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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, extracellular temperature 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. christenseniæ, A. novoguineensis, A. subnutans, A. transcarpathicus and A. wisconsinensis. A dichotomous key for the identification is provided.

Key words: Ascomycetes, Eurotales, Exotoxins, Multi-gene phylogeny, Subgenus Fumigati.
Taxonomic novelties: Aspergillus acidohumus A.J. Chen, Frisvad & Samson, A. christenseniæ 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. vinosobalbinus 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 (Masse 1914), later it was also found in soil in New Zealand (Neill 1939, di Menno et al. 2007), Malaysia and USA (Christensen & Fennell 1964, Christensen et al. 1964). This taxon was found to produce the quinol derivative terreterum and 3,6-dihydroxy-2,5-toluquinone (Elsohly et al. 1974). These authors stated that while the compound terreterum 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, Langdau 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 (Böss et al. 1997). Aspergillus nutans was originally found in soil in Australia (McLenman 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 terremutin (Pheobe et al. 1978). Aspergillus vinosobalbinus was isolated from a sweet feed 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 extracellular 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 colony colours, the production of soluble pigments and exudates were determined. Light microscope preparations were made from colony fragments. 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-R2 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) 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. subnautans, 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 1722 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 NNR 4897 (= CBS 122.56) together with other two strains (CBS 411.64 and CBS 122715) are described as A. christenseniae ssp. nov., while lineage NNR 2161 (= CBS 123896) and NNR 5027 (= CBS...
| Species | Strain no. | Source | GenBank accession nr. |
|---------|------------|--------|----------------------|
| Aspergillus acidohumus | CBS 14157T = CGMCC3.18217 = DTO 340-H1 = IKT 34346 | Acid soil, Guizhou, China | KX423646 KX423623 KX423634 KX423663 |
| A. cervinus | CBS 537.65T = DTO 054-D5 = ATCC 16915 = IKT 22087 = IMI 126542 = NRRL 5025 = QM 8875 = WB 5025 | Soil, tropical rain forest, near Kuala Lumpur, Malaysia | EF661288 EF661251 EF661261 EF661229 |
| | CBS 196.64 = ATCC 15508 = IMI 107684 = NRRL 3157 = IKT 22044 = WB 5026 | Soil, tropical rain forest, near Kuala Lumpur, Malaysia | EF661270 EF661250 EF661260 EF661228 |
| A. christenseniae | CBS 122.56T = DTO 022-C6 = IKT 22043 = IMI 1343732 = NRRL 12690 = NRRL 47474 = WB 4774 | Soil, Rietvlei, Pretoria, South Africa | FJ491613 FJ491639 FJ491608 |
| | CBS 196.64 = ATCC 15508 = IMI 107684 = NRRL 3157 = IKT 22044 = WB 5026 | Soil, Rietvlei, Pretoria, South Africa | EF661270 EF661250 EF661260 EF661228 |
| A. kanagawaensis | CBS 538.65T = DTO 054-F5 = ATCC 16143 = IKT 22077 = IMI 126690 = NRRL 4774 = WB 4774 = WB 4776 | Soil, Kanagawa, Japan | FJ491617 FJ491640 FJ491597 JN121531 |
| | CBS 129333 = DTO 022-A1 = IKT 22079 | Soil under Quercus sp., Wisconsin, USA | EF661270 EF661250 EF661260 EF661228 |
| A. novoguineensis | CBS 606.96T = DTO 021-G5 = IKT 29312 | Soil, Papua New Guinea | FJ491622 FJ491641 FJ491605 |
| | CBS 129386 = DTO 202-C3 = IKT 29314 | Soil under Tsuga canadensis, Wisconsin, USA | FJ491618 FJ491638 FJ491609 |
| A. parvulus | CBS 136.61T = DTO 021-G8 = IKT 22008 = ATCC 16911 = IMI 086588 = LSHE BB405 = NRRL 1846 = NRRL 4753 = QM 8159 | Soil under Pinus banksiana, Wisconsin, USA | JX528456 JX528454 JX528455 JX528453 |
| | CBS 133098 = NRRL 2667 = NRRL 5028 = IKT 22045 = WB 5028 | Soil, Georgia, USA | EF661271 EF661244 EF661258 EF661234 |
| A. subnuttans | CBS 129386T = DTO 202-C2 = WSF 445 = IKT 34352 | Soil under Tsuga canadensis, Wisconsin, USA | KX528456 KX528454 |
| A. transcarpathicus | CBS 423.68T = DTO 022-C7 = IKT 22080 = ATCC 16911 = IMI 134108 = VKM F-1331 | Transcarpathia, Ukraine | FJ491624 FJ491632 FJ491609 |
| | CBS 410.64 = IKT 22086 = USP 3141 = WSM 5750 | Sandy soil, of Salix nigra community, Wisconsin, USA | FJ491611 FJ491643 FJ491609 |
| A. wisconsinensis | CBS 413.64T = DTO 022-B1 = NRRL 5027 = IKT 22024 = IKT 22082 = WSM 380 = IKT 70-A5 = WB 5027 | Soil under Tsuga canadensis, Wisconsin, USA | FJ491618 FJ491638 FJ491609 |
| | CBS 129387 = DTO 202-C3 = IKT 34347 | Soil, Wisconsin, USA | KX423649 KX423633 KX423641 KX423673 |
| | CBS 129400 = DTO 202-D7 = IKT 34348 | Soil, Wisconsin, USA | KX423652 KX423630 KX423643 KX423674 |
| | CBS 126265 = DTO 195-E2 = IKT 34346 | Soil, Stephen Foster State Park, Florida, USA | KX423651 KX423632 KX423644 KX423675 |
| | CBS 127024 = DTO 196-F2 = IKT 34349 | Soil under Tsuga canadensis, Wisconsin, USA | KX423648 KX423629 KX423645 KX423672 |
| | CBS 123896 = NRRL 2161 = IKT 22041 = IKT 70-A3 | Soil, Australia | KX423656 KX423628 KX423640 KX423676 |
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.

