Two new aflatoxin producing species, and an overview of Aspergillus section Flavi

Varga, J.; Frisvad, Jens Christian; Samson, R. A.

Published in:
Studies in Mycology

Link to article, DOI:
10.3114/sim.2011.69.05

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Two new aflatoxin producing species, and an overview of Aspergillus section Flavi

J. Varga1,2*, J.C. Frisvad3 and R.A. Samson1

1CBS Fungal Biodiversity Centre, Uppsalalaan 8, NL-3584 CT Utrecht, the Netherlands; 2Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Közép fassor 52, Hungary; 3Center for Microbial Biotechnology, Department of Systems Biology, Building 221, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

*Correspondence: János Varga, j.varga@CBS.knaw.nl

Abstract: Aspergillus subgenus Circumdati section Flavi includes species with usually biseriate conidial heads, in shades of yellow-green to brown, and dark sclerotia. Several species assigned to this section are either important mycotoxin producers including aflatoxins, cyclopiazonic acid, ochratoxins and kojic acid, or are used in oriental food fermentation processes. Several species have been described in the past which were mainly based on traditional methods (morphological parameters, including colony diameter, colour and texture, size and texture of conidia and conidiophore structure; Klich 2002). However, species classification may be difficult due to extensive divergence of morphological characters produced by fungi. Aflatoxins are mainly produced by A. flavus and A. parasiticus, which coexist with and grow on almost any crop or food.

INTRODUCTION

Aspergillus section Flavi historically includes species with conidial heads in shades of yellow-green to brown and dark sclerotia. Isolates of the so-called domesticated species, such as A. oryzae, A. sojae and A. tamarii are used in oriental food fermentation processes and as hosts for heterologous gene expression (Campbell-Platt & Cook 1989). Genetically modified A. oryzae strains are used for the production of enzymes including lactase, pectin esterase, lipase, protease and xylanase (Pariza & Johnson 2001). Several species of section Flavi produce aflatoxins, among which aflatoxin B1 is the most toxic of the many naturally occurring secondary metabolites produced by fungi. Aflatoxins are mainly produced by A. flavus and A. parasiticus, which coexist with and grow on almost any crop or food.

Several species have been described in the past which were assigned to Aspergillus section Flavi mainly based on traditional methods (morphological parameters, including colony diameter, colour and texture, size and texture of conidia and conidiophore structure; Klich 2002). However, species classification may be difficult due to extensive divergence of morphological characters produced by fungi. Aflatoxins are mainly produced by A. flavus and A. parasiticus, which coexist with and grow on almost any crop or food.

Several species have been worked out to distinguish nine species of the section (Godet & Munaut 2010). In this study, we examined available isolates of the species proposed to belong to this section to clarify its taxonomic status. The methods used include sequence analysis of the ITS region (including intergenic spacer regions 1 and 2, and the 5.8 S rRNA gene of the rRNA gene cluster), and parts of the β-tubulin and calmodulin genes, macro- and micromorphological analysis, and analysis of extrolite profiles of the isolates. We also examined the presence of three aflatoxin biosynthetic genes in some aflatoxin-producing and non-producing isolates.

MATERIALS AND METHODS

Isolates

The strains used in this study are listed in Table 1. Sequence data of several other isolates available from GenBank database have also been used for constructing phylogenetic trees.

Morphological analysis

For macromorphological observations, Czapek Yeast Autolysate (CYA), Malt Extract Autolysate (MEA) agar, Yeast Extract Sucrose Agar (YES), Creatine Agar (CREA), and Aspergillus flavus/parasiticus Agar (AFPA) were used (Samson et al. 2004a). 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 with cotton blue from MEA colonies and a drop of alcohol was added to remove air bubbles and excess conidia.
Table 1. Aspergillus isolates examined.

<table>
<thead>
<tr>
<th>Name</th>
<th>Isolate</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. albertensis</td>
<td>NRRL 20602</td>
<td>Human ear, Alberta, Canada</td>
</tr>
<tr>
<td>A. alliaceus</td>
<td>CBS 542.65T</td>
<td>Soil, Australia</td>
</tr>
<tr>
<td></td>
<td>CBS 536.65</td>
<td>Dead blister beetle Macrobasis albida, Washington, CO, USA</td>
</tr>
<tr>
<td></td>
<td>CBS 612.78</td>
<td>Buenos Aires, Argentina</td>
</tr>
<tr>
<td>A. arachidica</td>
<td>CBS 117610T</td>
<td>Arachis glabrata leaf, CO, Argentina</td>
</tr>
<tr>
<td></td>
<td>CBS 117615</td>
<td>Arachis glabrata leaf, CO, Argentina</td>
</tr>
<tr>
<td>A. avenaceus</td>
<td>CBS 109.46T</td>
<td>Pisum sativum seed, UK</td>
</tr>
<tr>
<td></td>
<td>CBS 102.45</td>
<td>NCTC 6548</td>
</tr>
<tr>
<td>A. bombycis</td>
<td>CBS 117187</td>
<td>Frass in a silkworm rearing house, Japan</td>
</tr>
<tr>
<td>A. caelatus</td>
<td>CBS 763.97T</td>
<td>Soil, USA</td>
</tr>
<tr>
<td></td>
<td>CBS 764.97</td>
<td>Soil, USA</td>
</tr>
<tr>
<td>A. coremiiformis</td>
<td>CBS 553.77T</td>
<td>Soil, Ivory Coast</td>
</tr>
<tr>
<td>A. fasciculatus</td>
<td>CBS 110.55T</td>
<td>Air contaminant, Brazil</td>
</tr>
<tr>
<td>A. flavofuscatus</td>
<td>CBS 484.65T</td>
<td>Air contaminant, Brazil</td>
</tr>
<tr>
<td>A. flavus</td>
<td>CBS 100927T</td>
<td>Cellophane, South Pacific Islands</td>
</tr>
<tr>
<td></td>
<td>CBS 116.48</td>
<td>Unknown source, the Netherlands</td>
</tr>
<tr>
<td></td>
<td>CBS 616.94</td>
<td>Man, orbital tumor, Germany</td>
</tr>
<tr>
<td>A. flavus var. columnaris</td>
<td>CBS 485.65T</td>
<td>Butter, Japan</td>
</tr>
<tr>
<td></td>
<td>CBS 117731</td>
<td>Dipodomys spectabilis cheek pouch, New Mexico, USA</td>
</tr>
<tr>
<td>A. kambrensis</td>
<td>CBS 542.69T</td>
<td>Stratigraphic core sample, Japan</td>
</tr>
<tr>
<td>A. lanosus</td>
<td>CBS 650.74T</td>
<td>Soil under Tectona grandis, Gorakhpur, India</td>
</tr>
<tr>
<td>A. leporis</td>
<td>CBS 151.66T</td>
<td>Dung of Lepus townsendi, USA</td>
</tr>
<tr>
<td></td>
<td>CBS 349.81</td>
<td>Soil, Wyoming, USA</td>
</tr>
<tr>
<td>A. minisclerotigenes</td>
<td>CBS 117633</td>
<td>Arachis hypogaeae seed, FO, Argentina</td>
</tr>
<tr>
<td></td>
<td>CBS 117635T</td>
<td>Arachis hypogaeae seed, CD, Argentina</td>
</tr>
<tr>
<td>A. nomius</td>
<td>CBS 260.88T</td>
<td>Wheat, USA</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>CBS 100925T</td>
<td>Unknown source, Japan</td>
</tr>
<tr>
<td>A. parasiticus</td>
<td>CBS 100926T</td>
<td>Pseudococcus calceolariæ, sugar cane mealy bug, Hawaii, USA</td>
</tr>
<tr>
<td>A. parasiticus var. globosus</td>
<td>CBS 260.67T</td>
<td>Unknown source, Japan</td>
</tr>
<tr>
<td>A. panisclerotigenus</td>
<td>CBS 121.62T</td>
<td>Arachis hypogaeae, Nigeria</td>
</tr>
<tr>
<td>A. pseudocaelatus</td>
<td>CBS 117616</td>
<td>Arachis burkarti leaf, CO, Argentina</td>
</tr>
<tr>
<td>A. pseudonomius</td>
<td>CBS 119388</td>
<td>Diseased alkali bees, USA</td>
</tr>
<tr>
<td>A. pseudotamarii</td>
<td>CBS 766.97T</td>
<td>Soil, USA</td>
</tr>
<tr>
<td></td>
<td>CBS 765.97</td>
<td>Soil, USA</td>
</tr>
<tr>
<td>A. sojae</td>
<td>CBS 100928T</td>
<td>Soy sauce, Japan</td>
</tr>
<tr>
<td>A. subolivaceus</td>
<td>CBS 501.65T</td>
<td>Cotton, Lintafelt, UK</td>
</tr>
<tr>
<td>A. tamarii</td>
<td>CBS 104.13T</td>
<td>Activated carbon</td>
</tr>
<tr>
<td>A. terricola</td>
<td>CBS 620.95</td>
<td>WB4858</td>
</tr>
<tr>
<td></td>
<td>CBS 579.65T</td>
<td>USA</td>
</tr>
<tr>
<td>A. terricola var. americanus</td>
<td>CBS 580.65T</td>
<td>Soil, USA</td>
</tr>
<tr>
<td></td>
<td>CBS 119.51</td>
<td>Japan</td>
</tr>
<tr>
<td>A. terricola var. indicus</td>
<td>CBS 167.63T</td>
<td>Mouldy bread, Allahabad, India</td>
</tr>
<tr>
<td>A. thomii</td>
<td>CBS 120.51T</td>
<td>Culture contaminant</td>
</tr>
<tr>
<td>A. togoensis</td>
<td>CBS 272.89T</td>
<td>Seed, Central African Republic</td>
</tr>
<tr>
<td>A. toxicarius</td>
<td>CBS 822.72T</td>
<td>Arachis hypogaeae, Uganda</td>
</tr>
<tr>
<td></td>
<td>CBS 561.82</td>
<td>Löss deposit, Nebraska, USA</td>
</tr>
<tr>
<td>A. zhaoqingensis</td>
<td>CBS 399.93T</td>
<td>Soil, China</td>
</tr>
</tbody>
</table>

