TAXONOMÍA Y FILOGENIA DE AISLADOS CLÍNICOS Y AMBIENTALES DE PENICILLIUM Y TALAROMYCES

Marcela Isabel Guevara Suárez

UNIVERSITAT ROVIRA i VIRGILI

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MARCELA ISABEL GUEVARA SUÁREZ

TESIS DOCTORAL
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Dirigida por los doctores: José Francisco Cano Lira, Josepa Gené Díaz y Dania García Sánchez.

Departament de Ciències Mèdiques Bàsiques

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HAGO CONSTAR que el presente trabajo, titulado "**TAXONOMÍA Y FILOGENIA DE AISLADOS CLÍNICOS Y AMBIENTALES DE** *Penicillium* **y** *Talaromyces*.", que presenta **Marcela Isabel Guevara Suárez** para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Ciencias Médicas Básicas de esta universidad.

Reus, 09 de Enero de 2018

Los directores de la tesis

José F. Cano Lira   Josepa Gené Díaz   Dania García Sánchez

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A mi madre

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ÍNDICE

ÍNDICE DE ABREVIATURAS

ADN	Ácido desoxirribonucleico
BAL	Lavado broncoalveolar
BenA	Fragmento del gen β- tubulina
BI	Inferencia Bayesiana
CaM	Calmodulina
CBS	Colección internacional CBS-KNAW del *Westerdijk Fungal Biodiversity institute* (Holanda)
CMI	Concentración mínima inhibitoria
cm	Centímetro
CREA	Agar creatina sacarosa
CYA	Agar extracto de levadura Czapek
CYAS	Agar extracto de levadura Czapek con NaCl al 5%
CZ	Agar Czapek
DG18	Agar de dicloran-glicerol al 18%
diam	Diámetro
et al.	Y colaboradores
EE.UU.	Estados Unidos de América
FMR	Colección de cultivos de la *Facultat de Medicina de Reus*
g	Gramo
ICPA	Comisión Internacional de *Penicillium* y *Aspergillus*
ITS	Región espaciadora intergénica transcrita del ARNr (ARN ribosómico)
L	Litro
MALDI-TOF	*Matrix Assisted Laser Desorption Ionization – Time of Flight*
MEA	Agar extracto de malta
min	Minuto
ML	Máxima verosimilitud
mL	Mililitro
OA	Agar harina de avena
PCA	Agar patata zanahoria
PCR	Reacción en cadena de la polimerasa
PDA	Agar patata dextrosa
rpb1	ARN polimerasa subunidad I
rpb2	ARN polimerasa subunidad II
s. st	*sensu stricto*

sp. nov.	Nueva especie
UTHSCSA	*Fungus Testing Laboratory of the University of Texas Health Science Center in San Antonio* (EE.UU.)
YES	Agar extracto de levadura sacarosa
µg	Microgramo
µL	Microlitro
µm	Micrometro
ºC	Grados Celsius

1. INTRODUCCIÓN

1.1. Antecedentes históricos de *Penicillium* y *Talaromyces*

El género *Penicillium* fue introducido hace más de 200 años por Johann Heinrich Friedrich Link (1809), quien propuso dicho nombre por la semejanza de las estructuras de reproducción asexual (anamorfo) o conidióforos con un pequeño pincel —*Penicillium,* derivado del latín *penicillius* que significa 'pincel'—. Link describió tres especies: *P. candidum*, *P. expansum* y *P. glaucum*. Actualmente, *P. candidum* y *P. glaucum* se consideran especies sinónimas, y *P. expansum* se acepta como la especie tipo del género. En 1874, Julius Oscar Brefeld describió por primera vez las estructuras de reproducción sexual (teleomorfo) en *Penicillium*, consistentes en, ascomas cleistoteciales similares a esclerocios, con paredes duras y rígidas, dentro de los cuales se producían ascosporas elipsoidales de paredes ornamentadas. Estas estructuras pertenecían a *P. crustaceum*. En 1892, dicha especie fue transferida a *Eupenicillium* por Friedrich Ludwig, quien introdujo por primera vez un género teleomórfico para *Penicillium*.

Algunos de los aportes más importantes en la taxonomía del género fueron realizados por François Dierckx (1901) y Philibert Biourge (1923). En 1901, teniendo en cuenta los patrones de ramificación del conidióforo, Dierckx dividió a *Penicillium* en tres subgéneros (*Aspergilloides*, *Biverticillium* y *Eupenicillium*), además de describir 25 nuevas especies. Desafortunadamente, Dierckx no preservó material para ninguna de sus especies, contandose únicamente con los protólogos para la identificación de cada una de ellas. En 1923, Biourge publicó la primera monografía del género en la que se describieron 60 nuevas especies; asimismo, logró recuperar un número considerable de las especies descritas por Dierckx. Sin embargo, cabe destacar que a pesar de utilizar los mismos criterios de clasificación propuestos por Dierckx, Biourge cambió el nombre del subgénero *Aspergilloides* por el de *Monoverticillium*, y dividió el subgénero *Biverticillium* en dos secciones, una denominada *Coremigena* (especies sinematosas —conidióforos agrupados formando sinemas) y la otra *Simplicia* (especies mononematosas —conidióforos solitarios).

El concepto moderno de clasificación taxonómica basado en subgéneros y secciones en *Penicillium* deriva de las revisiones monográficas de Thom (1930) y de Raper y Thom (1949), realizadas por Pitt (1979), autor que divide a *Penicillium* en cuatro subgéneros: *Aspergilloides*, *Biverticillium, Furcatum* y *Penicillium,* así como un total de 11 secciones.

Charles Thom es considerado el padre de la taxonomía de *Penicillium*. En su primer trabajo, Thom describió únicamente las especies *P. camemberti* y *P. roqueforti* (Thom 1906), pero su siguiente publicación (Thom 1910) se destaca por incluir 13

nuevos taxones y por proponer la primera clave dicotómica para la identificación de las 36 especies conocidas hasta la fecha. Sin duda, otra de sus grandes contribuciones fue la de señalar la influencia de la temperatura en la distribución del género en la naturaleza, siendo además el primero en destacar la importancia del control de este parámetro para prevenir el deterioro de los alimentos almacenados. En este sentido, fue pionero también en relacionar las tasas de crecimiento de las diferentes especies con las temperaturas de incubación (Thom 1910). Posteriormente, en la monografía "*The Penicillia*" Thom publicó la primera revisión completa del género, aceptando e incluyendo las descripciones de 300 especies y las claves dicotómicas para la identificación de las mismas (Thom 1930). Siguiendo los criterios de Dierckx (1901), Thom subdividió al género en cuatro divisiones, 12 secciones y 18 subsecciones. Las especies que producían conidióforos biverticilados simétricos se clasificaron en la división *Biverticillata-Symmetrica* (Thom 1930), cuya mayoría se consideradan hoy especies del género *Talaromyces*.

Raper y Thom (1949), en su libro "*Manual of the Penicillia*", realizaron una revisión de las especies publicadas en la monografía de Thom, reduciendo a 137 el número total de especies aceptadas en el género. Dichas especies, fueron distribuidas en cuatro secciones y 41 series. Este manual fue el estándar para la clasificación e identificación de *Penicillium* por más de tres décadas; no obstante, sus propuestas no fueron aceptadas ampliamente al no tener en cuenta en su clasificación a los teleomorfos.

En 1955 —aproximadamente 80 años después de la primera descripción de un estado sexual en *Penicillium*—, Chester Ray Benjamin introdujo el segundo género teleomórfico para *Penicillium*, proponiendo el género *Talaromyces* para especies que producían ascomas cleistoteciales, de color típicamente amarillo, delimitados por una pared blanda y recibierta de un entremado de hifas, con ascos de ovalados a globosos y ascoporas espinosas. Cabe destacar sin embargo, que estas estrucutras sexuales había sido previamente observadas por Van Tieghem (1877) y Zukal (1889) en *P. aureum* y *P. luteum.* En consecuencia, Benjamin (1955) y posteriormente Udagawa y Takada (1966) transfirieron las especies de *Penicillium* con dichas características a *Talaromyces*. Más tarde, Stolk y Samson (1971, 1972) revisaron el género *Talaromyces* y lo limitaron a aquellas especies que formaban ascos en cadenas, proponiendo el género *Hamigera* para acomodar aquellas que producían ascos solitarios. Además, dichos autores, teniendo en cuenta la morfología del anamorfo, dividieron a *Talaromyces* en cuatro secciones: *Emersonii, Purpurea, Talaromyces* y *Thermophila*.

Entre los trabajos taxonómicos más importantes del siglo XX relacionados con el género *Penicillium* y sus teleomorfos, se encuentra el de Pitt (1979), quien en su monografía "*The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces*" describe e ilustra un total de 150 especies, concretamente 97 especies de *Penicillium* (distribuidas en cuatro subgéneros, 10 secciones y 21 series), 37 de *Eupenicillium* y 16 de *Talaromyces.* Pitt resaltó la importancia de los caracteres de las colonias (color, textura, producción de pigmentos, etc) para establecer las diferencias fenotípicas entre las especies, sugiriendo para su descripción las temperaturas de incubación 5, 25 y 37 °C a los siete días en diferentes medios de cultivo. Asímismo, observó la tendencia general de las especies del subgénero *Biverticillium* de ser morfológicamente más similares a las de *Talaromyces*, que a las especies de *Eupenicillium*, las cuales estas últimas producían conidióforos más semejantes a las especies clasificadas en los tres subgéneros restantes. Durante dicho siglo, se introdujeron más de 1000 nombres en el género *Penicillium.* Sin embargo actualmente, la mayoría de ellos no se aceptan por diferentes razones, entre ellas podemos destacar: 1) descripciones incompletas, 2) nombres publicados de forma no válida o 3) nombres de especies consideradas sinónimas de otras (Visagie *et al.* 2014a).

La clasificación de *Penicillium* y sus teleomorfos se actualizó a principios de los años 90, al incluir el concepto de "taxonomía polifásica", el cual se ha convertido en el estándar para la taxonomía de estos géneros al complementar la identificación morfológica con datos moleculares sobre el análisis de secuencias de diferentes genes, además de otros datos fenotípicos, tales como perfiles de extrolitos, crecimiento en diferentes medios a diferentes temperaturas, etc.

Berbee *et al.* (1995), mediante el análisis de secuencias de la subunidad menor del ARN ribosómico (SSU), demostraron que *Penicillium* era un género polifilético. Sus especies se distribuían en dos grandes grupos monofiléticos, uno incluía especies de *Talaromyces* y miembros del subgénero *Biverticillium*, mientras que el otro agrupaba las especies de *Eupenicillium* y especies de *Penicillium* encuadradas en los subgéneros *Aspergilloides, Furcatum* y *Penicillium.*

Boysen *et al.* (1996) fueron pioneros en utilizar un enfoque polifásico para delimitar especies de *Penicillium.* Estos autores combinaron el análisis de la región ITS (región espaciadora intergénica transcrita del ARNr), patrones de polimorfismos en la amplificación al azar del ADN, más conocida por el acrónimo en inglés RAPDs, y perfiles de extrolitos para la delimitación de las especies de la sección *Roqueforti.*

La más reciente y completa revisión taxonómica de *Penicillium* se realizó teniendo en cuenta los resultados presentados por Houbraken y Samson (2011) sobre

la revaluación de la familia *Trichocomaceae*. Los principales cambios taxonómicos y nomenclaturales realizados en este estudio se detallan en el apartado 1.2 de la presente memoria. La Tabla 1 muestra un resumen de la clasificación de *Penicillium* por secciones y sus respectivas especies tipo, propuesta en los priniclaples estudios taxonómicos del género realizados desde Pitt (1979) hasta la actualidad.

1.2.   Taxonomía y Nomenclatura

La identificación morfológica de los hongos pleomórficos y la posibilidad de atribuír nombres distintos a los estados anamórfico y teleomórfico de un mismo hongo ─Artículo 59 del Código Internacional de Nomenclatura Botánica (ICBN)─, condicionó durante muchos años la clasificación de una gran cantidad de micromicetes. Sin embargo, en las últimas décadas, los estudios moleculares, principalmente el análisis de secuencias del ADNr, han provocado cambios sustanciales no solo en la estructura taxonómica de estos organismos, sino también cambios nomenclaturales de los mismos. La posibilidad que clasificar un hongo independientemente de su morfología puso de manifiesto la incoherencia de la denominada "nomenclatura dual". Este hecho se trató, entre otros aspectos de la taxonomía fúngica, durante el Congreso Internacional de Botánica llevado a cabo en Julio de 2011 en Melbourne (Australia), y las propuestas que surgieron en el mismo condujeron a la creación del nuevo Código Internacional de Nomenclatura para Algas, Hongos y Plantas (McNeill *et al.* 2012). La propuesta más impactante fue la abolición del Artículo 59 sobre la nomenclatura dual de los hongos pleomórficos, la cual entró en vigencia el 1 de Enero de 2013.

El abandono de la nomenclatura dual dio lugar a cambios significativos en la taxonomía y la nomenclatura de *Penicillium, Talaromyces* y *Eupenicillium*. Diversos estudios sobre la filogenia de *Penicillium* evidenciaron que este género era polifilético (Berbee *et al.* 1995, LoBuglio *et al.* 1993, Ogawa y Sugiyama 2000). Houbraken y Samson (2011), basándose en una filogenia de cuatro genes, concretamente los correspondientes a los de las subunidades I y II de ARN polimerasa (*rpb*1 y *rpb*2), el factor de maduración del ribosoma (*Tsr*1) y la subunidad 8 de la chaperonina citoplasmática (*Cct*8), observaron que las especies de *Penicillium* se distribuían en dos clados principales: uno de ellos, definido por dichos autores como *Penicillium sensu stricto* (*s. st*), comprendía las especies de *Eupenicillium* y la mayoría de las especies previamente asignadas por Pitt (1979) a los subgéneros *Aspergilloides*, *Furcatum* y *Penicillium*; el otro clado incluía la mayoría de especies de *Talaromyces* y aquellas de *Penicillium* subgénero *Biverticillium*. Consecuentemente y siguiendo los conceptos de prioridad nomenclatural y nomenclatura única, las especies aceptadas de

Eupenicillium fueron transferidas a *Penicillium* y las de *Penicillium* subgénero *Biverticillium* al género *Talaromyces*.

El 14 de Abril de 2012, la Comisión Internacional de *Penicillium* y *Aspergillus* (ICPA) se reunió en Utrecht, Holanda, para discutir las implicaciones de la nomenclatura de nombre único en la taxonomía de los géneros *Aspergillus, Penicillium* y sus teleomorfos. Allí, se estableció por consenso la prioridad de uso del nombre anamórfico para especies clasificadas dentro de *Aspergillus s st.* y *Penicillium s st.*

Otro de los aportes del estudio de Houbraken y Samson (2011) fue la segregación de la familia *Trichocomaceae* en tres familias diferentes, situando a *Penicillium* y *Talaromyces* en familias distintas. Las nuevas familias propuestas en ese estudio fueron: *Aspergillaceae* (*Aspergillus, Hamigera, Leiothecium, Monascus, Penicilliopsis, Penicillium, Phialomyces, Sclerocleista, Warcupiella* y *Xeromyces*), *Thermoascaceae* (*Byssochlamys/Paecilomyces* y *Thermoascus*) y *Trichocomaceae* (*Rasamsonia, Sagenomella, Talaromyces, Thermomyces* y *Trichocoma*), .

Actualmente, *Penicillium* y *Talaromyces* están clasificados dentro del phylum *Ascomycota,* orden *Eurotiales,* clase *Eurotiomycetes*, y familias *Aspergillaceae* y *Trichocomaceae*, respectivamente (Figura 1). Los dos géneros cuentan con una clasificación por secciones basada en la identificación polifásica (Samson *et al.* 2010, Houbraken y Samson 2011, Visagie *et al.* 2014a). Es importante indicar que los nombres de las secciones utilizados en esta tesis corresponden a los aceptados en dichas publicaciones y no a los que actualmente se encuentran en el Index Fungorum.

Para clarificar y resolver las posibles cuestiones que se les puedan plantear a los investigadores sobre los cambios en la taxonomía de *Aspergillu*s, *Penicillium* y *Talaromyces*, la ICPA ha puesto a disposición pública una base de datos (http://www.aspergilluspenicillium.org). Esta base de datos proporciona listas de especies aceptadas para los tres géneros, incluyendo los números de acceso al *GenBank* de sus secuencias. Actualmente, el uso de análisis filogenéticos multilocus se aplica en la sistemática de *Penicillium* y *Talaromyces* y es indispensable para establecer los límites entre especies, utilizando el concepto de *Genealogical Concordance Phylogenetic Species Recognition* (GCPSR) propuesto por Taylor *et al.* (2000).

Tabla 1. Resumen de la clasificación por secciones en diferentes estudios de *Penicillium*. Adaptada de Houbraken y Samson (2011).

Pitt (1979)		Stolk y Samson (1985)		Houbraken y Samson (2011)		Clasificación actual[1]	
Sección	Especie tipo	Sección	Especie tipo	Sección	Especie tipo	Sección	Especie tipo
Aspergilloides	*P. aurantiobrunneum*	Aspergilloides	*P. glabrum*	Aspergilloides	*P. aurantiobrunneum*	Aspergilloides	*P. aurantiobrunneum*
Coremigenum	*P. duclauxii*	Biverticillium*	*P. minioluteum**	Brevicompacta	*P. olsonii*	Brevicompacta	*P. olsonii*
Coronatum	*P. olsonii*	Coremigenum	*P. duclauxii*	Canescentia	*P. canescens*	Canescentia	*P. canescens*
Cylindrosporum	*P. italicum*	Divaricatum	*P. janthinellum*	Charlesii	*P. charlesii*	Charlesii	*P. charlesii*
Divaricatum	*P. janthinellum*	Eladia	*P. sacculum*	Chrysogena	*P. chrysogenum*	Chrysogena	*P. chrysogenum*
Exilicaulis	*P. restrictum*	Geosmithia	*P. lavendulum*	Cinnamopurpurea	*P. cinnamopurpureum*	Cinnamopurpurea	*P. cinnamopurpureum*
Furcatum	*P. oxalicum*	Inordinate	*P. arenicola*	Citrina	*P. citrinum*	Citrina	*P. citrinum*
Inordinate	*P. arenicola*	Penicillium	*P. expansum*	Digitata	*P. digitatum*	Eladia	*P. sacculum*
Penicillium	*P. expansum*	Ramosum	*P. lanosum*	Eladia	*P. sacculum*	Exilicaulis	*P. restrictum*
Simplicium	*P. minioluteum*	Torulomyces	*P. lagena*	Exilicaulis	*P. restrictum*	Fasciculata	*P. viridicatum*
				Fasciculata	*P. viridicatum*	Fracta	*P. fractum*
				Fracta	*P. fractum*	Gracilenta	*P. gracilentum*
				Gracilenta	*P. gracilentum*	Lanata-divaricata	*P. janthinellum*
				Lanata-divaricata	*P. janthinellum*	Ochrosalmonea	*P. ochrosalmoneum*
				Ochrosalmonea	*P. ochrosalmoneum*	Osmophila	*P. osmophilum*
				Paradoxa	*P. paradoxus* (Antes *Aspergillus*)	Paradoxa	*P. paradoxus*
				Penicillium	*P. expansum*	Penicillium	*P. expansum*
				Ramigena	*P. cyaneum*	Ramigena	*P. cyaneum*
				Ramosum	*P. lanosum*	Ramosum	*P. lanosum*
				Roquefortorum	*P. roqueforti*	Robsamsonia	*P. robsamsonii*
				Sclerotiora	*P. sclerotiorum*	Roquefortorum	*P. roqueforti*
				Stolkia	*P. stolkiae*	Sclerotiora	*P. sclerotiorum*
				Thysanophora	*P. glaucoalbidum*** (Antes *Sclerotium*)	Stolkia	*P. stolkiae*
				Torulomyces	*P. lagena*	Thysanophora	*P. glaucoalbidum***
				Turbata	*P. turbatum*	Torulomyces	*P. lagena*
						Turbata	*P. turbatum*

[1]Clasificación actual de acuerdo a: Viagie *et al.* 2014a y Houbraken *et al.* 2016.
*Sección actualmente incluida en el género *Talaromyces* sección *Talaromyces* (*T. minioluteus*).
** Sin material tipo disponible.
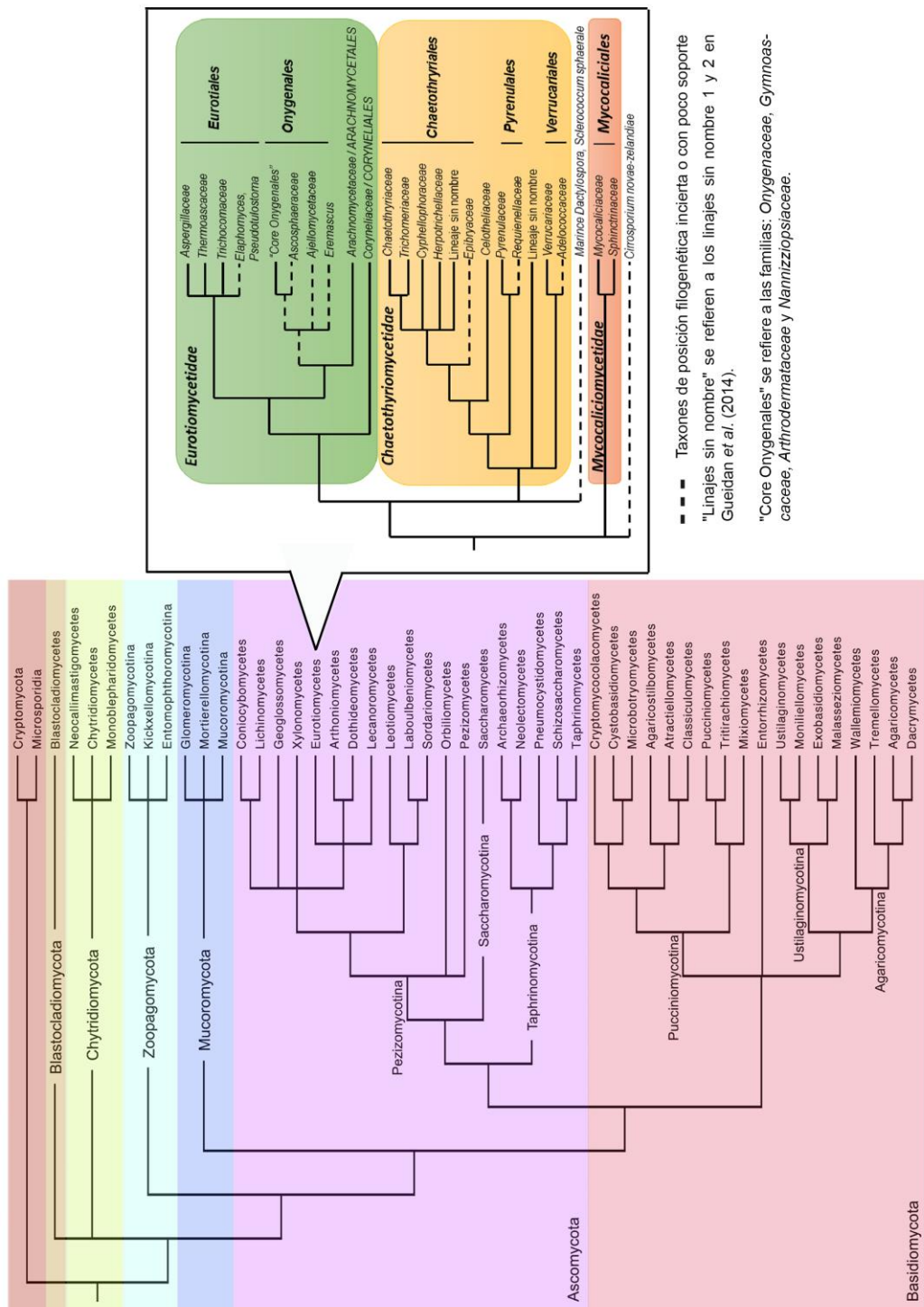
Figura 1. Representación esquemática de la ubicación taxonómica y de las relaciones filogenéticas dentro de los *Eurotiomycetes*. Adaptado de Geiser *et al.* 2015 y Spatafora *et al. 2017.*

De acuerdo a la última actualización de la base de datos de la ICPA, realizada en Marzo de 2017, el género *Penicillium* contiene más de 420 especies, distribuidas en 26 secciones (Figura 2a). Además, *Penicillium* tiene una clasificación adicional en dos subgéneros, *Aspergilloides* con 14 secciones y *Penicillium* con 12 secciones. Actualmente, el género *Talaromyces* incluye a más de 120 especies, distribuidas en siete secciones (Figura 2b).
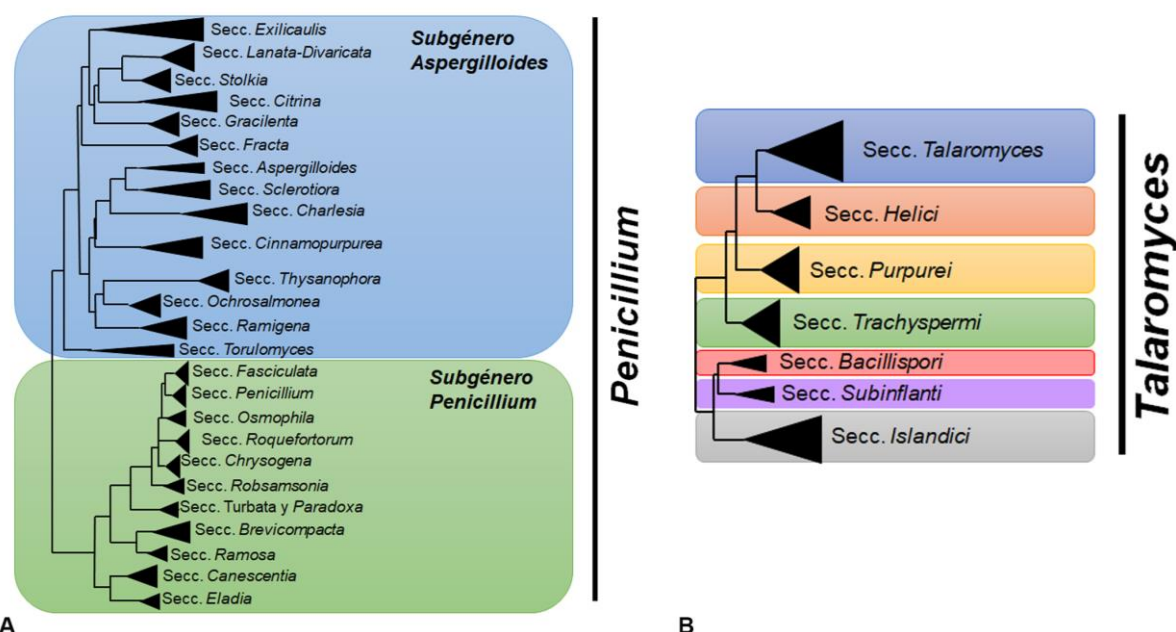
Figura 2. Representación esquemática de las relaciones filogenéticas en: **A.** *Penicillium*. **B.** *Talaromyces.* Adaptado de Kocsubé *et al.* 2016, Yilmaz *et al.* (2014).

La clasificación por secciones en dicho género se viene utilizado desde su introducción por Stolk y Samson (1972), aunque estos autores dividieron al género en cuatro secciones, teniendo principalmente en cuenta las diferencias morfológicas entre los estados anamórficos. Pitt (1979) aceptó esta clasificación por secciones e introdujo diferentes series para cada una de las secciones. En la Tabla 2 se muestra un resumen general de la clasificación por secciones de *Talaromyces* y sus especies tipo, derivado de los prinicipales estudios taxonómicos del género realizados en los últimos años.

Tabla 2. Resumen de la clasificación por secciones en diferentes estudios de *Talaromyces.* Adaptado de Yilmaz *et al.* (2014).

Stolk y Samson (1972)		Pitt (1979)		Yilmaz et al. (2014)	
Sección	**Especie tipo**	**Sección/Series**	**Especie tipo**	**Sección**	**Especie tipo**
Emersonii	T. emersonii	Penicillium secc. Simplicium ser. Islandica	P. islandicum	Bacillispori	T. bacillisporus
Purpurea	T. purpureus	Penicillium secc. Simplicium ser. Miniolutea	P. minioluteum	Helici	T. helicus
Talaromyces	T. flavus	Penicillium subgenus Biverticillium secc.	P. dendriticum	Islandici	T. islandicus
	(=T. vermiculatus)	Coremigenum ser. Dendritica			
Thermophila	T. thermophilus	Penicillium subgenus Biverticillium secc.	P. duclauxii	Purpurei	T. purpureus
		Coremigenum ser. Duclauxii			
		Purpureus/Purpurei	T. purpureus	Subinflati	T. subinflatus
		Talaromyces/Flavi	T. flavus	Talaromyces	T. flavus
		Talaromyces/Lutei	T. luteus	Trachyspermi	T. trachyspermus
		Talaromyces/Trachyspermi	T. trachyspermus		
		Thermophilus/Thermophili	T. thermophilus		

1.3. Características morfológicas generales

Durante años la identificación, clasificación y taxonomía de *Penicillium s. st.* y *Talaromyces* se basó exclusivamente en la observación de características morfológicas. En la actualidad, el estudio de características morfológicas se ha complementado con la inclusión de caracteres fisiológicos y genotípicos para darle a la identifcación un enfoque polifásico. Sin embargo, las características morfológicas siguen siendo relevantes en el momento de realizar la identificación de especies de los géneros estudiados la presente tesis. La morfología es el conjunto de características fenotípicas de un organismo que contempla aspectos macro y microscópicos de los mismos (Visagie *et al.* 2014a).

La gran mayoría de las especies de *Penicillium* y *Talaromyces* comparten características micromorfológicas similares (Figura 3). Los anamorfos de ambos géneros producen conidióforos compuestos por las siguientes estructuras: ***estipe****,* eje principal del conidióforo, generalmente largo y cilíndrico, relativamente estrecho (2–5 µm), y de paredes delgadas, en algunas especies se hincha apicalmente, y puede ser hialino o pigmentado con coloraciones de verde a marrón; ***métula****,* célula soporte de dónde se desarrollan las *células conidiógenas,* las métulas pueden estar ausentes cuando los conidióforos son simples (ver más adelante); ***células conidiógenas (fiálides)*** que pueden nacer directamente del ápice del estipe o de sus ramas, de forma asincrónica, disponiéndose en verticilios, suelen ser ampuliformes, raramente cilíndricas, y normalmente no superan los 15 µm de longitud; ***conidios****,* dispuestos en cadenas basípetas, no ramificadas, siempre son unicelulares, de forma globosa, elipsoidal o cilíndrica y con paredes lisas o ásperas, en masa suelen tener tonalidades verdes, oliva o marrón.
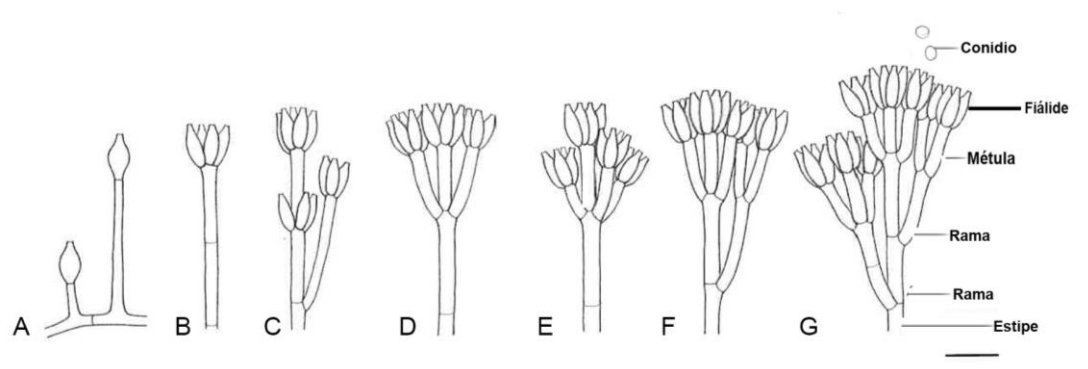
Figura 3. Tipos de conidióforo y patrones de ramificación de los mismos observados en *Penicillium*. **A.** Conidióforos con fialides solitarias. **B.** Conidióforo monoverticilado. **C.** Conidióforo divaricado. **D, E.** Conidióforos biverticilados. **F.** Conidióforo triverticilado. **G.** Conidióforo cuadriverticilado. Escala = 10 µm. Adaptado de Guarro *et al.* 2012.

Los ***conidióforos*** pueden presentar diferente grado de complejidad estructural, es decir, desde simples, con fiálides solitarias creciendo directamente de las hifas (Figura 3A), a conidióforos bien diferenciados y ramificados, con múltiples niveles de ramificación que dan lugar incluso a patrones de ramificación simétricos o asimétricos. Los ***conidióforos monoverticilados*** (Figura 3B) tienen un verticilio terminal de fiálides y, en algunas especies, la célula terminal del conidióforo está ligeramente hinchada o vesiculada; tales especies podrían confundirse con los diminutos conidióforos de algunas especies de *Aspergillus*, pero se diferencian generalmente por tener estipes septados, a diferencia de las especies de este último género. Los ***conidióforos divaricados***, antes también denominados irregulares (Figura 3C), se describen como conidióforos con un patrón de ramificación de simple a complejo con numerosas ramas subterminales. Los **conidióforos biverticilados** (Figura 3D y 3E) tienen un verticilo de tres o más métulas entre el extremo del estipe y las fiálides; las métulas pueden tener o no igual longitud, además de variar en su grado de divergencia, son habitualmente más o menos cilíndricas, pero también pueden ser claviformes o ligeramente vesiculadas. Los ***conidióforos triverticilados*** (Figura 3F) tienen otro nivel de ramificación entre el estipe y la métula, generalmente con una rama situada acontinuación del eje del estipe y una rama lateral. Finalmente, los ***conidióforos cuadriverticilados*** (Figura 3G) solo están presentes en un reducido número de especies y tienen un nivel extra de ramificación, más allá del patrón triverticilado. Los conidióforos triverticilados y cuadriverticilado tienden a ser claramente asimétricos. En colonias de muchas especies, especialmente cuando los cultivos comienzan a degenerar, puede haber más de un patrón de ramificación o formas intermedias, y puede ser difícil decidir qué patrón es típico o más abundante.

Como hemos mencionado anteriormente, los géneros teleomórficos históricamente asociados a *Penicillium sensu lato* (*s. l.*) han sido *Talaromyces* y *Eupenicillium* (este último actualmente es considerado sinónimo de *Penicillium s. st.*). Con relación a su morfología, las estructuras de reproducción sexual son distintas. En *Penicillium s. st.*, los ascomas son duros, de globosos a subglobsos, pseudoparenquimatosos o esclerenquimatosos, con paredes rígidas y gruesas, células isodiamétricas, madurando desde el centro hacia afuera y muy lentamente (pueden necesitar meses); blancos, amarillo pálidos, naranjas o marrones, ocasionalmente negruzcos o rojizos. A menudo, con ausencia de ascosporas (estériles). Ascos de elipsoidales a globosos, con un diámetro (diám) de 5–15 µm, y con 8 esporas. Ascosporas lenticulares, usualmente con crestas ecuatoriales, de 2–5 µm diám (Visagie *et al*. 2014a). Algunos de los caracteres morfológicos generales propios de las especies de *Penicillium* se muestran en la Figura 4.

En *Talaromyces*, los ascomas son frecuentemente de color amarillo, aunque también pueden ser blancos, de color crema, rosáceos o rojizos, generalmente presentan paredes peridiales delgadas y cubiertas por un entremado de hifas, y suelen madurar rápidamente (en pocas semanas). Ascos de globosos a elipsoidales, de 5–19 µm diám. Ascosporas unicelulares, a menudo con ornamentación superficial (espinas y/o crestas), de hialinas a amarillas, o rojizas pero solo en aquellas cepas productoras de abundante pigmento rojo (Yilmaz *et al*. 2014). Las características morfológicas generales de *Talaromyces* se presentan en la Figura 5.
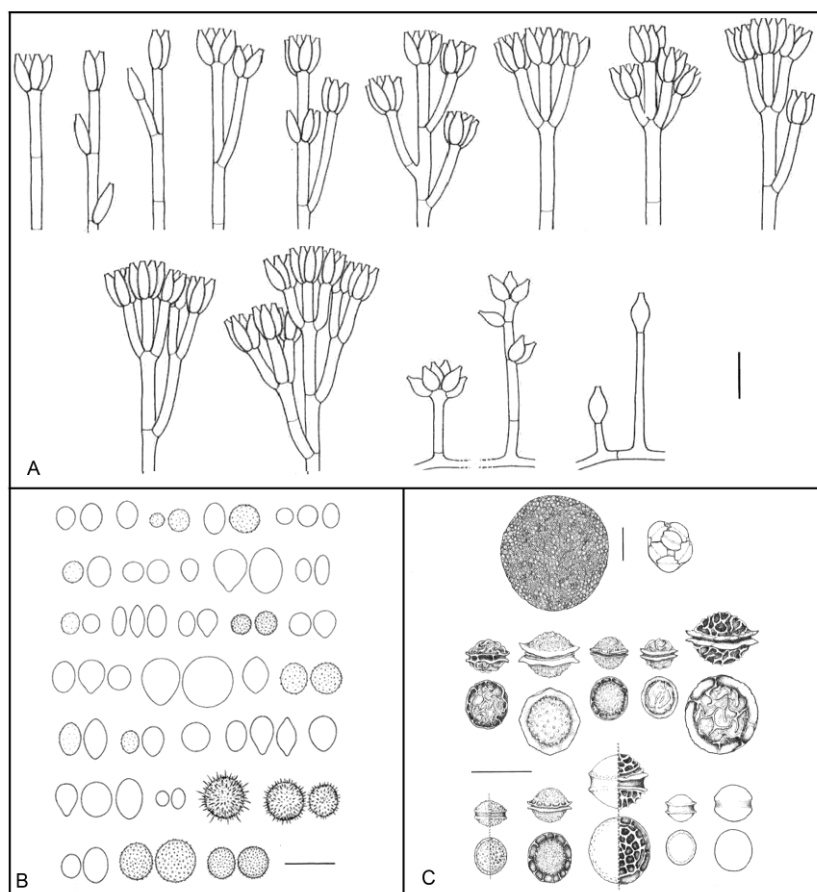
Figura 4. Características morfológicas generales de *Penicillium.* **A.** Conidióforos; **B.** Conidios; **C.** Ascoma, asco y ascosporas. Adaptado de: Guarro *et al.* 2012.
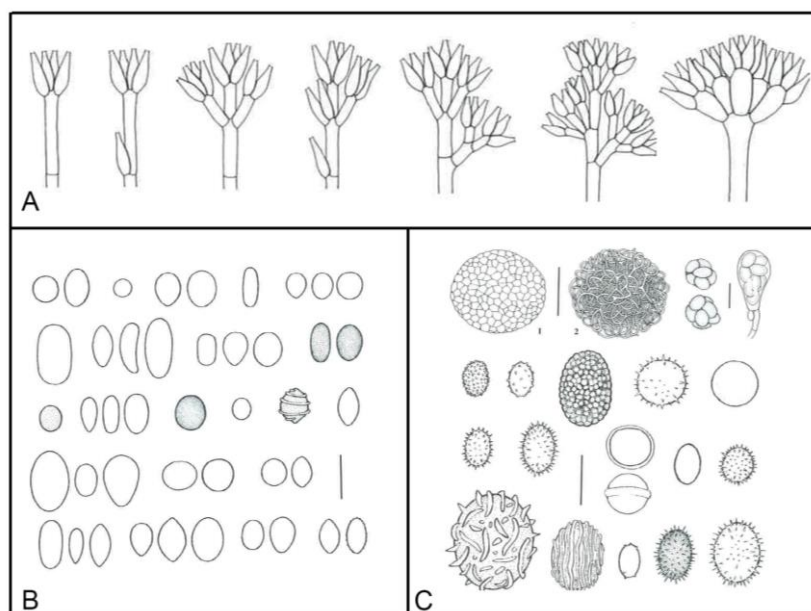
Figura 5. Características morfológicas generales de *Talaromyces.* **A.** Conidióforos; **B.** Conidios; **C.** Ascomas, ascos y ascosporas. Adaptado de: Guarro *et al.* 2012.

1.4. Identificación polifásica de *Penicillium* y *Talaromyces*

La identificación de una especie es un paso importante en la investigación biológica, un nombre correcto es vital para una comunicación óptima, ya que a menudo, éste el vínculo entre los estudios en diversos campos. Por lo tanto, es importante que la taxonomía sea clara y estable. Idealmente, la identificación debe ser inequívoca, precisa, simple e inmutable. La identificación polifásica se ha convertido en el estándar para la taxonomía de *Penicillium* y *Talaromyces,* y es recomendada, principalmente, para describir nuevas especies de estos géneros, incluyendo datos moleculares, morfológicos, fisiológicos y ecológicos (Figura 6).

1.4.1. Identificación fenotípica

Los caracteres fenotípicos (características macro y micromorfológicas y características fisiológicas), son importantes para la caracterización de las especies de *Penicillium* y *Talaromyces.* Recientemente, Visagie *et al.* (2014a) y Yilmaz *et al.* (2014) resumieron las principales características y los métodos que se deben tener en cuenta para el estudio fenotípico de aislados de estos dos géneros. Estos autores recomiendan como medios de cultivo estándar: agar extracto de malta (MEA), agar extracto de levadura Czapek (CYA), agar creatina sacarosa (CREA), agar harina de avena (OA; ideal para observar la producción de teleomorfos) y agar sacarosa extracto de levadura (YES); estos medios deberían incubarse idealmente a diferentes temperaturas (25, 30 y 37 ºC). Otros medios de cultivo alternativos recomendados para observar caracteres adicionales son: Blakeslee MEA, agar Czapek (CZ), agar de dicloran-glicerol al 18% (DG18) y CYA con NaCl al 5% (estos dos últimos con baja actividad de agua). Las placas con estos medios deben ser inoculadas en tres puntos equidistantes con suspensiones conidiales, estas últimas preparadas en medio de cultivo semisólido (0.2% de agar) con tween 80 al 0.05% (Pitt 1979). Para todos los cultivos se debe pemitir la aireación (Okuda *et al.* 2000), además de que estos sean preparados adicionando una solución de sulfato de zinc y sulfato de cobre como elementos traza (1 g de $ZnSO_4.7H_2O$ y 0.5 g de $CuSO_4.5H_2O$ en 100 mL de agua destilada) con el fin de favorecer una buena coloración de los conidios.
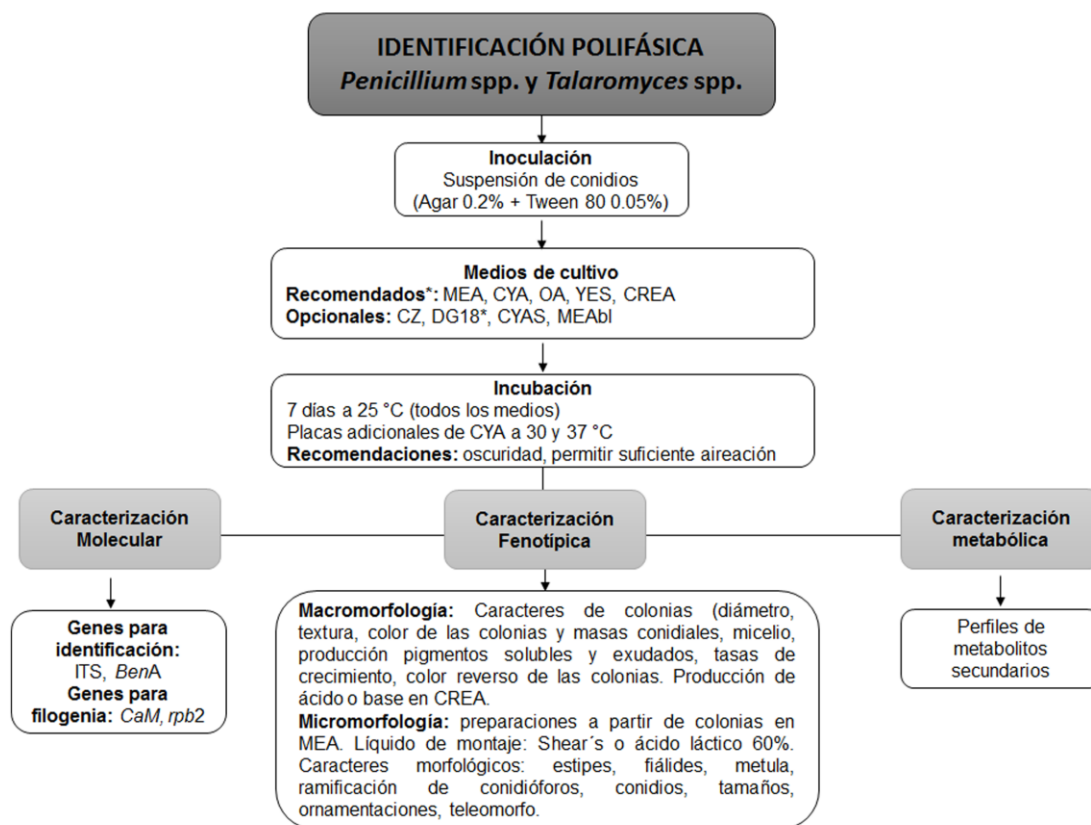
Figura 6. Diagrama de flujo que resume los métodos recomendados para la identificación y caracterización de especies de *Penicillium* y *Talaromyces*. Adaptado de Visagie *et al.* 2014a.

Para la caracterización macromorfológica, se deben tener en cuenta características como: tasas de crecimiento (diámetros de colonias), texturas de las colonias, grado de esporulación, color de las masas conidiales, presencia de pigmentos y exudados solubles, y color del reverso de las colonias. Para la caracterización micromorfológica se sugiere realizar preparaciones a partir de colonias en MEA con 7-10 días de incubación; cuando se observan abundantes masas conidiales es ideal realizar lavados con etanol al 70% que permitan la visualización de los conidióforos. Las principales características micromorfológicas (ver características micromorfológicas indicadas arriba) que deben tenerse en cuenta son: patrones de ramificación del conidióforo, dimensiones y pigmentación de todos los elementos del conidióforo (estipe, métula y fiálide), textura y ornamentación de la pared de los estipes y conidios (Visagie *et al.* 2014a). Para una mejor observación de la ornamentación conidial, se recomienda la utilización de microscopios ópticos con contraste interdiferencial (Nomarski) (Frisvad y Samson 2004, Samson *et al.* 2010, Visagie *et al.* 2014a).

1.4.2. Identificación molecular

La identificación de especies basada exclusivamente en caracteres morfológicos y fisiológicos es usualmente compleja y requiere personal bien entrenado. Además, el elevado número de especies actualmente aceptadas en estos géneros hace que esta tarea sea aún más difícil. Por ello, el uso de métodos moleculares representa una herramienta rápida y relativamente sencilla para la identificación a nivel de especie de *Penicllium* y *Talaromyces,* así como para las especies de otros géneros estrechamente relacionados. Recientemente, la región ITS fue aceptada como el primer código de barras para la identificación fúngica (Schoch *et al.* 2012). Sin embargo, varios estudios han demostrado que especies distintas de un mismo género pueden tener la misma secuencia de ITS (Viagie *et al.* 2014a, 2014b, Yilmaz *et al.* 2014).

Debido a las limitaciones asociadas a las secuencias de ITS en *Penicillium* y *Talaromyces*, con frecuencia, se necesita de otro marcador molecular para identificar de forma precisa a los aislados. Los requisitos que debe cumplir un marcador de identificación secundario son: ser fácil de amplificar, capaz de discriminar especies estrechamente relacionadas y, lo más importante, que el conjunto de datos de referencia debe ser completo, lo que significa que debe haber secuencias representativas y disponibles para todas las especies a comparar. Los genes codificantes de proteínas (genes estructurales) han sido ampliamente utilizados en micología para la identificación de especies al tener generalmente una mayor variabilidad interespecífica en comparación con la región ITS. Con base en estos criterios, se propuso el uso de un fragmento del gen β- tubulina (*Ben*A) como mejor marcador secundario para discriminar las especies de penicilia. Sin embargo, el *Ben*A presenta algunos inconvenientes, entre ellos: 1) los alineamientos en géneros con elevada diversidad interespecífica como *Penicillium* contiene un gran número de posiciones ambiguas, mayoritariamente localizadas en los intrones, lo que puede dificultar las filogenias; 2) existencia de genes parálogos en los *Eurotiales* (Peterson 2008, Hubka y Kolarik 2012, Peterson y Jurjević 2013). Otras alternativas de marcadores moleculares secundarios son: la calmodulina (*CaM*) o el *rpb*2, ambos genes tienen un poder discriminatorio similar al de *Ben*A. No obstante, el *rpb*2 tiene la ventaja de carecer de intrones en el amplicón, lo que permite una alineación robusta y fácil cuando se utiliza para filogenias, pero a veces es difícil de amplificar y la base de datos es más limitada. Del mismo modo, nos falta una base de datos completa para *CaM.* En resumen, *Ben*A es el marcador molecular por excelencia para la

identificación rutinaria de especies de *Penicillium* y *Talaromyces*, mientras que para la delimitación de nuevas especies se recomienda el uso combinado de ITS, *Ben*A y *CaM* o *rpb*2.

1.4.3. Perfiles de extrolitos

Los extrolitos o metabolitos secundarios son a menudo compuestos bioactivos, usualmente de bajo peso molecular, producidos como familias de compuestos relacionados con momentos particulares del ciclo de vida y suelen ser genotípicamente específicos para un grupo de especies (Keller *et al.* 2005, Hoffmeister y Keller 2007).

En los *Eurotiales,* se ha descrito que los extrolitos son usualmente producidos por el micelio y las estructuras esporulantes, además de evidenciar que los exudados, los pigmentos difusibles y los colores del reverso de las colonias son mezclas de metabolitos secundarios (Samson *et al.* 2011). Algunos de estos metabolitos son de relevancia industrial tal y como se explica en el apartado 1.5.1 de esta memoria. El estudio de los metabolitos secundarios dentro de los *Eurotiales* ha sido de gran importancia para complementar estudios taxonómicos (quimiotaxonomía) y ha permitido la caracterización de géneros y especies. Recientemente, se compararon los extrolitos de *Aspergillus*, *Penicillium* y *Talaromyces*, y se observó que los dos primeros compartían más de 90 familias biosintéticas, un número mayor a las que comparten *Penicillium* y *Talaromyces* (nueve familias); este hecho es congruente con los resultados de los análisis filogenéticos que muestran que *Aspergillus* y *Penicillium* forman parte de la familia *Aspergillaceae,* mientras que *Talaromyces* se ubica en la familia *Trichocomaceae* (Samson *et al.* 2011). A nivel de especie, la quimiotaxonomía ha sido útil para la identificación de especies dentro de los denominados complejos. Por ejemplo, el complejo de *P. aurantiogriseum* fue uno de los primeros en el que se logró discriminar entre especies mediante perfiles de extrolitos (Frisvad y Filtenborg 1983, 1989), diferenciación que mas tarde fue corroborada por el análisis de secuencias *Ben*A (Seifert y Louis-Seize 2000).

Para la caracterización de los metabolitos secundarios, usualmente, se utilizan colonias con 14 días de incubación a 25 °C; los medios de cultivo más usados para tal finalidad son los que contienen extracto de levadura y sacarosa, como el YES y el CYA (Viagie *et al.* 2014a). Los métodos para la caracterización dependerán de los equipos que tenga cada laboratorio destinado al procesamiento de muestras. En general, el proceso de caracterización incluye procesos de extracción, separación e identificación (Frisvad *et al.* 2008). Algunas de las metodologías de detección más

tradicionales son la cromatografía de capa fina (TLC; *Thin layer chromatography*) y la cromatografía de capa fina de alto rendimiento (HPLC; *High Performance Liquid Chromatography*) (Frisvad y Thrane 1987, 1993); mientras que dentro de las técnicas avanzadas encontramos la cromatografía líquida de alto rendimiento con detección de diodos y detección por espectrometría de masas de alta resolución (UHPLC-DAD-HRMS) (Kildgaard *et al.* 2014). A pesar de que se han establecido los patrones de metabolitos secundarios para un buén número de especies de *Penicillium* y *Talaromyces,* éstos no pueden ser usados como única herramienta para la identificación debido a que muchas de las especies propuestas recientemente carecen de perfiles de metabolitos secundarios descritos*.*

1.4.4. Genómica

La importancia de los miembros de las familias *Aspergillaceae* y *Trichochomaceae* a nivel biotecnológico se evidencia por el gran número de proyectos de secuenciación de genomas emprendidos. Actualmente en la base de datos de genomas del GenBank (https://www.ncbi.nlm.nih.gov/genome) se encuentran secuenciados los genomas de aproximadamente 34 especies de *Penicilllium* y 12 de *Talaromyces.* Sin embargo, varios de los genomas secuenciados corresponden a especies identificadas incorrectamente; un ejemplo lo tenemos en el genoma de la cepa Wisconsin 54-1255 que fue etiquetado como *P. chrysogenum*, sin embargo cicha cepa corresponde a *P. rubens* (Houbraken *et al.* 2011)*.* Por lo que la identificación correcta de las especies debería ser el primer paso en todos estos proyectos. (Houbraken *et al.* 2011, Samson *et al.* 2011). Para tal finalidad, se han realizado algunas recomendaciones antes del inicio de un proyecto de secuenciación de un genoma (Houbraken *et al.* 2014):

1. Las cepas deben de depositarse en dos o más colecciones públicas reconocidas. Esto garantiza que la cepa es fácilmente accesible para otros investigadores y para futuras investigaciones.

2. Realizar una identificación fiable de la cepa antes de la secuenciación del genoma. Idealmente, se sugiere ponerse en contacto con un taxónomo que pueda asesorar sobre la correcta identidad actual de la cepa.

3. Si el proyecto consiste en la secuenciación de un representante de una especie, la cepa seleccionada debe de responder a las características del protólogo. Las cepas tipo no son siempre la mejor opción porque generalmente han sido conservadas durante largos periodos de tiempo y por ello pueden estar deterioradas.

1.5. Importancia de *Penicillium* y *Talaromyces*

Tal y como se ha ido puntualizando, los géneros *Penicillium* y *Talaromyces* son importantes en diversos aspectos de la vida humana, ya sea en el campo de la biotecnología o industria (farmacéutica y alimentaria), o porque algunas de sus especies son de relevancia clínica por ser capaces de causar infecciones en humanos y animales.

1.5.1. Importancia indrustrial

En la industria farmacéutica, algunos de los compuestos más ampliamente conocidos y producidos por estos hongos son: la penicilina (antibiótico del grupo de los betalactámicos, *P. chrysogenum*), la grisofulvina (antifúngico, *P. griseofulvum*)*,* el ácido micofenólico (inmunosupresor, *P. brevicompactum*) o la compactina (agente anticolesterolémico, *P. brevicompactum*). En la industria alimentaria, se han utilizado en procesos de maduración de quesos (por ejemplo: queso camemberti, *P. camemberti*; queso roquefort, *P. roqueforti*) y salamis (*P. chrysogenum. P. nalgiovense* o *P. salami*) (Houbraken *et al*. 2014, Perrone *et al*. 2015). Sin embargo, algunas especies pueden producir micotoxinas como la patulina, la cual ha sido asociada al deterioro de frutas como: manzana, albaricoques, arándanos, cerezas, uvas, peras, melocotones y ciruelas. Algunas de las especies productoras de patulina son: *P. expansum*, *P. griseofulvum* y *P. novae-zeelandiae* (Dombrink-Kurtzman, Blackburn 2005). La patulina es estable en condiciones ácidas y resistente a la desnaturalización térmica, por lo que es difícil de eliminar de los alimentos que la contienen. Hay ciertas condiciones que pueden reducir el nivel de patulina detectada en algunos productos; por ejemplo, en el zumo de manzana la fermentación alcohólica puede reducir la patulina en más de un 99%, así como la adición de dióxido de azufre y la fortificación con vitamina C (Dombrink-Kurtzman, Blackburn 2005).

Por otra parte, la capacidad para producir enzimas y pigmentos solubles hace que *Talaromyces* y ,concretamente, las especies *T. funiculosus, T. pinophilus* y *T. purpurogenus* sean especies utilizadas en la producción de enzimas extracelulares (xilanasas y celulasas) y de pigmentos que se usan como colorantes naturales (Houbraken *et al*. 2014). La reciente revisión sobre la taxonomía de *T. purpurogenus*, mostró que esta especie es un complejo que consta de cuatro taxones: *T. purpurogenus, T. ruber*, *T. amestolkiae* y *T. stollii*. Desde un punto de vista biotecnológico, se recomienda utilizar *T. ruber* para la producción de enzimas, ya que *T. purpurogenus* produce cuatro tipos de micotoxinas y *T. amestolkiae* y *T. stollii* son

especies potencialmente patógenas para personas inmunocomprometidas (Yilmaz *et al.* 2012). Otra especie con potencial biotecnológico es *T. atroroseus,* recientemente descrita, al ser productora de pigmentos relacionados con la biosíntesis de mitorubrinas, además esta especie no produce micotoxinas (Frisvad *et al.* 2013).

En contraste, algunas especies también han sido usadas como agentes para el control biológico. Por ejemplo, *T. flavus* es uno de los antagonistas fúngicos más importantes utilizados como agente de control de patógenos transmitidos por el suelo, tales como *Verticillium dahliae*, *V. albo-atrum*, *Rhizoctonia solani* y *Sclerotinia sclerotiorum. Talaromyces thailandensis* también se ha descrito como agente biocontrolador inhibiendo aproximadamente el 70% de crecimiento micelial de *Fusarium oxysporum*, *Colletotrichum capsici*, *Phytophthora palmivora* y *Lasiodiplodia theobromae* (Yilmaz 2015)*.*

En la industria alimentaria, determinadas especies de *Talaromyces* destacan por el hecho de ser capaces de deteriorar los alimentos. Algunas de estas especies forman el teleomorfo y sus ascosporas son resistentes al calor, por lo que pueden persisitir en productos pasteurizados (Tournas 1994). Ejemplos de algunas de estas especies son *T. macrosporus, T. flavus, T. bacillisporus, T. helicus, T. stipitatus, T. trachyspermus and T. wortmannii*, las cuales se han identificado como responsables de alteraciones en zumos pasteurizados y otros productos a base de frutas (Pitt & Hocking 1997, Dijksterhuis 2007). Además, las ascosporas de *T. macrosporus* no solo son termonoresistentes sino que también son capaces de sobrevivir a tratamientos de ultrapresión (Dijksterhuis y Teunissen 2004).

1.5.2 Importancia clínica

A pesar de que las infecciones por especies de *Penicillium* y *Talaromyces* son poco frecuentes, se han descrito diversas especies como patógenas para el hombre y animales. Las manifestaciones clínicas de las infecciones ocasionadas por especies de ambos géneros incluyen micosis superficiales e infecciones invasivas, así como también alergias (Lyratzopoulos *et al.* 2002), pero el desarrollo de las mismas siempre suelen depender de la inmunidad del individuo (Yilmaz *et al.* 2012).

Dentro de *Talaromyces*, la especie de mayor relevancia clínica es *T. marneffei* (anteriormente *P. marneffei*). Se describe como agente causal de micosis sistémicas fatales principalmente en pacientes infectados por el virus de la inmunodeficiencia humana (VIH) del sureste de Asia, India y China (Yilmaz *et al.* 2014). A pesar del cambio nomenclatural de *T. marneffei*, las infecciones ocasionadas por este patógeno, se siguen reportando como "penicilosis". Otras especies de *Talaromyces* descritas

como oportunistas para el hombre son: *T. indigoticus,* causante de onicomicosis, y *T. piceus,* agente responsable de casos de infección pulmonar, osteomelitis y fungemia (Yilmaz *et al.* 2012, Yilmaz *et al.* 2015). Entre las especies patógenas oportunistas de *Penicillium* podemos citar a *P. chrysogenum* asociada a infecciones cutáneas e invasoras; a *P. citrinum* como responsable de queratitis, infecciones cutáneas y neumonía; a *P. decumbens* involucrada en infecciones paravertebrales; y, por último, a *P. oxalicum* responsable de micosis invasivas en pacientes con leucemia mieloide aguda, diabetes mellitus, y enfermedad pulmonar obstructiva crónica (de Hoog *et al.* 2001, Chowdhary *et al.* 2014).

En animales, existen muy pocos casos publicados de micosis ocasionadas por *Penicillium* y *Talaromyces*, y la mayoría son infecciones sistémicas y osteomielitis en perros. Algunas de las especies involucradas son: *P. brevicompactum*, *P. canis, T. helicus, T. purpurogenus, T. radicus* y *T. rugulosus* (Zanatta *et al.* 2006, Caro-Vadillo *et al.* 2007, Langlois *et al.* 2014).

El diagnóstico de las peniciliosis y talaromicosis (diferentes a las ocasionadas por *T. marneffei*), suele realizarse mediante el cultivo del hongo a partir de las muestras obtenidas de los posibles focos de infección, aunque un diagmostico presuntivo puede obtenerse mediante la observación de elementos fúngicos a partir de preparaciones directas de la muestra previamente tratada con KOH (muestras de tejido superficial) o preparaciones coloreadas a partir de biopsias y frotis de sangre (Kung *et al.* 2018). De todos modos, la identificación a nivel de especie rara vez se realiza en un laboratorio de rutina, y muchos de los casos son únicamente informados a nivel de género. No obstante, en la actualidad, el uso de herramientas moleculares representa una alternativa para llevar a cabo un diagnostico rápido y eficaz para la identificación de especies de *Penicillium* y *Talaromyces* asociadas a infecciones en humanos y animales (Yilmaz 2015). Además, en los últimos años, se vienen implementando técnicas alternativas para el diagnostico clínico, como el MALDI-TOF (*Matrix Assisted Laser Desorption Ionization – Time of Flight*), una tecnología basada en proteómica que se utiliza para caracterizar una amplia variedad de microorganismos incluyendo bacterias, hongos y virus (Croxatto *et al.* 2012). Recientemente, Masih *et al.* (2017) realizaron una caracterización por MALDI-TOF de especies de *Penicillium* y *Talaromyces* a partir de muestras respiratorias de pacientes con trastornos pulmonares. En este estudio se procesaron un total de 35 muestras, logrando identificar cinco especies de *Penicillium* (*P. chermesinum, P. chrysogenum, P. griseofulvum, P. citrinum* y *P. oxalicum*) y seis de *Talaromyces* (*T. beijingensis, T. cnidii, T. fusiformis, T. islandicus* y *T. stollii*). Este estudio demuestra el potencial de

esta técnica para la identificación de especies de estos dos géneros, aunque los autores resaltan que la base de datos comercial para especies de *Penicillium* y *Talaromyces* en el MALDI-TOF es muy limitada. No obstante, éste es un método efectivo y confiable para la identificación de especies de *Penicillium* y *Talaromyces,* pero siempre y cuando se disponga de una base de datos interna para las especies prevalentes a nivel local (Masih *et al.* 2017).

Cabe destacar que a excepción de *T. marneffei*, existen muy pocos datos sobre el perfil de sensibilidad antifúngica *in vitro* para especies *Penicillium* y *Talaromyces* frente a agentes antifúngicos clínicamente disponibles. Generalmente, los perfiles de sensibilidad disponibles provienen de casos clínicos puntuales o estudios en donde se ha ensayado un número muy reducido de cepas. En los mismos se observan resultados diferentes según la especie; por ejemplo *P. oxalicum* ha mostrado resistencia al tratamiento con voriconazol, teniéndose que recurrir al posaconazol como compuesto alternativo (Chowdhary *et al.* 2014), mientras que *P. citrinum,* ha presentado una resistencia general a los azoles (Mok *et al.* 1997). Recientemente, se ha publicado un caso de infección por *P. chrysogenum* en el que se describe resistencia a la anfotericina B liposomal, administrándose caspofungina como tratamiento alternativo (Avilés-Robles *et al.* 2016). Por otro lado, se ha observado también que las combinaciones de antifúngicos son una buena alternativa de tratamiento; un ejemplo es un caso de infección pulmonar por *P. capsulatum* cuyo paciente fue tratado satisfactoriamente con caspofungina y fluconazol (Chen *et al.* 2013). En el caso de *Talaromyces*, los estudios de sensibilidad antifúngica y las opciones de tratamiento se centran mayoritariamente en *T. marneffei*. En general, el tratamiento de las talaromicosis no está bien establecido, pero se ha observado que las concentraciones mínimas inhibitorias (CMI) en anfotericina B suelen ser variables y con una actividad antifúngica intermedia (Vanittanakom *et al.* 2006). En estos casos, los tratamientos alternativos suelen ser con voriconazol y caspofungina (Ouyang *et al.* 2017).

2. INTERÉS Y OBJETIVOS

El orden *Eurotiales* incluye numerosas especies de hongos microscópicos muy frecuentes en nuestro entorno y que pertenecen a las familias *Aspergillaceae* y *Trichocomaceae*. Los miembros de estas familias se caracterizan por ser hongos filamentosos, saprófitos, los cuales pueden encontrarse en numerosos sustratos y hábitats muy diversos. Algunos de los géneros incluidos en estas familias presentan características morfológicas similares, como es el caso de *Penicillium* y *Talaromyces,* y por ello, a menudo, nos referimos a estos como "penicilia", a pesar de ser géneros filogenéticamente distantes y bien delimitados (Visagie *et al.* 2014a, Yilmaz *et al.* 2014).

Penicillium y *Talaromyces*, junto con *Aspergillus,* son de los géneros fúngicos con mayor impacto económico para el hombre. Muchas de sus especies son importantes productoras de metabolitos secundarios de interés farmacéutico, biotecnológico, e industrial (Houbraken *et al.* 2014). Por ende, la investigación básica de estos géneros, en cuanto a la detección y propuesta de nuevas especies así como el depósito de sus cultivos en colecciones públicas, resulta un recurso esencial para la obtención de nuevas moléculas de potencial aplicación en distintos ámbitos. De ahí el interés del estudio de sustratos poco explorados, como los excrementos de herbívoros, cuya diversidad de especies de los géneros arriba indicados en este tipo de sustrato es aún muy poco conocida. A pesar de que los *Eurotiales* no se consideran predominantemente coprófilos (Krug *et al.* 2004), en el género *Penicillium* existe un grupo, representado por la sección *Robsamsonia*, que engloba principalmente especies coprófilas (Houbraken *et al.* 2016). Considerando el alto nivel de adaptación de los miembros de las familias *Aspergillaceae* y *Trichocomaceae* a diferentes sustratos con poca disponibilidad de agua (Kuthubutheen y Webster 1986), combinado con las características físico-químicas particulares que ofrecen las muestras de heces, podemos suponer que dicho sustrato es el escenario perfecto para la detección de potenciales nuevas especies en este grupo de hongos.

Cabe destacar también que algunas especies de *Talaromyces*, como *T. marneffei*, y, en menor medida, algunas de *Penicillium*, se han descrito como agentes causales de infecciones, tanto en animales como en humanos (de Hoog *et al.* 2011). Sin embargo, la diversidad o prevalencia en clínica de estos hongos ha sido escasamente estudiada, probablemente debido al hecho de que hay una cierta tendencia a considerarlos meros contaminantes ambientales. La profusa esporulación que suelen presentar las especies de *Penicillium* y *Talaromyces*, así como la facilidad de dispersión de sus conidios a través del aire, los convierte en hongos muy ubicuos, siendo, por ejemplo, éstos algunos de los géneros más comúnmente aislados en

ambientes cerrados (Pitt y Hocking 2009, Samson *et al.* 2010, Visagie *et al.* 2014a), como puede ser el intrahospitalario (Garcia-Hermoso *et al.* 2015). Sin embargo, cuando se aíslan de muestras clínicas, tal y como se ha indicado previamente, se descartan por considerarse cepas contaminantes (Garcia-Hermoso *et al.* 2015). Por otra parte, en los laboratorios clínicos rara vez se lleva a cabo la identificación a nivel de especie debido probablemente, a la complejidad de los métodos de estudio tanto fenotípicos como moleculares. En consecuencia, hoy en día no solo existe poca información acerca de la diversidad de sus especies en este ámbito, sino que también se desconocen los perfiles de sensibilidad antifúngica *in vitro* de las especies prevalentes y que pueden ser potencialmente patógenas, especialmente en un ambiente hospitalario con individuos inmunocomprometidos.

Aunque la biología molecular nos facilita la rápida identificación de especies, ésta nos obliga también al reestudio de muchos grupos fúngicos que, como en el caso de los hongos en los que nos centramos en la presente tesis, presentan una gran diversidad de especies, muchas de ellas definidas todavía a partir de caracteres fenotípicos. La estandarización de la metodología de estudio propuesta por Viagie *et al.* (2014a) y Yilmaz *et al.* (2014), basada en la combinación de caracteres morfológicos, fisiológicos y el análisis multilocus de secuencias, ha sido clave para dilucidar la estructura taxonómica de *Penicillium* y *Talaromyces*. Por consiguiente, en cualquier estudio sobre la biodiversidad de estos géneros, resulta imprescindible la aplicación de dicha metodología. Además, es preciso resaltar que existe todavía una larga lista de penicillia cuya posición taxonómica es incierta y que solo la obtención de nuevos aislados nos permitirá resolver dicha problemática.

Teniendo en cuenta lo expuesto, el **objetivo general** de esta tesis es **explorar la diversidad de especies de los géneros *Penicillium* y *Talaromyces* mediante una aproximación polifásica de aislados clínicos y ambientales.**

Para su consecución, se desarrollarán los siguientes objetivos específicos:

1. Identificar las cepas de ambos géneros aisladas a partir de muestras clínicas, de suelo y de heces de animales, mediante técnicas de identificación tradicionales y moleculares.

2. Determinar las relaciones filogenéticas de las especies identificadas mediante análisis multilocus de secuencias.

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

4. Determinar la sensibilidad antifúngica *in vitro* de las especies más frecuentemente aisladas de muestras clínicas.

3. MATERIALES Y MÉTODOS

3.1 Origen de los aislados

En los diferentes estudios de esta tesis se incluyeron un total de 314 aislamientos (Tabla 3), de los cuales: 118 (38%) se obtuvieron de muestras clínicas (108 de origen humano, seis de origen animal, uno de ambiente intrahospitalario, y tres de origen desconocido) y 196 (62%) de muestras ambientales (101 aislados de heces de animales y 95 de muestras de suelo) (Figura 7). Los aislados de origen clínico fueron proporcionados por el *Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio* (UTHSCSA, EE.UU.) y en su mayoría se aislaron de muestras del tracto respiratorio (86, 73%), principalmente de lavado broncoalveolar (BAL).
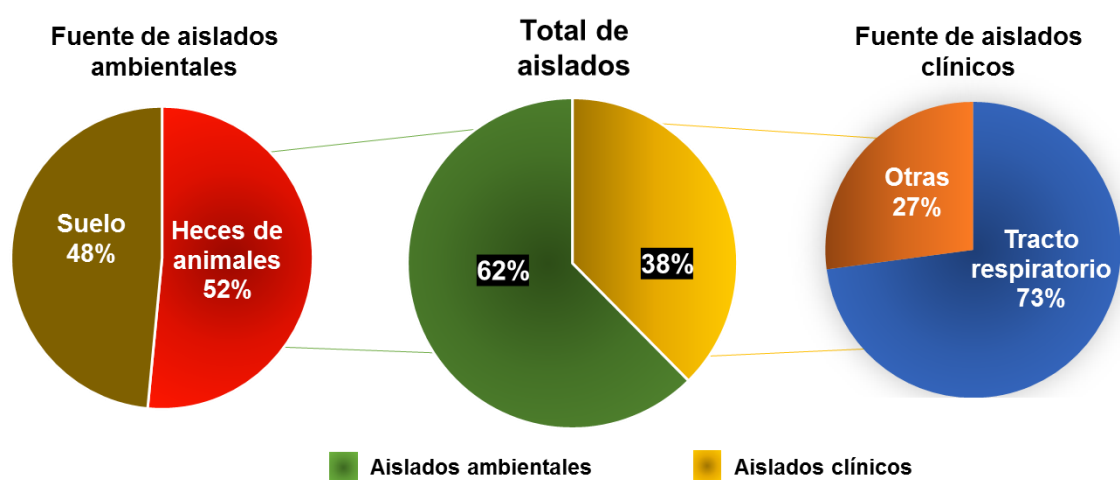
Figura 7. Porcentaje y origen de los aislados estudiados en esta tesis.

3.2 Origen de las muestras ambientales

Las muestras de heces de animales se colectaron mayormente durante el 2016 de diferentes regiones de España, como Andalucía, Baleares, Canarias, Cantabria, Castilla y León, Cataluña, Extremadura y Galicia. Se obtuvieron un total de 130 muestras de excrementos, en su mayoría heces de hervíboros como caballo, cabra, ciervo, conejo y oveja, aunque ocasionalmente también de otros animales cerdo salvaje y zorro.

Las muestras de suelo estudiadas se colectaron entre los años 1993 – 2016, las cuales se conservaron en cámara fría a 4 (± 2) °C durante todo ese tiempo. Estas muestras procedían de diversos países (Argentina, Brasil, Colombia, Costa Rica, EE.UU., España, México, Panamá, Portugal, Uruguay, Venezuela y Vietnam).

Tabla 3. Aislados de penicilia incluidos en esta tesis, identificación, orígen y números de acceso de las diferentes colecciones y de las secuencias depositadas en el GenBank.

