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New taxa in *Aspergillus* section *Usti*

R.A. Samson*,†*, J. Varga*,‡*, M. Meijer* and J.C. Frisvad

*CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, NL-3584 CT Utrecht, the Netherlands; ‡Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Kizülp fasor 52, Hungary; BioCentrum-DTU, Building 221, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

*Correspondence: Robert A. Samson, r.samson@CBS.knaw.nl

Abstract: Based on phylogenetic analysis of sequence data, *Aspergillus* section *Usti* includes 21 species, including two teleomorphic species *Aspergillus heterothallicus* (= *Emericella heterothallica*) and *Fennelia monodi*. *Aspergillus germanicus* sp. nov. was isolated from indoor air in Germany. This species has identical ITS sequences with *A. insuetus* CBS 119.27, but is clearly distinct from that species based on β-tubulin and calmodulin sequence data. This species is unable to grow at 37 °C, similarly to *A. keveii* and *A. insuetus*. *Aspergillus carlsbadensis* sp. nov. was isolated from the Carlsbad Caverns National Park in New Mexico. This taxon is related to, but distinct from a clade including *A. caudatus, A. pseudodefectus, A. insuetus* and *A. keveii* on all trees. This species is also unable to grow at 37 °C, and acid production was not observed on CREA. *Aspergillus californicus* sp. nov. is proposed for an isolate from chlamise charappal (*Adinostoma fasciculatum*) in California. It is related to a clade including *A. subseissilis* and *A. kasassensis* on all trees. This species grew well at 37 °C, and acid production was not observed on CREA. The strain CBS 504.65 from soil in Turkey showed to be clearly distinct from the *A. deflectus* ex-type strain, indicating that this isolate represents a distinct species in this section. We propose the name *A. turkensis* sp. nov. for this taxon. This species grew, although rather restrictedly at 37 °C, and acid production was not observed on CREA. Isolates from stored maize, South Africa, as a culture contaminant of *A. ustus*. In the third chemical group, *E. heterothallica* has been reported to produce emethallins, 5-hydroxyaveranthin, emetheterone, emesterones, 5-hydroxyaveranthin.

Key words: Ascomycetes, *Aspergillus* section *Usti*, ITS, calmodulin, extrtoles, β-tubulin, polyphasic taxonomy.


INTRODUCTION

*Aspergillus ustus* is a common filamentous fungus found in soils, food and indoor air environments (Samson et al. 2004). This species was considered as a relatively rare human pathogen that can cause invasive infection in immunocompromised hosts (Weiss & Thiemke 1983, Stiller et al. 1994, Verweij et al. 1999, Nakai et al. 2002, Pavie et al. 2005, Panacall et al. 2006, Yildiran et al. 2006, Krishnan-Natesan et al. 2008, Florescu et al. 2008, Vageli et al. 2008). However, recent studies clarified that infections attributed to *A. ustus* are caused in most cases by another species, *A. calidoustus* (Houbraken et al. 2007, Varga et al. 2008, Balajee et al. 2009, Peláez et al. 2010). This species is also common in indoor air (Houbraken et al. 2007, Slack et al. 2009) and is able to colonise water distribution systems (Hageskal et al. 2011). Other species related to *A. ustus* can also cause human or animal infections; *A. granulosus* was found to cause disseminated infection in a cardiac transplant patient (Fahik et al. 1995), while *A. deflectus* has been reported to cause disseminated mycosis in dogs (Jang et al. 1986, Kahler et al. 1990, Robinson et al. 2000, Schultz et al. 2008, Krockenberger et al. 2011).

Raper & Fennell (1965) classified *A. ustus* to the *Aspergillus ustus* species group (*Aspergillus section Usti* according to Gams et al. 1985) together with four other species: *A. panamensis, A. puniceus, A. conjunctus* and *A. deflectus*. Later, Kozakiewicz (1989) revised the taxonomy of the group, and included *A. ustus, A. pseudodefectus, A. conjunctus, A. puniceus, A. panamensis* and *A. granulosus* in the *A. ustus* species group, and established the *A. deflectus* species group including *A. deflectus, A. pulvinus* and *A. silvaticus* based on morphological studies. Klich (1993) treated *A. granulosus* as member of section *Versiclores*, and found that *A. pseudodefectus* is only weakly related to this section based on morphological treatment of section *Versiclores*. Peterson (2000) transferred *A. conjunctus, A. fumiculosus, A. silvaticus, A. panamensis* and *A. anthodesmis* to section *Sparsi*. More recently, Peterson (2008) examined the relationships of the *Aspergillus* genus using phylogenetic analysis of sequences of four loci, and assigned 15 species to this section (see below).

