Species diversity in *Penicillium* and *Talaromyces* from herbivore dung, and the proposal of two new genera of penicillium-like fungi in *Aspergillaceae*

M. Guevara-Suarez[1,2], D. García[1], J.F. Cano-Lira[1], J. Guarro[1], J. Gené[1]*

[1]Unitat de Micologia, Facultat de Medicina i Ciències de la Salut and IISPV, Universitat Rovira i Virgili, Reus, Spain
[2]Laboratorio de Micología y Fitopatología (LAMFU), Vicerrectoría de Investigaciones, Universidad de los Andes, Bogotá, Colombia

*Corresponding author: josepa.gene@urv.cat

Key words:
Aspergillaceae
coprophilous fungi
Eurotiales
phylogeny
Trichocomaceae

Abstract: Coprophilous fungi are saprotrophic organisms that show 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 of penicillium-like fungi isolated mainly from herbivore dung collected at different Spanish locations. From 130 samples, a total of 104 isolates were obtained, and 48 species were identified. Preliminary identifications were based on morphology and partial β-tubulin (*tub2*) gene sequences. Putative new taxa were characterized by a multi-gene sequencing analysis testing the *tub2*, the internal transcribed spacer rDNA (ITS), calmodulin (*cmdA*), 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 (GCPSR) method, we propose the new genera *Penicillago* (for *Penicillium nodositatum*) and *Pseudopenicillium* (for *Penicillium megasporum* and *P. giganteum*) in the family *Aspergillaceae,* and 11 new species, including seven *Penicillium*, three *Talaromyces* and one *Pseudopenicillium*. A lectotype and epitype are designed for *Penicillium nodositatum*. Our results show that the species diversity of penicillium-like fungi on herbivore dung has not been widely studied and that this substrate seems to be a good reservoir of interesting Eurotialean fungi.

INTRODUCTION

Herbivore dung is a complex substrate that has a high amount of readily available carbohydrates with high nitrogen content, water-soluble vitamins, growth factors and mineral ions (Bell 1983). Due to the nutritional properties, together with its physical structure, pH, and the varying moisture content, it is an excellent substrate for a great diversity of fungi to grow (Bell 1983, Richardson 2001, Herrera *et al.* 2011).

Ascomycetes accounts for the largest number of species found on dung from herbivorous animals; genera such as *Ascobolus*, *Lasiobolus, Podospora* or *Thelebolus* practically only occur on this substrate (Webster 1970, Bell 1983, Richardson 2001). On the contrary, other ascomycetes like Eurotialean penicillium-like fungi are not considered predominantly coprophilous (Krug *et al.* 2004), but they can be commonly found on this substrate when its soluble materials are exhausted, and its relative humidity has reduced to below 85 % (Kuthubutheen & Webster 1986). However, species diversity of this latter group has been poorly studied on herbivore dung. Although Seifert *et al.* (1983) provided a list of near 20 *Penicillium* species isolated from animal faeces, their identification was based exclusively on phenotypic features.

Traditionally, based on similarities in ecology, morphology and extrolite production, most of the *Penicillium* species considered coprophilous (*i.e. P. brevistipitatum*, *P. clavigerum*, *P. concentricum*, *P. coprobium*, *P. coprophilum*, *P. formosanum*, *P. glandicola* and *P. vulpinum*) were classified in series *Claviformia*

(Seifert & Samson 1985, Frisvad & Samson 2004, Wang & Zhuang 2005). Currently, the new concept of the genus, which is based on molecular taxonomy, coprophilous penicillia belongs in section *Robsamsonia* (Houbraken *et al.* 2016), which also includes other species previously assigned to series *Urticicolae* (Frisvad & Samson 2004). Furthermore, other species of *Penicillium* previously found on dung currently belong to the genus *Talaromyces* (Houbraken *et al.* 2016). Coprophilous species reported in this latter genus are *T. atroroseus, T. helicus, T. flavus, T. muroii* and *T. trachyspermus*, and two others *T. dupontii* and *T. emersonii*, which have been transferred to respectively, *Thermomyces* and *Rasamsonia* (Masunga *et al.* 2006, Frisvad *et al.* 2013, Yilmaz *et al.* 2014).

The taxonomy of penicillium-like fungi has changed dramatically due to phylogenetic data. Houbraken & Samson (2011) demonstrated that *Penicillium* is phylogenetically more-closely related to *Aspergillus* (family *Aspergillaceae*) than to *Talaromyces* (family *Trichochomaceae*). In the last decade, the species of these genera have been delineated on the basis of a polyphasic approach that has mainly included the evaluation of morphological and multi-locus sequence analyses testing with the internal transcribed spacer region rDNA (ITS), and the β-tubulin (*tub2*), calmodulin (*cmdA*), and/or the DNA-dependent RNA polymerase II largest subunit (*rpb2*) genes (Peterson 2008, Houbraken *et al.* 2014a, Samson *et al.* 2014, Visagie *et al.* 2014b, Yilmaz *et al.* 2014). In addition, *tub2* was recommended as an identification marker in *Penicillium* (Visagie *et al.* 2014b) and *Talaromyces* (Yilmaz *et al.* 2014).

The use of this already generalised approach has proven to be able to discriminate very close species and to discover and characterise new taxa (Visagie *et al.* 2014b, Guevara-Suarez *et al.* 2017, Barbosa *et al.* 2018).

Given the scarcity of data on species isolated from dung and identified by modern techniques, we consider this the perfect setting for discovering new taxa. Therefore, the present work focuses on the study of the species diversity of penicillium-like fungi from herbivore dung samples collected across Spain. Identification was made using both morphology and by comparing multi-locus sequences with a database of ex-type and verified reference strains. For multisequence analyses, the Genealogical Phylogenetic Species Recognition (GCPSR) method was applied to data in support of discovering putative new species (Taylor *et al.* 2000).

MATERIALS AND METHODS

Sampling and fungal isolation

Dung samples were collected in 2016 and 2017 from Spanish regions that have different climates and biodiversity, such as Andalusia, the Balearic and Canary Islands, Cantabria, Castilla-Leon, Catalonia, Extremadura and Galicia. The dung collected was mostly from herbivorous animals, such as rabbits, sheep, deer, goats, cattle and horses. However, some samples from other animals, such as wild pigs or foxes, and even soil mixed with dung was also studied. The samples were placed in individual paper or plastic bags and processed no later than 3 d 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 d. For the dilution series, approximately one gram of dung or associated soil was diluted 1:10 (w/v) 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, 1 000 mL distilled water). All media were supplemented with dieldrin in dimethyl-ketone (1 %). Petri dishes were incubated at room temperature for up to 30 d. The moist chambers and Petri dishes were examined at regular intervals with the aid of a stereo-microscope, and conidia from sporulating colonies were transferred to PDA supplemented with chloramphenicol.

Isolates identified morphologically as penicillium-like were preserved and deposited in the culture collection of the Faculty of Medicine, Reus (FMR). Cultures of interesting species as well as type material and cultures of the new species were deposited at the Westerdijk Fungal Biodiversity Institute (CBS, Utrecht, the Netherlands). Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al.* 2004).

Molecular identification and phylogenetic analysis

Isolates were cultured on PDA or malt extract agar (MEA; Difco, Detroit, USA) for 7–14 d at 25 °C. DNA was extracted using the FastDNA® kit protocol (MP Biomedicals, Solon, OH) and for the lysis step done with a FastPrep® FP120 cell disrupter (Thermo Savant, Holbrook, NY).

Preliminary species identifications were carried out BLASTing *tub2* DNA sequences using GenBank. In the case of putative new species, the ITS region, including the 5.8S rRNA gene, and fragments of *cmdA* and/or *rpb2* genes were also amplified and sequenced. The primer pairs used were: ITS5/ITS4 for ITS (White *et al.* 1990), Bt2a/Bt2b for *tub2* (Glass & Donaldson 1995), CMD5/CMD6 for *cmdA* (Hong *et al.* 2006), and RPB2-5F/RPB2-7Cr for *rpb*2 (Liu *et al.* 1999). The amplification protocol and PCR conditions were performed using methods and primers previously described (Peterson 2008, Houbraken & Samson 2011, Samson *et al.* 2014, Visagie *et al.* 2014b, Yilmaz *et al.* 2014). The amplified products were purified and sequenced at Macrogen Corp. Europe (Amsterdam, the Netherlands) with a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA). Consensus sequences were obtained using SeqMan v. 7.0.0 (DNASTAR, Madison, WI). Newly generated sequences and their GenBank/EMBL accession numbers are summarized in Table 1.

Sequences were retrieved from GenBank taking into account the last update of the database of the International Commission of *Penicillium* and *Aspergillus* (http://www.aspergilluspenicillium.org), which includes all the accepted species for *Penicillium* and *Talaromyces* (Samson *et al.* 2014, Visagie *et al.* 2014b, Yilmaz *et al.* 2014). Single and concatenated phylogenetic analyses were performed to delineate putative new species and the phylogenies corresponding to each section of those genera were properly reconstructed.

Data sets for each locus were aligned individually using ClustalW (Thompson *et al.* 1994), in MEGA v. 6.0 software (Tamura *et al.* 2013), refined with MUSCLE (Edgar 2004) under the same platform, and manually adjusted when needed. Larger alignments including different sections of each genus was performed using the MAFFT tool in the EMBL-EBI Web Services portal (https://www.ebi.ac.uk/Tools/msa/mafft/) and manually adjusted in MEGA v. 6.0. Phylogenetic reconstructions by maximum likelihood (ML) and Bayesian inference (BI) were carried out using MEGA v. 6.0 and MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003, Tamura *et al.* 2013), respectively. For ML analyses, the trees were inferred using Nearest-Neighbour-Interchange as a heuristic method and gaps were treated as partial deletion with a 95 % site coverage cut-off. Phylogenetic support for internal branches was assessed by 1 000 ML bootstrapped pseudoreplicates and bootstrap support (bs) ≥ 70 % was considered significant. The BI analyses were performed using five million Markov chain Monte Carlo (MCMC) generations, with two runs (one cold and three heated chains) and samples were stored every 1 000 generations. The 50 % majority-rule consensus tree and posterior probability values (pp) were calculated after discarding the first 25 % of the samples. A pp value ≥ 0.95 was considered significant. The best substitution models for each data partition were estimated using jModelTest v. 2.1.3 according to the Akaike criterion (Darriba *et al.* 2012, Guindon & Gascuel 2003). The length, number of variable and phylogenetic informative sites, and substitution models for each data partition are summarized in Table 2. The resulting trees were plotted using FigTree v. 1.3.1 (http://tree.

Table 1. Isolates of *Penicillium*, *Talaromyces* and related genera included in the study and their GenBank/EMBL accession numbers.

Genus/Species[1]	Section	Collection numbers[2]	Substrate and Origin	GenBank/EMBL accession numbers			
				ITS	**tub2**	**cmdA**	**rpb2**
P. arabicum	*Exilicaulis*	FMR 15298	Dung, Castile and Leon	–	LT898226	–	–
		FMR 15095	Dung, Catalonia	–	LT898225	–	–
P. atramentosum	*Paradoxa*	FMR 15309	Dung, Castile and Leon	–	LT898224	–	–
		FMR 15046	Dung, Catalonia	–	LT898221	–	–
		FMR 15092	Dung, Catalonia	–	LT898222	–	–
		FMR 15102	Dung, Catalonia	–	LT898223	–	–
P. balearicum	*Paradoxa*	FMR 15191[T] = CBS 143044	Dung, Balearic Islands	LT899762	LT898227	LT899758	LT899760
		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	–	–
		FMR 15313	Dung, Castile and Leon	–	LT898231	–	–
P. brasilianum	*Lanata-Divaricata*	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	–	–
		FMR 15647	Dung, Castile and Leon	–	LT898243	–	–
		FMR 15094	Dung, Catalonia	–	LT898239	–	–
		FMR 15486	Dung, Galicia	–	LT898240	–	–
		FMR 15520	Dung, Galicia	–	LT898241	–	–
P. concentricum	*Robsamsonia*	FMR 15195	Dung, Balearic Islands	–	LT898245	–	–
		FMR 15840	Dung, Castile and Leon	–	LT898246	–	–
P. coprobium	*Robsamsonia*	FMR 15201	Dung, Balearic Islands	–	LT898247	–	–
		FMR 15311	Dung, Castile and Leon	–	LT898248	–	–
P. coprophilum	*Robsamsonia*	FMR 15187	Dung, Balearic Islands	–	LT898249	–	–
P. cremeogriseum	*Lanata-Divaricata*	FMR 15487	Dung, Galicia	–	LT898250	–	–
		FMR 15488	Dung, Galicia	–	LT898251	–	–
P. crustosum	*Fasciculata*	FMR 15185	Dung, Balearic Islands	–	LT898259	–	–
		FMR 15186	Dung, Balearic Islands	–	LT898260	–	–
		FMR 15189	Dung, Balearic Islands	–	LT898261	–	–
		FMR 15194	Dung, Balearic Islands	–	LT898262	–	–
		FMR 15197	Dung, Balearic Islands	–	LT898263	–	–
		FMR 15200	Dung, Balearic Islands	–	LT898264	–	–
		FMR 15213	Dung, Balearic Islands	–	LT898265	–	–
		FMR 15034	Dung, Catalonia	–	LT898252	–	–
		FMR 15036	Dung, Catalonia	–	LT898253	–	–
		FMR 15037	Dung, Catalonia	–	LT898254	–	–
		FMR 15042	Dung, Catalonia	–	LT898255	–	–
		FMR 15043	Dung, Catalonia	–	LT898256	–	–
		FMR 15045	Dung, Catalonia	–	LT898257	–	–

Table 1. (Continued).

Genus/Species[1]	Section	Collection numbers[2]	Substrate and Origin	GenBank/EMBL accession numbers			
				ITS	*tub2*	*cmdA*	*rpb*2
		FMR 15098	Dung, Catalonia	–	LT898258	–	–
		FMR 15494	Dung, Galicia	–	LT898266	–	–
P. cvjetkovicii	*Cinnamopurpurea*	FMR 15310	Dung, Castile and Leon	–	LT898267	–	–
P. expansum	*Penicillium*	FMR 15097	Dung, Catalonia	–	LT898268	–	–
		FMR 15484	Dung, Galicia	–	LT898269	–	–
P. fimosum	*Paradoxa*	FMR 15104[T] = CBS 142991	Dung, Catalonia	LT970836	LT898273	LT970837	
P. flavigenum	*Chrysogena*	FMR 15096	Dung, Catalonia	–	LT898270	–	–
P. frequentans	*Aspergilloides*	FMR 15193	Dung, Balearic Islands	–	LT898271	–	–
		FMR 15212	Dung, Balearic Islands	–	LT898272	–	–
P. glabrum	*Aspergilloides*	FMR 15184	Dung, Balearic Islands	–	LT898274	–	–
		FMR 15190	Dung, Balearic Islands	–	LT898275	–	–
		FMR 15209	Dung, Balearic Islands	–	LT898276	–	–
P. griseofulvum	*Robsamsonia*	FMR 15203	Dung, Balearic Islands	–	LT898280	–	–
		FMR 15204	Dung, Balearic Islands	–	LT898281	–	–
		FMR 15207	Dung, Balearic Islands	–	LT898282	–	–
		FMR 15314	Dung, Castile and Leon	–	LT898283	–	–
		FMR 15029	Dung, Catalonia	–	LT898277	–	–
		FMR 15030	Dung, Catalonia	–	LT898278	–	–
		FMR 15093	Dung, Catalonia	–	LT898279	–	–
P. ibericum	*Paradoxa*	FMR 15040[T] = CBS 142992	Dung, Catalonia	LT899782	LT898285	LT899766	LT899800
		FMR 15107	Soil, Galicia	LT899783	LT898286	LT899767	LT899801
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	LT899784	LT898291	LT899768	LT899802
		FMR 15031 = CBS 142755	Dung, Catalonia	LT899785	LT898289	LT899769	LT899803
		FMR 15032	Dung, Catalonia	LT899786	LT898290	LT899770	LT899804
P. momoii	*Exilicaulis*	FMR 15208	Dung, Balearic Islands	–	LT898292	–	–
P. murcianum	*Canescentia*	FMR 15304	Dung, Andalusia	–	LT898293	–	–
		FMR 15305	Dung, Andalusia	–	LT898294	–	–
		FMR 15308	Dung, Andalusia	–	LT898295	–	–
		FMR 15491	Dung, Galicia	–	LT898296	–	–
		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	–	–
		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	–	–
		FMR 15521	Dung, Galicia	–	LT898305	–	–
P. synnematicola	*Robsamsonia*	FMR 15192[T] = CBS 142669	Dung, Balearic Islands	LT898167	LT898172	LT898137	LT898142
		FMR 15210	Dung, Balearic Islands	LT898168	LT898173	LT898138	LT898143
		FMR 15211	Dung, Balearic Islands	LT898169	LT898174	LT898139	LT898144

Table 1. (Continued).

Genus/Species[1]	Section	Collection numbers[2]	Substrate and Origin	GenBank/EMBL accession numbers			
				ITS	*tub2*	*cmdA*	*rpb2*
		FMR 16481 = CBS 143045	Soil, Catalonia	LT898170	LT898175	LT898140	LT898145
		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
	-	FMR 16442	Dung, Extremadura	LT899788	LT898313	LT899772	LT899806
Pse. cervifimosum	-	FMR 15299[T] = 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
		FMR 15490	Dung, Galicia	LT899792	LT898317	LT899774	LT899810
T. catalonicus	*Trachyspermi*	FMR 16441[T] = CBS 143039	Dung, Catalonia	LT899793	LT898318	LT899775	LT899811
T. coprophilus	*Talaromyces*	FMR 15199[T] = CBS 142756	Dung, Balearic Islands	LT899794	LT898319	LT899776	LT899812
T. muroii	*Talaromyces*	FMR 15496	Dung, Galicia	–	LT898321	–	–
T. pseudofuniculosus	*Talaromyces*	FMR 15307[T] = CBS 143041	Dung, Andalusia	LT899796	LT898323	LT899778	LT899814
		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	–	–

[1]*P.* = *Penicillium*; *Pgo.* = *Penicillago*; *Pse.* = *Pseudopenicillium*; *T.* = *Talaromyces*; new taxa proposed in this study are in **bold**; [T] = ex-type strain.

[2]CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; FMR: Facultat de Medicina i Ciències de la Salut, Reus, Spain.

bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator CS3. The alignments and trees were deposited in TreeBASE (www.treebase.org) under the submission number 21345.

## Phenotypic characterisation

Phenotypic characterization was carried out using standard growth conditions suggested by Visagie *et al.* (2014b) and Yilmaz *et al.* (2014). Briefly, isolates were cultured onto MEA 2 % (Samson *et al.* 2010), oatmeal agar (OA; Samson *et al.* 2010), Czapek yeast autolysate agar (CYA; Pitt 1979), yeast extract sucrose agar (YES; Frisvad 1981), creatine sucrose agar (CREA; Frisvad 1981) and dichloran 18 % glycerol agar (DG18; Hocking & Pitt 1980), incubated at 25 °C for 7 d in darkness. Colony growth rates were also measured after 7 d at 30 and 37 °C on MEA, CYA, YES and OA. Colours used for descriptions refer to Kornerup & Wanscher (1978). Microscopic features were examined on colonies grown on MEA after 1–2 wk, mounted on slides with Shear's solution or 60 % lactic acid, and excess conidia removed with 70 % ethanol. Microscopic features were captured with a Zeiss Axio-Imager M1 light microscope using Nomarski differential interference contrast and phase-contrast optics (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity x digital camera and DeltaPix InSight v. 5.3.11 software. Photoplates were assembled from separate photographs using PhotoShop CS3.1.

RESULTS

During the study, 130 dung samples were processed and 104 isolates of penicillium-like fungi were recovered, including three from soil (Table 1). Preliminary identification based on morphology and *tub2* sequences showed that most of the isolates belonged to *Penicillium* (n = 91; 87.5 %) and a few to *Talaromyces* (n = 9; 8.65 %), while four of them (3.85 %) could not be assigned to any genus despite exhibiting a penicillium-like morphology. To maximize the quality of the analyses, three separate *tub2* alignments were done for the different genera studied. We also carried out additional analyses of single-gene ITS, *cmdA* and *rpb2* and combined datasets from the different genes, corresponding to the genera or sections where putative new species were included (Table 2). The topology of the trees was similar for both methods, with ML trees used to represent the results. Bootstrap and BI posterior probability values are indicated on relevant branches. Final identification of the isolates, resulting from the phylogenetic and phenotypic characterization, is shown in Table 1.

