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New taxa of *Neosartorya* and *Aspergillus* in *Aspergillus* section *Fumigati*

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Abstract Three new species of *Neosartorya* and one new *Aspergillus* of section *Fumigati* are proposed using a polyphasic approach based on morphology, extrolite production and partial β-tubulin, calmodulin, and actin gene sequences. The phylogenetic analyses using the three genes clearly show that the taxa grouped separately from the known species and confirmed the phenotypic differences. *Neosartorya denticulata* is characterized by its unique denticulate ascospores with a prominent equatorial furrow; *N. assulata* by well developed flaps on the convex surface of the ascospores which in addition have two distinct equatorial crests and *N. galapagensis* by a funiculose colony morphology, short and narrow conidiophores and ascospores with two wide equatorial crests with a microtuberculate convex surface. *Aspergillus turcosus* can be distinguished by velvety, gray turquoise colonies and short, loosely columnar conidial heads. The four new taxa also have unique extrolite profiles, which contain the mycotoxins gliotoxin and viriditoxin in *N. denticulate*; apolar compounds provisionally named NEPS in *N. assulata* and gregatins in *N. galapagensis*. *A. turcosus* produced kotanins. *N. denticulata* sp. nov., *N. assulata* sp. nov., *N. galapagensis* sp. nov., and *A. turcosus* sp. nov. are described and illustrated.

Keywords Actin · *Aspergillus turcosus* · Calmodulin · Extrolites · DNA sequencing · *Neosartorya denticulata* · *N. assulata* · *N. galapagensis* · β-Tubulin

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Introduction

Aspergillus section *Fumigati* and its teleomorph *Neosartorya* include many species which are important because they can be pathogenic or allergenic to man (Brakhage and Langfelder 2002), cause food

spoilage and produce mycotoxins (Cole and Cox 1981). Certain species also produce interesting bioactive extrolites that are potential drug candidates (Turner and Aldridge 1983). Section *Fumigati* currently includes now 26 *Neosartorya* species and nine anamorph species (Pitt et al. 2000; Samson 2000; Horie et al. 2003; Hong et al. 2005, 2006).

During a survey of *Aspergillus* and *Penicillium* species from Korea, many isolates belonging to section *Fumigati* were isolated. These isolates were compared to known taxa and those present at the CBS and IBT culture collections which were atypical or unidentified, using a polyphasic approach (Frisvad and Samson 2004). We have examined the macro- and micromorphology, extrolite profiles and β-tubulin, calmodulin, and actin gene sequences of the isolates, and based on the above data, here we describe four new species in *Aspergillus* section *Fumigati*.

Materials and methods

Morphological examinations

For macro-morphological observations, isolates were cultivated on Czapek yeast autolysate (CYA), malt extract agar (MEA), CZ agar (CZA), and oatmeal agar (OA) (Samson et al. 2004). The isolates were inoculated at three points on each plate of each medium and incubated at 25°C in the dark for 7 days, and additionally at 37°C on CYA. For microscopic observations, mounts were made in lactic acid from MEA colonies; a drop of alcohol was added to remove air bubbles and excess conidia. Scanning Electron Microscopy (SEM) was performed using a Hitachi S570 electron microscope. For SEM, mature cleistothecia were transferred to aluminum stubs with double sided adhesive tape. A small drop of 10 mM ACES buffer containing 0.05% Tween-80 was added and the cleistothecia crushed. The suspension was air dried and coated with platinum.

DNA analyses

Isolates used for sequence analyses are listed in Table 1. Genomic DNA was extracted according to the procedure described by Lee and Taylor (1990). The 5′ portion of the β-tubulin gene (benA) was amplified using primers bt2a and bt2b (Glass and Donaldson 1995). Amplifications of the partial calmodulin and actin genes were set up as described previously (Hong et al. 2005). The amplified DNA fragments were purified by QIAquick PCR purification kits (Qiagene, Hilden, Germany). DNA sequences were determined using BigDye Terminator v3.1 Cycle Sequencing kit (ABI 0401041, Foster, CA, USA) and ABI 3100 DNA sequencer. Both strands of each fragment were sequenced using the same primers.

DNA sequences were edited with the DNASTAR computer package. Sequence alignments were performed by using CLUSTAL W (Thompson et al. 1994) and improved manually. The neighbor-joining (NJ) method was used for the phylogenetic analysis. Evolutionary distances between the sequences were calculated by Kimura's formula (Kimura 1980) using the program DNADIST of the PHYLIP program package (Felsenstein 1995). Phylogenetic trees were prepared by the NJ method (Saitou and Nei 1987) using the program NEIGHBOR of the PHYLIP package. Bootstrap values were calculated from 1,000 replications of the bootstrap procedure using programs SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE of the package (Felsenstein 1995).

For parsimony analysis, the PAUP* Version 4.0 software was used (Swofford 2002). 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 data sets 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 1,000 bootstrap replications (Hillis and Bull 1993). An *Aspergillus clavatus* isolate was used as outgroup in these experiments.

The β-tubulin, calmodulin and actin gene sequences, determined in this study, have been deposited in GenBank and the accession numbers are listed in Table 1.

