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Emericella venezuelensis, a New Species With Stellate Ascospores Producing Sterigmatocystin and Aflatoxin B_1

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Summary

Emericella venezuelensis is a new species, differing from two other species with stellate ascospores, *E. variecolor* and *E. pluriseminata,* by triangular flaps on the convex sides of the ascospores, and further from *E. variecolor* by producing an *Aspergillus* anamorph only on unconventional growth media. The three species also differ in their profiles of extrolites (secondary metabolites). *Emericella venezuelensis* produces aflatoxin B_1, sterigmatocystin, and terrein and compounds with chromophores of the shamixanthone, emerin and desertorin type of compounds. *E. variecolor* produces asteltoxin, shamixanthone, asperthecin, and terrein, in addition to metabolites unequivocally recorded in the literature or tentatively identified here as astellolide A & B, andibenin A, B, C, andilesin A, B, C, anditomin, astellatol, stellatic acid, stellatin, tajixanthone, radixanthone, najamxanthone, ajamxanthone, variecoxanthone A, B, C, isoemericellin, kojic acid, varitriol, varioxiran, dihydroterrein, 7-hydroxyemodin, avariquinone and stromemycin. *E. pluriseminata* produces several unknown specific extrolites. *E. venezuelensis* is the first organism of marine origin reported to produce aflatoxin. Aflatoxin production by *E. venezuelensis* makes this species an attractive model organism for the study of the regulation of this important type of carcinogenic mycotoxins in combination with the knowledge on sterigmatocystin production by *E. nidulans*, soon to be whole genome sequenced. The isolates were also analyzed cladistically using partial sequences of the β-tubulin gene. Since three species of *Emericella* have stellate ascospores, and the type material of *E. variecolor* is equivocal, this species is epitypified with CBS 598.65. *Emericella* species normally do not appear to cause problems for food safety, as they are most often found in litter and soil.

Key words: Aflatoxin – *Emericella venezuelensis* – *Emericella variecolor* – extrolites – sterigmatocystin

Introduction

Emericella is a genus containing species of considerable interest because of the well elucidated genetics of *E. nidulans* [44] and because some species produce penicillin [8, 9, 19]. Other species of *Emericella* produce the mycotoxin sterigmatocystin [11, 13, 28], and are therefore of interest for elucidating the biosynthesis of this mycotoxin and especially the important carcinogenic mycotoxin aflatoxin B_1. On the other hand only one species of *Emericella* have been reliably reported to produce aflatoxins [16]. The genetics and molecular biology of the aflatoxin biosynthesis is quite well known in *Aspergillus* section *Flavi* and even within this section regulation of aflatoxin production is variable, indicating several new species exist within *A. flavus* and *A. nomius* [10]. Phylogenetically quite unrelated species with aspergilla such as those in *Emericella* [51] would be of value in elucidating the general biology and genetics of aflatoxin biosynthesis. We here describe a new species in *Emericella* that produces aflatoxin. This new species differs from any known species described so far [2, 3, 4, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 42, 47, 55, 57, 58, 67, 69, 70], and is the second *Emericella* species reported to accumulate aflatoxin B_1 [14, 16].

Since the new aflatoxin producing species has stellate ascospores we also wanted to compare the extrome (all outwards directed metabolites of an organism) of all species with stellate ascospores in *Emericella*. Although this species is hitherto only represented by a single isolate, the combination of characters makes it unique and its description is therefore justified.

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Materials and Methods

Sample collection

The strain CBS 868.97 was isolated from a sponge in red mangrove surface water in Mochima Bay, Venezuela, as described earlier [5]. The isolate was grown on Czapek yeast autolysate (CYA) agar, malt extract agar (MA & MEA), Yeast extract sucrose (YES) agar, oat meal agar (OAT), G25N [52] and creatine-sucrose (CREA) agar at 25 °C and on CYA at 37 °C (Medium compositions in [59]). The new species was compared to *E. variecolor* CBS 136.55, CBS 273.65, CBS 597.65, CBS 598.65 and CBS 668.82, *E. pluriseminata* CBS 100523 and CBS 102705 and ex type strains of all other available *Emericella* species.

Isolation and maintenance of strains for sequencing

All strains were isolated and/or grown on malt extract agar (Oxoid CM59) at 25 °C. 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.

Emericella variecolor (CBS 136.55, CBS 668.82, CBS 598.65 and CBS 273.65), *E. astellata* (CBS 261.93, CBS 135.55), *E. nidulans* (CBS 589.65, CBS 288.95, CBS 121.35, CBS 114.63), *E. acristata* (CBS 119.55) and *E. echinulata* (CBS 120.55) were sequenced using the β-tubulin gene and compared to the new species *E. venezuelensis* (CBS 868.97) within the same region (all from Centraalbureau voor Schimmelcultures).

DNA Extraction, sequencing and analysis

The total fungal genomic DNA was isolated using FastDNA® Kit (Bio 101, Carlsbad, USA) according to the manufacturer's instructions. Amplification of the β-tubulin gene was performed using the primers Bt2a and Bt2b [20]. 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 [71] 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 1 µl of template DNA (10–15 ng/µl), 4 µl Dye terminator RR mix, 4 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 Yssel, 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 3700 Genetic Analyzer (AB Applied Biosystems, Nieuwerkerk a/d Yssel, The Netherlands). A concensus 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) version 4.0b10 [68]. Alignment gaps were treated as fifth character state, missing data were identified by '?', uninformative characters were excluded and all characters were unordered and equal-

