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Polyphasic taxonomy of *Aspergillus* section *Cervini*

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Abstract: Species belonging to *Aspergillus* section *Cervini* are characterised by radiate or short columnar, fawn coloured, uniseriate conidial heads. The morphology of the taxa in this section is very similar and isolates assigned to these species are frequently misidentified. In this study, a polyphasic approach was applied using morphological characters, extrolite data, temperature profiles and partial *BenA*, *CaM* and *RPB2* sequences to examine the relationships within this section. Based on this taxonomic approach the section *Cervini* is resolved in ten species including six new species: *A. acidohumus*, *A. christenseniae*, *A. novoguineensis*, *A. subnutans*, *A. transcarpathicus* and *A. wisconsinensis*. A dichotomous key for the identification is provided.

Key words: *Ascomycetes*, *Eurotiales*, Extrolites, Multi-gene phylogeny, Subgenus *Fumigati*.

Taxonomic novelties: *Aspergillus acidohumus* A.J. Chen, Frisvad & Samson, *A. christenseniae* A.J. Chen, Frisvad & Samson, *A. novoguineensis* A.J. Chen, Frisvad & Samson, *A. subnutans* A.J. Chen, Frisvad & Samson, *A. transcarpathicus* A.J. Chen, Frisvad & Samson, *A. wisconsinensis* A.J. Chen, Frisvad & Samson.

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INTRODUCTION

The section *Cervini* (Gams *et al.* 1985) of the genus *Aspergillus* includes species with radiate or short columnar, fawn coloured, uniseriate conidial heads. Phylogenetic analysis of multilocus sequence data showed that section *Cervini* belongs to *Aspergillus* subgenus *Fumigati* together with sections *Fumigati* and *Clavati* (Peterson 2008, Peterson *et al.* 2008).

Christensen *et al.* (1964) assigned four species to this section: *A. cervinus*, *A. kanagawaensis*, *A. nutans* and *A. parvulus*. Based on morphological similarities Samson (1979) proposed that *A. bisporus* described by Kwon-Chung & Fennell (1971) also belongs to section *Cervini*. However, molecular studies revealed that *A. bisporus* is distantly related to this section, and belongs to subgenus *Nidulantes*, section *Bispori* (Peterson 2000, 2008, Peterson *et al.* 2008, Chen *et al.* 2016). Udagawa *et al.* (1993) described *A. vinosobubalinus* in Japan belonging to this section and until now only five species are reported. Isolates assigned to section *Cervini* are frequently misidentified because they are morphologically similar.

Members of section *Cervini* are economically less important and not well-studied. *Aspergillus cervinus* has originally been isolated from African soil (Massee 1914), later it was also found in soil in New Zealand (Neill 1939, di Menna *et al.* 2007), Malaysia and USA (Christensen & Fennell 1964, Christensen *et al.* 1964). This taxon was found to produce the quinol derivative terremin and 3,6-dihydroxy-2,5-toluquinone (Elsohly *et al.* 1974). These authors stated that while the compound terremin showed a relationship with *A. terreus*, 3,6-dihydroxy-2,5-toluquinone indicated a relationship to *A. fumigatus*. *Aspergillus kanagawaensis* was originally isolated from soil in Japan (Nehira 1951) and later also found in soil in Wisconsin, USA (Christensen *et al.* 1964), Ukraine and Russia (Ushakova *et al.*

1974, Buiak *et al.* 1978), and on oak stumps in Poland (Kwasna 2001). This species secretes a range of proteases which have been studied in detail (Ushakova *et al.* 1974, Buiak *et al.* 1978, Landau *et al.* 1980), and also exhibits entomopathogenic properties against mosquito larvae (de Moraes *et al.* 2001). *Aspergillus parvulus* was originally isolated from soil in USA (Smith 1961), but also identified in feed ingredients in Argentina (Magnoli *et al.* 1998). This species has been found to exhibit a wide spectrum of antibiotic activities against a range of bacteria (Tsyganenko & Zaichenko 2004a), and phytotoxic activities (Tsyganenko & Zaichenko 2004b). This species has been found to produce parvulenone (Chao *et al.* 1979), naphthalenone (Bartman & Campbell 1979) and asparvenone derivatives (Bös *et al.* 1997). *Aspergillus nutans* was originally found in soil in Australia (McLennan *et al.* 1954), later in soil in Wisconsin, USA and South Africa (Christensen *et al.* 1964, Wicklow and Wittingham 1974), it was also reported to produce terremin (Phoebe *et al.* 1978). *Aspergillus vinosobubalinus* was isolated from a sweet flag bed in Shizuoka Prefecture, Japan (Udagawa *et al.* 1993) and it is characterised by sectional features as pinkish fawn, radiate, uniseriate conidial heads. However the ex-type culture CBM BF-33501 is unavailable for the further examination. Species of section *Cervini* have not been found to be important human pathogens, however Hubka *et al.* (2012) reported an isolate (closely related to *A. parvulus*) as the possible cause of human onychomycosis.

In this study, we examined the available isolates of the species belonging to *Aspergillus* section *Cervini* to clarify their taxonomic status. The methods used include phylogenetic analysis using internal transcribed spacer region (ITS), β -tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*), macro- and micro-morphological analysis, examination of temperature and extrolite profiles.

MATERIALS AND METHODS

Fungal strains

Strains used in this study were obtained from CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands; IBT, culture collection of the DTU Systems Biology, Lyngby, Denmark; CGMCC, China General Microbiological Culture Collection Centre, Beijing, China and DTO, working collection of the Applied and Industrial Mycology department housed at CBS-KNAW. An overview of strains is listed in [Table 1](#).

Morphological examinations

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 (DG 18), Oatmeal agar (OA) and Malt Extract agar (MEA, Oxoid malt) ([Samson et al. 2010](#)). To enhance the growth, the ex-type culture of *A. acidohumus* CBS 141577, isolated from acid soil from China, was additionally inoculated on Cherry Decoction agar (CHA) ([Crous et al. 2009](#)). The isolates were inoculated at three points on 90 mm Petri dishes and incubated for 7 d at 25 °C in darkness. In addition, CYA and MEA plates were incubated at 30 °C and 37 °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 determined. Light microscope preparations were made from 1 wk old colonies grown on MEA. Lactic acid (60 %) was used as mounting fluid. Ethanol (96 %) was used to remove excess conidia and prevent air bubbles. 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.

Analysis for secondary metabolites

The cultures were analysed according to the HPLC-diode array detection method of [Frisvad & Thrane \(1987, 1993\)](#) as modified by [Smedsgaard \(1997\)](#). The isolates were analysed on CYA and YES agar using three agar plugs ([Smedsgaard 1997](#)). The secondary metabolite production was confirmed by identical UV spectra with those of standards and by comparison to retention indices and retention times in pure compound standards.

DNA extraction, PCR amplification and sequencing

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

Data analysis

Sequence alignments were generated with MAFFT v. 7 ([Katoh & Standley 2013](#)). The most suitable substitution model was

determined based on Akaike Information Criterion (AIC) using FindModel ([Posada & Crandall 1998](#)). Maximum likelihood (ML) analyses including 500 bootstrap replicates were run using RAxML BlackBox web-server (Gamma model of rate heterogeneity) ([Stamatakis et al. 2008](#)). Bayesian analyses were performed with MrBayes v. 3.1.2 ([Ronquist & Huelsenbeck 2003](#)). A Markov Chain Monte Carlo (MCMC) algorithm of four chains was initiated in parallel from a random tree topology with a heating parameter set at 0.2. The MCMC analyses lasted until the average standard deviation of split frequencies were below 0.01. The sample frequency was set to 100 and the first 25 % of trees were removed as burn-in. *Aspergillus fumigatus* (CBS 133.61^T) was chosen as outgroup. The resulting trees were obtained with FigTree v1.4.2 and annotated using Adobe Illustrator CS5. Bayesian inference (BI) posterior probabilities (pp) values and bootstrap (bs) percentages of ML analysis are labelled at the nodes. Values less than 0.95 pp and less than 70 % bs are not shown. Branches with values more than 1 pp and 95 % bs are thickened. Newly obtained sequences were deposited in GenBank.

RESULTS AND DISCUSSION

Phylogenetic and morphological species recognition

The ITS sequences of section *Cervini* isolates do not contain sufficient variation for distinguishing the species. *Aspergillus acidohumus* is the only member that can be identified using ITS sequence, *A. cervinus*, *A. kanagawaensis*, *A. novoguineensis*, *A. nutans*, *A. parvulus*, *A. transcarpathicus* and *A. wisconsinensis* share identical ITS sequences, while *A. subnutans*, *A. christenseniae* show small difference with these seven species (99.8 % similarity, 427/428 bp). Therefore we examined the genetic relatedness using concatenated sequence data of three loci, *BenA*, *CaM* and *RPB2*, the aligned data set had a total length of 1 722 bp (*BenA*, 411 bp; *CaM*, 434 bp and *RPB2* 867 bp). The Maximum likelihood analyses including 500 bootstrap replicates were run using RAxML. For Bayesian analyses, the Kimura 2-parameter with gamma distributed (K2P+G) model was used for *BenA* and *RPB2*, while the General time reversible with gamma distributed (GTR+G) model was used for *CaM*. Based on multi-gene phylogenetic analysis, ten different clades are identified in *Aspergillus* section *Cervini* ([Fig. 1](#)). Four of these, *A. cervinus*, *A. parvulus*, *A. nutans*, *A. kanagawaensis*, have been described previously, while six others represent new species.

CaM performs well as the secondary identification maker for the identification of *Aspergillus* strains ([Peterson 2008](#), [Samson et al. 2014](#)). In section *Cervini*, all the ten species treated here have unique *CaM* sequences ([Fig. 2](#)). The *BenA* and *RPB2* data sets resulted in similar species delimitation ([Figs 3 and 4](#)).

[Peterson \(2008\)](#) studied the phylogenetic relationships within *Aspergillus* based on *BenA*, *CaM*, ITS, LSU rDNA (ID) and *RPB2*, and found that section *Cervini* formed a sister clade to sections *Fumigati* and *Clavati*. The section *Cervini* branch contained the four species placed in the group by [Raper and Fennel \(1965\)](#) and two additional lineages. These two lineages are also well resolved in our phylogeny, lineage NRRL 4897 (= CBS 122.56) together with other two strains (CBS 411.64 and CBS 122715) are described as *A. christenseniae* sp. nov., while lineage NRRL 2161 (= CBS 123896) and NRRL 5027 (= CBS

Table 1. Strains used in this study.

Species	Strain no.	Source	GenBank accession nr.			
			ITS	<i>BenA</i>	<i>CaM</i>	<i>RPB2</i>
<i>Aspergillus acidohumus</i>	CBS 141577 ^T = CGMCC3.18217 = DTO 340-H1 = IBT 34346	Acid soil, Guizhou, China	KX423646	KX423623	KX423634	KX423663
<i>A. cervinus</i>	CBS 537.65 ^T = DTO 054-D5 = ATCC 16915 = IBT 22087 = IMI 126542 = NRRL 5025 = QM 8875 = WB 5025	Soil, tropical rain forest, near Kuala Lumpur, Malaysia	EF661268	EF661251	EF661261	EF661229
	CBS 196.64 = ATCC 15508 = IMI 107684 = NRRL 3157 = IBT 22044 = WB 5026	Soil, tropical rain forest, near Kuala Lumpur, Malaysia	EF661270	EF661250	EF661260	EF661228
<i>A. christenseniae</i>	CBS 122.56 ^T = DTO 022-C8 = IBT 22043 = IBT 23735 = IMI 343732 = NRRL 4897 = WB 4897	Soil, Rietvlei, Pretoria, South Africa	FJ491613	FJ491639	FJ491608	EF661235
	CBS 411.64 = IBT 22076	Soil under <i>Quercus</i> sp., Wisconsin, USA	FJ491615	FJ491642	FJ491607	–
	CBS 122715 = DTO 022-A8 = DTO 349-F2 = IBT 29311	Soil, Atherton Tableland near little Malgrave, Queensland, Australia	FJ491612	FJ491644	FJ491594	KX423664
<i>A. kanagawaensis</i>	CBS 538.65 ^T = DTO 054-F3 = ATCC 16143 = IBT 22077 = IFO 6219 = IMI 126690 = NRRL 4774 = WB 4774	Soil, Kanagawa, Japan	FJ491617	FJ491640	FJ491597	JN121531
	NRRL 35642 = DTO 069-D6 = IBT 34350	Upland hardwood forest soil, Sumter County, Georgia, USA	EF661276	EF661240	EF661264	EF661237
	CBS 129333 = DTO 202-A5 = IBT 34351	Unknown	KX423647	KX423624	KX423637	KX528452
<i>A. novoguineensis</i>	CBS 906.96 ^T = DTO 021-G5 = IBT 29312	Humus, Papua New Guinea	FJ491622	FJ491641	FJ491605	KX423681
<i>A. nutans</i>	CBS 121.56 ^T = DTO 054-D3 = NRRL 575 = NRRL 4364 = NRRL A-6280 = ATCC 16914 = IFO 8134 = IMI 062874ii = IMI 62874 = QM 8159 = WB 4364 = WB 4546 = WB 4776	Soil, Australia	EF661272	EF661249	EF661262	EF661227
	CBS 122714 = DTO 349-F1 = IBT 29313	Soil, Barron Falls, Australia	FJ491614	FJ491630	FJ491600	KX423665
<i>A. parvulus</i>	CBS 136.61 ^T = DTO 021-G8 = IBT 22085 = ATCC 16911 = IMI 086558 = LSHB BB405 = NRRL 1846 = NRRL 4753 = QM 7955 = UC 4613 = WB 4753	Forest soil, USA	EF661269	EF661247	EF661259	EF661233
	CBS 412.64 = NRRL 5023 = WB 5023	Soil under <i>Pinus banksiana</i> , Wisconsin, USA	EF661274	EF661246	EF661256	EF661231
	CBS 262.67 = IBT 22079	Agricultural soil, Mansholtlaan, Wageningen, the Netherlands	KX423653	FJ491628	FJ491599	KX423668
	CBS 298.71 = IBT 22088 = IMI 151275	Soil, North Carolina, USA	FJ491616	FJ491635	FJ491602	KX423670
	CBS 123897 = NRRL 4220 = IBT 22039 = DTO 070-A4	Jackpine (<i>Pinus banksiana</i>) coniferous forest soil, Wisconsin, USA	KX423657	KX423625	KX423638	KX423666
	CBS 133109 = IBT 22046 = WB 4994 = DTO 070-A1 = NRRL A-3096	Pine forest soil, Scotland, UK	EF661267	EF661248	EF661257	EF661232
	DTO 189-G7 = IBT 34355	Soil, the Netherlands	KX423654	KX423627	KX423639	–
	CBS 133098 = NRRL 2667 = NRRL 5028 = IBT 22045 = WB 5028	Soil, Georgia, USA	EF661271	EF661244	EF661258	EF661234
<i>A. subnutans</i>	CBS 129386 ^T = DTO 202-C2 = WSF 445 = IBT 34352	Soil under <i>Tsuga canadensis</i> , Wisconsin, USA	KX528456	KX528454	KX528455	KX528453
<i>A. transcarpathicus</i>	CBS 423.68 ^T = DTO 022-C7 = IBT 22080 = IMI 134108 = VKM F-1331	Transcarpathia, Ukraine	FJ491624	FJ491632	FJ491610	KX423680
	CBS 410.64 = IBT 22086 = UPSC 3141 = WSF 5750	Sandy soil, of <i>Salix nigra</i> community, Wisconsin, USA	FJ491611	FJ491643	FJ491593	KX423678
	CBS 424.68 = IBT 22081	Forest soil, Zacarpathian region, Ukraine	FJ491626	FJ491631	FJ491603	KX423679
<i>A. wisconsinensis</i>	CBS 413.64 ^T = DTO 022-B1 = NRRL 5027 = IBT 22042 = IBT 22082 = WSF 380 = DTO 070-A5 = WB 5027	Soil under <i>Tsuga canadensis</i> , Wisconsin, USA	FJ491618	FJ491638	FJ491609	KX423671
	CBS 129387 = DTO 202-C3 = IBT 34347	Soil, Wisconsin, USA	KX423649	KX423633	KX423641	KX423673
	CBS 129400 = DTO 202-D7 = IBT 34348	Soil, Wisconsin, USA	KX423652	KX423630	KX423643	KX423674
	CBS 126265 = DTO 195-E2 = IBT 34346	Soil, Stephen Foster State Park, Florida, USA	KX423651	KX423632	KX423644	KX423675
	CBS 127024 = DTO 196-F2 = IBT 34349	Soil under <i>Tsuga canadensis</i> , Wisconsin, USA	KX423648	KX423629	KX423645	KX423672
	CBS 123896 = NRRL 2161 = IBT 22041 = DTO 070-A3	Soil, Australia	KX423656	KX423628	KX423640	KX423676

