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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 monodii. 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. 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 chamise chapparral (Adenostoma fasciculatum) in California. It is related to a clade including A. subseissilis and A. kasanusenii 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 Bipolaris sorokiniana from indoor air in Finland proved to be related to, but different from A. ustus and A. puniceus. The taxon is proposed as the new species A. pseudostoutus. 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 Fennellia 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 extracellular products. Aspergillus ustus, A. granulosus and A. puniceus produce ustic acid, while A. ustus and A. puniceus also produce austocystins and versicolorins. In the second chemical group, A. pseudodefectus produced drimans in common with the other species in this group, and also several unique unknown compounds. Aspergillus californicus isolates produced ophiothelins 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 emetillicins, 5-hydroxysteranin, emehterone, emesterones, 5-hydroxyvaneranin.

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

Taxonomic novelties: Aspergillus carlsbadensis Frisvad, Varga & Samson sp. nov., Aspergillus californicus Frisvad, Varga & Samson sp. nov., Aspergillus germanicus Varga, Frisvad & Samson sp. nov., Aspergillus monodii (Locquin-Linard) Varga, Frisvad & Samson comb. nov., Aspergillus pseudostoutus Frisvad, Varga & Samson sp. nov., Aspergillus turkensis Varga, Frisvad & Samson sp. nov.

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, 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, Vagefi 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 (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, Krokenberger 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 Versiculares, and found that A. pseudodefectus is only weakly related to this section based on morphological treatment of section Versiculares. Peterson (2000) transferred A. conjunctus, A. fumiculosus, A. silvaticus, A. panamensis and A. androspormis 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
| Species          | Strain No. | Source                                                                 |
|------------------|------------|------------------------------------------------------------------------|
| A. amylovorus    | CBS 600.67 = NRRL 5813 = IMI 129961 = VKM F-906 = IBT 23158          | Wheat starch, Ukraine                                                  |
| A. calidoustus   | CBS 112452  | Indoor air, Germany                                                    |
|                  | CBS 113228  | ATCC 38849; IBT 13091                                                  |
|                  | CBS 114380  | Wooden construction material, Finland                                  |
|                  | CBS 121601; 677 | Bronchoalveolar lavage fluid, proven invasive aspergillosis, Nijmegen, the Netherlands* |
|                  | CBS 121610; 91 | Post-cataract surgery endophthalmitis, Turkey                          |
| A. californicus  | CBS 123895T = IBT 16748 | 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. |
| A. carlsbadensis | CBS 123893 = IBT 16753 | Soil, Galapagos Islands, Ecuador                                      |
|                  | CBS 123894T = IBT 14493 | Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico, USA, D.E. Northup, 1992 |
|                  | CBS 123903 = IBT 18616 | Soil, Carthage, Tunesia                                               |
|                  | CBS 123903 = IBT 18616 | Soil, cave wall, Romania                                              |
| A. cavernicola   | CBS 117.76T = NRRL 6327 | Soil, Rio de Janeiro, Brazil                                          |
|                  | CBS 109.55T = NRRL 2206 = IBT 24665 | Potting soil                                                        |
|                  | CBS 4235 = IBT 25291 | Unknown                                                               |
|                  | CBS 121601; 91 | Post-cataract surgery endophthalmitis, Turkey                          |
|                 | NRRL 13131 = IBT 25254 | Unknown                                                               |
| A. egyptiacus    | CBS 123892 = IBT 16345 = RMF 9515 | Soil, Iraq                                                            |
|                  | CBS 656.73T = NRRL 5920 | Sandy soil, under Olea europaea, Ras-EHikma, Egypt                    |
|                  | CBS 991.72C | Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt            |
|                  | CBS 991.72A | Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt            |
|                  | CBS 991.72B | Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt            |
|                  | CBS 991.72F | Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt            |
|                  | CBS 991.72E | Bare ferruginous soil, Dahkla Oasis, Western desert, Egypt            |
| A. elongatus     | CBS 387.75T = NRRL 5176 | Alkaline Usar soil, Lucknow, India                                   |
| A. germanicus    | CBS 123887T = DTO 27-D9 = IBT 29365 | Indoor air, Stuttgart, Germany                                        |
| A. granulosus    | CBS 588.65T | Soil, Fayetteville, Arkansas, USA                                     |
|                  | CBS 119.58 | Soil, Texas, USA                                                      |
| A. heterothallicus | CBS 489.65T | Soil, Costa Rica                                                      |
|                  | CBS 488.65 | Soil, Costa Rica                                                      |
| A. insuetus      | CBS 107.25T = NRRL 279 | South Africa                                                          |
|                  | CBS 119.27 = NRRL 4876 | Soil, Iowa, USA                                                       |
|                  | CBS 102278 | Subcutaneous infection, Spain                                          |
| A. kassunensis   | CBS 419.69T = NRRL 3752 = IMI 334938 = IBT 23479 | Soil, Damascus, Syria                                                |
| A. kevei         | CBS 209.92 | Soil, La Palma, Spain                                                 |
|                  | CBS 561.65 = NRRL 1974 | Soil, Panama                                                          |
|                  | IBT 10524 = CBS 113227 = NRRL 1254 | Soil, Panama                                                        |
|                  | IBT 16751 | Soil at trail from Pelican Bay to inland, Isla Santa Cruz, Galapagos Islands, Ecuador, Tjtte de Vries and D.P. Mahoney, 1968 |
| A. lucknowensis  | CBS 449.75T = NRRL 3491 | Alkaline Usar soil, Lucknow, India                                   |
| A. monodii       | CBS 434.93 | Dung of Procavia sp. (daman), Darfur, Sudan                           |
|                  | CBS 435.93T | Dung of sheep, Ennedi, Chad                                            |
| A. pseudodeflectus | CBS 596.65 | Sugar, USA, Louisiana                                                 |
|                  | CBS 756.74T | Desert soil, Egypt, Western Desert                                    |
|                  | NRRL 4846 = IBT 25256 | Unknown                                                               |
| A. pseudostinus  | ATCC 36063 = NRRL 5856 = CSIR 1128 = CBS 123904T = IBT 28161 | Stored maize, South Africa                                           |
|                  | MRC 096 = IBT 31044 | Contaminant in a Bipolaris sorokiniana strain (MRC 093), South Africa |
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).

