Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*
A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins

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Abstract

Species in *Penicillium* subgenus *Penicillium* have terverticillate penicilli and are related to the ascomycete genus *Eupenicillium* series *Crustacea,* Many of its species are very common, being associated with stored foods of human beings and other animals, but also with animal dung and building materials, indoor air, and several other habitats. The taxonomy of this group has been regarded to be especially difficult, but here we propose a stable taxonomy of these species based on a polyphasic study of a large number of isolates. 58 species are accepted. Four new species, *P. cavernicola, P. freii, P. marinum* and *P. thymicola* are described and two new combinations are made: *P. melanoconidium* and *P. neoechinulatum.* The species are ordered in natural sections and series, i.e. series that are both ecologically and phylogenetically consistent. The sections are named *Coronata, Chrysogena, Roqueforti, Expansa, Digitata* and *Viridicata* and emended because they differ considerably from the circumscriptions made by Pitt (1979).

Some species with terverticillate penicilli, or rather twice biverticillate penicilli, including *P. arenicola*, *P. scabrosum, P. fennelliae* and *P. lanosum*, are regarded as phylogenetically and phenetically unrelated soil-borne forms and are not treated here. The phenotypic characters used include micro- and macro-morphology, physiology, including growth at 5, 15, 25, 30, 37ºC, growth at 5 % NaCl and 15 % sucrose, and growth inhibition in presence of 1 % propionic acid, nutritional characters, including growth on urea, nitrite and creatine. All species have been analyzed for secondary metabolites (extrolites) and the profiles of these extrolites are highly species specific, and often of high consistency. In general features based on fungal differentiation (morphology and extrolites) are most diagnostic and consistent, but the classification proposed is also supported by the physiological and nutritional characters. The ecology and biogeography of the species is discussed and data on extrolites, both mycotoxins and pharmaceutically active compounds, is listed. Descriptions and colour illustrations of the colonies and micromorphology of the 58 accepted species are given. Keys to the taxa in the various series are given, but for a more detailed electronic database including partial beta tubulin sequences reference is made to http: //www.cbs.knaw.nl/ penicillium.htm.

Introduction

One of the first species to be described in *Penicillium* was *P. expansum*, a species producing terverticillate smooth-walled penicilli, synnemata and capable of producing rot in apples. *P. expansum* nomenclaturally typifies the genus *Penicillium* and has easily been recognized by later taxonomists based on the above characteristics. Even though it was later shown that some of these features are actually variable, such as conidiophore stipe surface occasionally being rough (Raper and Thom, 1949, Berny and Hennebert, 1985) and synnemata not always being produced, *P. expansum* is still regarded by most taxonomists as an unique species. However such phenotypic plasticity has rendered the taxonomy of species with terverticillate penicilli very unstable and obviously features other than micromorphology and the few other characters usually employed are needed to stabilize the taxonomy and recognize and discover species. The introduction of DNA sequencing data has improved the situation, but it is clear that they are more suited for phylogenetic studies and is less satisfactory for classification and identification than phenotypic data.

In this paper we describe, illustrate and key out the 58 species in *Penicillium* subgenus *Penicillium.* As in the majority of fungal taxonomic monographs, a detailed phylogenetic and phenetic study of the spe-

cies is not presented, but will be published in additional papers in this volume and elsewhere as these two aspects, cladification and classification, are absolutely necessary in future monographs.

History

Several species of subgenus *Penicillium* were described by Dierckx (1901), Thom (1906, 1910), Westling (1911), Biourge (1923) and Zalesky (1927). Despite treatments of some of those species by Thom (1930) and Niethammer (1949), the first effective synthesis of the species was written by Raper and Thom (1949). They placed these species in their subsections *Asymmetrica-Lanosa*, *-Funiculosa*, *-Velutina* and *-Fasciculata*, with one species, *P. olsonii*, being placed in *Biverticillata-Symmetrica*. Abe (1956) mostly followed Raper and Thom (1949) and described some new varieties. Fassatiova (1977) also treated many of the fasciculate species in her emended description of the series *Expansa*. Samson *et al*. treated most of the terverticillate species in three studies (1976, 1977a & b) and Ramirez (1982) followed their mainly micromorphologically based taxonomy. Pitt (1973; 1979) reintroduced some physiological characters, such as growth rates at different temperatures and water activities and gathered the terverticillate Penicillia with flask shaped phialides in subgenus *Penicillium*. He placed *P. gladioli* as a synonym of *Eupenicillium crustaceum* and *P. sclerotigenum* in subgenus *Furcatum*, and included *P. fennelliae* and *P. lanosum* in subgenus *Penicillium*. This overall concept of subgenus *Penicillium* is very similar to the present day placement of species in the subgenus (as presented by Frisvad *et al*., 2000) or sequence based ribosomal DNA phylogeny (Peterson, 2000). The series classification of Pitt and Cruickshank (1990) based on colony diameters and micromorphology is, however, very different from that of Frisvad *et al*. (2000).

Secondary metabolites (extrolites), often recognisable as diffusible colours, colony reverse colours and exudate colours, have played a special role in fungal taxonomy. Usually colours, especially conidium colour, are regarded as part of morphology. These colours can be subdivided into melanin and protein melanin complexes that give fungal conidia their physical strength, hardiness and radiation protection *and* other colours (and volatiles) that often acts as ecological signals (Wicklow, 1986). Raper and Thom (1949) mentioned citrinin as a common extrolite in several *P. citrinum* strains, but did not ascribe any taxonomic value to it. Ciegler *et al*. (1973) used extrolites in their subdivision of one species, *P. viridicatum*, but concluded that" production of similar metabolic products does not provide an adequate basis for recognition of a new taxon", based on the advice of K.B. Raper. Frisvad (1981) was the first to suggest that extrolites could be used directly in *Penicillium* taxonomy and this was followed up by two studies on many of the species in subgenus *Penicillium* (Frisvad and Filtenborg, 1983; 1989, 1990a), where it was shown that extrolites are of particularly high value in a taxonomic sense (Frisvad *et al*., 1998). Later a series of studies with increasingly advanced instrumentation has confirmed the value of both non-volatile and volatile extrolites in taxonomy (Lund and Frisvad, 1994; Svendsen and Frisvad, 1994; Larsen and Frisvad, 1995 a & b; Smedsgaard and Frisvad, 1996).

Extracellular enzyme production was suggested for use in *Penicillium* taxonomy by Frisvad (1981). Profiles of isozymes were introduced by Cruickshank and Pitt (1987a & b) for subgenus *Penicillium*, but were later shown to be difficult to reproduce (Paterson *et al*., 1989). In some cases, isozyme profiles supported synonymies accepted by Samson *et al*. (1976) and Frisvad and Filtenborg (1983), e.g. the synonymy of *P. resticulosum* with *P. expansum* (Cruickshank and Pitt, 1987a), but rejected by Pitt (1979), in other cases for example the claimed synonymy of *P. aurantiovirens* with *P. expansum* (Pitt & Cruickshank, 1990) proved to be incorrect. In general the isozyme profiles appear to support the species series suggested in this paper. Isozyme profiles showed that *P. brevicompactum* and *P. olsonii* were closely related (Cruickshank & Pitt, 1987) in agreement with our emended series *Olsonii*, still Pitt and Cruickshank (1990) placed *P. brevicompactum* in series *Urticicola* and *P. olsonii* in series *Olsonii*. Using a more detailed protocol than that of Cruickshank (1983) and Cruickshank and Wade (1980), Banke *et al*. (1997) were able to classify isolates into species in the series *Chrysogena*. It seems that detailed analyses are needed to achieve resolution at the species level (Rosendahl and Banke, 1998). The latter authors also emphasize that variation within a species and statistics need to be considered. Filtenborg *et al*. (1996) suggested that extracellular enzymes may play an important role in the specific association of fungal species with their habitat, so these methods appear to be promising for future polyphasic taxonomic investigations.

Bridge *at al*. (1989 a & b) attempted to classify the terverticillate Penicillia by using a phenotypic approach. Their results were difficult to evaluate, because many isolates clustered tightly, even though they were actually very different. For example isolates of *P. expansum* and *P. aethiopicum* clustered even though they have no extrolites in common, while distinct taxa such as *P. coprophilum* had isolates placed in several different clusters (Frisvad and Filtenborg, 1989).

Table 1. Number of species accepted in different monographic treatments of *Penicillium* subgenus *Penicillium* (*P. arenicola, P. duclauxii, P. echinosporum, P. fagi, P. fennelliae, P. giganteum, P. isariiforme, P. kojigenum, P. lanosum, P. lavendulum, P. namyslowskii, P. oxalicum, P. pallidum, P. paxilli, P. putterillii*, and *P. skjabinii* not included).

Authors	Number of taxa accepted	New taxa described (accepted here)
Dierckx (1901)	14	10 (4)
Westling (1911)	31 (7?)	11 (4)
Biourge (1923)	64	23 (0)
Zaleski (1927)	-	10 (2)
Thom (1930)	64	7 (1)
Niethammer (1949)	64	0 (0)
Raper and Thom (1949)	43	1 (1)
Abe (1952)	-	6 (0)
Samson et al. (1976, 1977 a&b)	22	6 (1)
Fassatiova (1977)	-	4 (1)
Pitt (1979)	23	1 (0)
Ramirez (1982)	36	5 (0)
Bridge *et al*. (1989)	28	2 (0)
Frisvad & Filtenborg (1989)	38	12 (9)*
Pitt & Cruickshank (1990)	23	0 (0)
Frisvad et al. (2000)	50	2 (2)
Present work	58	6 (6)

* The three taxa not accepted here are two new combinations, one new variety was a synonym. Of the remaining nine taxa two were described as new species and seven have now been raised to species status.

Skouboe *at al*. (1996, 1999; 2000) and Boysen et al. (1996) sequenced the ITS1 and ITS2 region, including the 5.8 S region, of several terverticillate Penicillia and found rather few sequence differences among the species. *P. roqueforti, P. carneum* and *P. paneum* were quite different from the remaining species (Boysen et al., 1996), while morphologicaly different species such as *P. solitum* and *P. echinulatum* has no differences at all in this region (Skouboe *et al*., 2000).

Peterson (2000) also found few differences between terverticillate *Penicillium* species in the ribosomal DNA regions. Clearly the ribosomal DNA gene has too few informative differences to reveal the phylogeny of these Penicillia. Seifert & Louis-Seize (2000) used a part of the β-tubulin (exsons 3-6 of Ben A) gene to indicate a more resolved phylogeny of series *Viridicata* and related species. More than one gene may be necessary to elucidate the phylogeny of the terverticillate Penicillia, but at this point in time the β-tubulin gene seems to be most promising for a one-gene phylogeny.

The number of taxa accepted in these different taxonomic treatments is listed in Table 1. The number of species has had two peaks, one around Biourge (1923) and Thom (1930) and the next in the present work. Biourge (1923) was particularly unsuccessful in describing new species that have been accepted in this study, not one of his 23 new species is accepted here. 23 of the species accepted here have been described recently.

Materials and Methods

Isolates examined:
As many isolates as possible of each species were investigated in order to determine the variability of each taxon. Cultures ex type were always examined, but occasionally these were not in good condition after many years of maintenance in culture collections. Therefore typical cultures have been included for comparison and verification of identity of newly identified isolates. They are indicated with an **Y** in the description of each taxon. Eight isolates of each taxon were examined in depth. These are listed after the description of each species. Some species are presently only represented by one isolate as yet, including *P. formosanum* and *P. confertum*.

Media and incubation
The media were all modified by adding coppersulphate and zink-sulphate to ensure proper development of the green pigmentation of the conidial colour in *Penicillium* isolates (Smith, 1949; Filtenborg *et al*., 1990). All fungi were grown on the following media (all percentages are weight/volume):

Czapek-Dox (Cz) agar (Raper and Thom, 1949):

NaNO$_3$	0.3 %
Sucrose	3.0%
K$_2$HPO$_4\cdot$3H$_2$O	0.13%
MgSO$_4\cdot$7H$_2$O	0.05%
KCl	0.05%
FeSO$_4\cdot$7H$_2$O	0.001%
CuSO$_4\cdot$5H$_2$O.	0.0005%
ZnSO$_4\cdot$7H$_2$O	0.001%
Agar	1.5%

Distilled water, pH 6.3 ± 0.2

Czapek Yeast Autolysate (CYA) agar (Pitt, 1979):

NaNO$_3$	0.3%
Yeast extract (Difco) .	0.5%
Sucrose	3.0%
K$_2$HPO$_4$·3H$_2$O	0.13%
MgSO$_4$·7H$_2$O	0.05%
KCl	0.05%
FeSO$_4$·7H$_2$O	0.001%
CuSO$_4$·5H$_2$O .	0.0005%
ZnSO$_4$·7H$_2$O .	0.001%
Agar	1.5%

Distilled water, pH 6.3 ± 0.2

Blakeslee Malt Extract Autolysate (MEA) agar:(Raper and Thom, 1949)

Malt extract (Difco)	3.0%
Bacteriological peptone	0.1%
Glucose	2.0%
CuSO$_4$·5H$_2$O	0.0005%
ZnSO$_4$·7H$_2$O	0.001%
Agar	2.0%

Distilled water, pH 5.3 ± 0.3

2% malt extract (ME2) agar (Samson et al., 2002):

Malt extract	2.0%
CuSO$_4$·5H$_2$O	0.0005%
ZnSO$_4$·7H$_2$O	0.001%
Agar	1.5%

Distilled water, pH 5.4 ± 0.3

Oat meal (OAT) agar (Samson et al., 2002):

Oat meal	3.0%
CuSO$_4$·5H$_2$O	0.0005%
ZnSO$_4$·7H$_2$O	0.001%
Agar	1.5%

Distilled water

Yeast extract sucrose (YES) agar (Frisvad, 1981; Filtenborg et al., 1990):

Yeast extract (Difco)	2.0%
Sucrose	15.0%
MgSO$_4$·7H$_2$O	0.05%
CuSO$_4$·5H$_2$O.	0.0005%
ZnSO$_4$·7H$_2$O.	0.001%
Agar	2.0%

Distilled water

Creatine sucrose (CREA) agar (Frisvad, 1981, 1985, 1993):

Creatine·1H$_2$O	0.3 %
Sucrose	3.0%
K$_3$PO$_4$·7H$_2$O	0.16%
MgSO$_4$·7H$_2$O	0.05%
KCl	0.05%
FeSO$_4$·7H$_2$O	0.001%
CuSO$_4$·5H$_2$O.	0.0005%
ZnSO$_4$·7H$_2$O.	0.001%
Bromecresol purple	0.005%
Agar	1.5%

Distilled water, Adjust pH to 8.0 ± 0.2

UNO agar (new medium suggested here):

Urea	0.1%
NaNO$_2$	0.1%
Glucose	3.0%
K$_2$HPO$_4$·3H$_2$O	0.13%
MgSO$_4$·7H$_2$O	0.05%
FeSO$_4$·7H$_2$O	0.001%
CuSO$_4$·5H$_2$O	0.0005%
ZnSO$_4$·7H$_2$O	0.001%
Bromecresol purple	0.005%
Dichloran	0.0002%
Agar	1.5%

Distilled water, pH 6.3± 0.2

Czapek yeast autolysate with 5 % NaCl (CYAS) agar (medium suggested here):

NaNO$_3$	0.3%
NaCl	5.0%
Yeast extract (Difco)	0.5%
Sucrose	3.0%
K$_2$HPO$_4$·3H$_2$O	0.13%
MgSO$_4$·7H$_2$O	0.05%
KCl	0.05%
FeSO$_4$·7H$_2$O	0.001%
CuSO$_4$·5H$_2$O .	0.0005%
ZnSO$_4$·7H$_2$O .	0.001%
Agar	1.5%

Distilled water, pH 6.3 ± 0.2

Czapek with 1000 ppm propionic acid (CzP) agar (modified after Frisvad, 1981):

Sodium propionate .	0.1375%
NaNO$_3$	0.3 %
Sucrose	3.0%
K$_2$HPO$_4$·3H$_2$O	0.13%
MgSO$_4$·7H$_2$O	0.05%
KCl	0.05%
FeSO$_4$·7H$_2$O	0.001%
CuSO$_4$·5H$_2$O .	0.0005%
ZnSO$_4$·7H$_2$O .	0.001%
Agar	2.0%

Distilled water, after autoclaving adjust pH to 3.8 ± 0.1

Czapek with 50 ppm benzoic and sorbic acid (CzBS) agar (modified after Frisvad, 1981)

