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New species in *Aspergillus* section *Terrei*

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Abstract: Section *Terrei* of *Aspergillus* was studied using a polyphasic approach including sequence analysis of parts of the β-tubulin and calmodulin genes and the ITS region, macro- and micromorphological analyses and examination of extrolite profiles to describe three new species in this section. Based on phylogenetic analysis of calmodulin and β-tubulin sequences seven lineages were observed among isolates that have previously been treated as *A. terreus* and its subspecies by Raper & Fennell (1965) and others. *Aspergillus alabamensis, A. terreus* var. *floccosus*, *A. terreus* var. *africanus*, *A. terreus* var. *aureus*, *A. hortai* and *A. terreus* NRRL 4017 all represent distinct lineages from the *A. terreus* clade. Among them, *A. terreus* var. *floccosus*, *A. terreus* NRRL 4017 and *A. terreus* var. *aureus* could also be distinguished from *A. terreus* by using ITS sequence data. New names are proposed for *A. terreus* var. *floccosus*, *A. terreus* var. *africanus*, *A. terreus* var. *aureus,* while *Aspergillus hortai* is recognised at species level. *Aspergillus terreus* NRRL 4017 is described as the new species *A. pseudoterreus*. Also included in section *Terrei* are some species formerly placed in sections *Flavipedes* and *Versicolores*. A. clade including the type isolate of *A. niveus* (CBS 115.27) constitutes a lineage closely related to *A. carneus*. *Fennellia nivea*, the hypothesized teleomorph is not related to this clade. *Aspergillus allahabadii, A. niveus* var. *indicus*, and two species originally placed in section *Versicolores*, *A. ambiguus* and *A. microcysticus*, also form well-defined lineages on all trees. Species in *Aspergillus* section *Terrei* are producers of a diverse array of secondary metabolites. However, many of the species in the section produce different combinations of the following metabolites: acetylaranotin, asperphenamate, aspochalamins, aspulvinones, asteltoxin, asterric acid, asterriquinones, aszonalenins, atrovenetins, butyrolactones, citreoisocoumarins, citreoviridins, citrinins, decaturins, fulvic acid, geodins, gregatins, mevinolins, serantrypinone, terreic acid (only the precursor 3,6-dihydroxytoluquinone found), terreins, terrequinones, terretonins and territrems. The cholesterol-lowering agent mevinolin was found in *A. terreus* and *A. neoafricanus* only. The hepatotoxic extrolite citrinin was found in eight species: *A. alabamensis, A. allahabadii, A. carneus, A. floccosus, A. hortai, A. neoindicus, A. niveus* and *A. pseudoterreus*. The neurotoxic extrolite citreoviridin was found in five species: *A. neoafricanus, A. aureoterreus, A. pseudoterreus, A. terreus* and *A. neoniveus*. Territrems, tremorgenic extrolites, were found in some strains of *A. alabamensis* and *A. terreus*.

Key words: Ascomycetes, *Aspergillus* section *Terrei,* β-tubulin, calmodulin, citreoviridin, *Eurotiales*, extrolites, ITS, polyphasic taxonomy.
Taxonomic novelties: *Aspergillus aureoterreus* stat. et nom. nov., *Aspergillus floccosus* comb. et stat. nov., *Aspergillus neoafricanus* stat. et nom. nov., *Aspergillus neoindicus* stat. et nom. nov., *Aspergillus neoniveus* nom. nov., *Aspergillus pseudoterreus* sp. nov.

INTRODUCTION

Aspergillus section *Terrei* (Gams *et al.* 1985; *A. terreus* species group according to Raper & Fennell 1965) includes species with columnar conidial heads in shades of buff to brown. The most important species of this section is *A. terreus,* which is an ubiquitous fungus in our environment. Strains of this cosmopolitan species are frequently isolated from desert and grassland soils and compost heaps, and as contaminants of plant products like stored corn, barley and peanuts (Kozakiewicz 1989). *Aspergillus terreus* is an economically important species from a number of aspects. *Aspergillus terreus* isolates are used in the fermentation industry for the production of itaconic acid and itatartaric acid and for enzyme production (Bigelis & Arora 1992, Lowe 1992). *Aspergillus terreus* isolates produce a range of secondary metabolites, some of which have properties valuable for mankind, including lovastatin, a cholesterol lowering drug (Alberts *et al.* 1980), the antitumor metabolites terrein (Arakawa *et al.* 2008, Demasi *et al.* 2010), quadrone (Carlton *et al.* 1978) and asterriquinone (Kaji *et al.* 1998), acetylcholinesterase inhibitors like territrem B (Chen *et al.* 1999) and terreulactone, butyrolactones (Schimmel *et al.* 1998), and cyclosporine A (Sallam *et al.* 2003). Antiviral compounds such as acetylaranotin has also been reported from *Aspergillus terreus* (Miller *et al.* 1968, Kamata *et al.* 1983). Other secondary metabolites reported to be produced by *A. terreus* isolates are considered as mycotoxins, including citreoviridin (Franck & Gehrken, 1980), patulin (Kent & Heatley 1945, Draughon & Ayres 1980, Reddy & Reddy 1988), citrinin (Sankawa *et al.* 1983), emodin (Fujii *et al.* 1982), terretonin (Springer *et al.* 1979, Li *et al.* 2005), geodin (Kiriyama *et al.* 1977, Rønnest *et al.* 2011), territrems (Ling *et al.* 1979), gliotoxin (Lewis *et al.* 2005, Kupfahl *et al.* 2008), and cytochalasin E (Fujishima *et al.* 1979). *Aspergillus terreus* is also an important human pathogen, and often causes disseminated infection with increased lethality compared to other *Aspergillus* spp. (Tracy *et al.* 1983, Iwen *et al.* 1998, Lass-Florl *et al.* 2000, Walsh *et al.* 2003, Baddley *et al.* 2003, Steinbach *et al.* 2004, Balajee 2009). Recent data indicate that the accessory conidia produced by *A. terreus* can induce elevated inflammatory responses in a pulmonary model of aspergillosis (Deak *et al.* 2009, 2011). The importance of *A. terreus* to human health and industry is underlined by the fact that annotation of the full genome sequence of *A. terreus* isolate NIH 2624 is in progress (Birren *et al.* 2004, http://fungi.ensembl.org/Aspergillus_terreus/Info/Index), while whole-genome shotgun sequencing of isolate ATCC 20542 has also been carried out (Askenazi *et al.* 2003). Additionally, transcriptional and metabolite profiles (Askenazi *et al.* 2003) and the extracellular proteome of *A. terreus* have also been examined recently (Han *et al.* 2010).

Table 1. *Aspergillus* strains examined in this study.

Taxon	Strain No.	Origin
A. neoafricanus	CBS 130.55^T = NRRL 2399	*Aspergillus terreus* var. *africanus*; soil, Tafo, Ghana
	NRRL 4609	*Aspergillus terreus* var. *africanus*; soil, Panama
	IBT 13121	*Aspergillus terreus* var. *africanus*; soil, Japan
A. alabamensis	IBT 12702	Soil, New Mexico
	WB 1920 = IBT 22563	Soil, Cuba
	DTO 15-F8 = IBT 29084	Soil, Argentina
	DTO 15-F9 = IBT 29086	Soil, Argentina
	UAB 18	Sputum, Alabama, USA
	UAB 15	Sputum, Alabama, USA
	UAB 20T	Wound, Alabama, USA
	NRRL 29810 = IBT 29081	Soil, Florida, USA
A. allahabadii	CBS 164.63^T = NRRL 4539	Soil, India
	CCRC 32133 = IBT 21128	Soil, Taipei, Taiwan
A. ambiguus	CBS 117.58^T = NRRL 4737	Savannah soil, Somalia
A. aureoterreus	CBS 265.81	Wheat flour, India
	CBS 503.65^T = NRRL 1923	Soil, Texas, USA
A. carneus	CBS 494.65^T = NRRL 527 = IBT 13986	Culture contaminant, District of Columbia, USA
	NRRL 1928	Soil, Kansas, USA
	NRRL 298 = IBT 22569	Soil, Kansas, USA
A. floccosus	CBS 116.37^T = WB 4872 = IBT 22556	*Aspergillus terreus* var. *floccosus*; Waste cloth, Wuchang, China, isolated by Y.K. Shih as No. A 369
A. hortai	CBS 124230^T = NRRL 274 = IBT 26384	Clinical isolate, from ear, Brazil
	IBT 16744	Soil, Galapagos Islands
	IBT 16745	Soil, Galapagos Islands
	IBT 6271	Soil, Florida, USA
A. microcysticus	CBS 120.58^T = NRRL 4749	Savannah soil, Somalia
A. neoindicus	CBS 444.75^T = NRRL 6134	*Aspergillus niveus* var. *indicus*; Soil, Maharashta, India
A. neoniveus	CBS 261.73^T = NRRL 5299	Forest soil, Thailand
	CBS 262.73 = NRRL 5502	Forest soil, Thailand
	CBS 114.33 = NRRL 515	P. Biourge
	CBS 471.91 = NRRL 1955	Soil, Ontario, Canada
A. niveus	CBS 115.27^T = NRRL 5505	A. Blochwitz
	NRRL 4751^T	*Fennellia nivea* var. *bifida*; unknown
A. pseudoterreus	CBS 123890 = NRRL 4017	Soil, Argentina
A. terreus	IBT 26915	Capybara droppings, Gamboa, Panama
	CBS 601.65^T = NRRL 255	Soil, Connecticut, USA
	NRRL 260	Soil, College Station, Texas, USA
	NRRL 1913	Lung of pocket mouse, Arizona, USA
	IBT 6450	Corn, India
	IBT 14590 = UAMH 4733	Soil, Golf course, Japan
	IBT 24859	Saltern, Slovenia
	NRRL 680 = CBS 594.65 = IBT 6252	Soil, G. Ledingham
	CBS 117.37 = WB 4873	*Aspergillus terreus* var. *subfloccosus*; Air, Wuchang, China

