Novel *Neosartorya* species isolated from soil in Korea

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Phenotypic and genotypic characters of strains of *Neosartorya spinosa* and related taxa were analysed. *N. spinosa*, *Neosartorya botucatensis* and *Neosartorya paulistensis* had identical partial β -tubulin and calmodulin gene sequences and could not be differentiated on macro- and micro-morphological characteristics, including by scanning electron microscopy. Based on partial β -tubulin and calmodulin gene sequences and ascospore morphology, two separate groups are distinguished and are proposed as novel species. *Neosartorya laciniosa* sp. nov. has microtuberculate ascospores with two bent crests and two distinct equatorial rings of small projections. *Neosartorya coreana* sp. nov. has rugose to weak reticulate ascospores with two often bent crests, but without the equatorial rings of small projections. The type strain of *N. laciniosa* is CBS 117721^T (=NRRL 35589^T=KACC 41657^T) and the type strain of *N. coreana* is CBS 117059^T (=NRRL 35590^T=KACC 41659^T).

INTRODUCTION

Ascospore ornamentation is an important morphological character for distinguishing species of the ascomycete genus *Neosartorya* (Kozakiewicz, 1989). Based on ascospore ornamentation, Horie *et al.* (2003) distinguished homothallic *Neosartorya* species with white to pale-yellow ascomata and ascospores with two distinct equatorial crests into six species. In this group, *Neosartorya spinosa* and *Neosartorya glabra* have been separated on the basis of smooth (for *N. glabra*) or echinulate (for *N. spinosa*) ascospores, but many isolates possess intermediate patterns of ornamentation. Furthermore, *N. glabra* was also reported as highly polymorphic

based on secondary metabolite profiles (Samson *et al.*, 1990) and phenotypic and genotypic characteristics (Rinyu *et al.*, 2000).

During a survey of soil-borne *Aspergillus* and *Penicillium* in Korea, many *Neosartorya* strains were encountered and, in order to examine the taxonomic position of our isolates, we have re-examined *N. spinosa*, *N. glabra* and related taxa. We have used a polyphasic taxonomic approach in order to determine the delimitation and variability of the taxon. For phenotypic analyses, morphology was examined using macro- and micro-morphology and scanning electron microscopy (SEM) methods and growth temperatures were studied. For the phylogenetic analyses, β -tubulin and calmodulin gene sequences were used, which have proved to be useful for *Aspergillus* taxonomy (Feibelman *et al.*, 1998; Geiser *et al.*, 1998; Ito *et al.*, 2001; Varga *et al.*, 2000a, b).

METHODS

Strains examined are listed in Table 1. The strains were maintained on malt extract agar slants at 15 °C.

Morphology and extrolites. For macro-morphological observations, Czapek yeast autolysate (CYA), malt extract autolysate (MEA) agar and Czapek agar (CZA) were used (Samson *et al.*, 2004). Yeast extract sucrose (YES) agar, oatmeal agar (OA) and creatine sucrose agar (CREA) were also used (Frisvad & Samson, 2004). The isolates

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Abbreviations: NJ, neighbour-joining; SEM, scanning electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the β -tubulin and calmodulin gene sequences of the strains examined in this study are shown in Table 1.

A NJ phylogenetic tree based on calmodulin gene sequences and SEM images of ascospores of *Neosartorya* species are available as supplementary figures in IJSEM Online.

The Mycobank accession numbers for *Neosartorya laciniosa* sp. nov. and *Neosartorya coreana* sp. nov. are MB 500233 and MB 500234, respectively (<http://www.mycobank.org>).

Table 1. List of strains used in this study

CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; NRRL, Agricultural Research Service Culture Collection, Peoria, USA; IBT, Institute for Biotechnology, Lyngby, Technical University of Denmark; KACC, Korean Agricultural Culture Collection, Suwon, Korea.

