New *Talaromyces* species from indoor environments in China

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Abstract: *Talaromyces* contains both asexual and sexually reproducing species. This genus is divided in seven sections and currently has 105 accepted species. In this study we investigated the *Talaromyces* isolates that were obtained during a study of indoor air collected in Beijing, China. These indoor *Talaromyces* strains are resolved in four sections, seven of them are identified as *T. islandicus*, *T. aurantiacus*, *T. siamensis* and *T. albobiverticillius* according to *BenA* sequences, while 14 isolates have divergent sequences and are described here as nine new species. The new species are placed in four sections, namely sections *Helici*, *Islandici*, *Talaromyces* and *Trachyspermi*. They are described based on sequence data (ITS, *BenA*, *CaM* and *RPB2*) in combination with phenotypic and extrolite characters. Morphological descriptions and notes for distinguishing similar species are provided for each new species. The recently described *T. rubrifaciens* is synonymised with *T. albobiverticillius* based on presented phylogenetic results.

Key words: *Eurotiales*, Indoor air, Polyphasic taxonomy, *Talaromyces albobiverticillius*.

Taxonomic novelties: *Talaromyces aerius* A.J. Chen, Frisvad & Samson, *T. adpressus* A.J. Chen, Frisvad & Samson, *T. beijingensis* A.J. Chen, Frisvad & Samson, *T. cerinus* A.J. Chen, Frisvad & Samson, *T. chlamydosporus* A.J. Chen, Frisvad & Samson, *T. diversiformis* A.J. Chen, Frisvad & Samson, *T. fusiformis* A.J. Chen, Frisvad & Samson, *T. neorugulosus* A.J. Chen, Frisvad & Samson, *T. reverso-olivaceus* A.J. Chen, Frisvad & Samson.

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INTRODUCTION

The genus *Talaromyces* was introduced by Benjamin (1955) to accommodate teleomorphic *Penicillium* species with soft ascomata, which are usually white or yellowish and surrounded by multiple layers of interwoven hyphae. Phylogenetic studies revealed that *Penicillium* was polyphyletic and *Talaromyces* species and members of *Penicillium* subgenus *Biverticillium* belonged in a clade distinct from *Penicillium sensu stricto* (LoBuglio *et al.* 1993, LoBuglio & Taylor 1993, Berbee *et al.* 1995, Ogawa *et al.* 1997, Ogawa & Sugiyama 2000, Wang & Zhuang 2007, Houbraken & Samson 2011). Following the concept of nomenclatural priority and single name nomenclature, Samson *et al.* (2011) subsequently transferred all accepted species of *Penicillium* subgenus *Biverticillium* to *Talaromyces*.

Yilmaz *et al.* (2014) studied the taxonomy of *Talaromyces* in detail using a polyphasic approach. Based on multigene phylogeny, morphology and extrolites, 88 species were accepted and divided into seven sections: *Bacillispori*, *Helici*, *Islandici*, *Purpurei*, *Subinflati*, *Talaromyces* and *Trachyspermi*. Visagie *et al.* (2015) added five new species with ampulliform-like phialides to section *Talaromyces*. Later, Yilmaz *et al.* (2016b) resolved the taxonomy within section *Islandici* using a polyphasic approach and introduced four new species, *T. acaricola*, *T. crassus*, *T. infraolivaceus* and *T. subaurantiacus*. In the same year, eight new species, *T. amazonensis*, *T. columbiensis*, *T. francoae*, *T. neofusisporus*, *T. purgamentorum*, *T. qii*, *T. rubrifaciens* and *T. systylus*, were described from Argentina, China and Colombia (Luo *et al.* 2016, Romero *et al.* 2016, Wang *et al.* 2016, Yilmaz *et al.* 2016a).

In the last decades the interest in indoor mycobiota has grown because of their adverse health effects in humans (Samson *et al.* 1994, Prezant *et al.* 2008, Flannigan *et al.* 2011, Adan & Samson 2011). Samson *et al.* (2010) listed 100 common indoor fungal species which belong to 47 genera. *Talaromyces funiculosus*, *T. rugulosus* and *T. wortmanii* are among the most frequently encountered species in indoor environments. Visagie *et al.* (2014) analysed *Talaromyces* species from dust samples collected from nine countries, and based on ITS and *BenA* sequences, 18 *Talaromyces* species were identified including three new species: *T. oumae-annae*, *T. sayulitensis* and *T. yelensis*.

Various studies investigated the mycobiota of indoor environments in China. However, most surveys focused on total fungal counts and identified fungi to genera or species level based on phenotypic characters (Wu *et al.* 1982, Wu *et al.* 2000, Fang *et al.* 2005, Li *et al.* 2006, Si *et al.* 2007, Liu *et al.* 2014). Molecular based identifications are occasionally being performed and Luo *et al.* (2016) reported *T. rubrifaciens* as a new taxon from heating, ventilation and air conditioning systems in China.

During surveys of the mycobiota of indoor air in Beijing, China, numerous strains belonging to *Aspergillus*, *Cladosporium*, *Chaetomium*, *Penicillium* and other genera were isolated. Among them, 14 *Talaromyces* isolates could not be assigned to any described species. These strains are described here as nine new species based on multi-gene phylogenies based partial ITS, β-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II

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second largest subunit (*RPB2*) gene sequences, phenotype and extrolite data.

MATERIAL AND METHODS

Isolates

Isolates used in this study were collected by the sedimentation plate method on various media in the vicinity of air-conditioning exhausts. These strains were subsequently deposited in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China. In addition, isolates from the culture collection of CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, the Netherlands, and working collection of the Applied and Industrial Mycology department (DTO) housed at CBS-KNAW were used. An overview of strains is given in Table 1. For other strains used in the phylogenetic analyses, readers are referred to Yilmaz *et al.* (2014, 2016a, b), Visagie *et al.* (2014, 2015), Luo *et al.* (2016), Romero *et al.* (2016), and Wang *et al.* (2016).

DNA extraction, PCR amplification and sequencing

Strains were grown for 1 wk on malt extract agar (MEA, Oxoid malt) prior to DNA extraction. DNA was extracted using the Ultraclean™ Microbial DNA isolation Kit (MoBio, Solana Beach, U.S.A.) and stored at −20 °C. The ITS, *BenA*, *CaM*, and *RPB2* genes were amplified and sequenced using methods and primers previously described (Houbraken & Samson 2011, Yilmaz *et al.* 2014).

Phylogenetic analysis

A multi-gene phylogeny combining ITS, *BenA*, *CaM* and *RPB2* sequences was used to accommodate the new species of *Talaromyces* in the different sections. Prior combining the datasets, single gene alignments were generated with MAFFT v. 7 (Katoh & Standley 2013), and then trimmed at both ends. Aligned datasets were subsequently concatenated using Mesquite v 3.1 (Maddison & Maddison 2016). For each section, single gene phylogenies were generated to determine the phylogenetic relationship among species. The most suitable substitution model was determined using FindModel (Posada & Crandall 1998). Bayesian analyses were performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). The sample frequency was set to 100 and the first 25 % of trees were removed as burn-in. Maximum likelihood analyses including 500 bootstrap replicates were run using RAxML BlackBox webserver (Gamma model of rate heterogeneity) (Stamatakis *et al.* 2008). *Trichocoma paradoxa* (CBS 788.83^{T}) was used as an outgroup in the *Talaromyces* phylogeny. Sequences of *T. ucrainicus* (CBS 162.67^{T}), *T. subinflatus* (CBS 652.95^{T}), *T. dendriticus* (CBS 660.80^{T}) and *T. purpurogenus* (CBS 286.36^{T}) were used as outgroups in the *Talaromyces* sections *Helici*, *Islandici*, *Talaromyces* and *Trachyspermi* respectively. The resulting trees were visualised with FigTree v1.4.2 and edited in Adobe Illustrator CS5. Bayesian inference (BI) posterior probabilities (pp) values and bootstrap (bs) values are labelled on nodes. Values less than 0.95 pp and 70 % bs are not shown. Branches with posterior probability values of 1 and bootstrap values higher than 95 % are thickened. Newly obtained sequences were deposited in GenBank.

Table 1. Indoor *Talaromyces* strains used in this study.

Species name	Section	Strain no.	GenBank accession nr.			
			ITS	*BenA*	*CaM*	*RPB2*
Talaromyces diversiformis	*Helici*	CBS 141931^{T} = CGMCC3.18204 = DTO 317-E3	KX961215	KX961216	KX961259	KX961274
T. reverso-olivaceus	*Helici*	CBS 140672^{T} = CGMCC3.18195 = DTO 317-C3	KU866646	KU866834	KU866730	KU866990
T. reverso-olivaceus	*Helici*	CGMCC3.18216 = DTO 318-G2	KU866660	KU866847	KU866744	KU867004
T. cerinus	*Islandici*	CBS 140622^{T} = CGMCC3.18212 = DTO 318-A2	KU866658	KU866845	KU866742	KU867002
T. chlamydosporus	*Islandici*	CBS 140635^{T} = CGMCC3.18199 = DTO 317-D5	KU866648	KU866836	KU866732	KU866992
T. islandicus	*Islandici*	CGMCC3.18196 = DTO 317-C5	–	KX961217	–	–
T. neorugulosus	*Islandici*	CBS 140623^{T} = CGMCC3.18215 = DTO 318-A8	KU866659	KU866846	KU866743	KU867003
T. adpressus	*Talaromyces*	CBS 140620^{T} = CGMCC3.18211 = DTO 317-G4	KU866657	KU866844	KU866741	KU867001
T. aurantiacus	*Talaromyces*	CGMCC3.18198 = DTO 317-C9	–	KX961218	–	–
T. beijingensis	*Talaromyces*	CBS 140617^{T} = CGMCC3.18200 = DTO 317-D8	KU866649	KU866837	KU866733	KU866993
T. beijingensis	*Talaromyces*	CGMCC3.18201 = DTO 317-D9	KU866650	KU866838	KU866734	KU866994
T. beijingensis	*Talaromyces*	CGMCC3.18202 = DTO 317-E1	KU866651	KU866839	KU866735	KU866995
T. beijingensis	*Talaromyces*	CBS 140619 = CGMCC3.18208 = DTO 317-E9	KU866654	KU866841	KU866738	KU866998
T. fusiformis	*Talaromyces*	CBS 140637^{T} = CGMCC3.18210 = DTO 317-F4	KU866656	KU866843	KU866740	KU867000
T. fusiformis	*Talaromyces*	CBS 140636 = CGMCC3.18209 = DTO 317-F3	KU866655	KU866842	KU866739	KU866999
T. siamensis	*Talaromyces*	CGMCC3.18214 = DTO 318-B6	–	KX961219	–	–
T. aerius	*Trachyspermi*	CBS 140611^{T} = CGMCC3.18197 = DTO 317-C7	KU866647	KU866835	KU866731	KU866991
T. albobiverticillius	*Trachyspermi*	CGMCC3.18203 = DTO 317-E2	–	KX961222	–	–
T. albobiverticillius	*Trachyspermi*	CGMCC3.18205 = DTO 317-E4	–	KX961220	–	–
T. albobiverticillius	*Trachyspermi*	CGMCC3.18206 = DTO 317-E5	–	KX961221	–	–
T. albobiverticillius	*Trachyspermi*	CGMCC3.18207 = DTO 317-E6	–	KX961223	–	–

Morphological analysis

Macroscopic characters were studied on Czapek yeast autolysate 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) (Samson *et al.* 2010). Isolates were inoculated at three points on 90 mm Petri dishes and incubated for 7 d 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 d 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 noted. Acid production on CREA is indicated by a change in the pH sensitive bromocresol purple dye, from a purple to yellow colour in media surrounding colonies. For ascoma production, OA, MEA and CYA plates were incubated for up to four weeks.

Microscope preparations were made from 1 wk old colonies grown on MEA and ascomata, asci and ascospores were observed on OA. Lactic acid (60 %) was used as mounting fluid and 96 % ethanol was applied to remove the excess of conidia. A Zeiss Stereo Discovery V20 dissecting microscope and Zeiss AX10 Imager A2 light microscope equipped with Nikon DS-Ri2 cameras and software NIS-Elements D v4.50 were used to capture digital images.

Extrolites analysis

For extrolite extractions, three agar plugs (6 mm diam) were taken from colonies grown on CYA and YES (incubated for 1 wk at 25 °C), and combined in one Eppendorf vial. In addition, three plugs were taken from colonies grown on OA and Blakeslee's MEA (Blakeslee 1915), and combined in another Eppendorf vial. The plugs were ultrasonicated in ethylacetate/isopropanol (3:1) with 1 % formic acid for 50 min. After extraction, the liquid was transferred to another Eppendorf vial and thereafter evaporated. The remaining dry fraction was redissolved in 300 µl methanol and ultrasonicated for 10 min. The extract was centrifuged at 13 400 rpm in an Eppendorf centrifuge (Minispin), transferred to a V-formed vial with a 300 µl capacity, and subsequently injected into an Ultra high performance liquid chromatograph (UHPLC) via an autosampler. The Liquid chromatograph was a Dionex Ultimate 3000 UHPLC connected to a Dionex 3000 RS Diode array detector and an Agilent 1321A fluorescence detector. The column used was a Poroshell Phenyl hexyl 120 (100 mm × 2.1 mm) column with 2.7 µm particles (Agilent). The UHPLC gradient, injection volume and other conditions are given in Klitgaard *et al.* (2014). Standards of rugulosin, skyrin, rugulovasine, duclauxin and other *Talaromyces* derived extrolites were used as standards in the comparison of retention times and UV spectra.

RESULTS

Phylogeny

The phylogenetic relationships among the species in *Talaromyces* were studied using concatenated sequence data of four loci, ITS, *BenA*, *CaM* and *RPB2*. The total length of the aligned dataset was 2420 characters, and the single gene datasets consisted of 500, 493, 624 and 803 characters for ITS, *BenA*, *CaM* and *RPB2* respectively. The most optimal models for the concatenated and the single gene phylogenies are shown in Table 2. The multi-gene analysis reveals the presence of seven well-supported lineages in *Talaromyces* (Fig. 1), and these lineages correspond with sections *Bacillispori*, *Helici*, *Islandici*, *Purpurei*, *Subinflati*, *Talaromyces*, and *Trachyspermi*. Our indoor *Talaromyces* strains are resolved in four sections, seven of them are identified as *T. islandicus*, *T. aurantiacus*, *T. siamensis* and *T. albobiverticillius* based on *BenA* sequences, while 14 of them are described as nine new species: *T. diversiformis* and *T. reverso-olivaceus* in section *Helici*, *T. chlamydosporus*, *T. cerinus* and *T. neorugulosus* in section *Islandici*, *T. beijingensis*, *T. fusiformis* and *T. adpressus* in section *Talaromyces*, and *T. aerius* in section *Trachyspermi*.