Aspergillus terreus was the only species assigned to the *A. terreus* species group by Raper & Fennell (1965). Molecular studies have since indicated that this section should be expanded to include a number of other species (Peterson 2000, 2008, Varga *et al.* 2005). Besides *A. terreus* and its varieties, section *Terrei* also includes *A. niveus* (teleomorph: *Fennellia nivea*), *A. carneus*, *A. niveus* var. *indicus*, *A. allahabadii*, *A. ambiguus* and *A. microcysticus* (Peterson 2000, 2008, Varga *et al.* 2005). The first three species have previously been placed in section *Flavipedes* and the last three species were placed in section *Versicolores* (Raper & Fennell 1965, Samson 1979). *Aspergillus niveus* has been reported to cause pulmonary aspergillosis (Auberger *et al.* 2008), and recent data indicate that several isolates previously assigned to *A. terreus,* including clinical isolates causing aspergillosis, actually represent a separate species, *A. alabamensis* (Balajee *et al.* 2009). The last authors also indicated that *A. terreus* var. *aureus* should be recognised as distinct species, but they did not provide a formal description (Balajee *et al.* 2009).

In this study, we examined available isolates, which morphologically belong to section *Terrei,* to clarify the taxonomic

status of this section. We used the polyphasic approach including sequence analysis of parts of the β-tubulin and calmodulin genes and the ITS region, macro- and micromorphological analyses and examination of extrolite profiles of the isolates to clarify their taxonomic identity.

MATERIALS AND METHODS

Isolates

The fungi used in this study are listed in Table 1.

Morphological analysis

For macromorphological observations, Czapek Yeast Autolysate (CYA), Malt Extract (MEA) Agar, Yeast Extract Sucrose Agar (YES), Creatine Agar (CREA), and Oatmeal Agar (OA) were used (Samson *et al.* 2010). The isolates were inoculated at three points on each plate of each medium and incubated at 25 °C and 37 °C in the dark for 7 d. For micromorphological observations, microscopic mounts were made in lactic acid from MEA colonies and a drop of alcohol was added to remove air bubbles and excess conidia.

Extrolite analysis

The isolates were grown on CYA and YES at 25 °C for 7 d. Extrolites were extracted after incubation. Five plugs of each agar medium were taken and pooled together into same vial for extraction with 0.75 mL of a mixture of ethyl acetate / dichloromethane / methanol (3:2:1) (v/v/v) with 1 % (v/v) formic acid. The extracts were filtered and analysed by HPLC using alkylphenone retention indices and diode array UV-VIS detection as described by Frisvad & Thrane (1987, 1993), with minor modifications as described by Smedsgaard (1997). The column used was a 50 x 2 mm Luna C-18 (II) reversed phase column (Phenomenex, CA, USA) fitted with a 2 x 2 mm guard column.

Genotypic analysis

The cultures used for the molecular studies were grown on malt peptone (MP) broth using 1 % (w/v) of malt extract (Oxoid) 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. The ITS region and parts of the β-tubulin and calmodulin genes were amplified and sequenced as described previously (Varga *et al.* 2007a–c). Sequences have been deposited in GenBank under accession numbers FJ491703–FJ491731, and FJ531192–FJ531243.

Data analysis

The sequence data was optimised using the software package Seqman from DNAStar Inc. Sequence alignments were performed by MEGA v. 4.0 (Tamura *et al.* 2007) and improved manually. For parsimony analysis, the PAUP v. 4.0 software was used (Swofford 2002). 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 1 000 bootstrap replications (Hillis & Bull 1993). *Fennellia flavipes* NRRL 5504^T was chosen as outgroup in these analyses on the basis of prior studies (Peterson 2008).

RESULTS AND DISCUSSION

Phylogeny

Of the aligned β-tubulin sequences, a portion of 569 positions including 244 parsimony informative characters was selected for the analysis; MP analysis of the sequence data resulted in 46 679 similar, equally most parsimonious trees (tree length = 569 steps, consistency index = 0.7232, retention index = 0.9204), one of which is shown in Fig. 1. The calmodulin data set consisted of 764 characters including 299 parsimony informative sites; MP analysis resulted in 12 most parsimonious trees (length = 806, consistency index = 0.7122, retention index = 0.8704), one of which is presented in Fig. 2. The ITS data set consisted of 499 characters including 55 parsimony informative sites; MP analysis resulted in 36 equally most parsimonious trees (length = 102, consistency index = 0.8529, retention index = 0.9600), one of which is presented in Fig. 3.

Seven lineages were observed among isolates that have previously been treated as *A. terreus* and its subspecies by Raper & Fennell (1965) and others. *Aspergillus alabamensis, A. terreus* var. *floccosus*, *A. terreus* var. *africanus*, *A. terreus* var. *aureus* (*A. aureoterreus* according to Balajee *et al.* 2009), *A. hortai* and *A. terreus* NRRL 4017 all represent distinct lineages from the *A. terreus* clade based on phylogenetic analysis of calmodulin and β-tubulin sequences (Figs 1, 2). Among them, *A. terreus* var. *floccosus*, *A. terreus* NRRL 4017 and *A. aureoterreus* could also be distinguished from *A. terreus* by using ITS sequence data (Fig. 3). The *A. terreus* clade includes some other isolates which form well-defined subclades on the trees based on both β-tubulin and calmodulin sequence data. Further studies are needed to clarify if they represent separate species.

Also included in section *Terrei* are some species formerly placed in sections *Flavipedes* and *Versicolores*. A clade including the type isolate of *A. niveus* (CBS 115.27) constitutes a lineage closely related to *A. carneus*. *Fennellia nivea*, the hypothesised teleomorph is not related to this clade. *Aspergillus allahabadii, A. niveus* var. *indicus*, and two species originally placed in section *Versicolores*, *A. ambiguus* and *A. microcysticus* also form well-defined lineages on all trees (Figs 1–3).

Extrolites

Species in *Aspergillus* section *Terrei* are producers of a diverse array of secondary metabolites (Table 2). However, many of the species in the section produce different combinations of the following metabolites: acetylaranotin, asperphenamate, aspochalamins, aspulvinones, asteltoxin, asterric acid, asterriquinones, aszonalenins, atrovenetins, butyrolactones, citreoisocoumarins, citreoviridins, citrinins, decaturins, fulvic acid, geodins, gregatins, mevinolins, serantrypinone, terreic acid (only the precursor 3,6-dihydroxytoluquinone found), terreins,