We examined the evolutionary relationships among species assigned to section *Usti*. We have used a polyphasic taxonomic approach in order to determine the delimitation and variability of known and new species. For phenotypic analyses, macro- and micromorphology of the isolates was examined, and secondary...
<table>
<thead>
<tr>
<th>Species</th>
<th>Strain No.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. amylovorus</td>
<td>CBS 600.67T = NRRL 5813 = IMI 129961 = VKM F-906 = IBT 23158</td>
<td>Wheat starch, Ukraine</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>CBS 112452</td>
<td>Indoor air, Germany</td>
</tr>
<tr>
<td></td>
<td>CBS 113228</td>
<td>ATCC 38849; IBT 13091</td>
</tr>
<tr>
<td></td>
<td>CBS 114380</td>
<td>Wooden construction material, Finland</td>
</tr>
<tr>
<td></td>
<td>CBS 121601; 677</td>
<td>Bronchoalveolar lavage fluid, proven invasive aspergillosis, Nijmegen, the Netherlands</td>
</tr>
<tr>
<td></td>
<td>CBS 121610; 91</td>
<td>Post-cataract surgery endophthalmitis, Turkey</td>
</tr>
<tr>
<td>A. californicus</td>
<td>CBS 123895T = IBT 16748</td>
<td>Ex chamise chaparral (Adenostoma fasciculatum), in the foothills of the San Gabriel Mountains on Baldy Mountain Road near Shinn Road Intersection, North of Claremont and near San Antonio Dam, California, USA, Jeff S. La Favre, 1978. A wildfire occurred here 31/8 1975.</td>
</tr>
<tr>
<td>A. carlsbadensis</td>
<td>CBS 123893 = IBT 16753</td>
<td>Soil, Galapagos Islands, Ecuador</td>
</tr>
<tr>
<td></td>
<td>CBS 123894T = IBT 14493</td>
<td>Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico, USA, D.E. Northup, 1992</td>
</tr>
<tr>
<td>A. cavernicola</td>
<td>CBS 117.76T = NRRL 6327</td>
<td>Soil, cave wall, Romania</td>
</tr>
<tr>
<td>A. deflectus</td>
<td>CBS 109.55T = NRRL 2206 = IBT 24665</td>
<td>Soil, Rio de Janeiro, Brazil</td>
</tr>
<tr>
<td></td>
<td>NRRL 4235 = IBT 25291</td>
<td>Potting soil</td>
</tr>
<tr>
<td></td>
<td>NRRL 13131 = IBT 25254</td>
<td>Unknown</td>
</tr>
<tr>
<td>A. egyptiacus</td>
<td>CBS 123892 = IBT 16345 = RMF 9515</td>
<td>Soil, Iraq</td>
</tr>
<tr>
<td></td>
<td>CBS 656.73T = NRRL 5920</td>
<td>Sandy soil, under Olea europaea, Ras-El-Hikma, Egypt</td>
</tr>
<tr>
<td></td>
<td>CBS 991.72C</td>
<td>Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt</td>
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<tr>
<td></td>
<td>CBS 991.72A</td>
<td>Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt</td>
</tr>
<tr>
<td></td>
<td>CBS 991.72B</td>
<td>Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt</td>
</tr>
<tr>
