Aspergillus uvarum sp. nov., an uniseriate black *Aspergillus* species isolated from grapes in Europe

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A novel species, *Aspergillus uvarum* sp. nov., is described within *Aspergillus* section *Nigri*. This species can be distinguished from other black aspergilli based on internal transcribed spacers (ITS), β -tubulin and calmodulin gene sequences, by AFLP analysis and by extrolite profiles. *Aspergillus uvarum* sp. nov. isolates produced secalonic acid, common to other *Aspergillus japonicus*-related taxa, and geodin, erdin and dihydrogeodin, which are not produced by any other black aspergilli. None of the isolates were found to produce ochratoxin A. The novel species is most closely related to two atypical strains of *Aspergillus aculeatus*, CBS 114.80 and CBS 620.78, and was isolated from grape berries in Portugal, Italy, France, Israel, Greece and Spain. The type strain of *Aspergillus uvarum* sp. nov. is IMI 388523^T=CBS 127591^T=ITEM 4834^T=IBT26606^T.

INTRODUCTION

Black aspergilli (*Aspergillus* section *Nigri*; Gams *et al.*, 1985) have a significant impact on modern society. Many species cause food spoilage, and several are used in the fermentation industry to produce hydrolytic enzymes, such as amylases or lipases, and organic acids, such as citric acid and gluconic acid (Varga *et al.*, 2000). They can also be employed for genetic manipulation in the biotechnology industries, for example *Aspergillus niger* used under certain industrial conditions has been granted GRAS (Generally Regarded As Safe) status by the Food and Drug Administration of the US

Abbreviations: AFLP, amplified fragment length polymorphism; ITS, internal transcribed spacer; NJ, neighbour-joining; SEM, scanning electron microscopy; TBR, tree bisection and reconstruction; UPGMA, unweighted pair group method.

The GenBank/EMBL/DBJ accession numbers for the β -tubulin, ITS and calmodulin gene sequences of the strains examined in this study are shown in Table 1. The MycoBank accession number for *Aspergillus uvarum* sp. nov. is MB 510962 (<http://www.mycobank.org>).

Phylogenetic trees based on ITS and calmodulin gene sequence data and a dendrogram based on cluster analysis of AFLP data are available with the online version of this paper.

government. Although the main habitat of black aspergilli is soil, members of this section have been isolated from various other sources (Kozakiewicz, 1989; Abarca *et al.*, 2004; Samson *et al.*, 2004). Apart from their economical importance, black aspergilli are also important as ochratoxin-producing organisms which contaminate several agricultural products, including grape-derived products, coffee and cocoa (Cabañes *et al.*, 2002; Samson *et al.*, 2004; Battilani *et al.*, 2006 and other papers in this special issue).

In spite of their importance, the taxonomy of this section is still not completely resolved and the species distribution in food is often not clearly reported. Black aspergilli are one of the more difficult groups to classify and identify and the taxonomy of *Aspergillus* section *Nigri* has been studied by many taxonomists (Abarca *et al.*, 2004; Varga *et al.*, 2007). Nuclear and mitochondrial DNA (mtDNA) polymorphisms and PCR-based techniques led to the recognition of at least two species within the *A. niger* species complex (*A. niger* and *Aspergillus tubingensis*) (Kusters-van Someren *et al.*, 1991; Varga *et al.*, 1994). Phylogenetic analyses of sequences of the internal transcribed spacers region (ITS), the 5.8S rRNA gene and the D1–D2 region of the 28S rRNA gene indicated that,

in addition to *A. niger* and *A. tubingensis*, at least five other species belong to section *Nigri*: *Aspergillus heteromorphus*, *Aspergillus ellipticus*, *Aspergillus carbonarius*, *Aspergillus japonicus* and *Aspergillus aculeatus* (Varga *et al.*, 2000; Parenicova *et al.*, 2001). Several other black *Aspergillus* species have been described recently, including *Aspergillus vadensis* (de Vries *et al.*, 2005), *Aspergillus costaricensis*, *Aspergillus piperis*, *Aspergillus lacticoffeatus* and *Aspergillus sclerotioniger* (Samson *et al.*, 2004), *Aspergillus ibericus* (Serra *et al.*, 2006) and *Aspergillus brasiliensis* (Varga *et al.*, 2007).