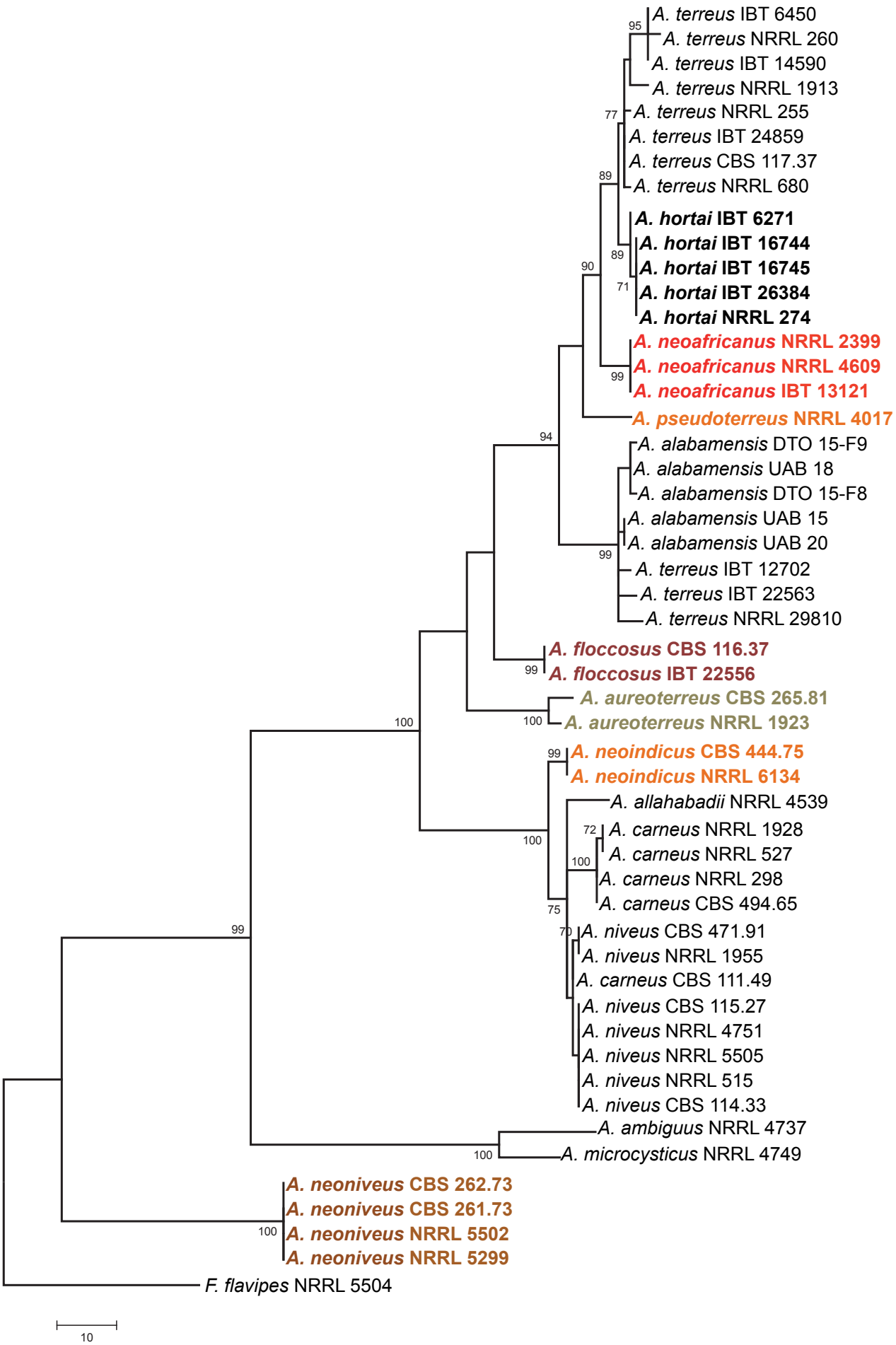

Fig. 1. The single MP tree obtained based on phylogenetic analysis of β-tubulin sequence data of *Aspergillus* section *Terrei*. Numbers above branches are bootstrap values. Only values above 70 % are indicated. *F.* = *Fennellia.*

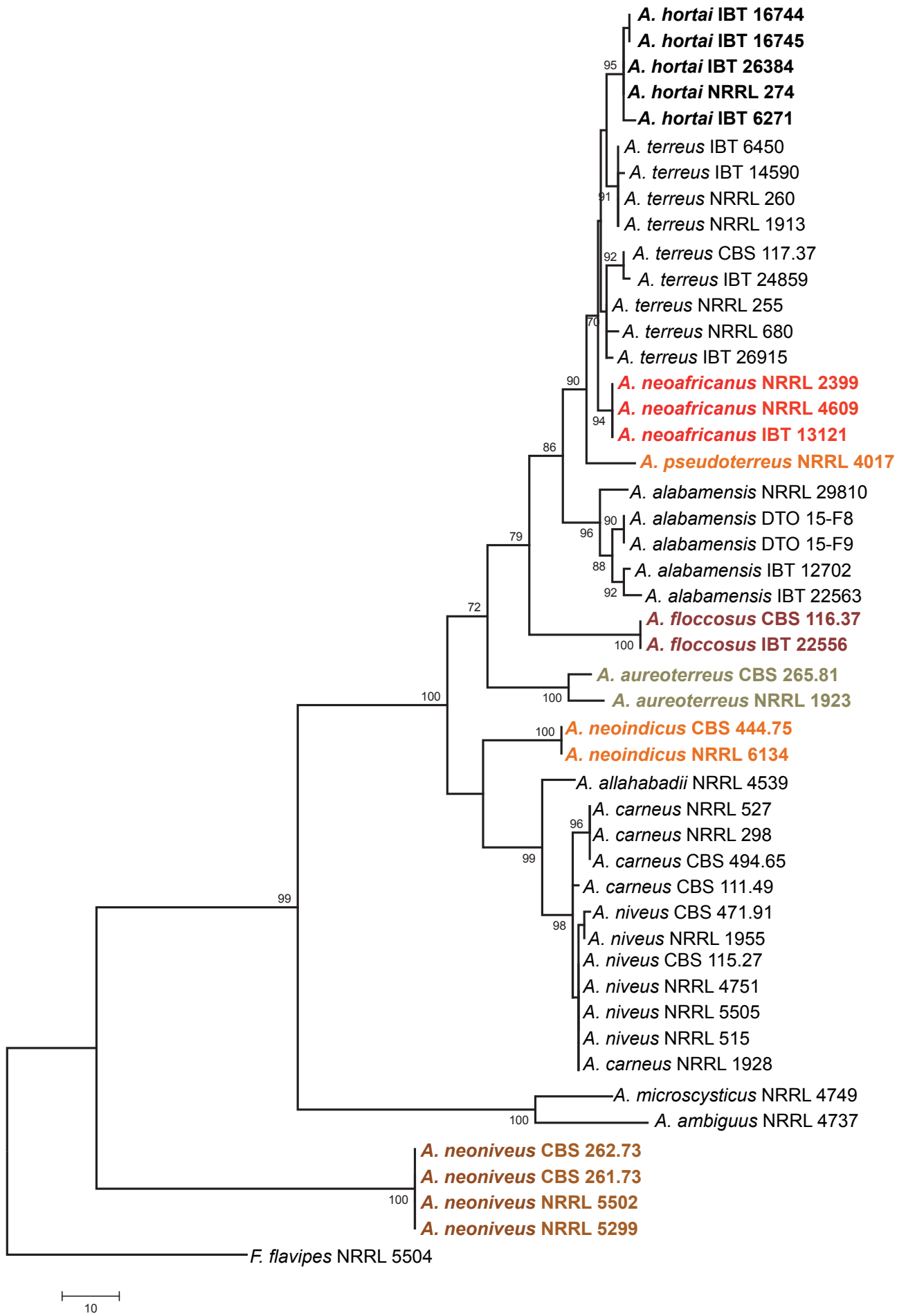

Fig. 2. One of the MP trees obtained based on phylogenetic analysis of calmodulin sequence data of *Aspergillus* section *Terrei*. Numbers above branches are bootstrap values. Only values above 70 % are indicated. *F.* = *Fennellia*.

