available online at www.studiesinmycology.org
doi:10.3114/sim.2007.59.11

STUDIES IN MYCOLOGY 59: 89–106. 2007.

# Taxonomic revision of *Aspergillus* section *Clavati* based on molecular, morphological and physiological data

J. Varga[1,3], M. Due[2], J.C. Frisvad[2] and R.A. Samson[1]

[1]*CBS Fungal Biodiversity Centre, Uppsalalaan 8, NL-3584 CT Utrecht, The Netherlands;* [2]*BioCentrum-DTU, Building 221, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark;* [3]*Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6701 Szeged, P.O. Box 533, Hungary*

**Correspondence*: János Varga, j.varga@cbs.knaw.nl

**Abstract:** *Aspergillus* section *Clavati* has been revised using morphology, secondary metabolites, physiological characters and DNA sequences. Phylogenetic analysis of β-tubulin, ITS and calmodulin sequence data indicated that *Aspergillus* section *Clavati* includes 6 species, *A. clavatus* (synonyms: *A. apicalis*, *A. pallidus*), *A. giganteus, A. rhizopodus*, *A. longivesica, Neocarpenteles acanthosporus* and *A. clavatonanicus*. *Neocarpenteles acanthosporus* is the only known teleomorph of this section. The sister genera to *Neocarpenteles* are *Neosartorya* and *Dichotomomyces* based on sequence data. Species in *Neosartorya* and *Neocarpenteles* have anamorphs with green conidia and share the production of tryptoquivalins, while *Dichotomomyces* was found to be able to produce gliotoxin, which is also produced by some *Neosartorya* species, and tryptoquivalines and tryptoquivalones produced by members of both section *Clavati* and *Fumigati*. All species in section *Clavati* are alkalitolerant and acidotolerant and they all have clavate conidial heads. Many species are coprophilic and produce the effective antibiotic patulin. Members of section *Clavati* also produce antafumicin, tryptoquivalines, cytochalasins, sarcins, dehydrocarolic acid and kotanins (orlandin, desmethylkotanin and kotanin) in species specific combinations. Another species previously assigned to section *Clavati*, *A. ingratus* is considered a synonym of *Hemicarpenteles paradoxus*, which is phylogenetically very distantly related to *Neocarpenteles* and section *Clavati*.

**Key words:** Ascomycetes, *Aspergillus* section *Clavati*, β-tubulin, calmodulin, *Dichotomomyces*, Eurotiales, *Hemicarpenteles*, ITS, mycotoxin, *Neocarpenteles*, patulin, polyphasic taxonomy.

## INTRODUCTION

Species in *Aspergillus* section *Clavati* are alkalitolerant, often dung-borne species that produce several mycotoxins such as patulin (Varga *et al*. 2003), cytochalasins (Demain *et al*. 1976; Steyn *et al*. 1982), tryptoquivalines and tryptoquivalones (Clardy *et al*. 1975; Büchi *et al*. 1977), and other bioactive natural products, including the sarcins (Cole & Cox 1981; Lin *et al*. 1994). Weisner (1942) and Bergel *et al*. 1943 found that *A. clavatus* produces patulin, and Florey *et al*. (1944) reported on patulin production by *Aspergillus giganteus* in 1944. Clavatol (Bergel *et al*. 1944) and ascladiol (Suzuki *et al*. 1971) were also isolates from *A. clavatus* as antibiotics. Cytochalasin E and K are also mycotoxins known from *Aspergillus clavatus* (Demain *et al*. 1976). *A. clavatus* was also reported to produce kotanin and xanthocillin X dimethylether (Büchi *et al*. 1977). Among the mycotoxins produced, patulin is receiving world-wide attention due to its frequent occurrence in apple juices (Harrison 1989; Beretta *et al.* 2000). *Aspergillus clavatus, A. giganteus* and *Neocarpenteles acanthosporus* isolates also produce ribotoxins, which are promising tools for immunotherapy of cancer (Martinez-Ruiz *et al.* 1999; Varga *et al.* 2003). The economically most important species of the section, *A. clavatus* is possibly a cosmopolitan fungus. It can be isolated mainly from soil and dung, but also occurs on stored products (mainly cereals) with high moisture content, e.g. inadequately stored rice, corn and millet (Flannigan & Pearce 1994). *A. clavatus* isolates appear to be particularly well adapted for growth during malting (Flannigan & Pearce 1994). *A. clavatus* was found to be responsible for an extrinsic allergic alveolitis known as malt worker"s lung, and in cases of mycotoxicoses of animals fed with by-products of malting (Flannigan & Pearce 1994; Lopez-Diaz & Flannigan 1997). The toxic syndromes observed in animals were suggested to result from the synergistic action of various mycotoxins produced by this species (Flannigan & Pearce 1994). Several species of section *Clavati* have phototrophic long conidiophores at temperatures around 20–23 °C (Fennell & Raper 1955; Trinci & Banbury 1967; Sarbhoy & Elphick 1968; Huang & Raper 1971; Yaguchi *et al.* 1993).

*Aspergillus* subgenus *Fumigati* section *Clavati* (Gams *et al.* 1985; Peterson 2000), formerly the *Aspergillus clavatus* group was recognised by Thom & Church (1926) with two species, *A. clavatus* and *A. giganteus*. *A. clavatonanicus* was added by Batista *et al.* (1955). After Raper & Fennell (1965) published their monograph on aspergilli, several new species or varieties assigned to section *Clavati* were described. These were summarised by Samson (1979), who recognised *A. longivesica* (Huang & Raper 1971) as the fourth species within the section. None of these have known teleomorphs. Another species, *A. rhizopodus* (Rai *et al.* 1975) was treated by Samson (1979) as a synonym of *A. giganteus*. *A. pallidus* Kamyschko has been treated as a white-spored synonym of *A. clavatus* by several authors (Peterson 2000; Varga *et al.* 2003). *A. acanthosporus* (Udagawa & Takada (1971), placed in subgenus *Ornati* (Samson 1979), was shown by Peterson (2000) to be more closely related to section *Clavati* than to section *Ornati*. Also, their major ubiquinone systems point in this direction as section *Clavati* and *A. acanthosporus* have Q10, while *H. ornatus* has Q9 ubiquinones (Tamura *et al.* 1999). Although its teleomorph was originally placed into the *Hemicarpenteles* genus, recently Udagawa & Uchiyama (2002) proposed the new ascomycete genus *Neocarpenteles* to accommodate this species, and excluded *N. acanthosporus* from section *Ornati*. Similar conclusions were drawn

**Table 1.** The *Aspergillus* section *Clavati* isolates examined in this study.

| Species | Strain No. | Origin |
|---|---|---|
| *A. clavatus* | CBS 104.45 | ATCC 9600; Czech Republic, Pribram |
| | CBS 105.45 | Church, No. Ac 87 |
| | CBS 106.45 | *Humulus lupulus* (Cannabaceae), G. Smith |
| | CBS 114.48 | Culture contaminant, Netherlands |
| | CBS 513.65$^T$ | ATCC 1007; IMI 015949; NRRL 1; Thom 107 |
| | CBS 514.65 | ATCC 10058; IMI 321306; NRRL 4; Thom 4754.3 |
| | CBS 470.91 | Toxic feed pellets, Hungary |
| | CBS 116685 | Milled rice, Netherlands |
| | CBS 118451 | Medicine, Germany |
| | DTO 6-F8 | Air, ciabatta factory, Netherlands |
| | DTO 27-C2 | Bakery, Netherlands |
| | SZMC 0918 | Soil, Hungary |
| | SZMC JV4 | Stored wheat, Hungary |
| | SZMC JV1.1 | Human mucosa, Hungary |
| | IMI 358435 | Feed pellet, Hungary |
| *A. giganteus* | CBS 117.45 | IMI 024256; P. Biourge |
| | CBS 119.48 | H. Burgeff, No. 382, Germany |
| | CBS 118.49 | Wood of ship (*Virola surinamensis*), Suriname |
| | CBS 122.53 | Tail borad, Nigeria |
| | CBS 117.56 | Wood in swimming pool, Netherlands |
| | CBS 101.64 | Unknown, Poland |
| | CBS 515.65$^T$ | ATCC 16439; IMI 235601; NRRL 7974; mouse dung, U.S.A. |
| | CBS 526.65 | ATCC 10059; IMI 227678; NRRL 10; Thom 5581.13A |
| | CBS 112.27 | A. Blochwitz |
| *A. rhizopodus* | CBS 450.75$^T$ | Usar soil, India, Lucknow |
| | IMI 351309 | Soil, Yugoslavia |
| *A. pallidus* | CBS 344.67$^T$ | ATCC 18327; IMI 129967; soil, Moldova |
| | SZMC JV6 | Culture contaminant, Hungary |
| *A. clavatonanicus* | CBS 474.65$^T$ | ATCC 12413; IMI 235352; WB 4741; finger nail lesion, Brazil |
| *A. longivesica* | CBS 530.71$^T$ | ATCC 22434; IMI 156966; soil, Nigeria |
| | CBS 187.77 | Soil, Ivory Coast, Tai |
| *A. apicalis* | CBS 236.81$^T$ | Wheat bran, India |
| *N. acanthosporus* | CBS 558.71$^T$ | Solomon Islands, Bougainville Island |
| | CBS 445.75 | Solomon Islands, Bougainville Island, Buin, Malapita |
| | CBS 446.75 | Solomon Islands, Bougainville Island, Buin, Batubatuai |
| | CBS 447.75 | Solomon Islands, Bougainville Island, Kieta |
| *D. cepjii* var. *cejpii* | CBS 761.96 | spent mushroom compost, Netherlands |
| *D. cepjii* var. *cejpii* | CBS 779.70 | Soil, Cincinatti, U.S.A. |
| *D. cepjii* var. *cejpii* | CBS 100192 | Soil, Bratislava, Slovakia |
| *D. cepjii* var. *cejpii* | CBS 474.77 | Soil, Egypt |
| *D. cepjii* var. *cejpii* | CBS 780.70 | Pasturised milk, Cincinatti, U.S.A. |
| *D. cepjii* var. *cejpii* | CBS 397.68 | Soil, South Africa |
| *D. cepjii* var. *cejpii* | CBS 345.68 | rhizosphere of *Hordeum vulgare*, Pakistan |
| *D. cepjii* var. *cejpii* | CBS 159.67 | Soil, Kominato, Japan |
| *D. cepjii* var. *cejpii* | CBS 157.66$^T$ | Orchard soil, Moldova, near Tiraspol |
| *D. cepjii* var. *spinosus* | CBS 219.67$^T$ | Soil, Kyoto, Japan |

by Varga *et al.* (2003) based on sequence analysis of the internal transcribed spacer regions and the 5.8 S rRNA gene (ITS region) of isolates belonging to *Aspergillus* section *Clavati*. Another species, *A. apicalis* Mehrotra & Basu (1976) (as *A. apica*), was placed in section *Ornati* by Samson (1979) because of morphological similarities to *H. paradoxus* (small clavate blue green aspergilla). Finally, *A. ingratus* has been described by Yaguchi *et al*. (1993), who stated that this sclerotium producing species belonged to section *Clavati*.

