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

**J. Varga**1,2*, 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 biserial 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 methods (morphological parameters, including colony diameter, colour and texture, size and texture of conidia and conidiophore 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; Klösch 2002). However, species classification may be difficult due to extensive divergence of morphological characters produced by a high level of genetic variability (Kumeda & Asao 1996). Despite intense investigation, the taxonomy of this group of fungi is still highly complex. Recent data indicate that several of the species assigned to section *Flavi* cannot be distinguished based on morphological features alone (Frisvad et al. 2005, Pildain et al. 2008). Recently, a six-step molecular strategy using real-time PCR, RAPD and Smal digestion of the nuclear DNA has 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 rDNA 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.

**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.

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**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.

| Name               | Isolate         | Source                                      |
|--------------------|-----------------|---------------------------------------------|
| A. albertensis     | NRRL 20602T = ATCC 58745 | Human ear, Alberta, Canada                  |
| A. alliaceus       | CBS 542.65T = NRRL 4181 | Soil, Australia                             |
|                    | CBS 536.65       | Dead blister beetle Macrobasis albida, Washington, CO, USA |
|                    | CBS 612.78 = NRRL 5181 | Buenos Aires, Argentina                      |
| A. arachidica      | CBS 117610T = IBT 25020 | Arachis glabrata leaf, CO, Argentina        |
|                    | CBS 117615 = IBT 27178 | Arachis glabrata leaf, CO, Argentina        |
| A. avenaceus       | CBS 109.46T = IBT 4376 | Pisum sativum seed, UK                     |
|                    | CBS 102.45       | NCTC 6548                                   |
| A. bombycis        | CBS 117187 = NRRL 26010T | Frass in a silkworm rearing house, Japan    |
| A. caelatus        | CBS 763.97T = NRRL 25528 | Soil, USA                                  |
|                    | CBS 764.97 = NRRL 25404 | Soil, USA                                  |
| A. coremiiformis   | CBS 553.77T = NRRL 13756 | Soil, Ivory Coast                           |
| A. fasciculatus    | CBS 110.55T      | Air contaminant, Brazil                     |
| A. flaviformis     | CBS 498.65T      | Air contaminant, Brazil                     |
| A. flavus          | CBS 100927T      | Cellophane, South Pacific Islands           |
|                    | CBS 116.48       | Unknown source, the Netherlands             |
|                    | CBS 616.94       | Man, orbital tumor, Germany                 |
| A. flavus var. columnaris | CBS 498.65T | Butter, Japan                                |
|                    | CBS 117731       | Dipodomys spectabilis cheek pouch, New Mexico, USA |
| A. kambrensis      | CBS 542.69T      | Stratigraphic core sample, Japan            |
| A. lanosus         | CBS 650.74T      | Soil under Tectona grandis, Gorakhpur, India|
| A. leporis         | CBS 151.66T      | Dung of Lepus townsendoti, USA              |
|                    | CBS 349.81       | Soil, Wyoming, USA                          |
| A. minisclerotigenes | CBS 117633     | Arachis hypogaeaea seed, FO, Argentina     |
|                    | CBS 117635T = IBT 27196 | Arachis hypogaeaea seed, CD, Argentina    |
| A. nomius          | CBS 260.88T = NRRL 13137 | Wheat, USA                                  |
| A. oryzae          | CBS 100925T      | Unknown source, Japan                       |
| A. parasiticus     | CBS 100926T      | Pseudococcus calceolariae, sugar cane mealy bug, Hawaii, USA |
| A. parasiticus var. globosus | CBS 260.67T | Unknown source, Japan                             |
| A. panisclerotigenus | CBS 121.62T   | Arachis hypogaeaea, Nigeria                  |
| A. pseudocaelatus  | CBS 117616       | Arachis buniartii leaf, CO, Argentina       |
| A. pseudonomius    | CBS 119388 = NRRL 3353 | Diseased alkali bees, USA                   |
| A. pseudotamarii   | CBS 766.97T = NRRL 25517 | Soil, USA                                  |
|                    | CBS 765.97       | Soil, USA                                   |
| A. sojae           | CBS 100928T      | Soy sauce, Japan                            |
| A. subolivaceus    | CBS 501.65T      | Cotton, Lintafelt, UK                       |
| A. tamarii         | CBS 104.13T      | Activated carbon                            |
| A. terricola       | CBS 620.95       | WB4858                                       |
|                    | CBS 579.65T      | USA                                          |
| A. terricola var. americanus | CBS 580.65T | Soil, USA                                   |
|                    | CBS 119.51       | Japan                                        |
| A. terricola var. indicus | CBS 167.63T | Mouldy bread, Allahabad, India              |
| A. thomii          | CBS 120.51T      | Culture contaminant                          |
| A. togoensis       | CBS 272.89T      | Seed, Central African Republic              |
| A. toxicarius      | CBS 822.72T      | Arachis hypogaeaea, Uganda                  |
|                    | CBS 561.82       | Lüöss deposit, Nebraska, USA                |
| A. zhaoqingensis   | CBS 399.93T      | Soil, China                                 |

