Mycoscience (2010) 51:253–262
DOI 10.1007/s10267-010-0037-8

FULL PAPER

Two new species of *Neosartorya* isolated from soil in Xinjiang, China

Takashi Yaguchi · Tetsuhiro Matsuzawa · Reiko Tanaka · Paride Abliz · Yan Hui · Yoshikazu Horie

Received: 10 November 2009 / Accepted: 16 December 2009 / Published online: 18 March 2010

Abstract Two new species, *Neosartorya shendaweii* and *N. tsunodae*, isolated from soil in Xinjing, China and in Pernambuco, Brazil, are described and illustrated. The first species is characterized by its ascospores with two widely separated equatorial crests and tuberculate to verrucose convex surfaces. This species has affinities with several known species of the genus, bearing ascospores with a similar ornamentation, but can be distinguished from these species by other morphological characteristics such as smaller cleistothecia and conidiophores, spathulate vesicles and rather ellipsoidal conidia. The second species is characterized by its unique ascospores with two low equatorial crests, an evident furrow as a deep depression, and finely reticulate convex surfaces. The validation of these new species is supported further by analyses of the β-tubulin, calmodulin and actin gene sequences.

Keywords *Aspergillus* section *Fumigati* · *Neosartorya shendaweii* · *Neosartorya tsunodae* · Phylogeny · Taxonomy

Electronic supplementary material The online version of this article (doi:10.1007/s10267-010-0037-8) contains supplementary material, which is available to authorized users.

T. Yaguchi (✉) · T. Matsuzawa · R. Tanaka
Medical Mycology Research Center, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba 260-8673, Japan
e-mail: t-yaguchi@faculty.chiba-u.jp

P. Abliz · Y. Hui
Department of Dermatology, The First Affiliated Hospital of Xinjiang Medical University, No. 1 Liyushan Road, Urumqi, Xinjiang 830053, China

Y. Horie
Natural History Museum and Institute, Chiba, 955-2 Aobacho, Chuo-ku, Chiba 260-8682, Japan

Introduction

The genus *Neosartorya* Malloch & Cain (anam. *Aspergillus* section *Fumigati*) in the Eurotiales was introduced from *Sartorya* Vuill. (Malloch and Cain 1972). Most members of *Neosartorya* are distributed worldwide and occur everywhere in soil, air, foods, organic materials and human habitations (Takada and Udagawa 1985; Takada et al. 1986, 2001; Kozakiewicz 1989; Udagawa et al. 1991, 1993, 1996; Horie et al. 1992, 1995a, b, 2001, 2003; Yaguchi et al. 1994; Someya et al. 1999; Hong et al. 2006, 2008; Samson et al. 2007). Some species of the genus are reported to be spoilage agents in fruit juices and other heat-processed food products. A few *Neosartorya* species are regarded as being mycotoxigenic and also are associated with disease, since they are the causative agents for aspergillosis, an opportunistic fungal infection (Peterson 1992; de Hoog et al. 2000; Guarro et al. 2002; Järv et al. 2004). Certain species of the genus are also used in the production of bioactive metabolites (Udagawa and Yaguchi 2005).

Recently, using several airborne strains of *Aspergillus fumigatus* Fresen., O'Gorman et al. (2009) set up crossing experiments with 12 isolates using all possible combinations of opposite mating types and a range of growth media and temperatures. After 6 months, cleistothecia producing viable ascospores which germinated to form the anamorphs were found in some pairings. The teleomorph belonged to the genus *Neosartorya* and the novel binomial *N. fumigata* (Fres.) C.M. O'Gorman, H.T. Fuller & P.S. Dyer was proposed for this. The recognition of a sexual stage for *A. fumigatus* enhanced the overall understanding of its biology, and provided a basis for the recombination already recognized in the fungus.

Ascospore ornamentation is an important morphological characteristic for distinguishing species within the genus.

Furthermore, polyphasic analysis based on phenotypic and molecular characteristics has been used for identification of species. Samson et al. (2007) described 23 *Neosartorya* species and 10 strictly anamorphic *Aspergillus* species.

In a survey of pathogenic and mycotoxigenic fungi in Xinjiang, China and in Pernambuco, Brazil, several isolates of unusual *Neosartorya* have been found in various types of soil. These isolates were identified by analyses of macro- and micromorphology and of β-tubulin, calmodulin and actin gene sequences. In this report, we describe two new species of *Neosartorya*, and compare them with other species in the genus.

Materials and methods

Isolates examined

The fungi isolated from soil in Xinjiang, China and in Pernambuco, Brazil and the strains used for comparison are listed in Table 1, along with their strain numbers and DNA Data Bank of Japan (DDBJ) accession numbers. The type specimens were placed in the Natural History Museum and Institute, Chiba, Japan (CBM) and freeze-dried culture ex-types were deposited in the Medical Mycology Research Center, Chiba University, Chiba, Japan (IFM) and the Department of Biotechnology, National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan (NBRC).

Incubation and observation

Each isolate was incubated at 25 and 37°C for 14–21 days on Czapek agar (CzA), malt extract agar (MEA) and oatmeal agar (OA). After the incubation, colonies were examined by a light microscope (LM) and a scanning electron microscope (SEM: Hitachi S3400, Tokyo, Japan). Colony colors were designated according to the Methuen Handbook of Colour (Kornerup and Wanscher 1978).

DNA extraction and sequencing analysis

DNA was prepared using the GenTorukun (Takara Bio Inc., Ltd., Otsu, Japan) from approximately 100 μl volume of fungal mass cultured at 37°C for 5 days on potato-dextrose agar (PDA) slants. The β-tubulin, calmodulin and actin genes were sequenced directly from PCR products using primer pair Bt2a and Bt2b (Glass and Donaldson 1995), primer pair cmd5 and cmd6 (Hong et al. 2005), and primer pair act-512 and ACT-783R (Carbone and Kohn 1999), respectively. The PCR products were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 3130ABI Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions.

Molecular phylogenetic analyses

DNA sequences were edited using ATGC Ver. 4 sequence assembly software (Genetyx Co., Tokyo, Japan). The alignments of the sequences and phylogenetic trees based on neighbor-joining (NJ) analysis (Saitou and Nei 1987) were performed using Clustal X software (Thompson et al. 1997). The distances between sequences were calculated using Kimura's two-parameter model (Kimura 1980). A bootstrap was conducted with 1,000 replications (Felsenstein 1985). The aligned dataset used in the analysis has been deposited with the TreeBASE under the accession number S2586.

Results and discussion

Morphological analyses

All isolates analyzed by microscopic examination exhibited white to pale yellow cleistothecia, bivalved ascospores, conidial heads in shades of grayish green, and short columnar, uniseriate aspergilla with spathulate vesicles fertile over the upper half, and broadly ellipsoidal and smooth conidia (Figs. 1, 2, 3–6, 7–10). They had similar colony appearances and growth rates on all media analyzed in this study, and all grew very fast at 37°C. These are typical morphological features associated with *Aspergillus* section *Fumigati* and the teleomorphic genus *Neosartorya* (Raper and Fennell 1965; Klich 2002).

