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Polyphasic taxonomy of *Aspergillus* section *Usti*

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Abstract: *Aspergillus ustus* is a very common species in foods, soil and indoor environments. Based on chemical, molecular and morphological data, *A. insuetus* is separated from *A. ustus* and revived. *A. insuetus* differs from *A. ustus* in producing drimans and ophiobolin G and H and *not* producing ustic acid and austocystins. The molecular, physiological and morphological data also indicated that another species, *A. keveii* **sp. nov.** is closely related but distinct from *A. insuetus*. *Aspergillus* section *Usti sensu stricto* includes 8 species: *A. ustus, A. puniceus*, *A. granulosus, A. pseudodeflectus, A. calidoustus*, *A. insuetus* and *A. keveii* together with *Emericella heterothallica*.

Taxonomic novelties: *Aspergillus insuetus* revived, *Aspergillus keveii* **sp. nov.**
Key words: actin, *Aspergillus*, β-tubulin, calmodulin, extrolite profiles, ITS, phylogenetics, polyphasic taxonomy.

INTRODUCTION

Aspergillus ustus is a very common filamentous fungus found in foods, soil and indoor air environments (Samson *et al.* 2002). This species is considered as a rare human pathogen that can cause invasive infection in immunocompromised hosts. However, *A. ustus* has been noted increasingly as causes of invasive aspergillosis in tertiary care centres in the US (Malani & Kaufman 2007). Up to date, 22 invasive aspergillosis cases have been reported to be caused by *A. ustus* (Verweij *et al.* 1999; Pavie *et al.* 2005; Panackal *et al.* 2006; Yildiran *et al.* 2006). Several studies indicate that *A. ustus* isolates are resistant to amphotericin B, echinocandins and azole derivatives (Verweij *et al.* 1999; Pavie *et al.* 2005; Gene *et al.* 2001; Garcia-Martos *et al.* 2005). Other species related to *A. ustus* can also cause human or animal infections. *Aspergillus granulosus* was found to cause disseminated infection in a cardiac transplant patient (Fakih *et al.* 1995), while *A. deflectus* has been reported to cause disseminated mycosis in dogs (Robinson *et al.* 2000; Kahler *et al.* 1990; Jang *et al.* 1986).

A. ustus is a variable species. Raper & Fennell (1965) stated that "not a single strain can be cited as wholly representative of the species as described". Indeed, *A. ustus* isolates may vary in their colony colour from mud brown to slate grey, with colony reverse colours from uncoloured through yellow to dark brown (Raper & Fennell 1965; Kozakiewicz 1989). Molecular data also indicate that this species is highly variable; RAPD analysis carried out in various laboratories could be used to detect clustering of the isolates (Rath *et al.* 2002; Panackal *et al.* 2006), and sequence analysis of parts of the ribosomal RNA gene cluster also detected variability within this species (Henry *et al.* 2000; Peterson 2000; Hinrikson *et al.* 2005).

We examined a large set of *A. ustus* isolates and related species originating from environmental and clinical sources to clarify the taxonomic status of the species, and to clarify the taxonomy of *Aspergillus* section *Usti*. The methods used include sequence analysis of the ITS region (intergenic spacer region and the 5.8 S rRNA gene of the rRNA gene cluster), and parts of the β-tubulin, calmodulin and actin genes, analysis of extrolite profiles, and macro- and micromorphological analysis of the isolates.

MATERIALS AND METHODS

Morphological examination. The strains examined are listed in Table 1. Both clinical and environmental strains were grown as 3-point inoculations on Czapek yeast agar (CYA), malt extract agar (MEA), creatine agar (CREA) and yeast extract sucrose agar (YES) at 25 °C, and on CYA at 37 °C for 7 d (medium compositions according to Samson *et al.* 2004). For micro morphological examination light microscopy (Olympus BH2 and Zeiss Axioskop 2 Plus) was employed.

Extrolite analysis. Extrolites were analysed by HPLC using alkylphenone retention indices and diode array UV-VIS detection as described by Frisvad & Thrane (1987), with minor modifications as described by Smedsgaard (1997). Standards of ochratoxin A and B, aflavinine, asperazine, austamide, austdiol, kotanin and other extrolites from the collection at Biocentrum-DTU were used to compare with the extrolites from the species under study.

Isolation and analysis of nucleic acids. The cultures used for the molecular studies were grown on malt peptone (MP) broth using 10 % (v/v) of malt extract (Brix 10) and 0.1 % (w/v) bacto peptone (Difco), 2 mL of medium in 15 mL tubes. The cultures were incubated at 25 °C for 7 d. DNA was extracted from the cells using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. Fragments containing the ITS region were amplified using primers ITS1 and ITS4 as described previously (White *et al.* 1990). Amplification of part of the β-tubulin gene was performed using the primers Bt2a and Bt2b (Glass 1995). Amplifications of the partial calmodulin and actin genes were set up as described previously (Hong *et al.* 2005). Sequence analysis was performed with the Big Dye Terminator

Table 1. Isolates in *Aspergillus* section *Usti* and related species examined in this study.

Species	Strain No.	Source
A. calidoustus	CBS 112452	Indoor air, Germany
A. calidoustus	CBS 113228	ATCC 38849; IBT 13091
A. calidoustus	CBS 114380	Wooden construction material, Finland
A. calidoustus	CBS 121601^{T}	Bronchoalveolar lavage fluid, proven invasive aspergillosis, Nijmegen, The Netherlands†
A. calidoustus	CBS 121602	Bronchial secretion, proven invasive aspergillosis, Nijmegen, The Netherlands†
A. calidoustus	CBS 121589	Autopsy lung tissue sample, proven invasive aspergillosis, Nijmegen, The Netherlands†
A. calidoustus	CBS 121603	Elevator shaft in hospital, Nijmegen, The Netherlands
A. calidoustus	CBS 121604	Patient room, Nijmegen, The Netherlands
A. calidoustus	CBS 121605	Laboratory, Nijmegen, The Netherlands
A. calidoustus	CBS 121606	Sputum, Nijmegen, The Netherlands
A. calidoustus	CBS 121607	Feces, Nijmegen, The Netherlands
A. calidoustus	CBS 121608	Bronchoalveolar lavage, Nijmegen, The Netherlands
A. calidoustus	7843	Pasteur Institute, Paris, France
A. calidoustus	8623	Oslo, Norway
A. calidoustus	9331	Mouth wash, Nijmegen, The Netherlands
A. calidoustus	9371	Mouth wash, Nijmegen, The Netherlands
A. calidoustus	9420	Bronchial secretion, Nijmegen, The Netherlands
A. calidoustus	9692	Hospital ward, Nijmegen, The Netherlands
A. calidoustus	V02-46	Tongue swab, Nijmegen, The Netherlands
A. calidoustus	V07-21	Bronchial secretion, Nijmegen, The Netherlands
A. calidoustus	V17-43	Bronchial secretion, Nijmegen, The Netherlands
A. calidoustus	V22-60	Skin biopsy, Nijmegen, The Netherlands
A. calidoustus	CBS 121609	Post-cataract surgery endophthalmitis, Turkey
A. calidoustus	907	Post-cataract surgery endophthalmitis, Turkey
A. calidoustus	908	Post-cataract surgery endophthalmitis, Turkey
A. calidoustus	64	Post-cataract surgery endophthalmitis, Turkey
A. calidoustus	67	Post-cataract surgery endophthalmitis, Turkey
A. calidoustus	CBS 121610	Post-cataract surgery endophthalmitis, Turkey
A. calidoustus	351	Osteorickets
A. calidoustus	482	Post-cataract surgery endophthalmitis
A. calidoustus	CBS 121611	Patient 4, Washington, U.S.A.
A. calidoustus	CBS 121616	Environmental, Washington, U.S.A.
A. calidoustus	FH 165	Patient 5b, Washington, U.S.A.
A. calidoustus	CBS 121614	Patient 5a, Washington, U.S.A.
A. calidoustus	CBS 121615	Patient 6, Washington, U.S.A.
A. calidoustus	CBS 121613	Patient 2, Washington, U.S.A.
A. calidoustus	CBS 121612	Patient 1, Washington, U.S.A.
A. calidoustus	FH 91	Patient 1a, Washington, U.S.A.
A. calidoustus	NRRL 26162	Culture contaminant, Peoria, U.S.A.
A. calidoustus	NRRL 281	Thom 5634
A. calidoustus	NRRL 277	Thom 5698.754, Green rubber
A. granulosus	CBS 588.65^{T}	Soil, Fayetteville, Arkansas, U.S.A.
A. granulosus	CBS 119.58	Soil, Texas, U.S.A.
A. granulosus	IBT 23478 = WB 1932 = IMI 017278iii = CBS 588.65	Soil, Fayetteville, Arkansas, U.S.A.
A. insuetus	CBS 107.25^{T}	South Africa
A. insuetus	CBS 119.27	Unknown
A. insuetus	CBS 102278	Subcutaneous infection left forearm and hand of 77-year-old woman
A. keveii	CBS 209.92	Soil, La Palma, Spain
A. keveii	CBS 561.65	Soil, Panama
A. keveii	IBT 10524 = CBS 113227 = NRRL 1254	Soil, Panama

Table 1. (Continued).

Species	Strain No.	Source
A. keveii	IBT 16751 = DMG 153	Galápagos Islands, Ecuador, D.P. Mahoney
A. pseudodeflectus	CBS 596.65	Sugar, U.S.A., Louisiana
A. pseudodeflectus	CBS 756.74 ^{T}	Desert soil, Egypt, Western Desert
A. puniceus	CBS 122.33	Unknown
A. puniceus	9377	Mouth wash, Nijmegen, Netherlands
A. puniceus	V41-02	Faeces, Nijmegen, Netherlands
A. puniceus	NRRL 29173	Indoor air, Saskatoon, Canada
A. puniceus	CBS 495.65 ^{T}	Soil, Zarcero Costa Rica
A. puniceus	CBS 128.62	Soil, Louisiana, U.S.A.
A. ustus	CBS 116057	Antique tapestries, Krakow, Poland
A. ustus	CBS 114901	Carpet, The Netherlands
A. ustus	CBS 261.67 ^{T}	Culture contaminant, U.S.A.
A. ustus	CBS 133.55	Textile buried in soil, Netherlands
A. ustus	CBS 239.90	Man, biopsy of brain tumor, Netherlands
A. ustus	CBS 113233	IBT 14495
A. ustus	CBS 113232	IBT 14932
A. ustus	NRRL 285	Soil, Iowa, U.S.A.
A. ustus	NRRL 280	Bat dung, Cuba
A. ustus	NRRL 1609	Bat dung, Cuba
A. ustus	NRRL 29172	Indoor air, Edmonton, Canada
E. heterothallica	CBS 489.65^{T}	soil, Costa Rica
E. heterothallica	CBS 488.65	soil, Costa Rica

[†]These samples were taken from the same patient (Verweij *et al.* 1999)

Cycle Sequencing Ready Reaction Kit for both strands, and the sequences were aligned with the MT Navigator software (Applied Biosystems). All the sequencing reactions were purified by gel filtration through Sephadex G-50 (Amersham Pharmacia Biotech, Piscataway, NJ) equilibrated in double-distilled water and analyzed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Data analysis. The sequence data was optimised using the software package Seqman from DNAStar Inc. Sequence alignments were performed by using CLUSTAL-X (Thompson *et al.* 1997) and improved manually. The neighbour-joining (NJ) method was used for the phylogenetic analysis. For NJ analysis, the data were first analysed using the Tamura–Nei parameter distance calculation model with gamma-distributed substitution rates (Tamura & Nei 1993), which were then used to construct the NJ tree with MEGA v. 3.1 (Kumar *et al.* 2004). To determine the support for each clade, a bootstrap analysis was performed with 1000 replications.