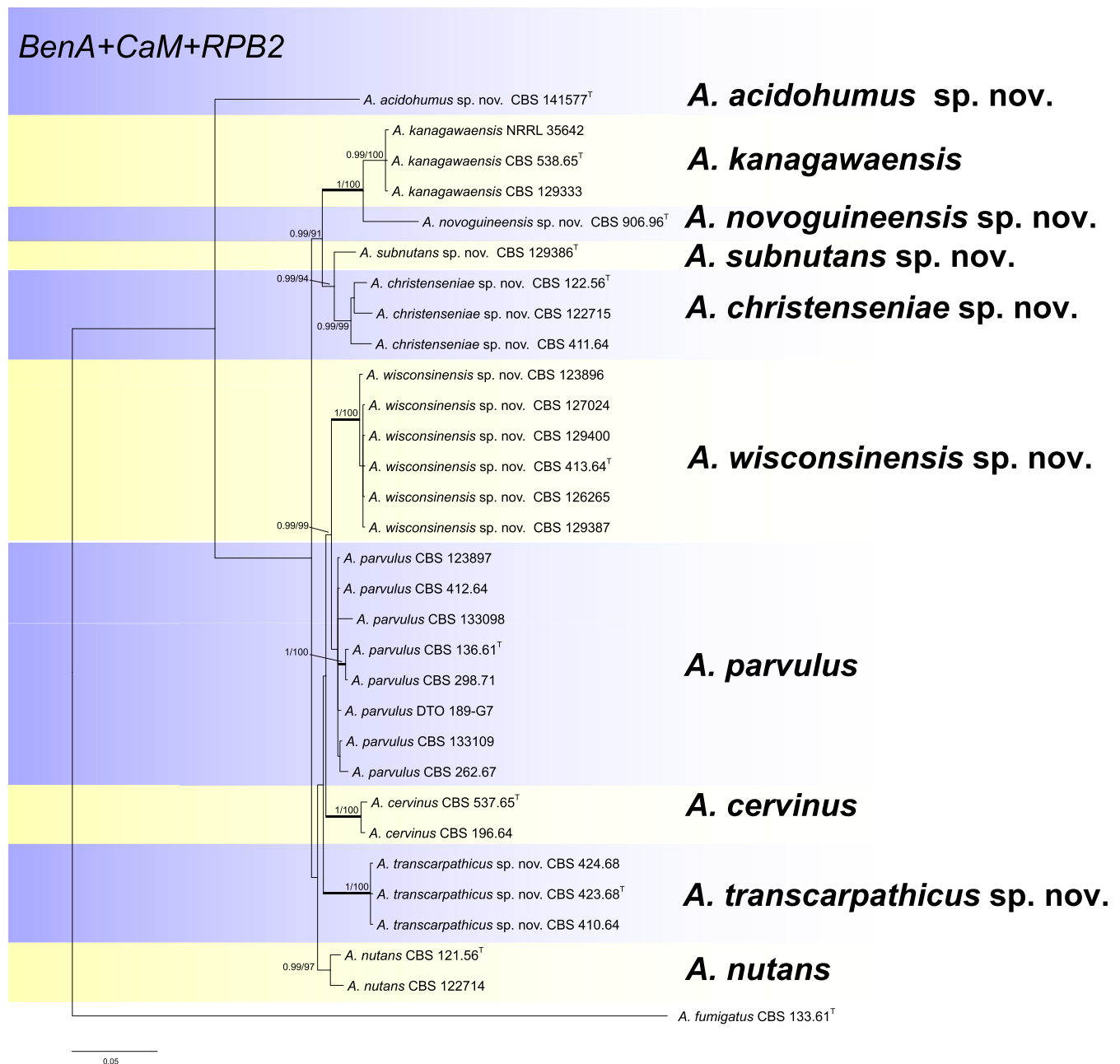


Fig. 1. Phylogenetic tree of section *Cervini* inferred from concatenated loci (*BenA*, *CaM* and *RPB2*). Branches with values more than 1 pp and 95 % bs are thickened. The phylogram is rooted with *Aspergillus fumigatus* (CBS 133.61^T).

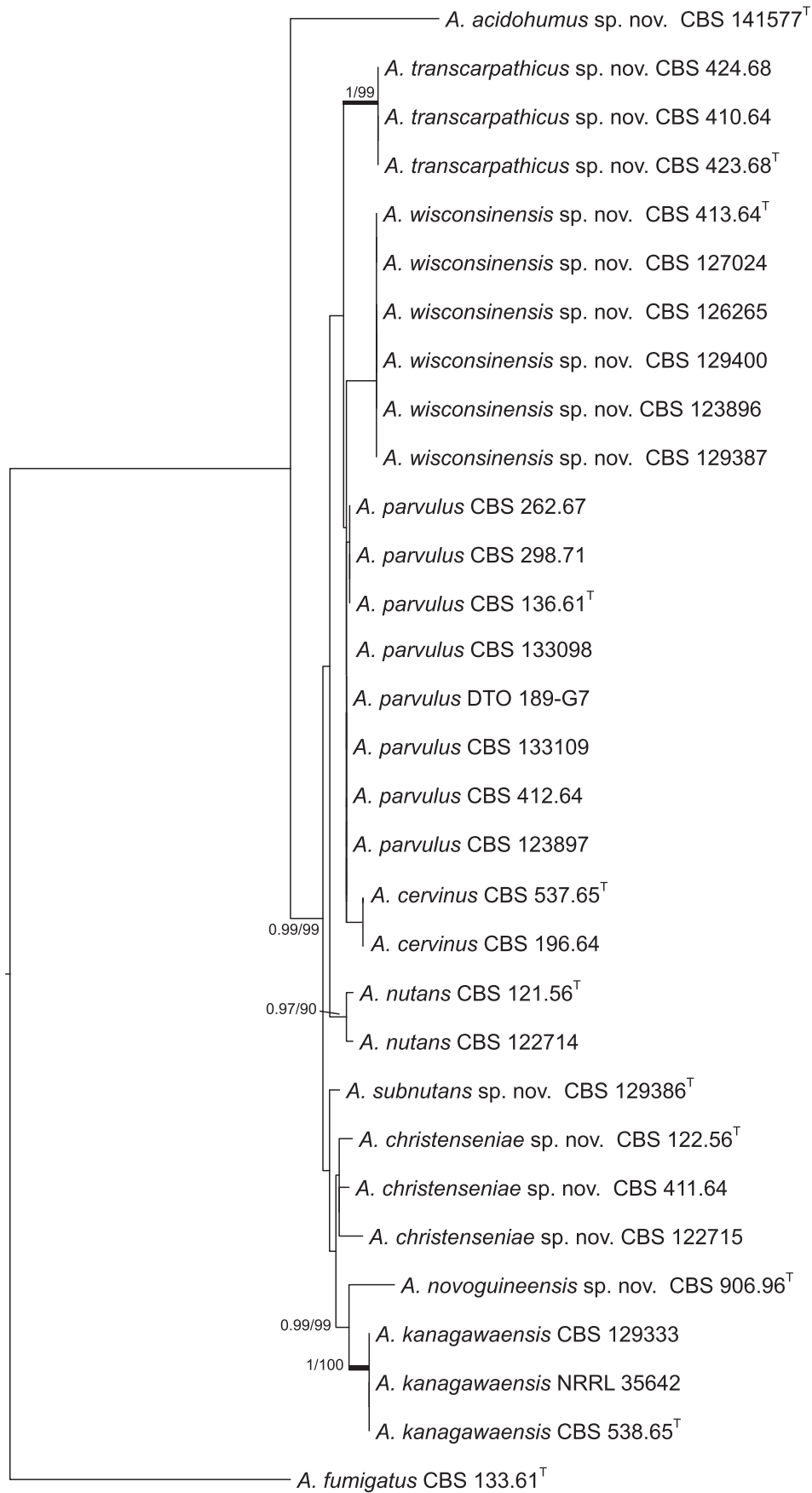
413.64) together with other four strains (CBS 127024, CBS 129387, CBS 126265 and CBS 129400) are described as *A. wisconsinensis* sp. nov.

Phylogenetically related species have morphological similarities, but there are some exceptions in section *Cervini*. For example, *A. parvulus* is phylogenetically related to *A. wisconsinensis*, *A. cervinus* and *A. transcarpathicus*, but morphologically this species has short conidiophores (< 100 µm) which resemble *A. nutans*, *A. christenseniae* and *A. subnutans* (Table 2). *Aspergillus wisconsinensis*, *A. cervinus* and *A. transcarpathicus* are phylogenetically and phenotypically closely related. These three species share the character of 100–300 µm long conidiophores, but there are small morphological differences within these three species. Thus *CaM* sequence analysis is recommended to facilitate species identification. *Aspergillus kanagawaensis* and *A. novoguineensis* form a well-supported lineage (100 % ML, 1 pp, Fig. 1). These two species produce extremely variable but long (100–800 µm)

conidiophores and they differ from each other by the growth profile at high temperature; *A. kanagawaensis* grows on CYA and MEA at 37 °C, while *A. novoguineensis* does not grow or grows restrictedly under the same condition (Table 2).

Aspergillus nutans is resolved in a separate branch and morphologically it resembles *A. christenseniae* and *A. subnutans*. All of these three species produce short conidiophores; *A. christenseniae* is characterised by subglobose to ellipsoidal conidia, while *A. subnutans* has upright and uncoloured conidial heads instead of strongly pigmented, nodding heads in *A. nutans*. *Aspergillus acidohumus*, isolated from acid soil from China, occupies a basal position in section *Cervini* without statistical support. This species grows very restrictedly on MEA, CYA, YES and OA. It grows better on CHA media (pH 4.7), but not on CREA, CYAS and DG 18. The species is characterised by extremely compact orange brown conidial heads and tightly connected conidia which have not been observed in other species of section *Cervini*.

CaM



0.09

Fig. 2. Phylogenetic tree of section *Cervini* inferred from CaM. Branches with values more than 1 pp and 95 % bs are thickened. The phylogram is rooted with *Aspergillus fumigatus* (CBS 133.61^T).

RPB2

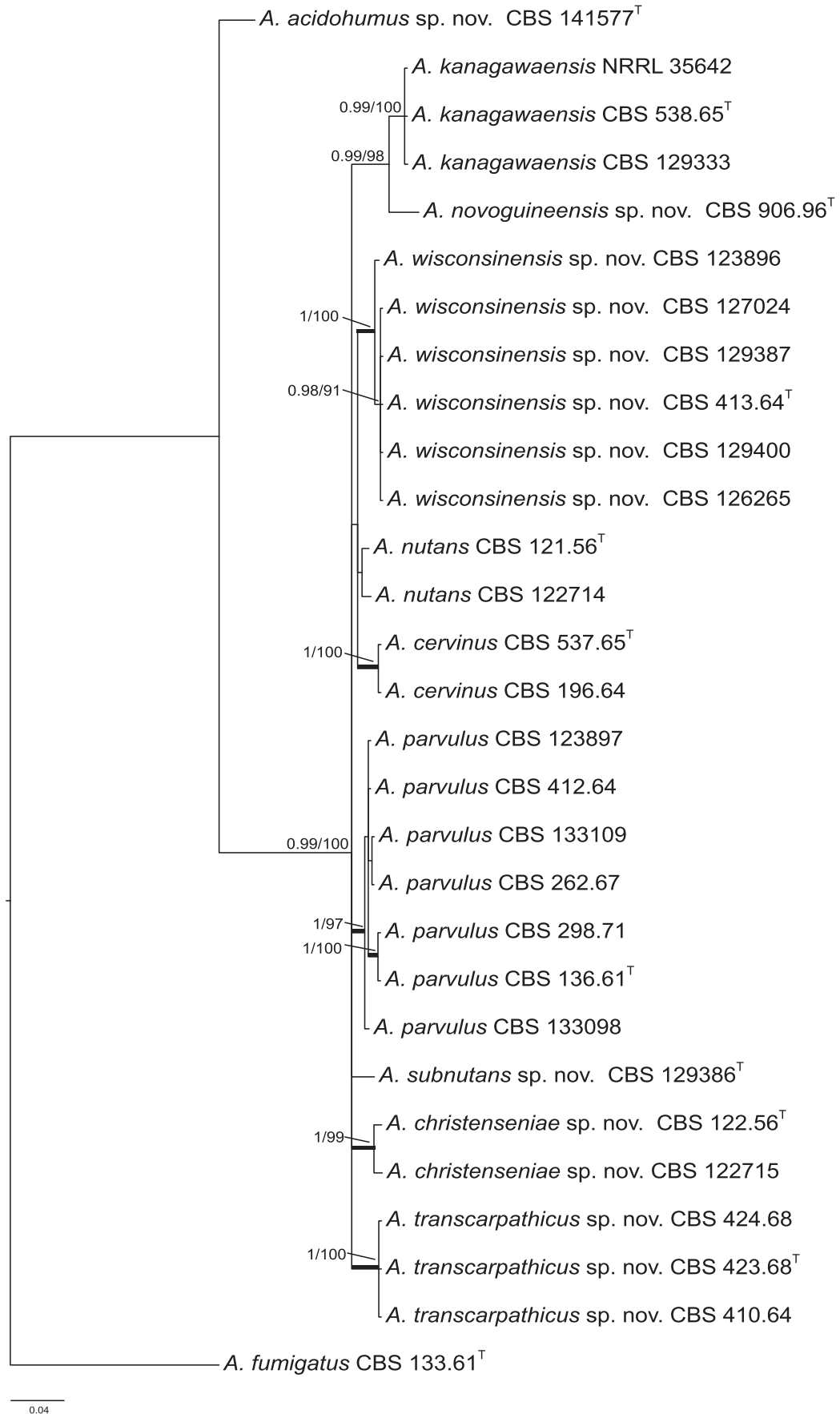


Fig. 3. Phylogenetic tree of section *Cervini* inferred from RPB2. Branches with values more than 1 pp and 95 % bs are thickened. The phylogram is rooted with *Aspergillus fumigatus* (CBS 133.61^T).