**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: 583.65).

| Species   | Strain No. | Source                                           |
|-----------|------------|--------------------------------------------------|
| A. pseudustus | IBT 22361  | Indoor air, Finland                              |
| A. puniceus    | CBS 495.65¹ | Soil, Zarcero, Costa Rica                        |
|              | CBS 128.62  | Soil, Louisiana, USA                             |
| A. subassilis | CBS 502.65¹ | Desert soil, Mojave desert, CA, USA              |
|              | CBS 988.72  | Desert soil, USA                                 |
| A. turkensis  | CBS 504.65¹ | Soil, Turkey                                     |
| A. ustus      | CBS 116057  | Antique tapestries, Krakow, Poland               |
|              | CBS 114901  | Carpet, The Netherlands                          |
|              | CBS 261.67¹ | Culture contaminant, USA                         |
|              | CBS 133.55  | Textile buried in soil, Netherlands              |
|              | CBS 239.90  | Man, biopsy of brain tumor, Netherlands          |
|              | CBS 113233 = IBT 14495 | Cave wall, Lechuguilla Cave, Carlsbad, New Mexico |
|              | CBS 113232 = IBT 14932 | Indoor air, Denmark                           |

*Table 1. (Continued).*
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 Fennellia 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). Fennellia 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 which was isolated outside Egypt.

Another new species in this section was isolated from indoor air in Germany. This species has identical ITS sequences with two-valved ascospores with low, wrinkled equatorial crests. The anamorph 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. amylolvorus and belongs to section Usti, while the A. varians type strain belongs to Aspergillus section Nidulantes (data not shown). Aspergillus amylolvorus 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. amylolvorus).

