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New taxa in *Aspergillus* section *Usti*

R.A. Samson¹, J. Varga², M. Meijer¹ and J.C. Frisvad³

¹CBS-KNAW Fungal Biodiversity Centre, Uppsalaalan 8, NL-3584 CT Utrecht, the Netherlands; ²Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Kőzép fasor 52, Hungary; ³BioCentrum-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 monodii*. *Aspergillus germanicus* spp. 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. pseudeffectus*, *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 caillouisicus* sp. nov. is proposed for an isolate from charisme chapparral (*Adinostoma fasciculatum*) in California. It is related to a clade including *A. subseasiliis* 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. turkenensis* 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. graminearum* were isolated from indoor air in Finland proved to be related to, but different from *A. ustus* and *A. puneceus*. The taxon is proposed as the new species *A. pseudoustus*.

Although supported only by low bootstrap values, *F. monodii* was found to belong to section *Usti* based on phylogenetic analysis of either loci BLAST searches to the GenBank database also resulted in closest hits from section *Usti*. 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 taxon. Species assigned to section *Usti* can be assigned to three chemical groups based on the extrolites. *Aspergillus ustus*, *A. granulosus* and *A. puneceus* produced ustic acid, while *A. ustus* and *A. puneceus* also produced ausocystins and versicolorins. In the second chemical group, *A. pseudeffectus* produced drimans in common with the other species in this group, and also several unique unknown compounds. *Aspergillus calidoustus* isolates produced ustic acid and ophioxolins in common with *A. insuetus* and *A. keveii*, but also produced austins. *Aspergillus insuetus* isolates also produced pergillin while *A. keveii* isolates produced nidulol. In the third chemical group, *E. heterothallicus* has been reported to produce emethallicins, 5-hydroxyvaneranin, emetherone, emesterones, 5-hydroxyvaneranin.

