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Cephalotrichum and related synnematous fungi with notes on species from the built environment

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Abstract: A recent taxonomic revision of *Microascaceae* with an emphasis on synnematous fungi enabled re-identification of previously isolated indoor strains of *Cephalotrichum*. All available *Cephalotrichum* strains from the culture collection of the Westerdijk Institute were studied, 20 originating from the built environment. Phylogenetic relationships were inferred from DNA sequence data from the internal transcribed spacer 1 and 2 and intervening 5.8S nrDNA (ITS), and parts of β-tubulin (*tub2*) and translation elongation factor 1-α (*tef1*) genes. Additionally, herbarium material of 14 *Cephalotrichum* species described from soil in China was studied, and the taxonomy of *C. album*, not considered in recent revisions, was reevaluated. Sixteen phylogenetic species in *Cephalotrichum* are distinguished, five described as new species: *C. domesticum*, *C. lignatile*, *C. telluricum*, *C. tenuissimum* and *C. transvaalense*. Five *Cephalotrichum* species occur in the built environment: *C. domesticum*, *C. gorgonifer* (formerly known as *Trichurus spiralis*), *C. microsporum*, *C. purpureofuscum*, and *C. verrucisporum*. Based on the number of isolates, *C. gorgonifer* (nine strains) is the most common indoor species. The study of the Chinese herbarium material resulted in the acceptance of three additional *Cephalotrichum* species: *C. casteneum*, *C. ellipsoideum*, and *C. spirale*. Four species are considered nomena dubia (*C. cylindrosporum*, *C. macrosporum*, *C. ovoideum*, and *C. robustum*), five are placed in synonymy with other *Cephalotrichum* species (*C. acutisporum*, *C. inflatum*, *C. longicollum*, *C. oblongum*, *C. terricola*) and one species, *C. verrucipes*, is probably a synonym of *Penicillium clavigerum*. *Cephalotrichum columnare*, former *Doratomyces columnaris*, is transferred to *Kernia*. *Cephalotrichum album*, formerly known as *Doratomyces putredinis*, is transferred to *Acaulium* and redescribed.

Key words: *Doratomyces*, Herbarium, *Microascaceae*, *Microascales*, *Sordariomycetes*, Synnematous hyphomycetes.
Taxonomic novelties: New combination: *Acaulium album* (Costantin) Seifert & Woudenb., *Kernia columnaris* (H.J. Swart) Woudenb. & Samson; **New species:** *Cephalotrichum domesticum* Woudenb. & Seifert, *C. lignatile* Woudenb. & Seifert, *C. telluricum* Woudenb. & Seifert, *C. tenuissimum* Woudenb. & Seifert, *C. transvaalense* Woudenb. & Seifert; **Typification: Epitypification (Basionyms):** *Synpenicillium album* Costantin.

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INTRODUCTION

The genus *Cephalotrichum* is characterised by the formation of dry-spored, indeterminate synnemata and enteroblastic percurrent conidiogenesis. No sexual morph is known. It was first described by Link (1809), for two species, *C. rigescens* and *C. stemonitis*. Hughes (1958) chose *C. stemonitis* as lectotype, anchoring the modern generic concept of *Cephalotrichum*. Later, *Doratomyces* was described with *D. neesii* as its type (Corda 1829, considered a synonym of *C. stemonitis* by Hughes 1958) and later, *Stysanus* with *S. stemonitis* as its type (Corda 1837). Consideration of the type or lectotype species of these three genera, *Cephalotrichum*, *Doratomyces* and *Stysanus*, leads to the conclusion that all are typified by the fungus originally described as *Isaria stemonitis* (Abbott 2000). A later genus, *Trichurus*, with *T. cylindricus* as its type, was distinguished by the presence of sterile setae on the synnemata (Clements & Pound 1896). In the unpublished Abbott (2000) thesis on holomorph studies in the *Microascaceae*, the synonymies of the three genera *Doratomyces*, *Stysanus* and *Trichurus* under *Cephalotrichum* were proposed, conclusions followed by Seifert *et al.* (2011). These synonymies were later confirmed based on analyses of the LSU and ITS rDNA subunits (Sandoval-Denis *et al.* 2016a, b). Within *Cephalotrichum* Sandoval-Denis *et al.* (2016b) described two new species, proposed five new combinations, and designated one neotype specimen, two lectotypes and four epitypes for accepted species. Although this provides a more stable taxonomy for synnematous *Microascaceae*, the papers also highlighted a large number of taxa that could not be studied because of the absence of living cultures. Their list of uncertain or excluded species included 43 *Cephalotrichum* spp., and seven *Doratomyces* spp. These included 14 new *Cephalotrichum* species described recently from China, mostly based on morphology characters alone (Jiang & Zhang 2008, Jiang *et al.* 2011). We were fortunate to obtain herbarium material of these latter species for study, allowing us to evaluate them in the broader context of the *Cephalotrichum* taxonomy established by Sandoval-Denis *et al.* (2016b).

Most *Cephalotrichum* species occur on decaying plant material, straw, dung, wood and in soil (Domsch *et al.* 2007). They are infrequently reported from the indoor or built environment. *Cephalotrichum microsporum* (previously known as *Doratomyces microsporus*) is the species most often reported from the indoor environment (Prezant *et al.* 2008, Samson *et al.* 2010, Flannigan *et al.* 2011), where it is mentioned as occurring especially on wet cellulose-containing substrates like wood. *Cephalotrichum purpureofuscum* has also been reported from indoor air (Abbott 2000, Sandoval-Denis *et al.* 2016b) as has *C. gorgonifer* (Abbott 2000, as *C. spirale*). *Cephalotrichum* species are not regarded as human pathogens, and not known as producers of

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mycotoxins. Strains have been isolated from clinical origins, mostly human respiratory systems, but are considered passive colonisers or sample contaminants rather than active pathogens (Sandoval-Denis *et al.* 2016b). *Cephalotrichum gorgonifer*, for example, has been isolated from human clinical samples and can grow at human body temperatures (Sandoval-Denis *et al.* 2016b). However such reports are scarce and clinical data is lacking. Given the amount of time we spend indoors, it is important to understand which microorganisms are co-habitants of this environment and what their potential implications may be to human health and to the design of the built environment. For that reason, we re-evaluated the identification of newly isolated strains from house dust and other indoor substrates, and other strains from the built environment in our collections.

The aim of our project was to construct an updated phylogenetic overview of the genus, taking into account the availability of the previously unavailable species described from China, and the strains from the built environment. Cultures and specimens were also examined of an anomalous coprophilous white species, included by Morton & Smith (1963) as *Doratomyces putredinis* then later renamed as *Cephalotrichum album* (De Beer *et al.* 2013), allowing us to complete the phylogenetic analysis of the classical species of this complex that are available in pure culture.

MATERIALS AND METHODS

Isolates and herbarium specimens

Seventy-two strains belonging to the genera *Acaulium*, *Cephalotrichum*, *Graphium*, *Kernia* and *Wardomyces* were included in this study (Table 1). They were obtained from the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, the Netherlands and the working collection of the Applied and Industrial Mycology department (DTO) at the Westerdijk Institute. Strains were grown on oatmeal agar (OA) (Samson *et al.* 2010).

Portions of fourteen holotype herbarium specimens, originally accessioned in the Plant Pathology Herbarium of the Shandong Agricultural University, China (HSAUP) were recently donated to the herbarium of the Westerdijk Institute (CBS-H) and were re-examined as part of this study (Table 2). For the holotypes of these species, we have indicated the original accession numbers for holotypes from the protologue, and consider the portions deposited in CBS-H to be isotypes, for which new accession numbers are published here with the following form: "**holotype** HSAUP xxxxx → **isotype** CBS-H yyyyy." Additional isotype were listed in the protologues in HMAS; we have not examined these, but include the accession numbers as listed by the authors.

DNA sequences from six strains maintained at the UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada were obtained from GenBank (Table 1).

DNA isolation, PCR and sequencing

DNA extractions were performed using the Ultraclean® Microbial DNA Isolation Kit (MoBio laboratories, Carlsbad, CA, USA), following manufacturer's instructions. The internal transcribed spacer 1 and 2 and intervening 5.8S nrDNA (ITS), and parts of the β-tubulin (*tub2*) and translation elongation factor 1-α (*tef1*) genes were amplified and sequenced as described in Woudenberg *et al.* (2017). Consensus sequences were assembled from forward and reverse sequences using Bionumerics v. 4.61 (Applied Maths, St-Martens-Latem, Belgium). All sequences generated were deposited in GenBank (Table 1).

Alignments and phylogenetic analyses

Individual sequence alignments of the ITS, *tub2* and *tef1* datasets were generated with MAFFT v. 7.271 (http://mafft.cbrc.jp/alignment/server/index.html) using the L-INS-i method. The best nucleotide substitution models were determined with Findmodel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). For both the single gene sequence alignments and the concatenated alignment, Bayesian and Maximum-likelihood analyses were performed as described in Woudenberg *et al.* (2017). An additional phylogenetic tree was constructed based on the ITS sequences of a broader selection of isolates representing all species recognized by Sandoval-Denis *et al.* (2016b) and in this study, together with ITS sequences from the Chinese herbarium specimens available in GenBank (Table 2). To demonstrate the placement of two species initially classified in *Cephalotrichum* outside the genus, an alignment and phylogenetic tree based on the ITS and LSU sequences of representative strains of the genera *Acaulium*, *Cephalotrichum*, *Kernia* and *Graphium* was assembled based on the sampling of Sandoval-Denis *et al.* (2016a). The resulting trees were printed with TreeView v. 1.6.6 (Page 1996) and, together with the alignments, deposited in TreeBASE (http://www.treebase.org).

Morphology

Cultures were incubated on oatmeal agar (OA, which favours synnema development), malt extract agar (MEA) and dichloran 18 % glycerol agar (DG18) plates (recipes from Samson *et al.* 2010) at 25 °C in the dark. After 14 d, growth rates were measured and colony characters noted. Colony colours were rated following the charts of Rayner (1970). Dried herbarium material was rehydrated in sterile water, which was then replaced by Shear's mounting media for photomicroscopy (Crous *et al.* 2009). Measurements and descriptions of microscopic structures were made from cultures grown on synthetic nutrient agar (SNA, Samson *et al.* 2010) at 25 °C in the dark for 14 d, mounted in 85 % lactic acid. Macroscopic photographs were made with a Nikon SMZ25 stereo microscope equipped with a Nikon DS-Ri2 high-definition colour camera head. Photomicrographs of diagnostic structures were made with a Zeiss Axio Imager A2 microscope equipped with a Nikon DS-Ri2 high-definition colour camera head, using differential interference contrast (DIC) optics and the Nikon software NIS-elements D v. 4.50.

RESULTS

Phylogeny

The concatenated, multi-gene *Cephalotrichum* phylogeny alignment included sequences of 62 strains and was 1 979 bp long, with the partitions being 566 characters for ITS (67 informative or unique), 884 for *tef1* (103) and 529 for *tub2* (227). The TrN model

Table 1. Isolates used in this study and their GenBank accession numbers. Bold accession numbers were generated in other studies.

Name	CBS Database	Strain number[1]	Substrate/host	Location	GenBank accession number			
					ITS	*tub2*	*tef1*	LSU
Acaulium acremonium		CBS 104.65^{ET}; ATCC 16282; DSM 1987; MUCL 8274	Wheat field soil	Germany	KY852468	**LN851109**	**LN851056**	KY852479
A. albonigrescens		CBS 109.69^{ET}; ATCC 18841; IHEM 18560	Litter, treated with urea	Japan	KY852469	**LN851111**	**LN851058**	KY852480
A. album comb. nov.	*Graphium putredinis*	CBS 378.64	Queen of bumble-bee	Denmark	KY852470			KY852481
	G. putredinis	CBS 212.73	Soil	Netherlands	KY852471			KY852482
	G. putredinis	CBS 257.82, ATCC 46569	Decaying *Coprinus micaceus*	Canada	KY852472			KY852483
	G. putredinis	CBS 539.85^{ET}	Hair in dung in pole cat	Netherlands	KY852473			KY852484
A. caviariforme		CBS 536.87^{T}; TRTC 50940	Decaying meat	Belgium	**LM652392**	**LN851112**	**LN851059**	**LN851005**
Cephalotrichum asperulum	*Doratomyces stemonitis*	CBS 127.22; DTO 170-B5; IMI 086947; LSHB Sc177; MUCL 4031	Seed	Netherlands	**LN850959**	**LN851113**	**LN851060**	
		CBS 215.49; DTO 334-G8; ATCC 11259	Unknown	Indonesia	KY249250	KY249291	KY249329	
	D. asperulus	CBS 582.71^{IT}; DTO 104-B7; ATCC 26885; LCP 73.2231	Soil	Argentina	**LN850960**	**LN851114**	**LN851061**	**KX924027**
C. brevistipitatum	*D. purpureofuscus*	CBS 157.57^{T}; DTO 334-H7; MUCL 4036	*Solanum tuberosum*	Netherlands	**LN850984**	**LN851138**	**LN851084**	
C. cylindricum	*Trichurus terrophilus*	CBS 646.70; DTO 335-A2	Soil	France	KY249251	KY249292	KY249330	
	T. terrophilus	CBS 587.77; DTO 335-A7	Soil	Turkey	KY249252	KY249293	KY249331	
		CBS 127136; DTO 335-C5; RMF 7618	Soil	USA	KY249253	KY249294	KY249332	
	T. cylindricus	UAMH 1348^{ET}	*Sorghum* seed	USA	**LN850965**	**LN851119**	**LN851066**	
C. dendrocephalum	*T. dendrocephalus*	CBS 528.85^{IT}; DTO 170-H4; MUCL 28855; NHL 2927	Cultivated soil	Iraq	**LN850966**	**LN851120**	**LN851067**	
C. domesticum sp. nov.	*D. purpureofuscus*	CBS 139.42; DTO 334-G6; IFO 7677; MUCL 4025	Manure	Netherlands	KY249277	KY249315	KY249357	
	D. purpureofuscus	CBS 255.50; DTO 334-G9; MUCL 4037	Mushroom compost	Netherlands	KY249278	KY249316	KY249358	
	D. purpureofuscus	CBS 395.67; DTO 336-C5	Indoor, plaster	Netherlands	KY249279	KY249317	KY249359	
		CBS 142035^{T}; DTO 077-D6	Indoor air, house	Netherlands	KY249280	KY249318	KY249360	
C. gorgonifer	*T. spiralis*	CBS 131.08; DTO 336-C2	Unknown	USA	**LN850974**	**LN851128**	KY249333	
	T. spiralis	CBS 104.15; DTO 338-G1; MUCL 9831	Unknown	UK	KY249254	KY249295	KY249334	
	T. terrophilus	CBS 368.53; DTO 334-H6	Treated wood	South Africa	**LN850976**	**LN851130**	**LN851076**	
	T. spiralis	CBS 496.62; DTO 338-G2; MUCL 9830	Compost ground domestic waste	Italy	KY249255	KY249296	KY249335	
	T. spiralis	CBS 877.68; DTO 334-I7; ATCC 16231	Wheat field soil	Germany	KY249256	KY249297	KY249336	
	T. spiralis	CBS 635.78^{ET}; DTO 170-G9	Human hair	Netherlands	**LN850977**	**LN851131**	**LN851077**	
	T. spiralis	CBS 120011; DTO 335-C2	Soil	South Africa	KY249257	KY249298	KY249337	
		CBS 124434; DTO 335-C3	Human foot	Denmark	KY249258	KY249299	KY249338	
	D. stemonitis	CBS 125064; DTO 335-C4	Mouldarray fungi	Denmark	KY249259	KY249300	KY249339	
		DTO 005-E7	Indoor	Germany	KY249260	KY249301	KY249340	
		DTO 054-I8	Indoor	Germany	KY249261	KY249302	KY249341	
		DTO 054-I9	Indoor	Germany	KY249262	KY249303	KY249342	
		DTO 055-C1	Indoor	Germany	KY249263	KY249304	KY249343	
		DTO 055-C2	Indoor	Germany	KY249264	KY249305	KY249344	
		DTO 055-D6	Indoor	Germany	KY249265	np	KY249345	
		DTO 090-A7	Indoor air, house	Netherlands	KY249266	KY249306	KY249346	
		DTO 164-D4	Indoor air, bakery	Netherlands	KY249267	KY249307	KY249347	

(*continued on next page*)

Table 1. (Continued).