Isolate	Source	Accession number	
		β -Tubulin	Calmodulin
<i>Aspergillus fumigatus</i> CBS 545.65	Unknown source, K. B. Raper	AY685151	AY689335
<i>Aspergillus lentulus</i> KACC 41391	Maize field soil, Yeongi, Korea	AY685170	AY689354
<i>Neosartorya aurata</i> CBS 466.65 ^T	Jungle soil, Brunei	AF057318*	AY870685
<i>Neosartorya botucatensis</i> CBS 114221 ^T	Soil, Brazil	AY870763	AY870723
<i>Neosartorya coreana</i>			
CBS 117057 (=IBT 22051)	Strawberries, Sydney, Australia	AY870757	AY870717
CBS 117059 ^T (=KACC 41659 ^T =NRRL 35590 ^T)	Tomato field soil, Buyeo, Korea	AY870758	AY870718
<i>Neosartorya fennelliae</i> CBS 411.89	Marine sludge, Japan	AY870727	AY870686
<i>Neosartorya fischeri</i>			
CBS 544.65 ^T	Canned apples	AF057322*	AY689370
CBS 317.89	Desert sand, Namibia	AY870728	AY870687
CBS 420.96	Maize field, Nepal	AY870729	AY870688
KACC 41668	Soil, Dominican Republic	AY870730	AY870689
KACC 41673	Soil, Dominican Republic	AY870731	AY870690
KACC 41674	Soil, Dominican Republic	AY870732	AY870691
KACC 41664	Sesame field soil, Yeongi, Korea	AY870733	AY870692
KACC 41665	Pepper field soil, Daejeon, Korea	–	–
<i>Neosartorya glabra sensu stricto</i>			
CBS 111.55 ^T	Rubber scrap from old tyre, Iowa, USA	AY870734	AY870693
CBS 165.63	Soil, Netherlands	AY870735	AY870694
CBS 582.90	Raspberry pulp, Netherlands	AY870736	AY870695
KACC 41654	Tomato field soil, Buyeo, Korea	AY870737	AY870696
KACC 41655	Tomato field soil, Buyeo, Korea	–	–
KACC 41656	Tomato field soil, Buyeo, Korea	–	–
<i>Neosartorya hiratsukae</i> CBS 294.93 ^T	Pasteurized aloe juice, Japan	AF057324*	AY870699
<i>Neosartorya laciniosa</i>			
KACC 41652	Perilla field soil, Daejeon, Korea	AY870747	AY870707
CBS 117065 (=IBT 6660)	Soil, Surinam	AY870748	AY870708
KACC 41644	Soil, Dominican Republic	AY870749	AY870709
KACC 41645	Soil, Dominican Republic	AY870750	AY870710
KACC 41646	Soil, Dominican Republic	AY870751	AY870711
KACC 41648	Soil, Kenya,	AY870752	AY870712
CBS 206.92	Strawberry pulp, Netherlands	AY870753	AY870713
CBS 315.89	Soil, Pakistan	AY870754	AY870714
CBS 585.90	Vineyard, USA	AY870755	AY870715
CBS 117059 ^T (=KACC 41657 ^T =NRRL 35589 ^T)	Tomato field soil, Buyeo, Korea	AY870756	AY870716
KACC 41658	Tomato field soil, Buyeo, Korea	–	–
KACC 41660	Pepper field soil Daejeon, Korea	–	–
<i>Neosartorya paulistensis</i> CBS 114216 ^T	Soil, Brazil	AY870764	AY870724
<i>Neosartorya pseudofischeri</i>			
KACC 41653	Tomato field soil, Buyeo, Korea	AY870740	AY870700
KACC 41661	Perilla field soil, Daejeon, Korea	AY870741	AY870701
CBS 208.92 ^T	Human vertebrae, USA	AY870742	AY870702
CBS 100504	Pectin, dairy food, Netherlands	AY870743	AY870703
CBS 110899	Human blood, Estonia	AY870744	AY870704
CBS 117063 (=IBT 6472)	Cherry filling, USA	AY870745	AY870705
CBS 117073 (=IBT 3002)	Soil, Denmark	AY870746	AY870706
<i>Neosartorya spinosa</i>			
CBS 483.65 ^T	Soil, Nicaragua	AF057329	AY689371
CBS 117058 (=IBT 3001)	Pineapple, Denmark	AY870759	AY870719

Table 1. cont.