Analysis for extrolites

The isolates were grown at 25°C for 1 week on CYA and YES in the dark and extracted according to Smedsgaard (1997). Extrolites were analyzed by

Table 1 *Aspergillus* section *Fumigati* isolates used in this study

Species	Isolate number	GenBank accession number			Source
		β-tubulin	Calmodulin	Actin	
A. brevipes	CBS 118.53^T	AF057311			Soil, Australia
A. duricaulis	CBS 481.65^T	AF057313			Soil, Buenos Aires, Argentina
A. fumigatiaffinis	IBT12703^T	DQ094885			Soil, USA
A. fumigatus	CBS 133.61	AY685150			Chicken lung, USA
A. fumisynnematus	IFM 42277^T	AB248076			Soil, Venezuela
A. lentulus	CBS 117887	AY738513			Man, USA
A. novofumigatus	IBT 16806^T	DQ094886			Soil, Ecuador
A. unilateralis	CBS 126.56^T	AF057316	AY689366	DQ094847	Rhizosphere, Australia
A. viridinutans	CBS 127.56^T	AF134779			Rabbit dung, Australia
A. turcosus sp. nov.	KACC 42090 = IBT 27920	DQ534142	DQ534147	DQ534178	Air conditioner, Inchen, South Korea
	KACC 42091^T = IBT 27921	DQ534143	DQ534148	DQ534179	Air conditioner, Seoul, South Korea
	KACC 41955 = CBS 117265 = IBT 3016	DQ534144	DQ534149	DQ534180	Car air conditioner, Seoul, South Korea
N. assulata sp. nov.	KACC 41691^T	DQ114123	DQ114131	DQ534189	Tomato soil, Buyeo, North Korea
N. aurata	CBS 466.65^T	AF057318	AY870685	DQ534112	Jungle soil, Brunei
N. aureola	CBS 105.55^T	AF057319			Soil, Tafo, Ghana
N. coreana	KACC 41659^T	AY870758			Tomato soil, Buyeo, North Korea
N. denticulata sp.nov.	CBS 652.73^T = KACC 41183	DQ114125	DQ114133	DQ534181	Soil under *Elaeis guineensis*, Suriname
	CBS 290.74 = KACC 41175	DQ114126	DQ114134	DQ534182	*Acer pseudoplatanus*, Netherlands
N. fennelliae	CBS 598.74^T	DQ114127	DQ114135	DQ534121	Eye ball of *Oryctolagus cuniculus*, USA
N. fischeri	CBS 544.65^T	AF057322			Canned apples
N. galapagensis sp. nov.	CBS 117522^T = IBT 16756 = KACC 41935	DQ534145	DQ534151	DQ534190	Soil, Ecuador
	CBS 117521 = IBT 16763 = KACC 41936	DQ534146	DQ534152	DQ534191	Soil, Ecuador
N. glabra	CBS 111.55^T	AY870734	AY870693	DQ534183	Rubber scrab from old tire, Iowa, USA
N. hiratsukae	CBS 294.93^T	AF057324	AY870699	DQ534184	Aloe juice, Tokyo, Japan
N. laciniosa	KACC 41657^T	AY870756			Tomato soil, Buyeo, North Korea
N. multiplicata	CBS 646.95^T	DQ114129	DQ114137	DQ534185	Soil, Mouli, Taiwan
N. nishimurae	IFM 54133	AB201360			Forest soil, Kenya
N. nishimurae	CBS 116047	DQ534075	DQ534150	DQ534186	Cardboard, Netherlands
N. pseudofischeri	CBS 208.92^T	AY870742	AY870702	DQ534187	Human vertebrate, USA
N. quadricincta	CBS 135.52^T	AF057326			Cardboard, York, UK
N. spathulata	CBS 408.89^T	AF057320			Soil under *Alocasia macrorrhiza*, Taiwan

Table 1 continued

Species	Isolate number	GenBank accession number			Source
		β-tubulin	Calmodulin	Actin	
N. spinosa	CBS 483.65^T	AF057329			Soil, Nicaragua
N. stramenia	CBS 498.65^T	AY870766	AY870726	DQ534188	Soil from maple-ash-elm forest, Wisconsin, USA
N. tatenoi	CBS 407.93^T	DQ114130	DQ114139		Soil of sugarcane, Timbauba, Brazil
N. udagawae	CBS 114217^T	AF132226			Soil, Brazil

CBS Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, *IBT* Institute for Biotechnology, Lyngby, Technical University of Denmark, *IFM* Institute for Food Microbiology (at present, the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University), Chiba, Japan, *KACC* Korean Agricultural Culture Collection, Suwon, South Korea, *T* type strain

HPLC using alkylphenone retention indices and diode array UV-VIS detection as described by Frisvad and Thrane (1987), as modified by Smedsgaard (1997).

Results and discussion

Molecular studies

For the phylogenetic analysis of β-tubulin sequences, most accepted species in section *Fumigati* except *Neosartorya indohii, N. sublevispora*, and *N. tsurutae* were included to determine the phylogenetic positions of the putatively new species (Fig. 1). For the calmodulin and actin datasets only sequences of closely related species were included (Suppl. Figs 1, 2). 468 nucleotides of the β-tubulin gene were analyzed. Among the 225 polymorphic sites, 126 were found to be phylogenetically informative. The topology of the NJ tree is the same as one of the 28 most parsimonious trees inferred by the PAUP program (length: 441 steps, consistency index: 0.7143, and retention index: 0.6993). The calmodulin data set included 538 characters, with 87 parsimony informative characters (tree length: 272, consistency index: 0.8051, and retention index: 0.7706). The actin data set included 394 characters, with 66 parsimony informative characters (tree length: 200, consistency index: 0.7900, and retention index: 0.7801).