Name	CBS Database	Strain number[1]	Substrate/host	Location	GenBank accession number			
					ITS	*tub2*	*tef1*	LSU
		DTO 240-B2	Indoor swab, archive	Netherlands	KY249268	KY249308	KY249348	
		UAMH 3585	Mushroom compost	Canada	**LN850978**	**LN851132**	**LN851078**	
C. hinnuleum	*D. stemonitis*	CBS 289.66^{T}; DTO 334-I1; IFO 8314	Dung of deer	Australia	**LN850985**	**LN851139**	**LN851085**	
C. lignatile sp. nov.	*D. microsporus*	CBS 209.63^{T}; DTO 170-D5	Timber in cave	Belgium	KY249269	KY249309	KY249349	
C. microsporum	*D. purpureofuscus*	CBS 523.63^{ET}; DTO 103-I7; ATCC 16224; IFO 31240; MUCL 4041	Wheat field soil	Germany	**LN850967**	np	**LN851068**	
	D. microsporus	CBS 132.68; DTO 055-I1	*Ligustrum vulgare*, dead twig	Netherlands	KY249270	KY249310	KY249350	
		DTO 152-C1	Indoor	Unknown	KY249271	KY249311	KY249351	
		DTO 152-D4	Indoor	Unknown	KY249272	np	KY249352	
		DTO 207-C6	Indoor	Germany	KY249273	KY249312	KY249353	
		UAMH 9365^{T}	Indoor air	Canada	**LN850968**	**LN851122**	**LN851069**	
C. nanum	*D. nanus*	CBS 188.60; DTO 103-H8; DTO 103-H9; MUCL 4042	Unknown	Italy	KY249274	KY249313	KY249354	
	D. nanus	CBS 191.61^{ET}; DTO 334-H8; IFO 8180; IFO 8184; IMI 068394; LSHB Sc14; LSHB Sc142; MUCL 4038	Dung of deer	UK	**LN850969**	**LN851123**	**LN851070**	
	D. nanus	CBS 882.68; DTO 104-B1; ATCC 16219; IFO 31239	Wheat field soil	Germany	KY249275	np	KY249355	
	D. nanus	CBS 139532; DTO 335-D3; WSF 5700	Forest soil	USA	KY249276	KY249314	KY249356	
		UAMH 9126	Dung of bison	Canada	**LN850970**	**LN851124**	**LN851071**	
C. purpureofuscum	*D. purpureofuscus*	CBS 174.68; DTO 055-I5	*Zea mays*, grain	Unknown	KY249281	KY249319	KY249361	
	D. stemonitis	CBS 116683; DTO 055-I3	Tunnelwall containing cellulose	Netherlands	KY249282	KY249320	KY249362	
		DTO 054-I1	Indoor	Germany	KY249283	KY249321	KY249363	
		DTO 055-H8	Indoor	Germany	KY249284	KY249322	KY249364	
		UAMH 9209	Indoor air	Canada	**LN850971**	**LN851125**	**LN851072**	
C. stemonitis	*D. stemonitis*	CBS 103.19^{NT}; DTO 170-B3; MUCL 6960	Seed	Netherlands	**LN850951**	**LN850954**	**LN850953**	**LN850952**
	D. stemonitis	CBS 180.35; DTO 334-F9; IMI 086946; LSHB Sc124	Unknown	Unknown	**LN850972**	**LN851126**	**LN851073**	
	D. stemonitis	CBS 127788; DTO 335-C6; RMF H 423	Soil	USA	KY249285	KY249323	KY249365	
		UAMH 1532	Unknown	Unknown	**LN850973**	**LN851127**	**LN851074**	
C. telluricum sp. nov.	*T. spiralis*	CBS 336.32^{T}; DTO 334-F7; MUCL 9829; UAMH 8882	Soil	Cyprus	KY249287	KY249325	KY249367	
	T. terrophilus	CBS 568.50; DTO 334-H1	Soil	Canada	KY249288	KY249326	KY249368	
C. tenuissimum sp. nov.	*D. microsporus*	CBS 127792^{T}; DTO 335-C7; RMF H 318	Soil	USA	KY249286	KY249324	KY249366	
C. transvaalense sp. nov.	*T. terrophilus*	CBS 448.51^{T}; DTO 170-C1; IFO 7660; IMI 046251; LSHB B344	*Eucalyptus saligna* timber, in cellar	South Africa	**LN850964**	**LN851118**	**LN851065**	
C. verrucisporum	*D. asperulus*	CBS 512.72; DTO 104-B9; DTO 104-C1; DTO 104-C2	Agricultural soil	Netherlands	KY249289	KY249327	KY249369	
	D. asperulus	CBS 187.78; DTO 336-C6	Sand dune soil	Netherlands	**LN850986**	**LN851140**	**LN851086**	
		DTO 055-D7	Indoor	Germany	KY249290	KY249328	KY249370	
Graphium penicillioides		CBS 102632^{ET}; JCM 10498	*Populus nigra*	Czech Republic	KY852474			KY852485
Kernia columnaris comb. nov.	*C. columnare*	CBS 159.66^{T}; IMI 116691	Dung of hare	South Africa	KY852475	KY852477	KY852478	KY852486
K. nitida		CBS 282.52; IFO 8200	*Chrysolina sanguinolenta*	France	KY852476			KY852487

Table 1. (Continued).

Name	CBS Database	Strain number[1]	Substrate/host	Location	GenBank accession number			
					ITS	*tub2*	*tef1*	LSU
Wardomyces inflatus		CBS 367.62^{NT}; DTO 170-D2; DAOM 84715; MUCL 669	Greenhouse soil	Belgium	**LN850994**	**LN851153**	**LN851099**	

[1] First strain number is of the examined isolate. ATCC: American Type Culture Collection, Manassas, VA, USA; CBS: Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; DAOM: Canadian National Mycological Herbarium, Agriculture and Agri-Food Canada, Ottawa, Canada; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; DTO: Working Collection of the Applied and Industrial Mycology Group of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands; IFO: Institute for Fermentation Culture Collection, Osaka, Japan; IHEM: Biomedical Fungi and Yeast Collection of the Belgian Co-ordinated Collections of Micro-organisms (BCCM), Brussels, Belgium; IMI: Culture Collection of CABI Europe-UK, Egham, UK; JCM: Japan Collection of Microorganisms, Microbe Division, RIKEN-BioResource Center, Koyadai, Tsukuba, Ibaraki, Japan; LCP: Laboratory of Cryptogamy, National Museum of Natural History, Paris, France; LSHB: London School of Hygiene and Tropical Medicine, London, UK; MUCL: (Agro)Industrial Fungi and Yeast Collection of the Belgian Co-ordinated Collections of Micro-organisms (BCCM), Louvain-la Neuve, Belgium; NHL: National Institute of Hygienic Sciences, Tokyo, Japan; RMF: Rocky Mountain Herbarium, Fungi, Univeristy of Wyoming, Laramie, WY, USA; TRTC: Royal Ontario Museum Fungarium, Toronto, Canada; UAMH: University of Toronto, UAMH Centre for Global Microfungal Biodiversity, Toronto, Canada; WSF: Wisconsin Soil Fungi Collection, Madison, WI, USA. Ex-epitype, -isotype, -type, and -neotype isolates are indicated with ^{ET}, ^{IT}, ^{T} and ^{NT}, respectively.

Table 2. Herbarium specimens studied with their GenBank accession numbers.

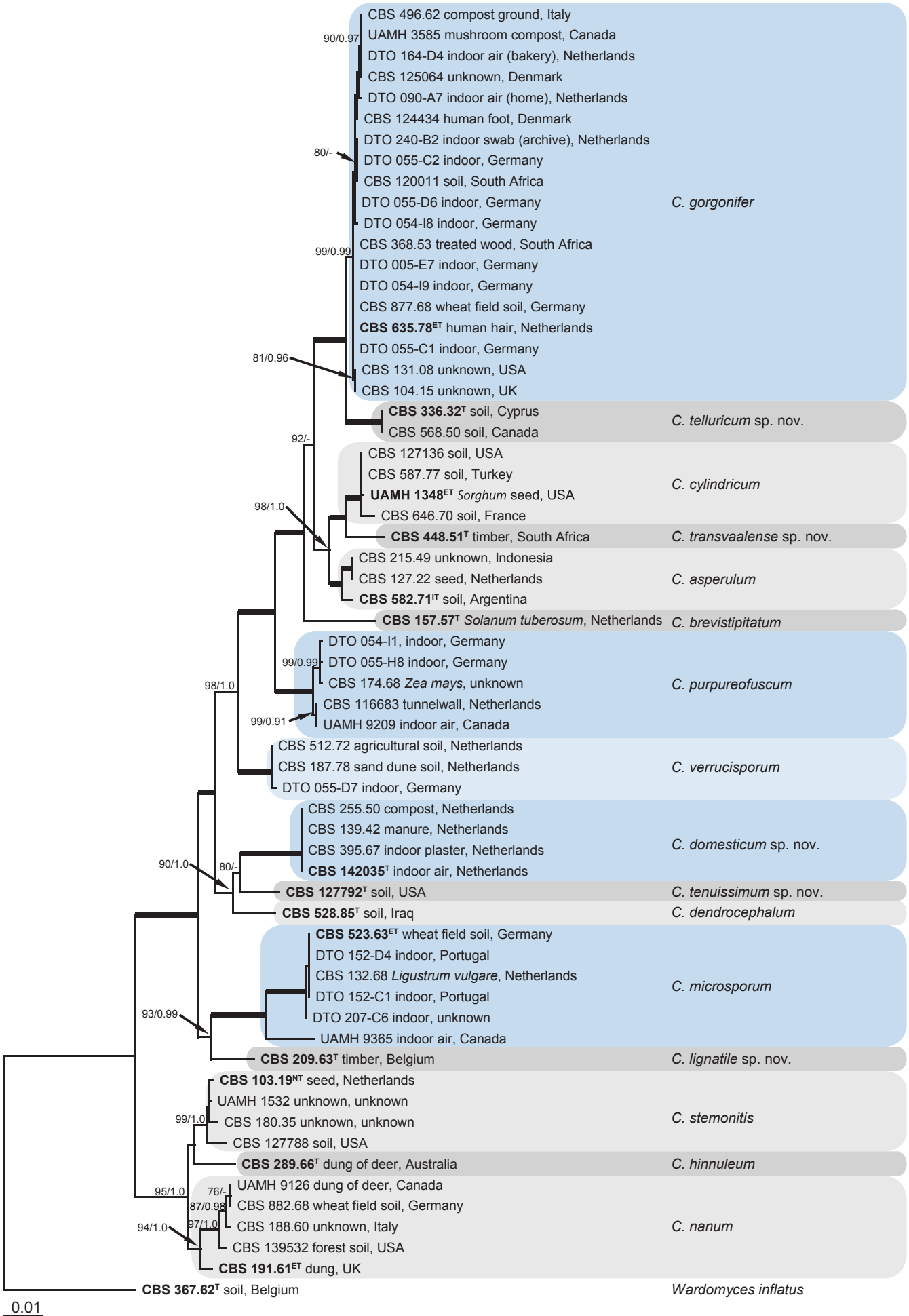
Original name	Collection #		ITS	After examination
C. acutisporum	HSAUPII$_{04}$2724	CBS H-22780	–	*C. purpureofuscum*
C. castaneum	HSAUPII$_{05}$1034	CBS H-22781	FJ914681	*C. castaneum*
C. cylindrosporum	HSAUPII$_{05}$2414	CBS H-22782	FJ914686	Nomen dubium
C. ellipsoideum	HSAUPII$_{07}$4053	CBS H-22783	–	*C. ellipsoideum*
C. inflatum	HSAUPII$_{05}$0918	CBS H-22784	FJ914676	*C. microsporum*
C. longicollum	HSAUPII$_{05}$0802	CBS H-22785	FJ914672	*C. purpureofuscum*
C. macrosporum	HSAUPII$_{05}$0878	CBS H-22786	FJ914675	Nomen dubium
C. oblongum	HSAUPII$_{04}$2723	CBS H-22787	FJ914667	*C. purpureofuscum*
C. ovoideum	HSAUPII$_{05}$0846	CBS H-22788	FJ914662	Nomen dubium
C. robustum	HSAUPII$_{05}$0875	CBS H-22789	FJ914674	Nomen dubium
C. spirale	HSAUPII$_{07}$4033	CBS H-22790	FJ914705	*C. spirale*
C. terricola	HSAUPII$_{05}$0924	CBS H-22791	FJ914677	*C. purpureofuscum*
C. verrucipes	HSAUPII$_{05}$0849	CBS H-22792	–	*Penicillium clavigerum*
C. verrucisporum	HSAUP$_{05}$1029	CBS H-22793	FJ914680	*C. verrucisporum*

with a gamma-distributed rate variation was suggested as the best model for the ITS and *tub2* alignments, and the GTR model with a gamma-distributed rate variation as the most suitable model for the *tef1* alignment. After discarding the burn-in phase trees, the multi-gene Bayesian analysis resulted in 2 020 trees from both runs, from which the majority rule consensus tree and posterior probabilities were calculated.

