A taxonomic review of *Penicillium* section *Charlesia*

Bing-Da Sun [2,3], Cobus M. Visagie[4], Amanda Juan Chen[1,*] and Jos Houbraken[5,*]

[1] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, People's Republic of China
[2] China General Microbiological Culture Collection Centre, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China
[3] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China
[4] Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield, Private Bag X20, Pretoria 0028, South Africa
[5] Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

*Correspondence authors:
Amanda Juan Chen. Email: amanda_j_chen@163.com
Jos Houbraken. Email: j.houbraken@wi.knaw.nl

Abstract

Penicillium section *Charlesia* was established based on a multigene phylogeny of *P. charlesii*, *P. coffeae*, *P. fellutanum*, *P. georgiense*, *P. indicum* and *P. phoeniceum*. Since then, three additional species were described in the section. Species can occur on a wide range of substrata including soil, corn, coffee, water, air, deteriorating cloth and clinical samples. The majority of species in section *Charlesia* grow restricted on Czapek yeast extract agar and produce smooth-walled, vesiculate, monoverticillate conidiophores. A limited number of studies have reviewed the taxonomy of this section. In the present study, available strains belonging to section *Charlesia* were evaluated in a multilocus phylogenetic analysis using the ITS rDNA region, partial β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) sequences. This analysis revealed 12 distinct species, including three that are newly described here as *Penicillium aspericonidium*, *P. fusiforme* and *P. longiconidiophorum*. The macromorphology on different media, vesicle width, stipe length and ornamentation, and conidial shape and size are important morphological characters for distinguishing species of section *Charlesia*.

Keywords: Ascomycetes; Eurotiales; Multigene phylogeny; Monoverticillate

Three new taxa: Penicillium aspericonidium B.D. Sun, A.J. Chen & Houbraken; P. fusiforme B.D. Sun, A.J. Chen & Houbraken; P. longiconidiophorum B.D. Sun, A.J. Chen & Houbraken

Introduction

In the phenotype-based classification system, prior to the use of molecular data and a consilient concept of species, *Penicillium* subgenus *Aspergilloides* (= *Monoverticillium* group, Raper and Thom 1949) comprised of species which predominantly produced well-defined, monoverticillate conidiophores (Pitt 1980). Pitt (1980) subdivided this subgenus into two sections, namely *Aspergilloides* and *Exilicaulis*; the former section for species having stipes with terminal swellings or (small) vesicles, the latter for species lacking these structures. Peterson (2000) used ITS rDNA sequence data to assess the infrageneric relationships in *Penicillium*. The majority of the Penicillia formed six groups, with Group 1 including two *Eupenicillium* species (*E. anatolicum* and *E. shearii*) and various species previously classified in subgenera *Aspergilloides* and *Furcatum*, showing the lack of congruency between molecular and phenotypic data. Houbraken and Samson (2011) assessed the phylogenetic relationships in *Penicillium* using four loci and based on this analysis, the genus was re-defined and subdivided into two subgenera and 25 sections, with subgenus *Aspergilloides* containing 14 sections. Recently, *Penicillium* was further expanded to 32 sections and 89 series (Houbraken et al. 2016, 2020). During the last decade, the taxonomy of several of these sections was revised, including sections *Aspergilloides*, *Cinnamopurpurea*, *Citrina*, *Chrysogena*, *Exilicaulis*, *Lanata-Divaricata*, *Osmophila*, *Robsamsonia*, *Sclerotiora* and *Torulomyces* (Visagie et al. 2013, 2015, 2016a, c; Houbraken et al. 2011, 2012, 2014, 2016; Peterson et al. 2015; Wang et al. 2017; Diao et al. 2018). Based on data of these and other studies, lists of accepted *Penicillium* species were compiled, modernizing the taxonomy of *Penicillium* (Visagie et al. 2014; Houbraken et al. 2020).

Members of *Penicillium* section *Charlesia* can occur on a wide range of substrata including soil, corn, coffee, water, air, deteriorating cloth, clinical samples and stroma of fungi (*Hypoxylon* and Pyrenomycetes). All of the known species reproduce asexually, but some can produce an eupenicillium morph (Pitt 1980; Peterson et al. 2005). Section *Charlesia*, typified with *Penicillium charlesii* Smith, encompasses species that grow restrictedly on Czapek yeast extract agar (except *P. indicum*) and typically produce monoverticillate conidiophores having an apical swelling. Species like *P. charlesii* and *P. fellutanum* can also produce irregularly branched biverticillate conidiophores (Houbraken and Samson 2011). Peterson et al. (2005) studied the phylogeny of this section using ITS, calmodulin and elongation factor 1α gene sequences and based on these data, *P. atrovirens* and *P. fellutanum* var. *nigrocastaneum* were synonymized with *P. charlesii*, *P. ebenbitarianum* was synonymized with *P. fellutanum*, and *P. charlesii* and *P. fellutanum* were accepted as separate species. Rivera and Seifert (2011) subsequently also showed that *P. multicolor* (CBS 501.73^{T}) should be considered a synonym of *P. fellutanum*. Currently, nine species are accepted in the section, distributed over four series: *Costaricensia (Penicillium costaricense*), *Fellutana* (*P. charlesii*, *P. fellutanum*), *Indica* (*P. chermesinum*, *P. cuddlyae*, *P. indicum*, *P. lunae*) and *Phoenicea* (*P. coffeae*, *P. phoeniceum*) (Houbraken et al. 2020). These species share several morphological features, but the macromorphology on various media (e.g. growth diam on CYA and MEA) proved to be useful for species differentiation (Peterson et al. 2005).

In this study, we delineate *Penicillium* section *Charlesia* and describe three new species namely *P. aspericonidium*, *P. fusiforme* and *P. longiconidiophorum* using a phylogenetic

analysis of a combined data set of ITS, β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) gene sequences. Subsequently, 12 species were accepted in this section based on the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept (Taylor et al. 2000). Macro- and micromorphological characters for differentiating species are provided.

Materials and methods

Strains

Strains examined in this study were obtained from the CBS, Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; ATCC, American Type Culture Collection; NRRL, Agricultural Research Service Culture Collection, Peoria, Illinois, USA; IBT, culture collection of the DTU Systems Biology, Lyngby, Denmark; DAOMC, Canadian Collection of Fungal Cultures, at the Ottawa Research and Development Centre Agriculture and Agri-Food, Ottawa, Canada; PPRI, The South African National Collections of Fungi housed at the Agricultural Research Council (ARC, Roodeplaat, Pretoria, South Africa) and the working collection of the Food and Indoor Mycology group (DTO), housed at the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. An overview of strains used in the phylogenetic analyses is listed in Table 1.

DNA extraction, PCR amplification and sequencing

To extract DNA, strains were grown on malt extract agar (MEA; Oxoid malt) at 25 °C for 1 week. The Ultraclean™ Microbial DNA isolation Kit (MoBio, Solana Beach, USA) was used for DNA extractions. The ITS region and parts of the *BenA*, *CaM* and *RPB2* genes were amplified and sequenced using primers and protocols previously described (Houbraken and Samson 2011; Houbraken et al. 2014; Visagie et al. 2014).

