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New ochratoxin A producing species of *Aspergillus* section *Circumdati*

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Abstract: *Aspergillus* section *Circumdati* contains species with yellow to ochre conidia and non-black sclerotia that produce at least one of the following extrolites: ochratoxins, penicillic acids, xanthomegnins or melleins. The exception to this is *A. robustus*, which produces black sclerotia, phototropic conidiophores and none of the extrolites listed above. Based on a polyphasic approach using morphological characters, extrolites and partial β-tubulin sequences 20 species can be distinguished, that, except for *A. robustus*, are phylogenetically and phenotypically strongly related. Seven new species are described here, *A. cretensis, A. flocculosus, A. neobridgeri, A. pseudoelegans, A. roseoglobulosus, A. steynii,* and *A. westerdijkiae*. Twelve species of section *Circumdati* produce mellein, 17 produce penicillic acid and 17 produce xanthomegnins. Eight species consistently produce large amounts of ochratoxin A: *Aspergillus cretensis, A. flocculosus, A. pseudoelegans, A. roseoglobulosus, A. westerdijkiae*, *A. sulphurous,* and *Neopetromyces muricatus*. Two species produce large or small amounts of ochratoxin A, but less consistently: *A. ochraceus* and *A. sclerotiorum*. Ochratoxin production in these species has been confirmed using HPLC with diode array detection and comparison to authentic standards. Four further species produce ochratoxin A inconsistently and in trace amounts according to the literature: *A. melleus*, *A. ostianus, A. petrakii*, and *A. persii*. The most important species regarding potential ochratoxin A production in coffee, rice, beverages and other foodstuffs are *A. ochraceus, A. westerdijkiae* and *A. steynii*.

Taxonomic novelties: *Aspergillus cretensis* Frisvad & Samson sp. nov., *Aspergillus flocculosus* Frisvad & Samson sp. nov., *Aspergillus neobridgeri* Frisvad & Samson sp. nov., *Aspergillus pseudoelegans* Frisvad & Samson sp. nov., *Aspergillus roseoglobulosus* Frisvad & Samson sp. nov., *Aspergillus steynii* Frisvad & Samson sp. nov., *Aspergillus westerdijkiae* Frisvad & Samson sp. nov.
Key words: *Aspergillus ochraceus,* BenA, *Circumdati*, extrolites, ochratoxin, penicillic acid, xanthomegnin, mellein.

INTRODUCTION

Members of *Aspergillus* section *Circumdati* (the *Aspergillus ochraceus* group of Raper & Fennell 1965) are important because of their production of several mycotoxins including ochratoxin A (van der Merwe *et al.* 1965a, b, Hesseltine *et al.* 1972, Ciegler 1972), penicillic acid (Ciegler 1972), xanthomegnin, viomellein and vioxanthin (Stack and Mislivec 1978, Robbers *et al.* 1978). Some of the species are also used in the bioindustry, especially for biotransformations (Singh *et al.* 1968, Miski & Davis 1988). The group has been taxonomically revised by Christensen & Raper (1970, 1982). Later Varga *et al.* (1998, 2000 a, b, c) presented molecular analysis of the genetic variability in species of this section. Rather few new species have been described since the revision by Christensen & Raper (1982): *A. sepultus* (Tuthill & Christensen 1986), *Neopetromyces muricatus* (Udagawa *et al.* 1994, Frisvad & Samson 2000), and *A. persii* (Zotti & Montemartini Corte 2002). Based on rDNA sequence data Peterson (2000) indicated that *Petromyces alliaceus*, *P. albertensis*, and *A. lanosus* belong to section *Flavi,* which was supported by phenotypic data (Frisvad & Samson 2000). Another species originally placed in section *Circumdati* was *A. campestris*. This species was transferred to section *Candidi* by Rahbaek & Beinholt (1999), Peterson (2000), and Rahbaek *et al.* (2000). Finally *A. dimorphicus* and *A. sepultus* were transferred to section *Wentii* (Peterson 1995, 2000, Frisvad & Samson 2000), leaving a homogeneous series of very closely related fungi in section *Circumdati* (Frisvad & Samson 2000), with only *A. robustus* being different from the remaining species in the section (Peterson 2000).

This group of organisms is especially well known for its production of ochratoxin A, named after the producer *A. ochraceus* CBS 263.67 (van der Merwe *et al.* 1965a, b) and has attracted much interest by its role in the ochratoxin contamination of coffee (Levi *et al.* 1974, Gallaz & Stalder 1976, Levi 1980, Tsubouchi *et al.* 1984, 1985, Studer-Rohr *et al.* 1995, Viga & Mercadier 1998, Mantle & Chow 2000, Taniwaki *et al.* 2001, Ahmad & Magan 2003, Batista *et al.* 2003, Martins *et al.* 2003, Taniwaki *et al.* 2003, Suárez-Quiroz *et al.* 2004). *Aspergillus ochraceus* and ochra-

toxin A have also been detected in rice (Miyaki *et al.* 1969, Uchiyama *et al.* 1976). On the other hand *A. ochraceus* and allied species are less common on wheat and corn (Wallace & Sinha 1962, Lopez & Christensen 1967, Christensen & Kaufman 1969). Mycotoxins from *A. ochraceus* can cause mycotoxicoses in mice and swine when grown on rice (Robbers *et al.* 1978, Zimmermann *et al.* 1979). Furthermore members of the section have been found on salted or dried fish (Hesseltine *et al.* 1972). Other members of section *Circumdati sensu stricto* have been reported to produce ochratoxin A: *A. auricomus* (Varga *et al*. 1996, Batista *et al*. 2003), *A. elegans* (Tsubouchi *et al*. 1985, Batista *et al*. 2003, Nasser *et al*. 2003), *A. insulicola* (Batista *et al*. 2003), *A. melleus* (Lai *et al*. 1970, Ciegler 1972), *A. ostianus* (Ciegler 1972, Batista *et al*. 2003, Nasser *et al*. 2003), *A. petrakii* (Ciegler 1972, Hesseltine *et al*. 1972, Batista *et al*. 2003), *A. sclerotiorum* (Ciegler 1972, Hesseltine *et al*. 1972, Varga *et al*. 1996, Batista *et al*. 2003, Nasser *et al*. 2003), *A. sulphureus* (Ciegler 1972, Hesseltine *et al*. 1972 Varga *et al*. 1996, Batista *et al*. 2003, Nasser *et al*. 2003) and *Neopetromyces muricatus* (Frisvad & Samson 2000). However, there is much confusion in the literature about the production of mycotoxins by other members of the section, as Ciegler (1972) and Hesseltine *et al*. (1972) indicated that many strains could be intermediate forms between known species. During our mycological investigations of various substrates we have collected several new taxa of the section, including some which have formerly been identified as *A. ochraceus*. These species are described here. Furthermore an overview of the mycotoxins produced by species assigned to this section is given.

MATERIALS AND METHODS

All cultures investigated in this study are listed in Table 1. The methods and media for isolation and identification are described by Samson *et al.* (2004). The names of colours are based on Kornerup & Wanscher (1984). The cultures used for the molecular study were grown on malt peptone (MP) broth using 10 % (v/v) of malt extract (Brix 10) and 0.1 % (w/v) bacto peptone (Difco) in 2 mL of medium in 15 mL tubes. The cultures were incubated at 25 °C for 7 d in light/darkness.

Extrolite analysis

Extrolites (includes secondary metabolites; for definition see Samson & Frisvad 2004) were analysed by HPLC using alkylphenone retention indices and diode array UV-VIS detection as described by Frisvad & Thrane (1987), with minor modifications by Smedsgaard (1997). Standards of ochratoxin A, B, antibiotic Y, diaporthin, orthosporin, TR-2, cycloechinulin, penicillic acid, xanthomegnin, viomellein, vioxanthin, circumdatin A, B, and C, mellein, and 4-hydroxymellein were available from the collection at Biocentrum-DTU (Kgs. Lyngby, Denmark) and used to compare with the extrolites found in extracts of members of *Aspergillus* subgenus *Circumdati* section *Circumdati*.

DNA Extraction, sequencing and analysis

The total fungal genomic DNA was isolated using FastDNA® Kit (Bio 101, Carlsbad, U.S.A.) according to the manufacturer's instructions. Amplification of β-tubulin gene was performed using the primers Bt2a and Bt2b (Glass 1995). PCR was performed in a 50 µL reaction mixture containing 1 µL of genomic DNA (10 ng/µL), 5 µL of PCR buffer, 30 µL of ultra pure sterile water, 10 µL dNTP (1 mM), 1 µL of each primer (50 pmol/µL) and 1 µL Taq polymerase (2.5 U/µL DNA) (SpaeroQ, Leiden, The Netherlands). Amplification was performed in a GeneAmp PCR system 9700 (AB Applied Biosystems, Nieuwerkerk a/d Yssel, The Netherlands); programmed for 5 cycles of 1 min denaturation at 94 °C followed by primer annealing 1 min 30 s at 68 °C and primer extension 2 min at 72 °C with a decrease of the annealing temperature 1 °C /cycle followed by 25 cycles of 1 min denaturation at 94 °C followed by primer annealing 1 min 30 s at 64 °C and primer extension 2 min at 72 °C and a final 10 min elongation step at 72 °C. After amplification of the β-tubulin gene, excess primers and dNTP's were removed from the reaction mixture using a commercial GFX column, PCR DNA Purification kit (Amersham Bioscience, Roosendaal, The Netherlands). The purified PCR fragments were resuspended in 50 µL of TE buffer. The PCR fragments were directly sequenced (White *et al.* 1990) in both directions with the primers Bt2a and Bt2b using a DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Bioscience, Roosendaal, The Netherlands). The sequence PCR reaction mixture, total reaction mix is 10 µL, contained 2 µL of template DNA (15–45 ng/µL), 4 µL Dye terminator RR mix, 3 µL ultra pure sterile water and 1 µL primer Bt2a or Bt2b (4 pmol/µL). The reaction was performed in a GeneAmp PCR system 9700 run in 9600 mode (AB Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands); programmed for 25 cycles of 10 s denaturation at 96 °C followed by primer annealing 5 s at 50 °C and primer extension 4 min at 60 °C. Sequencing products were purified according to the manufacturer's recommendations with Sephadex G-50 superfine column (Amersham Bioscience, Roosendaal, The Netherlands) in a multiscreen HV plate (Millipore, Amsterdam, The Netherlands) and with MicroAmp Optical 96-well reaction plate (AB Applied Biosystems, Nieuwerkerk a/d Yssel, The Netherlands). The samples were analyzed on an ABI PRISM

Table 1. List of cultures examined.