CBS = CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands. IBT = IBT Culture Collection of Fungi, Lyngby, Denmark. NRRL = USDA ARS Culture Collection, Peoria, USA. ATCC = American Type Culture Collection, Manassas, USA.
Extrolate analysis

The cultures were analysed according to the HPLC-diode array detection method of Frisvad & Thrane (1987, 1993) as modified by Smedsgaard (1997). The isolates were analysed on CYA and YES agar using three agar plugs (Smedsgaard 1997). 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), with minor modifications as described by Smedsgaard (1997).

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).

The presence of three genes taking part in aflatoxin biosynthesis has also been examined in some isolates. Part of the transcriptional regulator of aflatoxin biosynthesis, aflR, was amplified using the primers aflR-F (5'-GGATACCTGAGGTGTGCGCA-3') and aflR-R (5'-TGKGGCGACTGGAAYGGGT-3') developed based on previously identified aflR sequences in the GenBank database. Part of the norsolonic acid reductase (norA, aflE; Yu et al. 2004) gene was amplified using the primers nor1 (5'-ACCGTACGGCGACTTCGCGCA-3') and nor2 (5'-GTGGCGCCACGCTTACAGACG-3') developed by Geisen (1996). Part of the O-methyltransferase gene (omtA, aflP; Yu et al. 2004) was amplified using the primers omt1 (5'-GTGGACGGACCTAGTCCGACATCAC-3') and omt2 (5'-GTGCGCAGCGACTCGGTGGGGG-3') (Geisen 1996). Sequence analysis of the amplified products was carried out as described previously (Varga et al. 2007a).

DNA sequences were edited with the DNASTAR computer package. Alignments of the sequences were performed using MEGA v. 4 (Tamura et al. 2007). Phylogenetic analysis of sequence data was performed using PAUP v. 4.0b10 (Swofford 2000). Alignment gaps were treated as fifth character state, parsimony uninformative characters were excluded and all characters were unordered and equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option. To assess the robustness of the topology, 1000 bootstrap replicates were run by maximum parsimony (Hillis & Bull 1993). Other measures including tree length, consistency index, retention index and rescaled consistency index (Cl, RI and RC, respectively) were also calculated. Neopetromyces muricatus CBS 112808 was used as outgroup in the analyses of calmodulin, ITS and β-tubulin data sets, while A. versicolor SSRC 108 sequences were used as outgroups during analysis of aflR and norA sequences. No outgroup was used during the analysis of the omtA dataset, as sequences were not available from any other aflatoxin producing species outside Aspergillus section Flavi. Sequences were deposited at GenBank under accession numbers indicated on the figures.

RESULTS

Phylogenetic analysis

We examined the genetic relatedness of section Flavi isolates using sequence analysis of the ITS region of the ribosomal RNA gene cluster, and parts of the calmodulin and β-tubulin genes. During analysis of part of the β-tubulin gene, 561 characters were analysed, among which 223 were found to be phylogenetically informative. One of the 57 MP trees based on partial β-tubulin gene sequences is shown in Fig. 1 (tree length: 557, consistency index: 0.7279, retention index: 0.9051). The calmodulin data set included 583 characters, with 221 parsimony informative characters. One of the 485 MP trees based on partial calmodulin gene sequences is shown in Fig. 2 (tree length: 557, consistency index: 0.7181, retention index: 0.9026). The ITS data set included 496 characters with 58 parsimony informative characters. One of the 235 MP trees is shown in Fig. 3 (tree length: 193, consistency index: 0.8446, retention index: 0.8592).