Phylogenetic analysis

A concatenated alignment combining four loci (ITS, *BenA*, *CaM* and *RPB2*) was used to analyze the phylogenetic relationship within section *Charlesia*. Single gene phylograms were prepared to study the congruency between the datasets. Newly generated sequences together with sequences obtained from NCBI were aligned using MAFFT version 7 (Katoh et al. 2019). The most suitable substitution model was determined using MODELTEST version 3.06 (Posada and Crandall 1998). Maximum likelihood analyses including 1000 bootstrap replicates were run using RAxML-NG (Kozlov et al. 2019). Bayesian analyses were performed with MRBAYES version 3.2 (Ronquist et al. 2012). The sample frequency was set to 100 and the first 25% of trees were removed as burn-in. Sequences of *Penicillium chrysogenum* CBS 306.48 were used as an outgroup. The resulting trees were analyzed with FIGTREE version 1.4.2 and annotated in ADOBE ILLUSTRATOR CS5. BI posterior probabilities (pp) values and bootstrap (bs) percentages generated during the analysis are plotted at the nodes. Values less than 0.95 pp and less than 70% bs are not shown. Branches with 1.00 pp and a bootstrap support of 95% or higher are thickened.

Table 1. *Penicillium* section *Charlesia* strains used in this study

Species name	Strain no.	Substrate	GenBank accession no.			
			ITS	*BenA*	*CaM*	*RPB2*
Penicillium aspericonidium	CBS 141832 ^{T} = DTO 030-C5	Soil, wet forest, Atherton Tableland, Australia	**MT309657**	**MT302240**	**MT302209**	**MT302224**
Penicillium charlesii	CBS 304.48 ^{T} = ATCC 8730 = CBS 342.51 = CECT 2277 = FRR 778 = IMI 040232 = LSHBBB127 = LSHBP146 = NRRL 1887 = NRRL 778 = QM 6338 = QM 6838	Moldy corn (*Zea mays* L.), UK	AF033400	JX091508	AY741727	JN121486
	CBS 326.59 = NRRL 3464 = DTO 106-H6 (type of *P. atrovirens*)	Soil, Japan	KC411737	**MT302241**	AY741745	JN406571
	CBS 330.59 = NRRL 6208 (type of *P. fellutanum* var. *nigrocastaneum*)	Soil, Japan	KC411739	KX961230	KX961261	JN406570
	DTO 175-H7	Surface Water, Portugal	**MT309658**	**MT302242**	**MT302210**	**MT302225**
	CBS 100234	Unknown source, Italy	**MT309659**	**MT302243**	**MT302211**	**MT302226**
	DTO 058-H2	Corn kernels, imported from Brazil	**MT309660**	**MT302244**	**MT302212**	**MT302227**
Penicillium chermesinum	CBS 231.81 ^{T} = NRRL 2048	Deteriorating military equipment, Florida, USA	MH861332	KJ834441	AY741728	JN406600
	DTO 298-I8	Indoor hospital air, Turkey	**MT309661**	**MT302245**	**MT302213**	**MT302228**
	CBS 117279 = DTO 002-C8	Unknown source, the Netherlands	**MT309662**	**MT302246**	**MT302214**	**MT302229**
	CBS 305.48	Air, Panama	**MT309663**	**MT302247**	**MT302215**	JN406581
Penicillium coffeae	CBS 119387 ^{T} = IBT 27866 = NRRL 35363	Peduncle, *Coffea arabica*, Oahu, Aiea,Hawaii, USA	AY742702	KJ834443	AY741747	JN121436
	DTO 273-A7	Unknown	**MT309664**	**MT302248**	n.a	**MT302230**
Penicillium costaricense	DAOMC 250520 ^{T} = CBS 140998 = DTO 410-E3 = KAS 2597	Intestines of *Rothschildia lebeau*, Costa Rica	MN431396	KT887834	KT887795	MN969173
Penicillium cuddlyae	PPRI 26355 ^{T} = CMV016A6	Dog food, South africa	MK951942	MK951835	MK951908	MN418450

Penicillium fellutanum	CBS 229.81 ^{T} = CBS 326.48 = ATCC 10443 = FRR746 = IFO 5761 = IMI 039734 = IMI 039734iii = NRRL 746 = QM 7554	Unknown source, USA	AF033399	KJ834450	AY741753	JN121460
	CBS 501.73	Unknown source, USSR	JN799647	JN799645	JN799646	**MT302231**
	CBS 415.69 = NRRL 3760 (type of *P. ebenbitarianum*)	Unknown source, Syria	KC411759	**MT302249**	**MT302216**	**MT302232**
	CBS 172.44	Unknown source, the Netherlands	**MT309665**	**MT302250**	**MT302217**	**MT302233**
	CBS 152.45	Unknown	**MT309666**	**MT302251**	**MT302218**	**MT302234**
	CBS 118477	Unknown source, Belgium	**MT309667**	**MT302252**	**MT302219**	**MT302235**
Penicillium fusiforme	CBS 250.66 ^{T} = DTO 035-D7	Unknown source, The Netherlands	**MT309668**	**MT302253**	**MT302220**	**MT302236**
Penicillium indicum	CBS 115.63(Isotype) = NRRL3387 = ATCC 18324 = FRR 3387 = IFO 31744 = IMI 166620	Sputum, man, Delhi, India	AY742699	EU427263	AY741744	JN406640
	CBS 179.81 = IJFM 5967 = IMI 253804 = NRRL 35756 (type of *Penicillium gerundense*)	Unknown source, Spain	EU427291	EU427264	EU427283	EU427257
Penicillium longiconidiophorum	CBS 141831 ^{T} = DTO 088-C1	Alcohol treated soil, Madagascar	**MT309669**	**MT302254**	**MT302221**	**MT302237**
	DTO 092-C6	Alcohol treated soil, Madagascar	**MT309670**	**MT302255**	**MT302222**	**MT302238**
Penicillium lunae	PPRI 25881 ^{T} = CMV006E6	Banana, South Africa	MK450725	MK451088	MK451660	MK450863
Penicillium phoeniceum	CBS 249.32 ^{T} = ATCC 10481 = IJFM 5122 = IMI 040585 = NRRL 2070 = QM7608 = VKMF-321	Sooty mould on *Phoenix* sp. (palm)	KC411711	KJ834483	AY741729	JN406597
	DTO 259-B9	Unknown	**MT309671**	**MT302256**	**MT302223**	**MT302239**

New species described in this study and newly generated sequences are shown in bold (T=ex-type culture)

Morphological analysis

Macroscopic characters were studied on the agar media Czapek yeast extract agar (CYA), CYA supplemented with 5% NaCl (CYAS), yeast extract sucrose agar (YES), creatine sucrose agar (CREA), dichloran 18% glycerol agar (DG18), oatmeal agar (OA) and malt extract agar (MEA; Oxoid malt). The strains were inoculated at three equidistant points on 90 mm Petri dishes and incubated for 7 days at 25 °C in darkness. Additional CYA plates were incubated at 30 and 37 °C, while additional MEA plates were incubated at 30 °C. After 7 days of incubation, colony diameters were recorded. The colony texture, degree of sporulation, obverse and reverse colony colours, the production of soluble pigments and exudates were determined.

Microscope preparations were made from 1-week-old colonies grown on MEA. Lactic acid (60%) was used as mounting fluid and 70% ethanol was used to remove the excess of conidia. A Zeiss SteREO Discovery V20 dissecting microscope and Zeiss AXIO Imager A2 light microscope equipped with Nikon DS-Ri2 cameras were used to capture digital images.

