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**Original Article**

# Molecular Phylogenetics of Multiple Genes on *Aspergillus* Section *Fumigati* Isolated from Clinical Specimens in Japan

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**Abstract**

A phylogenetic study based on sequence analysis of the β-tubulin, hydrophobin and calmodulin genes was performed in 19 strains of *Aspergillus fumigatus* and related species isolated from clinical specimens in Japan. Correlations between detailed morphology and phylogeny were examined. Species in the section *Fumigati* were divided into five clades: clade I, typical strains of *A. fumigatus*; clade II, species including *A. lentulus* and *A. fumisynnematus*; clade III, species including *A. fumigatiaffinis* and *A. novofumigatus*, clade IV, atypical strains of *A. fumigatus* including *A. viridinutans*; and clade V, species including *A. brevipes*, *A. duricaulis* and *A. unilateralis*. Most of the examined strains from clinical specimens in Japan clustered together in clade I and exhibit globose conidia with lobate-reticulate ornamentation. Other strains from clinical specimens were divided into two clades (clades II and IV). The strains in clades II and the six strains in clade IV exhibit conidia with microtuberculate ornamentation, while *A. viridinutans*-complex in clades IV and the strains in clade V have conidia with lobate-reticulate ornamentation. The six strains are clearly distinguished from *A. viridinutans*-complex and are considered to be related to *Neosartorya udagawae*. The maximal growth temperatures of clades I, II, IV and V were above 50°C, 45°C, 42°C and 42°C, respectively. These data are useful for classification of species within the *Aspergillus* section *Fumigati*.

**Key words**: *Aspergillus* Section *Fumigati*, clinical isolates in Japan, molecular phylogenetics

## Introduction

Aspergillosis is a clinically important mycosis that comprises a wide variety of bronchopulmonary infections, such as invasive pulmonary aspergillosis, fungus ball in the lung cavity and allergic bronchopulmonary aspergillosis. The disease also manifests as corneal ulcer, nasal sinusitis with/without fungus balls and infections in the other organs and tissues. Among opportunistic fungal infections, invasive pulmonary aspergillosis is the most serious due to its high frequency and mortality rates. The causative agents are *Aspergillus fumigatus*, *A. flavus*, *A. niger* and other species in this genus. The most significant causative agent is *A. fumigatus* and it has been reported that cases of invasive infections are caused by species of a related teleomorphic genus, *Neosartorya*[1-3].

However, clinical isolates of the species are not necessarily morphologically uniform, and mistaken identifications of them by morphological characteristics have often happened. In order to develop diagnostic techniques, including DNA identification and assessment of sensitivity to antifungal agents, it is essential to clarify intra- and interspecies diversity in *A. fumigatus* and closely related species.

Several biochemical and molecular techniques have recently been applied to *A. fumigatus* and related species. Profiles of secondary metabolites produced by *A. fumigatus* and described varieties

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Table 1. List of taxa sequenced in this study and additional taxa included in the analysis