Taxon name	Strain number(s)	Substratum and origin	Sequence accession no
A. auricomus	CBS 613.78 = NRRL 397	Unknown	AY819983
	CBS 467.65 = ATCC 16890 = IMI 172277 = LCP 89.2596 = LSHB A41 = NRRL 391 = WB 391 **T**	Unknown	AY819982
A. bridgeri	CBS 350.81 = JB 26-1-2 = ATCC 44562 = IMI 259098 = NRRL 13000	Soil, Wyoming, U.S.A.	AY819991
A. cretensis	CBS 112805 = IBT 23283	*Citrus* sp., Israel	AY819978
	CBS 112802 = IBT 17505 **T**	Soil in the Samaria Cleft, Crete, Greece	AY819977
A. elegans	CBS 102.14 = ATCC 16886 = ATCC 13829 = CBS 543.65 = IFO 4286 = IMI 133962 = LCP 89.2585 = QM 9373 = WB 4850 = NRRL 4850 **T**	Unknown substratum, U.S.A.	AY819952
A. flavus	CBS 100927 = ATCC 16883 = CBS 569.65 = IMI 124930 = LCP 89.2565 = WB 1957	Cellophane, South Pacific Islands	AY819992
A. flocculosus	CBS 112798 = IBT 21076 = NRRL 5224	Unknown substratum, India	AY819954
	CBS 112799 = IBT 23406	Soil in Gauguin garden, Costa Rica	AY819957
	CBS 116336 = IBT 26255	Sector variant of CBS 112799, The Netherlands	AY819956
	CBS 112789 = IBT 22898	Internal infection of current grape, Greece	AY819958
	CBS 112784 = IBT 21104	Unknown substratum, Venezuela	AY819959
	CBS 112785 = IBT 23121 **T**	Saltern, Slovenia	AY819961
A. insulicola	CBS 382.75 = NRRL 6138 = ATCC 26220 **T**	Soil, Venezuela	AY819960
A. melleus	CBS 112786 = IBT 14265 = IBT 26262 = NRRL 386	Unknown	AY819964
	CBS 114.26	Unknown	AY819967
	CBS 546.65 = NRRL 5103 = ATCC 16889 = WB 5103 **T**	Soil, India	AY819965
A. neobridgeri	CBS 559.82 = IBT 14026 = NRRL 13078 = RMF 7127 **T**	soil, Nebraska, U.S.A.	AY819985
A. ochraceopetaliformis	CBS 123.55 = ATCC 12066 = IMI 211804 = QM 6955 = WB 4752 **T**	Scalp lesion of man, Brazil	AY819955
A. ochraceus	CBS 624.78 =IMI 313488 = IMI 016265ii = LSHB Ac83 = NCTC 3895 = NRRL 419	Unknown substratum, France	AY819970
	CBS 748.70 (Type strain of *Sterigmatocystis japonica*)	Unknown, Japan	AY819971
	CBS 108.08 = ATCC 1008; = CBS 547.65 = CECT 2093 = DSM 824 = IMI 016247 = IMI 016247iv = IMI 016247iii = LCP 89.2564 = LSHB Ac40 = NCTC 3889 = NRRL 398 = NRRL 1642 = QM 6731 = WB 398 **T**	Unknown	AY819973
A. ostianus	CBS 311.80 = IMI 237221	Pulses, India	AY819969
	CBS 548.65 = ATCC 16887 = CBS 103.07 = IMI 015960 = IMI 015960iii = LCP 89.2584 = LSHB Ac35 = NCTC 3788 = NRRL 420 = QM 4760 = WB 420 **T**	Unknown	AY819968
A. persii	CBS 112795 = IBT 22660; = MUCL 41970 **T**	Toenail of patient, Italy	AY819988
A. petrakii	CBS 640.78 = NRRL 4789 = WB4789	Unknown	AY819966
A. petrakii	CBS 105.57 = ATCC 16885 = IMI 172291 = LCP 89.2586 = QM 8041 = WB 4369 = WB 4777 **T**	*Leptinotarsa decemlineata*, Hungary	AY819972
A. pseudoelegans	CBS 112797 = IBT 23403	soil in Gauguin garden, Costa Rica	AY819963

	CBS 112796 = IBT 23402 **T**	soil in Gauguin garden, Costa Rica	AY819962
A. roseoglobulosus	CBS 112800 = IBT 14720 **T**	Decaying leave of *Rhizophora mangle*, Bahamas	AY819984
A. sclerotiorum	CBS 632.78 = NRRL 4901 = WB 4901	Unknown	AY819987
	CBS 549.65 = ATCC 16892 = DSM 870 = IFO 7542 = IMI 056732 = IMI 056673 = LCP 89.2594 = NRRL 415 = QM 6732 = WB 415 **T**	Fruit of *Malus sylvestris*, Oregon, U.S.A.	AY819986
A. steynii	CBS 112814 = IBT 23792	Green coffee bean, India	AY819953
	CBS 112812 = IBT 23096 **T**	Surface-disinfected green coffee bean, India	AY819951
A. sulphureus	CBS 385.75 = IMI 174453 = NRRL 5584 = WB 5280	Alkaline soil, India	AY819990
	CBS 550.65 = ATCC 16893 = IMI 211397 = LCP 89.2593 = WB 4077 **T**	Soil, India	AY819989
A. westerdijkiae	CBS 112791 = IBT 23783	Surface disinfected green coffee bean, India	AY819974
	CBS 112804 = IBT 24389 = EXF 618	Saltern, Slovenia	AY819975
	CBS 112803 = K804 = IBT 10738 = NRRL 3174 = ATCC 22947 = MUCL 39539 **T**	*Andropogon sorghum*, South Africa	AY819976
N. muricatus	CBS 112809 = FRR 3819 = IBT 22942	Peanuts, Australia	AY819979
	CBS 112810 = IBT 14266 = NRRL 5227	Unknown substratum, Indonesia	AY819980
	CBS 112808 = IBT 19374 = IMI 36852 1a **T**	Soil, grassland, Philippines	AY819981

T = ex-type culture.

3700 Genetic Analyzer (AB Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). A consensus was computed from the forward and reverse sequences with software package Seqman and Editseq from the lasergene package (DNAStar Inc., Madison, WI). The alignments of the partial β-tubulin gene sequence data were performed using the software package BioNumerics from Applied Maths and manual adjustments for improvement were made by eye where necessary. The phylogenetic analyses of sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2000). Alignment gaps were treated as fifth character state, missing data were identified by '?', uninformative characters were excluded and all characters were unordered and equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis & Bull 1993). Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) were also calculated. Sequences of strains obtained in this study and shown in Figure 1 were deposited at GenBank (Table 1).

RESULTS

All species in *Aspergillus* subgenus *Circumdati* section *Circumdati* produced at least one of the following important mycotoxins: ochratoxins (A and B), penicillic acids (penicillic acid, dehydropenicillic acid, orsellinic acid) and xanthomegnins (xanthomegnin, viomellein and vioxanthin), except one species: *A. robustus* (Table 2). The latter species is the only species left in the section with black sclerotia and phototropic conidiophores, and *A. robustus* appears to be outside the main clade of species in the section (Peterson 2000). Seven species produce all three polyketide families: *A. flocculosus*, *Neopetromyces muricatus*, *A. ochraceus*, *A. roseoglobulosus*, *A. sclerotiorum*, *A. westerdijkiae* and *A. sulphureus*. Eight species did not produce ochratoxins, but the two other polyketide families: *A. auricomus*, *A. bridgeri*, *A. insulicola*, *A. melleus*, *A. neobridgeri*, *A. ostianus*, *A. persii*, and *A. petrakii*.

However authentic strains from four of these species, *A. melleus* CBS 112786 = NRRL 386, *A. ostianus* NRRL 5225, *A. persii* NRRL 4901 and *A. petrakii* CBS 105.57 = NRRL 4369 have previously been reported to produce trace amounts of ochratoxin A (Hesseltine *et al.* 1972), so it is possible that 11 species of section *Circumdati* are capable of producing all three mycotoxin families. Only two species did not produce penicillic acid: *A. steynii* and *A. elegans*.

Table 2. Species in *Neopetromyces* and *Aspergillus* subgenus *Circumdati* section *Circumdati* and their mycotoxin and mellein production. Species names in bold are newly described in this paper.

Species	Sclerotia	Ochratoxins	Penicillic acid	Xanthomegnins	Mellein
(*A. robustus*)	+	–	–	–	–
A. auricomus	+	–[1]	+	+	+
A. bridgeri	+	–	+	+	–
A. cretensis	+	+	+	–	+
A. elegans	+	–	–	+	–
A. flocculosus	+	+	+	+	+
A. insulicola	–	–	+	+	+
A. melleus	+	–[2]	+	+	+
A. neobridgeri	–	–	+	+	–
A. ochraceus	+/–	+/–	+	+	+
A. ostianus	+/–	–[2]	+	+	+
A. persii	+	–[2]	+	+	–
A. petrakii	–	–[2]	+	+	+
A. pseudoelegans	+	+	+	–	+
A. roseoglobulosus	+	+	+	+	–
A. sclerotiorum	+	+/–	+	+	–
A. steynii	+	+	–	+	+
A. sulphureus	+	+	+	+	–
A. westerdijkiae	+	+	+	+	+
N. muricatus	+	+	+	+	–

[1]The strain *A. auricomus* FRR 3819, reported to produce ochratoxin A by Varga *et al*. (1996) was re-identified as *Neopetromyces muricatus*. [2]Type or authentic strains have been reported to produce trace amounts of ochratoxin A (Ciegler 1972, Hesseltine *et al.* 1972), but we have not been able to repeat the detection of OTA in those strains yet.