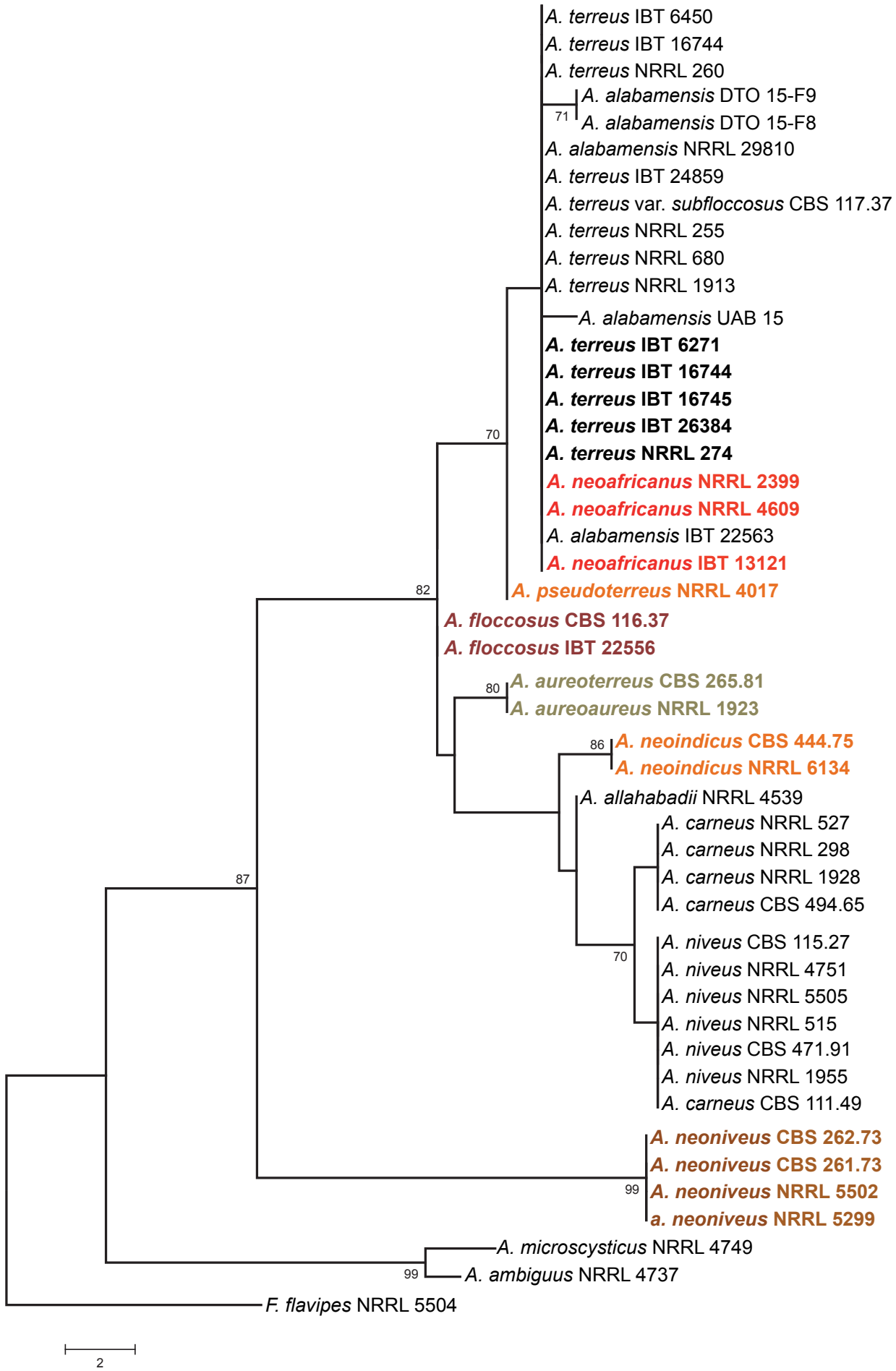

Fig. 3. One of the MP trees obtained based on phylogenetic analysis of ITS sequence data of *Aspergillus* section *Terrei*. Numbers above branches are bootstrap values. Only values above 70 % are indicated. *F.* = *Fennellia.*

Table 2. Extrolites produced by species in *Aspergillus* section *Terrei.*

Species	Strains examined	Extrolites
A. neoafricanus	CBS 130.55 = IBT 6266; ATCC 46560 = IFO 8835 = IBT 13121	Aspulvinone, asterriquinone, butyrolactones, citreoviridin, mevinolin, terrein, terrequinone A, (OSTO*). ATCC 46560 was reported to produce aspulvinones, asterriquinones, 3,6-dihydroxytoluquinone, emodin, α-oxo-β-(p-hydroxyphenyl)-γ-(p-hydroxy-m-3,3-dimethylallyl-benzyl)-γ-methoxycarbonyl-γ-butyrolactone = butyrolactone I, terrein (Kiriyama *et al.* 1977).
A. alabamensis	WB 1920 = IBT 22563; CDC UAB 28 = IBT 29083; IBT 29085 = CDC UAB1; 15-F8 = IBT 29084; 15-F9 = IBT 29086; IBT 12702; NRRL 28910 = IBT 29081	Asterriquinones, citrinin, terrein, terrequinone A, (NO2*) (in IBT 29084 = DTO 15-F8 additionally citreoviridin and a territrem). IBT 12702 produces asteltoxin, asterriquinones citrinin, fulvic acid, terrein, and NO2 and several unique extrolites only seen in this particular isolate.
A. allahabadii	IMI 139273 = IBT 23179; IBT 21128 = CCRC 32133	Asperphenamate, atrovenetins, butyrolactones, citrinin, gregatins, (SILKO*, ASPERGU*).
A. ambiguus	CBS 117.58^T	a butyrolactone, (ATROV*), terrequinone A.
A. auroterreus	CBS 503.6.5 = IBT 22090; IBT 23362	Citreoviridin, PR-toxin?, terrequinone A (AU*, AR*, AE*, SPA*).
A. carneus	NRRL 527. = IBT 13986; WB 298 = IBT 22569	Aszonalenin, asperphenamate, citrinin, dihydrocitrinone, gregatins.
		Reported in literature: the depsipeptides aspergillicins A-E, marcfortine A, acyl aszonalenin (Capon *et al.* 2003), citrinin, dihydrocitrinone and sclerin (Chien *et al.* 1977).
A. floccosus	CBS 116.37^T = IBT 10846 = WB 4872 = IBT 22556	Aszonalenin, austalides?, butyrolactones, citrinin, a decaturin, dihydrocitrinone, an isocoumarin, serantrypinone (NB*, NO22*, OSTO*, SOFL*).
A. hortai	NRRL 274^T = IBT 26384; IBT 16745; IBT 16744	Acetylaranotin, butyrolactones, citrinin, 3-methylorsellinic acid, terrein, terrequinone A.
A. microcysticus	IMI 139275 = IBT 23270 = CBS 120.58	Asperphenamate, butyrolactones, terrequinone A, (FUSI*, SORBA*, SOSTAI*, OLKA-1,2,3*); asposterol (Heberle *et al.* 1974), aspochalasins A-D (Kelle-Schierlein & Kupfer 1979).
A. neoindicus	CBS 444.75^T	Citrinin, naphthalic anhydride, atrovenetins, (SILKO).
A. niveus	CBS 115.27^T = IBT 10831, CBS 114.33 = IBT 19832; IMI 165060 = NRRL 1955 = IBT 13985 = CBS 471.91; IBT 16747; IBT 28598; IBT 28597; IBT 18418	Aszonalenine, butyrolactones, citrinin and gregatins, (SILKO*, SNOP*); CBS 115.27^T and CBS 114.33 only produce gregatins.
A. pseudoterreus	NRRL 4017 = IBT 29082 = 47-E6	Aspulvinones, asterriquinones, butyrolactones, citreoisocoumarin, citreoviridin, citrinin, 3-methylorsellinic acid, terrein, terrequinone A, (XANT*, AQ-1460*).
A. terreus	WB 255 = IBT 22562; CBS 601.65 = IBT 22089; IBT 24859; IBT 14590 = UAMH 4733; ATCC 20542; IBT 26974; IBT 6450; NRRL 1913 = IBT 26385; NRRL 260; CBS 117.37 = WB 4873 = IBT 22565	Acetylaranotin, aspulvinones, asterric acid, asterriquinones, aszonalenin (few strains), benzomalvins or related compounds, butyrolactones, citreoisocoumarin (in few strains), citreoviridin, 3,6-dihydroxytoluquinone (in some strains), erdin, geodin, geodoxin, 3-methylorsellinic acid, mevinolin, terrein, terrequinone A, terretonin (in some strains), territrems (in few strains), (GNOC*, GYAL*, SNIR*).
F. neonivea	IMI 171878 = NRRL 5299 = CBS 261.73^T	phenylahistin ?, paspalicine, an aspochalamin, many indol-alkaloids, (NB*); the cytochalasan derivatives aspochalamins A-D, aspochalasins D & Z, and citreoviridin A & B were isolated by Holtzel *et al.* (2004) and Gebhardt *et al.* (2004) from *A. niveus* LU 9575 (latter strain not available).
var. *curvatus*	CBS 265.81 = IBT 29947	Acyl aszonalenin, asperphenamate, terreic acid?

*Metabolites annotated with capitals had characteristic UV spectra, but their structure has not been elucidated as yet.

terrequinones, terretonins and territrems. The cholesterol-lowering agent mevinolin was found in *A. terreus* and *A. neoafricanus* only. The hepatotoxic extrolite citrinin was found in eight species: *A. alabamensis, A. allahabadii, A. carneus, A. floccosus, A. hortai, A. neoindicus, A. niveus* and *A. pseudoterreus*. The neurotoxic extrolite citreoviridin was found in five species: *A. neoafricanus, A. aureoterreus, A. pseudoterreus, A. terreus* and *Fennellia neonivea*. Territrems, tremorgenic extrolites, were found in some strains of *A. alabamensis* and *A. terreus*.

Species descriptions

Aspergillus aureoterreus Samson, S.W.Peterson, Frisvad & Varga, **stat. et nom. nov.** MycoBank MB560392. Fig. 5.
Basionym: *Aspergillus terreus* Thom var. *aureus* Thom & Raper – In A Manual of the Aspergilli: 198. 1945.

Type of *Aspergillus terreus* var. *aureus* from soil, Texas, USA.