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 133.61T).
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 133.61T).
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 133.61T).
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 terremutin, a precursor of the antiboically active extrolite terreic acid (Guo et al. 2014, Sharma et al. 2016). Producers of terremutin 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 terremutin 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 heteroextrolites 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 antinsect 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 heteroextrolite 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 *Kirschsteiniotheila* 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; CRE: 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. Ascomata not produced.

*Aspergillus cervinus* Masssee, 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 = IIB 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; CRE: 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;
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 133.61T).
mycelium white; texture velvety; sporulation dense, conidia en masse; 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.

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. Ascomata 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.

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 |
| Aspergillus acidohumus   | Weak growth No growth          | Radiate                                          |
| A. cervinus              | 26–27     | 25–26     | No growth 60–68 | 58–60    | No growth  |
| A. christenseniae        | 20–29     | 15–25     | No growth 42–55 | 37–52    | No or weak growth |
| A. kanagawaensis         | 17–20     | 20–24     | 10–14     | 45–55    | 51–62     | 14–25     | Radiate       | 100–800 5–8 5  |
| A. novoguineensis        | 20–21     | 19–21     | No growth 37–40 | 40–43    | No or weak growth |
| A. transcarpathicus      | 14–20     | 11–24     | 4–10      | 44–60    | 37–60     | 12–18     | Radiate       | 100–150 5.5–7  |
| A. vinosobubalinus       | 13–17     | 11–16     | No growth 51–53 | 40–43    | No growth  |

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

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

| Species                  | Extrolites                                                  |
|--------------------------|------------------------------------------------------------|
| Aspergillus acidohumus   | No extrolites detected                                      |
| A. cervinus              | Terremin, dihydroxy-2,5-toluquinone, cf. xanthocillin, slerin |
| A. christenseniae        | Cf. 4-hydroxymellein, terremin, orange-red anthraquinone, cf. chlorflavonin |
| A. kanagawaensis         | Few extrolites (two polar indol-alkaloids, one polar indol-alkaloid) |
| A. novoguineensis        | An asparvenone, sclerotigenin, terremin                     |
| A. transcarpathicus      | Terremin, some carotenoid-like extrolites                   |
| A. parvulus              | Asparvenones, parvalenones, 6-ethyl-7-methoxyjuglone, cf. cycloaspeptide, terremin, some carotenoid-like extrolites, cf. 4-hydroxymellein, orange-red anthraquinone |
| A. subnutans             | Cf. 4-hydroxymellein                                       |
| A. wisconsinensis        | Asparvenones, terremin, cf. 4-hydroxymellein, cf. xanthocillin |

white; single cells short, columnar, 8 μm wide, fertile over the three fourths; clavate, phialides hyaline, flask-shaped, 5–6.5 × 2.5–3.5 μm. Conidia globose, smooth, 2.5–4 μm. Ascomata not produced.
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. subnuntans, but can be distinguished by its subglobose conidioid conidia.