Penicillium phylogeny

The phylogenetic tree based on the *tub2* locus with the 91 isolates of *Penicillium* is shown in Fig. 1. The aligned dataset was 404 bp long, with 257 variable sites and 237 phylogenetic informative. The best substitution model for ML was K2+G, and for BI it was GTR+G+I. In general, the topology of the phylogenetic tree showed well-delimitated sections.
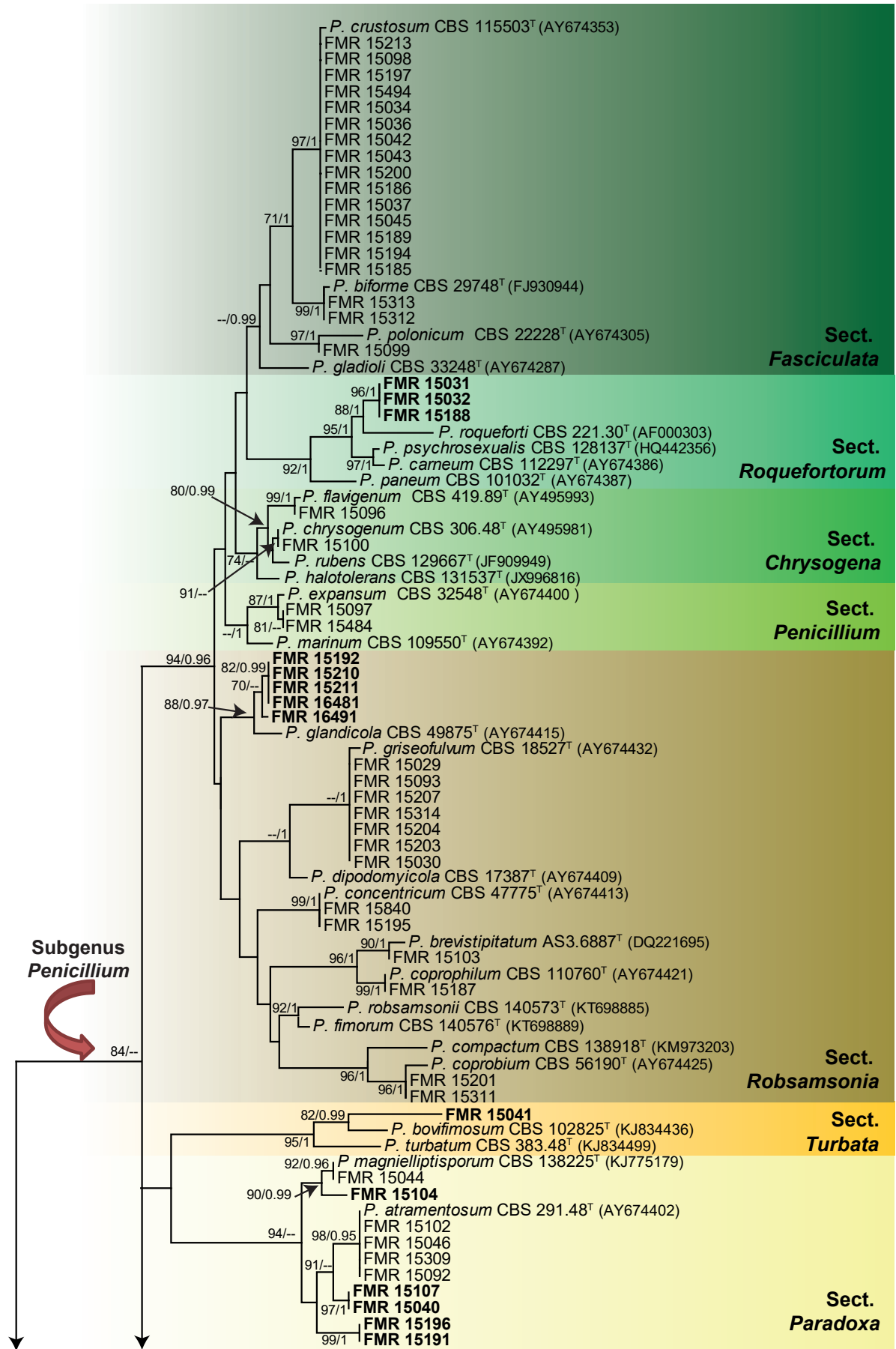
Fig. 1. ML tree of *Penicillium* inferred from *tub2* including the sections recovered from dung in this work. Branch lengths are proportional to phylogenetic distance. Some of the larger branches were condensed, with the proportions showed above the parallel diagonal lines. Bootstrap support values/Bayesian posterior probability scores above 69 %/0.94 are indicated on the nodes. Isolates corresponding to new species are shown in bold. Between parentheses, GenBank accession numbers of *tub2* sequences. The tree is rooted to *Talaromyces flavus* CBS 310.38 and *Talaromyces duclauxii* CBS 322.48. [T] = ex-type strain.
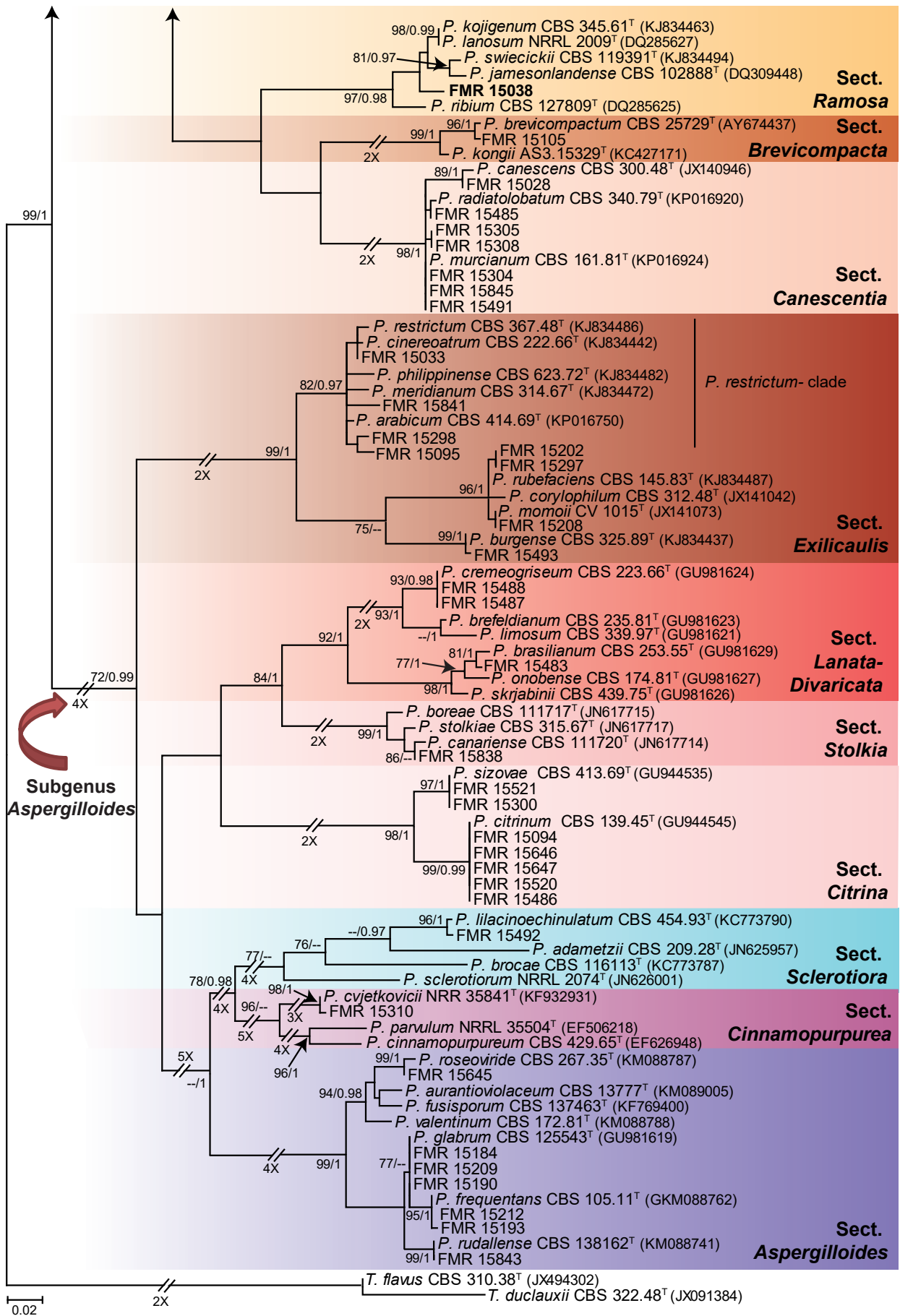
Fig. 1. (Continued).

Table 2. Overview and details used for phylogenetic analyses in sections of *Penicillium* and *Talaromyces*, and related taxa with taxonomic novelties proposed in the study.

| | | *Penicillium* | | | | *Talaromyces* | | Miscellaneous *Aspergillaceae* |
		Turbata and Paradoxa	*Ramosa*	*Robsamsonia*	*Roquefortorum*	*Talaromyces*	*Trachyspermi*	
ITS dataset	Length (bp)	504	485	428	506	360	474	440
	Pvar	29	47	207	24	72	119	124
	Pi	18	16	18	13	45	86	104
	Model*	K80+G	GTR+I	K80+G	TPM2uf+I	TrN+I+G	TIM2+I+G	TrN+I+G
tub2 dataset	Length (bp)	412	387	352	409	370	398	401
	Pvar	151	112	122	68	178	158	223
	Pi	100	64	67	29	140	104	187
	Model*	TIM2ef+G	TIM2ef+G	GTR+G	TIM1ef	HKY+I+G	TPM3uf+G	HKY+I+G
cmdA dataset	Length (bp)	492	544	486	501	475	482	–
	Pvar	195	223	164	50	250	247	–
	Pi	88	92	96	8	205	176	–
	Model*	TIM2+G	TIM2+G	GTR+I	TPM1+G	HKY+I+G	TPM1+I+G	–
rpb2 dataset	Length (bp)	915	–	804	915	–	–	956
	Pvar	239	–	243	106	–	–	463
	Pi	159	–	127	55	–	–	375
	Model*	TrN+I+G	–	GTR+I	TIM3ef+I	–	–	TrN+I+G
Concatenated dataset	Length (bp)	2323	1416	2068	2331	1205	1354	1797
	Pvar	614	382	574	248	503	524	810
	Pi	365	172	303	105	395	366	666

Pvar = variable sites; Pi = phylogenetic informative sites; * = substitution model for Bayesian inference

The analysis distributed the *Penicillium* isolates in at least 38 species, belonging to 17 sections. The two major clades coincided with the two subgenera currently accepted in *Penicillium*, *i.e. Aspergilloides* and *Penicillium* (Houbraken & Samson 2011). Subgenus *Penicillium* (84 % bs/-- pp) is represented by the sections that according to the numbers of isolates obtained are: *Fasciculata* and *Robsamsonia* with 18 isolates (19.78 %) each, followed by *Paradoxa* (n = 10; 11 %), *Canescentia* (n = 7; 7.69 %)*, Roquefortorum* (n = 3; 3.29 %), *Chrysogena* (n = 2; 2.19 %), *Penicillium* (n = 2; 2.19 %)*,* and *Brevicompacta, Ramosa,* and *Turbata* each with one isolate (1.09 %). Subgenus *Aspergilloides* (72 % bs/0.99 pp) is represented by the sections *Exilicaulis* (n = 8; 8.79 %)*, Aspergilloides* (n = 7; 7.69 %)*, Citrina* (n = 7; 7.69 %)*, Lanata-Divaricata* (n = 3; 3.29 %)*,* and *Sclerotiora* and *Stolkia* each with one isolate (1.09 %).

Isolates of section *Fasciculata* (--% bs/0.99 pp) were identified as *P. crustosum* (n = 15), *P. biforme* (n = 2), and *P. polonicum* (n = 1), the former being the most prevalent species (14.42 %) found in the study. In fact, *P. crustosum* is a relatively common species commonly isolated from nuts, meat, cheese, feeds, vegetables, and pomaceous and stone fruits (Sonjak *et al.* 2005).

In section *Roquefortorum* (92 % bs/1 pp) three isolates (FMR 15031, FMR 15032, FMR 15188) were clustered in the same terminal clade. This is a small section of closely related species (*i.e. P. carneum, P. paneum, P. psychrosexualis* and *P. roqueforti*) commonly associated with the cheese industry (Houbraken *et al.* 2010, 2016). Our isolates were related to *P. roqueforti*, but formed an independent and distant branch that could represent an undescribed species. In order to evaluate possible intra- and inter-specific variability within the species and confirm the phylogenetic position of our putative new species, we performed an additional analysis with *tub2* gene (see Fig. S1 in supplemental material) with more sequences of the species available in GenBank. This demonstrated that *P. roqueforti* is divided in two clades with an intra-specific variability of around 0.08 %, with our isolates forming a separate branch from the *P. roqueforti* complex. The concatenated analysis using ITS, *tub2*, *cmdA* and *rpb2* (Fig. 2) supported the novelty of these three isolates, being therefore proposed as the new species *P. mediterraneum*. Although, *P. mediterraneum* and *P. roqueforti* have
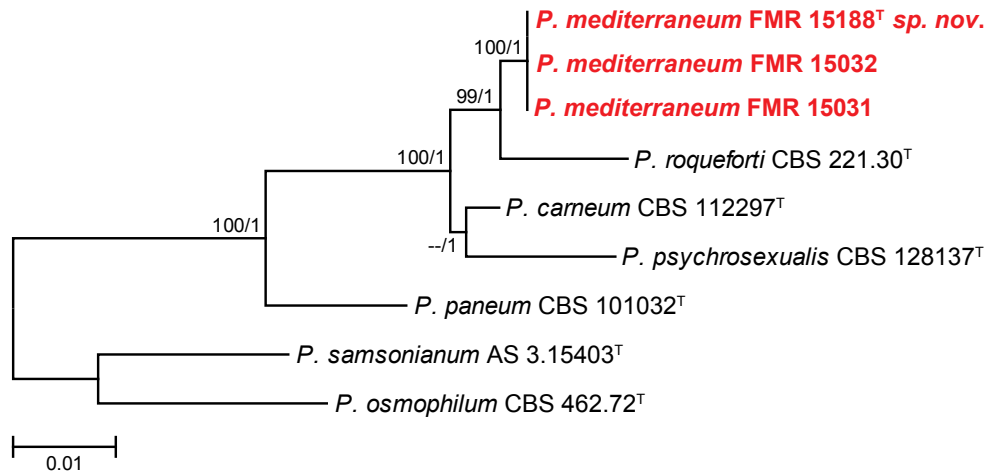
Fig. 2. ML tree of *Penicillium* section *Roquefortorum* inferred from the combined ITS, *tub2*, *cmdA*, and *rpb2* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.95 are indicated on the nodes. The tree is rooted to *P. samsonianum* AS 3.15403 and *P. osmophilum* CBS 462.72. The name in red is the new species described in this study. T = ex-type strain.

identical ITS barcodes, both species have unique *tub2*, *cmdA* and *rpb2* sequences.

In section *Chrysogena* (74 % bs/-- pp), which includes well-known species usually isolated from soil and also from indoor environments (Houbraken *et al.* 2012), two dung isolates were identified as *P. chrysogenum* and *P. flavigenum*, respectively. Whereas the two isolates of the section *Penicillium* (--% bs/1 pp), a group that currently mainly includes plant pathogenic penicillia (Houbraken *et al.* 2016), were both identified as *P. expansum*.

Isolates of section *Robsamsonia* (88 % bs/0.97 pp) were identified as *P. brevistipitatum* (n = 1), *P. concentricum* (n = 2), *P. coprobium* (n = 2), *P. coprophilum* (n = 1), and *P. griseofulvum* (n = 7); however, five isolates (FMR 15192, FMR 15210, FMR 15211, FMR 16481 and FMR 16491) could not be assigned to any known species. The preliminary *tub2* analysis, but also

the concatenate phylogeny (Fig. 3) including the currently accepted species in the section, showed that the five isolates grouped together in a supported and undescribed lineage closely related to the ex-type strain of *P. glandicola.* An additional *tub2* analysis (see Fig. S2 in supplemental material) with more sequences of *P. glandicola* showed that our isolates and *P. glandicola* were 97.4 % similar. The genetic differences and morphological peculiarities observed in this group of dung isolates justify the description of a new species, namely *P. synnematicola.*

The only isolate (FMR 15041) of section *Turbata* (95 % bs/1 pp) did not fit with any species of the section, although it was closely related to *P. bovifimosum*. In *Paradoxa* (94 % bs/-- pp) 10 isolates were included; four were identified as *P. atramentosum,* one as *P. magnielliptisporum*, and the rest (FMR 15040, FMR
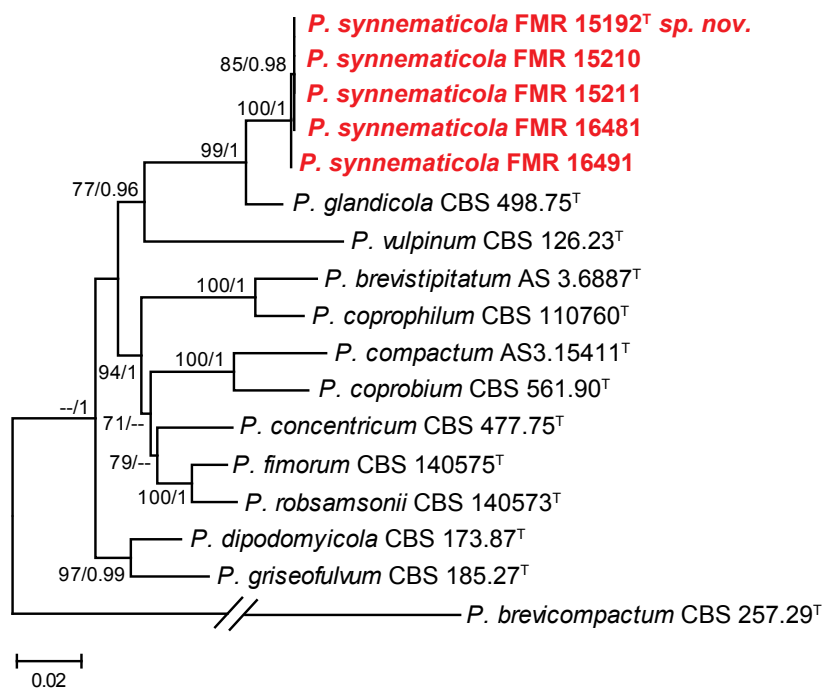
Fig. 3. ML tree of *Penicillium* section *Robsamsonia* inferred from the combined ITS, *tub2*, *cmdA*, and *rpb2* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.95 are indicated on the nodes. The tree is rooted to *P. brevicompactum* CBS 257.29. The name in red is the new species described in this study. T = ex-type strain.
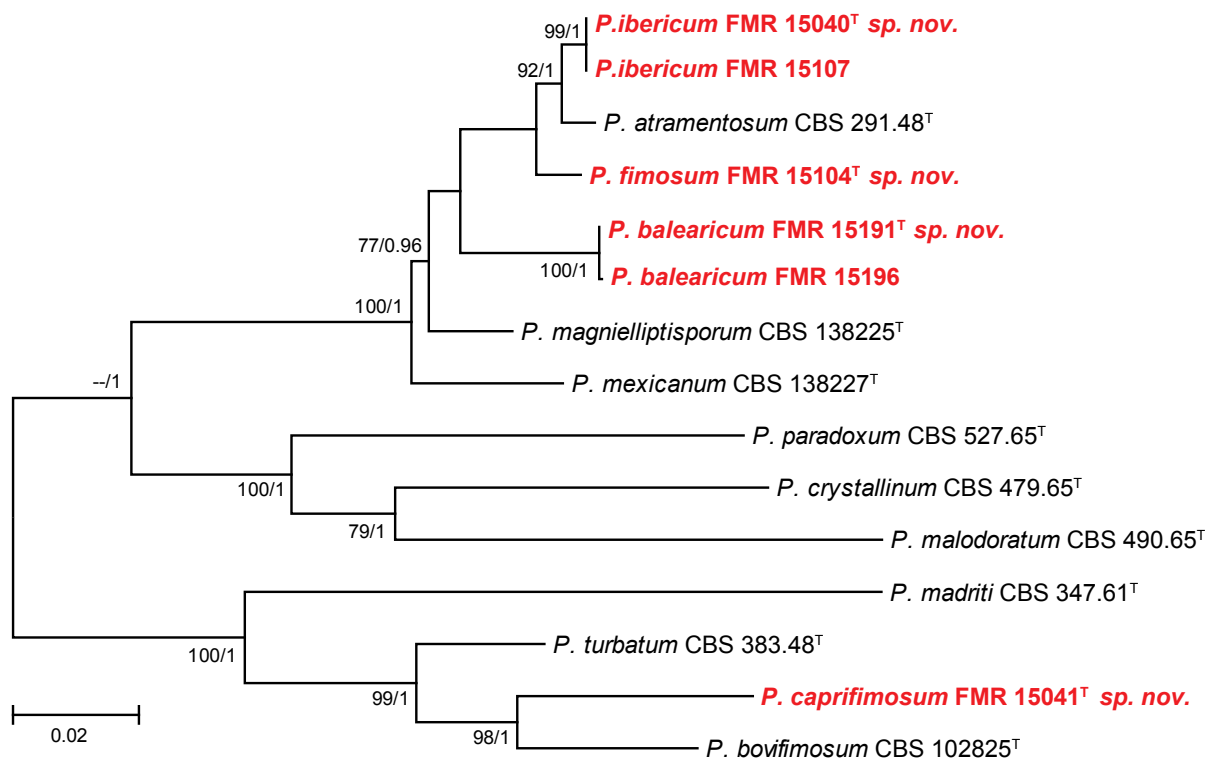
Fig. 4. ML tree of *Penicillium* sections *Paradoxa* and *Turbata* inferred from the combined ITS, *tub2* and *cmdA* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.95 are indicated on the nodes. The names in red are the new species described in this study. [T] = ex-type strain

15104, FMR 15107, FMR 15191 and FMR 15196) were allocated in three single branches which represent three putative new species for the genus. The concatenated phylogeny of sections *Paradoxa* and *Turbata* with the six unidentified isolates is shown in Fig. 4. In the former section, the isolates FMR 15107 and FMR 15040 formed a terminal clade separated from other one formed by FMR 15191 and 15196. The lineages of these two clades were in turn distant from the two terminal branches where FMR 15104 and the ex-type strain of *P. atramentosum* were located, respectively. Genetic differences of the three undescribed lineages with respect to *P. atramentosum* (97.07 % and 98.23 % similar with *tub2* and concatenate dataset for FMR 15107 and FMR 15040, respectively; 96.2 % and 96.2 % with *tub2* and concatenate dataset for FMR 15191 and FMR 15196; 94.68 % and 95 % with *tub2* and concatenate dataset for FMR 15104) justifies them as distinct taxa. The three species are proposed below as *P. ibericum, P. balearicum* and *P. fimosum.* An additional phylogenetic analysis with *tub2* including more sequences of the most closely related species confirms our proposal (see Fig. S3 in supplemental material). In section *Turbata, P. bovifimosum* and FMR 15041 were located in the same clade, but the latter formed a long terminal branch that proved to be a distinct species, described below as *P. caprifimosum.*

Section *Ramosa* (97 % bs/0.98 pp) only included the unidentified isolate FMR 15038. Currently, the section comprises 13 species (Visagie *et al.* 2014a, 2016a, Rong *et al.* 2016), although two of them, *P. lanosum* and *P. kojigenum*, were considered conspecific by Samson & Pitt (2000). The concatenate analysis performed with ITS, *tub2*, and *cmdA* (Fig. 5), *rpb2* was not included due to the lack of sequences for all species in the section, showed that FMR 15038 was placed in an independent branch between two clades, one with *P. kojigenum* and *P. lanosum*, and the other with *P. jamesonlandense* and *P.*

swiecickii. The similarity among our isolate and its phylogenetic sisters was 98.3 %, proving that our isolate is an undescribed species, which is proposed below as *P. beceitense*.

The only isolate of section *Brevicompacta* (99 % bs/1 pp) was identified as *P. brevicompactum*, a species commonly inhabiting in soil and decaying vegetation, but also described from food, cereals, textiles, clinical specimens and faeces of snake (Pitt 1979, Guevara-Suarez *et al.* 2016). The seven isolates classified in section *Canescentia* (98 % bs/1 pp) were identified as *P. canescens* (n = 1), *P. murcianum* (n = 3) and *P. radiatolobatum* (n = 3). Despite *tub2* being unable to discriminate between *P. radiatolobatum* and *P. murcianum*, these two species can be recognized with the *cmdA* and *rpb2* barcodes.