In this study, we examined the taxonomic assignment of these alkalitolerant species characterised by clavate aspergilla using molecular, morphological and chemotaxonomical methods. We also examined the relationships among teleomorphs of *Aspergillus* subgenus *Fumigati*, including *Neocarpenteles* and *Neosartorya* species to the *Dichotomomyces* genus using molecular approaches. Although the anamorphs of *Dichotomomyces* belong to the *Polypaecilum*, ascomata and ascospores of *Dichotomomyces* species have a similar morphology as those of *Neosartorya* and *Neocarpenteles* (Samson RA, unpubl. data).

## MATERIALS AND METHODS

### Source of microorganisms

The fungi examined included all species allocated to *Aspergillus* section *Clavati*, and some species assigned to section *Ornati* with clavate aspergilla (the *Aspergillus ornatus* group), which could possibly be related to *A. clavatus*. The strains examined are listed in Table 1.

### Morphology and physiology

The strains (Table 1) were grown for 7 d as 3-point inoculations on Czapek agar (CZA), Czapek yeast autolysate agar (CYA), creatine sucrose agar (CREA) and malt extract agar (MEA) at 25 °C in artificial daylight (medium compositions in Samson *et al*. 2004).

### Analysis for secondary metabolites

The cultures were analysed according to the HPLC-diode array detection method of Frisvad & Thrane (1987, 1993) as modified by Smedsgaard (1997). The isolates were analyzed on CYA and YES agar using three agar plugs (Smedsgaard 1997). The secondary metabolite production was confirmed by identical UV spectra with those of standards and by TLC analysis using the agar plug method, the TLC plates were eluted in toluene : ethylacetate:formic acid (6:3:1) and chloroform:acetone:2-propanol (85:15:20) (Filtenborg *et al*. 1983; Samson *et al*. 2004). Standards of patulin, cytochalasin E, kotanin, and nortryptoquivalin known to be produced by these fungi, were also used to confirm the identity of the compounds.

### Isolation and analysis of nucleic acids

The cultures used for the molecular studies were grown on malt peptone (MP) broth using 10 % (v/v) of malt extract (Brix 10) and 0.1 % (w/v) Bacto peptone (Difco), 2 mL of medium in 15 mL tubes. The cultures were incubated at 25 °C for 7 d. DNA was extracted from the cells using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. Fragments containing the ITS region were amplified using primers ITS1 and ITS4 as described previously (White *et al.* 1990). Amplification of part of the β-tubulin gene was performed using the primers Bt2a and Bt2b (Glass & Donaldson 1995). Amplifications of the partial calmodulin gene were set up as described previously (Hong *et al.* 2005). Sequence analysis was performed with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit for both strands, and the sequences were aligned with the MT Navigator software (Applied Biosystems). All the sequencing reactions were purified by gel filtration through Sephadex G-50 (Amersham Pharmacia Biotech, Piscataway, NJ) equilibrated in double-distilled water and analyzed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The unique ITS, β-tubulin, actin and calmodulin sequences were deposited at the GenBank nucleotide sequence database under accession numbers EU078624–EU078678 and EU076312–EU076343.

### Data analysis

The sequence data was optimised using the software package Seqman from DNAStar Inc. Sequence alignments were performed by using CLUSTAL-X (Thompson *et al.* 1997) and improved manually. 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 (Tamura & Nei 1993), which were then used to construct the NJ tree with MEGA v. 3.1 (Kumar *et al.* 2004). To determine the support for each clade, a bootstrap analysis was performed with 1000 replications.

For parsimony analysis, the PAUP v. 4.0 software was used (Swofford 2002). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option with 100 random taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis & Bull 1993). A *Neosartorya fischeri* isolate was used as outgroup in these experiments.

## RESULTS AND DISCUSSION

### Phylogeny

We examined the genetic relatedness of section *Clavati* isolates and their presumed relatives using sequence analysis of the ITS region of the ribosomal RNA gene cluster, and parts of the calmodulin and β-tubulin genes. During analysis of part of the β-tubulin gene, 468 characters were analyzed. Among the 174 polymorphic sites, 102 were found to be phylogenetically informative. The Neighbour-joining tree based on partial β-tubulin genes sequences is shown in Fig. 1. The topology of the tree is the same as one of the more than $10^5$ maximum parsimony trees constructed by the PAUP program (length: 233 steps, consistency index: 0.8798, retention index: 0.9728). The ITS data set included 448 characters with 8 parsimony informative characters. The Neighbour-joining tree shown in Fig. 2 has the same topology as one of the 4 maximum parsimony trees (tree length: 25, consistency index: 0.9600, retention index: 0.9896).

Phylogenetic analysis of β-tubulin sequence data indicated that *Aspergillus* section *Clavati* includes six species, namely: *A. clavatus*

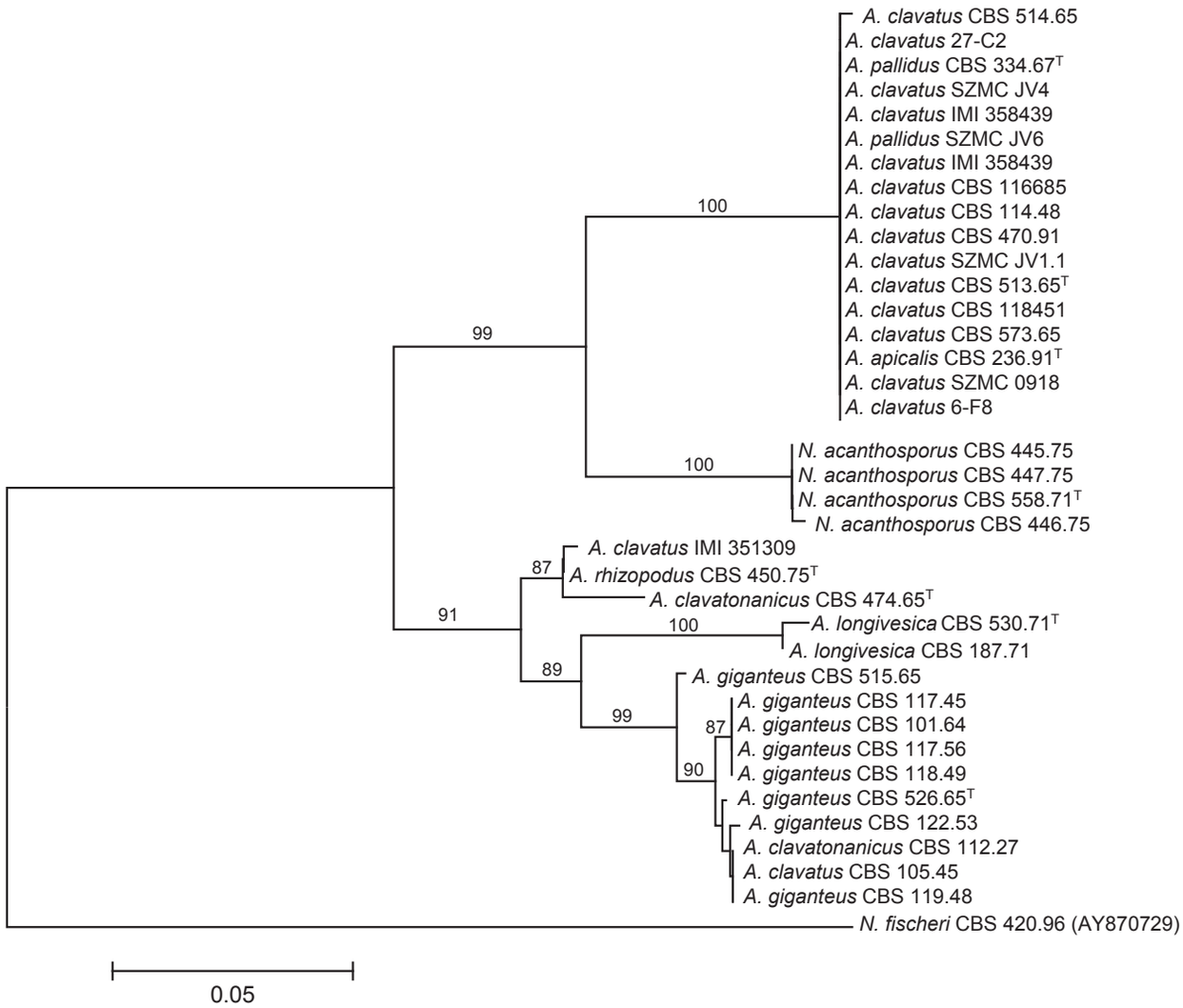


**Fig. 1.** Neighbour-joining tree based on β-tubulin sequence data of *Aspergillus* section *Clavati*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

(synonyms: *A. pallidus, A. apicalis*), *A. giganteus, A. longivesica, A. rhizopodus, A. clavatonanicus* and *N. acanthosporus*. Some misidentifications have also been clarified: isolates previously identified as *A. clavatus* (CBS 105.45) and *A. clavatonanicus* (CBS 112.27) were found to belong to the *A. giganteus* species, while one isolate originally identified as *A. clavatus* (IMI 351309) was found to belong to the *A. rhizopodus* species. The ITS sequences of *A. clavatonanicus* and *A. rhizopodus* isolates, and *A. giganteus* and *A. longivesica* isolates, respectively, were identical, indicating their close relationship.