Especie	Section	Números de colección	Sustrato	País/Localidad	Números de acceso GenBank			
					ITS	BenA	CaM	rpb2
P. adametzioides	*Sclerotiora*	FMR 13959 = UTHSCSA DI16-82	Cornea	EE.UU./Texas	LT558904	LT559022	-	-
P. allii	*Fasciculata*	FMR 14251 = UTHSCSA DI16-106	Senos Paranasales	EE.UU./Massachusetts	LT558928	LT559046	-	-
P. arabicum	*Exilicaulis*	FMR 14515	Suelo	España/Cataluña	-	-	-	-
		FMR 15095	Heces	España/Cataluña	-	LT898225	-	-
		FMR 15298	Heces	España/Castilla y León	-	LT898226	-	-
P. atramentosum	*Paradoxa*	FMR 15046	Heces	España/Cataluña	-	LT898221	-	-
		FMR 15092	Heces	España/Cataluña	-	LT898222	-	-
		FMR 15102	Heces	España/Cataluña	-	LT898223	-	-
		FMR 15309	Heces	España/Castilla y León	-	LT898224	-	-
		FMR 15510	Suelo	Brasil/São Paulo	-	-	-	-
P. balearicum	*Paradoxa*	FMR 15191 = CBS 143044	Heces	España/Islas Baleares	LT899762	LT898227	LT899758	LT899760
		FMR 15196	Heces	España/Islas Baleares	LT899763	LT898228	LT899759	LT899761
P. beceitense	*Ramosa*	FMR 15038 = CBS 142989	Heces	España/Cataluña	LT899780	LT898229	LT899764	LT899798
P. biforme	*Fasciculata*	FMR 15312	Heces	España/Castilla y León	-	LT898230	-	-
		FMR 15313	Heces	España/Castilla y León	-	LT898231	-	-
P. boreae	*Stolkia*	FMR 15640	Suelo	España/Islas Canarias	-	-	-	-
		FMR 15643	Suelo	España/Islas Canarias	-	-	-	-
P. brasilianum	*Lanata-Divaricata*	FMR 14296 = UTHSCSA DI16-117	Esputo	EE.UU./California	LT558939	LT559056	-	-
		FMR 15483	Heces	España/Galicia	-	LT898232	-	-
P. brefeldianum	*Lanata-Divariacata*	FMR 14716	Suelo	Colombia/Bogotá	-	-	-	-
P. brevicompactum	*Brevicompacta*	FMR 14024 = UTHSCSA DI16-88	Lesión Ósea	EE.UU./Texas	LT558910	LT559028	-	-

Especie	Sección	Cepa	Sustrato	Origen				
		FMR 14025 = UTHSCSA DI16-89	BAL	EE.UU./Illinois	LT558911	LT559029	-	-
P. burguense	*Exilicaulis*	FMR 14750	Suelo	México/-	-	-	-	-
		FMR 14751	Suelo	México/-	-	-	-	-
		FMR 15105	Heces	España/Cataluña	-	LT898233	-	-
		FMR 15103	Heces	España/Cataluña	-	LT898234	-	-
		FMR 15493	Heces	España/Galicia	-	LT898235	-	-
P. canariense	*Stolkia*	FMR 15838	Heces	España/Islas Canarias	-	LT898236	-	-
P. canescens	*Canescentia*	FMR 14475	Suelo	España/Islas Baleares	-	-	-	-
		FMR 14488	Suelo	España/Islas Baleares	-	-	-	-
		FMR 15028	Heces	España/Cataluña	-	LT898237	-	-
		FMR 15302	Suelo	España/Andalucía	-	-	-	-
		FMR 15651	Suelo	Argentina/-	-	-	-	-
P. caprifimosum	*Turbata*	FMR 15041 = CBS 142990	Heces	España/Cataluña	LT899781	LT898238	LT899765	LT899799
P. chrysogenum	*Chrysogena*	FMR 14008 = UTHSCSA DI16-53	Senos Paranasales	EE.UU./Minnesota	LT558875	LT558993	-	-
		FMR 14278 = UTHSCSA DI16-54	BAL	EE.UU./Massachusetts	LT558876	LT558994	-	-
		FMR 14480	Suelo	España/Asturias	-	-	-	-
		FMR 14493	Suelo	México/Cuernavaca	-	-	-	-
		FMR 14494	Suelo	México/Cuernavaca	-	-	-	-
		FMR 14499	Suelo	México/Merida	-	-	-	-
		FMR 14503	Suelo	-	-	-	-	-
		FMR 14505	Suelo	Brasil/São Paulo	-	-	-	-
		FMR 14940	Suelo	México/-	-	-	-	-
		FMR 15100	Heces	España/Cataluña	-	LT898244	-	-
P. cinereoatrum	*Exilicaulis*	FMR 14477	Suelo	España/Islas Baleares	-	-	-	-
		FMR 15033	Heces	España/Cataluña	-	LT898284	-	-
		FMR 14513	Suelo	España/Andalucía	-	-	-	-
P. citreonigrum	*Exilicaulis*	FMR 14250 = UTHSCSA DI16-86	BAL	EE.UU./Illinois	LT558908	LT559026	-	-

		Cepa	Origen	Procedencia				
P. citrinum	*Citrina*	FMR 13868 = UTHSCSA DI16-87	Esputo	EE.UU./Virginia	-	-	LT558909	LT559027
		FMR 15654	Suelo	España	-	-	-	-
		FMR 13878 = UTHSCSA DI16-62	BAL	EE.UU./Arizona	-	-	LT558884	LT559002
		FMR 13946 = UTHSCSA DI16-63	Senos Paranasales	EE.UU./Texas	-	-	LT558885	LT559003
		FMR 13956 = UTHSCSA DI16-64	Secreción Gástrica de Delfín	Bahamas	-	-	LT558886	LT559004
		FMR 13987 = UTHSCSA DI16-65	Senos Paranasales	EE.UU./Washington	-	-	LT558887	LT559005
		FMR 13997 = UTHSCSA DI16-66	Biopsia de Pulmón	EE.UU./Wisconsin	-	-	LT558888	LT559006
		FMR 14003 = UTHSCSA DI16-67	BAL	EE.UU./Minnesota	-	-	LT558889	LT559007
		FMR 14004 = UTHSCSA DI16-68	Senos Paranasales	EE.UU./Tennessee	-	-	LT558890	LT559008
		FMR 14007 = UTHSCSA DI16-69	Ojo	EE.UU./Nueva York	-	-	LT558891	LT559009
		FMR 14255 = UTHSCSA DI16-70	Senos Paranasales	EE.UU./Washington	-	-	LT558892	LT559010
		FMR 14262 = UTHSCSA DI16-71	Esputo	EE.UU./Virginia	-	-	LT558893	LT559011
		FMR 14264 = UTHSCSA DI16-72	BAL	EE.UU./Utah	-	-	LT558894	LT559012
		FMR 14285 = UTHSCSA DI16-73	Biopsia de Pulmón	EE.UU./Minnesota	-	-	LT558895	LT559013
		FMR 14309 = UTHSCSA DI16-74	Orina	EE.UU./Texas	-	-	LT558896	LT559014
		FMR 14313 = UTHSCSA DI16-75	BAL	EE.UU./Florida	-	-	LT558897	LT559015
		FMR 14717	Suelo	Colombia/Medellín	-	-	-	-
		FMR 14721	Suelo	Portugal	-	-	-	-
		FMR 14931	Suelo	Vietnam/-	-	-	-	-

Especie	Sección	Cepa	Sustrato	Procedencia						
P. commune	*Fasciculata*	FMR 15094	Heces	España/Cataluña	-	-	LT898239	-	-	-
		FMR 15486	Heces	España/Galicia	-	-	LT898240	-	-	-
		FMR 15497	Suelo	Brasil/São Paulo	-	-	-	-	-	-
		FMR 15505	Suelo	Brasil/São Paulo	-	-	-	-	-	-
		FMR 15507	Suelo	Brasil/São Paulo	-	-	-	-	-	-
		FMR 15515	Suelo	Brasil/São Paulo	-	-	-	-	-	-
		FMR 15520	Heces	España/Galicia	-	-	LT898241	-	-	-
		FMR 15636	Restos de Pingüino	Antartida	-	-	-	-	-	-
		FMR 15637	Suelo	España/desconocido	-	-	-	-	-	-
		FMR 15641	Suelo	España/Islas Canarias	-	-	-	-	-	-
		FMR 15646	Heces	España/Castilla y León	-	-	LT898242	-	-	-
		FMR 15647	Heces	España/Castilla y León	-	-	LT898243	-	-	-
		FMR 15648	Suelo	Brasil/São Paulo	-	-	-	-	-	-
		FMR 15649	Suelo	Argentina/-	-	-	-	-	-	-
		FMR 14497	Suelo	España/Andalucía	-	-	-	-	-	-
		FMR 14502	Suelo	España/Andalucía	-	-	-	-	-	-
P. concentricum	*Robsamsonia*	FMR 15195	Heces	España/Islas Baleares	-	-	LT898245	-	-	-
		FMR 15840	Heces	España/Castilla y León	-	-	LT898246	-	-	-
P. coprobium	*Robsamsonia*	FMR 15201	Heces	España/Islas Baleares	-	-	LT898247	-	-	-
		FMR 15311	Heces	España/Castilla y León	-	-	LT898248	-	-	-
P. coprophilum	*Robsamsonia*	FMR 13998 = UTHSCSA DI16-83	Senos Paranasales	EE.UU./Tennessee	-	-	LT559023	LT558905	-	-
P. coprophilum	*Robsamsonia*	FMR 15187	Heces	España/Islas Baleares	-	-	LT898249	-	-	-
P. corvianum	*Canescentia*	FMR 14753	Suelo	México/-	-	-	-	-	-	-
P. cremeogriseum	*Lanata-Diavricata*	FMR 15487	Heces	España/Galicia	-	-	LT898250	-	-	-
		FMR 15488	Heces	España/Galicia	-	-	LT898251	-	-	-
P. crustosum	*Fasciculata*	FMR 14300 = UTHSCSA DI16-100	Taburete intrahospitalario	EE.UU./Connecticut	-	-	LT559040	LT558922	-	-
		FMR 14298 = UTHSCSA DI16-101	Esputo	EE.UU./Tennessee	-	-	LT559041	LT558923	-	-

Cinnamopurpurea

Cepa	Sustrato	Origen				
FMR 13983 = UTHSCSA DI16-98	BAL	EE.UU./Montana	LT558920	LT559038	-	-
FMR 13957 = UTHSCSA DI16-99	Ganglio Linfático	EE.UU./Virginia	LT558921	LT559039	-	-
FMR 14491	Suelo	Uruguay/La Colonia	-	-	-	-
FMR 14929	Suelo	México/-	-	-	-	-
FMR 14930	Suelo	Vietnam/-	-	-	-	-
FMR 14932	Suelo	México/-	-	-	-	-
FMR 14933	Suelo	México/-	-	-	-	-
FMR 14938	Suelo	México/-	-	-	-	-
FMR 14941	Suelo	México/-	-	-	-	-
FMR 15034	Heces	España/Cataluña	-	LT898252	-	-
FMR 15036	Heces	España/Cataluña	-	LT898253	-	-
FMR 15037	Heces	España/Cataluña	-	LT898254	-	-
FMR 15042	Heces	España/Cataluña	-	LT898255	-	-
FMR 15043	Heces	España/Cataluña	-	LT898256	-	-
FMR 15045	Heces	España/Cataluña	-	LT898257	-	-
FMR 15098	Heces	España/Cataluña	-	LT898258	-	-
FMR 15106	Suelo	México/-	-	-	-	-
FMR 15108	Suelo	España/Andalucía	-	-	-	-
FMR 15185	Heces	España/Islas Baleares	-	LT898259	-	-
FMR 15186	Heces	España/Islas Baleares	-	LT898260	-	-
FMR 15189	Heces	España/Islas Baleares	-	LT898261	-	-
FMR 15194	Heces	España/Islas Baleares	-	LT898262	-	-
FMR 15197	Heces	España/Islas Baleares	-	LT898263	-	-
FMR 15200	Heces	España/Islas Baleares	-	LT898264	-	-
FMR 15205	Material vegetal	España/Cataluña	-	-	-	-
FMR 15213	Heces	España/Islas Baleares	-	LT898265	-	-
FMR 15494	Heces	España/Galicia	-	LT898266	-	-
FMR 15516	Suelo	Brasil/São Paulo	-	-	-	-
FMR 15517	Suelo	Brasil/São Paulo	-	-	-	-
FMR 15310	Heces	España/Castilla y León	-	LT898267	-	-

P. cvjetkovicii

Especie	Sección	Cepa	Sustrato	Origen				
P. decumbens	*Exilicaulis*	FMR 14319 = UTHSCSA DI16-84	BAL	EE.UU./Texas	LT558906	LT559024	-	-
P. echinulatum	*Fasciculata*	FMR 13945 = UTHSCSA DI16-97	Senos Paranasales	EE.UU./Tennessee	LT558919	LT559037	-	-
P. expansum	*Penicillium*	FMR 14754	Suelo	México/-	-	-	-	-
		FMR 15638	Suelo	España/Islas Canarias	-	-	-	-
		FMR 15097	Heces	España/Cataluña	-	LT898268	-	-
		FMR 15484	Heces	España/Galicia	-	LT898269	-	-
		FMR 15639	Suelo	España/Islas Canarias	-	-	-	-
P. fimosum	*Paradoxa*	FMR 15104 = CBS 142991	Heces	España/Cataluña	-	LT898273	-	-
P. flavigenum	*Chrysogena*	FMR 15096	Heces	España/Cataluña	-	LT898270	-	-
P. frequentans	*Aspergilloides*	FMR 14318 = UTHSCSA DI16-91	BAL	EE.UU./California	LT558913	LT559031	-	-
		FMR 15193	Heces	España/Islas Baleares	-	LT898271	-	-
		FMR 15212	Heces	España/Islas Baleares	-	LT898272	-	-
P. fuscum	*Aspergilloides*	FMR 14756	Suelo	México/-	-	-	-	-
P. glabrum	*Aspergilloides*	FMR 13995 = UTHSCSA DI16-92	Orina	EE.UU./Utah	LT558914	LT559032	-	-
		FMR 14252 = UTHSCSA DI16-93	Piel	EE.UU./Nueva York	LT558915	LT559033	-	-
		FMR 14257 = UTHSCSA DI16-94	Líquido senovial de Cadera	EE.UU./Michigan	LT558916	LT559034	-	-
		FMR 14292 = UTHSCSA DI16-95	Absceso Epidural	EE.UU./Minnesota	LT558917	LT559035	-	-
		FMR 14297 = UTHSCSA DI16-96	Tráquea Canina	EE.UU./California	LT558918	LT559036	-	-
		FMR 14481	Suelo	España/Asturias	-	-	-	-
		FMR 15184	Heces	España/Islas Baleares	-	LT898274	-	-
		FMR 15190	Heces	España/Islas Baleares	-	LT898275	-	-
		FMR 15209	Heces	España/Islas Baleares	-	LT898276	-	-
P. glycyrrhizacola	*Chrysogena*	FMR 15650	Suelo	Argentina/-	-	-	-	-

Especie	Sección	Cepa	Sustrato	Origen				
P. griseofulvum	*Robsamsonia*	FMR 15029	Heces	España/Cataluña	-	LT898277	-	-
		FMR 15030	Heces	España/Cataluña	-	LT898278	-	-
		FMR 15093	Heces	España/Cataluña	-	LT898279	-	-
		FMR 15203	Heces	España/Islas Baleares	-	LT898280	-	-
		FMR 15204	Heces	España/Islas Baleares	-	LT898281	-	-
		FMR 15207	Heces	España/Castilla y León	-	LT898282	-	-
		FMR 15314	Heces	España/Islas Baleares	-	LT898283	-	-
P. heteromorphum	*Exilicaulis*	FMR 14476	Suelo	España/Islas Baleares	-	-	-	-
		FMR 14478	Suelo	España/Castilla la Mancha	-	-	-	-
		FMR 14483	Suelo	España/Andalucía	-	-	-	-
P. ibericum	*Paradoxa*	FMR 15040 = CBS142992	Heces	España/Cataluña	LT899782	LT898285	LT899766	LT899800
		FMR 15107	Suelo	España/Galicia	LT899783	LT898286	LT899767	LT899801
P. janthinellum	*Lanata-Divaricata*	FMR 15514	Suelo	Brasil/São Paulo	-	-	-	-
P. kojigenum	*Ramosa*	FMR 14482	Suelo	España/Castilla la Mancha	-	-	-	-
P. lilacinoechinulatum	*Sclerotiora*	FMR 14720	Suelo	Portugal	-	-	-	-
P. magnielliptisporum	*Paradoxa*	FMR 15492	Heces	España/Galicia	-	LT898287	-	-
		FMR 15044	Heces	España/Cataluña	-	LT898288	-	-
P. mediterraneum	*Roquefortorum*	FMR 15031 = CBS 142755	Heces	España/Cataluña	LT899785	LT898289	LT899769	LT899803
		FMR 15032	Heces	España/Cataluña	LT899786	LT898290	LT899770	LT899804
		FMR 15188 = CBS 142754	Heces	España/Islas Baleares	LT899784	LT898291	LT899768	LT899802
P. melinii	*Exilicaulis*	FMR 15526	Suelo	Desconocido	-	-	-	-
P. meridianum	*Exilicaulis*	FMR 14514	Suelo	España/Cataluña	-	-	-	-
		FMR 14723	Suelo	E.E.U.U./Yosemite	-	-	-	-
P. momoii	*Exilicaulis*	FMR 15208	Heces	España/Islas Baleares	-	LT898292	-	-
P. murcianum	*Canescentia*	FMR 14511	Suelo	España/Islas Canarias	-	-	-	-
		FMR 15304	Heces	España/Andalucía	-	LT898293	-	-
		FMR 15305	Heces	España/Andalucía	-	LT898294	-	-

Especie	Sección	Cepa	Sustrato	Localidad				
		FMR 15308	Heces	España/Andalucía	-	LT898295	-	-
		FMR 15491	Heces	España/Galicia	-	LT898296	-	-
		FMR 15512	Suelo	Brasil/São Paulo	-	-	-	-
		FMR 15845	Heces	España/Galicia	-	LT898297	-	-
P. nigricans	Canescentia	FMR 14474	Suelo	España/Islas Baleares	-	-	-	-
P. oxalicum	Lanata-Divaricata	FMR 13953 = UTHSCSA DI16-108	BAL	EE.UU./Utah	LT558930	LT559048	-	-
		FMR 13960 = UTHSCSA DI16-109	Senos Paranasales	EE.UU./Minnesota	LT558931	LT559049	-	-
		FMR 13981 = UTHSCSA DI16-110	BAL	EE.UU./Texas	LT558932	LT559050	-	-
		FMR 14013 = UTHSCSA DI16-111	Esputo	EE.UU./Texas	LT558933	LT559051	-	-
		FMR 14249 = UTHSCSA DI16-112	BAL	Canada	LT558934	LT559052	-	-
		FMR 14261 = UTHSCSA DI16-113	Sangre	EE.UU./Missouri	LT558935	-	-	-
		FMR 14312 = UTHSCSA DI16-114	BAL	EE.UU./Minnesota	LT558936	LT559053	-	-
P. palitans	Fasciculata	FMR 14268 = UTHSCSA DI16-107	Desconocido	EE.UU./Washington	LT558929	LT559047	-	-
P. pancosmium	Citrina	FMR 13980 = UTHSCSA DI16-77	Líquido Peritoneal	EE.UU./Oklahoma	LT558899	LT559017	-	-
P. paneum	Roquefortorum	FMR 15524	Suelo	Desconocido	-	-	-	-
P. parvofructum	Exilicaulis	FMR 15047 = CBS 141690	Suelo	España/Cataluña	LT559091	LT627645	LT627646	-
P. pimiteouiense	Exilicaulis	FMR 14487	Suelo	Costa Rica	-	-	-	-
P. polonicum	Fasciculata	FMR 13965 = UTHSCSA DI16-102	Desconocido	EE.UU../Washington	LT558924	LT559042	-	-
		FMR 14259 = UTHSCSA DI16-103	Senos Paranasales	EE.UU./Tennessee	LT558925	LT559043	-	-
		FMR 14298 = UTHSCSA DI16-104	BAL	EE.UU./Washington	LT558926	LT559044	-	-
		FMR 13877 = UTHSCSA DI16-105	Sangre	EE.UU./Hawaii	LT558927	LT559045	-	-
		FMR 14934	Suelo	España/Andalucía	-	-	-	-

Especie	Cepa	Sección	Muestra	Origen						
					-	-	-	-	-	-
					-	-	-	-	-	-
P. radiolubatum	FMR 14936		Suelo	México/-	-	-	-	-		
P. raperi	FMR 15099	Canescentia	Heces	España/Cataluña	-	-	LT898298	-		
	FMR 15485		Heces	España/Islas Canarias	-	-	LT898299	-		
P. rolfsii	FMR 14500	Lanata-Divariacata	Suelo	Venezuela/-	-	-	-	-		
P. roqueforti	FMR 14307 = UTHSCSA DI16-115	Lanata-Divaricata	Bronquio	EE.UU./Texas	LT558937	LT559054				
	FMR 13867 = UTHSCSA DI16-116		BAL	EE.UU./California	LT558938	LT559055				
P. roseopurpureum	FMR 13869 = UTHSCSA DI16-79	Roquefortorum	Senos Paranasales	EE. UU/Tennessee	LT558901	LT559019				
	FMR 14269 = UTHSCSA DI16-80		Cadera	EE.UU./Minnesota	LT558902	LT559020				
	FMR 14295 = UTHSCSA DI16-81		Esputo	EE.UU./Washington	LT558903	LT559021				
P. roseoviride	FMR 14263 = UTHSCSA DI16-78	Citrina	Biopsia Nasal Canina	EE.UU./California	LT558900	LT559018				
P. rubefaciens	FMR 15645	Aspergilloides	Heces	España/Castilla y León	-	LT898300				
P. rubens	FMR 13947 = UTHSCSA DI16-85	Exilicaulis	BAL	EE.UU./Arizona	LT558907	LT559025				
	FMR 15202		Heces	España/Islas Baleares	-	LT898301				
	FMR 15297		Heces	España/Castilla y León	-	LT898302				
	FMR 13874 = UTHSCSA DI16-34	Chrysogena	Desconocido	EE.UU./Florida	LT558856	LT558974				
	FMR 13875 = UTHSCSA DI16-35		Cecal de pollo	EE.UU./Texas	LT558857	LT558975				
	FMR 13943 = UTHSCSA DI16-36		BAL	EE.UU./California	LT558858	LT558976				
	FMR 13967 = UTHSCSA DI16-37		Tejido cerebral	EE.UU./Pensilvania	LT558859	LT558977				
	FMR 13975 = UTHSCSA DI16-38		Cornea	EE.UU./Maryland	LT558860	LT558978				
	FMR 13989 = UTHSCSA DI16-39		Nasal	EE.UU./Carolina del Norte	LT558861	LT558979				

Sección / Especie	Cepa	Origen	País/Localidad				
	FMR 13991 = UTHSCSA DI16-40	Dedo del pie	EE.UU./Texas	LT558862	LT558980	-	-
	FMR 13999 = UTHSCSA DI16-41	Esputo	EE.UU./Virginia	LT558863	LT558981	-	-
	FMR 14015 = UTHSCSA DI16-42	Cornea	EE.UU./Texas	LT558864	LT558982	-	-
	FMR 14019 = UTHSCSA DI16-43	Senos Paranasales	EE.UU./Tennessee	LT558865	LT558983	-	-
	FMR 14245 = UTHSCSA DI16-44	Líquido Pericárdico	EE.UU./California	LT558866	LT558984	-	-
	FMR 14246 = UTHSCSA DI16-45	Biopsia de Pulmón	EE.UU./Pensilvania	LT558867	LT558985	-	-
	FMR 14256 = UTHSCSA DI16-46	Pleural	EE.UU./Minnesota	LT558868	LT558986	-	-
	FMR 14267 = UTHSCSA DI16-47	Válvula de aire	EE.UU./California	LT558869	LT558987	-	-
	FMR 14273 = UTHSCSA DI16-48	Nasal	EE.UU./California	LT558870	LT558988	-	-
	FMR 14279 = UTHSCSA DI16-49	Cateter	EE.UU./Texas	LT558871	LT558989	-	-
	FMR 14299 = UTHSCSA DI16-50	Esputo	EE.UU./Washington	LT558872	LT558990	-	-
	FMR 14314 = UTHSCSA DI16-51	BAL	EE.UU./Illinois	LT558873	LT558991	-	-
	FMR 14315 = UTHSCSA DI16-52	BAL	EE.UU./California	LT558874	LT558992	-	-
Aspergilloides — *P. rudallense*	FMR 13879 = UTHSCSA DI16-90	BAL	EE.UU./Massachusetts	LT558912	LT559030	-	-
Citrina — *P. sanguifluum*	FMR 15843	Heces	España/Islas Canarias	-	LT898303	-	-
	FMR 14485	Suelo	España/-	-	-	-	-
	FMR 14486	Suelo	España/-	-	-	-	-
	FMR 14489	Suelo	España/Islas Baleares	-	-	-	-
	FMR 14492	Suelo	Panamá/-	-	-	-	-
	FMR 14495	Suelo	Venezuela/-	-	-	-	-
	FMR 15301	Suelo	España/Andalucía	-	-	-	-
	FMR 15506	Suelo	Brasil/São Paulo	-	-	-	-

Especie	Sección	Cepa	Sustrato	Origen				
P. singorense	*Lanata-Divaricata*	FMR 15642	Suelo	España/Islas Canarias	-	-	-	-
P. sizovae	*Citrina*	FMR 14000 = UTHSCSA DI16-118	BAL	EE.UU./Texas	LT558940	LT559057	-	-
		FMR 15300	Heces	España/Castilla y León	-	LT898304	-	-
P. smithii	*Exilicaulis*	FMR 15521	Heces	España/Galicia	-	LT898305	-	-
P. sumatrense	*Citrina*	FMR 15525	Suelo	España/Islas Baleares	-	-	-	-
		FMR 14266 = UTHSCSA DI16-76	Nódulo Pulmonar	EE.UU./Wisconsin	LT558898	LT559016	-	-
P. synnematicola	*Robsamsonia*	FMR 15192 = CBS 142669	Heces	España/Islas Baleares	LT898167	LT898172	LT898137	LT898142
		FMR 15210	Heces	España/Islas Baleares	LT898168	LT898173	LT898138	LT898143
		FMR 15211	Heces	España/Islas Baleares	LT898169	LT898174	LT898139	LT898144
		FMR 16481 = CBS 143045	Suelo	España/Cataluña	LT898170	LT898175	LT898140	LT898145
		FMR 16491 = CBS 143046	Heces	España/Cataluña	LT898171	LT898176	LT898141	LT898146
P. terrigenum	*Citrina*	FMR 15500	Suelo	Brasil/São Paulo	-	-	-	-
P. ubiquetum	*Citrina*	FMR 15509	Suelo	Brasil/São Paulo	-	-	-	-
P. uruguayense	*Lanata-Divariacata*	FMR 14490 = CBS 143247	Suelo	Uruguay	LT904729	LT904699	LT904698	-
P. wotroi	*Lanata-Divariacata*	FMR 15518	Suelo	Brasil/São Paulo	-	-	-	-
P. yarmokense	*Canescentia*	FMR 14501	Suelo	E.E.U.U./Yosemite	-	-	-	-
		FMR 14722	Suelo	E.E.U.U./Yosemite	-	-	-	-
Penicillium sp.	*Chrysogena*	FMR 13876 = UTHSCSA DI16-55	Médula Ósea	EE.UU./Minnesota	LT558877	LT558995	-	-
		FMR 13942 = UTHSCSA DI16-56	BAL	EE.UU./California	LT558878	LT558996	-	-
		FMR 13949 = UTHSCSA DI16-57	Sangre	EE.UU./Texas	LT558879	LT558997	-	-
		FMR 13971 = UTHSCSA DI16-58	BAL	EE.UU./Texas	LT558880	LT558998	-	-
		FMR 14002 = UTHSCSA DI16-59	BAL	EE.UU./Minnesota	LT558881	LT558999	-	-
		FMR 14012 = UTHSCSA DI16-60	Esputo	EE.UU./Minnesota	LT558882	LT559000	-	-

Especie	Sección	Cepa	Muestra	Origen				
Penicillium sp.	*Exilicaulis*	FMR 14316 = UTHSCSA DI16-61	BAL	EE.UU./Minnesota	LT558883	LT559001	-	-
Penicillago nodositata		FMR 15841	Heces	España/Castilla y León	-	LT898311	-	-
Pgo. nodositata	-	FMR 15296 = CBS 142988	Heces	España/Islas Baleares	LT899787	LT898312	LT899771	LT899805
		FMR 16442	Heces	España/Extremadura	LT899788	LT898313	LT899772	LT899806
Pseudopenicillium coprobium	-	FMR 15299 = CBS 142670	Heces	España/Castilla y León	LT899789	LT898315	-	LT899807
Pse. giganteum	-	FMR 14718	Suelo	Suelo/desconocido	LT899790	LT898314	-	LT899808
Rasamsonia argillacea	-	FMR 14010 = UTHSCSA DI16-150	Biopsia de Pulmón	EE.UU./Tennessee	LT558972	LT559089	-	-
R. eburnea	-	FMR 14023 = UTHSCSA DI16-151	Biopsia de Pulmón	EE.UU./Nueva York	LT558973	LT559090	-	-
T. aculeatus	*Talaromyces*	FMR 15513	Suelo	Brasil/São Paulo	-	-	-	-
T. alveolaris	*Talaromyces*	FMR 13962 = UTHSCSA DI16-146	BAL	EE.UU./Texas	LT558968	LT559085	-	-
		FMR 13963 = UTHSCSA DI16-147 = CBS 142379	BAL	EE.UU./Utah	LT558969	LT559086	-	-
T. amestolkiae	*Talaromyces*	FMR 13941 = UTHSCSA DI16-129	BAL	EE.UU./Utah	LT558951	LT559068	-	-
		FMR 14260 = UTHSCSA DI16-130	Biopsia de Pulmón	EE.UU./Wisconsin	LT558952	LT559069	-	-
		FMR 14283 = UTHSCSA DI16-131	Esputo	EE.UU./Washington	LT558953	LT559070	-	-
		FMR 14290 = UTHSCSA DI16-132	BAL	EE.UU./Minnesota	LT558954	LT559071	-	-
		FMR 14304 = UTHSCSA DI16-133	BAL	EE.UU./Utah	LT558955	LT559072	-	-
		FMR 13951 = UTHSCSA DI16-134	BAL	EE.UU./Utah	LT558956	LT559073	-	-
		FMR 14294 = UTHSCSA DI16-135	BAL	EE.UU./Pensilvania	LT558957	LT559074	-	-
T. angelicus	*Talaromyces*	FMR 15489	Heces	España/Galicia	LT899791	LT898316	LT899773	LT899809
		FMR 15490	Heces	España/Galicia	LT899792	LT898317	LT899774	LT899810
T. atroroseus	*Trachyspermi*	FMR 14022 = UTHSCSA DI16-120	BAL	EE.UU./Utah	LT558942	LT559059	-	-

Especie	Sección	Cepa	Origen	Localización				
T. aurantiacus	*Talaromyces*	FMR 14286 = UTHSCSA DI16-127	Herida de Cuero Cabelludo	EE.UU./Minnesota	LT558949	LT559066	-	-
		FMR 13985 = UTHSCSA DI16-128	Pulmón Canino	EE.UU./California	LT558950	LT559067	-	-
T. catalonicus	*Trachyspermi*	FMR 16441 = CBS 143039	Heces	España/Cataluña	LT899793	LT898318	LT899775	LT899811
T. cnidii	*Talaromyces*	FMR 13970 = UTHSCSA DI16-138	BAL	EE.UU./Virginia	LT558960	LT559077	-	-
T. columbinus	*Islandici*	FMR 13944 = UTHSCSA DI16-119	Aspirado Traqueal	EE.UU./Nueva York	LT558941	LT559058	-	-
T. coprophilus	*Talaromyces*	FMR 15199 = CBS 142756	Heces	España/Islas Baleares	LT899794	LT898319	LT899776	LT899812
T. diversus	*Trachyspermi*	FMR 14283 = UTHSCSA DI16-121	Biopsia de Pulmón	EE.UU./Utah	LT558943	LT559060	-	-
T. funiculosus	*Talaromyces*	FMR 13986 = UTHSCSA DI16-139	BAL	EE.UU./Texas	LT558961	LT559078	-	-
T. gamsii	*Talaromyces*	FMR 15303 = CBS 143040	Suelo	España/Andalucía	LT899795	LT898320	LT899777	LT899813
T. georgiensis	*Helici*	FMR 14270 = UTHSCSA DI16-145 = CBS 142380	Líquido Senovial Animal	EE.UU./Georgia	LT558967	LT559084	-	-
T. kabodanensis	*Talaromyces*	FMR 13990 = UTHSCSA DI16-149	BAL	EE.UU./Washington	LT558971	LT559088	-	-
T. minnesotensis	*Trachyspermi*	FMR 14265 = UTHSCSA DI16-144 = CBS 142381	BAL	EE.UU./Minnesota	LT558966	LT559083	-	-
T. muroii	*Talaromyces*	FMR 15496	Oído	España/Galicia	-	LT898321	-	-
		FMR 14498	Suelo	Brasil/São Paulo	-	-	-	-
T. pinophilus	*Talaromyces*	FMR 13977 = UTHSCSA DI16-140	BAL	EE.UU./Montana	LT558962	LT559079	-	-
		FMR 13982 = UTHSCSA DI16-141	BAL	EE.UU./Montana	LT558963	LT559080	-	-
		FMR 14017 = UTHSCSA DI16-142	BAL	EE.UU./Arizona	LT558964	LT559081	-	-
T. pseudofuniculosus	*Talaromyces*	FMR 15035	Heces	España/Cataluña	LT899797	LT898322	LT899779	LT899815
		FMR 15307 = CBS 143041	Heces	España/Andalucía	LT899796	LT898323	LT899778	LT899814

Especie		Cepa	Fuente	Origen				
T. purpurogenus	Talaromyces	FMR 13973 = UTHSCSA DI16-122	BAL	EE.UU./Wisconsin	LT558944	LT559061	-	-
	Talaromyces	FMR 13988 = UTHSCSA DI16-123	BAL	EE.UU./Texas	LT558945	LT559062	-	-
	Talaromyces	FMR 14311 = UTHSCSA DI16-124	Biopsia de Pulmón	EE.UU./Wisconsin	LT558946	LT559063	-	-
	Talaromyces	FMR 14303 = UTHSCSA DI16-125	Esputo	EE.UU./Ohio	LT558947	LT559064	-	-
	Talaromyces	FMR 14308 = UTHSCSA DI16-126	BAL	EE.UU./Minnesota	LT558948	LT559065	-	-
T. rapidus	Talaromyces	FMR 14293 = UTHSCSA DI16-148 = CBS 142382	BAL	EE.UU./Ohio	LT558970	LT559087	-	-
T. ruber	Talaromyces	FMR 14001 = UTHSCSA DI16-136	Esputo	EE.UU./Minnesota	LT558958	LT559075	-	-
	Talaromyces	FMR 14288 = UTHSCSA DI16-137	Dedo	EE.UU./Washington	LT558959	LT559076	-	-
T. sayulitensis	Talaromyces	FMR 15839	Heces	España/Castilla y León	-	LT898324	-	-
	Talaromyces	FMR 15842	Heces	España/Islas Canarias	-	LT898325	-	-
T. subaurantiacus	Islandici	FMR 13996 = UTHSCSA DI16-143	BAL	EE.UU./Utah	LT558965	LT559082	-	-
T. tratensis	Islandici	FMR 14755	Suelo	México/-	-	-	-	-
T. wortmanii	Islandici	FMR 14512	Suelo	España/Andalucía	-	-	-	-

CBS: Colección –de cultivos del *Westerdijk Fungal Biodiversity Institute* (Utrecht, Holanda); FMR: Colección de cultivos de la *Facultat de Medicina de Reus* (España); UTHSCSA: *Fungus Testing Laboratory of the University of Texas Health Science Center in San Antonio* (EE.UU.); BAL: lavado broncoalveolar; ITS: región espaciadora intergénica transcrita del ARNr; *BenA*: fragmento del gen β- tubulina; *CaM*: calmodulina; *rpb2*: ARN polimerasa subunidad II; - : no disponible.

3.3. Técnicas de aislamiento de muestras de origen ambiental

Las muestras de heces fueron colectadas en bolsas individuales de plástico y se procesaron en un periodo máximo de tres días posteriores a la colecta. Una vez en el laboratorio, cada muestra era fraccionada y procesada a partir de dos metodologías: 1) en cámara húmeda, para la observación y aislamiento de las cepas directamente sobre sustrato natural (Richardson 2001), y 2) en dilusiones seriadas de acuerdo a Waksman (1922), ligeramente modificado. Las muestras de suelo fueron procesadas únicamente por el método de dilusiones seriadas.

Para las cámaras húmedas se utilizaron placas de Petri con papel de filtro estéril humedecido con agua destilada estéril y sobre el cual se colocaban algunos fragmentos de la muestra a estudiar. Las placas se incubadan a temperatura ambiente por un tiempo máximo de 60 días. Para el método de dilución se pesaba 1 g de muestra (heces o tierra), se suspendía en agua destilada estéril 1:10 (p / v) y se agitaba durante aproximadamente 10 min para conseguir la máxima homogeneización de la solución. A continuación, a dicha suspensión se le aplicaban factores de dilución 1:10 hasta conseguir una dilución de 10^{-2}. Finalmente, se depositaba un alicuota de 1 mL en una placa de Petri y se mezclaba con 20 mL de medio de agar fundido a 45 ºC aproximadamente. En total se preparaban dos placas por muestra diluida.

Los medios de cultivo utilizados para realizar el aislamiento primario fueron los siguientes: agar patata dextrosa (PDA; Pronadisa, Madrid), agar patata zanahoria (PCA; 20 g de patata, 20 g de zanahoria, 20 g de agar, 1000 mL de agua destilada), ambos suplementados con cloranfenicol (200 mg/L) para evitar el crecimientobacteriano, y agar dicloran rosa de bengala cloramfenicol (DRBC; 5 g de peptona, 10 g de glucosa, 1 g de dihidrógenofosfato de potasio [KH_2PO_4], 0,5 g de sulfato de magnesio [$MgSO_4$], 25 mg de rosa de bengala, 2 mg dicloran, 200 mg de cloranfenicol, 20 g de agar, 1000 mL de agua destilada). Todos los medios fueron suplementados con dieldrin disuelto en dimetil-cetona (1%) para evitar la proliferación de ácaros. Las placas se incubaron a temperatura ambiente durante 30 d y se revisaban periódicamente con ayuda del microscopio estereoscópico (Leica Wild 5A, con aumentos entre 4x y 50x). Cuando se observaban colonias con una morfología similar a penicilia creciendo sobre sustrato natural o de las placas de agar, con ayuda de una aguja hipodérmica previamente esterilizada, se transferían conidios en placas dePDA con cloranfenicol con la finalidad de obtener cultivos puros de las cepas a estudiar. Los conidios se inoculaban arrastrando la aguja sobre el agar formando una estría para obtener colonias aisladas.

3.4. Identificación de los aislados

Todos los aislados fueron identificados a nivel de especie a través de la secuenciación del gen *Ben*A. La identificación se realizó mediante la determinación del grado de similitud genética con secuencias disponibles en bases de datos públicas, CBS (http://www.westerdijkinstitute.nl) y GenBank (https://www.ncbi.nlm.nih.gov). Para las especies potencialmente nuevas o de interés taxonómico, la identificación se complementó realizando un estudio polifásico que incluyó caracterización morfológica, fisiológica, y molecular.

3.4.1. Caracterización morfológica y pruebas fisiológicas

La caracterización morfológica de los aislados se realizó siguiendo los métodos propuestos previamente por Visagie *et al.* (2014a) y Yilmaz *et al.* (2014) para los géneros *Penicillium* y *Talaromyces*, respectivamente. Para tal caracterización se utilizaron los siguientes medios de cultivo: MEA (Difco, Detroit, EE.UU.), OA (avena en copos 30 g, agar 20 g, agua destilada 1000 mL), CYA (Czapek concentrado 10 mL, sucrose 30 g, K_2HPO_4 1 g, agar 20 g, agua destilada 1000 mL), YES (extracto de levadura 20 g, sacarosa 150 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar 20 g, agua destilada 1000 mL), CREA (creatina 3 g, sacarosa 30g, $K_3PO_4 \cdot 7H_2O$ 1.6 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, purpura de bromocresol 0.05 g, agar 20 g, agua destilada 1000 mL) y DG18 (dicloran 31.5 g, glicerol 220 g, cloranfenicol 0.05 g, agar 20 g, agua destilada 1000 mL). Todos los medios fueron suplementados con 1 mL de solución de elementos traza ($CuSO_4 \cdot 5H_2O$ 0.5 g, $ZnSO_4 \cdot 7H_2O$ 0.1 g, agua destilada 100 mL). Cabe destacar que para la obtención de las formas sexuales, se sembraron placas adicionales de OA y CYA que se mantuvieron durante un período de incubación de hasta cuatro semanas.

Los especímenes en estudio se inocularon en tres puntos equidistantes sobre los medios de cultivo dispensados en placas de Petri de 90 mm. Las placas fueron incubadas a 25, 30 y 37 °C en condiciones de oscuridad. Las características macromorfológicas fueron evaluadas entre los 7–10 días de incubación y se consideraron los siguientes caracteres: diámetro de las colonias, coloración del anverso y reverso de la colonia, la textura, y la presencia de pigmentos difusibles y exudados. Los colores designados siguieron la nomenclatura establecida en el catálogo de colores de Kornerup y Wanscher (1978).

El estudio de las estructuras microscópicas, tanto del anamorfo como del teleomorfo, se realizó a partir de preparaciones de colonias con 7–10 días de crecimiento, utilizando como líquido de montaje el medio de Shear's (3 g de acetato de potasio, 150 mL de agua destilada, 60 mL de glicerina, 90 mL etanol 95 %) (Crous *et al.* 2009). En los casos en que la producción de conidios era profusa y muy densa, se realizaban lavados con etanol al 70% con el fin de eliminar el exeso de conidios (Visagie *et al.* 2014a). El examen microscópico de los ascomas se realizó mediante la técnica del squash para una mejor observación de las características del peridio, ascos y ascosporas.

El examen microscópico, incluyendo las mediciones de las estructuras, se realizó en un microscopio de campo claro Olympus CH-2 (Olympus Corporation, Japón), mientras que las microfotografías se obtuvieron con un microscopio automatizado Zeiss Axio-Imager M1 (Zeiss, Alemania) provisto de una cámara digital DeltaPix Infinity X21, utilizando óptica de contraste de fases y óptica Nomarski (óptica contraste interdiferencial), capturando y procesando las imágenes mediante el software DeltaPix InSight 5.0 (DeltaPix, Dinamarca).

3.4.2. Caracterización molecular

Depués de una identifiación morfológica preliminar se pasaba al estudio molecular con la finalidad decorroborar la identificación de los especímenes y dilucidar las relaciones filogenéticas de aquellos aislados de interés taxonómico. La caracterización molecular se realizó mediante la amplificación y secuenciación de diferentes marcadores genéticos: ITS, *Ben*A, *rpb*2, y *CaM.*

3.4.2.1. Extracción de ADN

Para la obtención de ADNnuclear, los aislados se cultivaron en placas de MEA durante 7–14 días a 25ºC. Posteriormente, se realizó la remoción del micelio aéreo y de las estructuras fértiles mediante el raspado superficial de la colonia utilizando un bisturí estéril. El ADN se extrajo mediante el kit comercial FastDNA® (MP Biomedicals, USA), usando el disgregador celular FastPrep FP120 (Thermo Savant, Holbrook, NY) y siguiendo las instrucciones del fabricante.

3.4.2.2. Amplificación y secuenciación

La caracterización molecular se realizó mediante la amplificación de los cuatro marcadores arriba indicados y recomendados propuestos para el análisis filogenético

de *Penicillium* y *Talaromyces* (Yilmaz *et al.* 2014, Visagie *et al.* 2014a). Los cebadores y programas de amplificación se detallan en las Tablas 4 y 5.

Las reacciones en cadena de la polimerasa (PCR) se realizaron en un volumen total de 25 µL, teniendo como componente principal EmeraldAmp® GT PCR Master Mix (Clontech, USA). El resto de componentes de las reacciones de PCR fueron añadidos según el protocolo sugerido por el fabricante. El termociclador usado para las amplificaciones fue 2720 *thermal cycler* (Applied Biosystems, USA). La visualización de los productos de amplificación se realizó mediante una electroforesis convencional en geles de agarosa al 1.5% o 2%. Finalmente, los productos de la PCR se enviaron para su purificación y secuenciación en ambas direcciones (*forward y reverse*) a Macrogen Corp. Europe (Ámsterdam, Holanda), utilizando los mismos pares de cebadores empleados para su amplificación.

Tabla 4. Cebadores utilizados para la amplificación y secuenciación.

Locus	Cebador	Dirección	Secuencia del Cebador	Referencia
ITS	ITS1	*Forward*	TCC GTA GGT GAA CCT GCG G	White *et al.* 1990
	ITS4	*Reverse*	TCC TCC GCT TAT TGA TAT GC	
BenA	Bt2a	*Forward*	GGT AAC CAA ATC GGT GCT GCT TTC	Glass y Donaldson 1995
	Bt2b	*Reverse*	ACC CTC AGT GTA GTG ACC CTT GGC	
CaM	CMD5	*Forward*	CCG AGT ACA AGG ARG CCT TC	Hong *et al.* 2006
	CMD6	*Reverse*	CCG ATR GAG GTC ATR ACG TGG	
*rpb*2	5F	*Forward*	GAY GAY MGW GAT CAY TTY GG	Liu *et al.* 1999
	7CR	*Reverse*	CCC ATR GCT TGY TTR CCC AT	

M: A, C; Y: T, C; W: A, T.

Tabla 5. Programas y ciclos térmicos utilizados para la amplificación.

Gen	Desnaturali- zación inicial	Ciclos	Desnaturali- zación	Anillaje	Elongación	Elongación Final
ITS, *BenA, CaM*	94 °C, 5 min	35	94 °C, 45 s	55 °C, 45 s	72 °C, 60 s	72 °C, 7 min
*rpb*2	94 °C, 5 min	5	94 °C, 45 s	50 °C, 45 s	72 °C, 60 s	
		5	94 °C, 45 s	52 °C, 45 s	72 °C, 60 s	
		30	94 °C, 45 s	55 °C, 45 s	72 °C, 60 s	72 °C, 7 min

3.4.2.3. Ensamblaje de secuencias

Los electroferogramas de las secuencias obtenidas se revisaron visualmente para determinar la calidad y fiabilidad de las mismas. El ensamblaje de las secuencias y la obtención de las secuencias consenso se realizaron utilizando el programa SeqMan, versión 7.0.0 (DNASTAR Lasergene, USA). Todas las secuencias generadas

en los estudios fueron depositadas en las bases de datos *GenBank* (http://www.ncbi.nlm.nih.gov/genbank) o *European Nucleotide Archive* (ENA) (http://www.ebi.ac.uk/ena), usando la plataforma Webin del *European Bioinformatics Institute* (EMBL-EBI) (http://www.ebi.ac.uk/ena).

3.4.2.4. Alineamiento de múltiples secuencias

Se realizaron alineamientos individuales con cada uno de los genes amplificados, incluyendo nuestras secuencias y secuencias de las cepas tipos de las especies aceptadas en la base de datos ICPA (https://www.aspergilluspenicillium.org/). Los alineamientos fueron realizados utilizando el algoritmo ClustalW (Thompson *et al.* 1994) incorporado en la plataforma *Molecular Evolutionary Genetic Analysis* (MEGA) versión 6.0 (Tamura *et al.* 2013). Los alineamientos fueron refinados usando el algoritmo *Multiple Sequence Comparison by Log-Expectation* (MUSCLE) (Edgar 2004) incluido en la misma plataforma, siendo finalmente, revisados y ajustados manualmente.

3.4.2.5. Concatenado de los alineamientos

Con el objetivo de obtener una mayor resolución y robustez de los análisis filogenéticos, se realizaron alineamientos concatenados utilizando una o más combinaciones de los genes seleccionados, siempre incluyendo secuencias de ITS y *Ben*A, dependiendo de la disponibilidad de secuencias de las cepas tipo. Para la evaluación de la concordancia filogenética entre los diferentes *loci*, se realizó una comparación visual de la topología de los clados basales y terminales que presentaban soporte estadístico significativo en las filogenias obtenidas para cada gen por separado y en el análisis concatenado (Wiens 1998). Los alineamientos y los árboles generados se depositaron en la base de datos TreeBASE (www.treebase.org).

3.4.2.6. Análisis filogenético

Las reconstrucciones de las hipótesis filogenéticas para cada gen y para los alineamientos concatenados se realizaron utilizando los métodos de Máxima verosimilitud (ML) y el análisis de inferencia Bayesiana (BI). Los análisis de ML se llevaron a cabo utilizando el software MEGA en su versión 6.0., con el uso de 1000 iteraciones, donde valores de Bootstrap (bs) ≥70% fueron considerados estadísticamente significativos. Para el análisis de BI, se utilizó el software MrBayes versión 3.1.2, el cual integra el modelo matemático Cadena de Markov y el método

Monte Carlo (MCMC) para calcular las probabilidades posteriores (pp) del árbol filogenético (Ronquist y Huelsenbeck 2003). Se realizaron simulaciones entre 1.000.000 a 5.000.000 generaciones, en dos series (cadenas) paralelas. El análisis era considerado óptimo cuando la convergencia de valores estadísticos para ambas cadenas era de <0,01. Para todos los análisis de BI, se eliminó el 25% de los primeros árboles obtenidos (*burn-in*) para, posteriormente, calcular el árbol consenso final (50%). La selección del modelo de sustitución nucleotídica más apropiado para cada gen se realizó utilizando la herramienta *Find Best DNA/Protein Model* incluida en el software MEGA para ML o, en su defecto, utilizando el software JModelTest (Posada 2008) a través de la plataforma CIPRES (https://www.phylo.org/).

3.5   Registro de novedades taxonómicas

Las nuevas propuestas taxonómicas (géneros, especies y combinaciones nuevas) fueron depositadas en la base de datos MycoBank (http://www.mycobank.org/) (Crous *et al.* 2004), siguiendo las recomendaciones del actual código de nomenclatura fúngica (McNeill *et al.* 2012).

3.6.   Almacenamiento y conservación de los aislados

Todos los aislamientos obtenidos e identificados en esta tesis se depositaron en la micoteca de la Facultad de Medicina de Reus (FMR) con un número de acceso determinado. Paralelamente, también se depositaron cultivos de las especies raras o especies nuevas para la ciencia en la colección del *Westerdijk Fungal Biodiversity Institute* (Utrecht, Holanda). Con el fin conservar en nuestro laboratorio las cepas vivas para futuros estudios, se utilizaron diferentes técnicas de conservación.

3.6.1   Conservación en agua

La conservación en agua de los aislamientos se realizó a partir de cultivos esporulados en medio PDA. El procedimiento consistió en cortar con ayuda de un bisturí estéril fragmentos de las colonias de aproximadamente 0.5 cm^3,. Seguidamente, dichos fragmentos se introdujeron en varios frascos de vidrio estériles conteniendo 2–3 mL de agua destilada estéril. Los frascos eran sellados con un tapón de caucho sintético y se almacenaban a temperatura ambiente.

3.6.2 Conservación en aceite mineral

La conservación en aceite mineral se realizó usando tubos de vidrio de 10 cm de longitud con agar inclinado y tapón de rosca. Los medios de cultivo utilizados fueron preferentemente OA, PCA o PDA.. Una vez inoculados los tubos y verificado el óptimo crecimiento y esporulación de las cepas, el cultivo se cubría con aceite mineral estéril y, a continuación, los tubos se guardaban en la oscuridad a temperatura ambiente (aproximadamente 25 °C).

3.6.3 Conservación por liofilización

Para el liofilizado de las cepas, se partía decultivados bien esporulados en placas de PDA incubadas a 25 ºC entre 7–14 d.. Posteriormente, se raspaban las colonias con ayuda de una asa estéril y la masa fúngica obtenida era depositada en un tubo de plástico estéril con 4 mL de medio *skim milk* (Difco, EE.UU.) hasta obtener una suspensión concentrada. La suspensión se homogeniizaba y distribuía en fracciones de 1–1.5 mL en tres frascos pequeños de vidrio estériles provistos de tapón de caucho hermético.

Los frascos se liofilizaban mediante el sistema automatizado *VirTis* Advantage 2.0 ES (SP Scientific, USA), utilizando el siguiente protocolo: congelación inicial a 45 ºC, seguida de la generación de vacío a 200 mTorr, desecación por sublimación a -30 ºC (240 min), -10 ºC (240 min), 10 ºC (300 min) y 30 ºC (300 min). Una vez finalizado el proceso de liofilización, se sellaban los frascos con un anillo de seguridad metálico. Para comprobar la viabilidad de la cepa y la pureza del cultivo, se seleccionaba al azar uno de los viales y se rehidrataba para posteriormente ser inoculado en placas de OA, PCA o PDA. Una vez comprobada la caliadad del producto liofilizado, los viales eran conservados a temperatura ambiente.

3.6.4 Almacenamiento mediante herborización

Las cepas seleccionadas como tipo de las nuevas especies propuestas en los diferents estudios, era herborizadas (holotipos e isotipos) de la siguiente forma: los aislamientos seleccionados se cultivabanpor duplicado en MEA o CYA durante 7–10 d a la temperatura óptima de incubación, hasta que alcanzaban su máximo crecimiento y esporulación. Una vez transcurrido dicho tiempo, se desecaban los cultivos en una estufa a 45–50 ºC y, posteriormente, cada cultivo seco se almacenaba en sobres de papel bien etiquetados. Un cultivo seco se depositaba como holotipo en el herbario del

Westerdijk Institute de Holanda y el otro cultivo desacado se guardaba en el herbario de nuestra colección como isotipo.

3.7 Sensibilidad antifúngica *in vitro*

Los perfiles de sensibilidad antifúngica *in vitro* solamente se determinaron para aquellas especies de *Penicillium* y *Talaromyces* más frecuentemente identificadas en nuestro set de aislados de origen clínico. Para tal fin, se siguió el protocolo descrito por el *Clinical and Laboratory Standards Institute* (CLSI) en el documento M38-A2 (CLSI 2008). Los fármacos ensayados incluyeron representantes de los principales grupos de antifúngicos de uso clínico: alilaminas (terbinafina, TRB), azoles (itraconazol, ITC; posaconazol, PSC; y voriconazol, VRC), equinocandinas (anidulafungina, AFG; caspofungina, CFG; micafungina, MFG); pirimidinas fluoradas (5-fluorocitosina, 5FC) y polienos (anfotericina B, AMB). Los rangos de concentraciones de antifúngicos ensayados fluctuaron entre 0,016–16 µg/mL.

Para la preparación de los inóculos, los aislados en estudio se sembraban en PDA y se incubaban a 25 ºC durante 7 d o hasta conseguir abundante esporulación. Posteriormente, se raspaba la superficie de las colonias con la ayuda de un bisturí, y la masa fúngica obtenida se suspendía en suero fisiológico estéril, el cual se filtraba mediante gasa o algodón estéril de forma sucesiva para eliminar los restos de hifas. Las suspensiones de conidios se cuantificaban a través de recuento en cámara de Neubauer y se ajustaban a una concentración de $3 \times 10^5 - 4 \times 10^6$ conidios/mL mediante dilución (factor de dilución 1/50) en medio de *Roswell Park Memorial Institute* (RPMI-1640, Gibco, UK). Las suspensiones conidiales se inoculaban en microplaca de 96 pocillos, las cuales contenían los antifúngicos a ensayar. Las microplacas se incubaban en la oscuridad, sin agitación, a 35 ºC durante 24–48 horas.

La lectura de la sensibilidad para las equinocandinas se realizó a las 24 y 48 horas de incubación, determinando la concentración mínima efectiva (CME). Este parámetro se define como la concentración mínima de antifúngico en la cual se observa un crecimiento aberrante del hongo, caracterizado por la formación de masas compactas, formadas por elementos miceliares de extremos redondeados, en comparación con el crecimiento normal aterciopelado o algodonoso observable en el pocillo control de la microplaca. La lectura de la sensibilidad para el resto de los antifúngicos se realizaba entre las 48 y 72 horas de incubación, y en estos casos el parámetro evaluado era la concentración mínima inhibitoria (CMI). Este parámetro se define como la mínima concentración de antifúngico que es capaz de conseguir el

100% de inhibición del crecimiento del hongo para AMB, ITC, PSC y VRC, el 80% de inhibición para TRB y el 50% de inhibición en el caso de 5FC. Las lecturas se llevaban a cabo visualmente con ayuda de un espejo invertido. Los perfiles de sensibilidad antifúngica *in vitro* siempre se realizaron por duplicado. Para el control de calidad de las pruebas, se utilizaban las cepas de *Aspergillus fumigatus* ATCC MYA-3626, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019 y *Paecilomyces variotii* ATCC MYA-3630.

4. RESULTADOS

En la presente tesis hemos examinado un total de 314 aislados (118 de origen clínico y 196 de origen ambiental), los cuales fueron identificados preliminarmente como penicilia. Un estudio más exhaustivo de los mismos, combinando caracteres morfológicos, fisiológicos y análisis de secuencias, nos permitió concluir que 263 aislados correspondían a *Penicillium* (83.75%), 45 a *Talaromyces* (33%) (Figuras 8 y 9) y dos a *Rasamsonia* (0.64%). Sin embargo, cuatro aislados, a pesar de su apariencia penicilioide, requirieron de un análisis filogenético más robusto para su identificación y ubicación taxonómica. Estos aislados han sido propuestos como dos géneros nuevos de la familia *Aspergillaceae*, denominados *Penicillago* y *Pseudopenicillium,* los cuales incluyen dos aislados (0.64%) en cada uno de ellos (ver apartado 4.3). La identificación final, su origen, y números de acceso, tanto de las cepas aisladas como de las secuencias obtenidas de las mismas, se presentan en la Tabla 3.
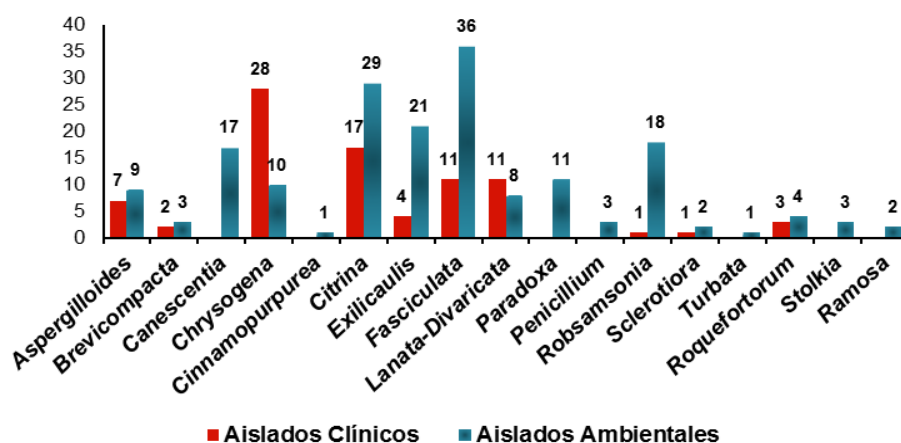
Figura 8. Gráfico que muestra el número de aislados de *Penicillium* estudiados y distribuidos por secciones. Las barras rojas corresponden a los aislados de origen clínico y las azules a los aislados de origen ambiental.
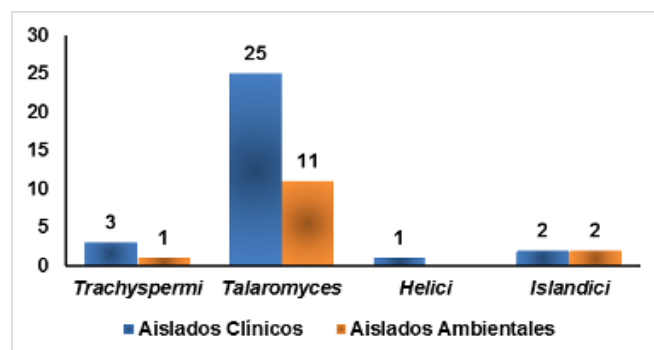
Figura 9. Gráfico que muestra el número de aislados de *Talaromyces* estudiados y distribuidos por secciones. Las barras azules corresponden a los aislados de origen clínico y las naranjas a los aislados de origen ambiental.

El análisis de secuencias de los marcadores ITS y *Ben*A nos permitió confirmar que los 1189 aislados clínicos se distribuían entre los géneros *Penicillium* (n = 85), *Talaromyces* (n = 31) y *Rasamsonia* (n = 2). En el género *Penicillium* se identificaron un total de 23 especies distribuidas en 10 secciones (Figura 8). Las especies con mayor prevalencia en nuestro set de aislados fueron *P. rubens* (n = 19; 22.4%) de la sección *Chrysogena* y *P. citrinum* (n = 14; 16.5%) de la sección *Citrina;* el resto de especies de *Penicillium* identificadas representaron entre 1 y 8% sobre el total de aislados clínicos del género estudiados (Figura 10). En *Talaromyces,* se identificaron 10 especies distribuidas en 4 secciones (Figura 9), siendo *T. amestolkiae* (n = 7; 22.6%) y *T. purpurogenus* (n = 5; 16.1%), ambas de la sección *Talaromyces,* las especies más frecuentemente identificadas (Figura 11). Cabe destacar que cinco de los aislados de *Talaromyces* no pudieron ser identificados con los marcadores anteriormente indicados y se requirió de un análisis adicional, combinando secuencias de ITS, *Ben*A, *rpb*2 y *CaM.* Los resultados demostraron que estas cepas correspondían a cuatro especies nuevas de *Talaromyces*, dos pertenecientes a la sección *Talaromyces* (*T. alveolaris* y *T. rapidus*)*,* una a la sección *Helici* (*T. georgiensis*) y una a *Trachyspermi* (*T. minnesotensis*). Los aislados relacionados con el género *Rasamsonia* se identificaron como *R. argillacea* y *R. eburnea.* Los resultados de la identificación de los aislados clínicos y la descripción de los cuatro nuevos taxones de *Talaromyces* fueron publicados en dos estudios (ver apartado 4.1 y 4.2).
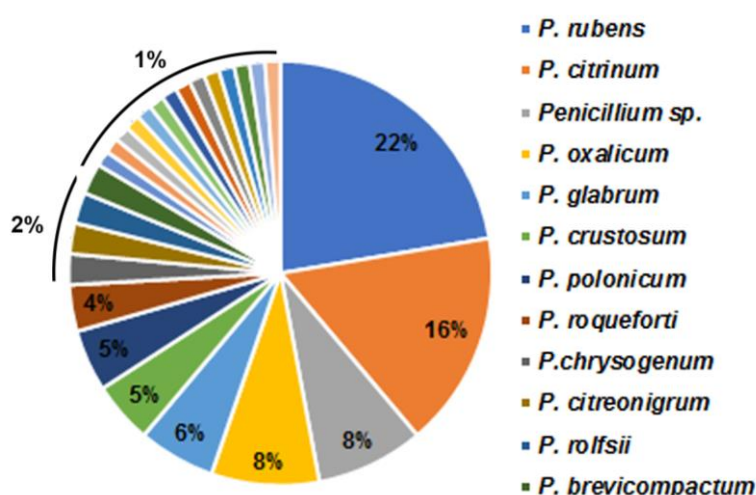
Figura 10. Gráfico que muestra las especies identificadas a partir de los aislados clínicos de *Penicillium* incluidos en esta tesis.
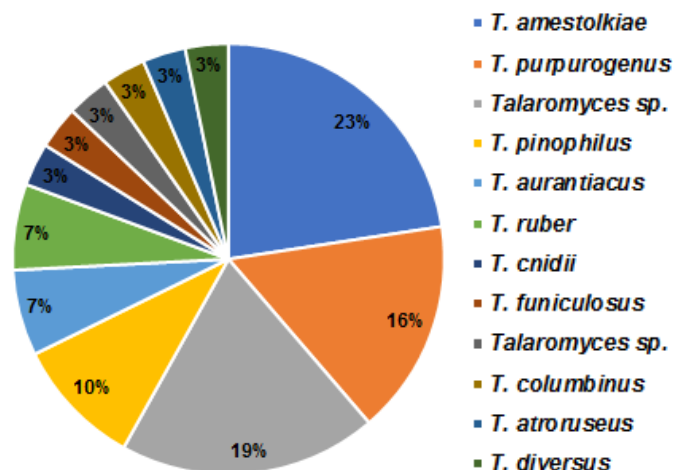
Figura 11. Gráfico que muestra las especies identificadas a partir de los aislados clínicos de *Talaromyces* incluidos en esta tesis.

La identificación molecular de aislados ambientales se llevó a cabo, principalmente, con el análisis de secuencias del gen *Ben*A, al ser el marcador por excelencia capaz de discriminar la mayoría de las especies de *Penicillium* y *Talaromyces.* Dicho análisis nos permitió concluir que la mayoría de los aislados ambientales correspondían principalmente a especies de *Penicillium* (n = 196; 91%) y menor medida a *Talaromyces* (n = 14; 7.1%). No obstante, cuatro aislados requirieron de un análisis adicional combinando secuencias de ITS, *Ben*A y *rpb*2, el cual nos permitió constatar que representaban dos nuevos linajes en la familia *Aspergillaceae* y que, tal y como se indicó arriba, se proponen como nuevos géneros para dicha familia. En el género *Penicillium* se identificaron 67 especies distribuidas en 17 secciones (Figura 8), siendo *P. crustosum* (n = 27; 15.16%) de la sección *Fasciculata* la especie más frecuentemente aislada, seguida de *P. citrinum* (n = 17; 9.55%) de la sección *Citrina*. A su vez, con el análisis combinado de los cuatro marcadores antes indicados, junto con un examen morfológico más exhaustivo de los aislados que no pudieron ser identificados con un solo marcador, se pudieron delimitar y caracterizar un total de nueve especies nuevas para la ciencia, siete de ellas procedentes de heces de animales (*P. balearicum*, *P. beceitense*, *P. caprifimosum*, *P. fimosum*, *P. ibericum*, *P. mediterraneum*, *P. synnematicola*) y dos de suelo (*P. parvofructum*, *P. uruguayense*). En *Talaromyces* se identificaron 11 especies distribuidas entre las secciones *Islandici, Talaromyces* y *Trachyspermi* (Figura 9); siete de ellas correspondían a especies ya conocidas, y cuatro a especies nuevas propuestas como *T. catalonicus, T. corophilus, T. pseudofuniculosus* y *T. gamsii.* Las tres primeras se han descrito a partir de heces

de herbívoro y la última de suelo. Gracias también al aislamiento a partir de muestras de estiércol e identificación molecular de especies de *Penicillium* poco frecuentes como *P. nodositatum* o *P. giganteum*, excluidas del género en anteriores estudios (Peterson *et al.* 2010, Visagie *et al.* 2013), hemos propuesto el género *Penicillago*, tipificado por *Pgo. nodositata* y el género *Pseudopenicillium*, con tres especies: *Pse. coprobium* como nueva para la ciencia y dos nuevas combinaciones, *Pse. giganteum* y *Pse. megasporum,* esta última propuesta como tipo para el género.

Es importante destacar que, con respecto a los aislados obtenidos de heces de animales en España, la región de donde se recuperó la mayoría de los penicilia fue Cataluña (31%), seguido de las Islas Baleares (27%). Cabe destacar que una de las secciones del género *Penicillium* con un mayor número de aislados de origen coprófilo correspondió a *Robsamsonia* (Figura 8), justamente la sección del género que se caracteriza por incluir la mayoría de especies de *Penicillium* definidas como coprófilas (Houbraken *et al.* 2016). En ésta hemos identificado un total de seis especies, una de ellas, *P. synnematicola*, descrita como nueva y caracterizada a partir de seis aislados recuperados principalmente de estiércol de cabra colectado tanto en diferentes localidades catalanas como de Mallorca.

Los resultados sobre el estudio taxonómico de los aislados ambientales procededentes de heces se muestran en el apartado 4.3 de esta tesis en formato de artículo. Asimismo, la descripción, la ilustración y los datos sobre la filogenia de los taxones de *Penicillium* encontradas en muestras de suelo se presentan en dos publicaciones cortas incluidas en el apartado 4.4.

4.1 Identification and Antifungal Susceptibility of Penicillium-Like Fungi from Clinical Samples in the United States.

Identification and Antifungal Susceptibility of Penicillium-Like Fungi from Clinical Samples in the United States

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Penicillium species are some of the most common fungi observed worldwide and have an important economic impact as well as being occasional agents of human and animal mycoses. A total of 118 isolates thought to belong to the genus Penicillium based on morphological features were obtained from the Fungus Testing Laboratory at the University of Texas Health Science Center in San Antonio (United States). The isolates were studied phenotypically using standard growth conditions. Molecular identification was made using two genetic markers, the internal transcribed spacer (ITS) and a fragment of the β-tubulin gene. In order to assess phylogenetic relationships, maximum likelihood and Bayesian inference assessments were used. Antifungal susceptibility testing was performed according to CLSI document M38-A2 for nine antifungal drugs. The isolates were identified within three genera, i.e., Penicillium, Talaromyces, and Rasamsonia. The most frequent species in our study were Penicillium rubens, P. citrinum, and Talaromyces amestolkiae. The potent in vitro activity of amphotericin B (AMB) and terbinafine (TRB) and of the echinocandins against Penicillium and Talaromyces species might offer a good therapeutic alternative for the treatment of infections caused by these fungi.

*P*enicillium is one of the largest fungal genera. It comprises some of the most commonly known filamentous fungi and can be found on numerous substrates, as well as in very diverse habitats (1). Apart from the species included in this genus, many other fungi, such as those included in the genera *Hamigera*, *Paecilomyces*, *Rasamsonia*, *Sagenomella*, *Talaromyces*, and *Trichocoma*, also show penicillium-like "little brush" structures. In spite of the morphological similarity of these fungi, recent phylogenetic studies have classified these genera into well-established families, i.e., *Aspergillaceae* (*Hamigera*, *Penicillium*), *Thermoascaceae* (*Paecilomyces*), and *Trichocomaceae* (*Rasamsonia*, *Sagenomella*, *Talaromyces*, *Trichocoma*) (2).

Despite the ubiquity of these fungi in air and in human habitats, their clinical significance is not well understood. Penicillium-like fungi are commonly recovered from clinical samples and in routine hospital air surveys; however, they are often discounted as mere contaminants. In addition, their identification to the species level is rarely made in routine laboratories due to the complexity of the phenotypic methods required for their *in vitro* study. Further, the high number of species currently accepted in these genera makes this task even more difficult (1, 3). The use of molecular methods does, however, represent a rapid and relatively simple approach for the identification of *Penicillium* species, as well as for species in other, closely related genera (4, 5).

Partly due to the aforementioned difficulties, the role of penicillium-like fungi in human pathology has been considered relatively unimportant. However, one species, *Talaromyces* (formerly *Penicillium*) *marneffei*, is notable for its clinical relevance as an agent of fatal systemic mycosis, mostly in HIV-infected patients, and mainly in southeast Asia, India, and China (6). A few other penicillium-like fungi are seen in the clinical setting, but with a considerably lower incidence. Some species of *Penicillium*, such as *P. chrysogenum*, *P. citrinum*, *P. commune*, *P. decumbens*, *P. piceum*, and *P. purpurogenum* (currently *Talaromyces*

picesus and *Talaromyces purpurogenus*, respectively), have been reported only rarely (7).

Clinical manifestations due to *Penicillium* species include superficial and invasive infections, as well as allergies (8). Infections in humans are mainly related to host immunity (9). There are very few data on animal infections by *Penicillium* species, and the few cases reported have been restricted to systemic diseases and fungal osteomyelitis in dogs. *Penicillium brevicompactum*, *P. purpurogenum*, and, recently, *P. canis* have also been reported in fungal infections in dogs (10–12). Antifungal susceptibility data for clinically available antifungal agents and treatment options for infections caused by *Penicillium* species are also poorly understood, apart from data published for *T. marneffei* (13).

The main goal of the present study was to identify, by molecular means, a large set of clinical and environmental isolates of *Penicillium* and related genera that had been isolated in the United States. The results can provide much-needed information on the diversity of species in that part of the world. Additionally, this study was designed to provide antifungal susceptibility data for the species identified, which will allow more-appropriate patient management of these infections.

MATERIALS AND METHODS

Sample collection. A total of 118 isolates identified morphologically as a *Penicillium* spp. were received from the Fungus Testing Laboratory at the University of Texas Health Science Center in San Antonio (UTHSCSA). The isolates were from different locations in the United States and comprised 108 clinical specimens that were isolated from humans, 6 that were isolated from animals, 1 that was isolated from a clinical environment, and 3 that were of unknown origin (see Table S1 in the supplemental material).

Phenotypic characterization. The isolates were subcultured onto malt extract agar (MEA; Difco Laboratories, Detroit, MI). Phenotypic identification was carried out using standard growth conditions as described previously (1). For microscopic observation, slides were made with Shear's solution using 7-to-10-day-old cultures. In addition, we evaluated the ability of the isolates to grow at 37°C.

DNA extraction, amplification, and sequencing. The isolates were grown on MEA for 7 to 14 days at 25°C prior to DNA extraction. DNA was extracted using a FastDNA kit and the kit protocol (MP Biomedicals, Solon, OH) with the homogenization step using a FastPrep FP120 cell disrupter (Thermo Savant, Holbrook, NY) according to the manufacturers' instructions. The DNA regions selected for sequencing were those recommended by Visagie et al. (1) for *Penicillium* identification. PCR was performed to amplify the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) and a fragment of the β-tubulin gene. The primer pairs used were ITS5/ITS4 for the ITS region (14) and Bt2a/Bt2b for β-tubulin (15).