This variety was proposed by Thom & Raper (1945) based on the slow growing colonies, which are bright yellow. It produces conidiophores tardily. As with the variety *africanus,* the ex-type isolate can be clearly distinguished based on our phylogenetic analysis. Therefore we propose to raise the taxon to species level.

Aspergillus floccosus Samson, S.W. Peterson, Frisvad & Varga, **comb. et stat. nov.** MycoBank MB560393. Fig. 6.
= *Aspergillus terreus* var. *floccosus* Shih, Lignan Sci Journal 15: 372. 1936.

Type of *Aspergillus terreus* var. *floccosus* from waste cloth, Wuchang, China, isolated by Y.K. Shih as No. A 369.

The ex-type culture shows white floccose colonies with some hyaline exudate on Czapek yeast agar. On MEA colonies are white with a light brown centre. Isolates of *A. terreus* may vary in colony morphology sometimes showing floccose colonies. The ex-type isolate of *A. terreus* var. *floccosus* CBS 116.37, which CBS directly received from Dr Shih, grouped in a distinct lineage, and therefore we propose to raise the variety to species level. Thom & Raper (1945) accepted the variety to accommodate colonies of *A. terreus* with strongly floccose colonies and conidial heads which are less compact and lighter in colour. Raper & Fennell (1965) however, synonymised var. *floccosus* under *A. terreus*, because

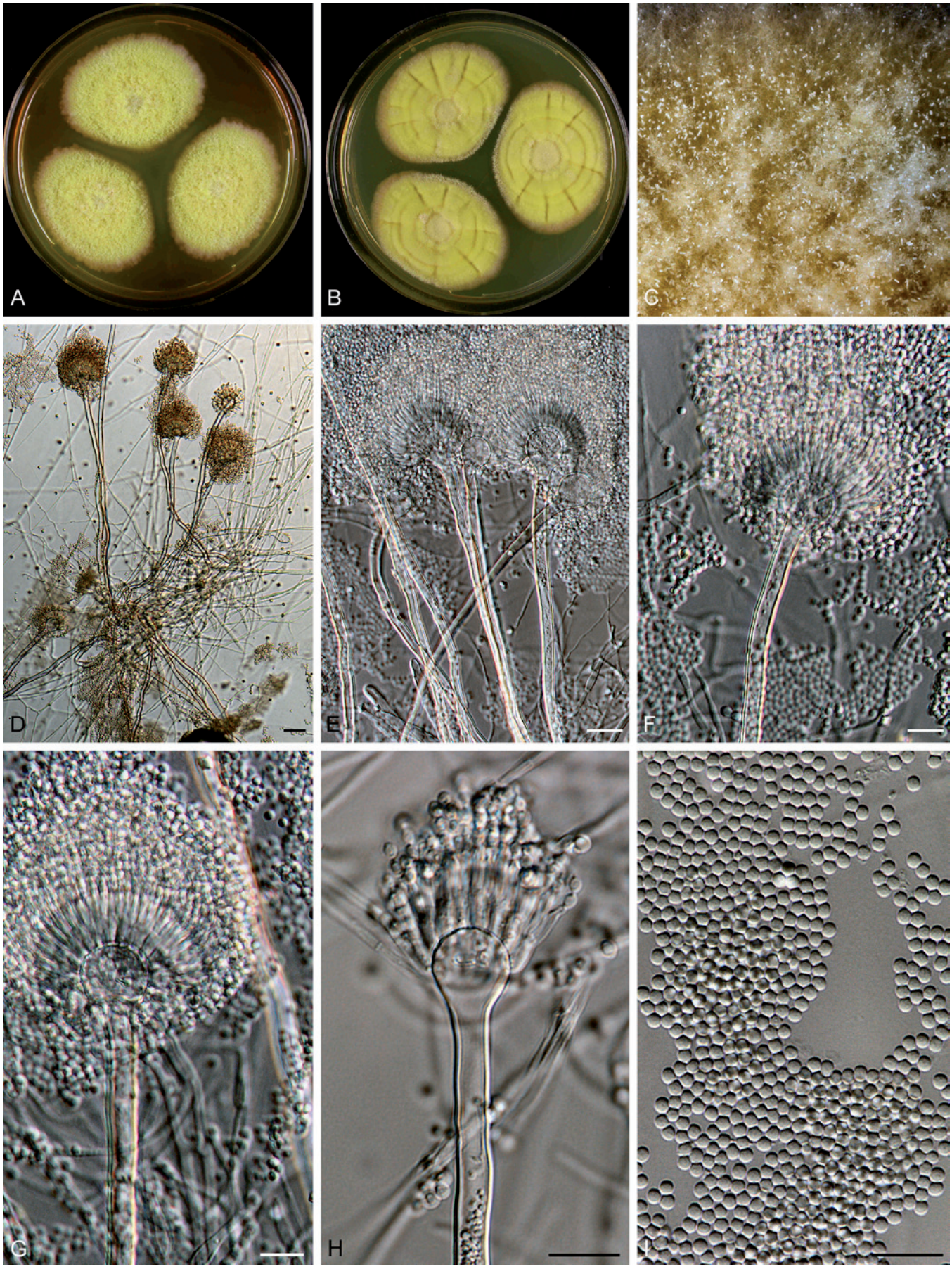

Fig. 4. *Aspergillus aureoterreus*. A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. CREA, D–I. Conidiophores and conidia. Scale bars = 10 µm.

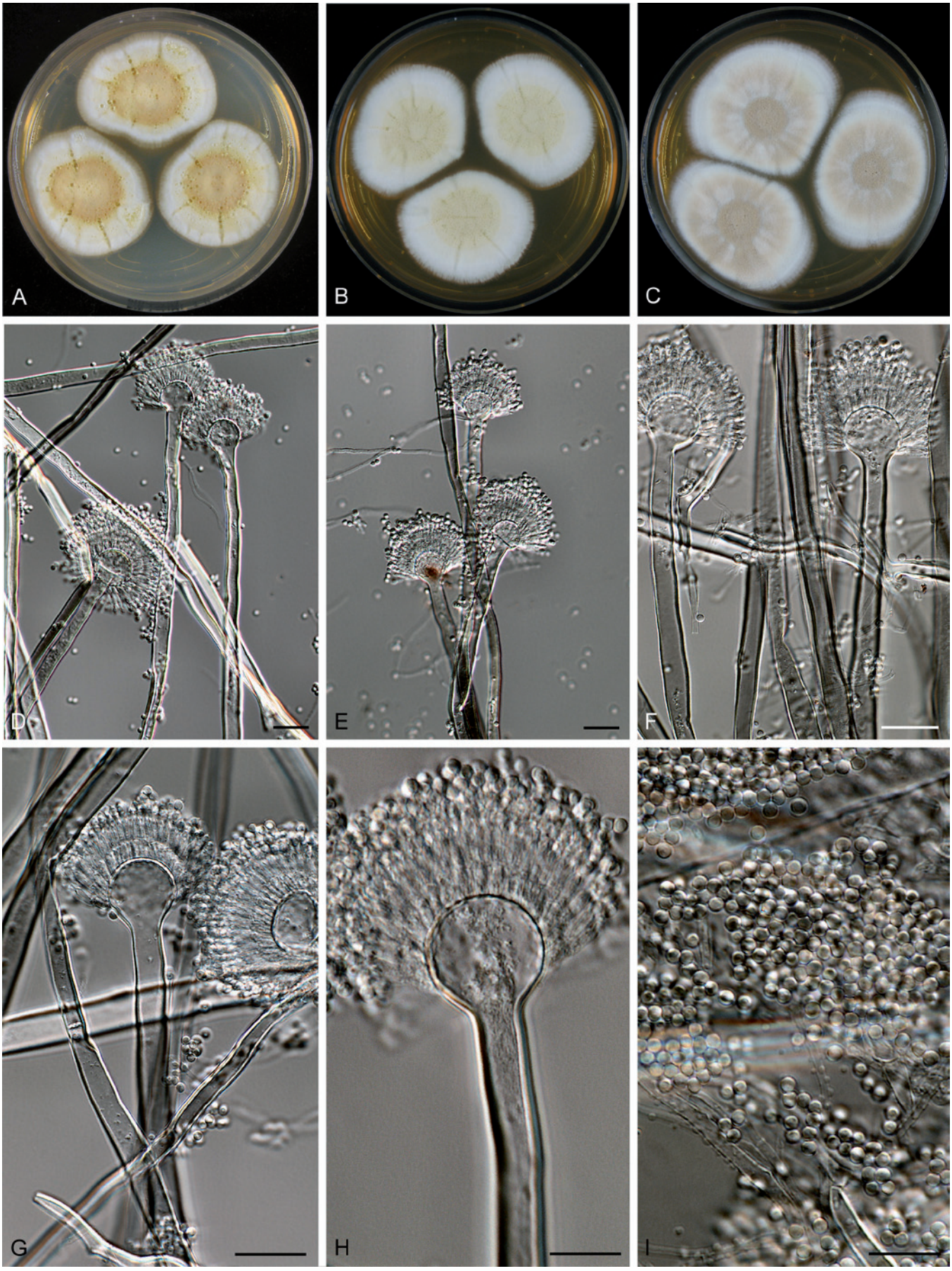

Fig. 5. *Aspergillus floccosus*. A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. CREA, D–I. Conidiophores and conidia. Scale bars = 10 µm.