Extrolites

Isolates of *Aspergillus* species usually produce a diverse range of secondary metabolites that are characteristic of the different sections of *Aspergillus*. In many cases extrolite profiles are species specific, which are very useful in identification of unknown species (Frisvad *et al.* 2004, 2011, Frisvad & Larsen 2015, Varga *et al.* 2011, Samson *et al.* 2014).

An overview of extrolites produced by *Cervini* species is provided in Table 3. Based on our results and other studies, several species in *Aspergillus* section *Cervini* produce terremitin, a precursor of the antibiologically active extrolite terreic acid (Guo *et al.* 2014, Sharma *et al.* 2016). Producers of terremitin include *A. parvulus*, *A. nutans*, *A. cervinus*, *A. transcarpathicus*, *A. novoguineensis* and *A. christenseniae*, and were formerly identified as the two first mentioned species (Elsohly *et al.* 1974, Phoebe *et al.* 1978). Since the soil-borne species *A. terreus* produces terremitin and terreic acid, it seems reasonable to speculate that terreic acid is an important antibiotic agent in soil, as members of *Aspergillus* section *Cervini* are also soil-borne. Soil-borne isolates of *A. fumigatus* are also known to produce fumigatin oxide, a compound very similar to terreic acid (Frisvad *et al.* 2009), indicating a relationship of section *Cervini* to section *Fumigati*. Another metabolite indicating the relationship to section *Terrei* is territrems, produced by *A. terreus* (Ling *et al.* 1984, Nong *et al.* 2014). The compound is reported here for the first time in *A. wisconsinensis* in section *Cervini*. The territrems are related to the heteroisoextrolites pyripropens from section *Fumigati* (Frisvad & Larsen 2015, 2016).

A strain identified as *A. cervinus* was reported to produce penicillic acid, 4R*,5S*-dihydroxy-3-methoxy-5-methylcyclohex-2-enone and 6-methoxy-5-dihydropenicillic acid (He *et al.* 2004). Unfortunately this strain is not available for study, but it may also be misidentified and be a member of *Aspergillus* section *Circumdati* containing several known producers of penicillic acid (Visagie *et al.* 2014).

The isolation of the antiinsectan extrolite sclerotigenin from *A. novoguineensis* and *A. wisconsinensis* is the first report of this compound from *Aspergillus*, as it was first found in *Penicillium* species (Joshi *et al.* 1999, Larsen *et al.* 2000). However the similar heteroisoextrolite auranthine was isolated from *A. lentulus* in section *Fumigati* (Larsen *et al.* 2007), again showing some chemosystematic similarities to section *Fumigati*.

The asparvenones are produced by *A. parvulus*, *A. transcarpathicus*, *A. novoguineensis* and *A. wisconsinensis*. These compounds have also been isolated from the unrelated fungi *Botryosphaeria australis* (Xu *et al.* 2011) and a *Kirschsteinothelia* species (Poch *et al.* 1992). One of the asparvenones (Chao *et al.* 1975, 1976, 1979), O-methylasparvenone, has been reported to be a serotonin antagonist (Bös *et al.* 1997). The asparvenones also have other promising biomedical properties (Poch *et al.* 1992, Xu *et al.* 2011).

TAXONOMY

Aspergillus acidohumus A.J. Chen, Frisvad & Samson, sp. nov. MycoBank MB817723. Fig. 5.

Etymology: Name refers to its origin, isolated from acid soil from China.

Diagnosis: *Aspergillus acidohumus* grows very restrictedly on MEA, CYA, YES and OA, produces extremely compact orange brown conidial heads and tightly connected conidia, these characters can easily distinguish this species from other section *Cervini* members.

Typus: China, Guizhou, soil, 2014, isolated by X.Z. Jiang (holotype CBS H-22730, culture ex-type CBS 141577 = CGMCC3.18217 = DTO 340-H1 = IBT 34346).

ITS barcode: KX423646. (Alternative markers: *BenA* = KX423623; *CaM* = KX423634; *RPB2* = KX423663).

Colony diam, 7 d (mm): CYA weak growth; CYA 30 °C No growth; CYA 37 °C No growth; MEA 18–19; MEA 30 °C 16–17; MEA 37 °C No growth; OA 6–7; YES 4–5; CREA No growth; CYAS No growth; DG18 No growth; CHA 21–22.

Colony characters: CYA 25 °C, 7 d: weak growth. MEA 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* dark fawn; soluble pigments absent; exudates absent; reverse yellowish brown. YES 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture floccose; sporulation absent; soluble pigments absent; exudates absent; reverse cream white. DG18 25 °C, 7 d: No growth. OA 25 °C, 7 d: Colonies low, plane; margins irregular; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* dark fawn to brown; soluble pigments olive; exudates absent; reverse olive. CREA 25 °C, 7 d: No growth. On CHA growth was better than on MEA but with less sporulation.

Micromorphology: Conidial heads radiate, extremely compact; conidiophores erect, walls smooth, 40–85 (–140) × 6.5–8.5 µm; vesicles orange brown coloured, globose, 17–23 µm wide, fertile over the entire vesicles; uniseriate, phialides coloured as vesicles, flask-shaped to cylindrical, 5–6.5 × 2–2.5 µm. Conidia globose, connected with each other, smooth, 3.5–4.5 µm. Ascospores not produced.

Aspergillus cervinus Masee, Bull. Misc. Inform. Kew 1914: 158. 1914. MycoBank MB211549. Fig. 6.

Typus: WT 540, culture ex-type: CBS 537.65 = DTO 054-D5 = ATCC 16915 = IBT 22087 = IMI 126542 = NRRL 5025 = QM 8875 = WB 5025.

ITS barcode: EF661268. (Alternative markers: *BenA* = EF661251; *CaM* = EF661261; *RPB2* = EF661229).

Colony diam, 7 d (mm): CYA 26–27; CYA 30 °C 25–26; CYA 37 °C No growth; MEA 59–60; MEA 30 °C 58–60; MEA 37 °C No growth; OA 35–40; YES 19–20; CREA No growth; CYAS No growth; DG18 21–23.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* light yellow to fawn; soluble pigments absent; exudates absent; reverse cream white. MEA 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire;

BenA

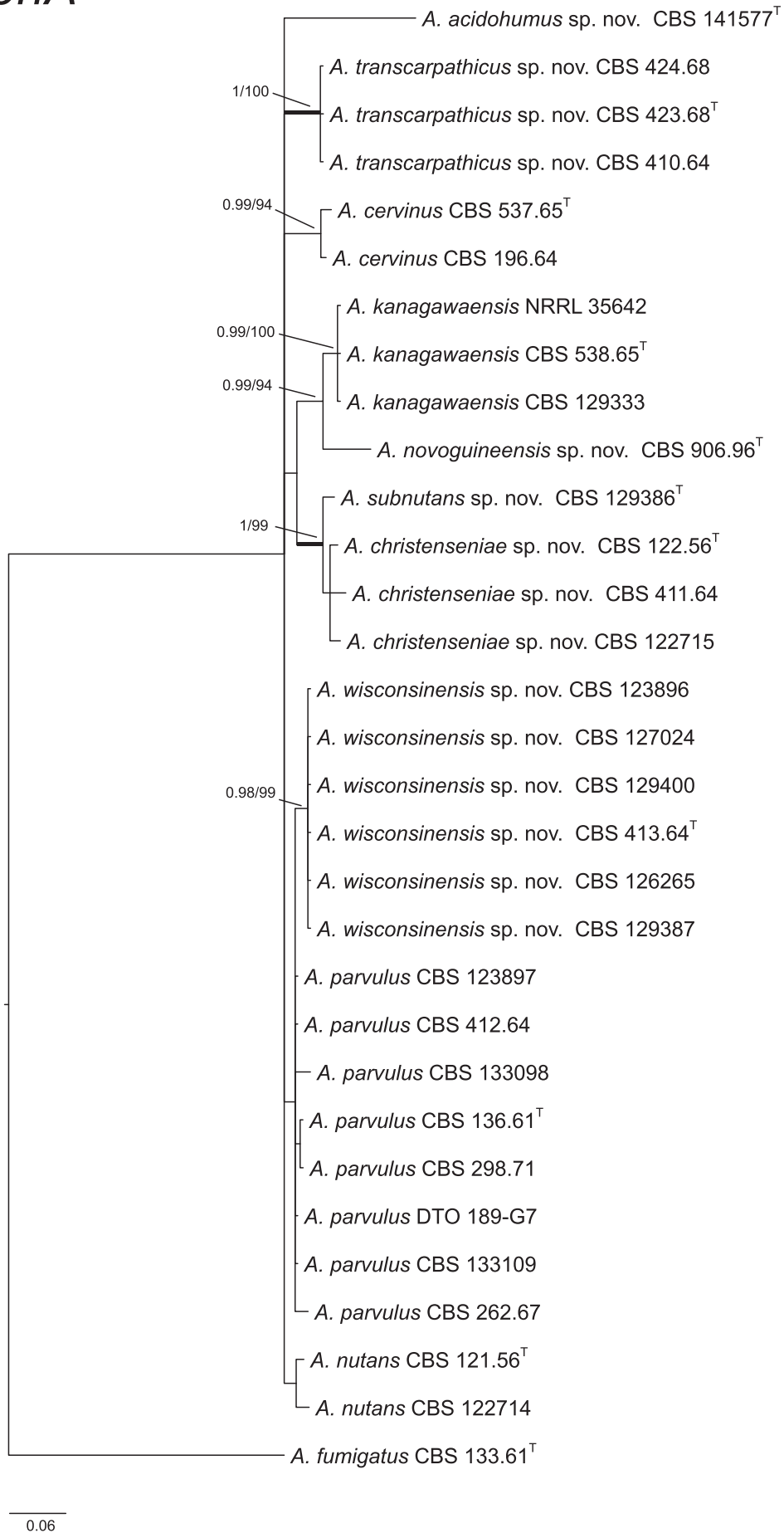


Fig. 4. Phylogenetic tree of section *Cervini* inferred from *BenA*. Branches with values more than 1 pp and 95 % bs are thickened. The phylogram is rooted with *Aspergillus fumigatus* (CBS 133.61^T).

Table 2. Most important morphological characters for species recognition in *Aspergillus* section *Cervini*.

Species	Macromorphology (7 d, in mm)						Micromorphology				
	CYA 25 °C	CYA 30 °C	CYA 37 °C	MEA 25 °C	MEA 30 °C	MEA 37 °C	Conidial heads	Conidiophores	Vesicles	Phialides	Conidia
<i>Aspergillus acidohumus</i>	Weak growth	No growth	No growth	18–19	16–17	No growth	Radiate	40–85 (–140) × 6.5–8.5	Globose, 17–23	5–6.5 × 2–2.5	Globose, 3.5–4.5
<i>A. cervinus</i>	26–27	25–26	No growth	59–60	58–60	No growth	Radiate	100–300 × 5–8	Globose, 15–20	5–6.5 × 2.5–3.5	Globose, 2.5–4
<i>A. christenseniae</i>	20–29	15–25	No growth	42–55	37–52	No or weak growth	Short columnar	8–45 × 3.5–5.5	Subclavate, 8–10.5	3.5–5 × 2–3.5	Subglobose to ellipsoidal, 4–4.5 × 3–3.5
<i>A. kanagawaensis</i>	17–20	20–24	10–14	45–55	51–62	14–25	Radiate	100–800 × 5–7.5	Globose, 20–25	5.5–7.5 × 3–3.5	Globose, 2.5–3.5
<i>A. novoguineensis</i>	20–21	19–21	No growth	37–39	40–43	No or weak growth	Radiate	210–550 × 5–7	Globose, 18–26	5–8 × 2.5–3.5	Globose, 2.5–3.5
<i>A. nutans</i>	15–16	17–19	Weak growth	38–42	46–47	6–8	Short columnar	25–80 × 2–4	Subclavate, 5–10	3.5–6 × 2–3	Globose, 2.5–3.5
<i>A. parvulus</i>	22–23	23–24	No growth	35–46	50–51	5–6	Radiate	17–75 × 2.5–3.5	Mainly globose, sometimes subclavate, 5–11	4–7 × 2–3	Globose, 2.5–4
<i>A. subnutans</i>	17–18	17–18	4–6	34–35	32–33	10–11	Short columnar	25–50 × 3.5–5	Subclavate, 7–13	5.5–7 × 2.5–3	Globose, 3–4
<i>A. transcarpathicus</i>	14–20	11–24	4–10	44–60	37–60	12–18	Radiate	100–150 × 5.5–7.5	Globose, 15–20	4.5–6.5 × 2.5–3	Globose, 3–4
<i>A. vinosobubalinus</i> ¹	85			63–64			Radiate, splitting into columns in age	550–1200 × 10–12.5	Globose to elongate, 30–45	5–7.5 (–10) × 2.5–3 (–5)	Globose to subglobose, 3–4.5 × 3–4
<i>A. wisconsinensis</i>	13–17	11–16	No growth	51–53	40–43	No growth	Radiate	100–200 × 4–7	Subclavate to globose, 14–20	5–7 × 2.5–3.5	Globose, 3–4.5

