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

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**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. apicillus*, *A. pallidus*), *A. giganteus*, *A. rhizopodus*, *A. longivesica*, Neocarpenetes acanthosporus and *A. clavatanonomicus*. Neocarpenetes acanthosporus is the only known teleomorph of this section. The sister genera to Neocarpenetes are Neosartorya and Dichotomomyces based on sequence data. Species in Neosartorya and Neocarpenetes have anamorphs with green conidia and share the production of tryptoquivalines, while Dichotomomyces were 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, sarms, 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 Hemicarpenetes paradoxus, which is phylogenetically very distantly related to Neocarpenetes and section Clavati.

**Key words:** Ascomycetes, Aspergillus section Clavati, β-tubulin, calmodulin, Dichotomomyces, Eurotiales, Hemicarpenetes, ITS, mycotoxin, Neocarpenetes, 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 sarms (Cole & Cox 1981; Lin et al. 1994). Weisner (1942) and Bergel et al. 1944 reported 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 isolated 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 xanthochillin 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 Neocarpenetes 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; Sarbhy & 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. clavatanonomicus* was added by Batista et al. (1955). After Raper & Fennell (1965) published their monograph on aspergili, 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 *Hemicarpenetes* genus, recently Udagawa & Uchiyama (2002) proposed the new ascomycete genus *Neocarpenetes* 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* (Cannabinaceae), G. Smith                            |
|                          | CBS 114.48 | Culture contaminant, Netherlands                                        |
|                          | CBS 513.65T| 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 356435| 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 515.65T| 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 450.75T| Usar soil, India, Lucknow                                               |
|                          | IMI 351309 | Soil, Yugoslavia                                                       |
| **A. pallidus**          | CBS 344.67T| ATCC 18327; IMI 129967; soil, Moldova                                  |
|                          | SZMC JV6  | Culture contaminant, Hungary                                            |
| **A. clavatonanicus**    | CBS 474.65T| ATCC 12413; IMI 235352; WB 4741; finger nail lesion, Brazil            |
| **A. longivesica**       | CBS 530.71T| ATCC 22434; IMI 156966; soil, Nigeria                                  |
|                          | CBS 187.77 | Soil, Ivory Coast, Tai                                                 |
| **A. apicalis**          | CBS 236.81T| Wheat bran, India                                                       |
| **N. acanthosporus**     | CBS 558.71T| Solomon Islands, Bougainville Island                                    |
|                          | CBS 445.75 | Solomon Islands, Bougainville Island, Buin, Malapita                   |
|                          | CBS 446.75 | Solomon Islands, Bougainville Island, Buin, Batubatua                  |
|                          | CBS 447.75 | Solomon Islands, Bougainville Island, Kietla                            |
| **D. cepii var. cepii**  | CBS 761.96 | spent mushroom compost, Netherlands                                     |
| **D. cepii var. cepii**  | CBS 779.70 | Soil, Cincinnati, U.S.A.                                               |
| **D. cepii var. cepii**  | CBS 100192 | Soil, Bratislava, Slovakia                                             |
| **D. cepii var. cepii**  | CBS 474.77 | Soil, Egypt                                                            |
| **D. cepii var. cepii**  | CBS 780.70 | Pasturised milk, Cincinnati, U.S.A.                                    |
| **D. cepii var. cepii**  | CBS 397.68 | Soil, South Africa                                                     |
| **D. cepii var. cepii**  | CBS 345.68 | rhizosphere of *Hordeum vulgare*, Pakistan                             |
| **D. cepii var. cepii**  | CBS 159.67 | Soil, Kominato, Japan                                                   |
| **D. cepii var. cepii**  | CBS 157.66T| Orchard soil, Moldova, near Tiraspol                                   |
| **D. cepii var. spinosus**| CBS 219.67T| 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. apicilis 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. ingranus 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 Neocarpteles and Neosartorya species to the Dichotomomyces genus using molecular approaches. Although the anamorphs of Dichotomomyces belong to the Polypaecium, ascomata and ascospores of Dichotomomyces species have a similar morphology as those of Neosartorya and Neocarpteles (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 analysed on CYA and YES agar using three agar plugs (Smidsgaard 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. 1997) and improved 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 10² 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...
(synonyms: A. pallidus, A. apicalis, A. giganteus, A. longipes, 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. longipes 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. longipes. 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 phosphodiester 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.
Neighbour-joining tree based on ITS sequence data of Aspergillus section Clavati. Numbers above branches are bootstrap values. Only values above 70% are indicated.