Isolate	Source	Accession number	
		β -Tubulin	Calmodulin
KACC 41647	Soil, Dominican Republic	AY870760	AY870720
KACC 41650	Soil, Kenya	AY870761	AY870721
KACC 41651	Soil, Kenya	AY870762	AY870722
KACC 41662	Tomato field soil, Buyeo, Korea	AY870765	AY870725
KACC 41663	Pepper field soil, Daejeon, Korea	—	—
<i>Neosartorya stramenia</i> CBS 498.65 ^T	Soil from maple–ash–elm forest, USA	AY870766	AY870726
<i>Neosartorya</i> sp. CBS 841.96	Bark of <i>Podocarpus</i> sp., Papua, New Guinea	AY870738	AY870697
<i>Neosartorya</i> sp. CBS 112.55	Garden soil, Australia	AY870739	AY870698

*Sequence obtained from GenBank.

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 micro-morphological 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. SEM was performed for every strain of *Neosartorya* used in this study using a Hitachi S570 microscope. For SEM preparation, mature cleistothecia were transferred to aluminium stubs using double-sided adhesive tape. A small drop of 10 mM ACES buffer containing 0.05 % Tween 80 was added and the cleistothecia were crushed. The suspension was air-dried and coated with platinum.

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

Phylogenetic analyses. Genomic DNA was extracted according to the procedure described by Lee & Taylor (1990). A fragment of the 5' portion of the β -tubulin gene was amplified using the primers bt2a and bt2b (Glass & Donaldson, 1995). The segment of the calmodulin gene was amplified using the primers cmd5 (5'-CCGAGTACAAGG-AGGCCTTC-3') and cmd6 (5'-CCGATAGAGGTCATAACGTGG-3') which were constructed in this study based on the complete *Aspergillus oryzae* sequence (GenBank accession number D44468). The amplified DNA fragments were purified using a QIAquick PCR purification kit (Qiagen). DNA sequences were determined using a BigDye Terminator v3.1 cycle sequencing kit (ABI) and an ABI 3100 DNA sequencer. Both strands of each fragment were sequenced.

DNA sequences were edited with the DNASTAR computer package and an alignment of the sequences was performed using the CLUSTAL W program (Thompson *et al.*, 1994). The neighbour-joining (NJ) method was used for the phylogenetic analysis. For NJ analysis, the data were first analysed using the Tamura–Nei parameter distance calculation model with gamma-distributed substitution rates, which were then used to construct the NJ tree with MEGA version 2.1 (Kumar *et al.*, 2001). To determine the support for each clade, a bootstrap analysis was performed with 1000 replications.

RESULTS

The primers bt2a and bt2b amplified about 550 bp of the 5' portion of the β -tubulin gene which contains introns 3, 4 and 5 and exons 3, 4, 5 and partial exon 6. The phylogenetic relationships of the β -tubulin gene sequences for the 46

strains were inferred from the NJ analysis and the resulting phylogenetic tree is presented in Fig. 1.

The five strains of *N. spinosa*, including the ex-type strain CBS 483.65^T, have identical partial β -tubulin gene sequences. Strain CBS 117058 differs by only two bases from the other *N. spinosa* strains, while *Neosartorya botucatensis* CBS 114221^T and *Neosartorya paulistensis* CBS 114216^T have identical sequences to *N. spinosa*. *N. spinosa* showed some gene sequence similarity to *N. glabra*, but showed stronger similarity to the two novel taxa, which are proposed as *Neosartorya coreana* sp. nov. and *Neosartorya laciniosa* sp. nov. In this study, strains previously identified as *N. glabra* based on ascospore characteristics were divided into five groups. The group *N. glabra sensu stricto* included the ex-type strain, CBS 111.55^T, and clustered with *Neosartorya aurata*, *Neosartorya stramenia*, *Neosartorya hiratsukae* and *Neosartorya fennelliae*, but was placed far away from *N. spinosa*. Strains CBS 112.55 and CBS 841.96 showed low sequence similarity with *N. glabra sensu stricto* and were not clustered within the species. *N. laciniosa*, which has ascospores with a finely rough convex surface and more or less straight crests, was located near to *N. spinosa*. *N. laciniosa* differed in three (two continuous base deletions at positions 327 and 328 after primer bt2a) of 466 bases in the partial β -tubulin gene sequence compared with *N. spinosa* and the bootstrap of the grouping was only 62 %. *N. coreana*, which has ascospores with more bent equatorial crests, was also located near *N. spinosa*, but separated by a 99 % bootstrap value.