The cladograms based on β-tubulin, calmodulin, and actin gene sequences revealed that isolates CBS 652.73 and CBS 290.74, which had identical sequences at each loci, were related to the heterothallic species *N. fennelliae*, but the similarity between this species and the two isolates was quite low (96.5% in the β-tubulin gene partition and 97.8–98.4% in the calmodulin gene partition). These two strains had unique ascospore ornamentations, with denticulate convex surfaces and a prominent equatorial furrow (Fig. 2) and could be easily microscopically differentiated from any other *Neosartorya* species (Samson et al. 1990; Horie et al. 2003). Both isolates produced gliotoxin, while CBS 652.73 also produced viriditoxin. Gliotoxin is also produced by *A. fumigatus* and *N. pseudofischeri*, but there were several differences in the profile of extrolites in *N. pseudofischeri* and these isolates (data not shown). *A. fumigatus* and *N. pseudofischeri* are among the

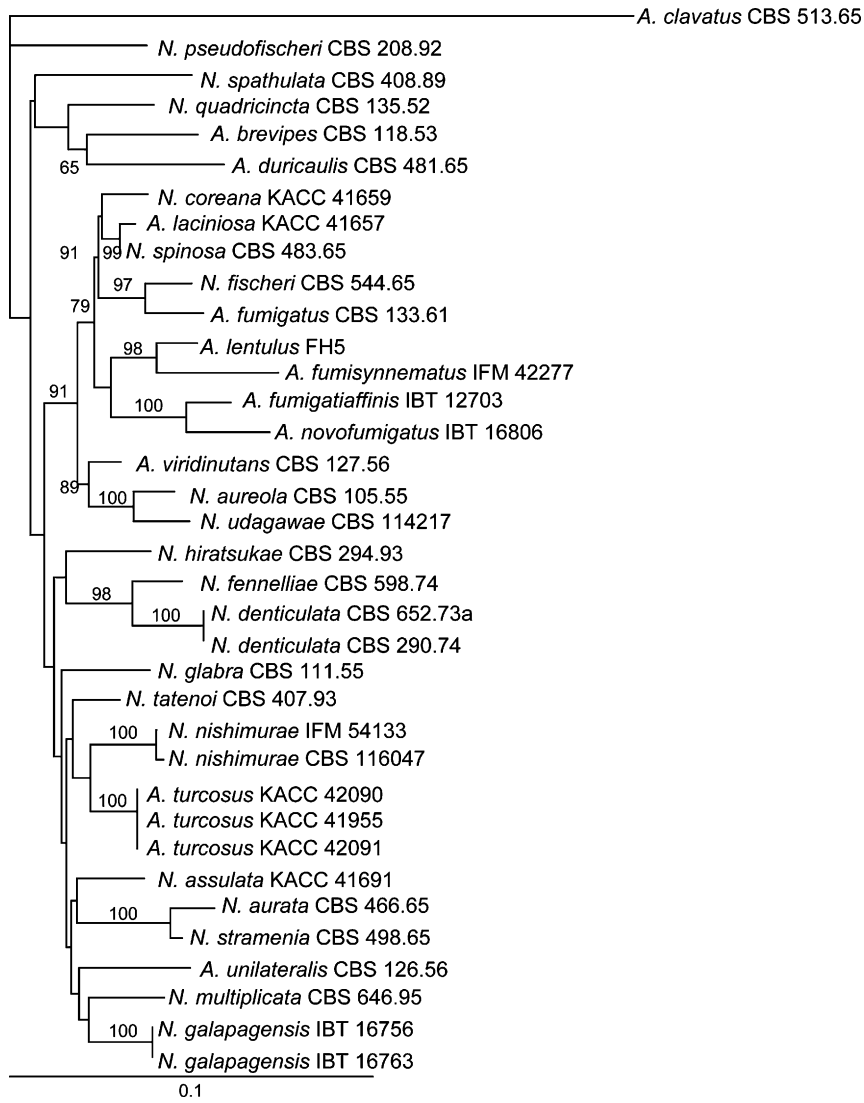

Fig. 1 Taxonomic position of some new species in *Aspergillus* section *Fumigati* inferred from Neighbor-Joining analysis of partial β-tubulin gene sequences. The *numbers* above/below the nodes represent bootstrap values of >60% (out of 1,000 bootstrap replications). The number of nucleotide changes is represented by branch length

most divergent species in the group (Geiser et al. 1998; Horie et al. 2003; Hong et al. 2005, 2006; Varga et al. 2000), yet they share the production of this mycotoxin. Here we describe CBS 652.73 and CBS 290.74 as *N. denticulata* sp. nov.

Isolate KACC 41691 did not show a clear relationship to any species in the β-tubulin phylogeny, but was closest to CBS 116047 based on calmodulin and actin sequence data (Suppl. Figs 1, 2). CBS 116047 is best accommodated as *N. nishimurae*. However, isolate KACC 41691 is homothallic, whereas *N. nishimurae* is heterothallic. This isolate has similar morphological characteristics to *N. pseudofischeri*, but our genotypic analyses indicate that they are phylogenetically distinct. KACC 41691 produces ascospores with several large flaps and two distinct equatorial crests (Fig. 3). These characteristics are similar to those of *N. pseudofischeri* in which the ascospores also have triangular flaps on a convex surface (Peterson 1992), but in KACC 41691 the flaps are more pronounced. Furthermore, KACC 41691 differs from *N. pseudofischeri* by its growth rates on MEA and CZA (after 7 days at 25°C colonies were 49–58 and 24–42 mm, respectively, for KACC 41691, and 90, and 60–70 mm for *N. pseudofischeri*). The ascomatal initials in *N. pseudofischeri* are