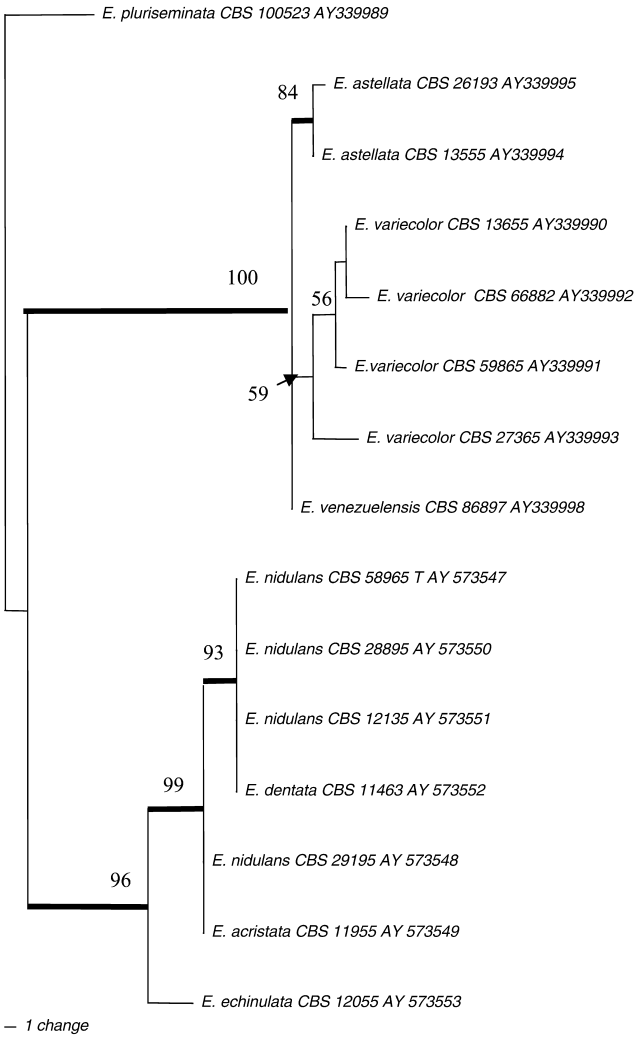

Fig. 1. Cladogram of *Emericella* species related to *E. venezuelensis* based on partial β-tubulin sequences. One of the 7 trees of 72 steps based on heuristic search partial β-tubulin sequences with *E. pluriseminata* as outgroup. The branches in bold are 100% in the 70% majority-rule consensus of equally parsimonious trees (CI = 0.889, Ri 0.966, Rc 0.859, Hi 0.111). The numbers represent bootstrap percentages >50%.

ly weighted. 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 [23]. Other measures including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC) were also calculated. The GenBank accession numbers for the β-tubulin sequences of the CBS strains are: AY339987, AY339988, AY339989, AY339990, AY339991, AY339992, AY339993, AY339994, AY339995, AY339996, AY339997, AY339998, AY339999, AY340000 (Fig. 1).

Chemical analysis

The cultures were analyzed according the HPLC-diode array detection method of Frisvad and Thrane [17,18] as modified by Smedsgaard [66]. The isolates were analyzed on CYA and YES agar using three agar plugs [66]. The production of aflatoxin on YES agar was confirmed by identical UV spectra and by HPLC-MS using electrospray ionization (Hewlett Packard HP 1100 LC/MSD instrument). An M+1 ion at 313 confirmed the presence of aflatoxin B_1 and the M+1 ion at 325 confirmed sterigmatocystin production. Aflatoxin production was confirmed by TLC analysis using the agar plug method, the TLC plates were eluted in toluene/ethyl acetate/formic acid (6:3:1) and chloroform/acetone/2-propanol (85:15:20) [12, 59]. Aflatoxin B_1, B_2, G_1, G_2 and sterigmatocystin (Sigma) were used as analytical standards. Standards of asperthecin, desertorin A, B and C and asteltoxin, known to be produced by *Emericella variecolor*, were also used to confirm the identity of those compounds.

The production of aflatoxin B_1 and sterigmatocystin was examined five independent times on YES and CYA agars at 25 °C using three agar plugs as recommended by Smedsgaard [66]. In one case other media were also examined for their stimulation of aflatoxin production: Czapek agar added trace metals and 3.2 ml Sigma R 7131 amino acid solution (CzAA), MEA and OAT. Aflatoxin B_1 was recorded using diode array detection at 210 nm and confirmed by its characteristic UV spectrum and the amount expressed as relative mAU (milli absorbtion units).

The production of aflatoxins was also confirmed by LC high resolution mass spectrometry. LC-MS analyses were done on an Agilent HP 1100 Liquid Chromatograph with a diode array detector (DAD) coupled to a LCT TOF (time of flight) mass spectrometer (Micromass, Manchester, UK) with a Z-spray electro spray source (ESI) and a LockSpray reference spray. The analysis was done on a HP Hypersil BDS-C18 column with 3 µm particles, 125 × 2 mm column with a 10 × 2 mm HP Supersphere 100 RP-18 guard column an (Waldbronn, Germany) at a flow rate of 0.3 ml/min water-acetonitrile gradient, starting with 15% acetonitrile-water going to 100% acetonitrile in 35 min, maintaining 100% acetonitrile for 5 min, before returning to the start conditions in 8 min 50 ppm (V/V) TFA was added to the water. UV spectra was collected by diode array detector every 0.4 sec from 200 to 700 nm with a resolution of 4 nm. The MS was operated in the positive ESI mode and tuned to a resolution >6000 (a half peak height) and maximal sensitivity. The mass spectrometer was calibrated from 100 to 900 Da/e on a PEG mixture. The capillary was held at 3000 V, sample cone at 35V and the extraction cone at 6 V. Desolvation temperature was 350 °C and desolvation gasflow at approx 450 L/hr and the source kept at 120 °C. Leucine-enkephalin solution (0.2 µg/ml in acetonitrile-water-formic acid (50:50:0.1)) was infused as a mass reference though the LockSpray probe at a 4 µl/min from a syringe pump. The $[M+H]^+$ ion at 556.2771 Da/e from leucine-enkephalin was used as mass reference. Data was collected as centroid data from m/z 100 to 900, with a scan time of 1 sec and an inter scan time of 0.1 sec, collecting every third scan as a reference [49].

Results and Discussion

Morphology and cladistic analysis of β-tubulin sequencing data

E. variecolor, *E. pluriseminata* and *E. venezuelensis* all produced stellate ascospores in contrast to any other species in *Emericella*. *E. variecolor* readily produced aspergilla, while production of aspergilla in *E. venezuelensis* was restricted to G25N and CREA agar. *E. pluriseminata* did not produce aspergilla on any media. All three species produced globose Hülle cells.