The primers cmd5 and cmd6 amplified about 580 bp of the calmodulin gene which contains introns 2, 3 and 4 and exons 2, 3, 4 and partial exon 5. The phylogenetic relationships of the calmodulin gene sequences for the 46 strains were inferred from NJ analyses (see Supplementary Fig. S1 in IJSEM Online). In the cladogram based on the calmodulin gene sequences, *N. laciniosa* was well separated from *N. spinosa* with a bootstrap value of 98 %. However, there were two subgroups in this taxon. Apart from these differences, the topology of the calmodulin gene sequence phylogenetic tree was similar to that of the β -tubulin gene sequence tree.

Fig. 1. Phylogenetic tree inferred from NJ analysis of partial β -tubulin gene sequences. Bootstrap values (1000 replications) are shown above or below the nodes. s. st., *sensu stricto*. Bar, 0.01 nucleotide changes between taxa.

On the basis of *Aspergillus* anamorph morphology, *N. spinosa*, *N. botucatensis*, *N. paulistensis*, *N. glabra*, *N. laciniosa* and *N. coreana* are very similar. The vesicle diameter of the six species ranges from 7.5 to 20 μ m and conidia are (sub)globose to ellipsoid, ranging from 2.5 to 4.0 μ m. The cleistothecia of *N. glabra* are yellow–white to pale yellow (2-3A2-3; Kornerup & Wanscher, 1978), while the other three species are pale yellow to light yellow (2-3A3-4). Based on light microscopy, the ascospores of *N. spinosa* are rough, those of *N. laciniosa* and *N. coreana* are finely roughened (Figs 2 and 3) and *N. glabra* has nearly smooth-walled ascospores. SEM observations showed that the ascospores of *N. spinosa* CBS 483.65^T have convex surfaces with distinct spines. The ascospores of *N. botucatensis* and *N. paulistensis* also have convex surfaces with spines (see Supplementary Fig. S2 in IJSEM Online). *N. glabra* ascospores have nearly smooth, convex surfaces with small microtuberculate

projections and two straight equatorial crests. Strains of *N. laciniosa* have microtuberculate ascospores with two distinct straight crests. In the furrow, two distinct equatorial rings with small projections are present (see Supplementary Fig. S2 in IJSEM Online). *N. coreana* has a rugose to weak reticulate ornamentation of convex ascospores with two distinct, and often bent, crests.

Our studies of extrolites showed that *N. spinosa*, *N. paulistensis* and *N. botucatensis* produced aszonalenins, 2-pyrovoylaminobenzamide and pseurotin (Table 2). In *N. coreana* and *N. laciniosa*, aszonalenins were also present, but the other compounds were absent. All strains of *N. laciniosa* also typically produced a large number of tryptoquivalins and tryptoquivalons. Aszonalenins were also found in strains of *Neosartorya fischeri*, but this species produced a different extrolite profile consisting of fischerin,

Fig. 2. *Neosartorya laciniosa* sp. nov. CBS 117074^T. (a) Colonies on MEA after 7 days at 25 °C; (b) ascomata; (c) asci; (d, e) conidial heads; (f) ascospores under a light microscope; (g) ascospores as seen by SEM. Bars, 100 μ m (b) and 10 μ m (c–g).

Table 2. Extrolites from *Neosartorya* species based on HPLC analysis of ex-type strains

The data were backed up by HPLC analyses of several further isolates of *N. glabra*, *N. spinosa*, *N. laciniosa*, *N. fischeri* and *N. pseudofischeri*.