Section *Exilicaulis* (99 % bs/1 pp) included eight of our isolates. It is noteworthy that the ML general tree based only on *tub2* presented some doubtful results in the species identification of some isolates, especially those belonging to the *P. restrictum*-clade. However, when we carried out a restricted alignment with only members of the section and reconstructing the ML tree, most of them were satisfactory identified (see Fig. S4 in supplemental material). These were *P. arabicum* (n = 2), *P. burgense* (n = 1), *P. cinereoatrum* (n = 1)*, P. momoii* (n = 1), and *P. rubefaciens* (n = 2). The only isolate that could not be identified was FMR 15841, precisely located in the *P. restrictum*-clade. The most recent review of the section by Visagie *et al.* (2016b) indicates that such a clade needs further revision due to the high variability between strains currently belonging to the clade. Thus, the identification of FMR 15841 remains uncertain until its taxonomy is resolved.

Section *Lanata-Divaricata* (92 % bs/1 pp) included three dung isolates. One of them was identified as *P. brasilianum*, a widespread species commonly found in soil (Pitt 1979) and recently also reported from human clinical specimens (Guevara-
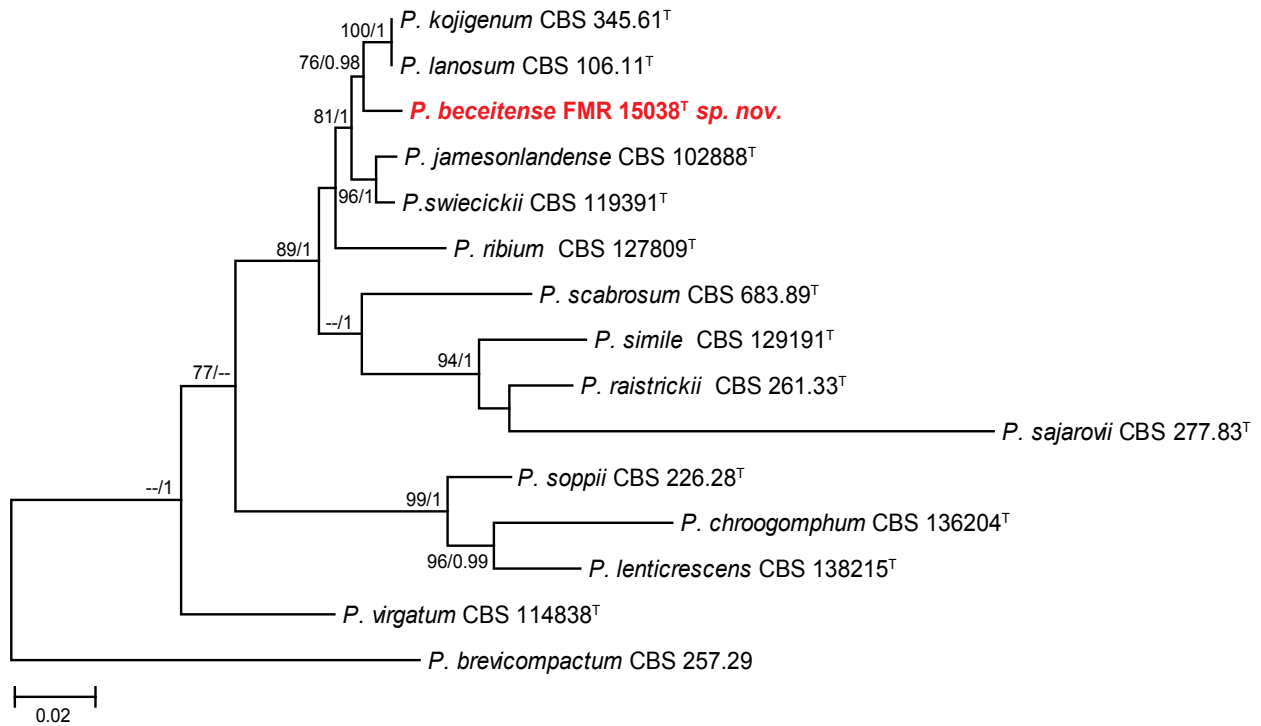
Fig. 5. ML tree of *Penicillium* section *Ramosa* inferred from the combined ITS, *tub2* and *cmdA* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.95 are indicated on the nodes. The tree is rooted to *P. brevicompactum* CBS 257.29. The name in red is the new species described in this study. [T] = ex-type strain.

Suarez *et al.* 2016). The other two isolates were identified as *P. cremeogriseum,* a species previously found in forest soil in Ukraine (Houbraken & Samson 2011, Visagie *et al.* 2016a). To our knowledge, this is the first report of *P. brasilianum* and *P. cremeogriseum* associated with herbivore dung.

The only species identified in our study belonging to section *Stolkia* (99 % bs/1 pp) was *P. canariense.* This species was described from soil in Canary Islands (Peterson & Sigler 2002), the same geographical origin as our isolate. This is the second isolate of the species found so far.

Isolates of section *Citrina* (98 % bs/1 pp) were identified as *P. citrinum* (n = 5) and *P. sizovae* (n = 2). Although we have not found previous records of these species from dung, they have a worldwide distribution, being isolated from soil, foodstuff, and many other types of substrates (Houbraken *et al.* 2011). This section comprises nearly 40 species, but only those identified here were included in the analysis to simplify the *tub2* phylogenetic tree (Fig. 1).

In sections *Sclerotiora* (77 % bs/-- pp) and *Cinnamopurpurea* (96 % bs/-- pp) *P. lilacinoechinulatum* (n = 1) and *P. cvjetkovicii* (n = 1) were respectively identified. Although the former species was previously considered synonym of *P. bilaiae*, they are currently recognised as distinct penicillia (Visagie *et al.* 2013). *P. lilacinoechinulatum* is an uncommon species which type was collected from Japanese soil (Abe 1956). *Penicillium cvjetkovicii* is a recently described from indoor air samples in the USA (Peterson *et al.* 2015).

Aspergilloides (99 % bs/1 pp) was the last section in *Penicillium* that included dung isolates. The species identified were *P. glabrum* (n = 3), *P. frequentans* (n = 2), *P. roseoviride* (n = 1), and *P. rudallense* (n = 1). Although the former two are currently accepted as distinct species, they were considered synonyms for a long time (Houbraken *et al.* 2014b). *Penicillium frequentans* together with *P. spinulosum* and *P. glabrum* are

generally the most common species in the section, being isolated from a wide range of substrates, including soil, food, bark, and indoor environments (Houbraken *et al.* 2014b).
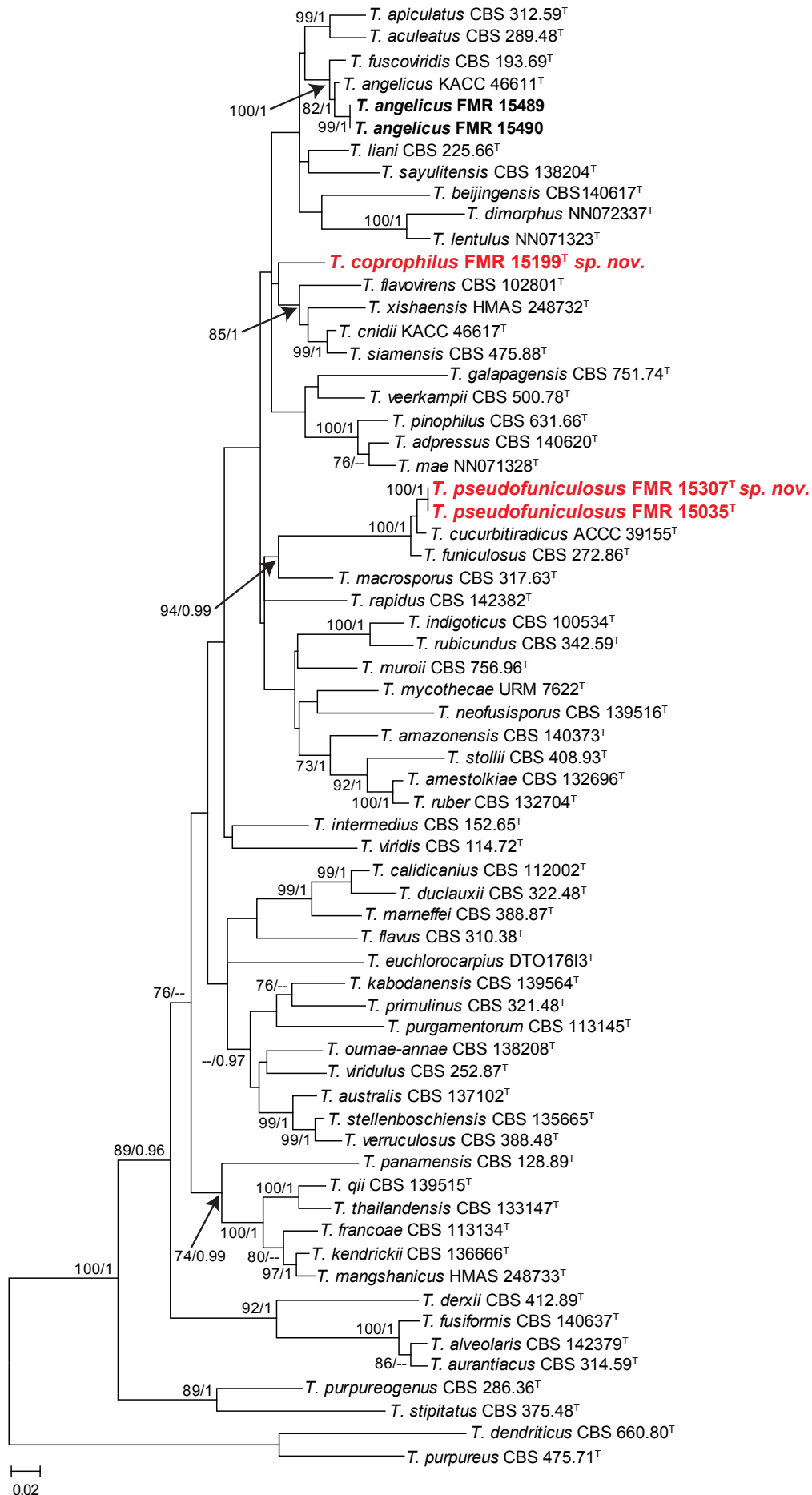
Talaromyces phylogeny

The phylogenetic tree based on the *tub2* locus (Fig. 6) shows the relationships of the nine *Talaromyces* isolates included in the study. The aligned dataset was 388 bp long, from which 217 were variable sites and 170 phylogenetic informative. The ML substitution model was K2+G+I, while for BI it was HKY+G+I. Two main clades were formed, representing sections *Talaromyces* (98 % bs/1 pp) and *Trachyspermi* (90 % bs/1 pp). Only three sect. *Talaromyces* species were identified, namely *T. muroii* (FMR 15496), *T. ruber* (FMR 15839) and *T. sayulitensis* (FMR 15842); the rest (FMR 15489, FMR15490, FMR 15199, FMR 15035 and FMR 15307) could not be identified with this single marker, just like the only isolate (FMR 16441) included in section *Trachyspermi*.

To resolve the taxonomy of these unidentified isolates, we calculated a phylogeny for each section using sequences of the ITS, *tub2* and *cmdA* genes from the accepted species in both sections, except for *T. amyrossmaniae* in section *Trachyspermi*. This is a recently described species that morphologically differs from our isolates and species of *Trachyspermi* by producing short determinate synnemata on both the natural substrate and *in vitro* (Rajeshkumar *et al.* 2019). Although *rpb2* was sequenced for all our isolates (Table 1), this marker was not incorporated in the concatenated analysis due to the lack of sequences for all ex-type strains. The combined phylogeny of the members of section *Talaromyces* is shown in Fig. 7. This analysis showed that the unidentified isolates FMR 15489 and FMR 15490 exhibited both a similarity of 99.42 % with respect to the ex-type strain of *T. angelicus*, a monotypic species described by Sang *et al.*
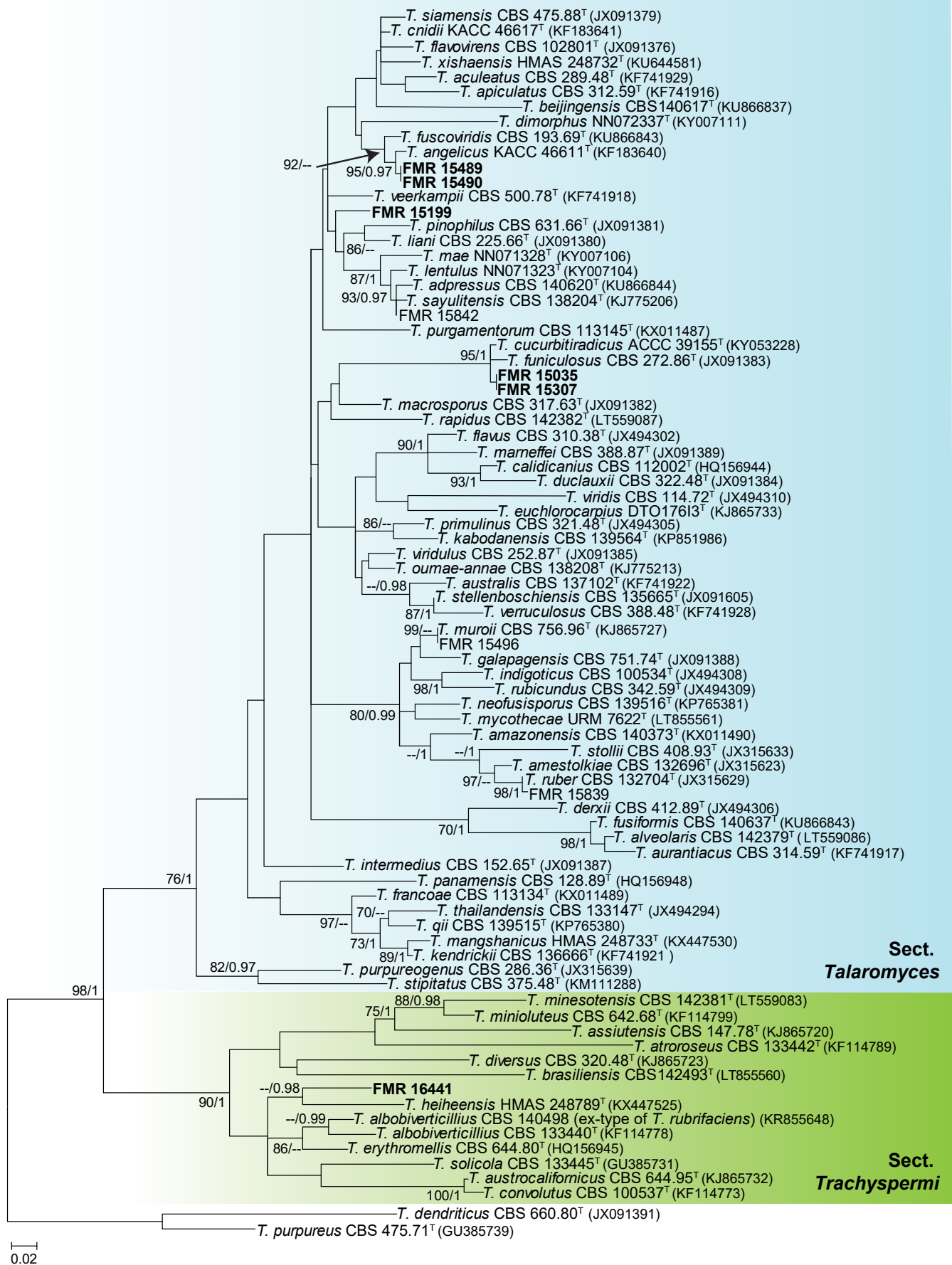
Fig. 6. ML tree of *Talaromyces* inferred from *tub2* including the sections recovered from dung in this work (*Talaromyces* and *Trachyspermi*). Branch lengths are proportional to phylogenetic distance. Some of the larger branches were condensed, with the proportions showed above the parallel diagonal lines. Bootstrap support values/Bayesian posterior probability scores above 69 %/0.95 are indicated on the nodes. Isolates corresponding to new species are shown in bold. Between parentheses, GenBank accession numbers of *tub2* sequences. The tree is rooted to *T. dendriticus* CBS 660.80 and *T. purpureus* CBS 475.71 (Section *Purpurei*). [T] = ex-type strain.

Fig. 7. ML tree of *Talaromyces* section *Talaromyces* inferred from the combined ITS, *tub2* and *cmdA* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.95 are indicated on the nodes. The tree is rooted to *T. dendriticus* CBS 660.80 and *T. purpureus* CBS 475.71 (Section *Purpurei*). The names in red are the new species described in this study. ᵀ = ex-type strain.

(2013). Although most of their morphological features matched with those of the protologue of *T. angelicus*, we observed some phenotypic variation with respect to the description of the species by Yilmaz *et al.* (2014); *i.e*, production of diffusible red pigment in one of our isolates (FMR 15490), the colonies of our isolates were deep turquoise and pastel red rather than yellow to white on MEA, they grew faster at 37 °C (on CYA 34–37 mm diam in 7 d *vs* 25–27 mm in Yilmaz *et al.* 2014), and exhibited stipes up to 100 µm long (up to 120 µm in Yilmaz *et al.* 2014). On the other hand, while FMR 15199 was located in an independent branch clearly separated from the other species of the section; FMR 15035 and FMR 15307 clustered together and were closely related to *T. curcubitiradicus* and *T. funiculosus,* but with enough genetic difference to be considered a distinct species (see also Fig. S5 in supplemental material). These genetic differences and the particular phenotypic features observed in the three FMR isolates allow us to propose the following new species, *T. coprophilus* (FMR 15199) and *T. pseudofuniculosus* (FMR 15035 and FMR 15307)*.* Both can be easily identified with the *tub2* marker.

The combined phylogenetic analysis of the section *Trachyspermi* (Fig. 8) showed that the isolate FMR 16441 was located in a single branch within the same clade as *T. albobiverticillius*, *T. erythromellis, T. heiheensis*, and *T. solicola* (88 % bs/0.99 pp). Considering the phylogenetic position of our isolate and the morphological differences observed, the new species *T. catalonicus* is proposed.

## Phylogeny of miscellaneous *Aspergillaceae*

According to the *tub2* phylogeny, isolates FMR 15296, FMR 16442, FMR 14718 and FMR 15299 were related to some penicillia currently excluded from the genus *Penicillium* (*i.e. P. giganteum, P. megasporum* and *P. nodositatum*), but belonging to the family *Aspergillaceae* (Fig. 9). Previous molecular data on these fungi suggested that they could represent distinct genera in the family (Peterson *et al.* 2010, Visagie *et al.* 2013). The aligned dataset of our analysis was 393 bp long, from which 242 were variable sites and 216 phylogenetic informative. The best substitution model for ML was K2 +G+I and for BI it was GTR +G+I. While FMR 15296 and FMR 16442 were located in a well-supported clade along with the ex-type strain of *P. nodositatum* (CBS 333.90), the other two (FMR 14718 and FMR 15299) were in a fully supported distant clade together with the ex-type strains of *P. giganteum* (NRRL 3553) and *P. megasporum* (NRRL 2232). The phylogeny inferred with sequences of the ITS, *tub2* and *rpb2* genes (Fig. 10), including the isolates under study and members of different genera of *Aspergillaceae*, confirms the proposal of two novel genera, which are described below as *Penicillago* (*Pgo.*)*,* typified by *Penicillium nodositatum*, and *Pseudopenicillium* (*Pse*.) with *Penicillium giganteum* and *Penicillium megasporum,* this latter species here selected as the type. The former genus is phylogenetically more related to *Penicillium,* whereas the latter to the genus *Hamigera*.