Talaromyces diversiformis and *T. reverso-olivaceus*, both belonging to section *Helici* are in the combined analysis related with *T. aerugineus* and *T. boninensis*, respectively (Fig. 1). *Talaromyces reverso-olivaceus* is in the *BenA*, *CaM* and *RPB2* analysis is a sister species of *T. boninensis* (>0.98 pp; >89 % bs). The phylogenetic relationship of *T. diversiformis* is more difficult to determine based on the single gene phylograms and this species appears to be related to *T. aerugineus* and *T. bohemicus* in the *BenA*, *CaM* and *RPB2* phylograms (>0.98 pp; >70 % bs). The ITS phylogram is poorly resolved. The *T. reverso-olivaceus* isolates cluster together (1 pp; 98 % bs) and these isolates are on a well-supported branch together with *T. helicus* and *T. boninensis*. The relationship of *T. diversiformis* is unresolved in the ITS phylogram (Fig. 2, Suppl. 1–3). *Talaromyces chlamydosporus* and *T. cerinus* are both members of section *Islandici* and are, with exception in the *CaM* analysis, related with high statistical support to *T. subaurantiacus* (Figs 1, 3, Suppl. 4–6). The multi-gene phylogeny and the ITS and *RPB2* phylograms show that *T. neorugulosus* is most closely related to *T. rugulosus*. This species is unresolved in the *CaM* analysis, and related to *T. rugulosus*, *T. infraolivaceus*, *T. atricola* and *T. acaricola* in the

Table 2. Sequence data sets and models used in phylogeny.

Section	Sequence data sets							
	ITS (bp)	Substitution model	*BenA* (bp)	Substitution model	*CaM* (bp)	Substitution model	*RPB2* (bp)	Substitution model
Overview *Talaromyces*	500	GTR+G	493	GTR+G	624	K2P+G	803	GTR+G
Section *Helici*	464	HKY+G	427	HKY+G	550	GTR+G	852	GTR+G
Section *Islandici*	502	GTR+G	474	GTR+G	458	K2P+G	803	GTR+G
Section *Talaromyces*	469	GTR+G	402	HKY+G	523	GTR+G	779	HKY+G
Section *Trachyspermi*	478	GTR+G	388	HKY+G	491	K2P+G	680	GTR+G

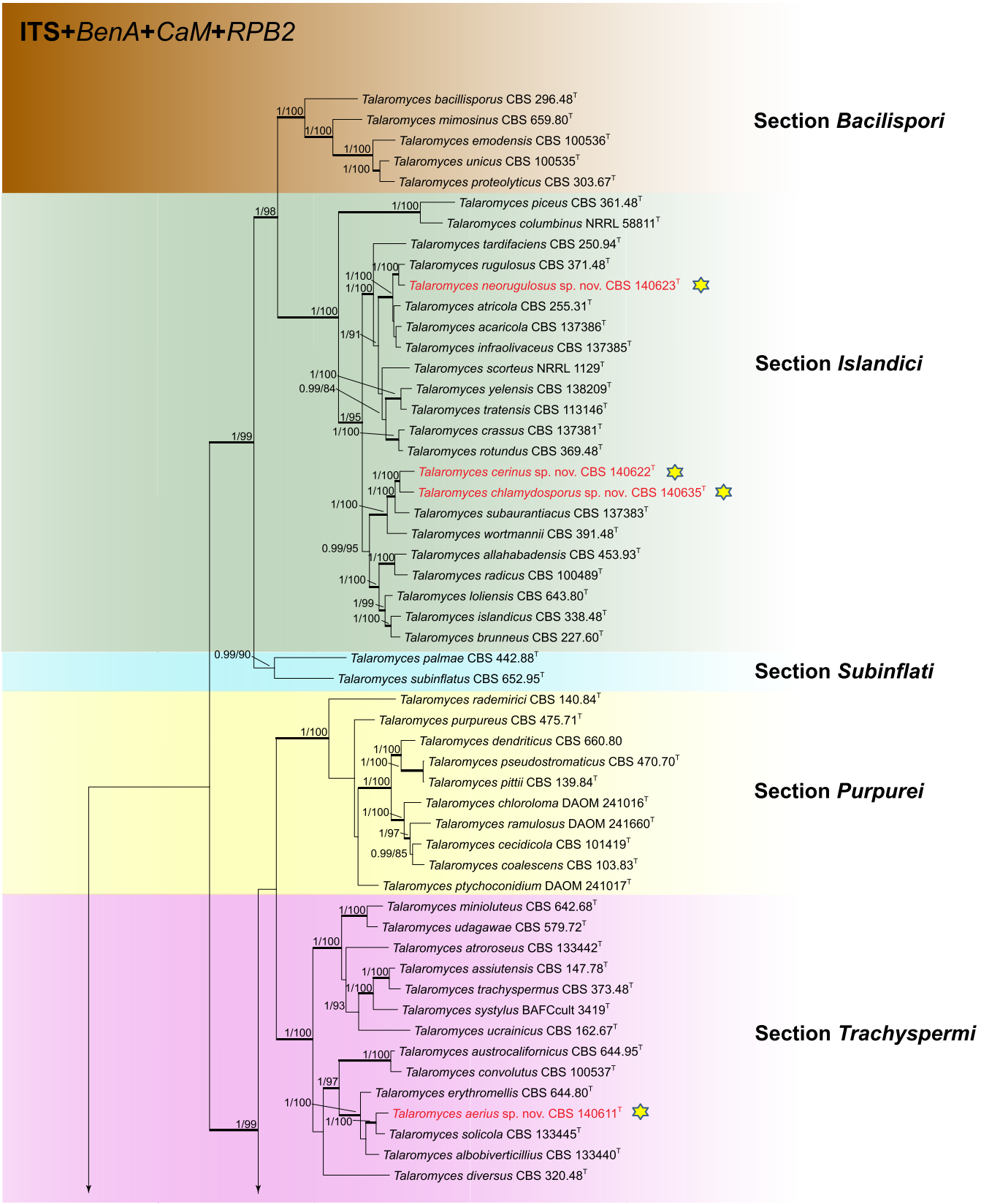


Fig. 1. Concatenated phylogeny of the ITS, *BenA*, *CaM* and *RPB2* gene regions of species from *Talaromyces*. Branches with 1 pp and bootstrap support values of more than 95 % are thickened. *Trichocoma paradoxa* was chosen as outgroup. Indoor isolates were marked with yellow star.

BenA phylogram (Fig. 3, Suppl. 4–6). Strains of *T. beijingensis* cluster together in a single clade, separate from other sect. *Talaromyces* species. The relationship of this species with other species is in all analysis (including the combined analysis) unresolved. Both strains of *T. fusiformis* form a single, separate clade in the four gene phylogenies, and they are a sister clade of *T. aurantiacus*. *Talaromyces adpressus* is in *BenA* phylogeny with statistical support related to *T. sayulitensis* (0.99 pp; 98 % bs), while it clusters with *T. pinophilus* in the *CaM* (1.00 pp; 98 % bs). The *RPB2* sequence of *T. sayulitensis* is unavailable thus cannot be compared here (Fig. 4, Suppl. 7–9). In section *Trachyspermi*, *T. aerius* clusters with statistical support with *T. solicola* in the three (*CaM*, *ITS* and *RPB2*) of the four single gene phylogenies (Fig. 5, Suppl. 10–12).

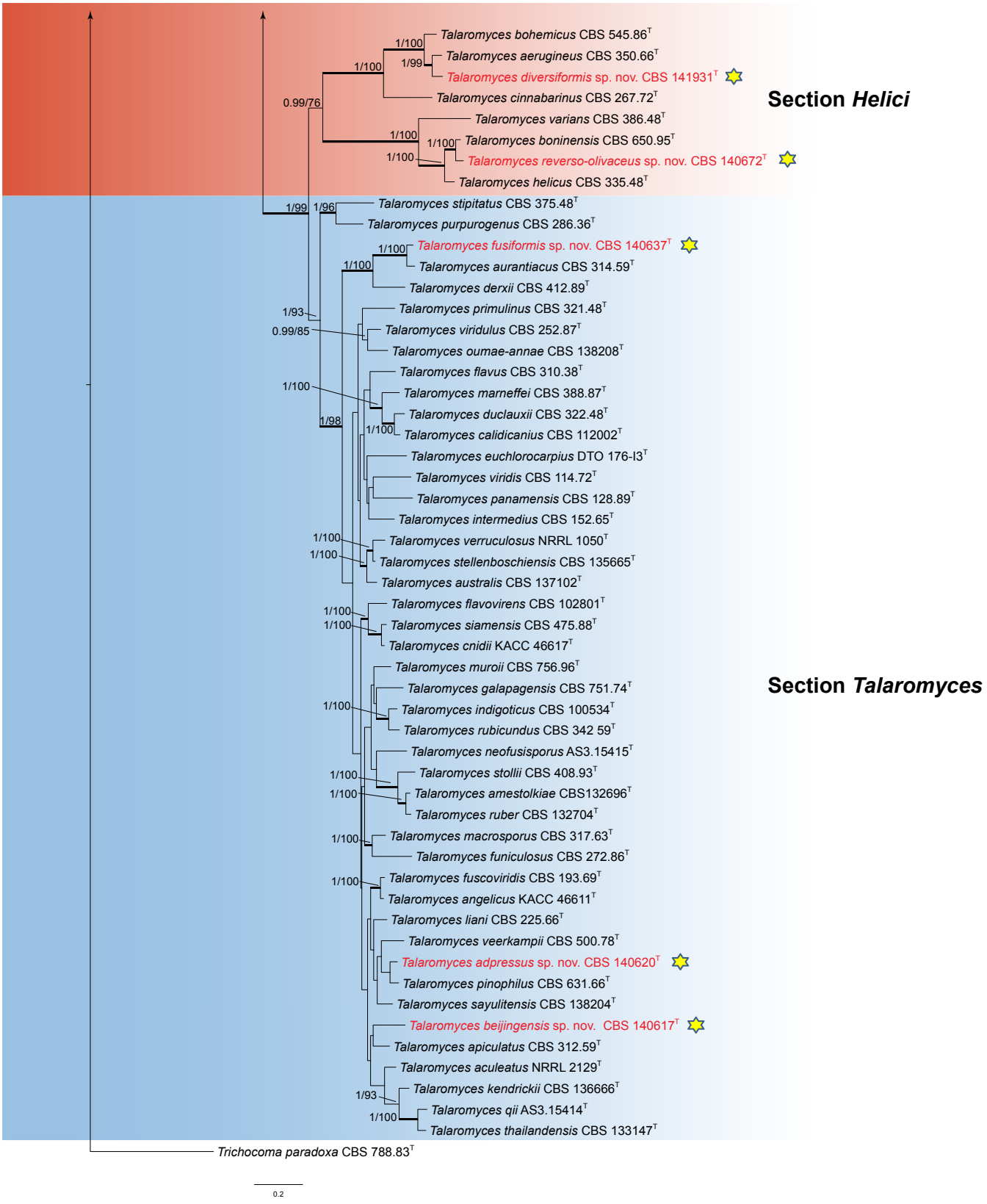


Fig. 1. (Continued).

Identification

All nine new species described here can be identified via *BenA*, *CaM* and *RPB2* sequences. Seven of them have unique ITS sequences. *Talaromyces neorugulosus* cannot be separated from *T. rugulosus* (strain CBS 285.37 and CBS 378.48) by its ITS sequence. *Talaromyces diversiformis* is similar to *T. aerugineus* (99.8 % similarity, 447/448 bp) and *T. ryukyuensis* (99.1 % similarity, 445/449 bp) by ITS sequences.

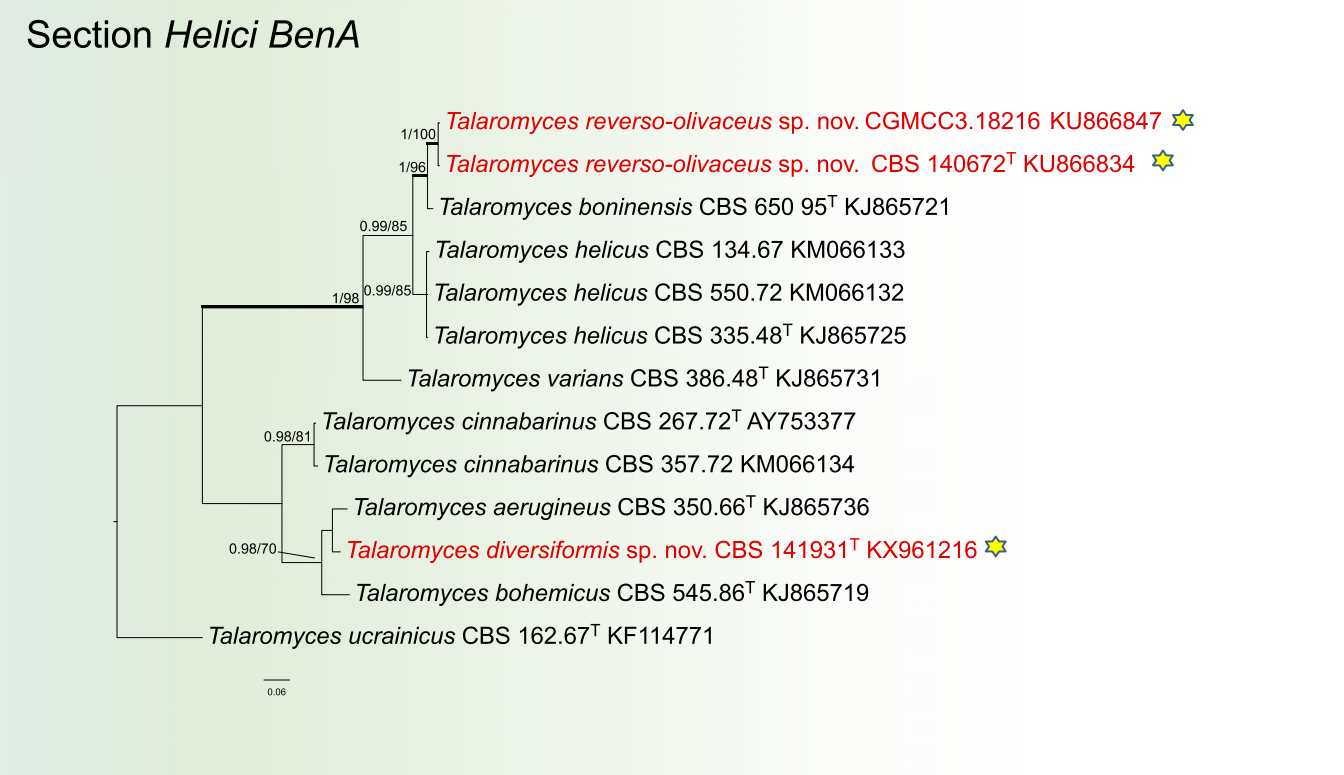


Fig. 2. Phylogeny of *BenA* for species classified in *Talaromyces* section *Helici*. Branches with 1 pp and bootstrap support values of more than 95 % are thickened. *Talaromyces ucrainicus* was chosen as outgroup. Indoor isolates were marked with yellow star.