Sodium benzoate	0.009%
Potassium sorbate	0.0067%
NaNO$_3$	0.3 %
Sucrose	3.0%
K$_2$HPO$_4$·3H$_2$O	0.13%
MgSO$_4$·7H$_2$O	0.05%
KCl	0.05%
FeSO$_4$·7H$_2$O	0.001%
CuSO$_4$·5H$_2$O	0.0005%
ZnSO$_4$·7H$_2$O	0.001%
Agar	2.0%

Distilled water, after autoclaving adjust pH to 3.8 ± 0.1

Nitrite sucrose (NO2) agar (Abe, 1956; Frisvad, 1981):

NaNO$_2$	0.3 %
Sucrose	3.0%
K$_2$HPO$_4$·3H$_2$O	0.13%
MgSO$_4$·7H$_2$O	0.05%
KCl	0.05%
FeSO$_4$·7H$_2$O	0.001%
CuSO$_4$·5H$_2$O .	0.0005%
ZnSO$_4$·7H$_2$O .	0.001%
Agar	2.0%

Distilled water, pH 6.3 ± 0.2

Raulin-Thom (RT) agar (Raper and Thom, 1949)

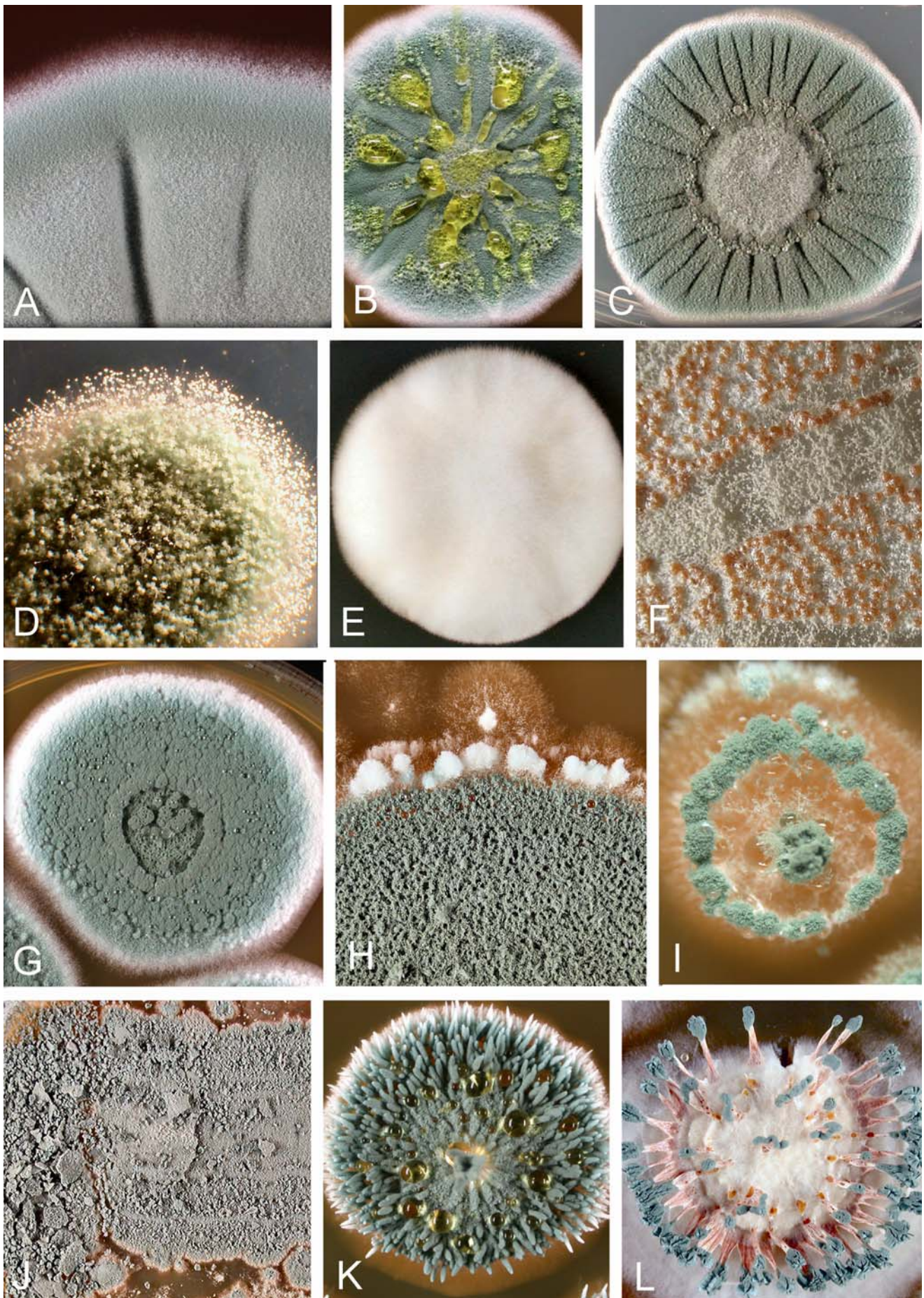|	
Ammoniumtartrate	0.26%
(NH$_4$)H$_2$PO$_4$	0.04%
(NH$_4$)$_2$SO$_4$	0.016%
Sucrose	3.0%
Tartaric acid	0.26%
Magnesium(OH)carbonate	0.028%
K$_2$CO$_3$	0.04%
FeSO$_4$·7H$_2$O	0.006%
CuSO$_4$·5H$_2$O .	0.0005%
ZnSO$_4$·7H$_2$O .	0.007%
Agar	2.0%

Distilled water

Fig. 1. A-B velvety colony of *P. persicinum* and *P. chrysogenym,* B. typical yellow exudate in *P. chrysogenum*, C. velvety colony of *P. commune* later becoming more fasciculate*,* D. large and compact conidial heads of *P, brevicompactum* E. floccose colony in *P. camemberti,* F. sclerotia in *P. olsonii,* G-I. fasciculate colonies of *P. expansum,* J. crusts of conidial masses of a 10 day old colony of P. crustosum, K-L. synnematous growth in *P. clavigerum and P. vulpinum*

Cultures were three-point inoculated on media in 9 cm plastic Petri dishes using a dense conidium suspension and incubated in the dark at 25°C, except where otherwise noted. Depending on the ventilation in the incubators, Petri dishes were incubated uncovered or in perforated plastic bags to retard drying out of the media. The fungi were also grown at 15, 30 and 37°C on CYA. The cultures were examined after 7 days of growth and further examined after 14 days. Colony diameters were measured using a ruler and colours were measured using a Minolta colourimeter and also subjectively evaluated. Fasciculation of the colonies was evaluated using a scale from 1 to 4.

All species were examined using oil immersion with an Olympus BHH microscope with Normarski interphase contrast at up to 1000 x magnification. Digital micrographs were taken with a Nikon Coopix 990 and 995.

Microscopic slides were prepared from malt based media (MEA and ME2) and 60 % lactic acid without colour dye was used as a mounting medium.

Morphology and other phenotypic characters

Colony patterns and growth
Colonies in species of subgenus *Penicillium* have various patterns. When freshly isolated, these patterns are consistent but the typical features may be lost after regular transferring and maintaining of the cultures. The following colony pattern can be found:

Velvety (Fig. 1 A-B). Conidiophores are produced singly and form a compact felt. Typical velvety taxa are: *P. persicinum*, *P. chrysogenum*, *P. aethiopicum*. In *P. brevicompactum* and *P. olsonii*, the colonies are velvety but the conidial heads are large and compact and resemble *Aspergillus* heads, which make the colony appearance more or less granular (**Fig. 1 D**).

Floccose (Fig. 1 E): *P. camemberti* and some strains of *P. nalgiovense* have colonies with white aerial and fluffy mycelium

Fasciculate (Fig. 1 G-I): Fasciculation occurs when conidophores are bundled together forming small tufts. These mostly are found at the edges of colonies. Typical fasciculate species are *P. expansum* and *P. concentricum.*

Synnematous (Fig. 1 K-L): The conidiophores are defined as synnematous when they consist of a distinct stalk and a head such as in *P. vulpinum, P. clavigerum*, *P. coprobium* and *P. formosanum.* Synnematous growth largely depends on the medium and typical synnema can often be found on OA or on MEA. In cultures fasciculate conidiophores can also be found repeatedly.

Crustose (Fig. 1 J): Fresh isolates of *P. crustosum* form a crust of conidial masses when they are 7 days and older. This character is typical for the species and can be used as an aid for identification.

Exudate: Several species produce distinct exudates droplets e.g. yellow in *P. chrysogenum* (**Fig 1 B**) and dark brown in *P. venetum.*

Reverse: Various pigments are more or less typical for the species. On YES agar the reverse colours are particularly pronounced. Colours vary from uncoloured, cream to yellow, yellow to brown or red

Conidiophores: In culture mature conidiophores are produced in 5-7 days old colonies. The penicillus of species of subgenus *Penicillium* are typically two staged branched (terverticillate). However, in some taxa the penicillus is often biverticillate. In other species, more branches are present and quaterverticillate conidiophores can be formed. *P. digitatum* deviates from the typical conidiophore branching, because it is often irregular and only biverticillate. It is important that conidophore branching and its elements can be best seen in microscopical slides made from MEA. On CYA, YES and other media the conidophores are often swollen and have an atypical branching pattern.

Stipe (Fig. 2 N-R): The stipes of most taxa are straight. Curved stipes are typical for *P. vulpinum* and *P. clavigerum.* The stipe of the conidiophore can either be smooth, rough-walled or tuberculate (warty). Typical smooth stipes are found in for example *P. mononematosum*, *P. vulpinum* and *P.olsonii*. In *P. chrysogenum* and *P. expansum* mostly smooth walled stipe are present, but some strains have rough-walled stipes. Rough-walled to echinulate stipes are typical for *P. glandicola* and *P. hirsutum*. Typical tuberculate stipes are found in *P. roqueforti, P. paneum* and *P. carneum.*

Often stipe ornamentation depends on the media and age of the culture. On Czapek and YES agar the conidiophores often do not have ornamented stipes, but they are produced on MEA. We have also observed that stipe ornamention depends on the availability of oxygen. In Petri dishes that are sealed with parafilm or in closed polyethylene bags the ornamentation is sometimes completely lacking. The lack of ornamentation in certain growth conditions is often evident in *P. roqueforti* cultures.

Phialides (Fig. 2 I-M): In subgenus *Penicillium,* the phialide shape can differ. Mostly the phialides are flask shaped consisting of a more or less cylindrical basal part with a short neck. The collarette of this neck can become thickened when conidia are produced. In *P. digitatum, P. ulaiense* and *P. italicum*, the cylindrical shape is more pronounced and the

phialides are longer. In *P. griseofulvum* and *P. dipodomyicola* the phialides are typical short.

Conidia (Fig. 2 A-H): Most taxa in subgenus Penicillium have globose smooth-walled conidia. Mainly ellipsoidal conidia are found in *P. formosanum* and *P. expansum*, while in *P. italicum, P. ulaiense,* and *P. persicinum,* they are typical cylindrical.

Sclerotia (Fig 1. F): In most species sclerotia are not produced. Only in *P. gladioli* and *P. sclerotigenum* are sclerotia present, while some isolates of *P. olsonii* from tropical soil also produce sclerotia. In 3 week old MEA colonies of *P. roqueforti,* soft sclerotium-like structures can be sometimes observed. In *P. italicum* large, white sclerotia at the margin of colonies growing on OA have been observed in cultures incubated in the dark at 0°C for three months. In old colonies of *P. persicinum,* sclerotia have been observed (Wang Long, personal communication). Sclerotial production is soon lost when cultures are transferred

Table 2. Degree of sporulation on YES after one week at 25ºC: 0: None or very thin and poor sporulation, 1: Sporulation in the centre of the colony, 2: Strong sporulation on more than 90% of the colony

Species	Sporulation	Reverse colour
P. aethiopicum	2	Yellow to curry yellow
P. albocoremium	0	Brownish yellow / orange
P. allii	2	Yellow brown to warm brown
P. atramentosum	2	Yellow brown to dark brown
(*P. aurantiocandidum*)	0	Yellow
P. aurantiogriseum	0/1/2	Yellow
P. bialowiezense	2	Cream to cream beige
P. brevicompactum	2	Cream to cream beige*
P. camemberti	0	Cream yellow
P. carneum	2	Cream beige
P. caseifulvum	2/(1)	Orange or orange yellow
P. cavernicola	2/(1)	Yellow to yellow orange
P. chrysogenum	2	Citrine yellow
P. clavigerum	0	Light to dark yellow brown
P. commune	0/1/(2)	Cream to cream yellow **
P. concentricum	2	Orange
P. confertum	2	Yellow cream to curry
P. coprobium	2	Yellow cream to yellow brown
P. coprophilum	2	Yellow brown to dark brown
P. crustosum	2	Yellow
P. cyclopium	0/(1)	Yellow

P. digitatum	2 (0 in old strains)	Cream yellow
P. dipodomyicola	2	Yellow olive to dark olive
P. dipodomyis	2	Orange to orange yellow
P. discolor	2	Orange to vivid orange red
P. echinulatum	2	Yellow
P. expansum	2/1/0	Cream yellow to orange brown
P. flavigenum	2	Citrine yellow
P. formosanum	0	Yellow to yellow orange
P. freii	0/(1)	Yellow
P. gladioli	0	Cream yellow **
P. glandicola	2	Orange red to red
P. griseofulvum	2	Cream yellow to beige
P. hirsutum	2/(0)	Orange yellow
P. hordei	0	Yellow
P. italicum	2	Orange to red brown
(*P. lumpi*)	0/1	Cream
P. marinum	1/(2)	Cream yellow
P. melanoconidium	2	Yellow
P. mononematosum	2	Cream to brown yellow
P. nalgiovense I	0	Dark yellow brown
P. nalgiovense II	2	Orange
P. nordicum	0/1/2	Cream to cream yellow
P. neoechinulatum	0	Yellow
P. olsonii	2	Yellow to yellow cream
P. palitans	2	Yellow
P. paneum	2	Cream yellow to beige*
P. persicinum	2	Red, with colour diffusing
P. polonicum	2	Yellow
P. radicicola	0/((1))	Deep to butter yellow
P. roqueforti	2	Dark blackish green
P. sclerotigenum	2	Cream yellow
P. solitum	2/1/0	Yellow to orange yellow
P. thymicola	0/1/2	Yellow to orange, diffusing
P. tulipae	0/1/2	Deep yellow to yellowish orange
P. tricolor	0	Brown yellow to honey
P. ulaiense	2	Cream yellow to brown
P. venetum	2	Yellow brown
P. verrucosum	0/1	Red brown to terracotta
P. viridicatum	0/1/(2)	Yellow
P. vulpinum	0/(2)	Cream yellow to beige