Maximum parsimony analysis of the sequence data resulted in 7 equally most parsimonious trees (TL = 72 steps, CI = 0.889, RI = 0.966, RC = 0.859), one of which is shown in Figure 1. The cladogram was rooted to *E. pluriseminata*. Bootstrap supports from 1000 replicates are shown at the nodes and are all quite high, except for *E. variecolor*.

The β-tubulin sequences suggest that *E. venezuelensis* is very closely related to *E. variecolor* and *E. astellata* (2–5 changes). This group is different from other species of *Emericella* as shown in the tree (cladogram) where *E. nidulans* and related species such as *E. dentata* and *E. acristata* appear to be closely related, but distantly related to the species with wide equatorial appendices whether stellate (*E. venezuelensis, E. variecolor*) or astellate (*E. astellata*), whereas the third stellate species, *E. pluriseminata*, is basal to the two former clades.

Production of mycotoxins and other extrolites by species with stellate ascospores

The culture ex type of *Emericella venezuelensis* produced aflatoxin B_1, sterigmatocystin, shamixanthones and a compound with an emerin chromophore. The production of aflatoxin B_1 and sterigmatocystin was confirmed by identical UV spectra, retention times and indices and by high resolution mass spectrometry as compared to standards. Apart for being phenotypically different, the two other species with stellate ascospores produced other profiles of extrolites (Table 1). *E. variecolor* and *E. pluriseminata* are among the few *Emericella* not producing sterigmatocystin or later biosynthetic derivatives, but the two latter species also differ in their ability to produce several other extrolites from *E. venezuelensis* (Table 1). Even though *E. venezuelensis, E. variecolor* and *E. astellata* differ in the production of a number of extrolites, they do share the following metabolites: asperthecin, shamixanthones, and terrein. Asperthecin and shamixanthone are also common in many other species of *Emericella*.

Strains of *E. variecolor* characteristically produced the following extrolites: asteltoxin, astellolide B, varitriols, shamixanthone, asperthecin and terrein. The same profile of extrolites was produced by CBS 598.65, CBS 668.82, CBS 136.55 & IMI 146289 (terrein could not be detected, however, in these latter two strains), IBT 12228, IBT 20986, and IBT 20997. UV spectra indicated that these strains of *E. variecolor* also produced desertorins,

Table 1. Production of extrolites by *Emericella* species with stellate ascospores: *E. variecolor*, *E. venezuelensis,* and *E. pluriseminata*.

Species	Secondary metabolites
E. variecolor:	Asteltoxin* [43] Asperthecin* [35, 48] Kojic acid and terrein* [54] Desertorins A, B and C, siderin [40, 50 for *E. desertorum*] Anditomin [65] Astellolide B** [21] Astellatol [56, 63] Stellatic acid [53] Stellatin [61] Andilesin A, B, C [62, 64] Andebenin A, B, C [62, 64] Shamixanthones: Shamixanthone*, tajixanthone, radixanthone, shahemxanthone, najamxanthome, ajamxanthone, variecoxanthone A, B, C, varixanthone, isoemericellin [1, 36, 37, 38, 39, 46] Varitriol* & varioxiran [46] Dihydroterrein & terrein* [46] 7-hydroxyemodin** & avariquinone [1] Stromemycin [1]
E. pluriseminata:	Several unusual unknown extrolites*
E. venezuelensis:	Aflatoxin B_1* Sterigmatocystin* Terrein* Compound with emerin chromophore** Compound with desertorin chromophore* Compounds with shamixanthone chromophore**

*Confirmed or detected using HPLC-DAD and authentic standards. ** Indicated by UV spectra similar to those from the literature.

kotanins, andibenin, andilesin, varioxiran and 7-hydroxyemodin. More detailed chemical analysis will show whether these latter compounds and the other compounds listed in Table 1 from *E. variecolor* are also produced by these strains. Asteltoxin appears to be a good chemotaxonomical marker for this particular species. Sterigmatocystin and B type aflatoxins have not been found in any of the strains of *E. variecolor* listed above. One strain, CBS 597.65, did produce traces of sterigmatocystin in addition to the characteristic profile of *E. variecolor*, however. It is therefore possible that the other strains will also have the genes required for sterigmatocystin production, even though they are not expressed phenotypically on the media and growth conditions used in this study.

Although species in the genus *Emericella* and related species are morphologically and chemically quite diverse, they are united by the production of aspergilla with pigmented stipes, rounded Hülle cells, presence of ascomata with orange-red to blue-violet pigmentation [55]. In a phylogenetic sense *Emericella* include *Aspergillus* subgenus *Nidulantes* sections *Nidulantes, Versicolores* and *Usti* [51]. Available phenotypic data support the relatedness of the sections in subgenus *Nidulantes,* including the production of Hülle cells and sterigmatocystin by many species [6, 13, 28, 55]. Species in subgenus *Nidulantes* either only have the *Aspergillus* anamorph, only the teleomorph or both morphs. Sterigmatocystin production has until now only been found in species with either the *Aspergillus* anamorph or both the *Aspergillus* and perfect state. Until now aflatoxin has not been reported in any species of *Emericella*, except some early and poorly substantiated reports that *E. rugulosa* [60] and *E. nidulans* [22] produced aflatoxins. This is the second substantiated report of an *Emericella* species producing aflatoxin. Aflatoxin production by *E. venezuelensis* was mentioned by Frisvad et al. in 1999, however [14]. *E. astellata* has previously been reported to produce aflatoxin B_1 and B_2 [16]. The strains of *E. venezuelensis* and *E. astellata* can thus be used in studies on the genetics and molecular biology of aflatoxin production as a third phylogenetic group which contains aflatoxin producers. The other two being *Petromyces*, with anamorphs in *Aspergillus* section *Flavi* [15] and the new *Aspergillus* section *Ochraceorosei* [Frisvad et al., in prep.] with no known connections to a named ascomycete genus. *Emericella* species are especially suited for genetic studies [44] because *E. nidulans* will be one of the first filamentous fungi to be full genome sequenced. The new species may be studied in order to find out why most species in the genus *Emericella* only accumulate sterigmatocystin, while a few have the ability to produce aflatoxin. The new species does not produce any of the other known extrolites in *Aspergillus* section *Flavi*, such a kojic acid, cyclopiazonic acid, aflatrem, aspernomine etc. and it only produces aflatoxin B_1 but none of the G type aflatoxins or parasiticol. In this respect *E. venezuelensis* resemble *A. ochraceoroseus*, which also produce both sterigmatocystin and aflatoxin B_1 [14, 41]. *A. ochraceoroseus* does not produce any other extrolites known from *Emericella*, however.