The multi-gene analysis divided the isolates among 16 species clades (Fig. 1) of which five are proposed as new and described in the Taxonomy section: *C. domesticum*, *C. lignatile*, *C. telluricum*, *C. tenuissimum* and *C. transvaalense*. The 20 strains isolated from indoor environment are distributed among five *Cephalotrichum* species (Fig. 1, blue coloured boxes), namely *C. gorgonifer* (n = 9), *C. microsporum* (n = 4), *C. domesticum* (n = 2), *C. purpureofuscum* (n = 4) and *C. verrucisporum* (n = 1). All 16 species can be identified with either *tef1* or *tub2* partial gene sequences. The only exception is strain CBS 191.61, which based on its *tef1* sequence clusters separately from the other *C. nanum* isolates (data not shown; all single gene phylogenies submitted to TreeBase). Based on ITS barcodes alone, *C. cylindricum* and *C. transvaalense* sp. nov. cannot be distinguished (Fig. 2).

A second ITS analysis included reference sequences for accepted species combined with sequences obtained from the herbarium specimens received from China, resulting in 28 sequences with a total length of alignment length of 564 bases, with 66 informative or unique sites. The TrN model with a gamma-distributed rate variation was suggested as the best model. After discarding the burn-in phase trees, the multi-gene Bayesian analysis resulted in 1 202 trees from both runs, from which the majority rule consensus tree and posterior probabilities were calculated (Fig. 2). Results of the phylogenetic analyses and data derived from the morphological observations of the herbarium species are discussed in the section, "Additional notes on *Cephalotrichum*" below.

A third phylogeny, based on LSU and ITS sequences, was used to demonstrate the placement of *C. album* and *C. columnare* outside *Cephalotrichum*. The alignment contained sequences from 12 isolates and had a total length of 1 453 characters, with respectively 78 informative or unique characters in the LSU, and 183 in the ITS. The TrN model with a gamma-distributed rate variation was suggested as the best model for the LSU and the GTR model with a gamma-distributed rate variation for the ITS. After discarding burn-in phase trees, the multi-gene Bayesian analysis resulted in 1 502 trees from both runs, from which the majority rule consensus tree and posterior probabilities were calculated


CBS 496.62 compost ground, Italy
UAMH 3585 mushroom compost, Canada
DTO 164-D4 indoor air (bakery), Netherlands
CBS 125064 unknown, Denmark
DTO 090-A7 indoor air (home), Netherlands
CBS 124434 human foot, Denmark
DTO 240-B2 indoor swab (archive), Netherlands
DTO 055-C2 indoor, Germany
CBS 120011 soil, South Africa
DTO 055-D6 indoor, Germany
DTO 054-I8 indoor, Germany
CBS 368.53 treated wood, South Africa
DTO 005-E7 indoor, Germany
DTO 054-I9 indoor, Germany
CBS 877.68 wheat field soil, Germany
CBS 635.78ET human hair, Netherlands
DTO 055-C1 indoor, Germany
CBS 131.08 unknown, USA
CBS 104.15 unknown, UK
C. gorgonifer
CBS 336.32T soil, Cyprus
CBS 568.50 soil, Canada
C. telluricum sp. nov.
CBS 127136 soil, USA
CBS 587.77 soil, Turkey
UAMH 1348ET Sorghum seed, USA
CBS 646.70 soil, France
C. cylindricum
CBS 448.51T timber, South Africa
C. transvaalense sp. nov.
CBS 215.49 unknown, Indonesia
CBS 127.22 seed, Netherlands
CBS 582.71IT soil, Argentina
C. asperulum
CBS 157.57T Solanum tuberosum, Netherlands
C. brevistipitatum
DTO 054-I1, indoor, Germany
DTO 055-H8 indoor, Germany
CBS 174.68 Zea mays, unknown
CBS 116683 tunnelwall, Netherlands
UAMH 9209 indoor air, Canada
C. purpureofuscum
CBS 512.72 agricultural soil, Netherlands
CBS 187.78 sand dune soil, Netherlands
DTO 055-D7 indoor, Germany
C. verrucisporum
CBS 255.50 compost, Netherlands
CBS 139.42 manure, Netherlands
CBS 395.67 indoor plaster, Netherlands
CBS 142035T indoor air, Netherlands
C. domesticum sp. nov.
CBS 127792T soil, USA
C. tenuissimum sp. nov.
CBS 528.85T soil, Iraq
C. dendrocephalum
CBS 523.63ET wheat field soil, Germany
DTO 152-D4 indoor, Portugal
CBS 132.68 Ligustrum vulgare, Netherlands
DTO 152-C1 indoor, Portugal
DTO 207-C6 indoor, unknown
UAMH 9365 indoor air, Canada
C. microsporum
CBS 209.63T timber, Belgium
C. lignatile sp. nov.
CBS 103.19NT seed, Netherlands
UAMH 1532 unknown, unknown
CBS 180.35 unknown, unknown
CBS 127788 soil, USA
C. stemonitis
CBS 289.66T dung of deer, Australia
C. hinnuleum
UAMH 9126 dung of deer, Canada
CBS 882.68 wheat field soil, Germany
CBS 188.60 unknown, Italy
CBS 139532 forest soil, USA
CBS 191.61ET dung, UK
C. nanum
CBS 367.62T soil, Belgium
Wardomyces inflatus
90/0.97
80/-
99/0.99
81/0.96
92/-
98/1.0
99/0.99
98/1.0
99/0.91
90/1.0
80/-
93/0.99
99/1.0
95/1.0
76/-
87/0.98
94/1.0
97/1.0
0.01

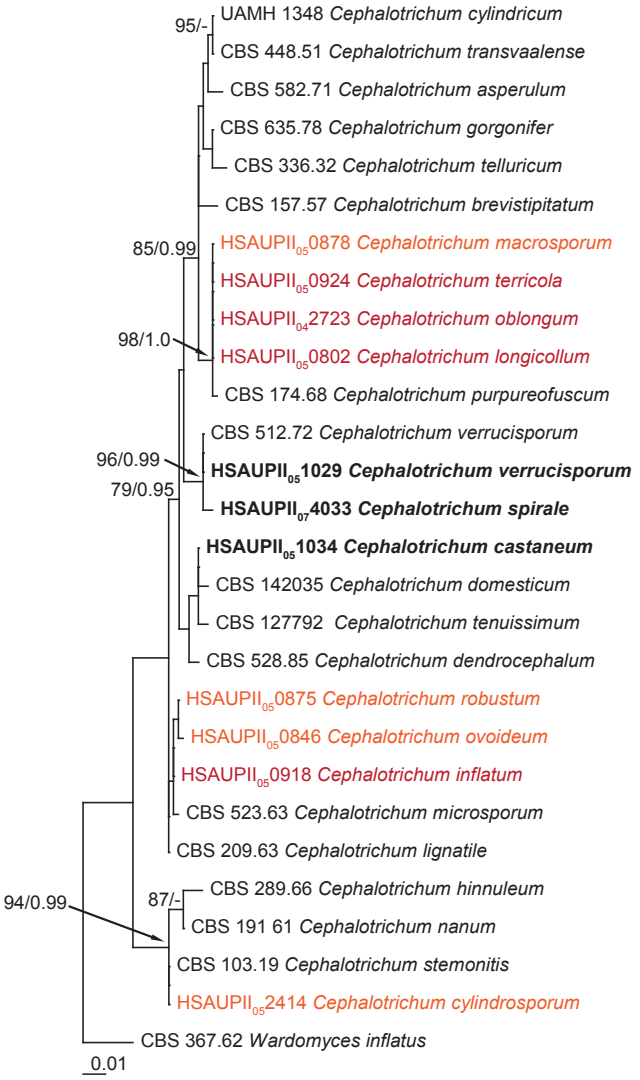


Fig. 2. Maximum likelihood tree based on the ITS sequences of 28 isolates. The RAxML bootstrap support values ≥75 % (BS) and Bayesian posterior probabilities ≥0.95 (PP) are given at the nodes. Thickened lines indicate a BS of 100 % and a PP of 1.0. Names in bold face represent accepted species names, names in orange are *nomen dubium*, names in red are names which are synonymised. The tree was rooted to *Wardomyces inflatus* (CBS 367.62).

(Fig. 3). As a result, new combinations are proposed below for these two species in the "Taxonomy" section.

Morphology

In most *Cephalotrichum* species, both mononematous conidiophores and synnemata occur, either in equal abundance or with one more prevalent, with the distinction between them not always clear. Mononematous conidiophores tend to be more highly branched than those in synnemata, but vary from (i) single, lateral conidiogenous cells to, (ii) monoverticillate conidiophores to, (iii) irregularly biverticillate to terverticillate (i.e. generally penicillate) structures with metulae and/or branches, or (iv) verticillate conidiophores with 2–4 levels of whorls of conidiogenous cells. In the branched conidiophores, conidiogenous cells tend to occur in whorls of 3–7 conidiogenous cells. In synnemata, the conidiophores are usually less branched than in the mononematous form, often arising in a palisade directly from the stipe of the hyphae, or more often with 2–3 conidiogenous cells arising from a lateral metula, and rarely with more levels of branching. Although we provide some observations on conidiophores in our descriptions of new species below, we have no evidence that the branching patterns of either mononematous or synnematous conidiophores have diagnostic value for species. As is common with many synnematous hyphomycetes, some strains have a reduced ability to produce well-developed synnemata with repeated transfer, and sometimes stop producing them completely.

Taxonomy

Acaulium album Seifert & Woudenb., **comb. nov.** MycoBank MB821421. Figs 4–5.

Basionym: *Synpenicillium album* Costantin, Bull. Soc. Mycol. Fr. 4: 62. 1888.

≡ *Coremium album* (Costantin) Sacc. & Traverso, Syll. fung. 22: 1444. 1913.

≡ *Cephalotrichum album* (Costantin) Seifert, CBS Biodiversity Series 12: 309. 2013.

Synonyms: *Penicillium costantini* Bainier, Bull. Soc. Mycol. Fr. 4: 67. 1888. [Non *Penicillium album* Preuss 1851] ≡ *Scopulariopsis costantini* (Bainier) Dale, Ann. Mycol. 12: 57. 1914.

Stysanus putredinis Corda, Icon. fung. (Prague) 3: 12. 1839 *fide* Morton & Smith 1963 ≡ *Doratomyces putredinis* (Corda) F.J. Morton & G. Sm., Mycol. Pap. 86: 83. 1963. Non *Graphium putredinis* (Corda) S. Hughes, Can. J. Bot. 36: 770. 1958 ≡ *Parascedosporium putredinis* (Corda) Lackner & de Hoog, IMA Fungus 2: 44. 2011.]

Acaulium fulvum Sopp, Skr. VidenskSelsk. Christiania, Kl. I, Math.-Natur., no. 11: 67. 1912 (*fide* Morton & Smith 1963, but synonymy rejected by Abbott (2000) because of discrepancies in spores sizes, and in the absence of a type specimen).

Conidiophores often mononematous in vitro, astipitate, or with a short stipe up to 250 µm tall, then monoverticillate, or irregularly biverticillate or terverticillate, or reduced to single conidiogenous cells; structures with similar dimensions to those in synnemata. *Synnemata* on the natural substrate scattered or caespitose, up to 500–700(–1 000) µm tall, stipes white, cream-coloured or eventually very pale brown, 10–45 µm wide, unbranched or with 1–3 side branches, conidial heads hyaline to white, divergent or feathery about 20–65 µm wide and tall. *Hyphae of stipe* hyaline, smooth walled, in two zones: an outer region of parallel hyphae 2.5–4.5 µm wide; surrounding a central broader hypha 7–11 µm wide, with individual cells (10–)20–45 µm long. *Setae* absent. *Conidiophores in synnemata* irregularly biverticillate or terverticillate, branches 16–22 × 3–4 µm, metulae 9–13 × 3–3.5 µm. *Conidiogenous cells* percurrent, ampulliform, hyaline, smooth-walled, 6–8.5 µm long, 2.5–3 µm broad at the widest part, with a distinct shoulder tapering to a cylindrical annellated zone 1.5–2.5 µm wide, up to 6.5 µm long, annellations inconspicuous; in terminal whorls of 3–6. *Conidia* obovoid, ellipsoidal to irregularly fusiform with a truncate base and rounded or bluntly

Fig 1. Maximum likelihood tree based on the ITS, *tub2* and *tef1* sequences of 62 isolates. The RAxML bootstrap support values ≥75 % (BS) and Bayesian posterior probabilities ≥0.95 (PP) are given at the nodes. Thickened lines indicate a BS of 100 % and a PP of 1.0. Ex-type strain numbers are in bold face and indicated with ^{T} (or $^{ET, IT, NT,}$ when ex-epitype, ex-isotype or ex-neotype respectively). The blue boxes indicate species which occur in the indoor environment. The tree was rooted to *Wardomyces inflatus* (CBS 367.62).

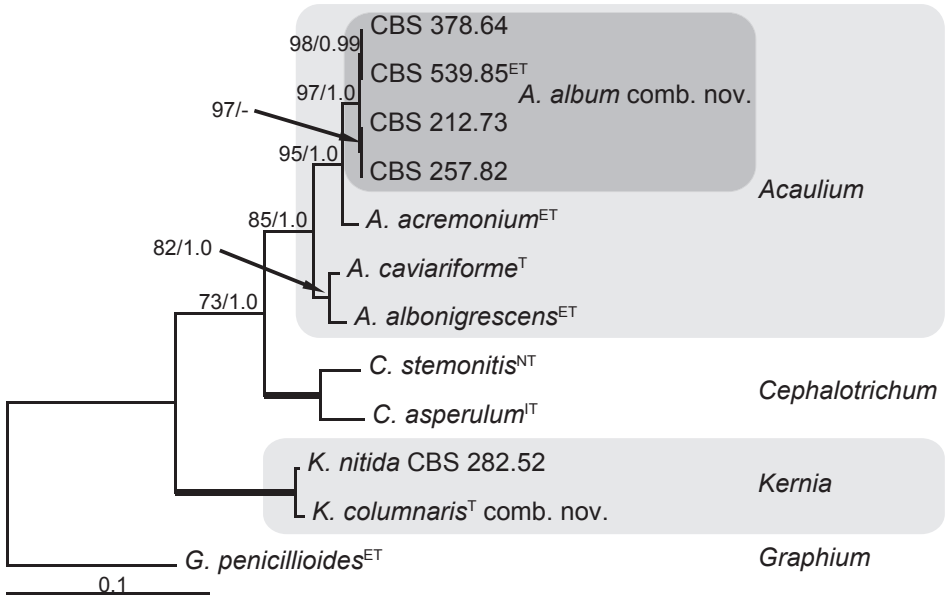


Fig. 3. Maximum likelihood tree based on the LSU and ITS sequences of 12 isolates. The RAxML bootstrap support values ≥75 % (BS) and Bayesian posterior probabilities ≥0.95 (PP) are given at the nodes. Thickened lines indicate a BS of 100 % and a PP of 1.0. Ex-type strain numbers are indicated with ^{T} (or $^{ET, NT,}$ when ex-epitype or ex-neotype respectively). The tree was rooted to *Graphium penicillioides* (CBS 102632).

pointed apex, 4.5–6.5(–7) × 2–3(–4) µm, hyaline, smooth and slightly thick-walled, in long, dry, basipetal chains, sometimes sticking laterally and forming columns up to 1 mm long. *Chlamydospores* abundant in culture, sometimes also in the synnema stipe, globose to ellipsoidal, 4–8 × 4–5 µm, single or in pairs, cyanophilous. Sexual morph not observed.