During a molecular survey of black *Aspergillus* isolates collected from 107 vineyards in different European countries within the EU project Wine-Ochra Risk (QLK1-CT-2001-01761), we discovered a group of uniseriate aspergilli morphologically very similar to the well-recognized species *A. japonicus* and *A. aculeatus*, but clearly separated by amplified fragment length polymorphism (AFLP) molecular analysis (Perrone *et al.*, 2006). In this paper, we analyse the relationships of these isolates to other aspergilli using molecular and chemotaxonomic approaches, and describe these isolates as a novel species, *Aspergillus uvarum* sp. nov.

METHODS

The strains of the novel species examined are listed in Table 1. The morphology of these strains was re-examined according to Raper & Fennell (1965) and Kozakiewicz (1989). The strains were maintained on malt extract autolysate (MEA) agar slants. The 28 *Aspergillus* section *Nigri* strains listed in Table 2 were used as reference strains for sequences and AFLP comparison analyses, and the type strain of *Aspergillus flavus* CBS 100927^{NT} was used as an outgroup.

Morphological analysis. For macromorphological observations, Czapek yeast autolysate (CYA), MEA agar, Czapek agar (CZA) (Samson *et al.*, 2004) and G25N agar (Pitt, 1979) were used. Czapek agar with 20 % sucrose (CZ20) and malt and yeast extract agar with 40 % sucrose (M40Y) were made as described previously (Raper & Fennell, 1965). Growth rates were studied and compared with reference strains for 7 days on CYA at 5, 25 and 37 °C, on G25N, CZA and MEA at 25 °C in the dark. Cultures were also grown on CZ20 and M40Y for 7 days at 25 °C in the dark to assess growth on media with reduced water activity. For micromorphological observations, microscopic mounts were made in lactic acid from MEA colonies and a drop of alcohol was added to remove air bubbles and excess conidia. Scanning electron microscopy (SEM) was performed with uncoated frozen samples and micrographs consisted of 30 averaged fast scans (SCAN 2 mode) in a JEOL 5600LV scanning electron microscope equipped with an Oxford CT1500 cryostation.

Extrolite analysis. Extrolites were analysed by HPLC using alkylphenone retention indices and diode array UV-VIS detection as described by Frisvad & Thrane (1987), with modifications as described by Smedsgaard (1997). Standards of ochratoxin A and B, aflavinine, asperazine, austriol, kotanin, asteric acid, secalonic acid D, neoxaline and roquefortine D from the collection at BioCentrum-DTU (Danish Technical University) were used for comparison with the extrolites from the strains under study.

Isolation and analysis of nucleic acids. Forty-four strains representative of the uniseriate black aspergilli isolated from grapes were analysed by sequence analysis together with 27 representative strains of section *Nigri*. Total nucleic acids were isolated as described

previously (Leach *et al.*, 1986). Fragments containing the region encoding the internal transcribed spacer 1 (ITS-1), 5.8S rDNA and internal transcribed spacer 2 (ITS-2) were amplified using primers ITS1 and ITS4 as described previously (White *et al.*, 1990). Amplification of part of the β -tubulin gene was performed using the primers Bt2a and Bt2b (Glass & Donaldson, 1995; Samson *et al.*, 2004). Amplifications of the partial calmodulin gene were set up as described previously (Perrone *et al.*, 2004). Sequence analysis was performed with the Big Dye Terminator Cycle Sequencing Ready reaction kit for both strands, and the sequences were aligned with the MT Navigator software (Applied Biosystems). All the sequencing reactions were purified by gel filtration through Sephadex G-50 (Amersham Pharmacia Biotech) equilibrated in double-distilled water and analysed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Representative ITS, β -tubulin and calmodulin sequences were deposited at the GenBank nucleotide sequence database (Table 1).