The analysis of ITS and *tub2* showed that the ex-type strain of *P. nodositatum* (CBS 333.90) and the isolates FMR 15296 and FMR 16442 have practically identical sequences. However, CBS 333.90 was not included in the concatenated phylogeny with the three markers (Fig. 10) since its *rpb2* sequence was not available for comparison. Despite this, we observed some cultural difference between our isolates respect to the protologue of *P. nodositatum* (Valla *et al.* 1989), microscopically they fit with the features of the species. Differences include better growth for FMR 15296 and FMR 16442 on CYA and MEA at 25 °C (40–44 mm
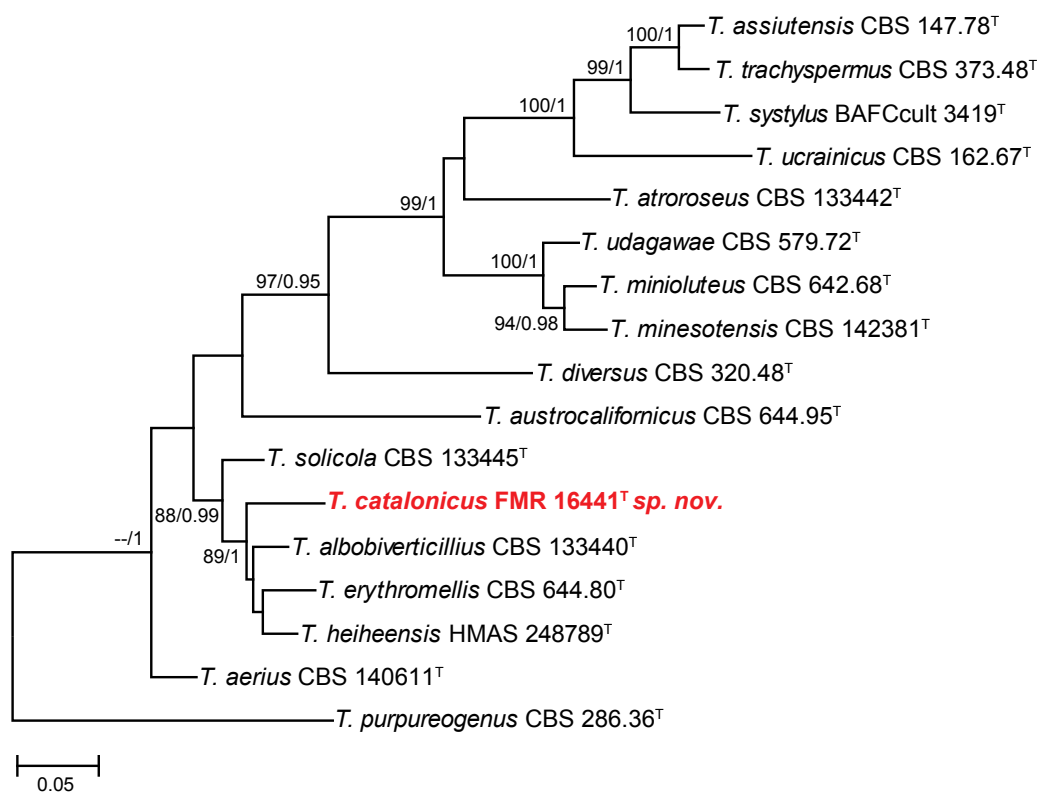
Fig. 8. ML tree of selected of *Talaromyces* section *Trachyspermi* inferred from the combined ITS, *tub2* and *cmdA* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.94 are indicated on the nodes. The tree is rooted to *T. purpureogenus* CBS 286.36 (Section *Talaromyces*). The name in red is the new species described in this study. ᵀ = ex-type strain.
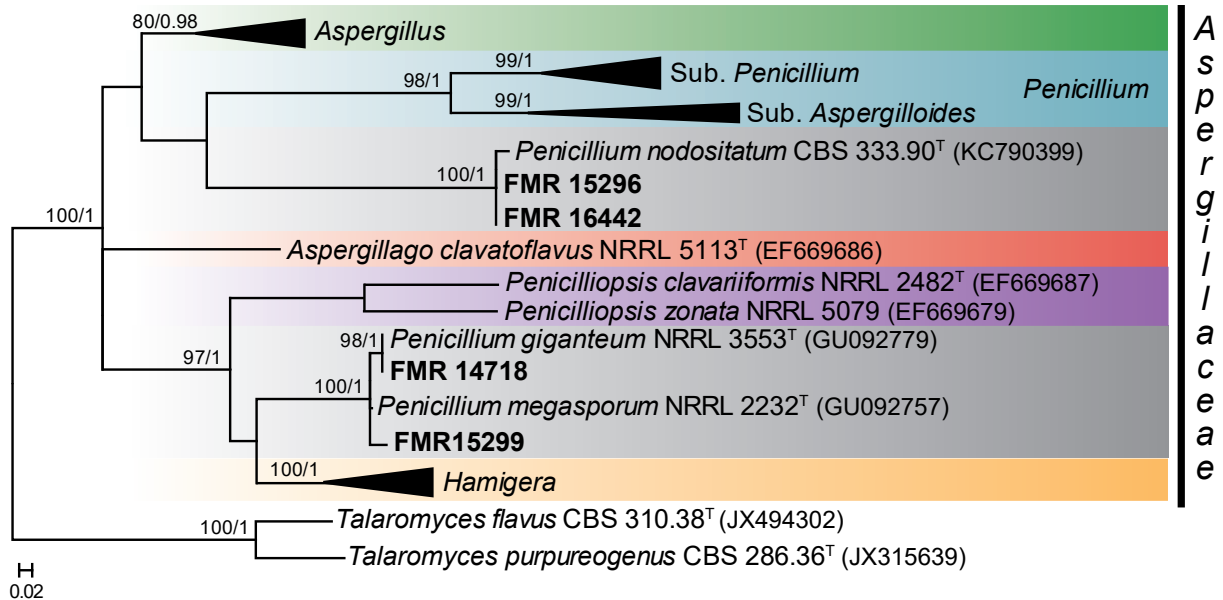
Fig. 9. ML tree of selected members of *Aspergillaceae* family inferred from *tub2*. Branch lengths are proportional to phylogenetic distance. Some clades were collapsed to facilitate layout and illustrate the phylogenetic relationships between the shown genera. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.95 are indicated on the nodes. Isolates corresponding to new or interesting taxa are shown in bold. Between parentheses, GenBank accession numbers of *tub2* sequences. The tree is rooted to *T. flavus* CBS 310.38 and *T. purpureogenus* CBS 286.36. [T] = ex-type strain.
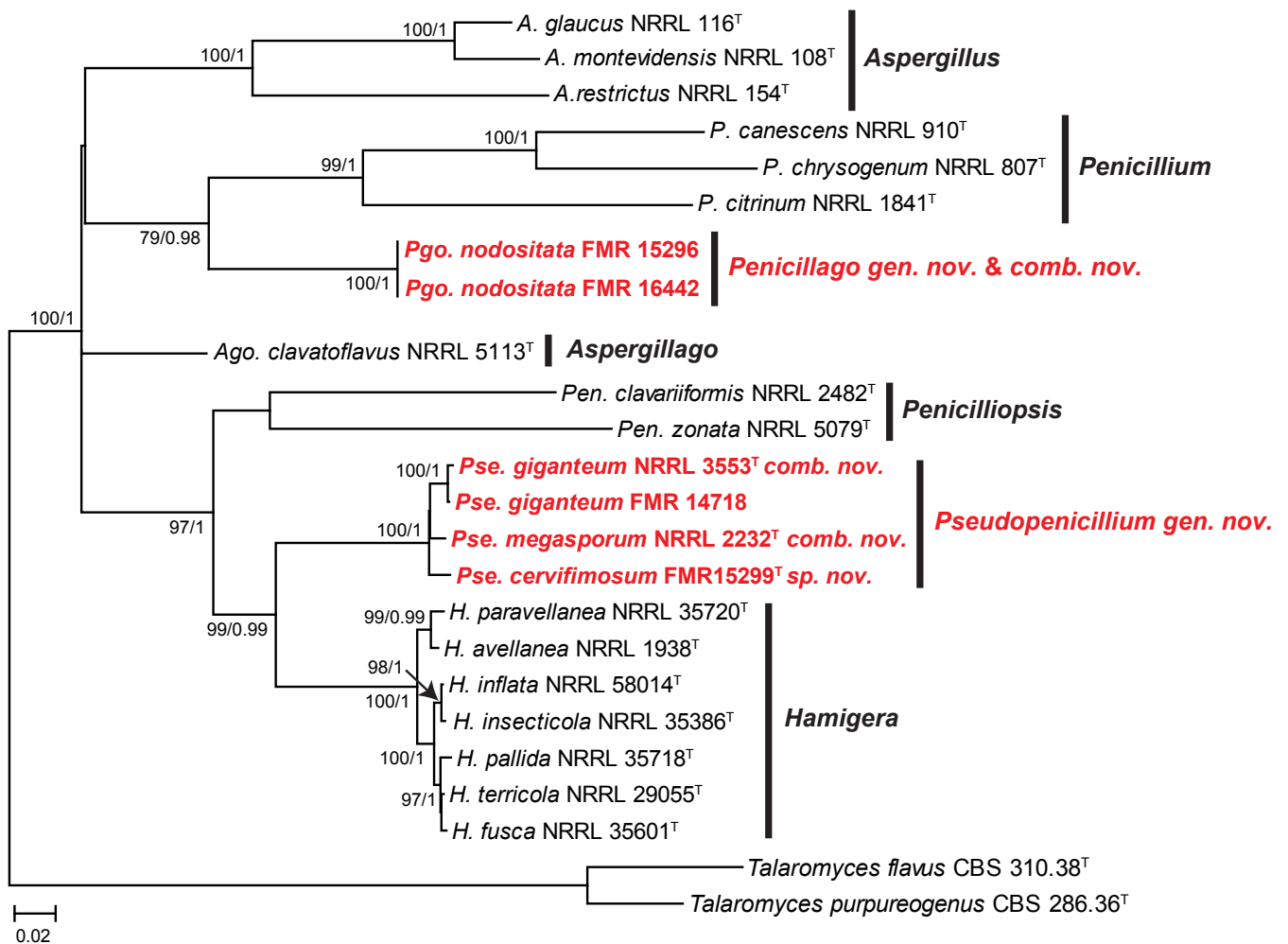
Fig. 10. ML tree of members of *Aspergillaceae* family inferred from the combined ITS, *tub2*, and *rpb2* loci. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.95 are indicated on the nodes. The tree is rooted to *Talaromyces flavus* CBS 310.38 and *Talaromyces purpureogenus* CBS 286.36. The names in red are the new taxa proposed in this study. [T] = ex-type strain.

and 34–36 mm diam in 7 d, respectively, *vs* 13–19 mm and 16–30 mm in CBS 333.90) and showed the ability to grow at 37 °C. Considering the phylogenetic position of our isolates and their morphological similarity with *P. nodositatum*, they were the base to propose the new combination *Pgo. nodositata.* Other fungus excluded from *Penicillium s. str.* and closely related to *Pgo. nodositata* was *P. kabunicum* (Visagie *et al.* 2013). Since only the ITS barcode was available for the ex-type strain of the latter species (CBS 575.90) in the GenBank database (MH862240), we constructed an ITS phylogenetic tree with different genera in *Aspergillaceae* showing that *P. kabunicum* clustered with *Pgo. nodositata* (see Fig. S6 in supplemental material).

The isolate FMR 14718 matched morphologically and molecularly with *P. giganteum*, whereas FMR 15299 was placed in a new lineage into the *Pseudopenicillium* clade (Figs 9, 10). The morphological differences observed, such as having smaller conidia (up to 6 µm) than those of the other two species in the genus (conidia up to 12 µm in *Pse*. *giganteum*, and up to 10 µm in *Pse*. *megasporum*), and the genetic differences obtained (98 % similar to *Pse*. *giganteum*, and 98.16 % similar to *Pse*. *megasporum*) allow us to propose the new species *Pse. cervifimosum.*

Taxonomy

Penicillium balearicum Guevara-Suarez, Cano & Gené, ***sp. nov.*** MycoBank MB822061. Fig. 11.

Etymology: Name refers to the geographical area (the Balearic Islands) where the species was found.

In: Section *Paradoxa.*
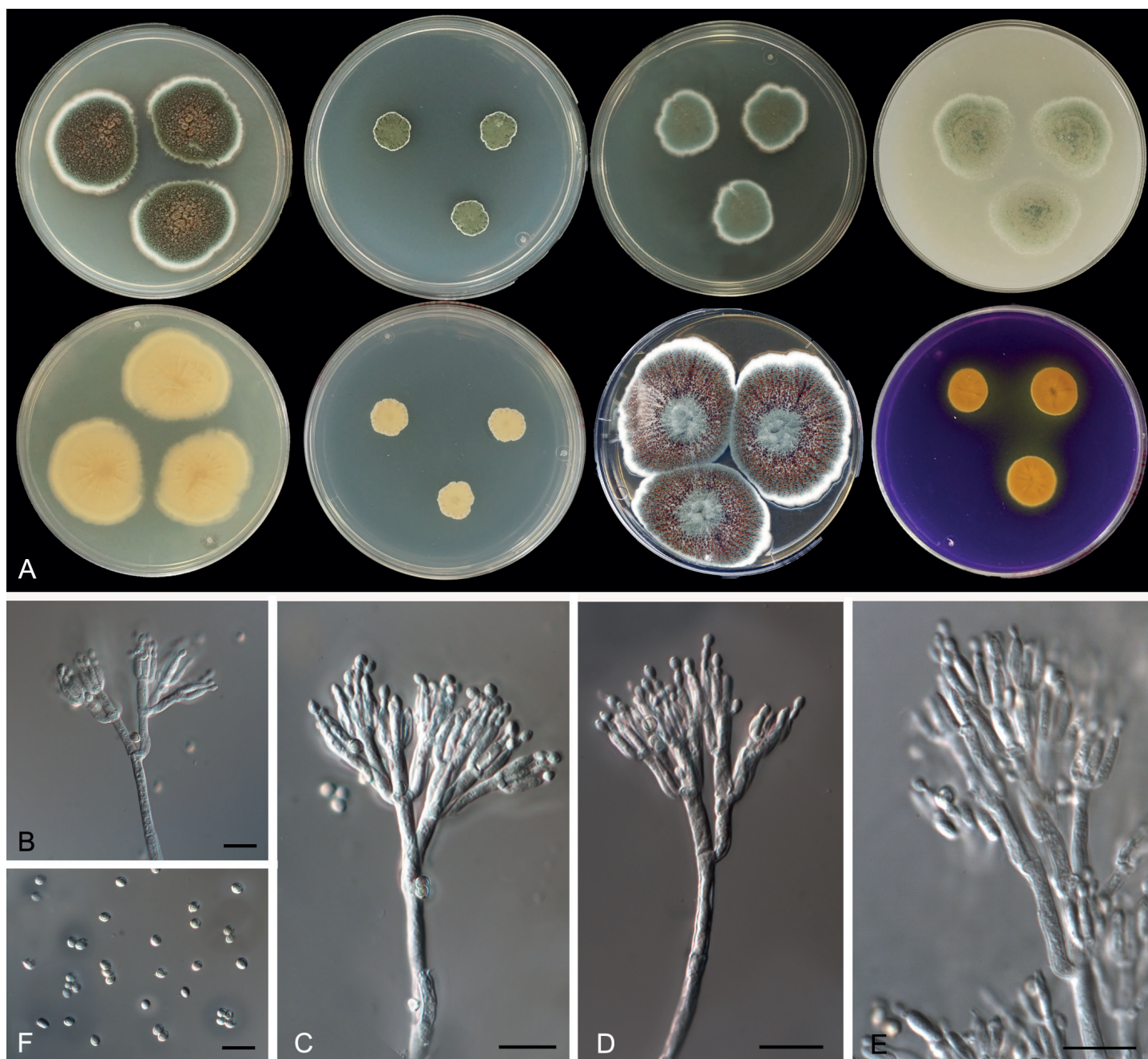
Fig. 11. Morphological characters of *Penicillium balearicum* (FMR 15191ᵀ). **A.** Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. **B–E.** Conidiophores. **F.** Conidia. Scale bars = 10 µm.

Typus: **Spain**, Balearic Islands, Mallorca, Pollença, from herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira* (**holotype** CBS H-23215; culture ex-type FMR 15191 = CBS 143044; ITS barcode LT899762, alternative markers: *tub2* LT898227, *cmdA* LT899758, *rpb2* LT899760).

Additional material examined: **Spain**, Balearic Islands, Mallorca, Pollença, from herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira* (FMR 15196).

Colony diameter in 7 d (mm): On CYA: 25 °C 25–36, 30 °C 5–6, 37 °C no growth; on MEA: 25 °C 14–17, 30 °C 4–6; on YES: 25 °C 36–45, 30 °C 4–6; on OA: 25 °C 26–30, 30 °C 2–6; on DG18: 25 °C 14–27, on CREA: 25 °C 14–20.

Colony characters at 25 °C in 7 d: On CYA, colonies velvety, flat, mycelium white, sporulation dense, conidial masses dark dull green (28E3), margin dentate; reverse pale orange (5A3); exudate present, with pale droplets; soluble pigment absent. On MEA, colonies velvety, elevated, sporulation dense, conidial masses dark dull green (28E3), margin lobate; reverse light orange (5A4); exudate and soluble pigment absent. On DG18, colonies flat and velvety, mycelium white, sporulation dense, conidial masses dull green (27D4); reverse greenish white (27B2). On YES, colonies cerebriform, raised at the centre, radially sulcate, mycelium white, sporulation dense at the centre, with conidial masses greenish white (27C2), margin lobate; reverse bright orange-red (8A8); exudate present, small brown (7E6-8) droplets; soluble pigment absent. On OA, colonies fasciculate, mycelium white, sporulation dense, with conidial masses greenish grey (28B2); exudate and soluble pigment absent. On CREA, acid production moderately strong.

Micromorphology: On MEA, conidiophores typically terverticillate, minor proportion biverticillate and quaterverticillate; stipes smooth, 250–500 × 2–2.5 µm; metula 3–4 per branch, divergent, 10–14 × 2–3 µm; phialides 3–4 per metula, ampulliform, 7–10 × 2–2.5 µm; conidia globose to subglobose, 2.5–3 × 2–3 µm, smooth-walled, brownish yellow.

Distinguishing characters: *Penicillium balearicum* is resolved in the *P. atramentosum*-clade containing six species, *P. atramentosum*, *P. balearicum*, *P. fimosum*, *P. ibericum* (the latter three described here), *P. magnielliptisporum* and *P. mexicanum* (Visagie *et al.* 2014a). *Penicillium atramentosum*, *P. balearicum*, *P. magnielliptisporum* and *P. mexicanum* have smooth-walled stipes and conidia, and the conidia of the latter two are larger (5 × 4 µm in *P. magnielliptisporum*, 4 × 3.5 µm in *P. mexicanum*); while *P. atramentosum* can be differentiated by its production of red soluble pigment on CYA and by the lack of growth at 30 °C (Pitt 1979, Frisvad & Samson 2004). *Penicillium balearicum* differs from *P. fimosum* and *P. ibericum* mainly by having longer stipes (up to 500 µm long), by the production of abundant brown exudate droplets on YES, and by the moderately strong acid production on CREA.

Penicillium beceitense Guevara-Suarez, Gené & Guarro, ***sp. nov***. MycoBank MB822063. Fig. 12.

Etymology: Name refers to Beceite, the vilage where the fungus was found.

In: Section *Ramosa*.

Typus: **Spain**, Aragon, Beceite, from herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez, E. Rosas* (**holotype** CBS H-23183; cultures ex-type FMR 15038 = CBS 142989; ITS barcode LT899780, alternative markers: *tub2* LT898229, *cmdA* LT899764, *rpb2* LT899798).

Colony diameter in 7 d (mm): On CYA: 25 °C 25–27, 30 °C no growth, 37 °C no growth; on MEA: 25 °C 23–25; on YES: 25 °C 26–28; on OA: 25 °C 26–30; on DG18: 25 °C 19–20, on CREA: 25 °C 3–5.

Colony characters at 25 °C in 7 d: On CYA, colonies sunken at the centre, slightly radially sulcate, velvety, mycelium white, sporulation dense, with conidial masses dull green (25E4), margin entire; reverse greyish green (28C5); exudate and soluble pigment absent. On MEA, colonies flat, velvety, mycelium white, sporulation dense, with conidial masses conidial masses dull green (25E4), margin entire; reverse orange (5A6) to greenish grey (30C2); exudate and soluble pigment absent. On DG18, colonies flat, velvety, mycelium white, sporulation dense, conidial masses dull green (26E4), margin entire; reverse greyish green (30B5). On YES, colonies radially sulcate, velvety, mycelium white, sporulation dense, with conidial masses dark green (27F14), margin lobate; reverse greyish yellow (4B5); exudate and soluble pigment absent. On OA, colonies flat, velvety, sporulation dense, conidial masses dark dull green (28D4), margin entire; reverse greenish grey (30C2); exudate and soluble pigment absent. On CREA, acid production absent.

Micromorphology: On MEA, conidiophores predominantly terverticillate, minor proportion biverticillate; stipes 190–250 × 3.5–4 µm, smooth-walled; branches 30–40 µm; metula 3–5 per branch, divergent, cylindrical, 10–12(–15) × 2.5–3 µm; phialides 4–6 per metula, ampulliform, 8–10 × 2.5–3 µm; conidia globose, 2.5–3 × 2.5–3 µm, smooth to finely roughened, greenish.

Distinguishing characters: *Penicillium beceitense* is closely related to *P. lanosum* and *P. kojigenum*, two species that are indistinguishable according to our phylogeny of the section *Ramosa* (Fig. 5). To date, there is no updated description for either *P. lanosum* or *P. kojigenum*. The protologue of *P. kojigenum* described conidia that are rough-walled, measuring 2.2–2.6 µm (Smith 1961). The absence of growth at 30 °C is the relevant feature to distinguish *P. beceitense* from other species of the section, such as *P. chroogomphum, P. jamesonlandense, P. lanosum, P. ribium*, and *P. soppii* (Rong *et al.* 2016).

Penicillium caprifimosum Guevara-Suarez, Dania García & Cano, ***sp. nov***. MycoBank MB822064. Fig. 13.

Etymology: From the Latin *capra* = "goat", and *fimosum* = "dung-dwelling", describing the substrate from where the species was isolated.

In: Section *Turbata*.

Typus: **Spain**, Catalonia, Els Ports Natural Park, from goat dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & E. Rosas* (**holotype** CBS H-23184; culture ex-type FMR 15041 = CBS 142990; ITS barcode: LT899781, alternative markers: *tub2* LT898238, *cmdA* LT899765, *rpb2* LT899799).
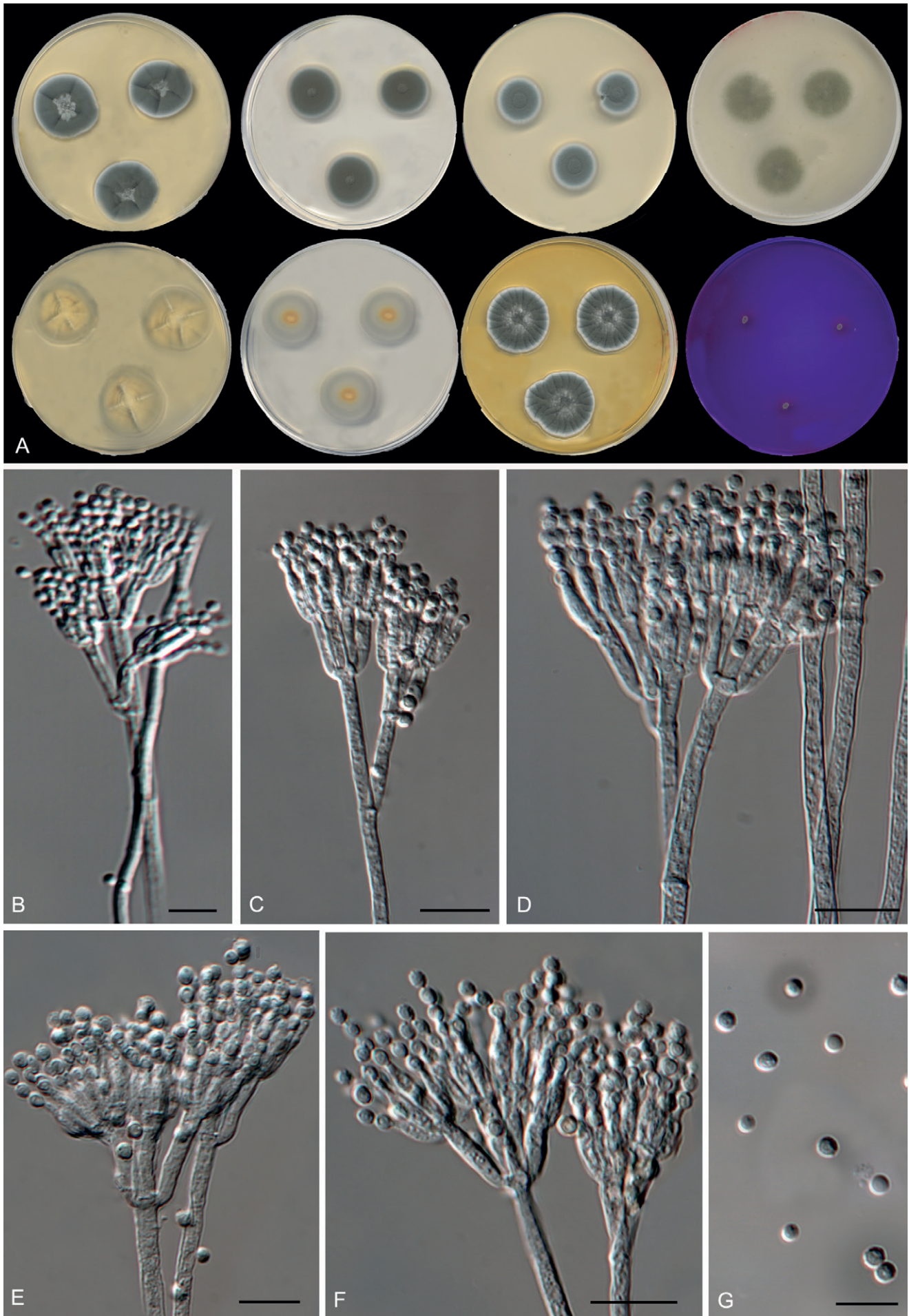
Fig. 12. Morphological characters of *Penicillium beceitense* (FMR 15038[T]). **A.** Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. **B–F.** Conidiophores. **G.** Conidia. Scale bars = 10 μm.