Results

Phylogeny

The phylogenetic relationships among section *Charlesia* species were studied using concatenated sequence data of the four loci ITS, *BenA*, *CaM* and *RPB2*; the total length of the aligned dataset was 2346 characters (ITS 713 bp, *BenA* 470 bp, *CaM* 543 bp, *RPB2* 915 bp, Treebase S27453). The best model for ITS, *BenA* and *CaM* was the General time reversible with gamma distributed (GTR + G), while the Kimura 2-parameter with gamma distributed (K2P + G) model was used for *RPB2*. In the multigene phylogram, the nine accepted species (*P. charlesii*, *P. chermesinum*, *P. coffeae*, *P. costaricense*, *P. cuddlyae*, *P. fellutanum*, *P. indicum*, *P. lunae*, *P. phoeniceum*) formed well-supported lineages corresponding to four series (Fig. 1). Furthermore, four strains in three lineages represent new species described here as *P. aspericonidium*, *P. fusiforme* and *P. longiconidiophorum*. *Penicillium longiconidiophorum* is phylogenetically sister to *P. coffeae*, and *P. aspericonidium* a sister species of the species in series *Phoenicea*. *Penicillium fusiforme* is phylogenetically closely related to *P. fellutanum* (Fig. 1).

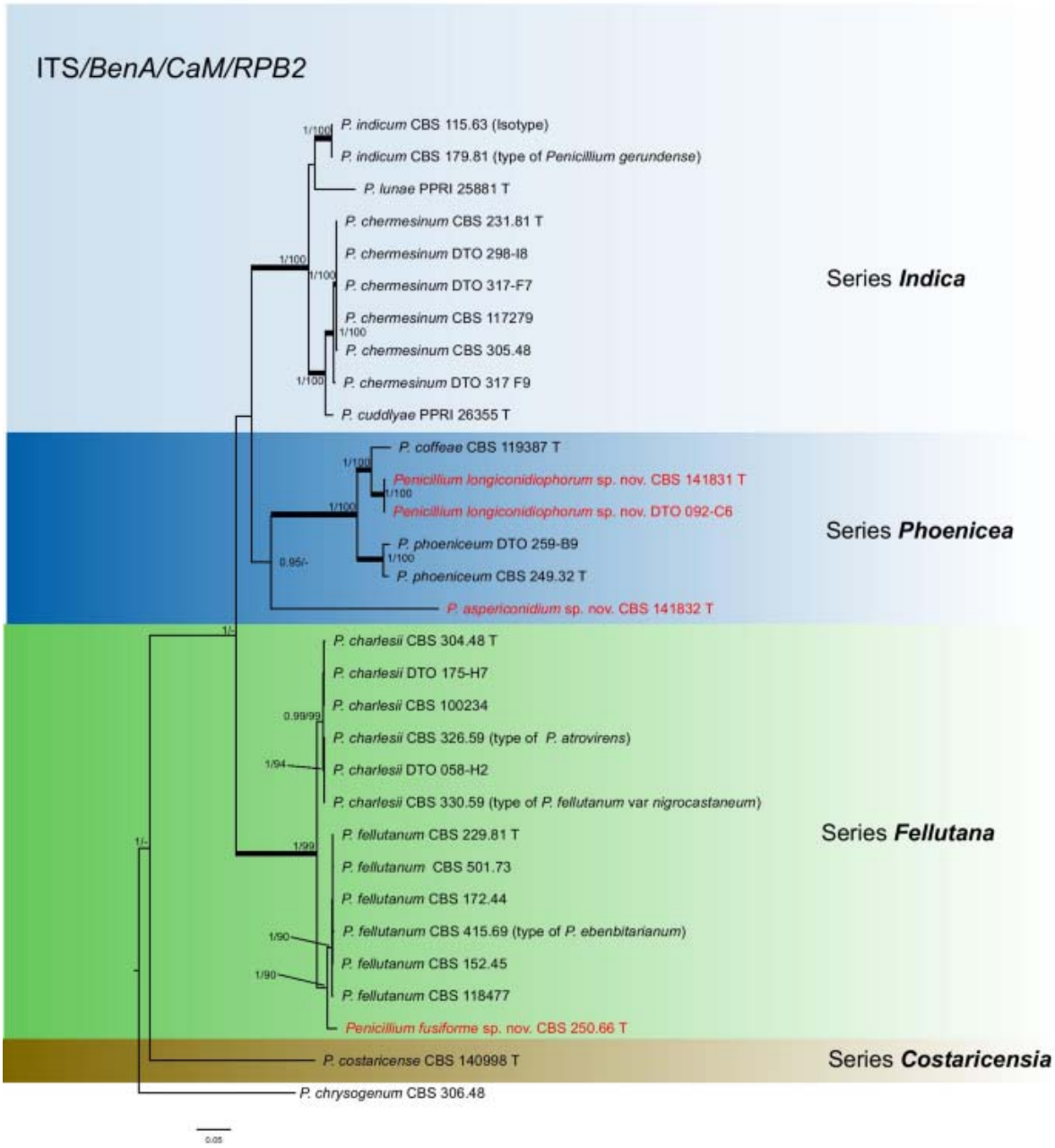

Fig. 1. Concatenated phylogeny of the ITS, *BenA*, *CaM* and *RPB2* gene regions of species from *Penicillium* section *Charlesia*. Branches with values more than 1 pp and 95% bs are thickened. *Penicillium chrysogenum* was chosen as an outgroup

In ITS and *CaM* phylograms, *P. aspericonidium* is sister to the species in ser. *Fellutana* and it is sister to ser. *Phoenicea* species in the *RPB2* phylogram. None of these positions has statistical support. The position of this species in the *BenA* phylogeny is unresolved. Based on the four-gene phylogram, this species clusters inside ser. *Phoenicea* with low support (0.95 pp/ < 70% bs). The position of *P. longiconidiophorum* is relatively stable and clusters with *P. coffeae* and *P. phoeniceum* in all four phylograms with high support. Similarly, *P. fusiforme* is closely related to *P. charlesii* and *P. fellutanum* (S. 1–4).

Molecular identification

As to ITS sequences, *P. aspericonidium* shows less than 96% similarity with other section *Charlesia* species, *P. longiconidiophorum* has a 98.8% similarity (492/498 bp) with *P. phoeniceum* (CBS 249.32 T) and *P. fusiforme* shows 98.7% (696/705 bp) and 99.0% (701/708 bp) similarity with the closely related species *P. charlesii* (CBS 304.48 T) and *P. fellutanum* (CBS 229.81 T), respectively. The new species *P. fusiforme*, *P. aspericonidium* and *P. longiconidiophorum* could be easily differentiated from the other section *Charlesia* species using *BenA*, *CaM* and *RPB2* sequences.

Taxonomy

Species descriptions

Penicillium aspericonidium B.D. Sun, A.J. Chen & Houbraken, sp. nov. Fig. 2

MycoBank: MB 838211.

Diagnosis: *Penicillium aspericonidium* produces distinctly roughened conidia, which is different from all other section *Charlesia* members.

Typification: AUSTRALIA, Atherton Tableland, wet forest, soil, Oct 2006, isolated by J. Houbraken and L. Janson (holotype CBS H-22830). Ex-type culture CBS 141832 = DTO 030-C5). GenBank: ITS = MT309657; *BenA* = MT302240; *CaM* = MT302209; *RPB2* = MT302224.

Etymology: Latin, *aspericonidium* refers to its distinctly roughened conidia.

Colony diam, 7 d (mm): CYA 14–16; CYA 30 °C No growth; CYA 37 °C No growth; MEA 16–18; MEA 30 °C No growth; OA 15–17; YES 20–22; CREA 3–4; CYAS 7–8; DG18 12–13.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture floccose; sporulation sparse; conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse yellowish brown. MEA 25 °C, 7 d: Colonies moderately deep, sulcate; margins slightly irregular; mycelium white; texture floccose; sporulation moderately dense; conidia *en masse* glaucous; soluble pigments absent; exudates absent; reverse brown. YES 25 °C, 7 d: Colonies moderately deep, sulcate; margins slightly irregular; mycelium white; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse straw yellow. DG18 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; mycelium white; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse straw yellow. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture floccose; sporulation dense; conidia *en masse* dark green; soluble pigments absent; exudates absent; reverse light yellow. CREA 25 °C, 7 d: Acid production absent.