* May become strawberry red with colour diffusing into the agar; ** Often turn to dark blackish brown with colour diffusing into the agar
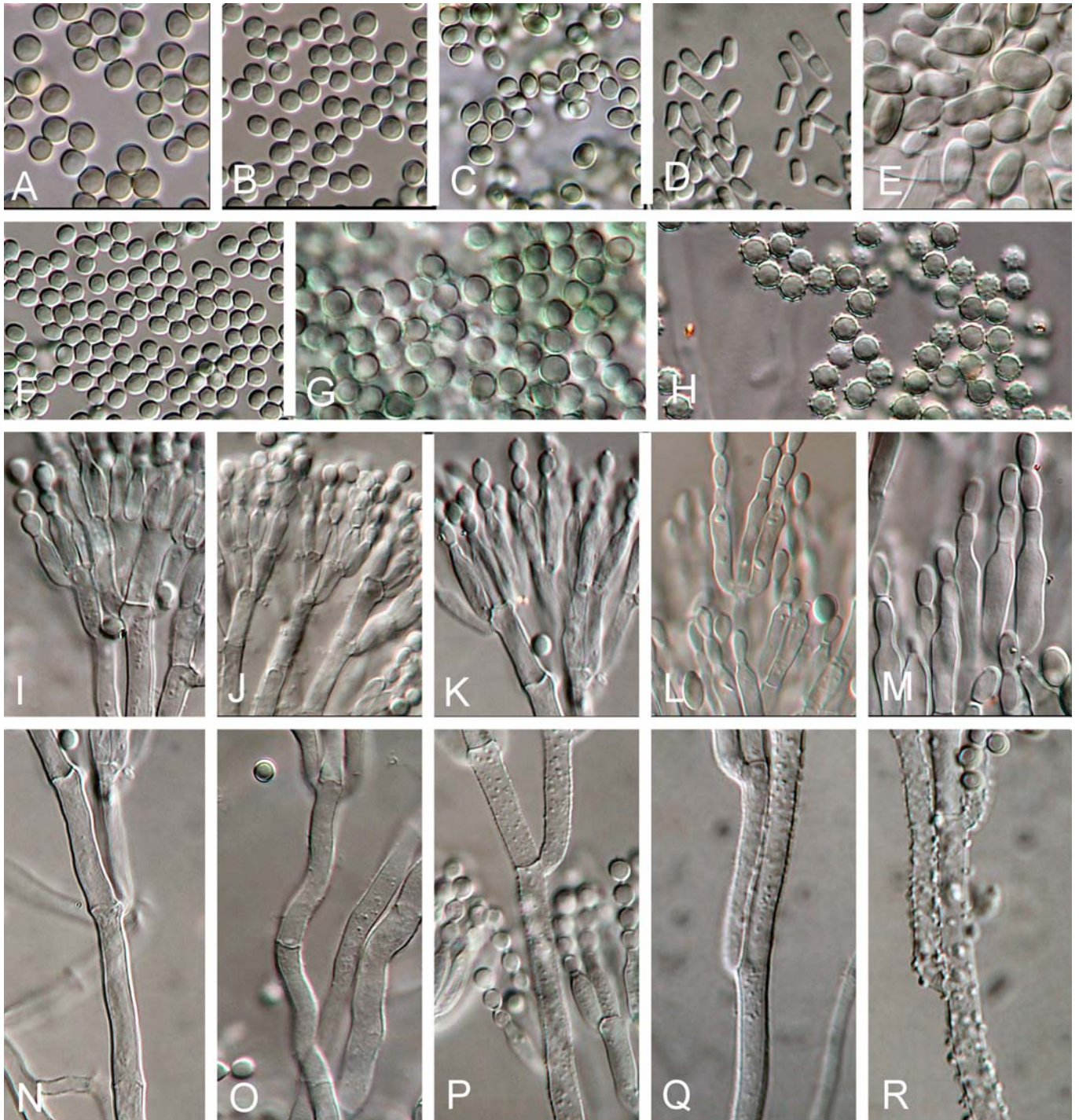
Fig 2. Morphological structures in *Penicillium* subgenus *Penicillium*. A-H. Conidia, A. Smooth, globose conidia in *P. roque-forti,* B. Globose to subglobose in *P. cyclopium,* C. ellipsoidal in *P. expansum,* D. cylindrical in *P. persicinum,* E. ellipsoidal to cylindrical in *P. digitatum,* F. subglobose to ellipsoidal in *P. confertum,* G. rough-walled conidia *P. discolor,* H. echinulate conidia in *P. echinulatum*. I-M. Phialide shape. I. flash-shaped in *P. chrysogenum*, J. flash-shaped but short in *P. griseofulvum*, K. flask-shaped but more elongated in *P. expansum,* L-M. phialides more or less cylindrical in *P. ulaiense* and P. *digitatum*, Conidiophore stipe, N. *P. expansum,* P. *P. clavigerum,* Q. *P. tulipae,* R. *P. roqueforti,* S. *P. glandicola*

Ehrlich test

All isolates were examined for production of cyclopi-azonic acid and other alkaloids reacting with Ehrlich reagent (Lund, 1995a) using a filter paper method. Ehrlich reagent consists of 2 g of 4-dimethylamino-benzaldehyde in 96% ethanol (85 ml) added 15 ml 10 N HCl. A *ca.* four mm agar plug is cut out of the center of a colony grown on CYA (incubated 5-9 days at 25ºC) and a round piece (1 cm diam.) of the wetted filter paper (Whatman No. 1) is placed on the mycelial side of the plug. If a violet ring appears after 2- 6 min. the culture contains cyclopiazonic acid or related alkaloids (**Fig. 4**). If the reaction comes after 7-10 min. it is regarded as weak. After 10 min the violet ring will fade away. Some fungi produce alkaloids that will react with Ehrlich reagent to give pink to red or yellow rings.
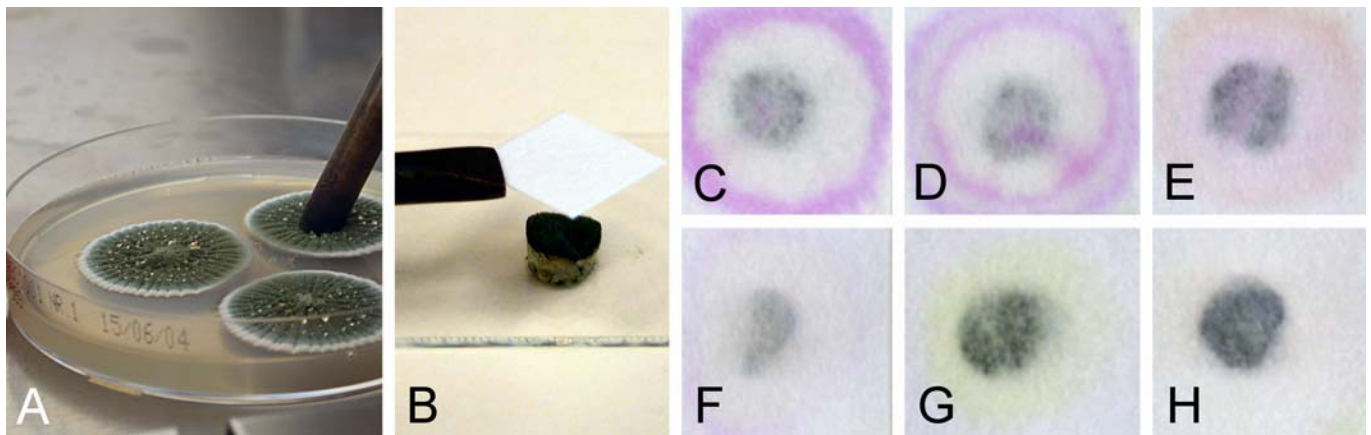
Fig 3. Ehrlich colour reactions. A. Taking a plug from a *Penicillium* colony. B. Adding a piece of filterpaper with Ehrlich solution. C. violet in *P. expansum*, D. violet in *P. palitans*, E. red brown in *P. allii*, F. weak violet in *P. discolor*, G. yellow in *P. olsonii*, H. no reaction in *P. italicum*

Table 3. Ehrlich results on CYA after one week at 25ºC

Species	violet	notes
P. albocoremium	(+)	
P. bialowiezense	(+)	
P. camemberti	(++)	(0 in old deteriorated cultures)
P. carneum	(+/++)	
P. caseifulvum	(+)	
P. commune	(++/+++)	
P. dipodomyicola	(+++)	
P. discolor	(+)	
P. expansum	(++/+++)	
P. griseofulvum	(+/++)	(0 in old deteriorated cultures)
P. hirsutum	(+/++)	(0 in some cultures)
P. hordei	(+)	
P. marinum	(++)	(0 in some mutants)
P. neoechinulatum	(+++,	red violet)
P. palitans	(+++)	
P. polonicum	(++/+)	
P. radicicola	(w/+)	
P. roqueforti	(+/++)	(yellow in some cultures)
P. tulipae	(+/++)	

Pink (to red) reaction		
P. allii		
P. aurantiogriseum		
P. cyclopium		(red brown to pink to yellow brown)
P. freii		(pink red)
P. lumpi		
P. melanoconidium		
P. viridicatum		(also yellow to brown)

Yellow reaction:

P. clavigerum		(yellow to violet in some cultures)
P. nordicum		(yellow green)
P. olsonii		
(*P. scabrosum*)		
P. thymicola		
P. viridicatum		(yellow pink brown)

Always negative (occasionally yellow reaction

P. aethiopicum	
P. atramentosum	
P. brevicompactum	(some cultures yellow, or faint yellow)
P. cavernicola	(yellow in some cultures)
P. chrysogenum	(some cultures yellow or faint yellow)
P. clavigerum	(**yellow, yellow to violet** in some cultures)
P. concentricum	(some cultures yellow, or faint yellow)
P. confertum	(faint yellow)
P. coprobium	(some cultures faint yellow)
P. coprophilum	
P. crustosum	(occasionally faint yellow)
P. digitatum	
P. dipodomyis	
P. echinulatum	
P. flavigenum	
P. formosanum	
P. gladioli	
P. glandicola	(some cultures yellow++ or faintly yellow)
P. italicum	
P. mononematosum	
P. nordicum	(**yellow green**)
P. nalgiovense	(occasionally faint yellow)
P. olsonii	(**yellow**)
P. paneum	(some cultures faintly violet)
P. persicinum	
P. sclerotigenum	(occasionally faintly yellow)
P. solitum	
P. thymicola	(**yellow green**)
P. ulaiense	
P. tricolor	
P. venetum	
P. verrucosum	(yellow in some cultures
P. vulpinum	(some cultures yellow or faintly violet)

9

Extrolite analysis
CYA and YES were used for extrolite analysis. Agar plugs (6 mm diameter) were cut out of 7 days old cultures and kept in a – 18°C freezer until extraction. The cultures were extracted according to the method of Smedsgaard (1987) using 500 µl ethylacetate / methanol / dichloromethane 3:2:1 (vol. / vol. / vol.) with 1 % formic acid and ultrasonicated for 10 minutes. The organic solvent was transferred to another vial and evaporated at 1 mbar in a Rotavapor centrifuge evaporator. The extract was redissolved in 400 µl methanol and analysed by HPLC with diode array detection (DAD) or electrospray mass spectrometric detection (ES-MS) (Frisvad and Thrane, 1987; 1993 and Smedsgaard, 1997; Nielsen and Smedsgaard, 2003). The extrolites were identified by their UV spectra and MS characteristics. Authentic analytical standards were employed for retention time and retention index comparison with the extrolites detected.

Taxonomy

Delimitation of *Penicillium* subgenus *Penicillium*
Penicillium subgenus *Penicillium* comprises species with terverticillate (two stage branched) conidiophores (**Fig. 4**). They all sporulate heavily and are often fasciculate. However the subgenus also appears to be a natural group, i.e. it is both phylogenetically and ecologically distinct. All species are related to animal nutrition and excretion and mans domesticated landscapes, e.g. they are found growing and sporulating on plant, algal, animal or fungal raw or processed materials and in dwellings of man and other animals. They all grow well at low temperatures and poorly, if at all, at 37°C. They also grow well at low water activities and at low pH values (Pitt and Hocking, 1998; Frisvad *et al*., 2000).

Excluded taxa
Some species in other subgenera with similar penicilli or ecology are excluded from subgenus *Penicillium* for the reasons discussed below.

Species in the other subgenera are mainly soil or plant root associated (subgenus *Aspergilloides* and *Furcatum*) or are often associated with wood and textiles (subgenus *Biverticillium*). These ecologically based subdivisions are strongly supported by DNA sequence data (Peterson, 2000). DNA sequence data also indicate that most species in *Eupenicillium* series *Crustacea*, except *E. shearii*, are related to *P. chrysogenum* (Peterson, 2000). Some species e.g. *Eupenicillium crustaceum E. egyptiacum* and *E. molle* (**Fig. 5**) have many terverticillate structures, however, and yet should be included in subgenus *Penicillium*. In the present revision we have not included the *Eupenicillium* species because a major revision using a

polyphasic approach and mulilocus DNA sequences is needed to resolve the taxonomy of this teleomorph genus. Occasionally biverticillate species include *P. digitatum*, *P. sclerotigenum* and certain isolates of *P. chrysogenum*. The extrolites produced by these species, the plant pathogenicity (of *P. digitatum* and *P. sclerotigenum*) and DNA sequence data (Peterson, 2000) clearly shows that these taxa should be placed in subgenus *Penicillium*.
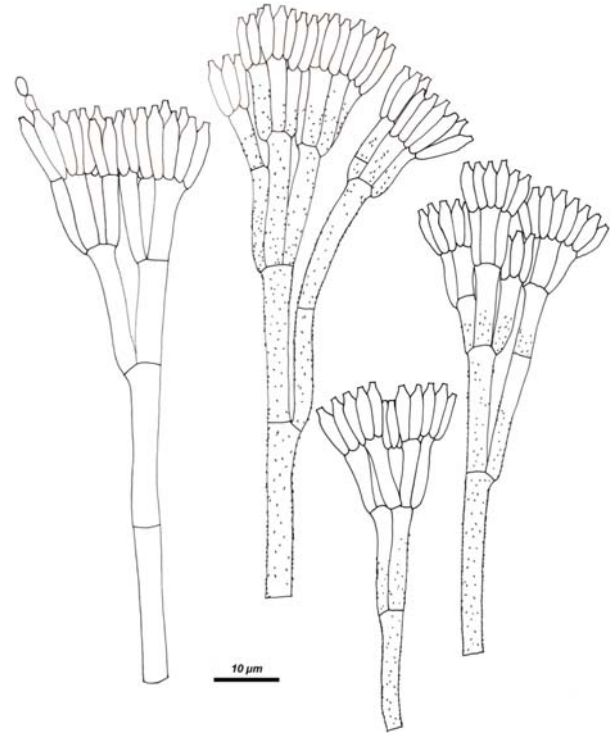

Fig 4. Conidiophore branching patterns in subgenus *Penicilllium*
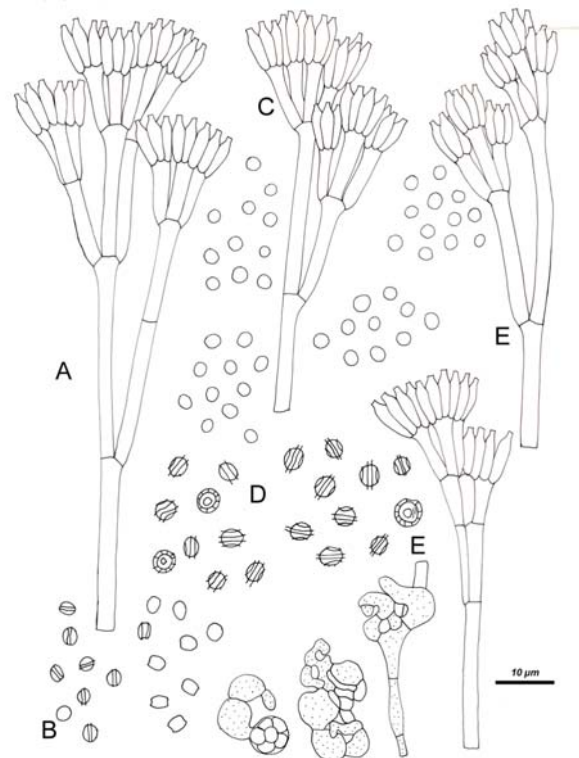

Fig 5. Conidiophores, conidia and ascogenous structures of a *Eupenicillium crustaceum* (A-B), *E. egyptiacum* (C-D) and *E. molle* (E).

P. oxalicum has biverticillate structures, but is pathogenic to cucumbers (Menzies *et al.*, 1995). It shows rich growth at 37 °C and is phylogenetically close to subgenus *Furcatum* (Peterson, 2000). On balance, this isolate have been excluded from subgenus *Penicillium* in this treatment. Other species in *Furcatum* are endophytes of plants; e.g. *P. nodusitatum* forms myconodules with elder trees (Valla *et al.*, 1989). This species is biverticillate and asymmetric and thus belong to subgenus *Furcatum*.

Several soil-borne species can produce a few asymmetric tervertricillate conidiophores, but in most cases, these can be recognized as twice biverticillate structures. The best examples of this are *P. lanosum* and *P. scabrosum*. *P. lanosum* (**Fig. 6**) was included among the asymmetric terverticillate Penicillia by Samson *et al.* (1976) and as a synonym of *P. puberulum* in subgenus *Penicillium* by Pitt (1979). *P. lanosum* and *P. scabrosum* produce extrolites that are both produced by subgenus *Furcatum* and subgenus *Penicillium* species (Frisvad *et al.*, 1990a & b). Based on phenetic and phylogenetic data we place the latter two species in subgenus *Furcatum*. This is in agreement with Domsch *et al.* (1980). These authors placed *P. scabrosum* (listed as *P. atrovenetum*, p. 545) and *P. lanosum* (p. 584) close to soil-borne Penicillia in subgenus *Furcatum*, *P. herquei* and *P. jensenii*, respectively.
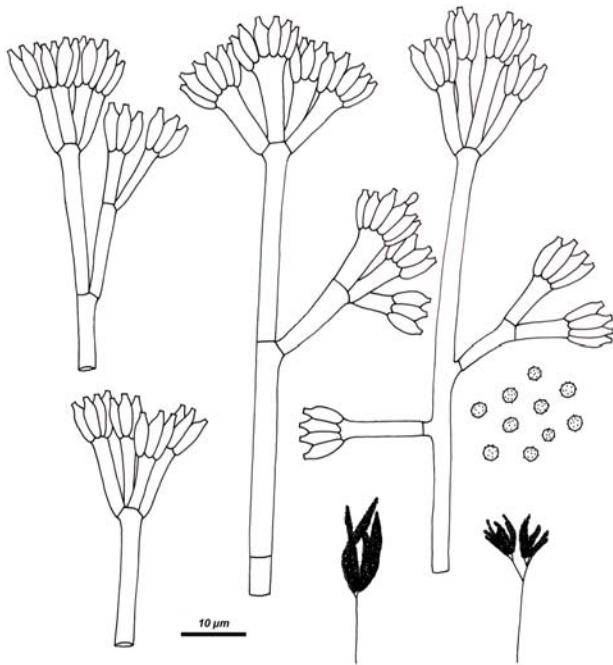

Fig. 6. Conidophores and conidia of *Penicillium lanosum*.