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 austocystsins and versicorolins. 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 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 insuetus 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-hydroxyverrucarin (Yabe et al. 1991), emetherone (Kawahara et al. 1988), emesterones A & B (Hosoe et al. 1998), 5-hydroxyverrucarin (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 desferritriacetylfusigen, which inhibits the growth of bacteria (Anke 1977), and deflectins, angular azaphilons, which have antibiotic properties, and exhibit lytic activities against bacteria and erythrocyes (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 (Zohn & Ismail 1994). Aspergillus egyptiacus produces fumitremorgins and verruculinog, 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 norgeamide / notoamide / aspargamide / steptacidi 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. pseudoustus NRRL 5856 = CSIR 1128, which was originally identified as A. ustus and the first strain from which austamides, austidols and austocystins (Table 2) were isolated (Steyn 1971, 1973, Steyn & Vleggar 1974, 1976a, b, Vleggar 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, (MTC-120B, ALTIN, FAAL, KNOK)
A. californicus An arugosin, (CANDU, SAERLO, SCAM, SEND, XANXU)
A. carlsbadensis 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)]
A. deflectus Desferribacetylfuscigen, deflectins A & B, emerin, a shamianthaxone, (FUMU, RED2)
A. egypiticus Fumitremorgin A, fumitremorgin B, verruculogen, (FYEN, UTSCAB, TOPLA, FUMU, PRUD, HØJV)
A. elongatus Fumitremorgin C, notoamide E, (DYK, SEXT, 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, emethelaterone, emetherones A & B and Mer-NF8054X, 5-hydroxyaveranthin, stellatin, sterigmatocystin, (DRI, NIDU)
A. insuetus Asperugins, drimans, ophiobolins G and H, pergillin-like compound, (AU, HETSCYT, INSU)
A. kastenateus Asperugins, Mer-NF8054X, (FYRT, SAERLO, SENSCAB, SENSTER)
A. keveii Asperugins, drimans, ophiobolins G and H, nidulol, (FUMU, HETSCYT, INSU, PUBO, SENSTER, UP)
A. lucknowensis An arugosin, (GULT, PULK, RED1)
A. monodii Terein, (DYVB, METK)
A. pseudodeflectus Drimans, (DRUL, SNOF, SLOT), asperugin in NRRL 4846
A. pseudodeflectus Asperugins, austamide, prolyl-2-(1',1'-dimethylallyl) tryptophyl diketopiperazine, 12,13-dihydroaustamide, 12,13-dehydroprolyl-2-1',1'-dimethylallyl)-tryptophyl diketopiperazine, 10,20-dehydro[12,13-dehydropropyl-2-1',1'-dimethylallyl]tryptophyl diketopiperazine, 12,13-dihydro-12-hydroxyaustamide, austidol, dihydrodeoxy-8-epi-austidol, austocystin A, B, C, D, E, F, G, H, I, norsolorinic acid, versicocerin C, averufin, (DRI, HETSCYT, SENSTER, UZ)
A. puniceus Ustic acids, austocystins (and versicolorins), phenylalanin, nidulol, (SENSCAB)
A. subsessilis Mer-NF8054X, (SENSCAB, VIRO)
A. turkensis An austocystin, deflectins, emerin, a shamianthaxone, (RED2)
A. ustus Ustic acids, austocystins (and versicolorins), austalides, nidulol, (SENSCER)

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.

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

Colonii flavo-brunnei, cum caespitulis ex conglomerationibus cellularum obtegentium (“Hülle”). Cellulis obtegentibus (“Hülle”) hyalinis, crassitunicatis, globosis vel late ellipsoidis, 15–30 μm. Conidiphoris biserialis, stipitibus plerunque levibus, brunnis, 4–5 μm latis. Vesiculis globosis, 10–14 μm diam. Conidios conspicue ornamentatis, echinulatis et verrucosis, ellipsoidis, 2.5–3.0 × 3.0–3.5 μm.

Typus: USA, from soil, Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico, isolated by D.E. Northup, 1992, (CBS H-30634 – holotypus, culture ex-type CBS 123894).