Single-band PCR products were purified and sequenced at Macrogen Corp. Europe 104 (Amsterdam, The Netherlands) with a 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). Sequence assembly and editing were performed using SeqMan v. 7.0.0 (DNASTAR, Madison, WI).

Phylogenetic reconstructions. Preliminary identification of the isolates to the genus level was performed by analysis of ITS sequences, using the BLAST algorithm implemented in the GenBank, CBS-KNAW, and MycoBank databases. Isolates were identified to the species level using the ITS and β-tubulin sequences. Multiple-sequence alignments were performed for each locus in MEGA v 6.0 software (16), using the CLUSTALW algorithm (17), refined with MUSCLE (18) and manually adjusted using the same software platform.

Phylogenetic analyses were made with the individual loci and combined genes using maximum likelihood (ML) in MEGA v. 6.0 (16) and Bayesian inference (BI) under MrBayes version 3.1.2 (19). For ML, support for internal branches was assessed by 1,000 ML bootstrapped pseudoreplicates of data. A bootstrap support (bs) value of ≥70 was considered significant. The phylogenetic reconstruction by BI was performed using 5 million Markov chain Monte Carlo (MCMC) generations, with two runs (one cold chain and three heated chains), and samples were stored every 1,000 generations. The 50% majority-rule consensus tree and posterior probability (pp) values were calculated after discarding the first 25% of the samples. A pp value of ≥0.95 was considered significant. The best substitution model for all gene matrices was estimated using jModelTest v.2.1.3 (20, 21).

Antifungal susceptibility testing. Antifungal susceptibility of the isolates was determined according to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution M38-A2 method for filamentous fungi (22). The *in vitro* activities of amphotericin B (AMB), voriconazole (VRC), itraconazole (ITC), posaconazole (PSC), terbinafine (TRB), anidulafungin (AFG), caspofungin (CFG), micafungin (MFG), and 5-fluorocytosine (5FC) were determined for those species with five or more isolates. The MIC was defined as the lowest concentration to inhibit 100% of growth on visual inspection for AMB, ITC, PSC, and VRC and to reduce growth by 80% for TRB compared to the drug-free control well. The minimal effective concentration (MEC) was defined as the lowest concentration seen to produce short, stubby, abnormally branched hyphae for the echinocandins. Both the MIC and the MEC parameters were determined at 48 h.

Candida krusei ATCC 6258, *Candida parapsilosis* ATCC 22019, *Paecilomyces variotii* ATCC MYA-3630, and *Aspergillus fumigatus* ATCC MYA-3626 were used as quality control strains for all tests.

Nucleotide sequence accession numbers. The nucleotide sequences obtained in this work were submitted to GenBank under accession numbers LT558856 to LT558973 for the ITS and LT558974 to LT559090 for β-tubulin (see Table S1 in the supplemental material).

RESULTS

On the basis of the results of the analysis of the ITS region, we discovered that the 118 isolates investigated corresponded to species belonging to *Penicillium* ($n = 85$), *Talaromyces* ($n = 31$), or *Rasamsonia* ($n = 2$). Identification of the isolates at the species level through phylogenetic analysis with the combination of ITS and β-tubulin sequences is summarized in Table 1.

We carried out a phylogenetic study for each of the three genera involved. The first objective was to identify *Penicillium* isolates by grouping them into their respective sections (see Fig. S1 in the supplemental material). The aligned data set was 919 bp long (ITS, 521 bp; β-tubulin, 398 bp), and Kimura's two-parameter (K2) model with gamma distribution (+G) was the model selected for each fragment. This analysis showed that our isolates corresponded to 23 species belonging to the following 10 sections: *Chrysogena* ($n = 28$), *Citrina* ($n = 17$), *Fasciculata* ($n = 11$), *Lanata-Divaricata* ($n = 11$), *Aspergilloides* ($n = 7$), *Exilicaulis* ($n = 4$), *Roquefortorum* ($n = 3$), *Brevicompacta* (n = 2), *Penicillium* ($n = 1$), and *Sclerotiora* ($n = 1$). The most frequently identified taxa were *P. rubens* (22.4%; $n = 19$; section *Chrysogena*) and *P. citrinum* (16.5%; $n = 14$; section *Citrina*). Additionally, within section *Citrina*, seven *Penicillium* sp. isolates could not be identified at the species level.

A second phylogenetic reconstruction was performed to identify the *Talaromyces* isolates (see Fig. S2 in the supplemental material). The aligned data set was 847 bp long (ITS, 474 bp; β-tubulin, 373 bp), and the model selected was the Tamura three-parameter (T92) model with gamma-distributed rates and the presence of invariant sites (G + I) for ITS and K2 + G + I for β-tubulin. In this genus, 10 species were identified belonging to four sections, i.e., *Talaromyces* ($n = 25$), *Trachyspermi* ($n = 3$), *Islandici* ($n = 2$), and *Helici* ($n = 1$). The most prevalent species were *T. amestolkiae* (22.6%; $n = 7$) and *T. purpurogenus* (16.1%; $n = 5$), both in section *Talaromyces*. A total of seven isolates could not be identified at the species level and are referred to here using the following six designations: *Talaromyces* sp. strain I ($n = 1$; section *Talaromyces*), *Talaromyces* sp. strain II ($n = 2$; section *Talaromyces*), *Talaromyces* sp. strain III ($n = 1$; section *Talaromyces*), *Talaromyces* sp. strain IV ($n = 1$; section *Helici*), *Talaromyces* sp. strain V ($n = 1$; section *Islandici*), and *Talaromyces* sp. strain VI ($n = 1$; section *Trachyspermi*).

The third phylogenetic analysis (see Fig. S3 in the supplemental material) was carried out to identify the *Rasamsonia* isolates. The aligned data set was 1,078 bp long (ITS, 599 bp; β-tubulin, 479 bp), and the selected models for each fragment were T92 and K2, with uniform rates used for ITS and β-tubulin, respectively. The isolates were identified as *R. argillacea* and *R. eburnea*.

The 118 isolates were mainly from the respiratory tract (72.9%), usually from human bronchoalveolar lavage (BAL) fluid

TABLE 1 Molecular identification and growth at 37°C of the isolates included in the study

Genus (total no. of isolates)	Species	Section	No. of isolates	Growth at 37°C (mm/7 days)
Penicillium (85)	P. rubens	Chrysogena	19	19
	P. citrinum	Citrina	14	14
	Penicillium sp.	Chrysogena	7	7
	P. oxalicum	Lanata-Divaricata	7	7
	P. glabrum	Aspergilloides	5	4
	P. crustosum	Fasciculata	4	0
	P. polonicum	Fasciculata	4	1
	P. roqueforti	Roquefortorum	3	0
	P. chrysogenum	Chrysogena	2	2
	P. citreonigrum	Exilicaulis	2	0
	P. rolfsii	Lanata-Divaricata	2	2
	P. brevicompactum	Brevicompacta	2	0
	P. sumatrense	Citrina	1	0
	P. pancosmium	Citrina	1	0
	P. roseopurpureum	Citrina	1	0
	P. decumbens	Exilicaulis	1	1
	P. rubefaciens	Exilicaulis	1	1
	P. allii	Fasciculata	1	0
	P. echinulatum	Fasciculata	1	0
	P. palitans	Fasciculata	1	0
	P. brasilianum	Lanata-Divaricata	1	1
	P. singorense	Lanata-Divaricata	1	1
	P. adametzioides	Sclerotiora	1	0
	P. coprophilum	Penicillium	1	0
	P. frequentans	Aspergilloides	1	0
	P. rudallense	Aspergilloides	1	1
Talaromyces (31)	T. amestolkiae	Talaromyces	7	7
	T. purpureogenus	Talaromyces	5	5
	T. pinophilus	Talaromyces	3	3
	T. aurantiacus	Talaromyces	2	2
	T. ruber	Talaromyces	2	2
	Talaromyces sp. I	Talaromyces	2	2
	Talaromyces sp. II	Talaromyces	1	1
	Talaromyces sp. III	Talaromyces	1	0
	T. cnidii	Talaromyces	1	1
	T. funiculosus	Talaromyces	1	1
	Talaromyces sp. IV	Helici	1	1
	T. columbinus	Islandici	1	1
	Talaromyces sp. V	Islandici	1	1
	T. atroroseus	Trachyspermi	1	1
	T. diversus	Trachyspermi	1	1
	Talaromyces sp. VI	Trachyspermi	1	0
Rasamsonia (2)	R. argillacea		1	1
	R. eburnea		1	1

(see Table S1 in the supplemental material). We carried out antifungal susceptibility testing of the most frequent species, i.e., a total of 51 isolates (39 *Penicillium* isolates and 12 *Talaromyces* isolates) representing seven species (Tables 2 and 3). Overall, TRB and the echinocandins showed the best *in vitro* activity against *Penicillium* species, with modes of <0.03 μg/ml for TRB, 0.06 μg/ml for CFG and AFG, and 0.125 μg/ml for MFG. Amphotericin B showed intermediate antifungal activity, with an overall mode of 2 μg/ml, while the azoles showed various levels of activity, with wide MIC ranges and modes of 0.5 μg/ml for PSC and ITC

and 2 μg/ml for VRC. The highest MIC values were observed for 5FC (Table 2). Terbinafine, the echinocandins, and AMB showed *in vitro* activity against *Talaromyces* species similar to that seen with the *Penicillium* species, while 5FC, with a mode of 0.125 μg/ml, showed good *in vitro* activity compared to the results seen with *Penicillium* species. In contrast, the azoles showed poor *in vitro* activity, with wide MIC ranges and modes of >16 μg/ml for PSC, VRC, and ITC (Table 3).

The results of growth at 37°C (Table 1) showed that 71.7% of the *Penicillium* isolates ($n = 61$) were able to grow at this temperature. All the isolates belonging to both section *Chrysogena* ($n = 28$) and section *Lanata-Divaricata* ($n = 11$) grew at 37°C, whereas, among the isolates belonging to section *Citrina*, only those identified as *P. citrinum* managed to grow at this physiologically relevant temperature. On the other hand, practically all isolates of the genus *Talaromyces* (99.6%; $n = 29$), as well as the two isolates of the genus *Rasamsonia*, grew at 37°C (Table 1).

DISCUSSION

Despite the uncertainty concerning the true role that penicillium-like fungi play in human pathology, there are several reports of infections that seem to have involved these fungi in the clinical setting, in particular, isolates from respiratory samples (23, 24). However, to our knowledge, the species diversity of a large collection of penicillium-like fungi from clinical origins has never been explored. Thus, this was the first study to investigate more than 100 isolates from clinical sources and to demonstrate, by using combined sequence analyses of the ITS region and β-tubulin gene, that three different genera of penicillium-like fungi (i.e., *Penicillium*, *Talaromyces*, and *Rasamsonia*) are, in fact, associated with these types of samples. As expected, *Penicillium* species were the most common (72%).

The most frequently identified species within *Penicillium* were *P. citrinum* and *P. rubens*. *P. citrinum* has been reported to be a human-opportunistic pathogen responsible for keratitis, cutaneous infections, and pneumonia (25–28).

Curiously, to date, *P. rubens* has not shown any link to clinical isolates, although it is a recently resurrected species, closely related to *P. chrysogenum* (29). In contrast, *P. chrysogenum* has already been identified as a human pathogen associated with cutaneous and invasive infections (8, 30, 31). A total of 28 of our isolates were classified within section *Chrysogena*, including 19 identified as *P. rubens*, 2 identified as *P. chrysogenum*, and 7 that were very similar to those but which will require additional phylogenetic markers to distinguish them properly (29). Although we were not able to demonstrate the pathogenic role of *P. rubens* and *P. chrysogenum*, the high number of strains of this species recovered and their ability to grow at 37°C highlight the clinical importance of these species.

Other *Penicillium* species found in our study were *P. glabrum* and *P. oxalicum*, which were the most frequent species after *P. rubens* and *P. citrinum*. While *P. glabrum* has not been associated with human infections, Chowdhary (32) reported three cases of invasive infections by *P. oxalicum* in patients with acute myeloid leukemia, diabetes mellitus, and chronic obstructive pulmonary disease. The lung was theorized to be the portal of entry for this pathogen in those three cases.

Several reports have recognized *P. decumbens* as the cause of a disseminated infection, a perioperative paravertebral infection, and a fungus ball (7). However, only one isolate of *P. decumbens*

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TABLE 2 Results of *in vitro* antifungal susceptibility testing of 39 isolates of *Penicillium* species

Species (no. of isolates tested)	Antifungal[a]	No. of isolates with antifungal MIC (µg/ml) of:											
		≤0.03	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	>16
P. citrinum (n = 10)	CFG		2	7	1								
	AFG		4	5	1								
	MFG		1	1	7	1							
	TRB	3	7										
	PSC				1			7	2				
	VRC												10
	ITC						1	4	1	1			3
	AMB							3	7				
	5FC								2	4	3	1	
P. rubens (n = 10)	CFG		1	2	4	3							
	AFG		1	4	1	4							
	MFG		1	2	5	2							
	TRB	8	1	1									
	PSC				2	2	6						
	VRC					1		2	4	1			2
	ITC			2		1	7						
	AMB				1		1	4	4				
	5FC							1	2	4	2		1
Penicillium sp. (n = 7)	CFG			1	6								
	AFG		1	1	1	4							
	MFG				3	3		1					
	TRB	3	4										
	PSC				1	1	4	1					
	VRC					1	1	2	3				
	ITC				2	2	2	1					
	AMB							1	6				
	5FC												7
P. oxalicum (n = 7)	CFG			2	1	2			1	1			
	AFG		1	2	1		1		2				
	MFG				2	2		1	1	1			
	TRB	5	2										
	PSC					4	2	1					
	VRC								7				
	ITC				1	1		1	4				
	AMB					2	4	1					
	5FC							1		1	4		1
P. glabrum (n = 5)	CFG		5										
	AFG		4		1								
	MFG		3	2									
	TRB	2		3									
	PSC				2	1	2						
	VRC							1	3	1			
	ITC			1	1		1	2					
	AMB			1	2	2							
	5FC							3	1	1			

[a] CFG, caspofungin; AFG, anidulafungin; MFG, micafungin; TRB, terbinafine; PSC, posaconazole; VRC, voriconazole; ITC, itraconazole; AMB, amphotericin B; 5FC, flucytosine.

was identified in our study; that isolate represents another rare species in the clinical setting (32). Some other *Penicillium* species, including many, such as *P. rubefaciens*, *P. brasilianum*, *P. singarense*, and *P. rudallense*, represented by only one isolate each in our study, have not been recognized previously in isolates from clinical specimens, but their ability to grow at 37°C suggests a potential pathogenicity.

It is relevant that a considerable number of our isolates were identified as *Talaromyces* species. Apart from *T. marneffei*, which

is the most clinically important member, this genus contains some opportunistic species of clinical importance such as *T. amestolkiae*, *T. indigoticus*, *T. piceus*, *T. purpurogenus*, *T. radicus*, *T. ruber*, *T. rugulosus*, *T. stollii*, and *T. verruculosus*. Most of these species were part of the genus *Penicillium* (3, 33). Nearly 80% of our isolates identified as *Talaromyces* species belong to section *Talaromyces*, which is the only section that includes both animal-pathogenic and human-pathogenic species (33). *T. amestolkiae* and *T. purpurogenus* were the species most frequent identified

UNIVERSITAT ROVIRA I VIRGILI
TAXONOMÍA Y FILOGENIA DE AISLADOS CLÍNICOS Y AMBIENTALES DE PENICILLIUM Y TALAROMYCES
Marcela Isabel Guevara Suárez

Penicillium-Like Fungi in Clinical Samples

TABLE 3 Results of *in vitro* antifungal susceptibility testing of 12 isolates of *Talaromyces* species

Species (no. of isolates tested)	Antifungal[a]	No. of isolates with antifungal MIC (µg/ml) of:											
		≤0.03	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	>16
T. amestolkiae (7)	CFG			2	3	1	1						
	AFG		1	3	3								
	MFG			3	2	2							
	TRB	6	1										
	PSC						2		1				4
	VRC									1			6
	ITC									1			6
	AMB				1	2	2	2					
	5FC			2	3		1		1				
T. purpurogenus (5)	CFG						1			1	1	2	
	AFG		1		3							1	
	MFG			2	1	2							
	TRB	1	2	1								1	
	PSC								1	1		1	2
	VRC								1	2	2		
	ITC												5
	AMB									3	2		
	5FC			3	2								

[a] CFG, caspofungin; AFG, anidulafungin; MFG, micafungin; TRB, terbinafine; PSC, posaconazole; VRC, voriconazole; ITC, itraconazole; AMB, amphotericin B; 5FC, flucytosine.

among our isolates. These, together with *T. ruber* and *T. stollii*, were recovered from pulmonary and invasive infections in humans (7, 9) and animals (8, 12). The production of a red diffusible pigment by the colonies of *T. purpurogenum* is a feature shared with *T. marneffei* and can lead to misidentification of those two species in diagnostic laboratories. Although the former can grow at 37°C, it is unable to develop a yeast morphology such as *T. marneffei* can (9).

We also identified two isolates belonging to *Rasamsonia*, one as *R. argillacea* and the other as *R. eburnea* (formerly *Talaromyces eburneus*). Recently, rates of infections by isolates within this genus appear to have increased in humans and animals, and those species are considered emerging pathogens (34–36). In 2011, nine cases of invasive infections by *Rasamsonia argillacea* were reported in patients with chronic granulomatous disease (37, 38) and, more recently, in a patient with graft-versus-host disease (39). Houbraken et al. (35) identified clinical isolates of *R. eburnea* from blood cultures, sputum, and peritoneal dialysis fluid from a patient with peritonitis. *Rasamsonia argillacea* and *R. eburnea* are phylogenetically close and have similar phenotypic characteristics, except that *R. eburnea* shows a blackish brown reverse (40).

Several cases have been reported in which the respiratory tract was the portal of entry of infections by penicillium-like fungi, with or without systemic dissemination. Although the majority of isolates in this study were obtained from respiratory specimens, it was not possible to establish the true pathogenic role of the identified species because of the nature of the samples and the absence of clinical data.

In vitro antifungal susceptibility profiles of penicillium-like species of fungi other than *T. marneffei* are currently based on very few studies and are mainly taken from case reports that have shown differing results (8, 27, 32). Our results show that TRB and the echinocandins are highly active *in vitro* against *Penicillium* and *Talaromyces* spp. However, these antifungals are not widely used

for treating invasive infections by these fungi (41). Terbinafine was chosen as a good alternative for long-term maintenance therapy for treatment of an infection associated with one isolate from a dog with osteomyelitis (11). In the present study, AMB also had intermediate antifungal activity, agreeing with previous studies (42, 43); however, the clinical experience reported in two cases of infections by species within this group revealed that the patients did not respond to this drug (8). Our susceptibility results show that the azoles have variable activity against *Penicillium* species and high MICs for *Talaromyces* species. In fact, ITC has been used as a prophylactic treatment for infections by *T. marneffei* (42). Chowdhary et al. (32) reported resistance to VRC in three cases of invasive infections by *P. oxalicum*, where the successful alternative treatment was PSC. We observed intermediate MIC values for VRC in our isolates of *P. oxalicum*, while the *P. citrinum* isolates showed resistance to this drug. This confirms the observations of Mok et al. (27), who reported high MIC values for the azoles against one isolate of *P. citrinum* from an acute leukemia patient with pneumonia and pericarditis.

In conclusion, although human and animal infections caused by penicillium-like fungi are infrequent, this study revealed that a relative wide range of species, all able to grow at 37°C, should be taken into account in the diagnosis of such infections. Identification at the species level remains difficult on the grounds that species of various genera share similar morphological characteristics. This supports the relevance of using DNA sequence data to identify them. More data are needed from both *in vitro* susceptibility studies and clinical outcomes in order to determine an effective treatment for infections caused by penicillium-like fungi.

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4.2 Four new species of *Talaromyces* from clinical sources.

Mycoses 2017; 60 (10):651–662.
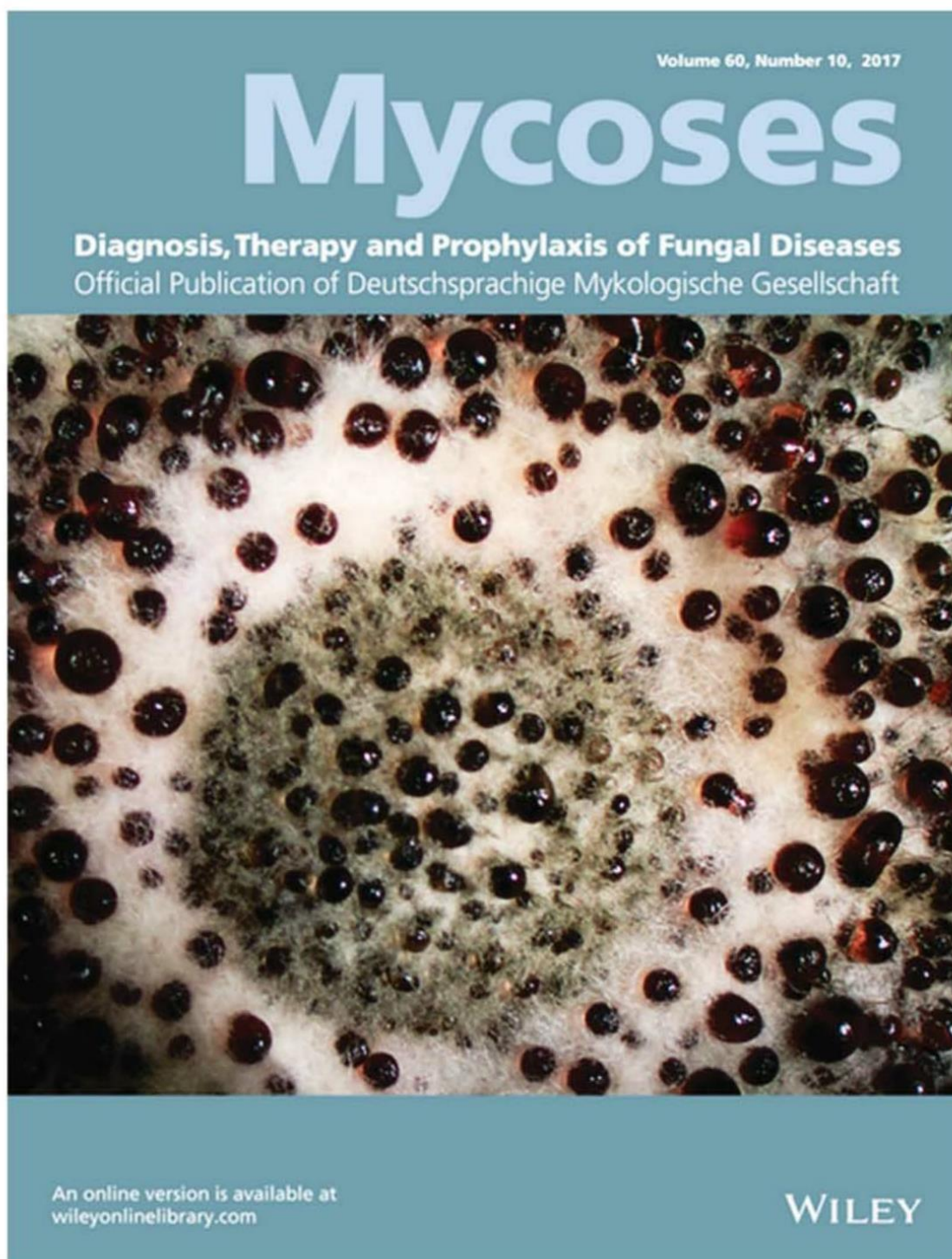
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ORIGINAL ARTICLE

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Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Four new species of *Talaromyces* from clinical sources

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Summary

The genus *Talaromyces* constitutes an important group of molds with species that are mainly found in soil, indoor environments and food products. Traditionally, it has been considered, together with *Eupenicillium*, the teleomorphic state of *Penicillium*. However, the taxonomy of these fungi has changed considerably, and *Talaromyces* currently includes sexually and asexually reproducing species. In a previous study of the occurrence of penicillium-like fungi from clinical samples in the USA, we used the combined phylogeny of the internal transcribed spacer (ITS) region of the rDNA and β-tubulin (*BenA*) gene to identify 31 isolates of *Talaromyces*, 85 of *Penicillium* and two of *Rasamsonia*. However, seven isolates of *Talaromyces* were assigned to the corresponding sections but not to any particular species. In this study, we have resolved the taxonomy of these isolates through a multilocus sequence analysis of the ITS, fragments of the *BenA*, calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) genes, and a detailed phenotypic study. As a result, four new species are described and illustrated, ie *Talaromyces alveolaris*, *T. georgiensis*, *T. minnesotensis* and *T. rapidus*.

KEYWORDS
clinical isolates, molecular identification, *Penicillium*, *Talaromyces*, taxonomy

1 | INTRODUCTION

The genus *Talaromyces* (*Trichocomaceae*, *Eurotiales*) has traditionally been characterised by its sexual morph having gymnothecial or cleistotecial ascomata, unitunicate 8-spored asci, and unicellular ascospores with or without equatorial crests. Their species were commonly associated with the asexual genus *Penicillium*, but also with other related genera such as *Geosmithia*, *Merimbla*, *Paecilomyces* and *Sagenomella*.[1,2] However, based on phylogenetic studies and following the abandonment of the dual nomenclature for pleomorphic fungi, *Penicillium* and *Talaromyces* have been separated into two distinct genera, including both sexually and asexually reproducing species, and Penicillia of the subgenus *Biverticillium* transferred to the latter genus.[3-5]

Although *Penicillium* and *Talaromyces* share many phenotypic features (ie micro- and macromorphology), the former is more related phylogenetically to the genus *Aspergillus* than the latter.[4] In addition, the species of *Talaromyces* grow particularly restricted on low

water activity media and have a quite different extrolite pattern than those of *Penicillium*.[6,7] It is noteworthy, however, that the identification of these fungi at the species level is currently a complex task. That requires to study morphological and physiological characters (ie growth on different culture media at different temperatures and extrolite profiles), as well as sequence data mainly of the β-tubulin (*BenA*) gene.[6,7] Nevertheless, to establish species boundaries or introduce new taxa, multilocus sequence analysis, including the internal transcribed spacer (ITS) region, *BenA* and fragments of the calmodulin (*CaM*) or the DNA-dependent RNA polymerase II largest subunit (*RPB2*), is necessary.[6,7]

Talaromyces currently includes around 110 accepted species, grouped into seven sections, ie *Bacillispori*, *Helici*, *Islandici*, *Purpurei*, *Subinflati*, *Talaromyces* and *Trachyspermi*.[8,9] Species within the genus have important biotechnological applications,[6] and have been reported to spoil pasteurised fruit juices and other fruit based products.[10,11] The genus also includes clinically relevant species such as *T. marneffei* (formerly *Penicillium marneffei*), which is considered an emerging pathogen

TABLE 1 *Talaromyces* strains used in this study

Species	Section	Strain no.	Source	GenBank accession number			
				ITS	*BenA*	CaM	*RPB2*
Talaromyces alveolaris	*Talaromyces*	UTHSC DI16-146	BAL	LT558968	LT559085	LT795594	LT795595
		UTHSC DI16-147 =CBS 142379[T]	BAL	LT558969	LT559086	LT795596	LT795597
Talaromyces georgiensis	*Helici*	UTHSC DI16-145 =CBS 142380[T]	Joint-fluid animal	LT558967	LT559084	-	LT795606
Talaromyces kabodanensis	*Talaromyces*	UTHSC DI16-149	BAL	LT558971	LT559088	LT795598	LT795599
Talaromyces minnesotensis	*Trachyspermi*	UTHSC DI16-144 =CBS 142381[T]	Ear	LT558966	LT559083	LT795604	LT795605
Talaromyces rapidus	*Talaromyces*	UTHSC DI16-148 =CBS 142382[T]	BAL	LT558970	LT559087	LT795600	LT795601
Talaromyces subaurantiacus	*Islandici*	UTHSC DI16-143	BAL	LT558965	LT559082	LT795602	LT795603

BAL, human bronchoalveolar lavage.
[T]Ex-type strain.

that causes fatal systemic mycosis in, mostly, immunosuppressed patients from Southeast Asia, India, and China.[12] Other species, such as *T. amestolkiae*, *T. indigoticus*, *T. piceus*, *T. purpurogenus*, *T. radicus*, *T. ruber*, *T. rugulosus*, *T. stollii* and *T. verruculosus* have also, more rarely, been reported to cause human disease.[2,8]

Although numerous penicillium-like fungi are commonly reported in the clinical environment, both the incidence and diversity of these species in clinical samples is poorly documented. A survey was carried out recently on the presence of such fungi in a large set of clinical isolates from a US reference laboratory. Using the sequences of the ITS region and of the *BenA* gene, we found that most of those isolates belonged to the genera *Penicillium*, *Talaromyces* and *Rasamsonia*.[13] In that study, we identified 31 isolates of *Talaromyces* recovered from human and animal clinical specimens; however, we were not able to identify seven of the isolates at the species level. The purpose of this study was to resolve the taxonomy of these unidentified isolates using a polyphasic approach, including further molecular markers and a detailed phenotypic study.

2 | MATERIALS AND METHODS

2.1 | Isolates and sequences

Seven *Talaromyces* isolates were investigated in this study (Table 1). They were provided by the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio (UTHSCSA). These specimens were part of a set of 118 clinical isolates of penicilium-like fungi included in Guevara-Suarez et al.[13] which had been assigned to the corresponding sections but not to particular species. Sequences of ex-type strains of all the species of the sections involved (ie, *Helici*, *Islandici*, *Talaromyces* and *Trachyspermi*) and reference strains of *T. aurantiacus* and *T. minioluteus*, mostly reported in different studies,[7-9,14-19] were retrieved from GenBank (Table S1) and included in the phylogenetic analyses.

2.2 | Morphological characterisation

Colony features were studied following Yilmaz et al.[7] Briefly, the isolates were cultured onto malt extract agar (MEA; Difco Inc., Detroit, USA),[20] oatmeal agar (OA),[20] Czapek yeast autolysate (CYA),[21] yeast extract sucrose agar (YES),[22] creatine sucrose agar (CREA),[22] and dichloran 18% glycerol agar (DG18),[23] incubated at 25°C for 7 days in darkness. Colony diameters were also measured after 7 days at 30 and 37°C on CYA, MEA, YES and OA. Colour notations in colony descriptions are from Kornerup and Wanscher.[24] For ascoma production, OA plates were incubated at 25°C for up to 4 weeks.

Microscopic characters were examined and measured from the isolates after 7 days of growth on MEA at 25°C and mounted on slides with Shear's solution. Photomicrographs were obtained using a Zeiss Axio-Imager M1 light microscope with Nomarski differential interference contrast and phase-contrast optics (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity X digital camera.

2.3 | DNA extraction, amplification and sequencing

DNA was extracted directly from colonies on MEA after 7-14 days at 25°C, using the FastDNA® kit protocol (MP Biomedicals, Solon, OH, USA) and for the homogenisation step a FastPrep® FP120 cell disrupter (Thermo Savant, Holbrook, NY, USA). We amplified the ITS region, including the 5.8S rDNA gene, and fragments of the *BenA*, *CaM* and *RPB2* genes proposed by Yilmaz et al.[7] for the phylogenetic studies in the genus *Talaromyces*. The primer pairs used were: ITS5/ITS4 for the ITS region,[25] Bt2a/Bt2b for *BenA* for most isolates and T10/Bt2b for one isolate of the section *Islandici*,[26] CMD5/CMD6 for *CaM*,[27] and RPB2-5F/RPB2-7Cr for *RPB2*.[28]

Single band PCR products were purified and sequenced at Macrogen Europe (Macrogen Inc., Amsterdam, The Netherlands) with a 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequence assembly and editing were performed using SeqMan v. 7.0.0

TABLE 2 Overview and details used for phylogenetic analyses

	Dataset			
	Section *Helici*	Section *Islandici*	Section *Talaromyces*	Section *Trachyspermi*
ITS dataset				
Length (bp)	465	462	466	477
Phylogenetic variable sites	102	102	84	107
Phylogenetic informative sites	41	56	47	83
Substitution model (ML)	T92+G	T92+G+I	TN93	T92+G
BenA dataset				
Length (bp)	410	412	404	382
Phylogenetic variable sites	162	179	199	153
Phylogenetic informative sites	104	128	136	94
Substitution model (ML)	K2+I	K2+G	K2+G	K2+G
CaM dataset				
Length (bp)	-	480	502	483
Phylogenetic variable sites	-	235	260	236
Phylogenetic informative sites	-	187	210	168
Substitution model (ML)	-	K2+G	K2+G	K2+G
RPB2 dataset				
Length (bp)	839	754	-	-
Phylogenetic variable sites	264	267	-	-
Phylogenetic informative sites	195	218	-	-
Substitution model (ML)	K2+G	K2+G	-	-
Concatenated data set				
Length (bp)	1714	2108	1372	1333
Phylogenetic variable sites	528	743	543	496
Phylogeneticin formative sites	340	543	393	345

(DNASTAR, Madison, WI, USA). GenBank accession numbers for the sequences newly generated in this study are listed in Table 1.

2.4 | Phylogenetic reconstructions

Sequences from each locus were aligned with MEGA v 6.0 software,[29] using the CLUSTALW algorithm,[30] refined with MUSCLE,[31] and visually adjusted using the same software platform. Phylogenetic analyses were made for each section with the individual locus and combined genes using maximum-likelihood (ML) in MEGA v. 6.0 and Bayesian inference (BI) under MrBayes version 3.1.2.[32] For the ML analysis, nearest-neighbour interchange (NNI) was used as the heuristic method for tree inference; support for internal branches was assessed by 1000 ML sets of data. A bootstrap support (bs) ≥70% was considered significant. The phylogenetic reconstruction by BI was carried out using five million Markov chain Monte Carlo (MCMC) generations, with two runs (one cold chain and three heated chains) and samples were stored every 1000 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 significant. The best substitution model for all gene matrices was estimated using jModelTest v.2.1.3.[33,34] Phylogenetic trees were edited for publication in Adobe Illustrator CS3.

3 | RESULTS

We carried out a phylogenetic study for the sections *Helici*, *Islandici*, *Talaromyces* and *Trachyspermi*. Phylogenies of each section were performed for the ITS region, *BenA*, *CaM* and/or *RPB2* loci (according to the availability of sequences of type strains for each section), as well as a concatenation of the three or four mentioned loci. The length, number of phylogenetic informative and variable sites, and substitution models (for ML) for each dataset are summarised in Table 2. The topologies of the trees of ML and BI analyses did not differ, therefore we used ML trees for representing results, with BI posterior probability values marked on relevant branches.

A first phylogeny concerning all currently accepted species in the section *Talaromyces*, including four unidentified clinical isolates, was
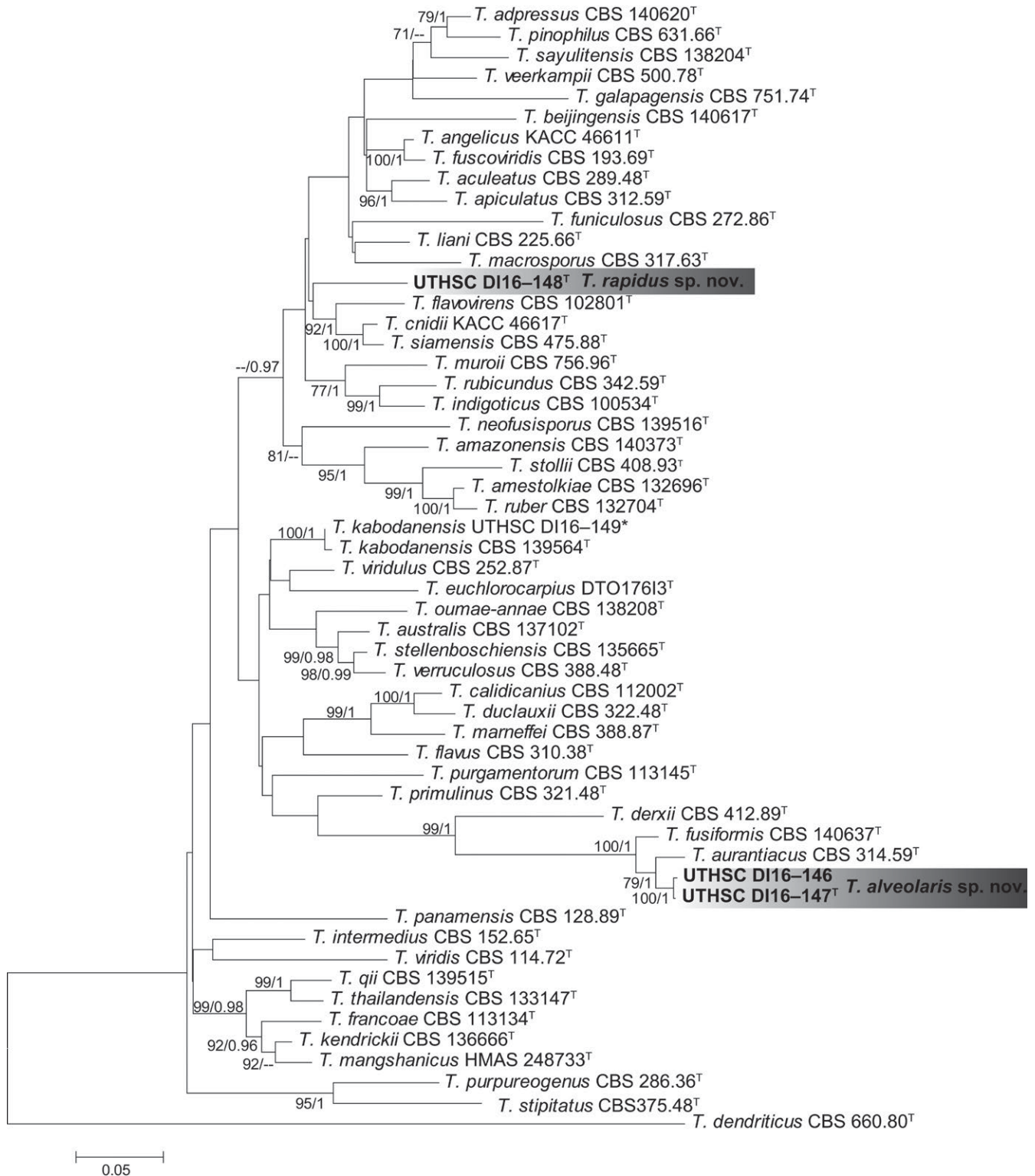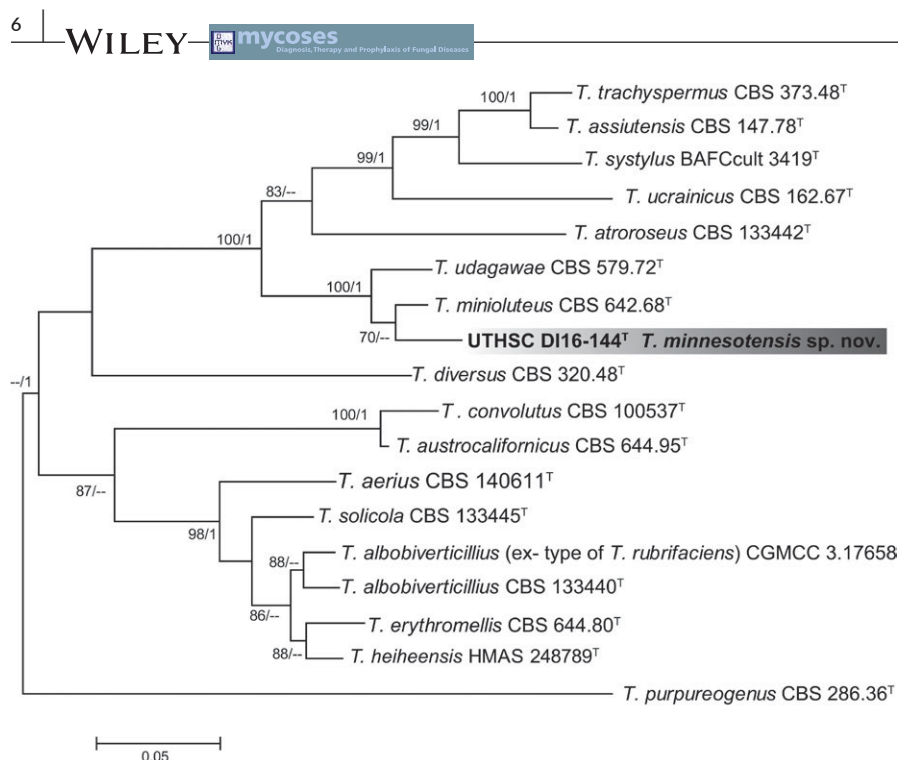
FIGURE 1 Phylogenetic tree of *Talaromyces* section *Talaromyces*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the combination of ITS (466 bp), *BenA* (404 bp) and *CaM* (502 bp) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces dendriticus* CBS 660.80 (Section *Purpurei*). T: type strain, *isolate identified in this study. The new species proposed are shown in dark box

performed using sequences of the ITS, *BenA* and *CaM* genes (Figure 1). The aligned data set was 1372 bp long (ITS 466 bp; *BenA* 404 bp; *CaM* 502 bp). In this section two putative new species could be well delineated. The isolate UTHSC DI16-148 formed an independent branch clearly distinct from the other species of the section, while UTHSC DI16-146 and UTHSC DI16-147 both formed a full-supported
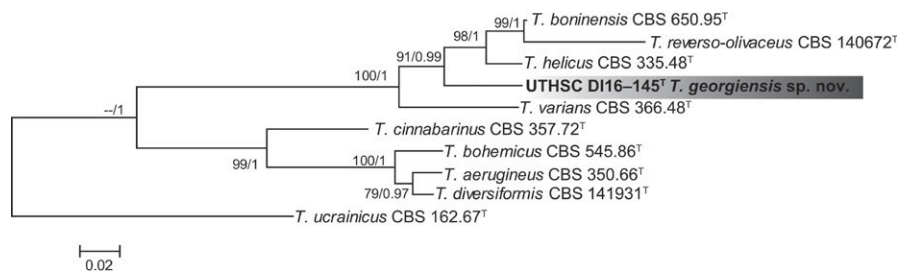
FIGURE 2 Phylogenetic tree of *Talaromyces* section *Helici*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the combination of ITS (465 bp), *BenA* (410 bp) and *RPB2* (839 bp) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces ucrainicus* CBS 162.67 (Section *Trachyspermi*). T: type strain. The new species proposed is shown in dark box
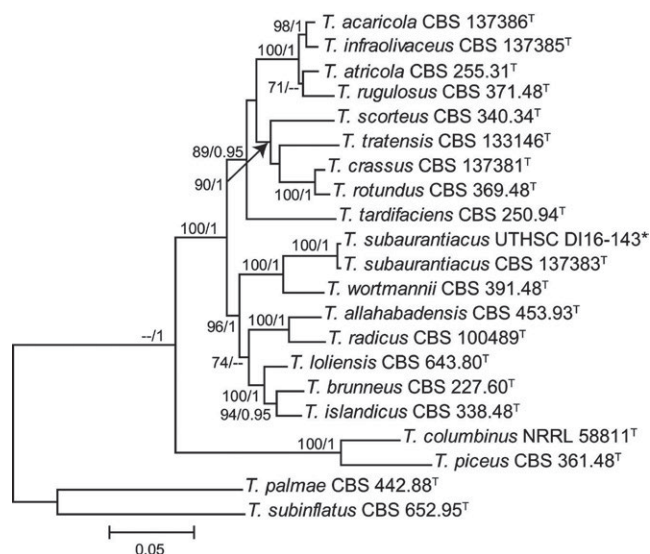
FIGURE 3 Phylogenetic tree of *Talaromyces* section *Islandici*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the combination of ITS (462 bp), *BenA* (412 bp) *RPB2* (754 bp), and *CaM* (480 bp) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/ posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces palmae* CBS 442.88 and *Talaromyces subinflatus* CBS 652.95 (Section *Subinflati*). T: type strain, *isolate identified in this study

clade closely related to *T. aurantiacus*. An additional analysis with the alternative barcode *BenA*, including all available GenBank sequences of *T. aurantiacus* (Figure S1), showed that the clade of the two clinical isolates was phylogenetically distant from the *T. aurantiacus* clade, with a similarity of 97.2% between them, and thus should be considered distinct taxa. The two new phylogenetic species are proposed below as *T. rapidus* and *T. alveolaris*, respectively. The isolate UTHSC DI16-149 matches morphologically and genetically with *T. kabodanensis*.[35]

A second phylogenetic reconstruction was performed for the section *Helici* (Figure 2), using ITS, *BenA* and *RPB2*. *CaM* was not included in the concatenated analysis because we were unable to get a reliable sequence of this locus from the isolate investigated. The aligned data set was 1714 bp long (ITS 465 bp; *BenA* 410 bp; *RPB2* 839 pb). Our phylogeny demonstrated that UTHSC DI16-145 was included in the

T. helicus-clade,[7] together with *T. boninensis*, *T. helicus*, *T. reverso-olivaceus* and *T. varians*. The concatenated analysis showed that our clinical isolate was located between *T. helicus* and *T. varians* in a separate and well-supported branch (91% bs/0.99 pp), representing a new lineage in the section and described below as *T. georgiensis*. The only species of the section not included in the present analysis was *T. ryukyuensis*, since only ITS sequences were available for comparison. However, according to the reported phylogeny, *T. ryukyuensis* is closely related to *T. aerugineus*, *T. bohemicus* and *T. cinnabarinus*, three species that formed a highly supported clade phylogenetically distant from the *T. helicus*-clade. A relevant feature of the species in section *Helici* is their ability to grow at 37°C, which was also observed in the new species.

The combined analysis of ITS (462 bp), *BenA* (412 bp), *CaM* (480 bp) and *RPB2* (754 bp) for the section *Islandici* (Figure 3) allowed for the identification of UTHSC DI16-143 as *T. subaurantiacus*.[8] This clinical isolate exhibited a phylogenetic distance of 0.3% with the ex-type strain of *T. subaurantiacus* (CBS 137383) and, phenotypically, it showed more restricted growth on all culture media and at the various temperatures tested (at 25°C on CYA 6-8 mm, MEA 14-15 mm, YES 11-12 mm; at 37°C 3-4 mm on CYA) compared with those described in the protologue of the species (at 25°C on CYA 16-18 mm, MEA 20-21 mm, YES 17-18 mm; at 37°C 7 mm on CYA).[8]

Finally, the phylogenetic relationship of the *Talaromyces* isolate UTHSC DI16-144 with the species of the section *Trachyspermi* was carried out using ITS, *BenA* and *CaM* (Figure 4). The aligned combined data set was 1333 bp long (ITS 477 bp; *BenA* 382 bp; *CaM* 474 bp). This isolate was included in a fully supported clade with *T. udagawae* and *T. minioluteus*, the latter being the closest species. Considering that *T. minioluteus* has been reported as a species complex by Visagie et al.[14] and to know whether our clinical isolate matches with any of the lineage delineated in the complex, we carried out an additional *BenA* analysis with reliable GenBank sequences of *T. minioluteus*, including those of some species considered synonyms, such as *Penicillium samsonii* (CBS 137.84) and *Penicillium purpurogenum* var. *rubrisclerotium* (CBS 270.35).[16] In that analysis (Figure S2), as in the combined analysis of the three mentioned loci data sets (Figure 4), our isolate was placed in a single branch distant from the clade where the ex-type strain of the species was included and from the main clade with the rest of *T. minioluteus* isolates, showing a similarity of 95.22% and 94.97% respectively to UTHSC DI16-144. Therefore,

FIGURE 4 Phylogenetic tree of *Talaromyces* section *Trachyspermi*, using Maximum-likelihood (ML) and Bayesian inference (BI), tree constructed with the combination of ITS (477 bp), *BenA*(382 bp) and *CaM* (474) sequence data. Support values are above branches, and represent bootstrap values >70% for ML/posterior probabilities >0.95 for BI. The phylogenetic tree was rooted with *Talaromyces purpurogenus* CBS 286.36 (Section *Talaromyces*). T: type strain. The new species proposed is shown in dark box

this isolate is considered representative of a distinct species and described below as *T. minnesotensis*. Distinctive features of this species were strong acid production on CREA, presence of a soluble red pigment on CYA, restricted growth on MEA at 25°C, and absence of growth at 37°C.

3.1 | Taxonomy

Talaromyces alveolaris Guevara-Suarez, Cano & Guarro, sp. nov. — MycoBank MB 820459; Figure 5.

Etymology. Referring to the clinical specimen where the fungus was isolated.

Specimens examined. USA, Utah, human bronchoalveolar lavage, 2010, D.A. Sutton (*holotype* CBS H-22999; ex-type cultures UTHSC DI16-147, CBS 142379, FMR 13963); Texas, human bronchoalveolar lavage, 2010, D.A. Sutton (UTHSC DI16-146, FMR 13962).

Colony diameter in 7 days (mm) — on CYA: 25°C 20-22, 30°C 29-31, 37°C 20-22; on MEA: 25°C 19-24, 30°C 16-19, 37°C 21-23; on YES: 25°C 25-28, 30°C 29-31, 37°C 36-40; on OA: 25°C 30-32, 30°C 30-35, 37°C 18-21; DG18 25°C no growth; CREA 25°C 8-10.

Colony characters at 25° in 7 days — On CYA, colonies with raised centre, floccose, white, margins entire; reverse greyish brown (8F3) to light orange (5A4); sporulation sparse; soluble pigment only at 30 and 37°C, brownish orange (6C8-6D8) to reddish brown (8E7); exudates hyaline droplets after 14 days. On MEA, colonies flat, velvety, mycelium white becoming pale green (30A3), margins lobate; reverse dark green (30F8) to yellowish green (30B8); sporulation moderate, with inconspicuous conidial masses; exudates and soluble pigments absent. On YES, colonies flat, slightly sulcate, floccose, mycelium light orange (5A5) to white, margins entire; reverse light orange (5A5); sporulation absent; soluble pigments brownish red (8C8) to reddish

brown (8F8) only at 30 and 37°C; exudates absent. On OA, colonies flat, velvety, white, margins entire; reverse pale orange (5A3); sporulation sparse; soluble pigments and exudates absent. On CREA, weak acid production.

Micromorphology on MEA — Conidiophores biverticillate; stipes smooth- walled, 85-130×2-3 μm; metulae two to four, divergent, cylindrical, 11-14×2-3 μm; phialides two to four per metulae, acerose, 10-13×2-3 μm; conidia smooth-walled, mostly subglobose to somewhat ellipsoidal, 2.5-3×2-2.5 μm. Ascomata not observed.

Notes —*T. alveolaris* is phylogenetically closely related to *T. aurantiacus* and *T. fusiformis*. Morphologically, *T. aurantiacus* mainly differs in having shorter stipes (up to 100 μm long) and cylindrical to ellipsoidal conidia (3-5×1.5-2.5 μm), and *T. fusiformis* in its ellipsoidal to fusiform conidia (3-4×2-3 μm) and absence of growth on CREA.[7,16]

Talaromyces georgiensis Guevara-Suarez, Sutton & Wiederhold, sp. nov. — MycoBank MB 820460; Figure 6.

Etymology. Referring to the State of Georgia in USA, where the fungus was isolated.

Specimen examined. USA, Georgia, Athens, from animal joint fluid, 2010, D.A. Sutton (*holotype* CBS H-23000; ex-type cultures UTHSC DI16-145, CBS 142380, FMR 14270).

Colony diameter in 7 days (mm) — on CYA: 25°C 29-31, 30°C 48-50, 37°C 47-50; on MEA: 25°C 28-31, 30°C 40-43, 37°C 43-45; on YES 25°C 28-30, 30°C 38-45, 37°C 40-43; on OA: 25°C 22-29, 30°C 38-40, 37°C 40-45; DG18 25°C 7-8; CREA 25°C 19-21.

Colony characters at 25°C in 7 days — On CYA, colonies with raised centre, flat towards the periphery, velvety, white, margins entire; reverse yellowish white (2A2); sporulation sparse, with conidial masses pale green (30A3); exudates absent; soluble pigments absent. On MEA, colonies cottony, mycelium white becoming greenish grey (29B2), margins entire; reverse greyish yellow (4B3); sporulation moderate, conidial
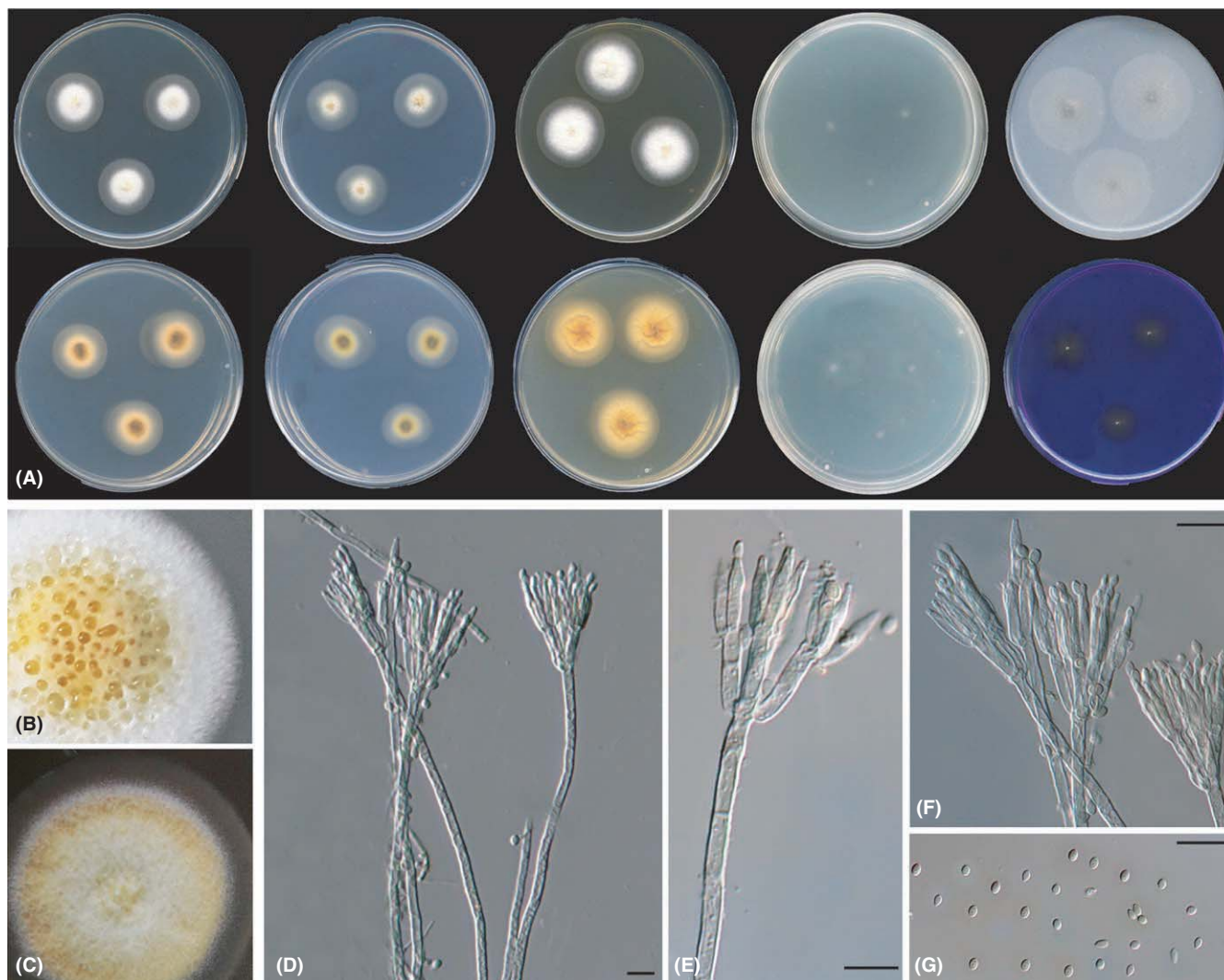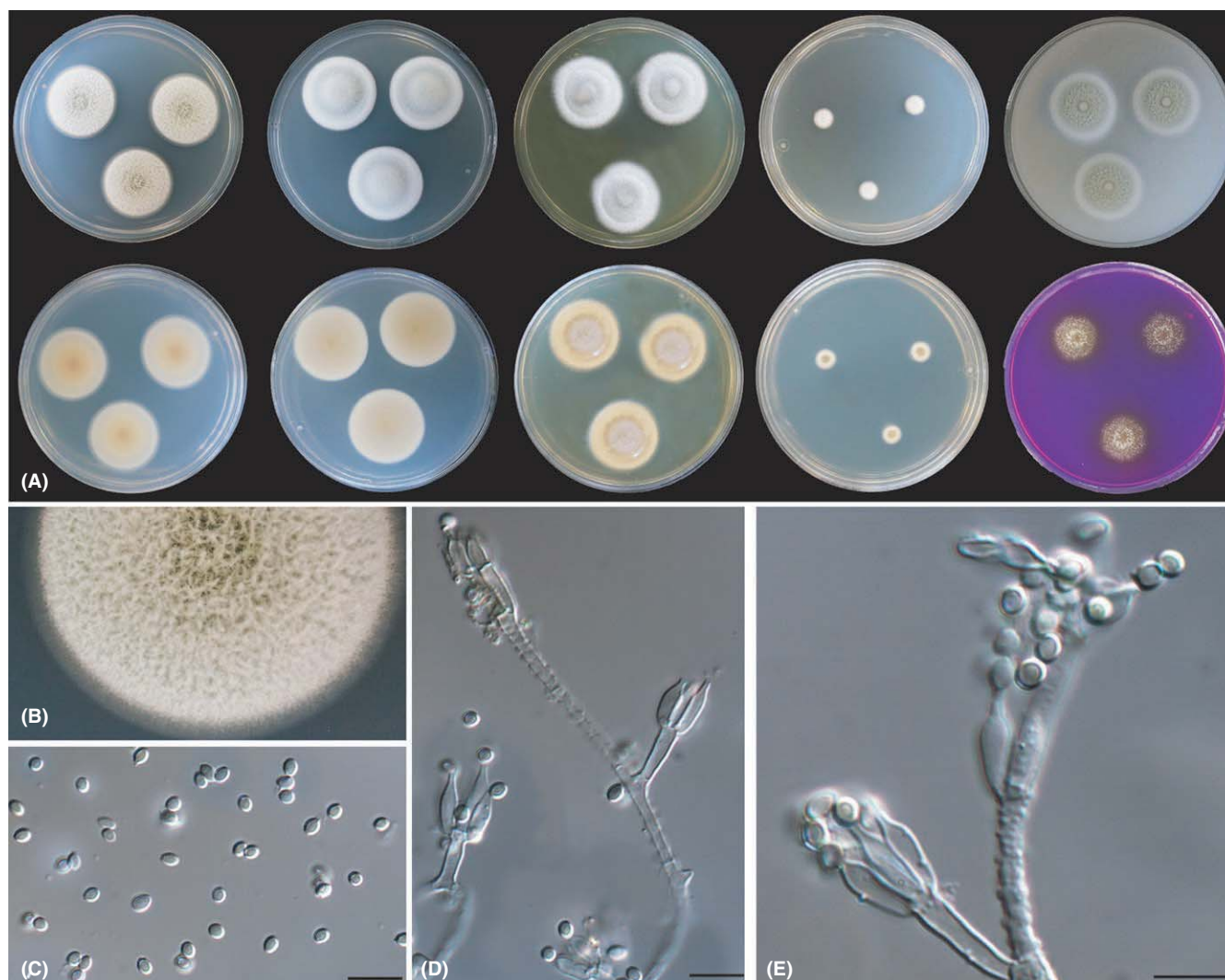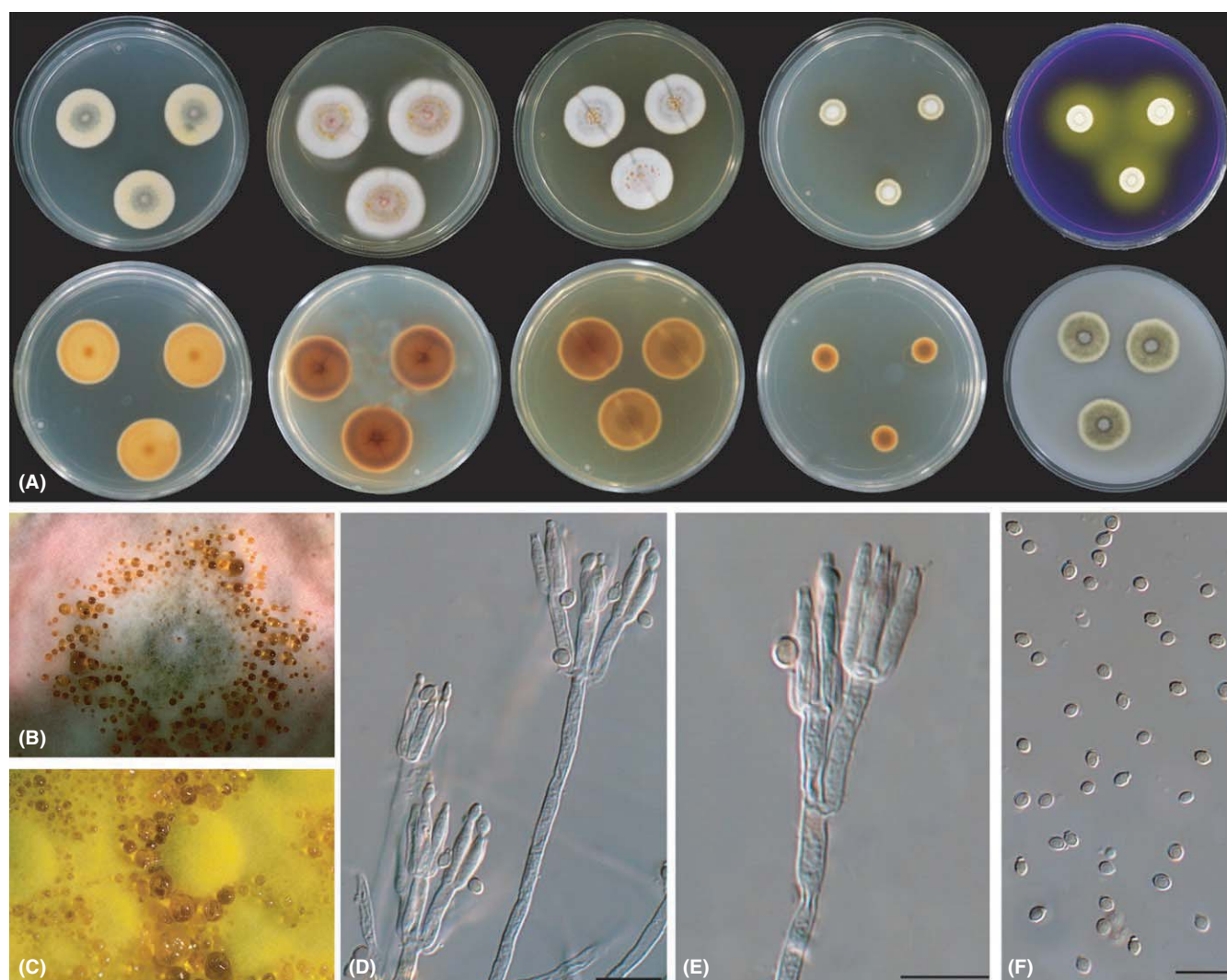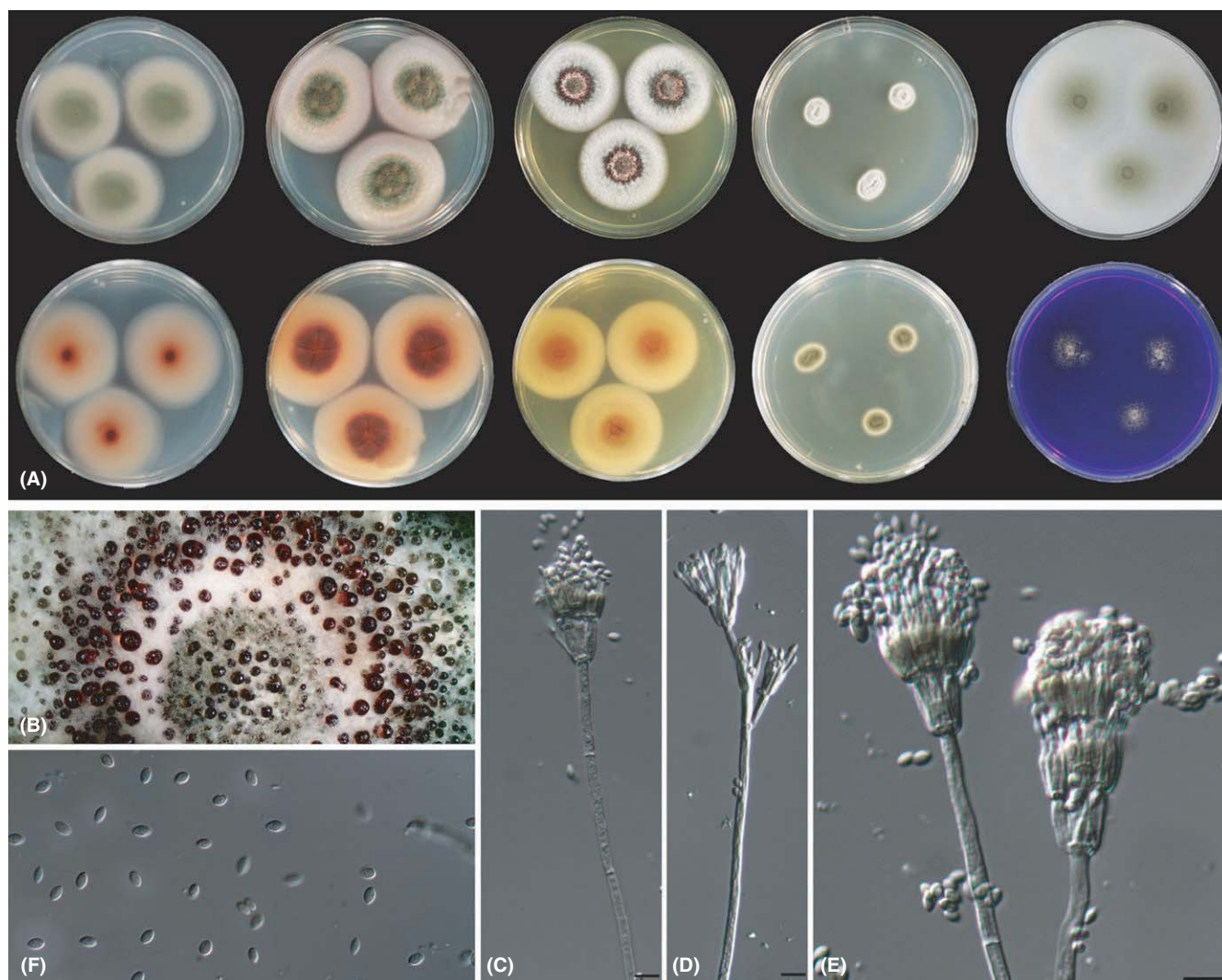
FIGURE 5 Morphological characters of *Talaromyces alveolaris* (UTHSC DI16-147[T]). (A) Colonies from left to right (top row) CYA, MEA, YES, DG18 and OA; (bottom row) MEA reverse, CYA reverse, YES reverse, DG18 reverse and CREA. (B) Colony texture on CYA at 25°C after 2-wk incubation. (C) Colony texture on CYA at 30°C after 1-wk incubation. (D-F) Conidiophores. (G) Conidia. Scale bars=10 μm

masses pale green (30A3); exudate absent; soluble pigment absent. On YES, colonies with a dome-shaped centre, cottony, white, margins entire; reverse pale yellow (4A3); sporulation absent; exudates and soluble pigments absent. On DG18, colonies flat, floccose, white, margins entire; reverse greyish green (30C5) to white; sporulation absent; exudates and soluble pigments absent. On OA, colonies flat, cottony, mycelium greyish green (28B3), margins entire; reverse yellowish white (2A2); sporulation moderate, conidial masses greenish white (29A2); exudate and soluble pigments absent. On CREA, weak acid production.

Micromorphology on MEA — Conidiophores mostly monoverticillate; stipes rough-walled, somewhat pigmented, 11-15×2.5-3 μm; metulae two to three, divergent, 12-15×2-2.5 μm; phialides two to four per metulae, acerose, 8-13(−20)×2.5-3 μm; conidia smooth-walled, globose to subglobose, 2.5-4(−4.5)×2.2-3 μm. Ascomata not observed.

Notes — *Talaromyces georgiensis* can be distinguished easily from its closely related species (ie *T. boninensis, T. helicus, T. reverso-olivaceus* and *T. varians*) by its profuse and improved growth at 30-37°C than at 25°C, by the acid production on CREA and by its rough-walled stipes. The maximum colony diameter reported for the species of the *T. helicus*-clade is 30 mm in 7 days at 37°C,[7,16] while the novel species can reach 50 mm. In addition, *T. georgiensis*, as well as *T. reverso-olivaceus* and *T. varians*, does not produce the sexual morph, which is present in *T. helicus* and *T. boninensis*.[7,16]

Talaromyces minnesotensis Guevara-Suarez, Cano & D. García, sp. nov. — MycoBank MB 820463; Figure 7.

Etymology. Referring to the State of Minnesota in USA, where the fungus was isolated.

Specimen examined. USA, Minnesota, from human ear, 2010, D.A. Sutton (*holotype* CBS H-23001; ex-type cultures UTHSC DI16-144, CBS 142381, FMR 14265).

Colony diameter in 7 days (mm) — on CYA: 25°C 24-26, 30°C 23-25, 37°C no growth; on MEA: 25°C 13-15, 30°C 19-21, 37°C no growth; on YES: 25°C 21-24, 30°C 24-26, 37°C no growth; on OA:25°C 19-20, 30°C 17-20, 37°C no growth; DG18 25°C 8-10; CREA 25°C 9-12.

FIGURE 6 Morphological characters of *Talaromyces georgiensis* (UTHSC DI16-145[T]). (A) Colonies from left to right (top row) MEA, CYA, YES, DG18 and OA; (bottom row) MEA reverse, CYA reverse, YES reverse, DG18 reverse and CREA. (B) Colony texture on MEA at 25°C after 1-wk incubation. (C) Conidia. (D,E) Conidiophores. Scale bars=10 μm

Colony characters at 25°C in 7 days — On CYA, colonies with a concave centre, radially sulcate to the periphery, velvety, mycelium white to pastel red (9A5), margins entire; reverse reddish brown (9D8); sporulation sparse; exudates orange (5B8) droplets only present at 30°C; soluble pigment reddish orange (7B8). On MEA, colonies flat, velvety, mycelium greenish grey (29C2) at the centre, yellowish white (2A3) towards the periphery, margins entire; reverse light orange (5A5); sporulation moderate, with conidial masses greyish green (27B4); exudates and soluble pigments absent. On YES, colonies flat, slightly concentrically sulcate and undulate, white; reverse brownish orange (7C7); sporulation absent; exudates with small clear droplets; soluble pigments absent. On DG18, colonies raised at centre, mycelium white to light yellow (3A4), margins entire; reverse orange (6B8); sporulation moderate; exudates and soluble pigments absent. On OA, colonies flat, velvety, mycelium olive (3D5) to pastel yellow (3A4), margins entire; reverse light yellow (4A5); sporulation abundant, with conidial masses greyish green (30D5); exudates and soluble pigments absent. On CREA, acid production strong.

Micromorphology on MEA — Conidiophores mostly biverticillate; stipes smooth-walled, 90-200 (−250)×2-3 μm; metulae two to five, divergent, 1015×23.5 μm; phialides three to five per metulae, acerose, 1013(−15)×2-3 μm; conidia smooth-walled, ellipsoidal, 2.5-3.5×2-3 μm. Ascomata not observed.

Notes — *Talaromyces minioluteus*, the species phylogenetically closest to *T. minnesotensis*, as mentioned before, differs in the lack of acid production on CREA and in having a more restricted growth on CYA (17-18 mm 7 days at 25°C).[7] *Talaromyces udagawae*, which is placed in the same clade as *T. minioluteus* and *T. minnesotensis*, can be differentiated easily by the production of ascomata.[7]

Talaromyces rapidus Guevara-Suarez, D. García & Gené, sp. nov. — MycoBank MB 820464; Figure 8.

Etymology. Referring to the fast growth in culture.

FIGURE 7 Morphological characters of *Talaromyces minnesotensis* (UTHSC DI16-144[T]). (A) Colonies from left to right (top row) MEA, CYA, YES, DG18 and CREA; (bottom row) MEA reverse, CYA reverse, YES reverse, DG18 reverse and OA. (B) Colony texture on MEA at 25°C after 1-wk incubation. (C) Colony texture on YES at 30°C after 1-wk incubation. (D,E) Conidiophores. (F) Conidia. Scale bars=10 μm

Specimen examined. USA, Ohio, human bronchoalveolar lavage, 2011, D.A. Sutton (*holotype* CBS H-23002; ex-type cultures UTHSC DI16-148, CBS 142382, FMR 14293).

Colony diameter in 7 days (mm) — on CYA: 25°C 44-46, 30°C 50-52, 37°C 20-21; on MEA: 25°C 39-42, 30°C 44-45, 37°C 25-27; on YES: 25°C 37-39, 30°C 45-47, 37°C 24-26; on OA 25°C 30-37, 30°C 40-44, 37°C 23-24; DG18 25°C 9-11; CREA 25°C no growth.