These species are also united by their common production of TR-2 and cycloechinulin and from a phylogenetic point of view they could be sister species. Only two species did not produce xanthomegnins: *A. cretensis* and *A. pseudoelegans*. Thus penicillic acid is produced by 85 % of the species, xanthomegnins are produced by 85 % of the species and ochratoxins are produced by 50–70 % of the species. It is entirely possible that the remaining species have parts of the genes required for production of these mycotoxins. The consistency in extrolite production among isolates within these species is high; in most cases 100 % of the isolates produce the extrolites characteristic of their species. *A. ochraceus* and *A. sclerotiorum* are the only exceptions; several isolates in these species do not produce ochratoxin A (Table 2). Furthermore the trace production of ochratoxin A in *A. melleus, A. ostianus, A. persii* and *A. petrakii* may be inconsistent. The extrolites mellein and 4-hydroxymellein, usually not regarded as a mycotoxins, are produced by 60 % of the species in *Circumdati*, but this fourth polyketide family of extrolites is apparently not directly linked to ochratoxin, penicillic acid or xanthomegnin production.

The parsimony analysis of the sequence data was restricted to 5000 equally most parsimonious trees MPT (TL = 517 steps, CI = 0.654 RI = 0.884, RC = 0.578), one of which is shown in a phylogram in Fig. 1**.** The tree was rooted to *A. flavus*. Bootstrap support from 1000 replicates is shown at the nodes**.** The phylogeny based on the partial β-tubulin sequences shows that *A. pseudoelegans* is a new species supported by consensus and bootstrap (95 %). It is different from *A. insulicola* (10 changes) and to the group of *A. flocculosus* (10–15 changes) this is supported by consensus and bootstrap (100 %). *Aspergillus flocculosus* is a new species but between the seven strains some variation (6 changes) exists but it is supported by consensus and bootstrap (78 %). Remarkably, also the ex-type culture of *A. ochraceopetaliformis* is part of this group. Morphological data and extrolite patterns showed that this species is distinct from *A. flocculosus* and close (or similar) to *A. insulicola*. *Aspergillus insulicola* and *A. ochraceopetaliformis* both share consistent production of insulicolide A and B and other extrolites and lack the production ochratoxin A and aspyrone, which are consistent produced by *A. flocculosus*. *Aspergillus steynii* forms together with *A. elegans* a separate clade. This clade is also supported by the extrolite analyses, where both species share the production of cycloechinuline. The consensus and the bootstrap (97 %) of the BenA sequences indicate that *A. steynii* is a distinct species and differs from the other species belonging to the section *Circumdati*. *A. melleus*, *A. petrakii*, *A. ostianus, A. ochraceus* and *A. westerdijkiae* form a separate clade.

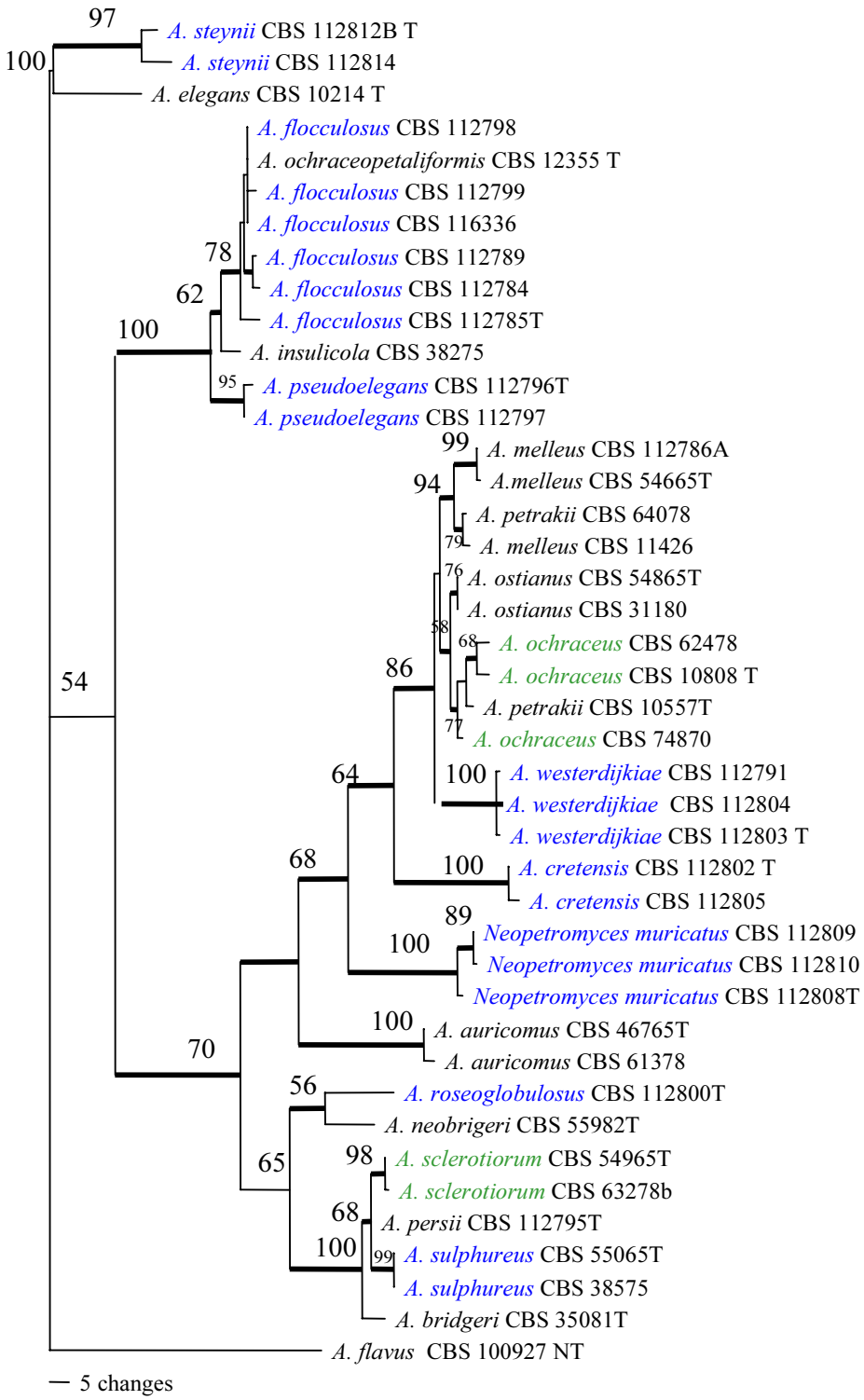

Fig. 1. One of the 5000 equally MPT of 517 steps based on heuristic search partial β-tubulin sequences with *A. flavus* as an outgroup. The branches in bold are 100 % in the 70 % majority-rule consensus of equally parsimonious trees. The numbers represent bootstrap percentages > 50 % (CI = 0.654, RI = 0.884 RC = 0.578, HI = 0.346). Taxa in blue are producers of ochratoxin, in green taxa which do not consistently produce ochratoxin.

The sequences of the three strains of *A. westerdijkiae* are almost identical (only 2 changes) and distinct from the other members in this group (also supported by consensus and bootstrap (100 %). The two strains of *A. cretensis* are also almost identical (2 base pair differences) and, supported by consensus and bootstrap (100 %), clearly different from the other members of the section *Circumdati*. *Aspergillus neobridgeri* and *A. roseoglobulosus* are related new species and this is supported by consensus, but poorly by bootstrap (56 %). There is a clear difference of 30 changes (the length of the branch) between these two species. The type culture of *A. petrakii* CBS 105.57 is close to the type of *A. ochraceus*, while the other strain of *A. petrakii* CBS 640.78 is close to *A. melleus*.

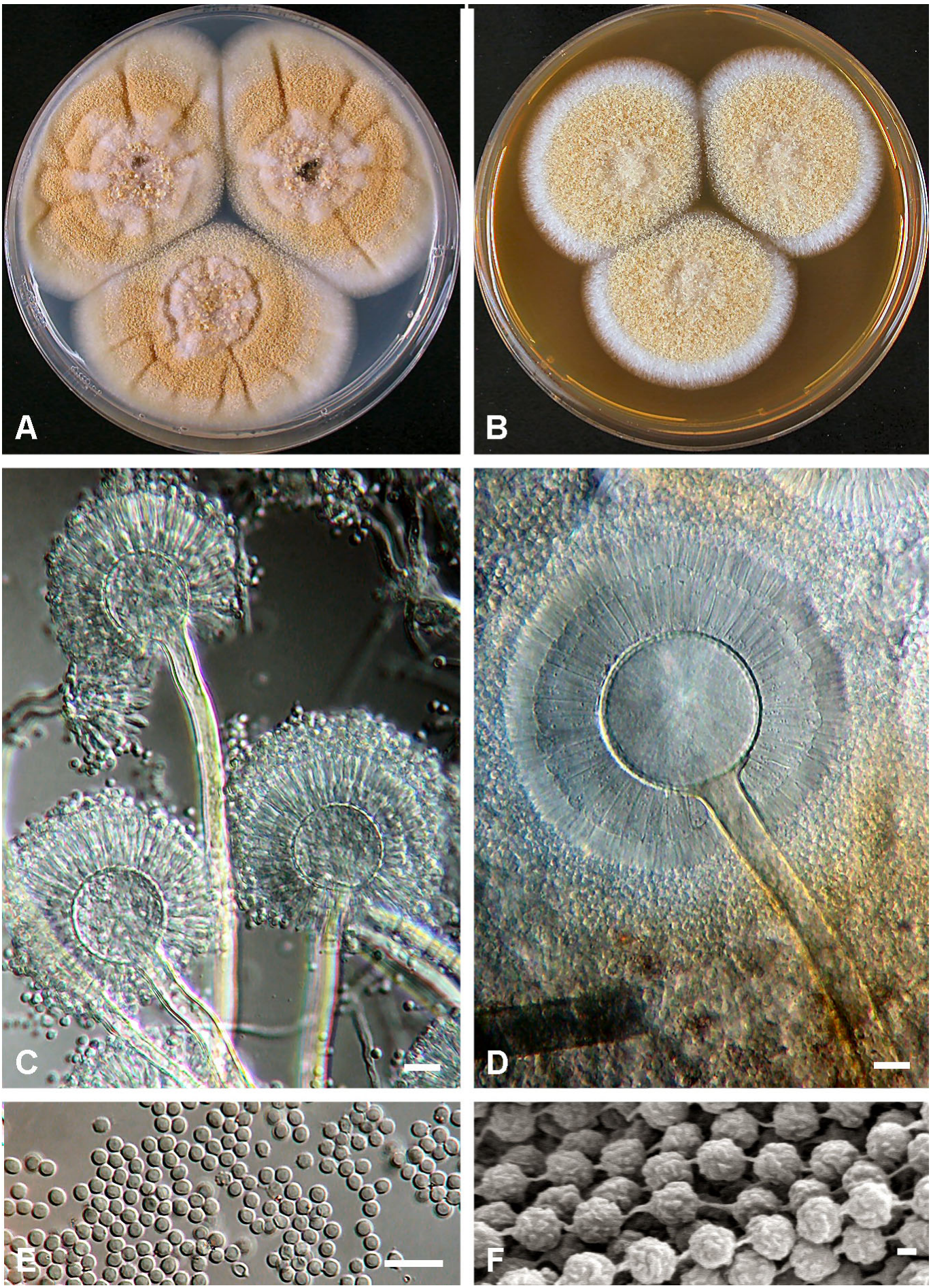

Fig. 2. *Aspergillus westerdijkiae*. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. E. Conidia. F. Scanning electron micrograph photo of conidia. Scale bars: C–E. = 10 µm, F = 1 µm.