Culture characteristics: Colonies on OA 46–47 mm diam after 14 d at 25 °C, planar to low convex, powdery, white to cream-coloured centre with hyaline outer ring, margin discrete, undulate. On MEA 44–45 mm diam, planar to low convex, powdery, white to cream with inconspicuous concentric rings about 1 mm apart, margin undulate.

Specimens and cultures examined: **Canada**, British Columbia, Vancouver, University of British Columbia (UBC), near University Golf Club, from decaying *Coprinus micaceus*, July 1981, J. A. MacKinnon, CBS 257.82 = ATCC 46569 (DAOM 230530); British Columbia, Vancouver, UBC Campus, 22 Oct. 1980, R.J. Bandoni, CBS H-3873; Same location, UBC chicken coop, 19 Jan. 1980, on chicken droppings, R.J. Bandoni & T. Thompson, CBS H-3874. Same location, UBC Experimental Garden, 2 Feb. 1981, on rotting potato, J.A. MacKinnon, CBS H-3872. Ontario, Ottawa-Carleton Twp, Bell's Corners, 9 May 1998, on bear dung (*Ursus americanus*), Keith A. Seifert no. 521 (DAOM 226656). **Czech Republic**, Prague, on rotting stems of *Echium* sp., 1838, Fieber (**holotype** of *Stysanus putredinis*, PR-C 155673). **Denmark**, from bumble-bee queen, collection date unknown, J.P. Skou, CBS 378.64. **Netherlands**, Hoogland, near Amersfoort, from hair in dung of pole cat (*Mustela putorius*), March 1984, H.A. van der Aa, (**epitype** designated here CBS H-12128, MBT376922, culture **ex-epitype** CBS 539.85); Wageningen, from soil, collection date unknown, J.H. van Emden, CBS 212.73. **USA**, Maine, Kittery Point, from decaying seaweed, 1918, R. Thaxter (FH).

Notes: De Beer *et al.* (2013, p. 309) briefly reviewed the history of the epithet '*putredinis*' and its contradictory use in *Graphium* and *Doratomyces*, which need not be repeated in detail here. Because the epithet *putredinus* is now used in *Parascedosporium* but previously was being used for two distinct species (one by Hughes 1958, the other by Morton & Smith 1963), it was necessary to transfer the next available epithet, i.e. from *Synpenicillium album*, to *Cephalotrichum*. The recent treatment by Sandoval-Denis *et al.* (2016b) noted morphological similarities between this species (as *C. album*) and other asexual species included in the *Acaulium* clade by molecular evidence, but did not redescribe or reclassify this white species. The phylogenetic analysis presented here (Fig. 3) shows that this species does not belong to *Cephalotrichum*, and is best classified as a synnematous species of *Acaulium* as suggested by Sandoval-Denis *et al.* (2016b).

Acaulium album is a relatively infrequently reported asexual fungus, but is broadly distributed in Europe and North America on heavily decayed organic material and various kinds of (often carnivore) dung. Apart from the white synnemata and spores, it is distinctive because of the broad hypha in the centre of the synnema stipe. Developmentally, the synnemata are rather odd. The cells of the broad central hypha produce narrower hyphae growing upward near the top of the cells or hyphae growing downward from the bottom of the same cells, all appressed to the central cylinder to make up the stipe. The downward growing hyphae anchor the conidiomata to the substrate; the upward growing hyphae branch to become the conidiophores. Similar downward growing hyphae, and broader core hyphae, are sometimes seen in the synnema stipes of true *Cephalotrichum* species (Lodha 1963, Swart 1964) but are more difficult to see because of the pigmentation of the cells.

Costantin (1888) described his fungus from panther dung from an unreported location, but probably from a zoo in Paris. Morton & Smith (1963) did not locate a type, but considered the identity of the fungus clear from the published illustration. There is a discrepancy in spore sizes, Costantin (1888) reporting dimensions of 7–13 × 3–6 µm, roughly double the size reported here, but given our observations of several specimens of the relatively common species described above, suspect this is probably a measurement error by Costantin (1888). We designate CBS H-12128, isolated from a hair in pole cat dung, as epitype above to further stabilise the species name.

Synpenicillium is an older name than *Acaulium* but has rarely been used after its original publication in 1888 (Costantin). *Acaulium* has generally been considered a synonym of *Scopulariopsis* but recently was re-instated as an accepted genus of *Microascaceae* with three species (Sandoval-Denis *et al.* 2016b). Although both names are relatively obscure, we see no reason to

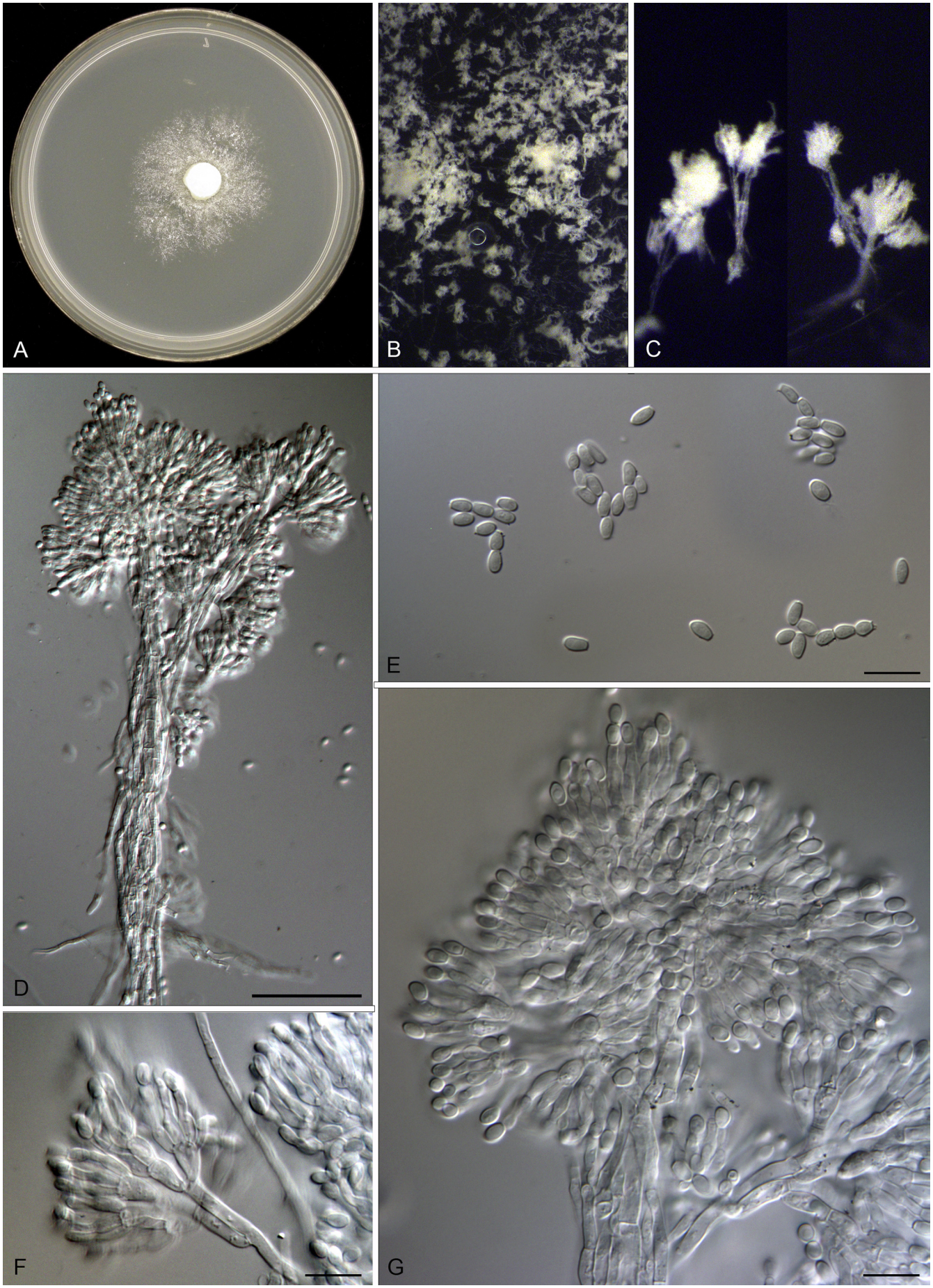


Fig. 4. *Acaulium album* DAOMC 226656. **A.** Four weeks old colony on cornmeal agar. **B.** Colony surface showing white conidial columns. **C.** Individual synnemata in side view. **D.** Synnema with apical conidiophores and broader central hypha. **E.** Conidia. **F.** Individual conidiophore on side of synnema. **G.** Top of synnema showing divergent conidiophores. Scale bars: D. 50 µm. E–G. 10 µm.

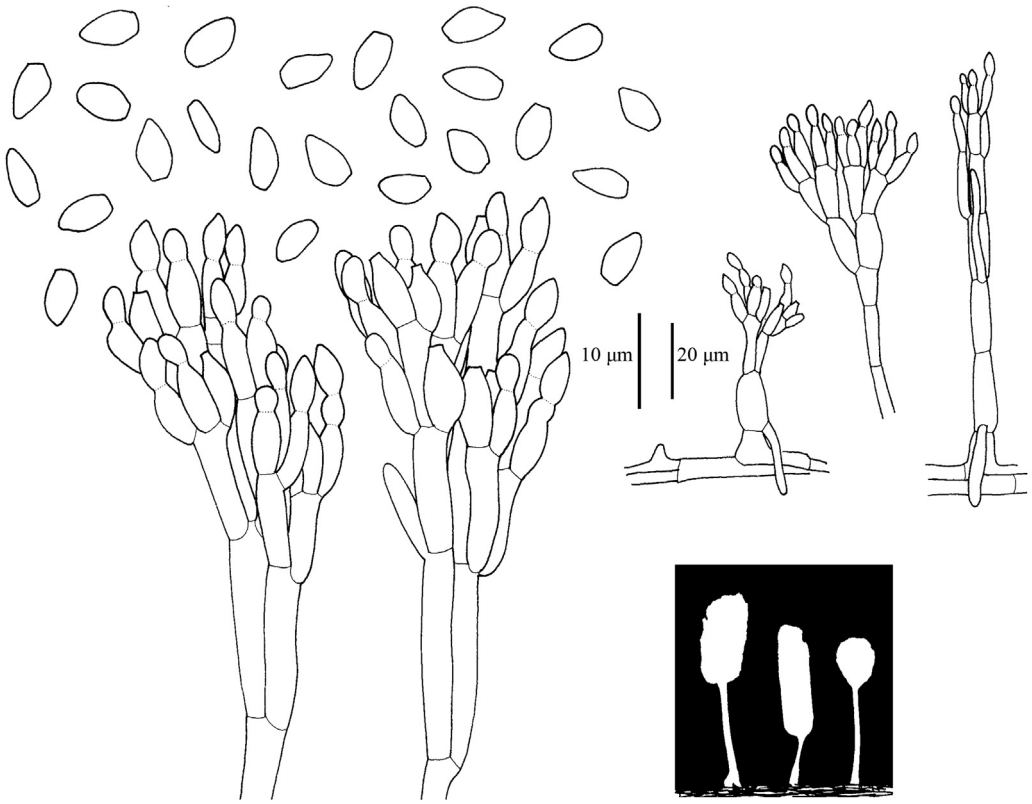


Fig. 5. Line drawings illustrating conidiophores and conidia of *Acaulium album*.

resurrect *Synpenicillium* for this clade. We will propose protection of *Acaulium* be added to the list of protected generic names now being discussed by the nomenclatural community.

Cephalotrichum domesticum Woudenb. & Seifert **sp. nov.** MycoBank MB819314. Fig. 6.

Etymology: The name refers to the usual occurrence of this species in the built environment, or other environments manipulated by humans, such as farms.

Mononematous conidiophores abundant among and intergrading with synnemata, hyaline to pale brown, monoverticillate, or irregularly biverticillate to terverticillate, sometimes 3–4- level verticillate, with a short stipe 7–25 µm tall, terminating in 3–5(–7) annellides on cylindrical, clavate or swollen metulae 5–8 × 2.5–3.5 µm; branches appressed or divergent, 10–12 × 2–3 µm. *Synnemata* 130–225(–245) µm tall, stipes pale brown, 7–10 µm wide, composed of rather loosely attached hyphae; conidial heads brown, subglobose to ellipsoidal. *Hyphae* of stipe parallel, 2–4 µm wide, pale-brown, slightly thick-walled. *Setae* absent. *Conidiophores in synnemata* monoverticillate, irregularly biverticillate or conidiogenous cells arising directly from the stipe hyphae, with all elements tightly appressed, metulae 5.5–8.5 × 2–2.5 µm. *Conidiogenous cells* ampulliform, (5.5–)6–7.5(–8) µm long, 2.5–3(–3.5) µm broad at the widest part, tapering gradually to a cylindrical annellate zone 1.5–2 µm wide, hyaline to pale brown, smooth-walled. *Conidia* ellipsoidal to cylindrical with truncate base and rounded or pointed apex, 5.5–6(–6.5) × 3–3.5(–4) µm, pale brown to brown, smooth and thick-walled, in basipetal chains. Sexual morph not observed.

Culture characteristics: Colonies on OA 52–55 mm diam after 14 d at 25 °C, low convex, felty, white with iron grey synnemata, margin crenate. On MEA 45–50 mm diam, low convex, finely felty, white with olivaceous grey to iron grey centre, margin uneven. On DG18 13–15 mm diam, planar, finely felty, white with iron grey zones, margin undulate.

Specimens examined: **Netherlands**, Limburg, from manure, Mar. 1942, P.J. Bels, CBS 139.42 = IFO 7677 = MUCL 4025; Utrecht, dried culture of strain isolated from indoor air of home (kitchen), 28 Aug. 2008, J. Houbraken, **(holotype** CBS H-22856, culture **ex-type** CBS 142035); from plaster, before Sept. 1967, H.J. Hueck, CBS 395.67; from mushroom compost, 1950, H.C. Bels-Koning, CBS 255.50 = MUCL 4037.

