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New taxa in *Aspergillus* section *Usti*

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Abstract: Based on phylogenetic analysis of sequence data, *Aspergillus* section *Usti* includes 21 species, including two teleomorphic species *Aspergillus heterothallicus* (= *Emericella heterothallica*) and *Fennellia 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. 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. *Aspergillus calicofusus* sp. nov. is proposed for an isolate from chamise chaparral (*Adenostoma fasciculatum*) in California. It is related to a clade including *A. subsexisiliis* and *A. kasasuensis* 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 *F. monodii*. This species obviously does not belong to the *Usti* section, 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 monodi* comb. nov. for this taxon. Species assigned to section *Usti* can be assigned to three chemical groups based on the extracellular alkaloids. *Aspergillus ustus*, *A. granulosus* and *A. puniceus* produced ustic acid, while *A. ustus* and *A. puniceus* also produced austyctins and versicolorins. In the second chemical group, *A. pseudodeflectus* produced drimans and ophiobolins in common with the other species in this group, and also several unique unknown compounds. *Aspergillus calidoustus* isolates produced ustic acid and ophiobolins in common with *A. insuetus* and *A. keveii*, but also produced austin. *Aspergillus insuetus* isolates also produced pergillin while *A. keveii* isolates produced nidulon. In the third chemical group, *E. heterothallica* has been reported to produce emethallicins, 5-hydroxyaveranthin, emeheterone, emesterones, 5-hydroxyaveran. 

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


INTRODUCTION

*Aspergillus ustus* is a common filamentous fungus found in soils, soil 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, Verwei et al. 1999, Nakai et al. 2002, Pavié et al. 2005, Panackal 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* (Houbreken et al. 2007, Varga et al. 2008, Balajee et al. 2009, Pelaez et al. 2010). This species is also common in indoor air (Houbreken 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 (Fakh 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. pseudodeflectus*, *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 *Versicolores*, and found that *A. pseudodeflectus* is only weakly related to this section based on morphological treatment of section *Versicolores*. Peterson (2000) transferred *A. conjunctus*, *A. funiculosus*, *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 polythetic 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><em>A. amylovorus</em></td>
<td>CBS 600:67T = NRRL 5813 = IMI 129961 = VKM F-906 = IBT 23158</td>
<td>Wheat starch, Ukraine</td>
</tr>
<tr>
<td><em>A. calidoustus</em></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><em>A. californicus</em></td>
<td>CBS 123895* = IBT 16748</td>
<td>Ex chamise chaparral (<em>Adenostoma fasciculatum</em>), 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><em>A. carlsbadensis</em></td>
<td>CBS 123893 = IBT 16753</td>
<td>Soil, Galapagos Islands, Ecuador</td>
</tr>
<tr>
<td></td>
<td>CBS 123894* = IBT 14493</td>
<td>Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico, USA, D.E. Northup, 1992</td>
</tr>
<tr>
<td></td>
<td>CBS 123901 = IBT 18616</td>
<td>Soil, Carthage, Tunesia</td>
</tr>
<tr>
<td><em>A. cavernicola</em></td>
<td>CBS 117.76* = NRRL 6327</td>
<td>Soil, cave wall, Romania</td>
</tr>
<tr>
<td><em>A. deflectus</em></td>
<td>CBS 109.55* = 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><em>A. egyptiacus</em></td>
<td>CBS 123892 = IBT 16345 = RMF 9515</td>
<td>Soil, Iraq</td>
</tr>
<tr>
<td></td>
<td>CBS 656.73* = NRRL 5920</td>
<td>Sandy soil, under Olea europaea, Ras-EHikma, Egypt</td>
</tr>
<tr>
<td></td>
<td>CBS 991.72C</td>
<td>Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt</td>
</tr>
<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 991.72E</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><em>A. elongatus</em></td>
<td>CBS 387.75* = NRRL 5176</td>
<td>Alkaline Usar soil, Lucknow, India</td>
</tr>
<tr>
<td><em>A. germanicus</em></td>
<td>CBS 123887* = DTO 27-D9 = IBT 29365</td>
<td>Indoor air, Stuttgart, Germany</td>
</tr>
<tr>
<td><em>A. granulosus</em></td>
<td>CBS 588.65*</td>
<td>Soil, Fayetteville, Arkansas, USA</td>
</tr>
<tr>
<td></td>
<td>CBS 119.58</td>
<td>Soil, Texas, USA</td>
</tr>
<tr>
<td><em>A. heterothallicus</em></td>
<td>CBS 489.65*</td>
<td>Soil, Costa Rica</td>
</tr>
<tr>
<td></td>
<td>CBS 488.65</td>
<td>Soil, Costa Rica</td>
</tr>
<tr>
<td><em>A. insuetus</em></td>
<td>CBS 107.25* = 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><em>A. kassunensis</em></td>
<td>CBS 419.69* = NRRL 3752 = IMI 334938 = IBT 23479</td>
<td>Soil, Damascus, Syria</td>
</tr>
<tr>
<td><em>A. kevei</em></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, Tjitte de Vries and D.P. Mahoney, 1968</td>
</tr>
<tr>
<td><em>A. lucknowensis</em></td>
<td>CBS 449.75* = NRRL 3491</td>
<td>Alkaline Usar soil, Lucknow, India</td>
</tr>
<tr>
<td><em>A. monodii</em></td>
<td>CBS 434.93</td>
<td>Dung of Procavia sp. (daman), Darfur, Sudan</td>
</tr>
<tr>
<td></td>
<td>CBS 435.93*</td>
<td>Dung of sheep, Ennedi, Chad</td>
</tr>
<tr>
<td><em>A. pseudodeflectus</em></td>
<td>CBS 596.65</td>
<td>Sugar, USA, Louisiana</td>
</tr>
<tr>
<td></td>
<td>CBS 756.74*</td>
<td>Desert soil, Egypt, Western Desert</td>
</tr>
<tr>
<td></td>
<td>NRRL 4846 = IBT 2526</td>
<td>Unknown</td>
</tr>
<tr>
<td><em>A. pseudostus</em></td>
<td>ATCC 36063 = NRRL 5856 = CSIR 1128 = CBS 123904* = 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.