Taxonomy

Aspergillus westerdijkiae Frisvad & Samson, **sp. nov.** MycoBank MB500000.

Etymology: Named in honour of a former director of the CBS, Prof. Dr Johanna Westerdijk.

Ab *Aspergillo ochraceo* sclerotiis cremeis, incremento absente 37 °C, et metabolito L-657.398 differt.

Typus: CBS 112803 (lyophilized culture).

Colony diameters after 7 d at 25 °C in mm: CYA25 49–57; MEA 42–47; no growth on CYA37. *Colony colours and textures*. Moderate to good conidia production on CYA25, pale to light to dull yellow (3B3–3A3); mycelium white, inconspicuous; sclerotia sparsely produced; pale yellow after 7 d, becoming dull orange at age. Reverse crème brown, no soluble pigment present. Good sporulation on MEA, velvety, pale to light or dull yellow (3A3–3B3) after 7 d; mycelium white, sclerotia sparsely formed, overgrown by conidial state and in shades of orange, reverse brown centre with yellow to medium–coloured edge. No growth on CYA37. Conidial heads radiate, splitting into columns; stipes up to 1800 µm in length, walls rough, uncoloured to yellow pigmented; vesicles globose to spathulate, (16–)20–35(–42) × (3–)3.5–5.7(–7.1)µm; biseriate; metulae covering the entire vesicle, measuring (10.5–)11 × 19(–23) µm; phialides (6.8–)7.3–9.7(–10.5) × (2–)2.1–3(–3.5) µm; conidia predominantly globose, finely roughened, (2.3–)2.5–3(–3.1) × (2.2–)2.3–2.8(–3.1) µm; sclerotia sparsely produced, white to cream, globose to subglobose, (460–)480–760(–840) × (430–)480–660 (–720) µm on CYA and (440–)450–720(–750) × (430–)430–650(–700) µm on OA.

Distinguishing features: This species is morphologically similar to *Aspergillus ochraceus*, though it is unable to grow at 37 °C. The white to cream sclerotia produced by *A. westerdijkiae* differ from the pink to vinaceous purple sclerotia of *A. ochraceus*.

Type: CBS 112803 in herb. CBS (cultures ex-type: CBS 112803 = K 804 = IBT 10738 = NRRL 3174 = ATCC 22947 = MUCL 39539, ex *Andropogon sorghum*, **South Africa**; the isolate from which ochratoxin was first discovered.

Other isolates: CBS 263.67 = CSIR 806 = IMI 132429, ex grain of *Andropogon sorghum*, **South Africa**, De B. Scott; PIL 657 = IBT 22338, ex rice, **China**; IBT 22648, ex wheat, **U.K.**; CBS 112804 = IBT 24389 = EXF 618, ex saltern, Secovlje, **Slovenia**, CBS 112791 = IBT 23783, ex surface disinfected green coffee bean, Karnataka, **India**; IBT 23791 & IBT 23971, ex Eilat salterns, 25 % salinity pond, **Israel**; IBT 24953, ex doorstep, dwelling, **Denmark**, IBT 24769 & IBT 21991, ex saltern, Secovlje, **Slovenia**; NRRL 5221 = IBT 13878, Australia; NRRL 5175, Texas, **U.S.A.**; NRRL 5228, New Jersey, U.S.A.; IBT 21930, ex green coffee bean, **Venezuela**, and numerous other samples from coffee in **Brazil** (21 isolates), **India** (15 isolates), **Indonesia** (five isolates), **Venezuela** (two isolates), **Kenya** (four isolates). All isolates listed produced large amounts of ochratoxin A and B, penicillic acid, xanthomegnin, viomellein and vioxanthin.

Extrolites: ochratoxin A & B, penicillic acid, mellein, 4-hydroxymellein, xanthomegnin, viomellein, vioxanthin, circumdatins (A–G) and asperloxins, aspergamides (= avrainvillamides = stephacidins), L-657,398 (Schwartz *et al*. 1988). The partially characterised extrolite NB1 is produced by all isolates, but not produced by any strain of *A. ochraceus*.

Aspergillus roseoglobulosus Frisvad & Samson, **sp. nov.** MycoBank MB500001.

Etymology: Named after colour and pattern of the colonies

Ab *Aspergillo ochraceo* mycelio roseo-rubro et concolori reverso coloniae in agaro CYA, incremento conspicuo 37 °C, melleinis et circumdatinis et aspergamidis absentibus differt.

Typus: CBS 112800 (lyophilized culture).

Colony diameters after 7 d of incubation, in mm: CYA25 46–55; MEA 47–52; YES: 72–78 mm, CYA, 37 ºC: 28–32 mm.
Colony colours and textures: Weak conidial production on CYA25, light yellow when young, dark brown exudates present, becoming greyish yellow to olive yellow at age; mycelium rose red, conspicuous; no sclerotia detected. Reverse rose red, (light) reddish soluble pigment present. No sporulation on MEA after 7 d, floccose; mycelium white, sclerotia sparsely produced; white after 7 d, in shades of brown at maturity, reverse brown to dark brown. Good growth at CYA37. Conidial heads radiate, splitting into two columns; stipes up to 1000 µm in length, walls rough, light yellow/brown pigmented; vesicles globose, (31–)32–37(–41) µm; biseriate; metulae covering the entire vesicle, measuring (11.6–)12.1–17.1(–17.6) × (3.7–)3.8–5.1(–5.3) µm; phialides (7.2–)7.8–10(–10.2) × (1.9–)2–2.9(–3) µm; conidia globose to subglobose, finely roughened, (1.9–)2.1–2.4(–2.6) µm; sclerotia only on MEA detected, sparsely, measuring (720–)740–990(–1020) × (600–)660–800(–830) µm on MEA.

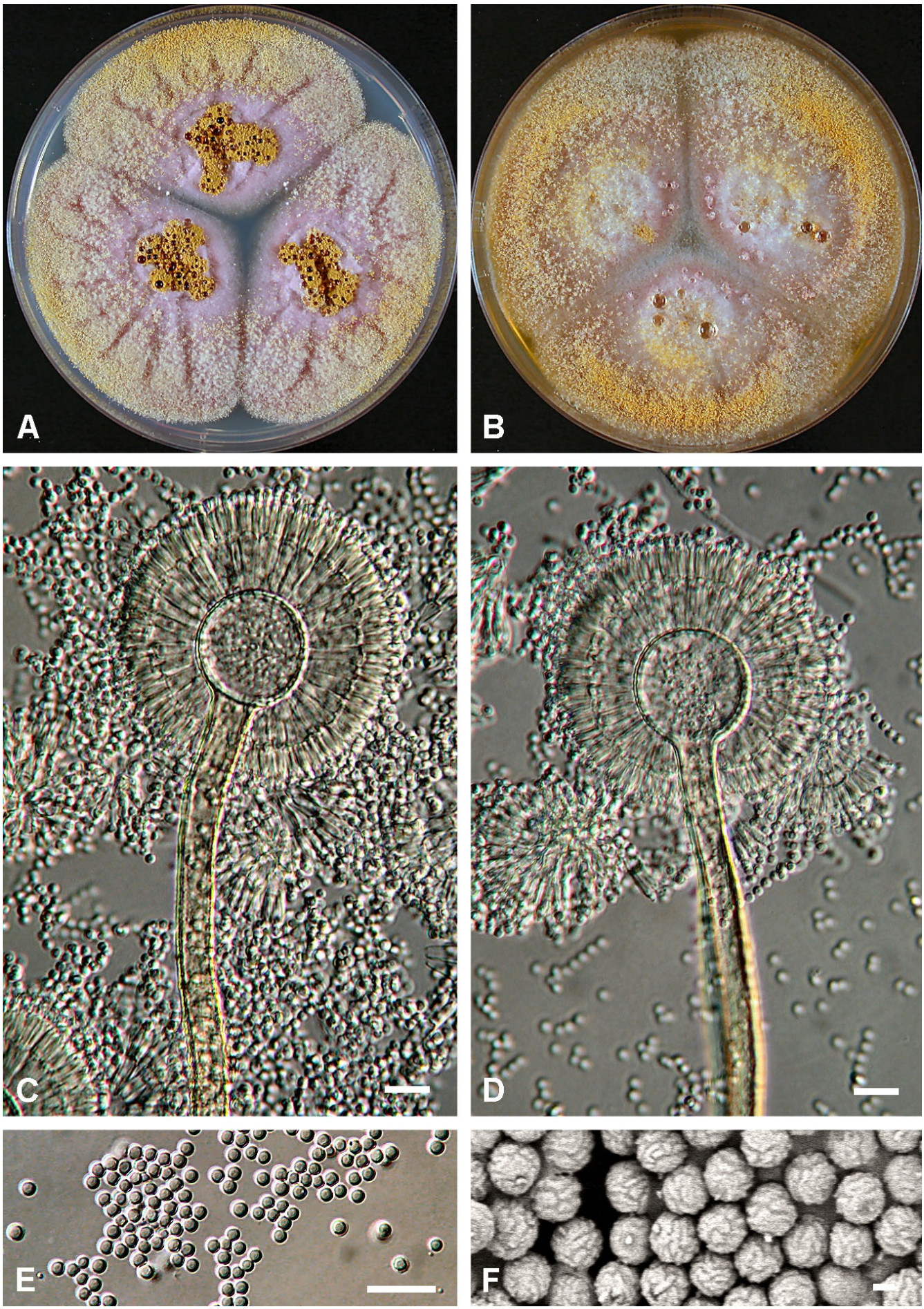

Fig. 3. *Aspergillus roseoglobulosus*. Fourteen-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. E. Conidia. F. Scanning electron micrograph photo of conidia. Scale bars: C–E = 10 µm, F = 1 µm.