The strains IFM 57610 and 57611 had lenticular ascospores with two widely separated equatorial crests and tuberculate to verrucose convex surfaces (Fig. 5). The ornamentation of ascospores suggested a relationship with "*N. glabra* (Fennell & Raper) Kozak.," *N. australensis* Samson, S.B. Hong & Varga, *N. galapagensis* Frisvad, S.B. Hong & Samson, *N. glabra* sensu stricto, *N. papuensis* Samson, S.B. Hong & Varga and *N. warcupii* Peterson, Varga & Samson (Hong et al. 2006, 2008; Samson et al. 2007). In addition to the common difference in smaller cleistothecia, the strains were distinguished from *N. australensis* in having ascospores with rougher convex surfaces and narrower conidiophores and vesicles; from *N. galapagensis* in having ascospores with rougher convex surfaces and ellipsoidal conidia; from *N. glabra* sensu stricto in having ascospores with rougher convex surfaces, shorter conidiophores and ellipsoidal conidia; from *N. papuensis* in having ascospores with rougher convex surfaces, shorter conidiophores and ellipsoidal conidia; and from *N. warcupii* in having ascospores with rougher

Table 1 *Neosartorya* and related isolates used in this study

Species	Isolate number	DDBJ accession number		
		β-Tubulin	Calmodulin	Actin
Aspergillus brevipes G. Sm.	CBS 118.53[T]	AF057311	AY689364	DQ094849
A. duricaulis Raper & Fennell	CBS 481.65[T]	AF057313	AY689368	DQ094854
A. fumigatiaffinis S.B. Hong, Frisvad & Samson	IBT 12703[T]	DQ094885	DQ094891	DQ094865
A. fumigatus Fresen.	CBS 545.65	AY685151	AY689335	DQ094851
A. fumisynnematus Y. Horie, Miyaji, Nishim., Taguchi & Udagawa	IFM 42277[T]	AB248076	AB259968	AB488769
A. lentulus Balajee & K.A. Marr	FH5[T]	AY738513	DQ094896	DQ094873
A. novofumigatus S.B. Hong, Frisvad & Samson	ITB 16806[T]	DQ094886	DQ094893	DQ094868
A. unilateralis Thrower	CBS 126.56[T]	AF067316	AY689366	DQ094847
A. turcosus S.B. Hong, Frisvad & Samson	KACC 42091[T]	DQ534143	DQ534148	DQ534179
A. viridinutans Ducker & Thrower	CBS 127.56[T]	AF134779	AY689362	DQ094862
Neosartorya assulata S.B. Hong, Frisvad & Samson	KACC 41691[T]	DQ114123	DQ114131	DQ534189
N. aurata (Warcup) Malloch & Cain	CBS 466.65[T]	AF057318	AY870685	DQ534112
N. aureola (Fennell & Raper) Malloch & Cain	CBS 105.55[T]	AF057319	AY689369	DQ094861
N. australensis Samson, S.B. Hong & Varga	CBS 112.55[T]	AY870739	AY870698	DQ534141
N. coreana S.B. Hong, Frisvad & Samson	KACC 41659[T]	AY870758	AY870718	DQ534116
N. denticulata Samson, S.B. Hong & Frisvad	CBS 652.73[T]	DQ114125	DQ114133	DQ534181
N. fennelliae Kwon-Chung & S.J. Kim	CBS 598.74[T]	DQ114127	DQ114135	DQ534121
N. ferenczii Varga & Samson	CBS121594[T]	EF669833	EU220285	EU220287
N. fischeri (Wehmer) Malloch & Cain	CBS 544.65[T]	AF057322	AY689370	DQ094863
N. galapagensis Frisvad, S.B. Hong & Samson	CBS 117522[T]	DQ534145	DQ534151	DQ534190
N. glabra (Fennell & Raper) Kozak.	CBS 111.55[T]	AY870734	AY870693	DQ534183
N. hiratsukae Udagawa, Tsub. & Y. Horie	NHL 3008[T]	AF057324	AY870699	DQ534184
N. indohii Y. Horie	CBM-FA-0934[T]	AB488757	AB488765	AB488774
N. laciniosa S.B. Hong, Frisvad & Samson	KACC 41657[T]	AY870756	AY870716	DQ534126
N. multiplicata Yaguchi, Someya & Udagawa	IFM 46955[T]	DQ114129	DQ114137	DQ534185
N. nishimurae Takada, Y. Horie & Abliz	CBS 116047	DQ534075	DQ534150	DQ534186
N. papuensis Samson, S.B. Hong & Varga	CBS 841.96[T]	AY870738	AY870697	DQ534140
N. paulistensis Y. Horie, Miyaji & Nishim.	CBM-FA-0690[T]	AB488758	AB488766	AB488775
N. pseudofischeri S.W. Peterson	CBS 208.92[T]	AY870743	AY870702	DQ534187
N. quadricincta (J.L. Yuill) Malloch & Cain	CBS 135.52[T]	AF057326	DQ114138	DQ534132
N. shendaweii sp. nov.	IFM 57610	AB488753	AB488761	AB488770
	IFM 57611[T]	AB488754	AB488762	AB488771
N. spathulata Takada & Udagawa	NHL 2948[T]	AF057327	DQ534173	DQ534138
N. spinosa (Raper & Fennell) Kozak.	CBS 483.65[T]	AF057329	AY689371	DQ094869
N. stramenia (R.O. Novak & Raper) Malloch & Cain	CBS 498.65[T]	AY870766	AY870726	DQ534188
N. sublevispora Someya, Yaguchi & Udagawa	IFM 53598[T]	AB488759	AB488767	AB488776
N. tatenoi Y. Horie, Miyaji, Koji Yokoy., Udagawa & Camp.-Takagi	CBM-FA-022[T]	DQ114130	DQ114139	DQ534135
N. tsunodae sp. nov.	IFM 57609[T]	AB488755	AB488763	AB488772
	IFM 53603	AB488756	AB488764	AB488773
N. tsurutae Y. Horie	CBM-FA-0933[T]	AB488760	AB488768	AB488777
N. udagawae Y. Horie, Miyaji & Nishim.	CBM-FA-0702[T]	AF132226	AY689372	DQ094858
N. warcupii Peterson, Varga & Samson	NRRL 35723[T]	EU220283	EU220284	EU220286
A. clavatus Desm.	CBS 513.65	AB489851	AB489852	AB489853

convex surfaces and narrower ridges, shorter conidiophores, narrower vesicles and ellipsoidal conidia. The ascospores of the strains were also similar to those of *N. paulistensis* Y. Horie, Miyaji & Nishim. (Horie et al. 1995a) and *N. laciniosa* S.B. Hong, Frisvad & Samson (Hong et al. 2006), but the smaller cleistothecia, shorter