Notes: Only the ex-type culture CBS 142035 produced synnemata in culture. This phenomenon has also been reported for *C. purpureofuscum*, where several colonial variants can be obtained by spontaneous sectoring (Domsch *et al.* 2007). Although morphologically *C. domesticum* resembles *C. purpureofuscum*, it can easily be separated from that based on any of the three genes studied here (see Discussion section notes regarding *C. purpureofuscum*). Given the similar morphology, frequently defined by lack of distinctive characters, examination of a larger number of isolates for *C. domesticum* and *C. purpureofuscum* is needed to adequately assess whether any consistent morphological features could be used to identify the species that are clearly distinct based on molecular data. Phylogenetically *C. domesticum* is closely related to *C. tenuissimum*. The smaller synnemata of *C. domesticum* (130–245 µm tall vs. 495–900 µm tall) and faster growth on OA (52–55 mm diam vs. 40 mm) and MEA (45–50 mm diam vs. 30 mm) at 25 °C can be used to distinguish the two species.

Cephalotrichum lignatile Woudenb. & Seifert **sp. nov.** MycoBank MB819309. Fig. 7.

Etymology: The name refers to the substrate of isolation, timber.

Mononematous conidiophores moderately abundant among synnemata, pale brown, mostly monoverticillate or biverticillate with a

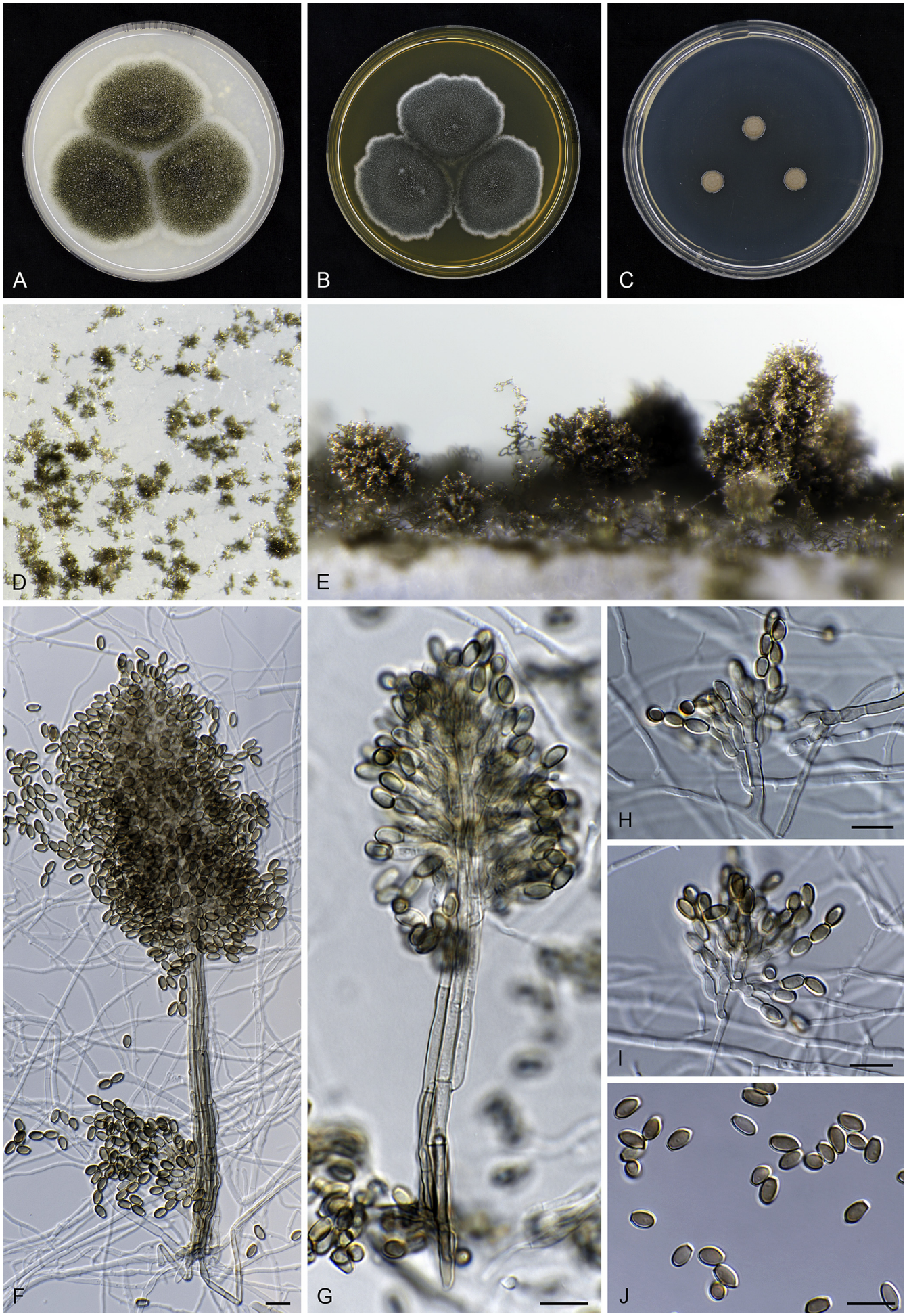


Fig. 6. *Cephalotrichum domesticum* sp. nov. CBS 142035. **A**–**C.** Fourteen day old colonies on OA (A), MEA (B) and DG18 (C). **D**–**G.** Synnemata. **H**–**I.** Conidiophores, conidiogenous cells and conidia. **J.** Conidia. Scale bars = 10 µm.

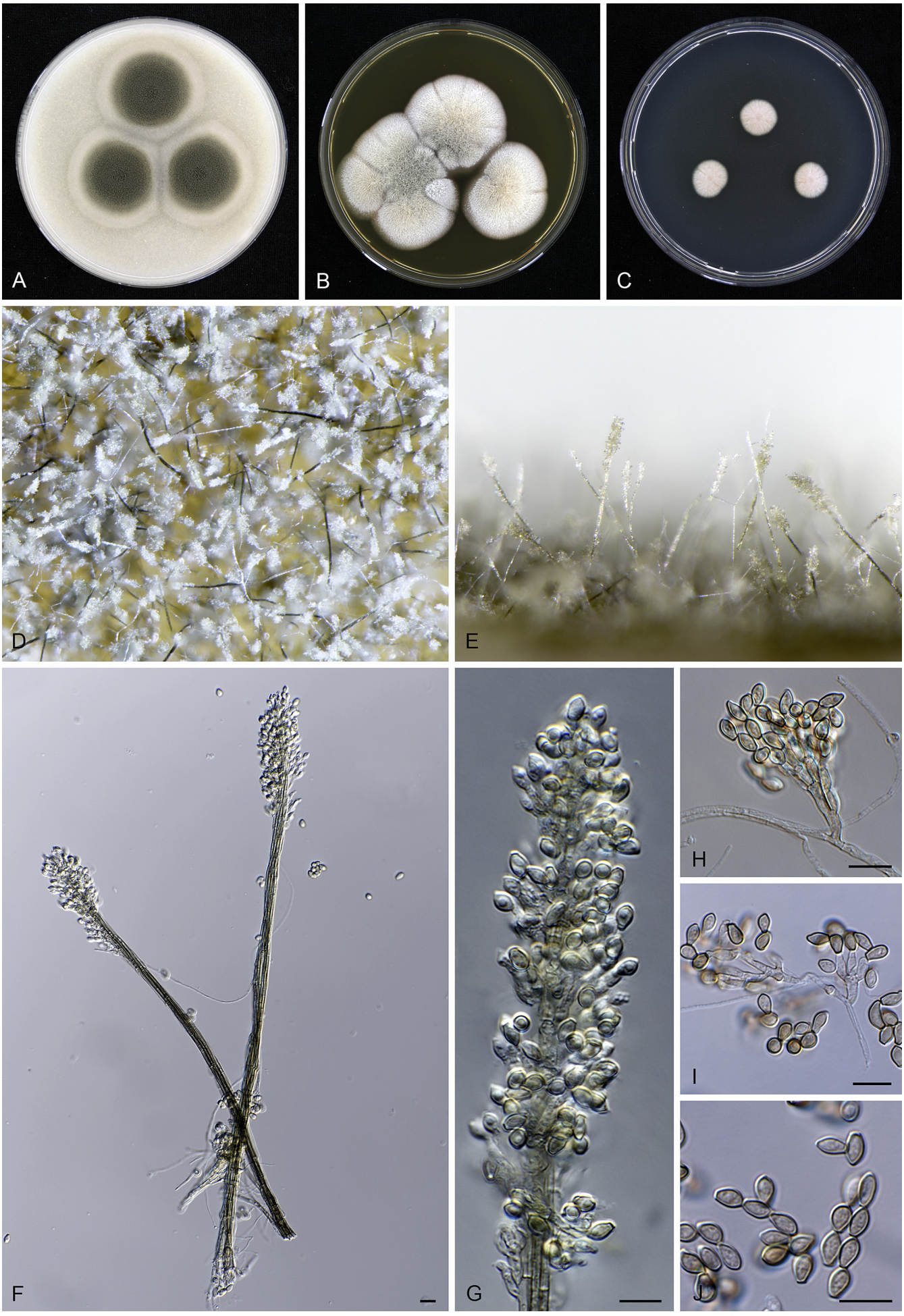


Fig. 7. *Cephalotrichum lignatile* sp. nov. CBS 209.63. **A**–**C.** Fourteen day old colonies on OA (A), MEA (B) and DG18 (C). **D**–**F.** Synnemata. **G.** Detail of the apical portion of a synnema. **H**–**I.** Conidiophores, conidiogenous cells and conidia. **J.** Conidia. Scale bars = 10 µm.

short stipe 4–10(–14) × 2–3 µm, sometimes 2-level verticillate, with (2–)3–5 annellides in compact whorls on slightly divergent, cylindrical to slightly clavate metulae 7–8 × 1.5–2.5 µm. *Synnemata* (280–)300–445(–465) µm tall, stipes pale brown to brown, (5.5–)6–8.5 µm wide, unbranched, conidial heads hyaline to pale brown, ellipsoidal or cylindrical. *Hyphae* of stipe parallel, 2–2.5 µm wide, pale-brown, slightly thick-walled. *Setae* absent. *Conidiophores in synnemata* irregularly monoverticillate, biverticillate or 2–3(–4)-level verticillate with all elements tightly appressed, metulae 6–8 × 2–3(–3.5) µm. *Conidiogenous cells* ampulliform, hyaline to pale brown, smooth-walled, (5.5–)6–7(–7.5) µm long, 2.5–3 µm broad at the widest part, tapering gradually to a cylindrical annellate zone (1–)1.5(–2) µm wide, annellations inconspicuous. *Conidia* obovoid to irregularly fusiform with truncate base and rounded or bluntly pointed apex, (4.5–)5–6(–6.5) × (2.5–)3–3.5(–4) µm, pale brown to brown, smooth and thick-walled, in basipetal chains. *Chlamydospores* absent. Sexual morph not observed.

Culture characteristics: Colonies on OA 36 mm diam after 14 d at 25 °C, planar to low convex, finely felty, dull green centre with white outer ring, margin uneven. On MEA 35 mm diam, low convex, felty, white to buff with pale olivaceous grey zones, radially striate, margin undulate. On DG18 14 mm diam, low convex, finely felty, buff with rosy buff centre, radially striate, margin uneven.

Specimen examined: **Belgium**, Han-sur-Lesse, dried culture of strain isolated from timber in a cave, 1959, G.L. Hennebert, (**holotype** CBS H-22852, culture **ex-type** CBS 209.63).

Notes: The sequences of CBS 209.63, which we describe here as *C. lignatile*, are identical to the sequences previously published for CBS 159.66 as *C. columnare* (Sandoval-Denis *et al.* 2016b). However, the morphology of CBS 209.63 does not match CBS 159.66. We also sequenced older batches of CBS 159.66 from the CBS collection to exclude the possibility of a mix-up, but all gave identical sequence results, placing *C. columnare* in the genus *Kernia*, where it is transferred below. The sequences associated with this strain by Sandoval-Denis *et al.* (2016b) seem to contain an error that we can't explain.

Cephalotrichum telluricum Woudenb. & Seifert **sp. nov.** MycoBank MB819318. Fig. 8.

Etymology: The name refers to the substrate of isolation, soil.

Mononematous conidiophores sparse among synnemata, hyaline, unbranched to monoverticillate or irregularly biverticillate, bearing 1–3 annellides on cylindrical or swollen metulae 7–12 × 2.5–3 µm. *Synnemata* 260–424(–490) µm tall, stipes pale brown to brown, 8.5–11.5(–12.5) µm wide, conidial heads pale brown to brown, subglobose or ellipsoidal. *Hyphae* of stipe parallel, 2–4 µm wide, pale-brown, slightly thick-walled, wider hyphae in the centre of the stipe near the base, downward growing branches occurring near the base of the synnemata. *Setae* coiled, simple, septate, pale brown, individually up to about 180 µm long, 3–4.5 µm wide, extending about 70–100 µm beyond the level of the conidiogenous cells with a rounded or acute apex. *Conidiophores in synnemata* solitary and lateral on stipe hyphae, or monoverticillate, metulae 6–8 × 2–3 µm. *Conidiogenous cells* ampulliform, (5.5–)6.5–8(–8.5) µm long, 2.5–3.5(–4) µm broad at the widest part, tapering gradually to a cylindrical annellate zone (1–)1.5–2 µm wide, hyaline, smooth-walled. *Conidia* ovoid to broad fusiform with truncate base, (5.5–)6–7.5(–8) × (3.5–)4–4.5 µm, pale brown, smooth, thick-walled, in basipetal chains. Sexual morph not observed.

Culture characteristics: Colonies on OA 65 mm diam after 14 d at 25 °C, planar, finely felty with tufts of mycelium, grey olivaceous with pale greenish grey mycelium and (pale) olivaceous grey sectors in the centre and a white outer ring, margin entire. On MEA 60–62 mm diam, planar to low convex, floccose, white with pale greenish grey ring and lavender grey to smoke grey regions, margin entire. On DG18 10–12 mm diam, raised, finely felty, cinnamon with olivaceous grey zones and white outer ring, margin undulate.

Specimens examined: **Canada**, Ontario, Vineland, from soil, Sep. 1949, R.F. Cain, CBS 568.50 = TRTC 12269. **Cyprus**, Nicosia, dried culture of strain isolated from soil, before Jan. 1932, R.M. Nattrass **(holotype** CBS H-22853, culture **ex-type** CBS 336.32 = MUCL 9829 = UAMH 8882).

Notes: Based on morphology and phylogeny, *C. telluricum* is closely related to *C. gorgonifer*. Both species have spirally coiled setae, but the synnemata of *C. telluricum* (<500 µm tall) are shorter than those of *C. gorgonifer* (500–1 000 µm). Based on sequence data *C. telluricum* can be distinguished from *C. gorgonifer* by all three genes, with ITS having 5 nt differences, *tub2* 15 nt, and *tef1* 3 nt between *C. telluricum* and the ex-epitype isolate of *C. gorgonifer*, CBS 635.78.