¹ Data derived from Udagawa *et al.* (1993).

mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates clear droplets; reverse yellowish brown to reddish brown. YES 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium

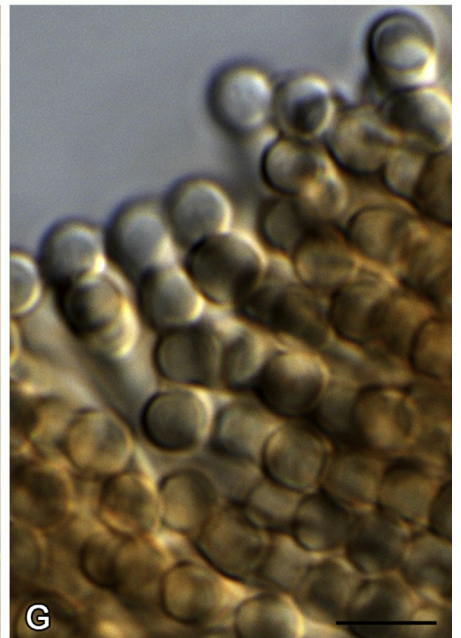
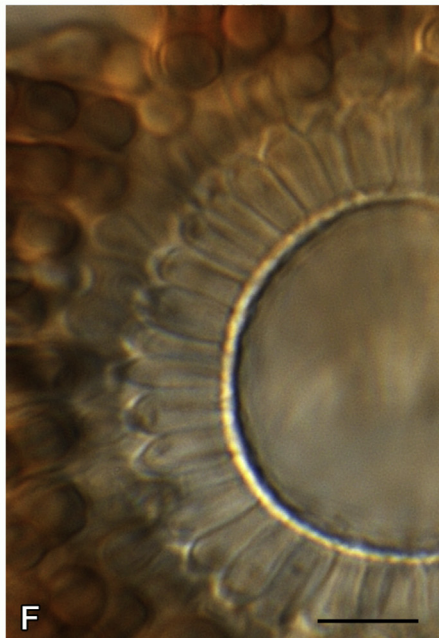
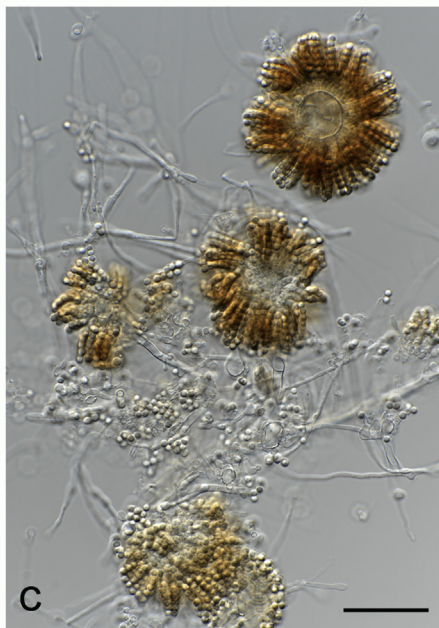
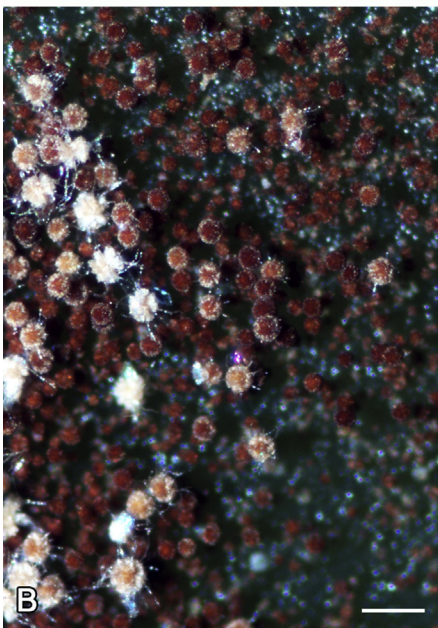
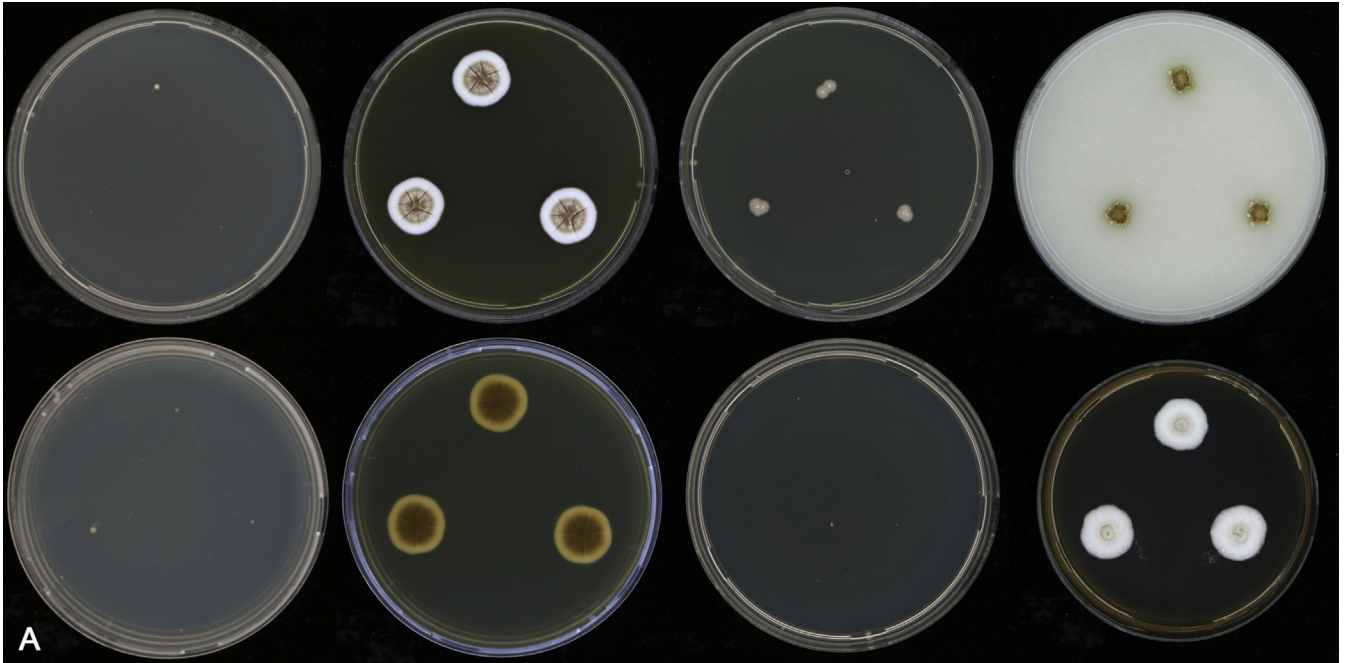
white; texture floccose; sporulation dense, conidia *en masse* light yellow to fawn; soluble pigments absent; exudates absent; reverse cream white to light buff. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* light yellow to fawn; soluble pigments absent; exudates absent; reverse cream white. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments olive brown; exudates clear droplets; reverse cream white. CREA 25 °C, 7 d: No growth.

Table 3. An overview of extrolites produced by section *Cervini* species.

Species	Extrolites
<i>Aspergillus acidohumus</i>	No extrolites detected
<i>A. cervinus</i>	Terremutin, dihydroxy-2,5-toluquinone, cf. xanthocillin, sclerin
<i>A. christenseniae</i>	Cf. 4-hydroxymellein, terremutin, orange-red anthraquinone, cf. chlorflavonin
<i>A. kanagawaensis</i>	Few extrolites (two polar indol-alkaloids, one polar indol-alkaloid)
<i>A. novoguineensis</i>	An asparvenone, sclerotigenin, terremutin
<i>A. nutans</i>	Terremutin, some carotenoid-like extrolites
<i>A. parvulus</i>	Asparvenones, parvulenones, 6-ethyl-7-methoxyjuglone, cf. cycloaspeptide, terremutin, some carotenoid-like extrolites, cf. 4-hydroxymellein, orange-red anthraquinone
<i>A. subnutans</i>	Cf. 4-hydroxymellein
<i>A. transcarpathicus</i>	Asparvenones, terremutin, cf. 4-hydroxymellein, cf. xanthocillin
<i>A. wisconsinensis</i>	An asparvenone, cf. 4-hydroxymellein, sclerotigenin, two territrems, cf. cycloaspeptide

Micromorphology: Conidial heads radiate; conidiophores erect and often terminally sinuous, walls smooth, light yellowish brown, 100–300 × 5–8 µm; vesicles hyaline to faintly coloured, globose, 15–20 µm wide, fertile over the three fourths; uniseriate, phialides hyaline, flask-shaped, 5–6.5 × 2.5–3.5 µm. Conidia globose, smooth, 2.5–4 µm. Ascospores not produced.

Distinguishing characters: Phylogenetically *A. cervinus* clusters with *A. parvulus* and *A. wisconsinensis* in combined phylogenetic analyses, but without statistical support, it can be distinguished from *A. parvulus* by longer conidiophores and from *A. wisconsinensis* by fast growth on CYA. Morphologically, *A. cervinus* is similar to *A. transcarpathicus*, but *A. transcarpathicus* can grow on CYA and MEA at 37 °C.



Aspergillus christenseniae A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817724. Fig. 7.

Etymology: Named in honour of Martha Christensen, who isolated the original culture.

Diagnosis: *Aspergillus christenseniae* is close to *A. nutans* and *A. subnutans*, but can be distinguished by its subglobose to ellipsoidal conidia.

Typus: **South Africa**, Pretoria, Rietvlei, soil, isolated by W.J. Lütjeharms (holotype CBS H-9217, culture ex-type: CBS 122.56 = DTO 022-C8 = IBT 22043 = IBT 23735 = IMI 343732 = NRRL 4897 = WB 4897).

ITS barcode: FJ491613. (Alternative markers: *BenA* = FJ491639; *CaM* = FJ491608; *RPB2* = EF661235).

Colony diam, 7 d (mm): CYA 20–29; CYA 30 °C 15–25; CYA 37 °C No growth; MEA 42–55; MEA 30 °C 37–52; MEA 37 °C No growth; OA 40–42; YES 9–11; CREA No growth; CYAS No growth; DG18 11–15.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation moderately dense, conidia *en masse* fawn; soluble pigments absent to olive brown; exudates clear droplets; reverse cream white to yellowish brown. MEA 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse reddish brown at centre, yellowish brown at edge. YES 25 °C, 7 d: Colonies moderately deep, plane to sulcate; margins entire; mycelium white to light fawn; texture floccose; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse yellowish brown to reddish brown. 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 low, plane; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* fawn; soluble pigments absent to light brown; exudates clear droplets; reverse reddish brown. CREA 25 °C, 7 d: No growth.

Micromorphology: Conidial heads short columnar; conidiophores erect or bent, sometimes nodding, walls smooth, strongly yellowish brown coloured, 8–45 × 3.5–5.5 µm; vesicles coloured as the stalks, subclavate, 8–10.5 µm wide, fertile over the upper half; uniseriate, phialides faintly coloured, flask-shaped, 3.5–5 × 2–3.5 µm. Conidia subglobose to ellipsoidal, smooth, 4–4.5 × 3–3.5 µm. Ascospores not produced.

Aspergillus kanagawaensis Nehira, J. Jap. Bot. 26: 109. 1951. MycoBank MB292847. Fig. 8.

Typus: IMI 126690, culture ex-type: CBS 538.65 = DTO 054-F3 = ATCC 16143 = IBT 22077 = IFO 6219 = IMI 126690 = NRRL 4774 = WB 4774.

ITS barcode: FJ491617. (Alternative markers: *BenA* = FJ491640; *CaM* = FJ491597; *RPB2* = JN121531).

Colony diam, 7 d (mm): CYA 17–20; CYA 30 °C 20–24; CYA 37 °C 10–14; MEA 45–55; MEA 30 °C 51–62; MEA 37 °C 14–25; OA 33–39; YES 15–18; CREA No growth to weak growth; CYAS 14–19; DG18 12–31.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* buff; soluble pigments absent; exudates clear droplets; reverse cream white to yellowish brown. MEA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates clear droplets; reverse yellowish brown. YES 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white to light buff; texture velvety; sporulation moderately dense, conidia *en masse* light buff; soluble pigments absent; exudates absent; reverse buff. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* buff to fawn; soluble pigments absent; exudates absent; reverse cream white to cream yellow. OA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* fawn; soluble pigments absent to light brown; exudates clear droplets; reverse yellowish brown to olive brown. CREA 25 °C, 7 d: No growth to weak growth.

Micromorphology: Conidial heads radiate; conidiophores erect and often terminally sinuous, smaller heads often nodding, walls smooth, light yellowish brown to light greyish, 100–800 × 5–7.5 µm; vesicles faintly coloured to light yellowish brown, globose, 20–25 µm wide, fertile over the three fourths to entire surface; uniseriate, phialides faintly coloured, flask-shaped, 5.5–7.5 × 3–3.5 µm. Conidia globose, smooth, 2.5–3.5 µm. Ascospores not produced.

Distinguishing characters: *Aspergillus kanagawaensis* is close to *A. novoguineensis*, but *A. novoguineensis* does not grow or grows very restrictedly on CYA and MEA at 37 °C.