*A. ingratus* (Yaguchi *et al.* 1993) was found to be the synonym of *H. paradoxus* based on sequence data, so it was excluded from section *Clavati* (data not shown). *H. paradoxus* isolates are only distantly related to section *Clavati*, with affinities to some *Penicillium* species (to be published elsewhere).

## Chemotaxonomy

The extrolites produced by species of *Aspergillus* section *Clavati* are listed in Table 2. Based on the common production of patulin, tryptoquivalins, tryptoquivalons and kotanins, most of the species appear to be closely related. *A. clavatus* produces patulin (= clavatin = clavacin) (Weisner 1942; Waksman *et al*. 1942, 1943; Hooper *et al*. 1944) and has been reported to cause mycotoxicosis in calves as early as 1954 (Forgacs *et al.* 1954). This mycotoxin was detected on YES agar in all isolates of *A. clavatus, A. giganteus* and *A. longivesica*. Previously the presence of the isoepoxydon dehydrogenase gene taking part in the biosynthesis of patulin has also been proved for *A. clavatonanicus* and *A. pallidus* isolates using primer pairs developed by Paterson *et al.* (2000) to identify potential patulin producing Penicillia (Varga *et al.* 2003). Other interesting metabolites produced by species of section *Clavati* are ribotoxins. Ribotoxins are a family of ribosome-inactivating proteins that have specific ribonucleolytic activity against a single phospodiester bond in the conserved sarcin/ricin domain of 26 S rRNA (Martinez Ruiz *et al.* 1999). Ribotoxins have recently been found in a number of *Aspergillus* species including *A. clavatus*, *A. giganteus*, *A. viridinutans*, *A. fumigatus*, *A. restrictus*, *A. oryzae* var. *effusus*, *A. tamarii* and *A. ostianus.* Anamorphs of *Neosartorya fischeri*, *N. glabra* and *N. spinosa* also produced ribotoxins (Lin *et al.* 1994; Martinez-Ruiz *et al.* 1999). Using the PCR probe developed by Lin *et al.* (1994), Varga *et al.* (2003) examined the presence of ribotoxin genes in isolates of *Aspergillus* section *Clavati;* a DNA fragment of about 600 bp was amplified in some *A. clavatus, A.*

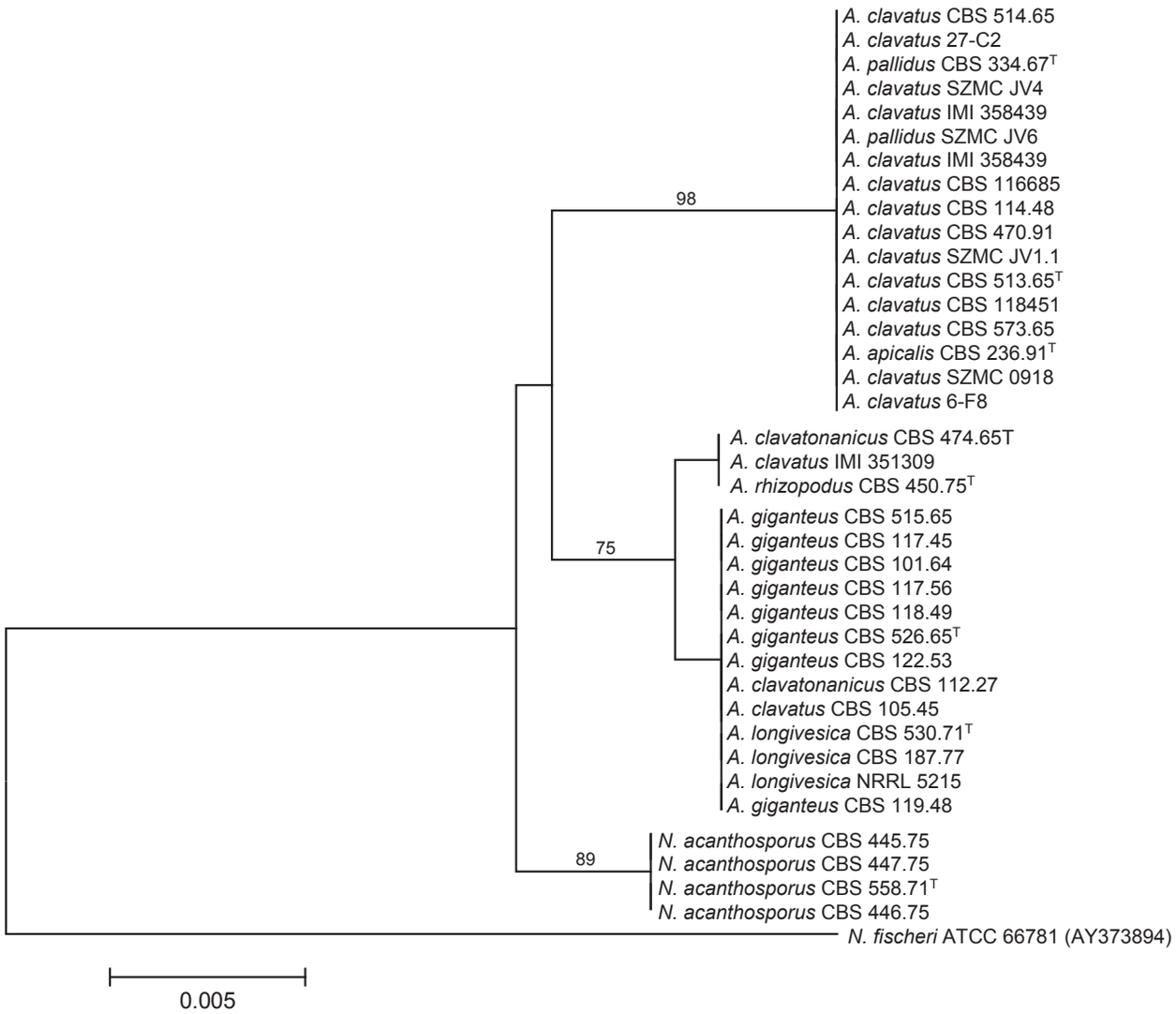


**Fig. 2.** Neighbour-joining tree based on ITS sequence data of *Aspergillus* section *Clavati*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

*giganteus, A. pallidus* and *N. acanthosporus* isolates, indicating that these isolates are able to synthesize ribotoxins (Varga *et al.* 2003). *Hemicarpenteles paradoxus*, however, including its synonym *A. ingratus* produces no secondary metabolites in common with these core species and appear to more distantly related to section *Clavati*. Thus this species appears to occupy a unique position in the *Aspergillus* genus with no obvious closely related species.

## Morphology

All the isolates except the ex type culture of *A. clavatonanicus*, produced numerous conidiophores with blue green conidia, hyaline conidiophore stipes and clavate aspergilla. The isolates in three species were phototropic producing very long conidiophores: *A. giganteus, A. rhizopodus* and *A. longivesica*. Another common phenotypic similarity was the alkalophilic tendency already described for *A. rhizopodus* which was isolated from soil with pH 8.5–9 and other species in the group (Raper & Fennell 1965; Rai *et al*. 1975). Several species have been isolated from dung which is also an alkaline substrate. This is further confirmed by the strong growth of all isolates on creatine-sucrose agar. This medium has an initial pH of 8 and creatine is an alkaline amino acid. Morphological and physiological data confirmed that *Neocarpenteles acanthosporus* and *Aspergillus* section *Clavati* are closely related.