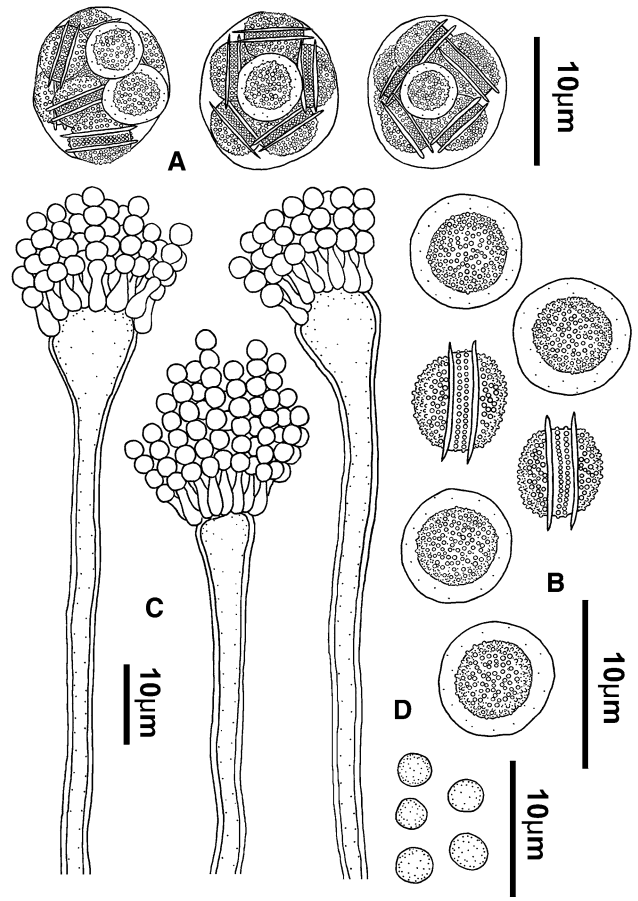


Fig. 1 *Neosartorya shendaweii*. *A* Asci, *B* ascospores, *C* aspergilla, *D* conidia

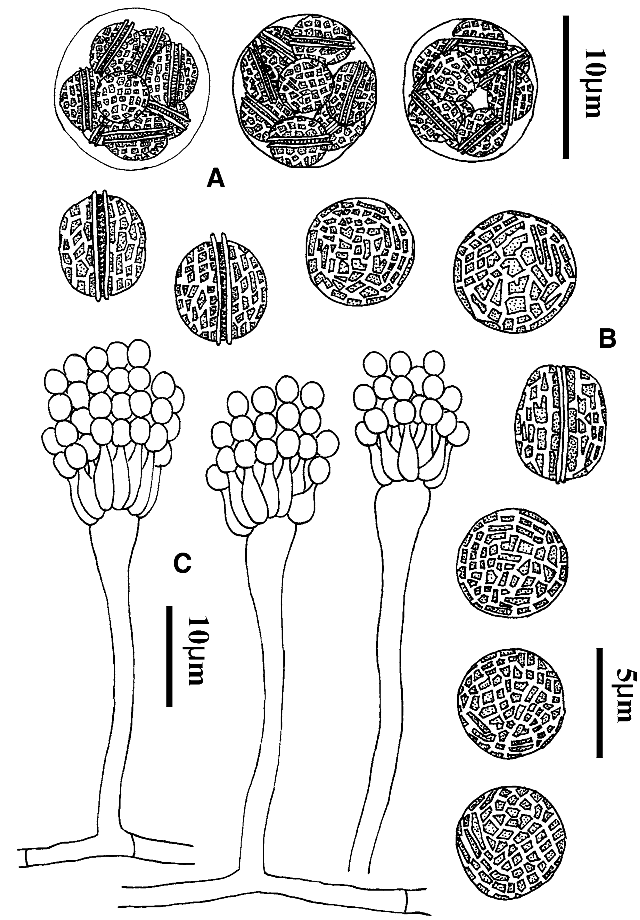


Fig. 2 *Neosartorya tsunodae*. *A* Asci, *B* ascospores, *C* aspergilla

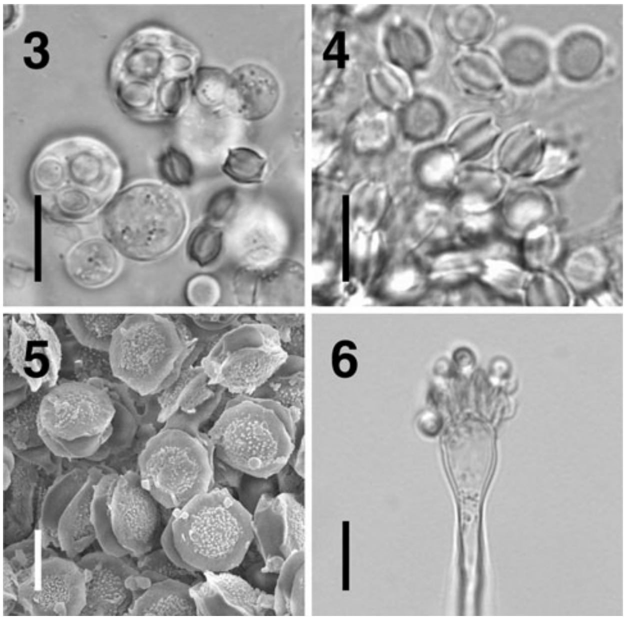
conidiophores, narrower vesicles and ellipsoidal conidia indicated that the latter species were different.

Ascospores of the strains IFM 53603 and 57609 were observed on the SEM to be elaborately reticulate on the convex surfaces (Fig. 9). The ornamentation of the ascospores was somewhat similar to those of *N. tatenoi* Y. Horie, Miyaji, Koji Yokoy., Udagawa & Camp.-Takagi (Horie et al. 1992), and *N. multiplicata* Yaguchi, Someya & Udagawa (Yaguchi et al. 1994). In the case of *N. tatenoi*, however, the ascospores are characterized by two prominent, thin and often recurved crests, a rather indistinct furrow and convex surfaces ornamented by a coarse network of anastomosing ridges. *Neosartorya multiplicata* is different in having almost globose ascospores which lack distinct equatorial crests and have ribbed ornamentation with several linear ridges. Reticulate ascospores are also observed in *N. fischeri* (Wehmer) Malloch & Cain (Raper and Fennell 1965), but this species can be separated from the IFM strains by ascospores having two ruffled equatorial crests and convex surfaces bearing a coarse network of rather irregularly anastomosing ridges (Kozakiewicz 1989; Samson et al. 2007).