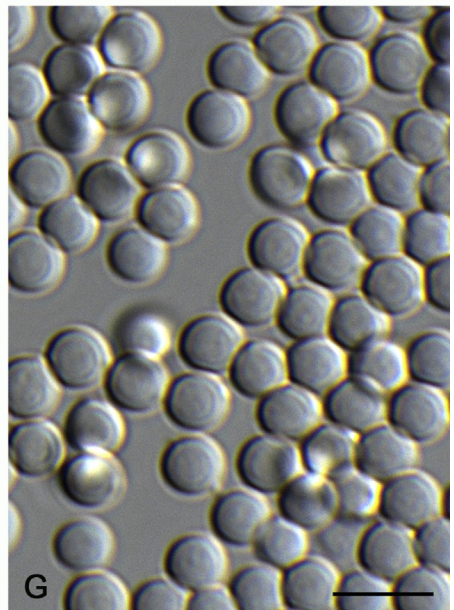
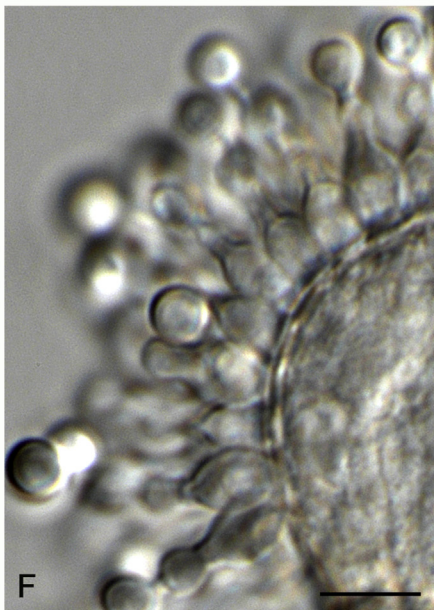
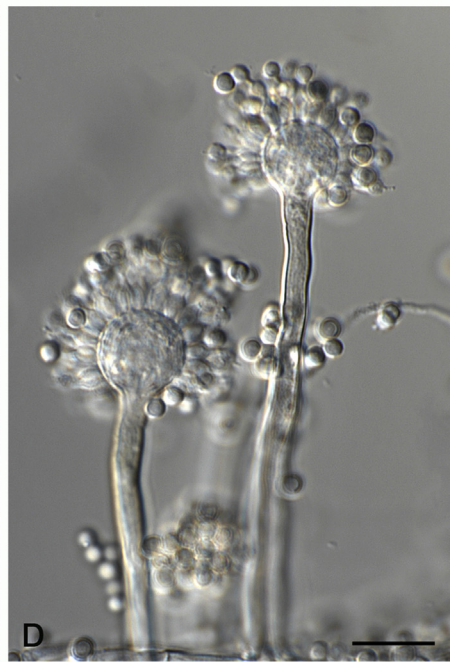
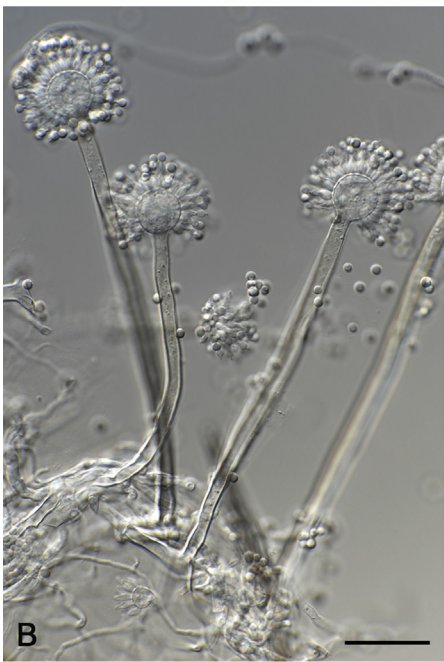
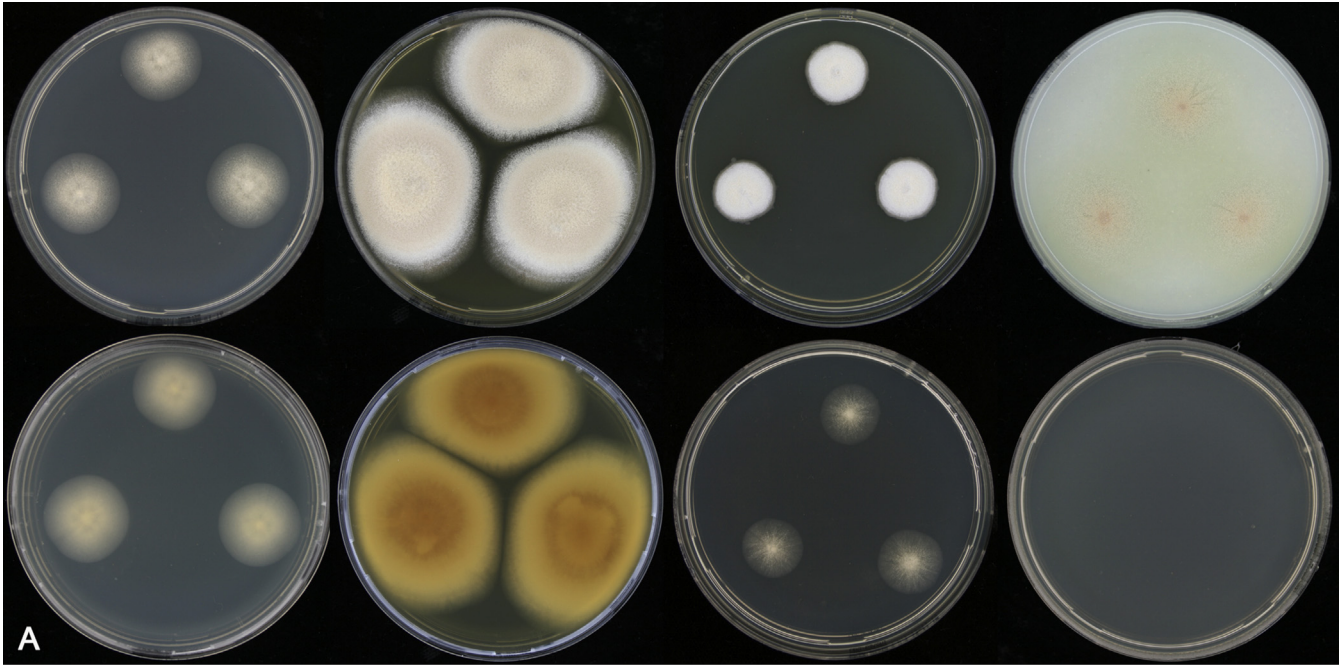
Notes: The ex-type culture (CBS 538.65) of *A. kanagawaensis* CBS 538.65 is degenerated and does not sporulate anymore; strain DTO 069-D6 was used for morphological observation and description.

Aspergillus novoguineensis A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817725. Fig. 9.

Etymology: Name refers to its origin, isolated from Papua New Guinea.

Diagnosis: *Aspergillus novoguineensis* is closely related to *A. kanagawaensis*, however *A. novoguineensis* does not grow or grows very restrictedly at 37 °C.

Fig. 5. Morphological characters of *Aspergillus acidohumus* (CBS 141577^T). **A.** Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CHA. **B.** Conidial heads on MEA after 2 wk incubation. **C–G.** Conidiophores and conidia. Scale bars: B = 1000 µm; C = 30 µm; D, E = 10 µm; F, G = 5 µm.



Typus: Papua New Guinea, Central province, Varirata national park near Port Moresby, humus, 1995, isolated by A. Aptroot (holotype: CBS H-22729, culture ex-type: CBS 906.96 = DTO 021–G5 = IBT 29312).

ITS barcode: FJ491622. (Alternative markers: *BenA* = FJ491641; *CaM* = FJ491605; *RPB2* = KX423681).

Colony diam, 7 d (mm): CYA 20–21; CYA 30 °C 19–21; CYA 37 °C No growth; MEA 37–39; MEA 30 °C 40–43; MEA 37 °C No or weak growth; OA 29–30; YES 20–21; CREA No growth; CYAS No growth; DG18 17–18.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse cream white to yellowish brown. MEA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates clear droplets; reverse yellowish brown. YES 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white to light buff; texture velvety; sporulation dense, conidia *en masse* buff; soluble pigments absent; exudates absent; reverse buff. DG18 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture floccose; sporulation moderately dense, conidia *en masse* buff to fawn; 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* fawn; soluble pigments olive brown; exudates clear droplets; reverse yellowish brown. CREA 25 °C, 7 d: No growth.

Micromorphology: Conidial heads radiate; conidiophores erect, or sometimes bent, walls smooth, 210–550 × 5–7 µm; vesicles globose, 18–26 µm wide, fertile over the two thirds to entire surface; uniseriate, phialides flask-shaped, 5–8 × 2.5–3.5 µm. Conidia globose, smooth, 2.5–3.5 µm. Ascospores not produced.

Aspergillus nutans McLennan & Ducker, Aust. J. Bot. 2: 355. 1954. Mycobank MB292850. Fig. 10.

Typus: IMI 62874ii, culture ex-type: CBS 121.56 = DTO 054-D3 = NRRL 575 = NRRL 4364 = NRRL A-6280 = ATCC 16914 = IFO 8134 = IMI 062874ii = IMI 62874 = QM 8159 = WB 4364 = WB 4546 = WB 4776.

ITS barcode: EF661272. (Alternative markers: *BenA* = EF661249; *CaM* = EF661262; *RPB2* = EF661227).

Colony diam, 7 d (mm): CYA 15–16; CYA 30 °C 17–19; CYA 37 °C Weak growth; MEA 38–42; MEA 30 °C 46–47; MEA 37 °C 6–8; OA 25–26; YES 9–12; CREA No growth; CYAS No growth; DG18 18–19.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* buff to fawn; soluble pigments

absent; exudates absent; reverse cream white to yellowish brown. MEA 25 °C, 7 d: Colonies moderately deep, slightly sulcate; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates clear droplets; reverse reddish brown at centre, yellowish brown at edge. YES 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture floccose; sporulation moderately dense to dense, conidia *en masse* white to fawn; soluble pigments absent; exudates absent; reverse cream white to buff. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse cream white. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* fawn; soluble pigments brown; exudates clear droplets; reverse brown. CREA 25 °C, 7 d: No growth.

Micromorphology: Conidial heads short columnar; conidiophores erect or bent, sometimes nodding, walls smooth, yellowish brown, 25–80 × 2–4 µm; vesicles coloured as the stalks, subclavate, sometimes borne at acute angles, 5–10 µm wide, fertile over the upper half to two thirds; uniseriate, phialides faintly coloured, flask-shaped, 3.5–6 × 2–3 µm. Conidia globose, smooth, 2.5–3.5 µm. Ascospores not produced.

Distinguishing characters: *Aspergillus nutans* can be distinguished from other *Cervini* members by columnar conidial heads, strongly pigmented, short, nodding conidiophores and globose conidia.

Aspergillus parvulus G. Sm., Trans. Brit. Mycol. Soc. 44: 45. 1961. MycoBank MB121074. Fig. 11.

Typus: IMI 86558, culture ex-type: CBS 136.61 = DTO 021-G8 = IBT 22085 = ATCC 16911 = IMI 086558 = LSHB BB405 = NRRL 1846 = NRRL 4753 = QM 7955 = UC 4613 = WB 4753.

ITS barcode: EF661269. (Alternative markers: *BenA* = EF661247; *CaM* = EF661259; *RPB2* = EF661233).

Colony diam, 7 d (mm): CYA 22–23; CYA 30 °C 23–24; CYA 37 °C No growth; MEA 35–46; MEA 30 °C 50–51; MEA 37 °C 5–6; OA 29–30; YES 10–11; CREA No growth; CYAS No growth; DG18 19–22.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates clear droplets; reverse reddish brown at centre, yellowish brown at edge. MEA 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates clear droplets; reverse reddish brown at centre, yellowish brown at edge. YES 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* fawn; soluble pigments

Fig. 6. Morphological characters of *Aspergillus cervinus* (CBS 537.65^T). **A.** Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. **B–G.** Conidiophores and conidia. Scale bars: B = 30 µm; C–E = 10 µm; F, G = 5 µm.

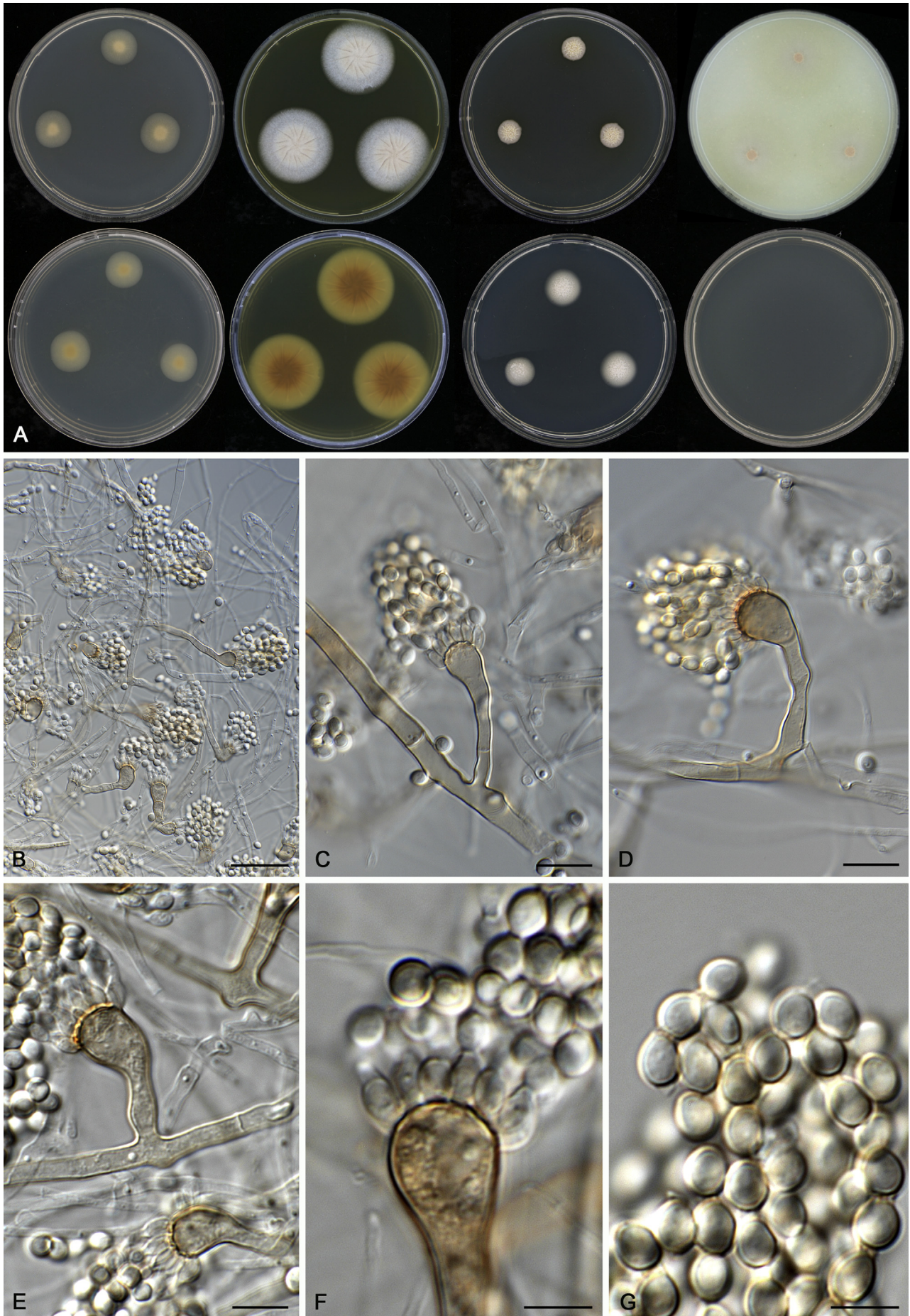


Fig. 7. Morphological characters of *Aspergillus christenseniae* (CBS 122.56^T). A. Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. B–G. Conidiophores and conidia. Scale bars: B = 30 μm; C–E = 10 μm; F, G = 5 μm.