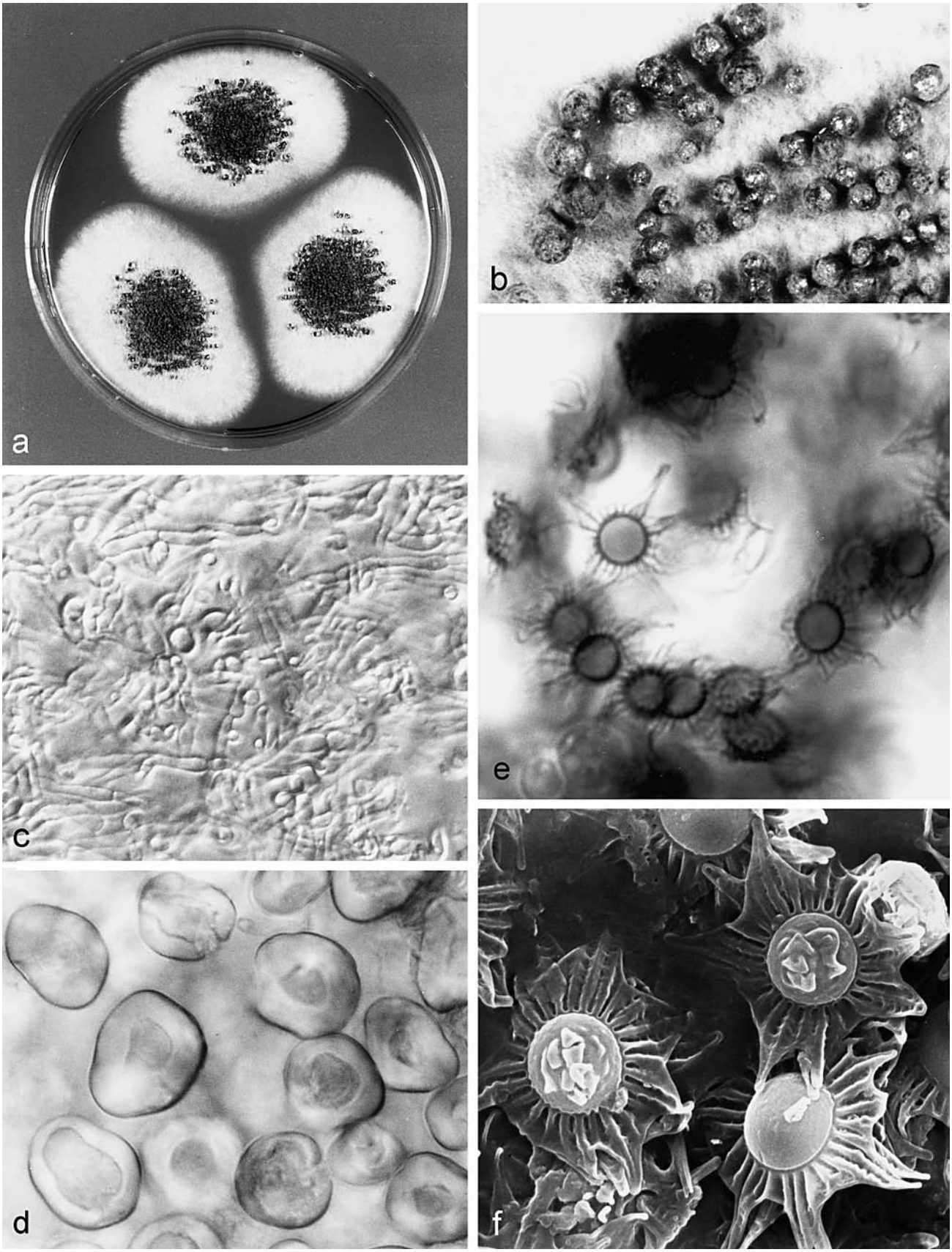

Fig. 2. **a.** Colonies of *Emericella venezuelensis* on CYA agar after 1 week at 25 °C. **b.** ascomata (100×), **c.** detail of the ascoma wall (400×), **d.** Hülle cells (1000×), **e.** stellate ascospores (1000×), **f.** Scanning electron micrograph of ascospores showing the triangular flaps on the convex sides (2000×).

Influence of growth media on aflatoxin and sterigmatocystin production

In the first experiment both aflatoxin B_1 and sterigmatocystin were produced, but with three times more aflatoxin B_1 than sterigmatocystin in relative peak height measurements on YES agar. In the next two experiments aflatoxin B_1 could be detected on both YES and CYA agar (2–33 mAU), but sterigmatocystin could not. In the fourth experiment aflatoxin B_1 was again detected on both YES and CYA (7–8 mAU), with no detection of sterigmatocystin. On MEA a trace of aflatoxin B_1 was formed but none on OAT. Large amounts of terrein were produced on MEA only. On CzAA on the other hand, large amounts of aflatoxin B_1 was produced (120 mAU) and also some sterigmatocystin (8 mAU). In the last experiment, the IBT strain 20956 had a sector with copious production of yellow pigment. After having recovered both the original *E. venezuelensis* type (IBT 24595) and the yellow type (IBT 24596), aflatoxin B_1 (and no sterigmatocystin) was recovered only on YES agar in IBT 24595. The yellow strain did not produce any aflatoxin or sterigmatocystin. In general *E. venezuelensis* consistently produces relatively more aflatoxin than sterigmatocystin on YES (and CYA) agar, but apparently no aflatoxin on oatmeal agar. This is in contrast to the other *Emericella* species producing aflatoxin B_1, *E. astellata* [16], which always produces most aflatoxin on oatmeal agar and approximately relative equal amounts of aflatoxin B_1 and sterigmatocystin. The regulation of aflatoxin production thus seems to be quite different in these two *Emericella* species. Both *Emericella* species are different from