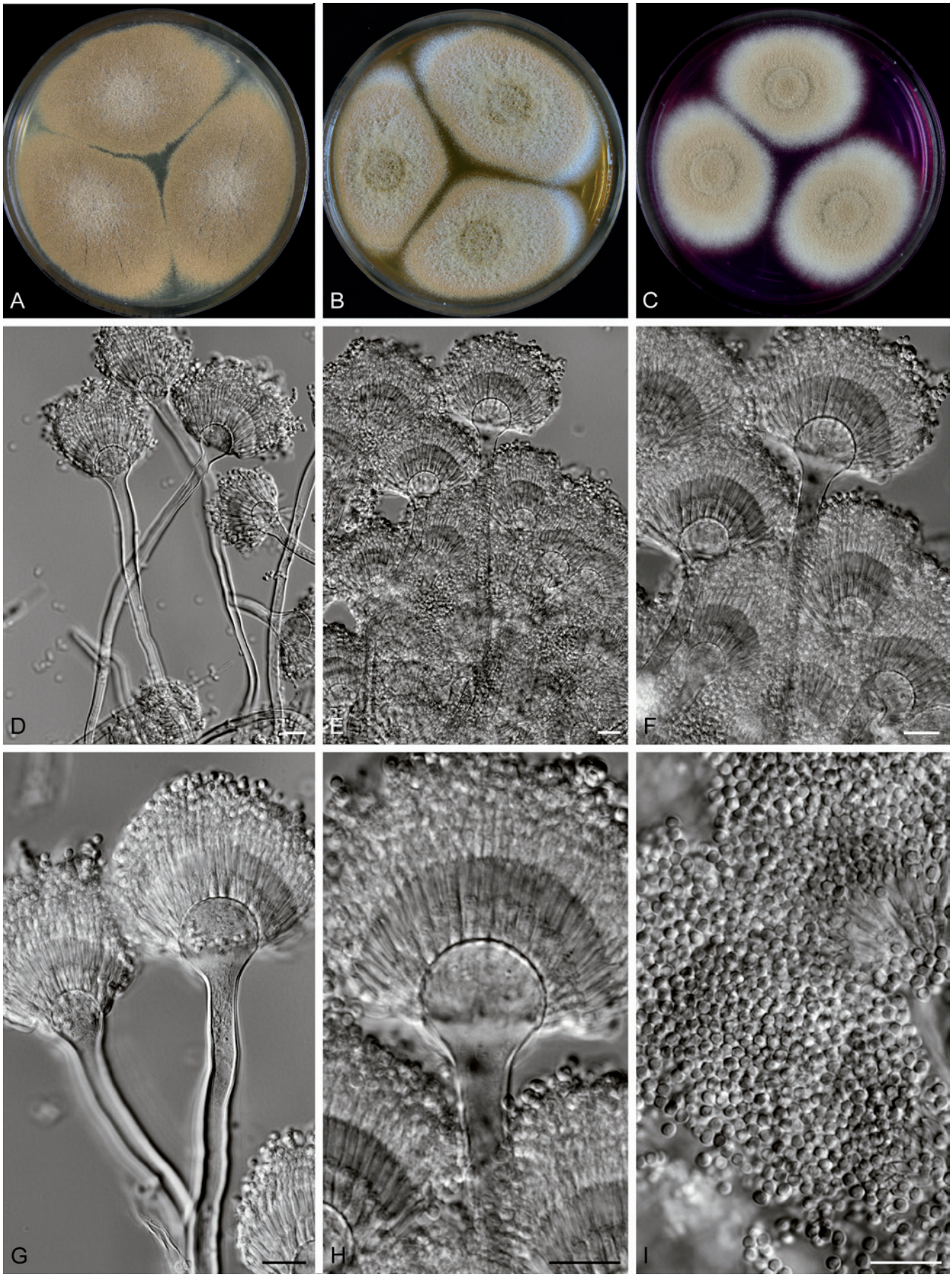

Fig. 6. *Aspergillus hortai*. A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. CREA, D–I. Conidiophores and conidia. Scale bars = 10 µm.

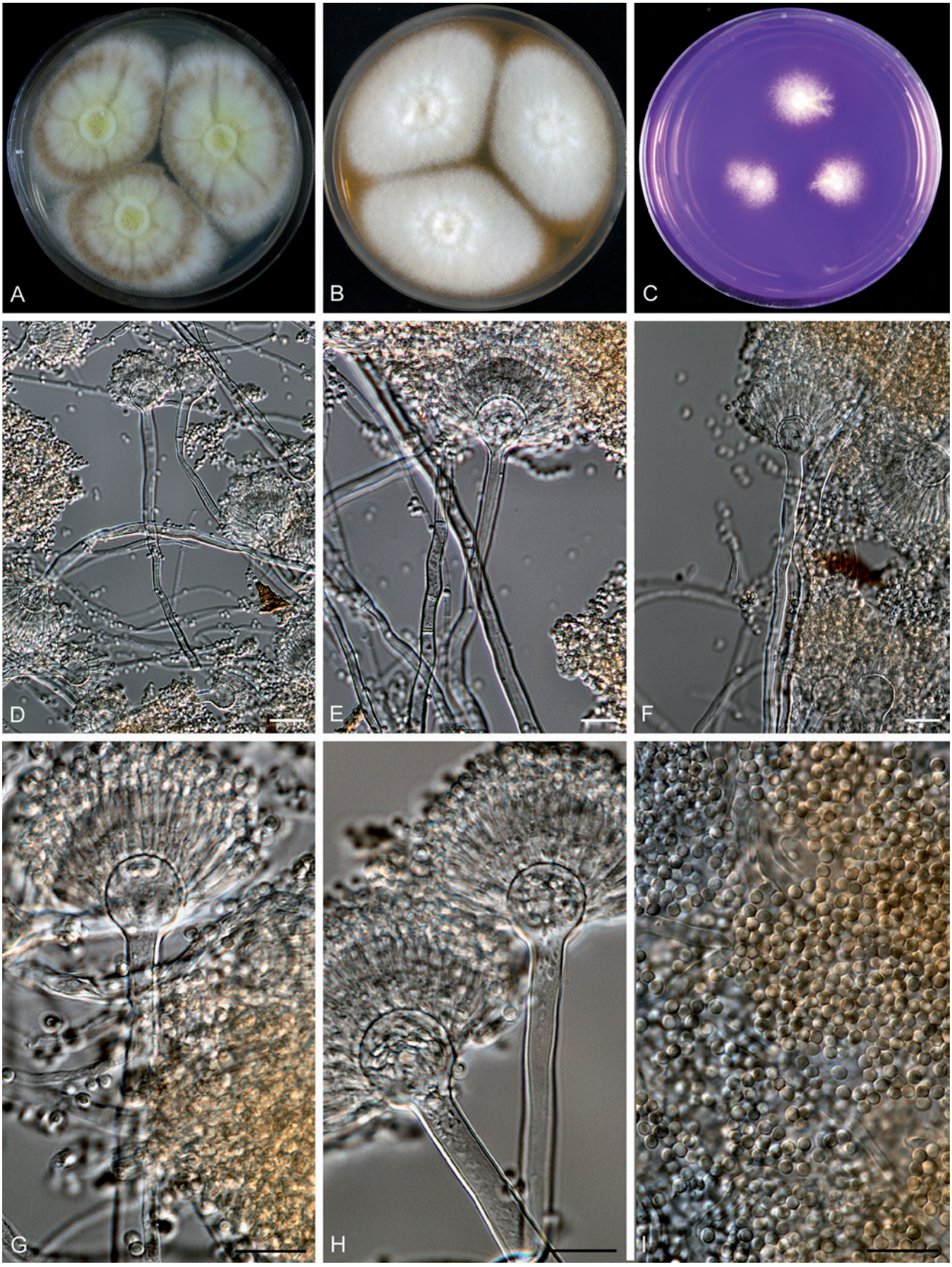

Fig. 7. *Aspergillus neoafricanus*. A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. CREA, D–I. Conidiophores and conidia. Scale bars = 10 µm.