Fig. 13. Morphological characters of *Penicillium caprifimosum* (FMR 15041ᵀ). **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.

Colony diameter in 7 d (mm): On CYA: 25 °C 30–35, 30 °C 35–38, 37 °C 18–20; on MEA: 25 °C 24–26, 30 °C 19–21, 37 °C 8–10; on YES: 25 °C 25–24, 30 °C 30–31; on OA: 25 °C 23–25, 30 °C 28–30, 37 °C 9–11; on DG18: 25 °C 16–21, on CREA: 25 °C 3–6.

Colony characters at 25 °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 centre, 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 entire; exudate and soluble pigment absent. On CREA, acid not produced.

Micromorphology: On MEA, conidiophores biverticillate, sometimes with subterminal branches; stipes smooth-walled, 100–250 × 3–3.5 µm; metula 3–4 per branch, appressed, 10–12 × 2–3 µm; phialides 3–4 per metula, ampulliform, 8–10 × 2–2.5 µm; conidia globose to subglobose, 2.5–3 × 2–2.5 µm, smooth-walled, brownish yellow.

Distinguishing characters: *Penicillium caprifimosum* can be differentiated from its closest relative, *P. bovifimosum* described from dry cow manure (Tuthill & Frisvad 2002), by its better growth at 25 and 37 °C on MEA and CYA. The maximum colony diameter reported for *P. bovifimosum* is 16–21 mm on MEA and 25–29 mm on CYA in 7 d at 25 °C and 6 mm on CYA at 37 °C (Tuthill & Frisvad 2002). In addition, this species produces cleistothecia in culture, which are absent in *P. caprifimosum*.

Penicillium fimosum Guevara-Suarez, Guarro & Dania García, ***sp. nov.*** MycoBank MB822069. Fig. 14.

Etymology: Name refers to the substrate from where the species was isolated.

In: Section *Paradoxa.*

Typus: **Spain**, Catalonia, Pratdip, from hervibore dung, Feb. 2016, *D. García* (**holotype** CBS H-23185; culture ex-type FMR 15104 = CBS 142991; ITS barcode: LT970836, alternative markers: *tub2* LT898273, *cmdA* LT970837).

Colony diameter in 7 d (mm): On CYA: 25 °C 31–34, 30 °C 3–4, 37 °C no growth; on MEA: 25 °C 25–30, 30 °C no growth; on YES: 25 °C 50–55, 30 °C 5–7; on OA: 25 °C 25–27, 30 °C no growth; on DG18: 25 °C 16–20, on CREA: 25 °C 20–23.

Colony characters at 25 °C in 7 d: On CYA, colonies velvety, slightly radially sulcate at the centre, mycelium white, sporulation dense, conidial masses dark dull green (26E4), margin slightly lobate and fimbriate; reverse greyish orange (6B4) at the centre, greyish green (28C3) towards the periphery; exudate

and soluble pigment absent. On MEA, colonies flat, velvety, sporulation dense, conidial masses dark dull green (28E3), irregular, margin slightly crenate; reverse light orange (5A4) to dull green (28D4); 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 centre, mycelium white, sporulation dense, with conidial masses dull green (26E5), margin slightly crenate; reverse bright orange-red (8A8) at the centre; 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 predominantly biverticillate, minor proportion terverticillate and some irregularly branched; stipes 70–130 × 2.5–3(–4) µm, smooth; metula 3–4 per branch, rather divergent, 8–14 × 2.5–3(–4) µm; phialides 3–4 per metula, ampulliform, (6–)8–10 × 2.5–3 µm; conidia globose to subglobose, 2.5–3 × 2.5–3(–4) µm, smooth, brownish yellow.

Distinguishing characters: *Penicillium fimosum* is closely related to *P. atramentosum* and *P. ibericum* (described below). *Penicillium fimosum* and *P. atramentosum* differ from *P. ibericum* by the absence or limited growth at 30 °C. *Penicilium atramentosum* can be distinguished by the production of reddish brown exudate, soluble pigment and colony reverse on CYA, features absent in *P. fimosum*. Moreover, *P. fimosum* has shorter stipes (70–130 µm) than *P. atramentosum* (300–500 µm) (Pitt 1979).

Penicillium ibericum Guevara-Suarez, Cano & Dania García, ***sp. nov.*** MycoBank MB822070. Fig. 15.

*Etymology*: Name refers to the occurrence of the species on the Iberian Peninsula.

In: Section *Paradoxa.*

Typus: **Spain**, Catalonia, Els Ports Natural Park, from herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & E. Rosas* (**holotype** CBS H-23186; culture ex-type FMR 15040 = CBS 142992; ITS barcode: LT899782, alternative markers: *tub2* LT898285, *cmdA* LT899766, *rpb2* LT899800).

Additional material examined: **Spain**, Galicia, Las Dunas de Corrubedo Natural Park, from soil, Feb. 2016, *D. García* (FMR 15107).

Colony diameter in 7 d (mm): On CYA: 25 °C 31–35, 30 °C 18–21, 37 °C no growth; on MEA: 25 °C 18–20, 30 °C 8–9; on YES: 25 °C 33–35, 30 °C 18–20; on OA: 25 °C 21–23, 30 °C no growth; on DG18: 25 °C 5–6; on CREA: 25 °C 21–22.

Colony characters at 25 °C in 7 d: On CYA, colonies flat, velvety, slightly radially sulcate, mycelium white, sporulation dense, conidial masses dark dull green (26E4), margin slightly lobate; reverse reddish brown (9E5) to reddish (9A2); exudate present, with pale red (10A3) droplets; soluble pigment reddish brown. On MEA, colonies, velvety and flat, sporulation dense, conidial masses dark dull green (28E3); reverse light orange (5A4), margin slightly irregular; exudate and soluble pigment absent.
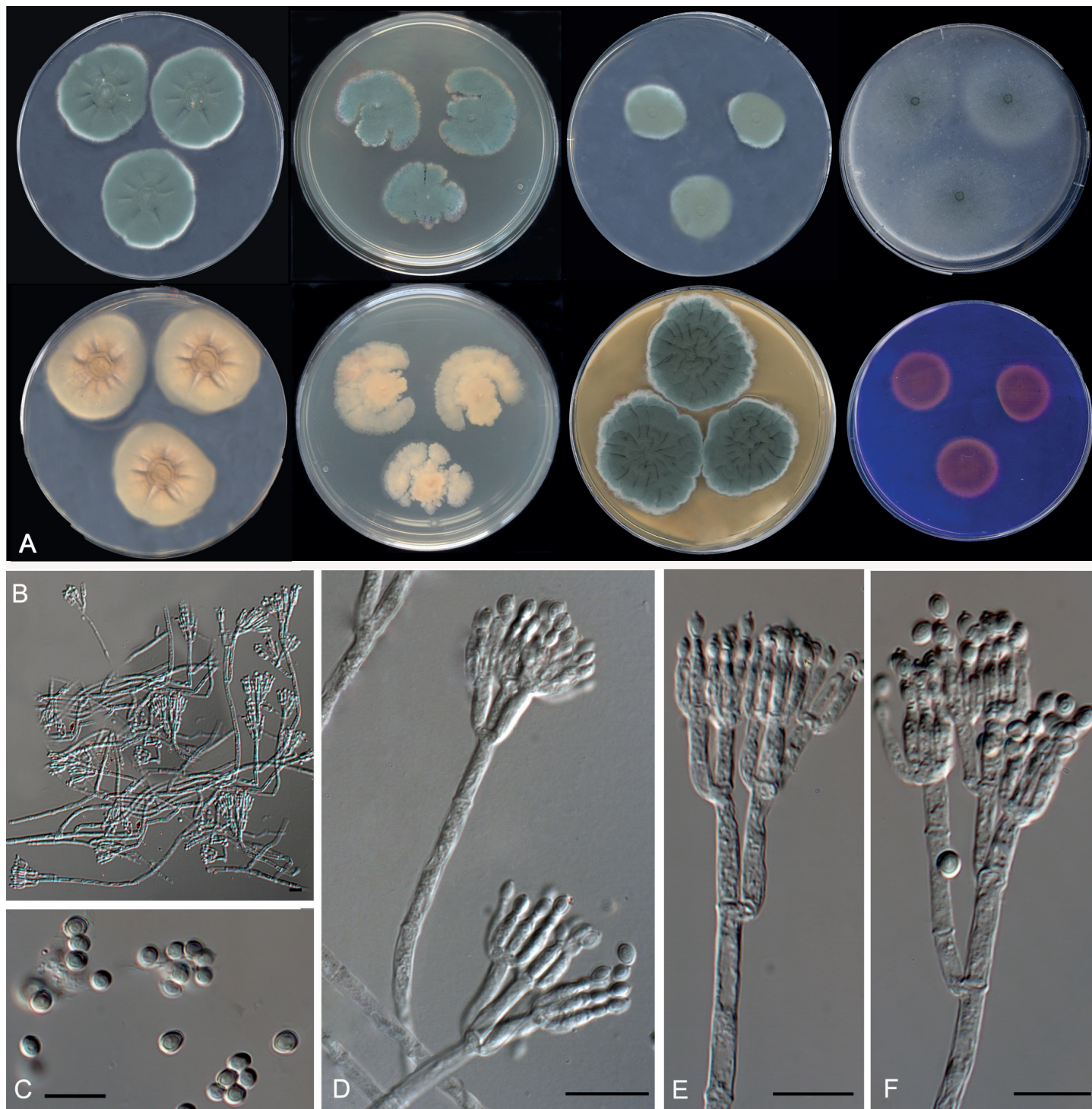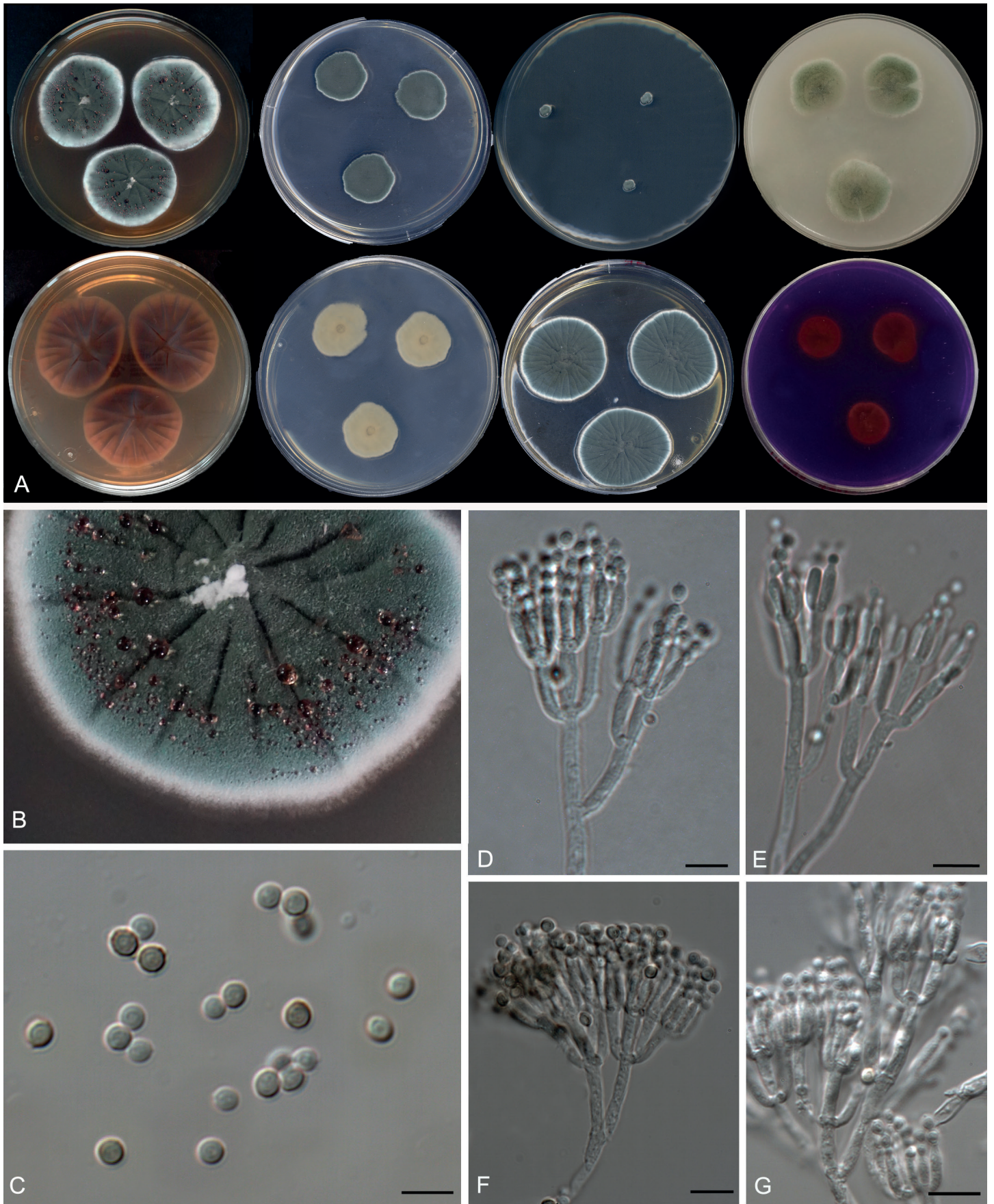
Fig. 14. Morphological characters of *Penicillium fimosum* (FMR 15104ᵀ). **A.** Colonies from left to right (top row) CYA, MEA, DG18 and OA; (bottom row) CYA reverse, MEA reverse, YES, and CREA. **B, D–F.** Conidiophores. **C.** Conidia. Scale bars = 10 μm.

On DG18, colonies sunken in the middle, velvety, mycelium white, sporulation moderate, conidial masses dull green (27D4); reverse greenish white (27B2). On YES, velvety, sulcate, mycelium white, sporulation dense, conidial masses dull green (26E5), margin crenate; reverse orange-grey (5B3); exudate and soluble pigment absent. On OA, colonies irregular, granulose, mycelium white, sporulation dense, conidial masses greyish green (28E7); exudate and soluble pigment absent. On CREA, moderate acid production.

Micromorphology: On MEA, conidiophores predominantly terverticillate, minor proportion quarterverticillate; stipes 70–

150 × 2–3(–4) μm, smooth; metula 2–4 per branch, divergent, 8–12 × 2–2.5 μm; phialides 3–4 per metula, ampulliform, 8–10 × 2.5–3 μm; conidia globose, 2.5–3 × 2.5–3 μm, smooth to finely roughened, subhyaline to pale green.

Distinguishing characters: *Penicillium ibericum* is closely related to *P. atramentosum* and *P. fimosum*. It can be distinguished by having shorter stipes (70–150 μm) than *P. atramentosum* (300–500 μm) (Pitt 1979), and by its more restricted growth on YES (33–35 mm diam) and DG18 (5–6 mm) compared to *P. fimosum* (YES 50–55 mm, DG18 16–20 mm).

Fig. 15. 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 °C after 1 wk incubation. **C.** Conidia. **D–G.** Conidiophores. Scale bars = 10 μm.

Fig. 16. 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.

Penicillium mediterraneum Guevara-Suarez, Gené & Cano, ***sp. nov.*** MycoBank MB822071. Fig. 16.

Etymology: Name refers to the geographical area where the species was found.
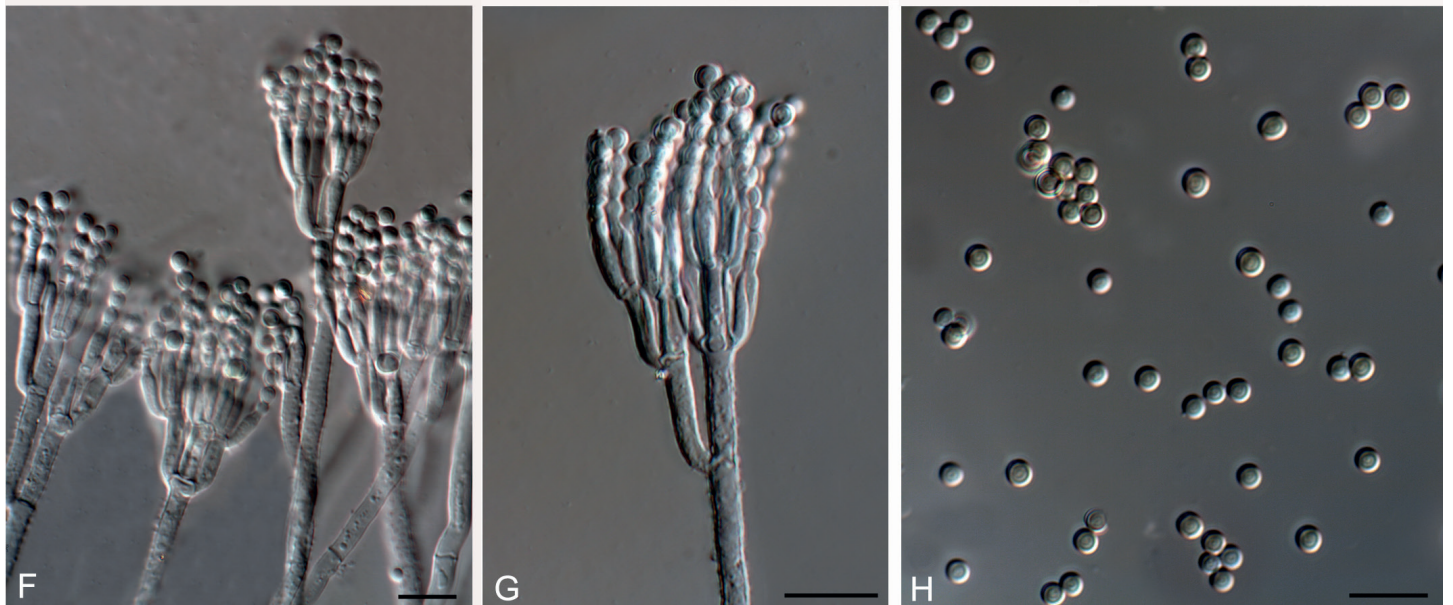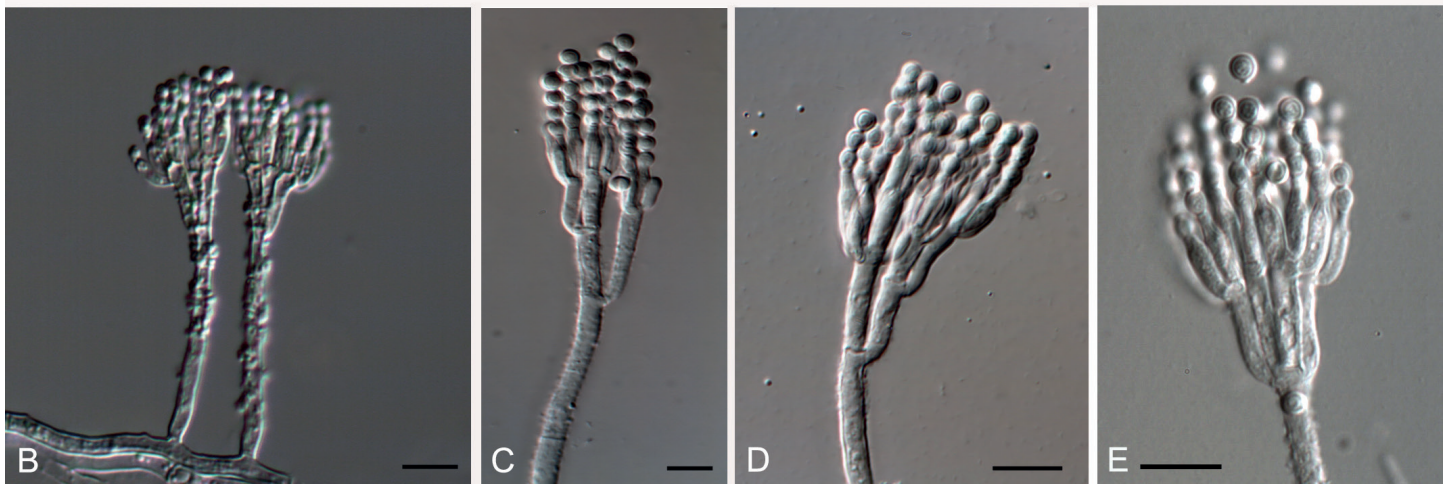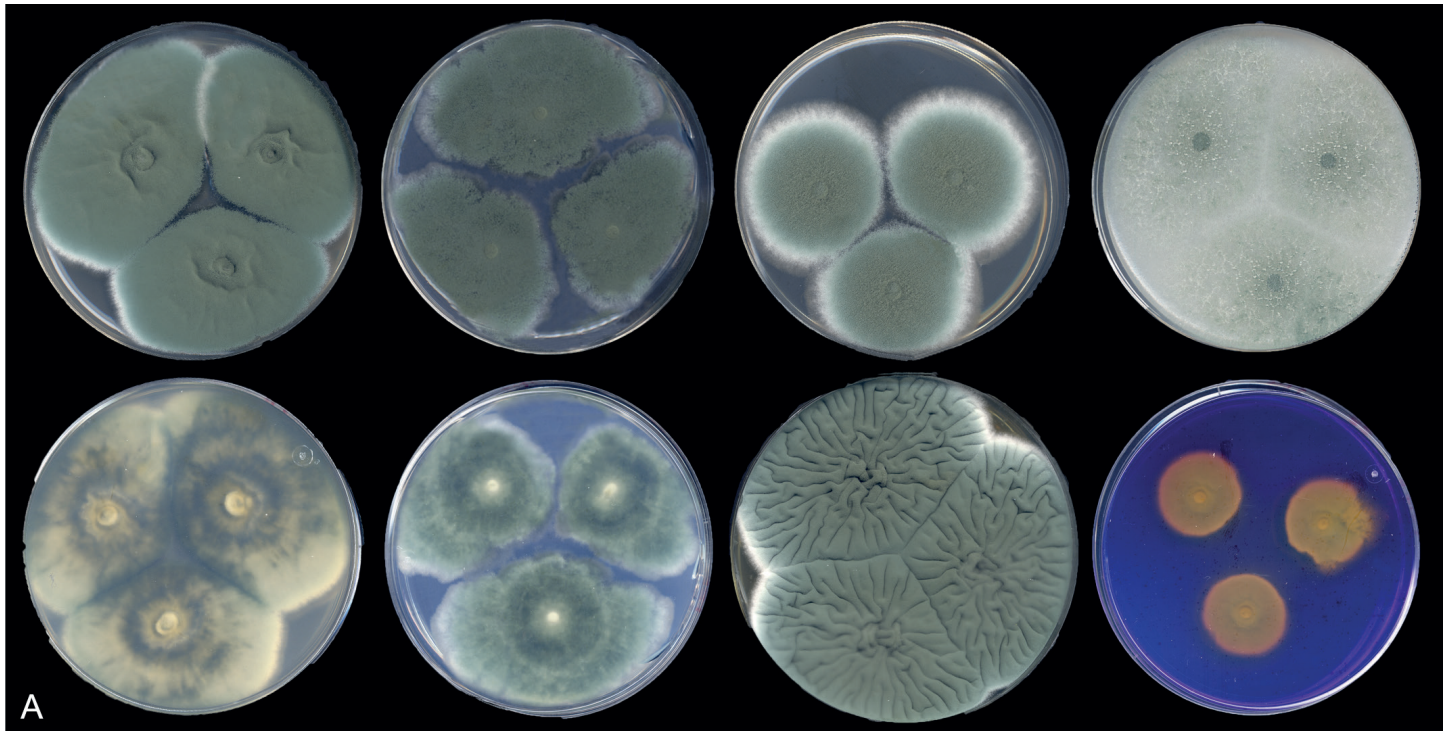
In: Section *Roquefortorum.*

Typus: **Spain**, Balearic Islands, Mallorca, Puigpuñert, from herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira* (**holotype** CBS H-23143; culture ex-type FMR 15188 = CBS 142754; ITS barcode: LT899784, alternative markers: *tub2* LT898291, *cmdA* LT899768, *rpb2* LT899802).