P. arenicola was so different from all other *Penicillium* species that Pitt (1979) set it apart in section *Inordinate* Pitt, series *Arenicola* Pitt, with *P. canadense* as a synonym. The very irregular penicilli, the golden blonde to olive brown conidia, the dark brown reverse, the production of canadensolide and the specific occurrence in forest soil all suggest an en-

tirely unique placement in *Penicillium* and no links to any species in subgenus *Penicillium*. The only features in common with species in subgenus *Penicillium* are the often tervertcillate Penicilli and the production of the extrolite asperphenamate. We have therefore omitted *P. arenicola* in this monograph.

Species concept
Many controversies exist regarding the infraspecific ranks of variety and subspecies. We have chosen to use only the species rank following the idea that varieties and subspecies are usually based on the neodarwinian idea that populations and races will gradually turn into new species, for example, after geographical separation and selection. As we do not subscribe to that mechanism as the only cause of speciation, in agreement with Schlichting and Pigliucci (1998), we here adopt the species level as the lowest formal taxonomic level.

We here adopt a phenotypic species concept in which each species is a homogeneous and distinct cluster in phenotypic space with a large distance to any other such cluster. Species discovered this way have proven to agree with other species concepts such as those based on ecology or phylogeny. The criteria applied are a **combination** of micromorphological, macromorphological, physiological and extrolite characters. Classifications and identifications based on any of those types of characters **alone** have been unsatisfactory among others because of the many taxa in *Penicillium*.

We will exemplify this with *Penicillium crustosum*. *P. crustosum* was described in 1930 by Thom and accepted in the *P. expansum* series by Raper and Thom (1949), even though a synonym of it, *P. terrestre* Jensen, was placed in the *P. terrestre* series based on slightly different colony texture. Samson *et al.* (1976) placed *P. crustosum* in synonymy with *P. verrucosum* var. *cyclopium* based on micromorphological similarities. Fassatiova (1977) placed *P. crustosum* close to *P. expansum* and reduced it to variety status as *P. expansum* var. *crustosum*. Pitt (1979b) accepted *P. crustosum*, but included isolates of *P. aurantiogriseum* (*P. australicum*) and *P. solitum* (*P. verrucosum* var. *melanochlorum*), inconsistent with the high growth rate claimed to be characteristic for *P. crustosum*, while *P. solitum* and *P. aurantiogriseum* grow very slowly. Other strains of *P. solitum* and *P. aurantiogriseum* were placed under the latter species by Pitt (1979b). Bridge *et al.* (1989) reduced *P. crustosum* to a variety of *P. solitum* as *P. solitum* var. *crustosum*. Frisvad and Filtenborg (1989) accepted *P. crustosum* based on its consistent production of penitrem A, roquefortine C, terrestric acid and cyclopenol in combination with its high growth rate and extraordinarily high production of conidia. Some of the similar and dissimilar features of the species above (see also page 50) show why it is important to

combine a suite of characters in order to classify or identify these fungi correctly. Based on a smaller subset of these characters many species could be placed in synonymy, but taken as a whole the species are indeed very different. Interestingly *P. crustosum* is not phylogenetically close to any of the species mentioned above, but is rather phylogenetically related to *P. commune* and *P. camemberti* (Skouboe *et al.*, 1996; Peterson, 2000).

The sectional classification of *Penicillium* subgenus *Penicillium*

Overview:

> **Section *Coronata*** –
> - Ser. *Olsonii*
> **Section *Roqueforti***
> - Ser. *Roqueforti*
> **Section *Chrysogena***
> - Ser. *Chrysogena*
> - Ser. *Mononematosa*
> - Ser. *Aethiopica*
> - Ser. *Persicina*
> **Section *Penicillium***
> - Ser. *Expansa*
> - Ser. *Urticicolae*
> - Ser. *Claviformia*
> - Ser. *Italica*
> - Ser. *Gladioli*
> **Section *Digitata***
> - Ser. *Digitata*
> **Section *Viridicata***
> - Ser. *Viridicata*
> - Ser. *Corymbifera*
> - Ser. *Verrucosa*
> - Ser. *Camemberti*
> - Ser. *Solita*

These six sections are all phenotypically distinct.

Section *Coronata*

Section *Coronata* includes species that all produce compact, often multiramulate penicilli with long stipes, and velutinous colonies. All species produces asperphenamate. Chemotaxonomically *Coronata* is most similar to sect. *Roqueforti*. *P. brevicompactum* share only few common extrolites with other species in subgenus *Penicillium*. Brevianamide A is also produced by *P. viridicatum* in section *Viridicata*. One or two of the three species produce a series of other unique extrolites including brevicompanins, brevi-ones, pebrolides, silvatins, and Raistrick phenols. *P. brevicompactum* produces botryodiploidin in common with *P. paneum* in Sect. *Roqueforti*. Furthermore *P. brevicompactum* and *P. bialowiezense* produce

mycophenolic acid in common with *P. roqueforti* and *P. carneum* in Sect. *Roqueforti*.

Species in section *Coronata* are able to grow both at very low water activities and at low temperatures, but do not tolerate high growth temperatures. The species occur worldwide, from the tropical to arctic regions. *P. olsonii* is found mostly in the tropics and these tropical isolates occasionally produce sclerotia. The species have been found on plants growing in greenhouses, especially on tomatoes, but they are also common in soil worldwide. All species in section *Coronata* are common species encountered in indoor environments. All species grow poorly on creatine as sole N-source and they produce no or little acid on CREA. All species tolerate nitrite well and can use it as sole N-source.

Section *Roqueforti*

Section *Roqueforti* is unique in its high tolerance to propionic acid, acetic acid, lactic acid and other acids and to high concentrations of carbondioxide and probably developed this resistance in competition or corporation with lactic acid bacteria during evolution. The species included in *Roqueforti* have large smooth-walled globose conidia, rough-walled stipes and low velutinous colonies, growing fast on almost all substrates. Roquefortine C is common to all species in the section *Roqueforti* but is also produced by several species in all other sections, except *Coronata*. PR-toxins, marcfortins, isofumigaclavins are only produce by (some) section *Roqueforti* species, whereas mycophenolic acid and botryodiploidin are also found in section Coronata. Penitrem A produced by *P. carneum* is also produced by *P. glandicola* and *P. clavigerum* in section *Expansa*, and *P. tulipae* and *P. melanoconidium* in section *Viridicata*. Patulin produced by *P. carneum* and *P. paneum* in *Roqueforti* is also produced by many species in section *Expansa*. All species in section *Roqueforti* grow well on creatine and nitrite as sole N-sources, and are poor or non-producers of acid on CREA. Section *Roqueforti* members grow relatively poorly at low water activities compared to species in other sections of subgenus *Penicillium*, but grow well at low temperatures, whereas growth at 37°C is nil.

Section *Chrysogena*

Section *Chrysogena* include species that have rather short broad phialides with broad collula and divaricate penicillus structures and two additional mono-typic series with unique morphologies (*Persicina* and *Aethiopica*). Most species share, as the only species in subgenus *Penicillium*, the ability to grow at 37°C. Penicilli can be biverticillate, terverticillate and/or quarterverticillate. All species have a velutinous to weakly floccose colony type and the all grow very or rather fast. They all grow very well at 30°C. Penicillin is common to all species in ser. *Chrysogena*, but is

also produced by *P. griseofulvum* in section *Expansa*. Anthraquinones and other yellow polyketides are produced by most species. Xanthocillins have been found in two species in *Chrysogena* (*P. chrysogenum* and *P. flavigenum*), in the related *Eupenicillium egyptiacum*, but only found in *P. italicum* in section *Expansa* outside *Chrysogena*. Chrysogine is also common in section *Chrysogena*, and only found in *P. tulipae* in section *Viridicata* outside *Chrysogena*. *P. mononematosum* is unique in this section, however, being characterized by the production of fumitremorgins, also found in *Eupenicillium crustaceum*, cyclopaldic acid (also found in *P. carneum* in section *Roqueforti* and *P. commune* in section *Viridicata*), and isochromantoxins (found in *P. steckii* in subgenus *Furcatum*). Roquefortine C and meleagrin is found in *P. chrysogenum*, but also by many other species in sections *Expansa* and *Viridicata*. Dipodazin has only been found in *P. dipodomyis* from this section and in *P. cavernicola* from section *Viridicata*. As mentioned earlier all species grow well at high temperatures, often producing colonies at 37°C. The species can all grow at very low water actitivities and high salt (NaCl) concentrations. Only species in section *Viridicata* seems to be more halotolerant. *P. aethiopicum*, as an exception, is not very halotolerant, however. No species use creatine well as sole N-source, but all species grow moderately well on nitrite as sole N-source and very well on UNO. The species are not resistant to acids, but grow well at relatively high pH values. Compared to other species in subgenus *Penicillium*, species in *Chrysogena* are those closest to *Eupenicillium* series *Crustacea* and other soil-borne Penicillia.

Section *Penicillium*

Series *Expansa* is characterized by smooth-walled ellipsoidal conidia, except *P. marinum* and *P. gladioli* which have globose to subglobose conidia. Most species have strongly fasciculate to coremiform colonies and conidiophores with smooth stipes and terverticillate to quarterverticillate structures. *P. gladioli* differs by having only slightly fasciculate colonies and rough-walled stipes.

In series *Urticicolae* the species are unique in having divaricate structures and very short phialides. Most species appear to be very competitive, producing patulin, griseofulvin, or fulvic acid or all of these (in *P. griseofulvum*). Furthermore all species in the section produce roquefortine C, except *P. gladioli, P. italicum* and *P. ulaiense*. Extrolites such as deoxybrevianamide E, italinic acid, cyclopiamide, cyclopiamine, communesins, expansolides, gladiolic acids, asperfuran, and pyripyropens are only known from section *Expansa* in subgenus *Penicillium*. *P. griseofulvum*, but also *P. commune* and *P. palitans* in section *Viridicata* produce cyclopiazonic acid. *P. expansum* produces chaetoglobosins, but these metabolites are also produced by *P. discolor* in section *Viridicata*. All species tolerate both quite acidic and alkaline conditions and can grow at relatively low water activities, albeit not as low as the other sections. No species can grow at 37°C. All species are psychrotolerant. All species in series *Expansa* and *Claviformia* grow well on creatine as sole N-source, whereas species in the other series in the section grow poorly on creatine. Several plant pathogenic species are found in section *Expansa*. *P. expansum* produces rots in pomaceous fruits and *P. italicum* and *P. ulaiense* produce rot in citrus fruits. *P. sclerotigenum* produces rot in yams and *P. gladioli* produces a destructive rot in *Gladiolus* corms. Species in series *Claviformia* are all coprophilic, creatine positive and synnemata producing.

Section *Digitata*

Section *Digitata* (and series *Digitata*) is only represented by one species, *P. digitatum*. This species is unique in its combination of features. Conidiophore and conidial structures are irregular and exceptionally large for *Penicillium*, biverticillate rather than terverticillate, divaricate and the conidia are olive-green. The conidia are large and ellipsoidal to cylindrical. The extrolites produced are tryptoquialanines, which it only shares with *P. aethiopicum* from series *Aethiopica* in section *Chrysogena*. The species grow poorly at low water activities and at higher temperatures, and it grows very poorly with no acid production on creatine as sole N-source. It is also the only species in subgenus *Penicillium* that grow poorly on Czapek agar. The species has only been found on rotting citrus fruits. It shares the citrus rotting ability and ellipsoidal to cylindroidal conidia with *P. italicum* and *P. ulaiense* from series *Italica* section *Expansa*, but shares no extrolites with those species. *P. digitatum* is the only species in subgenus *Penicillium* that cannot use nitrate as sole N-source.

Section *Viridicata*

Most species in section *Viridicata* have globose conidia and rough-walled conidiophore stipes, with *P. atramentosum* as an exception with smooth-walled stipes. However occasionally section *Viridicata* members do not produce rough-walled stipes. *Viridicata* also contain the only species with dark green rough walled conidia in subgenus *Penicillium*. Most species have a fasciculate colony texture and grow rather fast, except species in series *Verrucosa*, which grow slowly. Several extrolites are only found in section *Viridicata* in subgenus *Penicillium*: Xanthomegnins, penicillic acids, puberulic acids, ochratoxins, daldinin C, alantrypinone, anacins, verrucins, auranthine, aurantiamin, puberuline, verrucosidin, terrestric acids, rugulovasines, asteltoxin, territrems, arisugacins, palitantin, compactins, barceloneic acid, and atrovenetins. Verrucolone (arabenoic acid) is

produced by all species in series *Verrucosa*, but also by *P. italicum* in series *Italica* section *Expansa* and *P. olsonii* in section *Coronata*. Viridicatins are produced by many species in section *Viridicata* and outside this section only by *P. vulpinum* in section *Expansa*. The combination of roquefortine C and penitrem A is produced by *P. crustosum*, *P. melanoconidium* and *P. tulipae* in section *Viridicata*, but also by *P. glandicola* in section *Expansa*. In section *Viridicata* roquefortine C production is restricted to *P. crustosum* in series *Camembertii*, *P. melanoconidium* in series *Viridicata* and all species in series *Corymbifera*. Citrinin is produced by *P. verrucosum* and *P. radicicola* in section *Viridicata* but also by *P. expansum* in section *Expansa*. All species are psychrotolerant and grow well at low water activities. Section *Viridicata* species are common on stored or manufactured man-made foods. Series *Viridicata*, *P. verrucosum* and *P. hordei* are common on stored cereals, while series *Camemberti, Solita* and *P. nordicum* are common on cheese, nuts and other fat and protein rich substrates. Species in series *Corymbifera*, except *P. hordei*, are common on onions, root vegetables and flower bulbs.

Taxonomic and nomenclatoral notes on series, species and synonymy in *Penicillium* subgenus *Penicillium*

All holotypes, neotypes, epitypes listed below are those from the Names in Current Use (NCU) list (Pitt and Samson, 1993, Pitt et al., 2000) or otherwise indicated.