Phylogenetic analysis of ITS, calmodulin and β-tubulin sequence data indicated that the “A. caelatus” isolate CBS 117616 is closely related to, but phylogenetically distinct from A. caelatus (Figs 1–3). While all A. caelatus isolates known have come from soil, peanuts or tea fields located in Japan or USA, this isolate came from an Arachis burkartii leaf from Corrientes province, Argentina. This isolate also produces a set of different extrolites including aflatoxins B1, B2, G1, G2, kojic acid and cyclopazonic acid, while A. caelatus isolates produce kojic acid and asperglin. Another isolate, “A. nomius” CBS 119388 (= NRRL 3353) was found to form a distinct clade on the trees based on calmodulin and β-tubulin sequence data (Fig. 1, 2). This isolate was also found to be different from A. nomius and A. arachidicola by physiological means; it produces chrysogine, kojic acid and aflatoxin B1, similarly to A. arachidicola, which also produces aflatoxin G1. In addition, A. arachidicola produces paraistolide, dityropetenaline and metabolite “NO2”, the last one also being produced by isolate CBS 119388. Aspergillus nomius produces both B- and G-type aflatoxins, kojic acid, but not chrysogine. Based on phylogenetic analysis of calmodulin, β-tubulin, ITS and norsolonic acid reductase gene sequences, this new species includes several other isolates from insects and soil in Louisiana, Texas, Wyoming and Wisconsin in the USA (Peterson et al. 2001). Unfortunately, these isolates were not available for this study. The late C.W. Heseltine (NRRL, Peoria USA) indicated in a personal communication to J.C. Frisvad, that he considered NRRL 3353 morphologically different from other A. nomius, which was backed up by differences in tolerance to low water activity. These observations should be further investigated.

The presence of 3 genes taking part in aflatoxin biosynthesis has also been examined in a set of isolates, including isolate CBS 117616 and several A. caelatus isolates. While isolate CBS 117616 carried homologs of all three examined genes, the A. caelatus isolates did not carry homologs of aflR and norA (Fig. 4). During analysis of the aflR dataset, 514 characters wereanalysed, among which 113 were found to be phylogenetically informative. One of the 5 MP trees based on partial aflR genes sequences is shown in Fig. 5 (tree length: 464 steps, consistency index: 0.8836, retention index: 0.9339). The norA data set included 348 characters, with 40 parsimony informative characters. One of the 2 MP trees based on partial norA gene sequences is shown in Fig. 6 (tree length: 174, consistency index: 0.9138, retention index: 0.9302). The omtA
Fig. 1. Maximum parsimony tree based on β-tubulin sequence data of Aspergillus section Flavi. Numbers above branches are bootstrap values; only values above 70% are indicated. P. = Petromyces. N. = Neopetromyces.
A. kambarensis CBS 542.69° (EF203136)
A. flavus var. columnaris CBS 485.65° (EF203143)
A. subolivaceus CBS 501.65° (EF203144)
A. fasciculatus CBS 110.55° (EF203135)
A. flavus CBS 116.48 (EF203141)
A. oryzae CBS 100925° (EF203138)
A. flavus CBS 616.94 (EF203131)
A. flavus CBS 100927° (EF203132)
A. thomii CBS 120.51° (EF203133)
A. parvisclerosgenes CBS 117635T (EF203148)
A. minisclerosgenes CBS 117639 (EF203149)
A. parvisclerosgenes CBS 121.62° (EF203130)
A. minisclerosgenes CBS 117635T (EF203148)
A. minisclerosgenes CBS 117639 (EF203149)
A. toxiciaratus CBS 561.82 (EF203169)
A. toxiciaratus var. americanus CBS 580.65T (EF203157)
A. parvisclerosgenes CBS 260.67T (EF203156)
A. parvisclerosgenes CBS 115.37 (FJ491485)
A. parvisclerosgenes CBS 100926° (EF203155)
A. arachidicola CBS 117610° (EF203158)
A. arachidicola CBS 117615 (EF203161)
A. arachidicola CBS 117610° (EF203158)
A. arachidicola CBS 117615 (EF203161)
A. nomius CBS 260.88° (EF203120)
A. nomius NRRL 3161 (EF661493)
A. zhaoqingensis CBS 399.93 (FJ491478)
A. pseudonomius NRRL 6552 (EF661496)
A. pseudonomius NRRL 3533 (EF661495)
A. terricola CBS 620.95 (FJ491476)
A. bombycis CBS 117187T (EF203121)
A. tamarii CBS 104.13° (EF203123)
A. terricola CBS 579.65° (EF661472)
A. flavofurcatus CBS 484.65° (EF203124)
A. pseudotamarii CBS 766.97° (EF203125)
A. pseudotamarii CBS 765.97 (EF203126)
A. caelatus CBS 763.97° (EF203129)
A. caelatus NRRL 25566 (AF255035)
A. caelatus NRRL 25576 (AF255072)
A. caelatus NRRL 26100 (EF661471)
A. caelatus NRRL 25567 (FJ491483)
A. caelatus NRRL 25568 (FJ491484)
A. caelatus NRRL 25528 (EF661470)
A. pseudocaelatus CBS 117616° (EF203128)
A. leporis CBS 151.66° (EF203171)
A. leporis CBS 349.81 (FJ491475)
A. togoensis CBS 272.89° (FJ491477)
A. coremiiformis CBS 553.77° (EU014104)
A. coremiiformis NRRL 13756 (EU014105)
A. alliaceus NRRL 5108 (EF661469)
A. lanosus CBS 650.74° (FJ491479)
A. alliaceus CBS 542.65° (EF661466)
A. alliaceus NRRL 315 (EF661465)
A. albertensis NRRL 20602 (EF661464)
A. alliaceus NRRL 1206 (EF661463)
A. avenaceus CBS 109.46° (FJ491481)
A. avenaceus CBS 102.45 (FJ491480)
N. muricatus CBS 112808° (EF661355)

**Fig. 2.** Maximum parsimony tree based on calmodulin sequence data of Aspergillus section Flavi. Numbers above branches are bootstrap values; only values above 70% are indicated. N. = Neopetromyces.
Fig. 3. Maximum parsimony tree based on ITS sequence data of Aspergillus section Flavi. Numbers above branches are bootstrap values. Only values above 70 % are indicated. N. = Neopetromyces.
TWO NEW AFLATOXIN PRODUCING SPECIES, AND AN OVERVIEW OF ASPERGILLUS SECTION FLAVI

Fig. 4. PCR amplicons obtained using primer pairs developed for the aflR, norA and omtA genes in some isolates. M. 1 kb DNA ladder; 1. A. pseudocaelatus CBS 117616; 2–7. A. caelatus isolates (CBS 763.97, CBS 764.97, NRRL 25566, NRRL 25567, NRRL 25568 and NRRL 25569); 8. A. minisclerotigenes CBS 117633; 9. A. arachidicola CBS 117610; 10. A. parvisclerotigenus CBS 121.62; 11. A. bombycis NRRL 29236.

data set included 731 characters, with 136 parsimony informative characters. One of the 12 MP trees based on partial omtA gene sequences is shown in Fig. 7 (tree length: 386, consistency index: 0.7876, retention index: 0.8019). Isolate CBS 117616 was related to A. pseudotamarii based on aflR and omtA sequence data (Figs 5, 7), while the norA data set revealed that it is more closely related to A. caelatus (Fig. 6). Isolate CBS 119388 was related to, but distinct from A. nomius based on all trees. We propose the names Aspergillus pseudocaelatus and A. pseudonomius for these two new species.

Aspergillus pseudocaelatus Varga, Samson & Frisvad, sp. nov. MycoBank MB560397. Fig. 8.

Aspergillo caelato morphologicæ valde similis, sed aflatoxina (B & G), acor cyclopiazonicus et acor kojicus formantur.