Micromorphology: Conidiophores monoverticillate, occasionally biverticillate; stipes smooth-walled, 50–100 × 2.5–3.5 µm, vesicles 3–6 µm. Phialides ampulliform, 5–9 per stipe, 8–10 × 3–4 µm. Conidia globose, distinctly roughened, 2.5–3.5 µm.

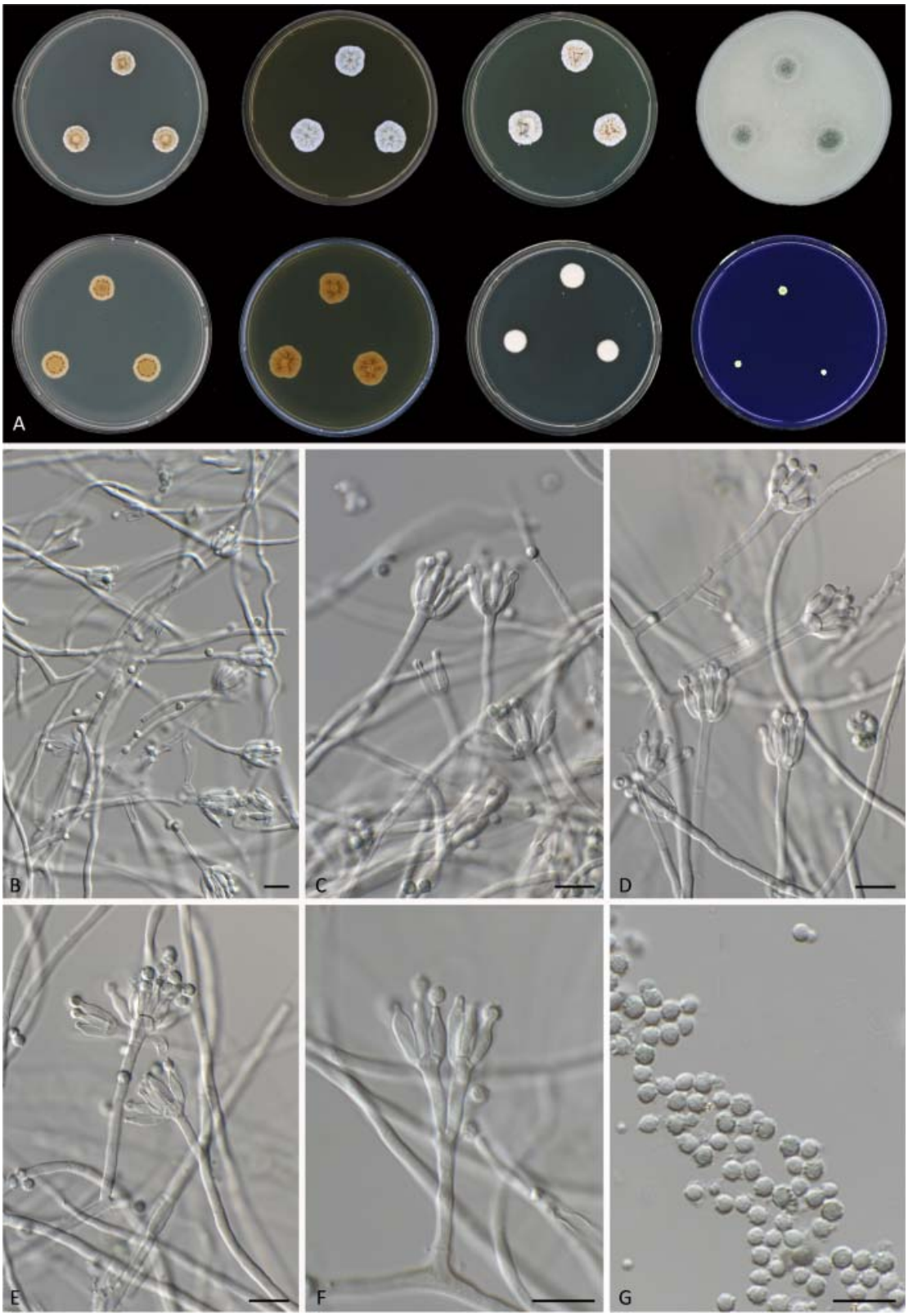

Fig. 2. *Penicillium aspericonidium* CBS 141832 ^{T}. **A** Colonies: top row left to right, obverse CYA, obverse MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CREA. Petri dish diameter: 9 cm. **B**–**G** Conidiophores and conidia. Scale bars: B–G = 10 µm

Notes: Both *P. aspericonidium* and *P. longiconidiophorum* do not grow at 30 and 37 °C, but can be differentiated by their conidium ornamentation (*P. longiconidiophorum* produces smooth conidia; *P. aspericonidium* rough-walled conidia).

Penicillium fusiforme B.D. Sun, A.J. Chen & Houbraken, sp. nov. Fig. 3.