Species	Extrolites
<i>N. hiratsukae</i>	Avenaciolide
<i>N. glabra</i>	Asperpentyn, avenaciolide, wortmannin-like compound
<i>N. spinosa</i>	Aszonalenins, chrysogine, 2-pyrovoylaminobenzamide, pseurotin
<i>N. paulistensis</i>	Aszonalenins, 2-pyrovoylaminobenzamide, pseurotin
<i>N. botucatensis</i>	Aszonalenins, 2-pyrovoylaminobenzamide, pseurotin
<i>N. laciniosa</i>	Aszonalenins, tryptoquivalins, tryptoquivalons
<i>N. coreana</i>	Aszonalenins
<i>N. fischeri</i>	Aszonalenins, fischerin, neosartorin, fiscalins, helvolic acid, fumitremorgins
<i>N. pseudofischeri</i>	Asperfuran, cytochalasin-like compound, fiscalin-like compound, pyripyropens, gliotoxin

neosartorin, fiscalins, helvolic acid and fumitremorgins. *N. glabra* differed from the other taxa by the production of asperpentyn, avenaciolide and wortmannin.

DISCUSSION

Raper & Fennell (1965) described *Neosartorya glabra* (Raper & Fennell) Kozakiewicz as having a smooth (or nearly so), convex ascospore surface and *N. spinosa* (Raper & Fennell) Kozakiewicz as having ascospores with spine-like projections ranging from $<0.5\ \mu\text{m}$ to as much as $2.0\ \mu\text{m}$. Peterson (1992) described the ascospores of *Neosartorya pseudofischeri* S.W. Peterson as having triangular flaps. Among the six species, *N. pseudofischeri* was clearly separated by the typical ascospore ornamentation, DNA–DNA hybridization (Peterson, 1992) and β -tubulin gene sequence (Geiser *et al.*, 1998; Varga *et al.*, 2000b). Our β -tubulin and calmodulin gene sequences confirmed that *N. pseudofischeri* is a well defined taxon. In addition, *N. pseudofischeri* produces a typical extrolite profile consisting of asperfuran, pyripyropens, gliotoxin and compounds which are similar to cytochalasins and fiscalins.

Six strains of *N. spinosa* (including CBS 483.65^T), CBS 114221 (ex-type strain of *N. botucatensis*) and CBS 114216 (ex-type strain of *N. paulistensis*) have almost the same β -tubulin and calmodulin gene sequences. Strain CBS 117058 differed in only two of 466 bases of the β -tubulin gene sequence when compared with the other strains. Strain KACC 41647 differed in only one of 522 bases of the calmodulin gene sequence when compared with the others. Horie *et al.* (1995) reported that the ascospores of *N. botucatensis* Horie *et al.* have convex surfaces with long spines up to 5 (maximum 7) μm long and *N. paulistensis* Horie *et al.*

have convex surfaces irregularly roughened by verruculose and small triangular projections up to 1 μm long. However, in our study, we have not observed that these two taxa differ significantly from *N. spinosa* with regard to their ascospore morphology and growth characteristics. Together with their phylogenetic similarity, we consider *N. botucatensis* and *N. paulistensis* to represent synonyms of *N. spinosa*.

Raper & Fennell (1965) described *N. spinosa* (as *Aspergillus fischeri* var. *spinusus*) with echinulate ascospores with projections ranging from $<0.5\ \mu\text{m}$ to as much as $2.0\ \mu\text{m}$. Our observations showed that *N. spinosa* has echinulate ascospores with spines ranging from $<0.5\ \mu\text{m}$ to 5 (7) μm long or with verruculose and small triangular, sometimes circularly arranged, projections.

Neosartorya takakii Horie *et al.* was described with ascospores bearing roughly circularly arranged projections (Horie *et al.*, 2001), a feature which has been also observed in other *N. spinosa* strains. This indicates that this species is a further synonym of *N. spinosa*. However, the ex-type strain of *N. takakii* could not be obtained in spite of numerous attempts. In order to establish the exact taxonomic position of *N. takakii*, a more detailed molecular and extrolite study of the type strain is required.