<td></td>
<td>CBS 991.72F</td>
<td>Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt</td>
</tr>
<tr>
<td></td>
<td>CBS 991.72E</td>
<td>Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt</td>
</tr>
<tr>
<td>A. elongatus</td>
<td>CBS 387.75T = NRRL 5176</td>
<td>Alkaline Usar soil, Lucknow, India</td>
</tr>
<tr>
<td>A. germanicus</td>
<td>CBS 123887T = DTO 27-D9 = IBT 29365</td>
<td>Indoor air, Stuttgart, Germany</td>
</tr>
<tr>
<td>A. granulosus</td>
<td>CBS 588.65T</td>
<td>Soil, Fayetteville, Arkansas, USA</td>
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<tr>
<td></td>
<td>CBS 119.58</td>
<td>Soil, Texas, USA</td>
</tr>
<tr>
<td>A. heterothallicus</td>
<td>CBS 489.65T</td>
<td>Soil, Costa Rica</td>
</tr>
<tr>
<td></td>
<td>CBS 488.65</td>
<td>Soil, Costa Rica</td>
</tr>
<tr>
<td>A. insuetus</td>
<td>CBS 107.25T = NRRL 279</td>
<td>South Africa</td>
</tr>
<tr>
<td></td>
<td>CBS 119.27 = NRRL 4876</td>
<td>Soil, Iowa, USA</td>
</tr>
<tr>
<td></td>
<td>CBS 102278</td>
<td>Subcutaneous infection, Spain</td>
</tr>
<tr>
<td>A. kassunensis</td>
<td>CBS 419.69T = NRRL 3752 = IMI 334938 = IBT 23479</td>
<td>Soil, Damascus, Syria</td>
</tr>
<tr>
<td>A. kevei</td>
<td>CBS 209.92</td>
<td>Soil, La Palma, Spain</td>
</tr>
<tr>
<td></td>
<td>CBS 561.65 = NRRL 1974</td>
<td>Soil, Panama</td>
</tr>
<tr>
<td></td>
<td>IBT 10524 = CBS 113227 = NRRL 1254</td>
<td>Soil, Panama</td>
</tr>
<tr>
<td></td>
<td>IBT 16751</td>
<td>Soil at trail from Pelican Bay to inland, Isla Santa Cruz, Galapagos Islands, Ecuador, Tijte de Vries and D.P. Mahoney, 1968</td>
</tr>
<tr>
<td>A. lucknowensis</td>
<td>CBS 449.75T = NRRL 3491</td>
<td>Alkaline Usar soil, Lucknow, India</td>
</tr>
<tr>
<td>A. monodii</td>
<td>CBS 434.93</td>
<td>Dung of Procavia sp. (daman), Darfur, Sudan</td>
</tr>
<tr>
<td></td>
<td>CBS 435.93T</td>
<td>Dung of sheep, Ennedi, Chad</td>
</tr>
<tr>
<td>A. pseudodeflectus</td>
<td>CBS 596.65</td>
<td>Sugar, USA, Louisiana</td>
</tr>
<tr>
<td></td>
<td>CBS 756.74T</td>
<td>Desert soil, Egypt, Western Desert</td>
</tr>
<tr>
<td></td>
<td>NRRL 4846 = IBT 25256</td>
<td>Unknown</td>
</tr>
<tr>
<td>A. pseudostaurus</td>
<td>ATCC 36063 = NRRL 5856 = CSIR 1128 = CBS 123904T = IBT 28161</td>
<td>Stored maize, South Africa</td>
</tr>
<tr>
<td></td>
<td>MRC 096 = IBT 31044</td>
<td>Contaminant in a Bipolaris sorokiniana strain (MRC 093), South Africa</td>
</tr>
</tbody>
</table>
metabolite profiles were studied. For genotypic studies, partial sequences of the β-tubulin and calmodulin genes and the ITS region of the rRNA gene cluster were analysed.