**Key words:** Ascomycetes, *Aspergillus* section *Usti*, ITS, calmodulin, extrolites, 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, 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* (Houbraken et al. 2007, Varga et al. 2008, Balajee et al. 2009, Pelaez 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 (Fakih 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. puneceus*, *A. conjunctus* and *A. deflectus*. Later, Kozakiewicz (1989) revised the taxonomy of the group, and included *A. ustus*, *A. pseudeffectus*, *A. conjunctus*, *A. puneceus*, *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. silvicolas* based on morphological studies. Klich (1993) treated *A. granulosus* as member of section *Versiculares*, and found that *A. pseudeffectus* is only weakly related to this section based on morphological treatment of section *Versiculares*. Peterson (2000) transferred *A. conjunctus*, *A. funiculosus*, *A. silvicolas*, *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><strong>A. amylovorus</strong></td>
<td>CBS 600.67 = NRRL 5813 = IMI 129961 = VKM F-906 = IBT 23158</td>
<td>Wheat starch, Ukraine</td>
</tr>
<tr>
<td><strong>A. calidoustus</strong></td>
<td>CBS 112452; CBS 113228; CBS 114380; CBS 121601; 677; CBS 121610; 91</td>
<td>Indoor air, Germany, Wooden construction material, Finland, Bronchoalveolar lavage fluid, proven invasive aspergillosis, Nijmegen, the Netherlands*, Post-cataract surgery endophthalmitis, Turkey</td>
</tr>
<tr>
<td><strong>A. californicus</strong></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><strong>A. carlsbadensis</strong></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><strong>A. cavernicola</strong></td>
<td>CBS 117.76 = NRRL 6327</td>
<td>Soil, cave wall, Romania</td>
</tr>
<tr>
<td><strong>A. deflectus</strong></td>
<td>CBS 109.55 = NRRL 2206 = IBT 24665; NRRL 4235 = IBT 25291; NRRL 13131 = IBT 25254</td>
<td>Soil, Rio de Janeiro, Brazil, Potting soil, Unknown</td>
</tr>
<tr>
<td><strong>A. egyptiacus</strong></td>
<td>CBS 123892 = IBT 16345 = RMF 9515; CBS 656.73 = NRRL 5920</td>
<td>Soil, Iraq</td>
</tr>
<tr>
<td></td>
<td>CBS 991.72C; CBS 991.72A; CBS 991.72B; CBS 991.72F; CBS 991.72E</td>
<td>Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt</td>
</tr>
<tr>
<td><strong>A. elongatus</strong></td>
<td>CBS 387.75 = NRRL 5176</td>
<td>Alkaline Usar soil, Lucknow, India</td>
</tr>
<tr>
<td><strong>A. germanicus</strong></td>
<td>CBS 123887 = DTO 27-D9 = IBT 29365</td>
<td>Indoor air, Stuttgart, Germany</td>
</tr>
<tr>
<td><strong>A. granulosus</strong></td>
<td>CBS 588.65; CBS 119.58</td>
<td>Soil, Fayetteville, Arkansas, Soil, Texas, USA</td>
</tr>
<tr>
<td><strong>A. heterothallicus</strong></td>
<td>CBS 489.65; CBS 488.65</td>
<td>Soil, Costa Rica</td>
</tr>
<tr>
<td><strong>A. insuetus</strong></td>
<td>CBS 107.25 = NRRL 279</td>
<td>Soil, Costa Rica</td>
</tr>
<tr>
<td></td>
<td>CBS 119.27 = NRRL 4876</td>
<td>Soil, Iowa, USA</td>
</tr>
<tr>
<td><strong>A. kassunensis</strong></td>
<td>CBS 419.69 = NRRL 3752 = IMI 334938 = IBT 23479; CBS 434.93</td>
<td>Soil, Damascus, Syria</td>
</tr>
<tr>
<td><strong>A. kevei</strong></td>
<td>CBS 209.92; CBS 561.65 = NRRL 1974; IBT 10524 = CBS 113227 = NRRL 1254</td>
<td>Soil, La Palma, Spain</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><strong>A. lucknowensis</strong></td>
<td>CBS 449.75 = NRRL 3491</td>
<td>Alkaline Usar soil, Lucknow, India</td>
</tr>
<tr>
<td><strong>A. monodii</strong></td>
<td>CBS 434.93; CBS 435.93</td>
<td>Dung of Procavia sp. (daman), Darfur, Sudan, Dung of sheep, Ennedi, Chad</td>
</tr>
<tr>
<td><strong>A. pseudodeflectus</strong></td>
<td>CBS 596.65; CBS 756.74 = IBT 25256</td>
<td>Sugar, USA, Desert soil, Egypt, Western Desert</td>
</tr>
<tr>
<td></td>
<td>NRRL 4846 = IBT 28161</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>A. pseudostustus</strong></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.

**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 583.65T 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:
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.
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. californicus* (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. punicus* produced ustic acids in common. *Aspergillus ustus* and *A. punicus* 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 dimans 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 pergillins (Cutler et al. 1980), while *A. keveii* isolates produced nidulol. In the third chemical group, *E. heterothallica* has been reported to produce emtelethacins A–F (Kawahara et al. 1989, 1990a, b), 5'-hydroxyaveranthin (Yabe et al. 1991), emetherone (Kawahara et al. 1988), emestroterones 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 emestroterones, and was also identified in an isolate identified as *A. ustus* (Mizuno et al. 1995). *Aspergillus deflectus* produces several antibiotics, including desferritriacetylfusigen, which inhibits the growth of bacteria (Anke 1977), and reflectins, 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. caesipitans* in that aspect. However, *A. caesipitans* 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 norgemamide / notoamide / aspergamide / 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. pseudodeflectus* NRRL 5856 = CSIR 1128, which was originally identified as *A. ustus* and the first strain from which ausstamides, ausstidiols and ausstycystins (Table 2) were isolated (Steyn 1971, 1973, Steyn & Vlieggaar 1974, 1976a, b, Vlieggaar et al. 1974). This very toxic species has, however, only been isolated from maize in South Africa twice, and once in indoor...
Table 2. Extrolites produced by species assigned to Aspergillus section Usti.