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.
Extrolite 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'-GGGATAGCTGTACGAGTTGTGCCAG-3') and aflR-R (5'-TGGKGGCCGACTCGAGGAAYGGGT-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 nor1R (5'-ACCGTACCGCCGACTCTCGGCA-3') and nor2L (5'-GTGGCCGCGACTTCCAGACACG-3') developed by Geisen (1996). Part of the O-methyltransferase gene (omtA, aflP; Yu et al. 2004) was amplified using the primers omt1F (5'-GGTCGCCGACCTAGTGCAGC-3') and omt2R (5'-GTCCGGCCACCGACTGGTGGG-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 (CI, RI and RC, respectively) were also calculated. Neopetromyces muciratus CBS 112808 was used as outgroup in the analyses of calmodulin, ITS and β-tubulin data sets, while A. versicolor SSCR 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 steps, consistency index: 0.7181, retention index: 0.9026). The ITS 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 burkittii 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 aspergillorin. 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 parasiticolide, dityrrophenaline 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. Hesseltine (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 were analysed, 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: 0.9051). 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. 1 (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).
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.
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.
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Table of Species:

| Species                                      | Accession | Additional Notes |
|----------------------------------------------|-----------|------------------|
| A. minisclerotigenes CBS 117616 (FJ491462)   |           |                  |
| A. oryzae BCRC 30229 (AY650941)              |           |                  |
| A. flavus BCRC 30019 (AY65036)               |           |                  |
| A. flavus var. columnaris BCRC 30433 (AY65042) |           |                  |
| A. sojae BCRC 33643 (AY650931)               |           |                  |
| A. parasiticus BCRC 30164 (AY650924)         |           |                  |
| A. parasiticus BCRC 30150 (AY65023)          |           |                  |
| A. arachidicola CBS 117610 (FJ491458)        |           |                  |
| A. parvisclerotigenus CBS 121.62 (FJ491457)  |           |                  |
| A. caelatus (CBS 763.97, CBS 764.97, NRRL 25566, NRRL 25568 and NRRL 25569) | | |
| A. arachidicola CBS 117610                    |           |                  |
| A. parvisclerotigenus CBS 121.62 (FJ491457)  |           |                  |
| A. bombycis NRRL 29236                        |           |                  |
| A. pseudotamarii NRRL 25517 (AF441428)       |           |                  |
| A. versicolor SRRC 108 (AY197609)            |           |                  |

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.

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.

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.

Aspergillus caealato morphologie 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. 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.
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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.

Extralites: strains of A. pseudocaelatus produce aflatoxins B₁, B₂ & G₁, G₂, cyclopiazonic acid and kojic acid.
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 nomio morphologicae valde similis, sed aflatoxinum B1 (neque aflatoxina typi G), chrysoginum 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 floccose with dominant aerial mycelium with poor sporulation. Reverse not coloured. Sclerotia not observed. Conidial heads uniseriate. Stipes hyaline, smooth, variable in length, mostly (250–)400–600(21000) μm; diam just below vesicles 5–8 mm. Vesicles globose to subglobose, 15–30 μm in diam, fertile upper 75 % of their surface; Conidia globose to subglobose, echinulate, greenish, 4–5 μm. Isolates grow well at 25, 37 and 42 °C.

Extrolites: strains of *A. pseudonomius* produce aflatoxin B1, chrysogine and kojic acid.