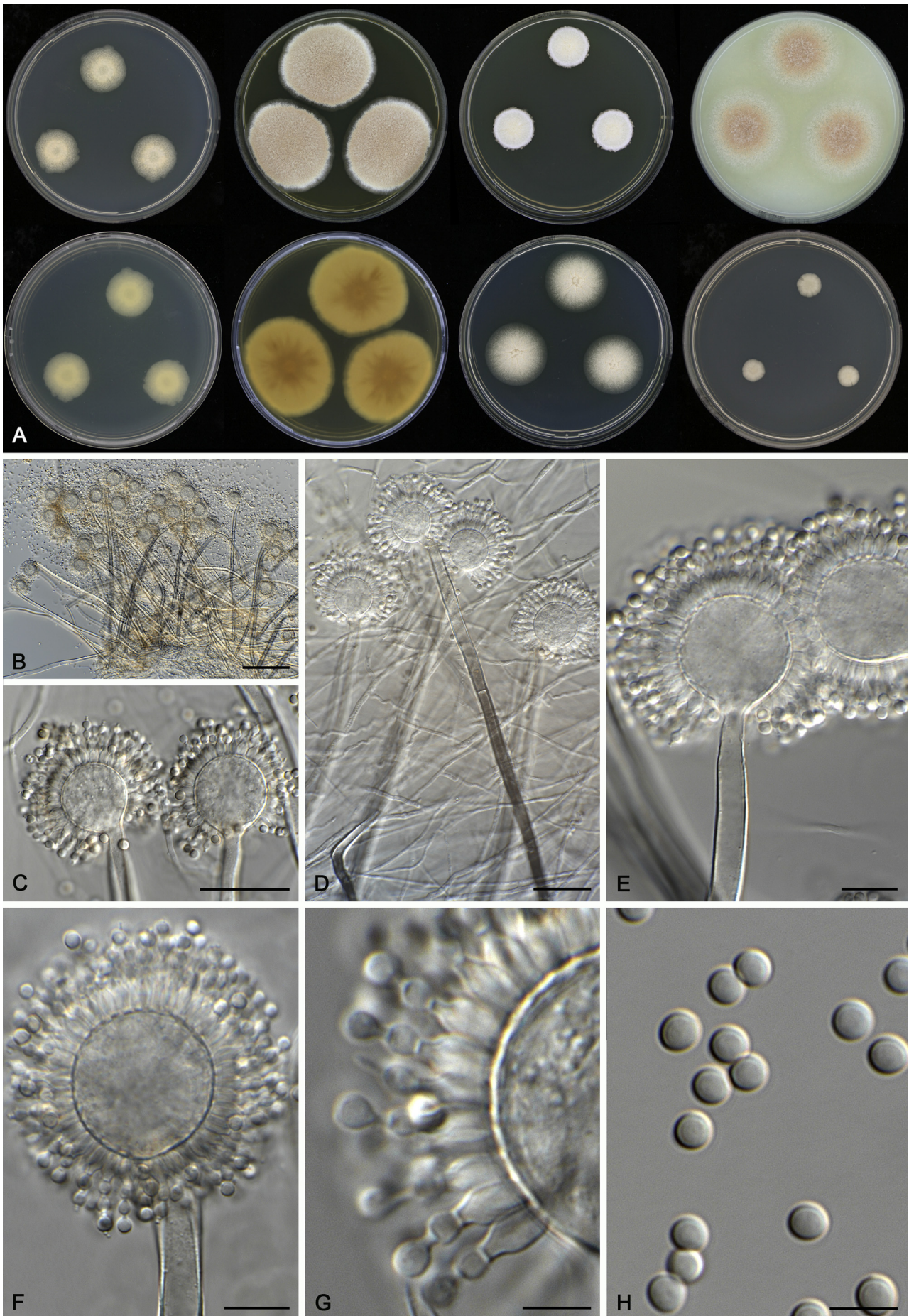


Fig. 8. Morphological characters of *Aspergillus kanagawaensis* (DTO 069-D6). **A.** Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. **B–H.** Conidiophores and conidia. Scale bars: B = 100 μ m; C, D = 30 μ m; E, F = 10 μ m; G, H = 5 μ m.

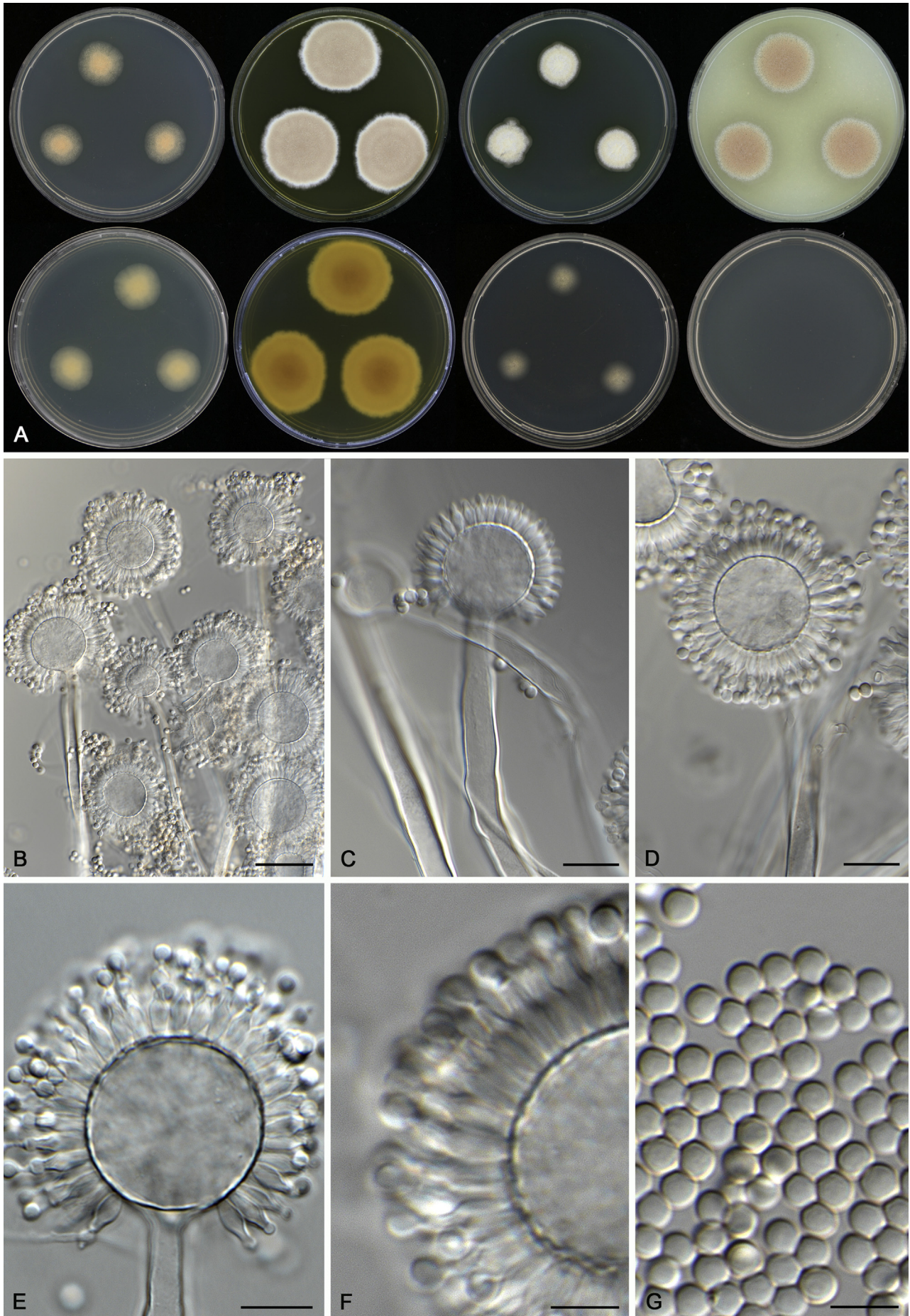


Fig. 9. Morphological characters of *Aspergillus novoguineensis* (CBS 906.96^T). **A.** Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. **B–G.** Conidiophores and conidia. Scale bars: B = 30 µm; C–E = 10 µm; F, G = 5 µm.

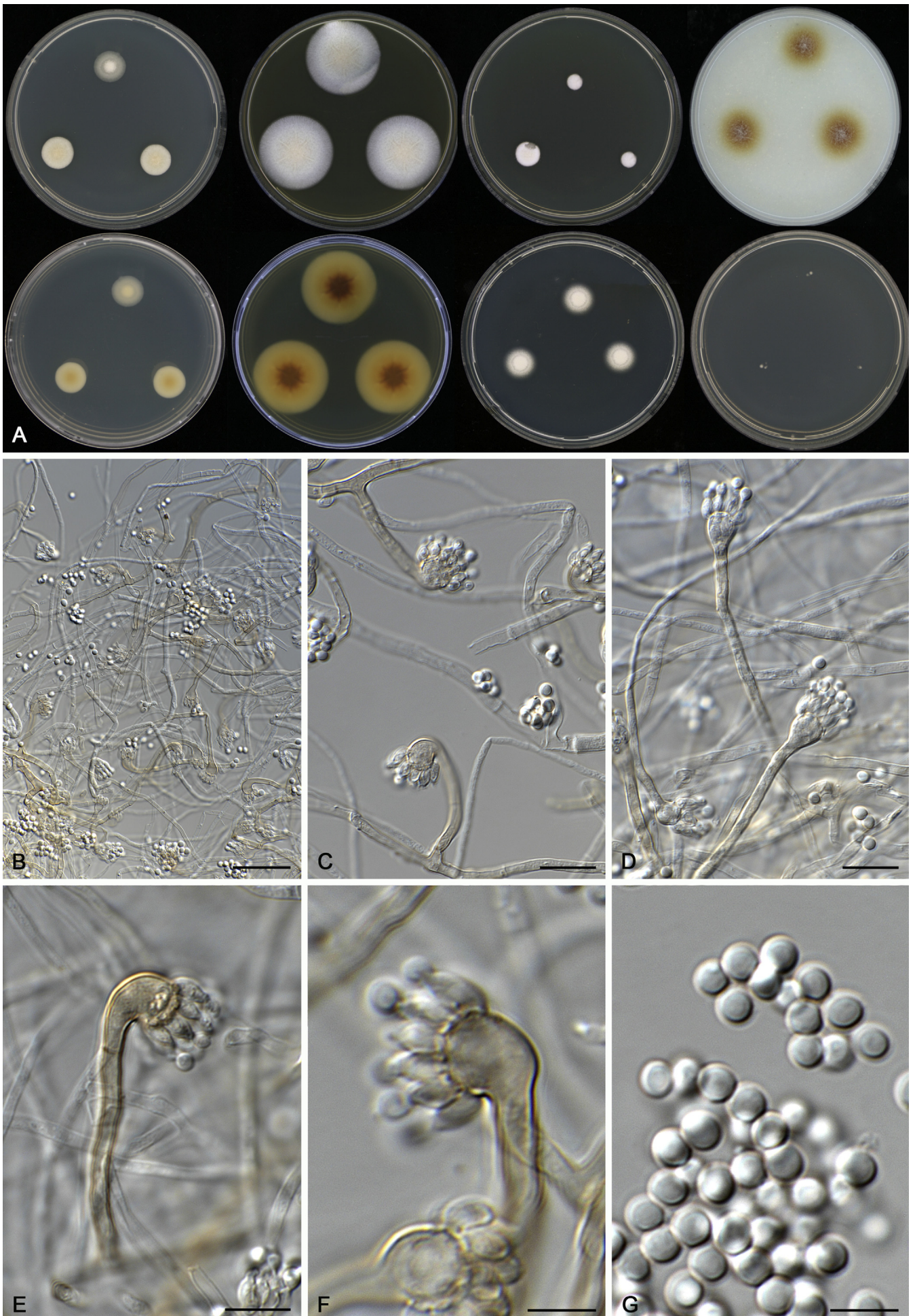
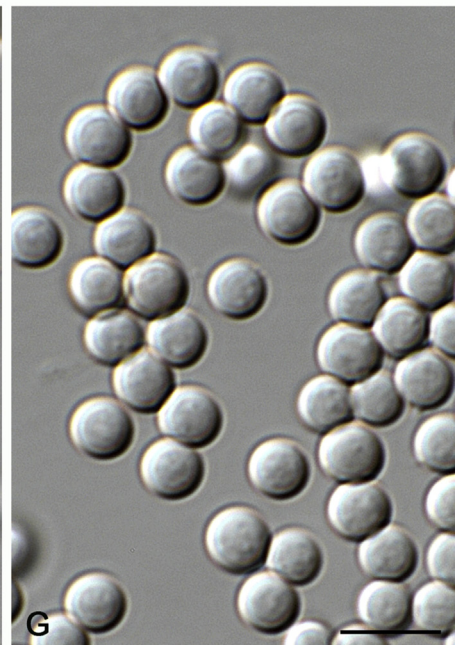
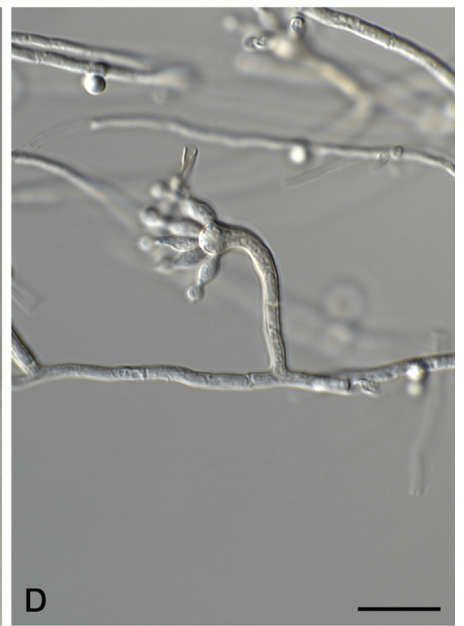
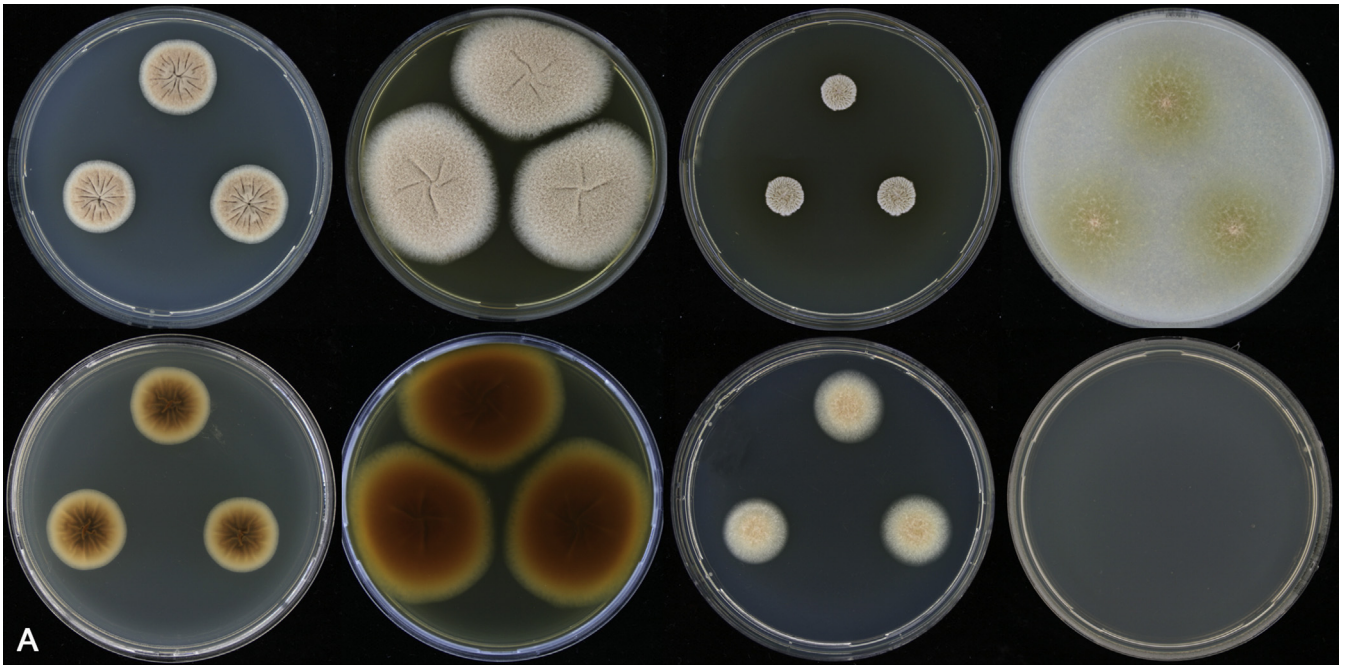