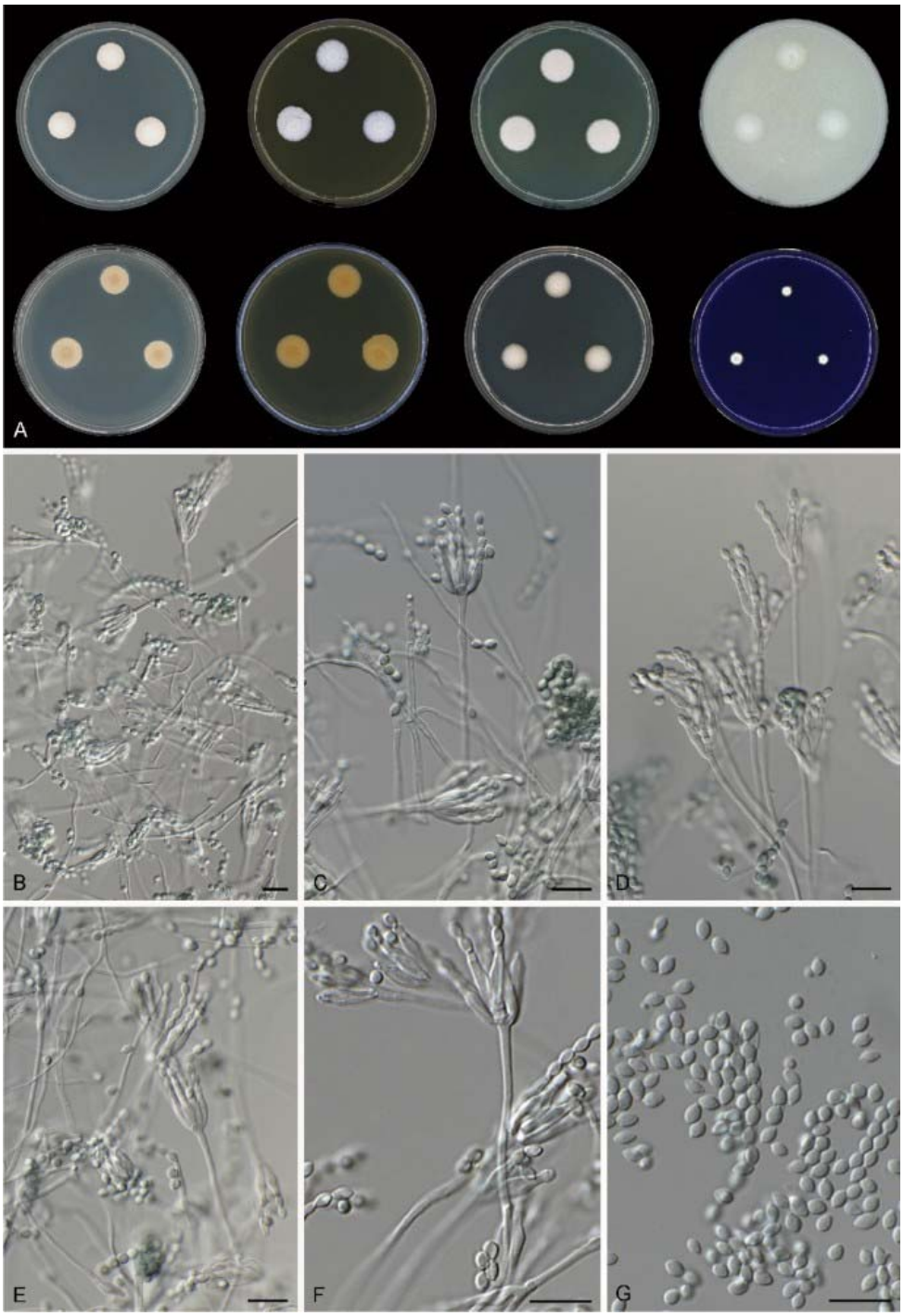

Fig. 3. *Penicillium fusiforme* CBS 255.66^{T}. **A** Colonies: top row left to right, obverse CYA, obverse MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CREA. Petri dish diameter: 9 cm. **B**–**G** Conidiophores and conidia. Scale bars: **B**–**G** = 10 µm

MycoBank: MB 838212.

Diagnosis: *Penicillium fusiforme* grows restrictedly on CYA and MEA at 30 °C, produces fusiform conidia.

Typification: THE NETHERLANDS, unknown source, Mar 1966, isolated by S. Bootsma (holotype CBS H-22840). Ex-type culture CBS 250.66 = DTO 035-D7. GenBank: ITS = MT309668; *BenA* = MT302253; *CaM* = MT302220; *RPB2* = MT302236.

Etymology: Latin, *fusiforme* refers to its fusiform conidia.

Colony diam, 7 d (mm): CYA 13–14; CYA 30 °C 7–9; CYA 37 °C No growth; MEA 16–18; MEA 30 °C 8–9; OA 15–17; YES 17–19; CREA 6–7; CYAS 12–13; DG18 13–15.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse salmon. MEA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation sparse; conidia *en masse* pale green; soluble pigments absent; exudates absent; reverse yellowish brown. YES 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium pinkish; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse yellowish brown. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse salmon. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture floccose; sporulation sparse; conidia *en masse* pale green; soluble pigments absent; exudates absent; reverse light yellow. CREA 25 °C, 7 d: Acid production absent.

Micromorphology: Conidiophores mostly monoverticillate, sometimes with divergent additional branches, stipes smooth-walled, 25–85 × 1.5–2.5 µm, vesicles 2–3.5 µm. Phialides ampulliform, 5–8 per stipe, 7–9 × 3–3.5 µm. Conidia fusiform, smooth, 3–4 × 2–3 µm.

Notes: *Penicillium fusiforme* is phylogenetically closely related and morphologically most similar to *P. charlesii* and *P. fellutanum*. *Penicillium charlesii* and *P. fellutanum* grow faster on CYA (25–27 mm for *P. charlesii*; 22–27 mm for *P. fellutanum*) and MEA (21–25 mm for *P. charlesii*; 24–27 mm for *P. fellutanum*) than *P. fusiforme* (CYA 13–14 mm, MEA 16–8 mm). Furthermore, *P. charlesii* and *P. fellutanum* produce globose to ellipsoidal conidia, while *P. fusiforme* produces fusiform conidia.

Penicillium longiconidiophorum B.D. Sun, A.J. Chen & Houbraken, sp. nov. Fig. 4

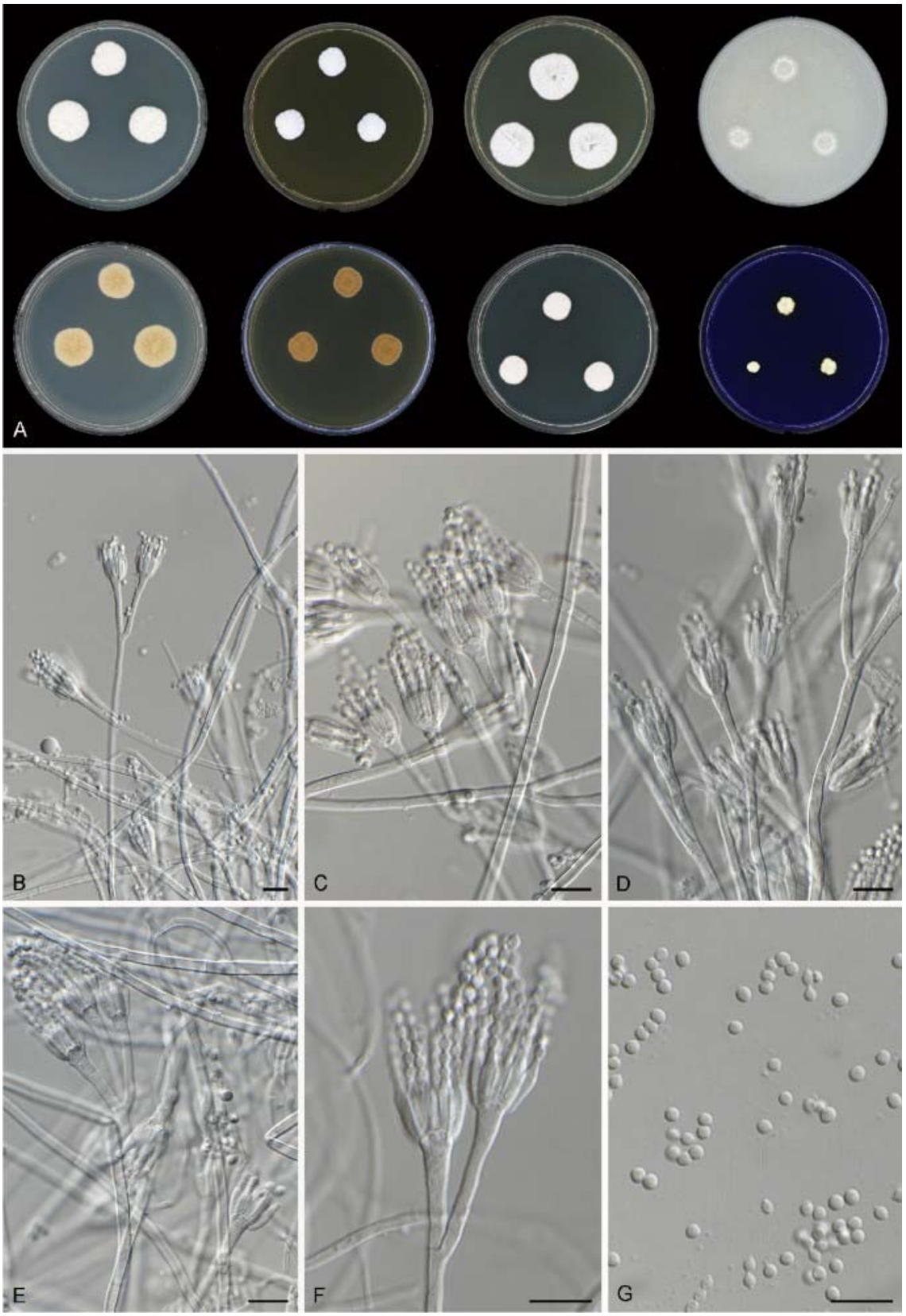

Fig. 4. *Penicillium longiconidiophorum* CBS 141831^{T}. **A** Colonies: top row left to right, obverse CYA, obverse MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CREA. Petri dish diameter: 9 cm. **B**–**G** Conidiophores and conidia. Scale bars: B–G = 10 µm

MycoBank: MB 838213.