N. glabra strains were divided into five groups based on partial β -tubulin and calmodulin gene sequences. Of the five groups, *N. glabra sensu stricto*, CBS 112.55 and CBS 841.96 clustered with *N. fennelliae*, *N. hiratsukae*, *N. stramenia* and *N. aurata* (Fig. 1 and Supplementary Fig. S1). *N. glabra sensu stricto* has smaller and white cleistothecia, relatively straight equatorial crests and smoother walled convex surfaces compared with *N. spinosa*, *N. laciniosa* and *N. coreana*. *N. glabra* grows somewhat slower (mean diameter of

Fig. 3. *Neosartorya coreana* sp. nov. CBS 117059^T. (a) Colonies on MEA after 7 days at 25 °C; (b) ascomata; (c) asci; (d, e) conidial heads; (f) ascospores under a light microscope; (g) ascospores as seen by SEM. Bars, 100 μm (b) and 10 μm (c–g).

42–51 mm within 7 days at 25 °C) than the other species (mean diameter 41–68 mm within 7 days at 25 °C). However, at 10 °C, strains of *N. glabra* grow (6 mm diameter in 7 days), but the other taxa do not show growth.

CBS 112.55 isolated from garden soil in Australia was identified as *N. glabra* by Raper & Fennell (1965), but with somewhat smaller ascospores and narrower, more widely separated crests. The strain produced secondary metabolites that were different from the ex-type strain of *N. glabra*, CBS 111.55^T (Samson *et al.*, 1990). Our study shows that CBS 112.55 has more than 5 % dissimilarity based on partial β -tubulin and calmodulin gene sequences and did not group with *N. glabra sensu stricto* (Fig. 1 and Supplementary Fig. S1). However, the isolate did not differ from *N. glabra* in phenotypic characters, except that it grew somewhat slower on CYA, MEA and CZA at 25 °C (mean diameter 25–29 mm in 7 days). From these results, the strain could represent a novel species, but more isolates should be available to determine its taxonomic position. Also CBS 841.96 had a special position in the cladograms based on β -tubulin and calmodulin gene sequences (Fig. 1 and Supplementary Fig. S1), but it resembled *N. glabra* based on phenotypic characteristics. As is the case for CBS 112.55, more isolates of CBS 841.96 will have to be studied to warrant its classification as a novel species.

Based on the β -tubulin and calmodulin gene sequence phylogeny, *N. laciniosa* was located distantly from *N. glabra*, but closer to *N. spinosa*. Its separation from *N. spinosa* was not well supported, with a bootstrap value of 62 % for the β -tubulin gene sequence, but it was well supported on the basis of the calmodulin gene sequence, with a bootstrap value of 98 %. *N. laciniosa* has microtuberculate ascospores with two distinct straight crests and two distinct equatorial rings with small projections, differing from *N. spinosa* which has ascospores with long spines or roughly circularly arranged projections on the convex walls. Based on the calmodulin gene sequences, two subgroups can be distinguished; one consists of strains KACC 41652, KACC 41657^T, CBS 206.92 and CBS 315.89, and the second consists of strains IBT 6660, KACC 41644, KACC 41645, KACC 41646, CBS 585.90 and KACC 41648. However, we do not consider them to represent two separate species according to the Genealogical Concordance Phylogenetic Species Recognition (Taylor *et al.*, 2000), because CBS 206.92 cross-located in the other subgroup in the tree based on the β -tubulin gene sequence. Furthermore, no phenotypic characteristics support the separation, apart from the observation that strain IBT 6660 produced asperfuran, in contrast to the strains of the first subgroup.

N. coreana was located near, but clearly separated from, *N. spinosa* based on the β -tubulin and calmodulin gene sequence trees (Fig. 1 and Supplementary Fig. S1). This species had similar phenotypic characteristics to *N. spinosa* and *N. laciniosa* but, when observed by SEM, the species had rugose to weak reticulate ornamentation of convex ascospore walls without the two equatorial rings of

microtuberculate projections and thereby differing from *N. spinosa* and *N. laciniosa*.