AFLP analysis. Seventy-nine strains, 54 uniseriate *Aspergillus* isolates from grapes and 25 isolates belonging to different species of *Aspergillus* section *Nigri*, were analysed by AFLP analysis (Tables 1 and 2). Fungal strain growth and relative DNA isolation were done according to Perrone *et al.* (2006).

Fluorescent AFLP was performed as described in the AFLP microbial fingerprinting kit protocol (Applied Biosystems). Four separate primer combinations were utilized for the selective amplifications: *EcoRI* + AC and *MseI* + CC, *EcoRI* + AT and *MseI* + CG, *EcoRI* + AC and *MseI* + CA and *EcoRI* + G and *MseI* + CT. GeneScan-500 (ROX) was used as internal size standard (Applied Biosystems). The product was separated by capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). After electrophoresis, the pattern was extracted with the GeneScan version 3.1.2 software (Applied Biosystems) and the fingerprints were automatically analysed with the Genotyper software (Applied Biosystems). To test the reproducibility of the method, DNA of five strains was isolated from three replicate cultures and tested separately in triplicate. DNA of remaining strains was tested in duplicate.

Peak height thresholds were set at 200. Genotyper software (Applied Biosystems) was set to medium smoothing. Bands of the same size in different individuals were assumed to be homologous and to represent the same allele. Bands of different size were treated as independent loci with two alleles. Data were analysed with the AFLP Manager database (ACGT BioInformatica) and were exported in a binary format with '1' for presence of a peak and '0' for its absence. For clustering, two different analyses were performed: fragments between 100 and 500 bp and fragments between 200 and 500 bp were analysed with NTSyspc software (Exeter Software) by using the Dice similarity coefficient and clustered by the unweighted pair group method (UPGMA) (Nei & Li, 1979). *A. flavus* CBS 100927^{NT} was used as an outgroup in these experiments.

Analysis of sequence data. The alignment of ITS regions, partial β -tubulin and calmodulin gene sequence data was performed using the software package BioNumerics (Applied Maths) and manual adjustments were made where necessary.

Phylogenetic trees were prepared by the neighbour-joining (NJ) method (Saitou & Nei, 1987) using the program NEIGHBOR of the PHYLIP package. Bootstrap values were calculated from 1000 replications of the bootstrap procedure using programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package (Felsenstein, 1985; 1995). For parsimony analysis, the PAUP* version 4.0 software was used (Swofford, 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum-parsimony analysis was performed for all datasets using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping

algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis & Bull, 1993). *A. flavus* CBS 100927^{NT} was used as an outgroup in these experiments.

RESULTS AND DISCUSSION

Several uniseriate black *Aspergillus* strains were isolated from grape berries in the Mediterranean area, mainly from Italy, France and Israel. They were found to be related to *A. japonicus*, based on morphological data, and none of these *A. japonicus*-related isolates produced ochratoxin A. Morphologically, *A. uvarum* sp. nov. is most closely related to *A. japonicus*. However, both vesicle and conidium sizes are somewhat smaller in *A. uvarum* sp. nov. For example, vesicle size in *A. uvarum* sp. nov. ranges from 20 to 30 µm diameter, whereas for *A. japonicus* they average 20–30 µm, but can be as large as 45 µm. Conidial shape is similar, namely subglobose to globose, but *A. japonicus* conidia are mostly 3.0–4.5 µm, compared with 3.0–4.0 µm for *A. uvarum* sp. nov. *A. aculeatus* has much larger conidial heads with vesicles ranging from 22 to 55 µm in diameter and conidia which are ellipsoidal in shape.

However, it is growth rates, particularly on CYA at 37 °C, which clearly separate *A. uvarum* sp. nov. from *A. japonicus* and *A. aculeatus*. Growth of *A. uvarum* sp. nov. is much slower, with colony diameters after 7 days growth ranging from 16 mm for isolates *A. uvarum* sp. nov. IMI 388537 and IMI 388671 to 23 mm for isolates IMI 388523, IMI 388537 and IMI 388671. Colony diameters for *A. japonicus* CBS 114.51^{NT} and *A. aculeatus* IMI 211388^T were 30 mm after 7 days. These morphological and physiological differences, together with the extrolite and molecular information discussed below, indicate that *A. uvarum* sp. nov. is a novel uniseriate species within section *Nigri*.