Additional materials examined: **Spain**, Catalonia, Els Ports Natural Park, herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & E. Rosas* (FMR 15031 = CBS 142755); Catalonia, Els Ports Natural Park, herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & E. Rosas* (FMR 15032).

Colony diameter in 7 d (mm): On CYA: 25 °C 45–60, 30 °C 24–26, 37 °C no growth; on MEA: 25 °C 45–60, 30 °C 23–26; on YES: 25 °C >60, 30 °C 35–40; on OA: 25 °C 31–38, 30 °C 8–15; on DG18: 25 °C 43–45; on CREA: 25 °C 22–25.

Colony characters at 25 °C in 7 d: On CYA, colonies flat, velvety, mycelium white, sporulation dense, conidial masses dull green (25E4), margin entire; reverse greyish green (28C5); exudate and soluble pigment absent. On MEA, colonies flat, velvety, mycelium white, margin crenate, sporulation dense, with conidial masses conidial masses dull green (25E4), margin irregular, slightly lobulate, reverse greyish green (28E5); exudate and soluble pigment absent. On DG18, colonies flat, velvety, mycelium white, sporulation dense, conidial masses dull green (26E4), margin entire; reverse greyish green (30C6). On YES, colonies velvety, raised at the centre, irregularly sulcate, mycelium white, sporulation dense, conidial masses dull green (26E4), margin entire and fimbriate; reverse greyish green (28C6); exudate and soluble pigment absent. On OA, colony cottony, mycelium white, sporulation dense, conidial masses dark dull green (28D4), margin entire; exudate and soluble pigment absent. On CREA, moderate acid production.

Micromorphology: On MEA, conidiophores bi- and terverticillate; stipes rough-walled, 50–100 × 2.5–3 µm; metula 2–3 per branch, appressed, cylindrical, 10–14 × 2–3 µm; phialides 2–4 per metula, ampulliform, 10–13 × 2–3 µm; conidia globose, 2.5–4 (–5) × 2–4 µm, smooth-walled, greenish.

Distinguishing characters: All the species in section *Roquefortorum* show fast growth on CYA and MEA and produce rough-walled conidiophores. *Penicillium roqueforti* is the closest-related species to *P. mediterraneum,* and it can be distinguished by a more restricted growth on CYA at 30 °C (5–15 mm diam) and by its blackish green colony reverse on YES (Frisvad & Samson 2004, Houbraken *et al.* 2010).

Penicillium synnematicola Guevara-Suarez, Dania García & Guarro, ***sp. nov.*** MycoBank MB822072. Fig. 17.

Etymology: Name refers to the formation of synnemata.

In: Section *Robsamsonia*.

Typus: **Spain**, Balearic Islands, Mallorca, Camí Vell d'Orient, from goat dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira* (**holotype** CBS H-23132, culture ex-type FMR 15192 = CBS 142669; ITS barcode: LT898167, alternative markers: *tub2* LT898172, *cmdA* LT898137, *rpb2* LT898142).

Additional materials examined: **Spain**, Mallorca, Escorca, from goat dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira* (FMR 15210); Mallorca, Camí Vell d'Orient, from goat dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira* (FMR 15211); Catalonia, Barcelona, Montseny Natural Park, from herbivore dung, Apr. 2017, *J. Gené, M. Guevara-Suarez & I. Iturrieta-González* (FMR 16481 = CBS 143045); Catalonia, Poblet, from herbivore dung, Mar. 2017, *J. Guarro, Guevara-Suarez & I. Iturrieta-González* (FMR 16491 = CBS 143046).

Colony diameter in 7 d (mm): On CYA: 25 °C 33–37, 30 °C 9–10, 37 °C no growth; on MEA: 25 °C 11–13, 30 °C 5–7; on YES: 25 °C 30–34, 30 °C 9–17; on OA: 25 °C 31–38, 30 °C 8–15; on DG18: 25 °C 17–19; on CREA: 25 °C 6–9.

Colony characters at 25 °C in 7 d: On CYA, colonies granular, raised at the centre, 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, colonies velvety, mycelium white, sporulation dense, conidial masses dull green (27E4); reverse greyish orange (5B3). On YES, colonies granular, raised at the centre, 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.

Micromorphology: On MEA, synnemata present, up to 1 mm long; conidiophores ter- to quaterverticillate, minor proportion biverticillate; stipes 100–218 × 3–4 µm (up to 1 mm long in synnemata), coarsely roughened; metula 3–4 per branch, rather appressed, cylindrical, smooth to conspicuously roughened, 10–14 × 2–3 µm; phialides 2–4 per metula, ampulliform, 10–13 × 2–3 µm; conidia globose, subglobose to broadly ellipsoidal, 2.5–3 × 2–2.5 µm, smooth-walled, subhyaline to pale green.

Distinguishing characters: Section *Robsamsonia* includes some species that produce synnemata, *e.g. P. coprophilum, P. glandicola* and *P. vulpinum. Penicillium coprophilum* and *P. vulpinum* can be differentiated from our new species by their smooth-walled stipes (Houbraken *et al.* 2016). *Penicillium synnematicola* is closely related to *P. glandicola*, a species with world-wide distribution isolated from oak trees and forest soil, including dungy soil and mouse dung (Houbraken *et al.* 2016), and both produce coarsely roughened synnematous

Fig. 17. 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 °C after 1 wk incubation. **C.** Colony texture on OA at 25 °C after 1 wk incubation. **D–G.** Conidiophores. **H.** Conidia. Scale bars = 10 µm.
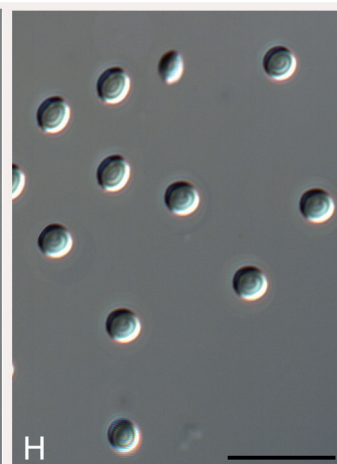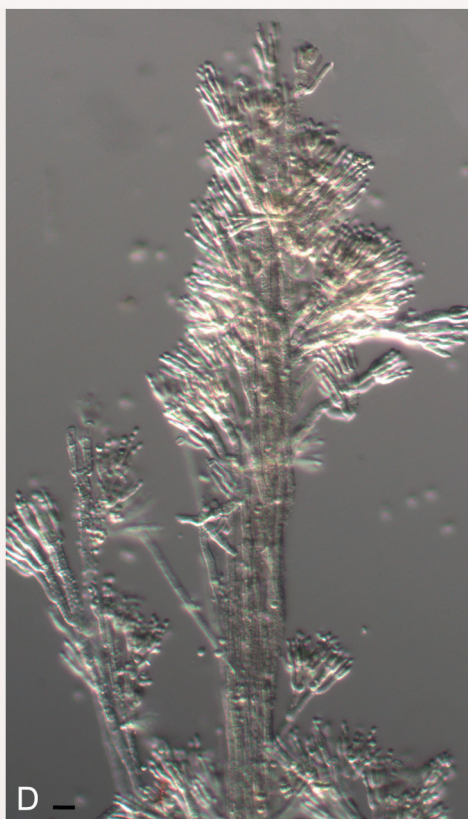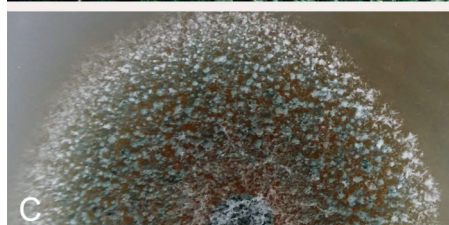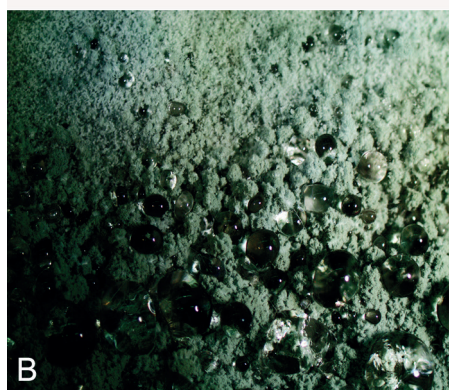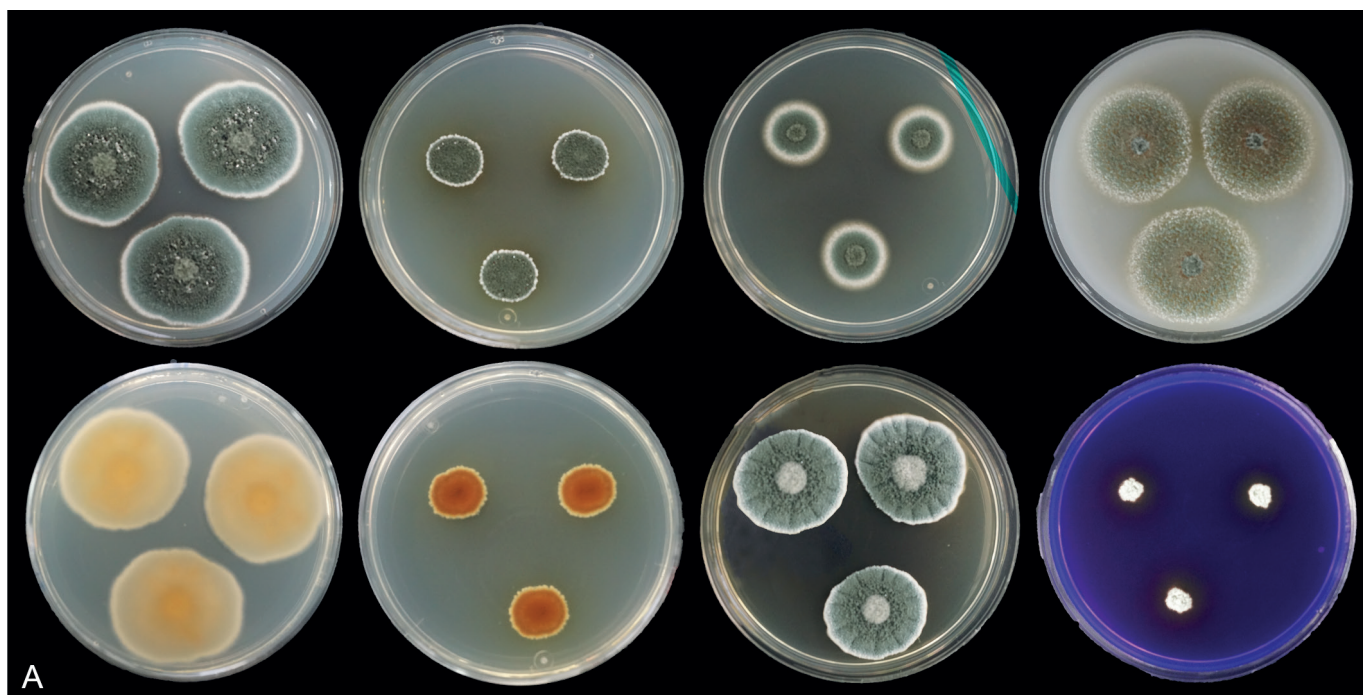
conidiophores. However, *P. glandicola* can be distinguished by having shorter phialides (7.5–10.5 µm) and ellipsoidal and yellow-green conidia. In addition, the colonies of *P. glandicola* show orange-brown to brown and bright orange-red reverse on CYA and YES, respectively, and tend to have a more restricted growth on both media (17–30 mm on CYA; 19–36 mm on YES) (Houbraken *et al.* 2016).

Penicillago Guevara-Suarez, Gené & Dania García, **gen. nov**. MycoBank MB822073.

Etymology: Name refers to the phylogenetic closeness to *Penicillium* (Fig. 10).

In: Family *Aspergillaceae.*

Diagnosis: Morphologically differs from *Penicillium* by its symmetrically biverticillate conidiophores, ampulliform to acerose phialides with a fine long neck, and conidia with conspicuous disjunctors. Conidiophore structure resembles *Talaromyces*, but phylogenetically distant.

Mycelium superficial and immersed, composed of septate, branched, canary yellow to chrome yellow hyphae. *Conidiophores* symmetrically branched, penicillium-like, composed of long, septate, hyaline stipes, often terminating in a small vesicle giving rise to a verticil of metulae, occasionally irregularly branched; metulae appressed to divergent, cylindrical to somewhat obpyriform, bearing 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 ellipsoid, coarsely echinulate, subhyaline to brown; conidial chains short to moderately long. *Sexual morph* unknown.

Type species: *Penicillago nodositata* (Valla) Guevara-Suarez, Gené & Dania García.

Notes: The genus *Penicillago* is introduced to accommodate *Penicillium nodositatum*. It is noteworthy that this species has had a confusing taxonomic history. Initially, Valla *et al.* (1989) classified *P. nodositatum* in the subgenus *Biverticillium* from its morphological affinity with members of the series *Islandica* (Pitt 1979). Later, based on molecular data, it was considered a member of the *Penicillium* section *Sclerotiora*, and tentatively placed in synonymy with *P. bilaiae* by Houbraken & Samson (2011), despite morphological differences in the conidiophore structure (biverticillate in *P. nodositatum vs* monoverticillate in *P. bilaiae*). However, Visagie *et al.* (2013) noticed that in the previous study that synonymy was based on a culture of *P. bilaiae* (CBS 330.90) mislabeled as *P. nodositatum*. The origin of the problem was probably introduced by Pitt & Samson (2000) since they erroneously designated on pag. 66 "No. 330.90 (CBS)" as neotype of *P. nodositatum*. In fact, the International Commission of *Penicillium* and *Aspergillus* (http://www.aspergilluspenicillium.org/) included the living ex-type strain of the herbarium specimen of *P. nodositatum* as CBS 333.90, this therefore being the correct ex-type strain for the species. Newly sequence data of that strain showed that *P. nodositaum* was closely related to *P. kabunicum*, and neither of them belonged in *Penicillium s. str.* (Houbraken & Samson 2011, Visagie *et al.* 2013). We observed that the only sequence available for *P. kabunicum* (CBS 574.90, ITS sequence GenBank MH862240) showed a 99.64 % of similarity with that of *P. nodositatum* (CBS 333.90, ITS sequence GenBank NR_103703) (Fig. S6 in supplemental material), suggesting they could be conspecific. However, the protologue of *P. kabunicum* (Baghdadi 1968) disagree with that of *P. nodosotatum* (Valla *et al.* 1989) in features such as the conidial morphology (globose, smooth-walled and hyaline in *P. kabunicum vs* ellipsoid to subglobose, echinulate and brown in *P. nodosotatum*). Therefore, further comparative studies will be needed to determine whether both species should be treated as synonym.

The possibility of examining two Spanish isolates that fit morphologically with *P. nodositatum* and their DNA sequences coincide with those of the ex-type strain of this species, has allowed us to delineate a new genus of penicillium-like fungi in the family *Aspergillaceae* (Figs 9, 10)*.* Because herbarium type material for *P. nodositatum* is lost according to Visagie *et al.* (2013), the illustrations included in the protologue of the species (Valla *et al.* 1989) are selected here as lectotype, and the metabolically inactive culture CBS 333.90 as epitype deposited in the Westerdijk Fungal Biodiversity Institute.

Penicillago nodositata (Valla) Guevara-Suarez, Gené & Dania García, **comb. nov.** MycoBank MB822074. Fig. 18.
Basionym: *Penicillium nodositatum* Valla, *Pl. Soil* **114**: 142. 1989.

Typus: **Lectotype designed here:** figs 1–4 in Valla *et al.* (1989), *Penicillium nodositatum* Valla, a new species inducing myconodules on *Alnus* roots, Plant & Soil 114: 142–146, MBT388228; **Epitype designed here:** metabolically inactive culture CBS 333.90, **France**, Isère, Col d'Ornon, from myconodules in roots of *Alnus incana*, 1986, *G. Valla*, MBT388229; culture ex-epitype CBS 333.90.

Additional materials examined: **Spain**, Balearic Islands, Mallorca, from wild pig dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira* (FMR 15296 = CBS 142988; ITS barcode: LT899787, alternative markers: *tub2* LT898312, *cmdA* LT899771, *rpb2* LT899805); Extremadura, Badajoz, Granja de Torrehermosa, from herbivore dung, Dec. 2016, *J. Cano-Lira* (FMR 16442).

Colony diameter in 7 d (mm): On CYA: 25 °C 40–44, 30 °C 34–36, 37 °C 7–8; on MEA: 25 °C 34–36, 30 °C 24–27, 37 °C 0–3; on YES: 25 °C 50–55, 30 °C 45–55, 37 °C 11–10; on OA: 25 °C 24–26, 30 °C 22–24, 37 °C 4–6; on DG18: 25 °C 2–3; on CREA: 25 °C 22–30.