Diagnosis: *Penicillium longiconidiophorum* does not grow on CYA and MEA at 30 °C, produces long conidiophores measuring 400–500 µm.

Typification: MADAGASCAR, alcohol treated soil, Dec 2008, isolated by J. Houbraken (holotype CBS H-22829). Ex-type culture CBS 141831 = DTO 088-C1. GenBank: ITS = MT309669; *BenA* = MT302254; *CaM* = MT302221; *RPB2* = MT302237.

Etymology: Latin, *longiconidiophorum* refers to its long conidiophores.

Additional strain: MADAGASCAR, near Antsiranana, alcohol-treated soil, Dec 2008, isolated by J. Houbraken, DTO 092-C6. GenBank: ITS = MT309670; *BenA* = MT302255; *CaM* = MT302222; *RPB2* = MT302238.

Colony diam, 7 d (mm): CYA 18–24; CYA 30 °C No growth; CYA 37 °C No growth; MEA 16–17; MEA 30 °C No growth; OA 14–15; YES 23–30; CREA 7–10; CYAS 11–21; DG18 13–18.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, sulcate; margins slightly irregular; mycelium white; texture floccose; sporulation sparse; conidia *en masse* pale green; soluble pigments absent; exudates absent; reverse saffron. MEA 25 °C, 7 d: Colonies deep, sulcate; margins slightly irregular; mycelium white; texture velvety; sporulation sparse; conidia *en masse* pale green; soluble pigments absent; exudates absent; reverse yellowish brown. YES 25 °C, 7 d: Colonies moderately deep, raise at centre, sulcate; margins entire; mycelium white; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse buff. DG18 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse rosy buff. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture floccose; sporulation sparse; conidia *en masse* pale green; soluble pigments absent; exudates absent; reverse buff. CREA 25 °C, 7 d: Acid production absent.

Micromorphology: Conidiophores monoverticillate, with divergent additional branches up to 35 µm; stipes smooth walled, 400–500 × 3–4 µm, vesicles 3–4.5 µm. Phialides ampulliform, 5–8 per stipe, 8–10.5 × 3–4 µm. Conidia globose, smooth, 2–3 µm.

Notes: Phylogenetically, *P. longiconidiophorum* is closely related to *P. coffeae*. However, *P. coffeae* can grow at 30 °C on CYA and MEA, while the new species does not grow at these temperatures. Morphologically, *P. longiconidiophorum* is similar to *P. aspericonidium*, but *P. aspericonidium* produces shorter stipes (50–100 µm) and distinctly roughened conidia.

Phenotype based identification of section *Charlesia* species.

A diagnostic key to species in Penicillium section Charlesia is provided below, as well as overviews of macro- and microscopic features (Figs. 5 and 6, Table 2).

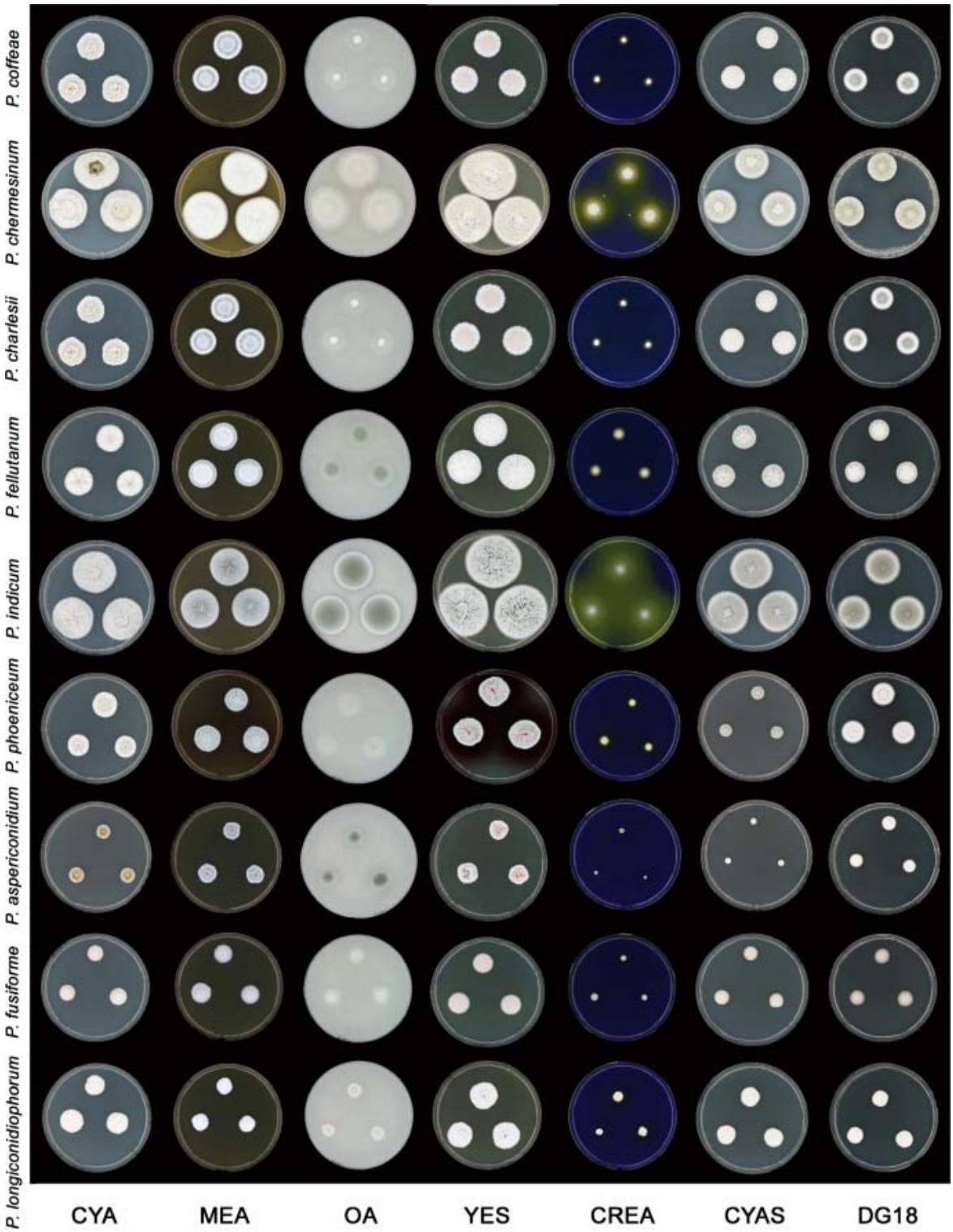

Fig. 5. Overview of growth characters of *Penicillium* section *Charlesia* species. Columns, left to right: CYA, MEA, OA, YES, CREA, CYAS, DG18. Petri dish diameter: 9 cm