Furthermore, these isolates could readily be distinguished from other uniseriate black aspergilli by AFLP analysis (Perrone *et al.*, 2006). We examined the genetic relatedness of some selected isolates (Table 1) to other black aspergilli, analysing nucleotide sequences of partial rDNA (ITS-1, 5.8S and ITS-2), calmodulin and β -tubulin genes. The β -tubulin dataset included 551 characters, with 161 parsimony-informative characters. The NJ tree based on partial β -tubulin gene sequences is shown in Fig. 1.

The topology of the β -tubulin tree is the same as that of a parsimony tree constructed by the PAUP* program (length: 437 steps, consistency index: 0.6865, retention index: 0.8880). The ITS dataset included 475 characters with 72 parsimony-informative characters. The NJ tree based on ITS sequence data, shown in Supplementary Fig. S1 (available in IJSEM Online), has the same topology as the ITS parsimony tree (tree length: 168, consistency index: 0.9048, retention index: 0.9615). The calmodulin dataset included 666 characters, with 272 parsimony-informative characters. The topologies of the calmodulin NJ tree (Supplementary Fig. S2) and the parsimony tree were the

same (tree length: 664, consistency index: 0.7229, retention index: 0.8999).

Although sequence analysis of the ITS region placed all of the uniseriate black aspergilli into the same clade, the β -tubulin and calmodulin sequence data both indicate the presence of three, closely related uniseriate taxa. In fact, these isolates formed a monophyletic clade supported by high bootstrap values on phylogenetic trees based on β -tubulin and calmodulin sequence data (Fig. 1 and Supplementary Fig. S2), despite a difference in topology of the two trees in the concordant branches which identified the closely related taxa *A. aculeatus* and *A. japonicus*. The grape-derived isolates were found to be closely related to the atypical '*A. aculeatus*' isolates CBS 114.80 and CBS 620.78. These isolates, together with recent isolates from Thai coffee beans, represent another novel species in section *Nigri* (P. Noonim, R. A. Samson and J. Varga, unpublished data).

The grape-derived isolates also formed a well-defined cluster on the UPGMA tree obtained from AFLP data (Supplementary Fig. S3) relative to four-primer combination assays. Our data indicate that these isolates are well separated from other black aspergilli, based on all molecular approaches used. Specific polymorphisms, within and between species, were observed by AFLP. Each primer combination consistently distinguished the 16 different species of *Aspergillus* species of section *Nigri*: similarity obtained among species analysed was less than 20 % (Supplementary Fig. S3). All the strains belonging to the same species shared more than 50 % of peaks. The *Aspergillus uvarum* cluster consists of a main group, including 48 strains clustered at 64 % similarity, and 6 strains (4958, 4959, 4961, 5287, 4997 and 4848), having 52 % similarity with the others (Supplementary Fig. S3).

Extrolite profiles of these isolates were characteristic and unique among the black aspergilli. The isolates of the new taxon proposed below produce secalonin acid D and F in common with *A. aculeatus*, but produced geodin, erdin, dihydrogeodin and asteric acid, which are not seen in any other black aspergilli. Geodin, erdin, dihydrogeodin and asteric acid are characteristic extrolites of *Aspergillus terreus* (Raistrick & Smith, 1936) and *Penicillium glabrum* (Mahmoodian & Stickings, 1964; Sato *et al.*, 2005) and are related to tryptacidin and monomethylsulochrin, produced by *Aspergillus fumigatus* (Turner, 1965; Larsen *et al.*, 2007).