Section *Coronata* Pitt, Gen. Penicil.: 392, 1979

Series *Olsonii* Pitt, Gen. Penicil.: 392, 1979
= Series *P. brevicompactum* Raper & Thom, Man. Penicillia: 404, 1949 (nom. inval., arts 21,36)

Type species: *P. olsonii*

Accepted species:

P. bialowiezense K. Zalesski, Bull. Int. Acad. Pol. Sci. Lett., Sér. B, 1927: 462, 1927.
Neotype : CBS 227.38

P. brevicompactum Dierckx, Ann. Soc. Scient. Brux. 25: 88, 1901.
= *P. griseobrunneum* Dierckx, Ann. Soc. Scient. Brux. 25: 88, 1901.
= *P. stoloniferum* Thom, Bull. Bur. Anim. Ind. US Dept. Agric. 118: 68, 1910.
= *P. tabescens* Westling, Ark. Bot. 11: 100, 1911.
= *P. szaferi* K.M. Zalessky, Bull. Int. Acad. Pol. Sci. Lett., Sér. B, 1927: 447, 1927.
= *P. hagemii* K.M. Zalessky, Bull. Int. Acad. Pol. Sci. Lett., Sér. B, 1927: 448, 1927.
= *P. patris-mei* K.M. Zalessky, Bull. Int. Acad. Pol. Sci. Lett., Sér. B, 1927: 496,1927.
= *P. brunneostoloniferum* Abe, J. Gen. Appl. Microbiol. 2: 104, 1956 (*nom. inval.*)
= *P. brunneostoloniferum* Abe ex Ramírez, Man. Atlas Pen.: 412, 1982.
Neotype: IMI 040225

P. olsonii Bain. & Sartory, Ann. Mycol. 10: 398, 1912.
= *P. monstrosum* Sopp, Skr. Vidensk. Selsk. Christiana 11: 150, 1912.
= *P. volgaense*, Beljakova & Mil'ko, Mikol. Fitopatol. 6: 147, 1972.
= *P. brevicompactum* var. *magnum* Ramírez, Man. Atlas Penicil.: 398, 1982.
Neotype: IMI 192502

Section diagnosis: Conidiophores strictly mononematous, with a long stipe, bearing a short, compact and broad, basically two-stage-branched penicillus, sometimes because of the septation of the branches, the penicilli become more complex. Branches 1-6 per branching point, rarely more, closely appressed. The penicilli of the section *Coronata* are shorter, broader

and more compact than those of the other sections in subgenus *Penicillium*: Characteristically, the number of branches per verticil is larger and the metulae and branches are shorter and appear clavate or swollen. Phialides have a broadly cylindrical base and a short, narrow neck. Looking superficially like *Aspergillus* heads in the stereomicroscope, the conidia adhere in divergent to radiating tangled chains, whereas in the other sections of subgenus *Penicillium* they develop in parallel chains, which may become somewhat tangled in age. Conidia subglobose, pear-shaped to broadly ellipsoidal, with walls finely roughened, sometimes appearing smooth. All species produce asperphenamate and the unknown metabolite O (Svendsen and Frisvad, 1994; Frisvad et al., 1990a). The species are common in all parts of the world, with *P. olsonii* being more common in tropical regions. Thriving in mountainous areas of the tropics, especially coffee estates, they also thrive in greenhouses and are common on tomatoes. *P. brevicompactum* and *P. bialowiezense* are also common on mushrooms, where they can produce conspicuous green colonies directly on the basidiocarps. The have also been found in yoghurts, liver patees and many other processed foods at low water activities. See also the description of the section *Coronata* above. This section only contains one series: *Olsonii.*

The series lacks known teleomorphs state, but few tropical strains of *P. olsonii* can produce large white sclerotia (see **Fig. 2 F).**
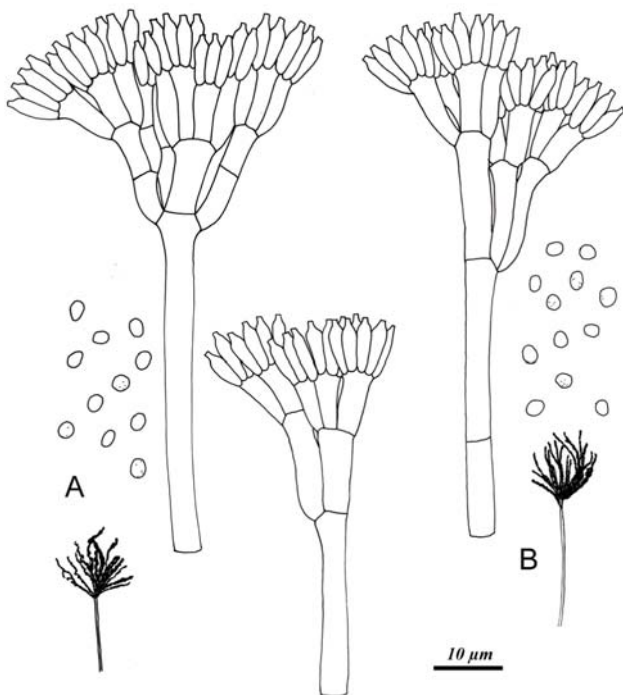

Fig. 7. Conidiophores and conidia of (A) *Penicillium olsonii* and (B) *P. brevicompactum.*

Series *Olsonii* contains only three closely related species: *P. olsonii*, *P. brevicompactum* and *P. bialowiezense*. They differ mainly in the complexity of their penicilli. In *P. brevicompactum* and *P. bialowiezense* branches are often single, although occasionally two to three of them may occur per branching point, whereas typical penicilli of *P. olsonii* produce a compact verticil of up to six branches, developing on the apex and sometimes also on the subapical part of the stipe. However, deteriorated strains of *P. olsonii* produce smaller verticils of branches.

Penicilli of *P. olsonii* are sometimes suggestive of the conidial structures from the section *Inordinata* (which contains only *P. arenicola*). The shape of the phialides and the brown colour of the colonies distinguish *Inordinate* from the section *Coronata* and we have excluded the former section from subgenus *Penicillium*.

P. brevicompactum has many synonyms. Most of these were described by Zaleski and one more well-known species, *P. stoloniferum*, was accepted by Raper and Thom (1949). We have examined ex type strains of *P. griseobrunneum* (NRRL 867), *P. stoloniferum* (CBS 236.51), *P. hagemii* (CBS 316.59), *P. patris-mei* (CBS 210.28) and *P. brunneostoloniferum* (CBS 317.59). They all have the typical morphology of *P. brevicompactum* and furthermore all produce mycophenolic acid, brevianamide A and the Raistrick phenols and are clearly synonyms of this common species. Strains of *P. tabescens* and *P. szaferi* were not available for study, so we follow Raper and Thom (1949) and Pitt (1979) in suggesting these as synonyms of *P. brevicompactum*.

P. volgaense (CBS 626.72) and *P. brevicompactum* var. *magnum* (IJFM 5954) were entirely typical of *P. olsonii*. *P. monstrosum* was unavailable for study, but Sopps protologue indicates that this was a *P. olsonii* rather than a *P. brevicompactum* as suggested by Raper and Thom (1949) and Pitt (1979).

Using multilocus DNA sequence analysis Peterson (2004) recognized *P. brevicompactum, P. olsonii* and a third clade which he assigned to P. *biourgeianum* Zaleski. Examination of the ex-type NRRL 865 of *P. biourgeianum* showed that it is identical with *P. bialowiezense* Peterson (2004) found that the culture NRRL 863 of *P. bialowiezense* is identical with *P. polonicum*. However, in our study we examined the ex-type of *P. bialowiezense* CBS 227.38, which was originally deposited at CBS by K. Zaleski. Therefore NRRL 863, which was sent later to C. Thom, can be considered a contaminant. Our examination of NRRL 863 showed that it has the typical extrolite production of *P. cyclopium*. It is somewhat different from *P. cyclopium* by its good sporulation on YES and the dark reverse on CYA.

Section *Roqueforti* Frisvad & Samson sect. nov.
Sectio generis *Penicillium* subgeneris *Penicillium*, penicillis asymmetrice terverticillatis, stipitibus rugosis,

conidiis obscure viridibus, levibus, globosis; coloniae celeriter crescentes, velutinae, creatinum vel nitritum velut substratum nitrogeni assimilantes; 0.5% acido acetico vel 1% propionico addito et in atmosphaera CO_2 bene crescentes; sed 37ºC non crescunt et 5% NaCl inhibuntur; roquefortinum formatur.

Typus *P. roqueforti* Raper & Thom

Series *Roqueforti* Raper & Thom ex Frisvad, Int. Mod. Meth. Pen Asp. Clas., 277, 2000.
    = Series *P. roqueforti* Raper & Thom, Man. Penicillia, 392, 1949 (nom. inval., arts 21,36)

Type species: *P. roqueforti*

Accepted species:

P. roqueforti Thom, Bull. Bur. Anim. Ind. US Dept. Agric. 82: 35, 1906.
    = *P. aromaticum casei* Sopp, Zentbl. Bakt. ParasitKde., Abt. II: 4: 164, 1898.
    = *P. vesiculosum* Bain., Bull. Trimest. Soc. Mycol. Fr. 23: 10, 1907.
    = *P. roqueforti* var. *weidemannii* Westling, Ark. Bot. 11: 71, 1911.
    = *P. atroviride* Sopp, Skr. Vidensk. Selsk. Christiana 11: 149, 1912.
    = *P. roqueforti* Sopp, Skr. Vidensk. Selsk. Christiana 11: 156, 1912.
    = *P. virescens* Sopp, Skr. Vidensk. Selsk. Christiana 11: 157, 1912.
    = *P. aromaticum* Sopp, Skr. Vidensk. Selsk. Christiana 11: 159, 1912.
    = *P. aromaticum-casei* Sopp ex Sacc., Syll. Fung. 22: 1278, 1913.
    = *P. suavolens* Biourge, Cellule 33: 200, 1923.
    = *P. gorgonzolae* Weidemann apud Biourge, Cellule 33: 204, 1923.
    = *P. weidemannii* (Westling) Biourge, Cellule 33: 204, 1923.
    = *P. stilton* Biourge, Cellule 33: 206, 1923.
    = *P. weidemannii* var. *fuscum* Arnaudi, Boll. Ist. Sieroter. Milan. 6: 27 (1928).
    = *P. biourgei* Arnaudi, Boll. Ist. Sieroter. Milan. 6: 27 (1928).
    = *P. roqueforti* var. *viride* Dattilo-Rubbo, Trans. Br. Mycol. Soc. 22: 178, 1938.
    = *P. conservandi* Novobranova, Nov. Sist. Niz. Rast. 11: 233, 1974.
Neotype: IMI 024313

P. carneum (Frisvad) Frisvad, Microbiology, UK, 142: 546, 1996.
    = *P. roqueforti* var. *carneum* Frisvad, Mycologia 81: 858, 1989.
Type: IMI 293204

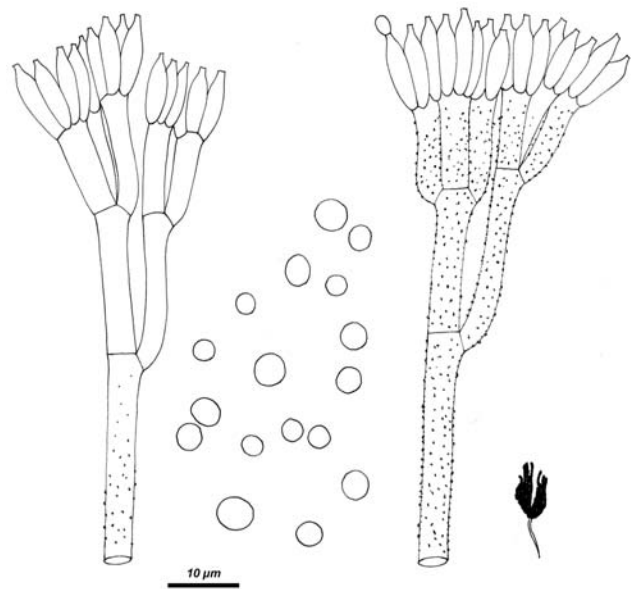**P. paneum** Frisvad, Microbiology (UK) 142: 546, 1996.
Holotype: C 25000

Fig. 8. Conidiophores and conidia of *P. roqueforti*

Section diagnosis: Conidiophores arising from submerged hyphae, up to 200 μm in length, relatively wide, and walls tuberculate (occasionally also smooth with stipe relatively short) bearing (one-) two-(three-)stage-branched penicilli with branches and usually also metulae tuberculate. All elements appressed. Phialides with a short, relatively wide neck. Conidia globose to subglobose, relatively large, smooth-walled, adhering in loose columns or in tangled chains. Conidial areas dark green or dark blue-green. Colony growth rate is fast for all species. All isolates in the series can grow at low pH values (for example on media containing 0.5 % acetic acid), at high alcohol concentrations and at elevated CO_2 levels. All species grow well on creatine and nitrite as the sole N-source. Roquefortine C is produced by all species. Isofumigaclavine and mycophenolic acid is produced by two of the three species. Members of the series appear to have a symbiotic relationship with lactic acid bacteria and certain acid-tolerant yeasts (Samson et al., 2002) .

The section and series *Roqueforti* is separated from the section *Viridicata* by rapid growth, thin, strictly velutinous colonies, tuberculate stipes and branches, as well as by relatively large, globose, smooth-walled conidia. The series includes three species, *P. roqueforti*, *P. carneum* and *P. paneum.*

The three species in series *Roqueforti* are closely related (Boysen *et al.*, 1996). *P. roqueforti* is the predominant mould occurring on cheeses of the Roquefort-type. Apart from blue-mould cheeses, *P. roqueforti* often occurs on other substrates, such as silage, rye bread and other acid preserved commodities. *P. roqueforti* produces small, soft white sclerotia-like structures after prolonged incubation (Samson *et al.*, 1977a). Furthermore, its dark green reverse is distinctive.

P. carneum is mainly distinguished from *P. roqueforti* by its dark blue-green conidial areas, pale brown colony reverse, lower average growth rate and the profiles of extrolites. This species does not occur on blue cheeses. It has been isolated from meat products, silage and other substrates. *P. carneum* produces geosmin, distinguishing it from the other species. *P. paneum* also has a pale brown colony reverse. It can be distinguished from *P. carneum* by the profile of volatiles and by the profile of other extrolites.

A number of other epithets have been given to blue cheese moulds. In agreement with other authors, all of them are regarded as synonyms of *P. roqueforti*. The ex-type cultures of *P. gorgonzolae* (NRRL 857), *P. roqueforti* var. *viride* (CBS 234.38) and *P. conservandi* (CBS 498.73) were examined and found to be entirely typical of *P. roqueforti*.

Section *Chrysogena* Frisvad & Samson, **sect. nov.**

= Series *Chrysogena* Raper & Thom ex Stolk & Samson, Adv. Pen. Asp. Syst.: 180, 1985 = Series *P. chrysogenum* Raper & Thom, Man. Penicillia: 355, 1949 (nom. inval., arts 21, 36)

Sectio generis *Penicillium* subgeneris *Penicillium*, penicillis raro biverticillatis, vulgo terverticillatis, stipitibus levibus; coloniae celeriter crescentes in substratis 15% sucrosi continentibus, velutinae; in creatino velut substrato nitrogeni parce crescentes; 37ºC plerumque sustinetur, sed 30ºC omnes species bene crescentes; 5% NaCl addito stimulantur (*P. aethiopico* excepto).
Typus *P. chrysogenum* Thom

Accepted species:

P. chrysogenum Thom, Bull. Bur. Anim. Ind. USDA 118: 58, 1910.
= *P. griseoroseum* Dierckx, Ann. Soc. Scient. Brux. 25: 86, 1901.
= *P. brunneorubrum* Dierckx, Ann. Soc. Scient. Brux. 25: 88, 1901.
= *P. citreoroseum* Dierckx, Ann. Soc. Scient. Brux. 25: 89, 1901.
= *P. baculatum* Westling, Svensk Bot. Tidskr. 14: 139, 1910.
= *P. notatum* Westling, Ark. Bot. 11: 95, 1911.
= *P. meleagrinum* Biourge, Cellule 33: 147, 1923.
= *P. flavidomarginatum* Biourge, Cellule 33: 150, 1923.
= *P. cyaneofulvum* Biourge, Cellule 33: 174, 1923.
= *P. roseocitreum* Biourge, Cellule 33: 184, 1923.
= *P. rubens* Biourge, Cellule 33: 265, 1923.
= *P. chlorophaeum* Biourge, Cellule 33: 271, 1923.
= *P. camerunense* Heim apud Heim, Nouvel & Saccas, Bull. Acad. R. Belg. Cl. Sci. 35: 42, 1949.
= *P. chrysogenum* var. *brevisterigma* Forster, Brit. Pat. 691: 242, 1953.

= *P. aromaticum* f. *microsporum* Romankova, Uchen. Zap. Leningr. Gos. Univ. (Ser. Biol. Nauk. 40:) 191: 102, 1955.
= *P. harmonense* Baghdadi, Nov. Sist. Niz. Rast. 5: 102, 1968.
= *P. verrucosum* var. *cyclopium* strain ananas-olens Ramírez, Man. Atlas. Penicil.: 457, 1982.
= *P. chrysogenum* mut. *fulvescens* Takashima, Arima & Abe ex Ramirez, Man. Atlas Penicil.: 365

Neotype: IMI 024314

P. flavigenum Frisvad & Samson, Mycological Research 101: 620, 1997.
Holotype: CBS 419.89

P. dipodomyis (Frisvad, Filt. & Wicklow) Banke, Frisvad and S. Rosendahl, Int. Mod. Meth. Pen. Asp. Clas., 270, 2000
= *P. chrysogenum* var. *dipodomyis* Frisvad, Filt. & Wicklow, Can. J. Bot. 65: 766, 1987.
= *P. dipodomyis* (Frisvad, Filt. & Wicklow) Banke, Frisvad & S. Rosendahl, Mycol. Res. 101: 622, 1997 (*nom. inval.*).