Typus: South Africa, Pretoria, Rietvlei, soil, isolated by W.J. Löthjeharms (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; CalM = FJ491608; RP2B = 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, sulphate; 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 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. Ascomata not produced.

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

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

ITS barcode: FJ491617. (Alternative markers: BenA = FJ491640; CalM = FJ491597; RP2B = 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 fawn; 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, sulphate; 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.

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 acidothumus (CBS 141577). 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 = 1 000 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 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 moderately dense, conidia en masse fawn; soluble pigments absent; exudates absent; reverse cream white to yellowish brown.

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. Ascomata 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 fawn 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.

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 brown; exudates clear droplets; reverse brown. CREA 25 °C, 7 d: No growth.
Fig. 7. Morphological characters of Aspergillus christenseniae (CBS 122.56T). 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 μm; C, D = 30 μm; E, F = 10 μm; G, H = 5 μm.
Fig. 9. Morphological characters of Aspergillus novoguineensis (CBS 906.96T). 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 121.56T). 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. 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 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 flask-shaped, 4–7 × 2–3 μm. Conidia globose, smooth, 2.5–4 μm. Ascomata not produced.

Distinguishing characters: The short conidiophores (< 100 μm) can distinguish Aspergillus parvulus from phylogenetically related species A. cervinus and A. transcarpathicus. 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 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 yellow 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.

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 transcarpathicus A.J. Chen, Frisvad & Samson, sp. nov. MycoBank MB817727. Fig. 13.

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

Diagnosis: Aspergillus transcarpathicus 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 = VFM F-1331).

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

Colonies: 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; CREAS No growth; CYAS No growth; DG18 8–23.

Colonies 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 yellowishbrown. 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 masse fawn.
Fig. 12. Morphological characters of Aspergillus subnutans (CBS 129386T). 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 transcarpathicus (CBS 423.68T). 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. Ascomata 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 Kornérup & 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. SAS) 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 cylindric, 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 = NRRL 5027 = IBT 22042 = IBT 22082 = WFS 380 = DTO 070-A5 = WB 5027).

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

**Colony diameter, 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 in masse fawn; soluble pigments absent; exudates absent; reverse yellowish brown. MEA 25 °C, 7 d: Colonies moderately deep, sulphate; margins entire; mycelium white; texture velvety; sporulation dense, conidia in masse fawn; soluble pigments absent; exudates clear droplets; reverse yellowish brown. YES 25 °C, 7 d: Colonies moderately deep, sulphate; margins entire; mycelium white; texture floccose; sporulation dense, conidia in masse white to light fawn; soluble pigments absent; exudates absent; reverse ochreous buff. DG18 25 °C, 7 d: Colonies moderately deep, plane; margins
Fig. 14. Morphological characters of Aspergillus wisconsinensis (CBS 413.64T). 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. natsus  
3b) Vesicles upright, uncoloured ............................................... A. subnatsus  
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. acidotomus  
5b) Vesicles not exceeding 15 μm in width ............................ A. parvulus  
6a) Conidiophores usually 100–300 μm in length ...................... 7  
6b) Conidiophores extremely variable, 1 001 200 μm in length ...... 9  
7a) Grow on CYA and MEA at 37 °C ................................. A. transcarpathicus  
7b) Does not grow on CYA and MEA at 37 °C ......................... 8  
8a) Slow growth (<20 mm, 25 °C, 7 d) on CYA ......................... A. wisconsinensis  
8b) Fast growth (>25 mm, 25 °C, 7 d) on CYA ........................ A. cervinus  
9a) Fast growth (>80 mm, 25 °C, 7 d) on CYA ...................... A. vinosobubalinus  
9b) Slow growth (<40 mm, 25 °C, 7 d) 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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