An examination of the extrolite profiles of the uniseriate black aspergilli showed that *A. aculeatus* produced secalonin acid D and F and physcion, *A. japonicus* isolates produced festuclavine and cycloclavin and the novel species produced secalonin acid D and F, asteric acid, erdin, geodin and dehydrogeodin. The isolate identified as an atypical *A. aculeatus*, CBS 114.80, produced okaramine A, B, H and I; *Aspergillus brunneoviolaceus* CBS 621.78^T produced okaramines, secalonin acid D and neoxaline and CBS 620.78 produced secalonin acid D and F, roquefortine D and some unique indole alkaloids. This is in agreement

Table 1. *Aspergillus uvarum* sp. nov. isolates from grapes examined

Isolate*	Origin	GenBank accession no.†		AFLP‡
		Calmodulin	β -tubulin	
ITEM 4685 (=IMI 387209)	Régua, Douro Region, Portugal	II	IV	+
ITEM 4690 (=IMI 387339)	Soave (VR), Veneto, Italy	I	V	+
ITEM 4691 (=IMI 387341)	Soave (VR), Veneto, Italy	I	V	+
ITEM 4692 (=IMI 387342)	Felizzano (CN), Piedmont, Italy	I	V	+
ITEM 4693 (=IMI 387343)	Carpaneto (PC), Lombardy, Italy	I	V	+
ITEM 4716 (=IMI 388182)	Bari, Apulia, Italy	NS	NS	+
ITEM 4727 (=IMI 388156)	Brindisi, Apulia, Italy	NS	NS	+
ITEM 4814 (=IMI 388506)	Montpellier, Vallée du Rhone, France	NS	NS	+
ITEM 4833 (=IMI 388522)	Brindisi, Apulia, Italy	I	V	+
ITEM 4834 ^T (=IMI 388523)	Brindisi, Apulia, Italy	I	V	+
ITEM 4835 (=IMI 388525)	Alghero, Sardinia, Italy	I	V	+
ITEM 4843 (=IMI 388532)	Verona, Veneto, Italy	II	V	+
ITEM 4844 (=IMI 388533)	Verona, Veneto, Italy	II	V	+
ITEM 4848 (=IMI 388537)	Piacenza, Emilia Romagna, Italy	NS	NS	+
ITEM 4850 (=IMI 388539)	Piacenza, Emilia Romagna, Italy	NS	NS	+
ITEM 4856 (=IMI 388545)	Ravenna, Emilia Romagna, Italy	NS	NS	+
ITEM 4857 (=IMI 388546)	Ravenna, Emilia Romagna, Italy	I	V	+
ITEM 4958 (=IMI 388711)	Spain	NS	NS	+
ITEM 4959 (=IMI 388712)	Spain	II	IV	+
ITEM 4960 (=IMI 388713)	Spain	NS	NS	+
ITEM 4961 (=IMI 388714)	Spain	NS	NS	+
ITEM 4962 (=IMI 388715)	Spain	III	IV	+
ITEM 4995 (=IMI 388669)	Lachish, Israel	III	VII	+
ITEM 4996 (=IMI 388668)	Gschur, Israel	III	VI	+
ITEM 4997 (=IMI 388670)	Lichron, Israel	II	VI	+
ITEM 4998 (=IMI 388671)	Polaia, Israel	III	VI	+
ITEM 4999 (=IMI 388667)	Zichron, Israel	III	VII	+
ITEM 5013 (=IMI 388656)	Alghero, Sardinia, Italy	NS	NS	+
ITEM 5019 (=IMI 388659)	Brindisi, Apulia, Italy	I	V	+
ITEM 5020 (=IMI 388660)	Brindisi, Apulia, Italy	I	V	+
ITEM 5021 (=IMI 388661)	Lecce, Apulia, Italy	I	V	+
ITEM 5023 (=IMI 388662)	Alghero, Sardinia, Italy	I	V	+
ITEM 5246 (=IMI 388906)	Nauosa, Xinamauro, Greece	NS	NS	+
ITEM 5287 (=IMI 388910)	Aghios Paulos, Greece	II	VII	+
ITEM 5321 (=IMI 389195)	Narbonne, Languedoc, France	III	VII	–
ITEM 5322 (=IMI 389196)	Narbonne, Languedoc, France	III	V	+
ITEM 5323 (=IMI 389197)	Narbonne, Languedoc, France	III	VII	+
ITEM 5324 (=IMI 389209)	Rives, Altes, Rousillon, France	III	V	+
ITEM 5325 (=IMI 389198)	Narbonne, Languedoc, France	III	VII	+
ITEM 5326 (=IMI 389199)	Narbonne, Languedoc, France	III	V	+
ITEM 5327 (=IMI 389200)	Narbonne, Languedoc, France	NS	NS	+
ITEM 5328 (=IMI 389201)	Narbonne, Languedoc, France	III	VII	+
ITEM 5330 (=IMI 389203)	Rivesaltes, Roussillon, France	III	VII	+
ITEM 5331 (=IMI 389204)	Rivesaltes, Roussillon, France	III	VII	+
ITEM 5332 (=IMI 389205)	Pierrefeu, Alpes Maritime, France	III	VII	+
ITEM 5333 (=IMI 389206)	Rivesaltes, Roussillon, France	III	VII	+
ITEM 5334 (=IMI 389207)	Rivesaltes, Roussillon, France	III	VII	+
ITEM 5335 (=IMI 389208)	Rivesaltes, Roussillon, France	II	V	+
ITEM 5337 (=IMI 389211)	Montpellier, Vallée du Rhone, France	III	VII	+
ITEM 5338 (=IMI 389212)	Montpellier, Vallée du Rhone, France	NS	NS	+
ITEM 5339 (=IMI 389213)	Montpellier, Vallée du Rhone, France	III	VII	+
ITEM 5340 (=IMI 389214)	Montpellier, Vallée du Rhone, France	III	VII	+
ITEM 5347 (=IMI 389163)	Kfar Daniel, Israel	III	VII	–
ITEM 5348 (=IMI 389164)	Pdaya, Israel	III	VII	+
ITEM 5349 (=IMI 389165)	Pdaya, Israel	NS	NS	+
ITEM 5350 (=IMI 389166)	Pdaya, Israel	III	VI	+