<table>
<thead>
<tr>
<th>Species</th>
<th>Extrolites produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. amylovorus</td>
<td>An asperugin, monosaccharin-like extrolites, (CANO, SCYT, SENSTER, STARM)</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>Austins, dirmans, ophiobolins G and H, TMC-120B, (ALTIN, FAAL, KNDF)</td>
</tr>
<tr>
<td>A. californicus</td>
<td>An arugosin, (CANDU, SAERLO, SCAM, SEND, XANXU)</td>
</tr>
<tr>
<td>A. carlsbadensis</td>
<td>Brevianamide A (only in IBT 14493), [An arugosin, DRI, TRITRA, TIDL (not in IBT 18753), GNI (only in IBT 18616), EMO (only in IBT 14493)]</td>
</tr>
<tr>
<td>A. deflectus</td>
<td>Desferriacetylfusigen, deflectins A &amp; B, emerin, a shamilxantheme, (FUMU, RED2)</td>
</tr>
<tr>
<td>A. egyptiacus</td>
<td>Fumitremorgin A, fumitremorgin B, verruculogen, (FYEN, UTSCABI, TOPLA, FUMU, PRUD, HØJV)</td>
</tr>
<tr>
<td>A. elongatus</td>
<td>Fumitremorgin C, notoamide E, (DYK, SENT, TERRET)</td>
</tr>
<tr>
<td>A. germanicus</td>
<td>Drimans, (DRUL, KNAT, SLOT, SNOF)</td>
</tr>
<tr>
<td>A. granulosus</td>
<td>Asperugins, ustic acids, nidulol, dirmans, (KMET, PUBO, SENSTER, SFOM)</td>
</tr>
<tr>
<td>A. heterothallicus</td>
<td>Emethallicins, emetherones and ustic acids (Table 2). Two of monascorubramin like red pigments, while A. austus, A. puniceus, 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 austamides, austidiol, austocystins, deflectins, dirmans, emethallicins, emetherones and ustic acids (Table 2). Two species produce red pigments, A. amylovorus produce a large number of monosaccharin-like red pigments, while A. turkensis produce few monosaccharin-like extrolites.</td>
</tr>
<tr>
<td>A. insuetus</td>
<td>Asperugins, dirmans, ophiobolins G and H, pergillin-like compound, (AU, HETSCYT, INSU)</td>
</tr>
<tr>
<td>A. kassunensis</td>
<td>Asperugins, Mer-NF8054X, (FYRT, SAERLO, SENSCAB, SENSTER)</td>
</tr>
<tr>
<td>A. kevei</td>
<td>Asperugins, dirmans, ophiobolins G and H, nidulol, (DRI, HETSCYT, INSU, PUBO, SENSTER, UP)</td>
</tr>
<tr>
<td>A. lucknowensis</td>
<td>An arugosin, (GULT, PULK, RED1)</td>
</tr>
<tr>
<td>A. monoidii</td>
<td>Terein, (DYVB, METK)</td>
</tr>
<tr>
<td>A. pseudodeflectus</td>
<td>Drimans, (DRUL, DRU, HUL, SLOT), asperugins in NRRL 4846</td>
</tr>
<tr>
<td>A. pseudoustus</td>
<td>An austocystin, deflectins, emerin, a shamilxantheme, (RED2)</td>
</tr>
<tr>
<td>A. puniceus</td>
<td>Ustic acids, austocystins (and versicolorins), phenylahistin, nidulol, (SENSTER)</td>
</tr>
<tr>
<td>A. subsessilis</td>
<td>Mer-NF8054X, (SENSCAB, VIRO)</td>
</tr>
<tr>
<td>A. subterraneus</td>
<td>Mer-NF8054X, (SENSCAB, VIRO)</td>
</tr>
<tr>
<td>A. turkensis</td>
<td>An austocystin, deflectins, emerin, a shamilxantheme, (RED2)</td>
</tr>
<tr>
<td>A. ustus</td>
<td>Ustic acids, austocystins (and versicolorins), austalides, nidulol, (SENSTER)</td>
</tr>
</tbody>
</table>

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.