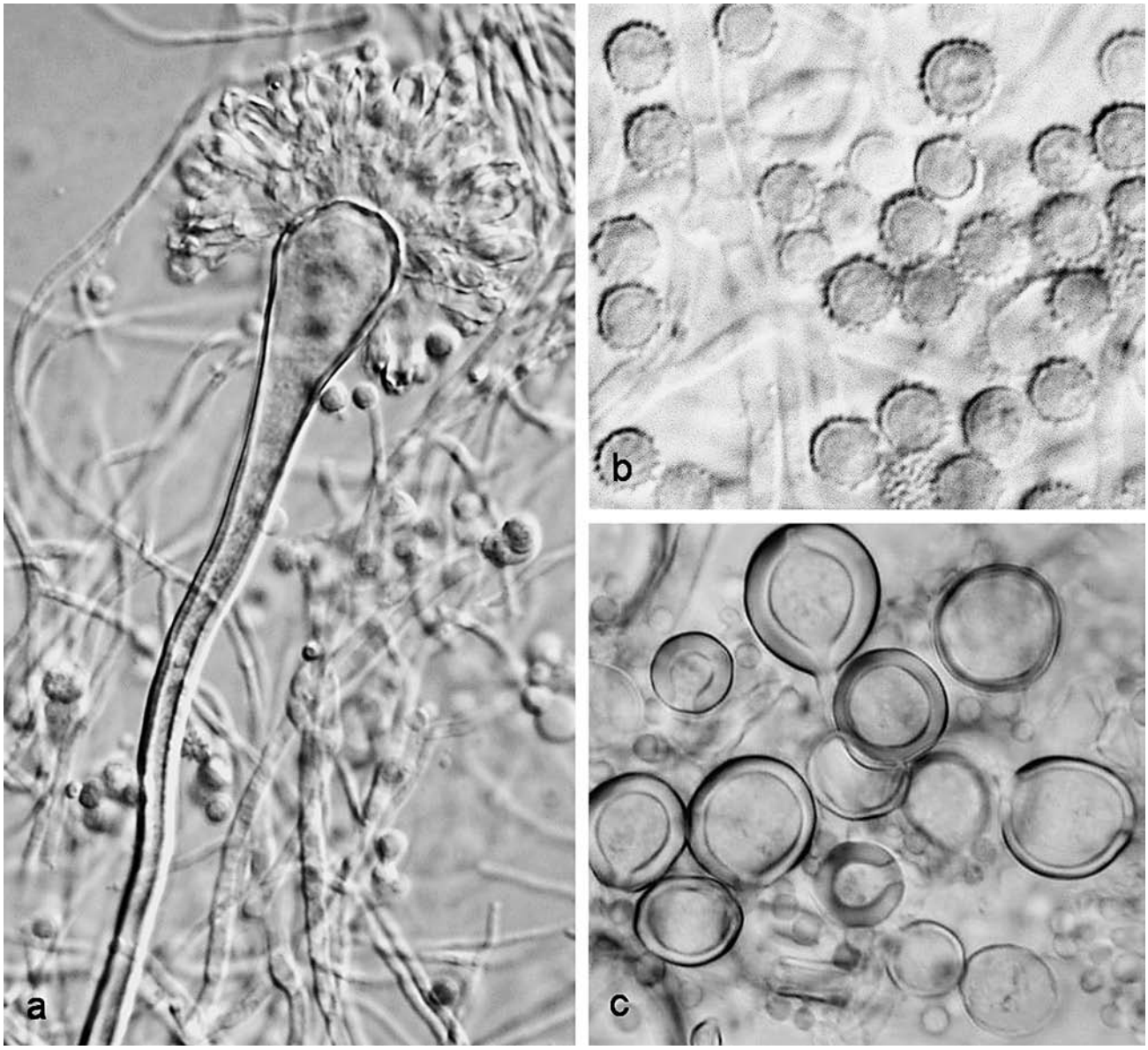

Fig. 3. Conidiophore of *Aspergillus venezuelensis*, **a.** conidiophore (1000×), **b.** conidia (2000×), **c.** Hülle cells (1000×).

members of *Aspergillus* section *Flavi* members. Species in the latter group do not accumulate sterigmatocystin, but aflatoxins and 3-methoxysterigmatocystin [14, 15, 41]. *E. venezuelensis* and *E. astellata* only produce rather small amounts of aflatoxin, and as they are not found in foods, they are not important regarding food safety. *E. venezuelensis* is the first marine micro organisms reported to produce any aflatoxins. The two other exotic aflatoxin producers (*A. ochraceoroseus*, *E. astellata*) have been isolated from the phylloplane in tropical regions. The known producers of aflatoxins, *A. flavus, A. parasiticus, A. pseudotamaii, A. nomius, A. toxicarius* and *A. bombycis* [15] are mostly found on domesticated cereals and other domesticated plants.

Taxonomic position

Emericella venezuelensis differs from *E. pluriseminata* by its ability to produce an *Aspergillus* anamorph but resembles *E. variecolor* and *E. pluriseminata* by its stellate ascospores. This is also shown in the cladogram with the partial β-tubulin sequences of taxa with stellate ascospores (Fig. 1). However it differs from all those species by the ascospore ornamentation (Fig. 2 d and e) and by the profiles of extrolites (Table 1). *E. venezuelensis* did not produce the *Aspergillus* anamorph on Czapek agar, CYA, YES or OAT, in contrast to most known species in *Emericella*, but aspergilla were produced on creatine sucrose agar, CYA + 40% sucrose and G25N.

Description of *Emericella venezuelensis* Frisvad & Samson sp. nov. (Fig. 2–3) (Stat. Anam. Aspergillus venezuelensis Frisvad & Samson, stat. nov.)

Coloniae in agaro CYA „Hülle“ cellulis abundantibus formantes, aurantio-brunneae, reversum aurantio-brunneum. Ascomata superficialia, non ostiolatae, brunnea, globosa, 100–300 µm diam, cum „Hülle“ cellulis numerosis, globosis vel irregularis, crassitunicatis, 10–20 µm diam, circumdata. Peridium 4–12 µm crassum, aurantiobrunneum. Asci 8-spori globosi vel elliopsoidei, 20–33 µm diam. Ascosporae unicellulares, brunneo-violaceae, lenticulares, 7–9 × 6–7 µm, cristis aequatorialibus duabus stelliformis praeditae, parte convexa con appendices triangularis ornatae. Capitula conidica viridia, radianta vel brevi-columnaria. Stipites conidiophorae, brunnea, 70–200 × 3–4.5 µm, vesiculae 7–10 µm, metulae 4.5–7 × 1.5–2 µm, phialides 5–7 × 1.5–2 µm. Conidia flavo-virida, globosa, 3–4 µm diam., spinulosa. Aflatoxinum B_1 et sterigmatocystinum producuntur.