For parsimony analysis, the PAUP v. 4.0 software was used (Swofford 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis & Bull 1993). An *Aspergillus versicolor* isolate was used as outgroup in these experiments. Unique sequences of the ITS, actin, calmodulin and β-tubulin gene sequences have been deposited in the GenBank under accession numbers EU076344–EU76377.

RESULTS

Phylogenetic analyses

For the molecular analysis, four genomic regions, the ITS region, and parts of the actin, calmodulin and β-tubulin genes were amplified and sequenced. Phylogenetic analysis of the data was carried out using the neighbour-joining technique and parsimony analysis. The trees obtained by the different approaches were identical, neighbour-joining trees based on the different data sets are shown in Figs 1–4. During analysis of part of the β-tubulin gene, 487 characters were analyzed, 111 of which were found to be parsimony informative. The topology of the tree is the same as that of one of the more than 10^4 maximum parsimony trees constructed by the PAUP program (length: 216 steps, consistency index: 0.8148, retention index: 0.9679). The calmodulin data set included 474 characters, with 172 parsimony informative characters (1 MP tree, tree length: 360, consistency index: 0.8083, retention index: 0.9550). The actin data set included 406 characters, with 161 parsimony informative characters (3 MP trees, tree length: 292, consistency index: 0.8870, retention index: 0.9633). The ITS data set included 482 characters, 26 of which were parsimony informative (>10^4 MP trees, tree length: 71, consistency index: 0.9155, retention index: 0.9781).

Molecular data revealed that *Aspergillus* section *Usti* consists of eight species: *A. ustus, A. puniceus*, *A. granulosus, A. pseudodeflectus, A. calidoustus*, *A. insuetus* and a new species including CBS 209.92 and some other isolates. We propose the name *A. keveii* **sp. nov.** for this set of isolates. The trees based on ITS, calmodulin and β-tubulin sequence data indicated that also *E. heterothallica* belongs to this section, although actin sequence data did not support this finding.

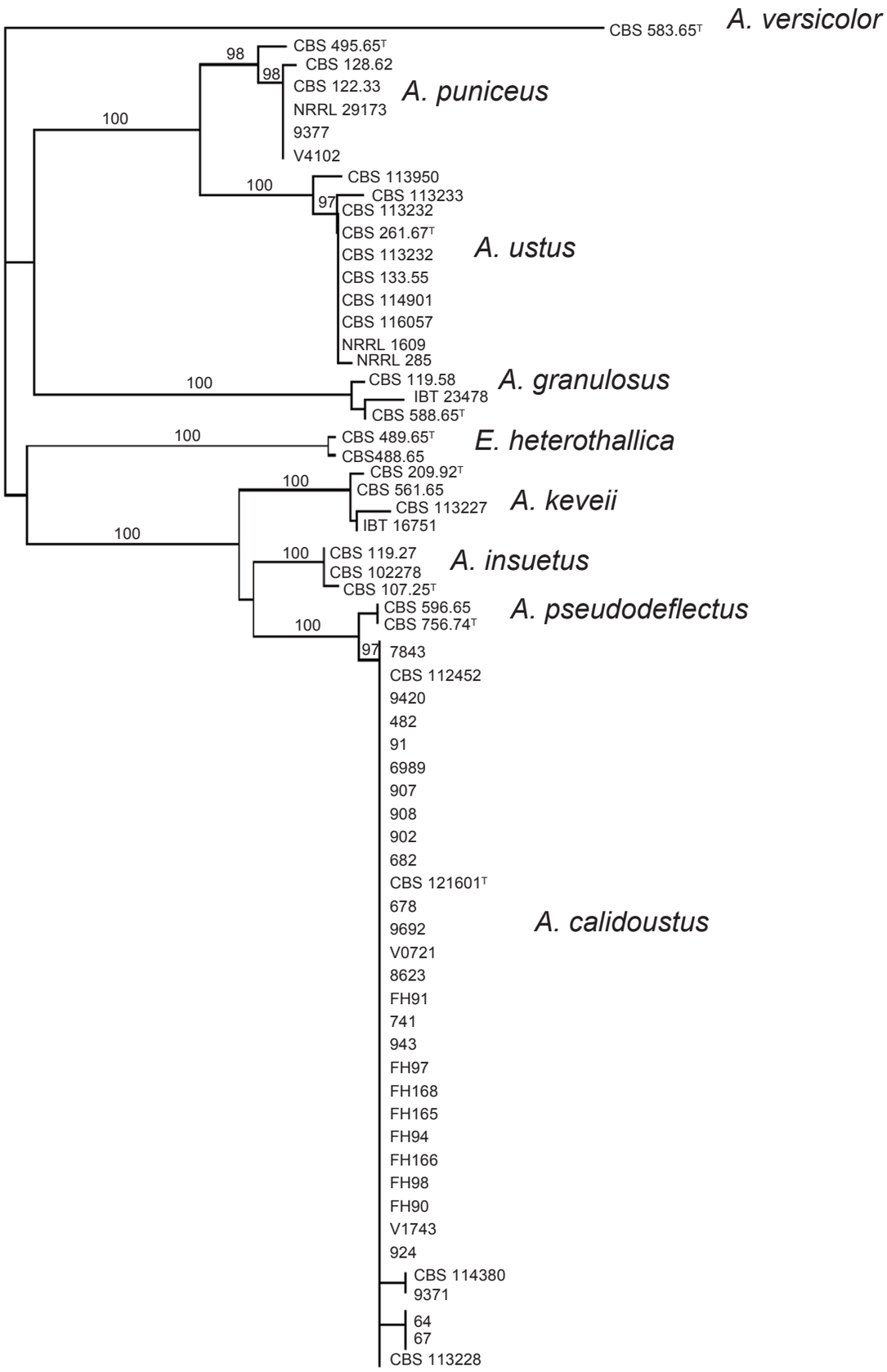

Fig. 1. Neighbour-joining tree based on β-tubulin sequence data of *Aspergillus* section *Usti*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

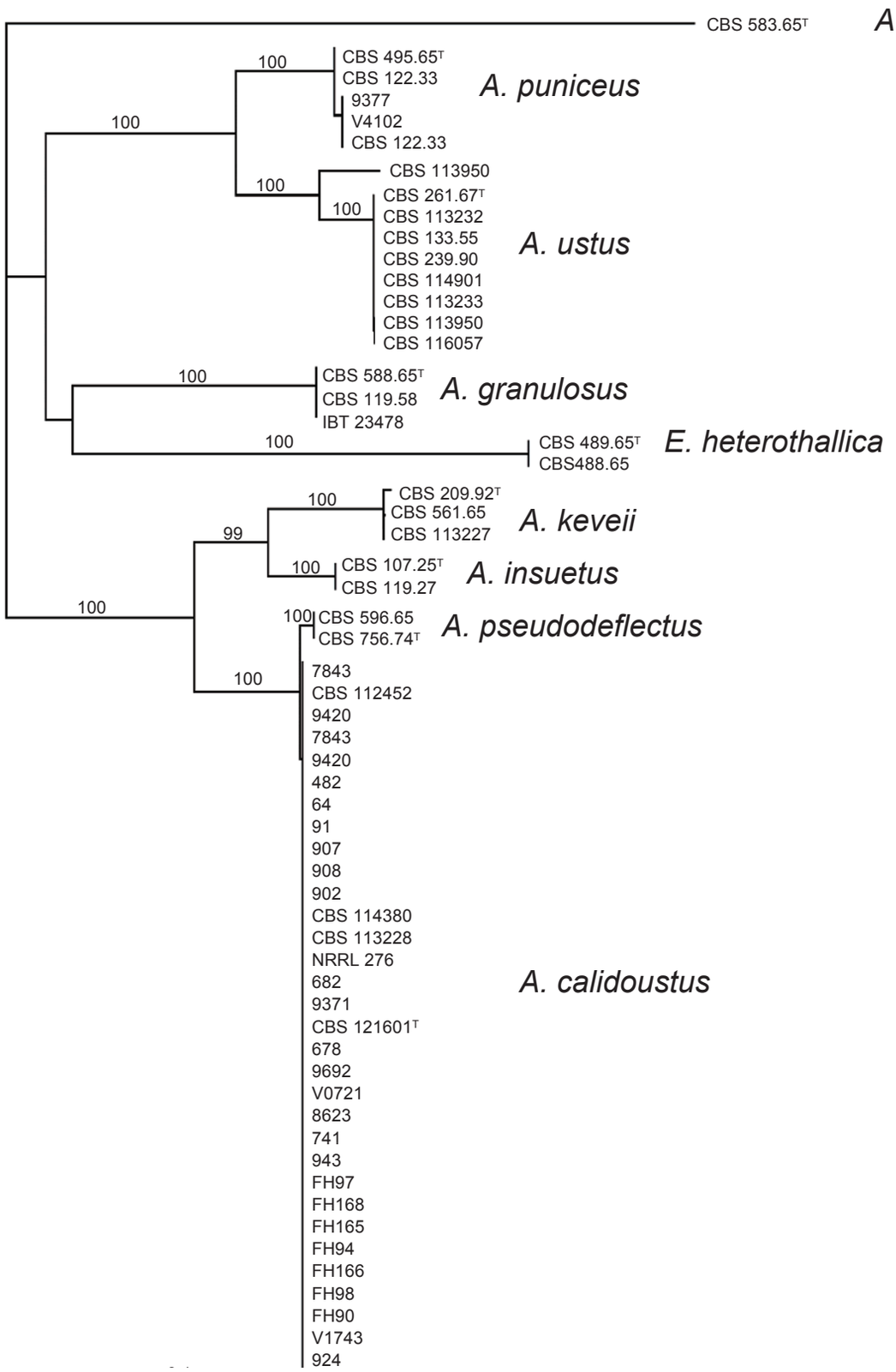

Fig. 2. Neighbour-joining tree based on calmodulin sequence data of *Aspergillus* section *Usti*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