MATERIALS AND METHODS

Isolates

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

Morphological analysis

For macromorphological observations, Czapek Yeast Autolysate (CYA), Malt Extract Autolysate (MEA) agar, Yeast Extract Sucrose Agar (YES), Creatine Agar (CREA), and Oatmeal Agar (OA) were used (Samson et al. 2004). 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.

Extralite analysis

The isolates were grown on CYA and YES at 25 °C for 7 d. Extralites were extracted after incubation. Five plugs of each agar medium were taken and pooled together into same vial for extraction with 0.75 mL of a mixture of ethyl acetate/dichloromethane/methanol (3:2:1) (v/v/v) with 1 % (v/v) formic acid. The extracts were filtered and analysed by HPLC using alkylphenone retention indices and diode array UV-VIS detection as described by Frisvad & Thrane (1987), 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 (Houbraken et al. 2007, Varga et al. 2007, 2008).

Data analysis

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, 1 000 bootstrap replicates were run by maximum parsimony (Hillis & Bull 1993). Other measures including tree length, consistency index and retention index (CI and RI, respectively) were also calculated. Aspergillus versicolor CBS 583.65™ was used as outgroup in these analyses. Sequences were deposited at GenBank under accession numbers FJ531124–FJ531191.

RESULTS AND DISCUSSION

Phylogenetic analysis

For the molecular analysis of the isolates, three genomic regions, the ITS region, and parts of the calmodulin and β-tubulin genes were amplified and sequenced. Phylogenetic analysis of the data was carried out using parsimony analysis. For the analysis of part of the β-tubulin gene, 589 characters were analysed, 197 of which were found to be parsimony informative. One of the 78 MP trees based on partial β-tubulin genes sequences is shown in Fig. 1 (tree length: 661 steps, consistency index: 0.6445, retention index: 0.8922). The calmodulin data set included 475 characters, with 266 parsimony informative characters. One of the 119 MP trees based on partial calmodulin gene sequences is shown in Fig. 2 (tree length: 355 steps, consistency index: 0.7556, retention index: 0.9187).
Fig. 1. The single MP tree obtained based on phylogenetic analysis of β-tubulin sequence data of Aspergillus section Usti. Numbers above branches are bootstrap values. Only values above 70% are indicated.
Fig. 2. One of the MP trees obtained based on phylogenetic analysis of calmodulin sequence data of Aspergillus section Usti. Numbers above branches are bootstrap values. Only values above 70% are indicated.
Fig. 3. One of the MP trees obtained based on phylogenetic analysis of ITS sequence data of Aspergillus section Usti. Numbers above branches are bootstrap values. Only values above 70% are indicated.
890, consistency index: 0.5753, retention index: 0.8788). The ITS data set included 541 characters with 100 parsimony informative characters. One of the 8 MP trees is shown in Fig. 3 (tree length: 224, consistency index: 0.7366, retention index: 0.9230).

Based on phylogenetic analysis of sequence data, Aspergillus section Usti includes now 21 species, at least two of which are able to reproduce sexually: Aspergillus heterothallicus (=Emericella heterothallica) and Fennelia monodii. Although supported only by low bootstrap values, *F. monodii* was found to belong to section *Usti* based on phylogenetic analysis of either loci (Figs 1–3). BLAST searches to the GenBank database also resulted in closest hits from section *Usti* (*A. pseudodeflectus* and *A. calidoustus* for the ITS and calmodulin sequence data, and *A. ustus* and *A. insuetus* for the β-tubulin sequences). *Fennelia monodii* was described in 1990 by Locquin-Linard from dung of herbivores in Tchad and Sudan. This species is characterised by two-valved ascospores with low, wrinkled equatorial crests. The anamorph of this species has not yet been observed in spite of repeated attempts using various media (data not shown). This species obviously does not belong to the *Fennelia* genus, instead it is a member of the *Emericella* genus. However, in accordance with the guidelines of the Amsterdam Declaration on fungal nomenclature (Hawksworth et al. 2011), and based on phylogenetic and physiological evidence, we propose the new combination *Aspergillus monodii* comb. nov. for this interesting species.

Another new species in this section was isolated from indoor air in Germany. This species has identical ITS sequences with *A. insuetus* CBS 119.27, but is clearly distinct from that species based on β-tubulin and calmodulin sequence data. This species is unable to grow at 37 °C, similarly to *A. keveii* and *A. insuetus*. We propose the name *A. germanicus* sp. nov. for this taxon.