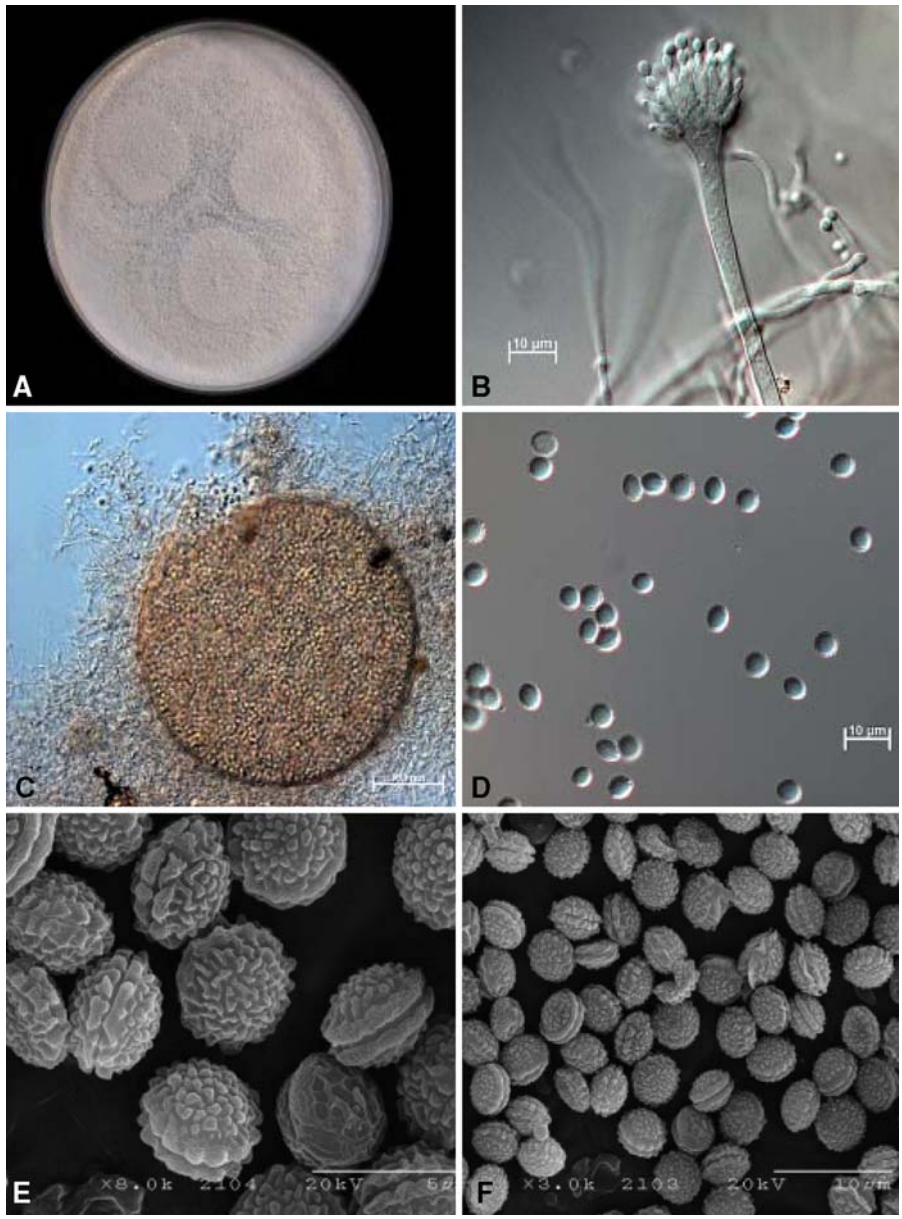

Fig. 2 *Neosartorya denticulata* sp. nov. (**A**) colonies on OA after 28 days of incubation, (**B**) aspergillum, (**C**) ascoma, (**D**) ascospores under a light microscope, (**E**) and (**F**) ascospores by SEM

characterized by many coiled hyphae whereas the initial in KACC 41691 is simpler. Ascospores are larger (5.1–6.0 µm in KACC 41691, while 4.5–5 µm in *N. pseudofischeri*). We could not detect any known extrolites in KACC 41691 but it produced partially characterized apolar compounds in common with several *Neosartorya* species. Here we propose the name *N. assulata* sp. nov. for isolate KACC 41691.

Isolates CBS 117522 and CBS 117521, both isolated from soil from the Galapagos Islands, were phylogenetically distinct from all other species within section *Fumigati* (Fig. 1, Suppl. Figs 1, 2). The colony texture of these two isolates is funiculose with conidiophores which arise from bundles of aerial hyphae. These conidiophore structures resemble as described by Horie et al. (1993) for *A. fumisynnematus*, but in this species the conidophores are longer,