| Group | Taxon | Strain number | Origin (substrate) |
|---|---|---|---|
| I | *A. fumigatus* | IAM 13869[NT] | USA (chicken) |
| | *A. fumigatus* | IFM 47447 | Japan (human) |
| | *A. fumigatus* | IFM 51745 | Japan (human) |
| | *A. fumigatus* | IFM 51925 | Japan (horse) |
| | *A. fumigatus* | IFM 51941 | Japan (human) |
| | *A. fumigatus* | IFM 51942 | Japan (human) |
| | *A. fumigatus* | IFM 51977 | Japan (human) |
| | *A. fumigatus* | IFM 51978 | Japan (human) |
| | *A. fumigatus* | IFM 53869 | Japan (human) |
| | *A. fumigatus* | IFM 53870 | Japan (human) |
| | *A. fumigatus* | IFM 53872 | Japan (human) |
| | *A. fumigatus* | IFM 54304 | Japan (human) |
| | *A. fumigatus* | CBS 112389 | Germany (indoor) |
| | *A. fumigatus* | CBS 110.46 | Unknown |
| | *A. fumigatus* | CBS 148.89 | France (corn oil) |
| | *A. fumigatus* | CBS 386.75 | India (soil) |
| | *A. fumigatus* var. *ellipticus* | CBS 487.65[T] | USA (human) |
| | *A. arvii* | CBM FD-0144[T] | Finland (cow) |
| II | *A. lentulus* | FH5[T] | USA (human) |
| | *A. lentulus* | FH7 | USA (human) |
| | *A. lentulus* | IFM 41090 | Venezuela (soil) |
| | *A. lentulus* | IFM 47063 | Japan (human) |
| | *A. lentulus* | IFM 47457 | Japan (human) |
| | *A. lentulus* | CBS 153.89 | USA (soil) |
| | *A. lentulus* | CBS 175.97 | Netherlands (dolphin) |
| | *A. lentulus* | CBS 116883 | Korea (soil) |
| | *A. lentulus* | KACC 41642 | Korea (soil) |
| | *A. lentulus* | MK245 | Australia (human) |
| | *A. fumisynnematus* | IFM 42277[T] | Venezuela (soil) |
| | *A. fumisynnematus* | 90-BP-70 | Brazil (soil) |
| | *A. fumisynnematus* | 90-BP-177 | Brazil (soil) |
| | *Aspergillus* sp. | JV3 | Unknown (cocoa beans) |
| III | *A. fumigatiaffinis* | CBS 117194[T] | USA (fungus) |
| | *A. novofumigatus* | CBS 117520[T] | Ecuador (soil) |
| IV | *Aspergillus* sp. | IFM 5058 | Japan (human) |
| | *Aspergillus* sp. | IFM 51744 | Japan (human) |
| | *Aspergillus* sp. | IFM 53867 | Japan (human) |
| | *Aspergillus* sp. | IFM 53868 | Japan (human) |
| | *Aspergillus* sp. | IFM 54302 | Japan (human) |
| | *Aspergillus* sp. | CBM FD-0143 | Japan (food) |
| | *Aspergillus* sp. | MK285 | Australia (human) |
| | *A. viridinutans* | IMI 062875 | Australia (dung) |
| | *A. viridinutans* | IMI 133982 | Russia (soil) |
| | *A. viridinutans* | IMI 182127 | Sri Lanka (plant) |
| | *A. viridinutans* | IMI 280490 | Zambia (soil) |
| | *A. viridinutans* | NRRL 6106 | Unknown |
| | *A. viridinutans* | CBS 127.56[T] | Australia (dung) |
| | *A. viridinutans* | IFM 54303 | Japan (human) |
| | *A. viridinutans* | FRR 1266 | Australia (soil) |
| | *Aspergillus* sp. | MK246 | Australia (human) |
| | *Aspergillus* sp. | MK284 | Australia (human) |
| V | *A. brevipes* | NRRL 2439[T] | Australia (soil) |
| | *A. duricaulis* | NRRL 4021[T] | Argentina (soil) |
| | *A. unilateralis* | NRRL 577 | Unknown |
| | *A. unilateralis* | CBS 126.56[T] | Australia (soil) |
| Others | *Aspergillus* sp. | NWS3 | Australia (human) |
| | *N. aureola* | NRRL 2244[T] | Ghana (soil) |
| | *N. fennelliae* (type A) | NRRL 5534[T] | USA (rabbit) |
| | *N. fennelliae* (type a) | NRRL 5535[T] | USA (rabbit) |
| | *N. fischeri* | NRRL 181[T] | Unknown |
| | *N. glabra* | NRRL 2163[T] | USA (rubber) |
| | *N. hiratsukae* | NRRL 20819 | Unknown |
| | *N. hiratsukae* | IFM 47035[T] | Japan (aloe juice) |
| | *N. pseudofischeri* | NRRL 20748[T] | USA (human) |
| | *N. spinosa* | NRRL 5034[T] | Nicaragua (soil) |
| | *N. udagawae* (type A) | CBM FA-0702[T] | Brazil (soil) |
| | *N. udagawae* (type a) | CBM FA-0703[T] | Brazil (soil) |
| Out group | *A. clavatus* | H522 | |
| | *A. oryzae* | RIB40 | |

T: type strain, NT: neotype strain

are homogeneous[4]. Isozyme electrophoretic patterns have been examined in the *A. fumigatus* complex by several groups[5-8]. The phylogenetic relationships among *A. fumigatus* and related species have also been analyzed by sequencing parts of the β-tubulin[9, 10] and cytochrome *b* genes[11].

In 2005, species of poorly sporulating *A. fumigatus* found among clinical isolates in Australia were determined to be atypical *A. fumigatus* based on DNA sequence analysis of 18S rDNA, and the alkaline protease and β-tubulin genes. Based on morphology, however, it was concluded that all isolates could be classified as *A. viridinutans*[12].

*Aspergillus lentulus* isolated from clinical specimens in USA was described as a new species[13]. It is not able to survive at 48℃, and is potentially drug resistant[14]. The clade comprising this species was distinct from the *A. fumigatus*-complex, which includes the varieties of *A. fumigatus*.

Variability within *A. fumigatus* and related species in Korea was recently examined using morphology, growth temperature regimens, extrolite patterns and DNA analyses of the partial β-tubulin, calmodulin and actin genes, and two new species, *A. fumigatiaffinis* and *A. novofumigatus*, were proposed[15].

We re-evaluated the identification of the *A. fumigatus* strains preserved at the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University (IFM), as causative agents of mycosis in human and animals. Because we identified morphologically atypical species of *A. fumigatus*, we then determined the phylogenetic relationships among *A. fumigatus* and related species, including *Neosartorya* species, by analyzing the DNA sequences of the partial β-tubulin, hydrophobin and calmodulin genes. Furthermore, correlations among detailed morphology, maximal growth temperatures, minimal inhibitory concentrations (MICs) of antifungal agents and phylogeny were analyzed.