Colonies on YES, MEA, OA and CYA attain a diam of 6–6.5 cm in 7 d at 25 °C; growing rapidly on CYA at 37 °C, with a diam of 6–7 cm. On CREA a typical acid production. Colony surface velvety with abundant conidial heads, olive to olive brown en masse. Reverse greenish yellow without diffusible pigments. Sclerotia not observed. Conidial heads uniseriate or biseriate. Stipes hyaline, smooth-walled, 5–8 µm wide variable in length, mostly (250–)400–600(21000) µm;

Fig. 5. Maximum parsimony tree based on aflR sequence data of Aspergillus section Flavi. Numbers above branches are bootstrap values; only values above 70 % are indicated.

Aspergillus minisclerotigenes CBS 117633; A. flavus var. columnaris BCRC 30433 (AY650426); A. sojae BCRC 33643 (AY650928); A. parasiticus BCRC 30164 (AY650924); A. arachidicola CBS 117610; A. parvisclerotigenus CBS 121.62; A. bombycis NRRL 29236.

A. pseudocaelatus CBS 117616 (FJ491459)

A. pseudonomius CBS 119388 (FJ491456)

A. nomius NRRL 13137 (AF441422)

A. bombycis NRRL 26010 (AF441414)

A. bombycis NRRL 29235 (FJ491460)

A. bombycis NRRL 29236 (FJ491461)

A. pseudonius CBS 159388 (FJ491456)

A. versicolor SRRC 108 (AY197609)
Fig. 6. Maximum parsimony tree based on norA sequence data of Aspergillus section Flavi. Numbers above branches are bootstrap values; only values above 70 % are indicated.

Fig. 7. Maximum parsimony tree based on omtA sequence data of Aspergillus section Flavi. Numbers above branches are bootstrap values; only values above 70 % are indicated.
Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*

Fig. 8. *Aspergillus pseudocaelatus* sp. nov. 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.

Vesicles globose to subglobose, 17–22 mm in diam. Conidia globose to subglobose, echinulate, greenish, 4.5–5 µm. Isolates grow well at 25, 37 and 42 °C.

Extrolites: strains of *A. pseudocaelatus* produce aflatoxins B₁, B₂ & G₁, G₂, cyclopiazonic acid and kojic acid.
Fig. 9. *Aspergillus pseudonomius* sp. nov. 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.
Aspergillus pseudocaelatus is represented by a single isolate collected from an Arachis burkarti leaf in Argentina. It is closely related to the non-aflatoxin producing A. caelatus, and produces aflatoxins B & G, cyclopiazonic acid and kojic acid. Aspergillus caelatus isolates produce kojic acid and aspirochlorin

Aspergillus pseudonomius Varga, Samson & Frisvad, sp. nov. MycoBank MB560398. Fig. 9.

Aspergillus nomius, USA. It is related to

An overview of Aspergillus section Flavi

In this study, we used sequence data from three loci to clarify the taxonomy of this section. Based on our phylogenetic analysis of calmodulin and ITS sequence data, Aspergillus section Flavi includes 7 main clades (Figs 1–3) with 20 or more taxa. The main clades isolates form well-defined subclades on the trees based on both β-tubulin and calmodulin sequence data. However, they are represented mostly by a single isolate e.g. A. coremiiformis. A. togoensis. Further collections and studies are needed to clarify if they represent separate species.

Figures 10–12 show the colonies of the accepted species on CYA, MEA and YES which are growing all well on these media, mostly reaching a diameter of 6 cm within 7 d. However the colony colour differences are distinct allowing to recognise the less common species from the typical yellow-green colonies of A. flavus (Fig. 10 A), A. arachdicicola (Fig. 10 D), A. caelatus (Fig. 10 E), A. pseudocaelatus (Fig. 11 C) and A. parasiticus (Fig. 11 H). Other species are brown (A. tamarii Fig. 12 E) or have a less pronounced colony colours due to poor sporulation or the presence of dark sclerotia. Conidial shape and ornamentation of the species are depicted in Figs 13, 14. Conidia of species in section Flavi are mostly globose and rough to echinulate. The conidial shape of most species is globose with rough to distinct ornamentation. The conidial shape of A. togoensis and A. coremiiformis is irregularly shaped, smooth-walled and larger than those produced by other taxa in section Flavi. The conidia of A. leporis, and Petromycyes alliaceus and P. albertensis are globose but relatively small. Aspergillus avenaceus is the most basal member of the section. Isolates of these species produce very long black sclerotia and long conidiophores (Kozakiewicz 1989), and have Q-10 as their main ubiquinones (Kuraiishi et al. 1990). Samson (1979) and Kozakiewicz (1989) suggested that A. avenaceus might be related to A. alliaceus based on morphological features; however, sequence data do not support this view. Aspergillus avenaceus has been found to produce avenacioclide, a water-insoluble bis-g-lactone antibiotic which possesses antifungal activity, and is a specific inhibitor of glutamate transport in rat liver mitochondria (Brookes et al. 1963, McGivan & Chapell 1970).

Another clade includes A. leporis isolates. This species is characterised by a Q-10 ubiquinone system, conidial heads in shades of olive, and white-tipped cinnamon coloured sclerotia (Christensen 1981, Kuraishi et al. 1990). Interestingly, isolates of this species produce sclerotia on rabbit dung, but not on CYA or MEA plates (Wicklow 1985). The sclerotia of A. leporis contain the antiniectan N-alkoxypryridone metabolite, leporin A (Tepaske et al. 1991), which has been found to be effective in controlling Lepidopteran insect pests (Dowd et al. 1994).

Aspergillus coremiiformis and A. togoensis are related based on all sequence data. The species are characterised by the formation of synnemata as illustrated by the ex-type strain of A. togoensis (CBS 272.89) (Fig. 15). The close relationship of A. coremiiformis to species of section Flavi was also suggested by Samson (1979), Christensen (1981), and Roquebert & Nicot (1985) based on morphological features. The latter authors stated that “Stilbothamnium nudipes (= A. coremiiformis) differs from A. tamarii only by having septate phialides” (Roquebert & Nicot 1984). Molecular data also indicated previously that these species have affinities to section Flavi (Dupont et al. 1990, Rigo et al. 2002, Frisvad et al. 2005). The observation that an A. togoensis isolate produces sterigmatocystin, an intermediate of the aflatoxin biosynthetic pathway also indicates that this species is a member of Aspergillus section Flavi (Wicklow et al. 1989). Recently, A. togoensis was also found to be able to produce aflatoxin B, and O-methyl-sterigmatocystin (Rank et al. 2011). There are only a few isolates of A. togoensis and A. coremiiformis known and more strains should be made available to elucidate the relationship between these two taxa.