## Teleomorph relationships in *Aspergillus* subgenus *Fumigati*

*Aspergillus* subgenus *Fumigati* includes section *Clavati* with the *N. acanthosporus* teleomorph, and section *Fumigati* with *Neosartorya* teleomorphs. We examined the relationships of these teleomorphs taxa to another ascomycete genus, *Dichotomomyces*. *Dichotomomyces cejpii* was originally described by Saito (1949) as *D. albus*, later validated as *D. cejpii* by Scott (1970). This species belongs to the Trichocomaceae family (although Malloch & Cain (1971) placed it to Onygenaceae). This species is characterised by the production of aleurioconidia on short branched conidiophores, and ascospores embedded in cleithothecia (Scott 1970; Udagawa 1970). Isolates of *D. cejpii* are highly heat resistant and can be found world-wide in soil, heat treated products and marine environments (Pieckova *et al.* 1994; Jesenska *et al.* 1993; Mayer *et al.* 2007). *D. cejpii* isolates has been claimed to produce a range of secondary metabolites including gliotoxin (Seigle-Murandi *et al.* 1990), xanthocillin X (Kitahara & Endo 1981), and several metabolites with

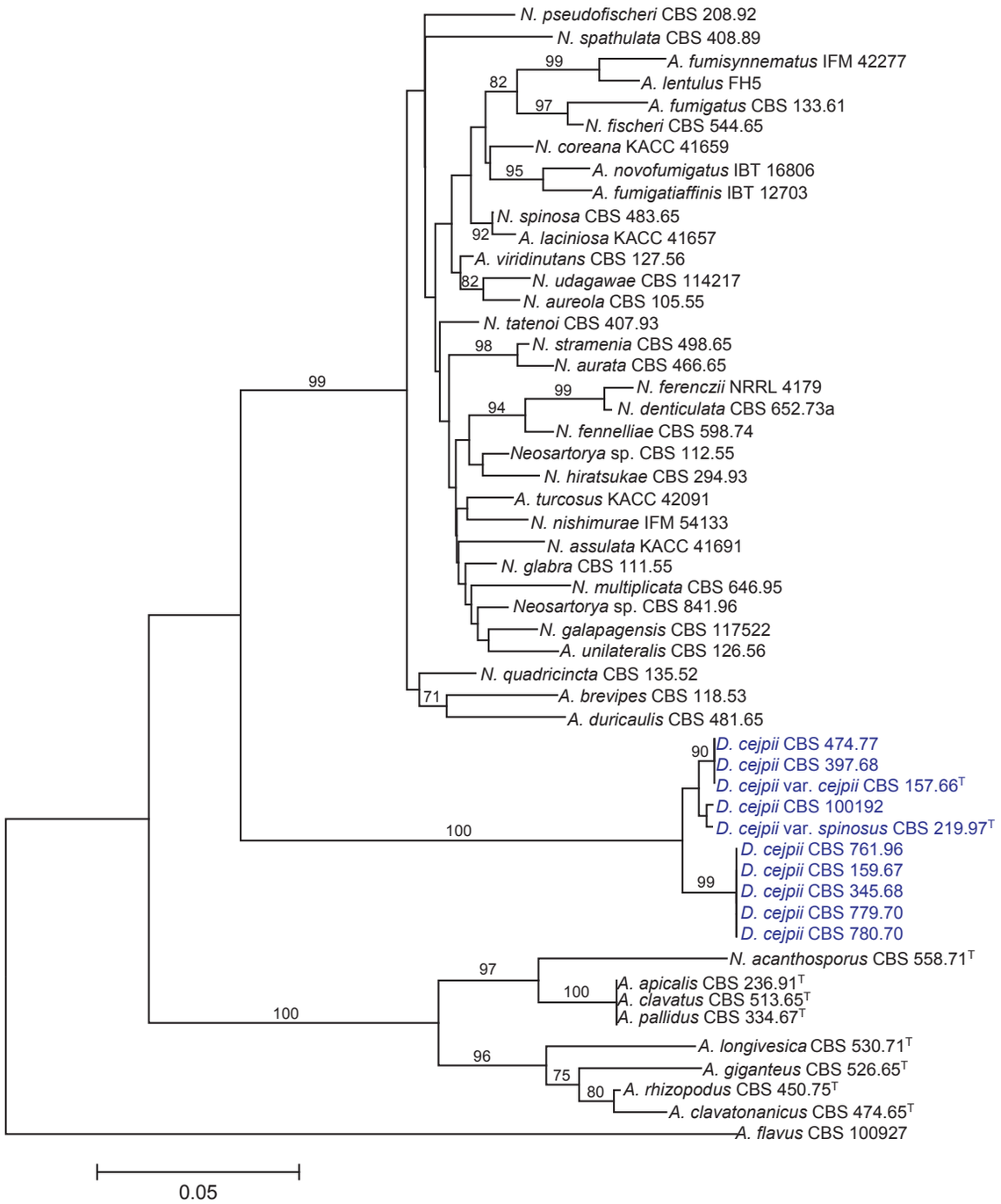


**Fig. 3.** Neighbour-joining tree based on β-tubulin sequence data of *Neosartorya*, *Neocarpenteles*, *Dichotomomyces* species and their asexual relatives. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

**Table 2.** Extrolite production of species assigned to *Aspergillus* section *Clavati* and *D. cejpii*. These toxins were all verified or found for the first time in the species listed, the ribotoxins (including α-sarcin) and xanthocillin X in *D. cejpii* were not verified, however.

| Species | Extrolites |
|---|---|
| *A. clavatonanicus* | antafumicins, glyanthrypine, kotanins, tryptoquivalines, tryptoquivalones |
| *A. clavatus* | patulin, cytochalasin E & K, kotanins, antafumicin, (dehydrocarolic acid), tryptoquivalones, tryptoquivalines, ascladiol, ribotoxins |
| *A. giganteus* | patulin, antafumicin, ascladiol, tryptoquivalones; tryptoquivalines, glyanthrypine, pyripyropen, α-sarcin and other ribotoxins |
| *A. longivesica* | patulin, tryptoquivalones, tryptoquivalines, antafumicins, pyripyropen |
| *A. rhizopodus* | pseurotins, dehydrocarolic acid, tryptoquivalines, tryptoquivalones, kotanins, cytochalasins |
| *N. acanthosporus* | kotanins, tryptoquivalines, tryptoquivalones, ribotoxins |
| *D. cejpii* | gliotoxin, tryptoquivalones, rubratoxins, (xanthocillin X) |

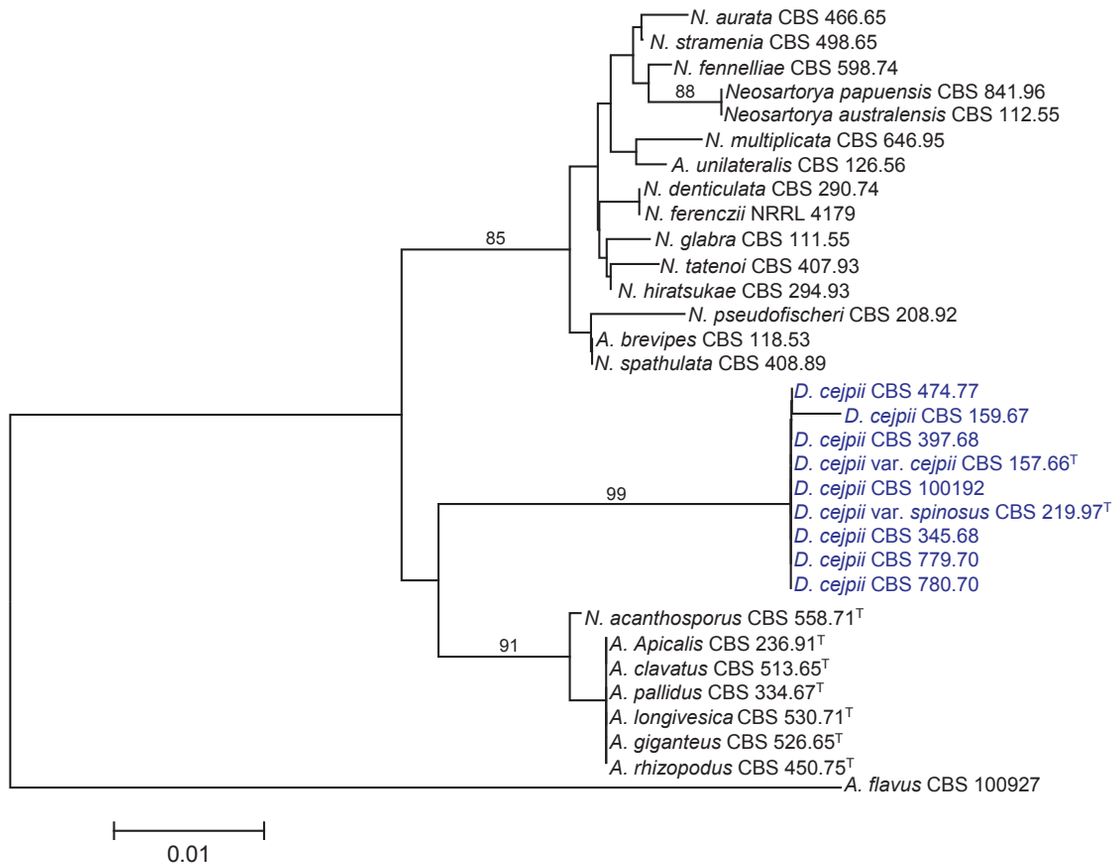


**Fig. 4.** Neighbour-joining tree based on ITS sequence data of *Neosartorya*, *Neocarpenteles*, *Dichotomomyces* species and their asexual relatives. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

antibiotic and ciliostatic properties (Pieckova & Jesenska 1997a, 1997b; Pieckova & Roeijmans 1999).