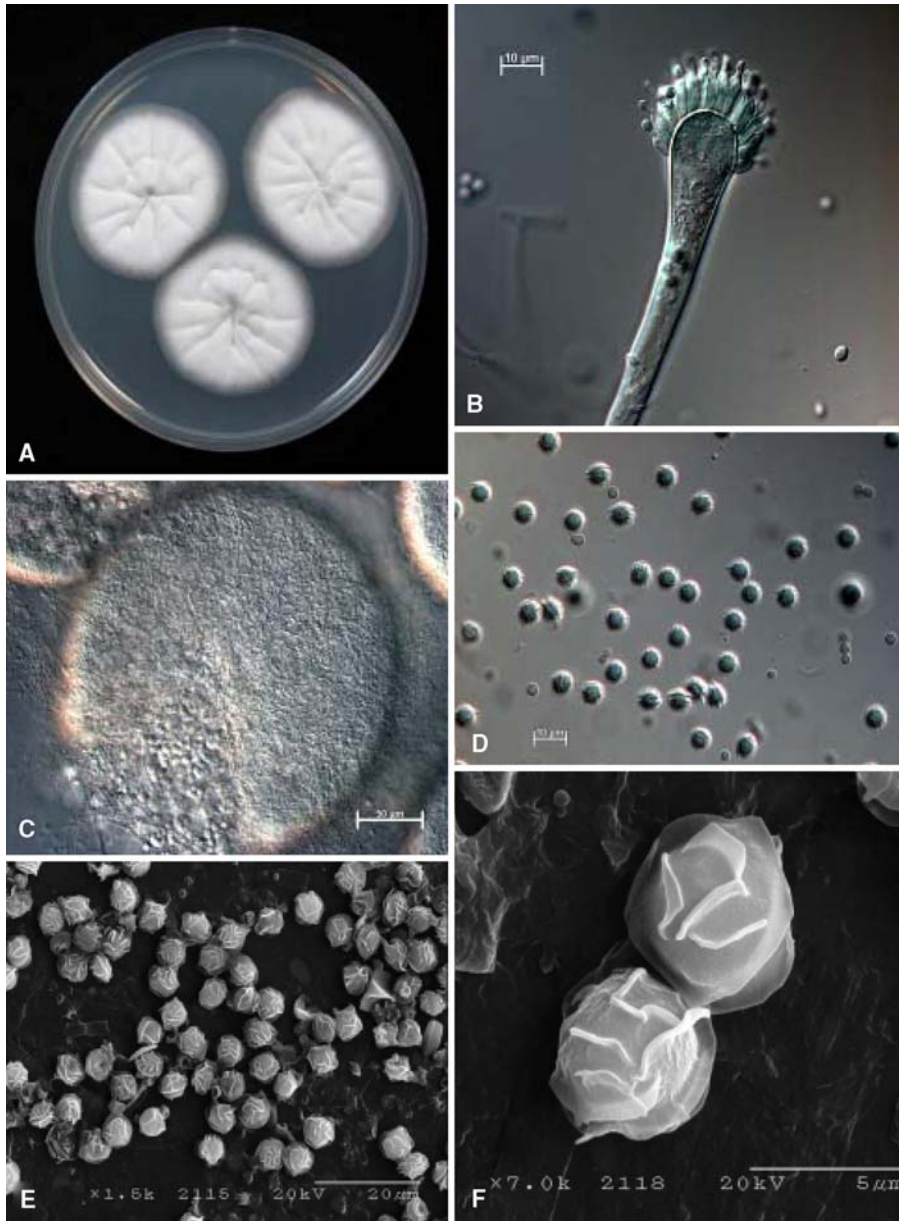

Fig. 3 *Neosartorya assulata* sp. nov. (**A**) colonies on CYA after 7 days of incubation, (**B**) aspergillum, (**C**) ascoma, (**D**) ascospores under a light microscope, (**E**) and (**F**) ascospores by SEM

up to 210 µm, with larger vesicles (16–20(25) µm in diam.). Ascomata were produced in 2 weeks-old colonies and ascospores were released after about 3 weeks. Ascospores resemble those of *N. glabra* and *N. laciniosa*, and have two conspicuous equatorial crests with a microtuberculate convex surface (Fig. 4). Isolates CBS 117522 and 117521 produced gregatins and several other extrolites not yet found in other *Neosartorya* or *Aspergillus* species, and appeared to be chemically unique. Gregatins have previously been found in *A. panamensis* in section *Sparsi* (Anke et al. 1980, 1988; Peterson 2000).

Here we describe isolates CBS 117522 and CBS 117521 as *N. galapagensis* sp. nov.

Isolates KACC 42090, KACC 42091, and KACC 41955 also showed a distinct taxonomic position