Cephalotrichum tenuissimum Woudenb. & Seifert **sp. nov.** MycoBank MB819317. Fig. 9.

Etymology: The name refers to the slender synnemata.

Mononematous conidiophores fairly frequent among synnemata, biverticillate to terverticillate but often irregular, bearing 3–5 annellides on cylindrical or swollen metulae 5–8.5 × 2–3 µm; branches divergent, 7.5–12.5 × 2–3 µm; stipe 5–20(–70) × 2–3 µm. *Synnemata* (495–)630–895(–900) µm tall, stipes pale brown to brown, (14–)15–21(–24.5) µm wide, conidial heads pale brown, obclavate. *Hyphae of stipe* parallel, brown, 1.5–2.5 µm wide. *Setae* absent. *Conidiogenous cells* ampulliform, (5–)6–8(–8.5) µm long, 2.5–3.5 µm broad at the widest part, tapering gradually to a cylindrical annellated zone 1–1.5(–2) µm wide, hyaline, smooth-walled, usually singly and arising at +/− right angles from hyphae of the stipe, sometimes in groups of 2–3 on short metulae. *Conidia* ellipsoidal with truncate base and rounded apex, (4.5–)5–6(–6.5) × (3–)3.5–4 µm, hyaline to pale green-brown, smooth, thick-walled, single or in short chains. Sexual morph not observed.

Culture characteristics: Colonies on OA 40 mm diam after 14 d at 25 °C, planar, felty, colourless with olivaceous grey synnemata, margin uneven. On MEA 30 mm diam, low convex to umbonate, felty, olivaceous grey with thin pale olivaceous grey ring, radially striate, margin crenated. On DG18 15 mm diam, crateriform, pale olivaceous grey with buff edge, radially striate, margin uneven.

Specimen examined: **USA**, Wyoming, Hanna, dried culture of strain isolated from soil, 1976, M. Christensen **(holotype** CBS H-22855, culture **ex-type** CBS 127792 = RMF H 318).

Notes: Based our phylogenies, *C. tenuissimum* is closely related to *C. domesticum* and *C. dendrocephalum*. Morphologically it is easily distinguished from *C. domesticum* (see notes under *C. domesticum*) and *C. dendrocephalum*, which forms characteristic, undulating, branched setae. The ex-type isolate of *C. tenuissimum* (CBS 127792) was originally identified in CBS as *C. microsporum*. However, *C. tenuissimum* can be distinguished

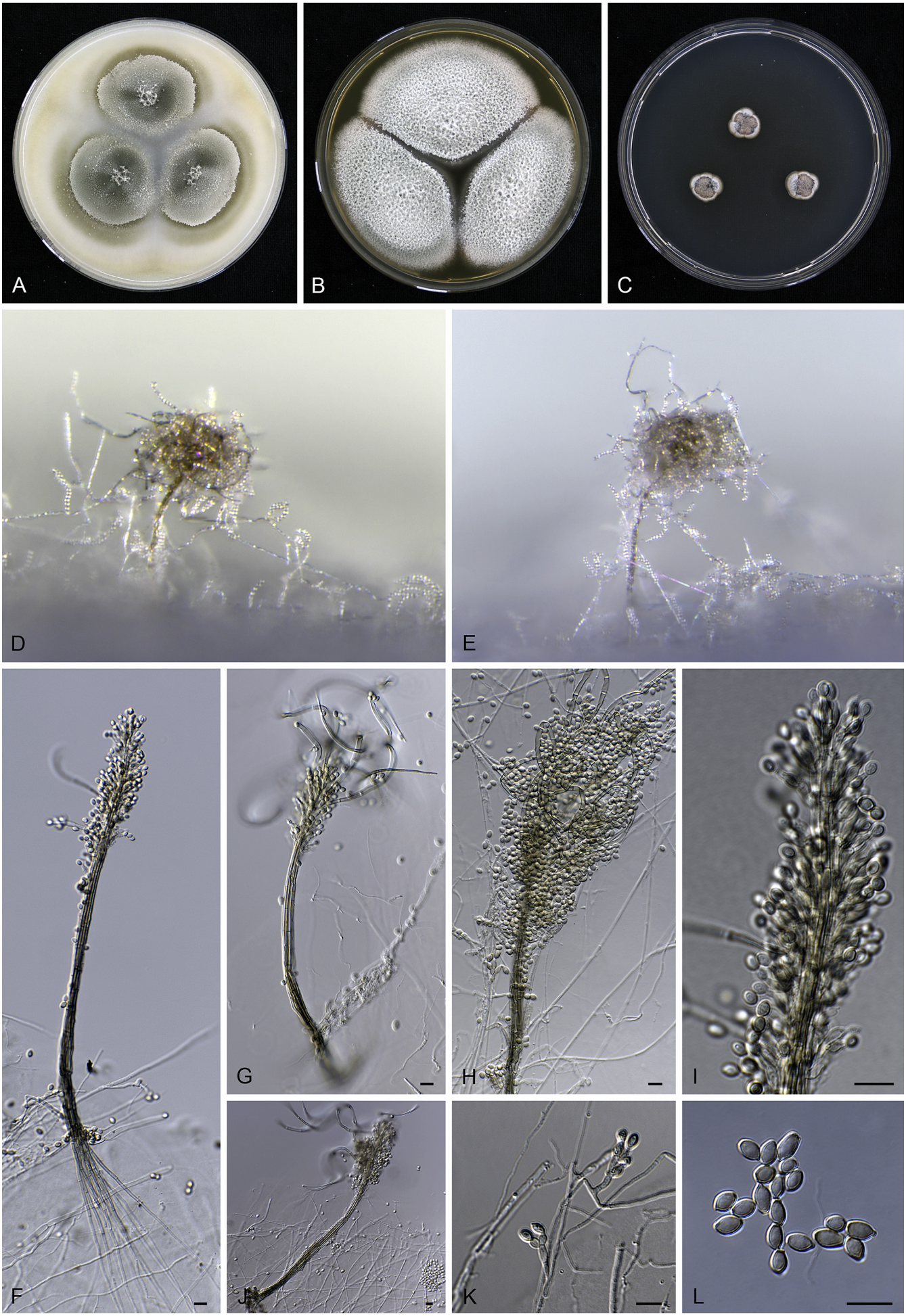


Fig. 8. *Cephalotrichum telluricum* sp. nov. CBS 336.32. **A**–**C.** Fourteen day old colonies on OA (A), MEA (B) and DG18 (C). **D**–**H, J.** Synnemata. **I.** Detail of the apical portion of a synnema. **K.** Conidiophores, Conidiogenous cells and conidia. **L.** Conidia. Scale bars = 10 µm.

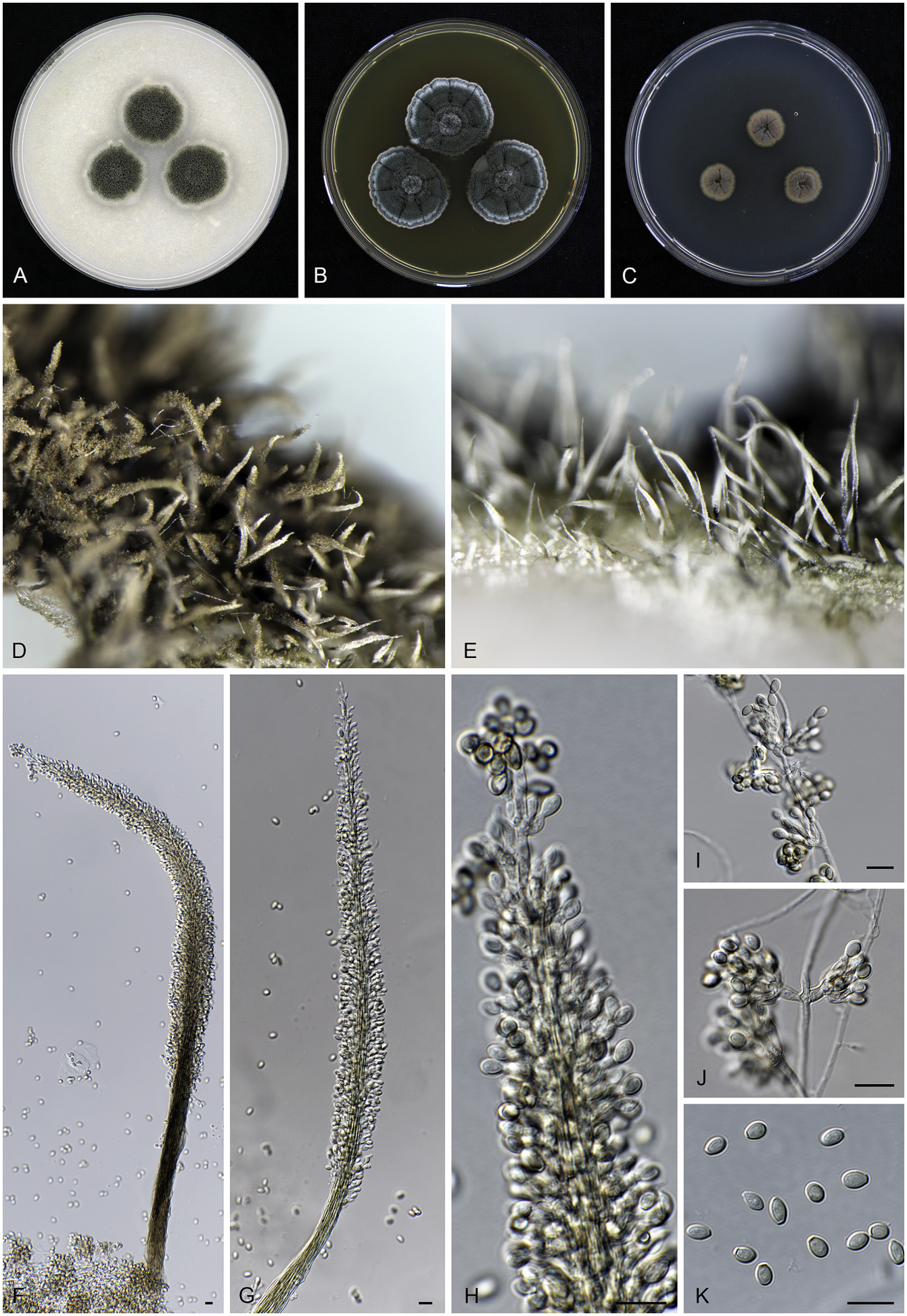


Fig. 9. *Cephalotrichum tenuissimum* sp. nov. CBS 127792. **A**–**C.** Fourteen day old colonies on OA (A), MEA (B) and DG18 (C). **D**–**G.** Synnemata. **H.** Detail of the apical portion of a synnema. **I**–**J.** Conidiophores, conidiogenous cells and conidia. **K.** Conidia. Scale bars = 10 µm.

from *C. microsporum* by the size of the conidia, 5–6 × 3.5–4 for *C. tenuissimum* vs. 3.5–5 × 2–3 µm for *C. microsporum*.

Cephalotrichum transvaalense Woudenb. & Seifert **sp. nov.** MycoBank MB819312. Fig. 10.

Etymology: The name refers to the Transvaal Province in South Africa, where the ex-type strain was first isolated.

Mononematous conidiophores monoverticillate to irregularly biverticillate, or 2–3-level verticillate. *Synnemata* 500–1 500 µm tall, stipes brown, 15–35 µm wide, unbranched, conidial heads pale brown to brown, ellipsoidal; sessile conidiomata lacking a stipe present in some transfers, forming brown to black, subglobose conidial tufts. *Hyphae* of stipe parallel, 2–4 µm wide, pale-brown, slightly thick-walled. *Setae* straight, aseptate, brown, (40–)70–110 µm long, 1–1.5 µm wide, base sometimes swollen to 2–2.5 µm wide, unbranched, bifurcate (90–120°) or with several layers of basal branching; sometimes arising from the same hyphae or metulae as conidiogenous cells. *Conidiophores in synnemata* mostly biverticillate, sometimes monoverticillate, metulae divergent, 5–8 × 2–3 µm. *Conidiogenous cells* ampulliform, 6–8(–10) µm long, 2–3 µm broad at the widest part, tapering abruptly to a cylindrical annellate zone 1–1.5 µm wide, pale brown, smooth-walled. *Conidia* ovoid to ellipsoidal with small truncate base and rounded apex, (4.5–)5–6.5 × 3–4.5 µm, pale brown, smooth, thick-walled, in basipetal chains. Sexual morph not observed.

Culture characteristics: Colonies on OA 53 mm diam after 14 d at 25 °C, planar, felty, white with olivaceous grey ring and pale olivaceous grey centre, margin uneven. On MEA 48 mm diam, low convex, wooly, white to (pale) olivaceous grey, margin entire. On DG18 15 mm diam, crateriform, planar, olivaceous grey with white to pale olivaceous grey centre, radially striate, margin entire.

Specimen examined: **South Africa**, Transvaal, dried culture of strain isolated from *Eucalyptus saligna* timber in cellar, 1951, leg. Bekker (**holotype** CBS H-22854, culture **ex-type** CBS 448.51 = IFO 7660 = IMI 046251 = LSH BB344 = UAMH 8848).

Notes: With the straight setae arising from the conidial head, *C. transvaalense* morphologically resembles *C. cylindricum* and the holotype strain was identified as this species in the past. IMI 46251 was used as the basis for the description of *C. cylindricum* (as *Trichurus terrophilus*) by Lodha (1963) and Swart (1964); their illustrations and descriptions indicate well-developed synnemata and some aspects of our description are adapted from these sources. Because the strain no longer makes well-developed synnemata, we have adapted measurements and details from these descriptions in our technical description above. *Cephalotrichum transvaalense* and *C. cylindricum* are closely related but distinct based on molecular data. The ITS sequences have no differences, but 20 nt differences in *tub2* and 9 nt differences in *tef1* sequences clearly distinguish the two species.

Kernia columnaris (H.J. Swart) Woudenb. & Samson **comb. nov.** MycoBank MB820690.
Basionym: *Doratomyces columnaris* H.J. Swart, Acta Bot. neerl. 15: 521. 1967 ≡ *Cephalotrichum columnare* (H.J. Swart) S.P. Abbott, Stud. Mycol. 83:206. 2016.

Descriptions and illustrations: Swart (1967), Abbott (2000), Sandoval-Denis *et al.* (2016a, b).

Specimen examined: **South Africa**, Johannesburg, Melville Koppies Nature Reserve, from dung of hare, 1964, H.J. Swart (culture **ex-type** CBS 159.66 = IMI 116691).