Typos: Argentina, Corrientes province; isolated from an *Arachis burkarti* leaf, isolated by B. Pildain (CBS H-20632 — holotypus, culture ex-type CBS 117616).

Aspergillus avenaceus is the most basal member of the section. Isolates of this species produce very long black sclerotia and long conidiophores (Kozakiewicz 1989), and have Q-10 as the antiinsectan N-alkoxypyridone 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 “Stilbothammium nudipes (= *A. coremiiformis*) differs from *A. tamarii* only by having septeal 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 B1 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. albentensis* 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 teleomorph *Petromyces* genus was assigned to *Aspergillus* section *Circumdati* (Gams et al. 1985, Samson 1994). Varga et al. (2000a, b) and Frisvad & Samson (2000) found that *A. lanosus*, and anamorphs of *Petromyces 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 albertensis* 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 homothallic, and produce ascospores in ascosporas embedded in stromata after relatively long incubation period (after about 8 wk in *A. albertensis*, shaped, smooth-walled and larger than those produced by other taxa in section *Flavi*. The conidia of *A. leporis*, and *Petromyces alliaceus* and *P. albertensis* are globose but relatively small.

Another clad 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 antiseptic N-alkoxypridone metabolite, leporin A (Tepaske et al. 1991), which has been found to be effective in controlling Lepidopteran insect pests (Dowd et al. 1994).
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 otorrhoea (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 B₁, 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 & Domer 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. Ascocarp 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 (=WB4858), which was Blochwitz’s strain of A. terricola (Raper & Fennell 1965), belongs to the A. bombycis species. Aspergillus zhaogingensis was isolated from soil in China (Sun & Qi 1991), and found to be able to produce kojic acid, aspergillic acid, aflatoxin B₂ and tenuazonic acid, like most strains of A. nomius (unpubl. data). Molecular data indicate that A. zhaogingensis is a synonym of A. nomius (Figs 1–3). Recent data indicate that A. nomius is a paralytic 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. 1990, Rigó et al. 2002). This clade includes A. tamarii and its synonyms A. terricola, A. terricola var. indicus and A. flavofoecatus, A. caelatus, and two aflatoxin producing species: A. pseudotamarii and A. pseudocaelatus. Aspergillus tamarii isolates are widely used