We examined the genetic variability and relationships of *Aspergillus* section *Clavati* and *Fumigati* isolates, *D. cejpii* var. *cejpii* and *D. cejpii* var. *spinosus* (Malloch & Cain 1971; originally described as *D. albus* var. *spinosus*; Udagawa 1970). Both the ITS region and part of the β-tubulin gene were amplified and sequenced, and phylogenetic analyses were carried out as described above. The trees based on both ITS and β-tubulin data indicate that *D. cejpii* forms a sister group with *Neosartorya* and *Neocarpenteles* species (Figs 3–4). During analysis of part of the β-tubulin gene, 469 characters were analyzed. Among the 270 polymorphic sites, 214 were found to be phylogenetically informative. The Neighbour-joining tree based on partial β-tubulin genes sequences is shown in Fig. 3. The topology of the tree is the same as one of the 22 maximum parsimony trees constructed by the PAUP program (length: 738 steps, consistency index: 0.6233, retention index: 0.8614). The ITS data set consisted of 446 nucleotides, with 45 parsimony informative sites. The topology of the Neighbour joining tree depicted in Fig. 4 was the same as one of the more than 105 maximum parsimony trees (length: 124 steps, consistency index: 0.7419, retention index: 0.9229). Both trees indicate that the *Dichotomomyces* genus should be transferred to *Aspergillus* subgenus *Fumigati*. Similar results were obtained during phylogenetic analysis of partial calmodulin gene sequences (data not shown). *D. cejpii* isolates have been found to produce gliotoxin in common with several species assigned to section *Fumigati* including some *Neosartorya* species (Larsen *et al.* 2007), tryptoquivalones also produced by several species assigned to sections *Clavati* and *Fumigati* (Hong *et al.* 2005), and rubratoxins, which are hepatotoxic mycotoxins produced by *P. crateriforme* (Frisvad 1989; Sigler *et al.* 1996; Richer *et al*. 1997) [misidentified as *Penicillium purpurogenum* (Natori *et al.* 1970) or *P. rubrum* (Moss *et al.* 1968)]. *D. cejpii* has also been claimed to produce xanthocillin X (Kitahara & Endo 1981), even though it could not be confirmed in our analyses. Xanthocillin and related compounds have also been found in *H. paradoxus* (Frisvad JC, unpubl. data) *A. candidus* (Rahbaek *et al.* 2000), *Eupenicillium crustaceum* (Turner & Aldridge 1983), *E. egyptiacum* (Vesonder 1979), *P. italicum* (Arai *et al.* 1989), *P. flavigenum* (Frisvad *et al.* 2004) and *P. chrysogenum* (Hagedorn *et al.* 1960; Achenbach *et al*. 1972; Pfeiffer *et al.* 1972; Frisvad *et al*. 2004; de la Campa *et al.* 2007). Since the anamorph of *Dichotomomyces* was earlier found to belong to *Polypaecilum*, further morphological and molecular studies are needed to clarify the significance of the morphology of the anamorph in the taxonomic placement of these species, and to clarify the taxonomy of *Polypaecilum* species.

In conclusion, the polyphasic approach applied to clarify the taxonomy of *Aspergillus* section *Clavati* led to the assignment of six species, namely: *A. clavatus* (synonyms: *A. pallidus, A. apicalis*), *A. giganteus, A. longivesica, A. rhizopodus, A. clavatonanicus* and *N. acanthosporum* to this section. *Hemicarpenteles paradoxus* (synonym: *A. ingratus*) was found to be unrelated to section *Clavati*, but more closely related to *Penicillium*. *Dichotomomyces* and *Neosartorya* were found to be sister clades to the genus *Neocarpenteles*. Further studies are needed to clarify the taxonomic status of *Dichotomomyces* species with *Polypaecilum* anamorphs.

***Aspergillus clavatonanicus*** Batista, Maia & Alecrim, Anais Fac. Med. Univ. Recife 15: 197. 1955. Fig. 5.

**Type**: CBS 474.65, from finger nail lesion, Recife, Brazil

**Other no. of the type**: ATCC 12413; DMUR 532; IMI 235352; WB 4741

**Description**
Colony diam (7 d): CYA25: 50–82 mm, MEA25: 45–78 mm, YES25: 57–82 mm, OA25: 49–60 mm, CYA37: 8–17 mm, CREA: very good growth and acid production in the margin of the colony
Colony colour: greyish blue green
Conidiation: abundant
Reverse colour (CZA): uncoloured to light brownish
Colony texture: floccose
Conidial head: clavate, up to 145–360 × 120–180 µm
Stipe: 40–470 × 6–16 µm, rough walled
Vesicle diam/shape: 22–125 × 5–22 µm, clavate
Conidium size/shape/surface texture: 5–8.5 × 5–6.5 µm, ellipsoid or cylindrical, smooth

**Cultures examined:** CBS 474.65 = IBT 12370 = IBT 24678, CBS 112.27 = IBT 12369 = IBT 24677

**Diagnostic features**: conidial heads smaller than 1 mm

**Similar species**: *A. clavatus*

**Distribution**: Brazil

**Ecology and habitats**: human

**Extrolites**: antafumicins, glyanthrypine, kotanin, tryptoquivalins, tryptoquivalons

**Pathogenicity**: isolated from nail lesion (Batista *et al.* 1955)

***Aspergillus clavatus*** Desmazières, Ann. Sci. Nat., Bot. 2: 71, 1834. Fig. 6.
= *Aspergillus pallidus* Kamyschko (1963)
= *Aspergillus apicalis* Mehrotra & Basu (1976)

**Type**: CBS 513.65, J. Westerdijk > 1909, C. Thom > NRRL

**Other no. of the type**: ATCC 1007; ATCC 9602; ATCC 9598; CECT 2674; DSM 816; IMI 015949; IMI 015949v; IMI 015949iv; IMI 015949iii; LSHB Ac86; LSHB Ac95; NCTC 978; NCTC 3887; NRRL 1; NRRL 1656; QM 1276; QM 7404; WB 1

**Description**
Colony diam (7 d): CYA25: 28–45 mm; MEA25: 25–44 mm, YES25: 29–45 mm, OA25: 31–47 mm, CYA37: 9–26 mm, CREA25: very good growth and moderate to very strong acid production (exceptions: CBS 514.65, NRRL 2, NRRL 8 and NRRL 2254 grow poorly on CREA and produce no or very little acid)
Colony colour: blue-green
Conidiation: abundant
Reverse colour (CZA): uncoloured to somewhat brownish with age in some isolates
Colony texture: velvety
Conidial head: clavate, commonly ranging from 300 to 400 µm by 150 to 200 µm when young, in age commonly splitting into two, three, or more divergent columns
Stipe: 1500–3000 × 20–30 µm
Vesicle diam/shape: 200–250 × 40–60 µm, clavate
Conidium size/shape/surface texture: 3–4.5 × 2.5–3 µm, elliptical, smooth

**Cultures examined:** CBS 104.45, CBS 105.45, CBS 106.45, CBS 114.48, CBS 513.65, CBS 514.65, CBS 470.91, CBS 116685, CBS 118451, DTO 6-F8, DTO 27-C2, SZMC 0918, SZMC JV4, SZMC JV1.1, IMI 351309, IMI 358435, CBS 117.45, CBS 119.48, CBS 118.49, CBS 122.53, CBS 117.56, CBS 101.64, CBS 515.65, CBS 526.65

**Diagnostic features**: conidial heads up to 4 mm in size

**Similar species**: *A. clavatonanicus*

**Distribution**: worldwide, mainly in tropical, subtropical and Mediterranean regions

**Ecology and habitats**: soil, cereals, malt, dung

**Extrolites**: Patulin, cytochalasin E, kotanins, antafumicin, (dehydrocarolic acid), tryptoquivalone, tryptoquivalines, ascladiol (all found in this study), ribotoxins (Lin *et al.* 1995, Huang *et al.* 1997)

**Pathogenicity**: caused endocarditis (Opal *et al.* 1986), responsible for an extrinsic allergic alveolitis known as malt worker"s lung (Grant *et al.* 1976; Lopez-Diaz & Flannigan 1997; Flannigan & Pearce 1994), and various toxic syndromes including neurological disorders (Shlosberg *et al.* 1991; McKenzie *et al.* 2004; Loretti *et al.* 2003; Gilmour *et al.* 1989; Kellerman *et al.* 1976) and other mycotoxicosis-related diseases (Byth & Lloyd 1971) observed in animals

**Notes**: some isolates carry dsRNA mycoviruses 35–40 nm in size (Varga *et al.* 2003)

***Aspergillus giganteus*** Wehmer, Mem. Soc. Phys. Genève 33 (2): 85. 1901. Fig. 7.

**Type**: CBS 526.65, dung of bat in cave, Yucatan, Mexico

**Other no. of the type**: ATCC 10059; DSM 1146; IFO 5818; IMI 227678; NRRL 10; QM 1970; WB 10; IBT 12368

**Description**
Colony diam: CYA25: (26–) 40–65 mm, MEA25: (29–) 43–65 mm, YES25: 40–80 mm, OA25: 31–75 mm, CYA37: 10–29 mm, CREA: very good growth and poor or no acid production
Colony colour: first white, becoming pale blue-green near light celandine green to slate-olive
Conidiation: usually abundant
Reverse colour (CZA): dull tan
Colony texture: velvety
Conidial head: splitting into 2 or more columns with age, blue green
Stipe: two types: 2–3(–4) mm; or several cm in length
Vesicle diam/shape: two types: 100–250 × 30–50 µm on short conidiophores, 400–600 × 120–180 µm on long ones, clavate
Conidium size/shape/surface texture: 3.5–4.5 × 2.4–3 µm, elliptical, thick-walled, smooth

**Cultures examined:** CBS 117.45, CBS 119.48, CBS 118.49, CBS 122.53, CBS 117.56, CBS 101.64, CBS 515.65

**Diagnostic features**: produces clavate vesicles in contrast with the elongate ones of *A. longivesica*; do not produce rhizoidal foot