TAXONOMY

Talaromyces aerius A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817398. Fig. 6.

Etymology: Latin, *aerius* refers to its origin, isolated from indoor air.

Diagnosis: This species produces smooth, ellipsoidal conidia; does not produce red pigments or red exudates on any of the used media.

In: *Talaromyces* section *Trachyspermi*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun (holotype CBS H-22506, culture ex-type CBS 140611 = CGMCC3.18197 = DTO 317-C7).

ITS barcode: KU866647. (*Alternative markers*: *BenA* = KU866835; *CaM* = KU866731; *RPB2* = KU866991).

Colony diam, 7 d (mm): CYA 17–18; CYA 30 °C 20–22; CYA 37 °C No growth; MEA 32–33; MEA 30 °C 34–36; DG18 11–12; CYAS 2–3; OA 28–30; CREA 2–4; YES 21–22.

Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, crateriform; margins entire; mycelium white; texture floccose; sporulation sparse, conidia *en masse* greyish green to olive green; soluble pigments absent; exudates absent; reverse saffron. MEA, 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse saffron. YES, 25 °C, 7 d: Colonies moderately deep, raise at centre, cricoid; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* olive green to greyish green; soluble pigments absent; exudates absent; reverse greyish olive. DG18, 25 °C, 7 d: Colonies moderately deep, raise at centre, slightly sulcate; margins entire; mycelium rosy buff; texture floccose; sporulation sparse, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse brown at centre, cream white at edge. OA, 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white and light yellow; texture velvety; sporulation dense, conidia *en masse* greyish green to yellow green; soluble pigments absent; exudates absent; reverse purplish red at centre, yellowish brown at edge. CREA, 25 °C, 7 d: Acid production absent.

Micromorphology: Conidiophores biverticillate, sometimes with extra subterminal branches; stipes smooth, 70–130 × 3–4 µm, extra branches 22–34 µm; metulae 3–5, divergent, 8–14 × 3–4 µm; phialides 4–6, acerose, 9–12 × 2–4 µm; conidia smooth, ellipsoidal, 2–3.5(–4.5) × 2–3 µm. Ascomata not observed.

Extrolites: Mitorubrinic acid.

Distinguishing characters: *Talaromyces aerius* is phylogenetically related to *T. solicola*, *T. albobiverticillius* and *T. erythromellis*. *Talaromyces solicola* produces rough conidia, *T. albobiverticillius* produces intense red soluble pigment on CYA and *T. erythromellis* grows restrictedly on CYA, MEA, YES and OA, and produces red exudates on MEA.

Talaromyces adpressus A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817397. Fig. 7.

Etymology: Latin, *adpressus* refers to its appressed metulae.

Diagnosis: This species produces white mycelium on MEA and OA; does not produce acid compounds on CREA and produces smooth, subglobose to ellipsoidal conidia measuring 2.5–4.5(–5) × 2–3.5 µm.

In: *Talaromyces* section *Talaromyces*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun (holotype CBS H-22507, culture ex-type CBS 140620 = CGMCC3.18211 = DTO 317-G4).

ITS barcode: KU866657. (*Alternative markers*: *BenA* = KU866844; *CaM* = KU866741; *RPB2* = KU867001).

Colony diam, 7 d (mm): CYA 32–33; CYA 30 °C 45–46; CYA 37 °C 35–38; MEA 42–43; MEA 30 °C 57–58; DG18 11–12; CYAS 1–2; OA 41–42; CREA 21–22; YES 42–43.

Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; mycelium white to buff, orange hyphae present at centre; texture floccose; sporulation sparse, conidia *en masse* greyish green; soluble pigments absent; exudates light droplets; reverse yellowish brown. MEA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture loosely funiculose to floccose; sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates clear droplets; reverse yellowish brown. YES, 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; white hyphae predominant, light coral red hyphae present; texture floccose; sporulation moderately dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse brown at centre, pale brown at edge. DG18, 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 light yellow at centre, cream white at edge. OA, 25 °C, 7 d: Colonies moderately deep, plane, margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates clear droplets; reverse pale buff. CREA, 25 °C, 7 d: Acid production present.

Micromorphology: Conidiophores biverticillate; stipes smooth, 100–200 × 3–4.5 µm; metulae 3–5, appressed, 10–15 × 3–4.5 µm; phialides 3–5, acerose, 9–14 × 2.5–3.5 µm; conidia smooth, subglobose to ellipsoidal, 2.5–4.5(–5) × 2–3.5 µm. Ascomata not observed.

Extrolites: Duclauxin, rugulovasine A.

Distinguishing characters: *Talaromyces adpressus* is closely related to *T. sayulitensis* and *T. pinophilus*; however, the latter two species produce large amounts of acid compounds on CREA. The micromorphology of these three species is identical, but they can be phylogenetically distinguished.

Talaromyces beijingensis A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817395. Fig. 8.

Etymology: Latin, *beijingensis* refers to its origin, isolated from Beijing, China.

Diagnosis: This species grows moderately on CYA and MEA, the colony reverse on CYA and MEA is peach coloured, and it produces smooth, subglobose to fusiform conidia.

In: *Talaromyces* section *Talaromyces*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun. (holotype CBS H-22508, culture ex-type CBS 140617 = CGMCC3.18200 = DTO 317-D8).

ITS barcode: KU866649. (*Alternative markers*: *BenA* = KU866837; *CaM* = KU866733; *RPB2* = KU866993).

Colony diam, 7 d (mm): CYA 27–28; CYA 30 °C 36–37; CYA 37 °C 26–28; MEA 35–39; MEA 30 °C 45–52; DG18 10–11; CYAS Weak growth; OA 34–37; CREA 19–20; YES 25–26.

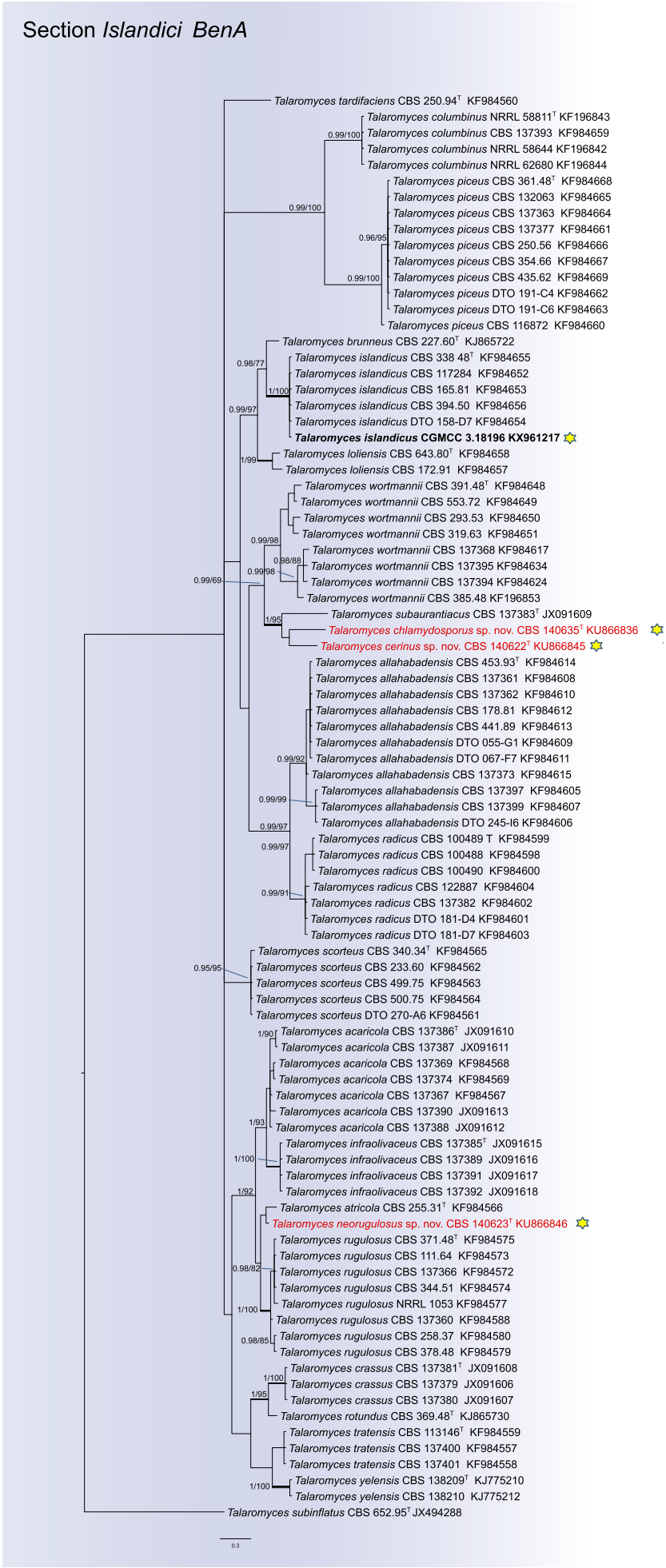
Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* yellow green to greyish green; soluble pigments absent; exudates clear droplets; reverse peach fading into rosy buff. MEA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* dark green; soluble pigments absent; exudates clear droplets; reverse peach fading into yellowish brown. YES, 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* greyish green to dark green; soluble pigments absent; exudates absent; reverse orange brown. DG18, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation sparse, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse brown to light yellow at centre, cream white at edge. OA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety to floccose; sporulation dense, conidia *en masse* dark green; soluble pigments absent; exudates clear droplets; reverse orange brown. CREA, 25 °C, 7 d: Acid production present.

Micromorphology: Conidiophores biverticillate, with symmetrical subterminal branches; stipes smooth, 91–175 × 3–4 µm, extra branches 18–20 µm; metulae 3–4, appressed, 11–14 × 2–4 µm; phialides 2–4, acerose, 9–12 × 2–3 µm; conidia smooth, ellipsoidal to fusiform, 3–4 × 2–3 µm. Ascomata not observed.

Extrolites: Duclauxin.

Distinguishing characters: Phylogenetically *T. beijingensis* belongs to section *Talaromyces*, but it cannot be assigned to any section members. Morphologically this species resembles *T. flavovirens* in having moderately growing, velvety, yellow green to greyish green colonies on CYA and MEA, biverticillate conidiophores and ellipsoidal to fusiform conidia. *Talaromyces flavovirens* can be differentiated by the production of synnemata (up to 750 µm) and yellow mycelium.

Talaromyces cerinus A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817393. Fig. 9.