Holotypus Herb. CBS 868.97.

Cleistothecia abundant, formed on the surface of the growth medium, spherical, brown, 100–300 µm diam., surrounded by numerous Hülle cells, globose to somewhat irregular, 10–20 µm diam., Cleistothecial peridium 4–12 µm, yellow brown. Asci 8-spored, spherical to subspherical, 20–33 µm diam., ascospores one celled, violet brown, lens-shaped, 7–9 × 6–7 µm, with two star-shaped equatorial crests and the convex sides covered with triangular flaps. The *Aspergillus* anamorph was observed on CREA agar, CYA + 40% sucrose and G25N. The conidiophores are brown to yellow brown and rather short 70–200 µm and (2.9–) 3.2 × 0.7 (–5.0) µm wide with small vesicles 7–10 µm. The metulae are 4.5–7 × 1.5–2 µm and the phialides are 5–7 × 1.5–2 µm. Conidia are globose and spinulose, (3.5–) 3.6 × 4.1 (–4.3) µm. The isolate ex type produces aflatoxin B_1, sterigmatocystin, and compounds with chromophores identical with those of desertorins, shamixanthone and emerin.

Colonies on CYA 24–32 mm diam. after one week at 25 °C, MEA: 36–39 mm diam. after one week at 25 °C, YES 38–42 mm diam. after one week at 25 °C, OAT 31–37 mm diam. after one week at 25 °C, poor growth on CREA and no acid production, CYA at 37 °C: 7–9 mm diam. after one week. Ascomata and Hülle cells were produced on all media.

Culture ex type CBS 868.97 = IBT 20956 isolated from a sponge (*Porifera*) in red mangrove, surface water (depth 0.5 m), 23 °C, Rojo, Mochima Bay, Mochima National Park, Sucre State, Venezuela, January 1977. A voucher specimen (Ven 97 M 75–14) is also kept at Departamento de Química, Universidad de Oriente, Cumaná, Venezuela.

Emericella variecolor Berk. & Broome, Intr. Crypt. Bot.: 340, 1857 (anamorph *Aspergillus stellifer* Samson & W. Gams), type: India, Bowenpilly, Secunderabad, 3 Jul 1855, *s.coll.* (K), epitype, designated here: CBS 598.65, because the type and the iconotype are insufficient to accurately describe the fungus and because the anamorph was not described by Berkeley and Broome.

The anamorph or holomorph has often incorrectly been cited as *Aspergillus stellatus* Curzi [7] or *A. variecolor* (Berk. & Broome) Thom & Raper [45] in the literature on extrolites from this fungus.

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References

1. Bringmann, G., Lang, G., Steffens, S., Günther, E., Schaumann, K.: Evariquinone, isoemericellin, and stromemycin from a sponge derived strain of the fungus *Emericella variecolor*. Phytochemistry 63, 437–443 (2003).
2. Christensen, M., Raper, K. B.: Synoptic key to *Aspergillus nidulans* group species and related *Emericella* species. Transactions of the British Mycological. Society 71, 177–191 (1978).
3. Christensen, M., Raper, K. B., States, J. S.: Two new *Aspergillus nidulans* group members from Wyoming soils. Mycologia 70, 332–342 (1978).
4. Christensen, M., States, J. S.: *Aspergillus nidulans* group: *A. navahoensis*, and a revised synoptic key. Mycologia 74, 226–235 (1978).