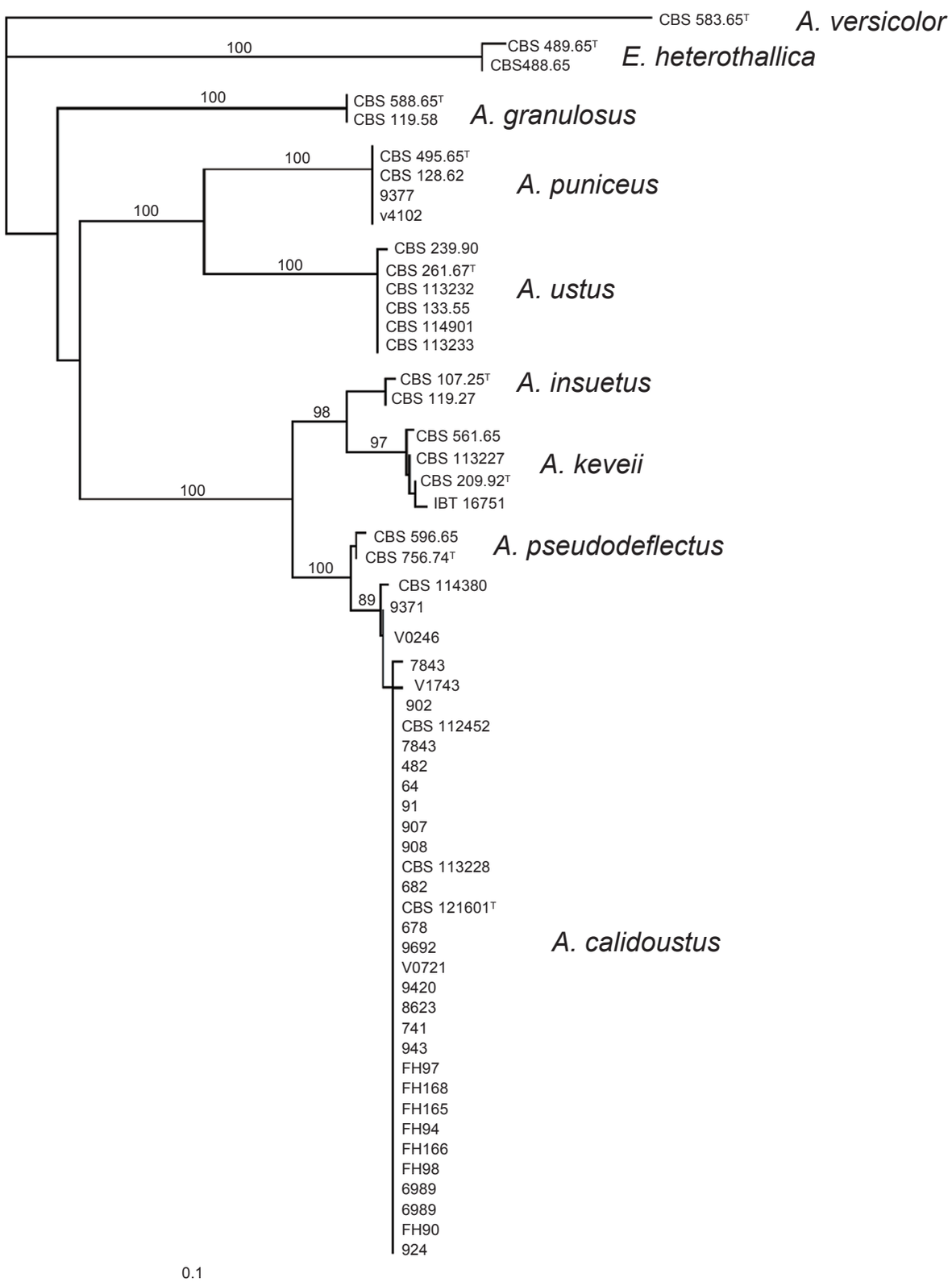

Fig. 3. Neighbour-joining tree based on actin sequence data of *Aspergillus* section *Usti*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

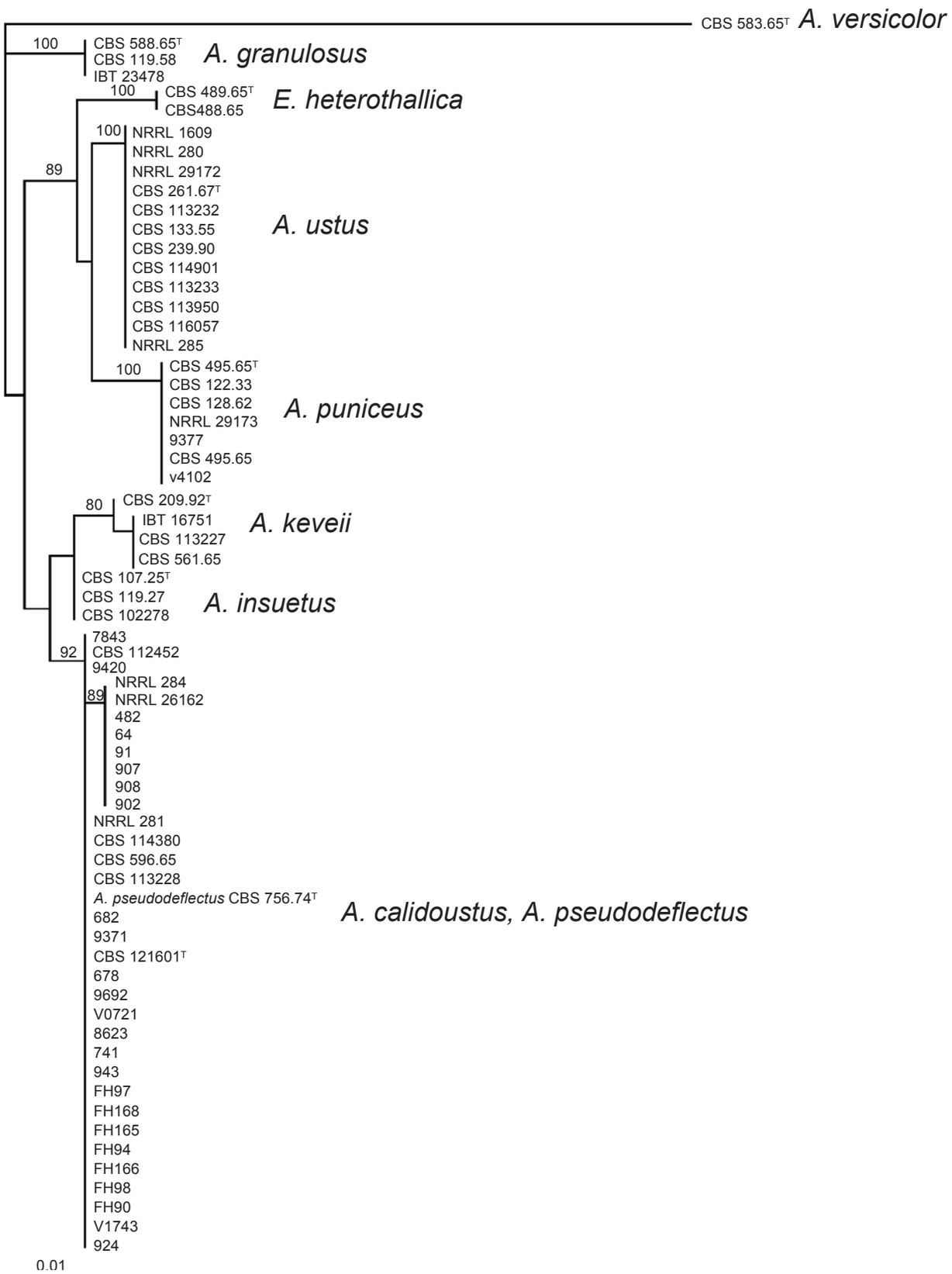

Fig. 4. Neighbour-joining tree based on ITS sequence data of *Aspergillus* section *Usti*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

Table 2. Overview of morphological criteria to differentiate between the members of *Aspergillus* section *Usti.*

Species	CYA37 (mm)	YES (mm)	Ehrlich reaction	Reaction on CREA	Conidial colour on MEA**
A. ustus	No growth	43–49	None	Good growth, faint yellow mycelium	Hair brown
A. puniceus	No growth	48–53	None	Moderate to good growth, yellow mycelium	Olive brown
A. calidoustus	20–35	36–41	Violet	Weak to moderate growth, hyaline mycelium	Brownish grey
A. insuetus	No growth	23–30	Violet	Good growth, hyaline mycelium	(Brownish) grey to light grey
A. keveii	No growth	40–46	Violet*	Good growth, hyaline mycelium	Brownish grey / pinkish brown
A. pseudodeflectus	15–20	20–30	None	Weak to moderate growth, hyaline mycelium	No sporulation
A. granulosus	30–35	35–40	Violet	Weak growth, hyaline mycelium	Buff to greyish brown
E. heterothallica	5–10	38–42	None	Weak growth, bright yellow mycelium	No sporulation

* All have violet reaction, except CBS 113227

** Colour according Methuen handbook of colours

Morphological and physiological studies

Phenotypic comparison of the different members of the section *Usti* showed that eight taxa could be distinguished. Various characters showed to be valuable for differentiation (see also Table 2). One of the main criteria is the growth rate on CYA at 37 °C. *A. calidoustus*, *A. pseudodeflectus* and *A. granulosus* had high growth rates at this temperature, while *E. heterothallica* only grew restrictedly. The other members of this section were unable to grow at 37 °C, which reduces the potential of these species to become opportunistic human pathogens. The growth rate and the mycelium colour on creatin agar (CREA) also proved to be a good tool to differentiate between the species examined. Some species, like *A. ustus*, *A. puniceus*, *A. insuetus* and *A. keveii* have a good growth on this medium. Since sporulation on this medium is often inhibited, this medium was also useful to determine the colour of the mycelium. The colours varied from bright yellow by *A. puniceus* and *E. heterothallica* to faint yellow in *A. ustus* to colourless in the other species. Another useful character was the use of the Ehrlich test to detect the presence of indol metabolites. This feature gave, with the exception of *A. keveii*, very clear-cut results. Besides these features, the colony diam on YES was also suitable to differentiate between *A. insuetus* and the other species.

Extrolite profiles

Aspergillus ustus has been claimed to produce a range of extrolites including austdiol (Vleggaar *et al.* 1974), Austin (Chexal *et al.* 1976), austocystins (Steyn & Vleggaar 1974; Kfir *et al.* 1986), brevianamide A (Steyn 1973), sterigmatocystin (Rabie *et al.* 1977), austalides (de Jesus *et al.* 1987), austamide (Steyn 1971), dehydroaustin (Scott *et al.* 1986), pergillin (Cutler *et al.* 1980), dehydropergillin (Cutler *et al.* 1981), phenylahistin (Kanoh *et al.* 1997), ophiobolins G & H (Cutler *et al.* 1984), drimans (Hayes *et al.* 1996), diacetoxyscirpenol (Tuomi *et al.* 2000) and ustic acid (Raistrick & Strickings 1951).