Isolate IBT 16753 from Galapagos Islands, Ecuador, and IBT 14493 isolated from Lechuguilla Cave, Carlsbad Caverns National Park in New Mexico, USA were found to be related to, but clearly distinct from a clade including *A. calidoustus*, *A. pseudodeflectus*, *A. insuetus* and *A. keveii* on all trees. This species is also unable to grow at 37 °C, and acid production was not observed on CREA. We propose the name *A. carlsbadensis* sp. nov. for this taxon.

Isolate IBT 16748 was isolated from chamise chaparral (Adenostoma fasciculatum) in California, USA in 1978. It was found to be related to a clade including *A. subsessilis* and *A. kassunensis* on all trees. This species grew well at 37 °C, and acid production was not observed on CREA. We propose the name *A. californicus* sp. nov. for this taxon.

The "A. deflectus" isolate CBS 504.65 came from soil in Turkey is clearly distinct from the *A. deflectus* type strain on all trees, indicating that this isolate represents a distinct species in this section. This species grew, although rather restrictively at 37 °C, and acid production was not observed on CREA. *Aspergillus versicolor* was not observed on CREA. We propose the name *A. versicolor* sp. nov. for this taxon.

Another new species in this section, tentatively called *A. pseudodeflectus* sp. nov., is represented by NRRL 5856 = IBT 28161, which was found to be related to, but clearly different from *A. ustus* and *A. puniceus* on all trees (Figs 1–3). This isolate came from Lechuguilla Cave, Carlsbad Caverns National Park in New Mexico, USA in 1978. It was found to produce several antibiotics, including desferritriacetylfusigens, which inhibits the growth of bacteria (Anke 1977), and deflectins, angular azaphilons, which have antibiotic properties, and exhibit lytic activities against bacteria and erythrocytes (Anke et al. 1981). *Aspergillus egyptiacus* has been suggested to be more closely related to *A. versicolor* based on its biochemical behavior (Zohri & Ismail 1994). *Aspergillus egyptiacus* produces fumitremorgins and verruculogen, thus resembling *A. caespitosus* in that aspect. However *A. caespitosus* is placed within *Aspergillus section Nidulantes* (Peterson 2008, J. Varga, unpubl. data). *Aspergillus elongatus* CBS 387.75 produced fumitremorgin C, but other fumitremorgins and verruculogen could not be detected in that strain. The same strain also produced a member of the norgemidine / notoamide / aspargamide / steptacidin family of secondary metabolites (notoamide E). This type of compound has also been found in a strain of *A. versicolor* (Greeshok et al. 2008).

In agreement with the data of Peterson (2008), *A. kassunensis*, which was treated as a synonym of *A. subsessilis* (Samson 1979, Samson & Moucchaca 2004), is also a valid species, related to *A. subsessilis* and *A. calidoustus* (Figs 1–3). *Aspergillus cavernicola* was treated as a synonym of *A. varians* by Samson (1979); however, based on sequence data, it is conspecific with *A. amylovorus* and belongs to section *Usti*, while the *A. varians* type strain belongs to *Aspergillus section Nidulantes* (data not shown). *Aspergillus amylovorus* was invalidly described (nom. inval., Art. 37) from wheat stalk (Panasenko 1964), and subsequently validated by Samson (1979), while *A. cavernicola* was described in 1969 from cave wall from Romania. This species was validly described and hence is the correct name for *A. cavernicola* (= *A. amylovorus*).