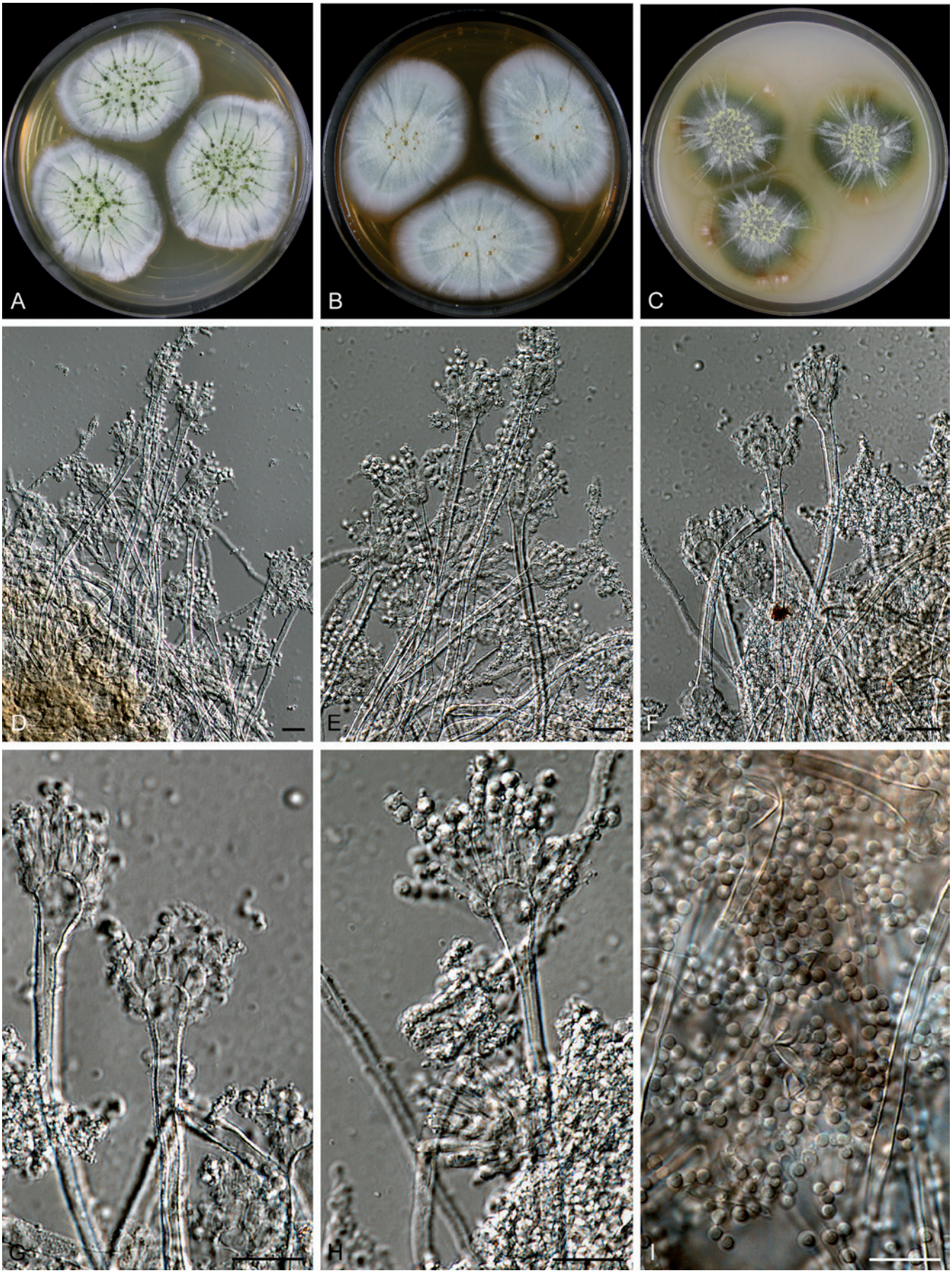

Fig. 8. *Aspergillus neoindicus*. A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. CREA, D–I. Conidiophores and conidia. Scale bars = 10 µm.

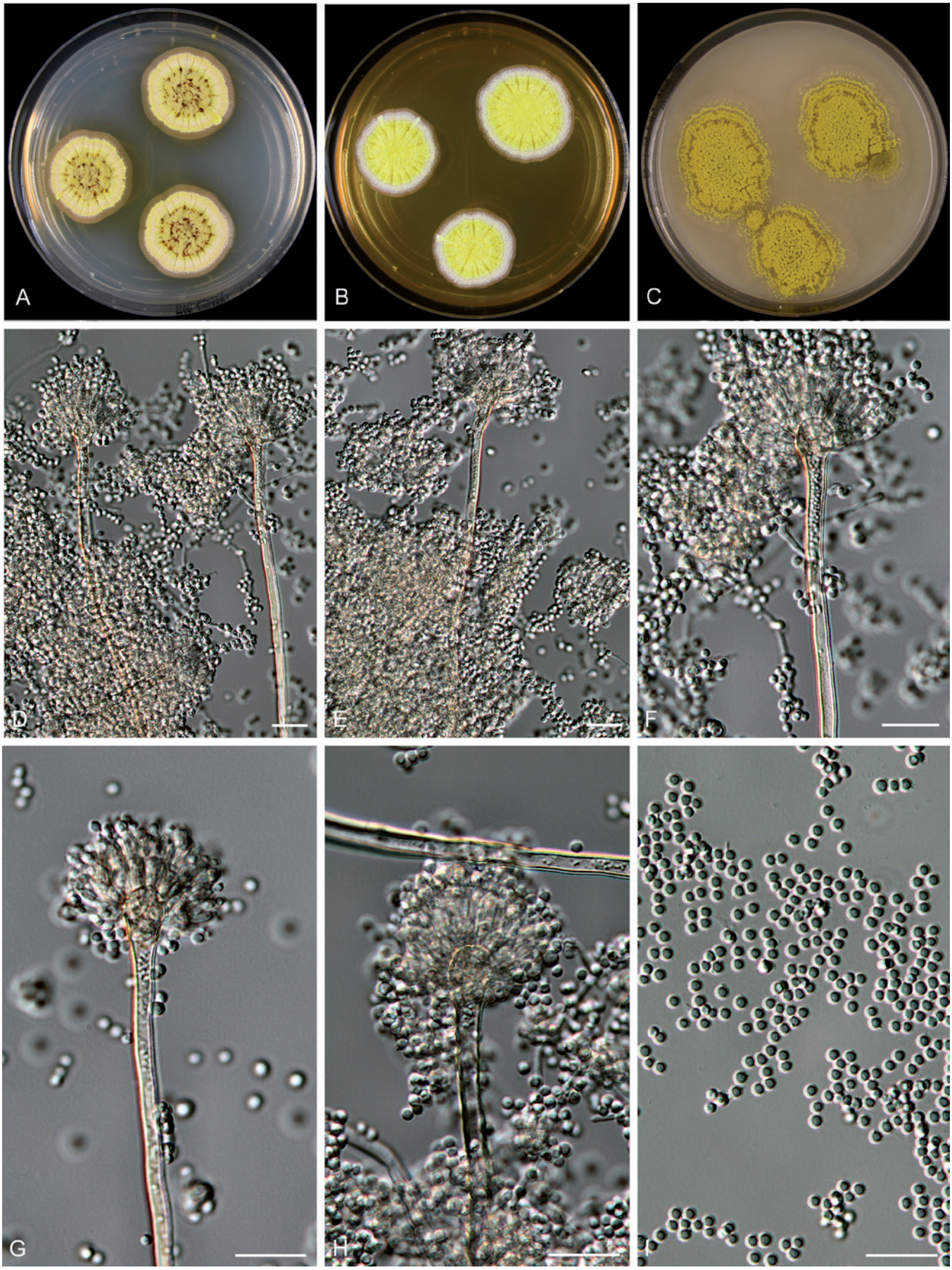

Fig. 9. *Aspergillus neoniveus* sp. nov. A–B. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. Crusts of Hülle cells, D–E, G–I. Conidiophores and conidia. F. Hülle cells. Scale bars = 10 µm, except F = 100 µm.