Section *Islandici* *BenA*
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Talaromyces columbinus NRRL 62680 KF196844
Talaromyces piceus CBS 361.48^{T} KF984668
Talaromyces piceus CBS 132063 KF984665
Talaromyces piceus CBS 137363 KF984664
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Talaromyces piceus CBS 250.56 KF984666
Talaromyces piceus CBS 354.66 KF984667
Talaromyces piceus CBS 435.62 KF984669
Talaromyces piceus DTO 191-C4 KF984662
Talaromyces piceus DTO 191-C6 KF984663
Talaromyces piceus CBS 116872 KF984660
Talaromyces brunneus CBS 227.60^{T} KJ865722
Talaromyces islandicus CBS 338 48^{T} KF984655
Talaromyces islandicus CBS 117284 KF984652
Talaromyces islandicus CBS 165.81 KF984653
Talaromyces islandicus CBS 394.50 KF984656
Talaromyces islandicus DTO 158-D7 KF984654
***Talaromyces islandicus* CGMCC 3.18196 KX961217**
Talaromyces loliensis CBS 643.80^{T} KF984658
Talaromyces loliensis CBS 172.91 KF984657
Talaromyces wortmannii CBS 391.48^{T} KF984648
Talaromyces wortmannii CBS 553.72 KF984649
Talaromyces wortmannii CBS 293.53 KF984650
Talaromyces wortmannii CBS 319.63 KF984651
Talaromyces wortmannii CBS 137368 KF984617
Talaromyces wortmannii CBS 137395 KF984634
Talaromyces wortmannii CBS 137394 KF984624
Talaromyces wortmannii CBS 385.48 KF196853
Talaromyces subaurantiacus CBS 137383^{T} JX091609
Talaromyces chlamydosporus sp. nov. CBS 140635^{T} KU866836
Talaromyces cerinus sp. nov. CBS 140622^{T} KU866845
Talaromyces allahabadensis CBS 453.93^{T} KF984614
Talaromyces allahabadensis CBS 137361 KF984608
Talaromyces allahabadensis CBS 137362 KF984610
Talaromyces allahabadensis CBS 178.81 KF984612
Talaromyces allahabadensis CBS 441.89 KF984613
Talaromyces allahabadensis DTO 055-G1 KF984609
Talaromyces allahabadensis DTO 067-F7 KF984611
Talaromyces allahabadensis CBS 137373 KF984615
Talaromyces allahabadensis CBS 137397 KF984605
Talaromyces allahabadensis CBS 137399 KF984607
Talaromyces allahabadensis DTO 245-I6 KF984606
Talaromyces radicus CBS 100489 T KF984599
Talaromyces radicus CBS 100488 KF984598
Talaromyces radicus CBS 100490 KF984600
Talaromyces radicus CBS 122887 KF984604
Talaromyces radicus CBS 137382 KF984602
Talaromyces radicus DTO 181-D4 KF984601
Talaromyces radicus DTO 181-D7 KF984603
Talaromyces scorteus CBS 340.34^{T} KF984565
Talaromyces scorteus CBS 233.60 KF984562
Talaromyces scorteus CBS 499.75 KF984563
Talaromyces scorteus CBS 500.75 KF984564
Talaromyces scorteus DTO 270-A6 KF984561
Talaromyces acaricola CBS 137386^{T} JX091610
Talaromyces acaricola CBS 137387 JX091611
Talaromyces acaricola CBS 137369 KF984568
Talaromyces acaricola CBS 137374 KF984569
Talaromyces acaricola CBS 137367 KF984567
Talaromyces acaricola CBS 137390 JX091613
Talaromyces acaricola CBS 137388 JX091612
Talaromyces infraolivaceus CBS 137385^{T} JX091615
Talaromyces infraolivaceus CBS 137389 JX091616
Talaromyces infraolivaceus CBS 137391 JX091617
Talaromyces infraolivaceus CBS 137392 JX091618
Talaromyces atricola CBS 255.31^{T} KF984566
Talaromyces neorugulosus sp. nov. CBS 140623^{T} KU866846
Talaromyces rugulosus CBS 371.48^{T} KF984575
Talaromyces rugulosus CBS 111.64 KF984573
Talaromyces rugulosus CBS 137366 KF984572
Talaromyces rugulosus CBS 344.51 KF984574
Talaromyces rugulosus NRRL 1053 KF984577
Talaromyces rugulosus CBS 137360 KF984588
Talaromyces rugulosus CBS 258.37 KF984580
Talaromyces rugulosus CBS 378.48 KF984579
Talaromyces crassus CBS 137381^{T} JX091608
Talaromyces crassus CBS 137379 JX091606
Talaromyces crassus CBS 137380 JX091607
Talaromyces rotundus CBS 369.48^{T} KJ865730
Talaromyces tratensis CBS 113146^{T} KF984559
Talaromyces tratensis CBS 137400 KF984557
Talaromyces tratensis CBS 137401 KF984558
Talaromyces yelensis CBS 138209^{T} KJ775210
Talaromyces yelensis CBS 138210 KJ775212
Talaromyces subinflatus CBS 652.95^{T} JX494288
0.99/100
0.99/100
0.96/95
0.99/100
0.98/77
1/100
0.99/97
1/99
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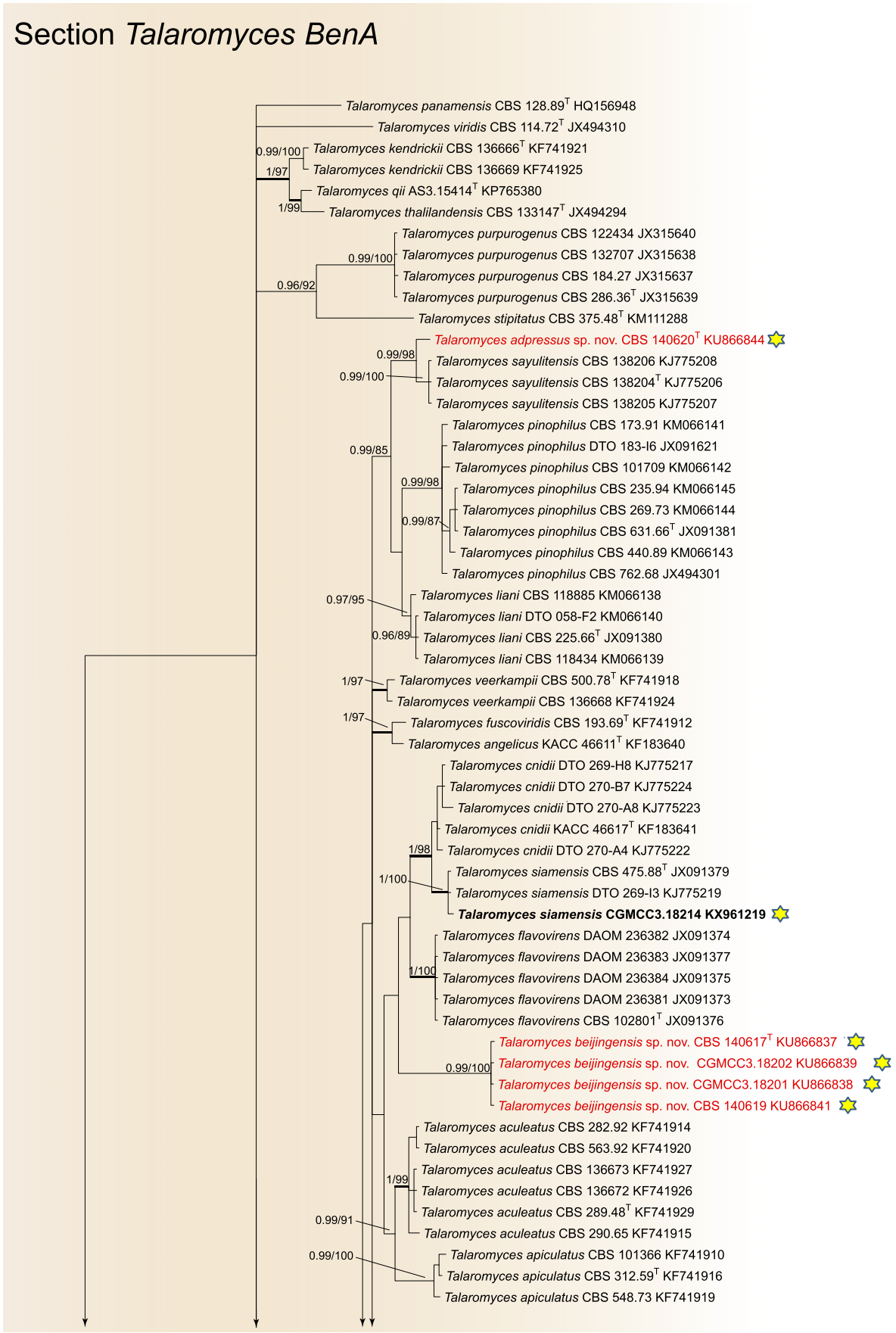

Fig. 4. Phylogeny of *BenA* for species classified in *Talaromyces* section *Talaromyces*. Branches with 1 pp and bootstrap support values of more than 95 % are thickened. *Talaromyces dendriticus* was chosen as outgroup. Indoor isolates were marked with yellow star.

Fig. 3. Phylogeny of *BenA* for species classified in *Talaromyces* section *Islandici*. Branches with 1 pp and bootstrap support values of more than 95 % are thickened. *Talaromyces subinflatus* was chosen as outgroup. Indoor isolates were marked with yellow star.

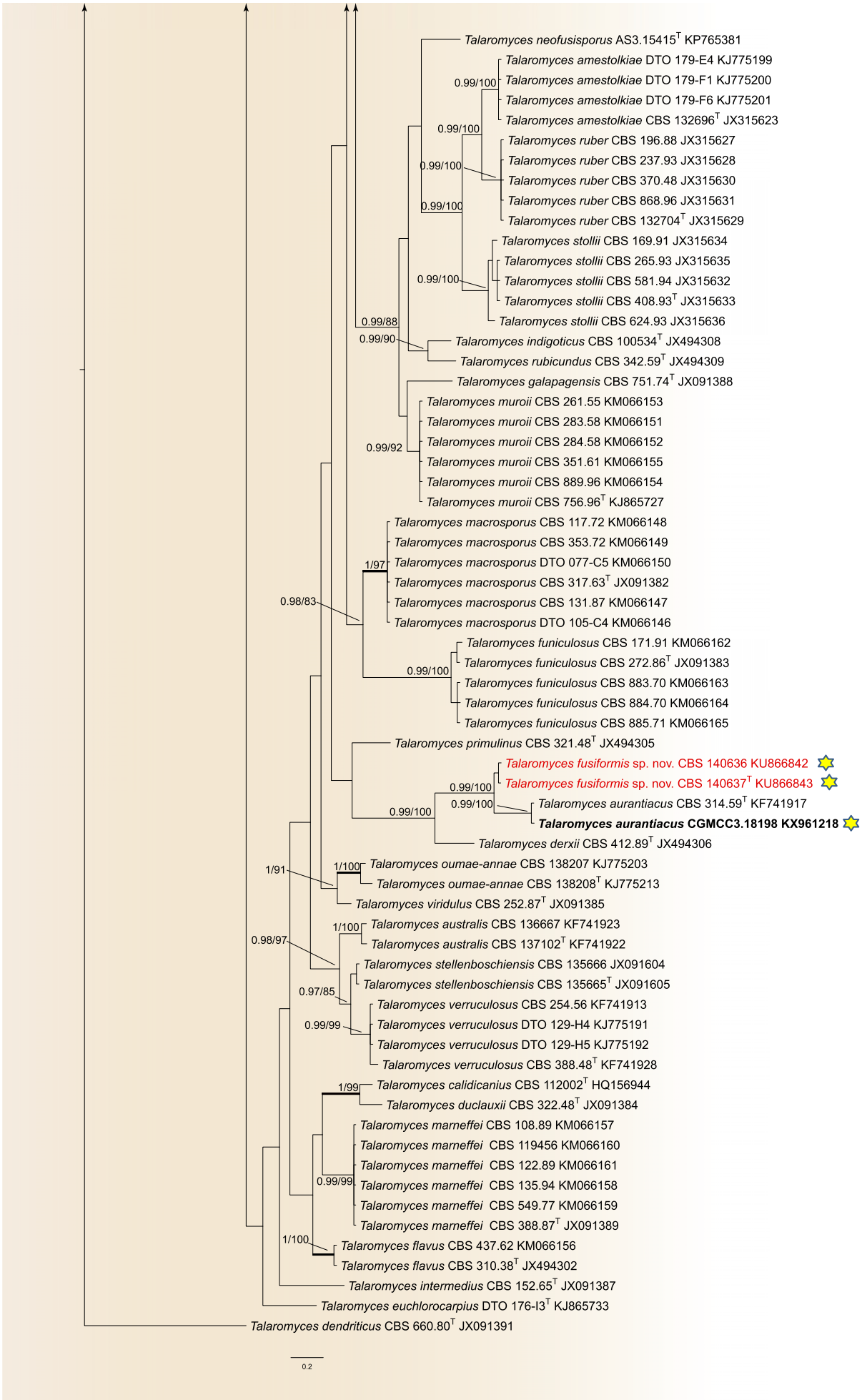

Fig. 4. (Continued).

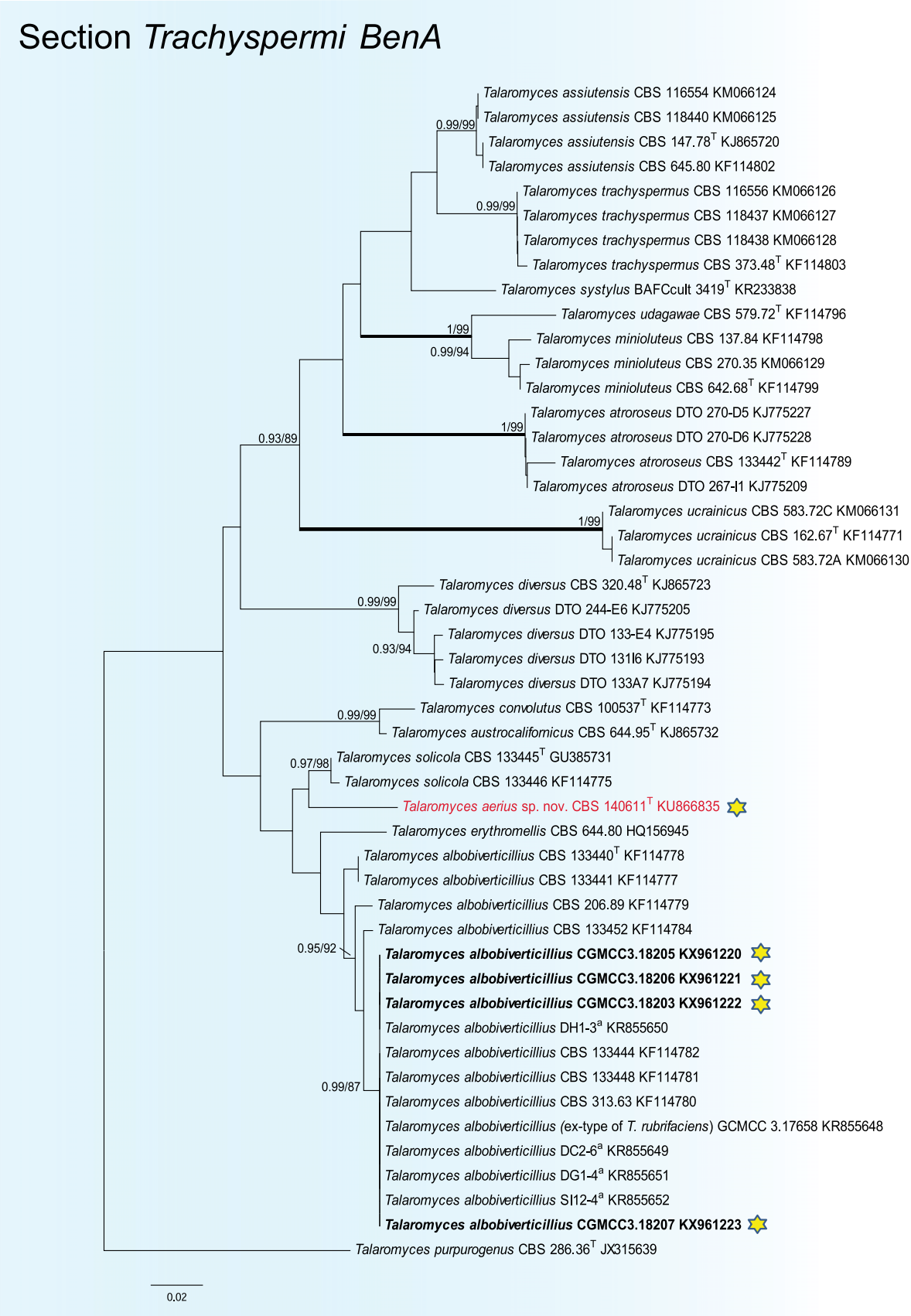

Fig. 5. Phylogeny of *BenA* for species classified in *Talaromyces* section *Trachyspermi*. Branches with 1 pp and bootstrap support values of more than 95 % are thickened. *Talaromyces purpurogenus* was chosen as outgroup. Indoor isolates were marked with yellow star. [a] indicates isolates previously identified as *T. rubrifaciens*.