## Materials and Methods

### Fungal isolates

Isolates were preserved at IFM and the Natural History Museum and Institute, Chiba, Japan (CBM), or were purchased from the Centraalbureau voor Schimmelcultures (CBS). Isolates are listed in Table 1.

### Incubation and observation

Cultures were grown in incubators at 25℃ or 37℃. Fungal structure of isolates grown for 14 days on Czapek (CzA) or malt extract (MEA) agar was examined under a light or scanning electron microscope (SEM: Hitachi S-800, Tokyo, Japan). Colony colors are as designated in the Methuen Handbook of Colour[16].

### Growth studies

The maximal growth temperatures of isolates were determined by the method of Balajee *et al.*[13]; 10 μl of conidial suspension ($10^5$ conidia/ml of sterile distilled water) was placed in the center of a CzA plate, which was incubated at 37, 42, 45, 48 or 50℃ for 3 days. The presence or absence of growth at the end of the incubation period was then recorded.

### Mating test

On oatmeal agar (1.5% oatmeal, 1.5% wheat germ and 2% agar), mating tests were conducted with mating types "A" and "a" of *N. udagawae* and isolates in clade IV (Table 1). Plates were incubated at 25℃ for 90 days, after which the presence or absence of ascomata formation was recorded. Mating between the "A" and "a" types of *N. udagawae* produced ascomata.

### Sequencing

The β-tubulin, hydrophobin and calmodulin genes were sequenced directly from PCR products using primer pair Bt2a (5'-AATAGGTGCCGC-TTTCTGG-3') and Bt2b (5'-AGTTGTCGGGAC-GGAAGAG-3')[17], primer pair rodA1 (5'-GCT-GGCAATGGTGTTGGCAA-3') and rodA2 (5'-A-GGGCAATGCAAGGAAGACC-3')[9], and primer pair cmd5 (5'-CCGAGTACAAGGAGGCCTTC-3') and cmd6 (5'-CCGATAGAGGTCATAACGTGG-3')[15], respectively. PCR products were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, Calif., USA) on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions.

### Molecular phylogenetic analysis

DNA sequences were edited using ATGC Ver. 4 sequence assembly software (Genetyx Co., Tokyo, Japan), and alignment of the sequences was performed using Clustal X software[18]. Maximum parsimony (MP) analysis[19] was performed by heuristic search with random addition sequences, branch swapping by tree bisection-reconnection (TBR) and MAXTREES set at 20000, using PAUP* 4b10[20]. Relative robustness of the individual branches was estimated by bootstrapping[21], with 1000