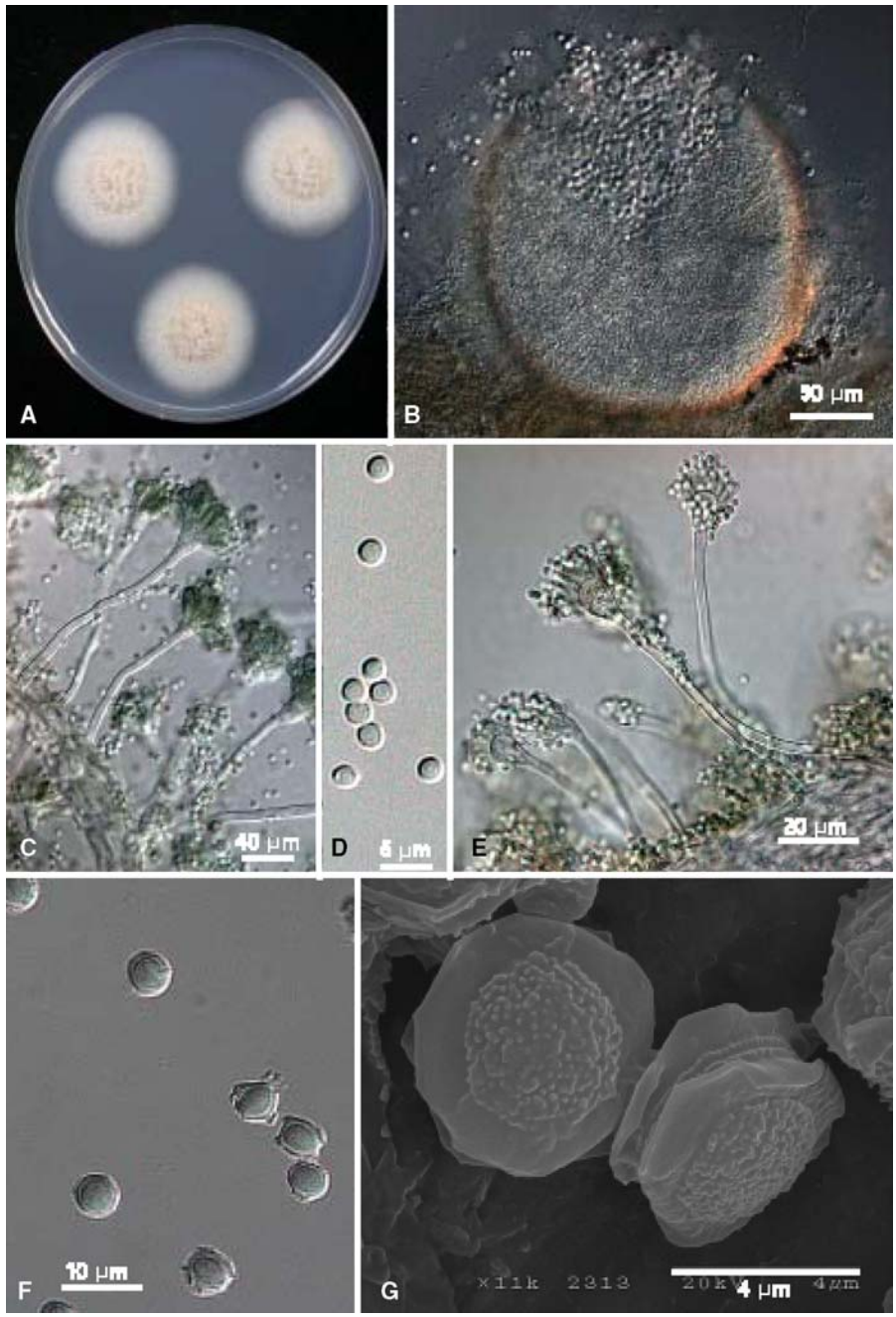

Fig. 4 *Neosartorya galapagensis* sp. nov. (**A**) colonies on CYA after 7 days of incubation, (**B**) ascoma, (**C**) and (**E**) stipes and conidial heads arisen from hyphal bundle, (**D**) conidia under a light microscope, (**F**) ascospores under a light microscope,(**G**) ascospores by SEM

within section *Fumigati* in the three gene phylogenies. The closest taxon to these three isolates in the β-tubulin and calmodulin gene phylogenies was the heterothallic species *N. nishimurae* (Fig. 1, Suppl. Fig. 1). However, the similarity of β-tubulin sequences between the two species was only 96.9% which is close to that observed between *N. fischeri* and *N. spinosa* (data not shown). Although *A. fumigatus*, *A. lentulus*, *A. viridinutans, A. fumigatiaffinis*, and *A. novofumigatus* share similar morphological

characteristics with these three isolates, these species showed comparatively low β-tubulin gene sequence similarities of 89.8, 91.6, 93.6, 92.3, and 92.7%, respectively. Isolates KACC 42090, 42091, and 41955 did not produce any teleomorph structure after incubation for 28 days on CYA, MEA, CZA, and OA at 25°C. During mating experiments, all of the pairings with *N. fennelliae*, *N. nishimurae*, *N. spathulata*, *N. udagawae*, and between conidial strains failed to yield cleistothecia. Some conidiophore characters suggest a similarity to *A. fumigatus* and *A. lentulus*, but these isolates are different from *A. fumigatus* by the vesicles which are fertile over the upper two-thirds and has short, loosely columnar conidial heads. These isolates are different from *A. lentulus* by their velvety and gray turquoise colonies. These isolates grow at 10 and 50°C on MEA and CZA. On the contrary, *A. fumigatus* does not grow at 10°C, while *A. lentulus*, *A. fumigatiaffinis*, and *A. novofumigatus* are unable to grow at 50°C (Hong et al. 2005). Isolates KACC 42091, 42090, and 41955 were also chemically unique. The extrolites produced by the isolates described here are typical for *Aspergillus* section *Fumigati* (data not shown). The three isolates also produced kotanins, previously found in species in less obviously related groups of *Aspergillus*, such as *A. niger* from section *Nigri* and *A. clavatus* from section *Clavati* (Turner and Aldridge 1983). Here we describe isolates KACC 42090, KACC 42091, and KACC 41955 as *A. turcosus* sp. nov.

The list of 26 known species of *Neosartorya* and nine anamorph species from the section *Fumigati* (Horie et al. 2003; Hong et al. 2005, 2006) is still expanding. With the species proposed here, there are now 29 *Neosartorya* species and 10 *Aspergillus* species in this group, 39 species in total. Unfortunately, some of the recently described species are not available for the scientific community, such as *N. indohii*, *N. nishimurae*, *N. otanii*, *N. sublevispora*, *N. takakii, and N. tsurutae.*

Taxonomy

Neosartorya denticulata Samson, S.B. Hong and Frisvad. sp. nov. (Fig. 2).

Species homothallica; ascomata superficialia, luteo-alba vel dilute lutea, globosa vel subglobosa, 140–230 μm in diam., hyphis hyalinis vel luteolis laxe textis circumdata. Asci 8-spori, globosi vel subglobosi, 12–14 μm diam. Ascosporae lenticulares, 4–5 μm diam, denticulatae. Conidiophora ex hyphis aeriis oriunda, 3–4.5 μm lata; conidiorum capitula columnaria sed brevia, uniseriata, vesiculae spathulatae vel subclavatae, 7–12 μm diam; phialides 7.5–9 × 2–3 μm, didimidium superius vesiculae occupantes. Conidia subglobosa vel late ellipsoidea, levia, 2–3 μm diam.

Holotype of *N. denticulata*, here designated as CBS 652.73^{T} (dried culture), isolated from soil in Suriname.