**Fig. 12.** Colonies of the various species of section Flavi on CYA, MEA and YES (7 d at 25 °C). A. Aspergillus nomius 260.88, B. A. pseudonomius C. A. fogoensis 272.89, D. Petromyces alliaceus 110.26, E. A. tamarii 104.13, F. P. albicans ATCC 55745.
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. flavofurcatus 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 an 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 has 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
| Species                  | Occurrence          | Extrolites produced                                                                 | Reference                          |
|--------------------------|---------------------|--------------------------------------------------------------------------------------|------------------------------------|
| *A. arachidicola*        | Argentina           | Aflatoxins B<sub>1</sub>, B<sub>2</sub> & G<sub>1</sub>, G<sub>2</sub>                | Pildain et al. (2008)              |
|                          |                     | Aspergillic acid                                                                     | Pildain et al. (2008)              |
|                          |                     | Chrysogine                                                                           | Pildain et al. (2008)              |
|                          |                     | Ditryptophenaline                                                                   | This study                         |
|                          |                     | Kojic acid                                                                           | Pildain et al. (2008)              |
|                          |                     | Parasiticolides                                                                      | Pildain et al. (2008)              |
| *A. avenaceus*           | UK, USA             | Avenaciolide                                                                         | Brookes et al. (1963)              |
|                          |                     | Aspirochlorine                                                                       | This study                         |
| *A. bombycis*            | Indonesia, Japan    | Aflatoxins B<sub>1</sub>, B<sub>2</sub> & G<sub>1</sub>, G<sub>2</sub>                | Peterson et al. (2001)             |
|                          |                     | Aspergillic acid                                                                     | This study                         |
|                          |                     | Kojic acid                                                                           | This study                         |
| *A. caelatus*            | Japan, USA          | Aspirochlorin                                                                        | Pildain et al. (2008)              |
|                          |                     | Kojic acid                                                                           | Frisvad & Samson (2000)            |
|                          |                     | Tenuazonic acid                                                                       | This study                         |
| *A. coremiformis*        | Ivory Coast         | Indol alkaloids (not structure elucidated)                                           | This study                         |
| *A. flavus*              | Worldwide           | Aflatoxins B<sub>1</sub>, B<sub>2</sub>                                              | Varga et al. (2009)                |
|                          |                     | Aflatrem                                                                              | Gallagher & Wilson (1978)          |
|                          |                     | Aflavarins                                                                           | TePaske et al. (1992)              |
|                          |                     | Allavazol                                                                             | TePaske et al. (1990)              |
|                          |                     | Aspergillic acid                                                                      | White & Hill (1943)                |
|                          |                     | Aspergillomarasmines A & B                                                            | Haenni et al. (1965)               |
|                          |                     | Cyclopiazonic acid                                                                   | Luk et al. (1977)                  |
|                          |                     | Ditryptophenaline                                                                    | Springer et al. (1977)             |
|                          |                     | Kojic acid                                                                           | Birkinshaw et al. (1931)           |
|                          |                     | Miyakamides*                                                                         | Shioji et al. (2002)               |
|                          |                     | 3-Nitropropionic acid                                                                 | Bush et al. (1951)                 |
|                          |                     | Paspalinine                                                                          | Cole et al. (1981)                 |
| *A. lanosus*             | India               | Ochratoxins A & B*                                                                    | Baker et al. (2003)                |
|                          |                     | Griseofulvin                                                                          | Frisvad & Samson (2000)            |
|                          |                     | Kojic acid                                                                           | Frisvad & Samson (2000)            |
| *A. leporis*             | USA                 | Antibiotic Y                                                                         | Frisvad & Samson (2000)            |
|                          |                     | Kojic acid, Leporin A                                                                 | Frisvad & Samson (2000)            |
|                          |                     | Pseurotin                                                                            | TePaske et al. (1991)              |
| *A. miniisclerotigenes*  | Argentina, Australia, Nigeria, USA         | Aflatoxins B<sub>1</sub>, B<sub>2</sub> & G<sub>1</sub>, G<sub>2</sub>                | Pildain et al. (2008)              |
|                          |                     | Aflavarins                                                                           | Pildain et al. (2008)              |
|                          |                     | Allatrem                                                                              | Pildain et al. (2008)              |
|                          |                     | Aflavinins                                                                           | Pildain et al. (2008)              |
|                          |                     | Aspergillic acid                                                                      | Pildain et al. (2008)              |
|                          |                     | Cyclopiazonic acid                                                                   | Pildain et al. (2008)              |
|                          |                     | Paspalinine                                                                           | Pildain et al. (2008)              |
| *A. nomius*              | Brazil, India, Japan, Thailand, USA        | Aflatoxins B<sub>1</sub>, B<sub>2</sub> & G<sub>1</sub>, G<sub>2</sub>                | Kurtzmann et al. (1987)             |
|                          |                     | Aspergillic acid                                                                      | Frisvad & Samson (2000)            |
|                          |                     | Aspemomine                                                                           | Staub et al. (1992)                |
|                          |                     | Kojic acid                                                                           | Frisvad & Samson (2000)            |
|                          |                     | Nominine                                                                             | Gloer et al. (1989)                |
**Table 2. (Continued).**