The mycotoxins and other extrolites found to be produced by the examined species in this study are listed in Table 3. Species assigned to section *Usti* could clearly be divided in three chemical groups based on the extrolites produced by them. *A. ustus, A. granulosus* and *A. puniceus* produced ustic acids in common. *A. ustus* and *A. puniceus* also produced austocystins and versicolorins. In the second chemical group, *A. pseudodeflectus* produced drimans (Hayes *et al.* 1996) in common with the other species in this group, and also several unique unknown compounds. *A. calidoustus* isolates produced drimans and ophiobolins in common with *A. insuetus* and *A. keveii*, but also produced austins not identified in other species of section *Usti*. *A. insuetus* isolates also produced pergillin, while *A. keveii* together with some other isolates produced nidulol. In the third chemical group, *E. heterothallica* has been reported to produce emethallicins A–F (Kawahara *et al.* 1989, 1990a, 1990b), 5"-hydroxyaveranthin (Yabe *et al*. 1991), emeheterone (Kawahara *et al.* 1988), emesterones A & B (Hosoe *et al.* 1998), 5"-hydroxyaveranthin (Yabe *et al.* 1991), Mer-NF8054X (Mizuno *et al.* 1995). This latter compound is an 18,22-cyclosterol derivative, and was also identified in an *A. ustus* isolate (Mizuno *et al.* 1995). Apart from this chemical similarity *Emericella heterothallica* appear to be quite different from the anamorphic species in section *Usti*, in agreement with actin sequencing data. Austamide, deoxybrevianamide E and austdiol could not be detected in any of the strains examined here and the strain producing these mycotoxins should be reexamined.

Comparing the extrolites profiles of section *Usti* with other sections within subgenus *Nidulantes,* nidulol and versicolorins are also produced by members of sections *Versicolores* and *Nidulantes* (Cole & Schweikert 2003). Interestingly, versicolorins and 5"-hydroxyaveranthin are intermediates of the aflatoxin biosynthetic pathway and also produced by species assigned to *Aspergillus* section *Flavi* and *Ochraceorosei* (Yabe *et al.* 1991; Frisvad *et al.* 2005). However, while the versicolorins are precursors of sterigmatocystin in section *Ochraceorosei*, *Versicolores* and *Nidulantes*, they are precursors of austocystins in section *Usti.*

Section *Usti* contains the only *Aspergillus* species known to produce pergillins, ophiobolins, austins, austocystins, ustic acids, drimans, Mer-NF8054X, austalides, deoxybrevianamides and austamide and thus this section is chemically unique. We have not examined the species for production of emethallicins, emesterones and emeheterones, as standards of these compounds were not available.

DISCUSSION

Raper and Fennell (1965) classified *A. ustus* in the *Aspergillus ustus* group together with four other species: *A. panamensis, A. puniceus, A. conjunctus* and *A. deflectus*. Later, Kozakiewicz (1989) revised the taxonomy of the group, and included *A. ustus*,

Table 3. Extrolites produced by species assigned to *Aspergillus* section *Usti*.

Species	Extrolites produced
Chemical group I	
A. ustus	Ustic acids, austocystins (and versicolorins), austalides, a compound related to sterigmatocystin, nidulol
A. granulosus	Ustic acids, a compound resembling sterigmatocystin, nidulol, drimans
A. puniceus	Ustic acids, austocystins (and versicolorins), phenylahistin, a compound related to sterigmatocystin, nidulol
Chemical group II	
A. pseudodeflectus	Drimans, unknown compounds
A. calidoustus	Drimans, ophiobolins G and H, austins
A. insuetus	Drimans, ophiobolins G and H, pergillin-like
A. keveii	Drimans, ophiobolins G and H, nidulol
Chemical group III	
E. heterothallica	Emethallicins A, B, C, D, E & F, emeheterone, emesterones A & B, 5"-hydroxyaveranthin, Mer-NF8054X, sterigmatocystin, versicolorins

A. pseudodeflectus, A. conjunctus, A. puniceus, A. panamensis and *A. granulosus* into the *A. ustus* species group, and established the *A. deflectus* group including *A. deflectus, A pulvinus* and *A. silvaticus* based on morphological studies. Klich (1993) treated *A. granulosus* as member of section *Versicolores,* and found that *A. pseudodeflectus* is only weakly related to this section based on morphological treatment of section *Versicolores*. Peterson (2000) transferred most species of section Usti to section *Nidulantes* based on sequence analysis of part of the 28 S rRNA gene. On his cladogram, *A. ustus*, *A. pseudodeflectus, A. granulosus* and *A. puniceus* form a well-supported branch closely related to *A. versicolor* and its allies, while *A. deflectus* is on another branch related to *A. elongatus* and *A. lucknowensis*. Peterson (2000) transferred *A. conjunctus, A. funiculosus, A. silvaticus, A. panamensis* and *A. anthodesmis* to section *Sparsi*. Recently Varga *et al*. (submitted) studied large numbers of isolates from clinical and other sources using molecular, morphological and physiological approaches. Phylogenetic analysis of partial β-tubulin, calmodulin, actin and ITS sequences indicated that none of the clinical isolates recognised previously as *A. ustus* belong to the *A. ustus* species. All but two of these isolates formed a well-defined clade related to *A. pseudodeflectus* based on sequence analysis of protein coding regions. Morphological and physiological examination of the isolates indicated that they are able to grow above 37 ºC, in contrast with *A. ustus* isolates, and give a positive Ehrlich reaction, in contrast with related species including *A. granulosus*, *A. ustus*, and *A. pseudodeflectus*. These isolates were described as *A. calidoustus.*

Aspergillus ustus (Bainier) Thom & Church was redescribed by Thom & Church (1926) based on *Sterigmatocystis usta* Bainier. In this manual, *A. insuetus* (Bainier) Thom & Church was also accepted based on *S. insueta* Bainier (Thom & Chuch, 1926), but later *A. insuetus* was abandoned (Thom and Raper, 1945) and included in the broad description of *A. ustus* in Raper and Fennell (1965). Our studies clarified that *A. insuetus* is a valid species which can be distinguished from *A. ustus* and other species assigned to *Aspergillus* section *Usti*. *A. insuetus* could be separated from the other members of the section *Usti* by various phenotypic characters. The most important one is the slower growth rate on YES agar and clear differences in extrolite profiles (Table 2). This finding was supported by all the different data sets used to characterise section *Usti*. The molecular data showed that this species is more related to *A. calidoustus* and *A. pseudodeflectus* than *A. ustus*. Also different extrolite patterns were observed. There were many differences between *A. ustus* and *A. insuetus*, and, like the molecular data, this species was mostly related to *A. calidoustus* and *A. pseudodeflectus*. The main difference between the latter species was the production of a pergillin-like compound by *A. insuetus* (Table 3).

Our polyphasic taxonomic approach revealed that *Aspergillus* section *Usti* includes eight species: *A. ustus, A. puniceus*, *A. granulosus, A. pseudodeflectus, A. calidoustus*, *A. insuetus* and *A. keveii* **sp. nov.** The phylogenetic trees based on ITS, calmodulin and β-tubulin sequence data indicated that *E. heterothallica* also belongs to this section. This species has similar morphology of the conidiophores and Hülle cells. In our study we were not able to observe ascospores by crossing the two mating strains but these are described by Raper and Fennell (1965: 502–503).

Aspergillus calidoustus Varga *et al*. Eukaryotic Cell submitted. Fig. 5.

Type: CBS 121604 from human, Netherlands

Other no. of the type: strain 677

Description strain
Colony diam, 7 d, in mm: CYA25 27–32; CYA37 20–35; MEA25 35–48; YES 36–41
Colony colour on CYA: blond/greyish yellow, brownish grey or greyish brown
Conidiation on CYA: abundant
Reverse colour (CYA): yellow with beige or olive brown centre
Colony texture: floccose
Conidial heads: loosely columnar
Stipe: 150–300 × 4–7 µm, smooth, brown
Vesicle diam/shape: 9–15 µm, pyriform to broadly spathulate
Conidium size/shape/surface texture: 2.7–3.5 x µm, globose, very rough ornamentation (0.5–0.8 µm high), inner and outer wall visible
Hülle cells: sparsely produced, irregularly elongated, in scattered groups
Ehrlich reaction: violet
Growth on creatine: weak to moderate growth with hyaline mycelium, no acid production

Diagnostic features: good growth at 37 °C, violet Ehrlich reaction, coarsely roughened to echinulate conidia

Cultures examined: CBS 121589, 121601–121616

Similar species: *A. pseudodeflectus*

Distribution: U.S.A., Turkey, Finland, Germany, Netherlands

Ecology and habitats: indoor air, rubber, construction material, human

Extrolites: Drimans, ophiobolins G and H, austins

Pathogenicity: pathogenic to humans (Verweij *et al.* 1999; Weiss & Thiemke 1983; Pavie *et al.* 2005; Panackal *et al.* 2006; Yildiran *et al.* 2006; Iwen *et al.* 1998)

Aspergillus granulosus Raper & Thom, Mycologia 36: 565. 1944. Fig. 6.

Type: CBS 588.65, from soil, Fayetteville, Arkansas, U.S.A.

Other no. of the type: ATCC 16837, NRRL 1932, WB 1932, CBS 452.93

Description
Colony diam, 7 d, in mm: CYA25 30–48; CYA37 30–51; MEA25 25–37; YES25 35–45; CZA25 17–25
Colony colour: buff to dull brown
Conidiation: moderate
Reverse colour (CYA): dull yellow to red brown
Colony texture: floccose, plane or irregularly furrowed
Conidial head: hemispherical to radiate
Stipe: 100–600 × 5.5–8 µm, thin-walled, smooth, straight, tan to light brown
Vesicle diam/shape: 15–25 × 12–18 µm, ovoid to elliptical
Conidium size/shape/surface texture: (3.3–)4–4.5(–5.5) µm, globose, delicately echinulate
Hülle cells: irregularly globose, ovoid to elongate, 12–30 µm, in colourless clusters at colony margins
Ehrlich reaction: violet
Growth on creatine: poor growth with inconspicuous mycelium, no acid production

Cultures examined: CBS 119.58, CBS 588.65, IBT 23478

Diagnostic features: small colourless clusters of irregularly globose Hülle cells, giving the colony a characteristic granular appearance, good growth at 37 °C and violet Ehrlich reaction

Similar species: -

Distribution: U.S.A.