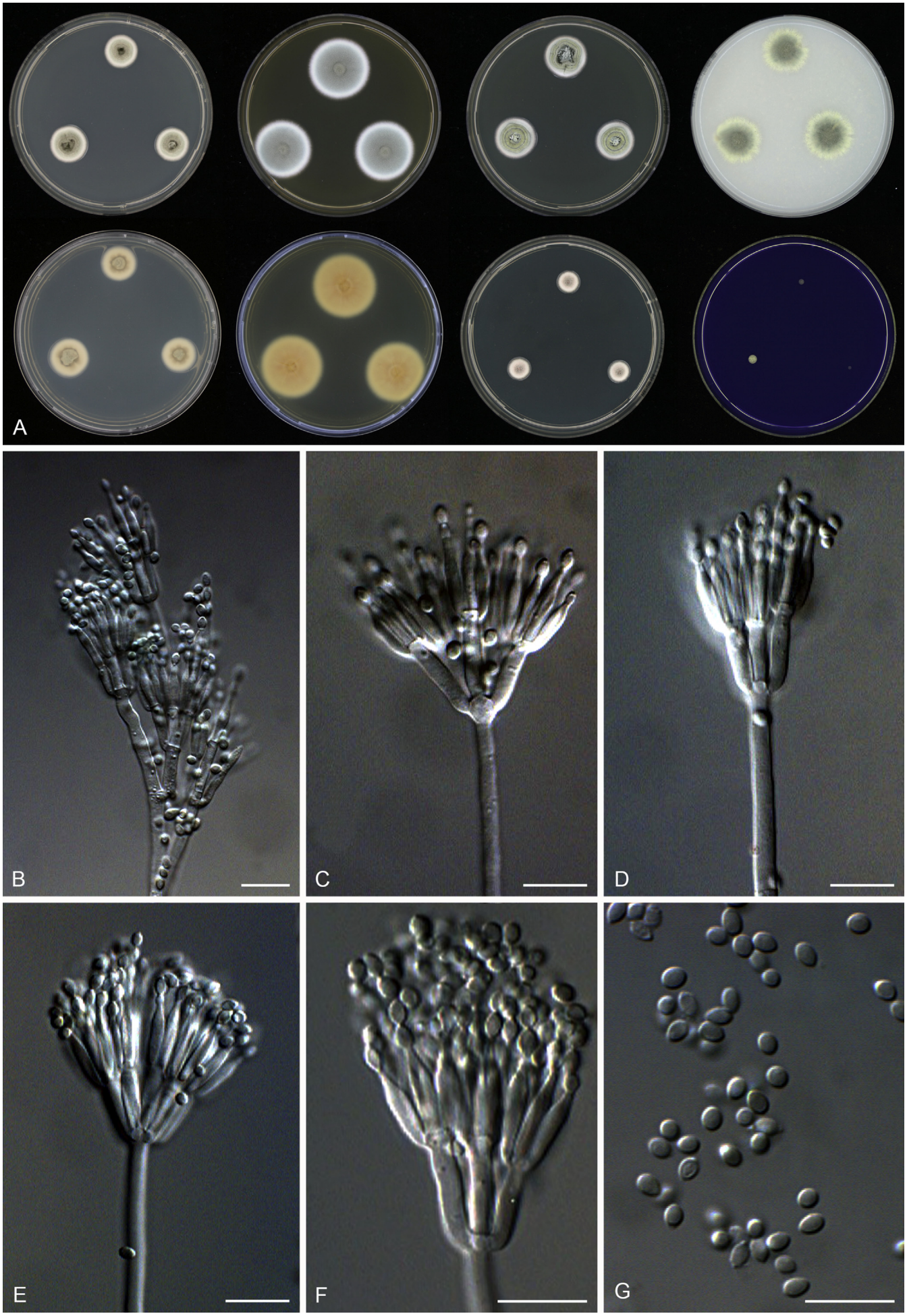

Fig. 6. Morphological characters of *Talaromyces aerius*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. conidiophores; G. Conidia. Scale bars = 10 µm.

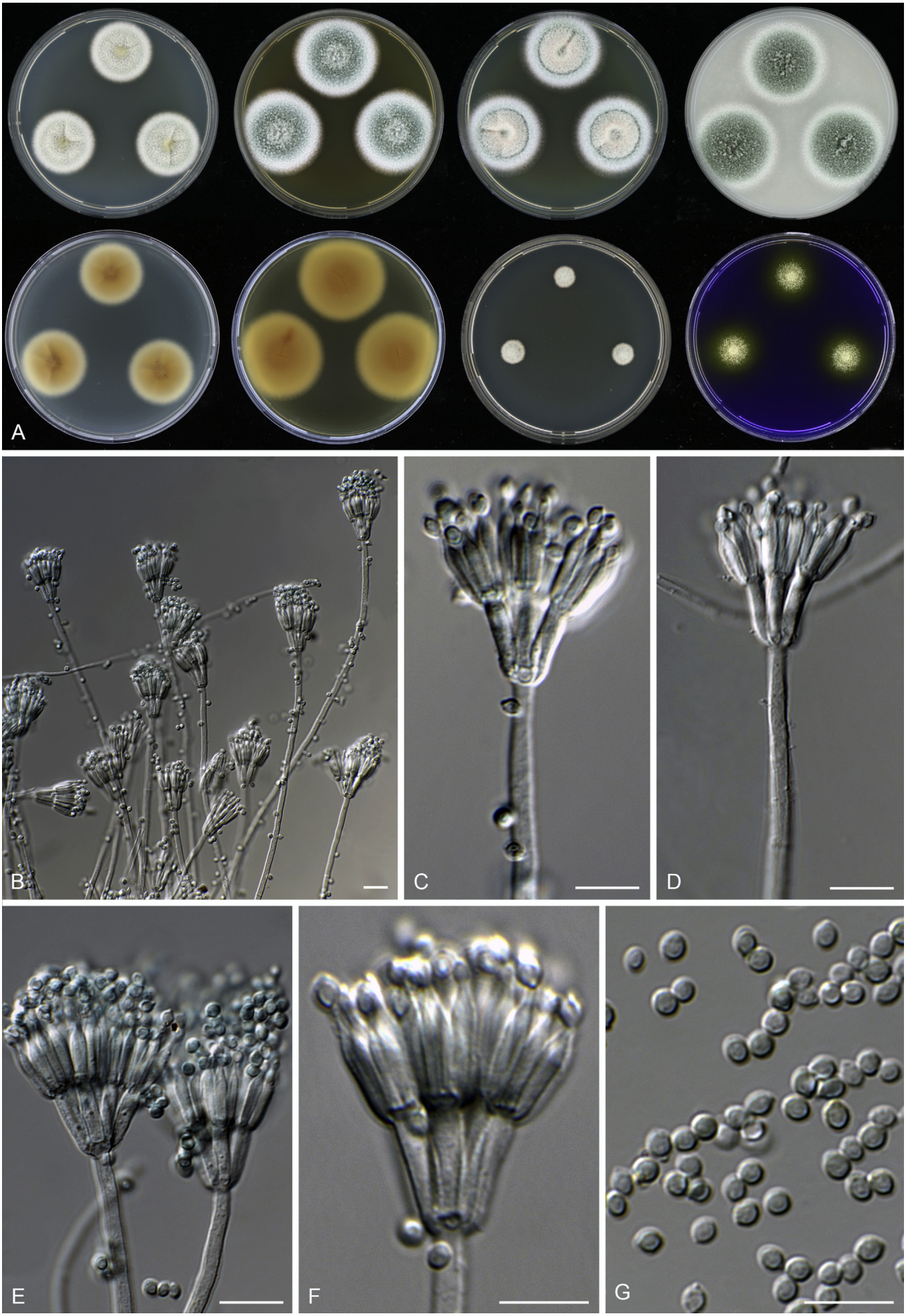

Fig. 7. Morphological characters of *Talaromyces adpressus*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. Conidiophores; G. Conidia. Scale bars = 10 µm.

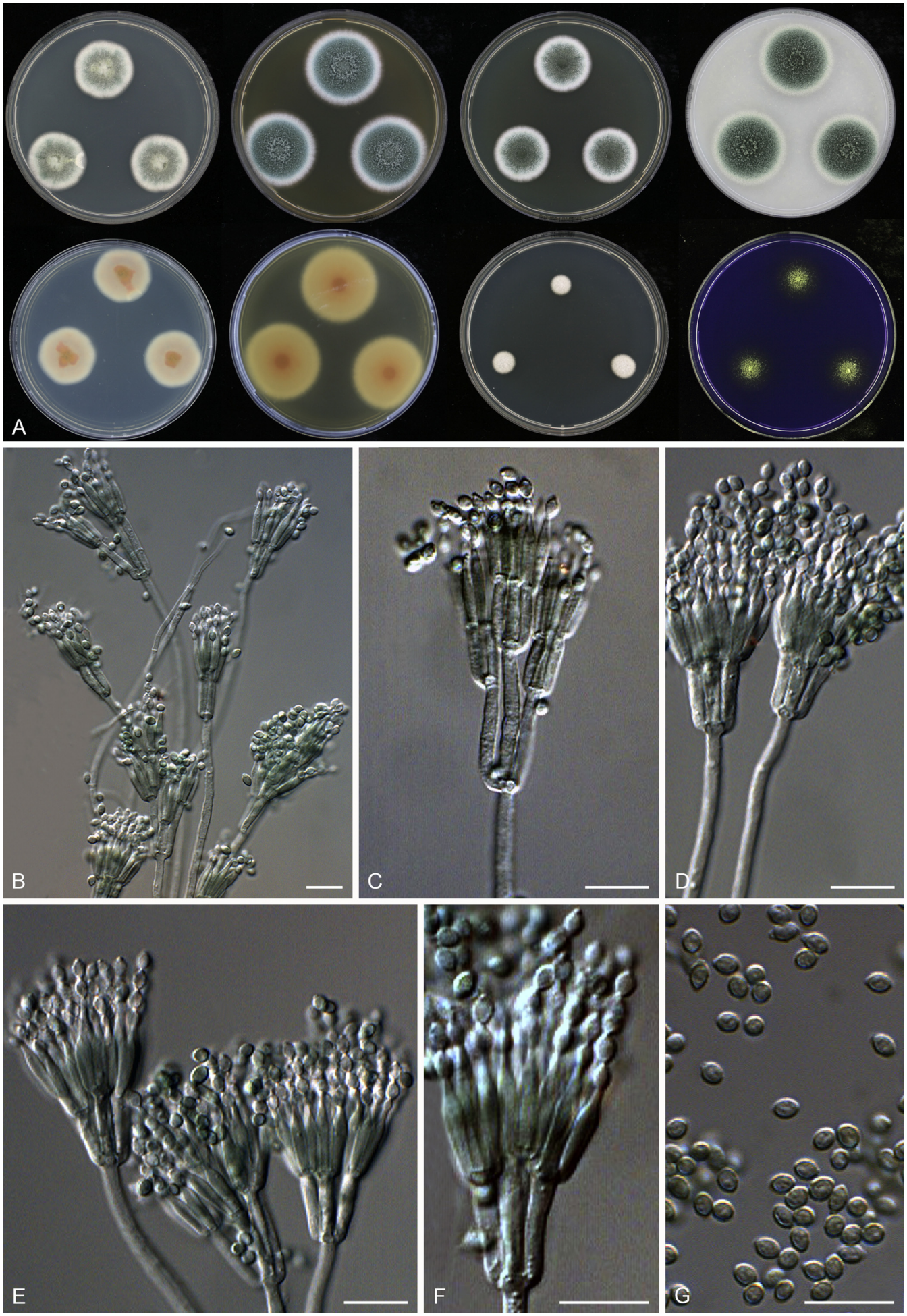

Fig. 8. Morphological characters of *Talaromyces beijingensis*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. Conidiophores; G. Conidia. Scale bars = 10 µm.

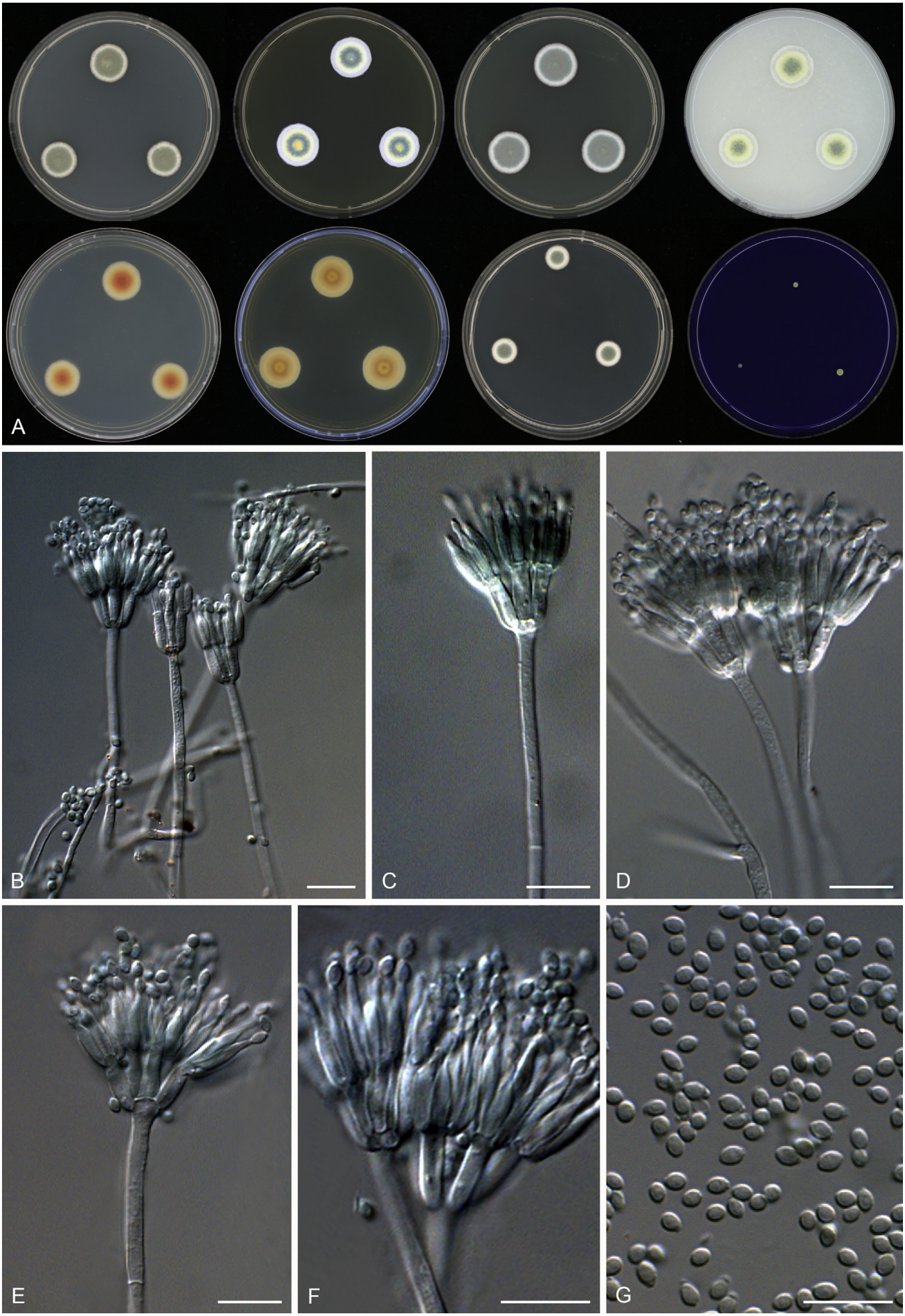

Fig. 9. Morphological characters of *Talaromyces cerinus*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. Conidiophores; G. Conidia. Scale bars = 10 µm.