Fig. 10. Morphological characters of *Aspergillus nutans* (CBS 121.56^T). **A.** Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. **B–G.** Conidiophores and conidia. Scale bars: B = 30 µm; C–E = 10 µm; F, G = 5 µm.



absent; exudates absent; reverse ochraceous buff. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse cream white to buff. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* fawn; soluble pigments olive brown; exudates clear droplets; reverse olive brown. CREA 25 °C, 7 d: No growth.

Micromorphology: Conidial heads radiate; conidiophores erect or bent, sometimes nodding, walls smooth, yellowish brown, 17–75 × 2.5–3.5 µm; vesicles faintly coloured to light yellowish brown, mainly globose, sometimes subclavate, 5–11 µm wide, fertile over the two thirds to three fourths; uniseriate, phialides faintly coloured, flask-shaped, 4–7 × 2–3 µm. Conidia globose, smooth, 2.5–4 µm. Ascospores not produced.

Distinguishing characters: The short conidiophores (< 100 µm) can distinguish *Aspergillus parvulus* from phylogenetically related species *A. cervinus* and *A. transcarchicus*. Morphologically *A. parvulus* resembles *A. nutans* and *A. subnutans*, but the latter two produce short columnar conidial heads.

Aspergillus subnutans A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817726. Fig. 12.

Etymology: Name refers to its resemblance with *A. nutans*.

Diagnosis: *Aspergillus subnutans* resembles *A. nutans* phylogenetically and morphologically, but differs in upright, uncoloured vesicles.

Typus: **USA**, Wisconsin, soil under *Tsuga canadensis*, 1960, isolated by M. Christensen (holotype: CBS H-22728, culture ex-type: CBS 129386 = DTO 202–C2 = WSF 445 = IBT 34352).

ITS barcode: KX528456. (Alternative markers: *BenA* = KX528454; *CaM* = KX528455; *RPB2* = KX528453).

Colony diam, 7 d (mm): CYA 17–18; CYA 30 °C 17–18; CYA 37 °C 4–6; MEA 34–35; MEA 30 °C 32–33; MEA 37 °C 10–11; OA 32–35; YES 15–16; CREA No growth; CYAS 7–8; DG18 14–19.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* white to fawn; soluble pigments absent; exudates clear droplets; reverse cream white. MEA 25 °C, 7 d: Colonies moderately deep, sulcate; margins irregular; mycelium white; texture floccose; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates clear droplets; reverse yellowish brown. YES 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture velvety; sporulation moderately dense to dense, conidia *en masse* white to light fawn; soluble pigments absent; exudates absent; reverse cream white to light buff. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins entire;

mycelium white; texture velvety; sporulation dense, conidia *en masse* light fawn; soluble pigments absent; exudates absent; reverse cream white. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* light fawn; soluble pigments olive brown; exudates clear droplets; reverse olive brown. CREA 25 °C, 7 d: No growth.

Micromorphology: Conidial heads short columnar; conidiophores erect or bent, walls smooth, 25–50 × 3.5–5 µm; vesicles subclavate, 7–13 µm wide, fertile over the upper half to two thirds; uniseriate, phialides flask-shaped, 5.5–7 × 2.5–3 µm. Conidia globose, smooth, 3–4 µm. Ascospores not produced.

Notes: The ex-type culture (CBS 129386 = DTO 202-C2 = WSF 445 = IBT 34352) was considered as an aberrant strain of *A. nutans* due to their high morphological similarity (Christensen *et al.* 1964). Our molecular results warrant it as a unique species.

Aspergillus transcarchicus A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817727. Fig. 13.

Etymology: Name refers to its origin, isolated from Transcarpathia, Ukraine.

Diagnosis: *Aspergillus transcarchicus* resembles *A. cervinus*, but differs by ability of growing on CYA and MEA at 37 °C.

Typus: **Ukraine**, Transcarpathia, soil, deposited by L.A. Belyakova (holotype: CBS H-22727, culture ex-type: CBS 423.68 = DTO 022-C7 = IBT 22080 = IMI 134108 = VKM F-1331).

ITS barcode: FJ491624. (Alternative markers: *BenA* = FJ491632; *CaM* = FJ491610; *RPB2* = KX423680).

Colony diam, 7 d (mm): CYA 14–20; CYA 30 °C 11–24; CYA 37 °C 4–10; MEA 44–60; MEA 30 °C 37–60; MEA 37 °C 12–18; OA 37–43; YES 10–17; CREA No growth; CYAS No growth; DG18 8–23.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse cream white to yellowish brown. MEA 25 °C, 7 d: Colonies moderately deep, plane to sulcate; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn or light yellow; soluble pigments absent; exudates clear droplets; reverse reddish brown at centre, yellowish brown at edge. YES 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* light yellow to fawn; soluble pigments absent; exudates absent; reverse cream white to light buff. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse cream white. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en*

Fig. 11. Morphological characters of *Aspergillus parvulus* (CBS 136.61^T). **A.** Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. **B–G.** Conidiophores and conidia. Scale bars: B = 30 µm; C–E = 10 µm; F, G = 5 µm.

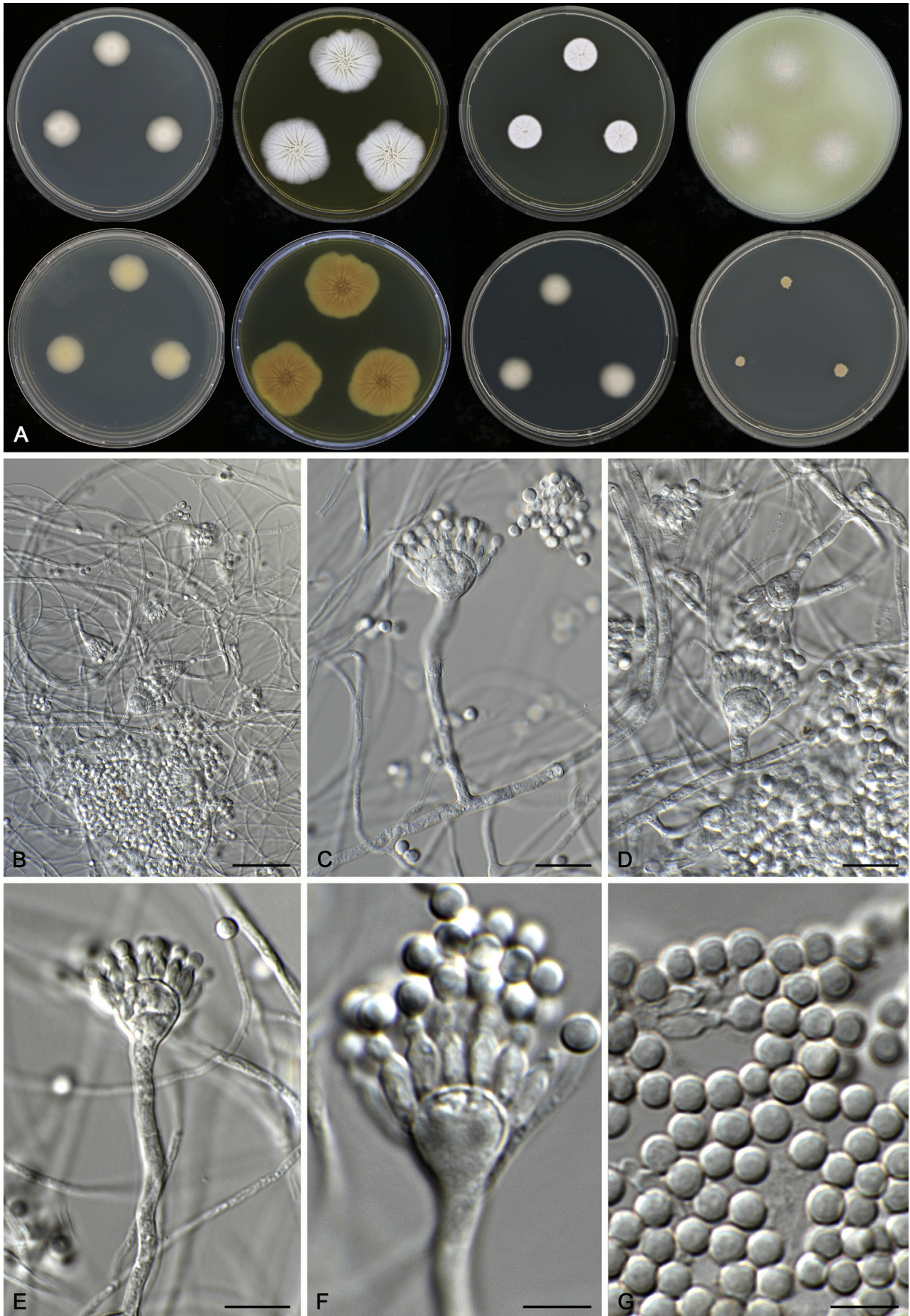


Fig. 12. Morphological characters of *Aspergillus subnutans* (CBS 129386^T). A. Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. B–G. Conidiophores and conidia. Scale bars: B = 30 µm; C–E = 10 µm; F, G = 5 µm.

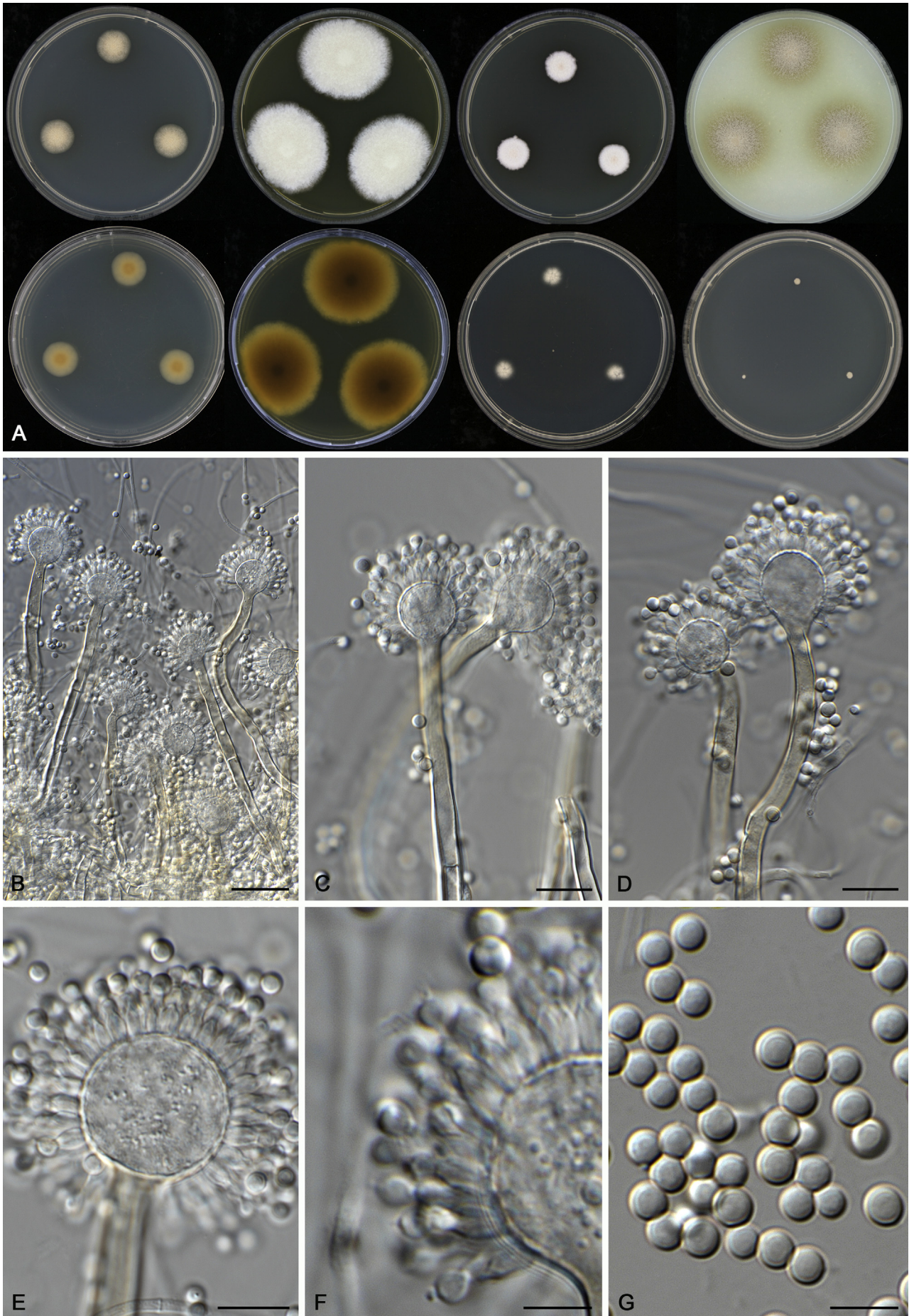


Fig. 13. Morphological characters of *Aspergillus transcarchicus* (CBS 423.68^T). **A.** Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. **B–G.** Conidiophores and conidia. Scale bars: B = 30 µm; C–E = 10 µm; F, G = 5 µm.

masse fawn; soluble pigments light brown to olive brown; exudates clear droplets; reverse cream white to olive brown to brown. CREA 25 °C, 7 d: No growth.