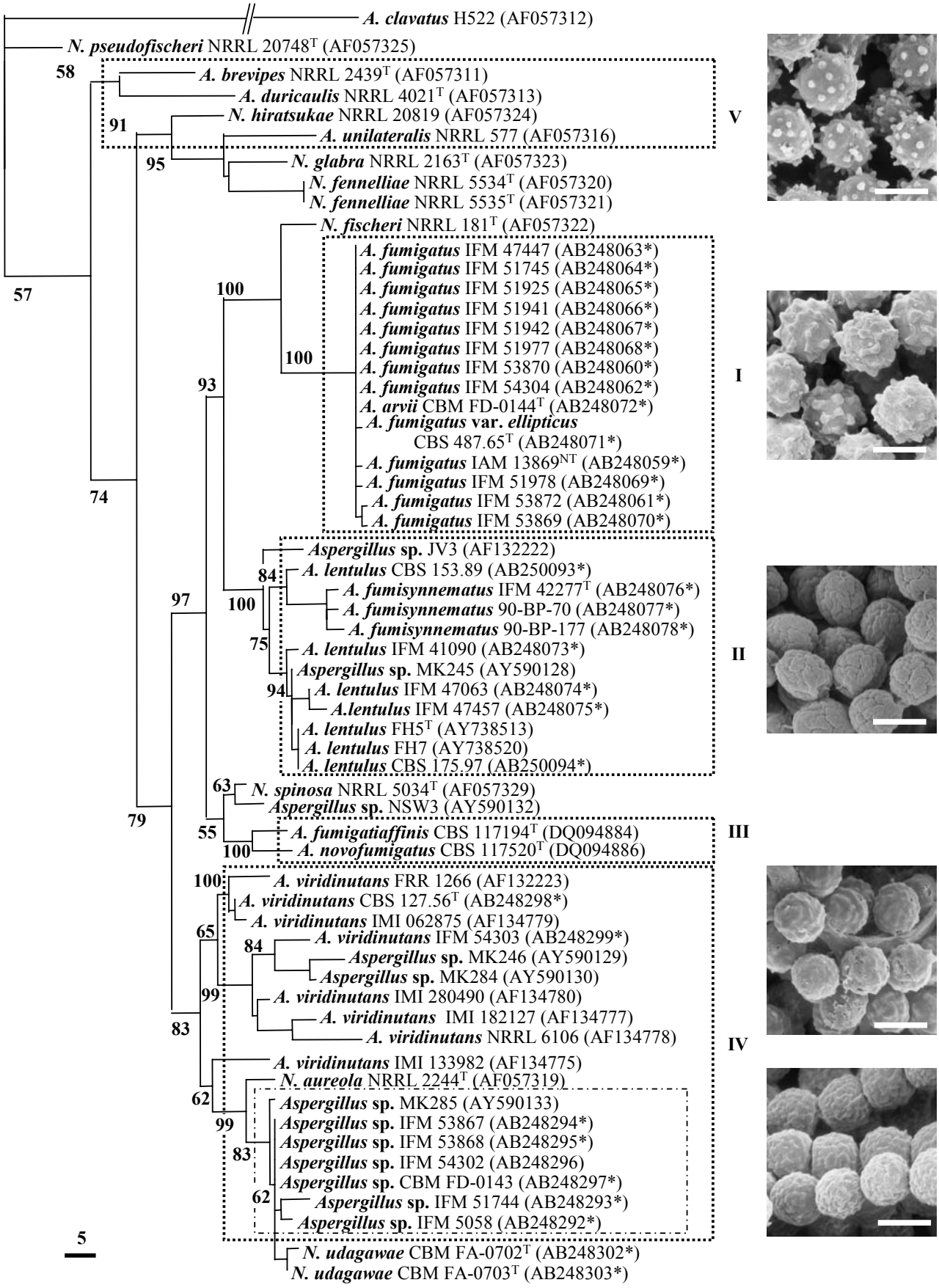


Fig. 1. One of 95 equally parsimonious trees obtained from analysis of the β-tubulin gene using PAUP. Trees were 459 steps in length with a CI of 0.721 and an RI of 0.879. Numbers above or below the nodes represent bootstrap values of >50% (out of 1000 bootstrap replications). *A. Aspergillus*; *N. Neosartorya*; *, this study. SEM photographs; conidia (scale bars=3 μm).