Extrtolite analysis

The isolates were grown on CYA and YES at 25 °C for 7 d. Extrtolites 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).

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:
**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.

A. pseudodeflectus CBS 756.74
A. pseudodeflectus CBS 596.65
A. calidoustus CBS 112452
A. calidoustus CBS 113228
A. calidoustus CBS 114380
A. calidoustus CBS 121610
A. calidoustus CBS 121601

A. carlsbadensis IBT 16753
A. carlsbadensis IBT 18616
A. carlsbadensis IBT 14493

A. insuetus CBS 107.25
A. insuetus CBS 119.27
A. keveii CBS 209.92
A. keveii NRRL 1974
A. keveii CBS 113227
A. keveii CBS 561.65

A. germanicus CBS 123887
A. elongatus CBS 387.75

A. turkensis CBS 504.65
A. turkensis NRRL 4993
A. lucknowensis CBS 449.75
A. deflectus NRRL 2206
A. deflectus CBS 109.55

A. granulosus CBS 119.58
A. granulosus CBS 588.65
A. heterothallicus CBS 489.65
A. heterothallicus CBS 488.65
A. pseudoustus IBT 28161

A. puniceus CBS 122.33
A. puniceus CBS 495.65
A. puniceus CBS 128.62
A. ustus CBS 261.67
A. ustus CBS 133.55
A. ustus CBS 239.90

A. monodii CBS 434.93
A. monodii CBS 435.93

A. kassunensis NRRL 3752
A. subessilis CBS 419.69
A. californicus IBT 16748

A. subessilis CBS 502.65
A. subessilis CBS 988.72
A. subessilis NRRL 4905
A. subessilis NRRL 4907

A. cavernicola CBS 117.76
A. amylovorus CBS 600.67

A. egyptiacus CBS 991.72E
A. egyptiacus IBT 16345
A. egyptiacus CBS 991.72F
A. egyptiacus CBS 656.73
A. egyptiacus CBS 991.72B
A. egyptiacus CBS 991.72C
A. egyptiacus CBS 991.72A

A. versicolor CBS 583.65
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.
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. pseudodefectus* 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. 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. 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. deflectus*, *A. pseudodefectus*, *A. insuetus* and *A. keveii* on all trees. This species was also unable to grow at 37 °C, and acid production was not observed on CREA. We propose the name *A. calidoustus* sp. nov. for this taxon.

The *“A. deflectus”* isolate CBS 504.65 came from soil in Turkey. It 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 well at 37 °C, and acid production was not observed on CREA. We propose the name *A. carlsbadensis* sp. nov. for this taxon.

Another new species in this section, tentatively called *A. pseudodefectus* 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 stored maize, South Africa. Other isolates belonging to this species include a culture contaminant of *Bipolaris sorokiniana* from South Africa (IBT 31044), and one isolate came from indoor air in Finland (IBT 22361).

Isolate IBT 16345 from soil, Iraq is a new isolate of *A. egyptiacus* based on all sequence data. The isolate grew well at 37 °C, and acid production was not observed on CREA. This is the first isolate of this species which was isolated outside Egypt.