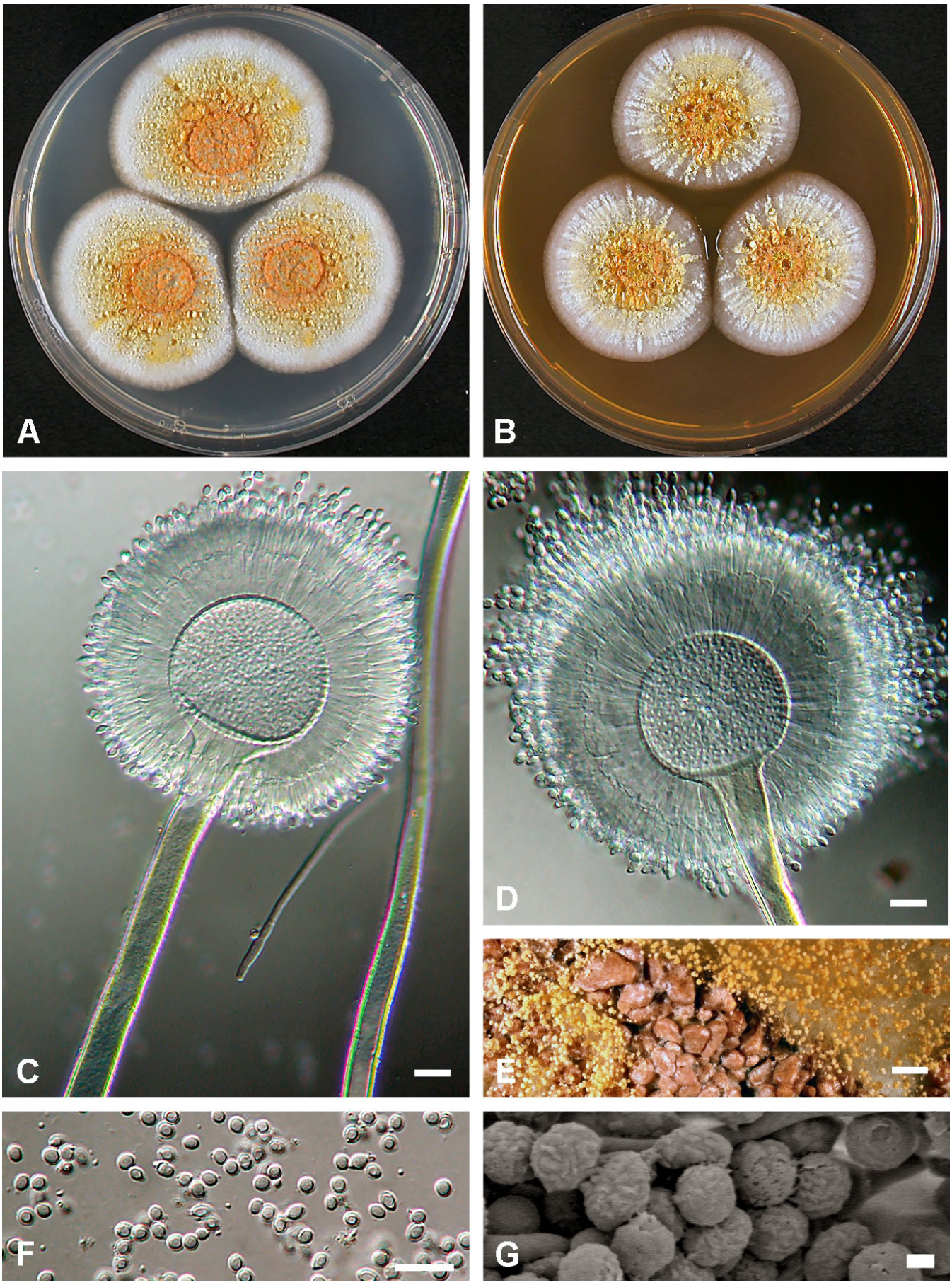

Fig. 4. *Aspergillus cretensis*. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. E. Detail of a 28-d-old colony showing sclerotia and conidial heads. F. Conidia. G. Scanning electron micrograph photo of conidia. Scale bars: C, D, F = 10 µm, E = 1 mm, G = 1 µm.

Type: CBS 112800 = IBT 14720, ex decaying leave of *Rhizophora mangle*. Little San Salvador Island, **Bahamas**, Jul. 1992, B.R. Rassing.

Distinguishing features: The rose red mycelium and rose red reverse on CYA make this a distinct species.

Extrolites: Ochratoxin A & B, penicillic acid, xanthomegnin, viomellein, a specific indol-alkaloid.

Aspergillus cretensis Frisvad & Samson, **sp. nov.** MycoBank MB500002.

Etymology: Named after the Greek island Crete, where the fungus was first found.

Ab *Aspergillo ochraceo* stipitibus longis conidiophororum, conidiis laete luteis, sclerotiis magnis discoideis, conidiis ellipsoideis, et melleinis, circumdatinis et aspergamidis absentibus differt.

Typus: CBS 112802 (lyophilized culture).

Colony diameters after 7 d of incubation, in mm: CYA25 38–46; MEA 33–40; no growth on CYA37. *Colony colours and textures.* Conidial production on CYA25 very sparsely in vivid yellow to pure yellow shades; mycelium white, inconspicuous; colony dominated by abundant sclerotia; white to light yellow after 7 d, becoming ash blond (CBS 112805) to reddish brown (CBS 112802). Reverse pale brown to pale yellow brown, no soluble pigment present. On MEA conidial production sparse, velvety, pastel yellow to light yellow after 7 d, becoming vivid yellow after prolonged incubation; mycelium white, sclerotia formed, ash blond (CBS 112805) to reddish brown (CBS 112802), reverse brown center with medium–brown edge. No growth on CYA37. Conidial heads radiate, splitting into columns; stipes long, up to 4000 µm in length, walls rough, uncoloured to yellow pigmented; vesicles large, globose to spathulate, (30–)40–50(–60) µm; biseriate; metulae covering the entire vesicle, measuring (11–)12–17(–23) × (3.1–)3.2–5.2(–6.3) µm; phialides (8.5–)9.5–11.5(–12.5) × (2–)2.2–3.2(–3.2)µm in length; conidia predominant broadly ellipsoid to ellipsoidal, occasionally fusiform, smooth to finely roughened, (2.3–)2.5–3(–3.1) × (1.9–)2–2.4(–2.5); sclerotia abundant, globose to subglobose, sometimes as flat disks, discoid, (450–)700–1000(–1300) × (420–)600–900(–1050) µm on CYA and (860–)940–1220(–1270) × (750–)780–1100(–1120) µm.

Type: CBS 112802 = IBT 17505, ex soil in the Samaria Cleft, Crete, **Greece**, May 1985, J.C. Frisvad.

Other isolates: CBS 112805 = IMI 001177 = IBT 23283, ex *Citrus* sp., **Israel**.

Distinguishing features: Long conidiophores (4 mm), vivid yellow conidia, large (discoid) sclerotia and broadly ellipsoidal to ellipsoidal conidia make this a distinctive species.

Extrolites: ochratoxin A & B, penicillic acid, mellein & 4-hydroxymellein, and several specific not yet structure elucidated compounds. One of these partly characterised compounds is also produced by *Aspergillus robustus* M. Chr. & Raper.

Aspergillus flocculosus Frisvad & Samson, **sp. nov.** MycoBank MB500003.

Etymology: Named after its very flocculent texture of the colony.

Ab *Aspergillo ochraceo* coloniis floccosis, conidiis parvis luteo-brunneis, asteltoxino exsudato, sed circumdatinis et aspergamidis absentibus differt.

Typus: CBS 112785 (lyophilized culture).

Colony diameters after 7 d of incubation, in mm: CYA25 38–51; MEA 42–51; CYA37 11–16. *Colony colours and textures*. No or weak sporulation on CYA25 after 7 d of incubation, olive (3D4) to brownish orange (5C4) conidia; mycelium white, pinkish or crème/grey, conspicuous floccose; sclerotia sparsely produced at prolonged incubation (>30 d), covered underneath mycelium and located as 'knits'; reverse red brown, occasionally orange brown, in some isolates produce a red brown soluble pigment. On MEA no to weak sporulation, prolonged incubation showed formation of conidiophores at the edge of the colony, dull yellow to greyish yellow (3B4) after 7 d and becoming light (yellow) brown (5D6–7) to brownish yellow (5C7–5D7) after prolonged incubation; white mycelium, sclerotia sparsely formed and covered by mycelium, reverse brown or red brown.
Good growth on CYA37, 11–16 mm. Conidial heads radiate, splitting into columns; stipes up to 1000–1500 µm in length, walls rough, light brown; vesicles globose to pyriform, with large variation(16–)20–44(–46) µm; biseriate; metulae covering the entire vesicle, measuring (7–)8–26(–28) × (2.3–)2.8–5.7(–6.1) µm; phialides (7–)7.6–10.5(–12) × (1.7–)1.9–3(–3.4) µm in length; conidia globose, predominantly smooth, relatively small (1.9–)2–2.5(–2.7) µm; sclerotia sparsely formed and covered by mycelium, (340–)360–500(–540) µm on CYA and on MEA (360–)400–590(–650) µm.