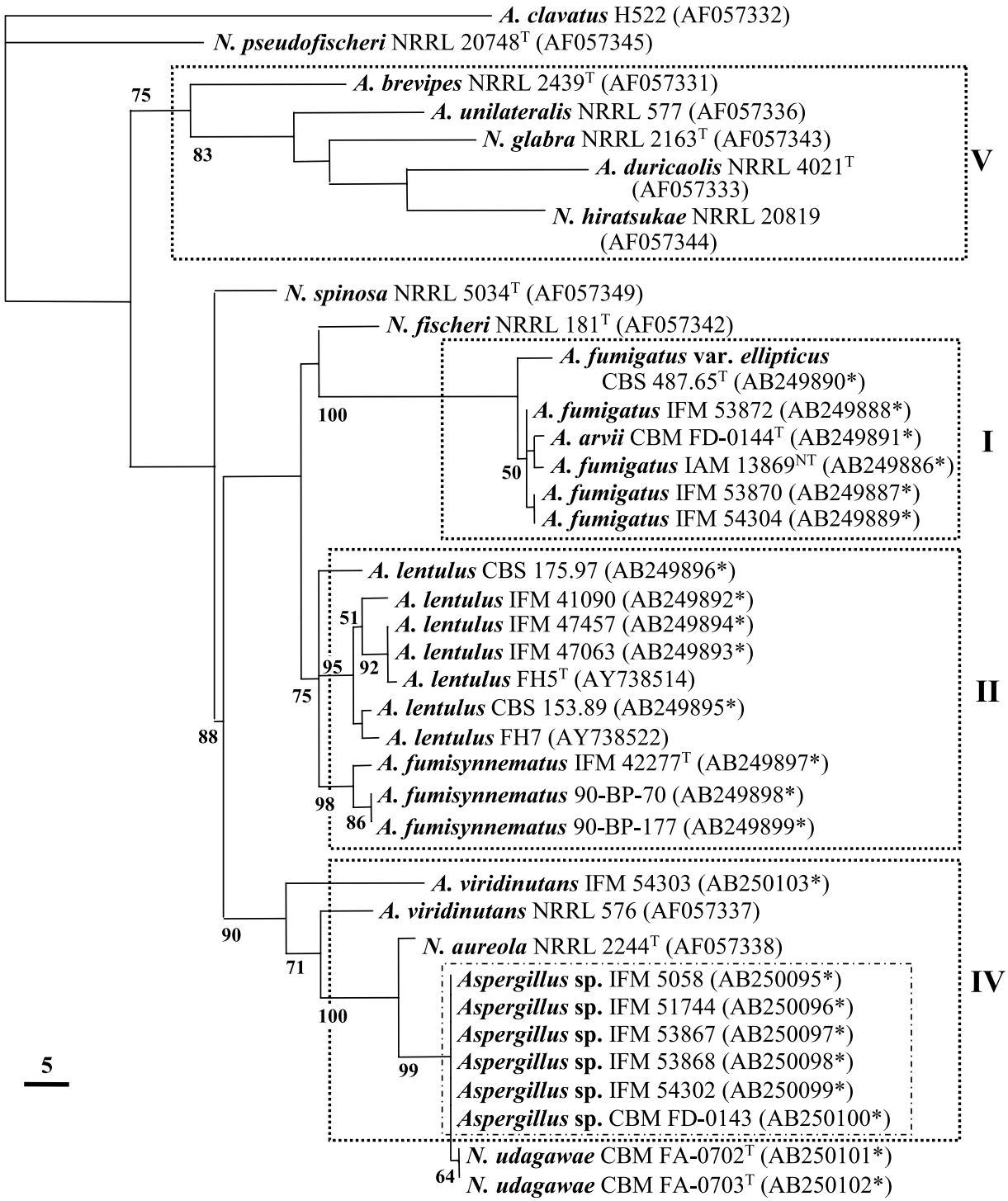


Fig. 2. One of six equally parsimonious trees obtained from analysis of the hydrophobin gene using PAUP. Trees were 368 steps in length with a CI of 0.690 and an RI of 0.819. Numbers above or below the nodes represent bootstrap values of >50% (out of 1000 bootstrap replications). *A. Aspergillus*; *N. Neosartorya*; *, this study.

replicates, using heuristic search and branch swapping by TBR and MAXTREES set at 100. For neighbor-joining (NJ) analysis[22], the distances between sequences were calculated using Kimura's two-parameter model[23].

**MICs of antifungal agents**

MICs of antifungal agents against the isolated fungi were measured by the microdilution method, which was proposed for filamentous fungi by the National Committee for Clinical Laboratory Standards (NCCLS M38-A)[24]. Microtiter plates (Dry Plate; Eiken Chemicals, Tokyo, Japan) containing lyophilized antifungals that were serially diluted two-fold were used according to the manufacturer's instructions, with slight