Holotype: IMI 296926

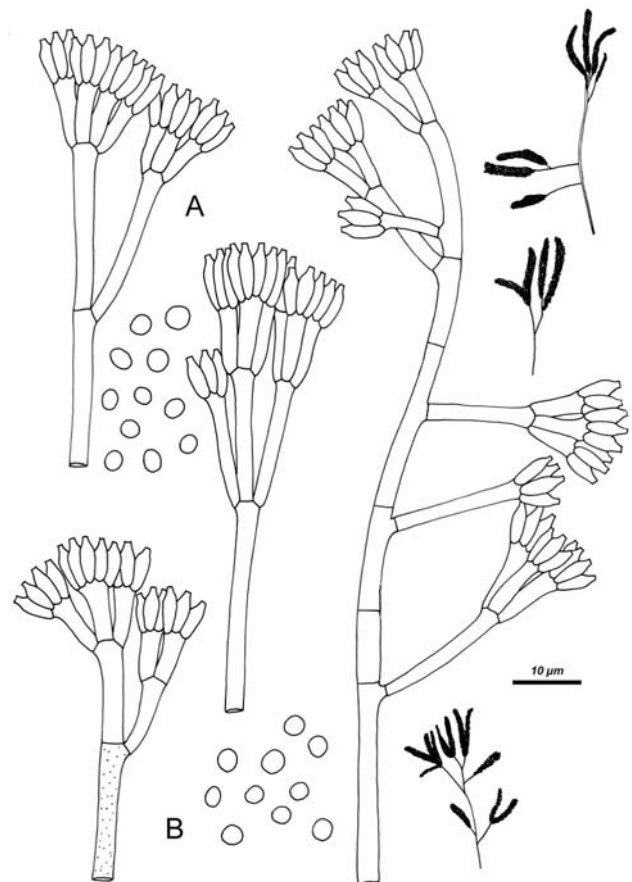**P. nalgiovense** Laxa, Zentbl. Bakt. ParasitKde, Abt. II 86: 162, 1932.
Neotype: CBS 352.48

Fig 9. Conidiophores and conidia of (A) *P. chrysogenum* and (B) *P. dipodomyis*.
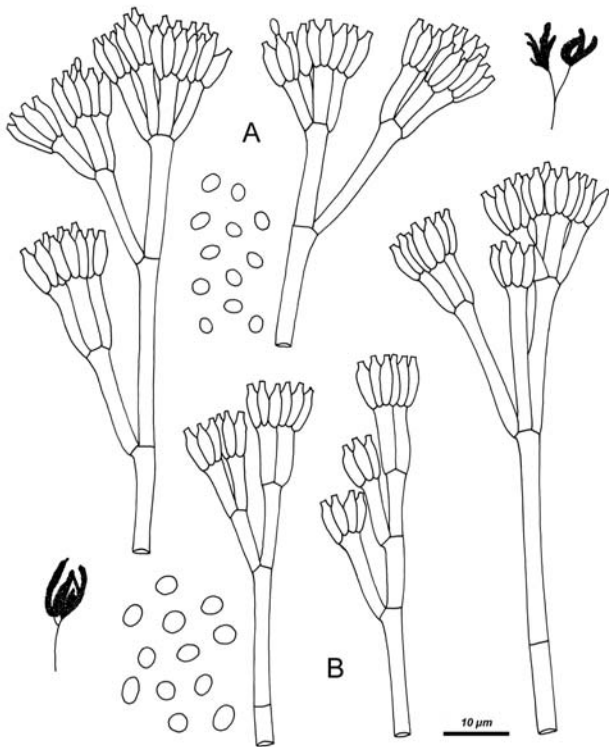
17

Fig. 10. Conidiophores and conidia of (A) *P. flavigenum* and (B) *P. atramentosum*

Section diagnosis: Conidiophores mononematous, (one-) two- or three-, occasionally more-stage-branched with the lower branches sometimes inter-grading with a variable number of single, strongly divergent, subterminal and/or intergrading branches (metulae), arising lower along the stipe. Stipes usually long, with walls smooth or nearly so, rarely very finely roughened. Metulae are in somewhat appressed verticils of 3-5. Branches are usually single and divergent; only when arising at the first septum below the verticil of metulae, do they occasionally occur in a somewhat appressed verticil of three. Phialides when typical, are relatively small (rarely longer up to 10 µm in lengh), with a broadly cylindrical base and a short, sometimes inconspicuous, narrowed neck. Conidia (broadly) ellipsoidal to subglobose or globose with walls smooth or very finely roughened, adhering in columns. Teleomorph and sclerotia absent, even though there is a close affinity with *Eupenicillium egyptiacum.*

Raper and Thom (1949) and Pitt (1979: 330) suggested that *P. chrysogenum* had some affinities with species in subgenus *Furcatum* (e.g. *P. citrinum*). Pitt actually placed *P. griseoroseum* (a synonym) in his subgenus *Furcatum*, but because of the two-to-three-stage-branched penicilli, he placed *P. chrysogenum* in subgenus *Penicillium*. Series *Chrysogena* is distinguished from series *Citrina* by the more complex conidiophores. The type strain of *P. griseoroseum* agrees in many respects with the type culture of *P. chrysogenum* but differs in producing one-stage-branched penicilli. According to Biourge's description (1923), *P. griseoroseum* was character-

ized by one- to two-stage-branched conidiophores, like those of *P. chrysogenum*. Consequently, the correct name of the present species should be *P. griseoroseum*. Since the name is in common use, *P. chrysogenum* was proposed for conservation (Frisvad *et al.*, 1990c; Kozakiewicz *et al*, 1992) and the Committee for Fungi and Lichens accepted this.

P. chrysogenum has many synonyms. Three of those were described before *P. chrysogenum* and therefore would have nomenclatural priority, but the name *P. chrysogenum* has been conserved (see above). The following ex type strains of synonyms of *P. chrysogenum* have been examined: *P. griseoroseum* (NRRL 820), *P. notatum* (CBS 355.48), *P. meleagrinum* (authentic, CBS 349.48), *P. cyaneofulvum* (CBS 314.48), *P. harmonense* (CBS 412.69), *P. roseocitreum* (NRRL 889), *P. rubens* (NRRL 822), *P. chlorophaeum* (NRRL 817), *P. camerunense* (CBS 339.58), *P. flourescens* (NRRL 819), *P. aromaticum* var. *microsporum* (CBS 302.67). All these isolates were indistinguishable from the ex type culture of *P. chrysogenum*, although there were some differences in the production of yellow pigment in the strains. *P. harmonense* differed in two kinds of extrolites and may be distinct, but more cultures of *P. harmonense* are needed to decide if this is the case. All the strains produce penicillin, roquefortine C and meleagrin.

The species in this section (Banke *et al.*, 1987) are united by their production of penicillin, their dry habitats, salt tolerance, strictly velutinous colony texture, divergent conidiophores and phialide shape, fast growth rates and production of yellow and orange pigments. Extrolite and isozyme data show that *P. chrysogenum* is most closely related to *P. flavigenum*, while *P. nalgiovense* (the starter culture strains) is more similar to *P. dipodomyis*.

Since strains of *P. chrysogenum* may develop up to five-, rarely more-stage-branched conidiophores, the species shows some morphological affinities with *P. griseofulvum* (series *Urticicolae*). However, the conidiophores of Section *Chrysogena* are generally less complicated and both the phialides and metulae are larger than those of *P. griseofulvum*.

Cultures of Section *Chrysogena* grow much more rapidly than those of the series *Tularensia* in *Eupenicillium* and they do not produce ascomata or sclerotia. Moreover, the conidial chains of the *Chrysogena* usually form columns, whereas those of the *Tularensia* adhere in parallel to tangled chains.

Three other species associated with dry habitats like deserts are included in the *Chrysogena*. *P. flavigenum* closely resembles *P. chrysogenum*. The conidia of *P. flavogenum* are a little more ellipsoidal and slightly smaller than those of *P. chrysogenum* and they adhere in at first loosely parallel, later tangled chains. The two species are mainly distinguished by their extrolites. *P. chrysogenum* and *P.*

dipodomyis are mainly distinguished by their extro-lites. Moreover, the stipes of *P. dipodomyis* are slightly rough-walled and the conidia of *P. dipodomyis* are darker green than those of *P. chrysogenum*. *P. nalgiovense* isolates from cheese are rather slow growing, produce large quantities of nalgiovensin and nalgiolaxin and only traces of penicillin, whereas the *P. nalgiovense* strains found on meat products are fast growing and produce large amounts of penicillin and smaller amounts of nalgiovensin and nalgiolaxin (Andersen and Frisvad, 1996). Starter cultures of *P. nalgiovense* have white conidia, because they have been selected for this character, but wild-type strains of *P. nalgiovense* from meat products have dark green conidia.

Scott et al. (*in press*) studied the phenotypic variation in *P. chrysogenum* from indoor environments and five unique multilocus haplotypes were revealed. Their phylogenetic analysis of allele sequences resolved in three strongly supported lineages. The majority of indoor isolates (90%) clustered together with the culture Alexander Flemming used for his penicillin experiments. A second clade contained the ex type cultures of *P. chrysogenum* and *P. notatum*. Scott et al. (*in press*) indicated that four taxa can be recognized with the *P. chrysogenum* complex and an expanded polyphasic study using strains from various substrates including multilocus sequence analysis is required to solve the delimitation of the taxa.

Series *Mononematosa* Frisvad, Int. Mod. Meth. Pen. Asp. Clas., 269, 2000.
Type species: *P. mononematosum*

Accepted species:

P. mononematosum (Frisvad, Filt. & Wicklow) Frisvad, Mycologia 81: 857, 1989.
    = *P. glandicola* var. *mononematosa* Frisvad, Filt. & Wicklow, Can. J. Bot. 65: 767, 1987.
    = *P. granulatum* var. *mononematosa* (Frisvad, Filt. & Wicklow) Bridge, Kozak. & R.R.M. Paterson, Myc. Pap. 165: 38, 1992.
Holotype: IMI 296925

P. confertum (Frisvad, Filt. & Wicklow) Frisvad, Mycologia 81: 852, 1989
    = *P. glandicola* var. *confertum*. Frisvad, Filt. & Wicklow, Can. J. Bot. 65: 769, 1987.
Holotype: IMI 296930

These non-fasciculate slow-growing species have complicated structures different from the usual appressed two-ramus structures seen in most other species in subgenus *Penicillium*. *P. mononematosum* has been found in deserts and warm salt marshes and usually in connection with burrows of rodents. The

only strain known of *P. confertum* has also been found in kangaroo rat burrows. These species were characteristic in the two numerical taxonomical studies where they have been included (Bridge *et al.*, 1989; Svendsen and Frisvad, 1994). The production of verrucologen and other fumitremorgins, cyclo-paldic acid, isochromantoxin, asteltoxin, viriditoxin and metabolite A (Frisvad and Filtenborg, 1989; Svendsen and Frisvad, 1994) by *P. mononematosum* clearly sets this species apart from other terverticillate Penicillia and even indicates that *P. mononematoseum* is more closely related to subgenus *Furcatum*. Both species bears some resemblance to *Chrysogena*, but in contrats to that series they do not produce penicillin and they are slow growing.
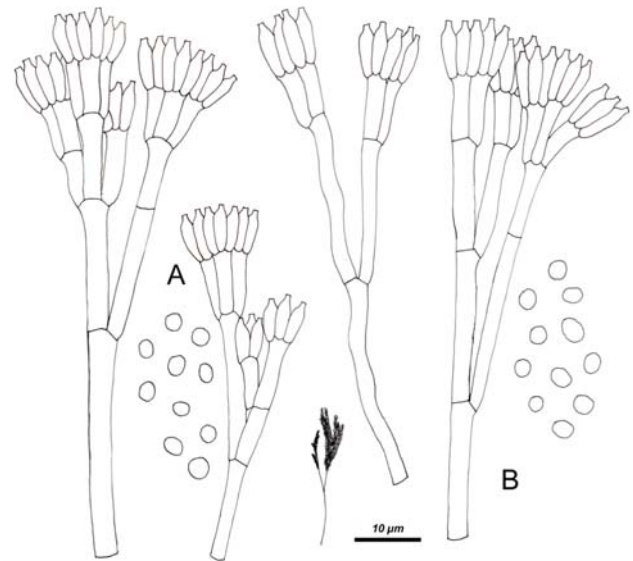
Fig. 11. Conidiophores and conidia of (A) *P. mononematosum* and (B) *P. confertum.*

Series *Aethiopica* Frisvad & Samson, **ser. nov**.
Series generis *Penicillium* subgeneris *Penicillium* sectionis *Chrysogena*, conidiophoris terverticillatis appressis, stipitibus levibus vel asperatis, conidiis levibus ellipsoideis; coloniae in reverso flavescentes, fasciculatae, 37ºC bene crescentes sed 5% NaCl addito inhibitae; pigmenta haud in agarum diffundentia.
Typus *P. aethiopicum*

P. aethiopicum Frisvad, Mycologia 81: 848, 1989.
Holotype: IMI 285524

Series *Persicina* Frisvad and Samson, **ser. nov**.
Series generis *Penicillium* subgeneris *Penicillium* sectionis *Chrysogena*, conidiophoris terverticillatis appressis, stipitibus levibus, conidiis ellipsoideis vel cylindricis; coloniae pigmento rubido diffundente, 37ºC bene crescunt.
Typus *P. persicinum* L. Wang, H. Zhou, Frisvad & Samson

P. persicinum L. Wang, H. Zhou, Frisvad & Samson, Ant. van Leeuwenhoek 86: 177
Holotype: HMAS 80638-1-4

These two species have only been found in warm climates. They both produce ellipsoidal conidia and griseofulvin. Growth at 37°C and production of chrysogine and roquefortine C by *P. persicinum* indicates an affinity with section *Chrysogenum*, while the ellipsoidal conidia and the production of griseofulvin by *P. percisinum* and *P. aethiopicum* indicate an affinity to section *Expansa*.

Section *Penicillium*

Series *Expansa* Raper & Thom ex Fassatiová, Acta Univ. Carol. Biol 12: 324, 1977

= Series *P. expansum* Raper & Thom, Man. Penicillia: 508, 1949 (nom. inval., arts 21,36)

= Series *P. terrestre* Raper & Thom, Man. Penicillia: 446, 1949 (nom. inval., arts 21,36)

Type species: *P. expansum*

Accepted species:

P. expansum Link, Obs. Mycol. 1: 16, 1809.

= *Coremium leucopus* Pers., Mycol. Eur. 1: 42, 1822.

= *Coremium glaucum* Link ex Pers., Mycol. Eu. 1: 42, 1822.

= *Floccaria glauca* Grev., Scot. Crypt. Fl. 6: 301, 1828.

= *Coremium alphitobus* Secr., Mycol. Suisse 3: 539, 1833.

= *Coremium vulgare* Corda, Pracht-Fl.: 54, 1839.

= *P. glaucum* var. *coremium* Sacc., Syll. Fung. 4: 78, 1886.

= *P. elongatum* Dierckx, Corda, Pracht-Fl.: 54, 1839.

= *P. glaucum* var. *coremium* Sacc., Syll. Fung. 4: 78, 1886.

= *P. elongatum* Dierckx, Ann. Soc. Scient. Brux. 25: 87, 1901.

= *P. musae* Weidemann, Zentralbl. Bakt. ParasitKde., Abt. II, 19: 687, 1907.

= *P. juglandis* Weidemann, Zentralbl. Bakt. ParasitKde., Abt. II, 19: 683, 1907.

= *P. variabile* Wehmer, Mykol. Zentralbl. 2: 195, 1913.

P. leucopus (Pers.) Biourge, C.R. Séanc. Soc. Biol. 82: 877, 1919.

= *P. plumiferum* Demelius, Verh. Zool.-Bot. Ges. Wien 72: 76, 1922.

= *P. aeruginosum* Demelius, Verh. Zool.-Bot. Ges. Wien 72: 76, 1922.

= *P. malivorum* Ciferri, Riv. Pathol. Veg., Padova 14: 77, 1924.

= *P. kap-laboratorium* Sopp apud Biourge, Cellule 36: 454, 1925.

= *P. resticulosum* Birkinshaw, Raistrick & G. Smith, Biochem. J. 36: 830, 1942.

Neotype: CBS 325.48

P. marinum Frisvad & Samson, **sp. nov.**

A *Penicillio expanso* conidiophoris divaricatis et conidiis subglobosis (2.5-3.2 μm) distinguitur; coloniae in omnibus substratis lentius crescentes, penostatinum formatur neque citrininum.