Colony characters at 25°C in 7 days — On CYA, colonies raised at centre, concentrically sulcate, floccose, mycelium greyish yellow (3B4) to light green (28B4) fading into white, margins plane, entire; reverse brownish red (9C8) centre fading to white; sporulation abundant, with conidial masses pastel green (28A4); exudates forming small red droplets; soluble pigments absent. On MEA, colonies flat, velvety, mycelium greyish green (29C6) to white, margins entire; reverse high red (10A8) to white; sporulation moderate; exudates and soluble pigments absent. On YES, colonies raised at the centre, with mycelium dark ruby (12F7) to bluish green (25C8), white towards the periphery, velvety, margins low, entire; reverse pastel red (7A4) centre fading to white; sporulation moderate; exudates violet brown droplets (11F7); soluble pigments absent. On DG18, colonies flat, floccose, white, margins entire; reverse yellowish green (30C8); sporulation absent. On OA, colonies flat, velvety, mycelium greyish green (30E6), margins entire; reverse colourless; sporulation abundant; exudates and soluble pigments absent. On CREA, acid production absent.

Micromorphology on MEA — Conidiophores mostly biverticillate, with a minor proportion having subterminal branches; stipes smooth-walled, 80-130×2.5-3 μm; metulae three to five, appressed, cylindrical 13-15×2-3 μm; phialides three to four per metulae, acerose almost flask-shaped, 9-13×2-3 μm; conidia smooth-walled, ellipsoidal to somewhat fusiform, 2.5-4×2-2.5 μm. Ascomata not observed.

Notes — *Talaromyces rapidus* is characterised by rapid growth on practically all media and at all temperatures tested, especially at 30°C. Phylogenetically, it forms an independent and distant branch

FIGURE 8 Morphological characters of *Talaromyces rapidus* (UTHSC DI16-148[T]). (A) Colonies from left to right (top row) MEA, CYA, YES, DG18 and OA; (bottom row) MEA reverse, CYA reverse, YES reverse, DG18 reverse and CREA. (B) Colony texture on MEA at 25°C after 1-wk incubation. (C-E) Conidiophores. (F) Conidia. Scale bars=10 μm

included in an unsupported clade together with the type species of *T. flavovirens*, *T. cnidii* and *T. siamensis* (Figure 1). Although the analyses of the ITS, *BenA* and *CaM* sequences (including the concatenated) did not resolve the relationship of this species with other species in this section *Talaromyces*, it did allow for the detection of this novel species. Morphologically, *T. rapidus* resembles *T. cnidii*, but the latter can be distinguished by the production of a red to yellow diffusible pigment on CYA at 25°C, and by its conidiophores with longer stipes (up to 230 μm) bearing divergent metulae.[7] *Talaromyces rapidus* does not produce diffusible pigments in any of the culture media tested, and its conidiophores have stipes up to 130 μm long bearing appressed metulae.

4 | DISCUSSION

The taxonomy of *Talaromyces* was redefined recently on the basis of DNA sequence data, extrolite profiles and other phenotypic features

including its morphology, resulting in a modern concept of the genus.[7] However, phylogenetic analyses of the ITS, *BenA*, *RPB2* and *CaM* genes are imperative for new species identification.[7] In this study, the multigene phylogeny proposed by Yilmaz et al.[7] allowed us to recognise four new species, and to identify two recently described *Talaromyces*, ie *T. kabodanensis* isolated from soil and also from clinical specimens in different countries,[35] and *T. subaurantiacus* recovered from a soil sample in South Africa.[8]

Two of these new species described here belong to the section *Talaromyces*, ie *T. alveolaris* and *T. rapidus*. In fact, most of the *Talaromyces* from clinical samples identified in our previous study belong to that section.[13] This is the largest section in the genus and includes nearly 50 species, 13 of them described in the last year from environmental samples.[9,15,16,19] The members of the section *Talaromyces* are closely related phylogenetically, based especially on their ITS sequences, but they also show a very similar morphology. Visagie et al.[15] reported numerous misidentifications in this section, mostly attributed to the

similarity of their conidiophores. However, they can be identified easily with the analysis of *BenA* sequences. Recently, Chen et al.[16] described nine new species of *Talaromyces* from indoor environments in China, three of them being assigned to the section *Talaromyces*, ie *T. fusiformis*, *T. adpressus* and *T. beijingensis*. *Talaromyces fusiformis* is closely related to *T. alveolaris* and forms a well-supported clade with *T. aurantiacus* and *T. derxii*. Interestingly, these four species are able to grow well at human body temperature, which is an important feature when considering their potential to cause disease. It is also noteworthy that *T. aurantiacus* was one of the species found in the set of penicillium-like clinical isolates previously studied, and found from a scalp wound and canine lung tissue.[13] *T. alveolaris* and *T. rapidus* have been recovered exclusively from the human respiratory tract.

Yilmaz et al.[7] introduced the section *Helici* for seven *Talaromyces* species, mostly isolated from soil, and more recently Chen et al.[16] added two more species from indoor air. The section was characterised by species with biverticillate conidiophores, occasionally consisting of solitary phialides, with stipes generally pigmented, colony reverse on CYA yellowish brown or dark green, usually growing at 37°C, and an absence of acid production on CREA. Our new species *T. georgiensis* shares all these features except the latter one; in fact, it is the only species in the section able to produce acid on CREA. This is phylogenetically related to the species of the *T. helicus*-clade (ie *T. boninensis*, *T. helicus*, *T. reverso-olivaceus* and *T. varians*),[7,16] and a common feature that distinguishes all them from the other species of the section is the production of conidiophores with pigmented stipes. Although this feature was not mentioned in the despription of *T. reverso-olivaceus*, the stipes are somewhat green coloured in its photomicrographs reported.[16] *Talaromyces georgiensis* can also be identified easily at the molecular level using the ITS barcode and *BenA*, however concatenated analysis with *RPB2* supports a better distinction of species within the section. It is noteworthy that *T. georgiensis* is the first species in the section found from clinical specimens.

Talaromyces atroroseus, *T. diversus*, and the novel species *T. minnesotensis* are, to date, the only species in the section *Trachyspermi* isolated from clinical specimens, having been recovered from lung samples.[13] However, considering the absence or the restricted growth at 37°C, the pathogenic potential of these fungi in immunocompetent individuals is probably limited. Species in this section can be distinguished easily by their growth rates on CYA, MEA and CREA.[7,16] Also, *T. minnesotensis* is the only species that shows a strong acid production on CREA. The ITS barcode as well as *BenA*, *CaM* and *RPB2* are good molecular markers for distinguishing the species in section *Trachyspermi*.

The section *Islandici* was re-evaluated recently by Yilmaz et al.[8] and currently includes 19 species. *Talaromyces subaurantiacus*, identified in this study from a BAL sample, was described as new in the abovementioned study from a Fynbos soil isolate. Therefore, our strain is only the second identification of this species so far. This clinical isolate shows practically the same phenotypic features as those described in the protologue, with restricted growth on all culture media, especially on CYA, and its ability to grow at 37°C. The combination of these phenotypic features distinguishes *T. subaurantiacus* from the other species

in the section.[8] *Talaromyces columbinus* is another species of the section *Islandici* previously found from a human clinical specimen.[13] Additionally, *T. piceus* has been reported as a causal agent of fungaemia[36] and osteomyelitis,[37] *T. radicus* as the etiologic agent of a fatal infection in a dog,[38] and *T. rugulosus* as responsible for a corneal ulcer.[39] All these species differs from *T. subaurantiacus* mainly by their good growth at 40°C;[8,40] *T. subaurantiacus* is unable to grow at this temperature.

This study expands the species diversity of *Talaromyces* in the clinical setting. Although the pathogenic role of the new species proposed has not been proven, *T. alveolaris*, *T. georgiensis* and *T. rapidus* demonstrate pathogenic potential by their ability to grow at human body temperature. Further studies are necessary, however, to understand both the distribution and the relevance of these new fungi in human and animal disease.

CONFLICT OF INTEREST

No conflict of interest declared.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

4.3 Species diversity in *Aspergillus, Penicillium* and *Talaromyces* from herbivore dung, and proposal of two new genera in *Aspergillaceae.*

Fungal diversity 2018 (Sometido)

Fungal Diversity

Species diversity in Aspergillus, Penicillium and Talaromyces from herbivore dung, and proposal of two new genera in Aspercillaceae
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Abstract:	Coprophilous fungi are saprotrophic organisms that show a great diversity, mainly on herbivore dung. The physico-chemical characteristics of this peculiar substrate combined with the high level of fungal adaptation to different environmental conditions offer the perfect setting for discovering new taxa. This study focused on the species diversity in Aspergillus and penicillium-like fungi isolated mainly from herbivore dung collected in different Spanish localities. From 130 samples, a total of 165 isolates were obtained and 90 species were identified. Preliminary identifiecations were based on morphology and partial β-tubulin (BenA) gene sequences. The new taxa were characterized by a multi-gene sequencing analysis testing the BenA, the internal transcribed spacer rDNA (ITS), calmodulin (CaM), and RNA polymerase II second largest subunit (RPB2) genes, and a detailed phenotypic study. Using this polyphasic approach and following the genealogical concordance phylogenetic species recognition concept (GCPSR), we propose the new genera Penicillago (for Penicillium nodositatum) and Pseudopenicillium (for Penicillium megasporum and P. giganteum) in the family Aspergillaceae, and 21 new species, including nine Aspergillus, seven Penicillium, four Talaromyces and one Pseudopenicillium. Our results show that the species diversity of Aspergillus, Penicillium and related genera on herbivore dung is poorly studied and that this substrate seems to be a good reservoir of interesting Eurotialean fungi.

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1   **Species diversity in *Aspergillus*, *Penicillium* and *Talaromyces* from**

2   **herbivore dung, and proposal of two new genera in *Aspercillaceae***

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14

Abstract

Coprophilous fungi are saprotrophic organisms that show a great diversity, mainly on herbivore dung. The physico-chemical characteristics of this peculiar substrate combined with the high level of fungal adaptation to different environmental conditions offer the perfect setting for discovering new taxa. This study focused on the species diversity in *Aspergillus* and penicillium-like fungi isolated mainly from herbivore dung collected in different Spanish localities. From 130 samples, a total of 165 isolates were obtained and 90 species were identified. Preliminary identifiecations were based on morphology and partial β-tubulin (*Ben*A) gene sequences. The new taxa were characterized by a multi-gene sequencing analysis testing the *Ben*A, the internal transcribed spacer rDNA (ITS), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB*2) genes, and a detailed phenotypic study. Using this polyphasic approach and following the genealogical concordance phylogenetic species recognition concept (GCPSR), we propose the new genera *Penicillago* (for *Penicillium nodositatum*) and *Pseudopenicillium* (for *Penicillium megasporum* and *P. giganteum*) in the family *Aspergillaceae*, and 21 new species, including nine *Aspergillus*, seven *Penicillium*, four *Talaromyces* and one *Pseudopenicillium*. Our results show that the species diversity of *Aspergillus*, *Penicillium* and related genera on herbivore dung is poorly studied and that this substrate seems to be a good reservoir of interesting Eurotialean fungi.

Key words: coprophilous; phylogeny; *Eurotiales*; *Trichocomaceae; Aspergillaceae*

Introduction

Fungi are able to grow and colonize a diverse range of substrates, especially those rich in nutrients, such as animal dung. This is a complex substrate with a high amount of readily available carbohydrates with high nitrogen content, water-soluble vitamins, growth factors and mineral ions (Bell 1983). In addition, the physical structure, pH, and varying moisture content of the dung make it a rich medium for fungal growth (Richardson 2001). A high diversity of fungal taxa, including a large number of *Ascomycota*, *Basidiomycota* and *Mucorales* species, considered primary saprotrophs, can be found on dung (Bell 1983; Richardson 2001).

Althougth some genera of ascomycetes are considered practically exclusively occurring on dung (e.g. *Ascobolus*, *Podospora*) and some are even restricted to a particular herbivore (e.g. *Lasiobolus cainii* only occurs on porcupine dung) (Webster 1970); most ascomycetes, and particularly the *Eurotiales,* are not considered predominantly coprophilous (Krug et al. 2004). However, they can be frequently found on this substrate when the soluble materials are exhausted, and a low water availability is present. More particularly, some reports indicate that species of *Aspergillus* and *Penicillium* are common in feces when the relative humidity decreases below 85 % (Kuthubutheen & Webster 1986). Although the literature on the presence of these groups of fungi on herbivore dung is scarce, some reports show that members of at least nine sections of *Aspergillus* can be present on this substrate. Species of sections *Nidulantes* and *Clavati* are occasionally found on dung samples (Varga et al. 2007a; Chen et al. 2016a). In addition, several *Aspergillus* species have been discovered from this substrate, among them *A.*

64 *latilabiatus, A. recurvatus, A. stercorarius* (section *Nidulantes*), *A. monodii*

65 (section *Usti*) or *A. viridinutans* (section *Fumigati*) (Raper & Fennell 1965;

66 Samson et al. 2011a; Chen et al. 2016a). Other species commonly found on

67 herbivore dung are *A. crystallinus, A. paradoxus* and *A. malodoratus*, although

68 they have recently been transferred to the genus *Penicillium* (Visagie et al.

69 2016).

70   In the past, some coprophilous penicillia, such as *P. brevistipitatum*, *P.*

71 *clavigerum*, *P. concentricum*, *P. coprobium*, *P. coprophilum*, *P. formosanum*, *P.*

72 *glandicola* and *P. vulpinum*, were classified in a particular group of *Penicillium,*

73 namely series *Claviformia* (Seifert & Samson 1985; Frisvad & Samson 2004;

74 Wang & Zhuang 2005). However, the most recent taxonomic classification

75 based on sections, most of the coprophilous *Penicillium* species have been

76 reclassified in section *Robsamsonia* (Houbraken et al. 2016), which includes,

77 apart from the mentioned species previously assigned to series *Claviformia*

78 others from *Urticicolae* (Frisvad & Samson 2004). Only a few studies on

79 coprophilous organisms have identified penicillium-like fungi at the species

80 level, and many of the fungi identified as *Penicillium* correspond in fact to other

81 morphologically similar genera, such as *Talaromyces* (Houbraken et al. 2016).

82 Coprophilous species reported in this latter genus are *T. atroroseus, T. dupontii,*

83 *T. emersonii, T. helicus, T. flavus, T. muroii* or *T. trachyspermus*, although

84 currently some of them, such as *T. dupontii* and *T. emersonii*, have been

85 transferred to the genera *Thermomyces* and *Rasamsonia*, respectively

86 (Masunga et al. 2006; Frisvad et al. 2013; Yilmaz et al. 2014).

87   The taxonomy of *Aspergillus* and penicillium-like fungi has changed

88 dramatically due to the phylogenetic data. Houbraken & Samson (2011)

89 demonstrated that *Penicillium* is phylogenetically more related to *Aspergillus*

90 (family *Aspergillaceae*) than to *Talaromyces* (family *Trichochomaceae*). In the

91 last decade, the species of *Aspergillus*, *Penicillium* and *Talaromyces* have been

92 delineated on the basis of a polyphasic approach that mainly includes the

93 evaluation of morphological and multilocus sequence analyses testing with the

94 internal transcribed spacer region rDNA (ITS), and the β-tubulin (*Ben*A),

95 calmodulin (*CaM*), and/or the DNA-dependent RNA polymerase II largest

96 subunit (*RPB*2) genes (Peterson 2008; Houbraken et al. 2014a; Samson et al.

97 2014; Visagie et al. 2014a; Yilmaz et al. 2014). In addition, the *BenA* gene has

98 been recommend as identification marker in *Penicillium* (Visagie et al. 2014a)

99 and *Talaromyces* (Yilmaz et al. 2014) and the *CaM* gene for *Aspergillus*

100 (Samson et al. 2014). The use of this, already generalized approach, has

101 demonstrated to be able to discriminate very close species and to discover and

102 characterize new taxa (Visagie et al. 2014a, Guevara-Suarez et al. 2017,

103 Siqueira et al. 2016, 2017).

104 Considering the animal dung as a perfect setting for detecting interesting

105 fungal species and the scarcity of data on fungal diversity in this substrate using

106 modern technichs for their identification, the present work focuses on the study

107 of the species diversity in *Aspergillus*, *Penicillium* and *Talaromyces* from

108 herbivore dung samples collected across Spain. Identifications were done using

109 morphology and comparing multilocus sequences with a database of ex-type

110 and verified reference strains. For multisequence analyses, the Genealogical

111 Phylogenetic Species Recognition (GCPSR) criterion was applied to data in

112 support of discovering putative new species (Taylor et al. 2000).

113

Materials and Methods

Sampling and fungal isolation

Dung samples were collected between 2016–2017 in different geographic regions from Spain. These have different climates and diverse fauna and flora, and included regions from Andalusia, Balearic and Canary Islands, Cantabria, Castile-Leon, Catalonia, Extremadura and Galicia. Collected dung were mostly from rabbit, fox, sheep, deer, and goats, although occasionally soil mixed with some of these substrates or even dung from other animals, as cattle, wild pig and horse were also studied. The samples were placed in individual paper or plastic bags and processed not later than three days after collection. Individual samples were divided into two parts; one processed using moist chambers (Richardson 2001) and the other by a modified Waksman (1922) dilution series method. For moist chambers, pieces of the sample were placed on moist filter paper with sterile distilled water in individual Petri dishes, and incubated at room temperature (22−25 °C) for up to 30 days. For dilution series, approximately one gram of dung or associated soil was 1:10 (w/v) diluted in sterile water, and shaken for approximately 10 min. Aliquots of the suspensions were pipetted into Petri dishes and mixed with 20 mL of melted cooled agar medium. Culture media used for isolation were potato dextrose agar (PDA; Pronadisa, Madrid, Spain), potato carrot agar (PCA; 20 g potatoes, 20 g carrot, 20 g agar, 1000 mL distilled water), both supplemented with chloramphenicol (200 mg/L), and dichloran rose-bengal chloramphenicol agar (DRBC; 5 g peptone, 10 g glucose, 1 g KH_2PO_4, 0.5 g $MgSO_4$, 25 mg rose-bengal, 2 mg dichloran, 200 mg chloramphenicol, 20 g agar, 1000 mL distilled water). All media were supplemented with dieldrin in dimethyl-ketone (1%). Petri dishes were

139   incubated at room temperature for up to 30 days. The moist chambers and Petri

140   dishes were examined at regular intervals with the aid of stereo microscope and

141   conidia from sporulating colonies were transferred to PDA supplemented with

142   chloramphenicol.

143   Isolates identified morphologically as belonging to *Aspergillus*,

144   *Penicillium* or *Talaromyces* were recovered and deposited in the culture

145   collection of the Medicine Faculty of Reus (FMR). Cultures of interesting

146   species, as well as type material and cultures of the new species were

147   deposited at the Westerdijk Fungal Biodiversity Institute (Utrecht, the

148   Netherlands). Nomenclatural novelties and descriptions were deposited in

149   MycoBank (Crous et al. 2004).

150

151   **Molecular identification and phylogenetic analysis**

152   Isolates were cultured on PDA or malt extract agar (MEA; Difco, Detroit, USA)

153   for 714 days at 25ºC. DNA was extracted using the FastDNA® kit protocol (MP

154   Biomedicals, Solon, OH) and for the lisis step done with a FastPrep® FP120

155   cell disrupter (Thermo Savant, Holbrook, NY).

156   Preliminary species identifications were carried out BLASTing *BenA* DNA

157   sequences using GenBank. In the case of putative new species, the ITS region,

158   including the 5.8S rRNA gene, and fragments of *CaM* and/or *RPB*2 genes were

159   also amplified and sequenced. The primer pairs used were: ITS5/ITS4 for ITS

160   (White et al. 1990), Bt2a/Bt2b for *Ben*A (Glass & Donaldson 1995),

161   CMD5/CMD6 for *CaM* (Hong et al. 2006), and RPB2-5F/RPB2-7Cr for *RPB*2

162   (Liu et al. 1999). The amplification protocol and PCR conditions were performed

163   using methods and primers previously described (Peterson 2008; Houbraken &

164   Samson 2011; Samsom et al. 2014; Visagie et al. 2014a; Yilmaz et al. 2014).

165   The amplified products were purified and sequenced at Macrogen Corp. Europe

166   (Amsterdam, the Netherlands) with a 3730XL DNA analyzer (Applied

167   Biosystems, Foster City, CA). Consensus sequences were obtained using

168   SeqMan v. 7.0.0 (DNASTAR, Madison, WI). Newly generated sequences and

169   their GenBank/EMBL accession numbers are summarized in Table 1.

170   Sequences were retrieved from GenBank taking into account the last

171   update of the database of the International Commission of *Penicillium* and

172   *Aspergillus* (http://www.aspergilluspenicillium.org), which includes all the

173   species accepted in those genera (Samsom et al. 2014; Visagie et al. 2014a;

174   Yilmaz et al. 2014). Single and concatenated phylogenetic analyses were

175   performed to delineate putative new species and the phylogenies corresponding

176   to each section of those genera were properly reconstructed.

177   Data sets for each locus were aligned individually using ClustalW

178   (Thompson et al. 1994), in MEGA v 6.0 software (Tamura et al. 2013), refined

179   with MUSCLE (Edgar 2004) under the same platform, and manually adjusted

180   when needed. Larger alignments including different sections of each genus was

181   performed using the MAFFT tool in the EMBL-EBI Web Services portal

182   (https://www.ebi.ac.uk/Tools/msa/mafft/) and manually adjusted in MEGA v 6.0.

183   Phylogenetic reconstructions by maximum likelihood (ML) and Bayesian

184   inference (BI) were carried out using MEGA v. 6.0 and MrBayes v. 3.1.2

185   (Huelsenbeck & Ronquist 2001), respectively. For ML analyses, the trees were

186   inferred using Nearest-Neighbour-Interchange as a heuristic method and gaps

187   were treated as partial deletion with a 95 % site coverage cut-off. Phylogeny

188   support for internal branches was assessed by 1,000 ML bootstrapped

189 pseudoreplicates and bootstrap support (bs) ≥ 70 was considered significant.

190 The BI analyses were performed using five million Markov chain Monte Carlo

191 (MCMC) generations, with two runs (one cold and three heated chains) and

192 samples were stored every 1,000 generations. The 50% majority-rule

193 consensus tree and posterior probability values (pp) were calculated after

194 discarding the first 25% of the samples. A pp value ≥0.95 was considered

195 significant. The best substitution models for each data partition were estimated

196 using jModelTest v.2.1.3 according to the Akaike criterion (Darriba et al. 2012;

197 Guindon & Gascuel 2003). The length, number of variable and phylogenetic

198 informative sites, and substitution models for each data partition are

199 summarized in Table 2. The resulting trees were plotted using FigTree v.1.3.1

200 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator CS3.

201 The alignments and trees were deposited in the TreeBASE (www.treebase.org)

202 under the submission number 21345.

203

204 **Phenotypic characterization**

205 Phenotypic characterization was carried out using standard growth conditions

206 described previously (Samson *et al.* 2014, Visagie *et al.* 2014a, Yilmaz *et al.*

207 2014). Briefly, isolates were cultured onto MEA 2% (Samson *et al.* 2010),

208 oatmeal agar (OA; Samson *et al.* 2010), Czapek yeast autolysate agar (CYA;

209 Pitt 1979), yeast extract sucrose agar (YES; Frisvad 1981), creatine sucrose

210 agar (CREA; Frisvad 1981) and dichloran 18 % glycerol agar (DG18; Hocking

211 and Pitt 1980), incubated at 25 °C for 7 d in darkness. Colony growth rates were

212 also measured after 7 d at 30 and 37 °C on MEA, CYA, YES and OA. Colors

213 used for descriptions refer to Kornerup & Wanscher (1978). Microscopic

214 features were examined on colonies grown on MEA after 1 -2 wk, mounted on

215 slides with Shear's solution or 60% lactic acid, and excess conidia removed with

216 70 % ethanol. Microscopic features were captured with a Zeiss Axio-Imager M1

217 light microscope using Nomarski differential interference contrast and phase-

218 contrast optics (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity X digital

219 camera and DeltaPix InSight v5.3.11 software. Photoplates were assembled

220 from separate photographs using PhotoShop CS3.1.

221

222 **Results and Discussion**

223 In this study, a total of 130 dung samples were processed. Preliminari

224 *Aspergillus* identification showed that members of sections *Fumigati*, *Flavi*, and

225 *Nigri* were very common on that substrate and their species diversity was

226 relatively poor. Considering that it is relatively easy to recognize the different

227 types of aspergillli directly on the substrate, we tried to select only *Aspergillius*

228 isolates belonging to sections different to those above mentioned. On the other

229 hand, a preliminary selection of penicillia was rather harder than in the case of

230 aspergilli, and therefore practically all penicillium-like isolates observed on the

231 samples were recovered. A total of 165 isolates, including five from soil, were

232 studied (Table 1). Preliminary identifications based on morphology and *Ben*A

233 sequences showed that the isolates recovered were *Aspergillus* (n = 60),

234 *Penicillium* (n = 91) and *Talaromyces* (n = 10), while four of them could not be

235 assigned to any of these genera in spite of exhibiting a penicillium-like

236 morphology. To maximize the quality of the analysis, four separate *Ben*A

237 alignments were done corresponding to the different genera studied. We also

238 carried out additional analyses of single genes ITS, *CaM* and *RPB2* and

239   combined datasets of the different genes corresponding to those genera or

240   sections where putative new species resulted included. The topologies of the

241   trees obtained by both methods were similar, the ML ones being used to

242   represent the results. Bootstrap values and BI posterior probability values were

243   marked on relevant branches.

244   Final identification of the isolates that resulted from the phylogenetic

245   analyses of the different genes and from the exam of their morphological

246   features is shown in Table 1.

247

248   ***Aspergillus* phylogeny**

249   The phylogenetic analysis inferred from the 60 *Aspergillus* isolates with the

250   *Ben*A sequences is shown in Figure 1. The alignment was 615 bp long, 408

251   from which resulted variable and 381 phylogenetic informative. ML substitution

252   model was Kimura 2-parameter (K2) and for BI was General Time Reversible

253   (GTR). Rates among sites were Gamma distributed (G) with invariant sites (I),

254   used for both methods. The 60 isolates represented 38 different species

255   belonging to the following nine sections *Aspergillus*, *Candidi*, *Circumdati*,

256   *Clavati*, *Cremei, Flavipedes*, *Nidulantes*, *Terreus*, and *Usti* forming seven well-

257   supported main clades (I – VII).

258   Clade I grouped the sections *Nidulantes* and *Usti*. In the first one (90%

259   bs/0.99 pp), eleven isolates were identified as *A. aureolatus* (n=1), *A.*

260   *austroafricanus* (n=1), *A. fructus* (n=1), *A. nidulans* (n=3), *A. rugulosus* (n=1), *A.*

261   *sydowii* (n=3), and *A. viridicatenatus* (n=1). The section *Nidulantes* included the

262   majority of species formerly known as *Emericella*, which were transferred to

263   *Aspergillus* by Samson et al. (2014) following the new critera for fungal

264   nomenclature. Note that the current concept of this section by Chen *et al.*

265   (2016a) also includes the members of the former section *Versicolores* (Jurjevic

266   *et al.* 2012; Siqueira *et al.* 2016).

267   The section *Usti* (93% bs/0.99 pp) comprised four isolates belonging to

268   three known species, i.e. *A. calidoustus* (n=1), *A. insuetus* (n=1) and *A.*

269   *pseudodeflectus* (n=2), and one isolate (FMR 15225) that could represent an

270   undescribed species. Single (data not shown) and concatenate (Figure 2)

271   analyses of most of the species currently accepted in this section (Samson et

272   al. 2014) show that FMR 15225 is closely related to *A. keveii*. However, some

273   phenotypic features, such as the ability of this isolate to grow at 37 ⁰C, and the

274   genetic distance (98 % similar with *Ben*A; 98.9 % in the concatenate dataset)

275   versus the ex-type strain of *A. keveii* allow to consider them distinct taxa, being

276   proposed below as *A. calidokeveii*. Species of section *Usti* are relatively

277   common in soil and indoor air, and only *A. monodii* and *A. ustus* have been

278   previously reported from dung (Samson et al. 2011a).

279   Clade II comprised the sections *Flavipedes* (91% bs/1 pp) and *Terrei*

280   (95% bs/1 pp), in which are included the largest number of *Aspergillus* isolates

281   studied here, i.e. 15 (25%) and 10 (16.7%) isolates, respectively. Members of

282   the sections *Flavipedes* and *Terrei* are commonly isolated from soil and dust

283   (Samson et al. 2011b; Hubka et al. 2015), but they have never been reported

284   from dung. In section *Flavipedes,* the species identified were *A. ardalensis*

285   (n=2), *A. iizukae* (n=2), *A. micronesiensis* (n=2), *A. spelaeus* (n=5) and *A.*

286   *templicola* (n=3); however, although FMR 15175 clearly belongs to this section,

287   it did not match with any of the species from this group. Both *Ben*A analysis

288   (Figure 1) and the concatenated phylogeny (Figure 3) with the currently

289   accepted species of the section (Arzanlou et al. 2016) showed that this isolate

290   formed a fully supported clade with the ex-type strain of *A. movilensis.*

291   However, both shows a genetic difference (97.1 % similar with *Ben*A; 98.5 %

292   with the concatenate alignment) enough to be considered distinct species.

293   Thus, FMR 15175 is described below as *A. albodeflectus* sp. nov. It is

294   noteworthy that the most frequent *Aspergillus* species isolated in this study is *A.*

295   *spelaeus* (8.33%), which it has recently been described by Hubka *et al.* (2015)

296   from cave sediment in Spain.

297   The section *Terrei* currently comprises 17 species (Samson *et al.* 2014)

298   with four of them identified in the present study, i.e. *A. albamenesis* (n=2), *A.*

299   *citrinoterreus* (n=1), *A. floccosus* (n=1), *A. hortai* (n=1), and *A. terreus* (n=1).

300   However, four isolates (FMR 15182, FMR 15228, FMR 15181 and FMR 15217)

301   did not fit morphologically either genetically with any species of the section. It is

302   noteworthy that the ML general tree based only on *BenA* presented some

303   doubtful results in the identification of *A. terreus* and *A. citrinoterreus* isolates

304   (Figure 1). However, when we carried out a restricted alignment with only

305   members of section *Terrei* and reconstructing the ML tree from this alignment,

306   both species could be satisfactory identified (see Figure S1 in supplemental

307   material). The concatenated sequence alignment of section *Terrei*, including the

308   four unidentified isolates mentioned above, confirmed that they represent three

309   undescribed phylogenetic species (Figure 4). Three species of this secton, i.e.

310   *A. ambiguous, A. microcysticus* and *A. neoniveus,* were not included in the

311   general alignment because of the long genetic distance versus the other

312   species of this group, and the first two acted as outgroups in the concatenate

313   phylogeny. Two major clades were observed, whereas the first one (98% bs/1

314 pp) included the isolates FMR 15228 and FMR 15182, which were

315 phylogenetically distant from *A. hortai* and *A. alabamensis*, respectively; the

316 second clade (100% bs/1 pp) included the isolates FMR 15181 and FMR

317 15217, which formed an independent fully supported lineage clearly distant from

318 the other species of the group. These aspergilli are described below as *A.*

319 *aurantiosulcatus* (FMR 15182), *A. fimeti-brunneus* (FMR 15228), and *A.*

320 *majoricus* (FMR 15181 and FMR 15217).

321 Clade III corresponded to section *Aspergillus* (95% bs/1 pp), which

322 included five isolates identified as *A. chevalieri* (n=1), *A. montevidensis* (n=2),

323 and *A. pseudoglaucus* (n=2). This section includes osmophilic organisms,

324 commonly found on organic materials, dust, and cereals (Kozakiewicz 1989).

325 Some members of this group had previously been reported from dung (Hubka

326 *et al.* 2013), showing that it may be a good reservoir for species of this section.

327 Particular conditions of the samples, as low water activity, may enhance the

328 growth of members of this section in such substrate.

329 Clade IV, representing to section *Candidi* (94% bs/1 pp), included eight

330 of our isolates, two of which were identified as *A. candidus* and one as *A.*

331 *dobrogensis*. Interestingly, the other five isolates represented at least three

332 undescribed phylogenetic species. Currently, this section comprises seven

333 species, three of them recently described, *A. pragensis* from toe nail (Hubka et

334 al. 2014), *A. subalbidus* from house dust (Visagie et al. 2014b) and *A.*

335 *dobrogensis* from indoor environments, caves and clinical material (Hubka et al.

336 2018). The concatenated sequence alignment of the seven species of the

337 section, using the four markers, showed that the unidentified isolates were

338 distributed in two well-supported clades (Figure 5). The first one (99% bs/1 pp)

339 encompassed *A. subalbidus* and *A. taichungensis*, which were basal to the two

340 new species proposed here, *A. canariensis* and *A. verruculosus*. Although these

341 two species were very closely related, with a similarity between each other of

342 98.2% in the concatenated alignment, their phenotypic differences support their

343 novelty. The second clade (96% bs/1 pp) corresponded to *A. campestris* and

344 two isolates recovered from deer dung which showed a genetic similarity of 98.3

345 % respect to the ex-type strain of the former species, and thus considered

346 distinct taxa. These two isolates are described below as *A. coprophilus*. It is

347 notewrthy our results show that the species diversity of sections *Candidi* and

348 *Terrei* on herbivore dung is poorly studied and that this substrate seems to be a

349 good reservoir of interesting aspergilli in both sections.

350 Clades V and VI corresponded to the sections *Circumdati* (96% bs/1 pp)

351 and *Clavati* (99% bs/1 pp), respectively. In the former, the two isolates identified

352 belonged to *A. affinis* and *A. subramanianii*, respectively, whereas in the latter

353 the two dung isolates were identified as *A. clavatus*. *Aspergillus affinis* is a rare

354 species only known from submerged leaf litter and soil in Italy and Macedonia,

355 respectively (Davolos et al. 2012). *Aspergillus subramanianii* is a widely-

356 distributed species recovered from different substrates and countries (Visagie et

357 al. 2014b, c; Siqueira et al. 2017), but it has never been reported from animal

358 dung. On the contrary, *A. clavatus* is frequently isolated from dung, soil and

359 even from other types of substrates (Varga et al. 2007a).

360 Two isolates resolved in section *Cremei* (clade VII, 94% bs/1 pp).

361 Whereas FMR 14605 could represent a putative new species, the isolate FMR

362 15216 was identified as *A. europaeus.* Despite the recent proposal of this new

363 latter species, it is reported as a common fungus in soil (Hubka *et al.* 2016). The

364   section *Cremei* currently comprises 17 species (Samson *et al.* 2104), although

365   only the ex-type strains of the ten species more closely related to the

366   unidentified isolate have been included in the final concatenated phylogeny of

367   the section presented here (Figure 6). The concatenated analysis showed that

368   FMR 14605 was closely related to *A. dimorphicus*, forming both a well-

369   supported terminal clade distant from the other species. Although the isolate

370   FMR 14605 and the ex-type strain of *A. dimorphicus* showed identical ITS, they

371   could be distinguished by *BenA* (98 % similar) and *CaM* (98.6 % similar)

372   sequences. Additional phylogenies including more sequences of *A. dimorphicus*

373   available in GenBank and of other closely related species, i.e. *A. chrysellus*, *A.*

374   *europaeus* and *A. wentii* (see Figures S2 to S6 in supplemental material)

375   supports the novelty of our isolate, which is described below as *A. esporlensis*.

376

377   ***Penicillium* phylogeny**

378   The phylogenetic tree based on the *Ben*A locus with the 91 isolates of

379   *Penicillium* is shown in Figure 7. The aligned dataset was 404 bp long, with 257

380   variable sites and 237 phylogenetic informative. The best substitution model for

381   ML was K2+G, and for BI it was GTR+G+I. In general, the topology of the

382   phylogenetic tree showed well-delimitated sections.

383   The analysis distributed the 91 isolates in at least 38 species belonging

384   to 16 sections represented by 14 clades (I-XIV). The two major clades coincided

385   with the two subgenera currently accepted in *Penicillium*, i.e. *Aspergilloides* and

386   *Penicillium* (Houbraken & Samson 2011). The former (84% bs/-- pp) includes

387   the following sections: *Brevicompacta, Canescentia, Chrysogena, Fasciculata,*

388   *Paradoxa, Penicillium, Ramosa, Robsamsonia, Roquefortorum* and *Turbata;*

389   and the latter (72% bs/0.99 pp) the sections *Aspergilloides, Cinnamopurpurea,*

390   *Citrina, Exilicaulis, Lanata-Divaricata, Sclerotiora* and *Stolkia.*

391   Clade I (--% bs/0.99 pp), representing section *Fasciculata*, included 18

392   isolates identified as *P. biforme* (n=2), *P. crustosum* (n=15) and *P. polonicum*

393   (n=1). *Penicillium crustosum* was the most frequently isolated species in this

394   study (14.28%). It is a relatively common species, frequently isolated from nuts,

395   meat, cheese, feeds, vegetables, and pomaceous and stone fruits (Sonjak *et al.*

396   2005). In our study, *P. crustosum* was mainly recovered from Mediterranean

397   areas (Catalonia and Balearic Islands), with the only exception of one isolate

398   that was from Galicia.

399   Clade II included members of the section *Roquefortorum* (92% bs/1 pp),

400   a section that comprises relevant species used in the cheese industry

401   (Houbraken et al. 2016). Within the clade clustered three dung isolates (FMR

402   15031, FMR 15032, and FMR 15188), that were related to *P. roqueforti* but

403   forming an independent and distant branch that could represent an undescribed

404   species. *Roquefortorum* is a small section of closely related species

405   (Houbraken *et al.* 2010). To evaluate possible intra- and inter-specific variability

406   within the species currently accepted, i.e. *P. carneum, P. paneum, P.*

407   *psychrosexualis* and *P. roqueforti,* and the phylogenetic position of our putative

408   new species, we performed an additional analysis with *Ben*A gene (see Figure

409   S7 in supplemental material) with more sequences of the species available in

410   GenBank. This demonstrated that *P. roqueforti* is divided in two clades with an

411   intra-specific variability of around 0.08%, with our isolates forming a separate

412   branch from *P. roqueforti* complex. The concatenated analysis using ITS, *Ben*A,

413   *CaM* and *RPB*2 (Figure 8) supported the novelty of these three isolates, being

414 therefore proposed as *P. mediterraneum*. Although, *P. mediterraneum* and *P.*

415 *roqueforti* show identical ITS barcode, both species have unique *Ben*A, *CaM*

416 and *RPB*2 sequences.

417 Clades III and IV were formed by members of the the sections

418 *Chrysogena* (74% bs/-- pp) and *Penicillium* (--% bs/1 pp), respectively. In the

419 former, two dung isolates were identified as the species *P. chrysogenum* and *P.*

420 *flavigenum*; whereas in the latter, other two were identified as *P. expansum*.

421 Until 2016, the species of the section *Penicillium* had been isolated from very

422 different substrates, including dung; however, this section was reevaluated

423 recently, and now mainly contains plant pathogenic species (Houbraken *et al.*

424 2016). By contrast, the species of the section *Chrysogena* are well known and

425 usually found in soil, with the exception of *P. chrysogenum*, *P. nalgiovense* and

426 *P. rubens* that commonly occur in indoor environments (Houbraken *et al.* 2012).

427 Clade V corresponded to *Robsamsonia*, a section recently introduced by

428 Houbraken et al. (2016) and that includes the majority of coprophilous species

429 described in *Penicillium*. A total of 18 isolates (19.78%) were included here;

430 thirteen of them identified as belonging to *P. brevistipitatum* (n=1), *P.*

431 *concentricum* (n=2)*, P. coprobium* (n=2)*, P. coprophilum* (n=1), and *P.*

432 *griseofulvum* (n=7); the five remaining ones (FMR 15192, FMR 15210, FMR

433 15211, FMR 16481 and FMR 16491) could not be assigned to any known

434 species. The preliminary *Ben*A analysis, but also the concatenate phylogeny

435 (Figure 9) including the currently accepted species in the section, showed that

436 the five isolates grouped together in a very supported and undescribed lineage

437 closely related to the ex-type strain of *P. glandicola.* An additional *Ben*A

438 analysis (see Figure S8 in supplemental material) with more sequences of *P.*

439 *glandicola* showed that our isolates and *P. glandicola* were 97.4% similar,

440 confirming that they were new taxa. The genetic differences and morphological

441 peculiarities observed in such group of isolates allowed to describe the novel

442 species *P. synnematicola.* Interestingly, this species seems to be a common

443 coprophilous fungus in the Mediterranean area, since most isolates have been

444 recovered from goat dung collected in Catalonia and in the Balearic Islands.

445 Clade VI comprised the sections *Turbata* (95% bs/1 pp) and *Paradoxa*

446 (94% bs/-- pp). In the former, the isolate FMR 15041 closely related to *P.*

447 *bovifimosum* did not fit with any species of the section. In *Paradoxa*, from a total

448 of 11 Spanish isolates, four were identified as *P. atramentosum* and one as *P.*

449 *magnielliptisporum*, the rest (FMR 15040, FMR 15104, FMR 15107, FMR

450 15191, and FMR 15196) were allocated in three single branches and could

451 represent three putative new species for the genus. The concatenated

452 phylogeny of sections *Paradoxa* and *Turbata* with the six unidentified isolates is

453 shown in Figure 10. The section *Paradoxa* was divided in two fully supported

454 clades, one with *P. crystallinum*, *P. malodoratum* and *P. paradoxum*, and the

455 other included the unidentified isolates of the section *Paradoxa* and the ex-type

456 strains of *P. atramentosum*, *P. magnielliptisporum* and *P. mexicanum*. The

457 isolates FMR 15107 and FMR 15404 clustered together, as well as FMR 15191

458 and 15196, and their respective single branches were separated from other two

459 terminal branches with FMR 15104 and the ex-type strain of *P. atramentosum.*

460 This latter species has been reported in fact as a species complex, from which

461 *P. mexicanum* and *P. magnielliptisporum* have recently been described (Visagie

462 et al. 2014b). The genetic differences showed between the lineage of FMR

463 15107 and FMR 15040 (97.07% similar with *Ben*A; 98.23% similar with

464   concatenate dataset), that of FMR 15104 (94.68% similar with *Ben*A; 95%

465   concatenate dataset), and that of FMR 15191 and 15196 (96.2% similar with

466   *Ben*A; 96.2% concatenate dataset) respect to *P. atramentosum*, the closest

467   species, allow to consider them distinct taxa. These isolates are described

468   below as *P. ibericum, P. fimosum* and *P. balearicum*, respectively. An additional

469   phylogenetic analysis with *Ben*A including more sequences of the most closely

470   related species confirms our proposal (see Figure S9 in supplemental material).

471   *Paradoxa* is the section of *Penicillium* with the highest number of new species

472   found in this study. In section *Turbata, P. bovifimosum* and FMR 15041 were

473   located in the same clade, but the latter formed a long terminal branch that

474   proved to be a distinc species. Thus, it is described as a new species, *P.*

475   *caprifimosum. Penicillium bovifimosum* is a monotypic species described from

476   dry cow manure by Tuthill & Frisvad (2002).

477   Section *Ramosa* (clade VII; 97% bs/0.98 pp) only included the

478   unidentified isolate FMR 15038. Currently, the section comprises 13 species

479   (Visagie et al. 2014a, Rong et al. 2016, Visagie et al. 2016a;), although *P.*

480   *lanosum* and *P. kojigenum* were considered conspecific by Samson & Pitt

481   (2000). In the concatenate analysis performed with three markers (ITS, *Ben*A,

482   and *CaM*), since *RPB*2 sequences were not available for all species in the

483   section (Figure 11), FMR 15038 was placed in an independent branch between

484   two clades, one with *P. kojigenum* and *P. lanosum*, and the other with *P.*

485   *jamesonlandense* and *P. swiecickii*. The similarity among our isolate and its

486   phylogenetic sisters was 98.3%, proving that our isolate is a distinguishing

487   species, proposed here as *P. beceitense*.

488   Clade VIII comprised the sections *Brevicompacta* (99% bs/1 pp) and

489   *Canescentia* (98% bs/1 pp). In the former, only one isolate was identified as *P.*

490   *brevicompactum*, a species commonly inhabiting in soil and decaying

491   vegetation, but previously described also from food, cereals, textiles, clinical

492   specimens and feces of snake (Pitt 1979, Guevara-Suarez et al. 2016). In

493   section *Canescentia,* seven isolates were identified as *P. canescens* (n=1), *P.*

494   *murcianum* (n=3) and *P. radiatolobatum* (n=3). The use of *BenA* for species

495   identification within this section is difficult, especially to distinguish *P.*

496   *radiatolobatum* and *P. murcianum*. Therefore, the analysis of *CaM* is

497   recommended to solve this problem (Visagie *et al.* 2016a)*.*

498   Clade IX corresponded to section *Exilicaulis* (99% bs/1 pp), in which

499   eight of our isolates were included. However, as observed in *Apergillus* section

500   *Terrei*, the general tree based only on *Ben*A was not useful to identify some

501   isolates of the section, especially those belonging to the *P. restrictum*-clade.

502   However, we confirmed the identification of *P. arabicum* (n=2), *P. burgense*

503   (n=1), *P. cinereoatrum* (n=1)*, P. momoii* (n=1), and *P. rubefaciens* (n=2) based

504   on *Ben*A analysis restricted to the section (see Figure S10 in supplemental

505   material). The only isolate located in *P. restrictum*-clade that could not be

506   identified at the species level was FMR 15841. In the most recent review of the

507   section, Visagie et al. (2016b) indicated that such clade needs further revision

508   since it could include some additional cryptic species. Thus, the identification of

509   our isolate remains uncertain until further studies clarifiying the taxonomic

510   structure about the *P. restrictum*-clade.

511   In section *Lanata-Divaricata* (clade X, 92% bs/1 pp), three isolates were

512   included. One of them was identified as *P. brasilianum*, a widespread species

513 commonly found on soil and recently also reported from human clinical

514 specimens (Pitt 1979, Guevara-Suarez et al. 2016); and the other two as *P.*

515 *cremeogriseum,* a species previously found on forest soil from Ukraine

516 (Houbraken & Samson 2011). To our knowledge, this is the first report of *P.*

517 *brasilianum* and *P. cremeogriseum* associated to herbivore dung. The section

518 has been recently revised by Visagie et al. (2016a), who described 7 new

519 species, mostly from soil.

520   The only species identified in our study belonging to section *Stolkia*

521 (clade XI, 99% bs/1 pp) was *P. canariense.* This species was described from

522 soil in Canary Islands (Peterson & Sigler 2002), the same geographical origin

523 as our isolate although from different substrates. This is the second isolate of

524 this species obtained so far.

525   In section *Citrina* (clade XII, 98% bs/1 pp), five isolates were identified as

526 *P. citrinum* and two as *P. sizovae.* Although we have not found previous records

527 of these species from dung, they have a worldwide distribution, being isolated

528 from soil, foodstuff, and many other types of substrates (Houbraken et al. 2011).

529 This section comprises nearly 40 species, but only those identified here were

530 included in the analysis to simplify the *Ben*A phylogenetic tree.

531   Clade XIII grouped the sections *Sclerotiora* (77% bs/-- pp) and

532 *Cinnamopurpurea* (96% bs/-- pp). In the former, one isolate was identified as *P.*

533 *lilacinoechinulatum* and in the latter another one as *P. cvjetkovicii.* Houbraken &

534 Samson (2011) revised the taxonomic position of *P. lilacinoechinulatum* and *P.*

535 *nodositatum* and considered both synonyms of *P. bilaiae.* However, they are

536 currently recognized as distinc species and *P. nodositatum* is even excluded

537   from the genus (Visagie et al. 2013). *Penicillium cvjetkovicii* has been recently

538   described from indoor air samples in the USA by Peterson et al. (2015).

539   The last clade XIV (99% bs/1 pp) emcompased *Penicillium* species

540   belonging to section *Aspergilloides*. The species identified were *P. glabrum*

541   (n=2)*, P. frequentans* (n=2)*, P. roseoviride* (n=2), and *P. rudallense* (n=1).

542   *Penicillium frequentans* together with *P. spinulosum* and *P. glabrum* are the

543   most common species in the section, being isolated from a wide range of

544   substrates, including soil, food, bark, and indoor environments (Houbraken et al.

545   2014b). Although currently *P. glabrum* and *P. frequentans* are accepted as

546   distinc species, they were considered synonyms for a long time (Houbraken et

547   al. 2014b).

548

549   ***Talaromyces* phylogeny**

550   The phylogenetic tree based on the *Ben*A locus (Figure 12) shows the

551   relationships of the 10 *Talaromyces* isolates included in the study. The aligned

552   dataset was 384 bp long, from which 193 were variable sites and 156

553   phylogenetic informative. The ML substitution model was K2 + G +I, while to BI

554   it was GTR + G +I. Two main clades were formed, representing sections

555   *Talaromyces* (97% bs/1 pp) and *Trachyspermi* (99% bs/1 pp). Six out of nine

556   isolates included in the first section (FMR 15489, FMR15490, FMR 15199, FMR

557   15035, FMR 15307 and FMR 15303) could not be assigned to any known

558   species of that group, whereas the other three were identified as *T. muroii* (FMR

559   15496), *T. ruber* (FMR 15839) and *T. sayulitensis* (FMR 15842). *Talaromyces*

560   *muroii* and *T. sayulitensis* are considered uncommon species mainly associated

561   to soil and indoor environments (Visagie et al. 2014b, Chen et al. 2016b). On

562   the other hand, only one isolate (FMR 16441) was allocated in section

563   *Trachyspermi*, which could not be identified.

564   To resolve the taxonomy of the unidentified isolates in both sections, we

565   performed the respective phylogeny using sequences of the ITS, *BenA* and

566   *CaM* genes and including all accepted species in each section. Although *RPB*2

567   was sequenced for all isolates (Table 1), this marker was not incorporated in the

568   concatenated analysis since there were not sequences available for all the type

569   strains of this section. The combined phylogeny of the members of section

570   *Talaromyces* is shown in Figure 13. *Talaromyces* is the largest section in the

571   genus and includes nearly 50 species, 15 of them described in the last year

572   from environmental or clinical samples (Cheng et al. 2016b, Guevara-Suarez et

573   al. 2017, Visagie et al. 2015, Wang et al. 2016, Yilmaz et al. 2016a,b). Our

574   concatenate dataset showed that the unidentified isolates FMR 15489 and FMR

575   15490 exhibited both a similarity of 99.42% respect to the ex-type strain of *T.*

576   *angelicus*, a monotypic species described by Sang *et al.* (2013) from roots of

577   *Angelica gigas* in Korea. Although most of their morphological features matched

578   with those of the protologue of *T. angelicus*, we observed some variation

579   respect to the description of this species by Yilmaz et al. (2014); i.e, production

580   of diffusible red pigment in one of our isolates (FMR 15490), the colonies of our

581   isolates were deep turquoise and pastel red rather than yellow to white on MEA,

582   they grew faster at 37 ºC (on CYA 34–37 mm diam in 7d vs 25–27 mm in

583   Yilmaz et al. 2014), and exhibited stipes up to 100 μm long (up to 120 μm in

584   Yilmaz et al. 2014). On the other hand, the isolate FMR 15199 was located in

585   an independent branch clearly distinct from the other species of the section; the

586   isolates FMR 15035 and FMR 15307 clustered together and were closely

587 related to *T. funiculosus* but with a genetic difference enough to be considered

588 distinct species (see also Figure S11 in supplemental material); and the isolate

589 FMR 15303 formed an independent branch close to the clade containing *T.*

590 *francoae, T. kendrickii, T. mangshanicus, T. qii* and *T. thailandensis.* Based on

591 concatenated phylogenetic analysis and supported by phenotypic differences

592 (see taxonomy section), we propose the following three new species, *T.*

593 *coprophilus, T. pseudofuniculosus*, and *T. gamsii*, respectively. These species

594 can be easily identified with the *Ben*A marker.

595 The combined phylogenetic analysis of *Talaromyces* section

596 *Trachyspermi* (Figure 14) showed that the isolate FMR 16441 was located in a

597 single branch within the same clade that *T. albobiverticillius*, *T. erythromellis, T.*

598 *heiheensis*, and *T. solicola* (88% bs/0.99 pp). Considering the unique

599 phylogenetic position and the morphological differences observed, we propose

600 the new species *T. catalonicus*. With the inclusion of this novel fungus, section

601 *Trachyspermi* currently comprises 16 species, the most recently described

602 being *T. aerius* from indoor air, *T. heiheensi* from rotten wood, and *T.*

603 *minnesotensis* from human clinical specimens (Yaguchi et al. 1996, Yilmaz et

604 al. 2014, Chen et al. 2016b, Wang et al. 2016, Guevara-Suarez et al. 2017). To

605 date, there are no reports of the presence of species of this section on dung

606 samples.

607

608 **Phylogeny of miscelaneous *Aspergillaceae***

609 According to *Ben*A phylogeny, four of our isolates (FMR 15296, FMR 16442, FMR

610 14718, FMR 15299) were related to some penicillia currently excluded from the genus

611 *Penicillium* (i.e. *P. giganteum, P. megasporum* and *P. nodositatum*) but belonging to

612   the family *Aspergillaceae* (Figure 15). The aligned dataset of this analysis consisted in

613   393 bp long, from which 242 were variable sites and 216 phylogenetic informative. The

614   best substitution model for ML was K2 +G+I and for BI it was GTR +G+I. Whereas

615   FMR 15296 and FMR 16442 were located in a well-supported clade along with the ex-

616   type strain of *P. nodositatum* (CBS 333.90), FMR 14718 and FMR 15299 were in a fully

617   supported distant clade together with the ex-type strains of *P. giganteum* (NRRL 3553)

618   and *P. megasporum* (NRRL 2232). Despite the morphology of these taxa resambles

619   *Penicillium*, based on a multigene phylogeny, Peterson et al. (2010) demonstrated that

620   the two latter species did not belong to *Penicillium* s. str.; and later, Visagie et al.

621   (2013) excluded *P. nodositatum* due to its genetic differences respect to the true

622   penicillia. Phylogenetically, these two clades where the mentioned species are

623   allocated could clearly represent two undescribed genera in *Aspergillaceae*. Our

624   phylogeny inferred with sequences of the ITS, *BenA* and *RPB*2 genes, including the

625   isolates under study and members of different genera of *Aspergillaceae*, confirms the

626   proposal of two novel genera (Figure 16), which are described below as *Penicillago*

627   (*Pgo.*), typified by *Penicillium nodositatum*, and *Pseudopenicillium* (*Pse.*) with

628   *Penicillium giganteum* and *Penicillium megasporum,* being this latter selected as the

629   type. The former genus being more phylogenetically related with *Penicillium,* whereas

630   the latter resulted a sibling genus of *Hamigera* such as it was previously suggested by

631   Peterson et al. (2010).

632   The analysis of ITS and *Ben*A showed that the ex-type strain of *P. nodositatum* and the

633   isolates FMR 15296 and FMR 16442 have practically identical sequences. However,

634   CBS 333.90 was not included in the concatenated analysis (Figure 16) since its *RPB2*

635   sequence was not available for comparison. Despite we observed some cultural

636   difference between our isolates respect to the protologue of *P. nodositatum* (Valla et al.

637   1989), microscopically they fit with the features of the species. FMR 15296 and FMR

638   16442 grow better on CYA and MEA at 25°C (40–44 mm and 34–36 mm diam in 7

639 days, respectively, vs 13–19 mm and 16–30 mm in CBS 333.90) and have the ability to

640 grow at 37°C. Considering the phylogenetic position of our isolates and their

641 morphological similariy with *P. nodositatum*, they were the base to propose the new

642 combination *Pgo. nodositata.*

643 The isolate FMR 14718 matched morphologically and molecularly with *P. giganteum*,

644 whereas FMR 15299 was placed in a new lineage into the *Pseudopenicillium* clade,

645 being more closely related to *Pse. megasporum* (Figures 15, 16). The morphological

646 differences observed, such as having smaller conidia (up to 6 μm) than those of the

647 other two species in the genus (conidia up to 12 μm in *Pse. giganteum*, and up to 10

648 μm in *Pse. megasporum*), and the genetic differences obtained (98% similar to *Pse.*

649 *giganteum,* and 98.16% similar to *Pse. megasporum*) allow us to propose it as a new

650 species named *Pse. coprobium.*

TAXONOMY

651 ***Aspergillus albodeflectus*** J.P.Z. Siqueira, Dania García & Gené, **sp. nov.**

652 MycoBank MB 821808. Figure 17.

653 Etymology — Name refers to the white color of the colonies and the

654 commonly observed bent conidial heads.

655 In — Section *Flavipedes*

656 Specimen examined — Spain, Balearic Islands, Mallorca, Pollença, from

657 herbivore dung, February 2016, J. Guarro, M. Guevara-Suarez, J.P.Z. Siqueira

658 (**holotype** CBS H-23128; culture ex-type FMR 15175 = CBS 142665; ITS

659 barcode LT798909, alternative markers: *Ben*A LT798936, *CaM* LT798937,

660 *RPB*2 LT798938).

Colony diameter in 7d (mm) — On CYA: 25 ⁰C 19–21, 30 ⁰C 20–21, 37 ⁰C 7–8; on MEA: 25 ⁰C 22–23; on DG18: 25 ⁰C 17–20; on YES: 25 ⁰C 20–23; on OA: 25 ⁰C 8–10; on CREA: 25 ⁰C 9–11.

Colony characters at 25 ⁰C in 7 d — On CYA, colonies powdery, forming concentric circles, mycelium white, margin entire; reverse pale yellow (3A3); sporulation dense, with conidial masses white; soluble pigment absent; exudate absent. On MEA, colonies powdery, radially sulcate, mycelium white, margin slightly lobulate; reverse light yellow (4A5); sporulation dense, with conidial masses white; soluble pigment absent; exudate absent. On DG18, colonies velvety, mycelium white, margin entire; reverse pale (2A2); sporulation moderately dense, with conidial masses white; soluble pigment absent; exudate absent. On YES, colonies powdery, radially sulcate, with a slightly elevated center, mycelium white, margin entire; reverse light yellow (4A5); sporulation dense, with conidial masses white; soluble pigment absent; exudate absent. On OA, colonies with floccose center, powdery towards the periphery, mycelium white, margin entire; reverse brownish yellow (5C7); sporulation dense, with conidial masses white; soluble pigment absent; exudate absent. On CREA, acid production absent.

Micromorphology — On MEA, conidiophores with conidial heads biseriate, often slightly to strongly bent, loosely columnar, white; stipes commonly septate, 150–550 x 5–8 µm, smooth, hyaline; vesicles globose to subglobose, 12–20 µm wide, hyaline; metula cylindrical, enlarged at apex, covering 50% to 75% of the vesicle, 6–9 x 3–6 µm, hyaline; phialides flask-shaped, 6–8.5 x 3–5 µm, hyaline; conidia globose, 2–4 µm diam, smooth, hyaline. Hülle cells and ascomata not observed.

686   Distinguishing characters — The new species is closely related to *A. lupii*

687 and *A. movilensis*. However, *A. lupii* produces bright yellow colonies while *A.*

688 *movilensis* produces faster growing colonies on MEA (25– 30 mm) and on CYA

689 at 37 ⁰C (10–17 mm) (Arzanlou et al. 2016). Additionally, colonies of *A.*

690 *movilensis* become light brown with age (Hubka et al. 2015) and both species

691 produce Hülle cells, absent in *A. albodefectus*. However, the production of Hülle

692 cells could be influenced by the composition of the culture media (Hubka et al.

693 2015).

694

695 ***Aspergillus aurantiosulcatus*** J.P.Z. Siqueira, Guarro & Dania García, **sp.**

696 **nov.** MycoBank MB 821811. Figure 18.

697   Etymology — Name refers to the orange color, characteristic of the

698 colonies and also to the furrows commonly observed, especially on CYA.

699   In — Section *Terrei*

700   Specimen examined — Spain, Balearic Islands, Mallorca, road near

701 Orient**,** from herbivore dung, February 2016, J. Guarro, M. Guevara-Suarez,

702 J.P.Z. Siqueira (**holotype** CBS H-23187; cultures ex-type FMR 15182 = CBS

703 142981; ITS barcode LT798912, alternative markers: *Ben*A LT798945, *CaM*

704 LT798946, *RPB*2 LT798947).

705   Colony diameter in 7 d (mm) — On CYA: 25 ⁰C 37–39, 30 ⁰C 56–57, 37

706 ⁰C 62–65; on MEA: 25 ⁰C 27–28; on DG18: 25 ⁰C 34–38; on OA: 25 ⁰C 20–21;

707 on YES: 25 ⁰C 55–61; on CREA: 25 ⁰C 24–26.

708   Colony characters at 25 ⁰C in 7 d — On CYA, colonies floccose, radially

709 and concentrically sulcate, mycelium white to pale yellow (3A3), margin entire;

710 reverse brownish orange (6C6); sporulation moderately dense, with conidial

masses light orange (6A4); soluble pigment yellowish brown (5D4); exudate colorless. On MEA, colonies floccose, mycelium white to greyish orange (5B3), margin entire; reverse brownish orange (6C6); sporulation moderately dense, with conidial masses white to yellowish brown (5F5); soluble pigment amber yellow (4B6); exudate yellowish white (4A2). On DG18, colonies floccose, mycelium white, margin entire; reverse light yellow (4A4) to orange (5A7); sporulation absent; soluble pigment absent; exudate absent. On YES, colonies cottony, irregularly sulcate, mycelium white, margin entire; reverse orange (6B7); sporulation moderately dense; with conidial masses white to light orange (6A5); soluble pigment absent; exudate absent. On OA, colonies powdery, dense at center, mycelium greyish orange (5B5), margin entire; reverse pale yellow (4A3) to orange (5A6); sporulation dense, with conidial masses light orange (6A5); soluble pigment absent; exudate absent. On CREA, acid production absent.

Micromorphology — On MEA, conidiophores with conidial heads biseriate, loosely radiate to columnar, white to brownish orange (5C6); stipes commonly septate, 110–350 x 2–8 µm, smooth, hyaline; vesicles globose to subglobose, 13–27 µm wide, hyaline; metula cylindrical, slightly enlarged at apex, 4.5–6.5 x 2–4 µm, hyaline; phialides flask-shaped, 4–6 x 1.5–3 µm, hyaline; conidia globose to subglobose, 1.5–3 µm diam, smooth, hyaline to shades of yellow. Accessory conidia not observed. Ascomata not observed.

Distinguishing characters — *Aspergillus aurantiosulcatus* is phylogenetically closely related to *A. alabamensis.* This latter species produces colonies yellowish-brown to cinnamon-brown, the conidial heads are densely columnar, and the vesicles subglobose (Balajee et al. 2009).

736

737 ***Aspergillus calidokeveii*** J.P.Z. Siqueira, Dania García & Gené, **sp. nov.**

738 MycoBank MB 821814. Figure 19.

739 Etymology — Name refers to the genetic similarity with *A. keveii* and the

740 ability to grow at 37°C.

741 In — Section *Usti*

742 Specimen examined — Spain, Castile and Leon, Palencia, San Juan

743 Valley, from herbivore dung, March 2016, J. Guarro, M. Guevara-Suarez

744 (**holotype** CBS H-23129; cultures ex-type FMR 15225 = CBS 142666; ITS

745 barcode LT798914, alternative markers: *Ben*A LT798951, *CaM* LT798952,

746 *RPB*2 LT798953).

747 Colony diameter in 7 d (mm) — On CYA: 25 ºC 36–40, 30 ºC 49–50, 37

748 ºC 23–25; on MEA: 25 ºC 32–34; on DG18: 25 ºC 24–25; on YES: 25 ºC 39–41;

749 on OA: 25 ºC 27–29; on CREA: 25 ºC 22–24.

750 Colony characters at 25 ºC in 7 d — On CYA, colonies floccose, slightly

751 sulcate, mycelium white to brownish grey (6D2), margin predominantly entire;

752 reverse light yellow (4A5) to yellow (3A7); sporulation dense, with conidial

753 masses olive brown (4E3); soluble pigment yellow (3A7); exudate colorless to

754 light yellow (4A5). On MEA, colonies floccose, mycelium white to brownish grey

755 (6D2), margin entire; reverse light yellow (4A5) to yellow (4A8); sporulation

756 dense, with conidial masses yellowish grey (3D2); soluble pigment yellow (3A7);

757 exudate absent. On DG18, colonies floccose, mycelium white, margin entire;

758 reverse yellowish white (3A2) to greyish yellow (3B5); sporulation sparse;

759 soluble pigment yellow (3A7) in 14 days; exudate absent. On YES, colonies

760 floccose, with raised center, mycelium white to brownish grey (5C2), margin

predominantly entire; reverse light yellow (4A4) to greyish orange (6B5); sporulation dense, with conidial masses pale grey (1B1) to medium grey (1E1); soluble pigment yellow (3A7) weakly produced; exudate absent. On OA, colonies floccose to cottony, mycelium white, margin entire; reverse light yellow (2A4 to 2A5); sporulation dense, with conidial masses olive brown (4A6); soluble pigment absent; exudate absent. On CREA, acid production absent.

Micromorphology — On MEA, conidiophores with conidial heads biseriate, radiate, pale grey (1B1) to olive brown (4E3); stipes commonly septate, (70)110–300 x 3–7.5 µm, smooth to verruculose, in shades of brown; vesicles globose, subglobose to spatulate, (8.5)11–19 µm wide, in shades of brown; metula cylindrical, covering 50% to 100% of the vesicle, 4.5–7 x 3–5 µm, hyaline to orange brown; phialides flask-shaped, 7–9 x 2–4 µm, hyaline to orange brown; conidia globose to subglobose, 2.5–5 µm diam, rough, in shades of brown. Hülle cells abundant, variably shaped, mostly elongated, 32–120 x 10–28 µm. Ascomata not observed.

Distinguishing characters — The present species is phylogenetically related to *A. keveii*, but they can be differentiated mainly by the absence of growth at 37 ºC in the latter (Houbraken et al. 2007). In addition, *A. keveii* has conidial heads loosely columnar with pyriform and smaller (9–13 µm) vesicles (Houbraken et al. 2007), while *A. calidokeveii* exhibits radiate conidial heads with subglobose to spatulate vesicles, measuring 11–19 µm wide.

Aspergillus canariensis J.P.Z. Siqueira, Gené & Guarro, **sp. nov.** MycoBank MB 821815. Figure 20.

785   Etymology — Name refers to the Canary Islands, where the fungus was

786   found.

787   In — Section *Candidi*

788   Specimens examined — Spain, Canary Islands, Gran Canaria, Santa

789   Brígida, from herbivore dung, September 2016, J. Cano-Lira, D. García, J.

790   Guarro, M. Guevara-Suarez (**holotype** CBS H-23188; cultures ex-type FMR

791   15736 = CBS 142982; ITS barcode LT798906, alternative markers: *Ben*A

792   LT798927, *CaM* LT798928, *RPB*2 LT798929); Teror, from herbivore dung, June

793   2016, J. Cano-Lira, D. García, J. Guarro, M. Guevara-Suarez (FMR 15733 =

794   CBS 142983).

795   Colony diameter in 7 d (mm) — On CYA: 25 ºC 17–20, 30 ºC 19–21, 37

796   ºC no growth; on MEA: 25 ºC 10–13; on DG18: 25 ºC 15–16; on YES: 25 ºC

797   22–23; on OA: 25 ºC 13–14; on CREA: 25 ºC 12–13.

798   Colony characters at 25 ºC in 7 d — On CYA, colonies floccose, with a

799   slightly granulose center, mycelium white, margin entire; reverse pale (5A3) to

800   light orange (5C4); sporulation dense, with conidial masses white to reddish

801   white (7A2); sclerotia absent; soluble pigment brown (6F7) in 14 d; exudate

802   absent. On MEA, colonies floccose, mycelium white, margin slightly lobulate;

803   reverse pale orange (5A3) to brownish orange (5C4); sporulation dense, with

804   conidial masses white to yellowish white (3A2); sclerotia absent; soluble

805   pigment absent; exudate colorless to yellowish white (2A2). On DG18, colonies

806   floccose to velutinous, with submerged mycelium, mycelium white to greenish

807   grey (2B2), margin lobulate; reverse yellowish white (3A2); sporulation

808   moderately dense, especially at borders, with conidial masses white; sclerotia

809   absent; soluble pigment absent; exudate absent. On YES, colonies floccose,

810 slightly sulcate, with elevated center, mycelium white, margin slightly lobulate;

811 reverse pale yellow (4A3) to brownish orange (5C6); sporulation dense, with

812 conidial masses white to greyish white (1B1); soluble pigment absent; exudate

813 absent. On OA, colonies slightly granulose to powdery, with submerged

814 mycelium, mycelium white, margin irregular; reverse yellowish white (3A2);

815 sporulation moderately dense, with conidial masses white; sclerotia absent;

816 soluble pigment absent; exudate colorless. On CREA, acid production absent.

817 Micromorphology — On MEA, conidiophores with conidial heads

818 biseriate, radiate, white to reddish white (7A2); stipes commonly septate, 200–

819 500 x 4–7.5 µm, smooth, hyaline; vesicles usually globose, (9–)12–27 µm wide,

820 hyaline; metula cylindrical, with the apex slightly wider than the bottom, 6–

821 15(21.5) x 5–8.5 µm, hyaline; phialides flask-shaped, 4–8.5 x 2–3 µm, hyaline;

822 conidia globose, 2–4 µm, smooth, hyaline. Ascomata not observed.

823 Distinguishing characters — *Aspergillus canariensis* is similar to *A.*

824 *candidus*, *A. subalbidus* and *A. verruculosus* in its white colonies, smooth

825 conidia and the inability to grow at 37 °C, but differs in the absence of

826 penicillium-like structures typically produced by *A. subalbidus* and *A. candidus*

827 (Visagie et al. 2014b), and also by the absence of sclerotia, which are

828 abundantly produced by *A. verruculosus* on CYA.

829

830 ***Aspergillus coprophilus*** J.P.Z. Siqueira, Dania García & Gené, **sp. nov.**

831 MycoBank MB 821816. Figure 21.

832 Etymology — Name refers to the substrate where the species was found.

833 In — Section *Candidi*

834   Specimens examined — Spain, Castile and Leon, Palencia, Monte el

835   Viejo, Deer Reserve Park**,** from deer dung, March 2016, J. Guarro, M. Guevara-

836   Suarez (**holotype** CBS H-23189; cultures ex-type FMR 15224, CBS 142984;

837   ITSbarcode: LT798902, alternative identification markers: *Ben*A LT798915,

838   *CaM* LT798916, *RPB*2 LT798917); Palencia, San Juan Valley, from herbivore

839   dung, March 2016, J. Guarro, M. Guevara-Suarez (FMR 15226 = CBS 142985).

840   Colony diameter in 7 d (mm) — On CYA: 25 ºC 18–21, 30 ºC 16–18, 37

841   ºC no growth; on MEA: 25 ºC 13–15; on DG18: 25 ºC 12–14; on YES: 25 ºC

842   26–28; on OA: 25 ºC 8–12; on CREA: 25 ºC 5–7.

843   Colony characters at 25 ºC in 7 d — On CYA, colonies floccose at the

844   center, granulose towards periphery, mycelium white, margin lobulated; reverse

845   light yellow (4A4) to becoming dark brown (9F5) after 14 d; sporulation dense,

846   with conidial masses yellowish white (4A2); sclerotia abundant, dark brown (9F5

847   to 7F4); soluble pigment dark purple (14F3) present after 14 d; exudate clear to

848   yellowish white (4A2). On MEA, colonies similar to those on CYA; sclerotia

849   absent; soluble pigment dark purple (14F3) after 14 d; exudate absent. On

850   DG18, colonies floccose, mycelium white to orange white (5A2), margin entire;

851   reverse colorless; sporulation absent; sclerotia absent; soluble pigment absent;

852   exudate absent. On YES, colonies floccose, slightly sulcate, mycelium white,

853   margin slightly lobulate; reverse pale yellow (4E3) to orange brown (4B7);

854   sporulation dense, with conidial masses white; sclerotia absent; soluble pigment

855   absent; exudate absent. On OA, colonies with aerial mycelium scarce, white,

856   margin entire and with submerged mycelium; reverse white; sporulation sparse,

857   only at the center and the periphery, with conidial masses white; sclerotia

858 absent; soluble pigment absent; exudate absent. On CREA, poor growth, acid

859 production absent.

860   Micromorphology — On MEA, conidiophores with conidial heads

861 biseriate, radiate, white; stipes commonly septate, 300–650 x 5–11 µm, smooth,

862 hyaline; vesicles globose to subglobose, occasionally diminutive, (5–)13–27 µm

863 wide, hyaline; metula cylindrical, with a slightly wider apex, 7–13 x 5–6 µm,

864 hyaline; phialides flask-shaped, 5–8 x 2–3 µm, hyaline; conidia subglobose to

865 ellipsoidal, 3–6 x 2–4 µm, smooth, hyaline; sclerotia usually globose to

866 subglobose, 300–1100 µm, dark brown (9F5 to 7F4). Ascomata not observed.

867   Distinguishing characters — *Aspergillus coprophilus* is closely related to

868 *A. campestris*, *A. candidus* and *A. dobrogensis*. *Aspergillus campestris* can be

869 distinguished by the absence of sclerotia and also by the production of sulphur

870 yellow colonies (Varga et al. 2007b). *Aspergillus candidus* differs by its whitish

871 or yellowish brown colony reverse on CYA after 14 d (Hubka et al. 2014), while

872 in *A. coprophilus* this is dark brown. *Aspergillus dobrogensis* differs by its larger

873 stipes, which are up to 3000 x 15 µm (Hubka et al. in press)

874

875 ***Aspergillus esporlensis*** J.P.Z. Siqueira, Gené & Guarro, **sp. nov.** MycoBank

876 MB 821817. Figure 22.

877   Etymology — Name refers to the town where the species was found.

878   In — Section *Cremei*

879   Specimen examined — Spain, Balearic Islands, Mallorca, Esporles, from

880 soil mixed with dung, Juny 2012, J. Gené (**holotype** CBS H-23139; cultures ex-

881 type: FMR 14605 = CBS 142750; ITS barcode LT798908, alternative markers:

882 *Ben*A LT798933, *CaM* LT798934, *RPB*2 LT798935).