### Extrolites

The mycotoxins and other secondary metabolites found to be produced by the examined species in this study are listed in Table 2. Species assigned to section *Usti* could clearly be assigned to three chemical groups based on the extrolites produced by them. *Aspergillus ustus*, *A. granulosus* and *A. puniceus* produced ustic acids in common. *Aspergillus ustus* and *A. puniceus* also produced austocystins and versicolorins. In the second chemical group, *A. pseudodeflectus* produced drimans (Hayes et al. 1996) in common with the other species in this group, and also several unique unknown compounds. *Aspergillus calidoustus* isolates produced drimans and ophibolins (Cutler et al. 1984) in common with *A. insuetus* and *A. keveii*, but also produced austins (Chemal et al. 1976) not identified in other species of section *Usti*. *Aspergillus insuetus* isolates also produced pergillin (Cutler et al. 1980), while *A. keveii* isolates produced niduliol. In the third chemical group, *E. heterothallica* has been reported to produce emethallicins A–F (Kawahara et al. 1989, 1990a, b), 5'-hydroxyaveranthin (Yabe et al. 1991), emetherone (Kawahara et al. 1988), emesterones A & B (Hosoe et al. 1998), 5'-hydroxyaveranthin (Yabe et al. 1991), Mer-NF8054X (Mizuno et al. 1995). This latter compound, an 18,22-cyclosterol derivative, is closely related to the emesterones, and was also identified in an isolate identified as *A. ustus* (Mizuno et al. 1995). *Aspergillus deflectus* produces several antibiotics, including desferritriacetylfusigens, which inhibits the growth of bacteria (Anke 1977), and deflectins, angular azaphilons, which have antibiotic properties, and exhibit lytic activities against bacteria and erythrocytes (Anke et al. 1981). *Aspergillus egyptiacus* has been suggested to be more closely related to *E. nidulans* than to *A. versicolor* based on its biochemical behavior (Zohri & Ismail 1994). *Aspergillus egyptiacus* produces fumitremorgins and verruculogen, thus resembling *A. caespitosus* in that aspect. However *A. caespitosus* is placed within *Aspergillus section Nidulantes* (Peterson 2008, J. Varga, unpubl. data). *Aspergillus elongatus* CBS 387.75 produced fumitremorgin C, but other fumitremorgins and verruculogen could not be detected in that strain. The same strain also produced a member of the norgemidine / notoamide / aspargamide / steptacidin family of secondary metabolites (notoamide E). This type of compound has also been found in a strain of *A. versicolor* (Greeshok et al. 2008).

Of particular interest is *A. pseudodeflectus* NRRL 5856 = CSIR 1128, which was originally identified as *A. ustus* and the first strain from which austamides, austidiols and austocystins (Table 2) were isolated (Steyn 1971, 1973, Steyn & Vleggaar 1974, 1976a, b, Vleggaar et al. 1974). This very toxic species has, however, only been isolated from maize in South Africa twice, and once in indoor...
Species Extrolites produced
A. amylovorus An asperugin, monascorubramin-like extrolites, (CANO, SCYT, SENSTER, STARM)
A. calidoustus Austins, drimans, ophiobolins G and H, TMC-120B, (ALTIN, FAAL, KNFO)
A. californicus An arugosin, (CANDU, SAERLO, SCAM, SEND, XANXU)
A. carlsbadensis Brevisaniame A (only in IBT 14493), [An arugosin, DRI, TRITRA, TIDL (not in IBT 18753), GNI (only in IBT 18616), EMO (only in IBT 14493)]
A. disflectus Desferriiactelyflusigen, deflectins A & B, emerin, a shamianthetape, (FUMU, RED2)
A. egyptiacus Fumitremorgin A, fumitremorgin B, verruculogen, (FYEN, UTSCABI, TOPLA, FUMU, PRUD, HØJV)
A. elongatus Fumitremorgin C, notoamide E, (DYK, SENT, TERRET)
A. germanicus Drimans, (DRUL, KNAT, SLOT, SNOF)
A. granulosus Asperugins, ustic acids, nidulol, drimans, (KMET, PUBO, SENSTER, SFOM)
A. heterothallicus Emethallicins A, B, C, D, E & F, emeheterones A & B and Mer-NF8054X, 5-hydroxyaveranthin, stellatin, sterigmatocystin, (DRI, NIDU)
A. insuetus Asperugins, drimans, ophiobolins G and H, pergilin-like compound, (AU, HETSCYT, INSU)
A. kassunensis Asperugins, Mer-NF8054X, (FYRT, SAERLO, SENSCAB, SENSTER)
A. kevei Asperugins, drimans, ophiobolins G and H, nidulol, (DRI, HETSCYT, INSU, PUBO, SENSTER, UP)
A. lucknowensis An arugosin, (GULT, PULK, RED1)
A. monodi Temein, (DYVB, METK)
A. pseudodeflectus Drimans, (DRUL, SLOT), asperugins in NRRL 4846
A. pseudoustus Asperugins, austamide, prolyl-2-(1',1'-dimethylallyl)-tryptophyldiketopiperazine, 12,13-dihydroaustamide, 12,13-dehydroprolyl-2-(1',1'-dimethylallyl)-tryptophyldiketopiperazine, 10,20-dehydro[12,13-dehydropropyl-2-1',1'-dimethylallyl]tryptophyldiketopiperazine, 12,13-dihydro-12-hydroxyaustamide, austidil, dihydrooexxy-8-epi-austidil, austocystin A, B, C, D, E, F, G, H, I, norsolorinic acid, versicolorin C, averuflin, (DRI, HETSCYT, SENSTER, UZ)
A. puniceus Ustic acids, austocystins (and versicolorins), phenylahistin, nidulol, (SENSTER)
A. subsessilis Mer-NF8054X, (SENSCAB, VIRO)
A. turkensis An austocystin, deflectins, emerin, a shamianthetape, (RED2)
A. ustus Ustic acids, austocystins (and versicolorins), australides, nidulol, (SENSTER)