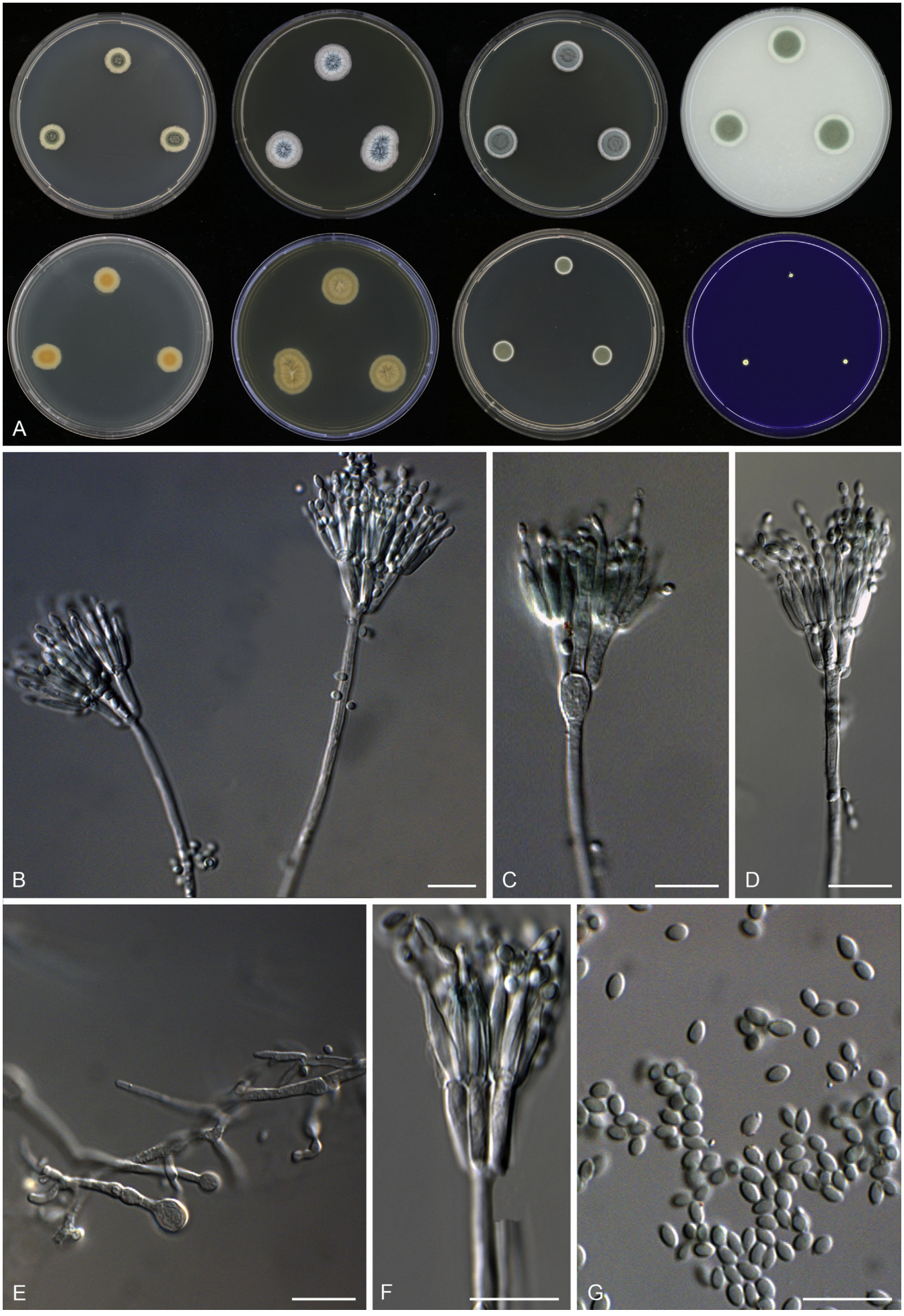

Fig. 10. Morphological characters of *Talaromyces chlamydosporus*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. Conidiophores; G. Conidia. Scale bars = 10 µm.

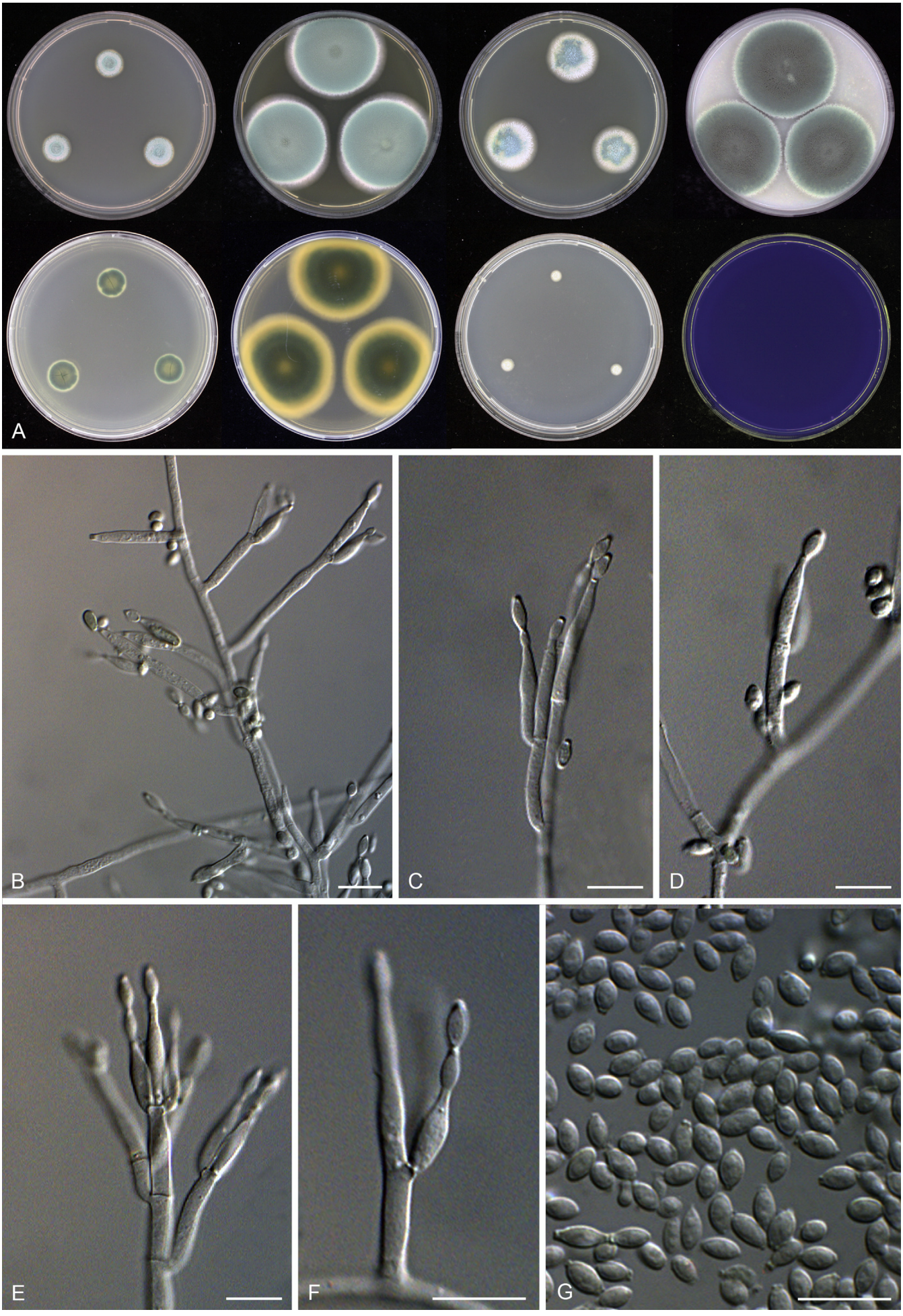

Fig. 11. Morphological characters of *Talaromyces diversiformis*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. Conidiophores; G. Conidia. Scale bars = 10 µm.

Etymology. Latin, *cerinus* refers to its yellow mycelium on MEA.

Diagnosis: This species produces yellow mycelium on MEA and orange centred reverse on CYA, does not grow on CYA at 37 °C.

In: *Talaromyces* section *Islandici*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun (holotype CBS H-22513, culture ex-type CBS 140622 = CGMCC3.18212 = DTO 318-A2).

ITS barcode: KU866658. (*Alternative markers*: *BenA* = KU866845; *CaM* = KU866742; *RPB2* = KU867002).

Colony diam, 7 d (mm): CYA 17–18; CYA 30 °C 20–21; CYA 37 °C No growth; MEA 19–20; MEA 30 °C 25–26; DG18 12–13; CYAS 10–11; OA 17–19; CREA 2–3; YES 19–21.

Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white to buff; texture floccose; sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse orange at centre, saffron at edge. MEA, 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; mycelium yellow at centre; texture floccose; sporulation moderately dense, conidia *en masse* yellow green; soluble pigments absent; exudates clear droplets; reverse saffron. YES, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse orange at centre, cream white at edge. DG18, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white to buff; texture velvety; sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse cream white. OA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white and light yellow; texture floccose; sporulation sparse, conidia *en masse* yellow green; soluble pigments absent; exudates clear droplets; reverse cream white. CREA, 25 °C, 7 d: Acid production absent.

Micromorphology: Conidiophores biverticillate, stipes smooth, 50–100 × 2.5–4 µm; metulae 3–5, 8–11 × 2.5–4 µm; phialides 3–7, acerose, 9–11(–14) × 2–3 µm; conidia smooth, ellipsoidal to fusiform, 2.5–4 × 2–3 µm. Ascomata not observed.

Extrolites: Emodin, mitorubrin, mitorubrinol, rugulosin, rugulovasine A, skyrin.

Distinguishing characters: *Talaromyces cerinus* resembles *T. subaurantiacus* and *T. chlamydosporus*, but *T. subaurantiacus* produces orange mycelium on CYA and MEA, and *T. chlamydosporus* produces globose to subglobose swollen cells resembling chlamydospores. In addition, *T. cerinus* does not grow on CYA at 37 °C.

Talaromyces chlamydosporus A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817392. Fig. 10.

Etymology: Latin, *chlamydosporus* refers to the globose to subglobose swollen cells resembling chlamydospores.

Diagnosis: This species grows restrictedly on CYA and MEA, reaches 3–4 mm on CYA at 37 °C after 7 days, and produces globose to subglobose swollen cells resembling chlamydospores.

In: *Talaromyces* section *Islandici*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun (holotype CBS H-22509, culture ex-type CBS 140635 = CGMCC 3.18199 = DTO 317-D5).

ITS barcode: KU866648. (*Alternative markers*: *BenA* = KU866836; *CaM* = KU866732; *RPB2* = KU866992).

Colony diam, 7 d (mm): CYA 12–13; CYA 30 °C 12–13; CYA 37 °C 3–4; MEA 18–19; MEA 30 °C 17–18; DG18 9–11; CYAS 9–10; OA 15–16; CREA 3–4; YES 16–17.

Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, raised at centre, plane; margins entire; mycelium buff; texture velvety; sporulation moderately dense, conidia *en masse* olive green to greyish green; soluble pigments absent; exudates absent; reverse orange at centre, yellowish brown at edge. MEA, 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins slightly irregular; mycelium white; texture floccose; sporulation moderately dense, conidia *en masse* blue green; soluble pigments absent; exudates clear droplets; reverse yellowish brown. YES, 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse yellowish brown. DG18, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium light yellow; texture velvety; sporulation moderately dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse yellowish brown. OA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* yellow green; soluble pigments absent; exudates absent; reverse yellowish brown. CREA, 25 °C, 7 d: Acid production absent.

Micromorphology: Conidiophores biverticillate; stipes smooth, 50–250 × 2.5–4 µm; metulae 3–5, appressed, sometimes irregularly swollen, 9–14 × 2–4(–6) µm; phialides 3–5, acerose, 9–14 × 2–3 µm; conidia smooth, ellipsoidal to fusiform, 2.5–4 × 1.5–3 µm. Swollen cells resembling chlamydospores globose to subglobose, 5–7 µm; Ascomata not observed.

Extrolites: Mitorubrin, mitorubrinol, mitorubrinol acetate, rugulosin, rugulovasin A & B, skyrin.

Distinguishing characters: *Talaromyces chlamydosporus* grows restrictedly and produce compact colonies on CYA and MEA, and can produce globose to subglobose swollen cells. These characters distinguish it from the closely related *T. subaurantiacus* and *T. cerinus*.

Talaromyces diversiformis A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB818696. Fig. 11.

Etymology: Latin, *diversiformis* refers to its diverse conidiophore branches.