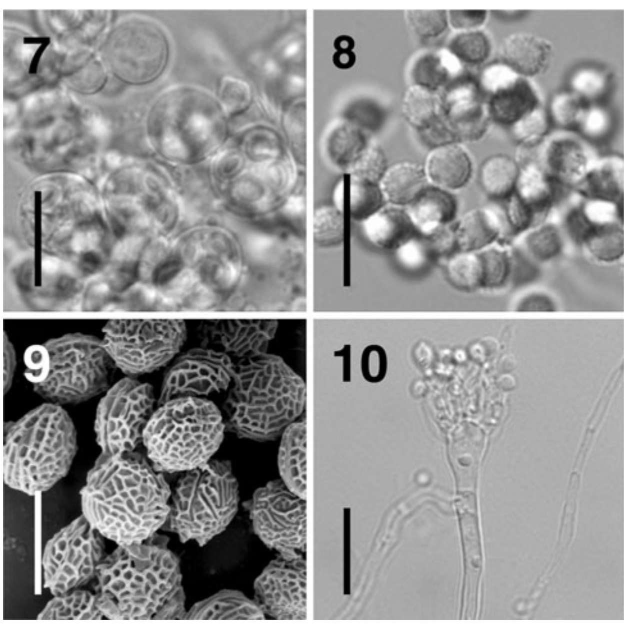
Phylogenetic analyses

The DNA sequences of the β-tubulin, calmodulin and actin genes in this study have been deposited in the DDBJ, and the accession numbers are listed in Table 1.

Strains IFM 57610 and 57611 showed identical β-tubulin and calmodulin gene sequences, and almost identical actin gene sequences (Figs. 11, 12, 13). The closest taxon to these strains based on β-tubulin and actin gene phylogenies was *N. galapagensis* (Figs. 11, 13). However, the similarity between this species and the two strains was quite low (95.5% in the β-tubulin gene partition and 93.6% in the actin gene partition). Furthermore, the similarity of calmodulin sequences between the species and the two strains was only 94.2% (Fig. 12). From the phylogenetic analyses of β-tubulin and calmodulin gene sequences, the two strains did not show a clear relationship to *N. australensis*, *N. glabra* sensu stricto, *N. papuensis* and *N. warcupii*. The two strains showed very low sequence similarity with *N. paulistensis* and *N. laciniosa* and were not clustered within these species. In fact, based on the β-tubulin, calmodulin and actin gene sequence phylogeny,



Figs. 3–6 *Neosartorya shendaweii*. **Fig. 3** Asci. **Fig. 4** Ascospores (LM). **Fig. 5** Ascospores (SEM). **Fig. 6** Aspergillum. *Bars* **3**, **4**, **6** 10 μm; **5** 5 μm



Figs. 7–10 *Neosartorya tsunodae*. **Fig. 7** Asci. **Fig. 8** Ascospores (LM). **Fig. 9** Ascospores (SEM). **Fig. 10** Aspergillum. *Bars* **7**, **8**, **10** 10 μm; **9** 5 μm

N. paulistensis and *N. laciniosa* were located distinctly from *N. glabra* sensu stricto, but closer to *N. spinosa* (Raper & Fennell) Kozak.

The sequences of strains IFM 53603 and 57609 were completely identical and clearly differed from *N. tatenoi* sequences (Figs. 11, 12, 13). The phylogenetic analyses of partial β-tubulin and calmodulin gene sequences indicate that the strains are most closely related to *N. multiplicata* (Figs. 11, 12). However, the strains were clearly separated from *N. multiplicata* based on the homologies of β-tubulin, calmodulin and actin gene sequences, which were 98.0, 96.8 and 95.6%, respectively.

When the evidence from morphology and these phylogenetic analyses are taken together, it is our conclusion that each of the two separate groups of the IFM strains should be distinct new species, *Neosartorya shendaweii* Yaguchi, Abliz & Y. Horie and *Neosartorya tsunodae* Yaguchi, Abliz & Y. Horie.

Phylogenetic assessment of the classification of some *Neosartorya*

Most *Neosartorya* and related anamorphic species in section *Fumigati* were defined based on morphology, with the additional consideration of molecular and extrolite data used in recent years (Geiser et al. 1998; Varga et al. 2000; Hong et al. 2005, 2006, 2008; Samson et al. 2007). To evaluate phylogenetic relationships between species and sections in *Aspergillus*, a four-locus DNA sequence study covering all major lineages (including most of the known and accepted species in section *Fumigati*) in the genus (Peterson 2008) was also carried out. The phylogenetic analysis, based on previous data (Samson et al. 2007; Peterson 2008) and the present results, indicated that *N. fischeri*, *N. coreana* S.B. Hong, Frisvad & Samson, *N. laciniosa*, *N. paulistensis* and *N. spinosa* are most clearly related to *A. fumigatus* (as the clade *Aspergillus fumigatus*). Ornamentation of the ascospores does not appear to be a reliable indicator of phylogenetic relatedness among *Neosartorya* species. Of the five *Neosartorya* species within the clade *A. fumigatus*, *N. fischeri* has only ascospores with convex surfaces bearing anastomosing ridges (reticulate), and *N. coreana* has rugose to weak reticulate ascospores without the equatorial rings of small projections. *Neosartorya paulistensis* and *N. laciniosa* have ascospores with two widely separated equatorial crests, distinct equatorial rings of small projections and tuberculate to verrucose convex surfaces, unlike *N. spinosa*, which has ascospores with long spines. *Neosartorya shendaweii* and *N. tsunodae* are distantly related to these species in the clade *A. fumigatus* (Figs. 11, 12, 13).

Synonymies of two species described previously by Hong et al. (2006) and Samson et al. (2007) were found during this study.

Neosartorya paulistensis is readily recognized by the production of white to pale yellow cleistothecia, broadly lenticular ascospores which have two widely separated equatorial crests, two rings of small projections in the equatorial furrow area, and verrucose convex surfaces