NS, not sequenced.

*IMI, CABI Bioscience Genetic Resource Collection, Egham, UK; ITEM, Agri-Food Toxigenic Fungi Culture Collection, Institute of Sciences of Food Production, Bari, Italy.

†ITS sequences are identical in all strains to ITEM 4834 (GenBank accession no. AM745757). Sequences identical to the deposited GenBank accession numbers are grouped as follows: I, AM745755; II, AM745754; III, AM745756; IV, AM745750; V, AM745751; VI, AM745752; VII, AM745753.

‡Strains subjected (+) or not (–) to AFLP analysis.

with the separation of these isolates suggested by molecular results (Fig. 1, Supplementary Figs S2 and S3). Thus both extrolite profiles and molecular data indicate that five species can be differentiated in the uniseriate black aspergilli. The isolates of *A. aculeatus* and *A. japonicus* examined and the new species described here were consistent in their production of extrolites, and thus can be easily differentiated chemically. Based on common production of secalonic acid D and F, the novel species (represented by ITEM 4834^T) appears to be more closely related to *A. aculeatus* than to *A. japonicus*.

Our findings were based on a polyphasic approach to the species concept in *Aspergillus* (Frisvad & Samson, 2004). A novel species is different from any other species in a diagnostic sense in both phenotypic and genotypic features. Here we have used morphological, physiological and chemotaxonomic features to characterize the phenotype and sequencing of three genes combined with AFLP results to characterize the isolates genotypically. Since the isolates were unique with regard to morphology, extrolite profiles and genotypic features, we propose the name *Aspergillus uvarum* sp. nov. for these isolates.

Description of *Aspergillus uvarum* Perrone, Varga et Kozakiewicz sp. nov. Fig. 2.