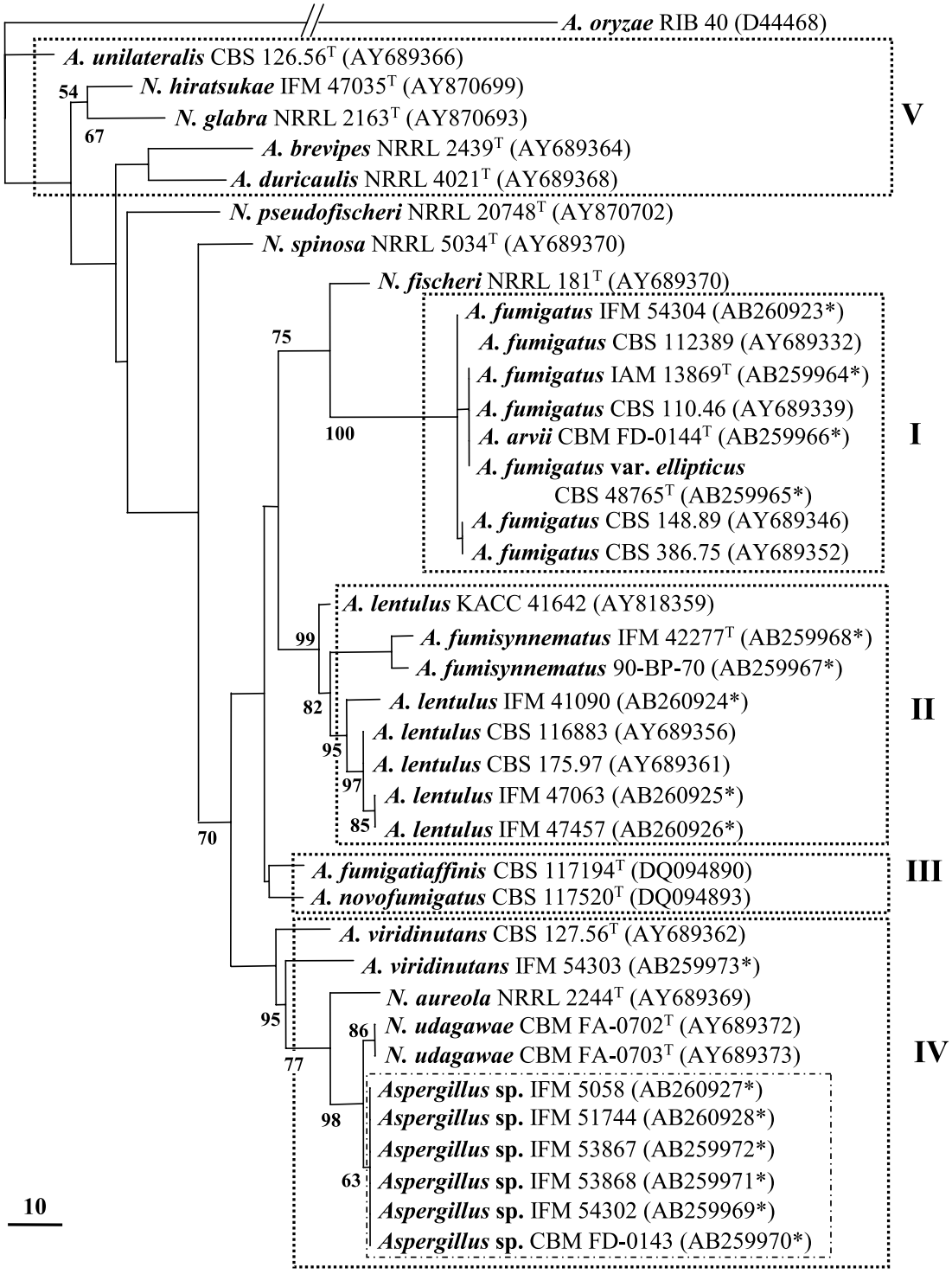


Fig. 3. One of 95 equally parsimonious trees obtained from analysis of the calmodulin gene using PAUP. Trees were 465 steps in length with a CI of 0.733 and an RI of 0.845. Numbers above or below the nodes represent bootstrap values of >50% (out of 1000 bootstrap replications). *A. Aspergillus*; *N. Neosartorya*; *, this study.

modification. The inoculum suspension was adjusted to $10^4$ CFU/m*l* in RPMI 1640 broth, added to the microtiter wells at 0.1 m*l*, and then incubated at 35℃ for 48 hours. The growth control included the same inoculum in the absence of antifungals, while the negative control was sterile RPMI 1640 broth. Visual examination of growth inhibition was performed at 48 hours, as further incubation at 72 hours revealed the same reading.

## Results

### Phylogenetic analysis

DNA sequences of the partial β-tubulin,

hydrophobin and calmodulin genes in the strains listed in Table 1 were determined. New sequences were deposited in the DNA Data Bank of Japan (DDBJ) and the accession numbers are listed in Figs. 1-3.

MP analysis of the β-tubulin gene sequences (Fig. 1) yielded 95 equally parsimonious trees based on 137 parsimony informative characters, 459 steps in length with a consistency index (CI) of 0.721 and a retention index (RI) of 0.879. That of the hydrophobin gene sequences (Fig. 2) yielded six equally parsimonious trees based on 110 parsimony informative characters, 368 steps in length with a CI of 0.690 and an RI of 0.819, and that of the calmodulin gene sequences (Fig. 3) yielded three parsimonious trees based on 145 parsimony informative characters, 465 steps in length with a CI of 0.733 and an RI of 0.845. No differences were seen between tree topologies from MP and NJ analyses (NJ trees not shown) of the β-tubulin, hydrophobin and calmodulin genes.

The three trees based on the three loci were found to be similar. The species of the section *Fumigati* were divided into five clades: clade I, typical strains of *A. fumigatus*; clade II, species including *A. lentulus* and *A. fumisynnematus*; clade III, species including *A. fumigatiaffinis* and *A. novofumigatus*; IV, atypical strains of *A. fumigatus* including *A. viridinutans*; and clade V, species including *A. brevipes*, *A. duricaulis* and *A. unilateralis*. Most of the examined strains, including ex-neotype strain IAM 13869, clustered together in the same clade (clade I), while *A. fumigatus* var. *ellipticus* and *A. arvii* were also placed here. Other strains from clinical specimens in Japan were divided into two clades (clades II and IV). Clade II formed a sister group with clade I and included *A. lentulus*, *A. fumisynnematus* and three strains, IFM 47063, 47475 and 41090. Clade III was related to clades I and II and no strains from Japanese clinical specimens belonged to this clade. Clade IV was separated from clades I, II and III. The six variant isolates, IFM 5058, 51744, 53867, 53868, 54302 and CBM FD-0143, belonged to this clade and were closely related to two species of *Neosartorya*, *N. udagawae* and *N. aureola*. This clade also included all strains of *A. viridinutans* and strain IFM 54303 from a clinical specimen in Japan. *A. brevipes*, *A. duricaulis* and *A. unilateralis* clustered together in clade V, which included no strains from clinical specimens.

**Morphology**

Conidium ornamentation on SEM in strains belonging to clade I was found to vary from almost smooth to echinulate; however, most strains were echinulate. This ornamentation was classified into the lobate-reticulate category[25]. *A. fumigatus* var. *ellipticus* exhibited ellipsoidal conidia with almost smooth ornamentation, while that of *A. arvii* was lobatereticulate.