Typus: CBS 109550

P. sclerotigenum Yamamoto, Scient. Rep. Hyogo Univ. Agric., Agric. Biol. Ser. 2, 1: 69, 1955.
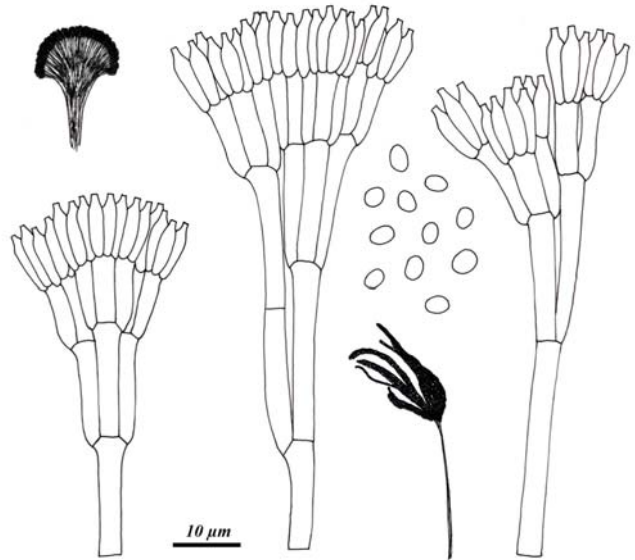
Lectotype: IMI 068616

Fig. 12. Conidiophores and conidia of *P. expansum*

Conidiophores predominantly mononematous, occasionally also synnematous, especially in marginal areas of fresh isolates (of *P. expansum*). Synnemata, when present consisting of a white, sterile stalk and a green, fertile capitulum. Stipes long, usually smooth-walled, occasionally on MEA slightly roughened, bearing a terminal, regularly two- to three-stage-branched penicillus, with elements appressed. In *P. sclerotigenum* several one stage branched structures can be found. Phialides are robust, with a broadly cylindrical basal part and a relatively short, slightly narrowed, comparatively wide neck. Conidia are ellipsoidal to subglobose, smooth-walled, adhering in parallel, sometimes slightly tangled chains, occasionally forming loose columns. In *P. sclerotigenum* the conidia at first are ellipsoidal, hyaline, smooth-walled, often later becoming globose to subglobose with walls pigmented and more or less roughened, adhering in parallel to tangled chains.

Teleomorph absent but sclerotia present in *P. sclerotigenum*.

The series *Expansa* contains three species; two closely related *P. expansum* and *P. marinum* in addition to *P. sclerotigenum*. They are characterized by large regularly two- to three-stage-branched conidiophores with smooth walls and large ellipsoidal or subglobose conidia. The well-developed coremia, occasionally present in marginal areas of fresh isolates of *P. expansum*, are lacking in the two other species. *P. expansum*, *P. marinum* and *P. sclerotigenum* are distinguished from the smooth-walled

species of the *Claviformia* by the more rapid growth of their colonies and the more robust phialides.

P. expansum causes a destructive rot of pomaceous fruits, on which it produces conspicuous concentric zones of crust-like coremia. Well-developed coremia occur only occasionally on agar media. They may develop in small numbers in marginal areas of fresh isolates. After a few years of maintenance, the capacity to produce well-defined coremia is usually lost. The synnemata may be induced by long maintenance at low temperatures, in apples or on media with toxic constituents. The ex type culture of *P. resticulosum*, regarded as floccose by Raper and Thom (1949), produced synnemata on malt extract agar after 3 month of storage at 0°C. This species is considered as a synonym of *P. expansum*. The cultures become velutinous, producing mononematous to slightly synnematous conidiophores.

P. sclerotigenum produces a rot in yam tubers and has only been found in habitats with yams. It is characterized by rapid growth and sclerotium production and a quite large proportion of biverticillate asymmetric penicilli. The latter feature leads Pitt (1979) to place *P. sclerotigenum* in *Furcatum*. Fresh isolates, however, have many terverticillate structures.

The three species in the series share the ability to produce patulin, roquefortine C and geosmin and general colony appearance. Pitt (1979) placed *P. chrysogenum* and *P. atramentosum* in this series, but his series were based more on facilitating identification than phylogenetic or overall phenetic similarity.

P. resticulosum (CBS 150.45) is clearly a synonym of *P. expansum*, despite its occasionally cylindrical conidia. The other synonyms listed above are not available as living cultures anymore.

Series *Urticicolae* Fassatiová, Acta Univ. Carol. Biol. 12: 324, 1977
= Series *P. urticae*, Raper & Thom, Man. Penicillia: 531, 1949 (nom. inval., arts 21,36)
Type species: *P. griseofulvum*

Accepted species:

P. griseofulvum Dierckx, Ann. Soc. Scient. Brux. 25: 88, 1901.
= *P. patulum* Bain., Bull. Trimest. Soc. Mycol. Fr. 22: 208, 1906.
= *P. urticae* Bain., Bull. Trimest. Soc. Mycol. Fr. 23: 15, 1907.
= *P. flexuosum* Dale apud Biourge, Cellule 33: 264, 1923.
= *P. maltum* Hori & Yamamoto, Jap. J. Bacteriol. 9: 1105, 1954.
= *P. duninii* Sidibe, Mikol. Fitopatol. 8: 371, 1974.
Neotype: IMI 075832
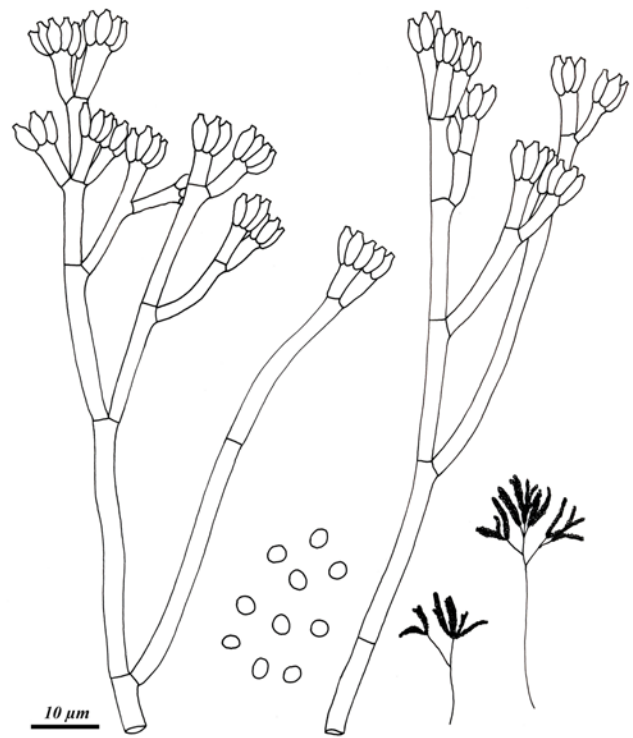
Fig. 13. Conidiophores and conidia of *P. griseofulvum*

P. dipodomyicola (Frisvad, Filt. & Wicklow) Frisvad, Int. Mod. Meth. Pen. Asp. Clas., 275.
P. griseofulvum var. *dipodomyicola* Frisvad, Filt. & Wicklow, Can. J. Bot. 65: 767, 1987.
Holotype: IMI 296935

Section diagnosis: Conidiophores mono- and synnematous. The stipes are long, often slightly sinuous, and smooth-walled. Penicilli are large, complicated, variable in pattern, loosely three- to five- or more-stage-branched, and all elements are smooth-walled. Branches single strongly divergent, bearing short, compact, one-(two-) stage-branched structures. Metulae 2-3 per verticil, comparatively short. Phialides very small, less than 6 μm in length, more or less swollen, with a very short or inconspicuous neck. Conidia ellipsoidal, smooth-walled, adhering in parallel chains, sometimes forming loose columns. Teleomorph and sclerotia absent.

Fassatiova (1977) proposed the series *Urticicola* to accomodate a single species: *P. urticae* (= *P. griseofulvum*). Pitt (1979) emended Fassatiova's concept by adding five species that like *P. griseofulvum* are characterized by relatively slow growth on CYA at 25°C. However, the added species produce regularly, two- to three-stage-branched penicilli and thus differ strongly from the complicated, loosely arranged, three- to five-stage-branched conidial structures, characteristic of *P. griseofulvum*. Moreover, the phialides are quite different. Because of its branched, synnematous conidiophores, most authors (Raper & Thom 1949, Fassatiova, 1977; Pitt, 1979;

Ramirez 1982) classified *P. griseofulvum* in Raper & Thom's subsection *Fasciculata*. However, in many respects, such as the complicated conidiophores, the divergent branches and the small metulae and phialides *P. griseofulvum* differs strongly from the section *Viridicata* and other series in *Expansa*. In fact *P. griseofulvum* and *P. dipodomyicola* represents unique species without very close relationships with other species. The three-to five-or more-stage-branched conidiophores are however, slightly reminiscent of *P. chrysogenum*. Penicillin production adds further to the similarity between these series. *P. griseofulvum* differs from the *Chrysogena* in producing synnematous conidiophores, which are more complicated and irregular in structure. Moreover *P. griseofulvum* and *P. dipodomyicola* are also distinguished from the *Chrysogena* by the very small phialides and metulae.

The series *Urticicolae*, as delimited here is restricted to only two closely related species, *P. griseofulvum* and *P. dipodomyicola*. The series *Urticicolae* is placed in section *Expansa* because of the strongly ellipsoidal smooth-waled conidia, the synnemata and the production of patulin. The two species differ from series *Expansa* by their inability to use creatine as sole N-source and their very short phialides. Both species produce griseofulvin, cyclopiazonic acid and patulin

P. griseofulvum and *P. dipodomyicola* have most often been found on dry cereals and seeds. Both species are distinct, however, as *P. dipodomyicola* produce predominantly bi- to rarely ter-verticillate structures while *P. griseofulvum* has ter- to quarter-verticillate structures. Each species consistently produce other species specific extrolites (Frisvad and Filtenborg, 1989; Svendsen and Frisvad, 1994; Smedsgaard and Frisvad, 1997).

The ex type cultures of *P. patulum* (NRRL 994), *P. urticae* (CBS 384.48) and *P. flexuosum* (CBS 124.14) were inseparable from *P. griseofulvum*.

Series *Claviformia* Raper & Thom ex Stolk, Samson & Frisvad, Int. Mod. Con. Pen. Asp. Clas.: 132, 1990
Type species: *P. vulpinum*

Accepted species:

P. clavigerum Demelius, Verh. Zool.-Bot. Ges. Wien 72: 74, 1922.
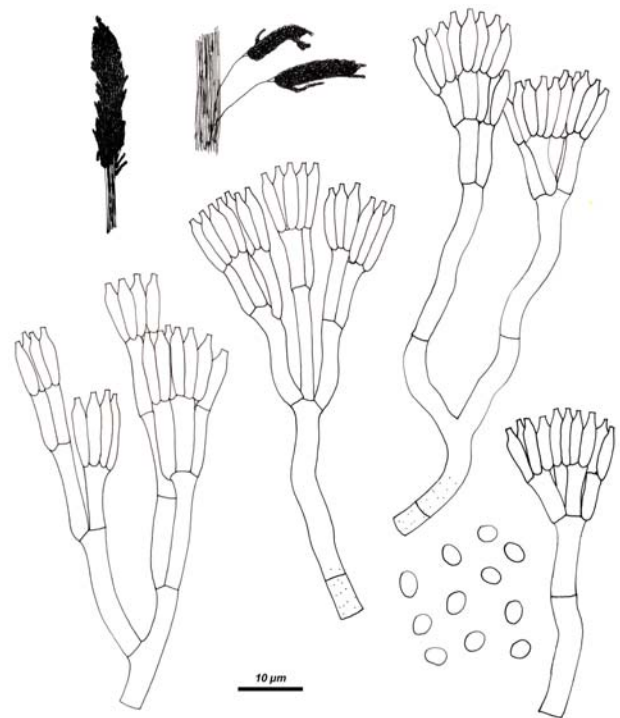Neotype: IMI 039807

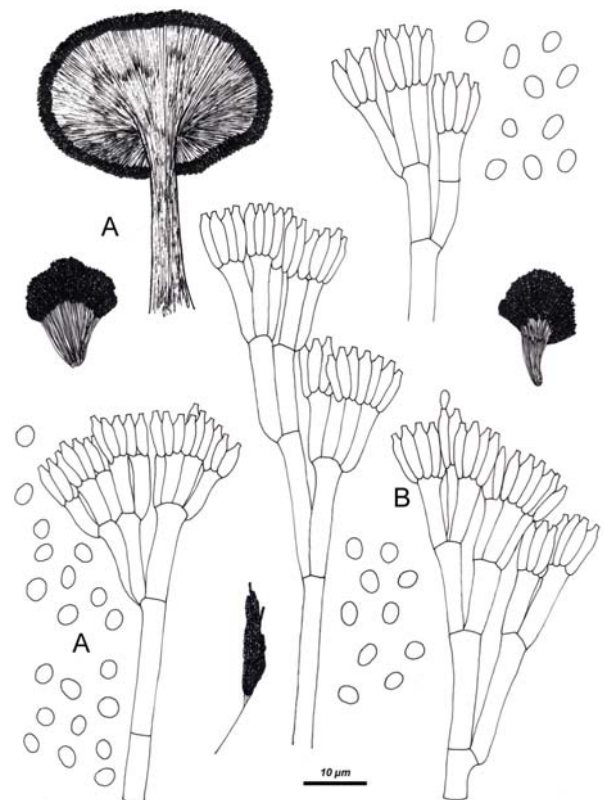**Fig 14**. Conidiophores and conidia of *P. clavigerum*

Fig. 15. Conidiophores and conidia of (A) *P. coprophilum* and (B). *P. coprobium*
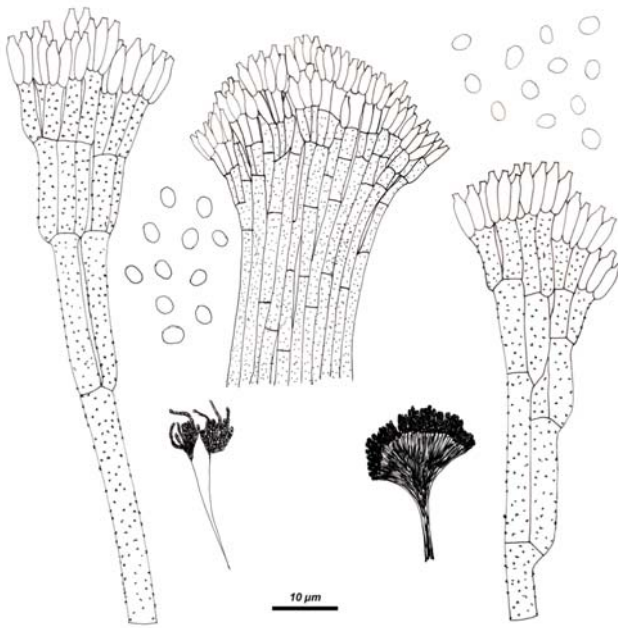
Fig. 16. Conidiophores and conidia of *P. glandicola*

P. concentricum, Samson, Stolk & Hadlok, Stud. Mycol. (Baarn) 11: 17, 1976.
   P. glandicola var. *glaucovenetum* Frisvad, Mycologia 81: 855, 1989.
Holotype: CBS 477.75

P. coprobium Frisvad, Mycologia 81: 853, 1989.
   Holotype: IMI 293209

P. coprophilum (Berk. & Curt.) Seifert & Samson, Adv. Pen. Asp. Syst.: 145, 1985.
Holotype: Cuba, Wright 666 (K). We designate CBS 110760 as epitype.

P. formosanum Hsieh, Su & Tzean, Trans. Mycol. Soc. R.O.C. 2: 159, 1987.
Holotype: PPEH 10001

P. glandicola (Oud.) Seifert & Samson, Adv. Pen. Asp. Syst.: 147, 1985.
   = *P. granulatum* Bain., Bull. Trimest Soc. Mycol. Fr. 21: 126, 1905.
   = *P. divergens* Bain. & Sartory, Bull. Trimest. Soc. Mycol. Fr. 28: 270, 1912.
   = *P. schneggii* Boas, Mykol. Zentralbl. 5: 73, 1914.
   = *P. granulatum* var. *globosum* Bridge, D. Hawksw., Kozak., Onions, R.R.M. Paterson, Sackin & Sneath, J. Gen. Microbiol. 135: 2957, 1989.
Holotype: Netherlands, Valkenburg, Jul 1901, *Rick* in herb. Oudemans (L), Epitype suggested here: CBS 498.75

P. vulpinum (Cooke & Massee) Seifert & Samson, Adv. Pen. Asp. Syst.: 144, 1985.
   = *Coremium claviforme* (Bain.) Peck, Bull. N.Y. St. Mus. 131: 16, 1909.
   = *Coremium silvaticum* Wehmer, Ber. Dt. Bot. Ges. 31: 373, 1914.