All designations in parenthesis with capital letters are secondary metabolites with characteristic chromophores (UV spectra) and retention-times, but their chemical structure is not yet known.

Species descriptions

Aspergillus carlsbadensis Frisvad, Varga & Samson, sp. nov. MycoBank MB560399 Fig. 4.

Colonii flavo-brunnei, cum caespitulis ex conglomerationibus cellularum obtengentium ("Hülle"). Cellulii obtengenti ("Hülle") hyalinitis, crassitunicatis, globosis vel late ellipsoideis, 15–30 μm. Conidiorum biserialis, stipitibus plerumque levibus, brunneis, 4–5 μm latis. Vesiculis globosis, 10–14 μm diam. Conidiis conspicue ornamentatis, echinulatis vel verrucosis, ellipsoideis, 2.5–3.0 × 3.0–3.5 μm.


CYA, 1 wk, 25 °C: 30–32 mm (poor to medium sporulation, cream yellow to dark brown reverse, Hülle cells), MEA, 1 wk, 25 °C: 7–29 mm (rather poor sporulation, light yellow to cream reverse), YES, 1 wk, 25 °C: 35–45 mm (no sporulation, yellow to curry yellow), OA, 1 wk, 25 °C: 25–32 mm (Hülle cells), CYA, 1 wk, 37 °C: no growth, CREA: good growth (18–22 mm) and no acid production.

Colonies yellow brown with white tufts of conglomerates of Hülle cells. Hülle cells hyaline, thick-walled, globose to broadly ellipsoidal, 15–30 μm. Conidiophores biseriate with typical smooth-walled, brown, 4–5 μm wide stipes. Vesicles globose, 10–14 μm in diam. Conidia, distinctly ornamented with spines or warts, ellipsoidal 2.5–3.0 × 3.0–3.5 μm.

Fig. 4. Aspergillus carlsbadensis Frisvad, Varga & Samson sp. nov. A–C. Colonies incubated at 25 °C for 7 d. A. CYA, B. MEA, C. Tufts of Hülle cells. D–E, G–I. Conidiophores and conidia. F. Hülle cells. Scale bars = 10 µm.
Fig. 5. Aspergillus californicus Frisvad, Varga & Samson 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.
The taxon is related to, but clearly distinct from a clade including *A. calidoustus*, *A. pseudodefectus*, *A. insuetus* and *A. kevei* on all trees. This species is also unable to grow at 37 °C, and acid production was not observed on CREA.

**Aspergillus calidoustus** Frisvad, Varga & Samson, **sp. nov.** MycoBank MB560400. Fig. 5.

Colonies claviformes, cum caespitis albidos ex conglomerationibus cellularum obtectentibus ("Hülle"). Cellulis obtectentibus ("Hülle") hyalinais, crassitunicatis, globosis vel late ellipsoides. Conidiophoris biseriatis, stipitibus levibus, clare brunneis, 3.5–5 µm latis. Vesiculis globosis, 11–16 µm in diam. Conidiospores levibus et subtiliter exasperatus, subglobosis vel globosis, hyalinais vel viridibus, 2.5–3.0 µm.