Aspergillus alliaceus together with A. lanosus and A. albertensis form another clade on all trees. Thom & Raper (1945) and Kozakiewicz (1989) assigned the A. alliaceus species to the A. wentii species group (Aspergillus section Wentii) based mainly on morphological features, while later the teleomorphic Petromycyes genus was assigned to Aspergillus section Circumdati (Gams et al. 1985, Samson 1994). Varga et al. (2000 a, b) and Frisvad & Samson (2000) found that A. lanosus, and anamorphs of Petromycyes alliaceus and P. albertensis are closely related to Aspergillus section Flavi. Aspergillus alliaceus is of world-wide distribution. This species was first identified as a wound parasite of onion bulbs (Raper & Fennell 1965), and is mainly isolated from grassland soils, nuts, and from air (Christensen & Tuthill 1985, Kozakiewicz 1989). Aspergillus alliaceus was isolated from a man’s ear swab in Canada (Tewari 1985). While A. alliaceus produces determinate ellipsoidal black stromata, A. albertensis produces indeterminate irregularly shaped grey stromata (Tewari 1985). Both A. alliaceus and A. albertensis are homothalic, and produce ascospores in ascocarps embedded in stromata after relatively long incubation period (after about 8 wk in A. alliaceus).
albertensis, and after 3–4 mo in A. alliaceus (Fennell & Warcup 1959, Tewari 1985). Ascospores were found to be smooth with a fine ridge (Tewari 1985). Sequence analyses of multiple loci indicate that A. albertensis is a synonym of A. alliaceus (Figs 1–3; Varga et al. 2000, Peterson 2000, McAlpin & Wicklow 2005, Peterson 2008). Several isolates of these species are able to produce ochratoxin A & B, and are considered to be responsible for ochratoxin contamination of figs (Varga et al. 1996, Bayman et al. 2002). Aspergillus alliaceus isolates
are also able to produce ochratoxins under “ex vivo” conditions (Klich et al. 2009). Consequently, ochratoxins were suggested to act as potential virulence factors during pathogenesis. Aspergillus alliaceus has also been encountered in human infections including ototinea (Koenig et al. 1985), invasive aspergillosis (Balajee et al. 2007) and pulmonary infection (Ozhak-Baysan et al. 2010). Aspergillus alliaceus was shown to exhibit reduced in vitro susceptibilities to amphotericin B and caspofungin (Balajee et al. 2007). Stromata of A. alliaceus strains contain compounds exhibiting insecticidal properties (Laakso et al. 1994, Nozawa et al. 1994), and asperlicins, potent cyclic peptide antagonists of cholecystokinin (Liesch et al. 1988). Aspergillus alliaceus strains are also used for steroid and alkaloid transformations (Burkhead et al. 1994, Sanchez-Gonzalez & Rosazza 2004), and for the production of pectin degrading enzyme preparations (Mikhailova et al. 1995).

Another clade includes A. nomius, A. pseudonomius and A. bombycis isolates. Aspergillus nomius and A. bombycis produce both aflatoxins B and G. A. pseudonomius produces only aflatoxin B, while none of them produce cyclopiazonic acid (Peterson et al. 2001, Table 2). Aspergillus bombycis was isolated from silkworm-rearing houses in Japan and Indonesia, while A. nomius is more widespread; it was originally isolated from mouldy wheat in the USA, and later from various substrates in India, Japan and Thailand. Aspergillus nomius is often associated with insects such as alkali bees (Hesseltine et al. 1970, Kurtzman et al. 1987) and termites (Rojas et al. 2001) and is frequently isolated from insect frass in silkworm-rearing houses in eastern Asia (Ito et al. 1998, Peterson et al. 2001). In addition soil populations in agricultural fields (Horn & Dorner 1998, Ehrlich et al. 2007) suggest that A. nomius might contribute to aflatoxin contamination of crops. Aspergillus nomius has been reported from tree nuts (Olsen et al. 2008, Doster et al. 2009), sugarcane (Kumeda et al. 2003) and an assortment of seeds and grain (Kurtzman et al. 1987, Pitt et al. 1993, Kumeda et al. 2003).

A recent study of soil samples from Thailand demonstrated that A. nomius is more widespread than may be commonly thought; it can be the predominant aflatoxin-producing Aspergillus species at certain geographic locations and must be considered a potential etiological agent of aflatoxin contamination events due to its ability to produce large quantities of aflatoxins (Ehrlich et al. 2007). For example, A. nomius accounted for > 9 % of section Flavi isolates from cornfield soils Iran (Razzaghi-Abyaneh et al. 2006). Recently, Olsen et al. (2008) have observed that A. nomius is an important producer of aflatoxins in Brazil nuts. Aspergillus nomius was recently identified from keratitis cases in India (Manikandan et al. 2009). Peterson et al. (2001) observed cryptic recombination in A. nomius populations using multilocus sequence data. Recently, Horn et al. (2010) identified the sexual state of A. nomius and named it as Petromyces nomius. An incubation period of 5 to 10 mo was needed for the formation of ascocarps within stromata. Ascospor and ascospore morphology in A. nomius were similar to that of A. flavus and A. parasiticus and differences between teleomorphs were insufficient for species separation. The majority of A. nomius strains were either MAT1-1 or MAT1-2, but several strains contained both genes. MAT1-1/MAT1-2 strains were self sterile and capable of mating with both MAT1-1 and MAT1-2 strains; hence, A. nomius appears to be functionally heterothallic (Horn et al. 2010).

Aspergillus pseudonomius has so far only been isolated from insects and soil in the USA. Aspergillus terricola isolate CBS 620.95 (=WB4855), which was Biochizit’s strain of A. luteovirescens (Raper & Fennell 1965), belongs to the A. bombycis species. Aspergillus zhaqingensis was isolated from soil in China (Sun & Qi 1991), and found to be able to produce kojic acid, aspergillus acid, aflatoxin B, and tenuazonic acid, like most strains of A. nomius (unpub. data). Molecular data indicate that A. zhaqingensis is a synonym of A. nomius (Figs 1–3). Recent data indicate that A. nomius is a paraphyletic group likely to contain several other species (Egel et al. 1994, Cotty & Cardwell 1999, Kumeda et al. 2003, Ehrlich et al. 2003, Peterson 2008, Doster et al. 2009). Based on sequence alignments for three DNA regions the A. nomius isolates could be separated into three well-supported clades (Ehrlich et al. 2007). Further studies on these clades are in progress.

The “A. tamarii” clade contains species with ubiquinone system Q-10(H2), and conidia in shades of olive to brown (Kuraishi et al. 1980, Rigó et al. 2002). This clade includes A. tamarii and its synonyms A. terricola, A. terricola var. indicus and A. flavofurcati, A. caelatus, and two aflatoxin producing species: A. pseudotamarii and A. pseudocaeatus. Aspergillus tamarii isolates are widely used
In the food industry for the production of soy sauce (known as red Awamori koji) (Jong & Birmingham 1992) and in the fermentation industry for the production of various enzymes, including amylases, proteases, and xylanolytic enzymes (Ferreira et al. 1999, Moreira et al. 2004). Recently, A. tamarii has also been identified as a cause of human keratitis in Southern India (Kredics et al. 2007), and A. tamarii spores were suggested as important sources of allergens present in the air (Vermani et al. 2010). Although A. caelatus was found to be very similar to A. tamarii morphologically, A. caelatus isolates were found not to produce cyclopiazonic acid, in contrast with A. tamarii isolates (Horn 1997, Ito et al. 1999). Aspergillus terricola and its subspecies were originally placed into section Wentii by Raper & Fennell (1965). Later A. terricola together with A. flavofurcatis and A. tamarii were placed into an “A. tamarii species group” by Kozakiewicz (1989). Sequence data indicate that these isolates belong to the same species. Aspergillus pseudotamarii (Ito et al. 2001) is an effective producer of B-type aflatoxins but the importance for mycotoxin occurrence in foods is unknown. The closely related species A. tamarii is not able to produce aflatoxins, despite several reports claiming this (Goto et al. 1996, Klich et al. 2000). Aspergillus pseudocaelatus is represented by a single isolate that came from a Arachis burkartii leaf from Argentina. This species produces both G- and B-type aflatoxins, and cyclopiazonic acid.