Ecology and habitats: soil

Extrolites: Ustic acids, a compound resembling sterigmatocystin, nidulol, drimans

Pathogenicity: pathogenic to humans (Fakih *et al.* 1995)

Aspergillus insuetus (Bainier) Thom & Church, Manual of the aspergilli: 153. 1929. Fig. 7.
= *Sterigmatocystis insueta* Bainier (1908)

Type: CBS 107.25, from South Africa, Sartory

Other no. of the type: ATCC 1033; IFO 4128; NRRL 279; NRRL 1726; Thom No. 4658.245

Description
Colony diam, 7 d, in mm: CYA 28–32; CYA37 no growth; MEA25 36–41; YES 23–30
Colony colour: almost black in center, shading through gray to white sterile floccose marginal areas
Conidiation on CYA: moderate to good
Reverse colour (CYA): yellow olive to blackish brown with age
Colony texture: floccose
Conidial head: radiate to hemispherical
Stipe: 300 × 4–8 µm, smooth, brown
Vesicle diam/shape: 11–16 µm, hemispherical to subglobose
Conidium size/shape/surface texture: 3.2–4 µm, globose, distinct roughened and inner and outer wall visible, fuligeneous, the colour mostly aggregated into echinulations of the cell-wall, and even forming bars and tubercules at times
Hülle cells: variously coiled or curved, in scattered groups
Ehrlich reaction: violet
Growth on creatine: good growth with hyaline mycelium, no acid production

Cultures examined: CBS 107.25, CBS 119.27, CBS 102278

Similar species: *A. keveii*

Distribution: South Africa, Spain

Diagnostic features: no growth at 37 °C, violet Ehrlich reaction, restricted growth on YES, coarsely roughened to echinulate conidia

Ecology and habitats: soil (?), human

Extrolites: Drimans, ophiobolins G and H, pergillin-like

Pathogenicity: caused subcutaneous infection (Gené *et al.* 2001)

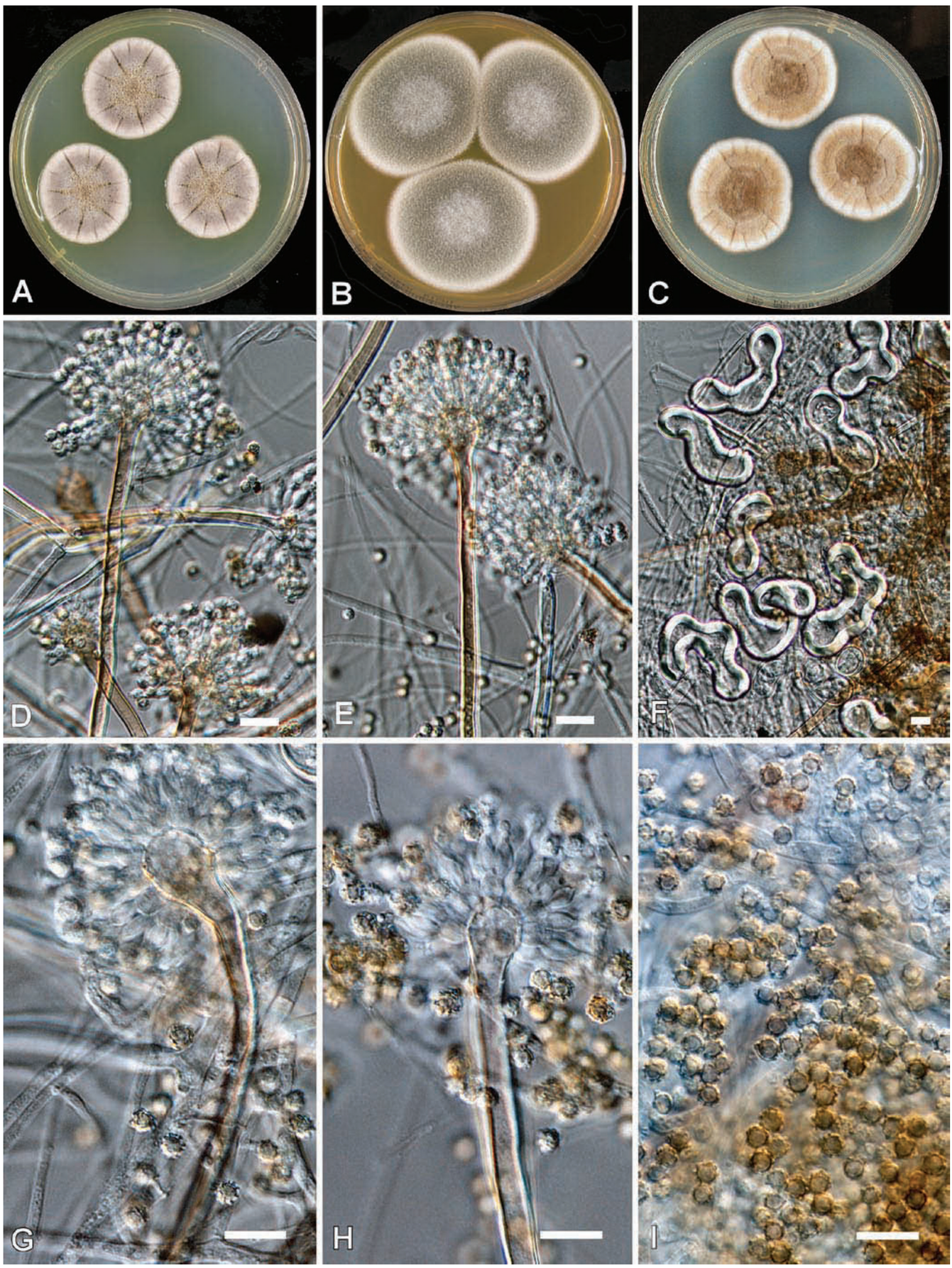

Fig. 5. *Aspergillus calidoustus*. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–E, G–H Conidiophores. F. Hülle cells. I. Conidia. Scale bars = 10 µm, except F = 30 µm

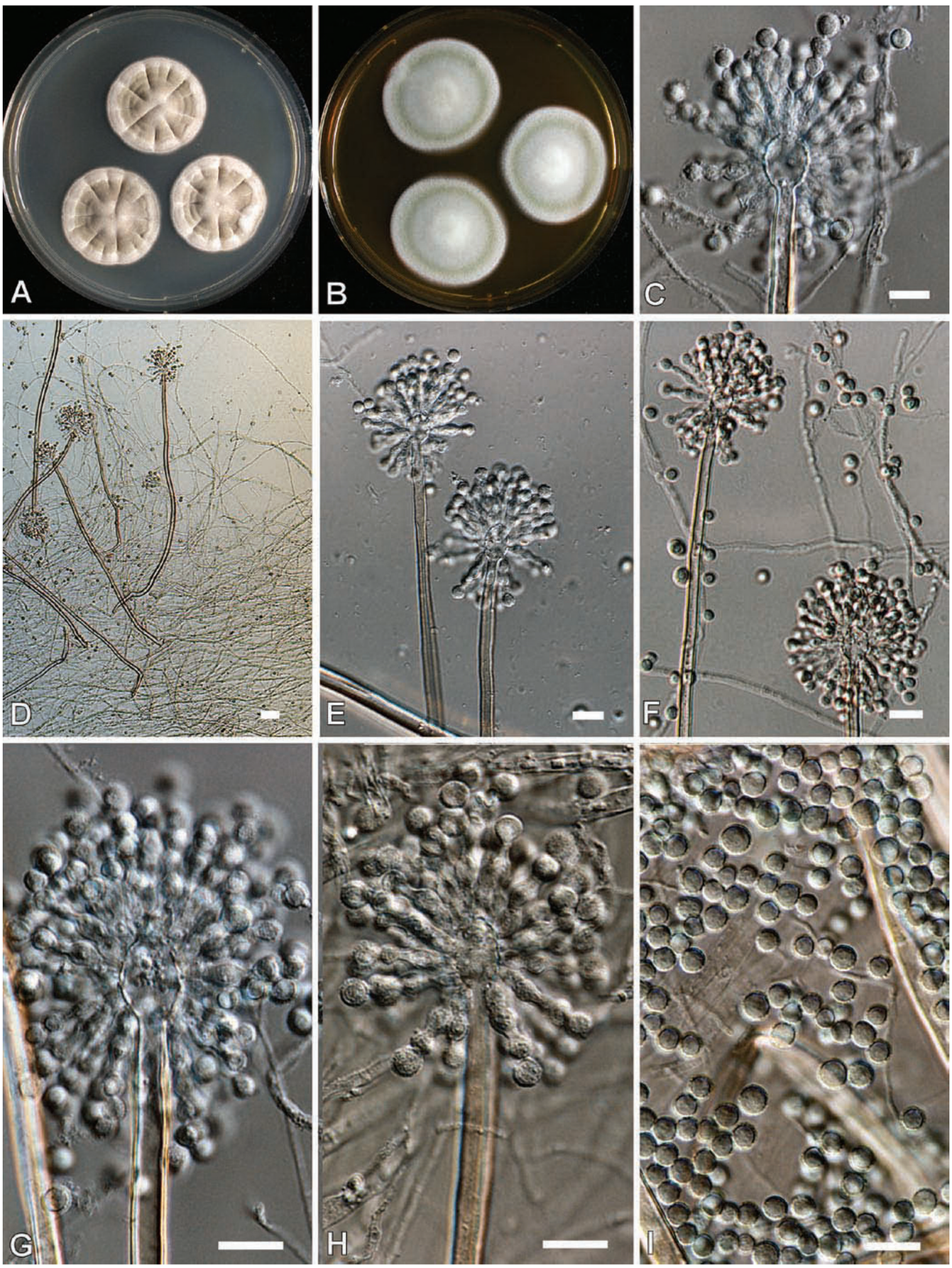

Fig. 6. *Aspergillus granulosus*. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. Conidia. Scale bars = 10 µm, except C = 30 µm.