Latin diagnosis of *Neosartorya laciniosa* S. B. Hong, Frisvad *et* Samson sp. nov.

Anamorph: *Aspergillus laciniosus* S. B. Hong, Frisvad *et* Samson sp. nov.

Coloniae in agaro malti 53–55 mm diam. post 7 dies 25 °C, *albae, planae, hyphis aeriis paucis superatae, margo irregularis, ascomata copiosa in zona marginali granulati. Species homothallica. Cleistothecia superficialia, alba vel dilute lutea, globosa vel subglobosa*, 300–400 μ m diam., post 10 hebdomades matura, *hyphis hyalinis vel flavidis*, 2–4 μ m latis *laxe circumdata*. Asci 8-spori, *globosi vel subglobosi vel ovoidei*, 11–13 μ m diam., *maturi evanescentes. Ascosporae late lenticulares*, 4–5 μ m, *duabus crestis aequatorialibus, distantibus, ad 2 μ m latis circumdatae, valvae leves vel asperulatae. Conidiophora ex hyphis aeriis oriunda, stipites 3–4 μ m lati; vesiculae hemisphaericae vel lageniformes*, 10–14 μ m diam. *Aspergilli uniseriati, phialides 7–8 $\times$ 2–3 μ m; conidia (sub-) globosa, nonnumquam late ellipsoidea, levia*, 2.5–3.5 μ m diam. *Massa conidiorum columnaris. Metabolita aszonaleninum, tryptoquivalina, tryptoquivalona.*

Typus: CBS 117721^T (=NRRL 35589^T=KACC 41657^T).

Description of *Neosartorya laciniosa* S. B. Hong, Frisvad & Samson sp. nov.

Anamorph: *Aspergillus laciniosus* S. B. Hong, Frisvad & Samson sp. nov.

Neosartorya laciniosa (la.cin.i.os'us. N.L. adj. *laciniosus* -a -um laciniate, divided into deep, narrow, irregular segments).

Rapidly growing colonies on MEA, 53–55 mm diameter in 7 days at 25 °C, white, plane, loosely overgrown by aerial hyphae, margin irregular, cleistothecia abundantly produced in marginal areas with granular appearance. Conidia heads few in number; reverse greyish orange (5B6) to yellowish orange (4B6). Colonies on CYA spread broadly, attaining a diameter of 56–58 mm in 7 days at 25 °C or more than 70 mm in 7 days at 37 °C, more or less sulcate, white to pale yellow (2A3), granular due to the abundant production of cleistothecia, loosely overgrown by aerial hyphae; conidial heads few in number, scattered; reverse light orange (6A45). Homothallic, cleistothecia superficial, white to light yellow, globose to subglobose, 300–400 μ m in diameter, surrounded by a loose covering of hyaline to yellowish white, 2–4 μ m wide hyphae. Cleistothecial peridium hyaline to light yellow, consisting of angular, 4–15 μ m diameter cells. Asci 8-spored, globose or subglobose to ovoid, 11–13 μ m diameter, evanescent at maturity. Ascospores broadly lenticular, spore body 4–5 μ m diameter, provided with two distinct equatorial crests which are up to 2 μ m wide with two distinct equatorial rings of small projections in the ascospore furrow; convex surfaces finely rough or smooth in light microscopy, appearing rugose to tuberculate in SEM (Fig. 2). Conidial

heads columnar. Conidiophores arising from aerial hyphae, 3–4 µm wide at the middle; vesicles subclavate, 10–14 µm in diameter. Aspergilli uniseriate, phialides 7–8 × 2–3 µm, covering the upper half of vesicle. Conidia, globose to subglobose, sometimes broadly elliptical, smooth, 2.5–3.5 µm diameter. Extrolites: aszonalenins and several tryptoquivalins, tryptoquivalons; strain IBT 6660 also produces asperfuran.