The “A. flavus” clade includes species characterised with Q-10(H2) as their main ubiquinone, and conidial colours in shades of green, and several isolates produce dark sclerotia. Aspergillus flavus is the most common species producing aflatoxins (Sargeant et al. 1961), occurring in most kinds of foods in tropical countries. This species is very common on maize, peanuts and cottonseed,


and produces only B-type aflatoxins. It has been estimated that only about 30–40 % of known isolates produce aflatoxin. Because of its small spores and its ability to grow at 37 °C, it can also be pathogenic to animals and humans. Infection by *A. flavus* has become the second leading cause of various forms of human aspergillosis (Hedayati et al. 2007, Pasqualotto & Denning 2008, Krishnan et al. 2009). *Aspergillus flavus* populations are genetically and phenotypically diverse (Geiser et al. 2000) with some isolates producing conidia abundantly, produce large (L) sclerotia, and variable amounts of aflatoxins, while another type produces abundant, small (S) sclerotia, fewer conidia and high levels of aflatoxins (Cotty 1989). The S-type isolates predominated in both soil and maize samples within aflatoxicosis outbreak regions, while the L strain was dominant in non-outbreak regions of Kenya (Probst et al. 2010). A related type, *A. oryzae* is atoxigenic and has been used as a source of industrial enzymes and as a koji (starter) mold for Asian fermented foods, such as sake, miso, and soy sauce (van den Broek et al. 2001). Although several lines of evidence suggest that *A. oryzae* and *A. sojae* are morphological variants of *A. flavus* and *A. parasiticus*, respectively, it was suggested that these taxa should be retained as separate species because of the regulatory confusion that conspecificity might generate in the food industry (Geiser et al. 1998b). *Aspergillus oryzae* isolates carry various mutations in the aflatoxin biosynthetic gene cluster resulting in their inability to produce aflatoxins (Tominaga et al. 2006). Particularly, the *aflR* gene is absent or significantly different in some *A. oryzae* strains compared to *A. flavus* (Lee et al. 2006). *Aspergillus oryzae* strains can be classified into three groups according to the structure of the aflatoxin biosynthesis gene cluster (Tominaga et al. 2006). Group 1 includes strains which have all aflatoxin biosynthesis gene orthologs, group 2 has the region beyond the *ver1* gene deleted, and group 3 has the partial aflatoxin gene cluster up to the *vbs* gene (Chang et al. 2009). Isolates assigned to groups 2 and 3 obviously cannot produce aflatoxins due to the loss of part of the gene cluster. Regarding group 1 isolates, the expression level of the *aflR* gene is extremely low, and no expression of several biosynthetic genes (*avnA, verB, omtA, vbs*) was observed. Recent studies clarified that amino-acid substitutions in *AflJ* gene induce inactivation at the protein level (Kiyota et al. 2011). Genome sequences of both *A. oryzae* and *A. flavus* are available (Machida et al. 2005, Chang & Ehrlich 2010, http://www.aspergillusflavus.org/genomics/). The genomes of both species are about 37 Mb and consist of 8 chromosomes. A comparative analysis of *A. oryzae* and *A. flavus* genomes revealed striking similarities between them. An
<table>
<thead>
<tr>
<th>Species</th>
<th>Occurrence</th>
<th>Extrolites produced</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. arachidicola</td>
<td>Argentina</td>
<td>Aflatoxins B&lt;sub&gt;1&lt;/sub&gt;–B&lt;sub&gt;2&lt;/sub&gt; &amp; G&lt;sub&gt;1&lt;/sub&gt;–G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergillic acid</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysogine</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ditryptophenaline</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parasiticolides</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td>A. avenaceus</td>
<td>UK, USA</td>
<td>Avenaciolide</td>
<td>Brookes et al. (1963)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirochlorine</td>
<td>This study</td>
</tr>
<tr>
<td>A. bombycis</td>
<td>Indonesia, Japan</td>
<td>Aflatoxins B&lt;sub&gt;1&lt;/sub&gt;–B&lt;sub&gt;2&lt;/sub&gt; &amp; G&lt;sub&gt;1&lt;/sub&gt;–G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Peterson et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergillic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>This study</td>
</tr>
<tr>
<td>A. caelatus</td>
<td>Japan, USA</td>
<td>Aspirochlorin</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tenuazonic acid</td>
<td>This study</td>
</tr>
<tr>
<td>A. coremiformis</td>
<td>Ivory Coast</td>
<td>Indol alkaloids (not structure elucidated)</td>
<td>This study</td>
</tr>
<tr>
<td>A. flavus</td>
<td>Worldwide</td>
<td>Aflatoxins B&lt;sub&gt;1&lt;/sub&gt; &amp; B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Varga et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aflatrem</td>
<td>Gallagher &amp; Wilson (1978)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aflavarins</td>
<td>TePaske et al. (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allavazol</td>
<td>TePaske et al. (1990)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergillic acid</td>
<td>White &amp; Hill (1943)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergilomarasmines A &amp; B</td>
<td>Haenni et al. (1965)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclopiazonic acid</td>
<td>Luk et al. (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ditryptophenaline</td>
<td>Springer et al. (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Birkinshaw et al. (1931)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Miyakamides*</td>
<td>Shiomi et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-Nitropropionic acid</td>
<td>Bush et al. (1951)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paspalinine</td>
<td>Cole et al. (1981)</td>
</tr>
<tr>
<td>A. lanosus</td>
<td>India</td>
<td>Ochratoxins A &amp; B&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Baker et al. (2003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td>A. leporis</td>
<td>USA</td>
<td>Antibiotic Y</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid,</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leporin A</td>
<td>TePaske et al. (1991)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseurotin</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td>A. miniscerotigenes</td>
<td>Argentina, Australia, Nigeria, USA</td>
<td>Aflatoxins B&lt;sub&gt;1&lt;/sub&gt;–B&lt;sub&gt;2&lt;/sub&gt; &amp; G&lt;sub&gt;1&lt;/sub&gt;–G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aflavasins</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aflatremns</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aflavinins</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergillic acid</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclopiazonic acid</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paspalinine</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td>A. nomius</td>
<td>Brazil, India, Japan, Thailand, USA</td>
<td>Aflatoxins B&lt;sub&gt;1&lt;/sub&gt;–B&lt;sub&gt;2&lt;/sub&gt; &amp; G&lt;sub&gt;1&lt;/sub&gt;–G&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Kurtzmann et al. (1987)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergillic acid</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspemomine</td>
<td>Staub et al. (1992)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nominine</td>
<td>Gloer et al. (1989)</td>
</tr>
<tr>
<td>Species</td>
<td>Occurrence</td>
<td>Extrolites produced</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>A. nomius</td>
<td></td>
<td>Paspaline</td>
<td>Staub et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseurotin</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tenuazonic acid</td>
<td>Frisvad &amp; Samson (2000)</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>China, Japan</td>
<td>Asperfuran</td>
<td>Pfefferle et al. (1990)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asperoerterin A &amp; B*</td>
<td>Matsuura et al. (1972)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirochlorin</td>
<td>Sakata et al. (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclopiazonic acid</td>
<td>Orth (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Birkinshaw et al. (1931)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojistatin*</td>
<td>Sato et al. (1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-nitropropionic acid</td>
<td>Nakamura &amp; Shimoda (1954)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sporogen AO-1*</td>
<td>Tamogami et al. (1996)</td>
</tr>
<tr>
<td>A. parasiticus</td>
<td>Australia, India, Japan, South America, Uganda USA</td>
<td>Aflatoxins B1, B2 &amp; G1, G2</td>
<td>Schroeder (1966)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergillic acid</td>
<td>Assante et al. (1981)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspersitin*</td>
<td>Hamasaki et al. (1975)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Birkinshaw et al. (1931)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parasperone and ustilaginoindin C*</td>
<td>Brown et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parasitenone*</td>
<td>Son et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parasiticolide</td>
<td>Bücki et al. (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sequoiatones*</td>
<td>Sierle et al. (1999, 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sequoiamonascins*</td>
<td>Sierle et al. (2003)</td>
</tr>
<tr>
<td>A. parvisclerotigenus</td>
<td>Nigeria</td>
<td>Aflatoxins B1, B2 &amp; G1, G2</td>
<td>Frisvad et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aflatem</td>
<td>Frisvad et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aflavarin</td>
<td>Frisvad et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirochlorin</td>
<td>Frisvad et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclopiazonic acid</td>
<td>Frisvad et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Frisvad et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paspaline</td>
<td>Frisvad et al. (2005)</td>
</tr>
<tr>
<td>A. pseudocaeltatus</td>
<td>Argentina</td>
<td>Aflatoxins B1, B2 &amp; G1, G2</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclopiazonic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>This study</td>
</tr>
<tr>
<td>A. pseudonomius</td>
<td>USA</td>
<td>Aflatoxin B1</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysogine</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>This study</td>
</tr>
<tr>
<td>A. pseudotamarni</td>
<td>Argentina, Japan</td>
<td>Aflatoxin B1, B2</td>
<td>Ito et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclopiazonic acid</td>
<td>Ito et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>This study</td>
</tr>
<tr>
<td>A. sojae</td>
<td>China, India, Japan</td>
<td>Asperfuran</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergillic acid</td>
<td>Pildain et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspirochlorin</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysogine</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>Tanaka et al. (2002)</td>
</tr>
<tr>
<td>A. tamarri</td>
<td>Worldwide (mostly warmer climates)</td>
<td>Aspirochlorin</td>
<td>Berg et al. (1976)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-)-canadensolide*</td>
<td>Berg et al. (1976)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclopiazonic acid</td>
<td>Dorner (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysogine</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kojic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fumigaclavine A*</td>
<td>Jahardhanan et al. (1984)</td>
</tr>
</tbody>
</table>
array based genome comparison found only 43 genes unique to A. flavus and 129 genes unique to A. oryzae (Georgianna & Payne 2009). A. oryzae sensu stricto has been isolated from koji fermentations used for miso, sake and other Japanese, Korean and Japanese fermented products. Sometimes the species has been reported from cereals, soil etc., and it is possible that all these isolates are just floccose variants of A. flavus. Therefore the report of aspergillomarasmmin, miyakamides, asperopterins etc. from A. oryzae, may actually be from Aspergillus flavus (see Table 2). The genome sequenced strain of A. oryzae (RIB 40) (Machida et al. 2005) was isolated from cereals and probably not from industrial settings, so it is possible that this isolate is a brownish to yellowish green spored variant of A. flavus too. Figure 16 illustrates the morphology of the ex-type strain of A. oryzae (CBS 100925) showing the typical feature of a floccose strain with less abundant sporulation. Conidiophores produce aberrant conidiogenous structures with elongated or inflated phialides and metulae. Conidia are smooth-walled and produce aberrant conidiogenous structures with elongated or inflated phialides and metulae.