| Species | Occurrence | Extrolites produced | Reference |
|---------|------------|---------------------|-----------|
| A. nomius | | Paspaline | Staub et al. (1993) |
| | | Pseurotin | Frisvad & Samson (2000) |
| | | Tenuazonic acid | Frisvad & Samson (2000) |
| A. oryzae | China, Japan | Asperfuran | Pfefferle et al. (1990) |
| | | Asperoeterin A & B* | Matsuura et al. (1972) |
| | | Asperichlorin | Sakata et al. (1983) |
| | | Cyclopiazonic acid | Orth (1977) |
| | | Kojic acid | Birkinshaw et al. (1931) |
| | | Kojistatin* | Sato et al. (1996) |
| | | 3-nitropropionic acid | Nakamura & Shimoda (1954) |
| | | Sporogen AO-1* | Nonoka et al. (1997) |
| | | TMC-2A, B, C* | Asai et al. (1998) |
| A. parasiticus | Australia, India, Japan, South America, Uganda USA | Aflatoxins B₁,B₂ & G₁,G₂ | Schroeder (1966) |
| | | Aspergillic acid | Assante et al. (1981) |
| | | Aspersitin* | Hamasaki et al. (1975) |
| | | Kojic acid | Birkinshaw et al. (1931) |
| | | Parasperone and ustilaginoïdine C* | Brown et al. (1993) |
| | | Parasitenone* | Son et al. (2002) |
| | | Parasiticolide | Büchi et al. (1983) |
| | | Sequoiatones* | Sierle et al. (1999, 2001) |
| | | Sequioamonsins* | Sierle et al. (2003) |
| A. parvisclerotigenus | Nigeria | Aflatoxins B₁,B₂ & G₁,G₂ | Frisvad et al. (2005) |
| | | Aflatrem | Frisvad et al. (2005) |
| | | Aflavorin | Frisvad et al. (2005) |
| | | Asperochlorin | Frisvad et al. (2005) |
| | | Cyclopiazonic acid | Frisvad et al. (2005) |
| | | Kojic acid | Frisvad et al. (2005) |
| | | Paspaline | Frisvad et al. (2005) |
| A. pseudocaelatus | Argentina | Aflatoxins B₁,B₂ & G₁,G₂ | This study |
| | | Cyclopiazonic acid | This study |
| | | Kojic acid | This study |
| A. pseudonomius | USA | Aflatoxin B₁ | This study |
| | | Chrysogine | This study |
| | | Kojic acid | This study |
| A. pseudotamarii | Argentina, Japan | Aflatoxin B₁,B₂ | Ito et al. (2001) |
| | | Cyclopiazonic acid | Ito et al. (2001) |
| | | Kojic acid | This study |
| A. sojae | China, India, Japan | Asperfuran | This study |
| | | Aspergillic acid | Pildain et al. (2008) |
| | | Asperochlorin | This study |
| | | Chrysogine | This study |
| | | Kojic acid | Tanaka et al. (2002) |
| A. tamarii | Worldwide (mostly warmer climates) | Asperochlorin | Berg et al. (1976) |
| | | (-)-canadensolide* | Berg et al. (1976) |
| | | Cyclopiazonic acid | Dorner (1983) |
| | | Fumigaclavine A* | Jahardhan et al. (1984) |
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 aspergillomarasmin, 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*. 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 floccose strain with less abundant sporulation. Conidiophores of *RIB 40* are typical bisteriate with regular shaped conidiogenous structures producing globose, smooth to finely roughened conidia. Phenotypically these two strains are distinct and it would be recommendable to genome sequence an *Aspergillus oryzae* strain used for koji fermentation also, for example the ex-type culture.

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. minisclerotigenes* 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 reproductively 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. 1998, 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 clone-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 *Petricymes 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 idiormorphs). 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. kambaresis*, *A. fusciculatus*, *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. kambaresis*, *A. fusciculatus*, *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 3851 = IBT 3851) was isolated from peanut in Nigeria, and this species has

### Table 2. (Continued).

| Species       | Occurrence                  | Extrolites produced                  | Reference                        |
|---------------|-----------------------------|--------------------------------------|----------------------------------|
| *A. tamarii*  | Central Africa              | Kojic acid                           | Birkinshaw et al. (1931)         |
| *A. togoensis*| Worldwide                   | Speradine A                          | Tsuda et al. (2003)              |
| *A. alliaceus*|                             | Aflatoxin B$_1$                       | Rank et al. (2011)               |
|               |                             | Sterigmatocystin                     | Wicklow et al. (1989)            |
|               |                             | Asperclins                           | Liesz et al. (1985)              |
|               |                             | Isokotanins                          | Laakso et al. (1994)             |
|               |                             | Nominine                             | Laakso et al. (1994)             |
|               |                             | Ochratoxin A & B                     | Ciegler (1972)                   |
|               |                             | Paspaline                            | Laakso et al. (1994)             |