Colony characters at 25 °C in 7 d: On CYA, colonies cottony, slightly sulcate, mycelium light yellow (3A5) to white, sporulation sparse after 7d, conidial masses yellowish brown (5E8) after 14 d, margin fimbriate; reverse greyish orange (5B4) at the centre, white to pale yellow towards the periphery; exudate and soluble pigment absent. On MEA, colonies slightly cottony, flat, mycelium light yellow (3A5) to white, sporulation dense after

Fig. 18. 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.
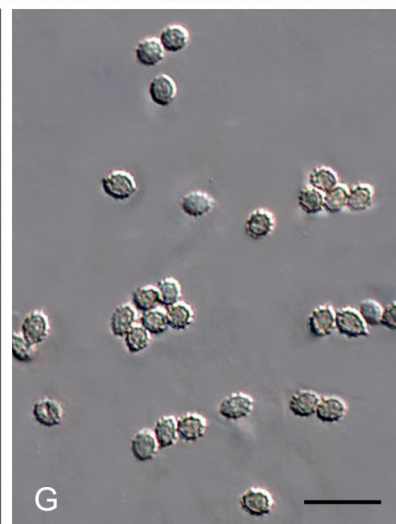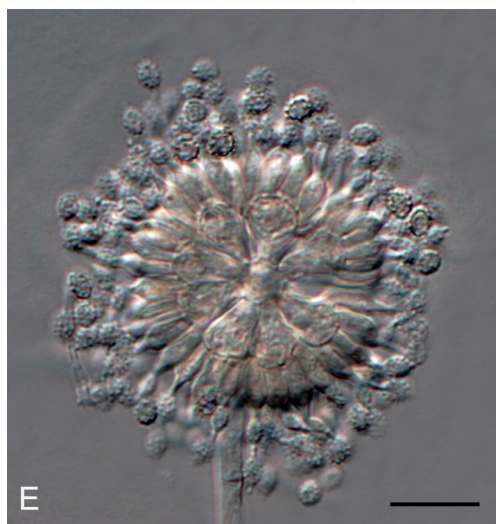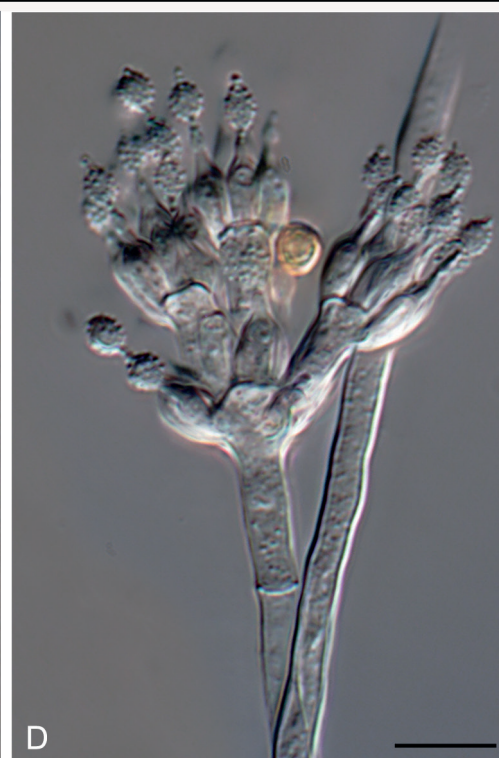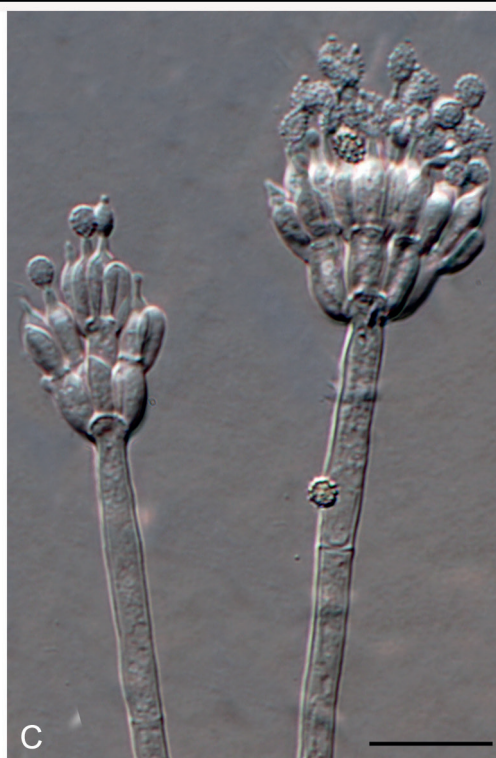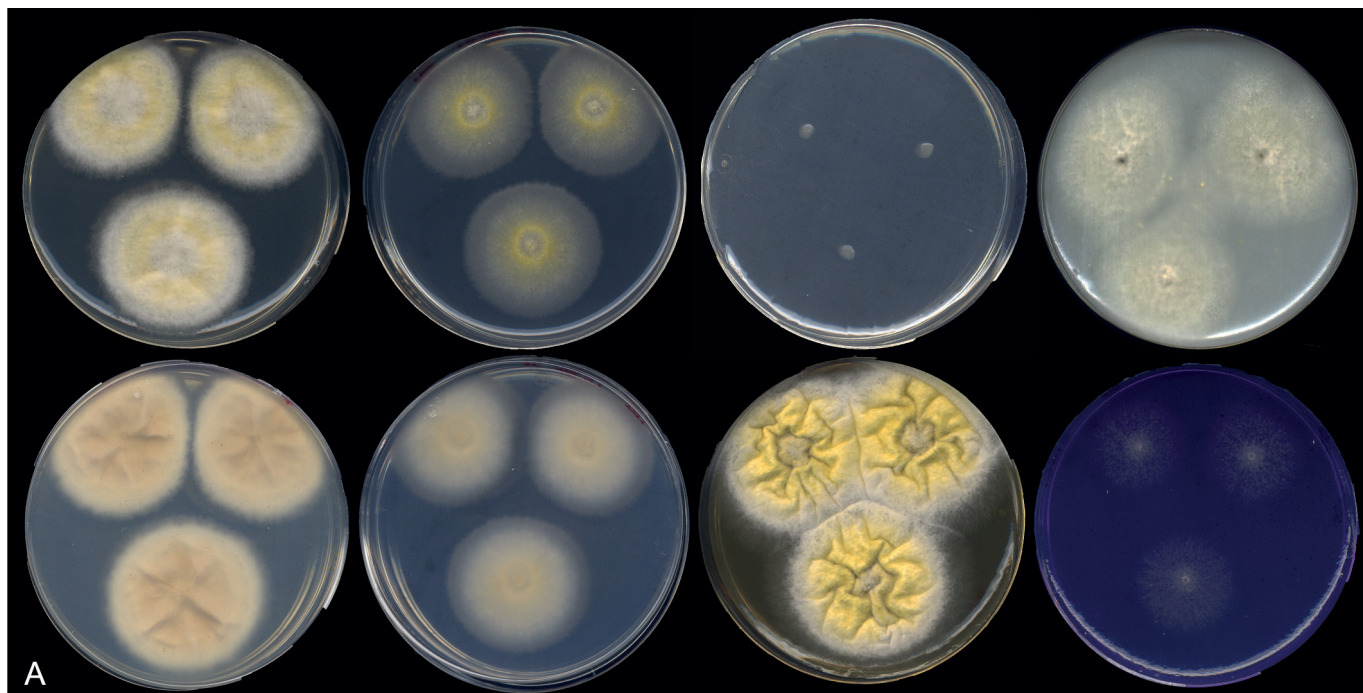
the 14 d, with conidial masses dark green (28F5), margin slightly fimbriate; reverse light yellow (4A4); exudate and soluble pigment absent. On DG18, colonies restricted, flat, mycelium white, sporulation absent, margin entire; reverse light yellow (4A4); exudate and soluble pigment absent. On YES, colonies velvety, raised at the centre, irregularly sulcate, mycelium yellow (3A7) to white, sporulation sparse, margin fimbriate; reverse greyish orange (5B5); exudate and soluble pigment absent. On OA, colony slightly cottony, mycelium white, sporulation sparse, margin entire; reverse yellowish white (4A2); exudate and soluble pigment absent. On CREA, acid production absent.

Micromorphology: On MEA, conidiophores symmetrically biverticillate, sometimes irregularly branched; stipes smooth, hyaline, often with a small vesicle at the apex, 3.5–5.5 µm wide, 200–300 × 2.5–3 µm; metulae divergent, cylindrical to somewhat obpyriform, 9–10(–15) × 3–4(–5) µm; phialides four to seven per metula, ampulliform or tending towards acerose, often tapering abruptly to a long, slender neck, 6–8(–9) × 2.5–3.5 µm; conidia in short to moderately long disorder chains, subglobose to broadly ellipsoidal, echinulate, 2.5–4 × 3–4(–5) µm, pale brown.

Pseudopenicillium Guevara-Suarez, Cano & Guarro, **gen. nov.** MycoBank MB822076.

Etymology: *Pseudo-* meaning "false"-, name refers to the morphological similarity but not belonging to *Penicillium*.

In: Family *Aspergillaceae*.

Diagnosis: Phylogenetic closeness to *Hamigera*, but differs in the lack of sexual morph and by its brown globose conidia with conspicuous disjunctors. Morphologically differs from *Penicillium* and *Penicillago* by its short and often irregularly branched conidiophores producing large conidia in short chains.

Mycelium superficial and immersed, composed of septate, branched, hyaline to subhyaline hyphae. *Conidiophores* undifferentiated, reduced to conidiogenous cells arising directly from the main hyphae, or differentiated composed of short to moderately long, aseptate or few septate, hyaline to brown stipes, terminating in an irregular penicilli; penicilli varying from monoverticillate to partly biverticillate, with few metulae bearing each a verticil of conidiogenous cells; metulae terminal or subterminal, generally divergent, cylindrical or apically inflated. *Conidiogenous cells* phialidic, ampulliform, with swollen base and tapering abruptly to a slender neck of variable length. *Conidia* dry, catenate, with conspicuous disjunctors, globose to subglobose, with spinulose walls, brown, dark in masses; conidial chains short. *Sexual morph* unknown.

Type species: *Pseudopenicillium megasporum* (P.A. Orpurt & D.I. Fennell) Guevara-Suarez, Cano & Gené

Notes: The genus *Pseudopenicillium* is proposed to accommodate a new species and two species previously classified in the genus *Penicillium*, *P. giganteum* and *P.* *megasporum*, the latter being selected as the type since it was the first described. *Penicillium giganteum* and *P. megasporum* were recovered from soil samples in India and the UK, respectively. Despite their different geographical origin, both were considered conspecific and classified in series *Megasporus* (Pitt 1979). *Penicillium asperosporum,* also included in this series, was later shown to be a synonym of *P. montanense* in section *Aspergilloides* (Houbraken *et al.* 2014b). Multi-gene phylogenies (Peterson *et al.* 2010, Houbraken & Samson 2011) demonstrated that *P. megasporum* and *P. giganteum* were distinct species, closely related to the genus *Hamigera*. Our phylogeny confirms this relationship and shows that *Hamigera* and the afore-mentioned *Penicillium* species comprise two sister clades with enough genetic differences to be considered distinct genera (Figs 9, 10).

Pseudopenicillium cervifimosum Guevara-Suarez, Dania García & Gené, **sp. nov.** MycoBank MB822079. Fig. 19.

Etymology: From the Latin cervus = "deer", and fimosum = "dung-dwelling", describing the substrate from where the species was isolated.

*Typus*: **Spain**, Castile and Leon, Palencia, Monte el Viejo, Deer Reserve Park, from deer dung, Mar. 2016, *J. Guarro & M. Guevara-Suarez* (**holotype** CBS H-23133; culture ex-type FMR 15299 = CBS 142670; ITS barcode: LT899789, alternative markers: *tub2* LT898315, *rpb2* LT899807).

Colony diameter in 7 d (mm): On CYA: 25 °C 26–30, 30 °C 30–32, 37 °C 6–9; on MEA: 25 °C 13–17, 30 °C 22–24, 37 °C 3–5; on YES: 25 °C 24–26, 30 °C 35–38, 37 °C 23–25; on OA: 24–26, 30 °C –34, 36 °C 14–16; on DG18: 25 °C 16–17; on CREA: 25 °C 18–20.

Colony characters at 25 °C in 7 d: On CYA, colonies flat, velvety, mycelium white, sporulation dense, central area with conidial masses dark green (29F3), margin entire; reverse pale yellow (4A3); exudate and soluble pigment absent. On MEA, colonies growing moderately, velvety, slightly umbonate, mycelium white, sporulation dense, with conidial masses olive-yellow (3C8) to olive (3F8), margin lobate; exudate and soluble pigment absent; reverse greyish yellow (2B4). On YES, colonies dome-shaped, velvety, mycelium white, sporulation absent, margin entire; reverse pale yellow (4A3); exudate and soluble pigment absent. DG18, colonies velvety, slightly sulcate, mycelium white, sporulation dense, with conidial masses dull green (28E3), margin entire; reverse yellowish white (4A3). On OA, colonies cottony, mycelium white, sporulation dense, with conidial masses dark green (29F3), margin fimbriate; exudate and soluble pigment absent. On CREA, strong acid production.

Micromorphology: On MEA, conidiophores short, arising as lateral branches from hyphae, biverticillate, sometimes monoverticillate; stipes 10–35 × 2.5–3 µm, cylindrical, without vesicle, smooth to finely verruculose, hyaline; metulae divergent, cylindrical, 9.5–10 × 2.5–3 µm; phialides in verticils of 2–4, ampulliform with swollen base, tapering abruptly to a

Fig. 19. Morphological characters of *Pseudopenicillium cervifimosum* (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 °C after 1 wk incubation. **C–H.** Conidiophores. **I.** Conidia. Scale bars = 10 µm.
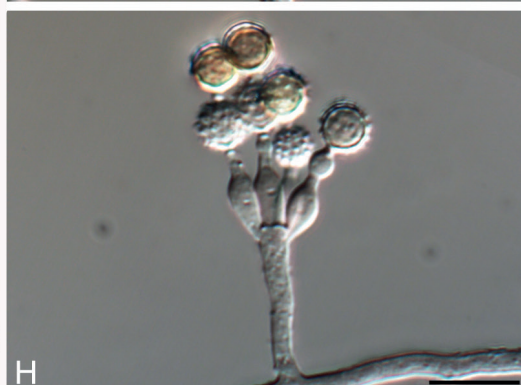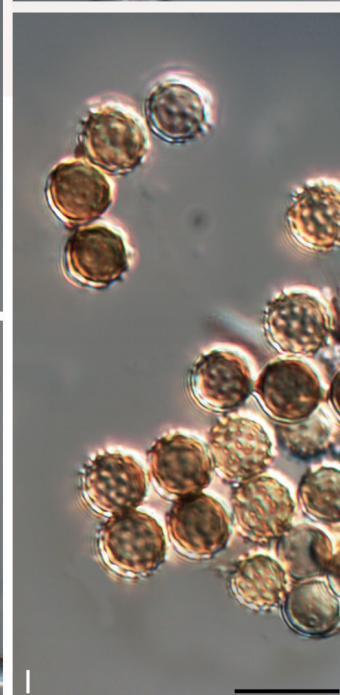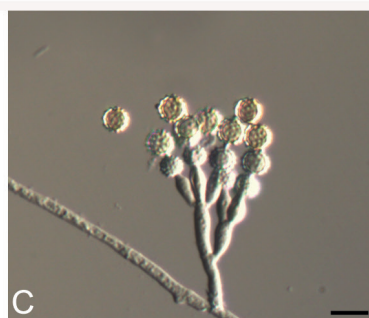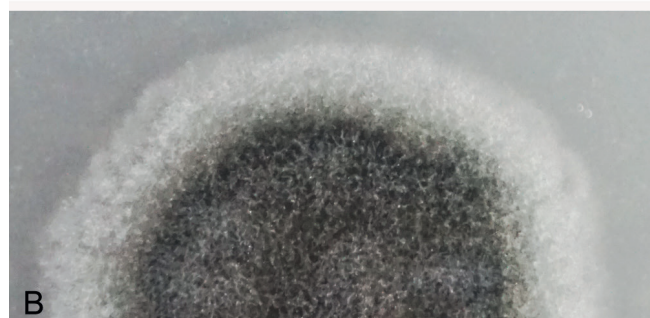
slender neck, (6–)7.5–9 × 2.5–3 µm; conidia globose, 5–5.5 × 6 µm, spinulose, thick-walled, pale brown to brown.

Distinguishing characters: *Pseudopenicillium cervifimosum* is closely related to *Pse. giganteum* and *Pse. megasporum.* This group of species have a similar conidiogenous apparatus and produce globose spinulose conidia. However, they can be distinguished by their conidial sizes (*i.e.*, up to 6 µm in *Pse. cervifimosum,* up to 10 µm in *Pse. megasporum* and up to 12 µm in *Pse. giganteum*). In addition, *Pse. megasporum* has a less abundant growth on CYA at 25 °C (15–25 mm) and shows vesiculate stipes (Pitt 1979, Peterson *et al.* 2010).

Pseudopenicillium giganteum (R.Y. Roy & G.N. Singh) Guevara-Suarez, Gené & Cano, ***comb. nov.*** MycoBank MB822077.
Basionym: *Penicillium giganteum* R.Y. Roy & G.N. Singh, *Trans. Br. Mycol. Soc*. **51**: 805. 1968.

Typus: **India**, West Bengal, Varanasi, Banaras Hindu University, Botanical Garden, from soil, Nov. 1967, *G.N. Singh* (**holotype** IMI 132774; cultures ex-type NRRL 3553 = ATCC 48996 = CBS 144.69 = FRR 535 = IMI 132774).

Descriptions and illustrations: See Roy & Singh (1968) and Peterson *et al.* (2010).

Additional material examined: **Spain**, unknown geographic region, from soil (FMR 14718).

Notes: The new isolate is the second specimen known for this taxon. In general, its morphological features are consistent with those of the species as described by Roy & Singh (1968).

Pseudopenicillium megasporum (P.A. Orpurt & D.I. Fennell) Guevara-Suarez, Cano & Gené, ***comb. nov.*** MycoBank MB822078.
Basionym: *Penicillium megasporum* P.A. Orpurt & D.I. Fennell, *Mycologia* **47**: 233. 1955.

Typus: **UK, England**, Suffolk, Lakenheath Warren, from heath soil, *J.H. Warcup* (**holotype** IMI 216904; cultures ex-type NRRL 2232 = ATCC 12322 = CBS 256.55 = FRR 2232 = MUCL 38804 = QM 6879 = WE 2232 = IMI 216904).

Descriptions and illustrations: See Orpurt & Fennell (1955), Pitt (1979) and Peterson *et al.* (2010).

Talaromyces catalonicus Guevara-Suarez, Gené & Guarro, ***sp. nov.*** MycoBank MB822080. Fig. 20.

Etymology: Name refers to the region (Catalonia) from where the fungus was isolated.

In: Section *Trachyspermi.*

Typus: **Spain**, Catalonia, Poblet, from herbivore dung, Feb. 2017, *J. Guarro, M. Guevara-Suarez & I. Iturrieta-González* (**holotype** CBS H-23212; culture ex-type FMR 16441 = CBS 143039; ITS barcode: LT899793, alternative identification markers: *tub2* LT898318, *cmdA* LT899775, *rpb2* LT899811).

Colony diameter in 7 d (mm): On CYA: 25 °C 35–40, 30 °C 38–40, 37 °C 15–18; on MEA: 25 °C 17–19, 30 °C 19–20, 37 °C 16–18;

on YES: 25 °C 45–50, 30 °C 50–55, 37 °C 25–30; on OA: 25 °C 40–45, 30 °C 40–42, 37 °C 12–14; on DG18: 25 °C 20–22, on CREA: 25 °C 17–19.

Colony characters at 25 °C in 7 d: On CYA, colonies velvety, radially sulcate, mycelium white, sporulation dense, conidial masses dull green (27E4), margin lobate; reverse greyish orange (5B4); exudate absent, soluble pigment only at 30 and 37 °C, light yellow (3A5) to light orange (5A6). On MEA, colonies velvety, flat, sporulation dense, with conidial masses greyish green (27E5), margin entire; reverse yellow (2B8); exudate and soluble pigment absent. On DG18, colonies flat, slightly cotton at the centre, velvety in the periphery, mycelium white, sporulation dense, conidial masses dull green (26E5), margin entire; reverse greyish green (28B4). On YES, colonies raised at the centre, irregularly sulcate, mycelium white, sporulation dense, with conidial masses dark green (30F3), margin entire; reverse orange-yellow (4B8); exudate and soluble pigment absent. On OA, colonies velvety, flat, mycelium white, sporulation dense, conidial masses dark green (27F3), margin entire; reverse greenish grey (29C2); exudate and soluble pigment absent. On CREA, weak acid production.

Micromorphology: On MEA, conidiophores biverticillate, sometimes irregularly branched, stipes (40–)100–130 × 2–2.5 µm, smooth-walled, hyaline; metulae divergent, 10–11 × 2–2.5 µm; phialides 4–6 per metula, ampulliform to acerose, (7–)8–10 × 2–2.5 µm; conidia globose to subglobose, 2–2.5 × 1.8–2 µm, smooth-walled, brownish yellow. Ascomata not observed.

Distinguishing characters: The most closely related species to *T. catalonicus* are *T. albobiverticillius, T. erythromellis, T. heiheensis,* and *T. solicola* (Fig. 8)*. Talaromyces catalonicus* is characterised by its ability to grow at 37 °C; in contrast, the above-mentioned species do not grow or grow very restricted at this temperature (Samson *et al.* 2011, Yilmaz *et al.* 2014).

Talaromyces coprophilus Guevara-Suarez, Cano & Dania García, ***sp. nov.*** MycoBank MB822088. Fig. 21.

Etymology: Name refers to the source of isolation of the fungus.

In: Section *Talaromyces.*

Typus: **Spain**, Balearic Islands, Mallorca, Escorca, from herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & J.P.Z. Siqueira* (**holotype** CBS H-23144; culture ex-type FMR 15199 = CBS 142756; ITS barcode: LT899794, alternative identification markers: *tub2* LT898319, *cmdA* LT899776, *rpb2* LT899812).

Colony diameter in 7 d (mm): On CYA: 25 °C 38–40, 30 °C 40–45, 37 °C 30–35; on MEA: 25 °C 33–35, 30 °C 30–35, 37 °C 21–24; on YES: 25 °C 45–50, 30 °C 50–55, 37 °C 38–42; on OA: 25 °C 35–40, 30 °C 40–45, 37 °C 25–26; on DG18: 25 °C no growth, on CREA: 25 °C 8–10.

Colony characters at 25 °C in 7 d: On CYA, colonies slightly raised at the centre, velvety, mycelium light yellow (4A5) to white, sporulation absent to sparse, with margin entire and fimbriate; reverse light orange (5A4); soluble pigment and exudate absent. On MEA, colonies slightly raised at centre, velvety, flat, mycelium light yellow (4A5) to white, conidial sporulation absent to sparse, young ascomata visible, margin fimbriate; reverse light
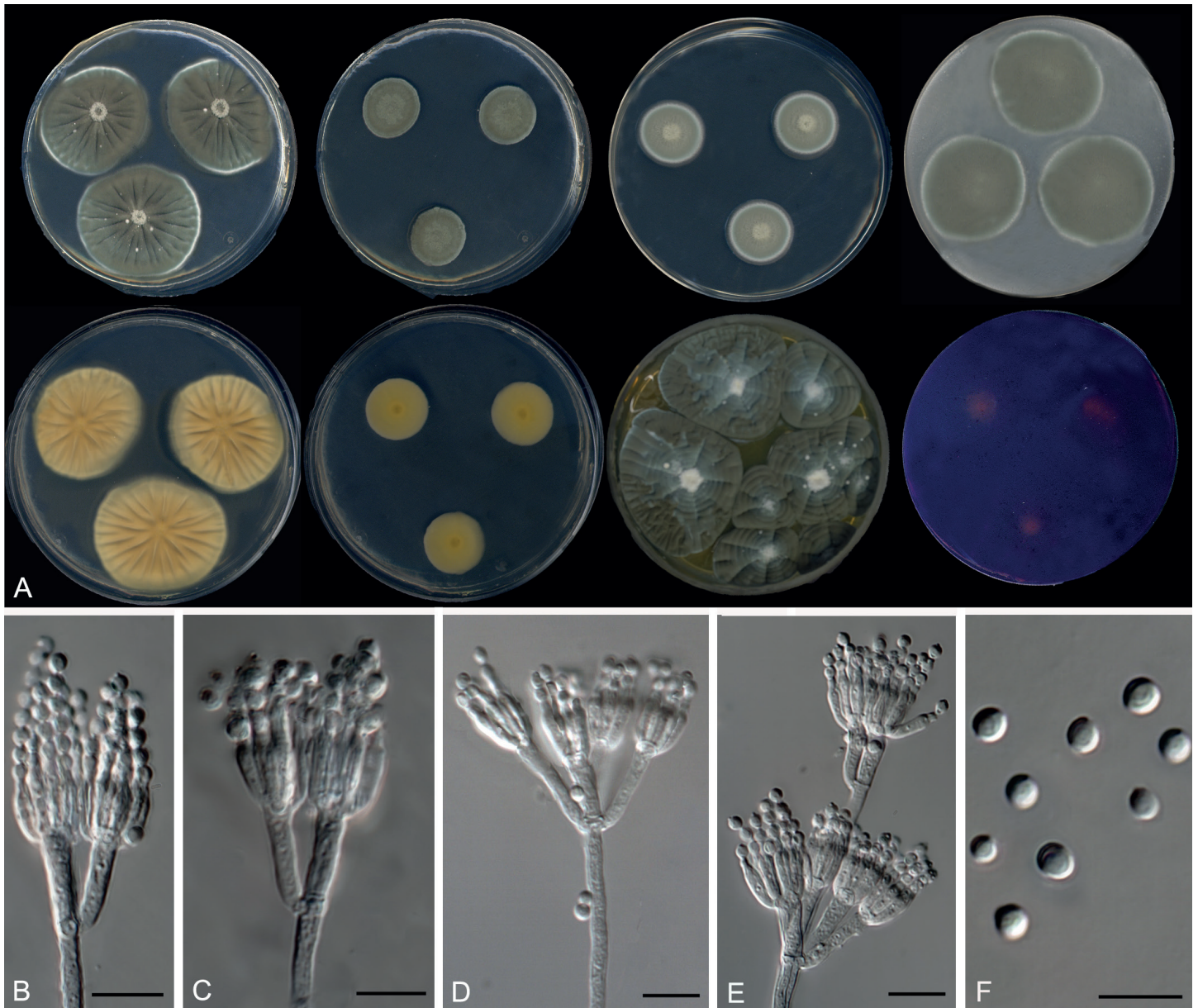
Fig. 20. Morphological characters of *Talaromyces catalonicus* (FMR 16441^T). **A.** Colonies from left to right (top row) CYA, MEA, DG18 and OA reverse; (bottom row) CYA reverse, MEA reverse, YES, and CREA. **B–E.** Conidiophores. **F.** Conidia. Scales bars = 10 μm.

orange (5A4); soluble pigment and exudate absent. On YES, colonies raised at centre, velvety, mycelium pinkish (7A3) to white, sporulation absent, with margin entire; reverse brownish orange (7C6); exudate absent and soluble pigment brownish red (10C7). On OA, colonies granular, flat, with young bright orange ascomata visible, conidial sporulation sparse, margin fimbriate; reverse greenish grey (28C3); exudate and soluble pigment absent. On CREA, strong acid production.