Colonis flavo-brunneis, cum caespiutulis ex conglomerationibus cellularum obtentum ("Hülle"). Cellulis obtentibus ("Hülle") hyalini, crassitunicati, globose vel late ellipsoideis, 15–30 μm. Conidiophoris biserialis, stipitibus plerumque levibus, brunnis, 4–5 μm latis. Vesiculis globosis, 10–14 μm diam. Conidios consipaci ornamentatis, echinulatis vel verrucosis, elongatis, 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 conglommates of Hülle cells. Hülle cells hyaline, thick-walled, globose to broadly ellipsoid, 15–30 μm. Conidiophores biseriate with typical smooth-walled, brown, 4–5 μm wide stipites. Vesicles globose, 10–14 μm in diam. Conidia, distinctly ornamented with spines or warts, ellipsoidai 2.5–3.0 × 3.0–3.5 μm.

air in Finland. All three strains examined produced austamides, austidiol and austocystins. The austocystins have been found in A. ustus, A. puniceus and A. pseudoustus and one austocystin has also been found in A. turkensis. The austocystins seem to be another biosynthetic family of secondary metabolites that are derived from the versicolorins. In other species in sections Aenei, Versicolores and Nidulantes, versicolorins are precursors of sterigmatocystin and in few species, the aflatoxins (Frisvad et al. 2005, Varga et al. 2009). Sterigmatocystin has not yet been found in any species in section Usti, but a related metabolite, listed as SENSTER in Table 2 is common in this section, and may be related to sterigmatocystin, as it has a similar UV spectrum.

Comparing the secondary metabolite profiles of section Usti with other sections within subgenus Nidulantes, nidulol, and versicolorins are also produced by members of sections Versicolores and Nidulantes (Cole & Schweikert 2003). Interestingly, versicolorin, sterigmatocystin and 5'-hydroxyaveranthin 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 austamides, austidiol, austocystins, deflectins, dirmans, emethallicins, emetherones and ustic acids (Table 2). Two species produce red pigments, A. amylovorus produce a large number of monosaccharin-like red pigments, while A. turkensis produce few monosaccharin-like extrolites.
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. kevei* on all trees. This species is also unable to grow at 37 °C, and acid production was not observed on CREA.

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

Colonies clare flavis, cum caespitulis abidis ex conglomerationibus cellularum obbogentium ("Hülle"). Cellulis obbogentibus ("Hülle") hyalinis, crassitunicatis, globosis vel late ellipsoides. Conidiophoris biseriatis, stipitibus levibus, clare brunneis, 3.5–5 μm latis. Vesiculis globosis, 11–16 μm in diam. Conidiospora levibus et sublititer exasperata, subglobosis vel globosis, hyalinis 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).

**CyA**, 1 wk, 25 °C: 18–20 mm (poor sporulation, yellow brown reverse, Hülle cells), **MEA**, 1 wk, 25 °C: 6–9 mm (rather poor sporulation, yellow brown reverse), **YES**, 1 wk, 25 °C: 23–26 mm (no sporulation, cream yellow reverse), **OA**, 1 wk, 25 °C: 18–21 mm (Hülle cells), **CYA**, 1 wk, 37 °C: no growth, **CREA**: good growth and no acid production.


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

Colonies in agar CYA brunneis et in agar MEA griseo-brunneis, cellulis tectegentibus ("Hülle") nullis. Conidiophoris biseriatis, stipitibus plerumque levibus, brunneis, 6–9 μm latis. Vesiculis spathuliformibus, 14–22 μm diam. Conidiospora levibus, globosis, brunneis, 3.5–5.0 μm diam.