5. Christophersen, C., Crescente, O., Frisvad, J. C., Gram, L., Nielsen, J., Nielsen, P. H., Rahbæk, L.: Antibacterial activity of marine-derived fungi. Mycopathologia 143, 135–138 (1999).
6. Cole, R. J., Cox, R. H.: Handbook of toxic fungal metabolites. Academic Press, New York (1981).
7. Curzi, M.: Un' aspergillacea ad ascospore stellate. Rendu Accademia Nazionale Lincei 19, 424–428 (1934).
8. Dulaney, E. L.: Some aspects of penicillin production by *Aspergillus nidulans*. Mycologia 39, 570–581 (1947).
9. Dulaney, E. L.: Penicillin production by the *Aspergillus nidulans* group. Mycologia 39, 582–586 (1947).
10. Ehrlich, K. C., Montalbano, B. G. and Cotty, P. J.: Sequence comparison of aflR from different *Aspergillus* species provides evidence for variability in regulation of aflatoxin production. Fungal Genetics and Biology 38, 63–74 (2003).
11. El-Khady, I. A., Abdel Hafez, S. I. I.: Production of sterigmatocystin by some species and varieties of *Aspergillus nidulans* group. Cryptogamie Mycologie 2, 239–244 (1981).
12. Filtenborg, O., Frisvad, J. C., Svendsen, J. A.: Simple screening method for moulds producing intracellular mycotoxins in pure cultures. Applied and Environmental Microbiology 45, 581–585 (1986).
13. Frisvad, J. C.: Secondary metabolites as an aid to *Emericella* classification, pp. 437–443. In: Advances in *Penicillium* and *Aspergillus* systematics (R. A. Samson, J. I. Pitt, eds.) Plenum Press, New York (1985).
14. Frisvad, J. C., Houbraken, J., Samson, R. A.: *Aspergillus* species and aflatoxin production: a reappraisal, pp. 125–126. In: Food Microbiology and food safety in the next millennium. (A. C. J. Tuijtelaars, R. A. Samson, F. M. Rombouts, S. Notermans, eds.) Foundation Food Micro '99, Zeist (1999).
15. Frisvad, J. C., Samson, R. A.: *Neopetromyces* gen nov. and an overview of teleomorphs of *Aspergillus* subgenus *Circumdati*. Studies in Mycology (Baarn) 45, 201–207 (2000).
16. Frisvad, J. C., Samson, R. A., Smedsgaard, J.: *Emericella astellata*, a new of aflatoxin B_1, B_2 and sterigmatocystin. Letters in Applied Microbiology 38, 440–445 (2004).
17. Frisvad, J. C., Thrane, U.: Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection). Journal of Chromatography 404, 195–214 (1987).
18. Frisvad, J. C., Thrane, U.: Liquid column chromatography of mycotoxins, pp. 253–272. In: Chromatography of mycotoxins: techniques and applications. (V. Betina, ed.) Journal of Chromatography Library 54, Elsevier, Amsterdam (1993).
19. Gill-Carey, D. E.: The nature of some antibiotics from Aspergilli. British Journal of Experimental Pathology 30, 119–122 (1949).
20. Glass, N. L., Donaldson, G. C.: Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61, 1323–1330 (1995).
21. Gould, R. D., Simpson, T. J., Wallinshaw, M. D. Isolation and X-ray crystal structures of astellolide A and astellolide B, sesquiterpenoid metabolites of *Aspergillus variecolor*. Tetrahedron Letters 22, 1047–1050 (1981).
22. Hanssen, E., Jung, M.: Control of aflatoxins in the food industry. Pure and Applied Chemistry 35, 239–250 (1973).
23. Hillis, D. M. & Bull, J. J.: An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42, 182–192. (1993)
24. Horie, Y.: A new species of *Emericella* from Indian herbal drug. Transactions of the Mycological Society of Japan 19, 313–317 (1978).
25. Horie, Y.: New or interesting *Emericella* from herbal drugs. Transactions of the Mycological Society of Japan 20, 481–491 (1979).
26. Horie, Y.: Ascospore ornamentation and its application to the taxonomic re-evaluation in *Emericella*. Transactions of the Mycological Society of Japan 21, 483–493 (1980).
27. Horie, Y., Miyaji, M., Nishimura, K., Udagawa, S.: *Emericella falconensis*, a new species from Venezuelan soil. Transactions of the Mycological Society of Japan 30, 257–263 (1989a).
28. Horie, Y., Miyaji, M., Nishimura, K., Taguchi, H., Yamaguchi, H., Udagawa, S.: Distribution of *Emericella* species in soil of South America, with special reference to their production of sterigmatocystin and the related metabolites. Proceedings of the Japanese Association of Mycotoxicology 29, 21–26 (1989b).
29. Horie, Y., Udagawa, S., Abdullah, S. K., Al-Bader, S. M.: *Emericella similis*, a new species from Iraqui soil. Transactions of the Mycological Society of Japan 31, 425–430 (1990).
30. Horie, Y., Udagawa, S.: *Emericella omanensis*, a new species from Oman soil. Mycoscience 35, 391–394 (1995).
31. Horie, Y., Miyaji, M., Nishimura, K., Franco, M. F., Coelho, K. I.: New and interesting species of *Emericella* from Brasilian soil. Mycoscience 37, 137–144 (1996a).
32. Horie, Y., Fukiharu, T., Nishimura, K., Taguchi, H., Wang, D. L., Li, R.: New and interesting species of *Emericella* from chinese soil. Mycoscience 37, 323–329 (1996b).
33. Horie, Y., Li, D. M., Fukiharu, T., Li, R., Abliz, P., Nishimura, K., Wang, D. L.: *Emericella appendiculata*, a new species from Chinese soil. Mycoscience 39, 161–165 (1998).
34. Horie, Y., Abliz, P., Hui, Y., Fukiharu, T., Nishimura, K., Li, D. L., Li, R.: *Emericella qinqixianii*, a new species from desert soil in China. Mycoscience 41, 183–187 (2000).
35. Howard, B. H., Raistrick, H.: Studies in the biochemistry of microorganisms. 94. The colouring matters of species in the *Aspergillus nidulans* group. I. Asperthecin, a crystalline colouring matter of *Aspergillus quadrilineatus* Thom and Raper; appendix by J. H. Birkinshaw: ultraviolet absorption spectra of some polyhydroxyanthraquinones. Biochemical Journal 59, 475–486 (1955).
36. Kamal, A., Husain, S. A., Noorani, R., Murtaza, N., Qureshi, I. H., Qureshi, A. A.: Biochemistry of microorganisms. XI. Isolation of tajixanthone, shamixanthone, ajamxanthone, shahenxanthone, najamxanthone, radixanthone, and mannitol from mycelium of *Aspergillus stellatus*. Pakistan Journal of Scientific and Industrial Research 13, 251–255 (1970).
37. Kamal, A., Shaheen, A., Qureshi, A. A.: Biochemistry of microorganisms. XXIV. Structure and stereochemistry of tajixanthone, a metabolic product of *Aspergillus stellatus*. Pakistan Journal of Scientific and Industrial Research 14, 90–103 (1971a).
38. Kamal, A., Husain, S. A., Qureshi, A. A.: Biochemistry of microorganisms. XXV. Structure and stereochemistry of shamixanthone and ajamxanthone, metabolic products of *Aspergillus stellatus*. Pakistan Journal of Scientific and. Industrial Research 14, 104–112 (1971b).
39. Kamal, A., Husain, S. A., Qureshi, A. A.: Biochemistry of microorganisms. XXVI. Structure and stereochemistry of shajhenxanthone and najamxanthone, metabolic products of *Aspergillus stellatus*. Pakistan Journal of Scientiific and Industrial Research 15, 49–57 (1972).