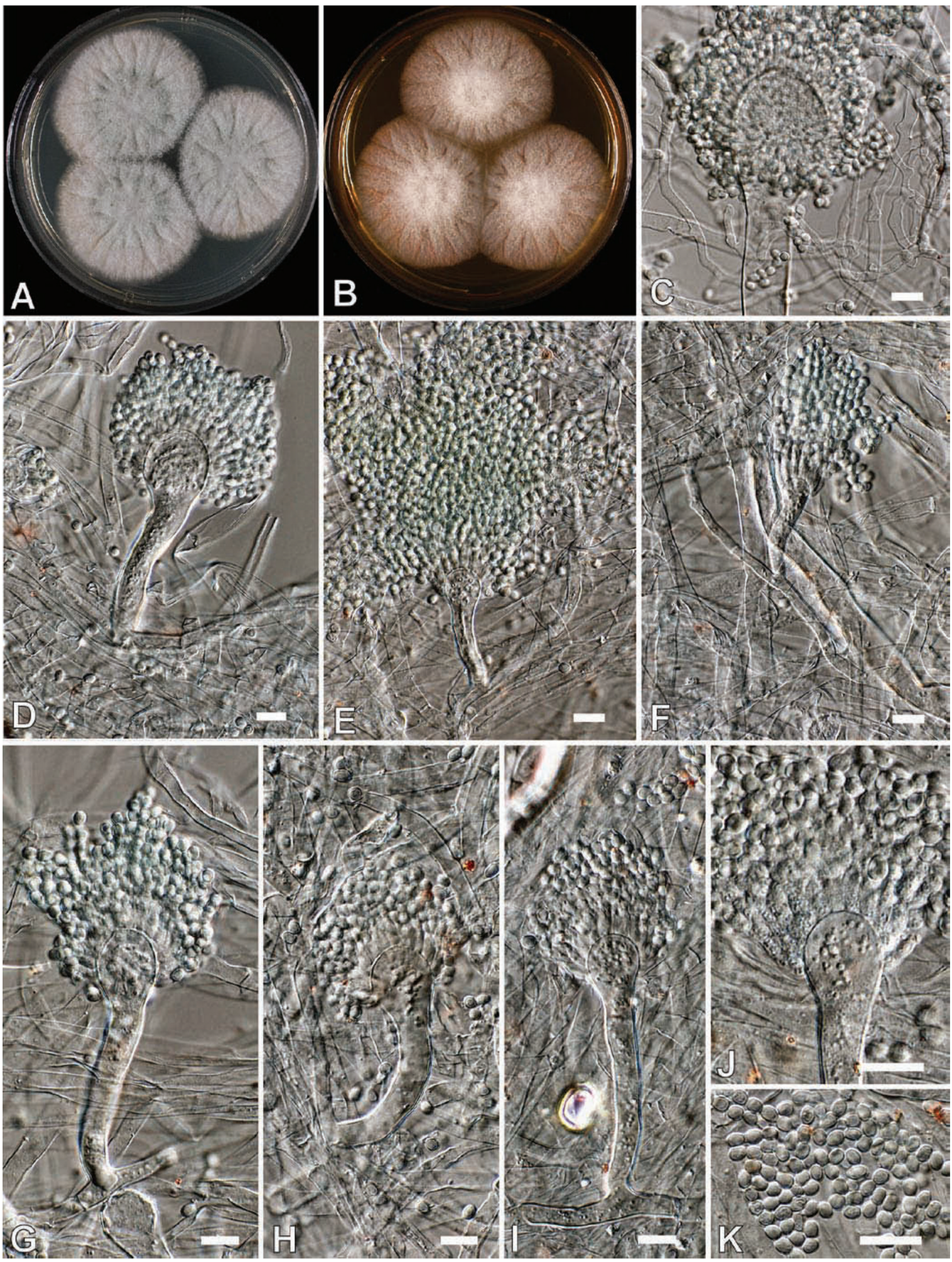


**Fig. 5.** *Aspergillus clavatonanicus*. A–B. Colonies after 7 d at 25 °C. A. CYA. B. MEA. C–J. Conidiophores. K. Conidia. Scale bars = 10 µm.

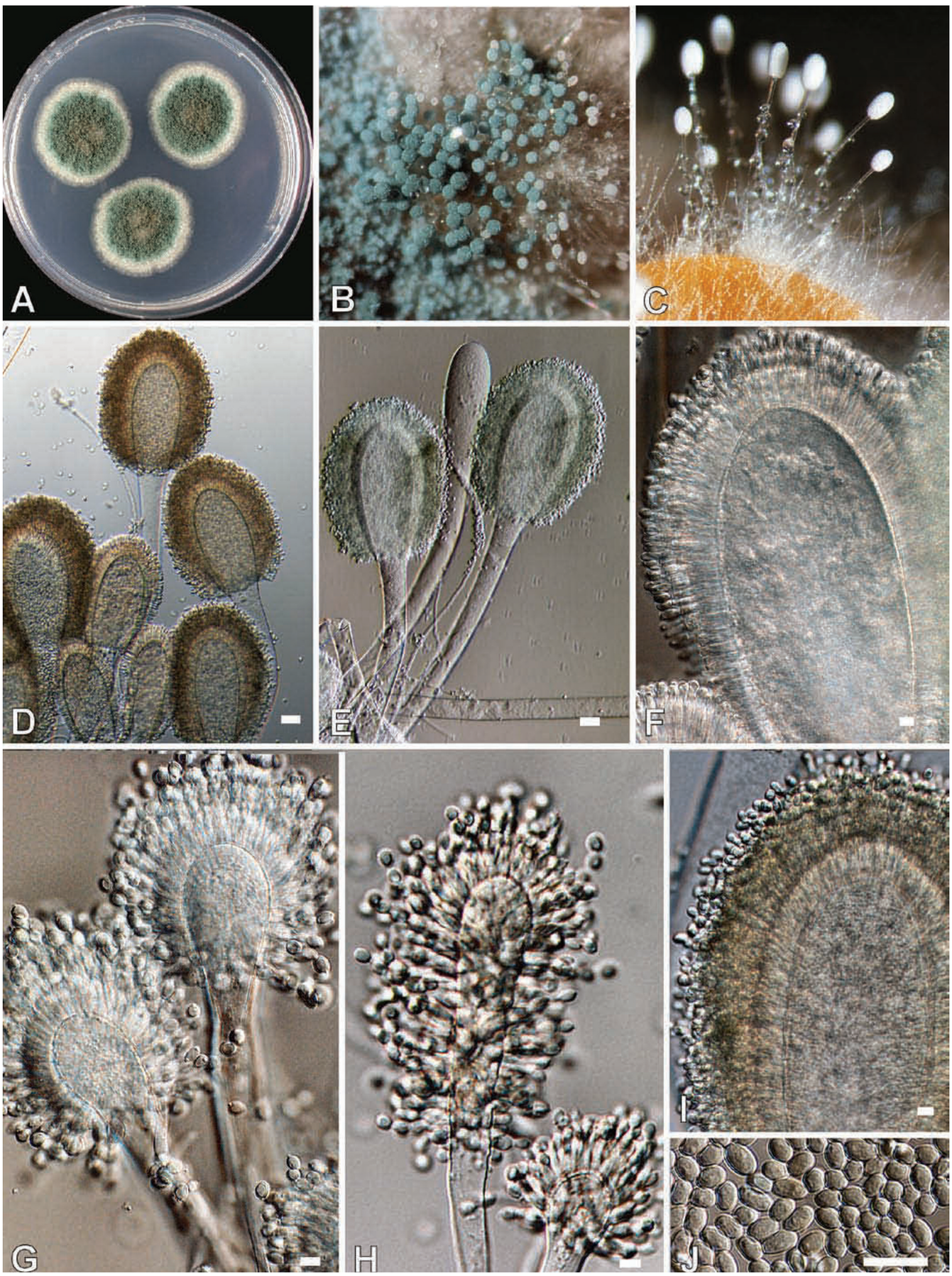


**Fig. 6.** *Aspergillus clavatus*. A. Colonies after 7 d at 25 °C on CYA. B–C. Macrophotograph of conidiophores. D–I. Conidiophores. J. Conidia. Scale bars = 10 µm, except D and E = 30 µm.