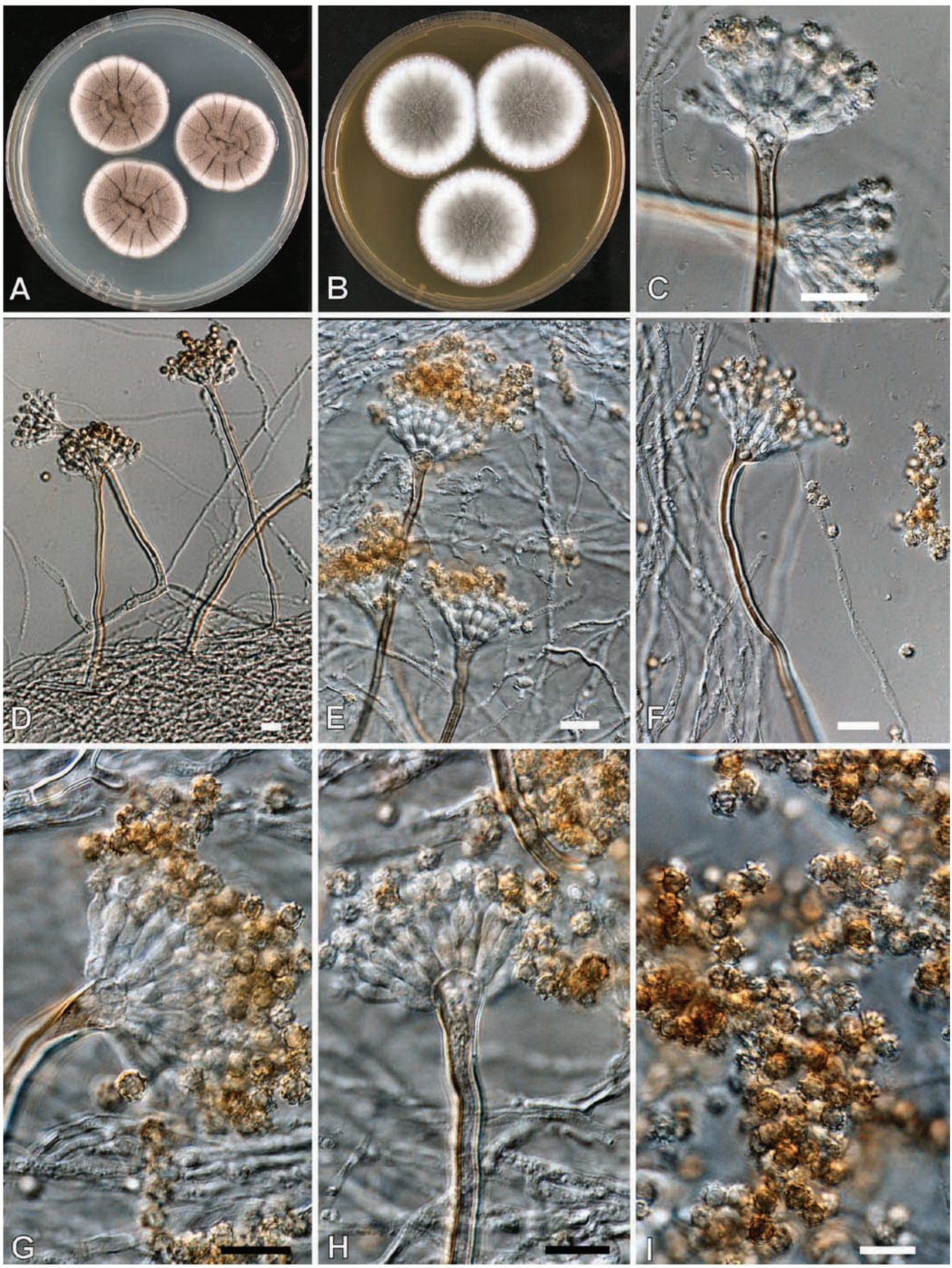

Fig. 7. *Aspergillus insuetus*. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. Conidia. Scale bars = 10 µm, except C = 30 µm.

***Aspergillus keveii* sp. nov.** Varga, Frisvad & Samson – MycoBank MB505570. Fig. 8.

Holotype of *Aspergillus keveii*, here designated as CBS 209.92^{T} (dried culture) isolated from soil, Las Palmas, Spain.

Coloniae in 7 dieibus et 25 °C in agaro MEA 36–41 mm, in CYA 30–39 mm, in YES 40–46 mm, in CREA 25–32 mm diam; auctus in 7 dieibus et 37 °C in agaro CYA nullus. Sporulatio in CYA abundans; colonia brunneogrisea vel subroseobrunnea; textura coloniae floccosa; colonia reversa flavide olivaceobrunnea vel atrobrunnea. Capitula conidialia laxe columnaria; stipites 150–300 × 4–6 µm, pariete laevi, brunneo; vesciculae pyriformes, 9–13 µm in lat., biseriatae; metulae 4.7–6.7 × 2.8–3.6 µm; phialides 5.7–7 × 2–3 µm; conidia globosa, 2.4–2.8 µm diam., ornamento exasperato vel echinulato. Cellulae "hülle" irregulariter elongatae, (10–) 25–40(–65) µm in long., in cumulis dispersis.

Colonies on MEA 36–41 mm, on CYA 30–39 mm, on YES 40–46 mm, on CREA 25–32 mm in diam. after 7 d at 25 °C, no growth on CYA after 7 d at 37 °C. Conidial heads abundant on CYA, colony colour brownish grey to pinkish brown, colony texture floccose, reverse yellow olive brown to dark brown. Conidial heads loosely columnar; stipes 150–300 × 4–6 µm, smooth walled, brown in colour; vesicles 9–13 µm wide, pyriform, biseriate; metulae covering the upper half to three-fourths of the vesicle, measuring 4.7–6.7 × 2.8–3.6 µm µm; phialides 5.7–7 × 2–3 µm; conidia globose 2.4–2.8 µm, coarsely roughened to echinulate. Hülle cells (10–)25–40(–65) µm, irregularly elongated, produced in scattered groups.

Etymology: named after Prof. Ferenc Kevei, eminent mycologist devoting his life to *Aspergillus* research.

Type: CBS 209.92

Ehrlich reaction: violet, with exception of CBS 113227

Growth on creatine: good growth with hyaline mycelium, no or weak acid production

Diagnostic features: no growth at 37 °C, good growth on CREA and YES, coarsely roughened to echinulate conidia; Hülle cells in scattered groups, violet Ehrlich reaction

Cultures examined: CBS 561.65, CBS 209.92 and CBS 113227

Similar species: *A. insuetus*

Distribution: U.S.A., Turkey, Finland, Germany, Netherlands

Ecology and habitats: indoor air, rubber, construction material, human

Extrolites: Drimans, ophiobolins G and H, nidulol

Pathogenicity: not reported

Notes: CBS 113227 is deviating in having larger conidial heads and small (2.6 µm), finely roughened pinkish brown coloured conidia

Aspergillus pseudodeflectus Samson & Mouchacca, Antonie van Leeuwenhoek 41(3): 325. 1975. Fig. 9.

Type: CBS 756.74, from desert soil, Western Desert, Egypt

Other no. of the type: IMI 278381

Description
Colony diam, 7 d, in mm: CYA25 43–49; CYA37 15–20; MEA25 35–45; YES 20–30; CZA25 25-26
Colony colour: white mycelial felt intermixed with brown conidiogenous structures
Conidiation: sparse
Reverse colour (CZA): yellow
Colony texture: velvety appearance, no sporulation
Conidial head: radiate, brown
Stipe: 35–200 × 2.5–3.5 µm, rough-walled with warty protuberances, brown
Vesicle diam/shape: 4–12 µm, globose to clavate
Conidium size/shape/surface texture: 3.5–5 µm, globose to ellipsoidal, brown, ornamented with small warts and colour bars
Hülle cells: absent
Ehrlich reaction: none
Growth on creatine: weak to moderate growth with hyaline mycelium, no acid production

Diagnostic features: Growth at 37 °C, curved brown conidiophores and the ornamented conidia, absence of Hülle cells

Cultures examined: CBS 756.74, CBS 596.65

Similar species: *A. calidoustus*

Distribution: Egypt, U.S.A.

Ecology and habitats: soil

Extrolites: Drimans (Hayes *et al.* 1996), unknown compounds

Pathogenicity: not reported

Aspergillus puniceus Kwon and Fennell, The genus *Aspergillus:* 547. 1965. Fig. 10.
= *A. ustus* var. *laevis* Blochwitz (1945)

Type: CBS 495.65, from soil, Zarcero, Costa Rica

Other no. of the type: ATCC 16800; IMI 126692; WB 5077

Description
Colony diam, 7 d, in mm: CYA 40–50; CYA37 no growth; MEA25 40–45; YES 48–53; CZA25: 40–50 mm
Colony colour: pinkish orange near vinaceous pink, with wine red exudate droplets
Conidiation: moderate
Reverse colour (CYA): dark yellow brown or crème brown
Colony texture: floccose
Conidial head: radiate to short columnar, dull green becoming light drab with age
Stipe: 150–250(–300) × 5.5–6(–8) µm, aerially borne stipes up to 135 × 3–4 µm, straight, smooth
Vesicle diam/shape: 8–16 µm (subglobose), 15–18 × 13–15 µm (elliptical)
Conidium size/shape/surface texture: 2.5–3.3 µm, globose, roughened
Hülle cells: elongate, crescent shaped or irregularly twisted, often aggregated into yellowish masses
Ehrlich reaction: no reaction
Growth on creatine: moderate to good growth with bright yellow mycelium, no acid production (in some isolates weak acid production under colony)

Cultures examined: CBS 495.65, CBS 122.33, CBS 128.62, 9377, V41-02, NRRL 29173

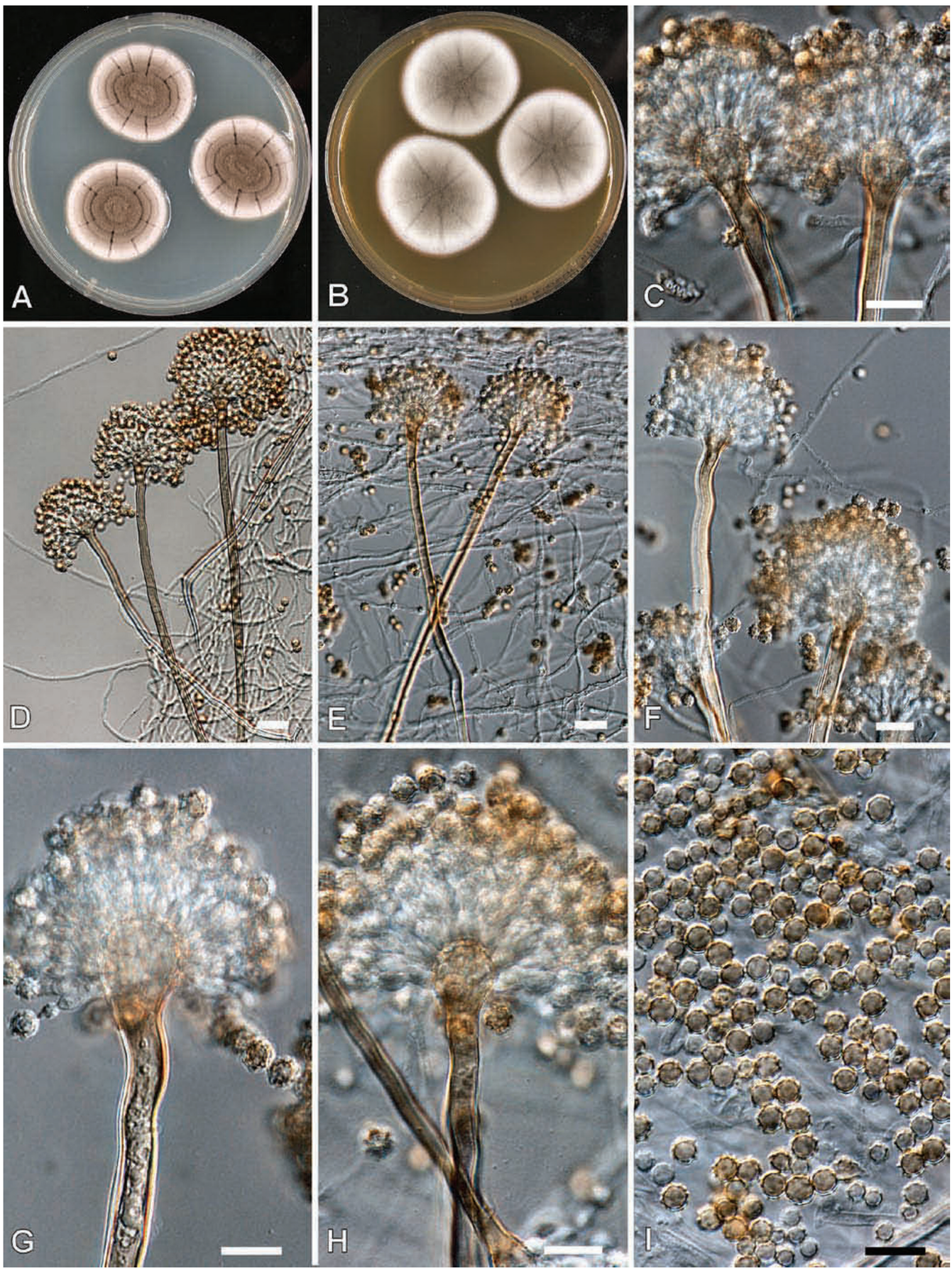

Fig. 8. *Aspergillus kerveii.* A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. Conidia. Scale bars = 10 µm.