Micromorphology: Conidial heads radiate; conidiophores erect and often terminally sinuous, walls smooth, yellowish brown, 100–150 × 5.5–7.5 µm; vesicles faintly coloured, globose, 15–20 µm wide, fertile over the two thirds to entire surface; uniseriate, phialides faintly coloured, flask-shaped, 4.5–6.5 × 2.5–3 µm. Conidia globose, smooth, 3–4 µm. Ascospores not produced.

Notes: Hubka *et al.* (2012) reported a isolation of section *Cervini* member from suspected onychomycosis. Their isolate CCF 3945 shows 94.2 % and 96.6 % similarity with *A. parvulus* in *BenA* and *CaM* sequences respectively. This isolate is identified as *A. transcarpathicus* here according to sequence data (*BenA* = FR775332, *CaM* = FR837972, *RPB2* = FR837980).

Aspergillus vinosobubalinus Udagawa, Kamiya & Kaori Osada, *Trans. Mycol. Soc. Japan* 34: 255. 1993. MycoBank MB361186.

Typus: CBM BF-33501. Culture ex-type: CBM BF-33501.

ITS barcode: n.a. (Alternative markers: *BenA* = n.a.; *CaM* = n.a.; *RPB2* = n.a.).

Colony characters: Fide Udagawa *et al.* (1993) Colonies on Czapek agar growing rapidly, attaining a diameter of 40–44 mm in diam within 7 days at 25 °C, velvety, loose-textured, more or less zonate, consisting of a thin basal felt from which conidiophores moderately arise, Purplish Gray (M. 13D2 after Kornerup & Wanscher 1978) or Fawn (Rayner 1970), becoming Brownish Gray (M. 8D2) or Vinaceous Buff (Rayner) in age; reverse uncoloured. Colonies on CYA spreading broadly, attaining a diameter of 85 mm within 7 days at 25 °C, velvety, zonate, furrowed in a radial pattern, consisting of a rather compact basal felt, usually producing abundant conidial heads, sometimes intermixed with Orange (M. 5A5) or saffron (Rayner) sclerotia on the felt, Purplish Gray (M. 14B2), becoming Brownish Gray (M. 8C2) or Vinaceous Buff (Rayner) in age; sclerotia produced more abundantly in granular appearance in the dark-incubated cultures; exudate lacking; reverse Pale Yellow (M. 4A3) or Buff (Rayner). Colonies on CYA with 20 % sucrose (CYA20S) spreading broadly, growth rate and other characters similar to those on CYA. Colonies on malt extract agar (MEA) growing rapidly, attaining a diameter of 63–64 mm within 7 days at 25 °C, velvety, plane, consisting of a thin submerged mycelium from which numerous conidiophores arise, Reddish Lilac (M. 14C3) to Dull Lilac (M. 15C3); exudate lacking; reverse uncoloured to Yellowish Gray (M. 4B2) or Smoke Gray (Rayner).

Micromorphology: Fide Udagawa *et al.* (1993) Conidial heads radiate, splitting into columns in age, 250–350 µm in diam. Conidiophores straight to terminally sinuous, sometimes nodding; stipes 550–1200 × 10–12.5 µm, with walls smooth and thickened up to 2 µm near the base, upper portion light yellowish brown; vesicles globose to more or less elongate, 30–45 µm in diam, brownish, fertile over the entire surface. Aspergilla

uniseriate with tightly packed phialides; phialides cylindrical, 5–7.5 (–10) × 2.5–3 (–5) µm. Conidia hyaline, pale yellowish brown in mass, globose to subglobose, 3–4.5 × 3–4 µm, at first echinulate and thin-walled, becoming verruculose with small warts, diminutive aspergilla sometimes present; conidiophores 150–350 × 3.5–5 µm; vesicles flask-shaped, 7.5–15 µm in diam, fertile over upper half to two-thirds; phialides 7.5–10 × 2.5–3 µm; conidia as described. Sclerotia greyish orange or saffron, mostly subglobose, sometimes elongate, 380–600 µm in diam, composed of angular, thick-walled, 15–30 × 10–24 µm cells; no evidence of asci through three months on CYA, CYA20S or MEA.

Distinguishing characters: The rapid growth on CYA (85 mm within 7 d), saffron-coloured sclerotia and wide vesicles (30–45 µm) can easily distinguish *A. vinosobubalinus* from other section *Cervini* members.

Notes: According to the original description, *A. vinosobubalinus* is characterised by fawn, radiate conidial heads, uniseriate, globose to elongate vesicles, which fit the morphological features of section *Cervini* (Udagawa *et al.* 1993). Unfortunately, the ex-type culture and sequence data of *A. vinosobubalinus* were not available for this study.

Aspergillus wisconsinensis A.J. Chen, Frisvad & Samson, **sp. nov.** MycoBank MB817728. Fig. 14.

Etymology: Name refers to its origin, isolated from soil under *Tsuga canadensis*, USA, Wisconsin.

Diagnosis: *Aspergillus wisconsinensis* resembles *A. cervinus* and *A. transcarpathicus*. It differs from *A. cervinus* by slow growth on CYA and from *A. transcarpathicus* by its lack of growth at 37 °C.

Typus: USA, Wisconsin, near Madison, soil under *Tsuga Canadensis*, isolated by M. Christensen (holotype: CBS H-9203, culture ex-type: CBS 413.64 = DTO 022–B1 = NRRL 5027 = IBT 22042 = IBT 22082 = WSF 380 = DTO 070-A5 = WB 5027).

ITS barcode: FJ491618. (Alternative markers: *BenA* = FJ491638; *CaM* = FJ491609; *RPB2* = KX423671).

Colony diam, 7 d (mm): CYA 13–17; CYA 11–16; CYA 37 °C No growth; MEA 51–53; MEA 30 °C 40–43; MEA 37 °C No growth; OA 25 °C 35–40; YES 14–23; CREA No growth; CYAS Weak growth; DG18 14–19.

Colony characters: CYA 25 °C, 7 d: Colonies moderately deep, plane; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse yellowish brown. MEA 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates clear droplets; reverse yellowish brown. YES 25 °C, 7 d: Colonies moderately deep, sulcate; margins entire; mycelium white; texture floccose; sporulation dense, conidia *en masse* white to light fawn; soluble pigments absent; exudates absent; reverse ochraceous buff. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins

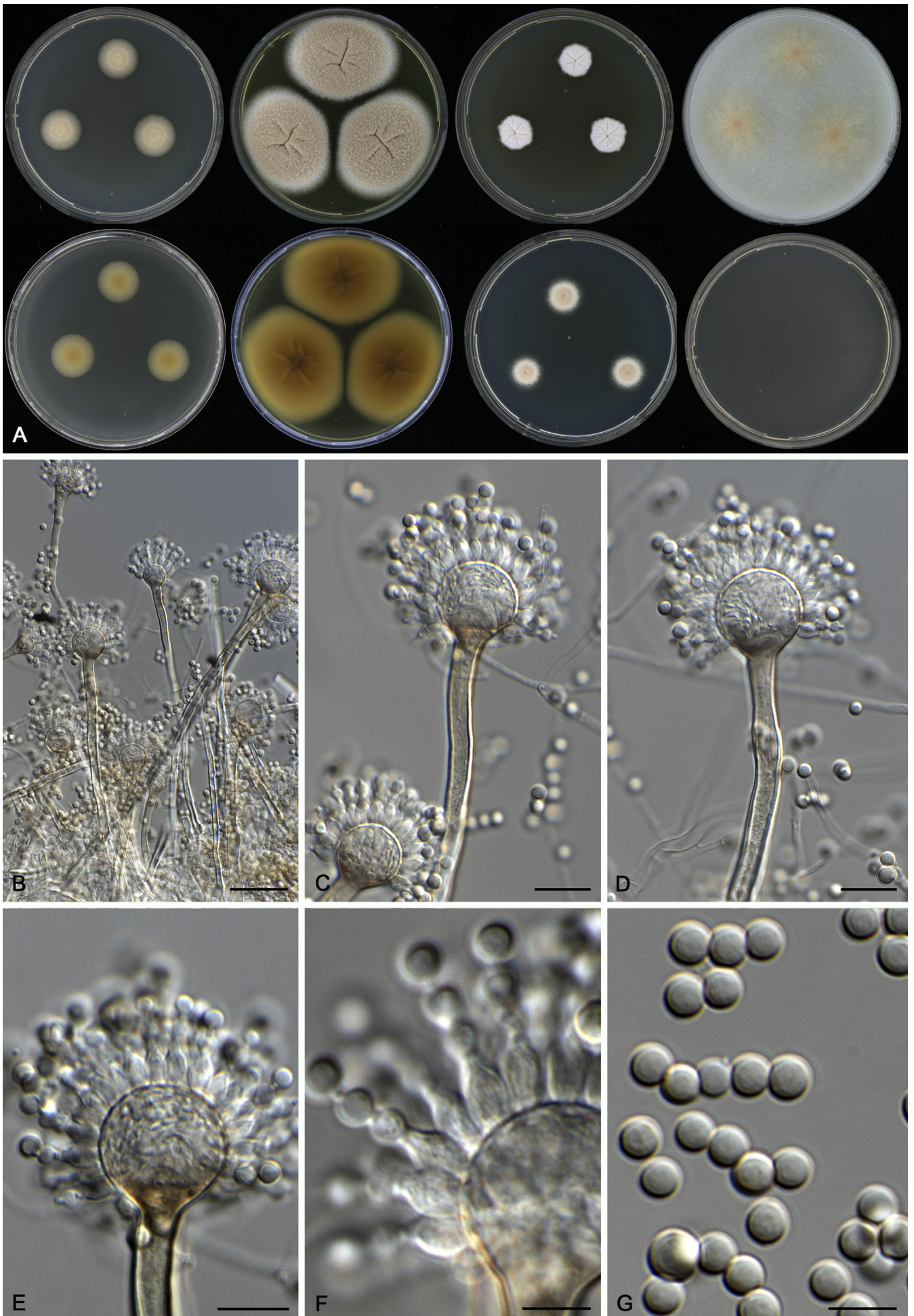


Fig. 14. Morphological characters of *Aspergillus wisconsinensis* (CBS 413.64^T). **A.** Colonies: top row left to right, CYA, MEA, YES and OA; bottom row left to right, reverse CYA, reverse MEA, DG18 and CYA 37 °C. **B–G.** Conidiophores and conidia. Scale bars: B = 30 µm; C–E = 10 µm; F, G = 5 µm.

entire; mycelium white; texture velvety; sporulation dense, conidia *en masse* fawn; soluble pigments absent; exudates absent; reverse yellowish brown at centre, cream white at edge. OA 25 °C, 7 d: Colonies low, plane; margins entire; mycelium white; texture velvety; sporulation moderately dense, conidia *en masse* fawn; soluble pigments olive brown; exudates clear droplets; reverse brown. CREA 25 °C, 7 d: No growth.

Micromorphology: Conidial heads radiate; conidiophores erect or bent, yellowish brown, 100–200 × 4–7 µm; vesicles coloured as the stalks, subclavate to globose, 14–20 µm wide, fertile over the two thirds; uniseriate, phialides faintly coloured, flask-shaped, 5–7 × 2.5–3.5 µm. Conidia globose, smooth, 3–4.5 µm. Ascomata not produced.

Dichotomous key to species from section *Cervini*

- 1a) Conidial heads short columnar 2
- 1b) Conidial heads radiate 4
- 2a) Conidia subglobose to ellipsoidal *A. christenseniae*
- 2b) Conidia globose 3
- 3a) Vesicles strongly pigmented, nodding *A. nutans*
- 3b) Vesicles upright, uncoloured *A. subnutans*
- 4a) Conidiophores mainly not exceeding 100 µm in length 5
- 4b) Conidiophores exceeding 100 µm in length 6
- 5a) Vesicles exceeding 15 µm in width *A. acidohumus*
- 5b) Vesicles not exceeding 15 µm in width *A. parvulus*
- 6a) Conidiophores usually 100–300 µm in length 7
- 6b) Conidiophores extremely variable, 100–200 µm in length 9
- 7a) Grow on CYA and MEA at 37 °C *A. transcarpaticus*
- 7b) Does not grow on CYA and MEA at 37 °C 8
- 8a) Slow growth (<20 mm, 25 °C, 7d) on CYA *A. wisconsinensis*
- 8b) Fast growth (>25 mm, 25 °C, 7d) on CYA *A. cervinus*
- 9a) Fast growth (>80 mm, 25 °C, 7d) on CYA *A. vinosobubalinus*
- 9b) Slow growth (<40 mm, 25 °C, 7d) on CYA 10
- 10a) No growth or restricted growth on CYA and MEA at 37 °C *A. novoguineensis*
- 10b) Grows on CYA and MEA at 37 °C *A. kanagawaensis*

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