883 Colony diameter in 7 d (mm) — On CYA: 25 ºC 25–27, 30 ºC 5–6; 37 ºC

884 no growth; on MEA: 25 ºC 19–20; on DG18: 25 ºC 33–35; on YES: 25 ºC 44–

885 48; on OA: 25 ºC 12–13; on CREA: 25 ºC 14–16.

886 Colony characters at 25 ºC in 7 d — On CYA, colonies cottony to

887 floccose, mycelium white to greyish yellow (4B4), margin slightly lobulate;

888 reverse pale yellow (4A3); sporulation dense, with conidial masses light yellow

889 (4A4) to golden (4C6), soluble pigment absent; exudate clear to yellowish white

890 (3A3). On MEA, colonies cottony, mycelium white to light brown (5D5), margin

891 predominantly entire; reverse greyish yellow (3B6); sporulation dense, with

892 conidial masses light yellow (3A5) to golden brown (5D7); soluble pigment

893 absent; exudate clear to yellowish white (3A3). On DG18, colonies cottony to

894 lanose, mycelium white, margin entire; reverse white; sporulation sparse, with

895 conidial masses white to yellow (3A6), soluble pigment absent; exudate absent.

896 On YES, colonies floccose, cottony towards periphery, mycelium white to light

897 brown (5D5), margin slightly lobulate; reverse pale yellow (3E3); sporulation

898 dense, with conidial masses light yellow (3A5) to brown (5E5); soluble pigment

899 absent; exudate absent. On OA, colonies floccose, mycelium white to light

900 brown (5D5), margin lobulate; reverse greyish orange (5B5); sporulation dense,

901 with conidial masse light orange (5A5) to brown (5E5); soluble pigment absent;

902 exudate absent. On CREA, acid production weak.

903 Micromorphology — On MEA, conidiophores with conidial heads biseriate,

904 radiate, white to golden brown (5D7); stipes commonly septate, 200–600 x 4.5–

905 10 µm, smooth, often verruculose towards the apical part, subhyaline; vesicles

906 globose to spatulate, 11.5–50 µm wide, hyaline; metula cylindrical, 7–15.5 x 5–

907 12 µm, hyaline; phialides flask-shaped, 8–11 x 3–5 µm, hyaline; conidia globose

908   to subglobose, sometimes ellipsoidal, 4–5.5 x 3–5.5 µm, rough, in shades of

909   brown. Ascomata not observed.

910   Distinguishing characters — The closest relative of *A. esporlensis* is *A.*

911   *dimorphicus.* Although the two species are genetically distinct, morphologicaly

912   they are rather similar. However, *A. dimorphicus* was described with swollen

913   metula and branched conidiophores (Mehrotra & Prasad 1969), features not

914   observed in A. *esporlensis.* Moreover, *A. dimorphicus* has very delicately

915   roughened conidia, while in *A. esporlensis* they are clearly roughened. Other

916   closely related species are *A. wentii* and *A. europaeus.* Colonies of *A. wentii* on

917   MEA tend to grow slowly and often with white masses of sterile hyphae on

918   colony surface (Raper & Fennell 1965); *A. europaeus* has mostly pyriform

919   vesicles and produces yellow soluble pigment on MEA (Hubka et al. 2016).

920   Other phylogenetically related species is *A. chrysellus* which by contrast

921   produces the sexual morph.

922

923   ***Aspergillus fimeti-brunneus*** J.P.Z. Siqueira, Gené & Dania García, **sp. nov.**

924   MycoBank MB 821818. Figure 23.

925   Etymology — Name refers to the substrate it was isolated from (dung)

926   and the brown color of the colonies.

927   In — Section *Terrei*

928   Specimen examined — Spain, Andalusia, Huelva, Doñana National Park,

929   near Rocina stream, from herbivore dung, March 2016, D. García (**holotype**

930   CBS H-23140; cultures ex-type FMR 15228 = CBS 142751; ITS barcode

931   LT798913, alternative markers: *Ben*A LT798948, *CaM* LT798949, *RPB*2

932   LT798950).

Colony diameter in 7 d (mm) — On CYA: 25 ºC 37–40, 30 ºC 62–65, 37 ºC 67–70; on MEA: 25 ºC 28–30; on DG18: 25 ºC 42–44; on YES: 25 ºC 65–67; on OA 29–32: 25 ºC; on CREA: 25 ºC 32–34.

Colony characters at 25 ºC in 7 d — On CYA, colonies floccose, cottony towards the periphery, radially sulcate, mycelium white to pale yellow (4A3), margin slightly lobulate; reverse brownish orange (6C6); sporulation dense, with conidial masses brown (6E6); soluble pigment greyish orange (5B6) weakly produced; exudate yellowish white (3A2). On MEA, colonies floccose, mycelium white to pale grey (1B1), margin entire; reverse golden (4C6); sporulation dense, with conidial masses white to dark brown (6F6); soluble pigment greyish orange (5B6) weakly produced; exudate colorless. On DG18, colonies floccose, dense at center, mycelium white, margin entire; reverse light yellow (3A5); sporulation moderately dense, with conidial masses white; soluble pigment absent; exudate absent. On YES, colonies floccose to cottony, irregularly sulcate, mycelium white to yellowish white (3A2), margin entire; reverse orange (5A7) to deep orange (5A8); sporulation moderately dense, with conidial masses white to pale white (1A3); soluble pigment absent; exudate absent. On OA, colonies powdery, dense at center, mycelium white to greyish orange (5B5), margin entire; reverse light orange (5A4); sporulation dense, with conidial masses brown (6D7); soluble pigment absent; exudate absent. On CREA, colonies floccose to powdery, mycelium white to yellowish white (3A2), margin entire; sporulation moderately dense; with conidial masses yellowish white (3A2) to light orange (5A4); acid production weak.

Micromorphology — On MEA, conidiophores with conidial heads biseriate, columnar, white to dark brown (6F6); stipes commonly septate, 120–

958 320 x 5–9 µm, smooth, hyaline; vesicles globose to subglobose, (9)16–24 µm

959 wide, hyaline; metula cylindrical, with a slightly wider apex, covering 50% to

960 75% of the vesicle, 5–7 x 2–3.5 µm, hyaline; phialides cylindrical to flask-

961 shaped, 6–9.5 x 1.5–2.5 µm, hyaline; conidia globose to ellipsoidal, sometimes

962 tear-shaped, 1.5–3 x 1.5–3 µm, smooth, hyaline. Accessory conidia commonly

963 present, borne sessile or on short stalks, globose, 3–5.5 µm. Ascomata not

964 observed.

965 Distinguishing characters — *Aspergillus fimeti-brunneus* is closely related

966 to *A. terreus*, *A. citrinoterreus*, *A. hortai* and *A. neoafricanus.* The novel species

967 and *A. citrinoterreus* can be distinguished from the others by the acid production

968 on CREA (Samson et al. 2011b). *Aspergillus citrinoterreus* differs in the

969 conspicuous production of a yellow soluble pigment (Guinea et al. 2015), while

970 in *A. fimeti-brunneus* it is greyish orange or absent.

971

972 ***Aspergillus majoricus*** J.P.Z. Siqueira, Gené & Guarro, **sp. nov.** MycoBank

973 MB 821820. Figure 24.

974 *Etymology* — Name refers to the Mallorca Island (Spain) where the

975 species was found.

976 In — Section *Terrei*

977 Specimens examined — Spain, Balearic Islands, Mallorca, Pollença,

978 from herbivore dung, February 2016, J. Guarro, M. Guevara-Suarez, J.P.Z.

979 Siqueira (**holotype** CBS H-23190; cultures ex-type FMR 15181 = CBS 142986;

980 ITS barcode LT798910, alternative markers: *Ben*A LT798939, *CaM* LT798940,

981 *RPB*2 LT798941); Mallorca, Pollença, from herbivore dung, February 2016, J.

982 Guarro, M. Guevara-Suarez, J.P.Z. Siqueira (FMR 15217 = CBS 142987).

Colony diameter in 7 d (mm) — On CYA: 25 ⁰C 31–36, 30 ⁰C 40–44, 37 ⁰C 42–50; on MEA: 25 ⁰C 24–27; on DG18: 25 ⁰C 23–27; on YES: 25 ⁰C 33–37; on OA: 25 ⁰C 22–25; on CREA: 25 ⁰C 22–26.

Colony characters at 25 ⁰C in 7 d — On CYA, colonies floccose, radially sulcate, mycelium white, margin slightly lobulate; reverse light yellow (4A4) to orange yellow (4A6); sporulation dense, with conidial masses white to pale yellow (3A3); soluble pigment yellowish brown (5D5) weakly produced; exudate colorless to pale yellow (2A3). On MEA, colonies floccose to cottony, mycelium white, margin entire; reverse light yellow (4A4) to yellowish brown (5D5); sporulation moderately dense, with conidial masses white to light yellow (4A4); soluble pigment absent; exudate colorless to orange white (5A2). On DG18, colonies floccose to velvety, with submerged mycelium towards the periphery, mycelium white, margin predominantly entire; reverse white to light yellow (3A5); sporulation dense, with conidial masses white; soluble pigment absent; exudate absent. On YES, colonies floccose, irregularly sulcate, with elevated center, mycelium white, margin lobulate; reverse light yellow (4A4), with brown (6E6) areas; sporulation dense, with conidial masses white; soluble pigment light yellow (4A4 to 4A5) weakly produced; exudate absent. On OA, colonies powdery, more dense at the center, with submerged mycelium towards the periphery, mycelium white, margin predominantly entire; reverse yellowish white (3A2), deep green (28D8) areas may be observed; sporulation dense, with conidial masses white; soluble pigment absent; exudate absent. On CREA, acid production absent.

Micromorphology — On MEA, conidiophores with conidial heads biseriate, columnar, occasionally loosely radiate, white to light yellow (4A4);

1008 stipes commonly septate, 130–650 x 2.5–6.5 µm, smooth, hyaline; vesicles

1009 subglobose to spatulate, (4.5–)10–18 µm wide, hyaline; metula cylindrical, 4.5–

1010 6.5 x 2–3.5 µm, hyaline; phialides flask-shaped, 3.5–5 x 1.5–2.5 µm, hyaline;

1011 conidia globose to subglobose, 2–3 µm diam, smooth, hyaline. Accessory

1012 conidia absent. Penicillium-like conidiophores often present. Ascomata not

1013 observed.

1014 Distinguishing characters — *Aspergillus majoricus* is phylogenetically

1015 related to *A. niveus*, *A. carneus* and *A. allahabadii*, but clearly constituting a

1016 distinct species. *Aspergillus carneus* differs in its vinaceous fawn colonies

1017 (Raper & Fennell 1965), in contrast to those of *A. majoricus* which remain light.

1018 *Aspergillus allahabadii* were reported with greenish glaucus-blue conidial heads

1019 (Mehrotra & Agnihotri 1962), wheares those of *A. majoricus* are white to light

1020 yellow. *Aspergillus niveus* mainly differs by its dark yellow through brown to

1021 greenish black colony reverse on Czapek agar, and by having longer

1022 conidiophores (up to 1000 µm long) (Raper & Fennell 1965).

1023

1024 ***Aspergillus verruculosus*** J.P.Z. Siqueira, Gené & Dania García, **sp. nov.**

1025 MycoBank MB 821821. Figure 25.

1026 Etymology — Name refers to the verruculose conidiophores commonly

1027 observed.

1028 In — Section *Candidi*

1029 Specimen examined — Spain, Canary Islands, Gran Canaria, North

1030 Coast, from herbivore dung, September 2016, J. Cano-Lira, D. García, J.

1031 Guarro, M. Guevara-Suarez (**holotype** CBS H-23130; cultures ex-type: FMR

15877 = CBS 142667; ITS barcode LT798907, alternative markers: *Ben*A LT798930, *CaM* LT798931, *RPB*2 LT798932).

Colony diameter in 7 d (mm) — On CYA: 25 ºC 18–19, 30 ºC 16–17, 37 ºC no growth; on MEA: 25 ºC 10–12; on DG18: 25 ºC 12–13; on YES: 25 ºC 25–27; on OA: 25 ºC 12–13; on CREA: 25 ºC 8–9.

Colony characters at 25 ºC in 7 d — On CYA, colonies granulose, with raised center, mycelium white, margin lobulate; reverse brownish orange (5C5); sporulation dense, with conidial masses white to orange white (5A2); sclerotia usually formed around the border of the colony, greyish yellow (4B3) to greyish orange (5B5), darker with age; soluble pigment dark brown (9F5) in 14 d; exudate colorless. On MEA, colonies floccose to cottony, mycelium white, margin lobulated; reverse light yellow (3A5); sporulation dense, with conidial masses white; sclerotia absent; soluble pigment absent; exudate clear to yellowish white (3A2). On DG18, colonies floccose to loosely cottony, mycelium white, margin predominantly entire; reverse yellowish white (2A3); sporulation moderately dense, with conidial masses white; sclerotia absent; soluble pigment absent; exudate absent. On YES, colonies floccose, with raised cottony center, slightly sulcate, mycelium white, margin slightly lobulate; reverse light yellow (4A4) to brownish orange (6C6), darker towards the center; sporulation dense, with conidial masses white; sclerotia reddish grey (7B2) to brownish orange (7C4); soluble pigment absent; exudate absent. On OA, colonies powdery to granulose, otherwise very similar to the colonies in DG18. On CREA, poor growth, acid production absent.

Micromorphology — On MEA, conidiophores with conidial heads biseriate, radiate, white; stipes commonly septate, (60–)190–500 x 3–6.5 μm, smooth to

1057   often verruculose, hyaline; vesicles globose to subglobose, (5–)9–18 µm wide,

1058   hyaline; metula cylindrical, with a slightly wider apex, covering 75% to 100% of

1059   the vesicle, 4.5–10 x 3.5–5.5(7) µm, hyaline; phialides flask-shaped, 4.5–8 x

1060   1.5–3 µm, hyaline; conidia globose, 2.5–3.5 µm, smooth, hyaline; sclerotia

1061   globose to elongate, 200–800 x 160–500 µm. Ascomata not observed.

1062   Distinguishing characters — This species is genetically related to *A.*

1063   *subalbidus* and *A. canariensis*. *Aspergillus verruculosus* is the only species

1064   exibiting verruculose stipes. It can be also distinguished by the production and

1065   color of the sclerotia; whereas in *A. canariensis* they are absent, those

1066   produced by *A. subalbidus* are purplish black (Visagie et al. 2014b).

1067

1068   ***Penicillium balearicum*** Guevara-Suarez, Cano & Gené, **sp. nov.** MycoBank

1069   MB 822061. Figure 26.

1070   Etymology — Name refers to the geographic area (the Balearic Islands)

1071   where the species was found.

1072   In — Section *Paradoxa*

1073   Specimens examined — Spain, Balearic Islands, Mallorca, Pollença,

1074   from herbivore dung, February 2016, J. Guarro, M. Guevara-Suarez, J.P.Z.

1075   Siqueira (**holotype** CBS H-23215; culture ex-type FMR 15191 = CBS 143044;

1076   ITS barcode LT899762, alternative markers: *Ben*A LT898227, *CaM* LT899758,

1077   *RPB*2 LT899760); Mallorca, Pollença, from herbivore dung, February 2016,

1078   Guarro, M. Guevara-Suarez, J.P.Z. Siqueira (FMR 15196).

1079   Colony diameter in 7 d (mm) — On CYA: 25 ºC 25–36, 30 °C 5–6, 37 °C

1080   no growth; on MEA: 25 ºC 14–17, 30 °C 4–6; on YES: 25 ºC 36–45, 30 ºC 4–6;

1081 on OA: 25 ºC 26–30, 30 ºC 2–6; on DG18: 25 ºC 14–27, on CREA: 25 ºC 14–

1082 20.

1083   Colony characters at 25º C in 7 d — On CYA, colonies velvety, flat,

1084 mycelium white, sporulation dense, conidial masses dark dull green (28E3),

1085 margin dentate; reverse pale orange (5A3); exudate present, with pale droplets;

1086 soluble pigment absent. On MEA, colonies velvety, elevated, sporulation dense,

1087 conidial masses dark dull green (28E3), margin lobate; reverse light orange

1088 (5A4); exudate and soluble pigment absent. On DG18, colonies flat and velvety,

1089 mycelium white, sporulation dense, conidial masses dull green (27D4); reverse

1090 greenish white (27B2). On YES, colonies cerebriform, raised at the center,

1091 concentrically sulcate, mycelium white, sporulation dense at the center, with

1092 conidial masses greenish white (27C2), margin lobate; reverse bright orange

1093 red (8A8); exudate present, small brown (7E8/7E6) droplets; soluble pigment

1094 absent. On OA, colonies fasciculate, mycelium white, sporulation dense, with

1095 conidial masses greenish grey (28B2); exudate and soluble pigment absent. On

1096 CREA, acid production moderately strong.

1097   Micromorphology — On MEA, conidiophores mostly terverticillate; stipes

1098 smooth, 250–500 x 2–2.5 µm; metula 3–4 per branch, divergent, 10–14 x 2–3

1099 µm; phialides 3–4 per metula, ampulliform, 7–10 x 2–2.5 µm; conidia globose to

1100 subglobose, smooth-walled, brownish yellow, 2.2–2.5 x 2–3 µm.

1101   Distinguishing characters — *Penicillium balearicum* is resolved in the *P.*

1102 *atramentosum*-clade containing six species, *P. atramentosum*, *P. balearicum*,

1103 *P. fimosum*, *P. ibericum* (the latter three described here), *P. magnielliptisporum*

1104 and *P. mexicanum. Penicillium atramentosum, P. balearicum, P.*

1105 *magnielliptisporum* and *P. mexicanum* have smooth-walled stipes and conidia,

1106   but the latter two have larger conidia (5 x 4 µm in *P. magnielliptisporum*, 4 x 3.5

1107   µm in *P. mexicanum*), while *P. atramentosum* can be differentiated by the

1108   production of red soluble pigment on CYA and by the absence of growth at 30

1109   ºC (Pitt 1979, Frisvad & Samson 2004). *Penicillium balearicum* differs from *P.*

1110   *fimosum* and *P. ibericum* mainly by having longer stipes (up to 500 µm long), by

1111   the production of abundant brown exudate droplets on YES, and by the

1112   moderately strong acid production on CREA.

1113

1114   ***Penicillium beceitense*** Guevara-Suarez, Gené & Guarro, **sp. nov.** MycoBank

1115   MB 822063. Figure 27.

1116   Etymology — Name referred to Beceite, the town where the fungus was

1117   found.

1118   In — Section *Ramosa*

1119   Specimens examined — Spain, Aragon, Beceite, from herbivore dung,

1120   February 2016, J. Guarro, M. Guevara-Suarez, E. Rosas, (**holotype** CBS H-

1121   23183; cultures ex-type FMR 15038 = CBS 142989; ITS barcode LT899780,

1122   alternative markers: *Ben*A LT898229, *CaM* LT899764, *RPB*2 LT899798).

1123   Colony diameter in 7 d (mm) — On CYA: 25 ºC 25–27, 30 °C no growth,

1124   37 °C no growth; on MEA: 25 ºC 23–25; on YES: 25 ºC 26–28; on OA: 25 ºC

1125   26–30; on DG18: 25 ºC 19–20, on CREA: 25 ºC 3–5.

1126   Colony characters at 25º C in 7 d — On CYA, colonies sunken at the

1127   center, slightly radially sulcate, velvety, mycelium white, sporulation dense, with

1128   conidial masses dull green (25E4), margin entire; reverse greyish green (28C5);

1129   exudate and soluble pigment absent. On MEA, colonies flat, velvety, mycelium

1130   white, sporulation dense, with conidial masses conidial masses dull green

1131 (25E4), margin entire; reverse orange (5A6) to greenish grey (30C2); exudate

1132 and soluble pigment absent. On DG18, colonies flat, velvety, mycelium white,

1133 sporulation dense, conidial masses dull green (26E4), margin entire; reverse

1134 greyish green (30B5). On YES, colonies radially sulcate, velvety, mycelium

1135 white, sporulation dense, with conidial masses dark green (27F14), margin

1136 lobate; reverse greyish yellow (4B5); exudate and soluble pigment absent. On

1137 OA, colonies flat, velvety, sporulation dense, conidial masses dark dull green

1138 (28D4), margin entire; reverse greenish grey (30C2); exudate and soluble

1139 pigment absent. On CREA, acid production absent.

1140 Micromorphology — On MEA, conidiophores mostly terverticillate, in a

1141 minor proportion monoverticillate; stipes 190–250 x 3.5–4 µm, smooth;

1142 branches 30–40 µm; metula 3–5 per branch, divergent, cylindrical, 10–12(–15)

1143 x 2.5–3 µm; phialides 4–6 per metula, ampulliform, 8–10 x 2.5–3 µm; conidia

1144 mostly globose, 2.5–3 x 2.5–3 µm, smooth-walled, dull-green.

1145 Distinguishing characters — *Penicillium beceitense* is closely related to

1146 *P. lanosum* and *P. kojigenum,* two species that are indistinguishable according

1147 to our phylogeny of the section *Ramosa* (Figure 11). To date, there is no

1148 updated description for either *P. lanosum* or *P. kojigenum.* The protologue of *P.*

1149 *kojigenum* described conidia rough-walled, measuring 2.2–2.6 µm (Smith 1961).

1150 A relevant feature that distinguishes *P. beceitense* from other species of the

1151 section, such as *P. chroogomphum, P. jamesonlandense, P. lanosum, P. ribium*

1152 and *P. soppii* (Rong et al. 2016), is the absence of growth at 30 ºC.

1153

1154 ***Penicillium caprifimosum*** Guevara-Suarez, Dania García & Cano, **sp. nov.**

1155 MycoBank MB 822064. Figure 28.

Etymology — From the Latin *capra*= 'goat', and *fimosum* = 'dung-dwelling', describing the subtrate from where the species was isolated.

In — Section *Turbata*

Specimens examined — Spain, Catalonia, Els Ports Natural Park, from goat dung, February 2016, J. Guarro, M. Guevara-Suarez, E. Rosas (**holotype** CBS H-23184; culture ex-type FMR 15041 = CBS 142990; ITS barcode: LT899781, alternative markers: *Ben*A LT898238, *CaM* LT899765, *RPB*2 LT899799)

Colony diameter in 7 d (mm) — On CYA: 25 ºC 30–35, 30 °C 35–38, 37 °C 18–20; on MEA: 25 ºC 24–26, 30 °C 19–21, 37 °C 8–10; on YES: 25 ºC 25–24, 30 ºC 30–31; on OA: 25 ºC 23–25, 30 ºC 28–30, 37 °C 9–11; on DG18: 25 ºC 16–21, on CREA: 25 ºC 3–6.

Colony characters at 25º C in 7 d — On CYA, colonies flat, velvety, mycelium white, sporulation dense, with conidial masses dull green (28E5), margin entire; reverse purplish grey (14B2); exudate absent; soluble pigment pinkish (12A2). On MEA, colonies flat, velvety, sporulation dense, conidial masses dark dull green (28E3), margin lobate; reverse pale yellow (4A3) to greenish grey (26B3); exudate and soluble pigment absent. On DG18, colonies velvety, flat, mycelium white, sporulation dense, conidial masses dull green (27D4); reverse greyish green (27B5). On YES, colonies velvety, raised at the center, cerebriform, mycelium greenish white (27A2), sporulation sparse, margin lobate; reverse light brown (7D5); exudate and soluble pigment absent. On OA, colonies granulose and flat, radially sulcate towards the periphery, mycelium white, sporulation dense, conidial masses dull green (28E2), margin

1180 entire; reverse colorless; exudate and soluble pigment absent. On CREA, acid

1181 not produced.

1182   Micromorphology — On MEA, conidiophores mostly biverticillate

1183 sometimes with subterminal branches; stipes smooth-walled, 100–250 x 3–3.5

1184 µm; metula 3–4 per branch, appressed, 10–12 x 2–3 µm; phialides 3–4 per

1185 metula, ampulliform, 8–10 x 2–2.5 µm; conidia mostly globose, 2–2.5 x 2.5–3

1186 µm, smooth-walled, brownish yellow.

1187   Distinguishing characters — *Penicillium caprifimosum* can be

1188 differentiated from the closest relative, *P. bovifimosum*, by its better growth at

1189 25 ºC and 37º C on MEA and CYA. The maximum colony diameter reported for

1190 *P. bovifimosum* is 16–21 mm on MEA and 25–29 mm on CYA in 7 days at 25

1191 ºC and 6 mm on CYA at 37 ºC. Furthermore, *P. bovifimosum* is characterized

1192 by producing cleistothecia (Tuthill & Frisvad 2002), which are absent in *P.*

1193 *caprifimosum*.

1194

1195 ***Penicillium fimosum*** Guevara-Suarez, Guarro & Dania García, **sp. nov.**

1196 MycoBank MB 822069. Figure 29.

1197   Etymology — Name refers to the substrate from where the species was

1198 isolated.

1199   In — Section *Paradoxa*

1200   Specimens examined — Spain, Catalonia, Pratdip, from hervibore dung,

1201 February 2016, D. García (**holotype** CBS H-23185; culture ex-type FMR 15104

1202 = CBS 142991; ITS barcode: LT970836, alternative markers: *Ben*A LT898273,

1203 *CaM* LT970837).

Colony diameter in 7 d (mm) — On CYA: 25 ºC 31–34, 30 °C 3–4, 37 °C no growth; on MEA: 25 ºC 25–30, 30 °C no growth; on YES: 25 ºC 50–55, 30 ºC 5–7; on OA: 25 ºC 25–27, 30 ºC no growth; on DG18: 25 ºC 16–20, on CREA: 25 ºC 20–23.

Colony characters at 25º C in 7 d — On CYA, colonies velvety, slightly radially sulcate at the center, mycelium white, sporulation dense, conidial masses dark dull green (26E4), margin lobate; reverse greyish orange (6B4) at the center, greyish green (28C3) towards the periphery; exudate and soluble pigment absent. On MEA, colonies flat, velvety, sporulation dense, conidial masses dark dull green (28E3), margin crenate; reverse light orange (5A4) to dull green (28D4) at the center, colorless towards the periphery; exudate and soluble pigment absent. On DG18, colonies velvety, flat, mycelium white, sporulation dense, conidial masses dull green (27D4); reverse greenish white (27B2). On YES, colonies irregularly sulcate, slightly raised at the center, mycelium white, sporulation dense, with conidial masses dull green (26E5), margin almost entire and fimbriate; reverse bright orange red (8A8) at the center; exudate and soluble pigment absent. On OA, colonies slightly granulose, mycelium white, sporulation dense, conidial masses greyish green (28E7); exudate and soluble pigment absent. On CREA, moderate acid production.

Micromorphology — On MEA, conidiophores mostly biverticillate, some irregularly branched; stipes 70–130 x 2.5–3(–4) µm, smooth; metula 3–4 per branch, rather divergent, 8–14 x 2.5–3(–4) µm; phialides 3–4 per metula, ampulliform, (6–)8–10 x 2.5–3 µm; conidia globose to subglobose, 2.5–3 x 2.5–3(–4) µm, smooth, brownish yellow.

1229   Distinguishing characters — *Penicillium fimosum* is closely related to *P.*

1230   *atramentosum* and *P. ibericum,* described here. *Penicillium fimosum* and *P.*

1231   *atramentosum* differ from *P. ibericum* by the absence or by having a very

1232   restrictive growth at 30ºC. *Penicilium atramentosum* can be distinguished by the

1233   production of exudate, soluble pigment and colony reverse on CYA of reddish

1234   brown color*,* features absent in *P. fimosum*. Moreover, *P. fimosum* has shorter

1235   stipes (70–130 µm) than *P. atramentosum* (300–500 µm) (Pitt 1979).

1236

1237   ***Penicillium ibericum*** Guevara-Suarez, Cano & Dania García, **sp. nov.**

1238   MycoBank MB 822070. Figure 30.

1239   Etymology — Name referred to the occurrence of the species in the

1240   Iberian Peninsula.

1241   In — Section *Paradoxa*

1242   Specimens examined — Spain, Catalonia, Els Ports Natural Park, from

1243   herbivore dung, February 2016, J. Guarro, M. Guevara-Suarez, E. Rosas

1244   (**holotype** CBS H-23186; culture ex-type FMR 15040 = CBS 142992; ITS

1245   barcode: LT899782, alternative markers: *Ben*A LT898285, *CaM* LT899766,

1246   *RPB*2 LT899800); Galicia, Las Dunas de Corrubedo Natural Park, from soil,

1247   February 2016, D. García (FMR 15107).

1248   Colony diameter in 7 d (mm) — On CYA: 25 ºC 31–35, 30 °C 18–21, 37

1249   °C no growth; on MEA: 25 ºC 18–20, 30 °C 8–9; on YES: 25 ºC 33–35, 30 ºC

1250   18–20; on OA: 25 ºC 21–23, 30 ºC no growth; on DG18: 25 ºC 5–6; on CREA:

1251   25 ºC 21–22.

1252   Colony characters at 25º C in 7 d — On CYA, colonies flat, velvety,

1253   sulcate, mycelium white, sporulation dense, conidial masses dark dull green

1254 (26E4), margin slightly lobate; reverse reddish brown (9E5) to reddish (9A2);

1255 exudate present, with pale red (10A3) droplets; soluble pigment absent. On

1256 MEA, colonies, velvety and flat, sporulation dense, conidial masses dark dull

1257 green (28E3); reverse light orange (5A4), margin slightly irregular; exudate and

1258 soluble pigment absent. On DG18, colonies sunken in the middle, velvety,

1259 mycelium white, sporulation moderate, conidial masses dull green (27D4);

1260 reverse greenish white (27B2). On YES, velvety, sulcate, mycelium white,

1261 sporulation dense, conidial masses dull green (26E5), margin crenate; reverse

1262 orange grey (5B3); exudate and soluble pigment absent. On OA, colonies

1263 irregular, granulose, mycelium white, sporulation dense, conidial masses

1264 greyish green (28E7); reverse colorless; exudate and soluble pigment absent.

1265 On CREA, moderate acid production.

1266 Micromorphology — On MEA, conidiophores ter- to quarterverticillate;

1267 stipes 70–150 x 2–3(–4) µm, smooth; metula 2–4 per branch, divergent, 8–12 x

1268 2–2.5 µm; phialides 3–4 per metula, ampulliform, 8–10 x 2.5–3 µm; conidia

1269 globose, 2.5–3 x 2.5–3 µm, smooth, dull green.

1270 Distinguishing characters — *Penicillium ibericum* is closely related to *P.*

1271 *atramentosum* and *P. fimosum*. It can be distinguished by having shorter stipes

1272 (70–150 µm) than *P. atramentosum* (300–500 µm) (Pitt 1979), and it grows

1273 more restricted on YES (33–35 mm diam) and DG18 (5–6 mm) compared to *P.*

1274 *fimosum* (YES 50–55 mm, DG18 16–20 mm).

1275

1276 ***Penicillium mediterraneum*** Guevara-Suarez, Gené & Cano, **sp. nov.**

1277 MycoBank MB 822071. Figure 31.

1278   Etymology — Name refers to the geographical area where the species

1279 was found.

1280   In — Section *Roquefortorum*

1281   Specimens examined: Spain, Balearic Islands, Mallorca, Puigpuñent,

1282 from herbivore dung, February 2016, J. Guarro, M. Guevara-Suarez, J.P.Z.

1283 Siqueira (**holotype** CBS H-23143; culture ex-type FMR 15188 = CBS 142754;

1284 ITS barcode: LT899784, alternative markers: *Ben*A LT898291, *CaM* LT899768,

1285 *RPB*2 LT899802); Catalonia, Els Ports Natural Park, herbivore dung, February

1286 2016, J. Guarro, M. Guevara-Suarez, E. Rosas, (FMR 15031 = CBS 142755);

1287 Catalonia, Els Ports Natural Park, herbivore dung, February 2016, J. Guarro, M.

1288 Guevara-Suarez, E. Rosas (FMR 15032).

1289   Colony diameter in 7 d (mm) — On CYA: 25 ºC 45–60, 30 °C 24–26, 37

1290 °C no growth; on MEA: 25 ºC 45–60, 30 °C 23–26; on YES: 25 ºC >60, 30 ºC

1291 35–40; on OA: 25 ºC 31–38, 30 ºC 8–15; on DG18: 25 ºC 43–45; on CREA: 25

1292 ºC 22–25.

1293   Colony characters at 25º C in 7 d — On CYA, colonies flat, velvety,

1294 mycelium white, sporulation dense, conidial masses dull green (25E4), margin

1295 entire; reverse greyish green (28C5); exudate and soluble pigment absent. On

1296 MEA, colonies flat, velvety, mycelium white, margin crenate, sporulation dense,

1297 with conidial masses conidial masses dull green (25E4), margin irregular,

1298 slightly lobulate, reverse greyish green (28E5); exudate and soluble pigment

1299 absent. On DG18, colonies flat, velvety, mycelium white, sporulation dense,

1300 conidial masses dull green (26E4), margin entire; reverse greyish green (30C6).

1301 On YES, colonies velvety, raised at the center, concentrically sulcate, mycelium

1302 white, sporulation dense, conidial masses dull green (26E4), margin entire and

1303 fimbriate; reverse greyish green (28C6); exudate and soluble pigment absent.

1304 On OA, colony cottony, mycelium white, sporulation dense, conidial masses

1305 dark dull green (28D4), margin entire; reverse colorless; exudate and soluble

1306 pigment absent. On CREA, moderate acid production.

1307 Micromorphology — On MEA, conidiophores bi- to terverticillate; stipes

1308 rough-walled, 50–100 x 2.5–3 µm; metula 2–3 per branch, appressed,

1309 cylindrical, 10–14 x 2–3 µm; phialides 2–4 per metula, ampulliform, 10–13 x 2–3

1310 µm; conidia mostly globose, 2.5–4 (–5) x 2–4 µm, smooth, dull-green.

1311 Distinguishing characters — All species of the section *Roquefortorum*

1312 show fast growth on CYA and MEA and produce rough-walled conidiophores.

1313 *Penicillium roqueforti* is the closest related species to *P. mediterraneum,* and it

1314 can be distinguished by a more restricted growth on CYA at 30ºC (5–15 mm

1315 diam.) and by its blackish green colony reverse on YES (Frisvad & Samsom

1316 2004, Houbraken et al. 2010).

1317

1318 ***Penicillium synnematicola*** Guevara-Suarez, Dania García & Guarro, **sp. nov.**

1319 MycoBank MB 822072. Figure 32.

1320 *Etymology* — Name referred to the formation of synnemata.

1321 In — Section *Robsamsonia*

1322 Specimens examined — Spain, Balearic Islands, Mallorca, Camí Vell

1323 d'Orient, from goat dung, February 2016, J. Guarro, M. Guevara-Suarez, J.P.Z.

1324 Siqueira (**holotype** CBS H-23132, culture ex-type FMR 15192 = CBS 142669;

1325 ITS barcode: LT898167, alternative markers: *BenA* LT898172, *CaM* LT898137,

1326 RPB2 LT898142); Mallorca, Escorca, from goat dung, February 2016, J.

1327 Guarro, M. Guevara-Suarez, J.P.Z. Siqueira (FMR 15210); Mallorca, Camí Vell

d'Orient, from goat dung, February 2016, J. Guarro, M. Guevara-Suarez, J.P.Z. Siqueira (FMR 15211); Catalonia, Barcelona, Montseny Natural Park, from herbivore dung, April 2017, J. Gené, M. Guevara-Suarez, I. Iturrieta-González (FMR 16481 = CBS 143045); Catalonia, Poblet, from herbivore dung, March 2017, J. Guarro, Guevara-Suarez, I. Iturrieta-González (FMR 16491 = CBS 143046).

Colony diameter in 7 d (mm) — On CYA: 25 ºC 33–37, 30 °C 9–10, 37 °C no growth; on MEA: 25 ºC 11–13, 30 °C 5–7; on YES: 25 ºC 30–34, 30 ºC 9–17; on OA: 25 ºC 31–38, 30 ºC 8–15; on DG18: 25 ºC 17–19; on CREA: 25 ºC 6–9.

Colony characters at 25º C in 7 d — On CYA, colonies granular, raised at the center, mycelium white, sporulation dense, conidial masses dark dull green (25E4), margin dentate; reverse light orange (5A5); exudate present, consisting of small hyaline droplets; soluble pigment absent. On MEA, colonies fasciculate due to the presence of small feathery synnemata, mycelium white, sporulation dense, with conidial masses dark green (28F5), margin crenate; reverse orange (6B8); exudate absent and soluble pigment deep orange (5A8). On DG18, colony velvety, mycelium white, sporulation dense, conidial masses dull green (27E4); reverse greyish orange (5B3). On YES, colonies granular, raised at the center, radially sulcate, mycelium white, sporulation dense, conidial masses dull green (27E4), margin dentate; reverse brownish orange (6C8); exudate and soluble pigment absent. On OA, colony strongly fasciculate, mycelium white, sporulation dense, conidial masses dark dull green (27E4), margin crenate; reverse yellowish white (4A); exudate and soluble pigment absent. On CREA, weak acid production.

1353   Micromorphology — On MEA, synnemata present, up to 1 mm long;

1354   conidiophores ter- to quaterverticillate; stipes 100–218 x 3–4 μm, coarsely

1355   roughened; metula 3–4 per branch, rather appressed, cylindrical, smooth to

1356   conspicuously roughened, 10–14 x 2–3 μm; phialides 2–4 per metula,

1357   ampulliform, 10–13 x 2–3 μm; conidia subglobose to broadly ellipsoidal, 2.5–3 x

1358   2–2.5 μm, smooth, hyaline.

1359   Distinguishing characters — Section *Robsamsonia* includes some

1360   species producing synnemata, e.g. *P. coprophilum, P. glandicola* and *P.*

1361   *vulpinum* (Houbraken et al. 2016)*. Penicillium synnematicola* can be easily

1362   differentiated from *P. coprophilum* and from *P. vulpinum* since both have

1363   smooth-walled conidiophores. The closest phylogenetic species is *P.*

1364   *glandicola,* which produces, similar to *P. synnematicola,* coarsely roughened

1365   conidiophores. However, the former can be distinguished by its orange brown to

1366   brown and bright orange-red colony reverse on CYA and YES, respectively, it

1367   tends to have a more restricted growth on CYA (17–30 mm) and YES (19–36

1368   mm), its phialides are shorter (7.5–10.5 μm long), and the conidia are yellow

1369   green (Houbraken et al. 2016).

1370

1371   ***Penicillago*** Guevara-Suarez, Gené & Dania García, **gen. nov.** MycoBank MB

1372   822073.

1373   Etymology — Name refer to the morphological similarity to *Penicillium*

1374   In — Family *Aspergillaceae*

1375 *Mycelium* superficial and immersed, composed of septate, branched, canary

1376   yellow to chrome yellow hyphae. *Conidiophores* composed of long, septate,

1377   hyaline stipes, often terminating in a small vesicle from which born a verticil of

metula, giving symmetrically biverticillate penicilli, occasionally irregulary branched; metula appressed to divergent, cylindrical to somewhat obpyriform, bearing in a compact verticil of conidiogenous cells. *Conidiogenous cells* phialidic, ampulliform to acerose, with a fine and long neck. *Conidia* dry, catenate, with conspicuous disjunctors, subglobose to ellipsoidal, coarsely equinulate, subhyaline to brown; conidial chains short to moderately long. *Sexual morph* unknown.

Type species — *Penicillago nodositata* (Valla) Guevara-Suarez, Gené & D. García.

Notes — The genus *Penicillago* is introduced to accommodate *Penicillium nodositatum*. It is noteworthy that the taxonomy of this species has been confuse. Initially, Valla *et al.* (1989) classified *P. nodositatum* in the subgenus *Biverticillium* since its morphological affinity with members of the series *Islandica* (Pitt 1979). Later, based on molecular data, it was considered as member of the *Penicillium* section *Sclerotiora* and tentatively placed in synonymy with *P. bilaiae* by Houbraken & Samson (2011), despite the morphological differences in the conidiophore structure (biverticillate in *P. nodositatum* vs monoverticillate in *P. bilaiae*). However, with the revaluation of both species and new sequence data, Visage et al. (2013) showed they were distinct species. These authors also noticed that *P. nodositaum* was related to *P. kabunicum*, however sequences of this latter species are not currently available for comparison. The possibility to examine two Spanish isolates that morphologically fit with *P. nodositatum* and their DNA sequences coinciding with those of the ex-type strain of this species allows us to delineate a new genus of penicillium-like fungi in the family *Aspergillaceae*.

1403

1404 **Penicillago nodositata** (Valla) Guevara-Suarez, Gené & Dania García, **comb.**

1405 **nov.** MycoBank MB 822074. Figure 33.

1406 *Basionym: Penicillium nodositatum* Valla, Pl. Soil 114: 142–146 1989.

1407 *Type culture*: CBS 333.90, France, Isère, Col d'Ornon, from myconodules in

1408 roots of *Alnus incana*, G. Valla.

1409 Specimens examined — Spain, Balearic Islands, Mallorca, from wild pig

1410 dung, February 2016, J. Guarro, M. Guevara-Suarez, J.P.Z. Siqueira (FMR

1411 15296 = CBS 142988; ITS barcode: LT899787, alternative markers: *BenA*

1412 LT898312, *CaM* LT899771, *RPB*2 LT899805). Extremadura, Badajoz, Granja

1413 de Torrehermosa, from herbivore dung, December 2016, J. Cano-Lira (FMR

1414 16442).

1415 Colony diameter in 7 d (mm) — On CYA: 25 ºC 40–44, 30 °C 34–36, 37

1416 °C 7–8; on MEA: 25 ºC 34–36, 30 °C 24–27, 37 °C 0–3; on YES: 25 ºC 50–55,

1417 30 ºC 45–55, 37 ºC 11–10; on OA: 25 ºC 24–26, 30 ºC 22–24, 37 ºC 4–6; on

1418 DG18: 25 ºC 2–3; on CREA: 25 ºC 22–30.

1419 Colony characters at 25º C in 7 d — On CYA, colonies cottony, slightly

1420 sulcate, mycelium light yellow (3A5) to white, sporulation sparse in 7d, conidial

1421 masses yellowish brown (5E8) after 14 d, margin fimbriate; reverse greyish

1422 orange (5B4) at the centre, colorless towards the periphery; exudate and

1423 soluble pigment absent. On MEA, colonies slightly cottony, flat, mycelium light

1424 yellow (3A5) to white, sporulation dense after the 14 d, with conidial masses

1425 dark green (28F5), margin slightly fimbriate; reverse light yellow (4A4); exudate

1426 and soluble pigment absent. On DG18, colonies restricted, flat, mycelium white,

1427 sporulation absent, margin entire; reverse colorless; exudate and soluble

1428 pigment absent. On YES, colonies velvety, raised at the center, irregularly

1429 sulcate, mycelium yellow (3A7) to white, sporulation sparse, margin fimbriate;

1430 reverse greyish orange (5B5); exudate and soluble pigment absent. On OA,

1431 colony slightly cottony, mycelium white, sporulation sparse, margin entire;

1432 reverse yellowish white (4A2); exudate and soluble pigment absent. On CREA,

1433 acid production absent.

1434 Micromorphology — On MEA, conidiophores symmetrically biverticillate,

1435 sometimes irregularly branched; stipes smooth hyaline, often with a small

1436 vesicle at the apice, 3.5-5.5 µm wide, 200–300 x 2.5–3 µm; metula divergent,

1437 cylindrical to somewhat obpyriform, 9–10(–15) x 3–4(–5) µm; phialides four to

1438 seven per metula, ampulliform, tapering abruptly to a long, slender neck, 6–8(–

1439 9) x 2.5–3.5 µm; conidia subglobose to broadly ellipsoidal, echinulate, 2.5–4 x

1440 3–4(–5) µm, pale brown.

1441

1442 *Pseudopenicillium* Guevara-Suarez, Cano & Guarro, **gen. nov.** MycoBank MB

1443 822076.

1444 Etymology — *Pseudo*- meaning "false"-, name referred to the

1445 morphological similarity but not belonging to *Penicillium*.

1446 In — Family *Aspergillaceae*

1447 *Mycelium* superficial and immersed, composed of septate, branched, hyaline to

1448 subhyaline hyphae. *Conidiophores* undifferentiated, reduced to conidiogenous

1449 cells arising directly from the main hyphae, or differentiated composed of short

1450 to moderately long, aseptate or few septate, hyaline to brown stipes, terminating

1451 in an irregular penicilli; penicilli varying from monoverticillate to partly

1452 biverticillate, with few metula bearing each a verticil of conidiogenous cells;

1453 metula terminal or subterminal, generally divergent, cylindrical or apically

1454 inflated. *Conidiogenous cells* phialidic, ampulliform, with swollen base and

1455 tapering abruptly to a slender neck of variable length. *Conidia* dry, catenate,

1456 with conspicuous disjunctors, globose to subglobose, with spinulose walls,

1457 brown, dark in masses; conidial chains short. *Sexual morph* unknown.

1458   Type species — *Pseudopenicillium megasporum* (P. A. Orpurt & D. I.

1459 Fennell) Guevara-Suarez, Cano & Guarro.

1460   Notes — The genus *Pseudopenicillium* is proposed to accommodate a

1461 new species and two species previously classified in the genus *Penicillium*, *P.*

1462 *giganteum* and *P. megasporum*, being this latter selected as the type since it

1463 was the firstly described. *Penicillium giganteum* and *P. megasporum* were

1464 recovered from soil samples in the UK and India, respectively. Despite their

1465 different geographical origin, both were considered conspecific and classified in

1466 series *Megasporus* (Pitt 1979). *Penicillium asperosporum,* also included in this

1467 series, was later shown to be a synonym of *P. montanense* in section

1468 *Aspergilloides* (Houbraken et al. 2014). Multigene phylogenies (Peterson et al.

1469 2010, Houbraken & Samson 2011) demonstrated that *P. megasporum* and *P.*

1470 *giganteum* were distinct species closely related to the genus *Hamigera*. Our

1471 phylogeny confirms this relationship, showing that *Hamigera* and the mentioned

1472 *Penicillium* species comprises two sister clades with enough genetic difference

1473 to be considered distinct genus (Figures 15, 16). Morphologically, the novel

1474 genus *Pseudopenicillium* differs from *Hamigera* in the lack of sexual morph and

1475 by its brown spinulose conidia, and from *Penicillium* and *Penicillago* mainly by

1476 its short and often irregularly branched conidiophores producing conidia in short

1477 chains.

1478

1479 ***Pseudopenicillium coprobium*** Guevara-Suarez, Dania García & Gené, **sp.**

1480 **nov.** MycoBank MB 822079. Figure 34.

1481   *Etymology* — Name refers to the source of isolation of the fungus.

1482   Specimen examined — Spain, Castile and Leon, Palencia, from

1483 herbivore dung, March 2016, J. Guarro, M. Guevara-Suarez (**holotype** CBS H-

1484 23133; culture ex-type FMR 15299 = CBS 142670; ITS barcode: LT899789,

1485 alternative markers: *BenA* LT898315, *RPB*2 LT899807).

1486   Colony diameter in 7 d (mm) — On CYA: 25 ºC 26–30, 30 °C 30–32, 37

1487 °C 6–9; on MEA: 25 ºC 13–17, 30 °C 22–24, 37 °C 3–5; on YES: 25 ºC 24–26,

1488 30 ºC 35–38, 37 ºC 23–25; on OA: 24–26, 30 ºC –34, 36 ºC 14–16; on DG18:

1489 25 ºC 16–17; on CREA: 25 ºC 18–20.

1490   Colony characters at 25º C in 7 d — On CYA, colonies flat, velvety,

1491 mycelium white, sporulation dense, central area with conidial masses dark

1492 green (29F3), margin entire; reverse pale yellow (4A3); exudate and soluble

1493 pigment absent. On MEA, colonies growing moderately, velvety, slightly

1494 umbonate, mycelium white, sporulation dense, with conidial masses olive-

1495 yellow (3C8) to olive (3F8), margin lobate; exudate and soluble pigment absent;

1496 reverse greyish yellow (2B4). On YES, colonies dome-shaped, velvety,

1497 mycelium white, sporulation absent, margin entire; reverse pale yellow (4A3);

1498 exudate and soluble pigment absent. DG18, colones velvety, slightly sulcate,

1499 mycelium white, sporulation dense, with conidial masses dull green (28E3),

1500 margin entire; reverse yellowish white (4A3). On OA, colonies cottony,

1501 mycelium white, sporulation dense, with conidial masses dark green (29F3),

1502   margin fimbriate; exudate and soluble pigment absent. On CREA, strong acid

1503   production.

1504   Micromorphology — On MEA, conidiophores short, arising as lateral

1505   branches from hyphae, monoverticillate, sometimes biverticillate; stipes 10–35 x

1506   2.5–3 µm, cylindrical, without vesicle, smooth to fine verruculose, hyaline;

1507   metula divergent, cylindrical, 9.5–10 x 2.5–3 µm; phialides in verticils of 2–4 on

1508   the stipe or per metula, ampulliform with swollen base, tapering abruptly to a

1509   slender neck, (6–)7.5–9 x 2.5–3 µm; conidia globose, 5–5.5 x 6 µm, spinulose,

1510   thick-walled, pale brown to brown.

1511   Differential diagnosis — *Pseudopenicillium coprobium* is closely related

1512   to *Pse. giganteum* and *Pse. megasporum.* This group of species have similar

1513   conidiogenous apparatus and produce globose spinulose conidia. However,

1514   they can be distinguished by their conidial sizes (i.e., up to 6 µm in *Pse.*

1515   *coprobium,* up to 10 µm in *Pse. megasporum* and up to 12 µm in *Pse.*

1516   *giganteum*). In addition, *Pse. megasporum* has a more poor growth on CYA at

1517   25 ºC (15–25 mm) and shows vesiculate stipes (Pitt 1979, Peterson et al.

1518   2010).

1519

1520   **Pseudopenicillium giganteum** (R.Y. Roy & G. N. Singh) Guevara-Suarez,

1521   Gené & Cano, **comb. nov.** MycoBank MB 822077.

1522   *Basionym: Penicillium giganteum* R.Y. Roy & G.N. Singh, Trans Br Mycol Soc

1523   51:805. 1968.

1524   *Type cultures:* NRRL 3553 = ATCC 48996 = CBS 144.69 = FRR 535 = IMI

1525   132774, West Bengal, Varanasi, Banaras Hindu University, Botanical Garden,

1526   from soil, Nov. 1967, G.N. Singh.

1527 *Descriptions and illustrations*: See Roy and Singh (1968) and Peterson et

1528 al. (2010).

1529 Specimen examined — Spain, unknown geographic region, from soil

1530 (FMR 14718).

1531 Notes — The isolate identified in the current study represents the second

1532 specimen of this taxa found so far. In general, its morphological features are

1533 consistent with those of the species giving by Roy and Singh (1968).

1534

1535 ***Pseudopenicillium megasporum*** (P. A. Orpurt & D. I. Fennell) Guevara-

1536 Suarez, Cano & Guarro, **comb. nov.** MycoBank MB 822078.

1537 *Basionym: Penicillium megasporum* P. A. Orpurt & D. I. Fennell. Mycologia 47:

1538 233, 1955.

1539 *Type cultures:* NRRL 2232 = ATCC 12322 = CBS 256.55 = FRR 2232 = IMI 216904 =

1540 MUCL 38804 = QM 6879, WE 2232. Egland, Suffolk, from heath soil, JH Warcup.

1541 *Descriptions and illustrations*: See Orpurt & Fennell (1955), Pitt (1979)

1542 and Peterson *et al.* (2010).

1543 ***Talaromyces catalonicus*** Guevara-Suarez, Gené & Guarro, **sp. nov.**

1544 MycoBank MB 822080. Figure 35.

1545 *Etymology* — Name refers to the region (Catalonia) from where the

1546 fungus was isolated.

1547 In — Section *Trachyspermi*

1548 Specimens examined — Spain, Catalonia, Poblet, from herbivore dung,

1549 February 2017, J. Guarro, M. Guevara-Suarez, I. Iturrieta-González (**holotype**

1550 CBS H-23212; culture ex-type FMR 16441 = CBS 143039; ITS barcode:

1551   LT899793, alternative identification markers: *Ben*A LT898318, *CaM* LT899775,

1552   *RPB*2 LT899811).

1553   Colony diameter in 7 d (mm) — On CYA: 25 ºC 35–40, 30 °C 38–40, 37

1554   °C 15–18; on MEA: 25 ºC 17–19, 30 °C 19–20, 37 °C 16–18; on YES: 25 ºC

1555   45–50, 30 ºC 50–55, 37 °C 25–30; on OA: 25 ºC 40–45, 30 ºC 40–42, 37 °C

1556   12–14; on DG18: 25 ºC 20–22, on CREA: 25 ºC 17–19.

1557   Colony characters at 25º in 7 d — On CYA, colonies velvety, radially

1558   sulcate, mycelium white, sporulation dense, conidial masses dull green (27E4),

1559   margin lobate; reverse greyish orange (5B4); exudate absent, soluble pigment

1560   only at 30 and 37°C, light yellow (3A5) to light orange (5A6). On MEA, colonies

1561   velvety, flat, sporulation dense, with conidial masses greyish green (27E5),

1562   margin entire; reverse yellow (2B8); exudate and soluble pigment absent. On

1563   DG18, colonies flat, slightly cotton at the center, velvety in the periphery,

1564   mycelium white, sporulation dense, conidial masses dull green (26E5), margin

1565   entire; reverse greyish green (28B4). On YES, colonies raised at the center,

1566   irregularly sulcate, mycelium white, sporulation dense, with conidial masses

1567   dark green (30F3), margin entire; reverse orange yellow (4B8); exudate and

1568   soluble pigment absent. On OA, colonies velvety, flat, mycelium white,

1569   sporulation dense, conidial masses dark green (27F3), margin entire; reverse

1570   greenish grey (29C2); exudate and soluble pigment absent. On CREA, weak

1571   acid production.

1572   Micromorphology — On MEA, conidiophores mono- to biverticillate,

1573   sometimes irregularly branched, stipes (40–)100–130 x 2–2.5 μm, smooth-

1574   walled, hyaline; metula divergent, 10–11 x 2–2.5 μm; phialides 4–6 per metula,

1575   acerose, (7–)8–10 x 2–2.5 µm; conidia globose to subglobose, 2–2.5 x 1.8–2

1576   µm, smooth, brownish yellow. Ascomata not observed.

1577   Distinguishing characters — The most closely related species to *T.*

1578   *catalonicus* are *T. albobiverticillius, T. erythromellis, T. heiheensis* and *T.*

1579   *solicola. Talaromyces catalonicus* is characterised by its ability to grow at 37 °C;

1580   in contrast, the above-mentioned species do not grow or grow very restricted at

1581   this temperature (Samson et al. 2011, Yilmaz et al. 2014).

1582

1583   ***Talaromyces coprophilus*** Guevara-Suarez, Cano & Dania García, **sp. nov.**

1584   MycoBank MB 822088. Figure 36.

1585   *Etymology* — Name referred to the source of isolation of the fungus.

1586   In — Section *Talaromyces*

1587   Specimens examined — Spain, Balearic Islands, Mallorca, Escorca, from

1588   herbivore dung, February 2016, J. Guarro, M. Guevara-Suarez, J.P.Z. Siqueira

1589   (**holotype** CBS H-23144; culture ex-type FMR 15199 = CBS 142756; ITS

1590   barcode: LT899794, alternative identification markers: *Ben*A LT898319, *CaM*

1591   LT899776, *RPB*2 LT899812).

1592   Colony diameter in 7 d (mm) — On CYA: 25 ºC 38–40, 30 °C 40–45, 37

1593   °C 30–35; on MEA: 25 ºC 33–35, 30 °C 30–35, 37 °C 21–24; on YES: 25 ºC

1594   45–50, 30 ºC 50–55, 37 °C 38–42; on OA: 25 ºC 35–40, 30 ºC 40–45, 37 °C

1595   25–26; on DG18: 25 ºC no growth, on CREA: 25 ºC 8–10.

1596   Colony characters at 25º C in 7 d — On CYA, colonies slightly raised at

1597   the center, velvety, mycelium light yellow (4A5) to white, sporulation absent to

1598   sparse, with margin entire and fimbriate; reverse light orange (5A4); soluble

1599   pigment and exudate absent. On MEA, colonies slightly raised at center,

1600 velvety, flat, mycelium light yellow (4A5) to white, conidial sporulation absent to

1601 sparse, young ascomata visible, margin fimbriate; reverse light orange (5A4);

1602 soluble pigment and exudate absent. On YES, colonies raised at center,

1603 velvety, mycelium pinkish (7A3) to white, sporulation absent, with margin entire;

1604 reverse brownish orange (7C6); exudate absent and soluble pigment brownish

1605 red (10C7). On OA, colonies granular, flat, with young ascomata visible, conidial

1606 sporulation sparse, margin fimbriate; reverse greenish grey (28C3); exudate

1607 and soluble pigment absent. On CREA, strong acid production.

1608 Micromorphology — On MEA, conidiophores mono- to biverticillate,

1609 stipes 40–70 x 2–2.5 µm, smooth, hyaline; metula rather appressed, 10–12 x

1610 2.5–3 µm; phialides 3–4 per metula, acerose, 9–12 (–14) x 2–2.5 µm; conidia

1611 ellipsoidal, 2–3 x 2.5–3 µm, smooth -walled, brownish yellow. Ascomata after 1–

1612 2 wk of incubation on OA and MEA at 25 °C, bright orange to orange-red,

1613 globose, 200–310 µm diam, with peridal hyphae branched and verruculose; asci

1614 globose to subglobose, 6–7 x 7–9 (10) µm; ascospores ellipsoidal, 3.5–4 × 3–4

1615 µm, spiny, thick-walled, golden yellow.

1616 Distinguishing characters — *Talaromyces coprophilus* is characterised by

1617 the production of orange-red ascomata with spiny ellipsoidal ascospores, by the

1618 lack of growth on DG18, and by having a rapid growth on the other culture

1619 media and temperatures tested. Phylogenetically, it forms an independent and

1620 distant branch in an unsupported clade along with the ex-type strains of *T.*

1621 *cnidii, T. flavovirens, T. funiculosus, T. macrosporus, T. pseudofuniculosus*

1622 (described here) *T. rapidus,* and *T. siamensis* are found (Figure 13).

1623 Morphologically, *T. coprophilus* resembles to *T. flavovirens* and *T. macrosporus*

1624 in the production of sexual morph*;* however, these two latter species mainly

1625 differs by having a slower growth on CYA at 25 ºC (19–20 mm in *T. flavovirens*,

1626 22–28 mm in *T. macrosporus*), and larger ascospores (4–7 × 3–4 µm in *T.*

1627 *flavovirens*, 5.5–6.5 × 4.5–5.5 µm in *T. macrosporus*) (Yilmaz et al. 2014).

1628

1629 ***Talaromyces gamsii*** Guevara-Suarez, Cano & Guarro, **sp. nov.** MycoBank

1630 MB 822089. Figure 37.

1631 *Etymology* — Named in tribute to the excellent mycologist Walter Gams.

1632 In — Section *Talaromyces*

1633 Specimens examined — Spain, Andalusia, La Rocina, Doñana National

1634 Park, from soil, March 2016, D. García (**holotype** CBS H-23213; culture ex-type

1635 FMR 15303 = CBS 143040; ITS barcode: LT899795, alternative markers: *Ben*A

1636 LT898320, *CaM* LT899777, *RPB*2 LT899813).

1637 Colony diameter in 7 d (mm) — On CYA: 25 ºC 13–15, 30 °C 11–15, 37

1638 °C no growth; on MEA: 25 ºC 25–29, 30 °C 26–30; on YES: 25 ºC 20–23, 30 ºC

1639 10–15; on OA: 25 ºC 12–15, 30 ºC 10–14; on DG18: 25 ºC no growth, on

1640 CREA: 25 ºC 8–10.

1641 Colony characters at 25º C in 7 d — On CYA, colonies slightly raised at

1642 the center, flat towards the periphery, velvety, mycelium white, sporulation

1643 dense, with conidial masses olive (1E8) to greenish yellow (1A7), margin entire;

1644 reverse dark orange (5D8) to yellowish orange (4A7); exudate absent; soluble

1645 pigment yellowish orange (4A8). On MEA, colonies velvety, flat, sporulation

1646 dense, conidial masses greyish green (30C7), margin slightly fimbriate and with

1647 submerged mycelium; reverse vivid red (9A8) to pale red (9C3); exudate and

1648 soluble pigment absent. On YES, colonies raised at the center, velvety, with

1649 mycelium pinkish (11A2) to white; sporulation sparse, margin entire; reverse

1650   brownish red (10D6) to pastel red (10B4); exudate absent; soluble pigment

1651   brownish red (10C7). On OA, colonies granular, mycelium white, sporulation

1652   dense, with conidial masses deep green (28D8), margin regular and slightly

1653   fimbriate; reverse greenish grey (28C3); exudate and soluble pigment absent.

1654   On CREA, acid production absent.

1655   Micromorphology — On MEA, conidiophores symmetrically biverticillate;

1656   stipes 150–220 x 2.5–3 µm, smooth-walled; metula 3–4 per branch, divergent,

1657   8–11 x 2–3.5 µm; phialides 4–6 per metula, acerose, 9–11 x 2–3 µm; conidia

1658   globose to subglobose, 2.5–3 x 2.5–3(–4) µm, finely rough-walled, brownish

1659   yellow. Ascomata not observed.

1660   Distinguishing characters — *Talaromyces gamsii* is related to *T.*

1661   *francoae*, *T. kendrickii, T. qii, T. mangshanicus* and *T. thailandensis*. All these

1662   species were characterized by the lack of growing at 37 ºC and by having

1663   ampulliform phialides. However, *T. gamsii* can be distinguished easily by the

1664   absence of growth on DG18 and by its restricted growth on CYA (13–15 mm).

1665   In addition to *T. gamsii* and *T. coprophilus* (also describe here), there are other

1666   *Talaromyces* species reported to be unable to grow on DG18, such as *T.*

1667   *subinflatus* and *T. udagawae* but they belong to other sections (Yilmaz *et al.*

1668   2014).

1669

1670   ***Talaromyces pseudofuniculosus*** Guevara-Suarez, Dania García & Gené, **sp.**

1671   **nov.** MycoBank MB 822090. Figure 38.

1672   *Etymology* — *–pseudo*, meaning "false"-, name referred to its

1673   phylogenetic relationship with *T. funiculosus*.

1674   In — Section *Talaromyces*

Specimens examined: Spain, Andalusia, La Rocina, Doñana National Park, from herbivore dung, March 2016, D. García (**holotype** CBS H-23214; culture ex-type FMR 15307 = CBS 143041; ITS barcode: LT899796, alternative markers: *Ben*A LT898323, *CaM* LT899778, *RPB*2 LT899814). Catalonia, Els Ports Natural Park, from herbivore dung, February 2016, J. Guarro, M. Guevara-Suarez & E. Rosas (FMR 15035).

Colony diameter in 7 d (mm) — On CYA: 25 ºC 36–43, 30 °C 45–40, 37 °C 31–35; on MEA: 25 ºC 24–29, 30 °C 43–45, 37 °C 23–26; on YES: 25 ºC 34–35, 30 ºC 40–44, 37 °C 32–34; on OA: 25 ºC 33–38, 30 ºC 42–45, 37 °C 27–30; on DG18: 25 ºC 12–15, on CREA: 25 ºC 2–4.

Colony characters at 25º C in 7 d — On CYA, colonies slightly raised at center, radially sulcate, cottony, mycelium white, sporulation dense, conidial masses greyish green (29D5), with margin entire; reverse dark orange (5D8); soluble pigments and exudate absent. On MEA, colonies velvety to cottony, flat, sporulation dense, conidial masses greyish green (28D5), margin entire to slightly crenate; reverse deep yellow (5A4) to greyish green (30C5); exudate and soluble pigment absent. On YES, colonies slightly raised at the center, sulcate, cottony, mycelium white, sporulation dense in the center, conidial masses greyish green (27D4), margin entire; reverse olive yellow (3C7); exudate and soluble pigments absent. On DG18, colonies flat and velvety, mycelium white; moderate sporulation, conidial masses dull green (27D4); reverse greenish white (27B2). On OA, colonies slightly granular, sporulation dense, conidial masses greenish grey (28B2), margin entre; reverse greyish green (30C5); exudate and soluble pigment absent. On CREA, acid production strong.

Micromorphology — On MEA, conidiophores mostly biverticillate; stipes 40–70 x 2.5–3 µm, smooth, brownish yellow; metula 3–4 per branch, mostly appressed, 8–10 x 2–3 µm; phialides 3–4 per metula, acerose, 8–10(–11) x 2–3 µm; conidia mostly ellipsoidal, 2–3 x 1.5–2(–2.5) µm, smooth. Ascomata not observed.

Distinguishing characters — *Talaromyces pseudofuniculosus* can be distinguished from its closely related species, *T. funiculosus,* by showing appressed metula (divergent in *T. funiculosus*) and by the pigmentation of the conidiophores, which is brownish yellow in *T. pseudofuniculosus,* and olivaceous in *T. funiculosus.* Moreover, *T. pseudofuniculosus* has a restricted growth on CREA (2–4 mm diam; 20–30 mm diam in *T. funiculosus*) and does not produce funiculose colonies, a typical feature of *T. funiculosus* mainly on MEA and OA (Yilmaz et al. 2014)*.*

CONCLUDING REMARKS

This is the first study focused on the molecular identification of *Aspergillus* and penicillium-like fungi isolated from dung samples. We identified 38 *Aspergillus*, (9 new; *A. albodeflectus*, *A. aurantiosulcatus*, *A. calidokeveii*, *A. canariensis*, *A. coprophilus*, *A. esporlensis*, *A. fimeti-brunneus*, *A. majoricus*, and *A. verruculosus*), 41 *Penicillium* species (7 new; *P. balearicum*, *P. beceitense*, *P. caprifimosum*, *P. fimosum*, *P. ibericum*, *P. mediterraneum*, and *P. synnematicola*), 8 *Talaromyces* species (4 new; *T. catalonicus*, *T. coprophilus*, *T. gamsii*, and *T. pseudofuniculosus*), and the new genera *Penicillago* and *Pseudopenicillium* with one new species in the latter, totaling 90 species of Eurotialean fungi isolated from dung samples in Spain.

It is remarkable the isolation of species rarely identified before and, never recovered from dung, i.e. *A. austroafricanus*, *A. fructus*, and *A. viridicatenatus* (section *Nidulantes*), *A. ardalensis* (section *Flavipedes*), *A. dobrogensis* (section *Candidi*), and *A. floccosus* (section *Terrei*), in *Aspergillus*; *P. canariense* (section *Stolkia*), *P. cremeogriseum* (section *Lanata-Divaricata)*, *P. momoii* (section *Exilicaulis*), and *P. roseoviride* (section *Aspergilloides*), in *Penicillium*; and *Penicillago nodositata*.

The Balearic Islands (n = 45, 27.27 %) and Catalonia (n = 42, 25.45%) were the regions that resulted with the highest number of isolates. Both were also the regions where more new species were found (i.e., four *Aspergillus*, three *Penicillium* and one *Talaromyces* in Balearic Islands, and two new *Penicillium* and two *Talaromyces* in Catalonia), followed by Castile and Leon with three novel *Aspergillus*. The new genera *Penicillago* and *Pseudopenicillium* were recovered in Balearic Islands and Castile and Leon, associated with dung of deer and wild pig, respectively.

Althought sequence analysis of different genes have been recommended for identificacion *Aspergillus* and penicillium-like fungi, in general, our results showed that *BenA* is a good molecular marker to identify these fungi; it is easy to amplify and sequencing, useful for the delimitation of sections, as well as for identification and detection of novel taxa. Our results with *Ben*A agree with other studies where large sets of isolates of these genera have been identified (Guevara-Suarez et al. 2016, Visagie et al. 2014b, Chen et al. 2016b).

Our study highlights that herbivore dung is a substrate with a great fungal diversity and that deserves more attention in future molecular taxonomic studies.

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Figure 1. ML tree of *Aspergillus* inferred from *Ben*A including the sections recovered from dung in this work. Branch lengths are proportional to phylogenetic distance. Some of the larger branches were condensed, with the proportions showed above the paralell diagonal lines. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. Isolates corresponding to potencially new species are shown in bold. Between paranthesis, GenBank accession numbers of *Ben*A sequences. The tree is rooted to *Penicillium paradoxum* CBS 527.65. [T] = type strain.

Figure 2. ML tree of selected *Aspergillus* section Usti species inferred from the combined ITS, *Ben*A, *CaM*, and *RPB*2 loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. elongatus* NRRL 5176. The name in red is the new species described in this study. [T] = type strain.

Figure 3. ML tree of *Aspergillus* section *Flavipedes* inferred from the combined ITS, *Ben*A, *CaM*, and *RPB*2 loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. janus* NRRL 1787. The name in red is the new species described in this study. [T] = type strain.

Figure 4. ML tree of selected *Aspergillus* section *Terrei* species inferred from the combined ITS, *Ben*A, *CaM*, and *RPB*2 loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. microcysticus* NRRL 4749 and *A. ambiguus* NRRL 4737. Names in red are the new species described in this study. [T] = type strain.

Figure 5. ML tree of *Aspergillus* section *Candidi* inferred from the combined ITS, *Ben*A, *CaM*, and *RPB*2 loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. niger* NRRL 326. Names in red are the new species described in this study. [T] = type strain.

Figure 6. ML tree of selected *Aspergillus* section *Cremei* species, inferred from the combined ITS, *Ben*A, *CaM*, and *RPB2* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. inflatus* CBS 682.79. The name in red is the new species described in this study. [T] = type strain.

Figure 7. ML tree of *Penicillium* inferred from *Ben*A including the sections recovered from dung in this work. Branch lengths are proportional to phylogenetic distance. Some of the larger branches were condensed, with the proportions showed above the paralell diagonal lines. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. Isolates corresponding to new species are shown in bold. Between

2038 paranthesis, GenBank accession numbers of *Ben*A sequences. The tree is
2039 rooted to *Talaromyces flavus* CBS 310.38 and *Talaromyces duclauxii* CBS
2040 322.48. [T] = type strain.
2041
2042 **Figure 8.** ML tree of *Penicillium* section *Roquefortorum* inferred from the
2043 combined ITS, *Ben*A, *CaM*, and *RPB*2 loci. Branch lengths are proportional to
2044 phylogenetic distance. Bootstrap support values/Bayesian posterior probability
2045 scores over 70/0.95 are indicated on the nodes. The tree is rooted to *P.
2046 samsonianum* AS 3.15403 and *P. osmophilum* CBS 462.72. The names in red
2047 is the new species described in this study. [T] = type strain.
2048
2049 **Figure 9.** ML tree of *Penicillium* section *Robsamsonia* inferred from the
2050 combined ITS, *Ben*A, *CaM*, and *RPB*2 loci. Branch lengths are proportional to
2051 phylogenetic distance. Bootstrap support values/Bayesian posterior probability
2052 scores over 70/0.95 are indicated on the nodes. The tree is rooted to *P.
2053 brevicompactum* CBS 257.29. The names in red is the new species described
2054 in this study. [T] = type strain.
2055
2056 **Figure 10.** ML tree of *Penicillium* sections *Turbata* and *Paradoxa* inferred from
2057 the combined ITS, *Ben*A and *CaM* loci. Branch lengths are proportional to
2058 phylogenetic distance. Bootstrap support values/Bayesian posterior probability
2059 scores over 70/0.95 are indicated on the nodes. The names in red are the new
2060 species described in this study. [T] = type strain
2061
2062 **Figure 11.** ML tree of *Penicillium* section *Ramosa* inferred from the combined
2063 ITS, *BenA* and *CaM* loci. Branch lengths are proportional to phylogenetic
2064 distance. Bootstrap support values/Bayesian posterior probability scores over
2065 70/0.95 are indicated on the nodes. The tree is rooted to *P. brevicompactum*
2066 CBS 257.29. The name in red is the new species described in this study. [T] =
2067 type strain.
2068
2069 **Figure 12.** ML tree of *Talaromyces* inferred from *BenA* including the sections
2070 recovered from dung in this work. Branch lengths are proportional to
2071 phylogenetic distance. Some of the larger branches were condensed, with the
2072 proportions showed above the paralell diagonal lines. Bootstrap support
2073 values/Bayesian posterior probability scores over 70/0.95 are indicated on the
2074 nodes. Isolates corresponding to new species are shown in bold. Between
2075 paranthesis, GenBank accession numbers of *Ben*A sequences. The tree is
2076 rooted to *T. dendriticus* CBS 660.80 and *T. purpureus* CBS 475.71 (Section
2077 *Purpurei*). [T] = type strain.
2078
2079 **Figure 13.** ML tree of *Talaromyces* section *Talaromyces* inferred from the
2080 combined ITS, *BenA*, *CaM*, and *RPB*2 loci. Branch lengths are proportional to
2081 phylogenetic distance. Bootstrap support values/Bayesian posterior probability
2082 scores over 70/0.95 are indicated on the nodes. The tree is rooted to *T.
2083 dendriticus* CBS 660.80 and *T. purpureus* CBS 475.71 (Section *Purpurei*). The
2084 names in red are the new species described in this study. [T] = type strain.
2085
2086 **Figure 14.** ML tree of selected of *Talaromyces* section *Trachyspermi* inferred
2087 from the combined ITS, *BenA* and *CaM* loci. Branch lengths are proportional to

phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *T. purpureogenus* CBS 286.36 (Section *Talaromyces*). The name in red are the new species described in this study. [T] = type strain.