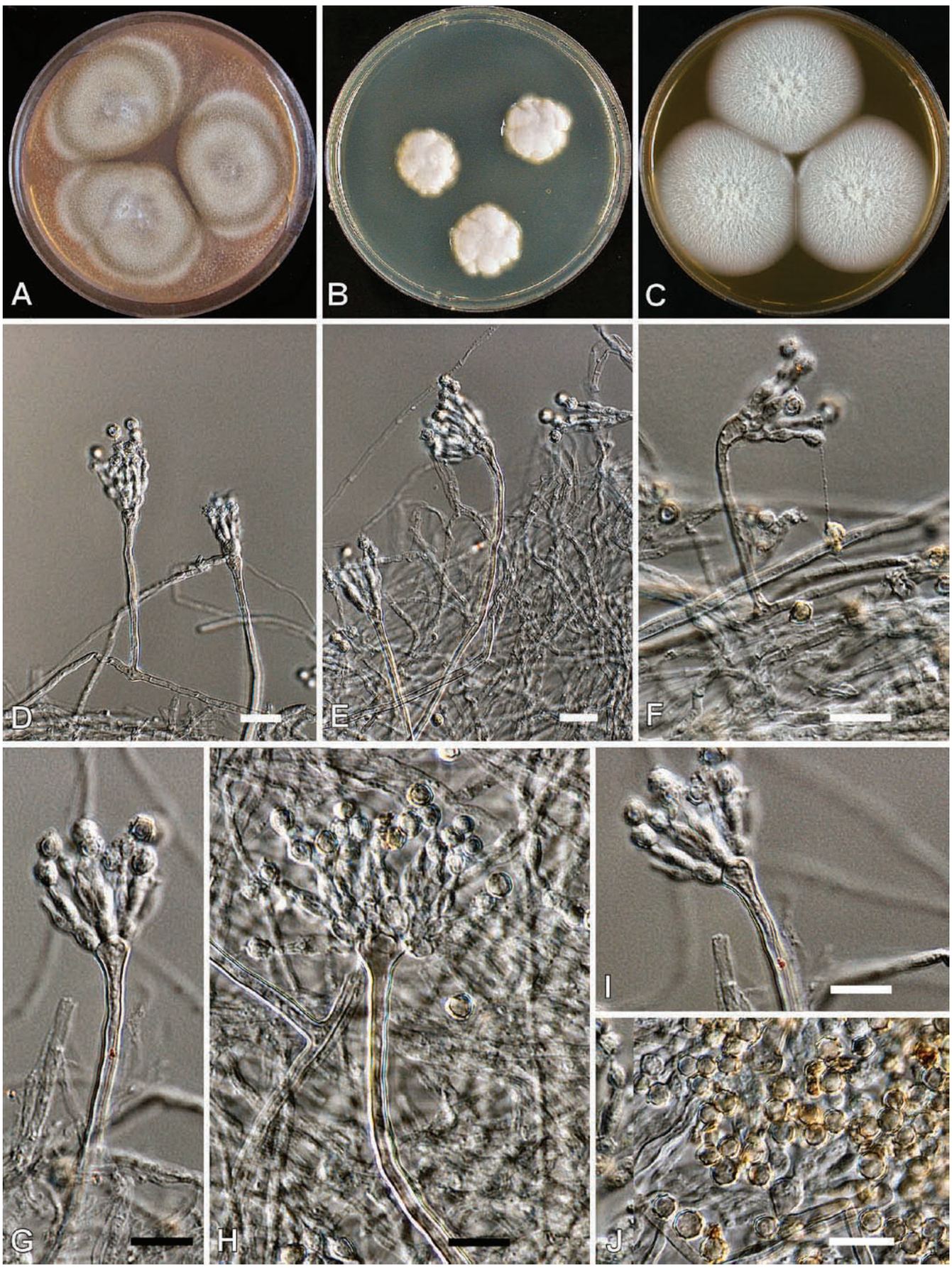

Fig. 9. *Aspergillus pseudodeflectus*. A–C. Colonies at 25 °C after 7 d. A. MEA + 40 % sucrose. B. CYA + 20 % sucrose. C. MEA. D–I. Conidiophores. H. Conidia. Scale bars = 10 µm.

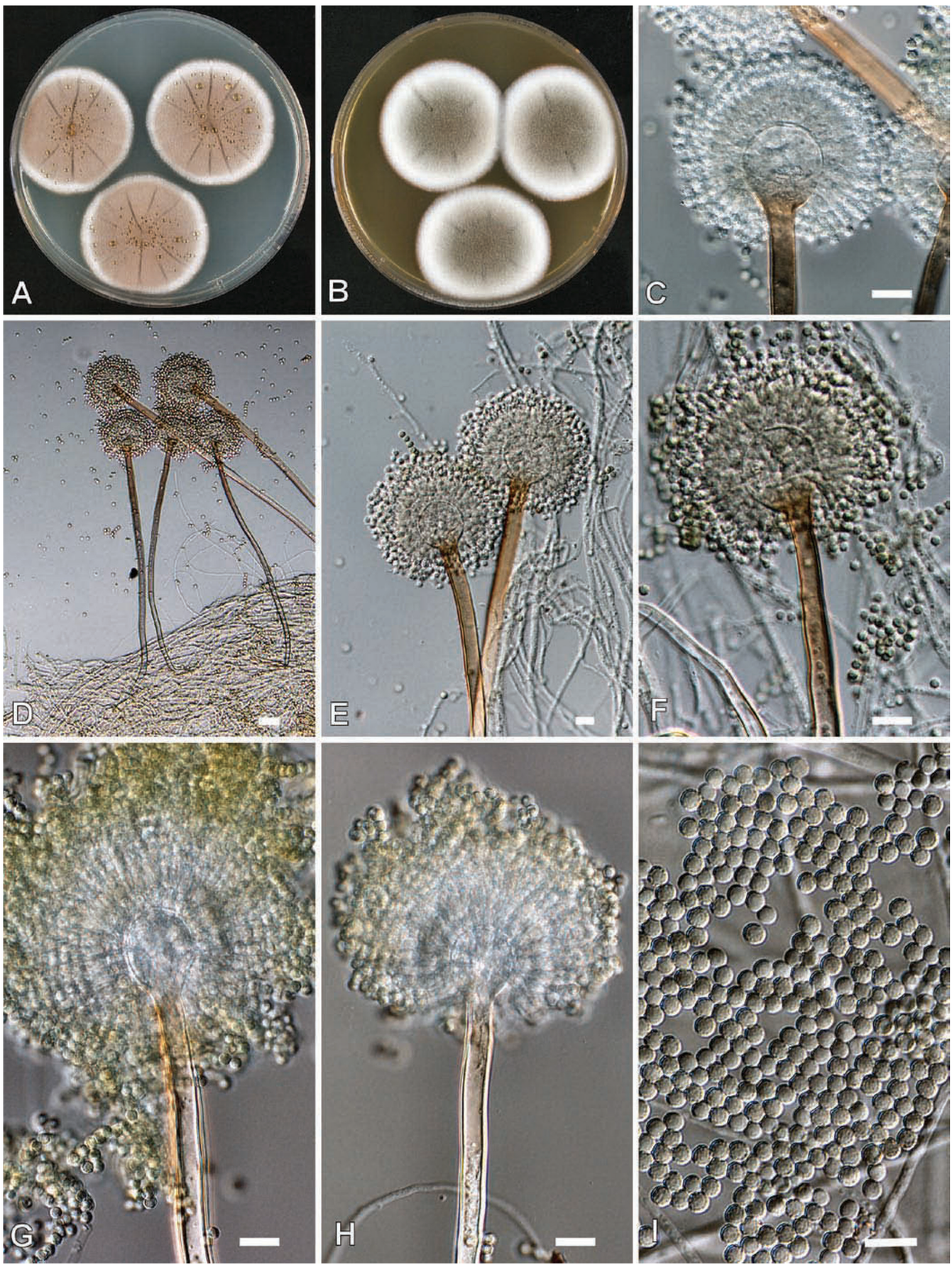

Fig. 10. *Aspergillus puniceus*. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. Sclerotia. J. Conidia. Scale bars = 10 µm, except D = 30 µm.

Diagnostic features: No growth at 37 °C, good growth on creatine with brightly pigmented yellow mycelium, Hülle cells aggregated into yellowish masses

Similar species: *A. ustus*

Distribution: Costa Rica, U.S.A., Canada, Netherlands

Ecology and habitats: soil, indoor air, human

Extrolites: ustic acids, austocystins, nidulol, versicolorins, phenylahistin, sterigmatocystin-related compound (in CBS 128.62)

Pathogenicity: isolated from mouth wash and faeces

Aspergillus ustus (Bainier) Thom & Church, The aspergilli: 152. 1924. Fig. 11.

= *Sterigmatocystis usta* Bainier (1881)
= *Aspergillus humus* Abbott (1926)

Type: CBS 261.67, culture contaminant, U.S.A.

Other no. of the type: ATCC 1041; ATCC 16818; IMI 211805; NRRL 275; QM 7477; WB 275; Thom 3556

Description
Colony diam, 7 d, in mm: CYA 36–43; CYA37 no growth; MEA25 39–46; YES 42–50
Colony colour: greyish brown to dark brown
Conidiation on CYA: moderate
Reverse colour (CZA): yellow-olive edge with olive brown centre
Colony texture: floccose, plane, sulcate or umbonate
Conidial head: radiate to hemispherical
Stipe: 400 × 3–6 µm, aerially borne stipes up to 125 × 2–5 µm, smooth, brownish
Vesicle diam/shape: 7–15 µm, hemispherical to subglobose
Conidium size/shape/surface texture: 3.2–4.5 µm, globose, roughened, greenish to dark yellow brown
Hülle cells: irregularly ovoid or elongate, usually scattered
Ehrlich reaction: no reaction
Growth on creatine: good growth with faint yellow mycelium, no acid production

Cultures examined: CBS 116057, CBS 114901, CBS 261.67, CBS 133.55, CBS 239.90, CBS 113233, CBS 113232, NRRL 285, NRRL 280, NRRL 1609, NRRL 29172

Diagnostic features: No growth at 37 °C; good growth on creatine with faint yellow pigmented mycelium; Hülle cells typically scattered or form irregular masses and not associated with pigmented mycelium

Similar species: *A. puniceus*

Distribution: U.S.A., Poland, Netherlands, Canada

Ecology and habitats: soil, indoor air, bat dung

Extrolites: Ustic acids, austocystins, versicolorins, austalides, a compound related to sterigmatocystin, nidulol

Pathogenicity: isolated from biopsy of man with brain tumour (CBS 239.90). However, this isolate does not grow at 37 °C on normal agar media and might therefore be a culture contamination.