Micromorphology: On MEA, conidiophores mono- to biverticillate, stipes 40–70 × 2–2.5 μm, smooth, hyaline; metulae rather appressed, 10–12 × 2.5–3 μm; phialides 3–4 per metula, acerose, 9–12(–14) × 2–2.5 μm; conidia ellipsoidal, 2–3 × 2.5–3 μm, smooth-walled, brownish yellow. Ascomata after 1–2 wk of incubation on OA and MEA at 25 °C, bright orange to orange-red, globose, 200–310 μm diam, with peridial hyphae branched and verruculose; asci globose to subglobose, 6–7 × 7–9(–10) μm; ascospores ellipsoidal, 3.5–4 × 3–4 μm, spiny, thick-walled, golden yellow.

Distinguishing characters: *Talaromyces coprophilus* is characterised by the production of orange-red ascomata with spiny ellipsoidal ascospores, the lack of growth on DG18, and a good growth on other culture media tested. Phylogenetically, it forms an independent and distant branch in an unsupported clade where the ex-type strains of *T. cnidii*, *T. flavovirens*, *T. siamensis*, and *T. xishaensis* are found (Fig. 7). Morphologically, *T. coprophilus* resembles *T. flavovirens* in the production of a sexual morph; however, the latter differs in slower growth on CYA at 25 °C (19–20 mm after 7d), and larger ascospores (4–7 × 3–4 μm) (Yilmaz *et al.* 2014).

Talaromyces pseudofuniculosus Guevara-Suarez, Dania García & Gené, ***sp. nov.*** MycoBank MB822090. Fig. 22.

Etymology: –*pseudo*, meaning "false"-, name refers to its phylogenetic relationship with *T. funiculosus*.

In: Section *Talaromyces*.
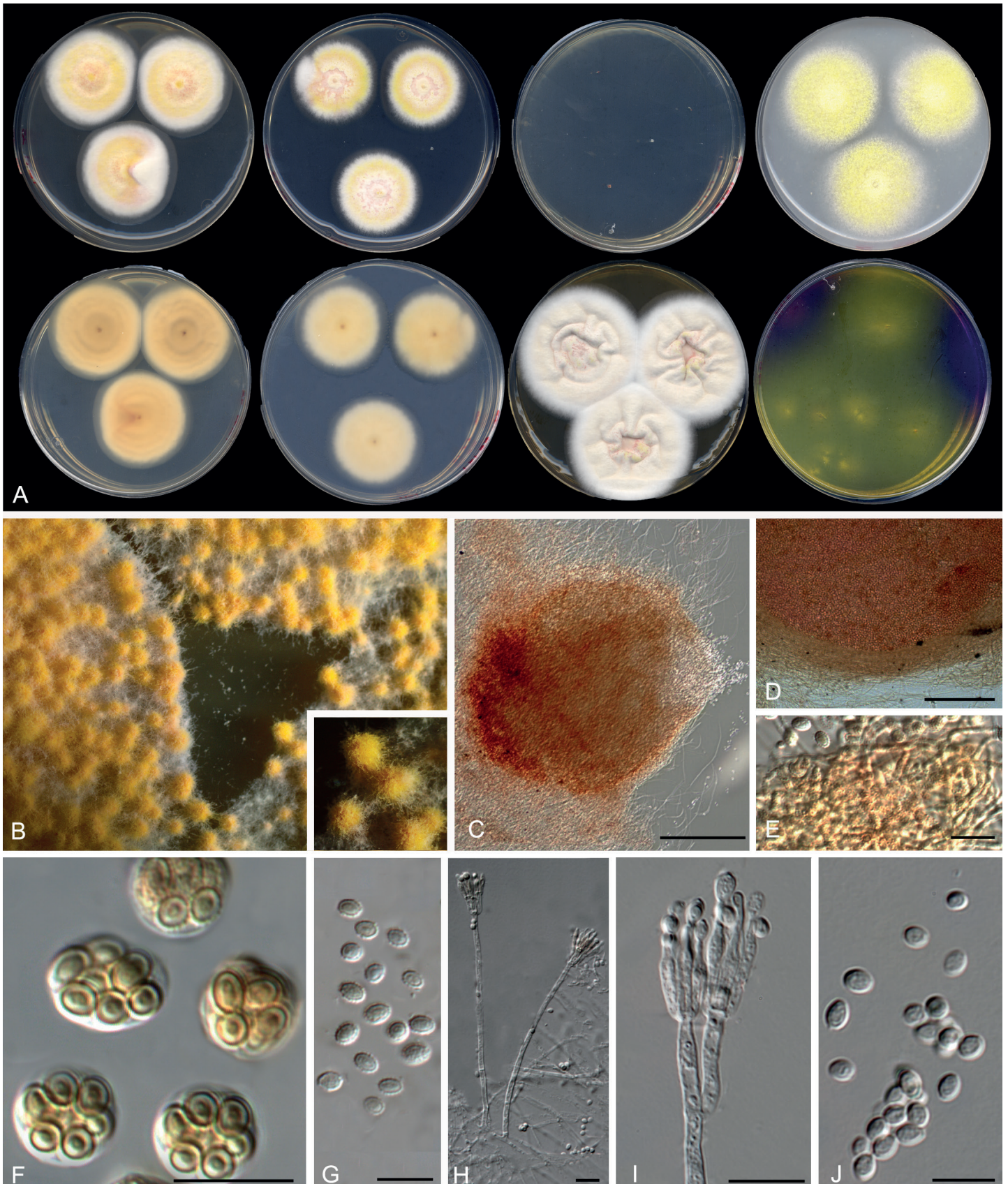
Fig. 21. Morphological characters of *Talaromyces coprophilus* (FMR 15199ᵀ). **A.** Colonies from left to right (top row) CYA, MEA, DG18 and OA reverse; (bottom row) CYA reverse, MEA reverse, YES, and CREA. **B.** Detail of the colony texture with ascomata on MEA after 2 wk incubation. **C.** Ascoma. **D, E.** Part of an ascoma and peridial hyphae. **F.** Asci. **G.** Ascospores. **H, I.** Conidiophores. **J.** Conidia. Scales bars C, D = 100 µm, E–J = 10 µm.

Typus: **Spain**, Andalusia, La Rocina, Doñana National Park, from herbivore dung, Mar. 2016, *D. García* (**holotype** CBS H-23214; culture ex-type FMR 15307 = CBS 143041; ITS barcode: LT899796, alternative markers: *tub2* LT898323, *cmdA* LT899778, *rpb2* LT899814).
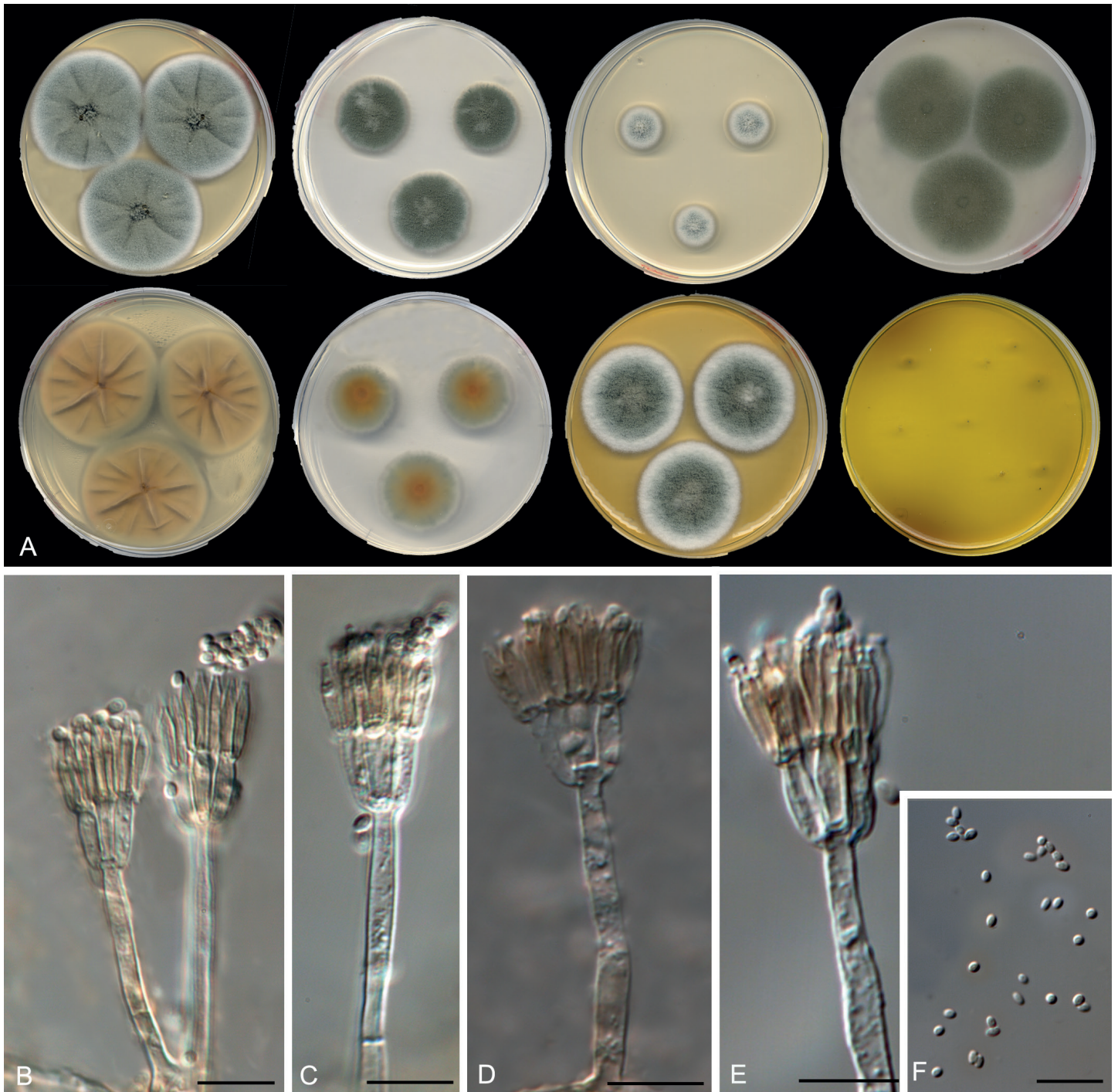
Fig. 22. Morphological characters of *Talaromyces pseudofuniculosus* (FMR 15307[T]). **A.** Colonies from left to right (top row) CYA, MEA, DG18 and OA reverse; (bottom row) CYA reverse, MEA reverse, YES, and CREA. **B–E.** Conidiophores. **F.** Conidia. Scale bars = 10 µm.

Additional material examined: **Spain**, Catalonia, Els Ports Natural Park, from herbivore dung, Feb. 2016, *J. Guarro, M. Guevara-Suarez & E. Rosas* (FMR 15035).

Colony diameter in 7 d (mm): On CYA: 25 °C 36–43, 30 °C 45–40, 37 °C 31–35; on MEA: 25 °C 24–29, 30 °C 43–45, 37 °C 23–26; on YES: 25 °C 34–35, 30 °C 40–44, 37 °C 32–34; on OA: 25 °C 33–38, 30 °C 42–45, 37 °C 27–30; on DG18: 25 °C 12–15, on CREA: 25 °C 2–4.

Colony characters at 25 °C in 7 d: On CYA, colonies slightly raised at centre, 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 centre, sulcate, cottony, mycelium white, sporulation dense in the centre, 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 biverticillate; stipes 40–70 × 2.5–3 μm, smooth-walled, brownish yellow; metulae 3–4 per branch, appressed, 8–10 × 2–3 μm; phialides 3–4 per metula, acerose, 8–10(–11) × 2–3 μm; conidia mostly ellipsoidal, 2–3 × 1.5–2(–2.5) μm, smooth-walled. Ascomata not observed.

Distinguishing characters: *Talaromyces cucurbitiradicus*, recently described by Su & Niu (2018), and *T. funiculosus* are the closest related species to *T. pseudofuniculosus.* The former can be distinguished from the other two by the production of chlamydospores and absence of growth at 37 °C. *Talaromyces funiculosus* differs from the new species by its divergent metulae (appressed in *T. pseodofuniculosus*) and by the pigmentation of the conidiophores, being olivaceous in *T. funiculosus* and brownish yellow in *T. pseudofuniculosus.* 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).

DISCUSSION

In recent years, the phylogeny of the genera *Penicillium, Talaromyces* and other members of family *Trichocomaceae sensu lato* was studied in order to clarify their taxonomic relationships. Subsequently, many species were re-classified and new taxonomic approaches proposed for species delineation and identification. Considering that several of these fungi have been discovered from specific and poorly studied substrates using modern taxonomic techniques (Houbraken *et al.* 2014b, Barbosa *et al.* 2018), we explored the species diversity of penicillium-like fungi from herbivore dung collected in different Spanish regions (Andalusia, Balearic and Canary Islands, Castile and Leon, Catalonia and Galicia) using the gene markers recommended for their study (Visagie *et al.* 2014b, Yilmaz *et al.* 2014).

Our results agree with other studies in which a large set of isolates of penicillium-like fungi could be identified with the *tub2* sequence analysis (Guevara-Suarez *et al.* 2016, Visagie *et al.* 2014a, Chen *et al.* 2016, Barbosa *et al.* 2018). It is a good marker, easy to amplify and sequence, and useful for the classification at the sectional and species level, and even for detection of putative undescribed species. However, in addition to the ITS and *tub2* barcodes, the characterization of penicillia requires multi-gene analyses along with other markers such as fragments of *cmdA* and/or *rpb2* loci. Based on this molecular approach, we were able to find a remarkable diversity of penicillium-like fungi that have not been described on herbivore dung so far. Among the 104 isolates recovered, we identified a total of 48 species, 38 *Penicillium* including seven new taxa (*i.e. P. balearicum, P. beceitense, P. caprifimosum, P. fimosum, P. ibericum, P. mediterraneum*, and *P. synnematicola*), seven *Talaromyces* including three new taxa (*T. catalonicus, T. coprophilus*, and *T. pseudofuniculosus*), and three species in the newly prosposed genera *Penicilago* (*Pgo. nodositata*) and *Pseudopenicillium* (*Pse. giganteum*, and the new species *Pse. cervifimosum*). In addition, of note is that the highest number of species identified (34 out of 48) and novel taxa described (9 out of 11) were recovered from samples collected in the Mediterranean area, namely Catalonia and the Balearic island of Mallorca (Table 1). This is not surprising if we consider that this area is recognized as one of the most species rich regions in Southern Europe (Cuttellot *et al.* 2008, Rundel *et al.* 2018).

Among *Penicillium* isolates, those of the sections *Fasciculata* and *Robsamsonia* were the most frequently isolated, accounting nearly 20 % of the isolates in each section. It is of note, however, that in *Fasciculata* most isolates identified were of *P. crustosum*, while in *Robsamsonia*, as expected, we found the most species diversity among the sections included here. This latter section currently comprises 11 species (Houbraken *et al.* 2016) and we identified five of these taxa, in addition to the new *P. synnematicola*. This species would seem to be a common coprophilous fungus in the Mediterranean area, as its isolates were recovered exclusively from samples collected in different locations from Mallorca and Catalonia. As mentioned before, Houbraken *et al.* (2016) restricted the majority of coprophilous *Penicillium* species in section *Robsamsonia*. However, our results clearly show that the coprophilous penicillia are distributed across different and phylogenetically distant sections of the genus (Fig. 1). In sections such as *Aspergilloides*, *Exilicatus* and *Paradoxa*, we even found a similar diversity to the section *Robsamsonia*. Of particular note is *Paradoxa* with the highest number of new penicillia proposed in this study (*P. ibericum*, *P. fimosum* and *P. balearicum*). However, we also identified *P. atramentosum* and *P. magnielliptisporum* as first records on dung. The former species is commonly associated to cheese and *P. magnielliptisporum* was described from house dust (Visagie *et sal.* 2014a). The only species in the section reported previously from animal faeces was *P. paradoxum*, an aspergillus-like *Penicillium* collected in different countries such as Denmark, India, New Zealand, the Netherlands and UK (Houbraken *et al.* 2014b, Rajeshkumar *et al.* 2016). *Paradoxa* is a very small section (nine species, including our new proposal) in comparison to sections *Aspergilloides* and *Exillicaulis*, which have 50 species each, but without any species reported from dung (Houbraken *et al.* 2014d, Visagie *et al.* 2016).

The most *Talaromyces* isolates found in our survey were from section *Talaromyces*. This is the largest section in the genus, with nearly 60 species, 22 of them described in the last four years from different substrates, including human clinical specimens (Visagie *et al.* 2015, Crous *et al.* 2016, Cheng *et al.* 2016, Wang *et al.* 2016a, b, 2017, Yilmaz *et al.* 2016, Guevara-Suarez *et al.* 2017, Barbosa *et al.* 2018, Jiang *et al.* 2018). We added two more new taxa in the section (*T. coprophilus* and *T. pseudofuniculosus*), but also identified several interesting species firstly reported from dung, such as *T. angelicus, T. muroii, T. ruber*, and *T. sayulitensis.* Unlike *T. ruber*, which is a world-wide species isolated from soil, air and fuel (Yilmaz *et al.* 2012), the other three are considered uncommon fungi previously found in the roots of *Angelica gigas* in Korea (Sang *et al.* 2013), in soil in Taiwan (Chen *et al.* 2016), and in house dust in Mexico (Visagie *et al.* 2014a), respectively. The other new *Talaromyces* species described is *T. catalonicus* in section *Trachyspermi.* This is a much smaller section than *Talaromyces*, with 19 species including our new one (Yaguchi *et al.* 1996, Yilmaz *et al.* 2014, Chen *et al.* 2016, Luo *et al.* 2016, Wang *et al.* 2016b, Guevara-Suarez *et al.* 2017, Barbosa *et al.* 2018, Rajeshkumar *et al.* 2019). The most recently described are *T. aerius* from indoor air in China (Chen *et al.* 2016), *T. amyrossmaniae* from decaying fruits in India (Rajeshkumar *et al.* 2019), *T. brasiliensis* from honey and nest of bee in Brazil (Barbosa *et al.* 2018), *T. heiheensi* from rotten wood in China (Wang *et al.* 2017), *T. minnesotensis* from human specimens in the USA (Guevara-Suarez *et al.* 2017), and *T. rubrifaciens* from air outlets of public buildings also in China (Luo *et al.* 2016), although this latter was considered a synonym

of *T. albobiverticillius* by Chen *et al.* (2016). To date, however, there are no reports of species in either of these two sections isolated from dung samples.

Penicilago nodositata seems to be a common fungus usually associated with the roots of *Alnus* in Europe (Valla 1989, Sequerra *et al.* 1997); it is not surprising therefore to recover this fungus from animal dung, in our case from a wild pig, which feeds on a wide variety of plant material including roots, shoots and fruit. Conversely, *Pse. giganteum* is a rare species only known from its type collection, a garden soil sample from India (Roy & Singh 1968). In addition to the latter species, we also recovered from herbivore dung other uncommon species of *Penicillium* and *Talaromyces*, which are monotypic or have very few isolates available for investigation. These are *P. canariense* (section *Stolkia*), *P. cremeogriseum* (section *Lanata-Divaricata*), *P. momoii* (section *Exilicaulis*), and *P. roseoviride* (section *Aspergilloides*), including the three *Talaromyces* species mentioned before from section *Talaromyces* (*i.e. T. angelicus, T. muroii* and *T. sayulitensis*). Therefore, our study highlights that herbivore dung is a good substrate for recovering interesting species of penicillium-like fungi, although their ecological role on that substrate remains unclear and would merit further investigation.

ACKNOWLEDGEMENT

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Fig. S1. ML tree of selected *Penicillium* section *Roquefortorum* species, inferred from *tub2*. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 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] = ex-type strain.

Fig. S2. ML tree of selected *Penicillium* section *Robsamsonia* species, inferred from *tub2*. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 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] = ex-type strain.

Fig. S3. ML tree of selected *Penicillium* section *Paradoxa* species, inferred from *tub2*. Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 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] = ex-type strain.

Fig. S4. ML tree of selected *Penicillium* section *Exilicaulis* species, inferred from *tub2*, 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 above 70 %/0.95 are indicated on the nodes. The tree is rooted to *P. trzebinskii* CBS 382.48. [T] = ex-type strain.

Fig. S5. ML tree of selected *Talaromyces* section *Talaromyces* species, inferred from *tub2*, 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 above 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] = ex-type strain.

Fig. S6. ML tree of selected members of *Aspergillaceae* family inferred from ITS, including the ex-type strain of *Penicillium kabuniccum* (CBS 575.90). Branch lengths are proportional to phylogenetic distance. Bootstrap support values/Bayesian posterior probability scores above 70 %/0.95 are indicated on the nodes. Isolates corresponding to novel taxa are shown in red. Between parentheses, GenBank accession numbers of ITS sequences. The tree is rooted to *T. flavus* CBS 310.38 and *T. purpureogenus* CBS 286.36. [T] = ex-type strain.