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. Conidiphores 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 clare flavis, cum caespitulis abidis ex conglomerationibus cellularum obtectegentium ("Hülle"). Cellulis obtectegentibus ("Hülle") hyalinis, crassitunicatis, globosis vel late ellipsoideis. Conidiophoris biseriatis, stipitibus levibus, clare brunneis, 3.5–5 μm latis. Vesiculis globosis, 11–16 μm in diam. Conidiospores vel subtiliter exasperati, subglobosis vel globosis, hyalinis vel viridibus, 2.5–3.0 μm.

Typus: **USA**, foothills of San Gabriel Mountains, California, ex chasmis chapparral (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, Hüle 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 (Hüle cells), CYA, 1 wk, 37 °C: no growth, CREA: good growth and no acid production.

Colonies light yellow with white tufts of conglomerates of Hüle cells. Hüle cells hyaline, thick-walled, globose to broadly ellipsoidal, 25–50 μm. Conidiophores biseriate with smooth-walled, light brown, 3.5–5 μm wide stipes. Vesicles globose, 11–16 μm in diam. Conidia, smooth to finely roughened, subglobe 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** Varga, Frisvad & Samson, sp. nov. MycoBank MB560401. Fig. 6.

Colonies in agar **CyA** cinnamomeo-brunneis et in agar MEA flavo-brunneis, cellulis obtectegentibus ("Hülle") nullis. Conidiophoris biseriatis, stipitibus plerumque levibus, brunneis, 6–9 μm latis. Vesiculis spathuliformibus, 14–22 μm diam. Conídiospores echinulati, globosis, brunneis, 3.5–5.0 μm diam.

Typus: **Germany**, ex indoor air, Stuttgart. Isolated by U. Weidner (CBS H-20636 — holotypus, culture ex-type CBS 123887).

**CyA**, 1 wk, 25 °C: 22–26 mm (poor to medium sporulation, yellow brown to orange reverse, pigment diffusing, Hüle 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 griseous brown. Hüle 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.

**Basionym:** *Fennelia monodii* Locquin-Linard, *Mycotaxon* 39: 10, 1990.

**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. Hüle cells forming the structure of the stromata, globose to ellipsoidal, 8–40 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 pseudoustus** Frisvad, Varga & Samson, sp. nov. MycoBank MB560403. Fig. 8.

Colonies in agar **CyA** cinnamono-brunneis et in agar MEA flavo-brunneis, cellulis obtectegentibus ("Hülle") nullis. Conidiophoris biseriatis, stipitibus plerumque levibus, brunneis, 3.5–5 μm latis. Vesiculis globosis, 10–14 μm diam. Conidiospores levibus distinct echinulati, globosis, brunneis vel viridibus, 2.5–3.0 μm.

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. Hüle 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 *Bipolaris sorokiniana*, isolated from maize, South Africa; IBT 22361, indoor air; Finland

**Aspergillus pseudoustus** sp. nov., is related to, but clearly different from *A. ustus* and *A. punicicus* 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 **CyA** clare brunneis et in agar MEA flavo-brunneis, cellulis obtectegentibus ("Hülle") nullis. Conidiophoris minute biseriatis, stipitibus plerumque levibus, brunneis, 6–9 μm latis. Vesiculis spathuliformibus, 14–22 μm diam. Conidiospores echinulati, globosis, brunneis, 3.5–5 μm diam.

Typus: **Turkey**, ex soil isolated by K.B. Raper in 1950 (CBS H-20638 — holotypus, culture ex-type CBS 504.65).

**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

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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 Hüße cells. D–E, G–I. Conidiophores and conidia. F. Hüße cells. Scale bars = 10 µm.
new taxa in *Aspergillus* section *Usti*

Fig. 7. *Aspergillus monodi* (Locquin-Linard) Varga, Fritsved & Samson comb. nov. A–B. Stromata containing ascomata, grown at 25 °C for 7 d, C. Mycelium with ascoma initials. D. Hüle cells, E–G. Asci and ascospores. 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. Hüle 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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Fig. 8. Aspergillus pseudoostus 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.
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