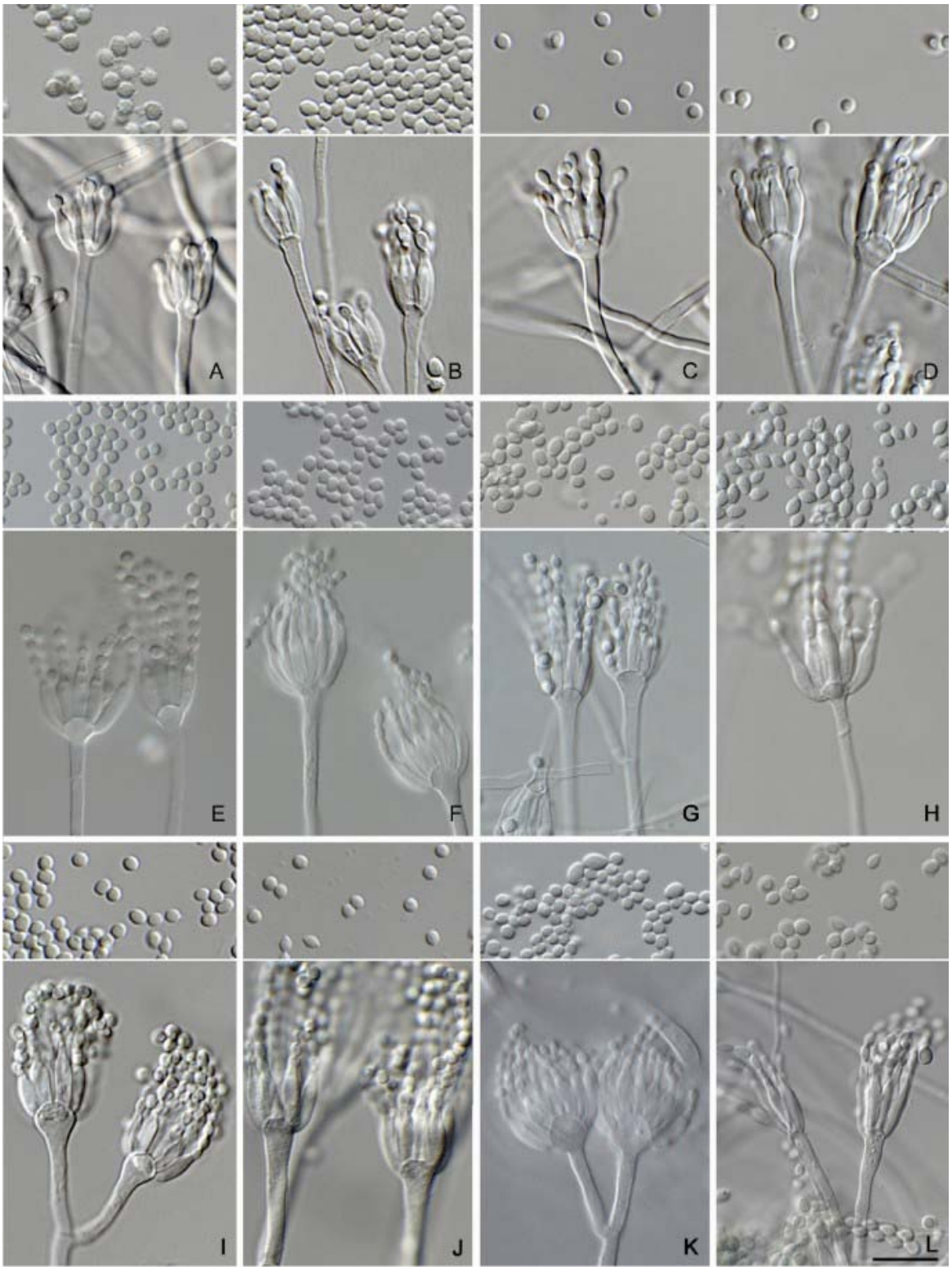

Fig. 6. Micromorphological characters of *Penicillium* section *Charlesia* species. **A** *P. aspericonidium* CBS 141832 ^{T}. **B** *P. charlesii* CBS 304.48 ^{T}. **C** *P. chermesinum* CBS 231.81 ^{T}. **D** *P. coffeae* CBS 119387 ^{T}. **E** *P. costaricense* DAOMC 250520 ^{T}. **F** *P. cuddlyae* PPRI 26355 ^{T}. **G** *P. fellutanum* CBS 229.81 ^{T}. **H** *P. fusiforme* CBS 250.66 ^{T}. **I** *P. indicum* CBS 115.63 ^{T}. **J** *P. longiconidiophorum* CBS 141831 ^{T}. **K** *P. lunae* PPRI 25881 ^{T}. **L** *P. phoeniceum* CBS 249.32 ^{T}. Scale bars = 10 µm, applies to A–L

Table 2. Selected phenotypic characters for distinguishing *Penicillium* section *Charlesia* species

		Macromorphology (mm)						Micromorphology (µm)			
		CYA	**CYA 30 °C**	**CYA 37 °C**	**MEA**	**MEA 30 °C**	**CREA**	**Vesicle width**	**Stipe length**	**Conidia ornamentation**	**Conidia shape and size**
Ser. *Costaricensia*	*P. costaricense**	15–17	n.a	No growth	19–21**	n.a	6–7	4–6.5	50–280	Smooth	Subglobose, 2.5–3 × 2–3
Ser. *Fellutana*	*P. charlesii*	25–27	21–26	No growth	21–25	15–20	9–12	2.5–4	50–100	Smooth	Ellipsoidal, 3–4 × 2.5–3
	P. fellutanum	22–27	15–21	No growth	24–27	13–21	10–11	4–6	100–200	Smooth	Globose to ellipsoidal, 2.5–4(–5) × 2–3.5
	P. fusiforme	13–14	7–9	No growth	16–18	8–9	6–7	2–3.5	25–85	Smooth	Fusiform, 3–4 × 2–3
Ser. *Indica*	*P. chermesinum*	32–39	30–31	32–33	41–42	44–47	20–23	2.5–3.5	20–50	Smooth	Globose to ellipsoidal, 2.5–3.5 × 1.5–3
	*P. cuddlyae**	24–26	31–33	19–21	21–23**	n.a	12–14	5–6	20–45	Smooth	Ellipsoidal to cylindrical, 2–3 × 2.5–2
	P. indicum	39–40	45–46	30–32	36–37	42–43	32–33	3–5	15–50	Smooth	Globose to ellipsoidal, 2–3 × 2–2.5
	*P. lunae**	34–36	28–29	No growth	25–26**	n.a	n.a	5–7	13–60	Smooth	Subglobose to broadly ellipsoidal, 2–3(–3.5) × 1.5–2(–2.5)
Ser. *Phoenicae*	***P. aspericonidium***	14–16	No growth	No growth	16–18	No growth	3–4	3–6	50–100	Distinctly roughened	Globose, 2.5–3.5
	P. coffeae	21–22	18–20	No growth	25–26	17–18	8–9	5–7	100–200	Smooth	Globose, 2–3
	P. longiconidiophorum	18–24	No growth	No growth	16–17	No growth	7–10	3–4.5	400–500	Smooth	Globose, 2–3
	P. phoeniceum	20–23	12–13	No growth	22–25	8–11	10–12	3–4.5	50–100	Smooth	Globose to ellipsoidal, 3–4 × 2–3

*Data from Visagie et al. (2016b) and Crous et al. (2019a, b)