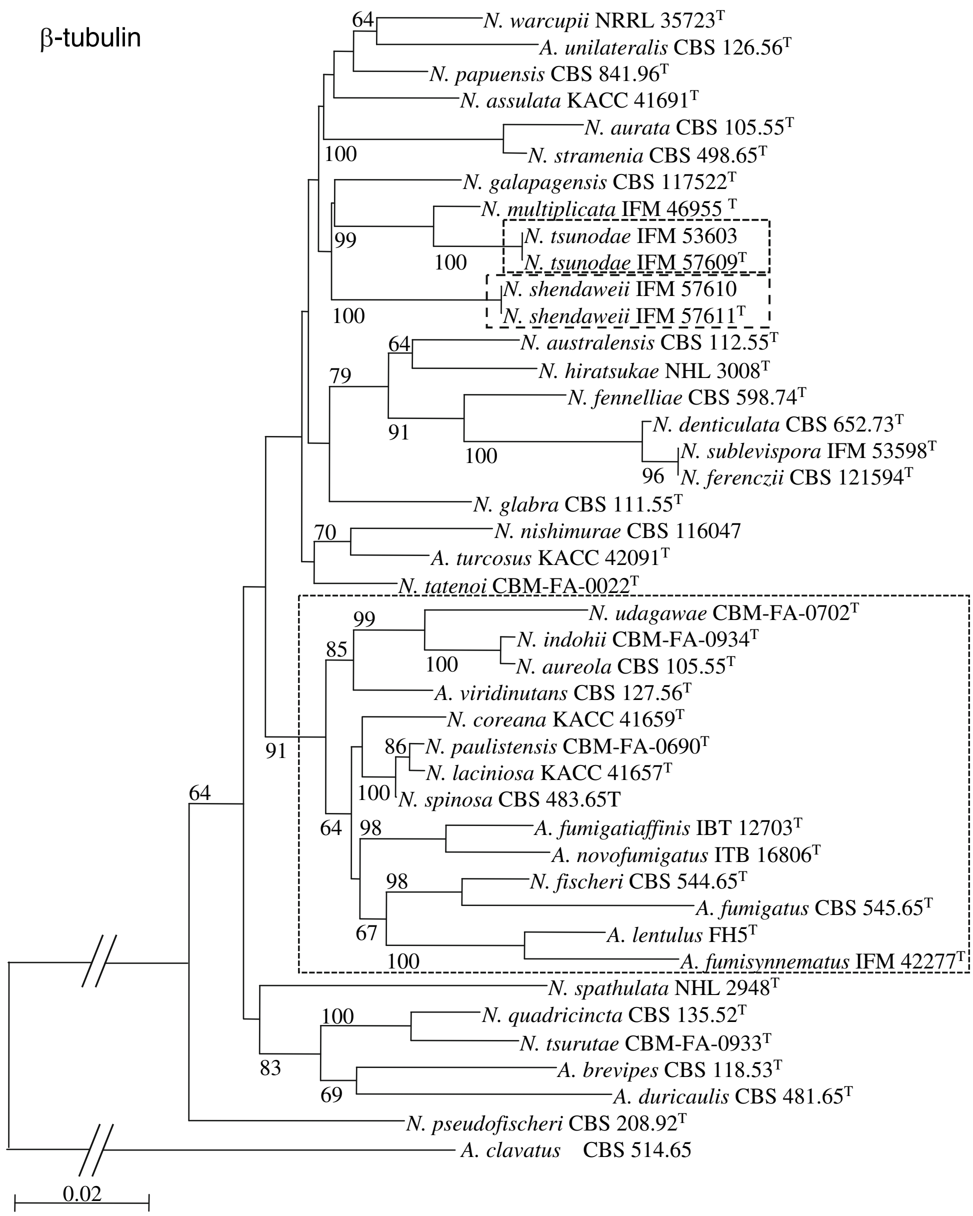


Fig. 11 Neighbor-joining tree from sequences of the *β*-tubulin gene. Each *number* indicates the percentage of bootstrap samplings, derived from 1000 samples, supporting the internal branches of 50% or higher

bearing tuberculate to small triangular projections up to 1.0 μm long (Horie et al. 1995a; "Electronic supplementary material" Fig. S1 A). These distinctive features, as well as the similarities in other phenotypic characteristics such as colony appearance and anamorph morphology, are strikingly identical to those of *N. laciniosa* ("Electronic supplementary material" Fig. S1 B). In their polyphasic taxonomic approach to the classification of section *Fumigati*, Samson et al. (2007) considered *N. paulistensis* to be a synonym of *N. spinosa*, while Hong et al. (2006) reported that *N. laciniosa* has microtuberculate ascospores with two distinct straight crests and two equatorial rings with small projections, unlike *N. spinosa*, which has ascospores with long spines or roughly circularly arranged projections on the convex surfaces. In addition, its separation from *N. spinosa* was well supported on the basis of the calmodulin gene sequence of *N. laciniosa*, with a bootstrap value of 98%. In fact, the spinelike ornamentation (ranging from <0.5 μm up to 7 μm long) of *N. spinosa* distinguishes it from these of *N. paulistensis*, *N. laciniosa*, or other closely related species.

Hong et al. (2006) stated that the five strains of *N. spinosa*, including the CBS 483.65 ex-type strain of *N. spinosa*, have identical partial *β*-tubulin and calmodulin genes sequences. They also found that CBS 114216, designated an ex-type strain of *N. paulistensis*, has spiny ascospores and identical sequences to *N. spinosa*. However, re-examination of the type specimen (CBM-FA-0690) of *N. paulistensis* in this study agreed well with the published description (Horie et al. 1995a). The sequence data based