Notes: As noted, sequences previously published for CBS 159.66 (Sandoval-Denis *et al.* 2016b) match sequences of CBS 209.63, which we describe above as *C. lignatile*. To exclude the possibility of mislabelling in the CBS collection, we also studied older preservation batches of CBS 159.66 from the CBS collection, which all yielded identical sequence results that convincingly place this species in the genus *Kernia* (Fig. 3). The affinity of this species with the latter genus rather than *Cephalotrichum* was already suggested by Abbott (2000), based on significantly discordant morphological characters recorded from several isolates, including its reduced conidiophores (50–700 µm), mostly mononematous or more rarely synnematous with poorly developed, hyaline stipes, which resemble more to those of the synnematous anamorphs of *Kernia hippocrepida* and *K. pachypleura* (Malloch & Cain 1971). Other relevant features of *K. columnaris* are: annellides subcylindrical to ampulliform, conidia ellipsoid, often slightly asymmetrical, apex rounded or bluntly pointed, smooth, 5–6 × 2.5–4 µm, commonly 5.5 × 3 µm, colonies grey to brown, slow growing (20–30 mm in 14 d at 25 °C). The mentioned characteristics match with the morphological treatment of the species by Sandoval-Denis *et al.* (2016a, b), suggesting that their illustrations are based on the actual ex-type strain of *K. columnaris*, while the sequence discrepancy most likely respond to a sequencing overlap.

ADDITIONAL NOTES ON *CEPHALOTRICHUM*

Recently, 14 new *Cephalotrichum* species were described based on isolates from soil from China (Jiang & Zhang 2008, Jiang *et al.* 2011). The status of those species, only known from their holotypes, could not be thoroughly evaluated by Sandoval-Denis *et al.* (2016b) because of the unavailability of material. Recently, portions of the holotypes materials were donated to the CBS herbarium collection by the authors of these names, and the species could be re-evaluated in our study. The conclusions derived from the morphological analysis of these specimens, and associated DNA sequences, are as follows:

Accepted species

Cephalotrichum castaneum (Y.L. Jiang & T.Y. Zhang) Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 224. 2011. Fig. 11.
Synonym: *Doratomyces castaneus* Y.L. Jiang & T.Y. Zhang, Mycotaxon 104: 131. 2008.

Specimen examined: **China**, Guizhou Province, Guiyang, Huaxi Park, dried culture isolated from grassland soil, Oct. 6. 2005, Y.L. Jiang (holotype of *D. castaneus* HSAUPII$_{05}$1034 → isotype CBS H-22781).

Notes: This species is morphologically very similar to *C. microsporum*. It produces synnemata of almost identical size and shape, and conidia of similar size. However, *C. castaneum* is easily identifiable by its dark brown, spherical to subspherical conidia, which contrast to the green-brown, oval to ellipsoidal conidia of *C. microsporum*. Our gene tree based on ITS sequences (Fig. 2) showed *C. castaneum* to be genetically distant from *C. microsporum*, clustering without strong bootstrap support as a sister species of *C. dendrocephalum*, *C. domesticum* and

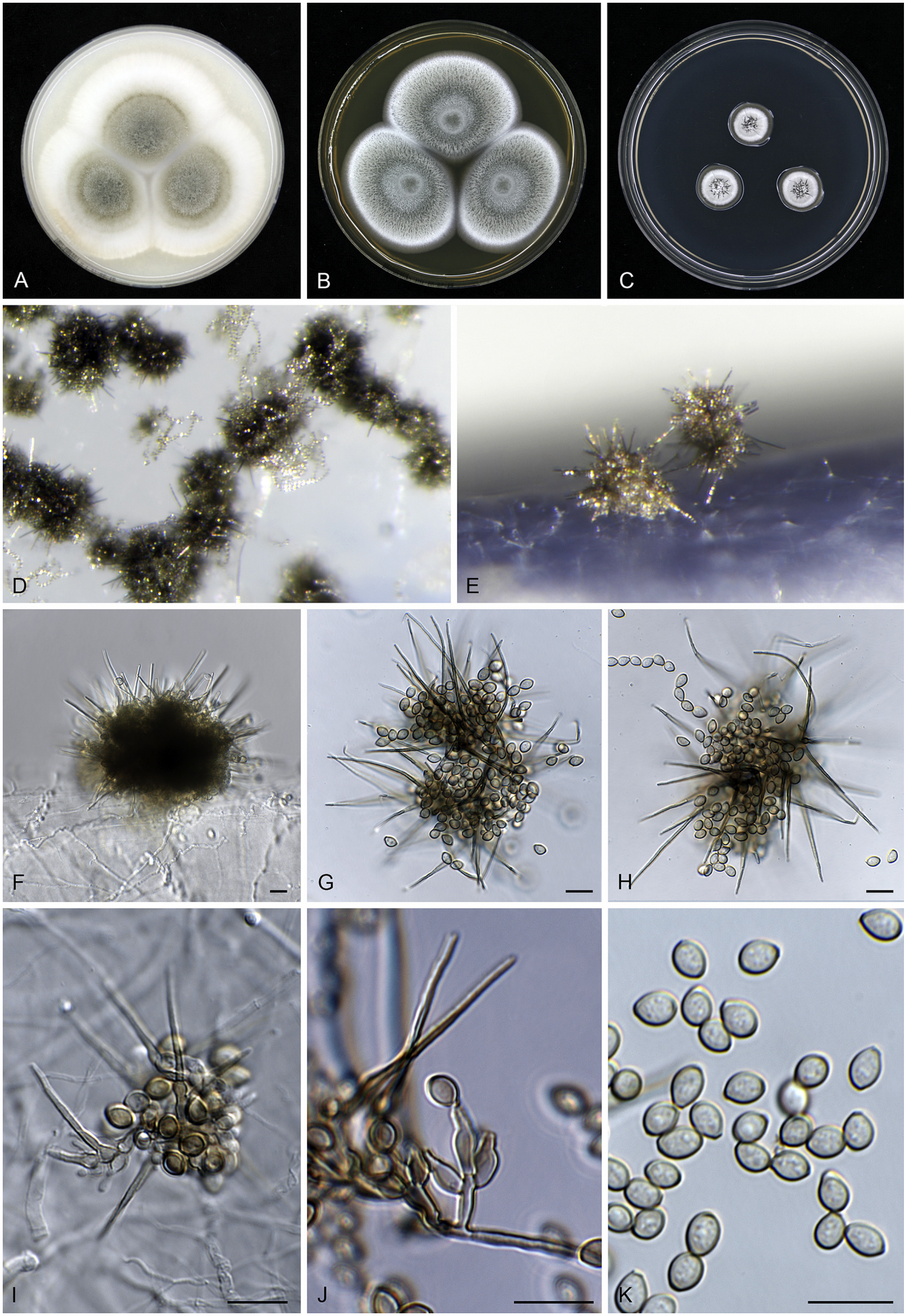


Fig. 10. *Cephalotrichum transvaalense* sp. nov. CBS 448.51. **A**–**C.** Fourteen day old colonies on OA (A), MEA (B) and DG18 (C). **D**–**H.** Synnemata. **I.** Detail of synnema setae. **J.** conidiophores, conidiogenous cells and conidia **K.** Conidia. Scale bars = 10 µm.

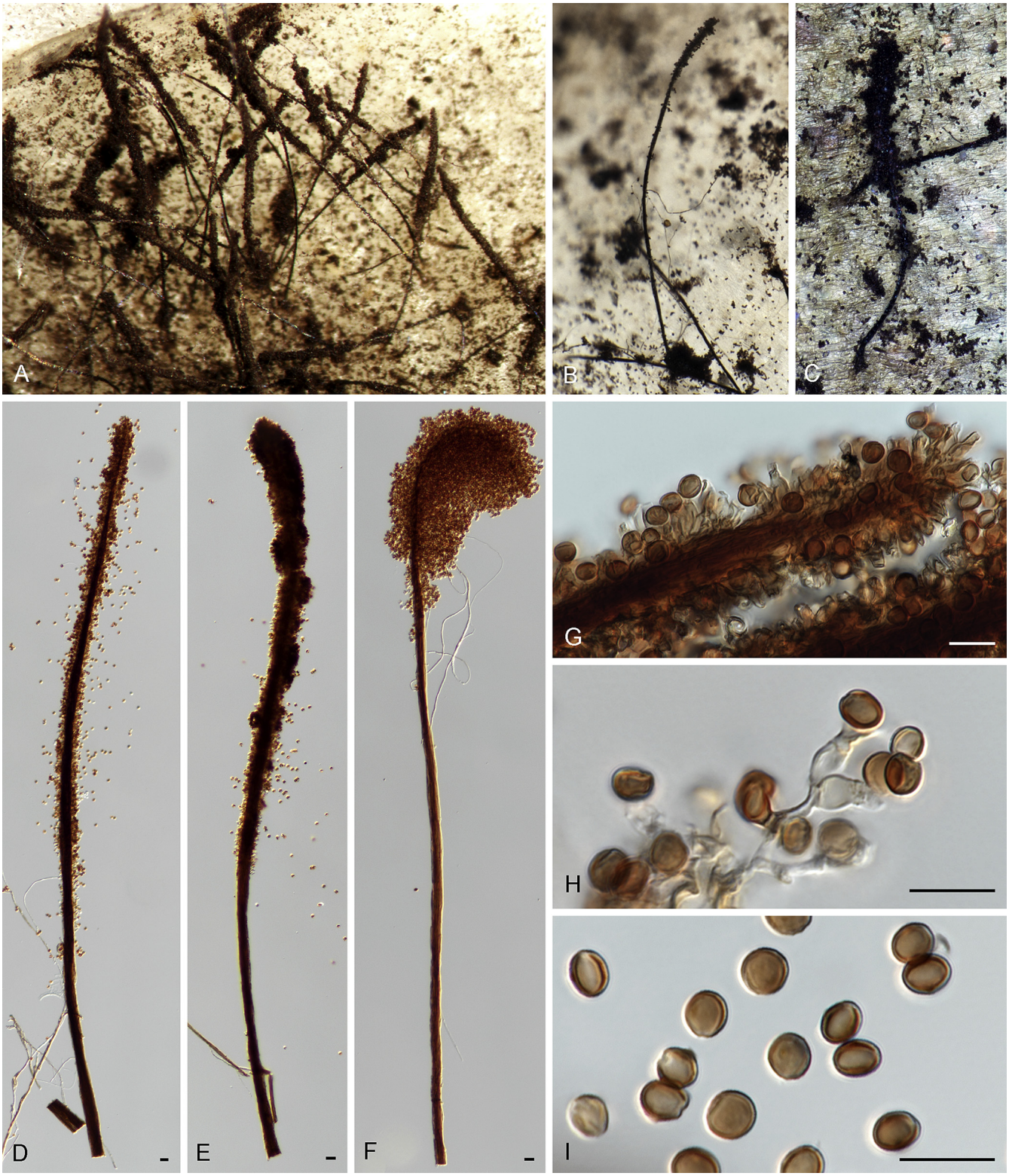


Fig. 11. *Cephalotrichum castaneum* isotype CBS H-22781. **A**–**F.** Synnemata. **G.** Detail of the apical portion of a synnema. **H.** Conidiophore, conidiogenous cells and conidia. **I.** Conidia. Scale bars = 10 µm.

C. tenuissimum. However, significant morphological differences exist among these species. The taller synnemata and conidial size and shape distinguish *C. castaneum* from *C. domesticum* and *C. tenuissimum*, while the absence of setae in the synnemata of *C. castaneum* differentiates it from *C. dendrocephalum*.

Cephalotrichum ellipsoideum H.Q. Pan & T.Y. Zhang, Mycotaxon 117: 211. 2011. Fig. 12.

Specimen examined: **China**, Qinghai Province, Maduo, dried culture isolated from grassland soil, Jun. 8. 2007, H.Q. Pan (holotype HSAUPII$_{07}$4053 → isotype CBS H-22783, additional isotype HMAS196224).

Notes: The dimensions of synnemata given in the protologue do not correlate with those observed in the type material. The length and robustness of the synnematal stipe and the shape of the conidial head are the main characters that distinguish this species from other *Cephalotrichum* spp. In *C. ellipsoideum*, the

Fig. 12. *Cephalotrichum ellipsoideum* isotype CBS H-22783. **A**–**D.** Synnemata. **E**. Detail of the apical portion of a synnema. **F.** Conidiogenous cells and conidia. **G.** Conidia. Scale bars = 10 µm.

synnemata are much more robust (<2 500 µm tall, with stipes <125 µm wide) with an obclavate and elongated conidial head, often tapering towards the apex. Other *Cephalotrichum* spp. with synnemata of similar size are *C. stemonitis* and *C. verrucisporum*; *C. ellipsoideum* differs from *C. stemonitis* by the absence of echinobotryum-like morph, and *C. verrucisporum* by its smooth conidia, in contrast to the markedly verrucose and pointed conidia of *C. verrucisporum*.

Cephalotrichum spirale H.M. Liu, H.Q. Pan & T.Y. Zhang, Mycotaxon 117: 220. 2011. Fig. 13.

Specimen examined: **China**, Qinghai Province, Dari County, dried culture isolated from grassland soil, Jun. 12. 2007, H.Q. Pan (holotype HSAUPII$_{07}$4033 → isotype CBS H-22790, additional isotype HMAS196233).

Notes: The type material contains two fungi, the synnematous *C. spirale* and a *Cladosporium* spp., the second probably a culture contaminant judging from its sparse presence.

Fig. 13. *Cephalotrichum spirale* isotype CBS H-22790. **A**–**C.** Synnemata. **D.** Detail of the apical portion of a synnema. **E.** Conidiophores, conidiogenous cells and conidia. **F.** Conidia. Scale bars = 10 µm.

Morphologically, *C. spirale* resembles *C. asperulum* and *C. nanum*, and all species have distinctly verrucose conidia. *Cephalotrichum spirale* differs from *C. asperulum* mainly by the conidial shape, with rounded apices in *C. spirale* vs. pointed apices in *C. asperulum*, and the degree of conidial roughness, which is not as pronounced in *C. asperulum*, which sometimes has conidia that are smooth. *Cephalotrichum spirale* differs from *C. nanum* by the size of its synnemata (<850 µm tall in *C. spirale* vs. <2 000 µm tall in *C. nanum*) as well as by conidial size and shape (5–7 × 3–4.5 µm, broadly ovoid to broadly ellipsoidal in *C. spirale* vs. 6–8 × 4.5–7.5 µm, subspherical to oval in *C. nanum*). *Cephalotrichum verrucisporum* is the closest relative genetically (ITS 2 nt difference, Fig. 2) but the two species are easily differentiated morphologically (see note under *C. verrucisporum* below). Note that this species is different from the well-known fungus described as *Trichurus spiralis*, but the coincidental epithets in *Cephalotrichum* led to the adoption of the later species name *C. gorgonifer* for the latter fungus.

Cephalotrichum verrucisporum (Y.L. Jiang & T.Y. Zhang) Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 223. 2011.
Synonym: *Doratomyces verrucisporus* Y.L. Jiang & T.Y. Zhang, Mycotaxon 104: 133. 2008.