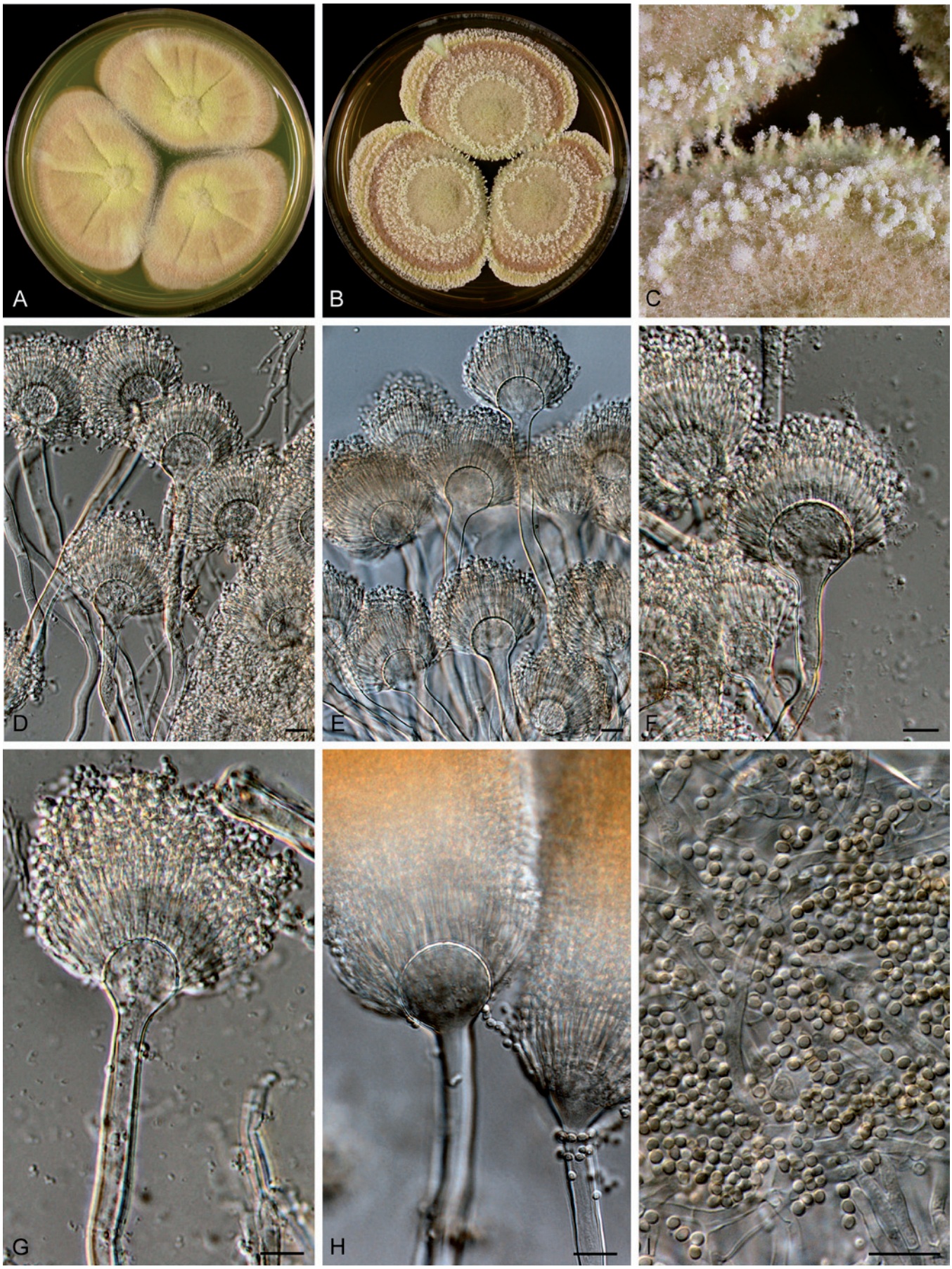

Fig. 10. *Aspergillus pseudoterreus*. A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. CREA, D–I. Conidiophores and conidia. Scale bars = 10 µm.

they examined the ex-type culture and believed that it belonged to *A. flavipes*. Interestingly they did not treat it as a synonym of this species, but left the name under *A. terreus.*

Aspergillus hortai (Langeron) Dodge, in Medical Mycology: 628. 1935. Fig. 7.
Basionym: *Sterigmatocystis hortai* Langeron, Bulletin Societé de Pathologie Exotique 15: 383. 1922.

Type of *Sterigmatocystis hortai* as clinical isolate, from ear, Rio de Janeiro, Brazil.

Langeron (1922) described this fungus from a human ear in Rio de Janeiro. Dodge (1935) noticed the resemblance of Langeron's fungus and transferred it to *Aspergillus*. However, Raper & Fennell (1965) considered it as a synonym of *Aspergillus terreus,* but our phylogenetic analysis clearly shows that it is distinct from *A. terreus* and therefore we accept it as a distinct species. *Aspergillus hortai* is known from the ex-type isolate and soil isolates from the Galapagos Islands and Florida (USA). The species show a strong morphological resemblance to *A. terreus*, but have a distinct extrolites profile.

Aspergillus neoafricanus Samson, S.W. Peterson, Frisvad & Varga, **comb. et stat. nov.** MycoBank MB560391. Fig. 4.
Basionym: *Aspergillus terreus* Thom var. *africanus* Fennell and Raper, Mycologia 47: 86. 1955.

Type of *Aspergillus terreus* var. *africanus* from soil, Tafo, Ghana.

The ex-type strain of *Aspergillus terreus* var. *africanus* is grouped in a distinct lineage from *Aspergillus terreus* and therefore we propose to raise the taxon to species level. Raper & Fennell (1965) considered this as a variety because they observed slow growing colonies on Czapek agar bright yellow vegetative mycelium. CBS 130.55 = NRRL 2399 derived from the type is now more or less floccose, with a yellow centre. Sporulation with yellow brown conidia occurs at the edges of the colonies. The degenerated condition of the culture also explains why we did not observe the sclerotium-like structures on malt extract agar + 20 % sucrose, which were reported by Raper & Fennell (1965).

Aspergillus neoindicus Samson, S.W. Peterson, Frisvad & Varga, **stat. et nom. nov.** MycoBank MB560394. Fig. 8.
Basionym: *Aspergillus niveus* var. *indicus* Lal & Sarbhoy, Indian Phytopathology 25: 309. 1973.

Type of *Aspergillus niveus* var. *indicus* from soil, Maharashta, India.

This species was described as a variety from soil in Maharastra by Lal & Sarbhoy (1973) and was considered by Samson (1979) as a synonym of *A. flavipes*. The species is phylogenetically distinct from *A. flavipes*, and is characterised by yellow green mycelial tufts. On OA a dark green pigment is diffusing into agar. The discrete masses of ellipsoidal and elongate Hülle cells described by Lal & Sarbhoy (1973) and Samson (1979) have not been observed in our current study.

Aspergillus neoniveus Samson, S.W. Peterson, Frisvad & Varga, **nom. nov.** MycoBank MB5603945. Fig. 9.
Basionym: *Emericella nivea* Wiley & Simmons, Mycologia 65: 934. 1973 (non *Aspergillus niveus* Blochwitz, 1929).
≡ *Fennellia nivea* (Wiley & Simmons) Samson, Stud. Mycol. 18: 5. 1979

Type from forest soil in Thailand.

Samson (1979) considered *Emericella nivea* distinct from the other *Emericella* species by the hyaline to pale yellow ascospores and the anamorph belonging to the *A. flavipes* group. The species is similar to *Fennellia flavipes* Wiley & Simmons and could be classified as the second species of *Fennellia.* However our phylogenetic analysis and those by Peterson (2008) and Peterson *et al*. (2008) showed that the isolates of *Emericella nivea* clustered separately from the isolates of *Aspergillus niveus* and hence it represents a different taxon. Following the need for an orderly transitition to a single-name nomenclatural system (Hawksworth *et al*. 2011) we have chosen to name this species in *Aspergillus* and not in its teleomorph genus, *Fennellia*.

Aspergillus pseudoterreus **S. W.** Peterson, Samson & Varga, **sp. nov.** MycoBank MB560396. Fig. 10.

Coloniis in agaro CYA aurantiaco-brunneis, caespitulis flavis usque ad 4 cm diam. Coloniis in agaro MEA cum marginibus clare aurantiacis. Conidiophoris aggregatis in synnematibus laxis, namque in coloniis vetustioribus (ad 14 d). Conidiophoris plerumque biseriatis, capitulis columnaribus efferentibus. Vesiculis globosis, 16–23 µm diam, stipitibus levibus, hyalinis, 4.5–7 µm latis. Conidiis levibus, hyalinis, globosis vel ellipsoideis, 1.5–2.2 × 1.8–2.5 µm.

Typus: from soil Argentina (CBS H-20631 -- holotypus, culture ex-type NRRL 4017).

Colonies on CYA orange brown with yellow tufts reaching a diameter of 4 cm. On MEA colonies are bright yellow with a clear orange edge. In older cultures of up to 14 d conidiophores are bundled in loose synnemata. Conidiophores typically biseriate, producing columnar heads. Vesicles globose 16–23 µm, stipe smooth, hyaline, 4.5–7 µm, conidia smooth, hyaline, globose to ellipsoidal, 1.5–2.2 × 1.8–2.5 µm.

This species was isolated from soil in Argentina and is characterised by a pronounced synnematal growth on MEA. The colony colour is reddish brown with biseriate conidiophores producing globose to ellipsoidal conidia.

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