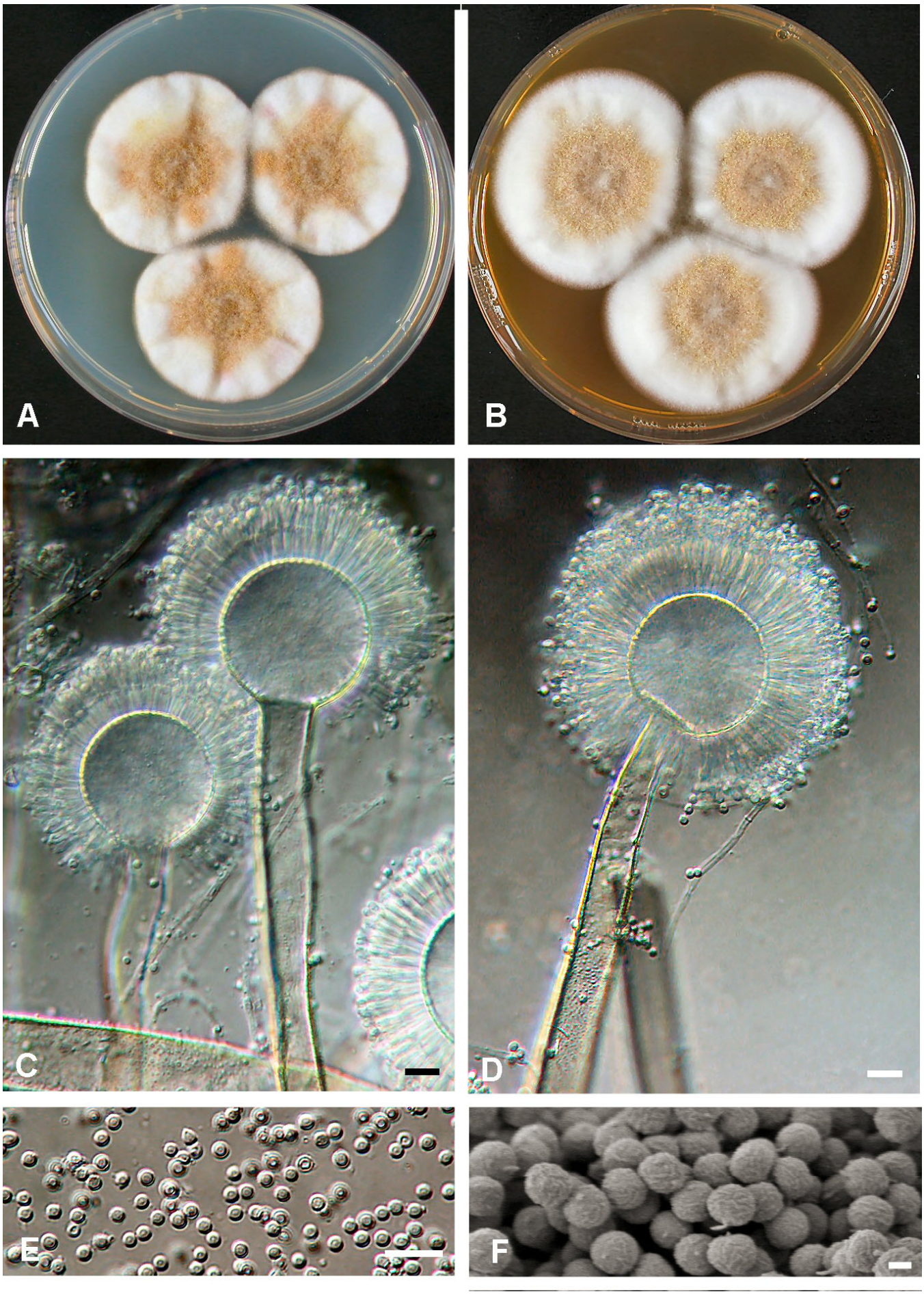

Fig.5. *Aspergillus flocculosus*. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. E. Conidia. F. Scanning electron micrograph photo of conidia. Scale bars: C–E = 10 µm, F = 1 µm.

Type: CBS 112785 = IBT 23121, ex saltern; Secovlje, **Slovenia**, N. Gunde-Cimerman.

Other isolates: CBS 112784 = IBT 21104, CBS 112789 = IBT 22898, CBS 112799 = IBT 23406 from same substrate as CBS 112785.

Distinguishing features: The strongly floccose colonies on CYA, (yellow) brown to brownish yellow conidia, which are relatively small sized makes this a distinct species.

Extrolites: ochratoxin A & B, penicillic acid, asteltoxin, xanthomegnin, viomellein, vioxanthin, 4-hydroxymellein, cf. dipodazin.

Aspergillus neobridgeri Frisvad & Samson, **sp. nov.** MycoBank MB500004.

Ab *Aspergillo bridgeri* vesiculis spathulatis, coloniis ex ambabus partibus brunneis in agaro CYA, in quo medio paucis conidiis formatis, differt.

Typus: CBS 559.82 (lyophilized culture).

Colony diameters after 7 d of incubation, in mm: CYA25 22–27; MEA 43–48; CYA37 38–44. *Colony colours and textures.* Weak sporulation on CYA25, yellowish white (3A2) after 7 d of incubation, becoming yellowish grey at age; mycelium white, inconspicuous; no sclerotia detected. Reverse yellow to yellow brown, no soluble pigment present. No sporulation on MEA after 7 d, becoming yellowish grey at age, velvety; mycelium white, no sclerotia produced. Higher growth at CYA37 than on CYA25 (38–44). Conidial heads radiate, splitting into columns; stipes up to 1800 µm in length, walls rough, light yellow/brown pigmented; vesicles distinct spathulate, (33–)34–41(–42) × (24–)25–32(–33) µm; biseriate; metulae covering the entire vesicle, measuring (9.4–)10.9–17.1(–21.1) × (3–)3.5–4.2(–4.6) µm; phialides (5.4–)6.1–7.9(–8.4) × (1.7–)1.9–2.4(–2.6) µm in length; conidia globose to subglobose, smooth, (1.8–)1.9–2.2(–2.4) µm; no sclerotia detected on MEA and CYA.

Type: CBS 559.82 = IBT 14026, ex soil, Nebraska, **U.S.A.**, M. Christensen.

Distinguishing features: The fast growth rate on CYA37 and the spathulate vesicles make this a distinct species.

Extrolites: Penicillic acid, xanthomegnin, viomellein, vioxanthin, several extrolites of unknown structure, some of which are also produced by *A. bridgeri*.

Aspergillus pseudoelegans Frisvad & Samson, **sp. nov.** MycoBank MB500005.

Ab *Aspergillo elegante* sclerotiis brunneo-griseis in agaro CYA formatis, conidiis dilute brunneis vel luteo-brunneis, multo ochratoxino A exsudato, sed xanthomegnino absente differt.

Typus: CBS 112796 (lyophilized culture).

Colony diameters after 7 d of incubation, in mm: CYA25 39–48; MEA 38–47; CYA37 0–8. *Colony colours and textures.* No conidia are produced on CYA25 after 7 d of incubation, light brown to yellowish brown (5D5–6) conidia are formed after prolonged incubation; mycelium white, inconspicuous; sclerotia abundantly present; white after 7 d, becoming brownish grey, (4C2–4D2) after 30 d of incubation. Reverse (light) red brown, light brown soluble pigment present. On MEA conidial production absent, prolonged incubation showed sparse production of conidiophores; sclerotia covered by mycelium, greyish beige (4C2) after 30 d of incubation, reverse light brown centre with medium-yellow edge. No or weak growth on CYA37, 0–8 mm. Conidial heads radiate, splitting into columns; stipes up to 1000–1200 µm in length, walls distinct rough, yellow to light brown; vesicles globose to spathulate, (26–)28–34(–36) µm; biseriate; metulae covering the entire vesicle, measuring (8–)9.5–17(–18) × (3.7–)4.1–6.1(–7) µm; phialides (6.6–)7–9.5(–10) × (1.7–)2.1–3(–3.6) µm in length; conidia globose to subglobose, smooth, (2–)2.1–2.5(–2.6); sclerotia abundant, globose to subglobose, (285–)300–430(–500) µm on CYA and somewhat larger on MEA (360–)400–590(–650) µm.

Type: CBS 112796 = IBT 23402, ex soil in Gauguin garden, Taboga Island, **Costa Rica**.

Other isolates: CBS 112797 = IBT 23403 = CR 18-1, ex soil in Gauguin garden, Taboga Island, **Costa Rica**.

Distinguishing features: Brownish grey sclerotia on CYA, light brown to yellowish brown conidia, which are relatively small sized, makes this a distinct species.

Extrolites: Ochratoxin A & B, penicillic acid, mellein, 4-hydroxymellein, cf. petromurin, antibiotic Y, specific extrolites to be structure elucidated.

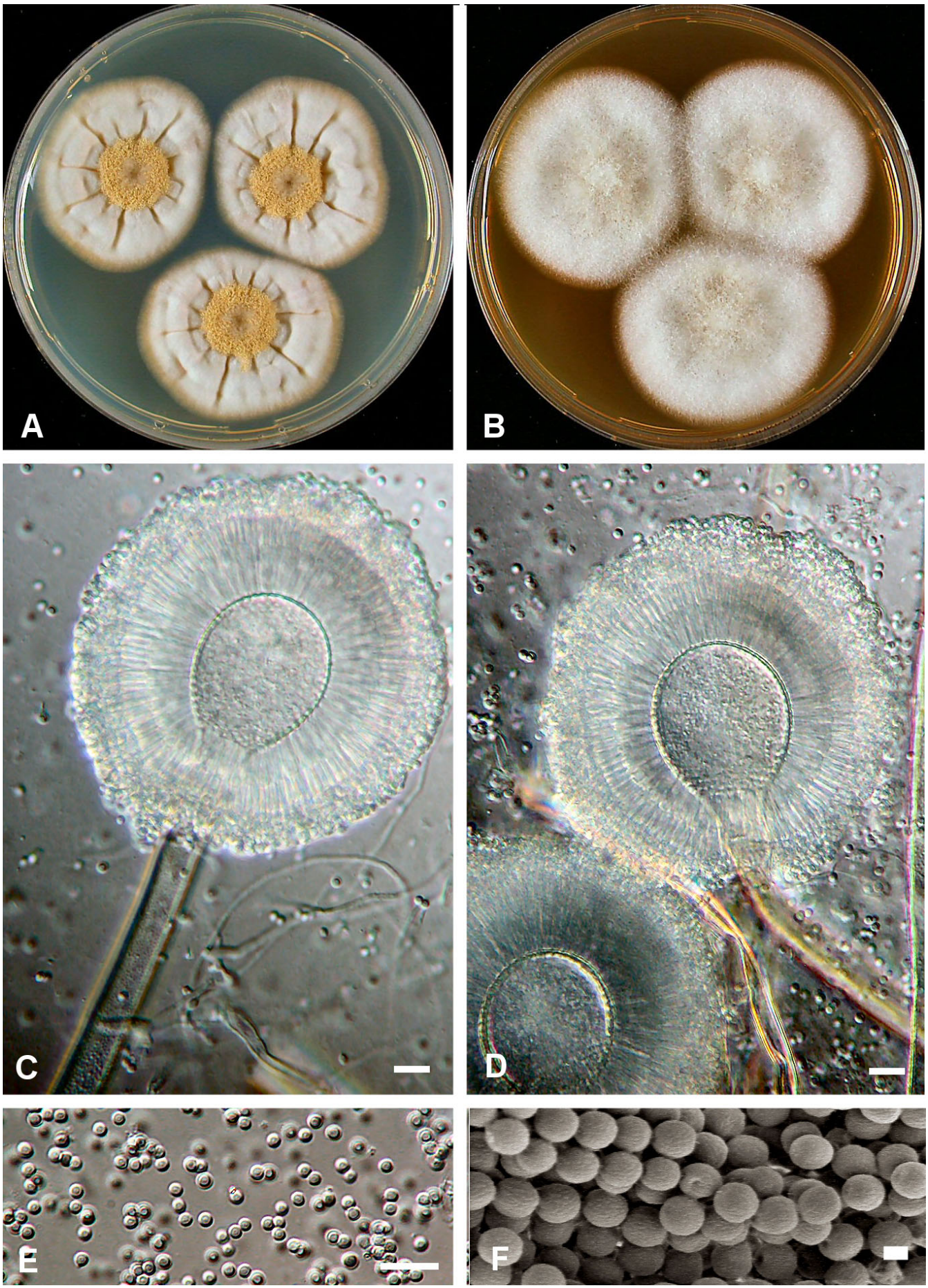

Fig. 6. *Aspergillus neobridgeri*. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. E. Conidia. F. Scanning electron micrograph photo of conidia. Scale bars: C–E = 10 µm, F = 1 µm.