Regarding the evolutionary origins of A. oryzae and A. flavus, Chang et al. (2009) suggested that, based on the genetic diversity in the region neighbouring the cyclopiazonic acid biosynthesis gene cluster, A. oryzae most likely descended from an ancestor that was the ancestor of A. miniscerotigenes or A. parvisclerotigenus producing both B- and G-type aflatoxins, while A. flavus descended from an ancestor of A. parasiticus.

Population genetic analyses of restriction site polymorphisms and DNA sequences of several genes indicated that A. flavus isolates fell into two reproducibly isolated clades (groups I and II). A lack of concordance between gene genealogies among isolates in group I suggested that A. flavus has a recombining population structure (Geiser et al. 1996, 2000). Regarding the distribution of the mating type genes in A. flavus populations, there was no significant difference in the frequency of the two mating types for A. flavus (and A. parasiticus) in either vegetative compatibility groups (VCG) or haplotype clonally corrected samples. The existence of both mating type genes in equal proportions in these populations together with the observed expression of these genes indicated the possible existence of a sexual state in A. flavus (Ramirez-Prado et al. 2008). The presence of mating type genes have also been observed in A. oryzae isolates (Chang & Ehrlich 2010). Recently the sexual stage of A. flavus has been described under the name of Petromyces flavus (Horn et al. 2009a, 2009b). However, in another study the distribution of mating type genes was uneven within an A. flavus population collected from maize fields in Southern Hungary, indicating that the given population reproduces primarily clonally (Tóth B. et al. in preparation). Indeed, population genetic analyses of molecular data confirmed that this population is a clonal one (data not shown). Sweany (2010) also observed uneven distribution of mating type genes in A. flavus isolates collected from maize with MAT1-2 being dominant (96 %), while the distribution of mating type genes was more balanced in soil isolates (48 % with MAT1-1, and 52 % with MAT1-2 idiomorphs). She also observed that the isolates belonging to different vegetative incompatibility groups of A. flavus almost exclusively carried either one or the other mating type gene (Sweany 2010). Differences between the corn and soil populations were suggested to indicate that not all soil isolates are as capable of infecting corn, and that some isolates have become specialised to infect corn.

Multilocus sequence data indicated that several species assigned to section Flavi are synonyms of A. flavus, including A. flavus var. columnaris, A. kambarenis, A. fasciculatus, A. thomii and A. subolivaceus (Figs 1–3). Although Peterson (2008) observed that A. subolivaceus formed a separate lineage distinct from A. flavus based on sequence data of two loci, it could not be distinguished by any other means from A. flavus isolates. Some of these species have also been found to be synonyms of A. flavus based on sequence analysis of part of their 18 S and 26 S rRNA genes (Nikkuni et al. 1998, Peterson 2000). Strains of A. flavus var. columnaris produce pronounced conidial columns, and most strains accumulate aflatoxin B1 only. It appears that certain mutations have induced this characteristic phenotype. The A. kambarenis, A. fasciculatus, A. thomii and A. subolivaceus ex-type strains could not produce aflatoxins, showing that aflatoxin ability can easily be lost in soil strains of A. flavus.