**CyA**, 1 wk, 25 °C: 22–26 mm (poor to medium sporulation, yellow brown to orange reverse, pigment diffusing, Hülle cells), **MEA**, 1 wk, 25 °C: 12–16 mm (good sporulation, light yellow to cream reverse), **YES**, 1 wk, 25 °C: 32–37 mm (some sporulation, yellow brown reverse), **OA**, 1 wk, 25 °C: 28–32 mm, **CYA**, 1 wk, 37 °C: 7–9 mm, **CREA**: good growth and no acid production.

Colonies on CYA brown, on MEA greyish brown. Hülle cells not observed. Conidiophores biseriate with typical smooth-walled, brown, 6–9 μm wide stipes. Vesicles spathulate, 14–22 μm diam. Conidia, distinctly echinulate, globose, brown, 3.5–5.0 μm.

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.

**Aspergillus monodii** (Locquin-Linard) Varga, Frisvad & Samson, **comb. nov.** MycoBank MB560402. Fig. 7.


**CyA**, 1 wk, 25 °C: 2–21 mm (no sporulation, white to cream reverse), **MEA**, 1 wk, 25 °C: 6–8 mm (ascomata, light yellow reverse), **YES**, 1 wk, 25 °C: 8–23 mm (no sporulation, yellow to red brown reverse, yellow obverse), **OA**, 1 wk, 25 °C: 9–19 mm (ascomata), **CYA**, 1 wk, 37 °C: 0–2 mm, **CREA**: poor growth and no acid production.

Colonies producing an orange brown crusts of stromata with ascomata 200–350 μm in diam. Hülle cells forming the structure of the stromata, globose to ellipsoidal, 8–40 μm diam. Asci 8–10 × 13–13 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 pseudoustus** Frisvad, Varga & Samson, **sp. nov.** MycoBank MB560403. Fig. 8.

Colonies in agar CYN cinnamomeo-brunneis et in agar MEA flavo-brunneis, cellulis obbogentibus ("Hülle") nullis. Conidiophoris biseriatis, stipitibus plerumque levibus, brunneis, 3.5–5 μm latis. Vesiculis globosis, 10–14 μm diam. Conidiospora brunneis, oblongis, 3.5–5.0 μm diam.

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

**CyA**, 1 wk, 25 °C: 30–32 mm (medium sporulation, yellow brown reverse), **MEA**, 1 wk, 25 °C: 15–25 mm (rather poor sporulation, light yellow reverse), **YES**, 1 wk, 25 °C: 35–45 mm (no sporulation, curry yellow to brown reverse), **OA**, 1 wk, 25 °C: 30–36 mm, **CYA**, 1 wk, 37 °C: no growth, **CREA**: 28–34 mm, no acid production.

Colonies on CYA cinnamon brown, on MEA yellow brown. Hülle cells not 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 greenish, 2.5–3.0 μm.

Other strains: MRC 096 = IBT 31044, contaminant in maize, South Africa; IBT 122861, indoor air; Finland

**Aspergillus turgidus** sp. nov., is related to, but clearly different from *A. ustus* and *A. puniceus* 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 turkensis** Varga, Frisvad & Samson, **sp. nov.** MycoBank MB560404. Fig. 9.

Colonies in agar CYN claris brunneis et in agar MEA flavo-brunneis, cellulis obbogentibus ("Hülle") nullis. Conidiophoris minute biseriatis, stipitibus plerumque levibus, brunneis, 2.5–3 μm latis. Vesiculis spathuliformibus, 5–8 μm diam. Conidiospora brunneis, oblongis, 2.5–3 μm diam.


**CyA**, 1 wk, 25 °C: 13–18 mm (poor sporulation, red orange reverse), **MEA**, 1 wk, 25 °C: 4–10 mm (rather poor sporulation, cream yellow reverse), **YES**, 1 wk, 25 °C: 35–45 mm (no sporulation, orange
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 tarkensis 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.