The type strain, CBS 117721^T (=NRRL 35589^T=KACC 41657^T) (dried culture), was isolated from soil from a tomato field in Buyeo, Korea.

Latin diagnosis of *Neosartorya coreana* S. B. Hong, Frisvad et Samson sp. nov.

Anamorph: *Aspergillus coreanus* S. B. Hong, Frisvad et Samson sp. nov.

Coloniae in agar multi 62–66 mm diam. post 7 dies 25 °C, albae vel luteo-albae, in medio elevatae, hyphis aeriis laxis superatae, margo irregularis, ascomata copiosa dispersa, aspectu granulati. Species homothallica. Cleistothecia superficialia, alba vel dilute lutea, post 3 hebdomades matura, globosa vel subglobosa, 200–300 µm diam., hyphis hyalinis vel flavidis, 2–3 µm latis laxe circumdata. Asci 8-sporei, globosi vel subglobosi vel ovoidei, 10–13 µm diam., maturi evanescentes. Ascospores lenticulares, 4–5 µm diam., duabus crestis aequatorialibus, distantibus, ad 2 µm latis, nonnumquam irregulariter aspectu stellari dissectis circumdatae, valvae rugosae vel parce reticulatae. Conidiophora ex hyphis aeriis oriunda, stipites 3–4 µm lati; vesiculae subclavatae vel lageniformes, 8–13(–15) µm diam. Aspergilli uniseriati, phialides 6–9 × 2–3 µm; conidia subglobosa vel late ellipsoidea, levia, 2.5–3.5 µm diam. Massa conidiorum columnaris. Metabolita aszonaleninum.

Typus: CBS 117059^T (=NRRL 35590^T=KACC 41659^T).

Description of *Neosartorya coreana* S. B. Hong, Frisvad & Samson sp. nov.

Anamorph: *Aspergillus coreanus* S. B. Hong, Frisvad & Samson sp. nov.

Neosartorya coreana (co.re.an'us. N.L. adj. *coreanus* -a -um pertaining to Korea).

Colonies grow rapidly on MEA, 62–66 mm in 7 days at 25 °C, white to yellowish white (1A2), centrally raised, loosely overgrown by aerial hyphae, irregular margin, cleistothecia produced in an irregular pattern with granular appearance. Conidial heads few in number, reverse greyish orange (5B6) to yellowish orange (4B6). Colonies on CYA spread broadly, attaining a diameter of 58–62 mm in 7 days at 25 °C or more than 70 mm in 7 days at 37 °C, radially sulcate, white to yellowish white (1A2). Cleistothecia produced on central part and along sulcate groove, loosely overgrown by aerial hyphae; conidial heads few in number, scattered, reverse pale to light orange (5A34). Homothallic.

Cleistothecia superficial, white to light yellow (34A45) in 3 weeks, globose to subglobose, 200–300 µm in diameter, surrounded by a loose covering of hyaline to yellowish white, 2–3 µm wide hyphae. Cleistothecial peridium hyaline to light yellow, membranaceous, consisting of angular, 4–18 µm diameter cells. Asci 8-spored, globose or subglobose to ovoid, 10–13 µm diameter, evanescent at maturity. Ascospores, spore body 4–5 µm diameter, provided with two well-separated, but often bent, equatorial crests which are up to 2 µm wide and sometimes irregularly shaped; convex surfaces finely roughened in light microscopy appearing rugose to weakly reticulate in SEM (Fig. 3). Conidial heads columnar. Conidiophores arising from aerial hyphae, smooth, more or less sinuous, 3–4 µm wide at the middle; vesicles subclavate, 8–13 (15) µm diameter. Aspergilli uniseriate; phialides, 6–9 × 2–3 µm, covering the upper half of vesicle. Conidia subglobose to broadly elliptical, smooth, 2.5–3.5 µm diam. Extrolites: aszonalenins, pseurotin in CBS 117057 and several further metabolites for which the structure has not yet been elucidated.

The type strain, CBS 117059^T (=NRRL 35590^T=KACC 41659^T) (dried culture), was isolated from soil from a tomato field in Buyeo, Korea.

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