*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 B$_1$, B$_2$, G$_1$, and G$_2$, aflatrem, aflavinines, aspergillic acid, cyclopiazonic acid, parasilicolides, kojic acid, aspergillic acid, paspaline, paspalinines 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, A. miniscerotigenes 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 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 A. flavus. Even though most strains of 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 1993, Frisvad et al. 2005). Isolates came from maize, almond and cocoa beans and assigned to 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 B1, B2, aflatoxin G1, G2, 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 Aspergillus section Flavi isolates collected in maize fields in Nigeria produce small sclerotia and both B- and G-type aflatoxins. These isolates which presumably belong to A. miniscerotigenes together with 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, 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 A. flavus. 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 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 in A. parasiticus populations was also indicative of the presence of a cryptic sexual stage (Ramirez-Prado et al. 2008). Recently, crosses between strains carrying opposite mating-type genes resulted in the development of ascospore-bearing ascocarps embedded within stromata. Sexually compatible strains belonged to different vegetative compatibility groups (Horn et al. 2009b). The sexual state of A. parasiticus has been described as Petromyces parasiticus (Horn et al. 2009c).

Nontoxigenic A. flavus and A. parasiticus isolates are used to control aflatoxin levels in various agricultural products. Great success in reducing aflatoxin contamination have been achieved by application of nontoxicogenic strains of A. flavus and A. parasiticus in fields of cotton, peanut, maize and pistachio (Brown et al. 1991, Pitt & Hocking 2006, Dorner 2008). Significant reductions in aflatoxin contamination in the range of 70%–90 % have been observed consistently by the use of nontoxicogenic A. flavus and A. parasiticus strains (Pitt & Hocking 2006, Dorner 2008, Yin et al. 2008). Actually, two products of nontoxicogenic strains have received U.S. Environmental Protection Agency (EPA) registration as biopesticides to control aflatoxin contamination in cotton and peanuts in several states of USA (Dorner 2008). This strategy is based on the application of nontoxigenic strains to competitively exclude naturally toxigenic strains in the same niche and compete for crop substrates. However, the discovery of a sexual cycle in A. flavus and in A. parasiticus raised concerns about the safety of these products. Indeed, Olarte et al. (2010) found that a single generation of sexual reproduction between a nonaflatoxigenic A. flavus isolate containing a single mutation in the aflatoxin biosynthesis gene cluster and an aflatoxigenic parent can restore aflatoxin production due to a crossing over within the aflatoxin biosynthesis gene cluster. In other crosses involving strains with either a partial aflatoxin gene cluster or strains missing the entire cluster and an aflatoxigenic A. flavus strain also regained toxicity via independent assortment of chromosomes, questioning the safety of using non-aflatoxigenic A. flavus or A. parasiticus strains for lowering aflatoxin levels in agricultural products. Aspergillus toxicarius, which also produces B- and G-type aflatoxins (Murakami et al. 1966, Murakami 1971), was suggested to be conspecific with A. parasiticus by Kozakiewicz (1989), which view is supported by the sequence data. Aspergillus terricola var. americanus (which does not produce aflatoxins!) and A. parasiticus var. globosus (which produces all the known aflatoxins) could also not be distinguished from A. parasiticus by neither phylogenetic analysis of multilocus sequence data nor by extrolite profiles indicating that these are also synonyms of A. parasiticus (Figs 1–3). Aspergillus sojae is the domesticated variety of A. parasiticus, which can hardly be distinguished from it apart from its inability to produce aflatoxins (Rigò et al. 2002, Chang et al. 2007). The lack of aflatoxin-producing ability of some A. sojae isolates results primarily from an early termination point mutation in the pathway-specific AfiR regulatory gene, which causes the truncation of the transcriptional activation domain of AfiR and the abolishment of interaction between AfiR and the AfiJ co-activator. In addition, a defect in the polyketide synthase gene also contributes to its nonaflatoxigenicity (Chang et al. 2007). Recently, Garber et al. (2010) identified 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 A. parasiticus, A. terricola var. americanus, and the domesticated forms (A. sojae) cannot produce aflatoxins similar to the examples in A. flavus.

Aspergillus arachidcola was isolated from leaves of Arachis glabrata in Argentina, and produce aflatoxins B1, B2, G1, and G2, aspergillaric acid, chrysogine, asperilicin, parasilicolidine, diftyrophane and the extentrile NO2. All strains had a floccose colony texture, a conidium colour similar to A. flavus but, except for the production of chrysogine by most isolates, they exhibited extrolite profiles similar to those of A. parasiticus isolates (Pildain et al. 2008, Table 2).

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