Emericella heterothallica (Kwon-Chung, Fennell & Raper) Malloch & Cain [anamorph: *A. compatibilis* Samson & Gams], Can. J. Bot. 50: 62. 1972. Fig. 12.

Type: CBS 489.65, from soil, Costa Rica

Other no. of the type: ATCC 16824; IHEM 2064; IMI 139278; RV 34434; WB 5097; IBT 22604

Description
Colony diam, 7 d, in mm: CYA25 35–39; CYA37 5–8; MEA25 40–42; YES25 38–42
Colony colour: cream to yellow to orange
Conidiation: limited
Reverse colour (CYA): yellow to orange to pink becoming dark reddish brown
Colony texture: floccose
Conidial head: hemispherical to short columnar
Stipe: 185–410 × 5–11 µm, generally sinuous, brownish with age, smooth
Vesicle diam/shape: 13–20 µm
Conidium size/shape/surface texture: 2.5–4 µm, globose, echinulate, yellow green
Hülle cells: 600–700(–1000) µm, pyriform to oval to elongate to twisted, in globose to subglobose masses
Cleistothecia: produced in a heterothallic manner, 270–510 µm, cinnamon to dark purple, surrounded by Hülle cells
Ascospores: 4–4.5 × 3.5–4 µm, lenticular, orange brown in colour, with two pleated equatorial crests (1.5–2 µm), with convex smooth
Ehrlich reaction: none
Growth on creatine: weak growth with yellow coloured mycelium, no acid production

Diagnostic features: heterothallic species, weak growth at 37 °C

Cultures examined: CBS 489.65, CBS 488.65 = IBT 22607

Similar species: -

Distribution: Costa Rica

Ecology and habitats: soil

Extrolites: Found in this study: Sterigmatocystin, versicolorins, Mer-NF8054X. Literature data: emethallicins A–F (Kawahara *et al.* 1989, 1990a), 5"-hydroxyaveranthin (Yabe *et al*. 1991), emeheterone (Kawahara *et al.* 1988), emesterones A & B (Hosoe *et al.* 1998), 5"-hydroxyaveranthin (Yabe *et al.* 1991), Mer-NF8054X (Mizuno *et al.* 1995).

Pathogenicity: not reported

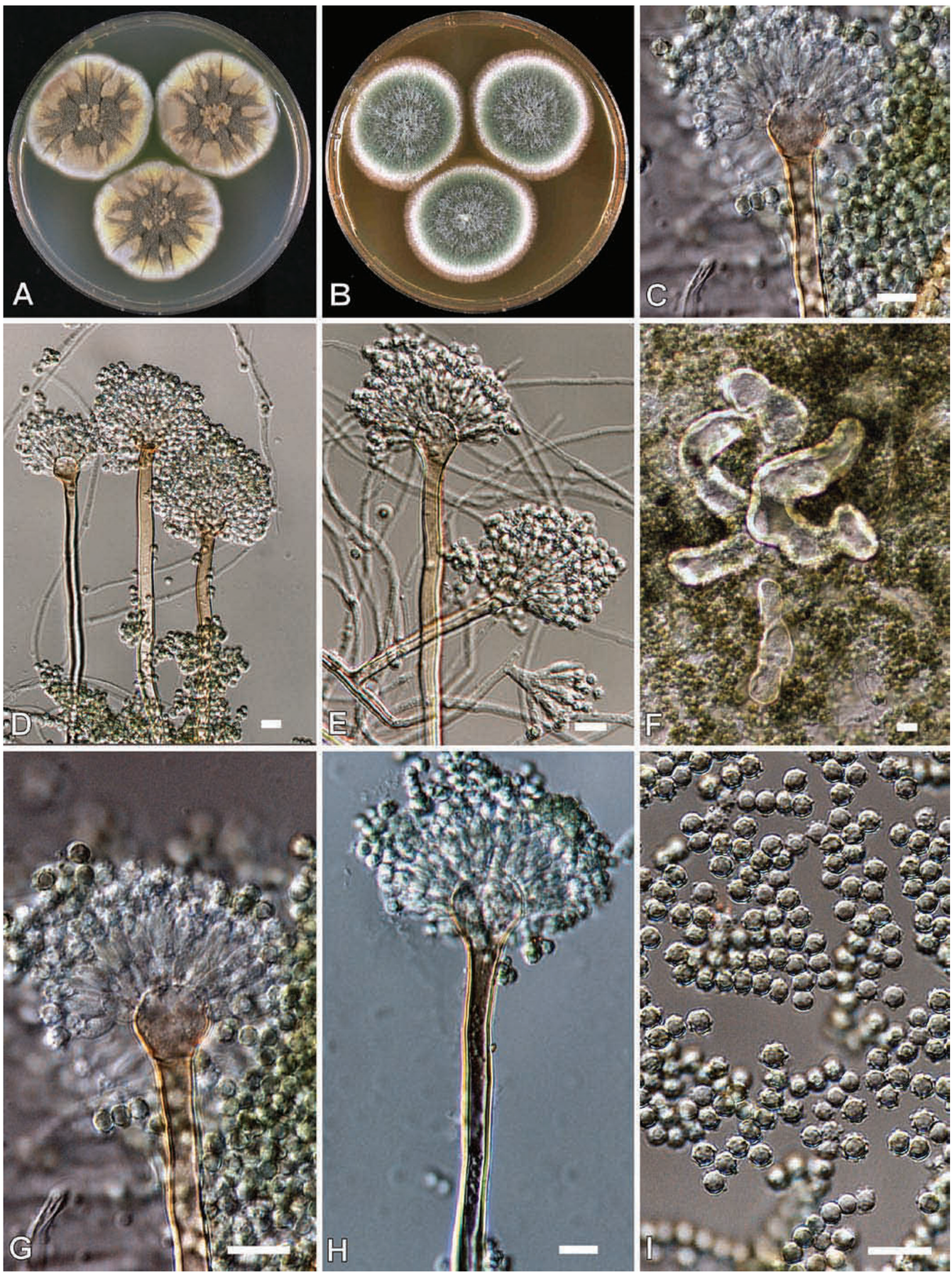

Fig 11. *Aspergillus ustus*. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–E. G–H Conidiophores. F. Hülle cells. I. Conidia. Scale bars = 10 µm, except F = 30µm.

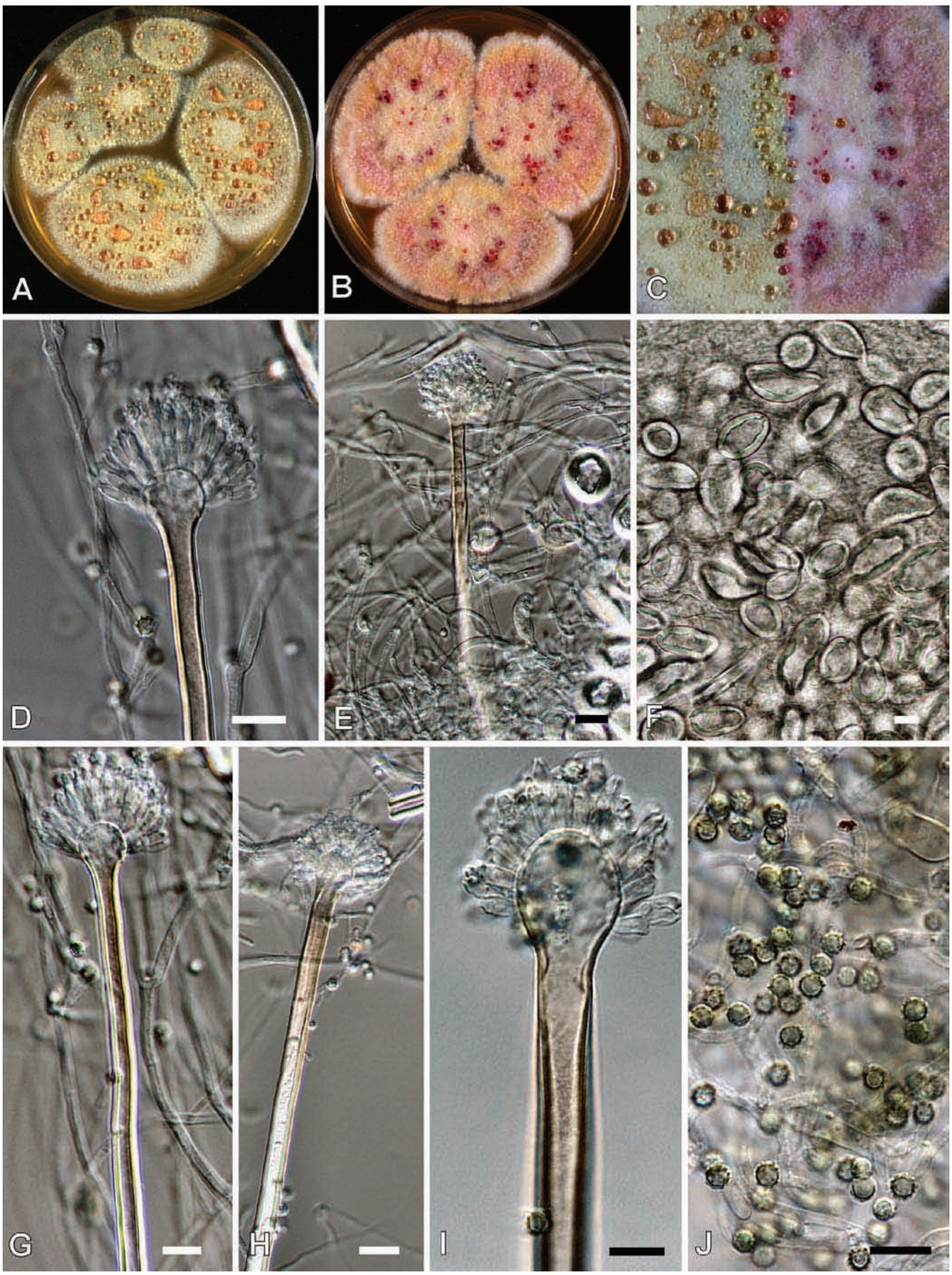

Fig. 12. *Emericella heterothallica*. A–C. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C. Crossing of mating strains. D–E, F–H. Conidiophores. I. Conidia. Scale bars = 10 µm.

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