Strains IFM 47063, 47475 and 41090, which exhibited almost the same alignment as *A. lentulus* FH 5, were found to have subglobose conidia with microtuberculate ornamentation[25]. Strains IFM 42277, 90-BP-70 and 177, which were in clade II and were identified as *A. fumisynnematus*, had ellipsoidal conidia with microtuberculate ornamentation and their colonies were floccose and grayish green.

The six strains in clade IV (IFM 5058, 51744,

Table 2. Maximum growth temperatures and MICs on species of *Aspergillus* section *Fumigati*

| Group | Species | Strain | Maximum growth temperature (°C) | MIC (mg/ml)* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | AMPH | 5-FC | FCZ | ITZ | MCZ | MCFG |
| I | *A. fumigatus* | IAM 13869 | >50 | 1 | >64 | >64 | 0.5 | 2 | >16 |
| | *A. fumigatus* | CBS 110.46 | >50 | 0.5 | >64 | >64 | 0.5 | 4 | >16 |
| | *A. fumigatus* | IFM 59125 | >50 | 1 | >64 | >64 | 0.5 | 4 | >16 |
| | *A. arvii* | CBM FD-0144 | >50 | 1 | >64 | >64 | 0.5 | 2 | >16 |
| | *A. fumigatus* var. *ellipticus* | CBS 478.65 | >50 | 1 | 32 | >64 | 0.5 | 2 | >16 |
| II | *A. lentulus* | IFM 47457 | 45 | 2 | >64 | >64 | 0.5 | 4 | >16 |
| | *A. lentulus* | IFM 47063 | 45 | 2 | >64 | >64 | 1 | 32 | >16 |
| | *A. lentulus* | CBS 175.97 | 45 | 2 | >64 | >64 | 1 | 4 | >16 |
| | *A. fumisynnematus* | IFM 42277 | 45 | 1 | >64 | >64 | 1 | 2 | >16 |
| IV | *Aspergillus* sp. | IFM 51744 | 42 | 1 | 64 | >64 | 0.5 | 2 | >16 |
| | *Aspergillus* sp. | IFM 53868 | 42 | 1 | 64 | >64 | 1 | 32 | >16 |
| | *Aspergillus* sp. | IFM 54302 | 42 | 1 | >64 | >64 | 1 | 2 | >16 |
| V | *A. viridinutans* | CBS127.56 | 42 | 1 | 64 | >64 | 0.5 | 1 | >16 |
| | *A. viridinutans* | IFM 54303 | 42 | 1 | >64 | >64 | 2 | >32 | >16 |

*MICs shown were determined by the NCCLS methods. AMPH=amphotericin B, 5-FC=flucytosine, FCZ=fluconazole, ITZ=itraconazole, MCZ=miconazole, MCFG=micafungin.

53867, 53868, 54302 and CBM FD-0143) had globose conidia with microtuberculate ornamentation, while the ex-type strain of *A. viridinutans* CBS 127.56 had finely echinulate conidia classified as lobate-reticulate. Therefore, these strains were distinguished from *A. viridinutans* by conidia ornamentation. Furthermore, most strains of *A. viridinutans* possessed nodding vesicles.

The strains in clade VI had globose conidia with lobate-reticulate ornamentation, which were identical to those of typical *A. fumigatus*.

**Maximal growth temperature**

Strains belonging to clade I, including *A. fumigatus* var. *ellipticus* and *A. arvii*, grew at more than at 50℃. Strains belonging to clade II grew poorly at 45℃ and did not grow at 48℃, while those belonging to clades IV and V grew well at 42℃ but did not grow at 45℃ (Table 2).

**Mating test**

Mating between the six strains in clade IV (IFM 5058, 51744, 53867, 53868, 54302 and CBM FD-0143) and mating types "A" and "a" of *N. udagawae* did not occur.

**MIC of antifungal agents**

MICs of antifungal agents against the isolates are listed in Table 2. The MIC of amphotericin B (AMPH) in *A. lentulus* was high when compared with other species.

## Discussion

Our results concurred with those of Katz *et al.*[12] and Hong *et al.*[15] regarding the outline of phylogenetic trees based on DNA sequences of the β-tubulin gene. Namely, typical strains of *A. fumigatus* clustered in clade I (2a by Katz *et al.*, *A. fumigatus* s. str. by Hong *et al.*) and all strains of *A. viridinutans* clustered in clade IV (1a and 1b by Katz *et al.*, *A. viridinutans*-complex by Hong *et al.*). Varga *et al.*[26] showed that all strains of *A. viridinutans* belonged to a cluster based on the β-tubulin gene. However, we found six unknown strains in clade IV. The sequences of the hydrophobin and calmodulin genes gave trees with identical topology as that based on the β-tubulin gene. These strains are very closely related to *N. udagawae*, a heterothallic species, isolated from soil in Brazil[27]. However, mating between the strains and *N. udagawae* did not occur. It is often difficult to do successful mating experiments on clinical isolates and fungi that have been routinely sub-cultured. Therefore these strains need to be further investigated before they are identified as the anamorphic state of *N. udagawae*. The strains have conidia with microtuberculate ornamentation, while *A. viridinutans* has conidia with lobate-reticulate ornamentation. The maximal growth temperature of this species is 42℃, which differs from those of *A. fumigatus* and *A. lentulus*. This phenotype is important and helpful to rapidly classify the species in the section *Fumigati*.