Specimens examined: **China**, Guizhou Province, Guiyang, Huaxi Park, dried culture isolated from mountain soil, Oct. 6. 2005, Y.L. Jiang (holotype of *D. verrucisporus* HSAUP$_{05}$1029 → isotype CBS H-22793); **Germany**, from indoor environment, 2007, DTO 055-D7; **Netherlands**, Katwijk, from sand dune (50 cm depth), Mar. 1978, W. Gams, CBS 187.78; Wageningen, from agricultural soil, Jul. 1972, J.W. Veenbaas-Rijks, CBS 512.72.

Notes: This species was recently accepted as distinct and was illustrated by Sandoval-Denis *et al.* (2016b) on the basis of the identity of an ITS derived from the type with an available culture (CBS 187.78). Our study of type material confirms that application of this name (Fig. 2), showing this species to be a closely related lineage to *C. spirale*. Both species produce verrucose conidia. *Cephalotrichum verrucisporum* can be differentiated by its taller synnemata (<3 000 µm tall vs. <850 µm tall in *C. spirale*) and its somewhat larger and pointed conidia (6–9 × 3–5.5 µm vs. 5–7 × 3–4.5 µm, with a rounded apex in *C. spirale*). *Cephalotrichum verrucisporum* has ovoid conidia that are a bit darker than the oval to ellipsoidal pale brown conidia of *C. asperulum*, and synnemata that are longer than those of *C. asperulum*, which are usually ~1 000 µm tall (Sandoval-Denis *et al.* 2016b).

Doubtful and excluded species

Cephalotrichum acutisporum J.J Xu & T.Y. Zhang, Mycotaxon 117: 208. 2011.

Specimen examined: **China**, Fujian Province, Zhangping, dried culture isolated from soil of a park, Oct. 22. 2004, J.J. Xu (holotype HSAUPII$_{04}$2724 → isotype CBS H-22780, additional isotype HMAS196222).

Notes: This species is a synonym of *C. purpureofuscum*. Synnemata and conidiogenous cells in the isotype material are much larger than indicated in the protologue. According to our observations synnemata are (600–)640–955 µm tall, with conidiogenous cells (8.5–)9.5–10.5 × (2.5–)3–4 µm. Also, the conidial shape, originally described and illustrated as markedly pointed is not a consistent character; nearly half of the conidia observed in the isotype have rounded apices, a pattern commonly observed in *C. purpureofuscum*.

Cephalotrichum cylindrosporum Y.L. Zhang & T.Y. Zhang, Mycotaxon 117: 209. 2011.

Specimen examined: **China**, Hainan Province, Tunchang, dried culture isolated from rice field soil, Nov. 1. 2005, Y.L. Jiang (holotype HSAUPII$_{05}$2414 → isotype CBS H-22782, additional isotype HMAS196223).

Notes: The fungus on the isotype is morphologically indistinguishable from *C. purpureofuscum*. As noted by Sandoval-Denis *et al.* (2016b), this is a serious discrepancy from the ITS sequence derived from the ex-type (FJ914686), which matches the epitype of *C. stemonitis*, and hence cannot represent *C. purpureofuscum* (Fig. 2). It is probable that mislabelling or cross contamination of the original culture occurred, and the name must be regarded as a *nomen dubium*.

Cephalotrichum inflatum Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 213. 2011.

Specimen examined: **China**, Sichuan Province, Mianyang, dried culture isolated from mountain soil, Aug. 8. 2005, Y.L. Jiang (holotype HSAUPII$_{05}$0918 → isotype CBS H-22784, additional isotype HMAS196226).

Notes: This species is a synonym of *C. microsporum*. The main distinctive feature of *C. inflatum* was the presence of distinctly swollen cells at the top of the synnemata, from which the conidiogenous cells arise. When cultures were grown on PDA, this feature was also observed in our *C. microsporum* isolates. Although the ITS sequence of *C. inflatum* has 1 nt difference from the ex-type strain of *C. microsporum* (CBS 523.63), it is identical to that of *C. microsporum* strain DTO 207-C6.

Cephalotrichum longicollum Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 213. 2011.

Specimen examined: **China**, Sichuan Province, Emei Mountain, dried culture isolated from soil, Aug. 9. 2005, Y.L. Jiang (holotype HSAUPII$_{05}$0802 → isotype CBS H-22785, additional isotype HMAS196228).

Notes: This species is a synonym of *C. purpureofuscum*. The original description is inaccurate, according to our observations, with conidiogenous cells 8–11(–12.5) × (2.5–)3–4 µm, and conidia (4–)5–6 × 3–3.5(–4) µm. The isotype material contains only short synnemata 475–500 µm tall; however, synnemata of this stature are not uncommon in *C. purpureofuscum*. Morton & Smith (1963) examined isolates of *C. purpureofuscum* with reduced synnemata, as little as 50 µm tall. The morphological identity with *C. purpureofuscum* is confirmed by DNA data. The ITS sequence from the ex-type of *C. longicollum* has 1 nt difference from the reference strain of *C. purpureofuscum* (CBS 174.68, Fig. 2), but is identical to the ITS sequences of the other *C. purpureofuscum* isolates included in this study (data not shown).

Cephalotrichum macrosporum Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 214. 2011.

Specimen examined: **China**, Sichuan Province, Jiuzhaigou, dried culture isolated from forest soil, Aug. 18. 2005, Y.L. Jiang (holotype HSAUPII$_{05}$0878 → isotype CBS H-22786, additional isotype HMAS196229).

Notes: The isotype is in poor condition. Only fragments of synnema stipes and conidia can be seen, and the fungus is unidentifiable. The conidial dimensions, (4–)5.5–7.5(–10) × (2.5–)3.5–4(–4.5) µm, and unusual conidial shapes, cylindrical, irregularly cylindrical, curved or cashew-nut shaped, reflect the degraded state of the material. The ITS sequence from the ex-type has 1 nt difference with the reference strain of *C. purpureofuscum* (CBS 174.68, Fig. 2), but is identical to the ITS sequences of the other *C. purpureofuscum* isolates included in this study (data not shown). Provisionally, *C. macrosporum* seems to be a synonym of *C. purpureofuscum*. Fresh isolations of this fungus may clarify this status, or the remainder of the holotype will need to be examined to observe undamaged synnemata.

Cephalotrichum oblongum J.J. Xu & T.Y. Zhang, Mycotaxon 117: 216. 2011.

Specimen examined: **China**, Yunnan Province, Pingbian County, dried culture isolated from soil, Oct. 11. 2004, J.J. Xu (holotype HSAUPII$_{04}$2723 → isotype CBS H-22787, additional isotype HMAS196230).

Notes: This is a synonym of *C. purpureofuscum*. The isotype material contains elements of two fungi, synnemata of *C. purpureofuscum* and numerous mesoconidia and macroconidia of a *Fusarium* spp. The original description deviates from our observations of the synnemata on the type material. Synnemata are taller than reported, (305–)325–650 µm tall, the conidiogenous cells are shorter and wider, 5.5–8 × 3–4 µm, and the conidia are larger, (4.5–)5–6(–6.5) × (2.5–)3–3.5(–4) µm,

all dimensions that fit well with *C. purpureofuscum*. The ITS sequence has 1 nt difference from the reference strain of *C. purpureofuscum* (CBS 174.68, Fig. 2), but is identical to the ITS sequences of the other *C. purpureofuscum* isolates included in this study (data not shown).

Cephalotrichum ovoideum Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 217. 2011.

Specimen examined: **China**, Sichuan Province, Jiuzhaigou, dried culture isolated from forest soil, Aug. 18. 2005, Y.L. Jiang (holotype HSAUPII$_{05}$0846 → isotype CBS H-22788, additional isotype HMAS196231).

Notes: Based on the original publication, this species could be synonymised with *C. microsporum*. However, the isotype material is in poor condition. Only conidia were observed, while synnema stipes and conidial heads were not well preserved. The ITS phylogeny suggests a close affinity with both *C. microsporum* and *C. robustum*. Until the remainder of the holotype can be examined, or a fresh isolate obtained, *C. ovoideum* should be considered a provisional synonym of *C. microsporum*.

Cephalotrichum robustum Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 218. 2011.

Specimen examined: **China**, Sichuan Province, Jiuzhaigou, dried culture isolated from forest soil, Aug. 18. 2005, Y.L. Jiang (holotype HSAUPII$_{05}$0875 → isotype CBS H-22789, additional isotype HMAS196232).

Notes: This fungus morphologically resembles *C. microsporum*, *C. purpureofuscum* and *C. ovoideum*, but conclusive comparisons are impossible because of the poor condition of the isotype material (see notes on *C. ovoideum* above). The ITS phylogeny shows a relationship with *C. microsporum*, from which *C. robustum* differs morphologically by its shorter synnemata and longer conidia. This species is provisionally synonymised with *C. microsporum* until the holotype material of fresh isolations can be examined.

Cephalotrichum terricola Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 221. 2011.

Specimen examined: **China**, Sichuan Province, Panzhihua, dried culture isolated from mountain soil, Aug. 13. 2005, Y.L. Jiang (holotype HSAUPII$_{05}$0924 → isotype CBS H-22791, additional isotype HMAS196227).

Notes: This is a synonym of *C. purpureofuscum*. The isotype contains two fungi, synnemata of *Cephalotrichum* and second hyaline fungus, probably an *Aspergillus* sp. The synnemata show some differences from the original description. The conidial surface is not smooth as reported but presents some fine roughness. Most conidia are finely pointed, but conidia with rounded apices were also commonly observed. The synonymy is supported by sequence data. The ITS sequence from the ex-type of *C. terricola* has 1 nt difference with the reference strain of *C. purpureofuscum* (CBS 174.68), but is identical to ITS sequences of other *C. purpureofuscum* isolates included in this study (data not shown).

Cephalotrichum verrucipes Y.L. Jiang & T.Y. Zhang, Mycotaxon 117: 223. 2011.

Specimen examined: **China**, Sichuan Province, Jiuzhaigou, dried culture isolated from forest soil, Aug. 19. 2005, Y.L. Jiang (holotype HSAUPII$_{05}$0849 → isotype CBS H-22792, additional isotype HMAS196234).

Note: This is a synnematous *Penicillium* species closely matching morphologically with *P. clavigerum*. Apart from the reported percurrent rather than phialidic conidiogenesis, the protologue of *C. verrucipes* is more or less consistent with this synonymy.

DISCUSSION

This study presents a molecular phylogenetic study of species of the genus *Cephalotrichum*, clarifying the identity of species recently described from China, reclassifying the white synnematous species as *Acaulium album*, and confirming the identity of the species occurring in the built environment.

Cephalotrichum purpureofuscum has a worldwide distribution and is mainly isolated from soil, dung and wood (Domsch *et al.* 2007) and as noted below is also common indoors. The absence of clear diagnostic morphological characters (e.g. smooth conidia, absence of setae) can be used to identify an isolated as belonging to the *C. purpureofuscum* species complex (Sandoval-Denis *et al.* 2016a, b). The newly described *C. domesticum* morphologically resembles *C. purpureofuscum*, but molecular data can easily separate the two species, using any of the three genes studied here. All *C. domesticum* isolates were initially identified as *C. purpureofuscum* at CBS based on their morphology. Several of the recently described species from China fall into the broad concept of *C. purpureofuscum*, and are synonymised here (*C. acutisporum*, *C. longicollum*, *C. oblongum* and *C. terricola*, with *C. cylindrosporum* and *C. macrosporum* possible synonyms). *Cephalotrichum microsporum* also resembles *C. purpureofuscum* and *C. domesticum*, with smooth conidia and the lack of setae, but can be distinguished but its smaller conidia and synnemata. Morphologically, the group of species that would previously have been included in *Trichurus* are easily recognized, with two species with coiled setae being distinguished by the length of the synnemata, 500–1 000 µm for *C. gorgonifer* and <500 µm for the newly described *C. telluricum*. The two species with straight setae, *C. cylindricum* and the newly described *C. transvaalense*, are morphologically very similar but easily distinguished by DNA sequences.

All 16 phylogenetic species of *Cephalotrichum* recognised can be identified with *tef1* and *tub2* partial gene sequences. One anomaly is the strain *C. nanum* CBS 191.61, which does not group with other *C. nanum* isolates based on *tef1* (data not shown, all single gene phylogenies are submitted to TreeBase). Based on ITS alone, *C. cylindricum* and *C. transvaalense* cannot be distinguished (Fig. 2), but morphology and *tub2* and *tef1* sequences clearly differentiate them. The lack of discriminating power of ITS barcodes, in combination with the poor quality of some of the isotype herbarium specimens examined here, prevented us from conclusively characterizing four of the recently described Chinese species, leaving them as nomena dubia. This highlights the importance of depositing living material in internationally accessible culture collections for taxonomic and biodiversity studies.

In this study, five *Cephalotrichum* spp. were confirmed for the built environment, the newly described *C. domesticum*, *C. gorgonifer* (previously reported by Abbott 2000), *C. microsporum* (Prezant *et al.* 2008, Samson *et al.* 2010, Flannigan *et al.* 2011), *C. purpureofuscum* (Abbott 2000, Sandoval-Denis *et al.* 2016b), and newly reported *C. verrucisporum*. *Cephalotrichum gorgonifer* (formerly mainly known as *Trichurus spiralis*) seems to be the most common species in the indoor environment, with *C. purpureofuscum* and *C. microsporum* as other commonly

isolated species. *Cephalotrichum gorgonifer* and *C. microsporum* both have a worldwide distribution, and have mostly been isolated from cultivated soils (Domsch *et al.* 2007). They both degrade cellulose (Domsch *et al.* 2007), and the latter may decay wood (Nilsson 1973). Some isolates of *C. microsporum* also decompose xylan (Domsch & Gams 1969), and may produce extracellular keratinase capable of hydrolysing keratinous materials such as wool, hair, nails and skin (Gradišar *et al.* 2000).

Only one indoor strain of *C. verrucisporum* was isolated (DTO 055-D7), for which we unfortunately do not have more information other than that it was isolated from the indoor environment in Germany. The species is morphologically similar to *C. asperulum* and *C. spirale*, as all have rough-walled conidia. Both *C. verrucisporum* isolates were originally identified as *Doratomyces asperulus* (*C. asperulum*) at CBS. Additional isolates are necessary to determine whether *C. verrucisporum* actually occurs consistently in the indoor environment, or whether this was just an incidental, single isolation.

During the publication process of this manuscript, three new *Cephalotrichum* species are described from a carbonate cave in China (Jiang *et al.* 2017). Phylogenetic comparison places the new species *C. oligotriphicum* and *C. laeve* as sister species of *C. verrucisporum*. The new species *C. guizhouense* is closely related to *C. dendrocephalum* and our newly described species *C. tenuissimum* and *C. domesticum*. With the now up-to-date phylogeny of the genus *Cephalotrichum*, identification of (new) *Cephalotrichum* species is made much easier. We expect more *Cephalotrichum* species to be discovered and described.

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