40. Kawai, K., Shiro, M., Nozawa, K.: Absolute chemistry of detertorins, bicoumarins from *Emericella desertorum*. Journal of Chemical Research, Synopsis 1993 (3), 92–93 (1995).
41. Klich, M., Mullaney, E. J., Daly, C. B., Cary, J. W.: Molecular and physiological aspects of aflatoxin and sterigmatocystin biosynthesis by *Aspergillus tamarii* and *A. ochraceoroseus* Applied. Microbiology and Biotechnology 53, 605–609 (2000).
42. Kong, H., Qi, Z.: A species of *Emericella*. Acta Mycologica Sinica 5, 211–214 (1986).
43. Kruger, G. J., Steyn, P. S., Vleggaar, R., Rabie, C. J.: X-ray crystal structure of asteltoxin, a novel mycotoxin from *Aspergillus stellatus* Curzi. Journal of the Chemical Society Chemical Communications 1979 (10), 441–442 (1979).
44. Maccabe, A. P., Orejas, M., Ramón, D.: *Aspergillus nidulans* as a model organism for the study of the expression of genes encoding enzymes of relevance in the food industry, pp. 239–265. In: Applied Mycology and Biotechnology Vol. 1. Agriculture and Food Production (G. G. Khachatourians, D. K. Arora, eds). Elsevier, Amsterdam (2001).
45. Malloch, D., Cain, R. F.: New species and combinations of cleistothecial ascomycetes. Canadian Journal of Botany 50, 61–72 (1972).
46. Malmstrøm, J., Christophersen, C., Barrero, A. F., Oltra, J. E., Justicia, J., Rosales, A.: Bioactive metabolites from a marine-derived strain of the fungus *Emericella variecolor*. Journal Natural Products 65, 364–367 (2002).
47. Mehrotra, B. S., Prasad, R.: *Aspergillus dimorphicus* and *Emericella cleisto-minuta* spp. nov. from Indian soils. Transactions of the British Mycological Society 52, 331–336 (1969).
48. Neelakantan, S., Pockern, A., Raistrick, H.: Studies in the biochemistry of microorganisms. 101. The colouring matters of species in the *Aspergilus nidulans* group. Part 2. Further observations on the structure of asperthecin. Biochememical Journal 66, 234–237 (1957).
49. Nielsen, K. F., Smedsgaard, J.: Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. Journal of Chromatography A 1002, 111–136 (2003).
50. Nozawa. K., Seyea, H., Nakajima, S., Udagawa, S., Kawai, K.: Studies on fungal products. Part. 10. Isolation and structures of novel bicoumarins, desertorins A, B, and C, from *Emericella desertorum*. Jornal of the Chemical Society, Perkin Trans. 1 (1987), 1735–1738 (1987).
51. Peterson, S. W.: Phylogenetic relationships in *Aspergillus* based on rDNA sequence analysis, pp. 323–355. In: Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* classification (R. A. Samson, J. I. Pitt, eds.) Harwood Academic Publishers, Amsterdam (2000).
52. Pitt, J. I.: The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, New York (1979).
53. Qureshi, I. H., Husain, S. A., Noorani, R., Murtaza, N., Iitaka, Y., Iwasaki, S., Okuda, S.: Stellatic acid: a new class of sesterpenoid; X-ray crystal structure. Tetrahedron Letters 21, 1961–1962 (1980).
54. Qureshi, I. H., Kamal, A., Noorani, R., Aziz, S., Husain, S. A.: Biochemistry of microorganisms. VII. Terrein and kojic acid; metabolic products of *Aspergillus stellatus*. Pakistan Journal of Scientific and Industrial Research 11, 367–369 (1968).
55. Raper, K. B., Fennell, D. I.: The genus *Aspergilllus*. Williams and Wilkins, Baltimore, (1965).
56. Sadler, I. H., Simpson, T. J.: The determination by NMR methods of the structure and stereochemistry of astellatol, a new and unusual sesterpene. Journal of the Chemical Society Chemical Communications 1989 (21), 1602–1604 (1989).
57. Samson, R. A., Mouchacca, J.: Some interesting species of *Emericella* and *Aspergillus* from Egyptian desert soil. Antonie van Leeuwenhoek 40, 121–131 (1974).
58. Samson, R. A., Mouchacca, J.: Additional notes on species of *Aspergillus, Eurotium* and *Emericella* from Egyptian desert soil. Antonie van Leeuwenhoek 41, 343–351 (1975).
59. Samson, R. A., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O. (eds.), Introduction to food- and airborne fungi. 6th ed. 2nd print. Centraalbureau voor Schimmelcultures, Utrecht, (2002).
60. Schroeder, H. W., Kelton, W. H.: Production of sterigmatocystin by some species of the genus *Aspergillus* and its toxicity to chicken embryos. Applied and Environtal Microbiology 30, 589–591 (1975).
61. Simpson, T. J.: Use of long range proton-carbon-13 couplings in structure determination: stellatin, a novel dihydroisocoumarin from *Aspergillus variecolor*. Journal of the Chemical Society Chemical Communications 1978 (14), 627–628 (1978).
62. Simpson, T. J. Studies on fungal metabolites. 1. Structures of andibenin A and andibenin C, and andilesin A, andilesin B, and andilesin C. Journal of the Chemical Society Perkin Transactions 1 (1979), 2118–2121 (1979).
63. Simpson, T. J.: Biosynthesis of astellatol, a novel rearranged sesterterpenoid metabolite of *Aspergillus variecolor*. Journal of the Chemical Society Perkin Transactions 1 1994 (21), 3055–3056 (1994).
64. Simpson, T. J., Ahmad, S. A., Mcintyre, C. R., Scott, F. E., Sadler, I. H.: Biosynthesis of polyketide-terpenoid (meroterpenoid) metabolites andibenin B and andilesin A in *Aspergillus variecolor*. Tetrahedron 53, 4013–4034 (1997).
65. Simpson, T. J., Walkinshaw, M. D. Anditomin, a new C-25 metabolite from *Aspergillus variecolor*. Journal of the Chemical Society Chemical Communications 1981, 914–915 (1981).
66. Smedsgaard, J.: Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures. Journal of Chromatography A 760, 264–270 (1997).
67. Stchigl, A. M., Guarro, J.: A new species of *Emericella* from Indian soil. Mycologia 89, 937–941 (1997).
68. Swofford, D. L.: *PAUP* 4.0: phylogenetic analysis using parsimony*. Sinauer Associates, Sunderland, MA (2000)
69. Udagawa, S., Horie, Y.: A new species of *Emericella*. Mycotaxon 4, 535–539 (1976).
70. Udagawa, S., Muroi, T.: Some interesting species of Ascomycetes from imported spices. Transactions of the Mycological Society of Japan 20, 13–22 (1979).
71. White, T. J., Bruns, T., Lee S., Taylor, J.: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315–322. In: PCR protocols: a guide to methods and applications (M. S. Innis, D. H. Gelfand, eds.) Academic Press, New York (1990).

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