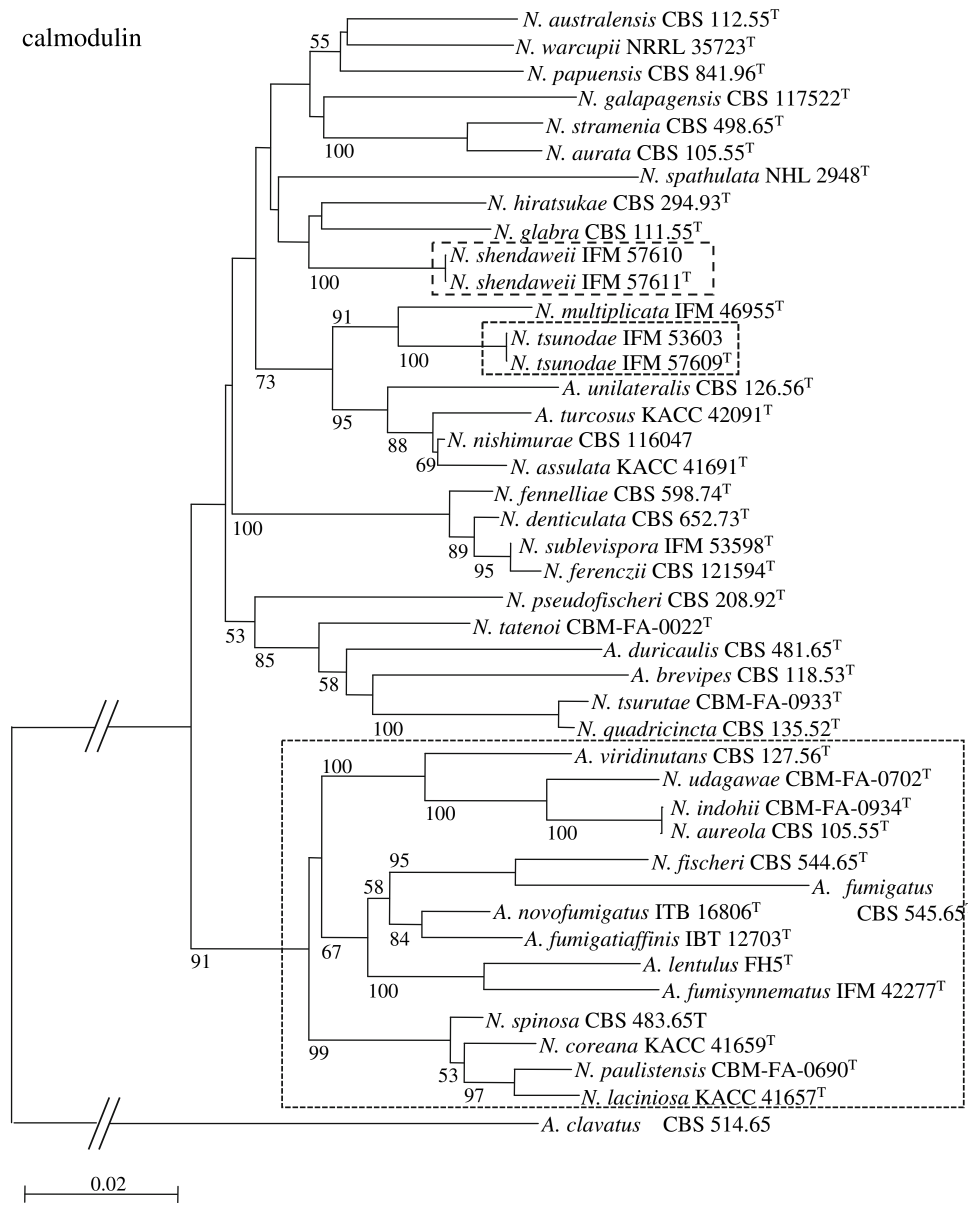


Fig. 12 Neighbor-joining tree from sequences of the calmodulin gene. Each *number* indicates the percentage of bootstrap samplings, derived from 1000 samples, supporting the internal branches of 50% or higher

on CBS-FA-0690 for the β-tubulin and calmodulin genes have not only been distinguished from those of CBS 114216, but are also quite similar to those of *N. laciniosa*. In addition, the β-tubulin gene sequence of *N. paulistensis* was identical to the sequence data for AF132231, as determined by Varga et al. (2000). The clade formed by *N. paulistensis* and 10 strains of *N. laciniosa* in the trees inferred from the analysis of these two loci received high bootstrap support ("Electronic supplementary material" Figs. S2 and S3). From the results of the re-examination of the type specimen of *N. paulistensis*, the morphological features and the sequence data based on CBS 114216 strain (AY870764 and AY870724) appear to be incorrect and should not be accepted as characteristic of *N. paulistensis*. These data confirm that *N. paulistensis* is valid as the accepted species, while *N. laciniosa* should be considered a synonym of the former species.

Neosartorya paulistensis Y. Horie, Miyaji & Nishim., Mycoscience 36: 163. 1995.

= *N. laciniosa* S.B. Hong, Frisvad & Samson, Int J Syst Evol Microbiol 56: 484. 2006.

Neosartorya sublevispora Someya, Yaguchi & Udagawa is characterized by cleistothecia covered loosely with a pale yellowish hyphal envelope, lenticular ascospores with two low equatorial crests and subglobose conidia (Someya et al. 1999). The ornamentation of ascospores, which is composed of two closely appressed crests and small rounded projections on their convex

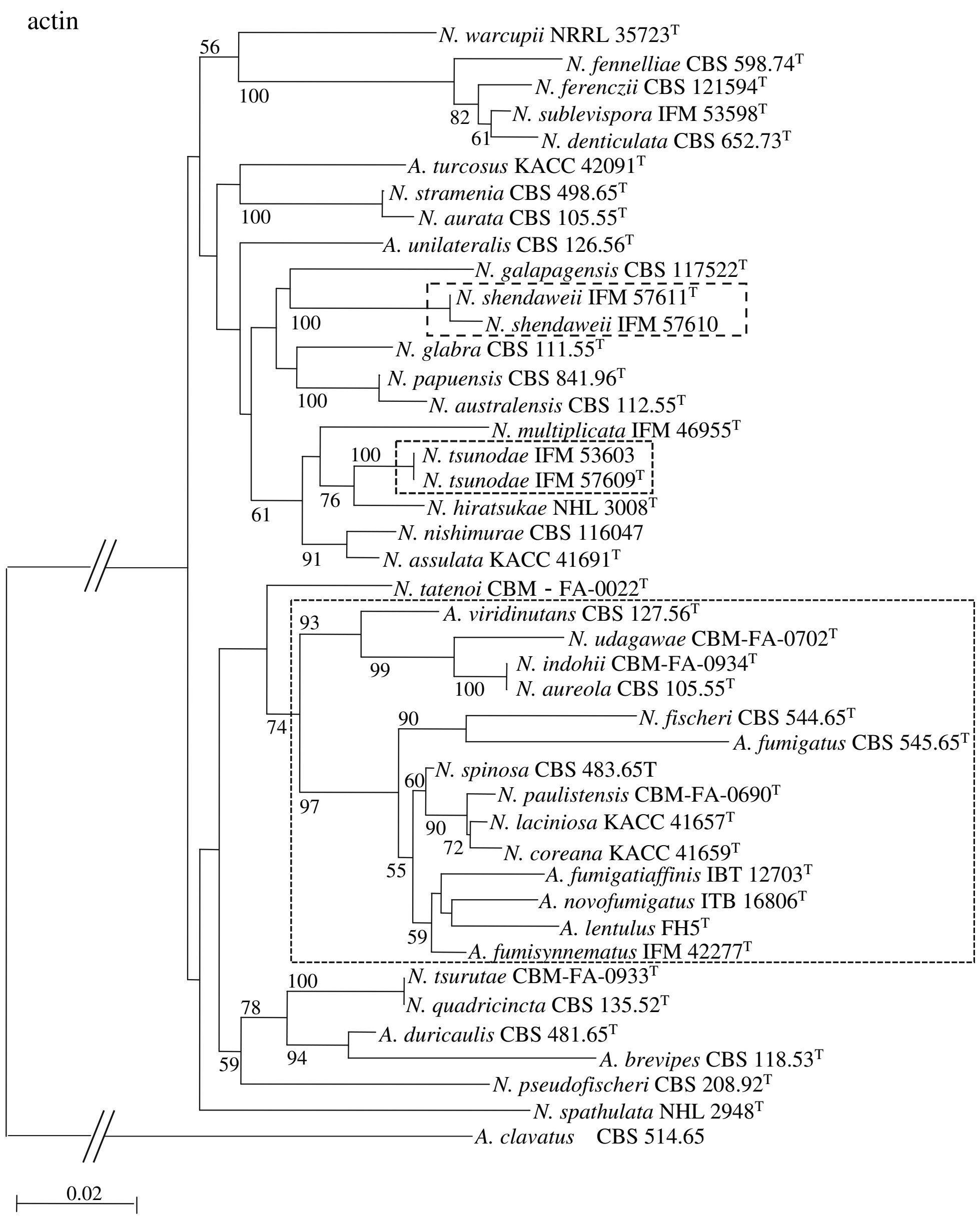


Fig. 13 Neighbor-joining tree from sequences of the actin gene. Each *number* indicates the percentage of bootstrap samplings, derived from 1000 samples, supporting the internal branches of 50% or higher

surfaces, particularly serves to distinguish this species from other recognized species (Someya et al. 1999; "Electronic supplementary material" Fig. S1 C). These morphological characteristics are shared by *N. ferenczii* Varga & Samson (Samson et al. 2007; "Electronic supplementary material" Fig. S1 D). Furthermore, the sequences for the β-tubulin and calmodulin genes from the type specimen CBS 121594 were almost identical to those of *N. sublevispora* ex-type culture (Figs. 11, 12). Thus *N. ferenzii* should be considered to be synonymous with *N. sublevispora*.