Strain CBM FD-0143 belonging to clade IV was isolated from food in Japan and was found to produce the neurotropic mycotoxins fumitremorgin A and B[28]. Strain IFM 5058[29] was isolated from a corneal ulcer and showed invasiveness in a mouse brain infection model, and it was also found to belong to this clade. *Aspergillus* sp. MK 285[12] isolated from the respiratory tract of a cat in Australia was included in this clade based on β-tubulin gene sequence, and is considered to be closely related to those strains. Additional study is necessary to confirm the identity of the Australian isolate.

Strains IFM 47063 and 47457 from clinical specimens in Japan were identified as *A. lentulus*, which was proposed as a new species demonstrating low *in vitro* susceptibility to antifungal drugs, including amphotericin B and itaconazole[13, 14]. Our MP tree based on the β-tubulin gene had the same topology of the ML tree by Balajee *et al.*[13] among *A. fumigatus*, *A. lentulus* and *N. fischeri*. These strains had smaller conidial heads than *A. fumigatus*, did not survive at 48℃ and had the same susceptibilities as *A. lentulus* (Table 2). Therefore, they are considered to belong to *A. lentulus* based on phylogeny, and morphological and physiological characteristics. According to de Hoog *et al.*[30], the MICs of AMPH for *A. fumigatus* ranged from 0.125 μm/m*l* to 2 μm/m*l*. It was confirmed that Japanese isolates of *A. lentulus* had lower susceptibility to AMPH than typical isolates of *A. fumigatus*. Strain IFM 41090 from soil in Venezuela was also identified as *A. lentulus* and this species was reported to be isolated from clinical specimens in USA and Australia, soils in Korea and a dolphin in the Netherlands[13, 15]. Therefore, *A. lentulus* is widely distributed in the world and the number of mycoses caused by this species is expected to increase from now.

*Aspergillus fumigatus* var. *ellipticus* is known to be a human pathogen and is distinguished from *A. fumigatus* by light yellow/green, short conidial heads and ellipsoidal, smooth or nearly so conidia, while *A. fumigatus* has dark green,

long conidial heads and globose echinulate conidia[31]. In 1989, Kozakiewicz[25] promoted this variety to an identical species, *A. neoellipticus*, which resulted in much debate regarding species independence. Our results showed that for three different genes, the ex-type strain of *A. fumigatus* var. *ellipticus* (CBS 487.65) could not be distinguished from the strains identified as *A. fumigatus*, including the ex-neotype strain IAM 13869. Similar data were seen for β-tubulin and hydrophobin gene analysis[9, 13] and mitochondrial cytochrome *b* gene analysis[11]. However, there has been no discussion regarding molecular phylogenetic analysis and morphology examined under SEM. The ornamentation of conidia on *A. fumigatus* var. *ellipticus* is almost smooth, while that of *A. fumigatus* is lobate-reticulate. Therefore, this taxon should remain as a variety of *A. fumigatus*, and *A. fumigatus* var. *ellipticus* is a suitable name.

In 1994, *A. arvii* was isolated from liver lesions in a dairy cow in Finland and was reported as a new species classified in section *Fumigati* based on its buff color on all standard media[32]. This characteristic is not found in the original descriptions of *A. fumigatus*. However, this taxon has the same alignment with typical species of *A. fumigatus* in three different genes. Moreover, the shape and ornamentation of the conidia are the same as those of *A. fumigatus*. Therefore, it was concluded that this taxon is a variety of *A. fumigatus*.

*Aspergillus fumigatiaffinis* and *A. novofumigatus* were recently proposed as two new species[15] clustered together with *N. spinosa* on the β-tubulin gene. We have not found any strains related to the two species. These taxa were isolated from soils in Korea and Ecuador. Therefore, additional isolates identified as these species will be identified with further research.

*Aspergillus brevipes*, *A. duricaulis* and *A. unilateralis* were very distant from other members of the section *Fumigati*. All three species are known from their type strains or very few isolates[25], and so are not considered clinically important.

In conclusion, we found that species of *Aspergillus* section *Fumigati* were divided into five clades, and there were correlations regarding phylogeny, morphology and physiological characteristics. The present polyphasic analysis demonstrates its ability to classify pathogenic fungi and its great potential as a tool in developing diagnostic techniques and in clarifying pathogenesis and epidemiology.

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