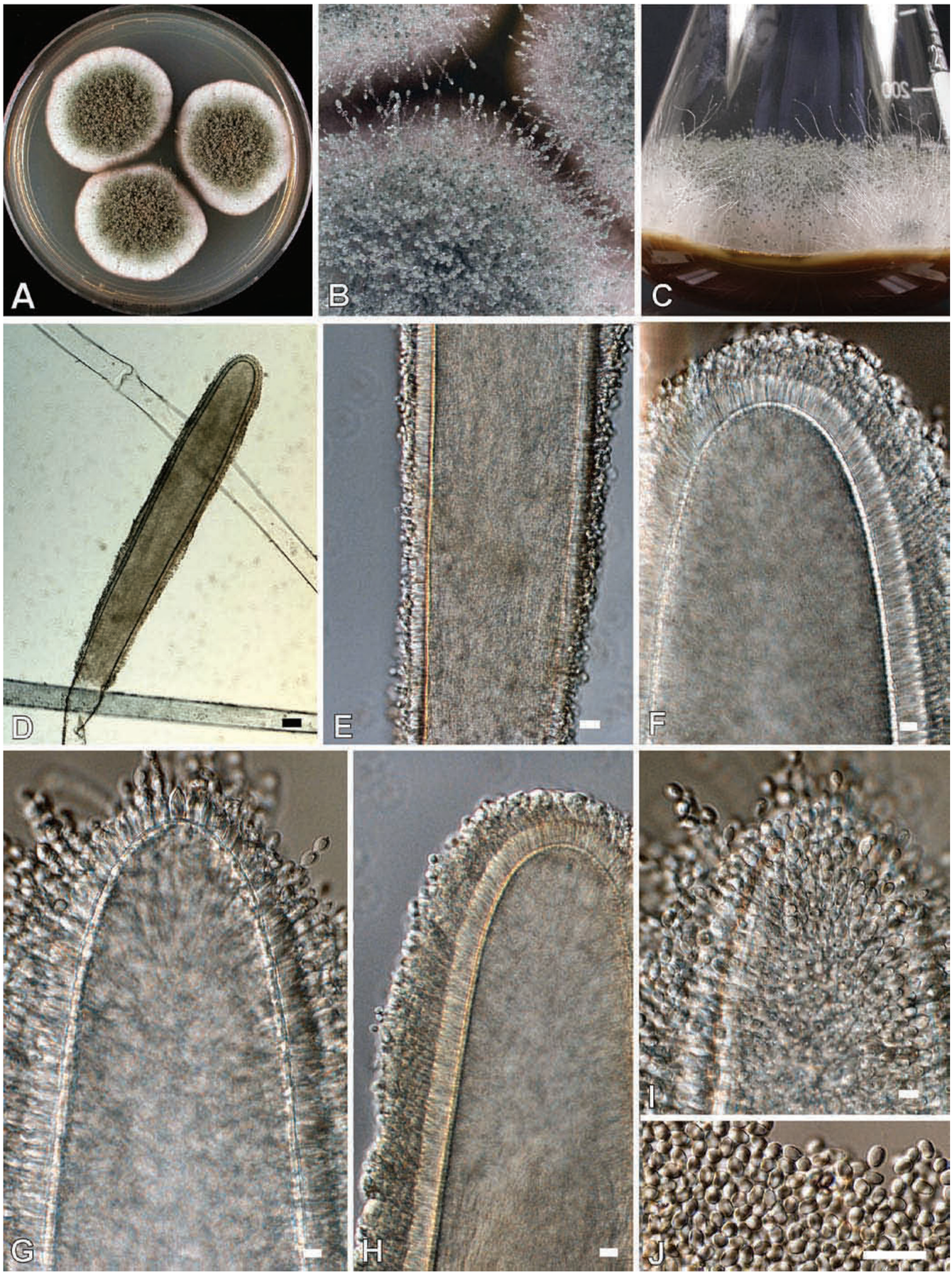


**Fig. 7.** *Aspergillus giganteus*. A. Colonies after 7 d at 25 °C on CYA. B–C. Macrophotograph of conidiophores. D–I. Conidiophores. J. Conidia. Scale bars = 10 µm, except D and E = 30 µm.

cells characteristic to *A. rhizopodus*; conidial heads can be up to 1–5 cm long

**Similar species**: *A. rhizopodus, A. longivesica*

**Distribution**: Nigeria, U.S.A., Egypt, Mexico, Panama, Germany, Suriname, Netherlands, Poland

**Ecology and habitats**: dung, soil, wood

**Extrolites**: patulin, antafumicin, ascladiol, tryptoquivalone; tryptoquivalines, glyanthrypine, pyripyropen (found in this study), α-sarcin and other ribotoxins (Olson & Goerner 1965; Olson *et al.* 1965; Lin *et al.* 1995; Wirth *et al.* 1997; Martinez-Ruiz *et al.* 1999). Carotens are also produced (van Eijk *et al.* 1979)

**Pathogenicity**: not reported

**Note**: two types of conidial structures: (1) conidiophores commonly 2 to 3 mm, rarely exceeding 4 mm in height, bearing clavate heads 200 to 350 µm in length; (2) conidiophores one to several centimeters in length, bearing heads up to 1 mm in length; longer conidiophores are phototropic, and only elongate in the presence of light

***Aspergillus longivesica*** Huang & Raper, Mycologia 63(1): 53. 1971. Fig. 8.

**Type**: CBS 530.71, from soil, rain forest, Nigeria

**Other no. of the type**: ATCC 22434; IMI 156966; QM 9698

**Description**
Colony diam: CYA25: 31–51 mm; MEA25: 48–56 mm; YES25: 60–74 mm; OA25: 52–60 mm, CYA37: 0 mm, CREA25: weak growth and no acid production (CBS 187.77 grow very well on CREA, however)
Colour: white to cream
Conidiation: abundant, rarely less abundant
Reverse colour (CYA): pale cinnamon buff
Colony texture: thin floccose
Conidial head: elongate, splitting into divergent columns with age, greyish blue green
Stipe: two types: 80–420 × 7–11.2 µm, or 1.5–4.5 cm long, thick walled (5.6–7 µm)
Vesicle diam/shape: two types: 2.2–3.2 mm × 130–200 µm, elongate, clavate, thick-walled, or 18–36 µm, globose to flask-shaped, thin-walled
Conidia length/shape/surface texture: two types: 4.2–16.8 × 2.8–7 µm, globose to elliptical, or 3.5–5.2 × 2.5–3.5 µm, elliptical or pyriform

**Cultures examined:** CBS 530.71, CBS 187.77

**Diagnostic features**: produces longer and wider conidiophores, longer vesicles and larger conidia than *A. giganteus*; vesicles are elongate to fusoid-clavate for the long conidiophre and globose for the samml ones, while those of *A. giganteus* are clavate

**Similar species**: *A. giganteus*

**Distribution**: Nigeria, Ivory Coast

**Ecology and habitats:** soil

**Extrolites**: patulin, tryptoquivalone, tryptoquivalines, antafumicins, pyripyropens (found in this study)

**Pathogenicity**: not reported

**Note**: longer conidiophores are phototropic, and only elongate in the presence of light

***Aspergillus rhizopodus*** Rai, Wadhwani & Agarwal, Trans. Br. Mycol. Soc. 64: 515. 1975. Fig. 9

**Type**: CBS 450.75, from usar soil, Lucknow, India

**Other no. of the type**: IMI 385057; WB5442

**Description**
Colony diam (7 d): CZA30: 40 mm; CYA25: 38–42 mm; MEA25: 50–55 mm; YES25: 68–72 mm; OA25: 43–47 mm; CYA37: 17–19 mm; CREA25: rather good growth and no acid production
Colony colour: blue green
Conidiation: abundant
Reverse colour (CZA): colourless
Colony texture: slightly furrowed
Conidial head: short columnar
Stipe: two types: 208–800 × 11–32 µm, or 5–22 mm × 36 µm, thick walled, smooth
Vesicle diam/shape: two types: 40–176 × 11–32 µm, or 288 × 79 µm, clavate
Conidium size/shape/surface texture: 4–5.5 × 2.5–3 µm, ellipsoidal, smooth

**Cultures examined:** CBS 450.75, IMI 351309

**Diagnostic features**: produces variously shaped foot cells with finger-like projections

**Similar species**: *A. giganteus, A. longivesica*

**Distribution**: India, Yugoslavia

**Ecology and habitats**: soil

**Extrolites**: pseurotins, dehydrocarolic acid, tryptoquivalines, tryptoquivalones, kotanins and cytochalasin (found in this study)

**Pathogenicity**: not reported

**Note**: large conidial heads formed only in the presence of light

***Dichotomomyces cejpii*** (Milko) D.B. Scott, Trans. Brit. Mycol. Soc. 47: 428, 1970. Fig. 10.
= *Talaromyces cejpii* Milko (1964)
= *Dichotomomycs albus* Saito (1949)
= *Royella albida* Dwiveli (1960)

**Type**: CBS 157.66, from orchard soil, near Tiraspol, Moldova

**Description**
Colony diam (7 d): CYA25: 25–47 mm; MEA25: 35–58 mm; YES25: 47–50 mm; OA25: 38–48; CYA37: 24–32 mm; CREA: poor growth and noa cid production
Colony colour: white to cream coloured
Conidiation: sparse
Reverse colour (CZA):
Colony texture: floccose, granular
Conidium size/shape/surface texture: 5–10 µm, subglobose to pyriform, smooth
Homothallic
Cleistothecia: variable in size, spherical, white to cream coloured


**Fig. 8.** *Aspergillus longivesica*. A. Colonies after 10 d at 25 °C on CYA. B–C. Macrophotograph of conidiophores. D–I. Conidiophores. J. Conidia. Scale bars = 10 µm, except D and E = 30 µm.