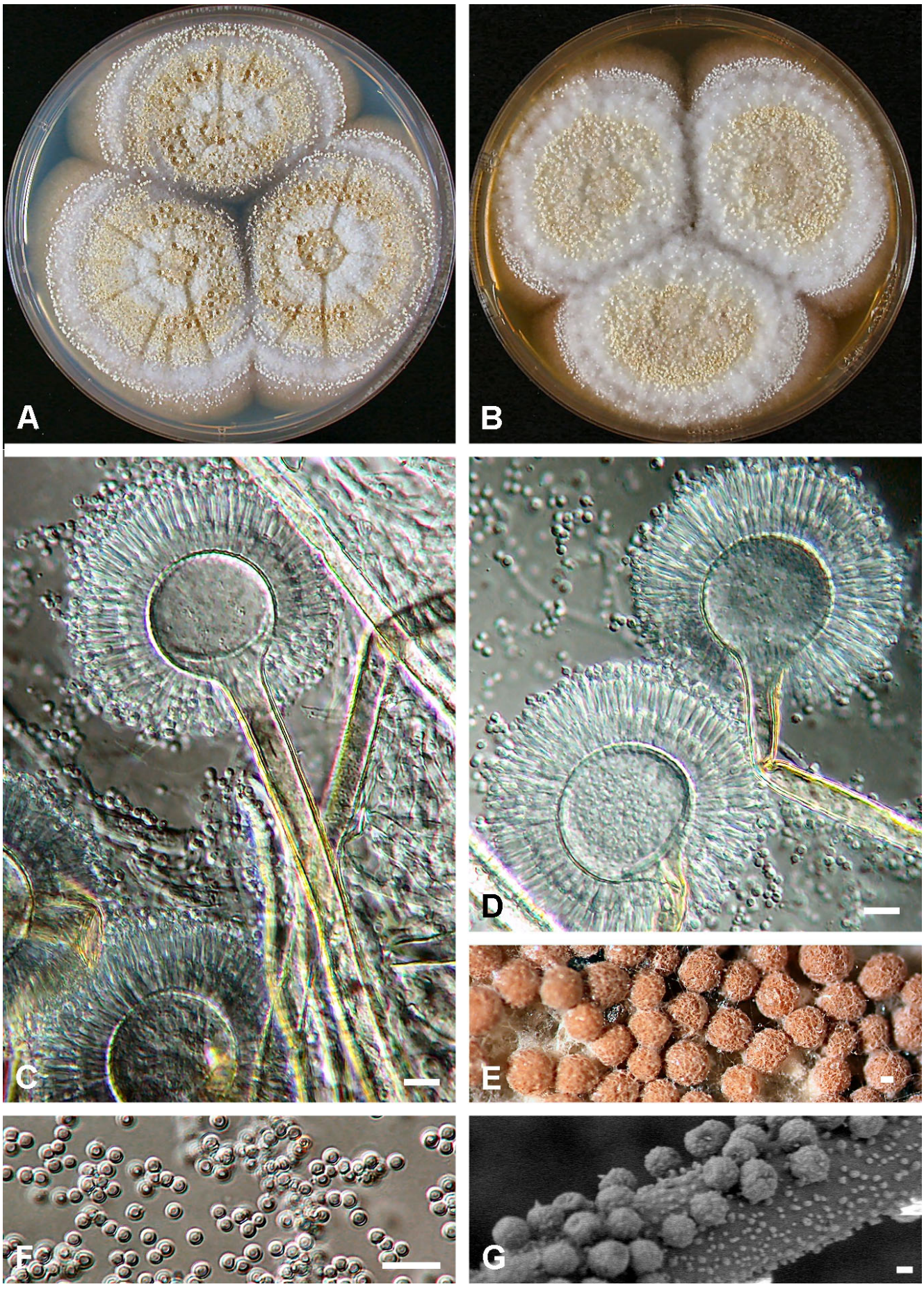

Fig 7. *Aspergillus pseudoelegans*. Fourteen-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. E. Detail of a 28-d-old colony showing sclerotia. F. Conidia. G. Scanning electron micrograph photo of conidia. Scale bars: C, D, F = 10 µm, E = 100 µm, G = 1 µm.

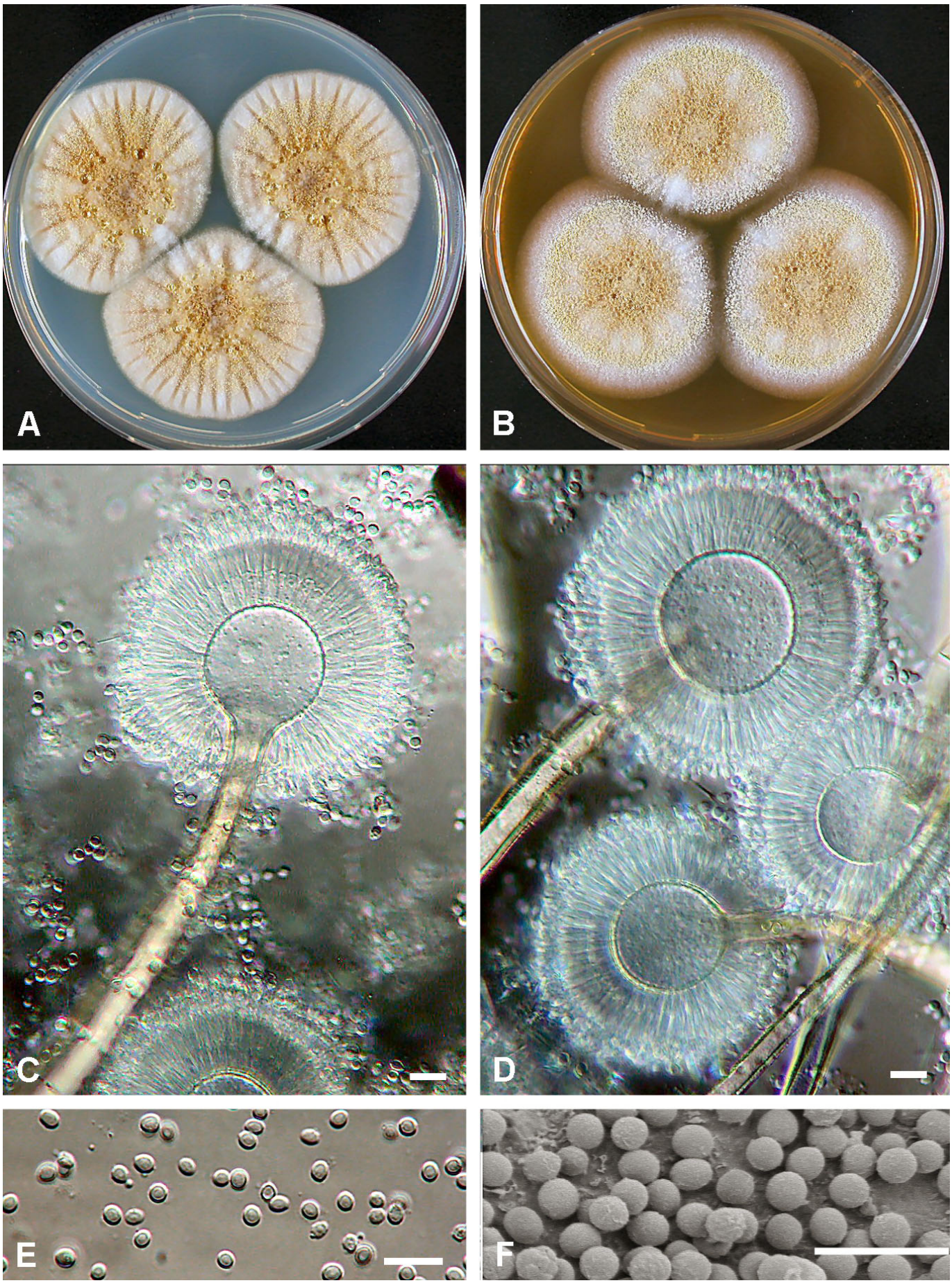

Fig. 8. *Aspergillus steynii*. Seven-day-old cultures on A. CYA and B. MEA. C, D. Conidiophores. E. Conidia. F. Scanning electron micrograph photo of conidia. Scale bars = 10 µm.

Aspergillus steynii Frisvad & Samson, **sp. nov.** MycoBank MB500006.

Etymology: Named in honour of Pieter S. Steyn for all his excellent work on ochratoxin A and other mycotoxins.

Ab *Aspergillo elegante* conidiis dilute flavis ellipsoideis et multo ochratoxino A exsudato differt.

Typus: CBS 112812 (lyophilized culture).