Homothallic, cleistothecia superficial, yellowish white to pale yellow, globose to subglobose, 140–230 μm in diam., surrounded by a loose covering of hyaline to yellowish white hyphae. Asci 8-spored, globose to subglobose 12–14 μm, evanescent at maturity. Ascospores, 4–5 μm, denticulate with a prominent equatorial furrow. Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads short, columnar. Conidiophores arising from aerial hyphae, uniseriate, stipes 3–4.5 μm wide; vesicles spathulate to subclavate, 7–12 μm in diam.; phialides 7.5–9 × 2–3 μm, covering the upper half of vesicle. Conidia subglobose to broadly elliptical, smooth, 2–3 μm. Colonies on MEA growing rapidly, 35–40 mm in 7 days at 25°C, white. Conidial heads produced only in colony margins. Colonies on CYA, 22–24 mm in 7 days at 25°C, 35–38 mm in 7 days at 37°C, white, loosely overgrown by aerial hyphae in center, weakly sulcate in marginal area. Conidial heads few in number. Reverse yellowish white to pale yellow (12A23) (Kornerup and Wanscher 1978).

Extrolites: The two isolates produced the mycotoxin gliotoxin. CBS 652.73 was a particularly strong producer, and also produced the mycotoxin viriditoxin. Furthermore, the two isolates produced some unique, yet unelucidated secondary metabolites.

Additional isolates: CBS 290.74 = KACC41175, from *Acer pseudoplatanus*, The Netherlands.

Distinguishing features: Denticulate ascospores with a prominent equatorial furrow and the production of gliotoxin.

Neosartorya assulata S.B. Hong, Frisvad and Samson. sp. nov. (Fig. 3).

Species homothallica; ascomata superficialia, alba vel luteo-alba, globosa vel subglobosa, 150–250 μm diam, hyphis hyalinis vel luteolis laxe textis circumdata.

Asci 8-spori, globosi vel subglobosi, 14–16 μm diam. Ascosporae lenticulares, 5–6 μm diam, duabus cristis distantibus praeditae, valvis nonnullis distinctis longis intumescentii ornamentatae. Conidiophora hyalina, 3–7.5 μm lata; conidiorum capitula columnaira, brevia, uniseriata; vesicula subclavata, 10–18 μm diam. Phialides 7–9 × 2–3 μm, didimidium superius vesiculae occupantes. Conidia subglobosa vel late ellipsoidea, levia, 2–3 μm diam.

Holotype of *N. assulata*, here designated as KACC 41691^{T} (dried culture), isolated from soil, tomato field, Buyeo, North Korea.

Homothallic, cleistothecia superficial, white to yellowish white, globose to subglobose, 150–250 μm in diam. Asci 8-spored, globose to subglobose 14–16 μm, evanescent at maturity. Ascospores lenticular, spore body 5.0–6.0 μm, with two well-separated equatorial crests and convex surface decorated with several large, round flaps. Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads short, columnar. Conidiophores arising from aerial hyphae and substrate, 3–7.5 μm wide; vesicles subclavate, 10–18 μm in diam., uniseriate, phialides 7–9 μm, covering the upper half of vesicles. Conidia, subglobose to broadly elliptical, ovoid, smooth, 2–3 μm. Colonies on MEA, 49–58 mm in 7 days at 25°C, white, radially weak sulcate. Conidial heads aerial, numerous. Colonies white on CYA, 37–41 mm at 25°C, 64–68 mm at 37°C in 7 days. Radially and roundly sulcate, with some clear exudates. Conidial heads aerial, abundant. Reverse yellowish white (1A2) to pale yellow (1A3) (Kornerup and Wanscher 1978).

Extrolite profile: This species is characterized by relatively weak production of secondary metabolites. It does produce some indole alkaloids and some apolar metabolites.

Distinguishing features: Large, round flaps on convex surface of ascospores with two distinct equatorial crests.

Neosartorya galapagensis Frisvad, S.B Hong and Samson. sp. nov. (Fig. 4).

Species homothallica; ascomata luteo-alba, globosa vel subglobosa, 90–200 μm diam,. hyphis hyalinis vel luteolis laxe textis circumdata. Asci 8-spori, globosi vel subglobosi, 12–15 μm diam; ascosporae late lenticulates, *ca*. 5 μm diam, duabus cristis distantibus 1–2 μm latis praeditae, valvis exigue tuberculatis. Conidiophora singula vel funiculosa, levia, 2–4 μm lata; conidiorum capitula columnaria, brevia, uniseriata; vesiculae subclavatae, 4–11 μm diam. Phialides lageniformes, 5–7 × 2–3 μm, dimidium superius vesiculae occupantes. Conidia globosa vel subglobosa, levia vel exigue asperulata, 2.3–3.0 μm diam.

Holotype of *N. galapagensis*, here designated as CBS 117522^{T} (IBT 16756 = KACC 41935) (dried culture), isolated from soil, Galapagos Islands, Ecuador, D. Mahooney.

Colonies on MEA 28–35 mm in diam. after 7 days at 25°C and more than 70 mm after 7 days at 37°C, funiculose in texture, yellowish white (3A12). Conidiophores sparse, cleistothecial initials produced after *ca*. 10 days of incubation. Colonies on CYA 27–40 mm in diam. after 7 days at 25°C and 61–65 mm after 7 days at 37°C, strongly funiculose in texture and white with a golden yellow (5B78) reverse without diffusible pigment. Conidial heads columnar. Conidiophores arising from bundles of aerial hyphae or the basal mycelium, smooth walled, up to 100 μm long, 2–4 μm in width; vesicles 4–11 μm (sub)clavate with 5–7 μm flask-shaped phialides which are fertile on the upper half to two-thirds of the surface. Conidia 2.3–3.0 μm, globose to subglobose and the surface usually smooth. Cleistothecia yellowish white (4A2), globose to subglobose, 90–200 μm in diam., surrounded by a loose covering of aerial hyphae. Peridium consisting of angular cells, 3–8 μm in diam.; asci 8-spored, globose to subglobose, 12–15 μm in diam.; ascospores broadly lenticular, spore body *ca*. 5 μm in diam. with two distinct equatorial crests 1–2 μm wide, convex surface of ascospores microtuberculate.