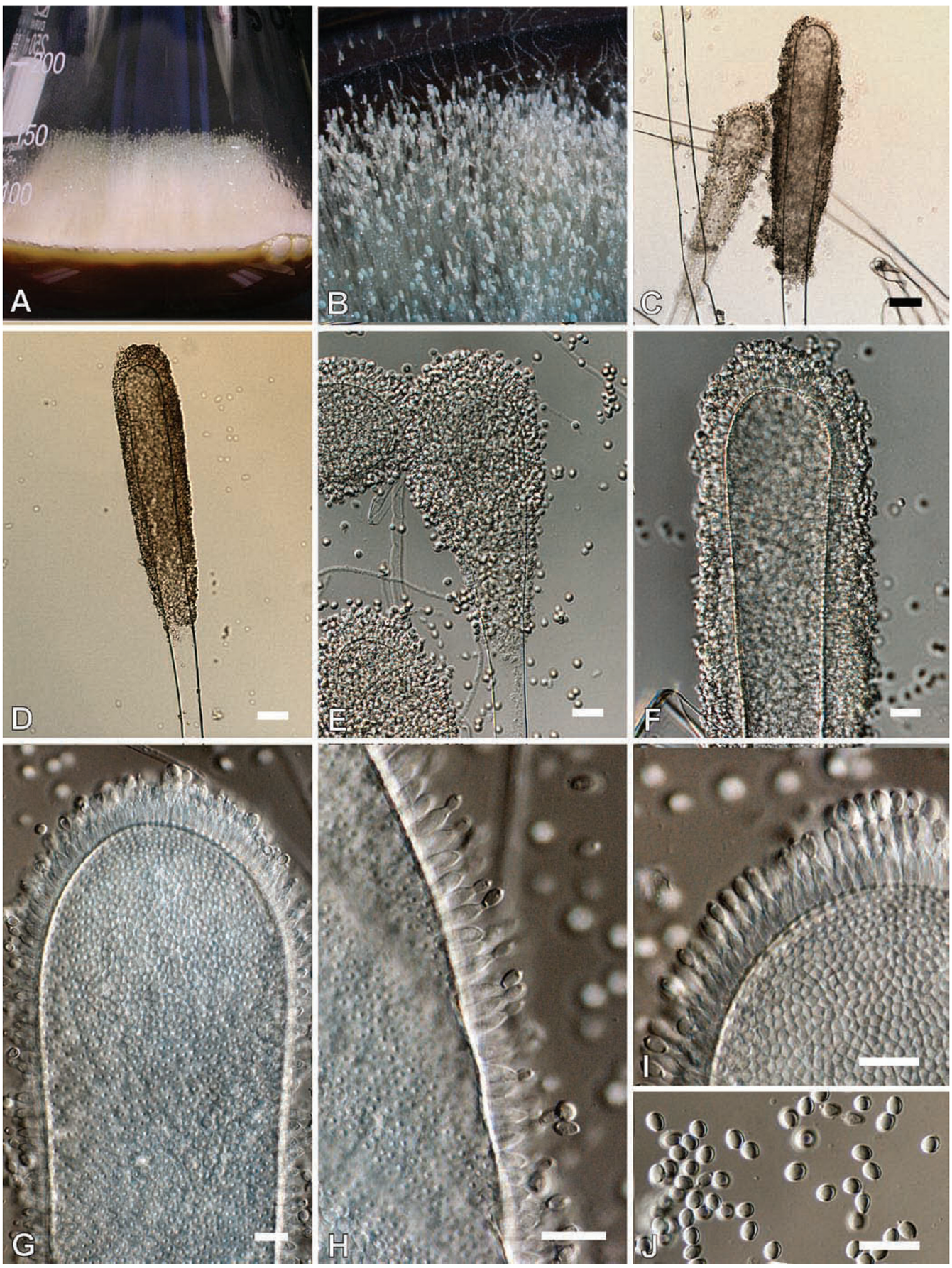


**Fig. 9.** *Aspergillus rhizopodus*. A. Colonies after 10 d at 25 °C on CYA. B. Macrophotograph of conidiophores. C–I. Conidiophores. J. Conidia. Scale bars = 10 µm, except D and E = 30 µm.

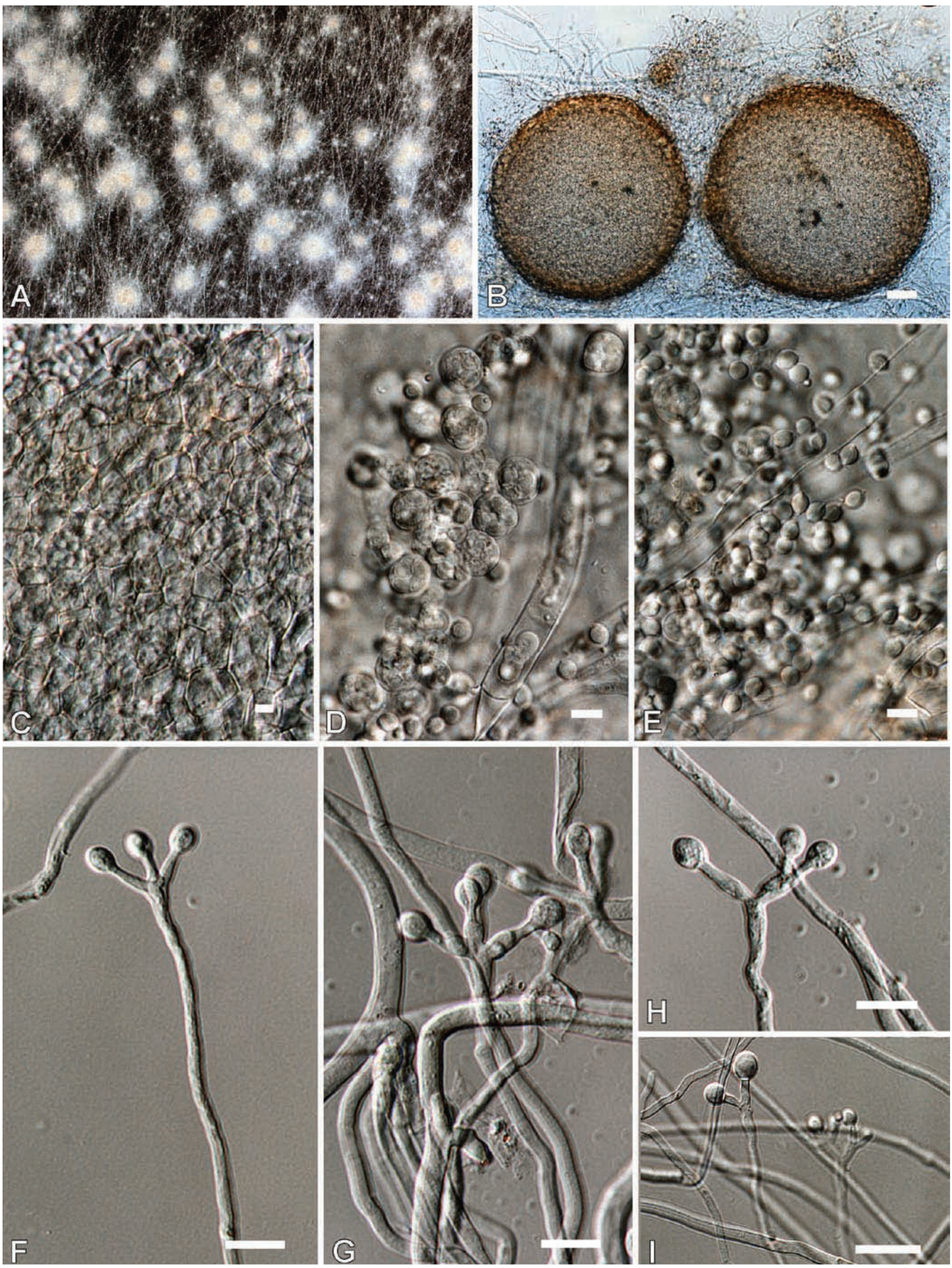


**Fig. 10.** *Dichotomomyces cejpii*. A–B. Ascomata on MEA after 10 d at 25 °C. C. Ascomata wall. D–E. Asci and ascospores. F–I conidiophores and conidia. Scale bars = 10 µm, except B and C = 30 µm.

Ascospores: 3–3.5 × 4–4.5 µm, lenticular, with two closely appressed very thin equatorial crests and convex walls smooth

**Cultures examined:** CBS 761.96, CBS 779.7, CBS 219.67, CBS 100192, CBS 474.77, CSB 780.70, CBS 397.68, CBS 345.68, CBS 159.67, CBS 157.66, CBS 212.50

**Diagnostic features**: conidiophore apices are dichotomously branched, and conidia are produced from these branches (*Polypaecilum* anamorph); racquet hyphae are frequently produced; vegetative hyphae often bear rhizomorphs

**Similar species**: -

**Distribution**: Slovakia, Netherlands, Egypt, U.S.A., South Africa, Pakistan, Japan, Moldova, India

**Ecology and habitats**: soil, compost, pasteurised products

**Extrolites**: gliotoxin (Seigle-Murandi *et al.* 1990, confirmed in this study), tryptoquivalons (found in this study), rubratoxins (found in this study), xanthocillin X (Kitahara & Endo 1981; could not be confirmed in this study), and several metabolites with antibiotic and ciliostatic properties (Pieckova & Roeijmans 1999; Pieckova & Jesenska 1997a, 1997b)

**Pathogenicity**: not reported

**Note:** this species is reported as a heat resistant fungus causing food spoilage (Pieckova *et al.* 1994; Jesenska *et al.* 1993; Mayer *et al.* 2007)

***Neocarpenteles acanthosporus*** (Udagawa & Takada) Udagawa & Uchiyama [anamorph: *A. acanthosporus* Udagawa & Takada], Mycoscience 43(1): 4. 2002.
= *Hemicarpenteles acanthosporus* Udagawa & Takada (1971)

**Type**: CBS 558.71, from soil, Bougainville Island (Solomon Islands), Papua New Guinea

**Other no. of the type**: ATCC 22931; IMI 164621; NHL 2462

**Description**
Colony diam (7 d): CYA25: 37–47 mm; MEA25: 72–85: mm; YES25: 62–82; OA25: 40–49 mm; CYA37: 0 mm; CREA: poor growth and no acid production
Colour: white to brownish orange
Conidiation: sparse
Reverse colour (CYA): greyish-orange
Colony texture: floccose
Conidial head: radiate to loosely columnar
Stipe: (50–)100–400 × 5–12 µm, smooth, septate
Vesicle diam /shape: 10–26 µm, flask shaped
Conidia length/ shape/ surface texture: 4.5–7 µm, globose to subglobose, spinulose
Homothallic
Cleistothecia: 350–1000 × 250–850 µm, sclerotioid, subglobose to ovoid, fawn, covered with dense aerial hyphae
Ascospores: 4–4.5 × 3.5–4 µm, lenticular, with two thin equatorial crests and convex walls ornamented with raised flaps

**Cultures examined:** CBS 558.71, CBS 445.75, CBS 446.75, CBS 447.75

**Diagnostic features**: small dull green readiate conidial heads, short conidiophores with small flask-shaped vesicle, production of ascospores, and large globose conidia distinguish this species from other members of section *Clavati*

**Distribution**: Papua New Guinea (Bougainville Island), Japan

**Ecology and habitats**: soil

**Extrolites:** kotanins, tryptoquivalines, tryptoquivalones (found in this study), ribotoxins (Varga *et al.* 2003). (+)-isoepoxydon has also been reported (Kontani *et al*. 1990)

**Pathogenicity**: not reported

**Note:** not illustrated here, for detailed description and illustration see Udagawa & Takada (1971); no growth at 37 °C

## ACKNOWLEDGEMENTS

We thank Ellen Kirstine Lyhne for technical assistance and the Danish Technical Research Council for financial support to Programme for Predictive Biotechnology.

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