Colony diameters after 7 d of incubation, in mm: CYA25 32–46; MEA 35–46; no growth on CYA37. *Colony colours and texture.* Moderate to good conidia production on CYA25, greyish yellow (3B5–4B5); mycelium white, inconspicuous; sclerotia sparsely produced; white to pale yellow after 7 d, becoming greyish yellow at age. Reverse (light) olive brown to blackish brown, no soluble pigment present. Good sporulation on MEA, velvety, light yellow (4A4–4B4), occasionally pale yellow (2A3) after 7 d; mycelium white, in some isolates sclerotia formed abundantly, greyish yellow to greyish orange (4B5–5A4), reverse brown to dark brown. Conidial heads radiate, splitting into columns; stipes up to 1500 µm in length, walls rough, light yellow pigmented; vesicles globose to slightly spathulate, (20–)22–44(–45) µm; biseriate; metulae covering the entire vesicle, measuring (7–)7.5–18(–22.5) × (3.2–)3.5–5.1(–6) µm; phialides (6.6)8–11.5(12) × (1.8–)2–3(–3.3) µm in length; conidia broadly ellipsoidal, smooth walled, (2.4–)2.5–3.1(–3.2) × (2.1–)2.2–2.8(–3) µm; sclerotia in some isolates abundantly produced, white to cream, globose to subglobose, (380–)410–590(–630) × (380–)410–590(–630) µm on CYA and (380–)480–705(–730) × (390–)420–600(–640) µm on MEA.

Distinguishing features: The absence of growth at 37 °C, broadly ellipsoidal conidia and pale yellow colour of the conidia *en masse* on MEA make this a distinct species. Hesseltine *et al.* (1972) mentioned that *A. steynii* NRRL 3519, identified by them as *A. ochraceus*, had bright yellow conidia, with the conidia suggesting *A. elegans* and the sclerotia suggesting *A. melleus*. NRRL 3519 was the best producer of OTA they found in their screening only equalled by *A. ochraceus* NRRL 3174, now identified as *A. westerdijkiae*.

Type: CBS 112812 (ex-type CBS 112812 = IBT 23096, ex surface disinfected green coffee bean, Chamumdeshuran Estate, district Giris, Karnataka, **India**, J.M. Frank).

Other strains: CBS 112813 = IMI 371128 = IBT 21755; CBS 112814 = IBT 23792, ex green coffee bean, **India**; PIL 661 = IBT 22339, ex rice, **China**; FRR 3846 = IBT 22941; I224i; I 110; I176i; D2306 = IBT 19554, unknown source, **Australia**; NRRL 6423 = NRRL A-19242 = IBT 14586; NRRL 5223 = IBT 13879, Canal Zone, **Panama**; NRRL 3519 = IBT 14309, ex soil, **Argentina**; CBS 348.80 = IBT 14023 = IMI 231047, ex *Arecha catechu*, **Sri Lanka**; B204i; FRR 3846 = IBT 22941, ex mouldy soy beans, Inverell, New South Wales, **Australia**, A.D. Hocking.

*Extrolites***:** Ochratoxin A & B, xanthomegnin, viomellein, vioxanthin, mellein, 4-hydroxymellein, diaporthin, orthosporin, cycloechinulin, ochrindol A-D, TR-2.

DISCUSSION

Aspergillus subgenus *Circumdati* section *Circumdati* and its associated *Neopetromyces* teleomorph is generally a uniform assemblage of species indicating that *Neopetromyces* is a natural ascomycete genus. This is backed up by morphological, physiological and extrolite characters and by DNA sequence data (Peterson 2000, Frisvad & Samson 2000). The conidium colour criterion that was used by Raper & Fennell (1965) and Christensen & Raper (1982) as a major distinguishing factor from other *Aspergillus* groups or sections is only partly useful. Several species with ochre or yellow conidia may belong to other subgenera within subgenus *Circumdati*, including *Petromyces alliaceus*, *P. albertensis*, *A. lanosus*, *A. sepultus*, *A. dimorphicus*, *A. taichungensis* and *A. campestris*. The first three species belong to section *Flavi* (Frisvad & Samson 2000), the next two species belong in section *Wentii* (Peterson 1995, Frisvad & Samson 2000) and the last two species belong in section *Candidi* (Rahbaek *et al*. 2000). *Aspergillus robustus* is unique in section *Circumdati*, but the remaining 19 species, including the seven new species described here, represent a very homogeneous group (Table 2).

The ochratoxin A production reported from *A. wentii* and *A. auricomus* by Varga *et al*. (1996) was based on misidentified or contaminated strains. *Aspergillus wentii* IMI 017295 proved to be a typical *Petromyces alliaceus* (Frisvad & Samson 2000), while *A. wentii* IMI 371128 was re-identified by us as *A. steynii*. *Aspergillus auricomus* FRR 3819 proved to be a *Neopetromyces muricatus*.

Ochratoxin A production is usually high in YES agar (Ciegler 1972, Lund & Frisvad 2003), but trace metals and magnesium sulphate have to be added to this medium to support ochratoxin A production (Filtenborg *et al.* 1990). Thus for example Bayman *et al.* (2002), did not find ochratoxin A in several isolates that are regarded very good producers, for example *Petromyces alliaceus* NRRL 315 and NRRL 4181 (see their table 1) and in none of their strains of *A. ochraceus* isolated from figs. It is very important to use several growth conditions and media in order to be sure that a member from section *Circumdati* is not a producer of ochratoxin A. It remains to be seen

whether *A. auricomus, A. bridgeri, A. elegans, A. insulicola, A. neobridgeri* and *A. robustus* are really incapable of producing OTA, but we have seen no signs of OTA production in those species yet.

Many of the species described here are apparently rare and may not be important concerning potential mycotoxin production in foods and beverages. The most important ochratoxin A producing species in this regard appears to be *A. ochraceus*, *A. westerdijkiae* and *A. steynii*. Isolates of these species are very common and most of the isolates in these species produce large amounts of ochratoxin A. They have often all been identified as *A. ochraceus*. For example the original OTA producing strain (NRRL 3174) (van der Merwe *et al.* 1965a, b) is now identified as *A. westerdijkiae*. These two species are closely related and a lot of work has been done on *A. westerdijkiae*, rather than *A. ochraceus sensu stricto* (Trenk *et al.* 1971, Davis *et al.* 1972, Madhyastha *et al.* 1990, Cheelak *et al.* 1991a, b, Blank *et al.* 1995, Xiao *et al.* 1996, Blank *et al.* 1998, Lee & Magan 1999, Lee & Magan 2000, Pardo *et al.* 2004). The work on biosynthesis of ochratoxin A by Harris & Mantle (2001a) and the detection of diaporthin and orthosporin (Harris & Mantle 2001b) was based on an isolate of *A. steynii* (D2306 = IBT 19554). This isolate is from Australia (Connole *et al.* 1981) and has been used in toxicological studies (Tapia & Seawright 1984, Stander *et al.* 2000, Stoev *et al* 2000). It may, however, be important which other extrolites these species produce as for example penicillic acid and ochratoxin A have synergistic effects (Lindenfelser *et al.* 1973, Stoev *et al.* 2001). For example, in addition to ochratoxin A, penicillic acid and xanthomegnins, *A. steynii* produces cycloechinulin (Hansen *et al.* 2001) and diaporthin plus orthosporin (Harris & Mantle 2001b), while *A. westerdijkiae* and *A. ochraceus* produce among several other extrolites circumdatins = asperloxins (Rahbaek *et al.* 1999, Dai *et al.* 2001) and aspergamides (Fenical *et al.* 2000, Sugie *et al.* 2001, Bode *et al.* 2002, Qian-Cutrone *et al.* 2002), in addition to the known toxins. The interaction of these mycotoxins and other extrolites is unknown. The aspergamides = avrainvillamides = stephacidins have potent activities against different kinds of cancer and so the correct identification of strains of these species is important. For example isolates that do not produce ochratoxin A may be used for large scale production of the aspergamides.

In this study, partial β-tubulin sequences of 42 strains, representing isolates of known species of *Aspergillus* subgenus *Aspergillus* section *Circumdati*, were determined. In general, parsimony analyses of the alignments provided excellent markers at species level. Previous taxonomic studies on species belonging to the section *Circumdati* have been carried out. Among other approaches, sequencing of parts of the ribosomal operon (Peterson 2000, Varga *et al.* 2000c) and mitochondrial DNA restriction and random amplified polymorphic DNA profiles (Varga *et al*. 2000a) were applied. Peterson (2000) showed that *A. ostianus*, *A. ochraceus*, *A. melleus* and *A. petrakii* have identical LSU-rDNA sequences. Partial β-tubulin data confirms that these species are closely related and a one-gene approach might not be sufficient to separate those taxa. The other species only have a few variable sites, making the use of this part of the rDNA not useful as species markers. Despite the low resolution of the LSU-rDNA, differences between the type of *A. bridgeri* (NRRL 13000 = CBS 350.81) and another isolate of *A. bridgeri* (= *A. neobridgeri,* NRRL 13078 = CBS 559.82) could be observed. This is also supported by the partial β-tubulin sequences and provides evidence that, together with the extrolite and morphological data, they are distinct species. ITS sequences (Varga *et al.* 2000a, b, c) revealed more variation and provided a well resolved phylogram, showing *A. ochraceus* (= *A. westerdijkiae)* NRRL 3174, as a separate entity from the type strain of *A. ochraceus*. Besides differences in ITS sequence, the separation of *A. westerdijkiae* and *A. ochraceus* as two species could also be observed in the results of mtDNA RFLP and RAPD analyses of *A. ochraceus* strains (Varga *et al.* 2000a). These data support the β-tubulin data showing *A. westerdijkiae* as new species. Two main clades could be identified with ITS sequencing (Varga *et al.* 2000c), these clades could also be observed within the partial β-tubulin gene phylogram, with the differences that 1. *N. muricatus* did not belong to the group of *A. ochraceus*, *A. melleus*, *A. petrakii*, *A. ostianus* and *A. westerdijkiae*; 2. *A. insulicola* is not part of the group containing the type species of *A. sulphureus*, *A. bridgeri* and *A. sclerotiorum*, and *A. persii* should be added to this clade. *Aspergillus robustus* is excluded from the phylogenetic study, since it appears to be outside the main clade of species in the section (Peterson 2000). A multi-gene approach is needed, to establish the relationship of this species with other member of the sections *Eurotium* and *Circumdati*.

Species in section *Circumdati* are generally very efficient producers of three classes of mycotoxins and thus the whole section should be revised and keys should be made in order to be able to identify these closely related species. The present paper is a step in that direction.

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