Typus: **USA**, foothills of San Gabriel Mountains, California, ex chamise chaparral (*Adenostoma fasciculatum*), Jeff S. La Favre, 1978 (CBS H-20635 -- holotypus, culture ex-type CBS 123895).

**Aspergillus germanicus** Varga, Frisvad & Samson, **sp. nov.** MycoBank MB560401. Fig. 6.

Colonies claviformes, cum caespitis albidos ex conglomerationibus cellularum obtectentibus ("Hülle"). Cellulis obtectentibus ("Hülle") hyalinais, crassitunicatis, globosis vel late ellipsoides. Conidiophoris biseriatis, stipitibus plerumque levibus, clare brunneis, 3.5–6 µm latis. Vesiculis globosis, 11–16 µm in diam. Conidiospores levibus et subtiliter exasperatus, subglobosis vel globosis, hyalinais vel viridibus, 2.5–3.0 µm.


**Aspergillus pseudoustus** Frisvad, Varga & Samson, **sp. nov.** MycoBank MB560403. Fig. 8.

Colonies in agar CYN cinnamomeo-brunneis et in agar MEA flavo-brunneis, cellulis obtectentibus ("Hülle") nullis. Conidiophoris biseriatis, stipitibus plerumque levibus, brunneis, 6–9 µm latis. Vesiculis spathuliformibus, 14–22 µm diam. Conidiospores levibus, globosis, brunneis, 3.5–5 µm diam.

Typus: **South Africa**, ex stored maize (CBS H-20637 -- holotypus, culture ex-type CBS 123904).

**Aspergillus turkensis** Varga, Frisvad & Samson, **sp. nov.** MycoBank MB560404. Fig. 9.

Colonies in agar CYN cinnamomeo-brunneis et in agar MEA flavo-brunneis, cellulis obtectentibus ("Hülle") nullis. Conidiophoris biseriatis, stipitibus plerumque levibus, brunneis, 6–9 µm latis. Vesiculis spathuliformibus, 14–22 µm diam. Conidiospores levibus, globosis, brunneis, 3.5–5 µm diam.


This species grows well at 37 °C, and acid production was not observed. Conidiophores biseriate with typical smooth-walled, light brown, 3.5–5 µm wide stipes. Vesicles globose, 11–16 µm in diam. Conidia, smooth to finely roughened, subglobose to globose, hyaline to greenish, 2.5–3.0 µm.

This species grows well at 37 °C, and acid production was not observed. Conidiophores biseriate with typical smooth-walled, light brown, 3.5–5 µm wide stipes. Vesicles globose, 11–16 µm in diam. Conidia, smooth to finely roughened, subglobose to globose, hyaline to greenish, 2.5–3.0 µm.

This species occurs on dung and found on sheep dung in Chad and daman dung in Soudan.
Fig. 6. Aspergillus germanicus Varga, Frisvad & Samson sp. nov. A–C. Colonies incubated at 25 °C for 7 d, A. CYA, B. MEA, C. Tufts of Hüle cells. D–E, G–I. Conidiophores and conidia. F. Hüle cells. Scale bars = 10 µm.

yellow reverse, yellow obverse), OA, 1 wk, 25 °C: 14–17 mm (yellow reverse and obverse), CYA, 1 wk, 37 °C: 6–14 mm, CREA: weak growth and no acid production.

Colonies on CYA light brown, on MEA pale yellow brown. Hülle cells not observed. Conidiophores small biseriate with typical smooth-walled, light brown, 2.5–3 μm wide stipes. Vesicles spathulate, 5–8 μm diam. Conidia, smooth-walled, globose, hyaline, 2.5–3.0 μm.

Isolate CBS 504.65 is distinct from the *A. deflectus* ex-type strain on all trees, indicating that this isolate represents a distinct species in this section. This species grew, although rather restrictedly at 37 °C, and acid production was not observed on CREA.

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REFERENCES


Fig. 8. Aspergillus pseudoustus Frisvad, Varga & Samson 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.
Fig. 9. Aspergillus turkensis Varga, Frisvad & Samson 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.


NEW TAXA IN ASPERGILLUS SECTION USTI