Many reports indicate that certain A. flavus strains, including micro-sclerotial strains, and strains listed as intermediate between A. flavus and A. parasiticus can also produce type G aflatoxins (Codd et al. 1963, Hesseltine et al. 1970, Cotty & Cardwell 1999, Begum & Samajpati 2000). One group of these isolates have been named previously as A. flavus var. parvisclerotigenus (Saito et al. 1986, Saito & Tsuruta 1993), and later raised to species status as A. parvisclerotigenus (Frisvad et al. 2005). The type strain of A. parvisclerotigenus (CBS 121.62 = NRRL A-11612 = IBT 3651 = IBT 3851) was isolated from peanut in Nigeria, and this species has

### Table 2. (Continued).

<table>
<thead>
<tr>
<th>Species</th>
<th>Occurrence</th>
<th>Extrtoles produced</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. tamani</td>
<td>Central Africa</td>
<td>Kojic acid</td>
<td>Birkinshaw et al. (1931)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Speradine A</td>
<td>Tsuda et al. (2003)</td>
</tr>
<tr>
<td>A. togoensis</td>
<td>Worldwide (Argentina, Australia, Canada, Egypt, France, Greece, Hungary, Lybia, Mexico, Netherlands, New Zealand, Russia, Saudi Arabia, Spain, Tunisia, Turkey, UK, USA)</td>
<td>Aflatoxin B1, Sterigmatocystin</td>
<td>Rank et al. (2011), Wicklow et al. (1989)</td>
</tr>
<tr>
<td>A. alliaceus</td>
<td></td>
<td>Asperinics</td>
<td>Liesz et al. (1985)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isokotanins</td>
<td>Laakso et al. (1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nomine</td>
<td>Laakso et al. (1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ochratoxin A &amp; B</td>
<td>Ciegler (1972)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paspaline</td>
<td>Laakso et al. (1994)</td>
</tr>
</tbody>
</table>

*We did not detect these compounds in any strains examined in this study.*
also been identified in grain samples came from Nigeria and Ghana (Perrone et al. 2009).

Another group of A. flavus-related isolates producing both B- and G-type aflatoxins has also been described as A. minisclerotigenes. This species was originally isolated from Argentinean peanuts and had small sclerotia and produced aflatoxins B₁, B₂, G₁, G₂, aspergillic acid, cyclopiazonic acid, kojic acid, parasiticolides and several other extrolites (Pildain et al. 2008, Table 2). One of the strains
listed by Hesseltine et al. (1970), NRRL A-11611 = NRRL 6444 also produced aflatoxins B₁, B₂, G₁ and G₂, aflatrem, aflavinines, aspergillic acid, cyclopiazonic acid, parasiticolides, kojic acid, aspergillic acid, paspaline, paspalinine and eminole SB and is an A. minisclerotigenes. Aspergillus parvisclerotigenus has an extrolite profile very similar to that of A. minisclerotigenes, but in contrast
with the Argentinean strains, it also produces parasiticolides, and compound A 30461 (aspirochlorin = oryzachlorin; Table 2). Based on the molecular studies, \textit{A. minisclerotigenes} seems to be quite widespread occurring in Argentina, USA, Nigeria and Australia as well (Pildain et al. 2008). Recently, Damann et al. (2010) observed sexual recombination between compatible partners of Australian isolates assigned to \textit{A. flavus} groups I and II by Geiser et al. (1998). Further studies are needed to clarify the significance of these findings.

A third group of microsclerotial strains, represented by NRRL 3251, actually produces only B-type aflatoxins, but are, except being the S-type, typical \textit{A. flavus}. Even though most strains of \textit{A. flavus} produce large sclerotia, a smaller number of strains can produce small sclerotia. Thus at least three taxa can produce small sclerotia.

Many other isolates producing both aflatoxins B and G and bearing small sclerotia have been reported to date (Bayman & Cotty 1993, Saito & Tsurota 1993, Egel 1994, Pitt & Hocking 2006, Dorner 2008). Isolates came from maize, almond and cocoa beans and assigned to \textit{A. flavus} based on either morphological or ITS sequence data have also been found to belong to different chemotypes based on their abilities to produce aflatoxins B$_1$, B$_2$, aflatoxin G$_1$, G$_2$, and cyclopiazonic acid (Razzaghi-Abyaneh et al. 2006, Giorni et al. 2007, Sanchez-Hervas et al. 2008, Rodrigues et al. 2009). Recently, Donner et al. (2009) found that about 8 % of the \textit{Aspergillus} section \textit{Flavi} isolates collected in maize fields in Nigeria produce small sclerotia and both B- and G-type aflatoxins. These isolates which presumably belong to \textit{A. minisclerotigenes} together with \textit{A. parasiticus} were suggested to be the greatest contributors to aflatoxin contamination of maize in regions where they occurred (Donner et al. 2009). Further studies are necessary to assign these isolates to species.

Another important aflatoxin producer, \textit{Aspergillus parasiticus} occurs rather commonly in peanuts, and almonds (Rodrigues et al. 2009), but is apparently quite rare in other foods (e.g. on dried figs; Oktay et al. 2009). It is more restricted geographically as compared to \textit{A. flavus}. \textit{Aspergillus parasiticus} produces both B- and G-type aflatoxins (Sargeant et al. 1983), and virtually all known isolates are toxigenic. Linkage disequilibrium analyses of variation across 21 intergenic regions also revealed several distinct recombination blocks in \textit{A. parasiticus}, and recombination events have also been observed between different vegetative compatibility groups (Carbone et al. 2007). The even distribution of the mating type genes resulted primarily from an early termination point mutation in the pathway-specific \textit{AflR} regulatory gene, which causes the truncation of the transcriptional activation domain of \textit{AflR} and the abolishment of interaction between \textit{AflR} and the \textit{AflJ} co-activator. In addition, a defect in the polyketide synthase gene also contributes to its nonaflatoxicigenicity (Chang et al. 2007). Recently, Garber et al. (2010) identified \textit{A. parasiticus} lineages associated with maize and peanut cultivation in USA, Asia and Africa, and a presumably new species with an ancient, global and almost exclusive association with sugarcane (Saccharum sp.). Again a soil-borne form of \textit{A. parasiticus}, \textit{A. terricola} var. \textit{americanus}, and the domesticated forms (\textit{A. sojae}) cannot produce aflatoxins similar to the examples in \textit{A. flavus}.

\textit{Aspergillus arachidicola} was isolated from leaves of \textit{Arachis hypogaea} in Argentina, and produce aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$, aspergillic acid, chrysogine, asporoclin, parasalicilide, dityrophalenine and the extrortile NO2. All strains had a floccose colony texture, a conidium colour similar to \textit{A. flavus} but, except for the production of chrysogine by most isolates, they exhibited extrortile profiles similar to those of \textit{A. parasiticus} isolates (Pildain et al. 2008, Table 2).

Aflatoxins have been shown to be produced by \textit{A. flavus}, \textit{A. parasiticus} (Codner et al. 1963, Schroeder 1966), \textit{A. nomius} (Kurtzman et al. 1987), \textit{A. pseudotomamii} (Ito et al. 2001), \textit{A. bombycis} (Peterson et al. 2001), \textit{A. toxicarius} (Murakami 1971, Murakami et al. 1982, Frisvad et al. 2005), \textit{A. parvisclerotigenus} (Salt & Tsuruta 1993, Frisvad et al. 2005), \textit{A. minisclerotigenes}, \textit{A. arachidicola} (Pildain et al. 2007) and \textit{A. pseudonomius} and \textit{A. pseudocaelatus} in \textit{Aspergillus} section \textit{Flavi}. Aflatoxin-producing species are scattered throughout the phylogenetic trees indicating that aflatoxin-producing ability was lost (or gained) several times during evolution.
ACKNOWLEDGEMENTS