Coloniae in agar Czapekii celeriter crescentes in septem dies 25 °C, 90 mm diametro attigentes, granulosa, superficie brunnea-nigra, facie inferiore primo alba, deinde obscure ochracea; exudata non conspicuosa, con solubis pigmentis ochraceis. Sclerotia producta, brunnea usque nigra, globosa vel verticale elongata. Conidiogenesis abundanta. Capitula conidica radiantia, in columnas aegre formatas fissantia, 200–300 µm diametro. Stipites leves incolores, superne pallide ochracei, 500–1000 µm longissimi, parie plerumque 5–10 µm crassis; vesiculae globosae vel ellipticae 20–30 µm diametro, per toto fertilibus. Aspergilla uniseriata; phialides 5–7 × 2.5–3.0 µm. Conidia brunnea-nigra oculo nudo visa, globosa vel subglobosa, 3–4 µm, conspicue spinulosa in maturitate et spinis 0.5 µm projecti.

Typus siccus in herb. IMI 388523^T (=CBS 127591^T=ITEM 4834^T=IBT26606^T) *et ex-typus vivus, isolatus e botrys, Brundisium, Italia.*

Description of *Aspergillus uvarum* Perrone, Varga et Kozakiewicz sp. nov.

Aspergillus uvarum (u.va'rum. L. masc. adj. *uvarum* of grapes).

Colonies grow rapidly on Czapek agar, attaining 90 mm diameter within 7 days at 25 °C, granular, upper surface brown-black; reverse white, wrinkled, becoming dull yellow with age with black colony centres; a soluble pale-yellow pigment is produced; exudate not conspicuous. Sclerotia produced in some fresh isolates, dark brown to black, globose to vertically elongated. Abundant conidiogenesis.

Table 2. *Aspergillus* section *Nigri* strains used in this study

CBS, Centraalbureau voor Schimmelfcultures, Utrecht, The Netherlands; JHC, James H. Croft's culture collection, Birmingham, UK; IMI, CABI Bioscience Genetic Resource Collection, Egham, UK; ITEM, Agri-Food Toxigenic Fungi Culture Collection, Institute of Sciences of Food Production, Bari, Italy; IBT, Center for Microbial Biotechnology, BioCentrum-DTU, Lyngby, Denmark; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural, Utilization Research, Peoria, IL, USA.

Isolate
<i>Aspergillus brasiliensis</i> ITEM 7048 ^T =IMI 381727 ^T =CBS 101740 ^T =JHC 614 ^T
<i>Aspergillus tubingensis</i> ITEM 4500 ^T =IMI 172296 ^T =CBS 115.29 ^T ITEM 7040 ^T =CBS 134.48 ^T
<i>Aspergillus niger</i> ITEM 4501 ^T =IMI 050566 ^T =CBS 554.65 ^T ITEM 4506=IMI 015954=CBS 126.48
<i>Aspergillus carbonarius</i> ITEM 4503 ^T =IMI 016136 ^T =CBS 556.65 ^T ITEM 4504=IMI 041875=CBS 420.64 ITEM 4556=IMI 387242
<i>Aspergillus ibericus</i> ITEM 4776 ^T =IMI 391429 ^T ITEM 6600=IMI 391428
<i>Aspergillus foetidus</i> ITEM 4508=IMI 175963=CBS 565.65 ITEM 4507=IMI 104688=CBS 564.65
<i>Aspergillus ellipticus</i> ITEM 4505 ^T =IMI 172283 ^T =CBS 482.65 ^T CBS 707.79 ^{NT} =IMI 278384 ^{NT}
<i>Aspergillus aculeatus</i> ITEM 7047=CBS 621.78=IMI 312981* ITEM 7038=CBS 114.80 ITEM 7046 ^T =CBS 172.66 ^T =IMI 211388 ^T CBS 620.78=NRRL 2053
<i>Aspergillus japonicus</i> ITEM 4497=IMI 211387=CBS 568.65 ITEM 7034 ^T =CBS 114.51 ^T
<i>Aspergillus heteromorphus</i> ITEM 7045=CBS 117.55=IMI 172.288
<i>Aspergillus vadensis</i> ITEM 7561 ^T =CBS 113.365 ^T =IMI 313.493 ^T
<i>Aspergillus costaricensis</i> ITEM 7555 ^T =CBS 115574 ^T =IBT 23401 ^T
<i>Aspergillus sclerotioniger</i> ITEM 7560 ^T =CBS 115572 ^T =IBT 22905 ^T
<i>Aspergillus lacticoffeatus</i> ITEM 7557=CBS 101884=IBT 22030 ITEM 7558=CBS 101886=IBT 22032 ITEM 7559 ^T =CBS 101883 ^T =IBT 22031 ^T
<i>Aspergillus homomorphus</i> ITEM 7556 ^T =CBS 101889 ^T