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. clavatonicus, 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. rhizopus and A. longivesica. Another common phenotypic similarity was the alkalophilic tendency already described for A. rhizopus 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 teleomorph 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
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), tryptoquivalines, tryptoquivalines, ascladiol, ribotoxins |
| A. giganteus       | patulin, antafumicin, ascladiol, tryptoquivalones, tryptoquivalines, glyanthrypine, pyripyropen, α-sarcin and other ribotoxins |
| A. longivesica     | patulin, tryptoquivalones, antafumicins, pyripyropen                         |
| A. rhizopodus      | pseudotoxins, dehydrocarolic acid, tryptoquivalines, kotanins, cytochalasins |
| N. acanthosporus   | kotanins, tryptoquivalones, ribotoxins                                       |
| D. cejpii          | gliotoxin, tryptoquivalones, rubratoxins, (xanthocillin X)                  |
antibiotic and ciliostatic properties (Pieckova & Jesenska 1997a, 1997b; Pieckova & Roeljans 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 Penicillum 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 et al. 1979), P. italicum (Ara 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. acanthosporus 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:** soil, cereals, malt, dung

**Extrtolites:** antafumicins, glyanthrypine, kotanin, tryptoquivalins, tryptoquivalones

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

Aspergillus clavatus  Desmazières, Ann. Sci. Nat., Bot. 2: 71, 1834. Fig. 6.

= Aspergillus pallidus Karnychko (1963)
= Aspergillus apicalis Mehrtra & Basu (1976)

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

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

**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: 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 104.45, CBS 105.45, CBS 106.45, CBS 114.48, CBS 513.65, CBS 514.65, CBS 470.91, CBS 116885, CBS 118451, D TO 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

**Extrtolites:** Patulin, cytochalasin E, kotanins, antafumicin, (dehydropyrocarmic 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; Lorette et al. 2003; Gilmore 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 mm 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, CBS 526.65

**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). Carotenoids 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 (7 d): CYA25: 31–51 mm; MEA25: 48–56 mm; YES25: 60–74 mm; OA25: 52–60 mm, CYA37: 0 mm, CREA25: rather good 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 (CZA): colourless

Colony texture: floccose, granular

Conidium size/shape/surface texture: 5–10 µm, subglobose to pyriform, smooth

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

**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

**Dichotomomycos cejpii** (Milko) D.B. Scott, Trans. Brit. Mycol. Soc. 47: 428, 1970. Fig. 10.

= *Talaromyces cejpii* Milko (1964)
= *Dichotomomycos 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 no acid 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 cepii. A–B. Ascomata on MEA after 10 d at 25 °C. C. Ascomata wall. D–E. Ascii and ascospores. F–I conidiophores and conidia. Scale bars = 10 µm, except B and C = 30 µm.
Ascosporas: 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

Extritolites: gliotoxin (Seigle-Murandi et al. 1990, confirmed in this study), tryptoquivalines (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)

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: 35–47 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

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 / surface texture: 4.5–7 μm, globose to subglobose, spinulose

Homothallic

Cleistothecia: 350–1000 × 250–850 μm, sclerotoid, subglobose to ovoid, fawn, covered with dense aerial hyphae

Ascosporas: 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 radiate conidial heads, short conidiophores with small flask-shaped vesicle, production of ascosporas, and large globose conidia distinguish this species from other members of section Clavati

Distribution: Papua New Guinea (Bougainville Island), Japan

Ecology and habitats: soil

Extritolites: 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

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