In agreement with the data of Peterson (2008), *A. kassunensis*, which was treated as a synonym of *A. subsessilis* (Samson 1979, Samson & Mouchaca 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. pseudodefectus* 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 ophiobolins (Cutler et al. 1984) in common with *A. insuetus* and *A. keveii*, but also produced austins (Cheval et al. 1976) not identified in other species of section *Usti*. *Aspergillus ustus* isolates also produced pergillin (Cutler et al. 1980), while *A. keveii* isolates produced nidulol. 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. 1991c), 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 desferricatetly fusigen, 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. caesipitosus* in that aspect. However *A. caesipitosus* is placed within the *Nidulantes* section (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 norgeamide / notoamide / aspermamide / stephacidin family of secondary metabolites (notoamide E). This type of compound has also been found in a strain of *A. versicolor* (Greshock et al. 2008).

Of particular interest is *A. pseudodefectus* NRRL 5856 = CSIR 1128, which was originally identified as *A. ustus* and the first strain from which austamides, austdiols and austocystins (Table 2) were isolated (Steyn 1971, 1973, Steyn & Vleggaa 1974, 1976a, b, Vleggaa et al. 1974). This very toxic species has, however, only been isolated from maize in South Africa twice, and once in indoor...
SaMSon of monascorubramin like red pigments, while in section sections. On the other hand, several metabolites have only been found in subgenus austins and the metabolite DRI are present in species of the different in other sections in subgenus SENSTER in Table 2 is common in this section, and may be related to sterigmatocystin, as it has a similar UV spectrum.

Species Extrolites produced
A. amyllovorus An asperugin, monoscarubramin-like extrolites, (CANO, SCYT, SENSTER, STARM)
A. calidoustus Austins, drimans, ophiobolins G and H, TMC-120B, (ALTIN, FAAL, KNOF)
A. californicus An arugosin, (CANDU, SAERLO, SCAM, SEND, XANXU)
A. carlsbadensis Brevianamide A (only in IBT 14493), [An arugosin, DRI, TRIRTA, TIDL (not in IBT 18753), GNI (only in IBT 18616), EMO (only in IBT 14493)]
A. deflectus Desferririacetylfsugen, deflectins A & B, emerin, a shamixanthone, (FUMU, RED2)
A. eggyptiacus Fumigermorn A, fumitremorin B, verruculogen, (FYEN, UTSCABI, TOPLA, FUMU, PRUD, HØJV)
A. elongatus Fumigermorn C, notoamide E, (DYK, SENT, TERRET)
A. germanicus Drimans, (DRUL, KNAT, SLOT, SNOF)
A. granulosus Asperugins, austic acids, nidulol, drimans, (KMET, PUBO, SENSTER, SFOM)
A. heterothallicus Emethallicins A, B, C, D, E & F, emeheterone, emesterones A & B and Mer-NF8054X, 5-hydroxyaeveranthin, stellatin, sterigmatocystin, (DRI, NIDU)
A. insuetus Asperugins, drimans, ophiobolins G and H, perglinil-like compound, (AU, HETSCYT, INSU)
A. kassenensis Asperugins, Mer-NF8054X, (FYRT, SAERLO, SENSCAB, SENSTER)
A. keveii Asperugins, drimans, ophiobolins G and H, nidulol, (DRI, HETSCYT, INSU, PUBO, SENSTER, UP)
A. lucknowensis An arugosin, (GULT, PULK, RED1)
A. monodi Terein, (DYVB, METK)
A. pseudodefectus Drimans, (DRUL, SLT, 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, austidol, dihydrodeoxy-8-epi-austalidol, austocystin A, B, C, D, E, F, G, H, I, norsolorinic acid, versicolorin C, averufin, (DRI, HETSCYT, SENSTER, UZ)
A. puniceus Ustic acids, austocystins (and versicolorins), phenylahistin, nidulol, (SENSER)
A. subsessilis Mer-NF8054X, (SENSCAB, VIRO)
A. turkensis An austocystin, deflectins, emerin, a shamixanthone, (RED2)
A. ustus Ustic acids, austocystins (and versicolorins), austalides, nidulol, (SENSER)

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.