*Type of *A. brunneoviolaceus*.

Fig. 1. Phylogenetic tree based on β -tubulin sequence data of *Aspergillus* section *Nigri*. Numbers next to nodes are bootstrap values. Only values above 70 % are indicated. Bar, changes per nucleotide position.

Conidial heads radiate, splitting into poorly defined columns as they age, 200–300 μ m in diameter. Stipes smooth, 500–1000 $\times$ 5–10 μ m, uncoloured, upper portion light yellowish-brown; vesicles globose to elliptical, 20–30 μ m, fertile over the entire surface. Aspergilli uniseriate; phialides 5–7 $\times$ 2.5–3.0 μ m. Conidia brown–black when seen with the naked eye, globose to subglobose, 3–4 μ m, conspicuously spinose at maturity with spines projecting 0.5 μ m.

Colonies grown for 7 days at 25 °C on CYA overgrow a 90 mm plate. Sclerotia produced by some fresh isolates, usually coloured black. Abundant conidiogenesis. Conidial heads brown–black, mycelium white, usually inconspicuous; exudate inconspicuous; reverse pale or dull yellow with black colony centres; pale-yellow soluble pigment produced. When grown at 37 °C on CYA colonies attain (14) 16–22 mm diameter and are identical to colonies grown at 25 °C. When grown on G25N, colonies attain 13–17 mm diameter but otherwise are identical to those on CYA at 25 °C. No growth or germination is observed at 5 °C. On M40Y and CZ20 colonies are overgrown (90 mm plates) in 7 days at 25 °C, and are identical to those grown on CYA at 25 °C. Colonies on MEA are overgrown (90 mm plates) after 7 days at 25 °C; conidia brown–black, mycelium inconspicuous; reverse orange-brown, otherwise as on CYA.

Colony colours and texture. On MEA conidiophores are abundantly produced, conidial areas are dark brown; sclerotia absent. Reverse on MEA light yellow, furrowed.

Sequence data. All *Aspergillus uvarum* sp. nov. strains examined have high sequence similarity inside the group:

Fig. 2. *Aspergillus uvarum* sp. nov. IMI 388523^T. (a) Colonies on CYA, (b) colonies on MEA, (c) colonies on oatmeal agar, (d–h) conidiophores, (i) conidia under light microscopy, (j) conidia as seen using SEM. Bars, 10 μ m (e–h), 100 μ m (d) and 5 μ m (j).

100 % for ITS, 99.5 % (four groups of individual variability) for the β -tubulin gene and 99.8 % (three groups of individual variability) for the calmodulin gene. The interspecific sequence divergences in the ITS region between these isolates and *A. aculeatus*, *A. japonicus* and *Aspergillus homomorphus* are 0.3, 0.5 and 6.5 %, respectively. In the β -tubulin dataset the differences are higher, in the range 10–18 % for *A. aculeatus* and *A. japonicus* and 25 % for *A. homomorphus*; while, in the calmodulin dataset, the differences are in the range 4–7 % for *A. aculeatus* and *A. japonicus* and 15.8 % for *A. homomorphus*.

Extrolites. All isolates produced secalonic acid D and F, asteric acid, geodin, erdin and dihydrogeodin.

Holotype. Dried colonies of IMI 388523 after 7 days growth on CZA, deposited in the Herbarium of CABI (UK Centre), Egham, UK, formerly International Mycological Institute. Ex-type culture: IMI 388523.

Known distribution. Mediterranean basin, Europe.

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