Neosartorya sublevispora Someya, Yaguchi & Udagawa, Mycoscience 40: 405. 1999.

= *Neosartorya ferenczii* Varga & Samson, Stud Mycol 59: 178. 2007.

Taxonomy

Neosartorya shendaweii Yaguchi, Abliz & Y. Horie, sp. nov. Figs. 1, 3–6.

MycoBank no.: MB 513151.

Cleistothecia dilute flavo-brunnea vel laete flava, globosa vel subglobosa, 55–135 μm diam., cum hyphis aeriis laxe intricatis circumdata; peridium tenue, membranaceum. Asci octospori, globosi vel late elliptici, 11–13 × 10–12 μm, evanescentes. Ascosporae hyalinae vel dilute flavo-brunneae, late lenticulares, sine cristis 5–6 × 4–5 μm, duabus cristis aequatorialibus 0.5–1 μm latis praeditae, superficies convexae tuberculatae vel verrucosae.

Capitula conidica griseo-viridia, brevi-columnaria, usque 140 × 20–35 μm. Conidiophora ex mycelio basali vel hyphis aeriis orientia, stipites hyalini vel dilute flavo-brunnei, usque 100 µm longi, medio 3.5–5 µm crassi, leves; vesiculae spathulatae, 8–12 µm diam. Aspergilla uniserialia; phialides 4–6 × 1–1.5 µm. Conidia hyalina, ovoidea vel late ellipsoidea, 2.5–3 × 2–2.5 µm, levia.

Etymology: named in memory of the Prof. Shen Dawei, Xinjiang Medical University, eminent dermatologist.

Colonies on CzA growing rather restrictedly, attaining a diamter of 29–33 mm in 14 days at 25°C, at first white, later becoming pale yellow (4A3, after Kornerup and Wanscher 1978) to orange white (5A2), velvety to floccose, consisting of a thin mycelial felt and loose aerial hyphae; cleistothecia and conidiogenesis few in number; reverse pale yellow (4A3) to orange white (5A2). Colonies on MEA spreading broadly, attaining a diameter of 57–65 mm in 14 days at 25°C, white to yellowish white (4A2), consisting of a thin mycelial felt and very abundant cleistothecia; conidiogenesis few in number; reverse light yellow (4A4) to brownish yellow (5C7).

Cleistothecia superficial, pale yellowish brown to light yellow, globose to subglobose, 55–135 µm in diameter, surrounded by a loose covering of hyaline to pale yellow aerial hyphae; peridium pale yellowish brown to light yellow, thin, membranaceous, consisting of angular cells. Asci 8–spored, globose to broadly ellipsoidal, 11–13 × 10–12 µm, evanescent at maturity. Ascospores hyaline to pale yellowish brown, broadly lenticular, spore body 5–6 × 4–5 µm, provided with two widely separated equatorial crests measuring 0.5–1 µm wide, with convex surfaces tuberculate to verrucose (LM and SEM).

Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads grayish green, short columnar, up to 140 × 20–35 µm. Conidiophores arising from the basal mycelium or aerial hyphae, hyaline to pale yellowish brown, up to 100 µm long, 3.5–5 µm wide at the middle, smooth-walled; vesicles hyaline to pale yellowish brown, spathulate, 8–12 µm in diameter. Aspergilla uniseriate; phialides hyaline to pale yellowish brown, covering the upper half of the vesicle, ampulliform, 4–6 × 1–1.5 µm. Conidia hyaline, ovoid to broadly ellipsoidal, 2.5–3 × 2–2.5 µm, smooth.

At 37°C, growth is more rapid than at 25°C.

Specimen examined: CBM-FA-0958 (holotype), a dried culture derived from an isolate from wasteland soil near Moor pagoda, Artux, Kizulsukirgiz autonomous province, Xinjiang Uygur autonomous region, China, 7 Aug. 2000. Ex-type culture: IFM 57611, NBRC 106417.

Additional specimen examined: CBM-FA-0959, from corn field soil at Krakax village, Karakax (Moyu) prefecture, Hotan district, Xinjiang Uygur autonomous region, China, 4 Aug. 2000; IFM 57610.

Neosartorya tsunodae Yaguchi, Abliz & Y. Horie, sp. nov. Figs. 2, 7-10.

MycoBank no.: MB 513152.

Cleistothecia alba, globosa vel subglobosa, usque 240 µm diam., cum hyphis aeriis laxe intricatis circumdata; peridium tenue, membranaceum. Asci octospori, globosi vel subglobosi vel ovoidei, 10–12.5 × 9–11 µm, evanescentes. Ascosporae hyalinae vel dilute flavo–brunneae, late lenticulares, sine cristis 4.5–5.5 × 4–5 µm, duabus cristis aequatorialibus 0.5 µm latis praeditae, superficies convexae subtiliter reticulatae.

Capitula conidica griseo-viridia, radiantia vel laxe columnaria. Conidiophora ex mycelio basali vel hyphis aeriis orientia, stipites hyalini vel dilute flavo–brunnei, 18–32 µm longi, medio 2–3.5 µm crassi, leves; vesiculae spathulatae, 4–6 µm diam. Aspergilla uniserialia; phialides 6–7 × 2–3 µm. Conidia hyalina vel dilute griseo-viridia, late ellipsoidea, 3–4 × 2.5–3 µm, levia.

Etymology: named in memory of the late Prof. Hiroshi Tsunoda, eminent phytopathologist who was best known as an authority on mycotoxigenic fungi of storage cereals and mycotoxin research.

Colonies on CzA spreading broadly, attaining a diameter of 55–65 mm in 14 days at 25°C, white to yellowish white (4A2), floccose, consisting of a thin mycelial felt; cleistothecia and conidiogenesis few in number; reverse uncolored to yellowish white (4A2). Colonies on MEA spreading broadly, attaining a diameter of 75–76 mm in 14 days at 25°C, white to greenish gray (1B2), consisting of a thin mycelial felt; cleistothecia very abundantly produced, granular in appearance; conidiogenesis few in number; reverse yellowish gray (2B2).

Cleistothecia superficial, white, globose to subglobose, up to 240 µm in diameter, surrounded by a loose covering of hyaline aerial hyphae; peridium hyaline to pale yellow, thin, membranaceous, consisting of angular cells. Asci 8-spored, globose to subglobose or ovoid, 10–12.5 × 9–11 µm, evanescent at maturity. Ascospores hyaline to pale yellowish brown, lenticular, spore body 4.5–5.5 × 4–5 µm, provided with two low equatorial crests measuring up to 0.5 µm wide and an evident furrow as a deep depression, with convex surfaces finely reticulate (SEM).