Comparing the secondary metabolite profiles of section Usti with other sections within subgenus Nidulantes, nidulol, and versicolorins are also produced by members of sections Versicoleus and Nidulantes (Cole & Schweickert 2003). Interestingly, versicolorin, sterigmatocystin and 5'-hydroxyaeveranthin are intermediates of the aflatoxin biosynthetic pathway and also produced by species assigned to Aspergillus sections Flavi and Ochraceorosei (Yabe et al. 1991, Frisvad et al. 2005). Other extrolites found in species in section Usti are also found in other sections in subgenus Nidulantes: arugosins, asperugins, austins and the metabolite DRI are present in species of the different sections. On the other hand, several metabolites have only been found in section Usti, including austamide, austidol, austocystins, deflectins, drimans, emethallicins, emetherones and ustic acids (Table 2). Two species produce red pigments, A. amyllovorus produce a large number of monoscarubramin like red pigments, while A. turkensis produce few monoscarubramin-like extrolites.

Species descriptions

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

Colonii flavo-brunneis, cum caespitiis ex conglomerationibus cellularum obtectum ("Hülle"). Cellulis obtectis ("Hülle") hyaliniis, crassitunicatiis, globosis vel late ellipsoideis, 15–30 μm. Conidiospores biserialis, stipitibus plerumque levibus, brunneis, 4–5 μm latis. Vesiculli globosi, 10–14 μm diam. Conidios conspicui ornamentati, echinulati vel verrucosi, 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. Conidiofarcis 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 Hüle cells. D–E, G–I. Conidiophores and conidia. F. Hüle 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. keveii* 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 clare flavis, cum caespitulis abidis ex conglomerationibus cellularum obtectis obtegentibus ("Hülle"). Cellulis obtectis ("Hülle") hyalinis, crassitunicatis, globosis vel late ellipsoideis. Conidiophoris biseriatis, stipitis levibus, clare brunneis, 3.5–5 μm latis. Vesiculis globosis, 11–16 μm in diam. Conidia levibus vel subtiliter exasperatas, subglobosis vel globosis, hyalinis vel viridibus, 2.5–3.0 μm.

Typus: **USA**, foothills of San Gabriel Mountains, California, ex chamisae chaparri (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 in agar CYA brown, on MEA greyish brown. Hülle cells 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 grew well at 37 °C, and acid production was not observed on CREA. It was found to be related to species in a clade including *A. subsessilis* and *A. kassunensis*.

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

Colonies in agar CYA cinnamon brown, on MEA yellow brown. Hülle cells observed. Conidiophores biseriate with typical smooth-walled, brown, 3.5–5 μm wide stipes. Vesicles globose, 10–14 μm in diam. Conidia, smooth to distinctly echinulate, globose, brown to orange reverse, 2.5–3.0 μm.

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

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

Colonies in agar CYN cinnamon-brunneis et in agar MEA flavo-brunneis, cellulis obtectis ("Hülle") nullis. Conidiophoris biseriatis, stipitis plerumque levibus, brunneis, 6–9 μm latis. Vesiculis spathuliformibus, 14–22 μm diam. Conidia conspicue echinulatis, globosis, brunneis, 3.5–5 μm diam. Asci 8–10 × 10–13 μm. Ascospores 3.0–3.5 × 4.5–5.0 μm, hyaline, smooth-walled with two equatorial rings. **Aspergillus** anamorph not observed on various media and after cultivation at different temperatures.

This species occurs on dung and found on sheep dung in Chad and daman dung in Soudan.

**Aspergillus turkensis** Varga, Frisvad & Samson. sp. nov. MycoBank MB560404. Fig. 7.

Colonies in agar CYN cinnamon-brunneis et in agar MEA flavo-brunneis, cellulis obtectis ("Hülle") nullis. Conidiophoris biseriatis, stipitis plerumque levibus, brunneis, 3.5–5 μm latis. Vesiculis globosis, 10–14 μm diam. Conidia, distinctly echinulate, globose, brown to orange, 2.5–3.0 μm.

Other strains: MRC 096 = IBT 31044, contaminant in maize, South Africa; IBT 22361, indoor air; Finland

**Aspergillus pseudoustus** sp. nov., is related to, but clearly different from *A. ustus* and *A. punices* on all trees. This isolate came from stored maize, South Africa. Other isolates belonging to this species include a culture contaminant of *Bipolaris sorokiniana* from South Africa (IBT 31044), and one isolate came from indoor air in Finland (IBT 22361).

**Aspergillus subcamosus** sp. nov. MycoBank MB560404. Fig. 9.

Colonies in agar CYN clare brunneis et in agar MEA flavo-brunneis, cellulis tectebratis ("Hülle") nullis. Conidiophoris biseriatis, stipitis plerumque levibus, brunneis, 6–9 μm latis. Vesiculis spathuliformibus, 14–22 μm diam. Conidia conspicue echinulatis, globosis, brunneis, 3.5–5.0 μm diam.

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.
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 paecilomus 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* turkezensis 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.