**Growth was recorded on Blakeslee's malt extract agar (MEAbl)

1. Conidia smooth.....2

1. Conidia distinctly roughened.....*P. aspericonidium*

2. Stipe length mostly > 400 µm.....*P. longiconidiophorum*

2. Stipe length mostly < 400 µm.....3

3. Stipe length mostly < 100 µm.....4

3. Stipe length mostly between 100 and 400 µm.....10

4. Growth on CYA at 37°C 5

4. No growth on CYA at 37°C 7

5. Colony diameter > 25 mm after 7 d on CYA at 37°C 6

5. Colony diameter < 25 mm after 7 d on CYA at 37°C .....*P. cuddlyae*

6. Colony diameter > 40 mm after 7 d on CYA at 30°C .....*P. indicum*

6. Colony diameter < 40 mm after 7 d on CYA at 30°C ..... *P. chermesinum*

7. Colony diameter > 15 mm after 7 d on CYA at 30°C 8

7. Colony diameter < 15 mm after 7 d on CYA at 30°C 9

8. Colony diameter > 30 mm after 7 d on CYA at 25 °C, vesicle width 5–7 µm .....*P. lunae*

8. Colony diameter < 30 mm after 7 d on CYA at 25 °C, vesicle width 2.5–4 µm .....*P. charlesii*

9. Colony diameter > 20 mm after 7 d on CYA at 25 °C, conidia globose to ellipsoidal ..... *P. phoeniceum*

9. Colony diameter < 20 mm after 7 d on CYA at 25 °C, conidia fusiform
.....*P. fusiforme*

10. Colony diameter > 20 mm after 7 d on CYA at 25°C
..... 11

10. Colony diameter < 20 mm after 7 d on CYA at 25°C
..... *P. costaricense*

11. Colony diameter > 22 mm after 7 d on CYA at 25 °C, conidia globose to ellipsoidal, 2.5–4(–5) × 2–
3.5 µm.....*P. fellutanum*

11. Colony diameter < 22 mm after 7 d on CYA at 25 °C, conidia globose, 2–3 µm
..... *P. coffeae*

Discussion

Penicillium is one of the largest fungal genera, now spanning more than 483 accepted species (Houbraken et al. 2020). A list of "Names in Current Use" (NCU) for the *Trichocomaceae* was compiled in 1993 (Pitt & Samson 1993) and was updated in 2000 (Pitt et al. 2000). Visagie et al. (2014a) provided a list of accepted *Penicillium* species (354 species) based on phylogenetic data. They released reference sequence data for all ex-types, suggested *BenA* as identification marker and suggested clear guidelines to working methods for *Penicillium*. Since then, the number of accepted species increased rapidly, with the most recent review accepting 483 species, an increase of 129 (Houbraken et al. 2020). Subgeneric classifications have a long tradition in *Penicillium* and are valuable for working with such a large genus. Even though these were traditionally based on morphological characters, which has since been shown to have resulted in superficial subgeneric classifications, they have been modernized and are currently based on phylogenetic clades supported by multiple gene regions. Houbraken and Samson (2011) were the first to redefine subgeneric classifications using this approach and at the time accepted 25 sections. Subsequently, the two new sections *Osmophila* and *Robsamsonia* were introduced in subgenus *Penicillium*, while section *Digitata* was synonymized with section *Penicillium*, resulting in 26 sections in the genus (Houbraken et al. 2016). Houbraken et al. (2020) provided an overview of *Eurotiales* and introduced an updated subgeneric, sectional and series classification for *Aspergillus* and *Penicillium*, subsequently dividing *Penicillium* into two subgenera, 32 sections and 89 series.

The species in *Penicillium* section *Charlesia* share the production of monoverticillate, vesiculate conidiophores (Houbraken and Samson 2011). The macromorphology on different media was recommended for species differentiation (Peterson et al. 2005), and this approach was also confirmed in this study (Fig. 5). Houbraken et al. (2020) suggested colony growth rates and conidiophore complexity as distinguishing characters. Four series were introduced for this section. Series *Fellutana* includes *P. charlesii*, *P. fellutanum* and *P. fusiforme*: all of these species grow restrictedly on CYA and cannot grow at 37 °C on it.

Series *Indica* includes *P. chermesinum*, *P. cuddlyae*, *P. indicum* and *P. lunae*. These species generally grow faster than series *Fellutana* species. The one exception is *P. cuddlyae*, which grows restrictedly on CYA (24–26 mm in 7 days); however, it grows faster at 30 °C (31–33 mm in 7 days) (Crous et al. 2019b). Except for *P. lunae*, all series *Indica* species can grow at 37 °C (Crous et al. 2019a). Series *Phoenicae* includes *P. coffeae*, *P. phoeniceum* and the two species described in this study: *P. aspericonidium* and *P. longiconidiophorum*. All four series *Phoenicae* species cannot grow on CYA at 37 °C, *P. coffeae* and *P. phoeniceum* can grow on CYA at 30 °C, while *P. aspericonidium* and *P. longiconidiophorum* cannot. Series *Phoenicae* species grow restrictedly on all media, similar to series *Fellutana* species. *Penicillium costaricense* forms an independent branch outside series *Fellutana*, *Indica* and *Phoenicae*, cannot grow on CYA at 37 °C and grows restrictedly on CYA (15–17 mm) and MEA (19–21 mm) (Visagie et al. 2016b).

Penicillium charlesii was reported to produce citreoviridin (Cole et al. 1981) and Wicklow (1984) re-identified this citreoviridin-producing strain (NRRL 13013) as *P. citreoviride* (= *P. citreonigrum*). NRRL 13013 was included in the taxonomic study of section *Cinnamopurpurea* and was designated as the ex-type strain of the newly introduced species *P. colei* (Peterson et al. 2015). Another *Penicillium charlesii* strain (ATCC 20841) is reported to produce paraherquamide, a potent and broad-spectrum anthelminthic. Paraherquamide producers have been found in *Penicillium* section *Lanata-Divaricata*, *Brevicompacta* and *Canescentia* (Klas et al. 2018; Houbraken et al. 2020). The identity of ATCC 20841 needs confirmation using sequence data to confidentially link paraherquamide production to *P. charlesii*.

Three new species *P. fusiforme* (ser. *Fellutana*), *P. aspericonidium* and *P. longiconidiophorum* (ser. *Phoenicae*) were introduced in this study. All of them can be identified based on phylogenetic data. According to our observations, the macromorphology on different media, vesicle width, stipe length and conidial ornamentation, shape and size are important distinguishing morphological characters (Table 2). *Penicillium charlesii* and *P. fellutanum* have subtle phenotypic differences: *P. charlesii* produces elliptical conidia compared to spherical conidia in *P. fellutanum* (Peterson et al. 2005). We observed both elliptical and globose conidia in *P. fellutanum*; however, the conidial size of *P. fellutanum* is more variable, and it produces relatively wider vesicles (Fig. 6). Other siblings are *P. indicum* and *P. chermesinum*, and Pitt et al. (2000) considered *P. indicum* to be a synonym of *P. chermesinum.* Peterson et al. (2005) treated them as separate species based on phylogenetic differences in three loci (ITS, calmodulin and elongation factor-1α) and found that *P. indicum* grows faster on CYA and MEA. We found that the growth rates of these two species on CYA are nearly the same (Fig. 6); the growth rates of *P. indicum* on MEA are slightly slower than those of *P. chermesinum*. In addition, we found that *P. indicum* grows much faster on CYA at 30 °C and on CREA compared to *P. chermesinum*, which can be used as useful distinguishing characters.

Data availability

All available via Amanda Juan Chen amanda_j_chen@163.com.

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Contributions

Conceptualization, JH and AJC; methodology, BDS and CMV; formal analysis, BDS and AJC; investigation, BDS; resources, JH and CMV; original draft preparation, BDS and AJC; review and editing, CMV and JH; supervision, JH; funding acquisition, BDS. All authors have read and agreed to the published version of the manuscript.

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