Diagnosis: This species produces solitary phialides, biverticillate conidiophores, which have in some cases extra subterminal

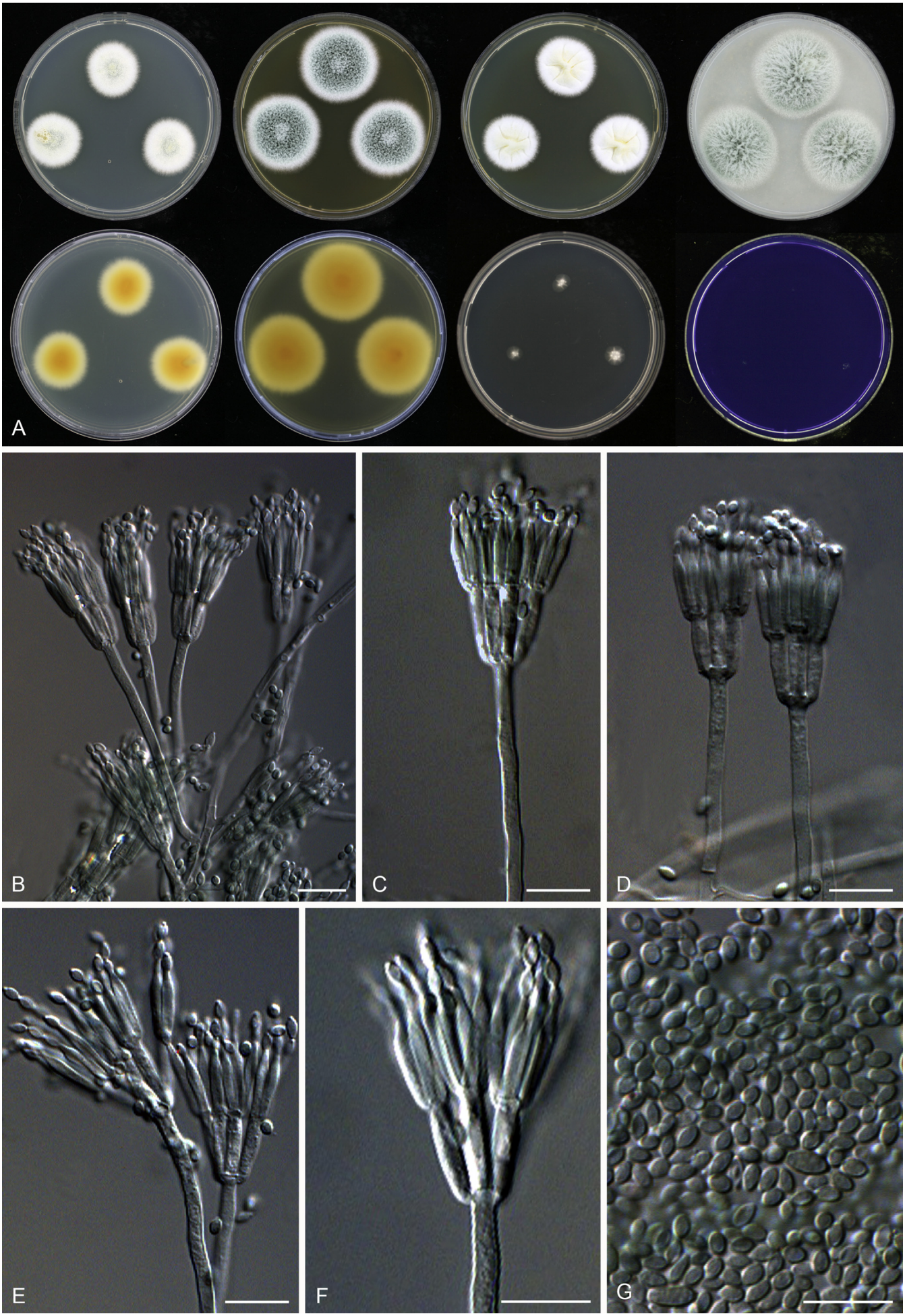

Fig. 12. Morphological characters of *Talaromyces fusiformis*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. Conidiophores; G. Conidia. Scale bars = 10 µm.

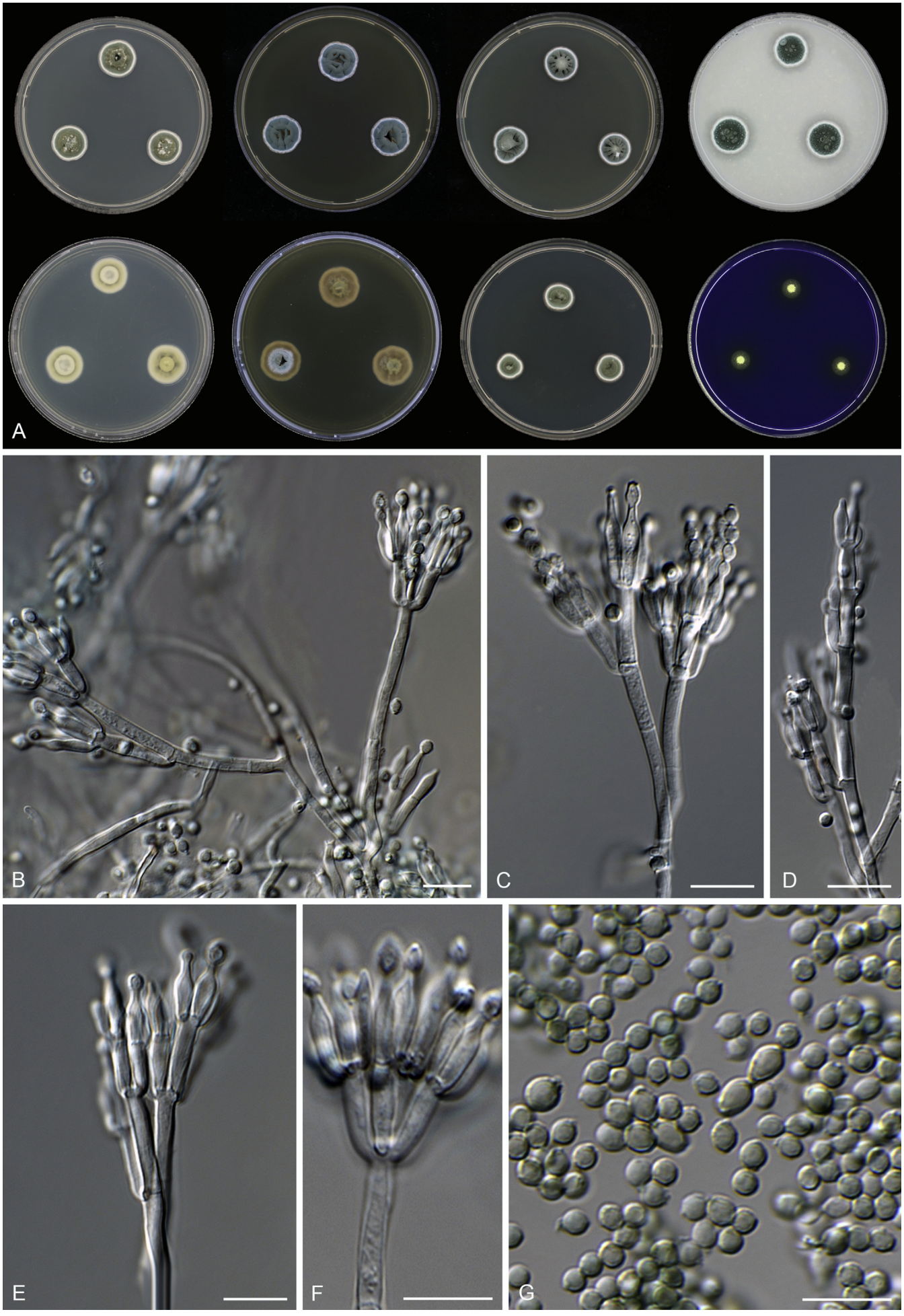

Fig. 13. Morphological characters of *Talaromyces neorugulosus*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. Conidiophores; G. Conidia. Scale bars = 10 µm.

branches. This species furthermore produces large, ellipsoidal to fusiform conidia measuring 4–6(–8) × 2–4 µm.

In: *Talaromyces* section *Helici*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun (holotype CBS H-22841, culture ex-type CBS 141931 = CGMCC 3.18204 = DTO 317-E3).

ITS barcode: KX961215. (*Alternative markers*: *BenA* = KX961216; *CaM* = KX961259; *RPB2* = KX961274).

Colony diam, 7 d (mm): CYA 13–14; CYA 30 °C 14–16; CYA 37 °C 17–19; MEA 45–48; MEA 30 °C 56–57; DG18 5–6; CYAS No growth; OA 52–53; CREA No growth; YES 22–24.

Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation moderately dense, conidia *en masse* blue green; soluble pigments absent; exudates absent; reverse yellowish brown fading into greyish green. MEA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse dark olive green fading into yellowish brown. YES, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture funiculose; moderately dense, conidia *en masse* blue green, soluble pigments absent; exudates absent; reverse yellowish green at centre, cream white at edge. DG18, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse cream white. OA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* dark green; soluble pigments absent; exudates absent; reverse dark greenish glaucous. CREA, 25 °C, 7 d: No growth.

Micromorphology: Conidiophores with solitary phialides, or biverticillate, or with extra subterminal branches; stipes smooth, 13–70 × 2.5–4 µm; metulae 2–3, 16–18 × 3–4 µm; phialides 1–3, flask shaped to acerose, (8–)16–18(–23) × 3–4.5 µm; conidia ellipsoidal to fusiform, smooth, 4–6(–8) × 2–4 µm. Ascomata not observed.

Extrolites: no extrolites detected.

Distinguishing characters: *Talaromyces diversiformis* is phylogenetically closely related to *T. aerugineus* and *T. bohemicus*. This species has, compared with *T. aerugineus*, more complex branched conidiophores. *T. bohemicus* produces light brown mycelium which turns to cinnamon brown (Fassatiová & Pêčková 1990).

Talaromyces fusiformis A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817396. Fig. 12.

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

Diagnosis: This species produces funiculose colonies on OA, does not grow on CREA, produces smooth, ellipsoidal to fusiform conidia measuring 3–4(–6) × 2–3 µm.

In: *Talaromyces* section *Talaromyces*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun (holotype CBS H-22510, culture ex-type CBS 140637 = CGMCC3. 18210 = DTO 317-F4).

ITS barcode: KU866656. (*Alternative markers*: *BenA* = KU866843; *CaM* = KU866740; *RPB2* = KU867000).

Colony diam, 7 d (mm): CYA 28–29; CYA 30 °C 26–28; CYA 37 °C 24–25; MEA 37–38; MEA 30 °C 49–51; DG18 8–9; CYAS No growth; OA 39–40; CREA No growth; YES 30–31.

Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium light yellow at centre; texture floccose; sporulation sparse, conidia *en masse* yellow green; soluble pigments absent; exudates light yellow droplets; reverse orange at centre, yellowish brown at edge. MEA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture loosely funiculose; sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates clear droplets; reverse orange at centre, yellowish brown at edge. YES, 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; mycelium light yellow at centre, white at edge; texture floccose; sporulation absent to sparse, conidia *en masse* pale green; soluble pigments absent; exudates light yellow droplets; reverse yellowish brown. DG18, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation sparse, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse white. OA, 25 °C, 7 d: Colonies deep, plane; margins entire; mycelium white, texture funiculose, sporulation dense, conidia *en masse* greyish green; soluble pigments absent; exudates clear droplets; reverse yellowish green. CREA, 25 °C, 7 d: No growth.

Micromorphology: Conidiophores biverticillate, with symmetrical subterminal branches; stipes smooth, 42–70 × 2.5–4 µm, extra branches 28–37 µm; metulae 3–4, appressed, 12–15 × 2.5–4 µm; phialides 3–4, acerose, 11–15 × 2–3 µm; conidia smooth, ellipsoidal to fusiform, 3–4(–6) × 2–3 µm. Ascomata not observed.

Extrolites: A purpactin, secalonic acid D, a chrodrimanin = thailandolide.

Distinguishing characters: *Talaromyces fusiformis* is close to *T. aurantiacus* and *T. funiculosus*, but *T. aurantiacus* does not sporulate on CYA, MEA and YES and produces cylindrical to ellipsoidal conidia. *T. funiculosus* produces strong acid on CREA.

Talaromyces neorugulosus A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817394. Fig. 13.

Etymology: Latin, *neorugulosus* refers to its resemblance with *T. rugulosus*.

Diagnosis: This species produces compact, velvety, olive green to dark green colony, produces globose, subglobose to ellipsoidal conidia measuring 3–4(–5) × 2–3(–4) µm, phylogenetically distinct from *T. rugulosus*.

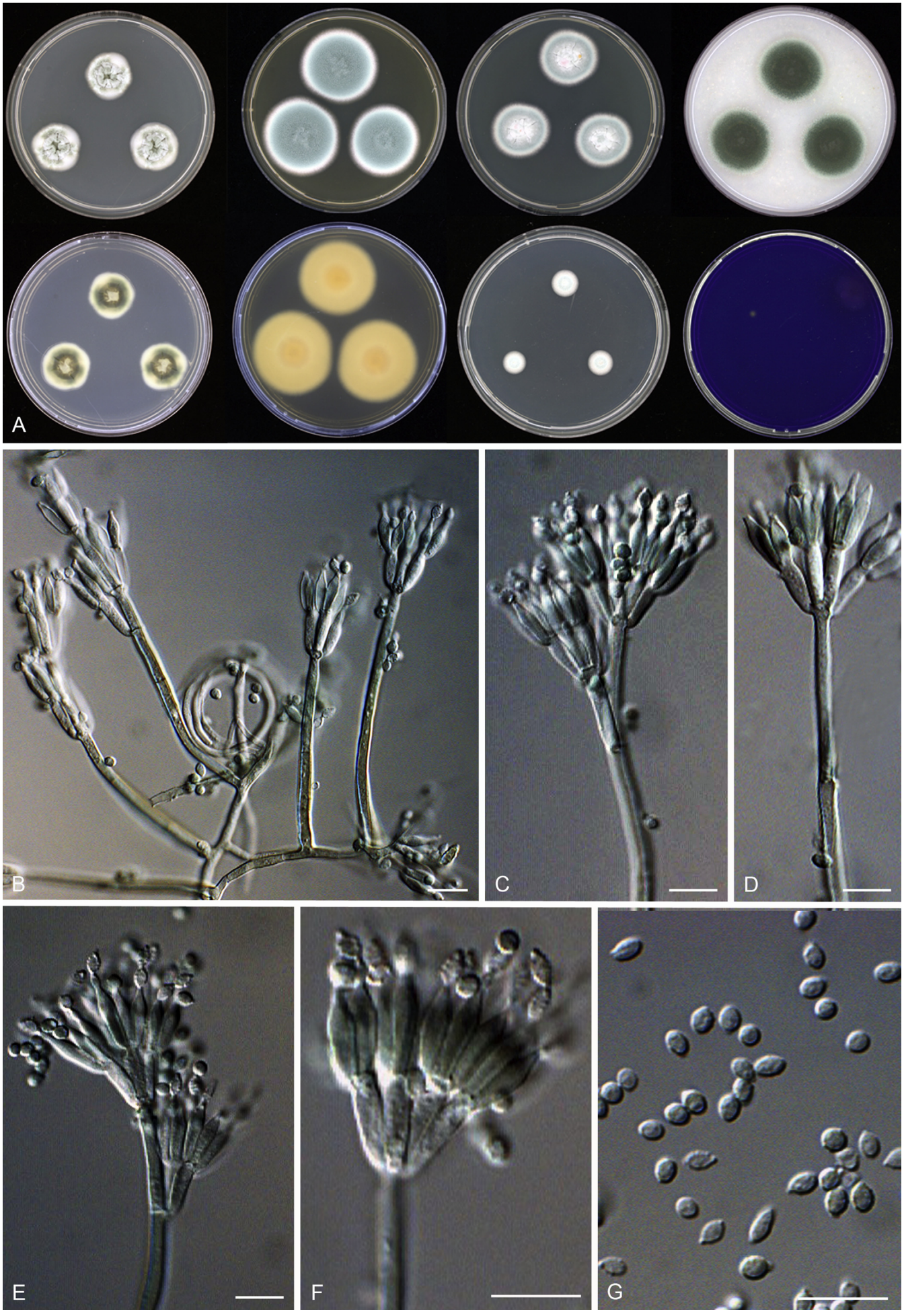

Fig. 14. Morphological characters of *Talaromyces reverso-olivaceus*. A. Colonies from left to right (top row) CYA, MEA, YES and OA; (bottom row) CYA reverse, MEA reverse, DG 18 and CREA; B–F. Conidiophores; G. Conidia. Scale bars = 10 µm.