Figure 15. ML tree of selected members of *Aspergillaceae* family inferred from *BenA*. Branch lengths are proportional to phylogenetic distance. Some of the larger branches were condensed, with the proportions showed above the paralell diagonal lines. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. Isolates corresponding to new species are shown in bold. Between paranthesis, GenBank accession numbers of *Ben*A sequences. The tree is rooted to *T. flavus* CBS 310.38 and *T. purpureogenus* CBS 286.36. [T] = type strain.

Figure 16. ML tree of members of *Aspergillaceae* family inferred from the combined ITS, *BenA*, and *RPB*2 loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *Talromyces flavus* CBS 310.38 and *Talaromyces purpureogenus* CBS 286.36. The names in red are the new species described in this study. [T] = type strain.

Figure 17. Morphological characters of *Aspergillus albodeflectus* (FMR 15175[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–F. Conidiophores. G. Conidia. Scale bars = 10 µm.

Figure 18. Morphological characters of *Aspergillus aurantiosulcatus* (FMR 15182[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Conidial heads in detail. C–F. Conidiophores. G, H. Conidia. Scale bars = 10 µm.

Figure 19. Morphological characters of *Aspergillus calidokeveii* (FMR 15225[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–E. Conidiophores. F. Conidia. G. Hülle cells. Scale bars: B = 50 µm, C–G = 10 µm.

Figure 20. Morphological characters of *Aspergillus canariensis* (FMR 15736[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–F. Conidiophores. G. Conidia. Scale bars = 10 µm.

Figure 21. Morphological characters of *Aspergillus coprophilus* (FMR 15224[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Sclerotia on CYA at 25 °C after 7 d. C–E. Conidiophores. F. Conidia. G. Diminutive vesicle. Scale bars = 10 µm.

Figure 22. Morphological characters of *Aspergillus esporlensis* (FMR 14605[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–E. Conidiophores. F, G. Conidia. Scale bars = 10 µm.

Figure 23. Morphological characters of *Aspergillus fimeti-brunneus* (FMR 15228[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–D. Conidiophores. E, F. Accessory conidia. G. Conidia. Scale bars = 10 µm.

Figure 24. Morphological characters of *Aspergillus majoricus* (FMR 15181[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–E. Conidiophores. F. Conidia. Scale bars = 10 µm.

Figure 25. Morphological characters of *Aspergillus verruculosus* (FMR 15877[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Sclerotia on CYA at 25 ºC after 7 d. C, E–G. Conidiophores. D. Conidia. Scale bars = 10 µm.

Figure 26. Morphological characters of *Penicillium balearicum* (FMR 15191[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Colony texture on YES at 25 ºC after 1-week incubation. C–E. Conidiophores. F. Conidia. Scale bars = 10 µm.

Figure 27. Morphological characters of *Penicillium beceitense* (FMR 15038[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–F. Conidiophores. G. Conidia. Scale bars = 10 µm.

Figure 28. Morphological characters of *Penicillium caprifimosum* (FMR 15041[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–F. Conidiophores. G. Conidia. Scale bars = 10 µm.

Figure 29. Morphological characters of *Penicillium fimosum* (FMR 15104[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Conidiophores. C. Conidia. D-F. Conidiophores. Scale bars = 10 µm.

Figure 30. Morphological characters of *Penicillium ibericum* (FMR 15040[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Colony texture on CYA at 25 ºC after 1-week incubation. C. Conidia. D–G. Conidiophores. H. Scale bars = 10 µm.

Figure 31. Morphological characters of *Penicillium mediterraneum* (FMR 15188[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–G. Conidiophores. H. Conidia. Scale bars = 10 µm.

Figure 32. Morphological characters of *Penicillium synnematicola* (FMR 15192[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA;

(bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Colony texture on CYA at 25 ºC after 1-week incubation. C. Colony texture on OA at 25 ºC after 1-week incubation. D–G. Conidiophores. H. Conidia. Scale bars = 10 µm.

Figure 33. Morphological characters of *Penicillago nodositata* (FMR 15296). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–F. Conidiophores. G. Conidia. Scale bars = 10 µm.

Figure 34. Morphological characters of *Pseudopenicillium coprobium* (FMR 15299[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA reverse; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Colony texture on OA at 25 ºC after 1-week incubation. C–H. Conidiophores. I. Conidia. Scale bars = 10 µm.

Figure 35. Morphological characters of *Talaromyces catalonicus* (FMR 16441[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA reverse; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–D. Conidiophores. E. Conidia. Scales bars C–D= 100 µm, E–I = 10 µm.

Figure 36. Morphological characters of *Talaromyces coprohilus* (FMR 15199[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA reverse; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B. Detail of the colony texture with ascomata on MEA after 2 wk incubation. C. Ascoma. D, E Part of a ascoma and peridial hyphae. F. Asci. G. Ascospores. H, I. Conidiophores. J. Conidia. Scales bars C–D= 100 µm, E–I = 10 µm.

Figure 37. Morphological characters of *Talaromyces gamsii* (FMR 15303[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA reverse; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–E. Conidiophores. F. Conidia. Scale bars = 10 µm.

Figure 38. Morphological characters of *Talaromyces pseunofuniculosus* (FMR 15307[T]). A. Colonies from left to right (top row) CYA, MEA, DG18 and OA reverse; (bottom row) CYA reverse, MEA reverse, YES, and CREA. B–E. Conidiophores. F. Conidia. Scale bars = 10 µm.

SUPPLEMENTARY MATERIAL

Figure S1. ML tree of selected *Aspergillus* section *Terrei* species inferred from *Ben*A, including the isolates belonging to this section recovered in this work. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. microcysticus* NRRL 4749 and *A. ambiguus* NRRL 4737. Names in bold are the new species described in this study. ^{T} = type strain.

Figure S2. ML tree of selected *Aspergillus* section *Cremei* species, inferred from ITS. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. inflatus* CBS 682.79. The name in red is the new species described in this study. ^{T} = type strain.

Figure S3. ML tree of selected *Aspergillus* section *Cremei* species, inferred from *Ben*A. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. inflatus* CBS 682.79. The name in red is the new species described in this study. ^{T} = type strain.

Figure S4. ML tree of selected *Aspergillus* section *Cremei* species, inferred from *CaM*. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. inflatus* CBS 682.79. The name in red is the new species described in this study. ^{T} = type strain.

Figure S5. ML tree of selected *Aspergillus* section *Cremei* species, inferred from *RPB*2. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. inflatus* CBS 682.79. The name in red is the new species described in this study. ^{T} = type strain.

Figure S6. ML tree of selected *Aspergillus* section *Cremei* species, inferred from the combined ITS, *Ben*A, *CaM*, and *RPB2* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *A. inflatus* CBS 682.79. The name in red is the new species described in this study. ^{T} = type strain.

Figure S7. ML tree of selected *Penicillium* section *Roquefortorum* species, inferred from *Ben*A. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *P. sansonianum* AS 3.5403 and *P. osmophilum* CBS 462.72. The name in red is the new species described in this study. ^{T} = type strain.

Figure S8. ML tree of selected *Penicillium* section *Robsamsonia* species, inferred from *Ben*A. Branch lengths are proportional to phylogenetic distance.

Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *P. brevicompactum* CBS 257.29. The name in red is the new species described in this study. ^{T} = type strain.

Figure S9. ML tree of selected *Penicillium* section *Paradoxa* species, inferred from *Ben*A. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to species of section *Tubata*. The names in red are the new species described in this study. ^{T} = type strain.

Figure S10. ML tree of selected *Penicillium* section *Exilicaulis* species, inferred from *Ben*A, including the isolates belonging to this section recovered in this work. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *P. trzebinskii* CBS 382.48. Names in bold are the new species described in this study. ^{T} = type strain.

Figure S11. ML tree of selected *Talaromyces* section *Talaromyces* species, inferred from *Ben*A, including the isolates belonging to this section recovered in this work. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores over 70/0.95 are indicated on the nodes. The tree is rooted to *T. dendriticus* CBS 660.80 and *T. purpureus* CBS 475.71. The names in red are the new species described in this study. ^{T} = type strain.

Table 1. Isolates of *Aspergillus*, *Penicillium*, *Talaromyces*, and related genera included in the study and their GenBank/EMBL accession numbers.

Genus/Species*	Section	Collection number	Substrate and Origin	GenBank/EMBL accession number			
				ITS	BenA	CaM	RPB2
A. affinis	Circumdati	FMR 15602	Dung, Galicia		LT798961		
A. alabamensis	Terrei	FMR 15731	Dung, Canary Islands		LT798985		
A. alabamensis	Terrei	FMR 15412	Dung, Galicia		LT798984		
A. albodeflectus	Flavipedes	FMR 15175ᵀ = CBS 142665	Dung, Balearic Islands	LT798909	LT798936	LT798937	LT798938
A. ardalensis	Flavipedes	FMR 15057	Dung, Catalonia		LT798966		
A. ardalensis	Flavipedes	FMR 15058	Dung, Catalonia		LT798967		
A. aurantiosulcatus	Terrei	FMR 15182ᵀ = CBS 142981	Dung, Balearic Islands	LT798912	LT798945	LT798946	LT798947
A. aureolatus	Nidulantes	FMR 15442	Dung, Galicia		LT798994		
A. austroafricanus	Nidulantes	FMR 15174	Dung, Balearic Islands		LT798995		
A. calidokeveii	Usti	FMR 15225ᵀ = CBS 142666	Dung, Castile and Leon	LT798914	LT798951	LT798952	LT798953
A. calidoustus	Usti	FMR 15609	Dung, Castile and Leon		LT798990		
A. canariensis	Candidi	FMR 15733 = CBS 142983	Dung, Canary Islands	LT798905	LT798924	LT798925	LT798926
A. canariensis	Candidi	FMR 15736ᵀ = CBS 142982	Dung, Canary Islands	LT798906	LT798927	LT798928	LT798929
A. candidus	Candidi	FMR 15218	Dung, Balearic Islands		LT798960		
A. candidus	Candidi	FMR 15172	Dung, Catalonia		LT798959		
A. chevalieri	Aspergillus	FMR 15878	Dung, Extremadura		LT798954		
A. citrinoterreus	Terrei	FMR 15876	Dung, Canary Islands		LT798989		
A. clavatus	Clavati	FMR 15610	Dung, Castile and Leon		LT798963		
A. clavatus	Clavati	FMR 15611	Dung, Castile and Leon		LT798964		
A. coprophilus	Candidi	FMR 15224ᵀ = CBS 142984	Dung, Castile and Leon	LT798902	LT798915	LT798916	LT798917
A. coprophilus	Candidi	FMR 15226 = CBS 142985	Dung, Castile and Leon	LT798903	LT798918	LT798919	LT798920
A. dobrogensis	Candidi	FMR 15444 = CBS 142752	Dung, Galicia	LT798904	LT798921	LT798922	LT798923
A. esporlensis	Cremei	FMR 14605ᵀ = CBS 142750	Soil, Balearic Islands	LT798908	LT798933	LT798934	LT798935
A. europaeus	Cremei	FMR 15216	Dung, Balearic Islands		LT798965		
A. fimeti-brunneus	Terrei	FMR 15228ᵀ = CBS 142751	Dung, Andalusia	LT798913	LT798948	LT798949	LT798950
A. floccosus	Terrei	FMR 15061	Dung, Catalonia		LT798986		
A. fructus	Nidulantes	FMR 15728	Dung, Canary Islands		LT798996		
A. hortai	Terrei	FMR 15227	Dung, Andalusia		LT798987		
A. iizukae	Flavipedes	FMR 15606	Dung, Castile and Leon		LT798969		
A. iizukae	Flavipedes	FMR 15051	Dung, Catalonia		LT798968		

Species	Section	Strain	Origin				
A. insuetus	*Usti*	FMR 15322	Dung, Andalusia		LT798991		
A. majoricus	*Terrei*	FMR 15181[T] = CBS 142986	Dung, Balearic Islands	LT798910	LT798939	LT798940	LT798941
A. majoricus	*Terrei*	FMR 15217 = CBS 142987	Dung, Balearic Islands	LT798911	LT798942	LT798943	LT798944
A. micronesiensis	*Flavipedes*	FMR 15214	Dung, Balearic Islands		LT798970		
A. micronesiensis	*Flavipedes*	FMR 15737	Dung, Canary Islands		LT798971		
A. montevidensis	*Aspergillus*	FMR 15608	Dung, Castile and Leon		LT798956		
A. montevidensis	*Aspergillus*	FMR 15738	Dung, Extremadura		LT798955		
A. nidulans	*Nidulantes*	FMR 15229	Dung, Andalusia		LT798981		
A. nidulans	*Nidulantes*	FMR 15219	Dung, Balearic Islands		LT798980		
A. nidulans	*Nidulantes*	FMR 15377	Dung, Balearic Islands		LT798982		
A. pseudodeflectus	*Usti*	FMR 15376	Dung, Andalusia		LT798992		
A. pseudodeflectus	*Usti*	FMR 15727	Dung, Canary Islands		LT798993		
A. pseudoglaucus	*Aspergillus*	FMR 15607	Dung, Castile and Leon		LT798957		
A. pseudoglaucus	*Aspergillus*	FMR 15612	Dung, Castile and Leon		LT798958		
A. rugulosus	*Nidulantes*	FMR 15173	Dung, Catalonia		LT798983		
A. spelaeus	*Flavipedes*	FMR 15176	Dung, Balearic Islands		LT798972		
A. spelaeus	*Flavipedes*	FMR 15178	Dung, Balearic Islands		LT798973		
A. spelaeus	*Flavipedes*	FMR 15180	Dung, Balearic Islands		LT798974		
A. spelaeus	*Flavipedes*	FMR 15215	Dung, Balearic Islands		LT798975		
A. spelaeus	*Flavipedes*	FMR 15223	Dung, Balearic Islands		LT798976		
A. subramanianii	*Circumdati*	FMR 15729	Dung, Canary Islands		LT798962		
A. sydowii	*Nidulantes*	FMR 15880	Dung, Canary Islands		LT798999		
A. sydowii	*Nidulantes*	FMR 15603	Dung, Castile and Leon		LT798997		
A. sydowii	*Nidulantes*	FMR 15618	Dung, Galicia		LT798998		
A. templicola	*Flavipedes*	FMR 15179	Dung, Balearic Islands		LT798979		
A. templicola	*Flavipedes*	FMR 15055	Dung, Catalonia		LT798977		
A. templicola	*Flavipedes*	FMR 15059	Dung, Catalonia		LT798978		
A. terreus	*Terrei*	FMR 15054	Dung, Catalonia		LT798988		
A. verruculosus	*Candidi*	FMR 15877[T] = CBS 142667	Dung, Canary Islands	LT798907	LT798930	LT798931	
A. viridicatenatus	*Nidulantes*	FMR 15446	Dung, Galicia		LT799000		
P. arabicum	*Exilicaulis*	FMR 15298	Dung, Castile and Leon		LT898226		
P. arabicum	*Exilicaulis*	FMR 15095	Dung, Catalonia		LT898225		
P. atramentosum	*Paradoxa*	FMR 15309	Dung, Castile and Leon		LT898224		
P. atramentosum	*Paradoxa*	FMR 15046	Dung, Catalonia		LT898221		
P. atramentosum	*Paradoxa*	FMR 15092	Dung, Catalonia		LT898222		
P. atramentosum	*Paradoxa*	FMR 15102	Dung, Catalonia		LT898223		

Species	Section	Strain	Locality				
P. balearicum	Paradoxa	FMR 15191^T = CBS 143044	Dung, Balearic Islands	LT899762	LT898227	LT899758	LT899760
P. balearicum	Paradoxa	FMR 15196	Dung, Balearic Islands	LT899763	LT898228	LT899759	LT899761
P. beceitense	Ramosa	FMR 15038^T = CBS 142989	Dung, Catalonia	LT899780	LT898229	LT899764	LT899798
P. biforme	Fasciculata	FMR 15312	Dung, Castile and Leon		LT898230		
P. biforme	Fasciculata	FMR 15313	Dung, Castile and Leon		LT898231		
P. brasilianum	Lanata-Diavricata	FMR 15483	Dung, Galicia		LT898232		
P. brevicompactum	Brevicompacta	FMR 15105	Dung, Catalonia		LT898233		
P. brevistipitatum	Robsamsonia	FMR 15103	Dung, Catalonia		LT898234		
P. burguense	Exilicaulis	FMR 15493	Dung, Galicia		LT898235		
P. canariense	Stolkia	FMR 15838	Dung, Canary Islands		LT898236		
P. canescens	Canescentia	FMR 15028	Dung, Catalonia		LT898237		
P. caprifimosum	Turbata	FMR 15041^T = CBS 142990	Dung, Catalonia	LT899781	LT898238	LT899765	LT899799
P. chrysogenum	Chrysogena	FMR 15100	Dung, Catalonia		LT898244		
P. cinereoatrum	Exilicaulis	FMR 15033	Dung, Catalonia		LT898284		
P. citrinum	Citrina	FMR 15646	Dung, Castile and Leon		LT898242		
P. citrinum	Citrina	FMR 15647	Dung, Castile and Leon		LT898243		
P. citrinum	Citrina	FMR 15094	Dung, Catalonia		LT898239		
P. citrinum	Citrina	FMR 15486	Dung, Galicia		LT898240		
P. citrinum	Citrina	FMR 15520	Dung, Galicia		LT898241		
P. concentricum	Robsamsonia	FMR 15195	Dung, Balearic Islands		LT898245		
P. concentricum	Robsamsonia	FMR 15840	Dung, Castile and Leon		LT898246		
P. coprobium	Robsamsonia	FMR 15201	Dung, Balearic Islands		LT898247		
P. coprobium	Robsamsonia	FMR 15311	Dung, Castile and Leon		LT898248		
P. coprophilum	Robsamsonia	FMR 15187	Dung, Balearic Islands		LT898249		
P. cremeogriseum	Lanata-Diavricata	FMR 15487	Dung, Galicia		LT898250		
P. cremeogriseum	Lanata-Diavricata	FMR 15488	Dung, Galicia		LT898251		
P. crustosum	Fasciculata	FMR 15185	Dung, Balearic Islands		LT898259		
P. crustosum	Fasciculata	FMR 15186	Dung, Balearic Islands		LT898260		
P. crustosum	Fasciculata	FMR 15189	Dung, Balearic Islands		LT898261		
P. crustosum	Fasciculata	FMR 15194	Dung, Balearic Islands		LT898262		
P. crustosum	Fasciculata	FMR 15197	Dung, Balearic Islands		LT898263		
P. crustosum	Fasciculata	FMR 15200	Dung, Balearic Islands		LT898264		
P. crustosum	Fasciculata	FMR 15213	Dung, Balearic Islands		LT898265		
P. crustosum	Fasciculata	FMR 15034	Dung, Catalonia		LT898252		
P. crustosum	Fasciculata	FMR 15036	Dung, Catalonia		LT898253		

Species	Section	Strain	Origin			
P. crustosum	*Fasciculata*	FMR 15037	Dung, Catalonia			LT898254
P. crustosum	*Fasciculata*	FMR 15042	Dung, Catalonia			LT898255
P. crustosum	*Fasciculata*	FMR 15043	Dung, Catalonia			LT898256
P. crustosum	*Fasciculata*	FMR 15045	Dung, Catalonia			LT898257
P. crustosum	*Fasciculata*	FMR 15098	Dung, Catalonia			LT898258
P. crustosum	*Fasciculata*	FMR 15494	Dung, Galicia			LT898266
P. cvjetkovicii	*Cinnamopurpurea*	FMR 15310	Dung, Castile and Leon			LT898267
P. expansum	*Penicillium*	FMR 15097	Dung, Catalonia			LT898268
P. expansum	*Penicillium*	FMR 15484	Dung, Galicia			LT898269
P. fimosum	*Paradoxa*	FMR 15104^T = CBS 142991	Dung, Catalonia	LT970837	LT970836	LT898273
P. flavigenum	*Chrysogena*	FMR 15096	Dung, Catalonia			LT898270
P. frequentans	*Aspergilloides*	FMR 15193	Dung, Balearic Islands			LT898271
P. frequentans	*Aspergilloides*	FMR 15212	Dung, Balearic Islands			LT898272
P. glabrum	*Aspergilloides*	FMR 15184	Dung, Balearic Islands			LT898274
P. glabrum	*Aspergilloides*	FMR 15190	Dung, Balearic Islands			LT898275
P. glabrum	*Aspergilloides*	FMR 15209	Dung, Balearic Islands			LT898276
P. griseofulvum	*Robsamsonia*	FMR 15203	Dung, Balearic Islands			LT898280
P. griseofulvum	*Robsamsonia*	FMR 15204	Dung, Balearic Islands			LT898281
P. griseofulvum	*Robsamsonia*	FMR 15207	Dung, Balearic Islands			LT898282
P. griseofulvum	*Robsamsonia*	FMR 15314	Dung, Castile and Leon			LT898283
P. griseofulvum	*Robsamsonia*	FMR 15029	Dung, Catalonia			LT898277
P. griseofulvum	*Robsamsonia*	FMR 15030	Dung, Catalonia			LT898278
P. griseofulvum	*Robsamsonia*	FMR 15093	Dung, Catalonia			LT898279
P. ibericum	*Paradoxa*	FMR 15040^T = CBS142992	Dung, Catalonia	LT899800	LT899782	LT898285
P. ibericum	*Paradoxa*	FMR 15107	Soil, Galicia	LT899801	LT899783	LT898286
P. lilacinoechinulatum	*Sclerotiora*	FMR 15492	Dung, Galicia			LT898287
P. magnielliptisporum	*Paradoxa*	FMR 15044	Dung, Catalonia			LT898288
P. mediterraneum	*Roquefortorum*	FMR 15188^T = CBS 142754	Dung, Balearic Islands	LT899802	LT899784	LT898291
P. mediterraneum	*Roquefortorum*	FMR 15031 = CBS 142755	Dung, Catalonia	LT899803	LT899785	LT898289
P. mediterraneum	*Roquefortorum*	FMR 15032	Dung, Catalonia	LT899804	LT899786	LT898290
P. momoii	*Exilicaulis*	FMR 15208	Dung, Balearic Islands			LT898292
P. murcianum	*Canescentia*	FMR 15304	Dung, Andalusia			LT898293
P. murcianum	*Canescentia*	FMR 15305	Dung, Andalusia			LT898294
P. murcianum	*Canescentia*	FMR 15308	Dung, Andalusia			LT898295
P. murcianum	*Canescentia*	FMR 15491	Dung, Galicia			LT898296

Species	Section	Strain no.	Substrate, locality	GenBank	GenBank	GenBank	GenBank
P. murcianum	*Canescentia*	FMR 15845	Dung, Galicia		LT898297		
P. polonicum	*Fasciculata*	FMR 15099	Dung, Catalonia		LT898298		
P. radiolubatum	*Canescentia*	FMR 15485	Dung, Canary Islands		LT898299		
P. roseoviride	*Aspergilloides*	FMR 15645	Dung, Castile and Leon		LT898300		
P. rubefaciens	*Exilicaulis*	FMR 15202	Dung, Balearic Islands		LT898301		
P. rubefaciens	*Exilicaulis*	FMR 15297	Dung, Castile and Leon		LT898302		
P. rudallense	*Aspergilloides*	FMR 15843	Dung, Canary Islands		LT898303		
P. sizovae	*Citrina*	FMR 15300	Dung, Castile and Leon		LT898304		
P. sizovae	*Citrina*	FMR 15521	Dung, Galicia		LT898305		
P. synnematicola	*Robsamsonia*	FMR 15192ᵀ = CBS 142669	Dung, Balearic Islands	LT898167	LT898172	LT898137	LT898142
P. synnematicola	*Robsamsonia*	FMR 15210	Dung, Balearic Islands	LT898168	LT898173	LT898138	LT898143
P. synnematicola	*Robsamsonia*	FMR 15211	Dung, Balearic Islands	LT898169	LT898174	LT898139	LT898144
P. synnematicola	*Robsamsonia*	FMR 16481 = CBS 143045	Soil, Catalonia	LT898170	LT898175	LT898140	LT898145
P. synnematicola	*Robsamsonia*	FMR 16491 = CBS 143046	Dung, Catalonia	LT898171	LT898176	LT898141	LT898146
Penicillium sp.	*Exilicaulis*	FMR 15841	Dung, Castile and Leon		LT898311		
Pgo. nodositata	-	FMR 15296 = CBS 142988	Dung, Balearic Islands	LT899787	LT898312	LT899771	LT899805
Pgo. nodositata	-	FMR 16442	Dung, Extremadura	LT899788	LT898313	LT899772	LT899806
Pse. coprobium	-	FMR 15299ᵀ = CBS 142670	Dung, Castile and Leon	LT899789	LT898315		LT899807
Pse. giganteum	-	FMR 14718	Soil, unknown	LT899790	LT898314		LT899808
T. angelicus	*Talaromyces*	FMR 15489	Dung, Galicia	LT899791	LT898316	LT899773	LT899809
T. angelicus	*Talaromyces*	FMR 15490	Dung, Galicia	LT899792	LT898317	LT899774	LT899810
T. catalonicus	*Trachyspermi*	FMR 16441ᵀ = CBS 143039	Dung, Catalonia	LT899793	LT898318	LT899775	LT899811
T. coprophilus	*Talaromyces*	FMR 15199ᵀ = CBS 142756	Dung, Balearic Islands	LT899794	LT898319	LT899776	LT899812
T. gamsii	*Talaromyces*	FMR 15303ᵀ = CBS 143040	Soil, Andalusia	LT899795	LT898320	LT899777	LT899813
T. muoii	*Talaromyces*	FMR 15496	Dung, Galicia		LT898321		
T. pseudofuniculosus	*Talaromyces*	FMR 15307ᵀ = CBS 143041	Dung, Andalusia	LT899796	LT898323	LT899778	LT899814
T. pseudofuniculosus	*Talaromyces*	FMR 15035	Dung, Catalonia	LT899797	LT898322	LT899779	LT899815
T. ruber	*Talaromyces*	FMR 15839	Dung, Castile and Leon		LT898324		
T. sayulitensis	*Talaromyces*	FMR 15842	Dung, Canary Islands		LT898325		

* A. = *Aspergillus*; P. = *Penicillium*; Pgo. = *Penicillago*; Pse. = *Pseudopenicillium*; T. = *Talaromyces*; new taxa proposed in this study are in **bold**; ᵀ = type strain.

Table 2. Overview and details used for phylogenetic analyses of *Aspergillus*, *Penicillium*, *Talaromyces*, and the related genera.

		Aspergillus					Penicillium				Talaromyces		Miscellaneous Aspergillaceae
	Section	Candidi	Cremei	Flavipedes	Terrei	Usti	Turbata and Paradoxa	Ramosa	Robsamsonia	Roquefortorum	Talaromyces	Trachyspermi	
ITS dataset	Length (bp)	490	532	556	564	481	504	485	428	506	361	473	440
	Pvar	49	58	67	54	50	29	47	207	24	73	110	124
	Pi	4	27	32	43	19	18	16	18	13	42	80	104
	Model*	GTR+I	GTR+I	GTR+I	GTR+I+G	GTR+I	K80+G	GTR+I	K80+G	TPM2uf+I	TrN+I+G	TIM2+I+G	TrN+I+G
BenA dataset	Length (bp)	473	474	560	531	440	412	387	352	409	402	381	401
	Pvar	192	195	241	216	151	151	112	122	68	212	160	223
	Pi	58	106	140	159	75	100	64	67	29	166	98	187
	Model*	GTR+G	SYM+G	HKY+I+G	HKY+I	K80+I	TIM2ef+G	TIM2ef+G	GTR+G	TIM1ef	HKY+I+G	TPM3uf+G	HKY+I+G
CaM dataset	Length (bp)	554	500	560	560	465	492	544	486	501	480	474	-
	Pvar	134	211	257	223	214	195	223	164	50	246	233	-
	Pi	34	121	164	144	122	88	92	96	8	212	185	-
	Model*	SYM+G	SYM+I	SYM+G	GTR+I	GTR+I	TIM2+G	TIM2+G	GTR+I	TPM1+G	HKY+I+G	TPM1+I+G	-
RPB2 dataset	Length (bp)	900	1014	965	1013	904	915	-	804	915	-	-	956
	Pvar	221	290	265	226	244	239	-	243	106	-	-	463
	Pi	92	173	165	175	142	159	-	127	55	-	-	375
	Model*	GTR+I	SYM+G	SYM+I+G	SYM+G	SYM+I+G	TrN+I+G	-	GTR+I	TIM3ef+I	-	-	TrN+I+G
Concatenated dataset	Length (bp)	2417	2520	2641	2668	2290	2323	1416	2068	2331	1243	1329	1797
	Pvar	596	754	830	719	659	614	382	574	248	531	503	810
	Pi	188	427	501	521	358	365	172	303	105	420	336	666