Mycelium composed of hyaline, branched, septate, smooth-walled hyphae. Conidial heads grayish green, radiate to loosely columnar, 50–75 × 15–20 µm. Conidiophores arising from the basal mycelium or aerial hyphae, hyaline to pale yellowish brown, 18–32 µm long, 2–3.5 µm wide at the middle, smooth-walled; vesicles hyaline to pale yellowish brown, spathulate, 4–6 µm in diameter. Aspergilla uniseriate; phialides hyaline, covering the upper half of the vesicle, ampulliform, 6–7 × 2–3 µm. Conidia hyaline to pale grayish green, broadly ellipsoidal, 3–4 × 2.5–3 µm, smooth.

At 37°C, growth is more rapid than at 25°C.

Specimen examined: CBM-FA-0950 (holotype), a dried culture of an isolate from desert soil in Tazhong (central area of Taklimakan desert), Qarqan, Bayingolin-mongol autonomous province, Xinjiang Uygur autonomous region, China, 3 Aug. 2000. Ex-type culture: IFM 57609, NBRC 106416.

Additional specimen examined: CBM-FA-949 isolated from soil in an orchard of an experimental farm in Serra Talhada, Pernambuco State, Brazil, Nov. 1996; IFM 53603.

Acknowledgments We are grateful to Dr. Shun-ichi Udagawa, Tama Laboratory, the Japan Food Research Laboratories, for his kind advice and for reviewing the manuscript. This work was supported in part by the Grant-in-Aid for Scientific Research (B-18405005) from the Japan Society for the Promotion of Science.

References

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

De Hoog GS, Guarro J, Gené J, Figueras MJ (2000) The genus *Neosartorya*. In: Atlas of clinical fungi, 2nd edn. Centraalbureau voor Schimmelcultures, Utrecht, pp 291–296

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Geiser DM, Frisvad JC, Taylor JW (1998) Evolutionary relationships in *Aspergillus* section *Fumigati* inferred from partial β-tubulin and hydrophobin DNA sequences. Mycologia 90:831–845

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330

Guarro J, Kallas EG, Godoy P, Karenina A, Gene J, Stchigel A, Colombo AL (2002) Cerebral aspergillosis caused by *Neosartorya hiratsukae*, Brazil. Emerg Infec Dis 8:989–991

Hong S-B, Go S-J, Shin H-D, Frisvad JC, Samson RA (2005) Polyphasic taxonomy of *Aspergillus fumigatus* and related species. Mycologia 97:1316–1329

Hong S-B, Cho H-S, Shin H-D, Frisvad JC, Samson RA (2006) Novel *Neosartorya* species isolated from soil in Korea. Int J Syst Evol Microbiol 56:477–486

Hong S-B, Shin H-D, Hong J-B, Frisvad JC, Nielsen PV, Samson RA (2008) New taxa of *Neosartorya* and *Aspergillus* in *Aspergillus* section *Fumigati*. Antonie van Leeuwenhook 93:87–98

Horie Y, Miyaji M, Yokoyama K, Udagawa S, Campos-Takagi GM (1992) *Neosartorya tatenoi*, a new species from Brazilian soil. Trans Mycol Soc Jpn 33:395–399

Horie Y, Miyaji M, Nishimura K, Franco MF, Coelho KIR (1995a) Two new species of *Neosartorya* from Brazilian soil. Mycoscience 36:159–165

Horie Y, Miyaji M, Nishimura K, Franco MF, Coelho KIR (1995b) New and interesting species of *Neosartorya* from Brazilian soil. Mycoscience 36:199–204

Horie Y, Abliz P, Fukushima K, Okada K, Gusmao NB (2001) *Neosartorya takakii*, a new species from soil in Brazil. Mycoscience 42:91–95

Horie Y, Abliz P, Fukushima K, Okada K, Campos-Takaki GM (2003) Two new species of *Neosartorya* from Amazonian soil, Brazil. Mycoscience 44:397–402

Järv H, Lehtmaa J, Summerbell RC, Hoekstra ES, Samson RA, Naaber P (2004) Isolation of *Neosartorya pseudofischeri* from blood: first hint of pulmonary aspergillosis. J Clin Microbiol 42:925–928

Kimura M (1980) A simple method for estimation evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Klich MA (2002) Identification of common *Aspergillus* species. Centraalbureau voor Schimmelcutures, Utrecht

Kornerup A, Wanscher JH (1978) Methuen handbook of colour, 3rd edn. Eyre Methuen, London

Kozakiewicz Z (1989) *Aspergillus* species on stored products. Mycol Pap 161:1–188

Malloch D, Cain RF (1972) The Trichocomataceae: Ascomycetes with *Aspergillus*, *Paecilomyces* and *Penicillium* imperfect states. Can J Bot 50:2613–2628

O'Gorman CM, Fuller HT, Dyer PS (2009) Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature 457:471–474

Peterson SW (1992) *Neosartorya pseudofischeri* sp. nov. and its relationship to other species in *Aspergillus* section *Fumigati*. Mycol Res 96:547–554

Peterson SW (2008) Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia 100:205–226

Raper KB, Fennell DI (1965) The *Aspergillus fumigatus* group. In: The genus *Aspergillus*. Williams & Wilkins, Baltimore, pp 238–268

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Samson RA, Hong S-B, Peterson SW, Frisvard JC, Varga J (2007) Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*. Stud Mycol 59:147–203

Someya A, Yaguchi T, Udagawa S (1999) *Neosartorya sublevispora*, a new species of soil-borne Eurotiales. Mycoscience 40:405–409

Takada M, Udagawa S (1985) A new species of heterothallic *Neosartorya*. Mycotaxon 24:395–402

Takada M, Udagawa S, Norizuki K (1986) Isolation of *Neosartorya fennelliae* and interspecific parings between *N. fennelliae*, *N. spathulata*, and *Aspergillus fumigatus*. Trans Mycol Soc Jpn 27:415–423

Takada M, Horie Y, Abliz P (2001) Two new heterothallic *Neosartorya* from African soil. Mycoscience 42:361–367

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Udagawa S, Yaguchi T (2005) *Neosartorya* (Eurotiales): taxonomy and significance in applied mycology. In: Deshmukh SK, Rai MK (eds) Biodiversity of fungi: their role in human life. Oxford & IBH, New Delhi

Udagawa S, Tsubouchi H, Horie Y (1991) *Neosartorya hiratsukae*, a new species of food-borne Ascomycetes. Trans Mycol Soc Jpn 32:23–29

Udagawa S, Toyazaki N, Tsubouchi H (1993) *Neosartorya primulina*, a new species of food-borne Ascomycetes. Mycotaxon 47:359–366

Udagawa S, Tsubouchi H, Toyazaki N (1996) Isolation and identification of *Neosartorya* species from house dust as hazardous indoor pollutants. Mycoscience 37:217–222

Varga J, Vida Z, Toth B, Debets F, Horie Y (2000) Phylogenetic analysis of newly described *Neosartorya* species. Antonie van Leeuwenhoek 77:235–239

Yaguchi T, Someya A, Udagawa S (1994) A new species of *Neosartorya* from Taiwan soil. Mycoscience 35:309–313