Extrolite profile: All isolates examined in the species produce several gregatins and several partially characterized secondary metabolites. This species is chemically very distinct and different from the other species in section *Fumigati* or *Neosartorya* species. The gregatins have also been found in *A. panamensis* (Anke et al. 1980, 1988). The latter species was placed in *Aspergillus* section *Usti* by Raper and Fennell (1965).

Additional isolates: CBS 117521 = IBT 16763 = KACC 41936, ex soil, Galapagos Islands, Ecuador.

Distinguishing features: The *Aspergillus* anamorph arising in bundles of aerial hyphae and the ascospores with two wide conspicuous equatorial crests and microtuberculate convex surface.

Aspergillus turcosus S.B. Hong, Frisvad and Samson. sp. nov. (Fig. 5).

Coloniae in agaro maltoso ad 42–51 mm diam post-seven dies 25°C, 70 mm diam 37°C. Coloniae velutinae, griseo-glaucae vel griseo-virides, plerumque planae; reversum luteo-aurantium vel griseo-aurantium. Conidiophora levia, 4–7 μm lata. Conidiorum capitula columnaria, brevia, uniseriata; vesiculae globosae vel subclavatae, 15–25 μm diam. Phialides lageniformes, 6–8 × 2–3 μm, duo tertia superiora vesiculae occupantes. Conidia subglobosa vel ovoidea, levia, 2.5–3.5 μm diam.

Holotype of *A. turcosus*, here designated as KACC 42091^{T} (=IBT 27921) (dried culture) isolated from home air conditioner, Seoul, South Korea.

Colonies on MEA 42–51 mm in diam. after 7 days at 25°C and more than 70 mm after 7 days at 37°C. Colony texture velvety, gray-turquoise to gray-green (24-25B3-5) and usually plane. In reverse, colonies are yellowish orange (4B6) to grayish orange (5B6). Colonies on CYA attain a diam. of 38–41 mm after 7 days at 25°C and more than 70 mm after 7 days at 37°C. Colony texture is velvety. Colony texture and color similar to that on MEA. In reverse, colonies are deep yellow (4A78). Conidial heads loose and short columnar. Conidiophores smooth-walled, up to 80 μm long and 4–7 μm wide. Vesicles are 15–25 μm in diam., flask-shaped to globose, bearing 6–8 μm flask-shaped phialides over two-thirds of the surface. Conidia are subglobose, ovoid and smooth, 2.5–3.5 μm in diam.

Extrolite profile: Kotanins and several unique compounds but not yet elucidated secondary metabolites.

Additional isolates: KACC 42090 = IBT 27920, KACC 41955 = IBT 3016.

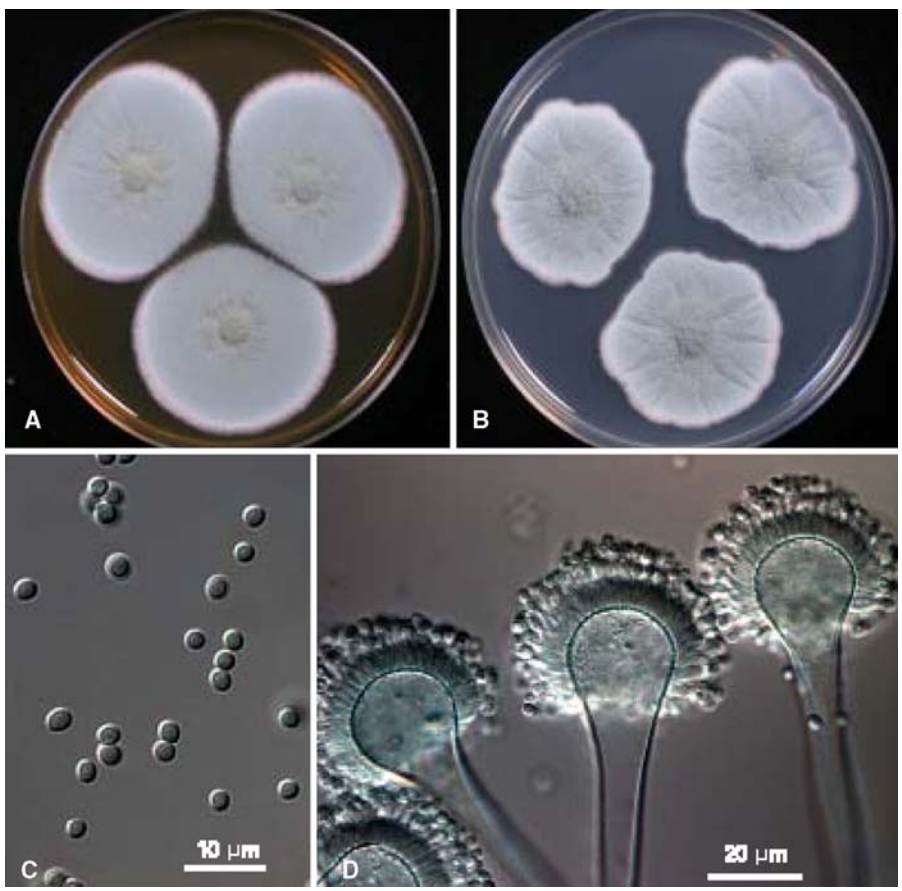

Fig. 5 *Aspergillus turcosus* sp. nov. (**A**) and (**B**) colonies on MEA (**A**) and CYA (**B**) after 7 days at 25°C, (**C**) conidia under a light microscope, (**D**) conidial heads

Distinguishing features: Velvety colony, gray-turquoise (green) color on CYA, phialides over two-third of the vesicle and growth at 10 and 50°C are distinctive characteristics of the species.

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