Pvar = variable sites; Pi = phylogenetic informative sites; * = substitution model for Bayesian inference
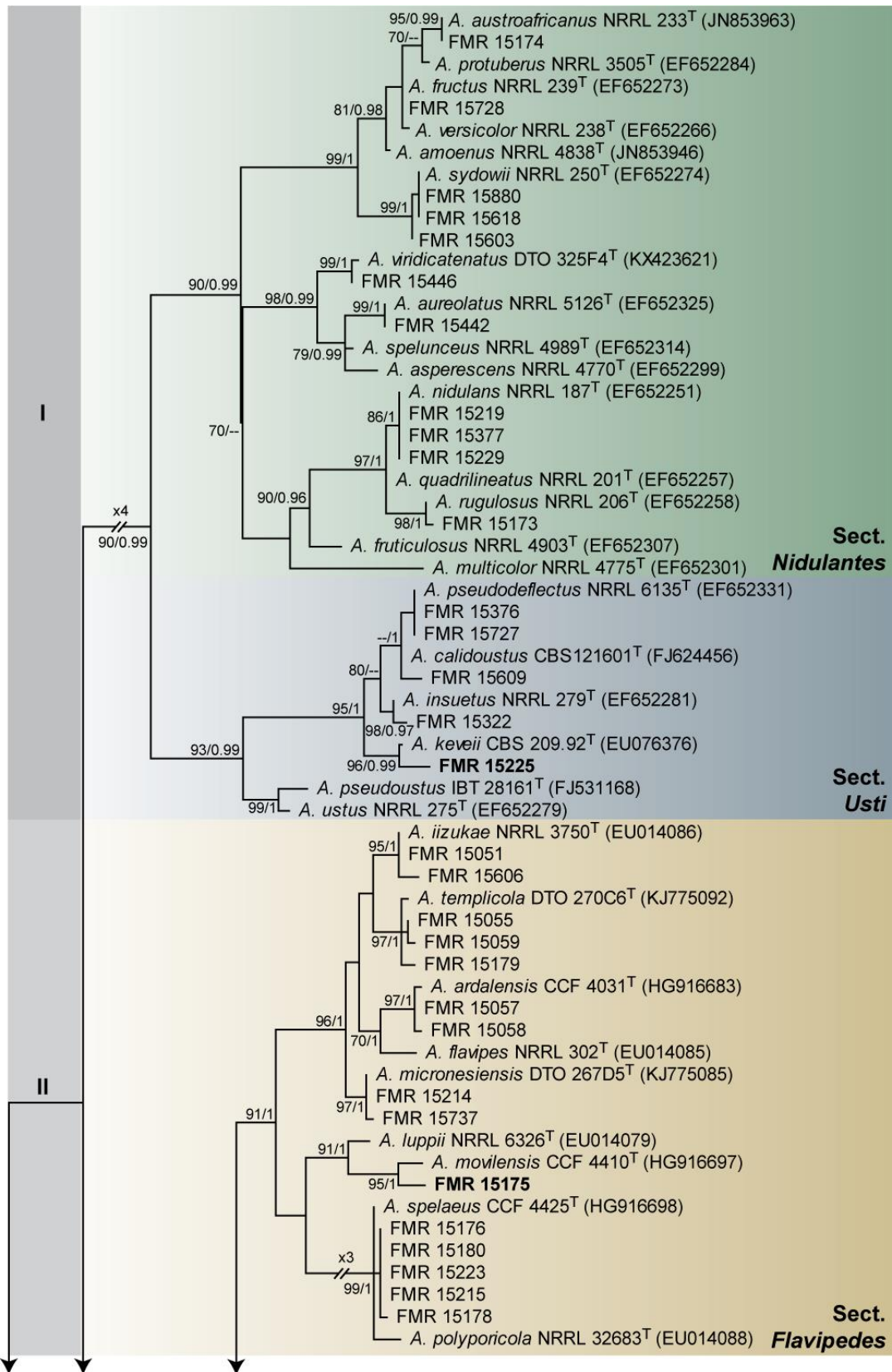
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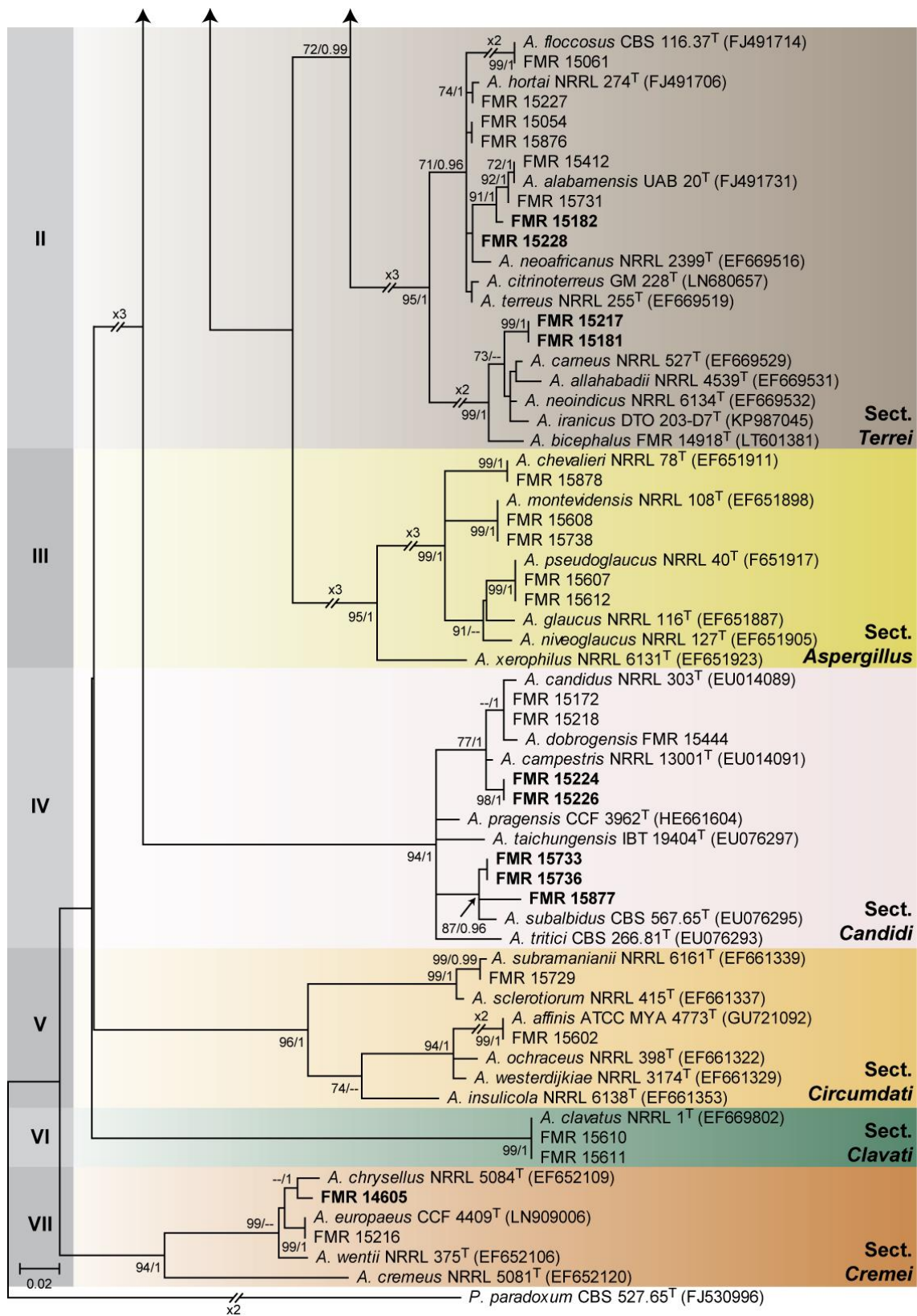
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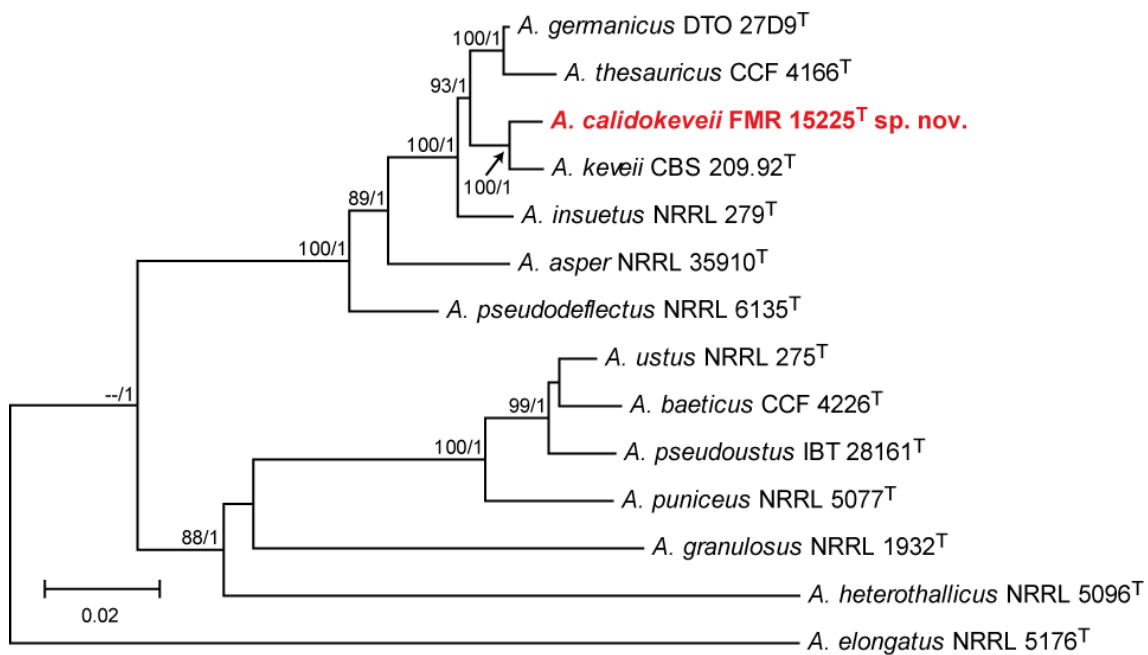
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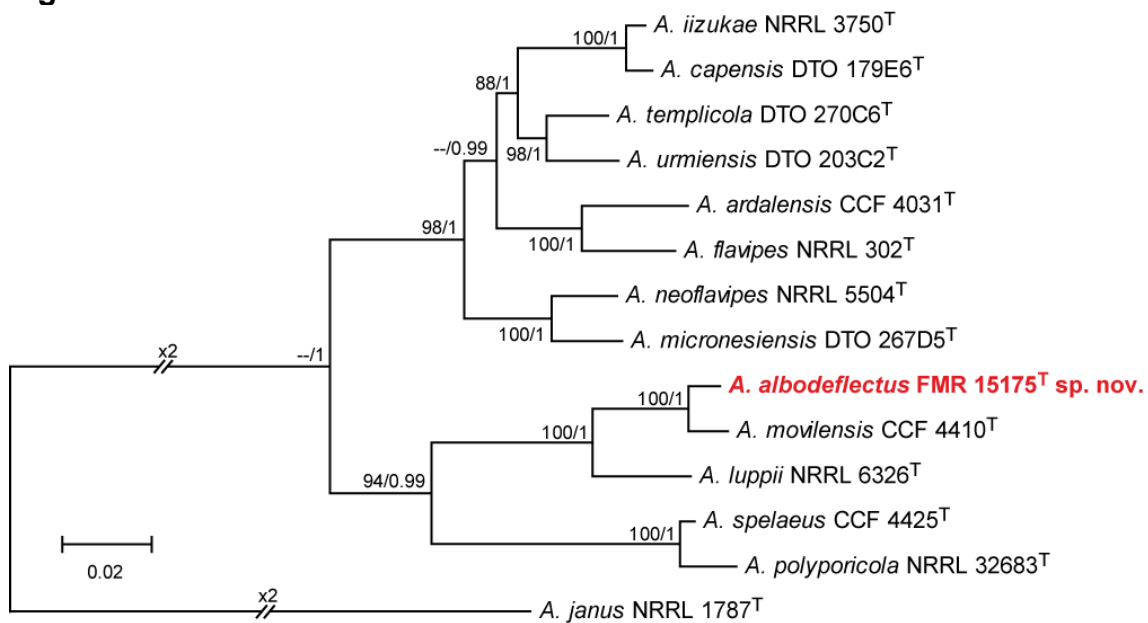
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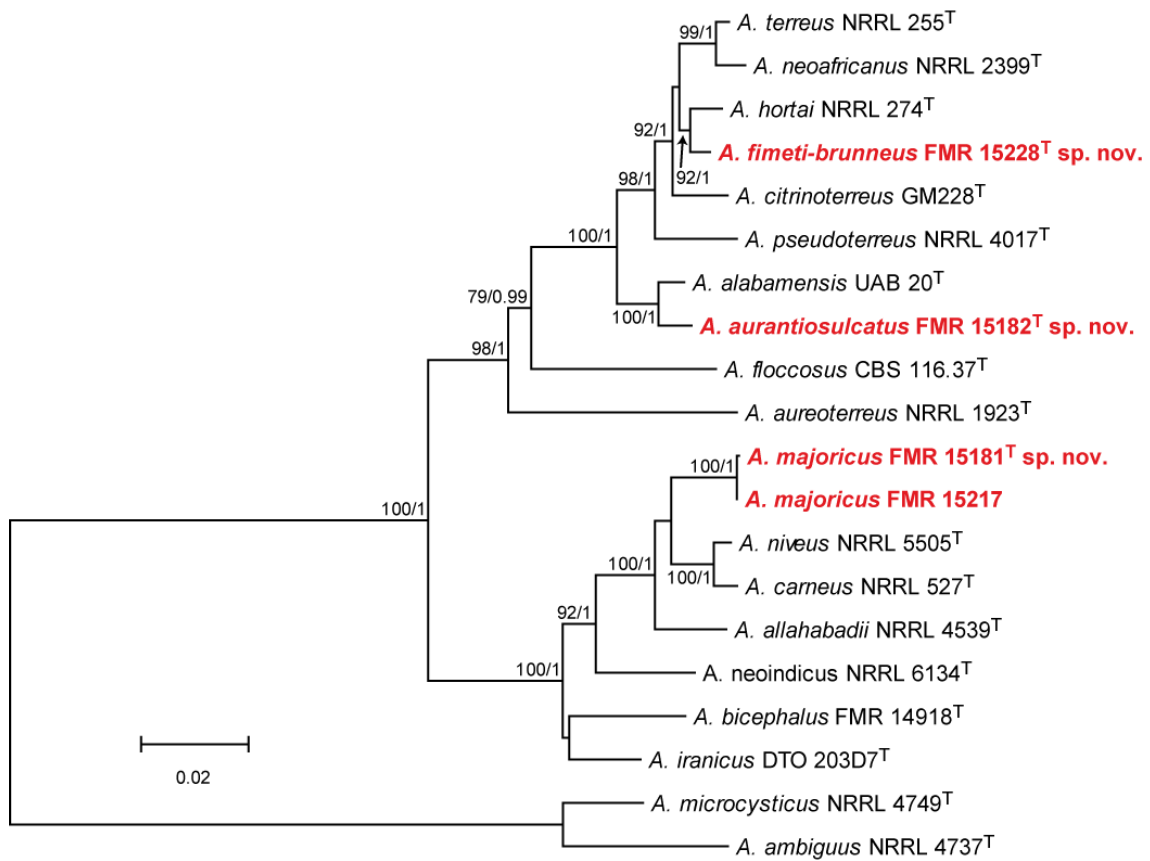
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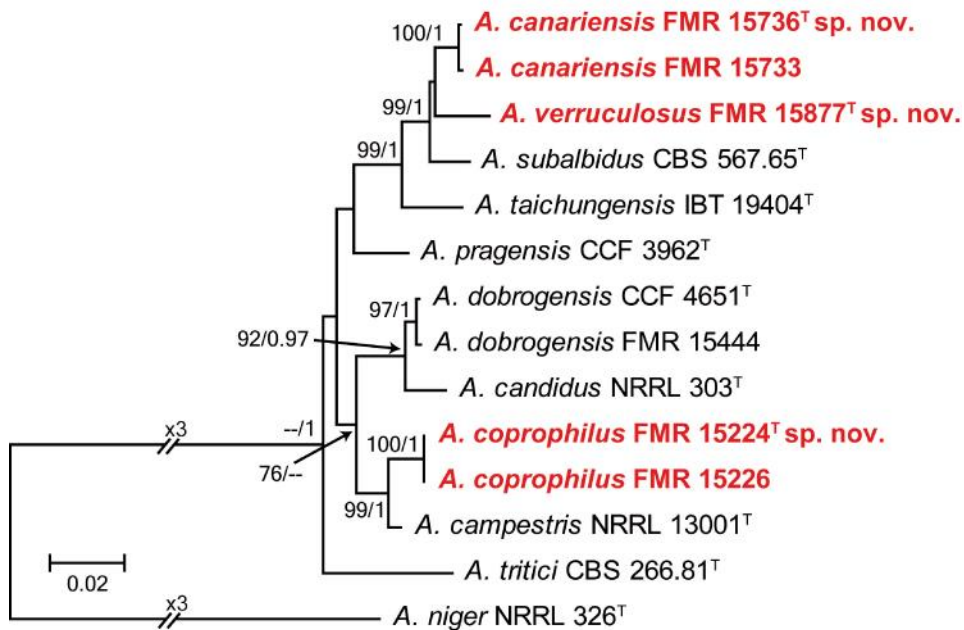
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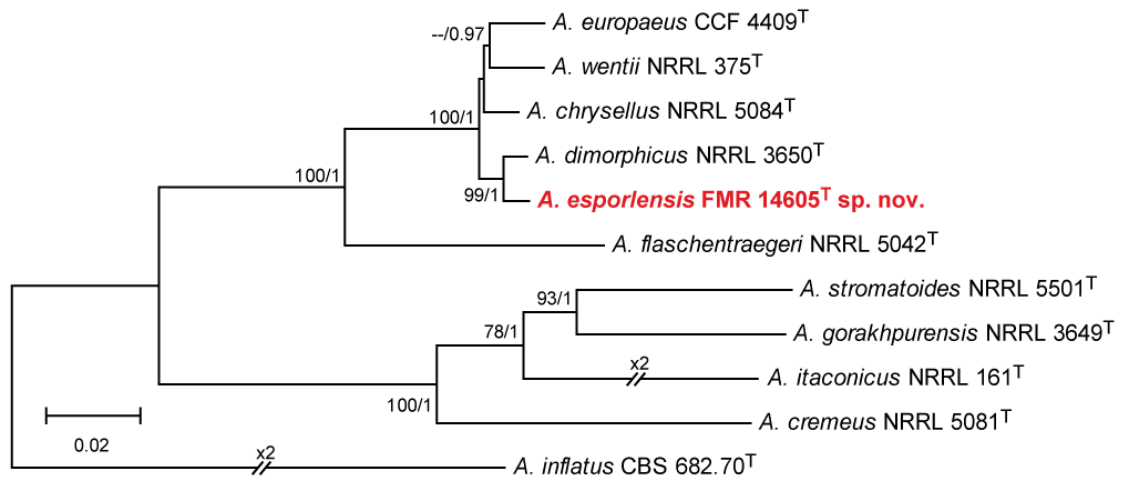
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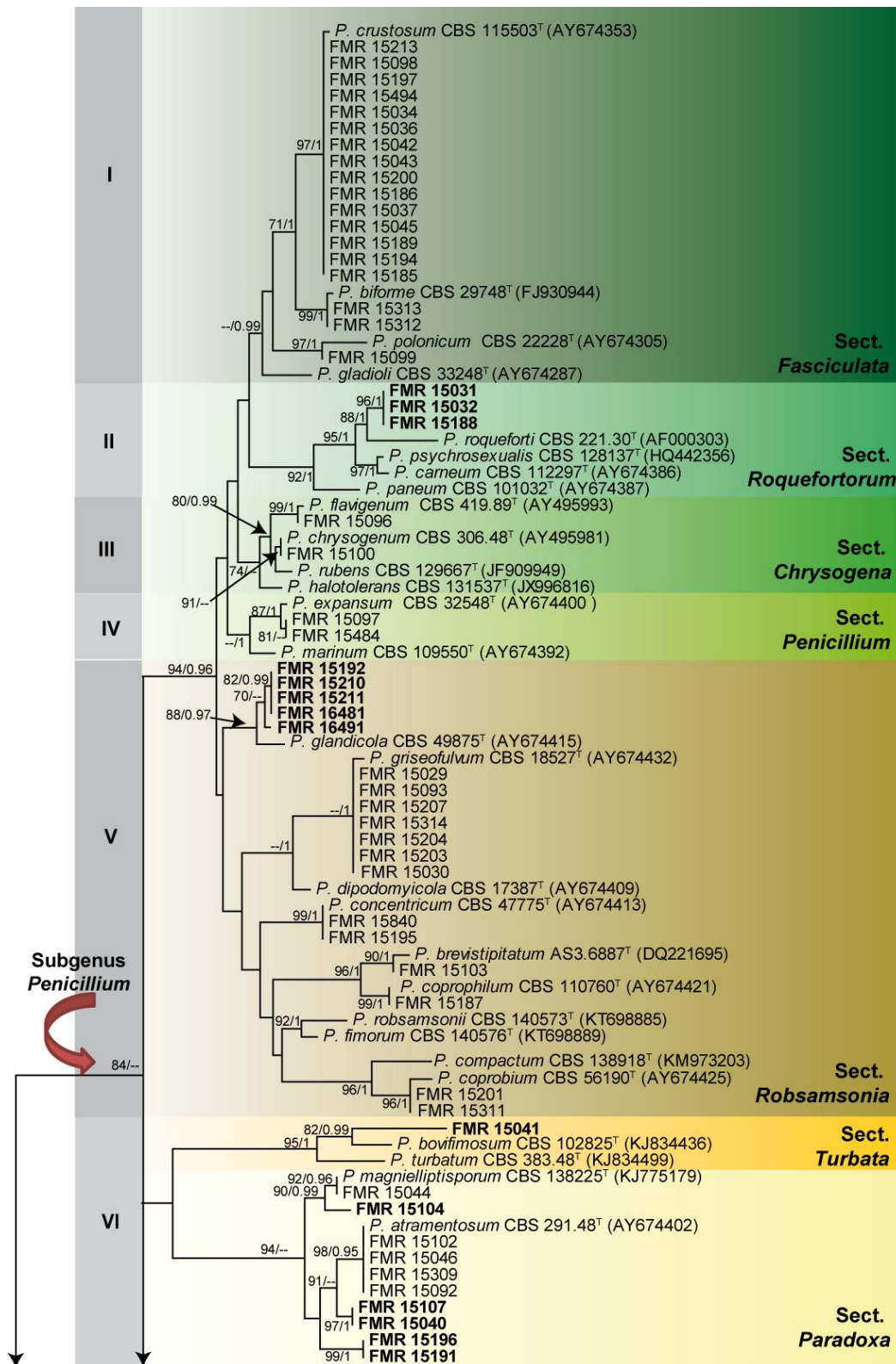
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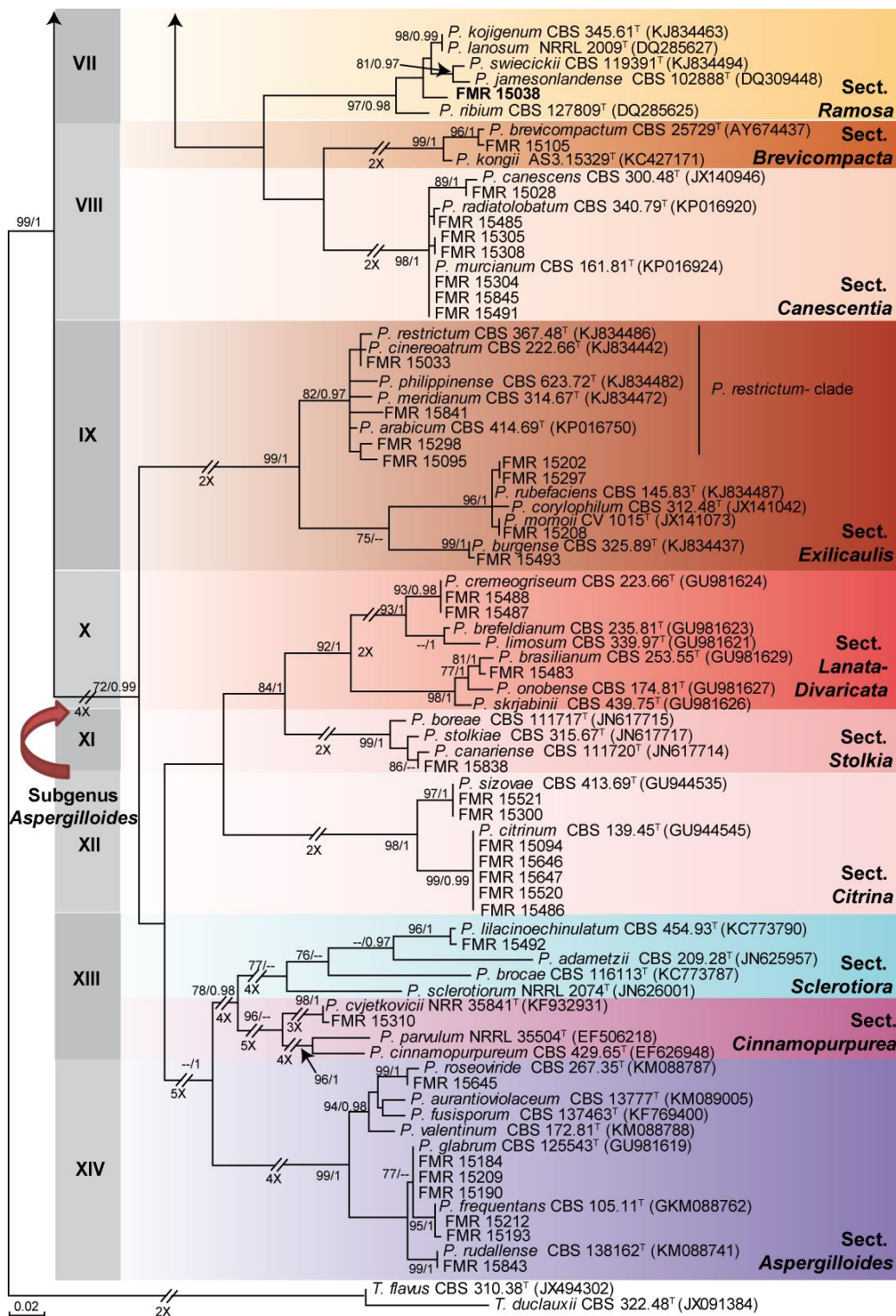
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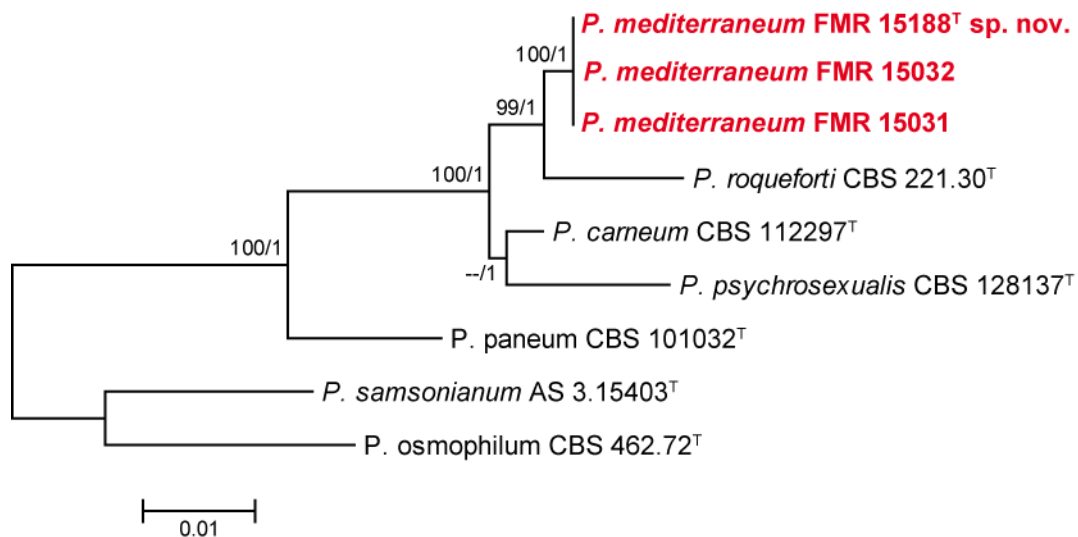
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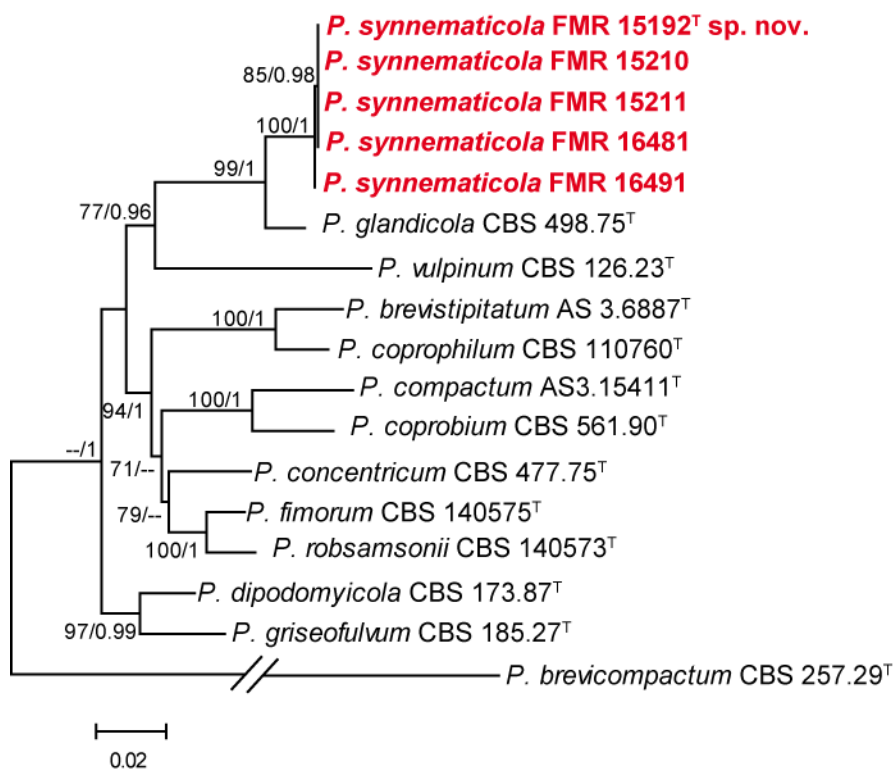
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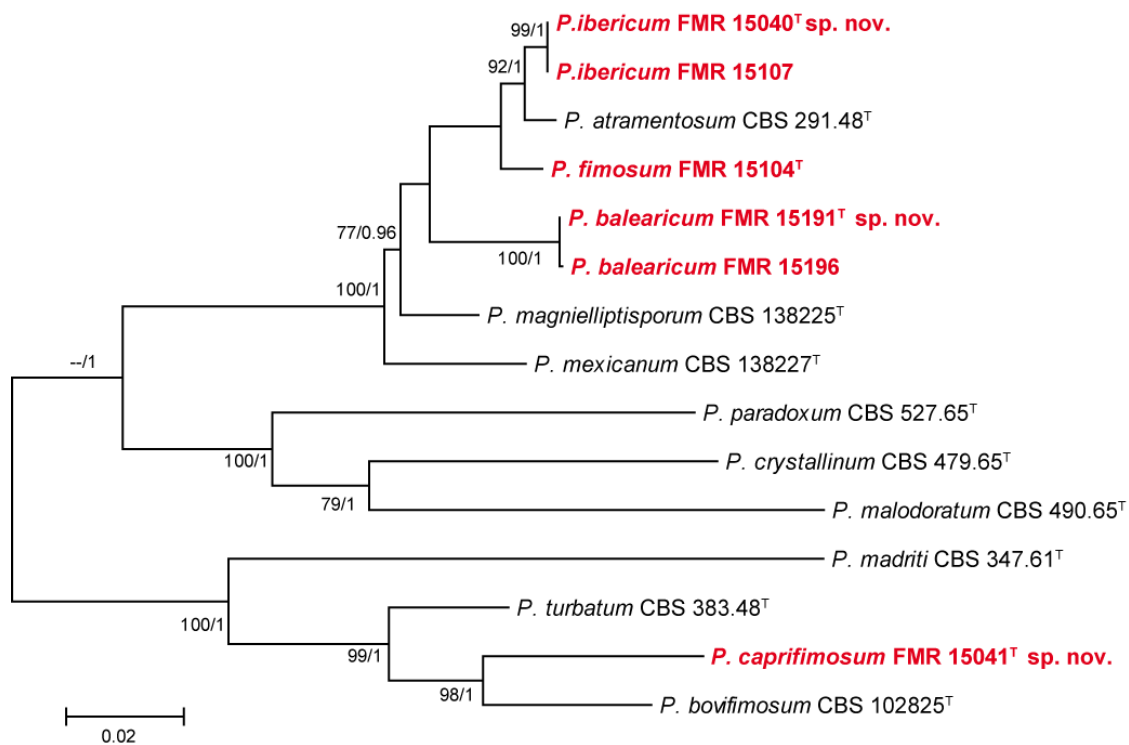
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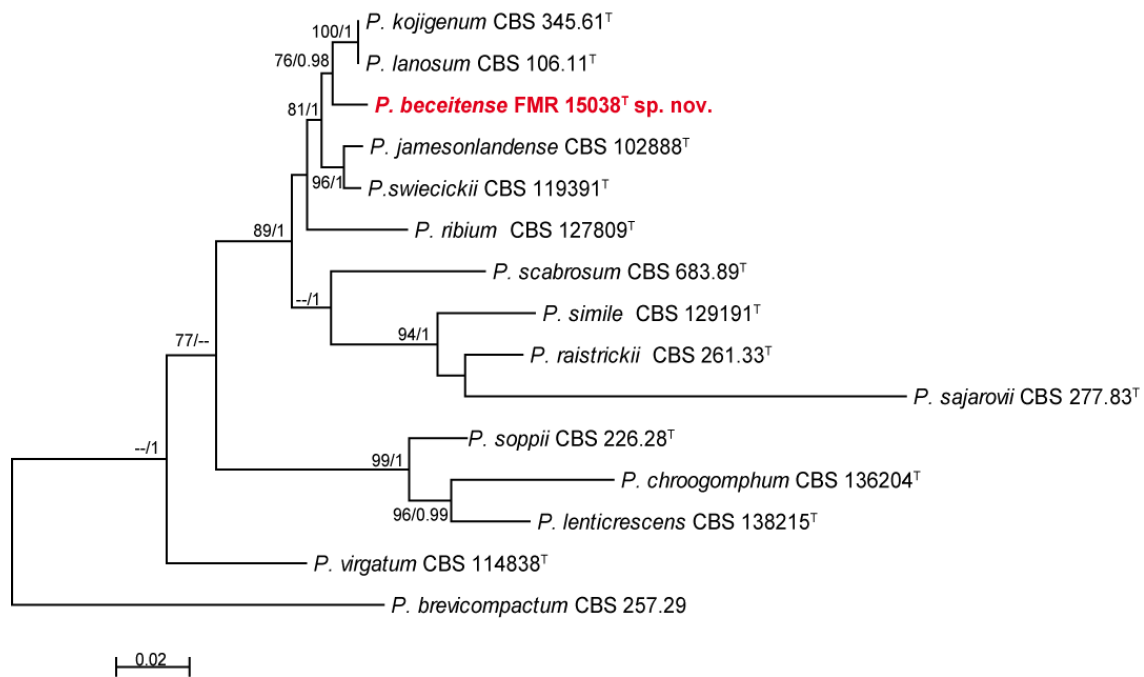
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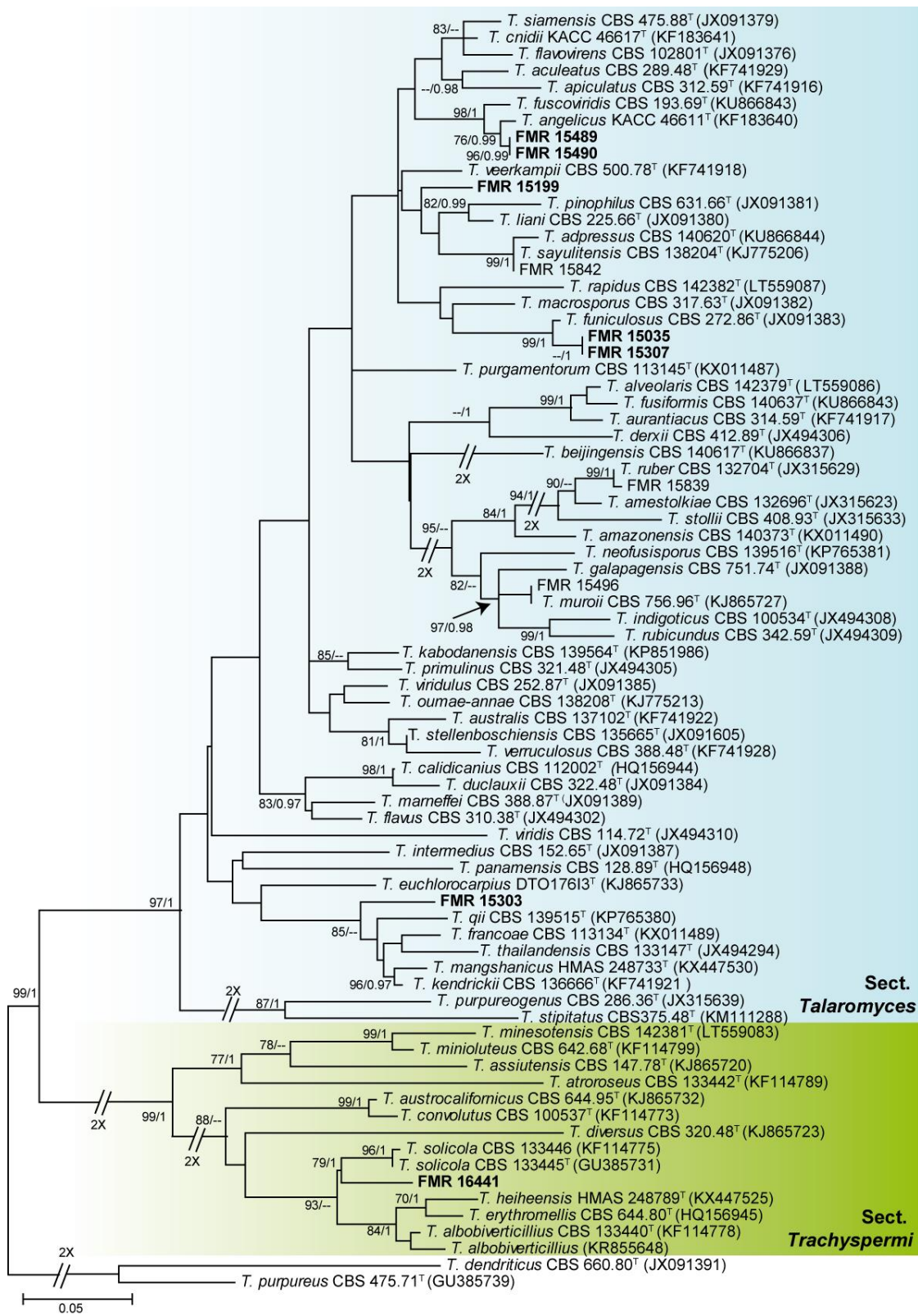
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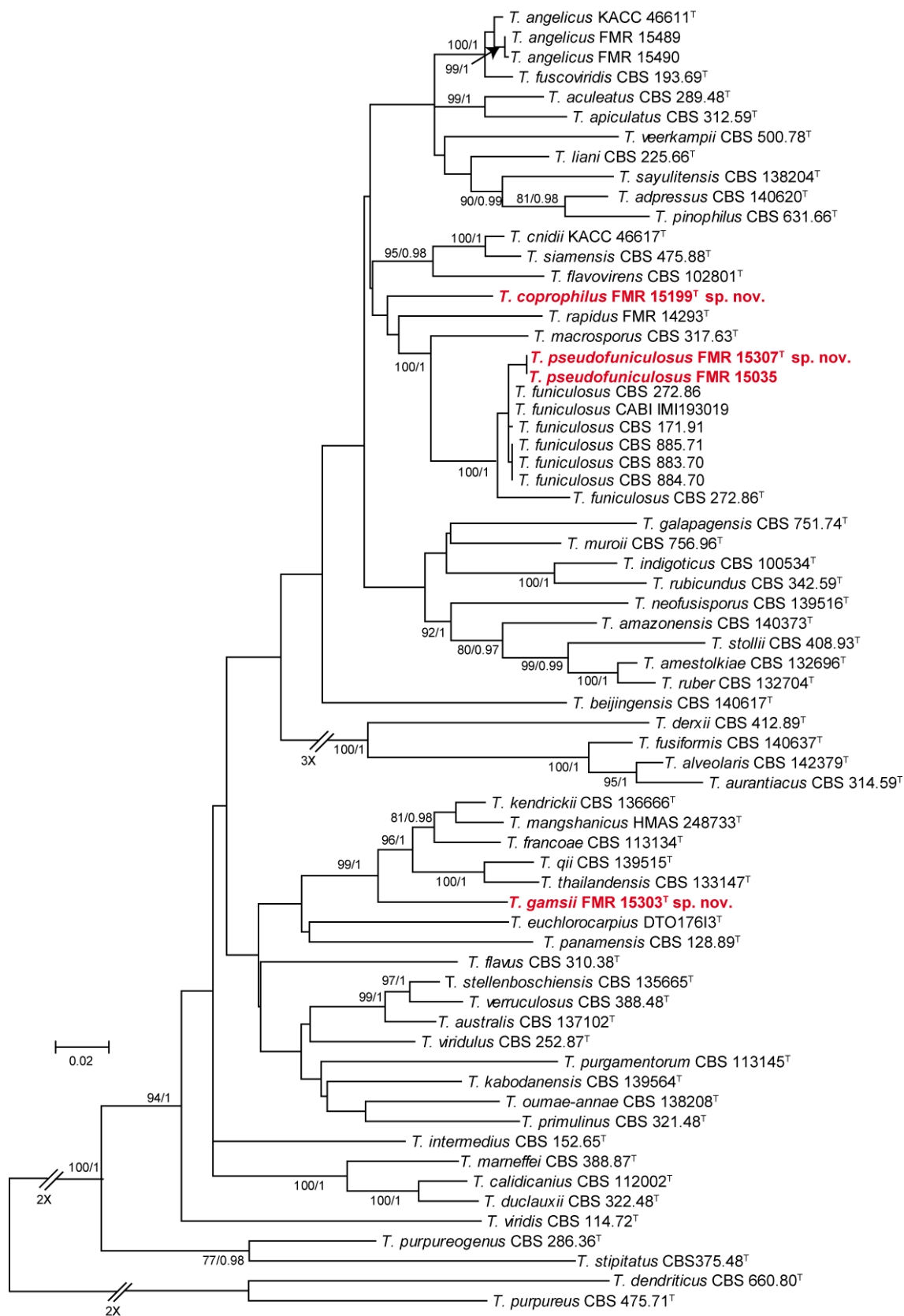
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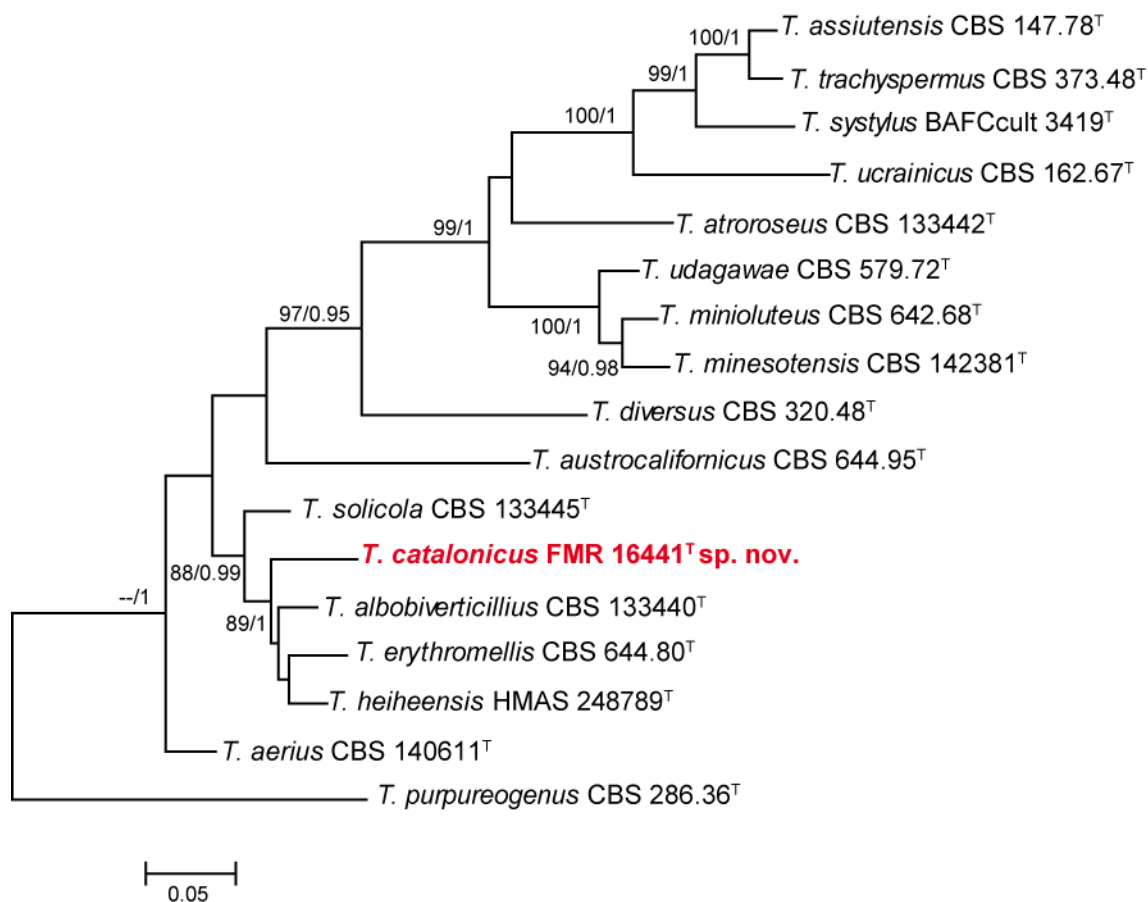
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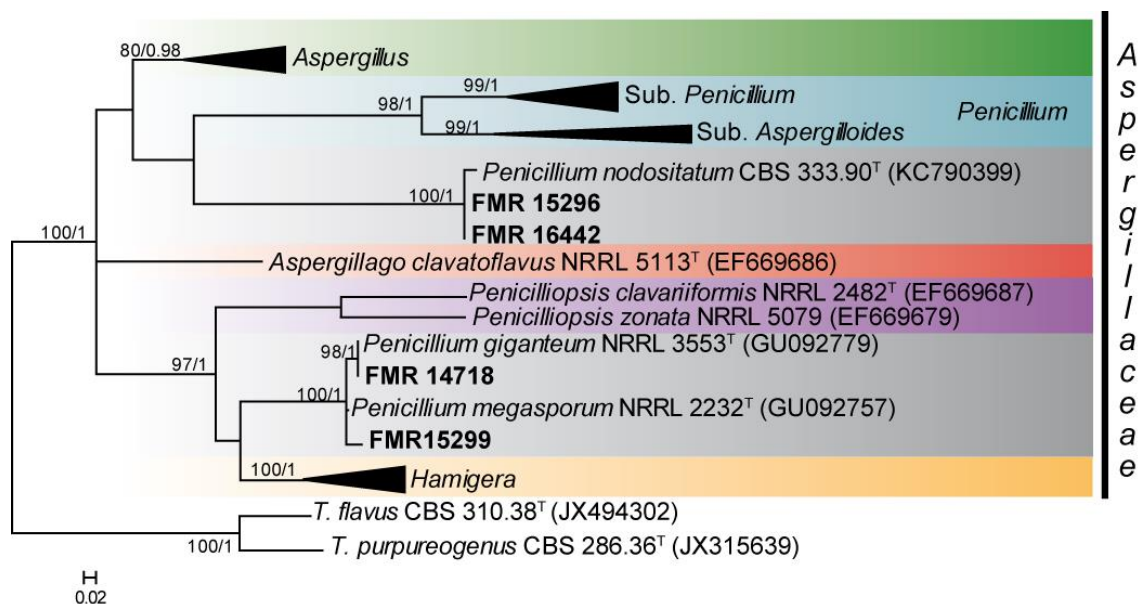
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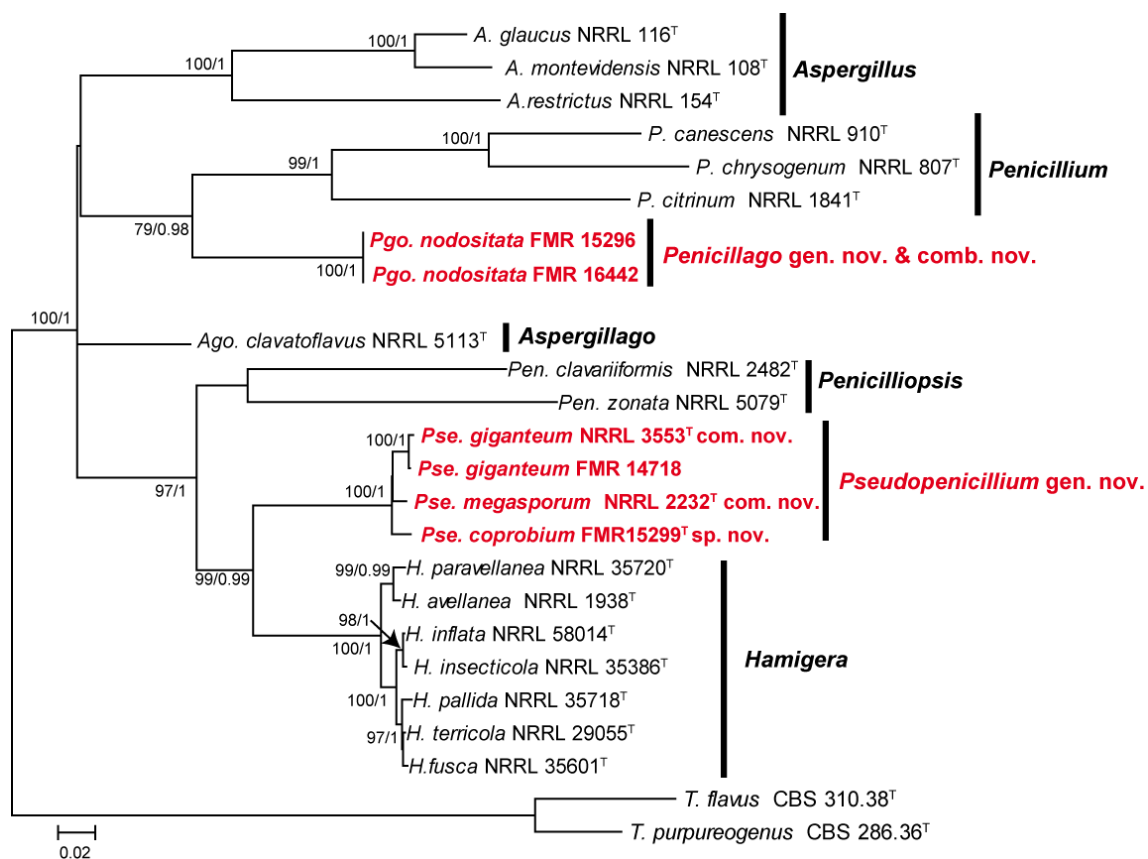
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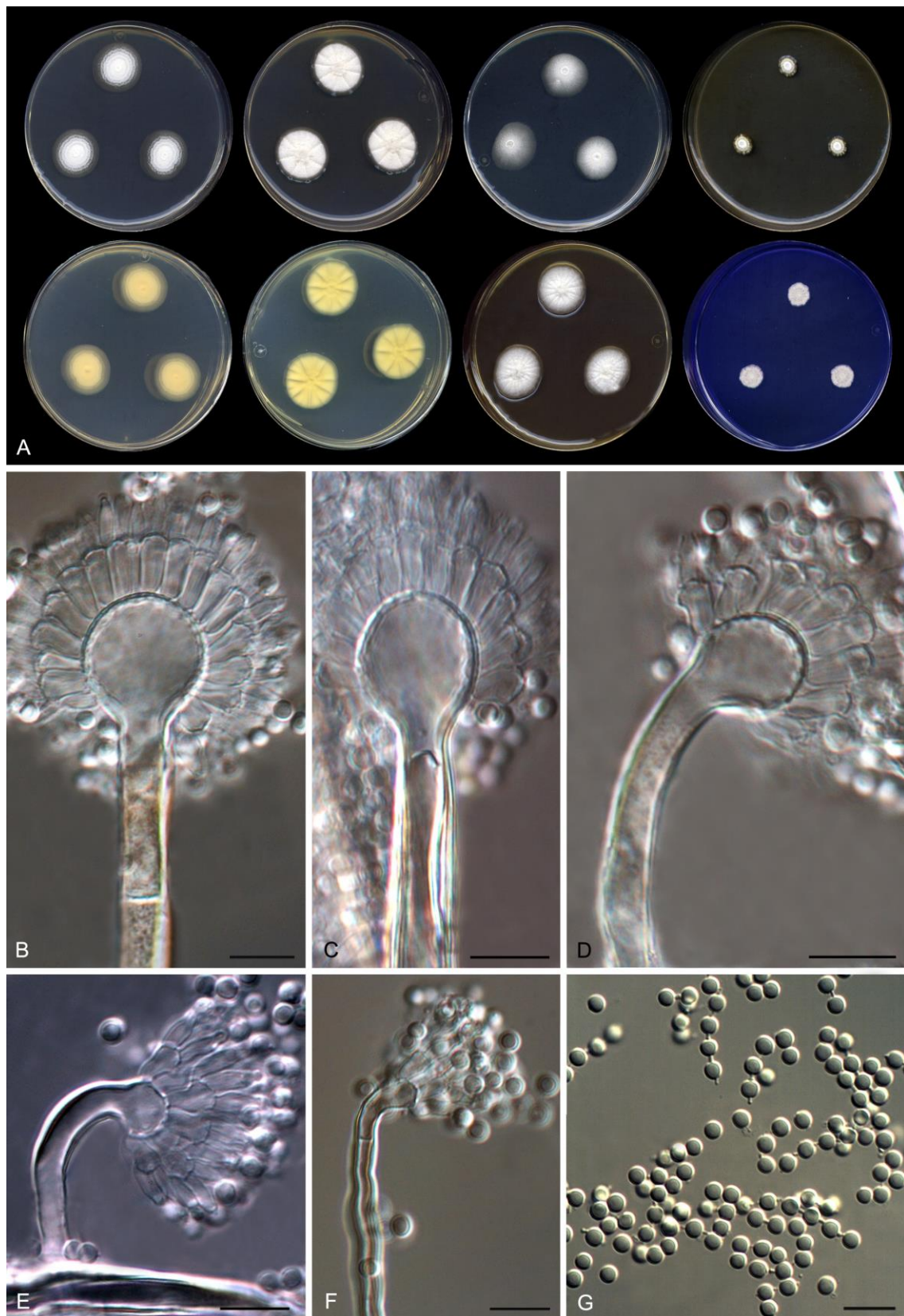
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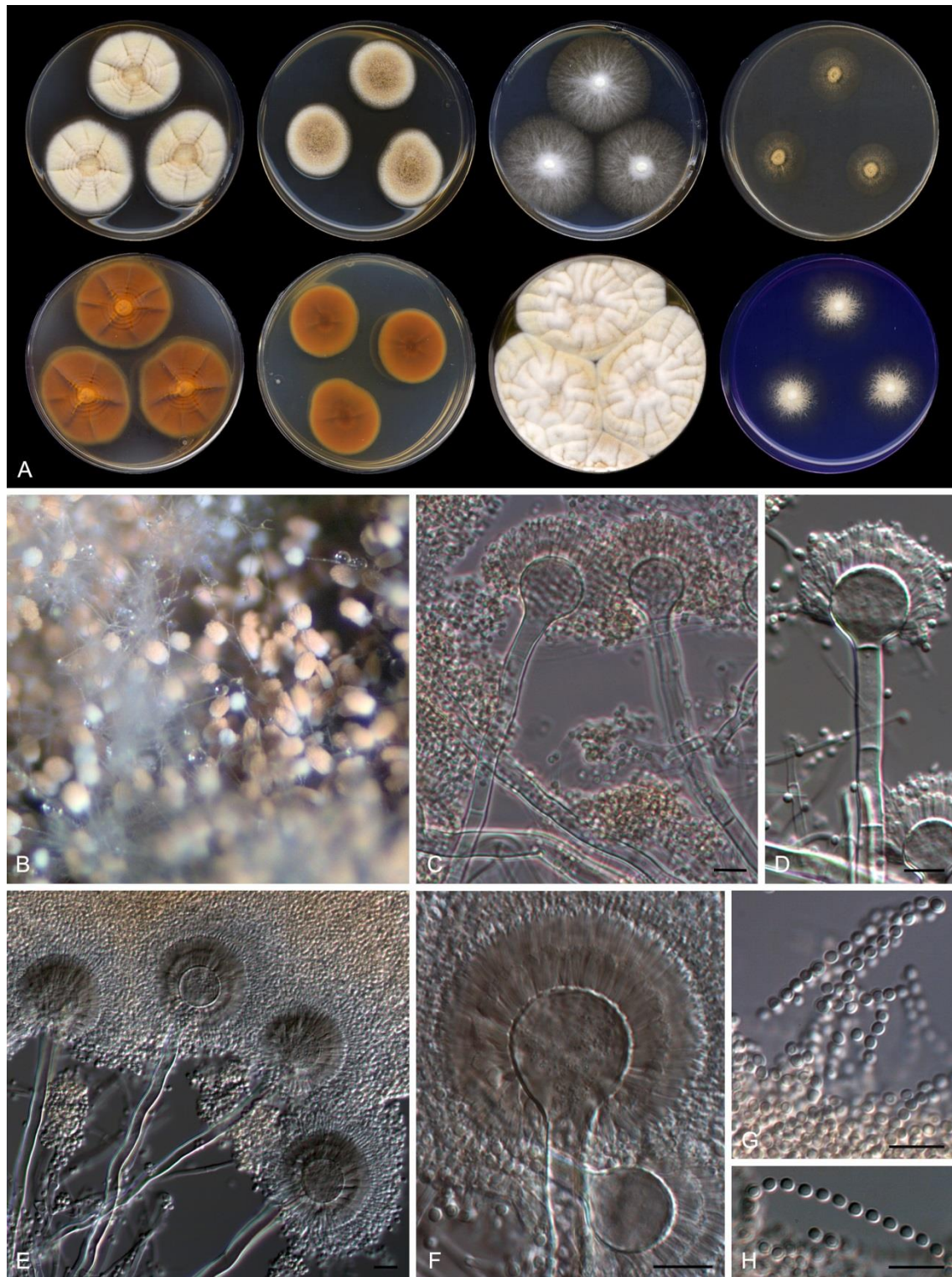
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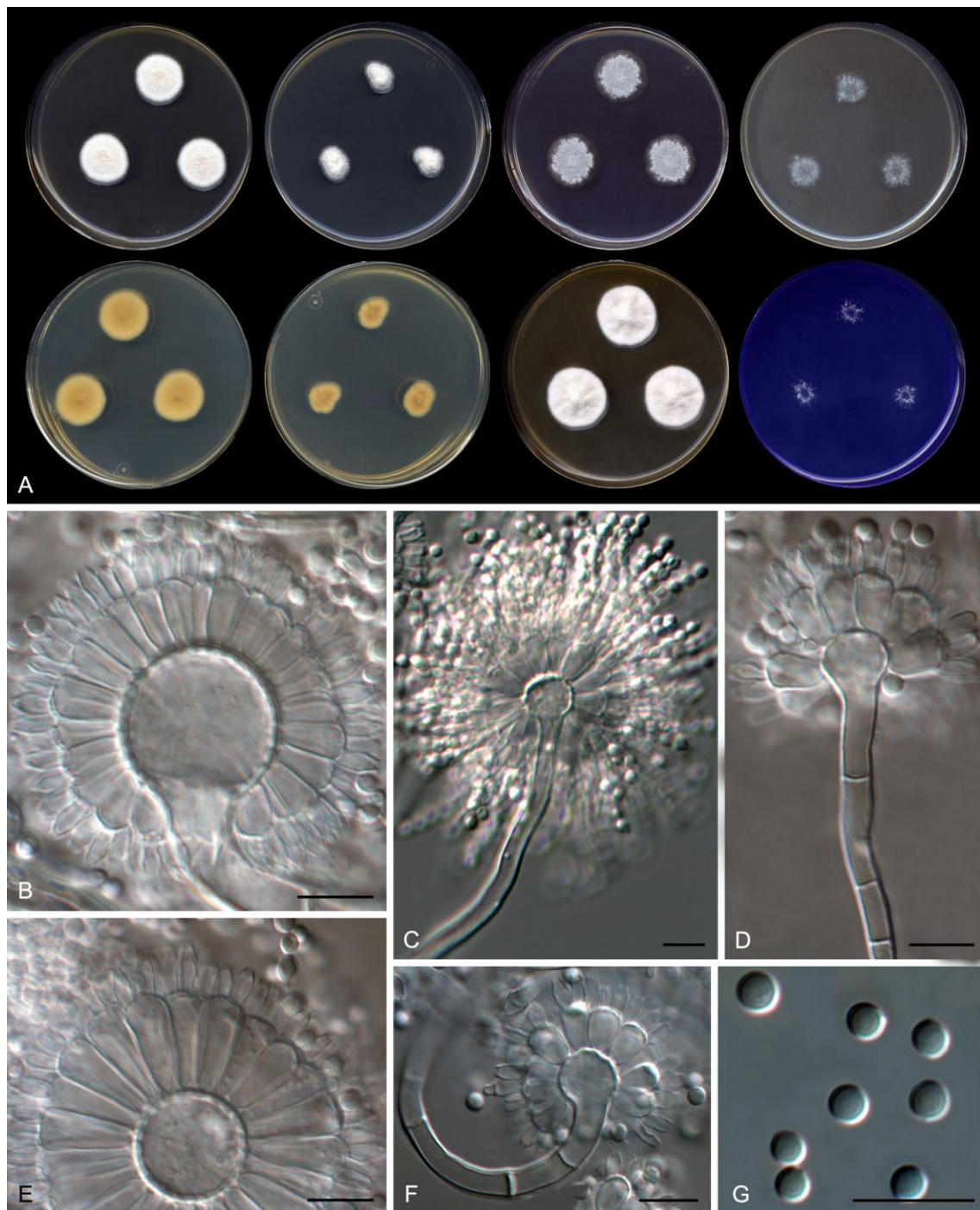
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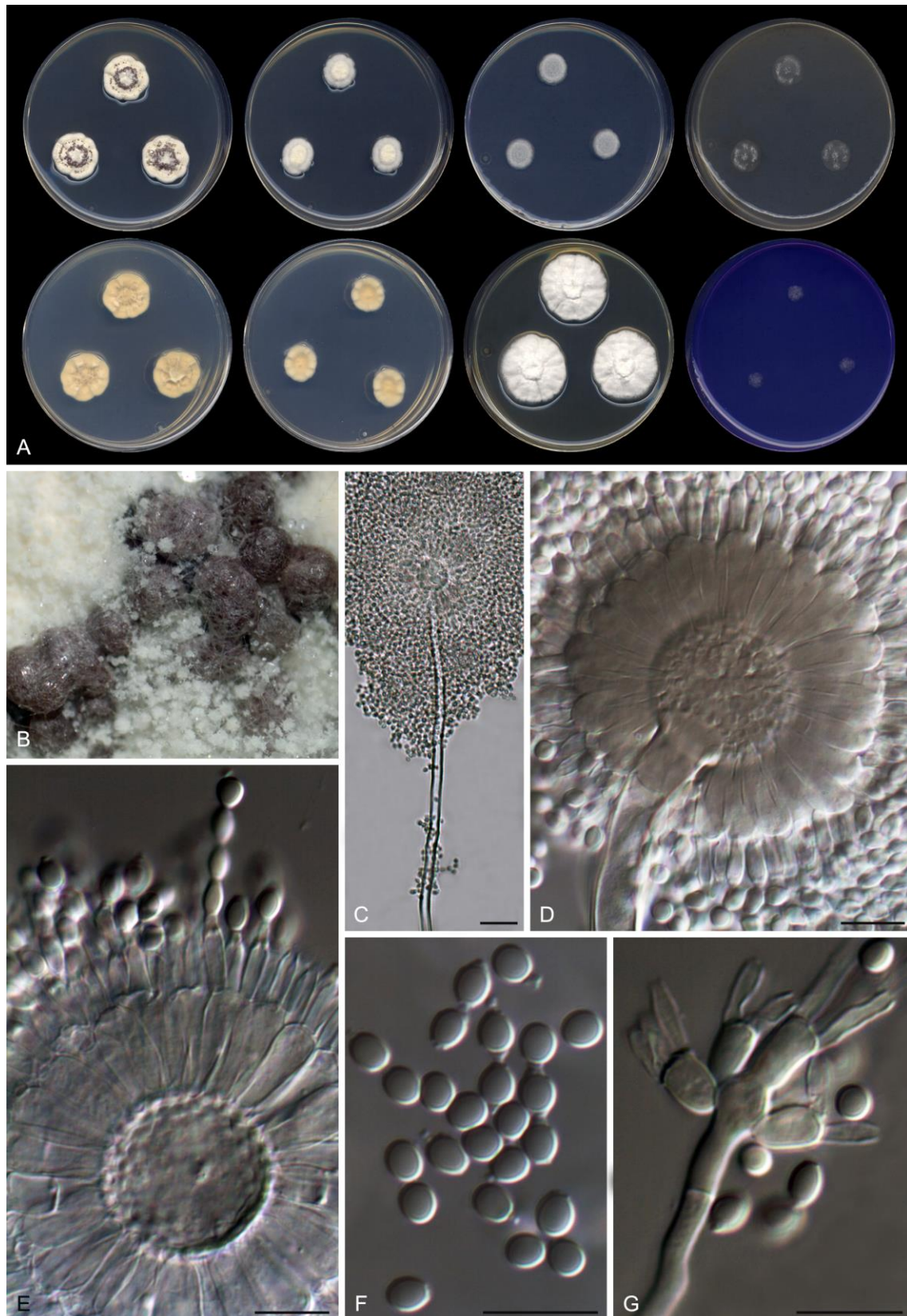
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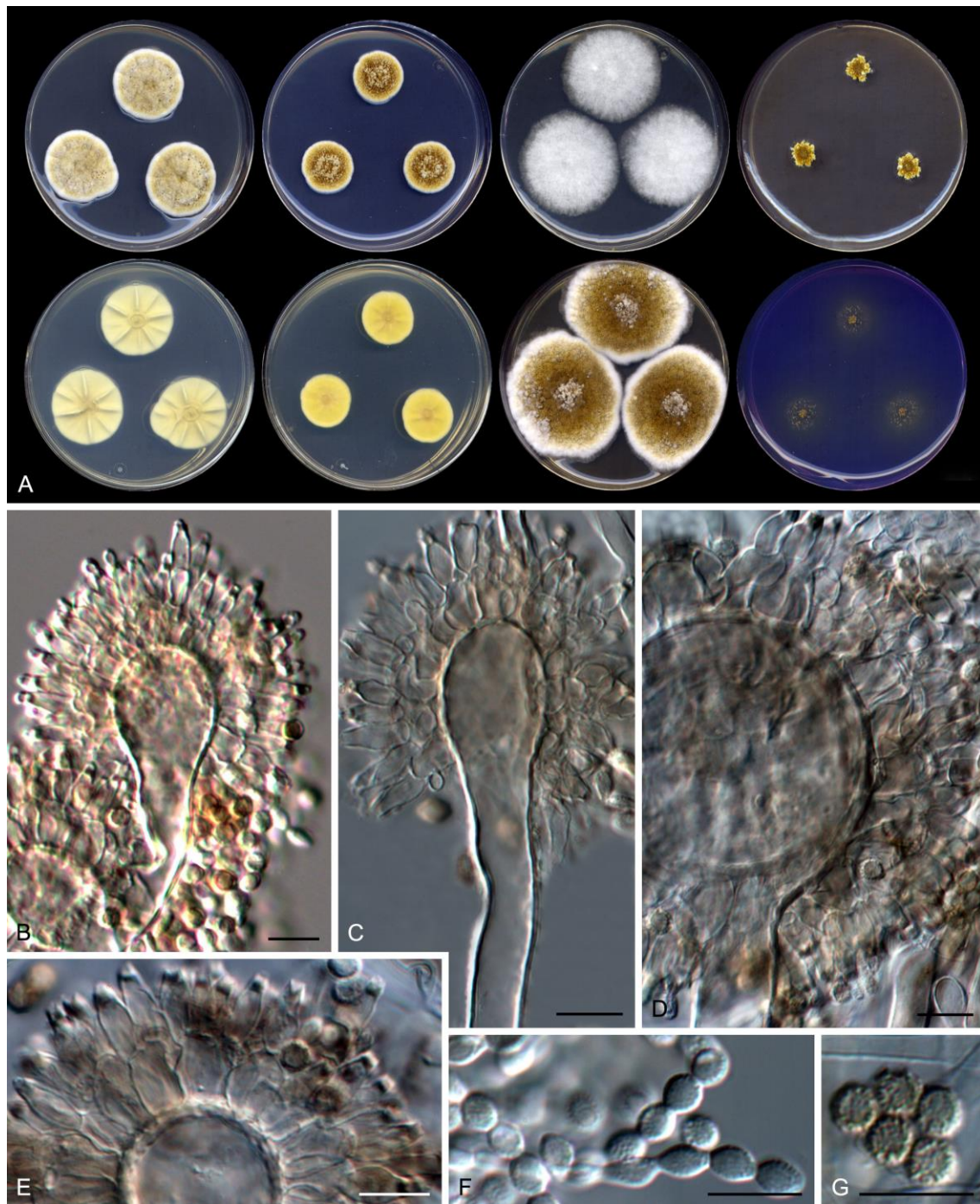
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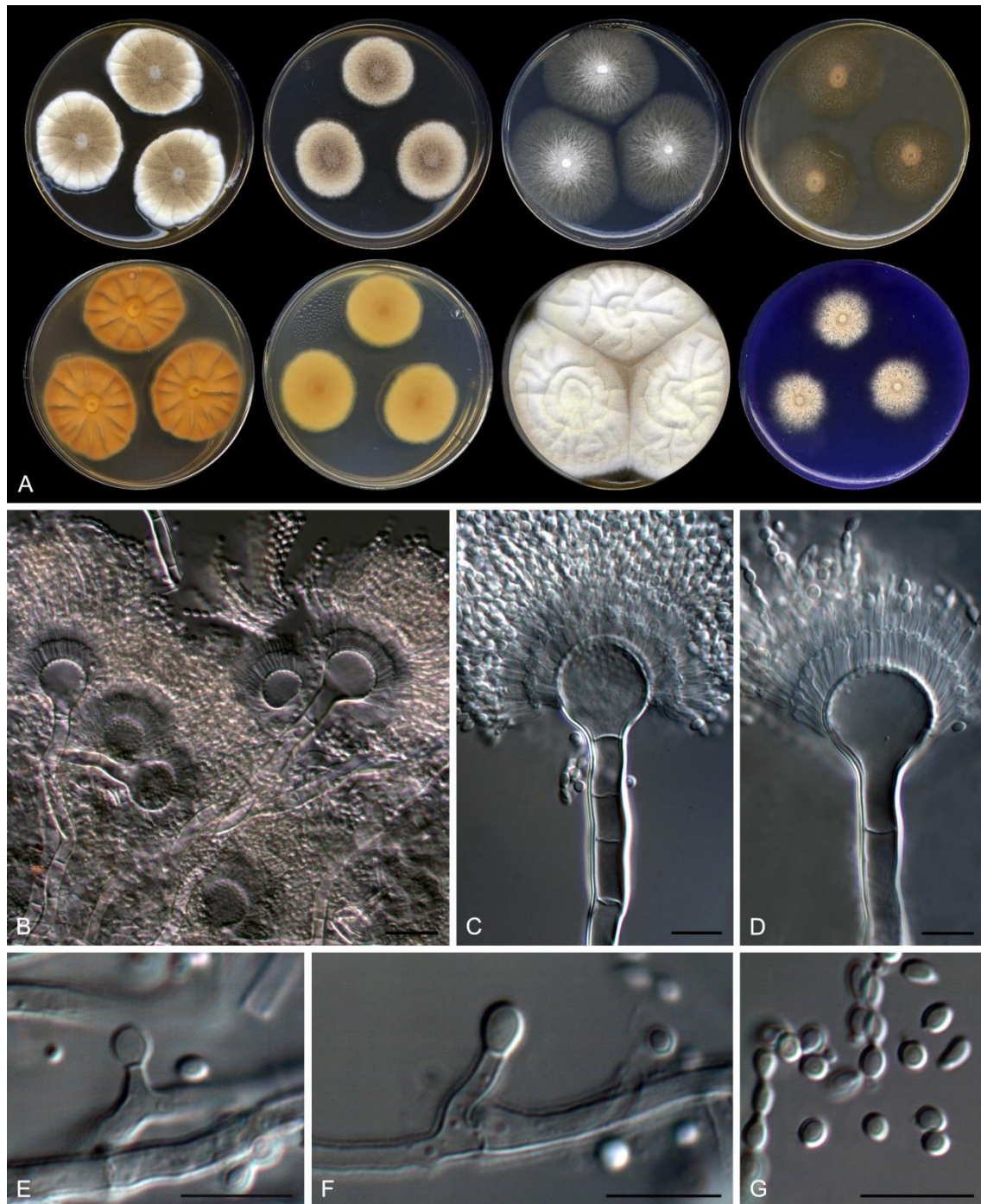
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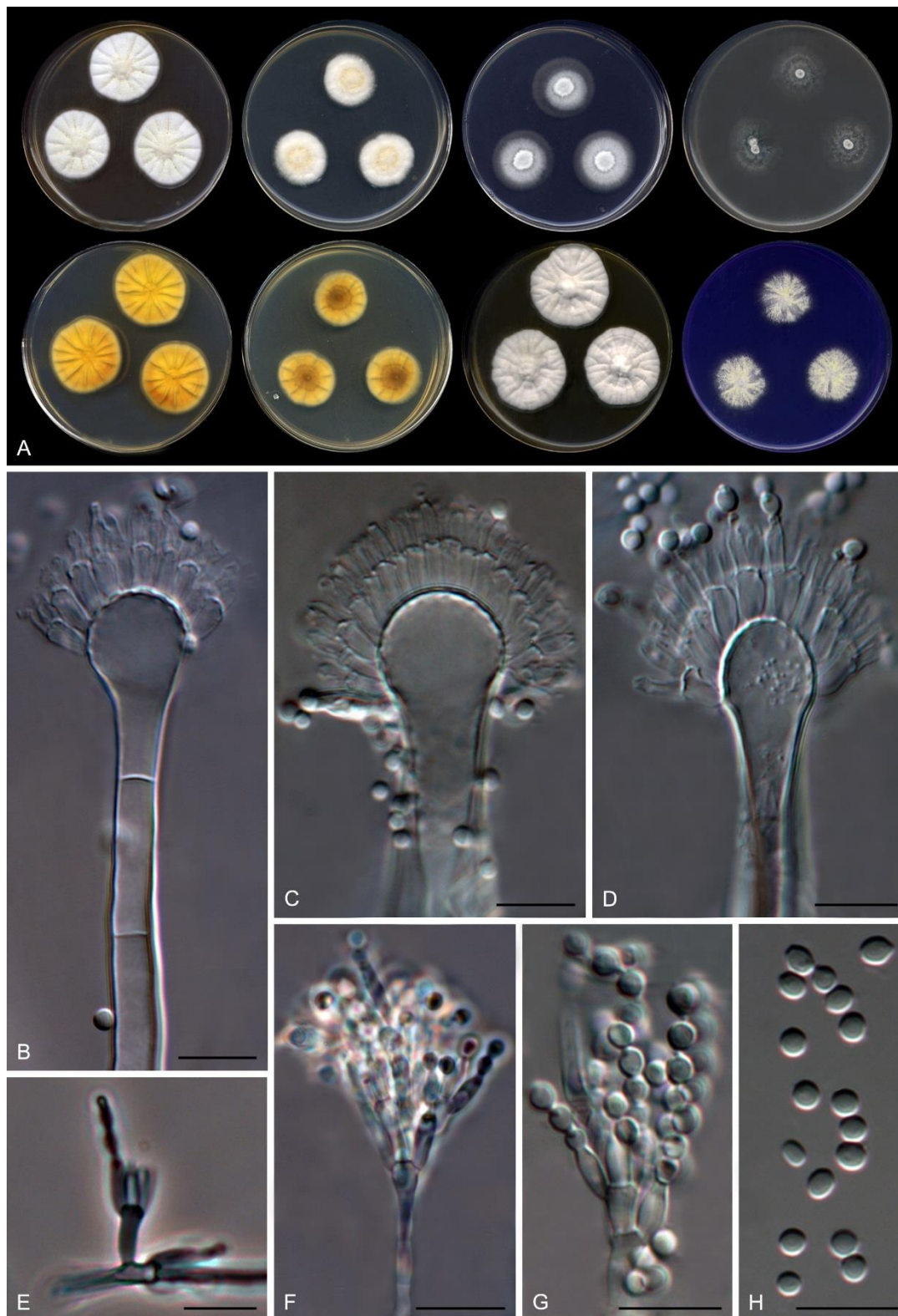
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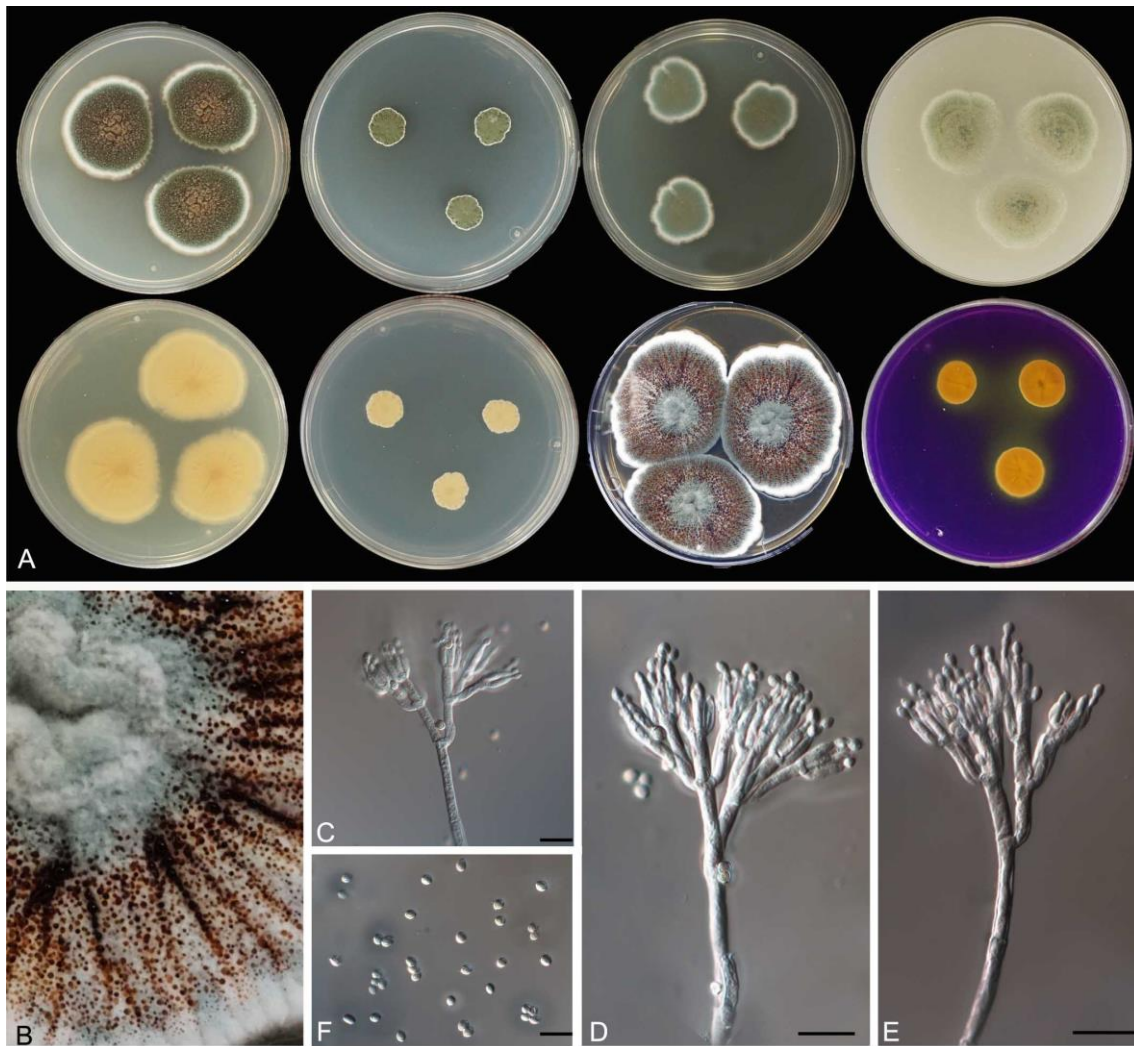
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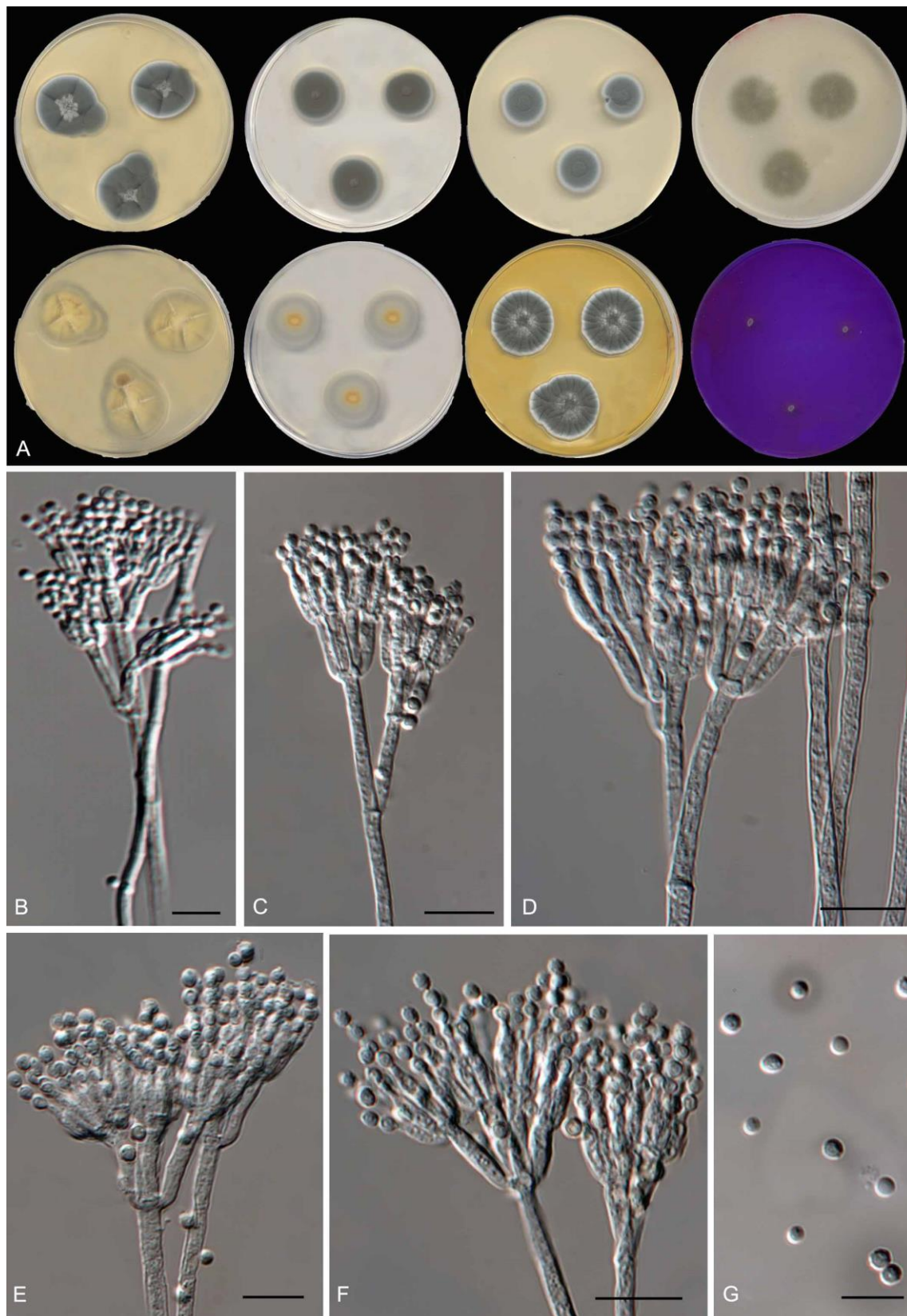
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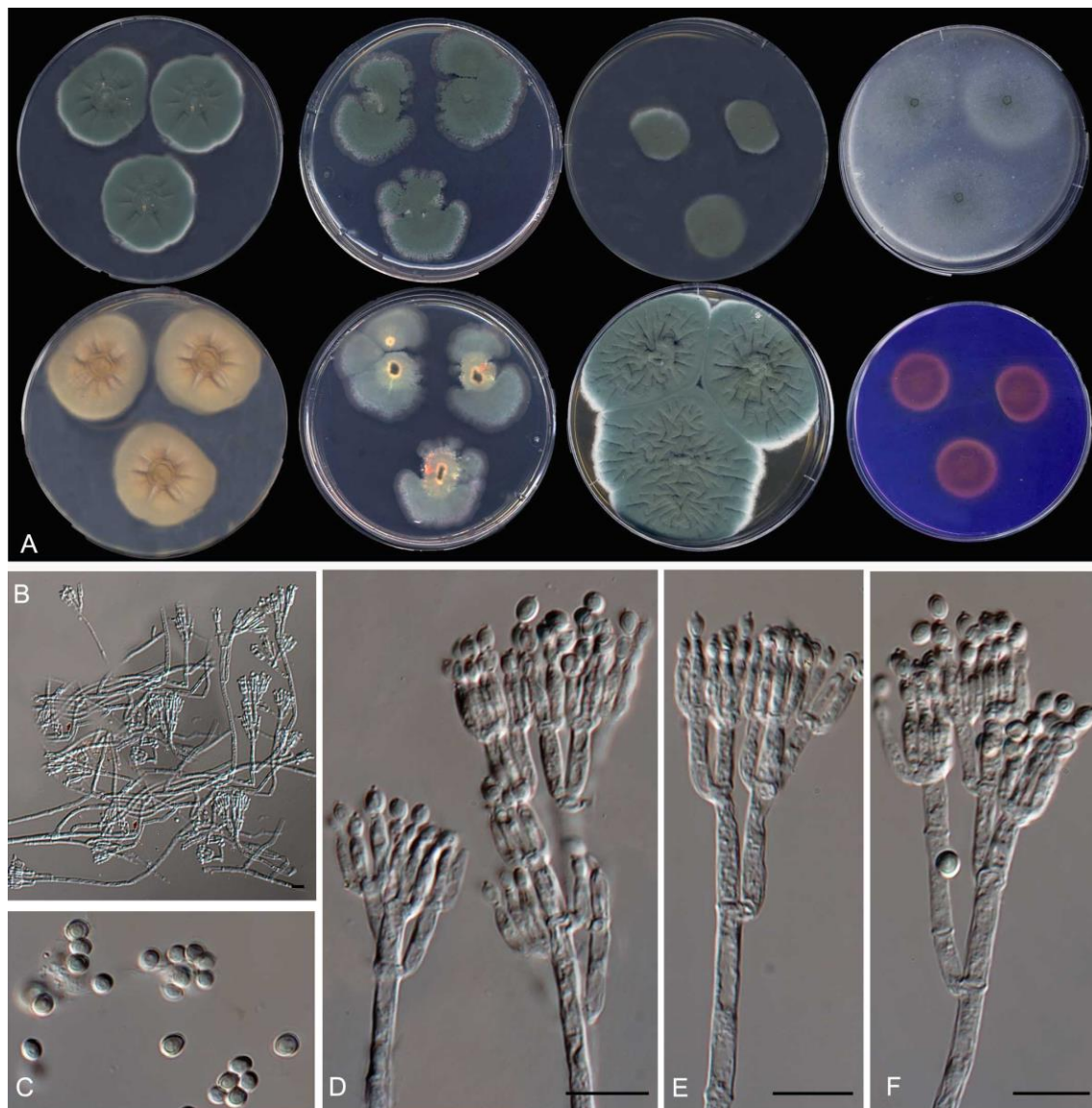
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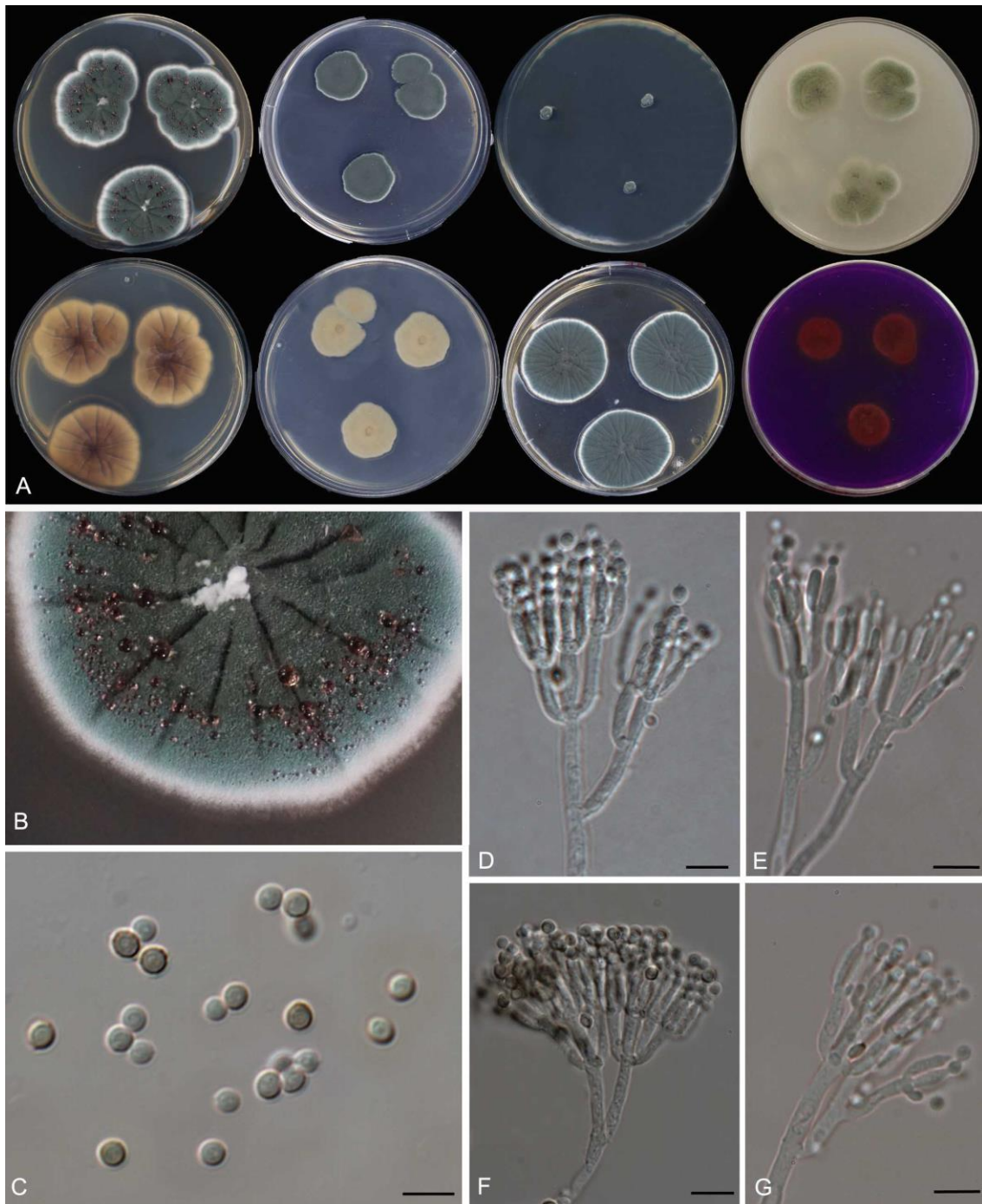
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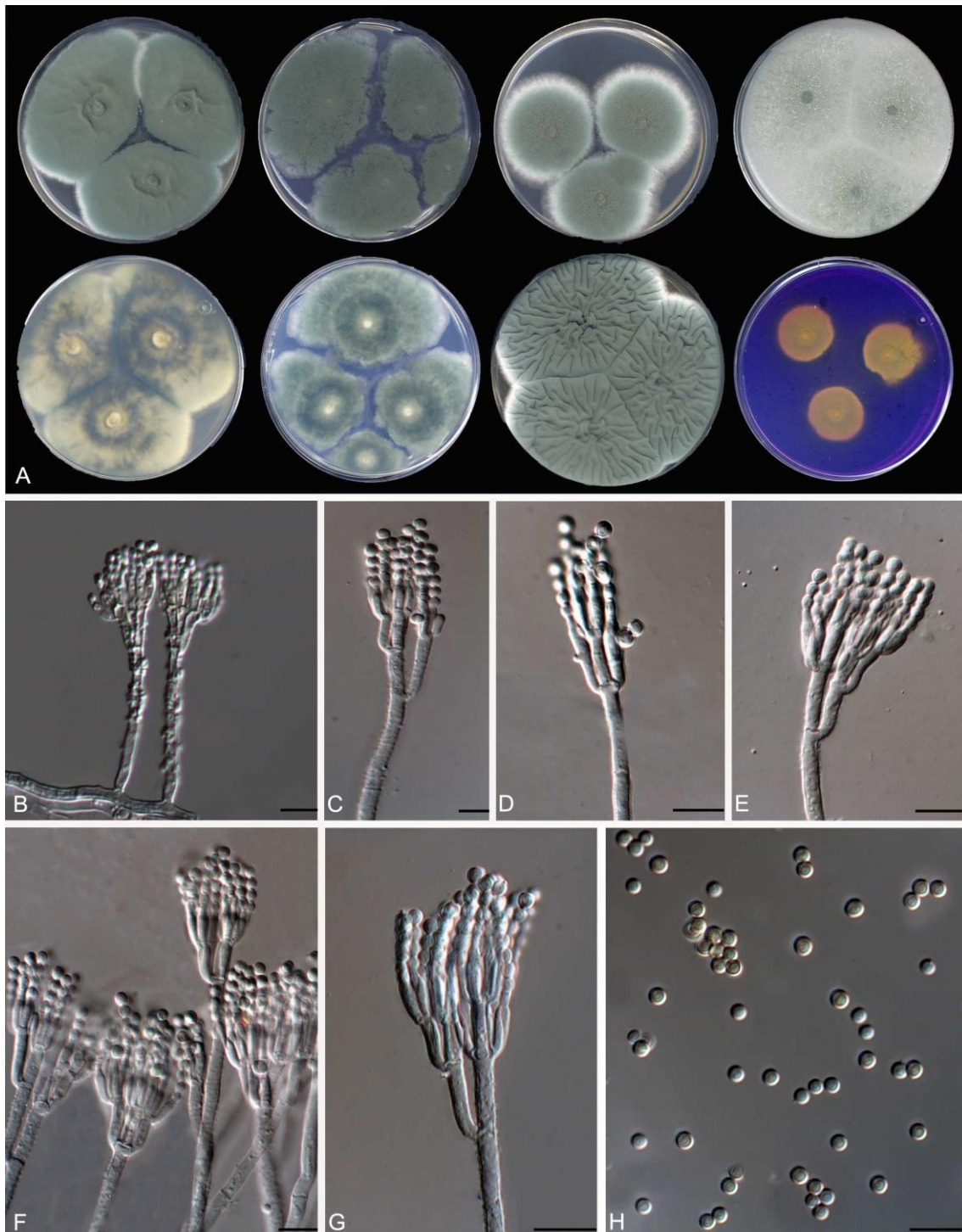
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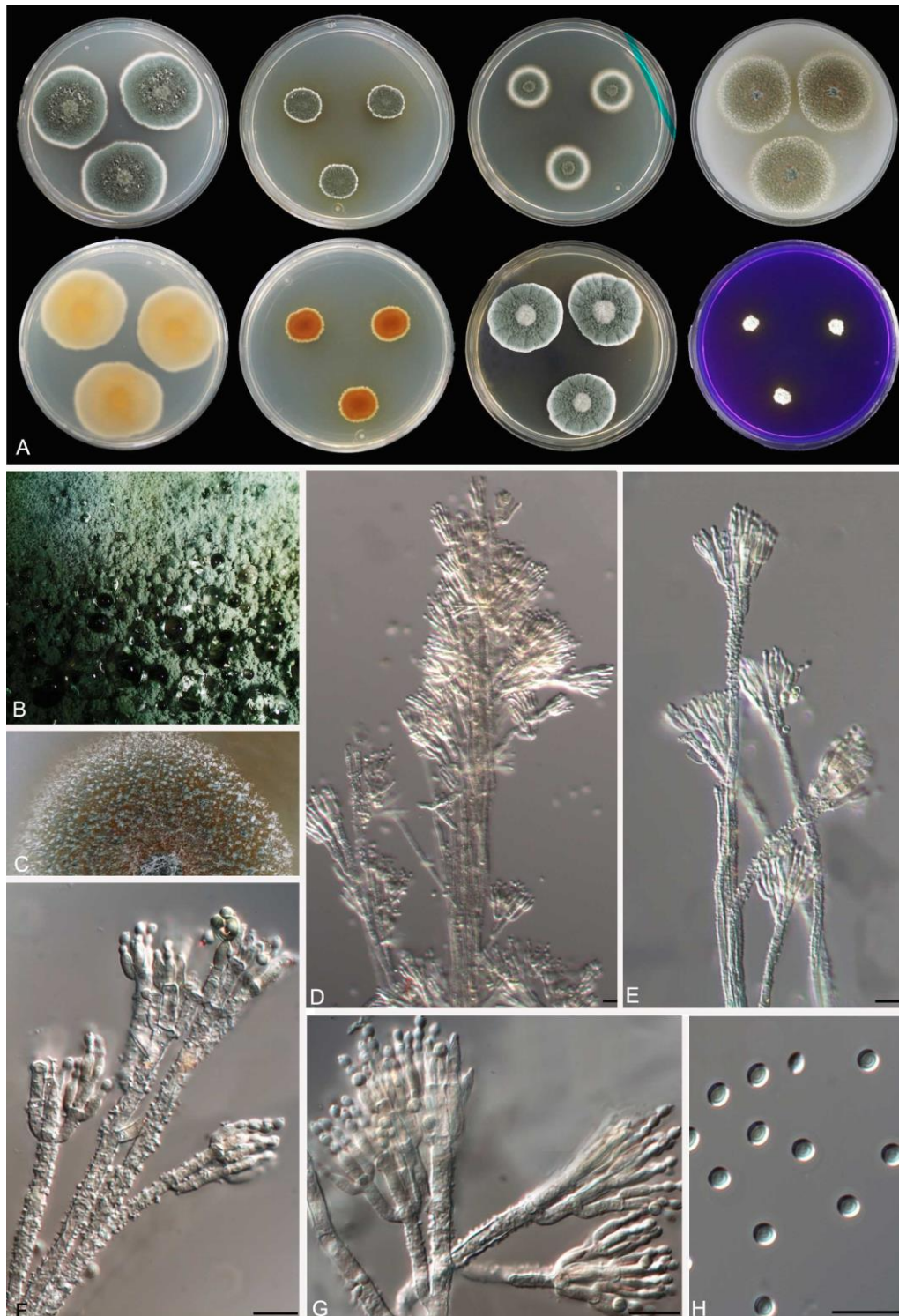
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Figure 33.