In: *Talaromyces* section *Islandici*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun (holotype CBS H-22511, culture ex-type CBS 140623 = CGMCC3.18215 = DTO 318-A8).

ITS barcode: KU866659. (*Alternative markers*: *BenA* = KU866846; *CaM* = KU866743; *RPB2* = KU867003).

Colony diam, 7 d (mm): CYA 17–18; CYA 30 °C 20–21; CYA 37 °C No growth; MEA 19–20; MEA 30 °C 25–26; DG18 12–13; CYAS 10–11; OA 17–19; CREA 2–3; YES 19–21.

Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; mycelium light yellow; texture velvety; sporulation moderately dense, conidia *en masse* olive green; soluble pigments absent; exudates absent; reverse buff. MEA, 25 °C, 7 d: Colonies moderately deep, sulcate, crateriform, sunken at centre; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* dark green; soluble pigments absent; exudates clear droplets; reverse cinnamon. YES, 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* greyish green to olive green; soluble pigments absent; exudates absent; reverse olive green to olive buff. DG18, 25 °C, 7 d: Colonies moderately deep, sunken at centre and crateriform; margins entire; mycelium light yellow; texture velvety; sporulation dense, conidia *en masse* greyish green to olive green; soluble pigments absent; exudates absent; reverse olive buff. OA, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* dark green; soluble pigments absent; exudates clear droplets; reverse cream white. CREA, 25 °C, 7 d: Acid production present.

Micromorphology: Conidiophores biverticillate, sometimes with additional branches; stipes smooth, 15–100 × 2–4 µm, extra branches 14–30 µm; metulae 3–5, divergent, 8–14 × 3–4 µm; phialides 2–5, flask shaped, 7–12 × 2–4 µm; conidia smooth, globose, subglobose to ellipsoidal, 3–4(–5) × 2–3(–4) µm. Ascomata not observed.

Extrolites: Ukulactones = pruginosins.

Distinguishing characters: *Talaromyces neorugulosus* is close to *T. rugulosus*, *T. atricola* and *T. scorteus*. However, *T. scorteus* grows more restrictedly on CYA, MEA, YES and OA, *T. atricola* is characterised by floccose colonies and poor sporulation. Morphologically, *T. neorugulosus* resembles *T. rugulosus* and only small differences were found of the colony colour: *T. rugulosus* produces dull to dark green colonies on CYA, YES and DG18 and *T. neorugulosus* in shades of olive green. Phylogenetically, these two species can be distinguished by *BenA*, *CaM* and *RPB2* sequences.

Talaromyces reverso-olivaceus A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817391. Fig. 14.

Etymology: Latin, *reverso-olivaceus* refers to the olive centred reverse.

Diagnosis: This species produces olive green centred reverse on CYA and saffron reverse on MEA, produces ellipsoidal to fusiform, finely roughed conidia measuring 2.5–4.5 × 2.5–3 µm.

In: *Talaromyces* section *Helici*

Typus: **China**, Beijing, indoor air, May 2014, isolated by B.D. Sun (holotype CBS H-22512, culture ex-type CBS 140672 = CGMCC 3.18195 = DTO 317-C3).

ITS barcode: KU866646. (*Alternative markers*: *BenA* = KU866834; *CaM* = KU866730; *RPB2* = KU866990).

Colony diam, 7 d (mm): CYA 19–23; CYA 30 °C 23–27; CYA 37 °C 18–20; MEA 34–37; MEA 30 °C 46–49; DG18 9–12; CYAS 4–6; OA 33–36; CREA No growth; YES 25–26.

Colony characters: CYA, 25 °C, 7 d: Colonies moderately deep, sulcate, raised at centre; margins entire; mycelium white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse olive green at centre fading into white. MEA, 25 °C, 7 d: Colonies moderately deep, plane to light sulcate; margins entire; mycelium white; texture velvety to floccose; sporulation dense, conidia *en masse* blue green; soluble pigments absent; exudates absent; reverse saffron. YES, 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* blue green to greyish green; soluble pigments absent; exudates absent; reverse saffron. DG18, 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation absent to sparse, conidia *en masse* greyish green; soluble pigments absent; exudates absent; reverse dark green at centre fading into white. OA, 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* dark green; soluble pigments absent; exudates absent; reverse light buff. CREA, 25 °C, 7 d: No growth.

Micromorphology: Conidiophores biverticillate, sometimes with extra subterminal branches; stipes smooth, 50–100 × 2.5–4 µm; branches 12–15 × 2–3 µm; metulae 3–5, 10–13 × 3–4 µm; phialides 3–5, acerose, 10–12(–14) × 2.5–3 µm; conidia ellipsoidal to fusiform, finely roughed, 2.5–4.5 × 2.5–3 µm. Ascomata not observed.

Extrolites: rugulovasine A.

Distinguishing characters: Phylogenetically, *Talaromyces reverso-olivaceus* clusters in section *Helici*, related to *T. boninensis* and *T. helicus*. *Talaromyces boninensis* sporulates poorly on CYA and MEA, has a light orange reverse on CYA and white to orange reverse on MEA, and produces globose to subglobose ascospores. *Talaromyces helicus* produces greyish red to yellowish brown reverse on CYA, brownish orange reverse on MEA, and produces ellipsoidal ascospores. In addition, *T. reverso-olivaceus* produces finely roughed conidia, while *T. boninensis* and *T. helicus* produce smooth-walled conidia.

DISCUSSION

The genus *Talaromyces* was recently monographed (Yilmaz *et al.* 2014), accepting seven sections and 88 species. This study promoted the taxonomy of this genus, and since more than 17 new species were described (Visagie *et al.* 2015, Yilmaz *et al.*

2016a, b, Luo *et al.* 2016, Romero *et al.* 2016, Wang *et al.* 2016). In this study, *Talaromyces* isolates obtained from indoor air in China were studied. These isolates can be classified in four sections, and nine species are described here as new based on a polyphasic approach.

Talaromyces section *Helici* includes two clades (Yilmaz *et al.* 2014), and two of our new species fall into these two distinct clades. *Talaromyces reverso-olivaceus* clusters in the main clade containing *T. helicus*, *T. boninensis* and *T. varians*, and these species share the production of pigmented conidiophores. The other new species, *T. diversiformis*, clusters with the monoverticillate species *T. aerugineus* and *T. bohemicus*. This branching complexity of *T. diversiformis* is variable and both monoverticillate and biverticillate (occasionally with subterminal branches) conidiophores are observed.

The majority of species belonging to *Talaromyces* section *Islandici* grow restrictedly on most media, produce yellow mycelium and characteristic mycotoxins (Yilmaz *et al.* 2014, 2016b). The three new *Islandici* species grow restrictedly and produce yellow mycelium on DG18, confirming their relationship with other member of this section. Furthermore, *T. neorugulosus* produces pruginosins, and *T. chlamydosporus* and *T. cerinus* produce emodin, mitorubrin, mitorubrinol, rugulosin, skyrin and rugulovasine A. These extrolites are commonly shared by members of section *Islandici*. Besides shared characteristics, these species can also be distinguished based on morphological and physiological characters. *Talaromyces chlamydosporus* produces globose to subglobose swollen cells resembling chlamydospores and *T. cerinus* does not grow on CYA incubated at 37 °C, in contrast to the closely related species *T. chlamydosporus* and *T. subaurantiacus*. *Talaromyces neorugulosus* is morphologically similar to *T. rugulosus*. These species can be distinguished on their conidial colour on CYA and DG18; however, *BenA*, *CaM* or *RPB2* sequencing is recommended for accurate identification.

Talaromyces section *Talaromyces* was initially introduced for species producing yellow, white, creamish, pinkish or reddish ascomata and yellow ascospores (Stolk & Samson 1972), and this section currently contains both asexual and sexual species (Samson *et al.* 2011, Yilmaz *et al.* 2012, Manoch *et al.* 2013, Sang *et al.* 2013, Yilmaz *et al.* 2014, Visagie *et al.* 2014, Wang *et al.* 2016). Morphologically, the two new *Talaromyces* species proposed in this section (*T. beijingensis* and *T. fusiformis*) can be distinguished from related species by mycelial colour, conidial shape and ornamentation (see notes in Taxonomy section).

Talaromyces aerius resembles other species of section *Trachyspermi* by restricted growth on CYA, YES and DG18, a slightly faster growth rate on MEA, and poor growth on CREA. *Talaromyces aerius* differs from the phylogenetically related species *T. solicola* by its conidial ornamentation (smooth-walled in *T. aerius vs*. rough-walled in *T. solicola*). The two recently described *Trachyspermi* members, *T. systylus* and *T. rubrifaciens*, were included in the phylogenetic analyses. *Talaromyces systylus* is well-separated from its sister species *T. trachyspermus* and *T. assiutensis*, while all of *T. rubrifaciens* strains cluster together with *T. albobiverticillius*. The *BenA* gene, which is recommended as the identification marker in *Talaromyces* (Yilmaz *et al.* 2014), is identical for these two species. *Talaromyces albobiverticillius* has a large intraspecies sequence variation (Frisvad *et al.* 2013). For the description of *T. rubrifaciens*, Luo *et al.* (2016) included a limited number of *T. albobiverticillius* sequences in their phylogenetic analyses. This selection did not fully represent the sequence diversity within this species. The noticeable features like the formation of restricted colonies on MEA and CYA, soluble red pigment production on YES and MEA and green coloured conidia are common in *T. albobiverticillius* (Frisvad *et al.* 2013, Yilmaz *et al.* 2014). Other reported characters to distinguish *T. rubrifaciens* from *T. albobiverticillius* are the number of metulae (9–15) and phialides (6–10). These characters were, however, not depicted in the original figures (Luo *et al.* 2016). Based on molecular and morphological characters, we consider *T. rubrifaciens* a synonym of *T. albobiverticillius*. Interestingly, four of our indoor isolates were also identified as *T. albobiverticillius*. Visagie *et al.* (2014) reported this species in house dust from Thailand and South Africa, and all of these results indicate a widespread occurrence of this species in indoor environments.

The research on airborne fungi started in China in 1957, when Wu *et al.* (1982) compared the outdoor fungal concentration from 1957 to 1982 in Beijing, China. The predominant genera found were *Cladosporium*, *Aspergillus*, *Alternaria* and *Penicillium*. Later, several investigations were conducted on airborne fungi in different cities and seasons. In most studies only the fungal propagules were quantified and if identification was performed, then it was based on morphology (Wu *et al.* 2000, Li *et al.* 2006, Si *et al.* 2007, Liu *et al.* 2014). Fang *et al.* (2005) analysed the culturable airborne fungi in outdoor environments in Beijing, China. *Talaromyces funiculosus* (= *Penicillium funiculosum*), *T. pinophilus* (= *P. pinophilum*), *T. ruber* (= *P. rubrum*), *T. wortmannii* (= *P. variabile*), and *T. flavus* were identified using the Biolog Microstation System (Biolog, Hayward, CA). Li *et al.* (2006) analysed the indoor and outdoor *Penicillium* population in Nanchang city, Jiangxi province, and using morphological identification, *T. islandicus* (= *P. islandicum*) was found to be one of the predominant species. During a study on indoor fungi in Beijing, Fang *et al.* (2013) identified their isolates on genus level using ITS sequences, and three of them belong to *Talaromyces*. In Flora Fungorum Sinicorum v35 *Penicillium* et teleomorphi cognati, Kong (2007) described the most complete records of *Penicillium* and its teleomorphs in China. *Talaromyces funiculosus* (= *Penicillium funiculosum*), *T. verruculosus* (= *Penicillium verruculosum*) and *T. flavus* were recorded from air.

Talaromyces contains several important etiologic agents. *Talaromyces marneffei*, the only known dimorphic species in *Talaromyces*, has been considered to be exclusively associated with acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) infections (Supparatpinyo *et al.* 1994). Nowadays the epidemiology of *T. marneffei* infection has changed significantly with the improved treatment of HIV, and an increasing number and proportion of cases have been reported in non-HIV-infected patients, who had other immunocompromising conditions (Tang *et al.* 2010, Lee *et al.* 2012, Lee *et al.* 2014, Chan *et al.* 2016). In our survey of indoor fungi in China we did not detect *T. marneffei*.

Other medically important *Talaromyces* species including *T. indigoticus*, *T. piceus*, *T. indigoticus*, *T. helicus*, *T. rugulosus*, *T. purpurogenus*, *T. radicus* and *T. verruculosus* have been reported in superficial or disseminated, fatal infections (Neuhann 1976, Swietliczkowa *et al.* 1984, de Hoog *et al.* 2000, Horré *et al.* 2001, Santos *et al.* 2006, de Vos *et al.* 2009, Weisenborn *et al.* 2010, Tomlinson *et al.* 2011). Among the nine new species described here, *T. adpressus*, *T. beijingensis*,

T. diversiformis, *T. fusiformis* and *T. reverso-olivaceus* grow well at 37 °C, thus are more risky for human health.

In our study, 13 species were identified including *T. islandicus*, *T. aurantiacus*, *T. siamensis*, *T. albobiverticillius* and nine new species. The main focus of our study was to describe the indoor *Talaromyces* diversity in houses in Beijing, China, and further research is needed to study the ecology of these species. The "old" *Penicillium* and *Talaromyces* concepts and morphological identification are still used in China nowadays, and it is expected that with the broad application of molecular diagnostics, the number of indoor *Talaromyces* species in China will increase.

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APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.simyco.2016.11.003.

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