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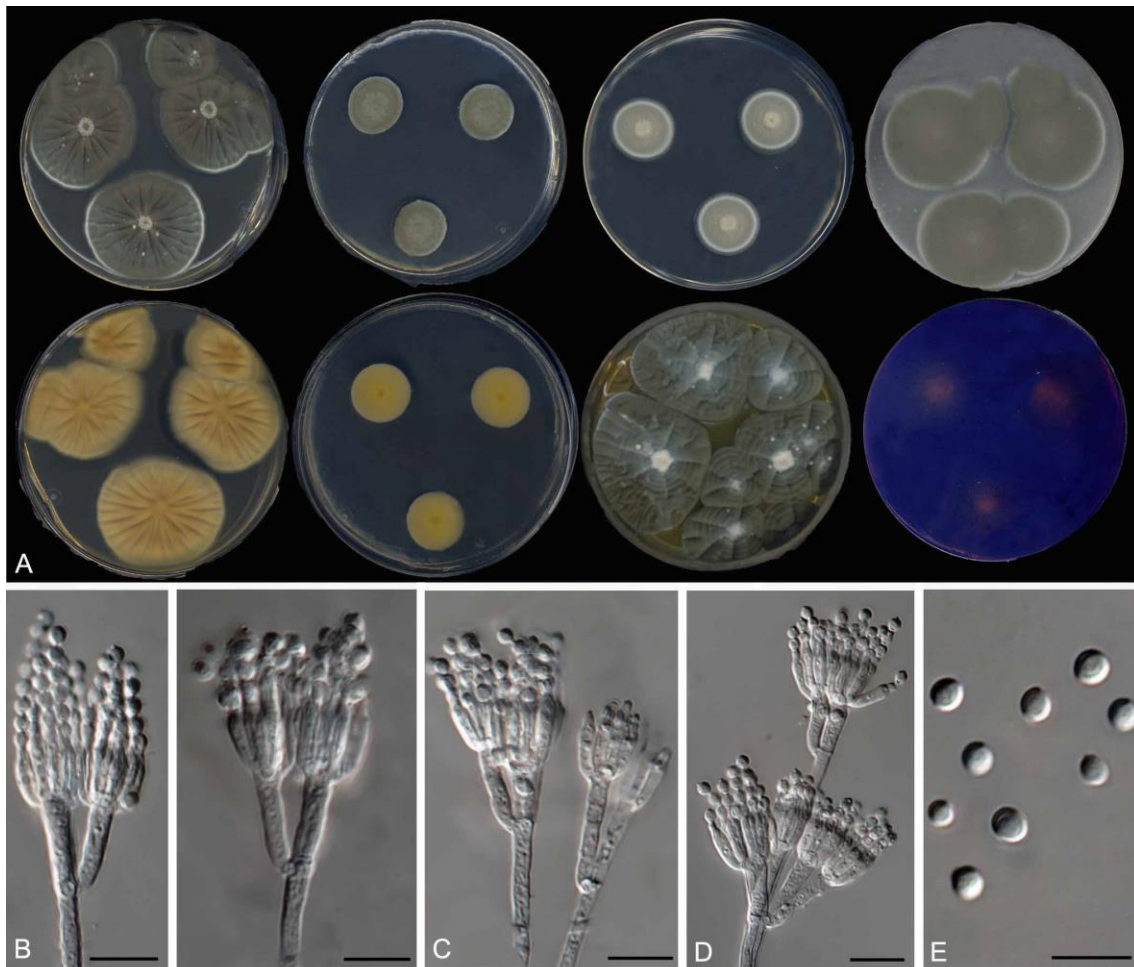
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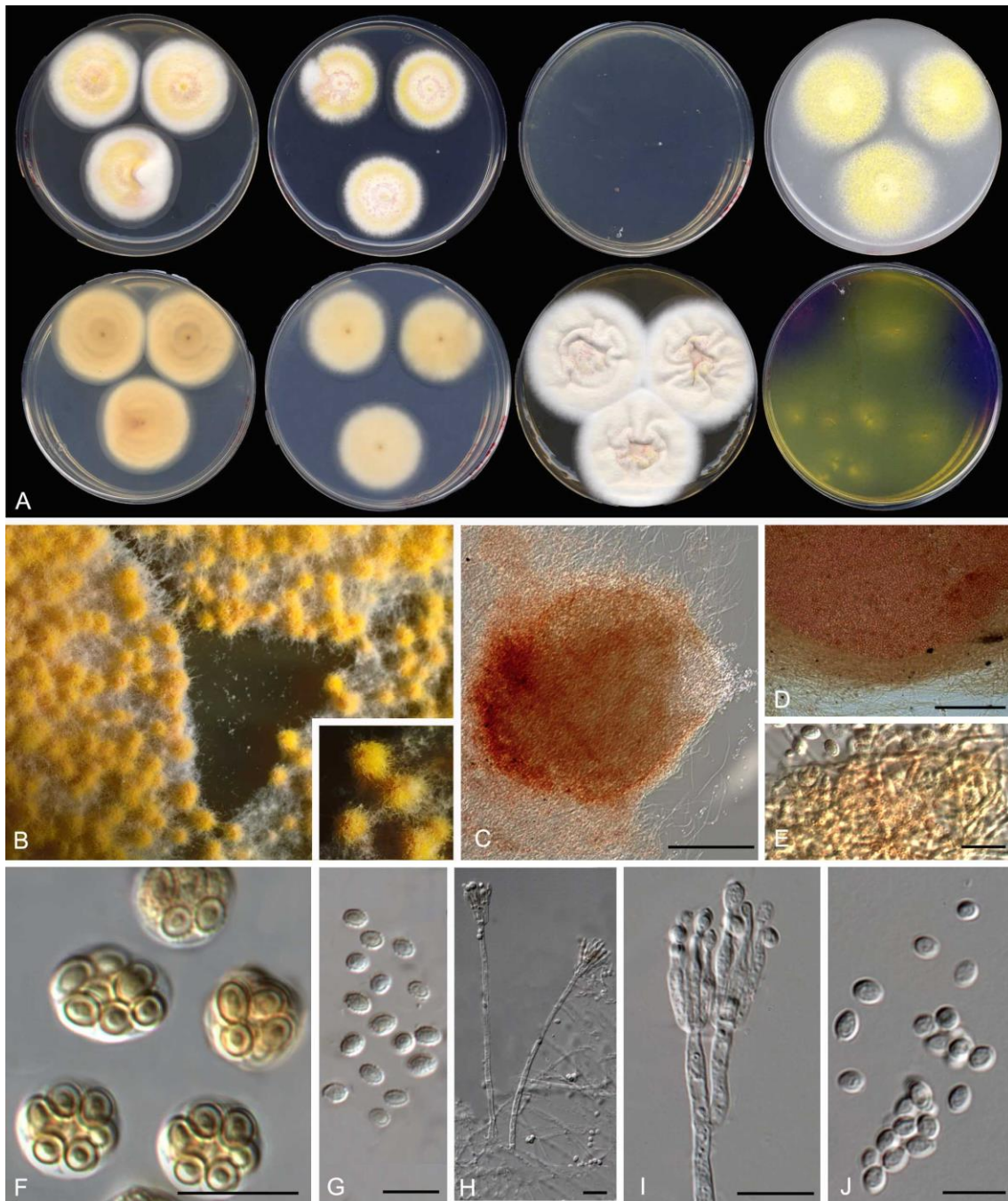
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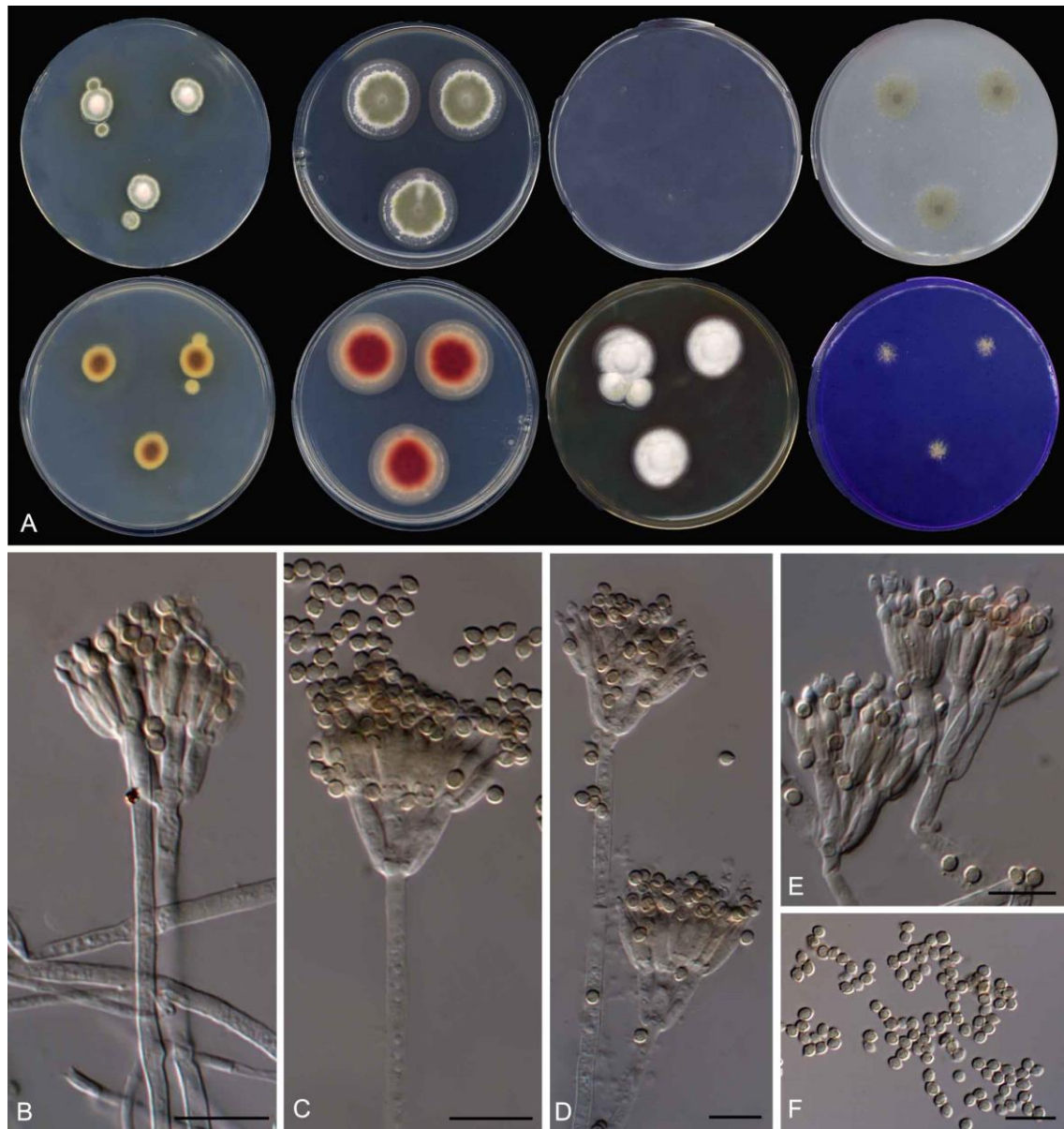
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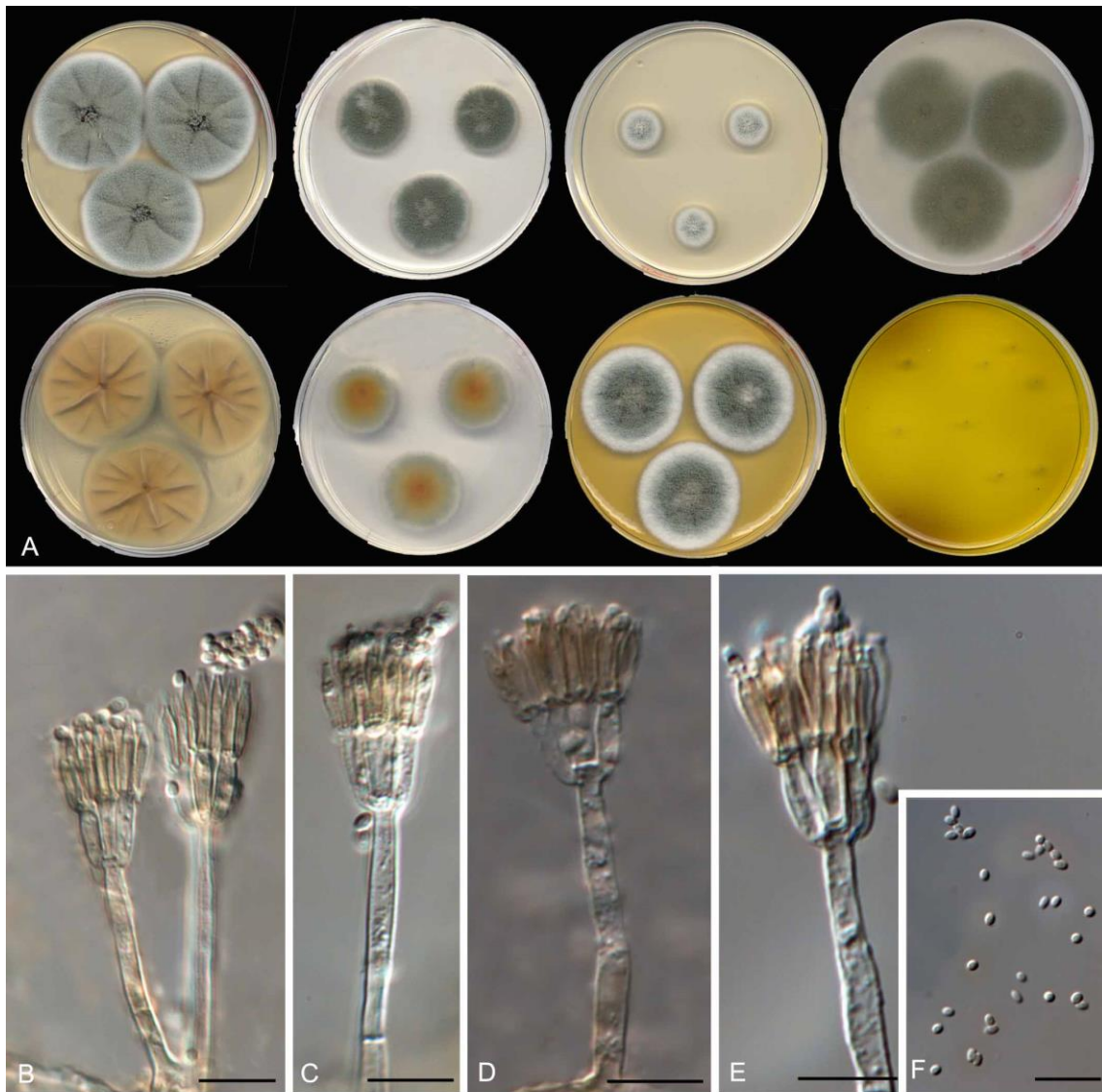
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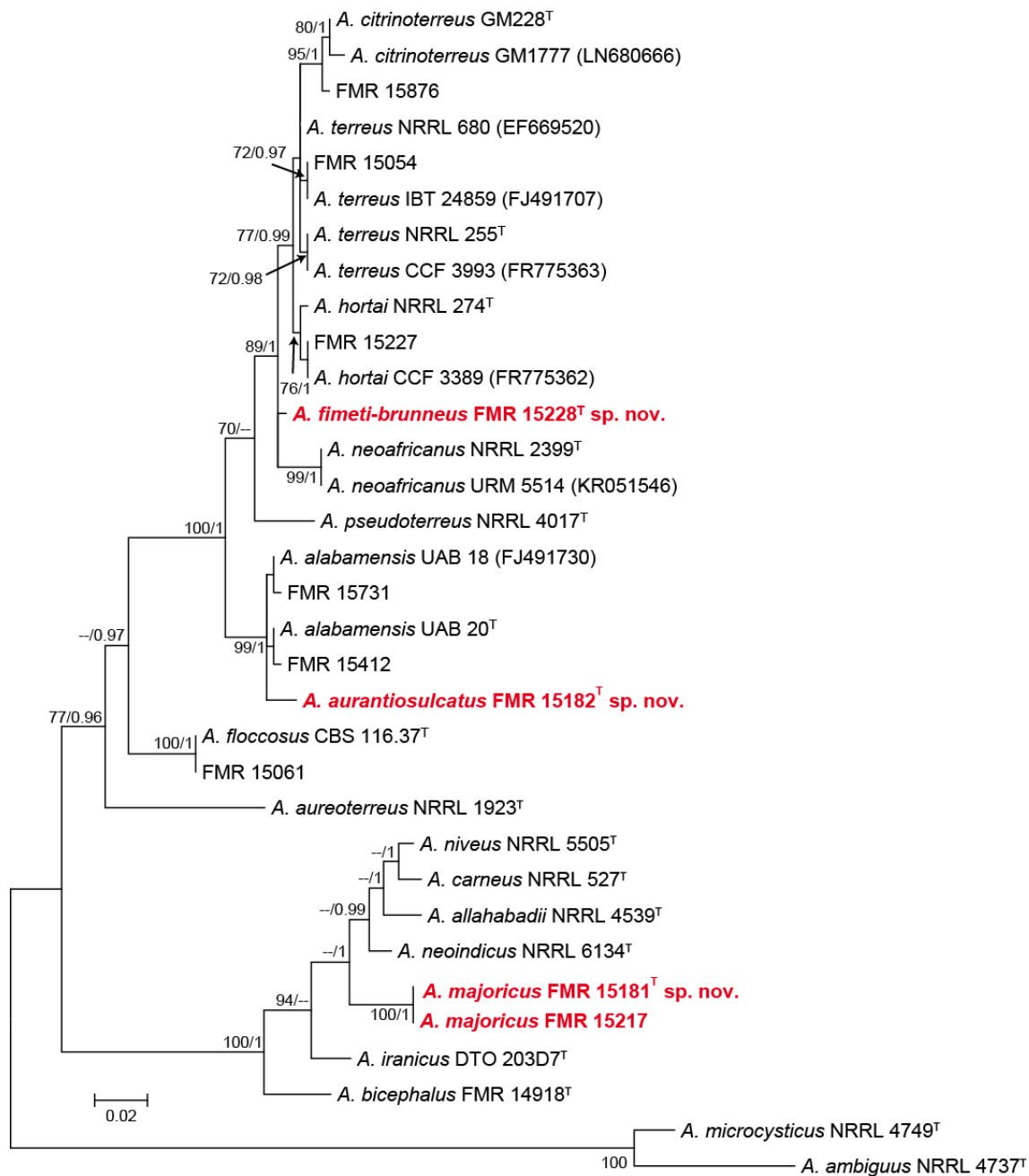
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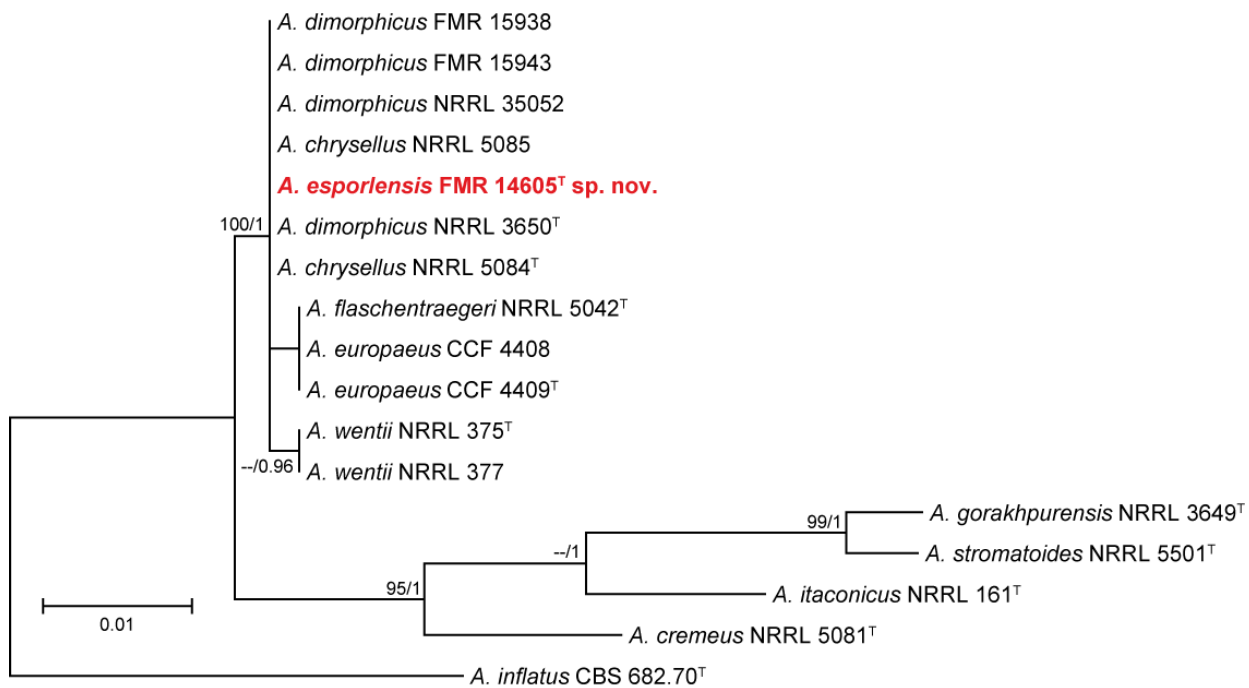
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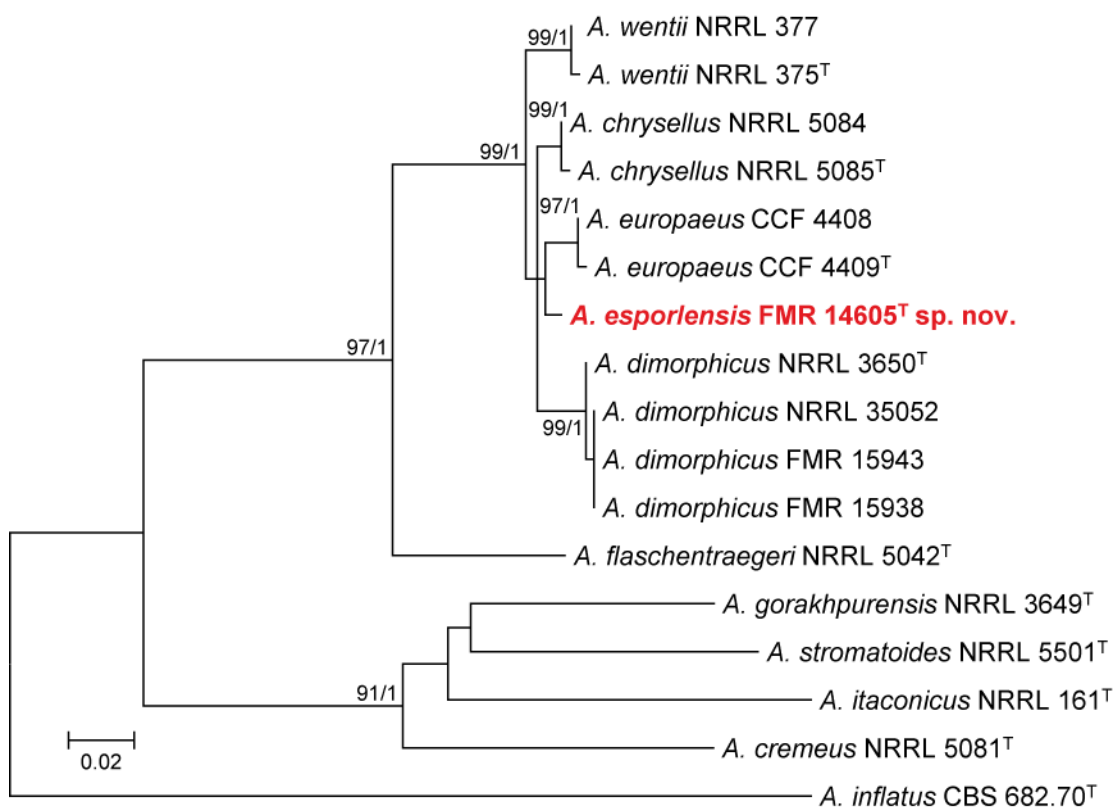
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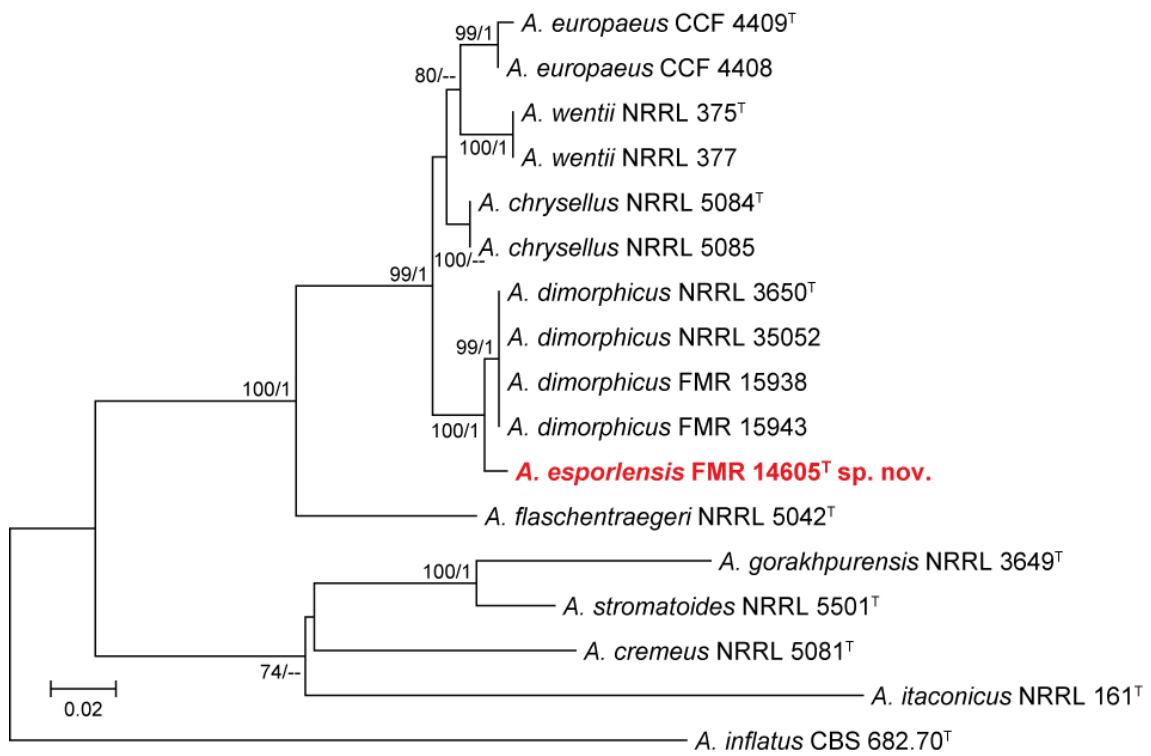
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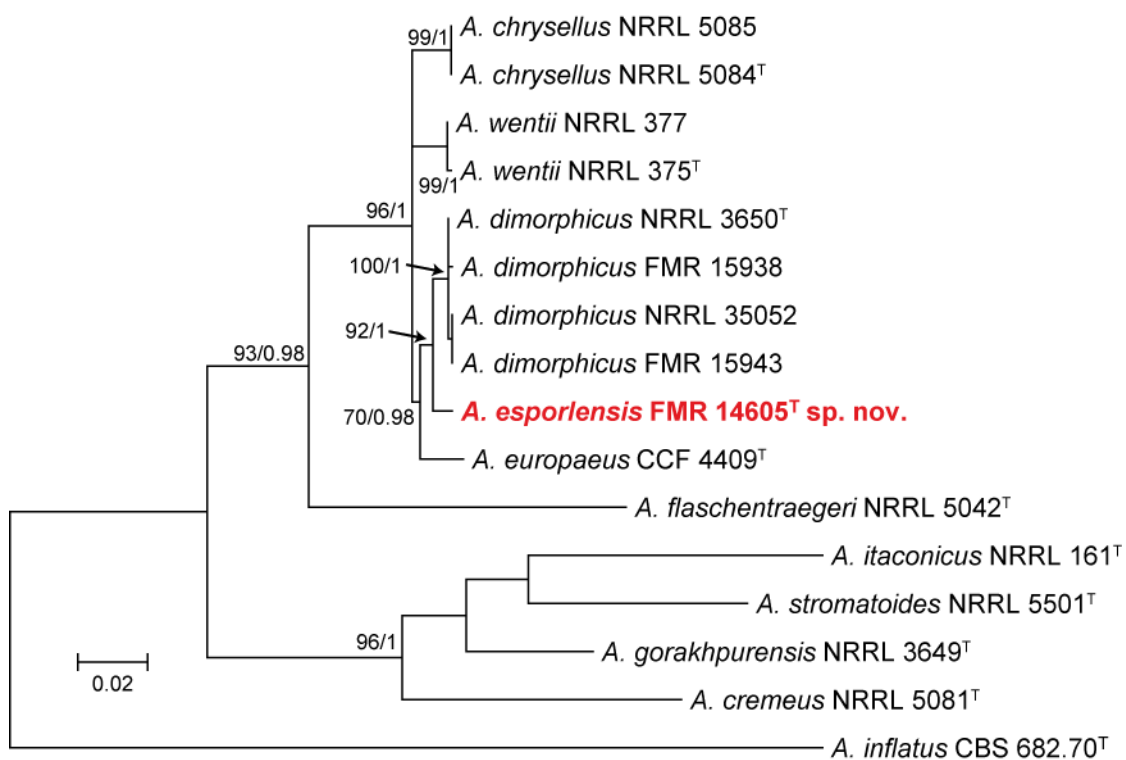
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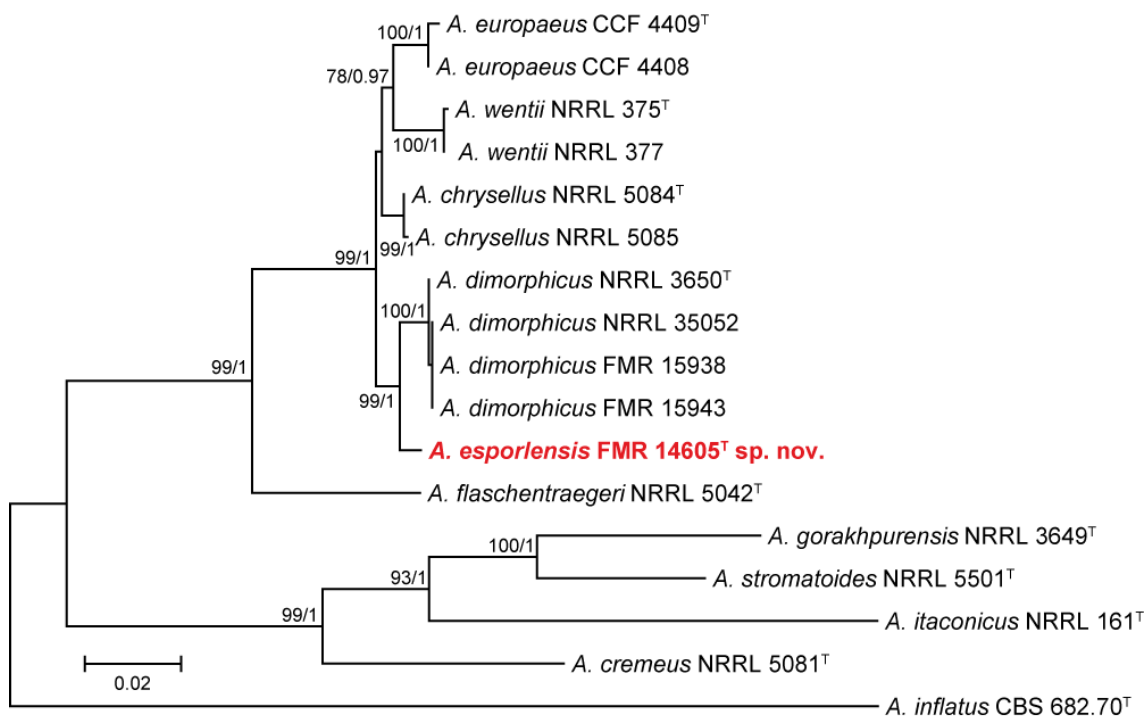
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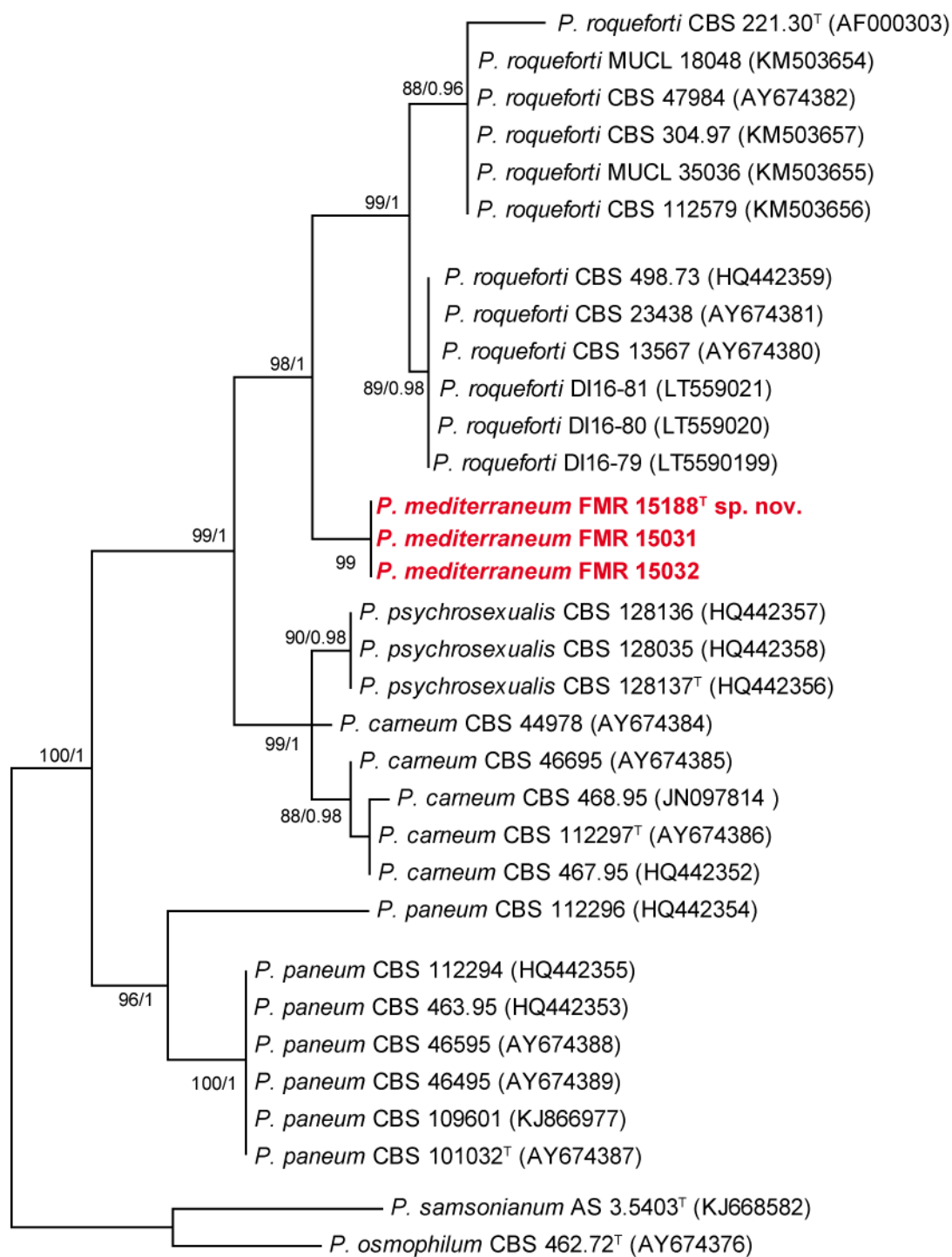
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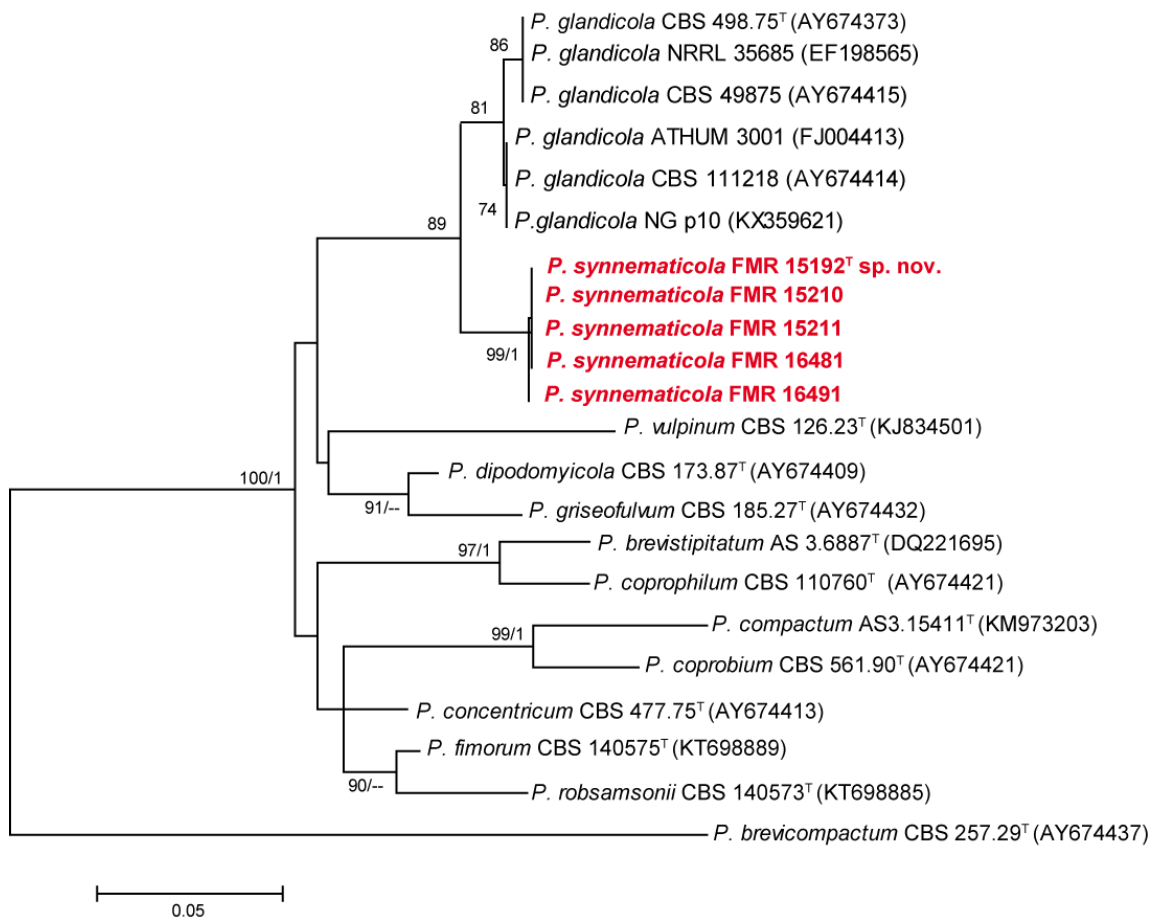
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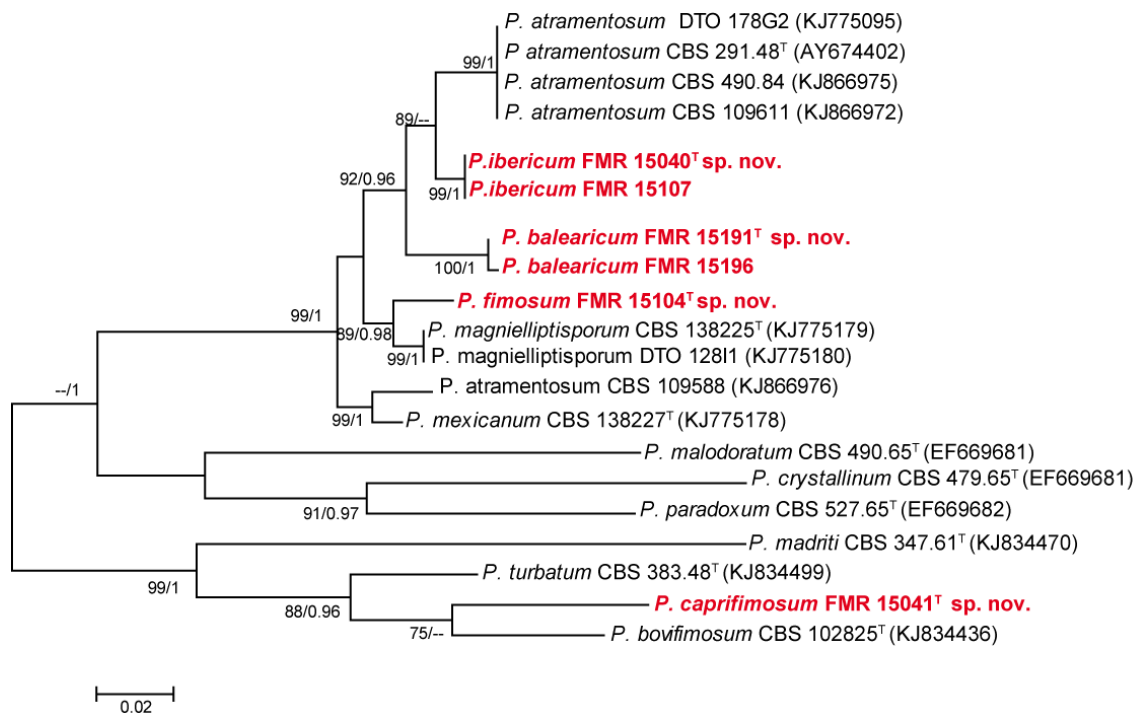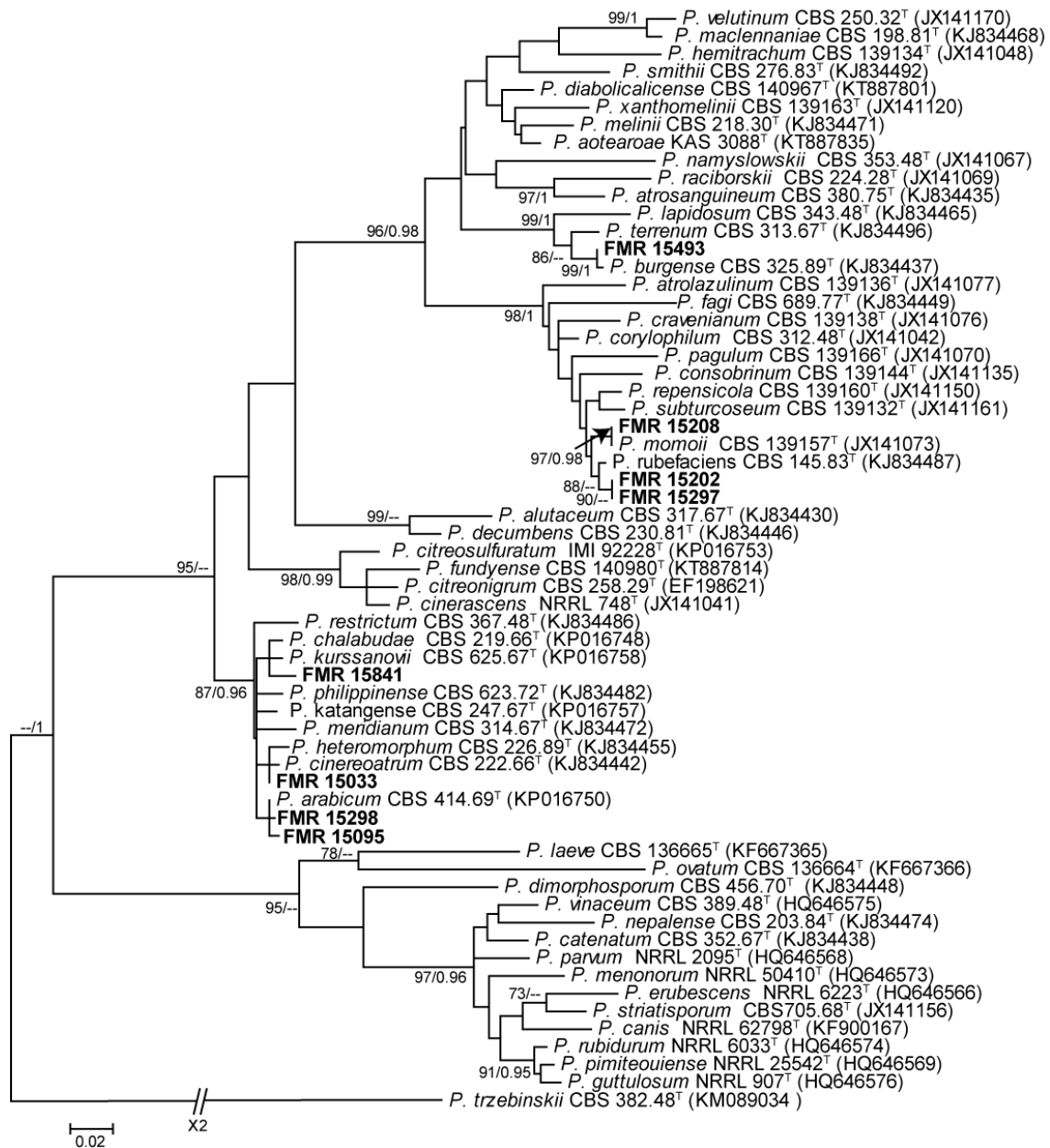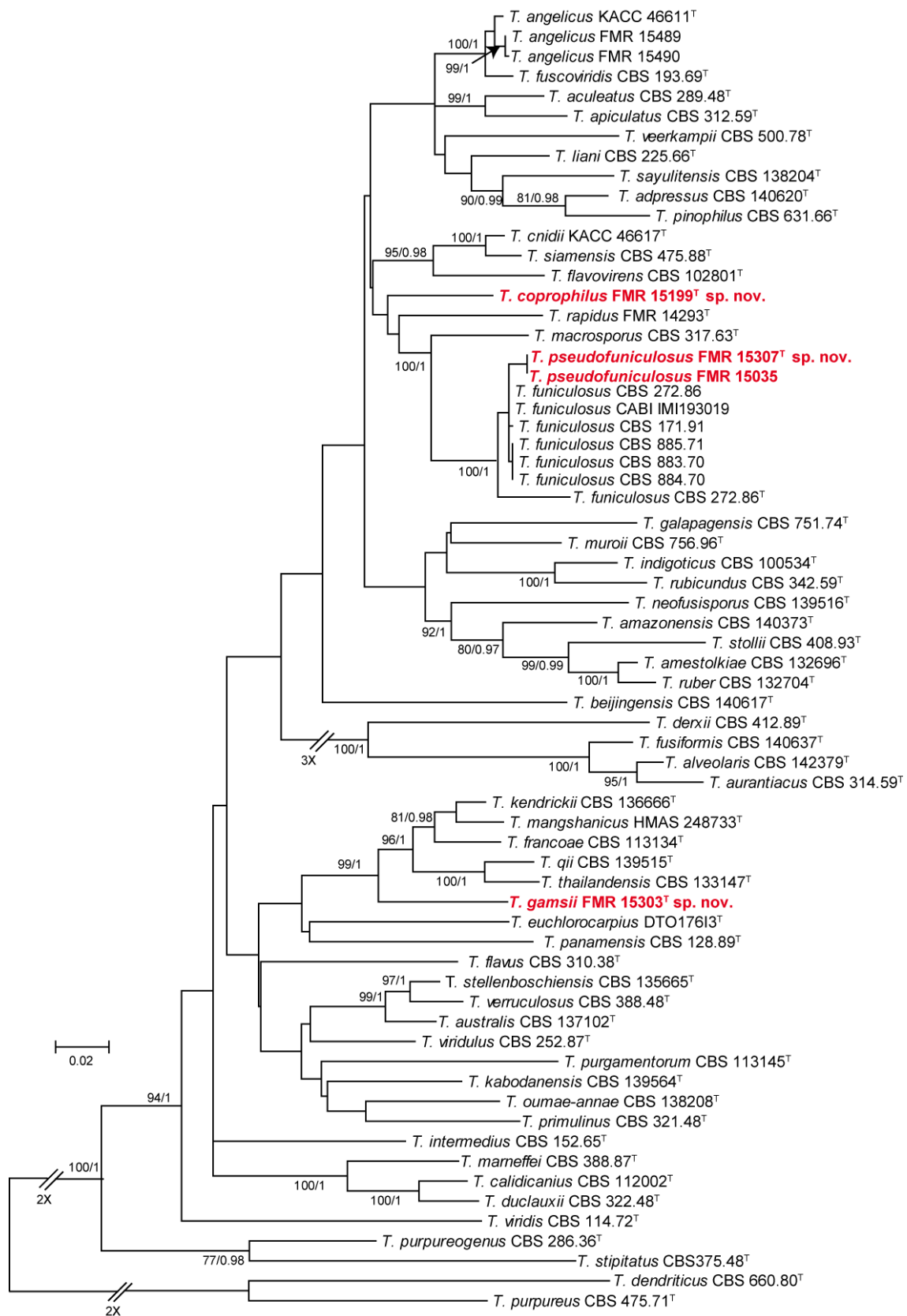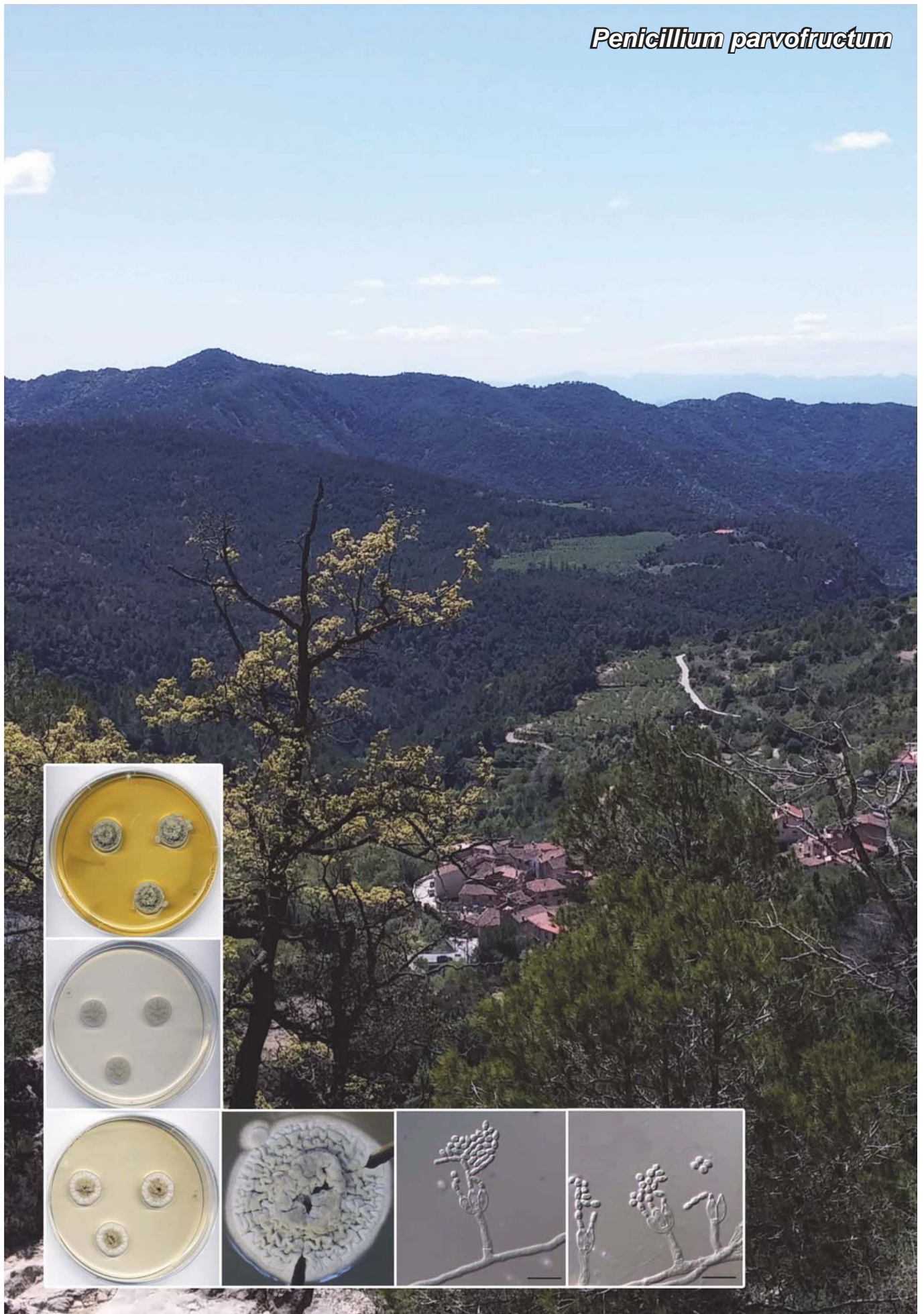**Figure S9.**

Figure S10.

Figure S11.

4.4 Nuevas especies de *Penicillium* de suelo.

Persoonia, Fungal Planet description sheets, 2017.

4.4.1 *Penicillium parvofructum* sp.nov. (38: 352-353).

4.4.2 *Penicillium uruguayense* sp.nov. (39: 322-323).

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Penicillium parvofructum

UNIVERSITAT ROVIRA I VIRGILI
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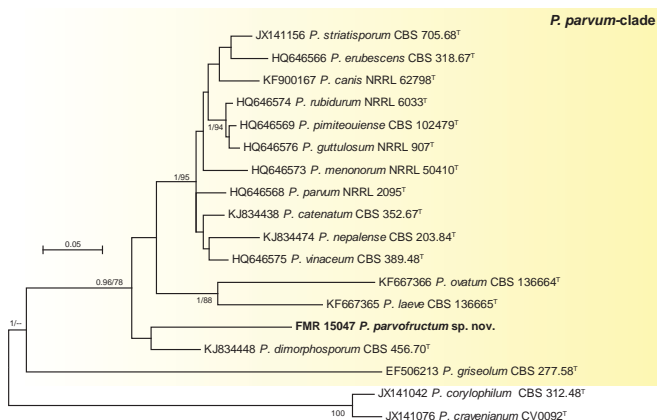# *Penicillium parvofructum* Guevara-Suarez, Cano-Canals, Cano & Stchigel, *sp. nov.*

Etymology. From Latin *parvum-*, small, and *-fructum*, fruit, in reference to the small size of the conidiophores.

Classification — *Aspergillaceae*, *Eurotiales*, *Eurotiomycetidae*, *Eurotiomycetes*.

Mycelium sparse, uncoloured, septate, branched. *Conidiophores* typically monoverticillate; stipes 13–18 × 2.5–3 µm, smooth-walled, hyaline. *Conidiogenous cells* phialidic, solitary to in verticils of up to 3, ampulliform, 8–10 × 1.5–2.5 µm, smooth-walled, hyaline. *Conidia* in chains, broadly ovoid to bacilliform, 3–3.5 × 2–2.5 µm, smooth-walled, hyaline to subhyaline.

Culture characteristics — (after 7 d at 25 °C in darkness). On MEA colonies attaining 13–15 mm diam, flat, with a raised centre and a concave edge, radially sulcate, margins entire, whitish (M.2A1); sporulation absent; exudate and soluble pigment absent. On CYA colonies attaining 17–19 mm diam, similar to those on MEA, but light yellow (M.2A5) centrally and at the margins; sporulation poor; exudate and soluble pigment absents. On YES colonies attaining 15–17 mm diam, cerebriform, of raised centre with a concave edge, margins entire edge, greenish grey (M.1B2) at the margins and centrally greyish yellow (M.1B5); sporulation moderately abundant; exudate and soluble pigment absents. Optimum temperature of growth 30–37 °C (CYA at 30 °C, 21–25 mm diam; CYA at 37 °C, 23–26 mm diam; MEA at 30 °C, 19–20 mm diam; MEA at 37 °C, 18–19 mm diam; YES at 30 °C, 21–25 mm diam; YES at 37 °C, 25–28). Does not grow at/above 40 °C.

Typus. Spain, Tarragona province, Prades, from a forest soil sample, 13 June 2015, *J. Cano-Canals* (holotype CBS H-22733, cultures ex-type FMR 15047 = CBS 141690; ITS, LSU, *BenA*, and *CaM* sequences GenBank LT559091, LT559092, LT627645, and LT627646; MycoBank MB819947).

P. parvum-clade

JX141156 *P. striatisporum* CBS 705.68ᵀ
HQ646566 *P. erubescens* CBS 318.67ᵀ
KF900167 *P. canis* NRRL 62798ᵀ
HQ646574 *P. rubidurum* NRRL 6033ᵀ
HQ646569 *P. pimiteouiense* CBS 102479ᵀ
HQ646576 *P. guttulosum* NRRL 907ᵀ
HQ646573 *P. menonorum* NRRL 50410ᵀ
HQ646568 *P. parvum* NRRL 2095ᵀ
KJ834438 *P. catenatum* CBS 352.67ᵀ
KJ834474 *P. nepalense* CBS 203.84ᵀ
HQ646575 *P. vinaceum* CBS 389.48ᵀ
KF667366 *P. ovatum* CBS 136664ᵀ
KF667365 *P. laeve* CBS 136665ᵀ
FMR 15047 *P. parvofructum* sp. nov.
KJ834448 *P. dimorphosporum* CBS 456.70ᵀ
EF506213 *P. griseolum* CBS 277.58ᵀ
JX141042 *P. corylophilum* CBS 312.48ᵀ
JX141076 *P. cravenianum* CV0092ᵀ

Colour illustrations. Prades, Tarragona, Spain; colonies after 7 d at 25 °C on YES, MEA and CYA, respectively; texture of colonies on YES at 37 °C; conidiophores with conidia. Scale bars = 10 um.

Notes — According to a sequence comparison with available data (ITS, *BenA* and *CaM*), *P. parvofructum* is most closely related with *P. dimorphosporum* in the *P. parvum* clade, section *Exilicaulis* (Visagie et al. 2016).

ITS. Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the ITS sequence are *Penicillium dimorphosporum* (GenBank NR 121271; Identities = 534/553 (97 %), Gaps = 4/553 (0 %)), *Penicillium erubescens* (GenBank NR 121245; Identities = 532/551 (97 %), Gaps = 6/551 (1 %)), and *Penicillium rubidurum* (GenBank NR 121243; Identities = 531/551 (96 %), Gaps = 5/551 (0 %)).
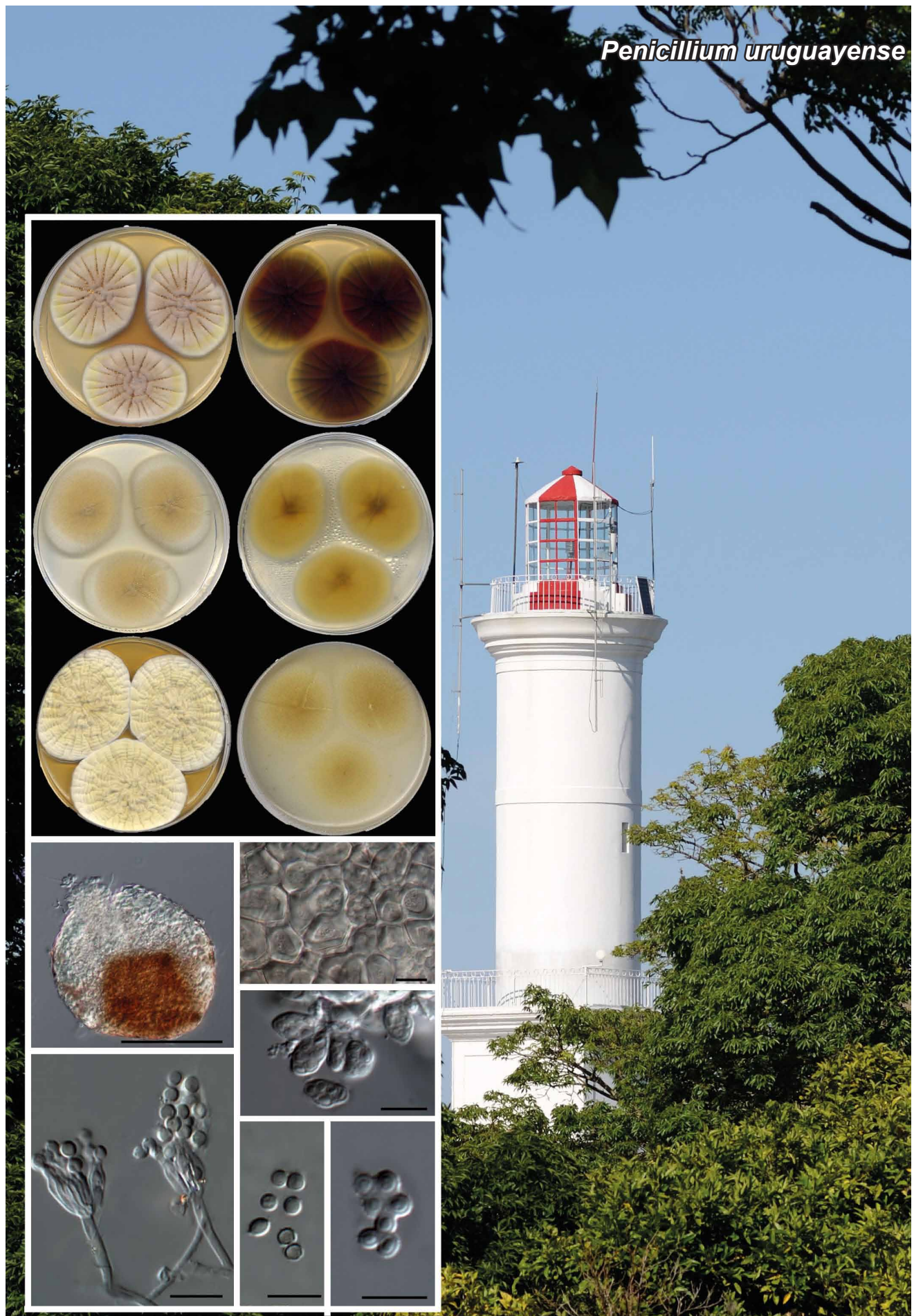
BenA. Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the *BenA* sequence are *Penicillium rubidurum* (GenBank HQ646574; Identities = 408/466 (88 %), Gaps = 4/466 (0 %)), *Penicillium dimorphosporum* (GenBank KF900165; Identities = 383/429 (89 %), Gaps = 5/429 (1 %)), and *Penicillium pimiteouiense* (GenBank KC344994; Identities = 406/467 (87 %), Gaps = 7/467 (1 %)).

CaM. Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using the *CaM* sequence are *Penicillium dimorphosporum* (GenBank KF900176; Identities = 472/544 (87 %), Gaps = 13/544 (2 %)), *Penicillium vinaceum* (GenBank AY678543; Identities = 452/544 (83 %), Gaps = 18/544 (3 %)), and *Penicillium pimiteouiense* (GenBank HQ646580|; Identities = 454/548 (83 %), Gaps = 25/548 (4 %)).

Penicillium parvofructum differs from *P. dimorphosporum* (the species phylogenetically more closely related) in the size of the stipes of the conidiophores (13–18 µm long in *P. parvofructum* vs 15–30 µm long in *P. dimorphosporum*), in the morphology of the conidia (*P. parvofructum* produces hyaline to subhyaline, smooth-walled, broadly ovoid to bacilliform conidia, which turn brown, ornamented and globose with age in *P. dimorphosporum*) and in the optimum temperature of growth (*P. parvofructum* displays the best growth at 37 °C, while the optimum temperature for *P. dimorphosporum* is 25 °C).

Phylogenetic tree built by using *BenA* (401 bp) nucleotide sequences of *Penicillium* section *Exilicaulis* belonging to the *P. parvum* clade, using Maximum-likelihood and Bayesian inference. The tree was built by using MEGA v. 6. Posterior probabilities and/or bootstrap support values higher than 0.95 and 70 %, respectively, are indicated at the nodes. *Penicillium corylophilum* and *Penicillium cravenianum* were chosen as outgroup. Ex-type strains of the different species are indicated with ᵀ. The new species is indicated in **bold**. The alignment and tree is available in TreeBASE (ID 19427).

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e-mail: marcelita726@gmail.com, albertomiguel.stchigel@urv.cat & jose.cano@urv.cat
Julia Cano-Canals, I.E.S Gabriel Ferrater i Soler, Ctra. de Montblanc, 5-9, 43206 Reus, Tarragona, Spain; e-mail: july_cc_98@hotmail.com

Penicillium uruguayense

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Penicillium uruguayense Guevara-Suarez, Dania García, Cano & Gené, *sp. nov.*

Etymology. Name refers to Uruguay, where the fungus was isolated.

Classification — *Aspergillaceae*, *Eurotiales*, *Eurotiomycetidae*, *Eurotiomycetes*.

Colony diam in 7 d (mm) — On CYA: 25 °C 48–50, 30 °C 54–56, 37 °C 39–50; on MEA: 25 °C 44–47, 30 °C 50–52, 37 °C 42–45; on YES: 25 °C 53–55, 30 °C 54–56; 37 °C 50–53; on OA: 25 °C 40–45, 30 °C 50–60, 37 °C 40–45; on DG18: 25 °C 10–12; on CREA: 25 °C 10–12, weak acid production.

Colony characters at 25 °C in 7 d — On CYA, colonies velvety, slightly raised at the centre, radially sulcate, mycelium brownish grey (7C2) to yellow (3A7), margin entire; reverse reddish brown (9F8) to greyish yellow (3B5); conidial sporulation absent; abundant production of cleistothecia; exudate present, consisting of small hyaline to yellow droplets along the sulcus; soluble pigment golden yellow (5B7). On MEA, colonies granular, flat, mycelium white, margin entire; reverse greyish yellow (4B5); abundant cleistothecia; conidial sporulation absent; exudate and soluble pigment absent. On YES, colonies somewhat cerebriform at the centre, radially sulcate towards the periphery, mycelium white; reverse brown (6E8); sporulation absent; exudate and soluble pigment absent. On OA, colonies granular, flat, mycelium white, margin entire; reverse light yellow (2A5); abundant production of cleistothecia; conidial sporulation scarce, with conidial masses dull green; exudate and soluble pigment absent.
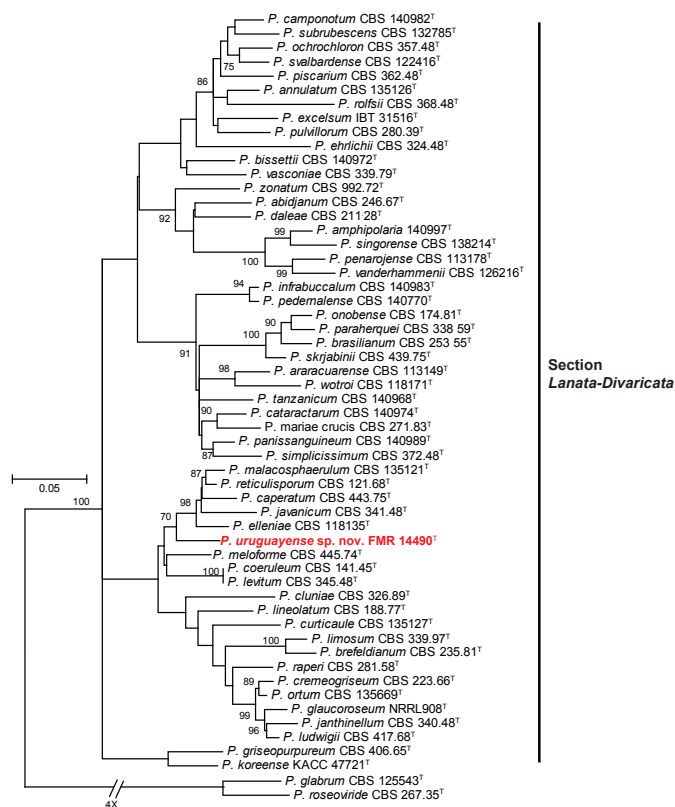
Micromorphology — *Conidiophores* only observed on OA, monoverticillate; stipes 20–50 × 2–2.5 µm, smooth-walled, hyaline. *Phialides* in verticils of 3–4 per verticil, ampulliform, 8–10 × 2–2.5 µm. *Conidia* globose to subglobose, 2.5–3 × 2–3 µm, smooth-walled. *Cleistothecia* ripen after 1–2 wk on CYA, MEA and OA at 25 °C, superficial, globose, 90–210 µm diam, greyish yellow. *Asci* clavate to ovoid, 7–9 × 5–7 µm. *Ascospores* mostly subglobose, 3–4 × 3–3.5 µm, finely spiny, with conspicuously pleated subequatorial crests.

Typus. Uruguay, Colonia Del Sacramento, from soil, 2007, collector unknown (holotype FMR H-14490, cultures ex-type CBS 143247 = FMR 14490; LSU, ITS, *Ben*A and *Ca*M sequences GenBank LT904730, LT904729, LT904699 and LT904698, MycoBank MB822920).

Maximum likelihood (ML) tree obtained from the analysis of ITS, *Ben*A and *Ca*M sequence data. Bootstrap support values above 70 % are shown at the nodes. The alignment included 1 387 bp (ITS 490 bp, *Ben*A 444 bp, *Ca*M 453 pb) and was performed with ClustalW and MUSCLE. Kimura-2-parameter (K2P) with Gamma distribution and invariant sites (G+I) was used as the best nucleotide substitution model. Both the alignment and tree were constructed with MEGA v. 6.06 (Tamura et al. 2013). The name in red is the new species described. [T] = type strain.

Notes — *Penicillium uruguayense* belongs to sect. *Lanata-Divaricata*. Phylogenetically, it is located in a basal branch in the *P. javanicum* clade (Visagie et al. 2015). This clade includes other sexually reproducing species, i.e., *P. caperatum*, *P. elleniae*, *P. javanicum*, *P. malacosphaerulum* and *P. reticulisporum*. *Penicillium uruguayense* is characterised by having good growth at 37 °C on all media tested, by the production of acid on CREA and by its restrictive growth on DG18. Within the *P. javanicum* clade, only *P. elleniae* and *P. caperatum* produce acid on CREA. However, the latter two species have ascospores with two longitudinal flanges or equatorial ridges (Visagie et al. 2015), which are inconspicuous and subequatorial in *P. uruguayense*. In addition, *P. caperatum* has smooth ascospores, whereas in *P. elleniae* and *P. uruguayense* they are spinose. The latter two can be differentiated by their conidial ornamentation, i.e., spinose in *P. elleniae* and smooth in *P. uruguayense*. *Penicillium malacosphaerulum* and *P. reticulisporum* also have smooth conidia, but their ascospores are finely rough-walled with two longitudinal flanges. *Penicillium javanicum* and *P. uruguayense* are the only species in the clade showing ascospores with an inconspicuous longitudinal furrow, but unlike the other related species, *P. javanicum* produces roughened stipes.

Colour illustrations. Lighthouse at Colonia Del Sacramento, Uruguay (image credit: Rosa Cabecinhas and Alcino Cunha); colonies growing on CYA observe, CYA reverse, MEA observe, MEA reverse, YES observe and OA observe, after 15 d at 25 °C; ascoma, peridial wall; asci; conidiophores; conidia; ascospores. Scale bars: 100 µm (ascoma), 10 µm (all others).

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5. DISCUSIÓN GENERAL

Las especies de *Penicillium*, *Talaromyces* y géneros relacionados son hongos filamentosos de distribución mundial capaces de colonizar sustratos de hábitats muy diversos. A su vez, éstos son géneros de gran interés para el hombre, ya que, además de incluir una gran diversidad de especies motivo de estudio desde el punto de vista taxonómico, algunas de ellas tienen un gran impacto económico en la industria alimentaria o farmacéutica. Además, son la base de estudio en campos como la biotecnología, la ecología y la genómica (Visagie *et al.* 2014, Yilmaz *et al.* 2014). Cabe destacar que gran parte de la expansión del número de especies en *Penicillium* y géneros relacionados se remonta a la época de la revolución de los antibióticos, debido principalmente al interés que suscitó el hecho de encontrar cepas productoras de estas moléculas simplemente a partir de muestras de suelo (Visagie *et al.* 2016a).

La identificación clásica de los penicilia, así como de la mayoría de los hongos filamentosos, se basa fundamentalmente en el estudio morfológico de las estructuras sexuales o asexuales productoras de esporas, permitiendo incluso la distinción entre géneros teleomórficos y anamórficos. En el caso de los penicilia, los nombres de *Eupenicillium, Talaromyces* y *Penicillium* eran un reflejo de esa taxonomía morfológica, identificándose los dos primeros cuando se observaban estructuras sexuales y el último nombre genérico para aquellos que mostraban estructuras asexuales. Sin embargo, la biología molecular, en especial la secuenciación del ADNr, puso de manifiesto el carácter artificial de dicha taxonomía y permitió evidenciar la extraordinaria diversidad de especies existentes, incluso en hongos con una gran similitud morfológica como los penicilia. En los últimos años, el estudio filogenético de los hongos ha propiciado la reorganización de su sistemática y ha sido el motivo principal del cambio de criterios sobre la nomenclatura fúngica recogidos en el Código Internacional de Nomenclatura para las algas, los hongos y las plantas (McNeill *et al.* 2012). La biología molecular no sólo ha conducido a cambios nomenclaturales, no siempre bien acogidos por los investigadores (Houbraken *et al.* 2014), sino que ha creado la necesidad imperiosa de revisar la diversidad de especies asociadas a los diversos ámbitos al permitir una correcta identificación de las mismas y, por tanto, de llevar a cabo estudios más fiables sobre su prevalencia y entender mejor el rol de las especies presentes en los diferentes sustratos o ambientes.

Gracias a la reciente revisión molecular de *Penicillium*, *Talaromyces* y géneros afines, y a la estandarización de la metodología para la delimitación y caracterización de sus especies llevada a cabo por Visagie *et al.* (2014a) y Yilmaz *et al.* (2014), actualmente, disponemos de datos fenotípicos (micro y macromorfológicos, fisiológicos

y perfiles de extrolitos) y de secuencias de código de barras de ADN para casi todas las especies descritas y aceptadas en dichos géneros. A pesar de su complejidad, dicha metodología no sólo nos permite realizar una identificación fiable de las especies conocidas, sino también detectar y describir nuevos taxones en este grupo de hongos.

Estudios de diversidad sobre penicilia de origen clínico son prácticamente inexistentes, pero, además su aislamiento en muestras humanas y de veterinaria siempre ha tenido escaso valor clínico. De hecho, a pesar de que son hongos que se aíslan con frecuencia a partir de especímenes humanos o muestras asociadas a entornos intrahospitalarios, a menudo, son descartados como contaminantes ambientales (Garcia-Hermoso *et al*. 2015) y su identificación no va más allá del mero *Penicillium* sp. Sin embargo, existen evidencias de la posible implicación de los penicilia en infecciones humanas, principalmente de origen respiratorio (Garcia-Hermoso *et al.* 2015). No obstante, la identificación de los aislados clínicos sigue siendo mayoritariamente a nivel de género. Ello, sin duda, se debe no sólo a la complejidad de los métodos de estudio *in vitro* propuestos (Visagie *et al*. 2014a, Yilmaz *et al.* 2014), sino también al elevado número de especies existentes en estos géneros, lo que en conjunto dificulta la identificación de estos hongos en los laboratorios clínicos que probablemente adolecen de personal entrenado y de tiempo para llevar a cabo dichos estudios. Por lo tanto, podemos decir que esta tesis es uno de los primeros estudios sobre diversidad de especies asociada a muestras clínicas, en donde se han identificado molecularmente más de 100 aislados de dicho origen. Sin embargo, cabe resaltar que no ha sido posible establecer el rol de estos aislados con las infecciones de los individuos de donde provenían las muestras, principalmente por falta de datos clínicos.

Todos los aislados clínicos recibidos estaban etiquetados como *Penicillium* sp. No obstante, los resultados obtenidos nos permitieron concluir que éstos se distribuían en tres géneros diferentes: *Penicillium, Talaromyces* y *Rasamsonia*. Como era de esperar, las especies de *Penicillium* fueron las más comunes (72%). Entre ellas, podemos destacar a *P. rubens* y a *P. citrinum* como las más frecuentes, aunque a la fecha *P. rubens* no ha sido relacionado como patógeno en humanos o animales. Esto puede deberse a que esta última especie es muy similar y está estrechamente relacionada con *P. chrysogenum*, la cual se ha catalogado en diversas ocasiones como responsable de infecciones cutáneas e invasoras (Hoffman *et al.* 1992, Lyratzopoulos *et al.* 2002), aunque sus cepas bien podrían estar mal identificadas (Houbraken *et al.* 2011).

Dentro del género *Talaromyces,* a pesar de que ninguno de nuestros aislados correspondió a *T. marneffei*, se identificaron especies descritas anteriormente como patógenas oportunistas humanas. Entre ellas, podemos citar a *T. amestolkiae, T. indigoticus, T. piceus, T. purpurogenus, T. radicus, T. ruber, T. rugulosus, T. stollii* y *T. verruculosus*. Es preciso señalar que el 80% de las especies identificadas son de la sección *Talaromyces*, lo cual no es de extrañar en la medida en que ésta es la sección del género con mayor número de especies descritas y la que incluye la mayoría de las especies patógenas reportadas en este género (Yilmaz *et al.* 2014).

Por otro lado, en *Rasamsonia,* se identificaron dos especies, *R. argillacea* (*Geosmithia argillacea*) y *R. eburnea* (*Talaromyces eburneus*). Este género fue recientemente propuesto para acomodar algunas especies termotolerantes y termofílicas de *Geosmithia* y *Talaromyces* (Houbraken *et al.* 2012). Mientras que *R. argillacea* es una de las especies más comunes del género y se ha descrito como responsable de infecciones invasivas en pacientes con enfermedad granulomatosa crónica (De Ravin *et al.* 2011, Machouart *et al.* 2011), *R. eburnea* se ha aislado repetidamente de muestras de hemocultivos, esputo, y líquido de diálisis peritoneal (Houbraken *et al.* 2013). Aunque las especies de *Rasamsonia* se consideran patógenos poco comunes, el número de infecciones, en humanos y animales, ocasionadas por dichos hongos ha ido en aumento en los últimos años, por lo que actualmente se consideran patógenos emergentes en paciente inmunocomprometidos (Doyon *et al.* 2013, Houbraken *et al.* 2013, Matos *et al.* 2015). Uno de los casos más recientes es el presentado por Hong *et al.* (2017), en el cual se describe una infección sistémica causada por *R. aegroticola* en un paciente con trasplante de pulmón.

Éste es el primer estudio sobre sensibilidad antifúngica *in vitro* de un buen número de especies de *Penicillium* y *Talaromyces* con cepas de origen clínico identificadas mediante secuenciación. Nuestros resultados mostraron que los antifúngicos con mejor actividad frente a especies de ambos géneros fueron la terbinafina y las equinocandinas. Sin embargo, estos fármacos no se usan como primera línea de tratamiento en infecciones invasivas (Hu *et al.* 2013). Un ejemplo lo tenemos en el reciente caso de infección diseminada por *P. chrysogenum,* el cual se trató inicialmente sin éxito con anfotericina B pero que remitió totalmente al ser tratado con caspofungina (Avilés-Robles *et al.* 2016). Este hecho concuerda con nuestros resultados al observar que anfotericina B tenía una actividad antifúngica intermedia frente a dichos hongos. La actividad antifúngica de los azoles frente a las especies de *Penicillium* fue muy variable, mientras que para *Talaromyces* obtuvimos CMI altas para

las diferentes especies estudiadas. El tratamiento con voriconazol de tres casos de infección invasiva por *P. oxalicum* resultó ineficaz, siendo el posaconzol el tratamiento alternativo de éxito para todos ellos (Chowdhary *et al*. 2014). Los escasos estudios existentes sobre sensibilidad antifúngica *in vitro* en *Penicillium* y *Talaromyces* hace que el tratamiento de las infecciones ocasionadas por esto hongos sea empírico y, a menudo, poco eficiente (Mok *et al*. 1997, Chowdhary *et al*. 2014, Avilés-Robles *et al*. 2016). Considerando el incremento de casos de infección por este grupo de hongos entre nuestra población, es evidente la necesidad de más estudios en este sentido para intentar paliar los efectos de dichas infecciones desde el principio de su detección.

Aunque los aislados de origen clínico mostraron una diversidad de géneros, secciones y especies que no esperábamos, dicha diversidad fue mucho mayor en los aislados de origen ambiental a partir de los cuales pudimos detectar y caracterizar un buen número de taxones nuevos para la ciencia. En los últimos años, y haciendo uso de los nuevos enfoques dentro de *Penicillium* y *Talaromyces,* se ha descrito un gran número de especies a partir de aislados de origen ambiental (Chen *et al*. 2016, Yilmaz *et al.* 2016, Visagie *et al*. 2016a, Visagie *et al.* 2016b), hecho que los ubica entre los géneros con mayor diversidad dentro de los *Ascomycota*. Sin embargo, la cantidad de especies que podrían llegar a albergar estos géneros todavía es un tema controvertido. Considerando el ritmo de descripción de nuevas especies en estos últimos años y los muchos hábitats inexplorados o poco muestreados existentes en el mundo, se estima que, por ejemplo, tan sólo *Penicillium* podría incluir un total de 1000 especies (Visagie *et al.* 2016b).

En este sentido, en esta tesis se ha explorado la diversidad de especies en *Penicillium* y *Talaromyces* asociadas a muestras de suelo y excrementos, principalmente de herbívoros. Se han estudiado muestras de suelo procedentes de 12 países, la mayoría del continente americano (Argentina, Brasil, Colombia, Costa Rica, EE.UU., España, México, Panamá, Portugal, Uruguay, Venezuela y Vietnam). En total, se identificaron 41 especies de *Penicillium*, incluyendo dos nuevas especies, *P. parvofructum* y *P. uruguayense*. Entre las especies identificadas podemos destacar a *P. crustosum* como la más frecuente, aislada en casi todas las muestras de suelo estudiadas. De hecho, *P. crustosum* ya ha sido descrita como una de las especies más comunes del género, frecuentemente aislada, además, de material vegetal, frutas, nueces y piensos (Sonjak *et al*. 2005). Sin embargo, el número de especies de *Talaromyces* aisladas de suelo ha sido mucho más inferior; tan solo se han

identificado cinco especies de este género, incluyendo la nueva especie propuesta *T. gamsii.*

Tal y como se ha indicado en el apartado 3.2, todas las muestras de excrementos de animales incluidas en nuestro estudio proceden deEspaña, incluyendo algunas de las Islas Baleares y Canarias. El estiércol se considera un sustrato complejo con grandes cantidades de carbohidratos y nitrógeno. La presencia de micronutrientes, la estructura física de este sustrato, su pH y el contenido de humedad variable lo hacen un medio idóneo para el crecimiento de hongos interesantes (Richardson 2001). A pesar de que existen trabajos monográficos sobre hongos coprófilos (Webster 1970, Bell 1983, Richardson 2001, Sarroco 2016), en ninguno de ellos se han aplicado técnicas moleculares para el estudio de su biodiversidad. Considerando las características excepcionales de las heces como sustrato y la ausencia de trabajos taxonómicos con identificación molecular, creímos interesante la caracterización genotípica de especies del orden *Eurotiales* procedentes de dicho sustrato (ver apartado 4.3), aunque en la presente memoria, tal y como se ha podido apreciar, sólo se profundiza en el estudio de los penicilia.

Dentro del género *Penicilium* se han identificado 38 especies, las cuales se encuentran distribuidas en 16 secciones. Entre ellas, se incluyen las siete nuevas especies halladas en heces, aunque sólo una de estas pertenece a la sección *Robsamsonia*, la cual es exclusiva de hongos coprófilos dentro del género (Houbraken *et al.* 2016). Cabe destacar que a partir de dicho sustrato también se han identificado especies de *Penicillium* poco comunes. Entre estas podemos citar a *P. canariense* (sección *Stolkia*), *P. cremeogriseum* (sección *Lanata-Divaricata*), *P. momoii* (sección *Exilicaulis*), o *P. roseoviride* (sección *Aspergilloides*), siendo interesantes por ser especies monotípicas o especies con pocas cepas disponibles en las bases de datos y colecciones de cultivos debido, en muchos casos, a su reciente descripción. En *Talaromyces,* se identificaron siete especies. seis pertenecientes a la sección *Talaromyces* y una a la sección *Trachyspermi*. Como en el caso de las muestras de suelo, el número de especies de *Talaromyces* identificadas sobre heces ha sido mucho menor respecto al número de especies de *Penicillium.* Este hecho nos confirma la gran diversidad y la amplia distribución de las especies de este último género con respecto a las de *Talaromyces*. Sin embargo, es importante destacar que las muestras de excremento parecen ser un buen reservorio de especies interesantes de *Talaromyces* ya que, de las siete identificadas, tres resultaron ser nuevas para la ciencia. Además, el estudio de este sustrato nos ha permitido aislar especies de

Penicillium excluidas del mismo a partir de los estudios filogenéticos llevados a cabo principalmente por Visage *et al.* (2013, 2014a), concretamente nos referimos a *P. nodositatum, P. giganteum* y *P. megasporum.* Dichas especies han sido la base para la propuesta de dos nuevos géneros *Penicillago,* con *Pgo. nodositata* como especie tipo y *Pseudopenicillium,* con *Pse. megasporum* como tipo, *Pse. giganteum* y la nueva especie *Pse. croprophilum* aislada de excrementos de ciervo colectados en Palencia. Nuestros resultados filogéneticos mostraron que ambos géneros pertenecían a la familia *Aspergillaceae,* y que *Pseudopenicillium* estaba relacionado con *Hamigera,* pero con suficiente diferencia genética para ser considerado un género distinto, mientras que *Penicillago* formó un linaje independiente entre los miembros de dicha familia. Morfológicamente, *Pseudopenicillium* difiere de *Hamigera, Penicillium* y *Penicillago* por tener cadenas cortas de conidios con paredes espinosas de color marrón, conidióforos cortos irregularmente ramificados y por la ausencia de estado teleomórfico. Los análisis filogenéticos obtenidos nos permiten concluir que *rpb*2 es el marcador molecular más adecuado para la identificación de especies en *Penicillago* y *Pseudopenicillium.*

De todos modos, en general, nuestros resultados demuestran que *BenA* es un buen marcador para discriminar entre especies de *Penicillium*, *Talaromyces* y géneros relacionados. Se trata de un marcador fácil de amplificar y secuenciar que permite distinguir a su vez la mayoría de las secciones, así como detectar nuevas especies en los géneros estudiados. La filogenia con este marcador concuerda con lo publicado por otros autores, quienes también estudiaron un buen número de aislados de estos géneros (Visagie *et al.* 2014b, Chen *et al.* 2016). Por lo tanto, con los datos aportados en la presente tesis, sin duda, hemos contribuido a tener un mejor conocimiento de la biodiversidad de este grupo de hongos procedentes de sustratos clínicos y ambientales, y de la propia estructura taxonómica de la familia *Aspergillaceae* con la descripción de los nuevos géneros arriba indicados.

6. CONCLUSIONES

La presente tesis ha contribuido al mejor conocimiento de la diversidad de los hongos genéricamente conocidos como penicilia a partir del estudio de muestras tanto de origen clínico como ambiental. La identificación y clasificación de los aislados se ha realizado siguiendo los criterios de una taxonomía polifásica que combina estudios fenotípicos y moleculares para la delimitación de los taxones. Sobre esta base, se proponen 22 nuevos taxones, concretamente nueve de *Penicillium*, ocho de *Talaromyces* y los nuevos géneros *Penicillago* y *Pseudopenicillium*, que incluyen entre ambos una nueva especie, así como tres nuevas combinaciones. Además, se ofrecen los perfiles de sensibilidad antifúngica *in vitro* de las especies más frecuentemente identificadas en nuestro set de aislados clínicos.

Teniendo en cuenta lo expuesto, las principales conclusiones de este trabajo son las siguientes:

Del estudio de aislados de origen clínico concluimos que:

1. Las especies de *Penicillium* con mayor representación en las muestras examinadas pertenecen a las secciones *Chrysogena* (33%), *Citrina* (20%), *Fasciculata* (13%) y *Lanata-Divaricata* (13%), mientras que en *Talaromyces,* el mayor número de especies identificadas pertenecen a una única sección (81%), concretamente la correspondiente a *Talaromyces.*

2. Se identificaron un total de 23 especies de *Penicillium* y 14 especies de *Talaromyces,* siendo en este último cuatro de ellas nuevas para la ciencia (*T. alveolaris T. georgiensis, T. minnesotensis* y *T. rapidus*)*.*

3. Las especies identificadas con mayor frecuencia entre los aislados clínicos fueron *P. rubens* (16.1%), *P. citrinum* (11.9%), *T. amestolkiae* (6%), y *T. purpurogenus* (4.2%), las tres últimas descritas anteriormente como patógenos oportunistas humanos.

4. Se identificaron por primera vez asociadas a muestras clínicas un total de 23 especies. Entre estas podemos desatacar como las más prevalentes a *P. rubens, P. glabrum, T. pinophilus.*

5. En general, la terbinafina y las equinocandinas mostraron una buena actividad antifúngica *in vitro* frente a las especies de *Penicillium* y *Talaromyces* evaluadas, mientras que la anfotericina B mostró tener una actividad antifúngica intermedia.

Del estudio de los aislados obtenidos de muestras de excrementos concluimos que:

6. Las especies de *Penicillium* con mayor representación en las muestras examinadas pertenecen a las secciones *Fasciculata* (20.22%) y *Robsamsonia* (19.1%), esta última exclusiva de especies coprófilas*.* En *Talaromyces,* el mayor número de especies identificadas sigue siendo las pertenecientes a la sección *Talaromyces* (89%)*.*

7. Se ha identificado por primera vez sobre dicho sustrato un total de 50 especies de penicilia, algunas de ellas correspondientes a especies raras de las que prácticamente sólo se contaba hasta la fecha con el material tipo. Entre éstas podemos citar a *P. canariense*, *P. cremeogriseum, P. momoii* o *P. roseoviride*.

8. El mayor número de nuevos taxones propuestos en esta tesis se aislaron a partir de excrementos de herbívoros, lo que sugiere que este sustrato es un buen reservorio de especies de interés taxonómico para *Penicillium*, *Talaromyces*, y otros géneros del orden *Eurotiales*.

9. Se describen un total de 10 nuevas especies para la ciencia: siete de *Penicillium* (*P. balearicum, P. beceitense, P. caprifimosum, P. fimosum, P. ibericum, P. mediterraneum,* y *P. synnematicola*)*,* tres de *Talaromyces* (*T. catalonicus, T. coprophilus,* y *T. pseudofuniculosus*)*.*

10. Se proponen dos géneros nuevos para la familia *Aspergillaceae: Penicillago* tipificado por *Pgo. nodositata* comb. nov. y *Pseudopenicillium* con *Pse. coprobium* como nueva especie y dos nuevas combinaciones, *Pse. giganteum* y *Pse. megasporum*, esta última seleccionada como especie tipo para el género.

Del estudio de aislados obtenidos de suelos:

11. Se proponen *P. parvofructum*, *P. uruguayense* y *T. gamsii* como nuevas especies para la ciencia.

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