   = *Penicillium silvaticum* (Wehmer) Biourge, Celule 33: 1056, 1923.
   = *P. silvaticum* (Wehmer) Gäumann, Vergl. Morph. Pilze: 177, 1926.
   = *P. claviforme* mut. candicans Abe & Uro ex Ramirez, Man, Atl. Penicil.: 488, 1982
   = *P. claviforme* mut. olivicolor Abe & Uro ex Ramirez, Man, Atl. Penicil.: 490, 1982
Holotype: "on dung", *s.coll*., in herb. Cooke (K), Epitype suggested here CBS 126.23
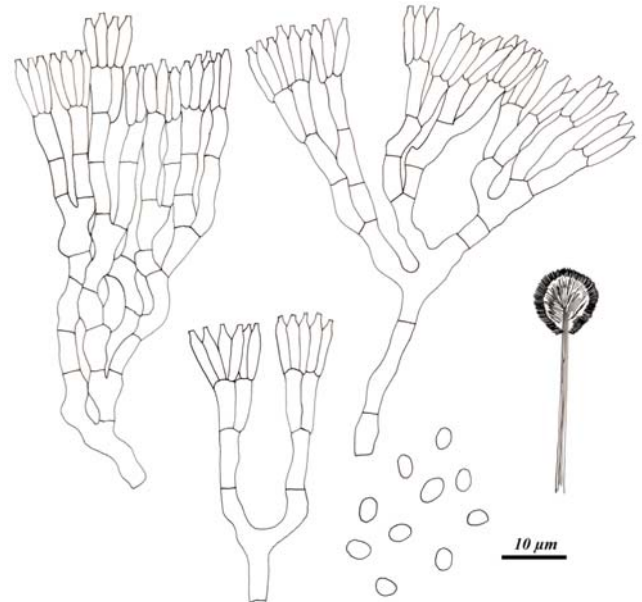
Fig, 17. Conidiophores and conidia of *P. vulpinum*

Series diagnosis: Cultures strongly fasciculate to coremiform, with a majority of the conidiophores aggregated onto loose or well-defined coremia. Mononematous structures in varying numbers also present. Coremia, developing commonly in concentric zones, usually consisting of a stalk, comprised of the stipes of conidiophores and an apical, feathery capitulum comprised of separate, diverging conidiophores, occasionally acicular with penicilli borne over nearly the entire length, though commonly more concentrated on the terminal part. Stipes of varying length, with walls smooth or roughened. Penicilli large, two- to three-stage-branched, with elements typically appressed, occasionally slightly divergent. Phialides with a cylindrical base and a short, but distinct, slightly narrowed neck. Conidia ellipsoidal to subglobose, smooth-walled, adhering in parallel chains, sometimes forming columns. Sclerotia rarely present.

Species of the *Claviformia* are characterized by more or less restricted colonies, producing loose to well-defined coremia in concentric zones. The coremia consist usually of a sterile stalk and a fertile, feathery capitulum, occasionally they are acicular with the penicilli covering nearly the entire stalk, though especially the terminal part (e.g. *P. clavigerum*).

In contrast to *P. vulpinum* the conidiophores of the remaining species of the series retain their individuality and they are somewhat divergently arranged, giving the coremia their feathery appearance. The coremia of *P. vulpinum* are compact. Generally the coremia of the *Claviformia* are larger and better developed on the natural substrate than on agar media.

In *P. clavigerum* colonies are strongly synnematous. Synnemata are well-developed, acicular, with a pointed top, often not showing a clear differentiation into stalk and conidium-bearing area, usually simple, but sometimes branched near the top; conidiophores borne over nearly the entire length of the stalk, though usually more concentrated on the terminal area, one- to two-stage-branhced with elements commonly appressed and smooth-walled. The stipes are variable in length, often long, sinuous, compressing the body of the synnemata. The phialides have a cylindrical basal part and a short, narrowed neck. Conidia are ellipsoidal and smooth-walled. *P. clavigerum* is mainly separated from the other species of the series *Claviformia* by the structure of the synnemata. The coremia of most synnematous species of the subgenus *Penicillium* consist of a more or less, sterile stalk and a fertile capitulum, whereas the synnemata of *P. clavigerum* show no clear differentiation into stalk and capitulum. They are acicular and composed of a stalk, covered over nearly the entire length with conidiophores.

Pitt (1979) regarded *P. clavigerum* as a synonym of *P. duclauxii*, since the structure of their synnemata is alike. Yet, *P. clavigerum* does not produce the acerose phialides of the series *Duclauxii* (subgenus *Biverticillium*) but is characterized by the flask-shaped phialides typical of the subgenus *Penicillium* and consequently belongs in the latter subgenus. Moreover the penicilli and conidia, as well as the cultural appearance are quite different. *P. clavigerum* is placed here in the *Claviformia* (subgenus *Penicillium*) because of its morphological affinities with *P. coprophilum* and its biochemical affinities with *P. glandicola*. This classification based on morphology is supported by chemotaxonomic evidence (Samson *et al*., 1989, p. 140). *P. duclauxii* produce duclauxin and other secondary metabolites typical of the genus *Talaromyces* and subgenus *Biverticillium* (Frisvad *et al*., 1990d), and have no extrolites in common with *P. clavigerum* at all. In contrast to *P. vulpinum*, the conidiophores of *P. clavigerum*, like those of the remaining members of *Claviformia*, retain their individuality.

In *P. vulpinum*, the cultures are strongly coremiform. Coremia consisting of a sterile stalk and a subglobose to ellipsoidal, fertile capitulum, comprised of interwoven, anastomosing penicilli forming a hymenium-like layer. Conidiophore stipes of varying length, somewhat sinuous and interlaced, smooth-walled. Penicilli dichotomously two- to four-stage-branched, with both branches per branching point nearly equal in length, all elements smooth-walled. Branches sinuous, gnarled, strongly interwoven, the ultimate branches bearing 2-3 metulae. Phialides slender, with a relatively long, cylindrical basal part and a short, slightly narrowed neck. Conidia ellipsoidal, smooth-walled, adhering in well-defined columns.

The characteristic, well-developed coremia and the dichotomously two- to four-stage-branhced conidiophores distinguish *P. vulpinum* from all other species of *Penicillium*. Raper & Thom (1949) proposed the series *P. claviforme* to include the two species: *P. claviforme* (= *P. vulpinum*) and *P. clavigerum*. They included their *P. claviforme* series in the subsection *Fasciculata* since both species produce well-defined coremia. This assigment was accepted by Samson *et al*. (1976) and Ramirez (1982). According to Pitt (1979) *P. claviforme* and *P. clavigerum* should be classified in the series *Duclauxii* of the subgenus *Biverticillium*. However, the phialides of both species are characterized by a short, truncate neck. Acuminate necks, as occurring in the subgenus *Biverticillium* were not observed by us. Using DNA sequence data, LoBuglio *et al.*, 1994 also showed that these two strongly synnematous species belong in subgenus *Penicillium*.

P. coprophilum, *P. coprobium* and *P. concentricum* resemble one another closely. Coremia, conidiophores, conidia as well as cultural characters (e.g. rate of growth and zonation of the cultures) are identical. Their profiles of metabolites are, however, quite different. Moreover they show differences in the colouration of their colony reverses. *P. coprobium* can produce white sclerotia, which are absent in the other two species. *P. concentricum* shows because of its conidia and smooth-walled stipes more affinities with *P. coprophilum* than with *P. glandicola*. It is regarded here as a separate species near *P. coprophilum*.

The *Claviformia* are separated from the *Corymbifera* by their ellipsoidal conidia and their better-developed coremia. They differ from the *Expansa* by their more restricted growth and the more prominent coremia. In addition the phialides of the *Claviformia* are slender as compared with the robust conidia - bearing structures of *P. expansum*.

This series is here amended to include all known synnematous coprophilic species of *Penicillium*. They all have predominantly smooth walled ellipsoidal conidia and all species produce patulin. The members of this group have several features in common with species in series *Expansa* and *Urticicolae* including production of patulin and/or griseofulvin and roquefortine C, smooth walled stipes and smooth-walled ellipsoidal conidia and the production of synnemata.

Ex type cultures of *P. granulatum* (CBS 333.48), *P. schneggii* (NRRL 985) and *P. granulatum* var. *globosum* (IMI 299049) were all examined and found to agree with *P. glandicola*.

The ex type cultures of *P. claviforme* (CBS 126.23) and *P. silvaticum* (NRRL 1001) were both found to agree in all features with *P. vulpinum*.

Series *Italica* Raper & Thom ex Pitt, Gen. Penicil.: 381, 1979

= Series *P. italicum* Raper & Thom, Man. Penicillia: 523, 1949 (nom. inval., arts 21,36)
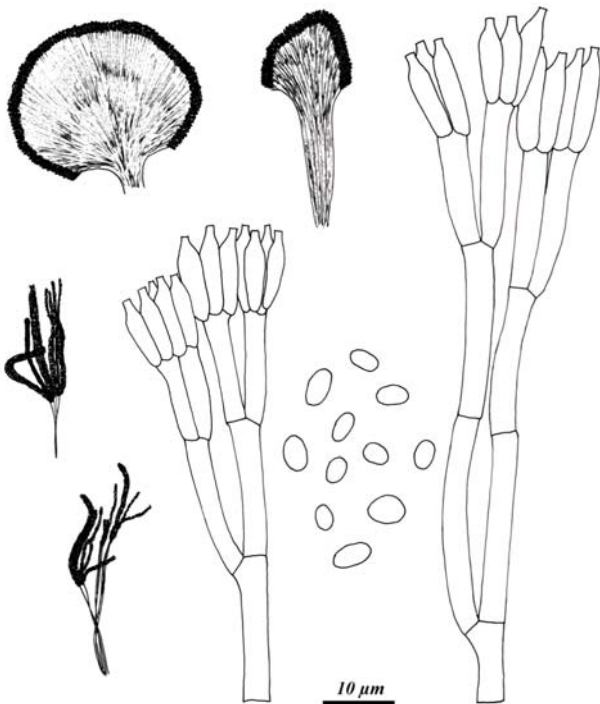
Type species: *P. italicum*

Fig. 18. Conidiophores and conidia of *P. italicum*

Accepted species:

P. italicum Wehmer, Hedwigia 33: 211, 1894.
= *Oospora fasciculata* (Grev.) Sacc. & Vogl. apud Sacc., Syll. Fung. 4: 11, 1886.
= *P. aeruginosum* Dierckx, Ann. Soc. Scient. Brux. 25: 87, 1901.
= *P. ventruosum* Westling, Ark. Bot. 11: 112, 1911.
= *P. digitatum* var. *latum* Abe, J. Gen. Appl. Microbiol. 2: 97, 1956.
= *P. japonicum* G. Smith, Trans. Br. Mycol. Soc. 46: 333, 1963.
= *P. italicum* var. *avellaneum* Samson & Gutter, Stud. Mycol. (Baarn) 11: 30, 1976.
Neotype: CBS 339.48

P. ulaiense Hsieh, Su & Tzean, Trans. Mycol. Soc. R.O.C. 2: 161, 1987.
Holotype: PPEH 29001.87

This is a natural series adapted to citrus fruits (Holmes *et al.*, 1994). The species share several extrolites, yet each species both produce species specific extrolites. The series has been emended to only include *P. italicum* and *P. ulaiense*, as the other species included in it were *P. fennelliae, P. digitatum* and *P. resticulosum* (Pitt, 1979) and these latter species are very different from the core species *P. italicum*.

Section diagnosis: Conidiophores mononematous to definitely synnematous, especially in marginal areas, one- to two-stage-branched, with all elements appressed and smooth-walled. Synnemata typically consisting of short to long stalks, up to 1 mm or more in length, erect or ascending, sometimes arising well below the agar surface, bearing terminal fertile parts. Stipes long sometimes slightly sinuous, forming the stalks of the synnemata. Metulae in verticils of 2-4. Phialides parallel, large, robust with a broad, cylindrical base and a wide neck, varying in length and merging almost imperceptibly into the conidial chain. Conidia cylindrical to ellipsoidal, smooth-walled, adhering in parallel chains occasionally forming loose columns. Colonies have greyish blue-green shades (rarely white or avellaneous). Teleomorph and sclerotia probably absent. Wehmer (1894) and Thom (1910) described sclerotia in *P. italicum*, but failed to find ascospores. Schwarz (1927) reported on a strain of *P. italicum*, which produced sclerotia upon oranges. After 12 weeks a few sclerotia developed asci. The smooth ascospores were provided with an equatorial ring, they measured 3.9 x 2.6 μm. Since then no ascomatal or sclerotial strain of *P. italicum* has been reported. Schwarz's description suggests that his strain represented a species of *Eupenicillium*, possibly *E. baarnense*.

The series is represented by two species, *P. italicum* and *P. ulaiense*. They cause a characteristic blue rot of citrus fruits. *P. italicum* grows much faster and has a more strongly coloured red-brown reverse on Cz based agars as compared to *P. ulaiense*. *P. italicum* and *P. ulaiense* are distinguished from *P. digitatum* by the synnematous conidiophores, the better developed one- to two-stage-branched penicilli as well as by the greyish, blue-green colonies. White or avellaneous mutants of *P. italicum* may occasionally be encountered.

P. italicum is somewhat reminiscent of *P. expansum* (series *Expansa*). However, *P. italicum* produces (one-) two-stage-branched conidiophores, whereas in *P. expansum* the penicilli range from two- to three-stage-branched. Moreover, the penicillic elements of *P. italicum* are larger, the conidia are also larger and more definitely ellipsoidal and the colonies differ strongly in appearance. In addition, *P. italicum* causes a blue rot of Citrus fruit, whereas *P. expansum* causes a rot of pomaceous fruits. *P. resticulosum*,

placed by Pitt (1979) in the *Italica*, seems in spite of its cylindrical to pyriform conidia, to be better classified in series *Expansa*. It is regarded to be an extreme variant of *P. expansum*. *P. japonicum* (CBS 327.59), placed by Pitt (1979) under *P. resticulosum*, is a typical *P. italicum*.

Series *Gladioli* Raper & Thom ex Stolk & Samson, Adv. Pen. Asp. Syst.: 183, 1985
= Series *P. gladioli*, Man. Penicillia: 471, 1949 (nom. inval., arts 21,36)
Type species: *P. gladioli*

Accepted species:

P. gladioli McCulloch & Thom, Science, N.Y. 67: 217, 1928.
= *P. gladioli* Machacek, Rep. Queb. Soc. Prot. Pl. 19: 77, 1928
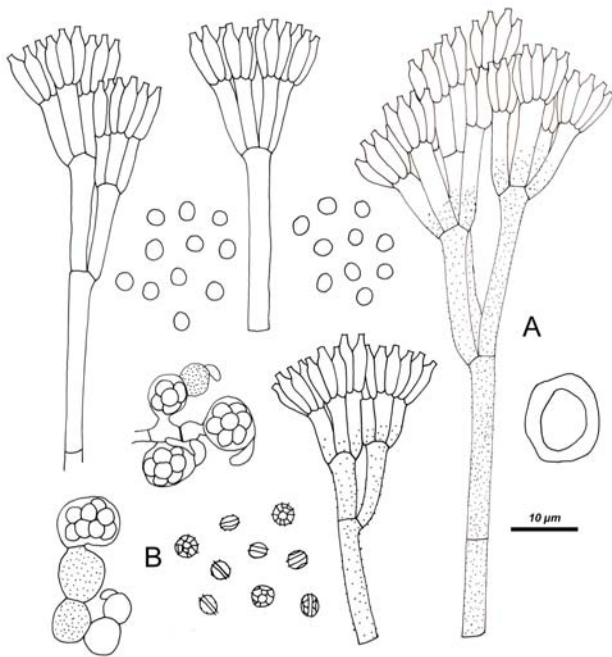Neotype: IMI 034911

Fig. 19. Conidiophores and conidia of (A) *P. gladioli* and (B) *P. sinaicum*

Series diagnosis: Conidiophores are mononematous, occasionally slightly synnematous. Stipes are varying in length, but are typically long. They are bearing terminal, slender one-two-three-stage-branched penillus, but with the lower branch often somewhat divergent, and stipes often rough walled. Metulae in verticils of 2-5; one to two, usually single, lower branches, each one with 2-4 apical metulae. Phialides consisting of a cylindrical base and a relatively short, narrowed neck. In *P. gladioli* conidia globose to subglobose (occasionally at first ellipsoidal), with walls smooth, adhering in parallel or tangled chains, sometimes forming ill-defined columns. Sclerotia

often present. There are no teleomorphs known, but *E. crustaceum* was regarded as the teleomorph of *P. gladioli* by Pitt (1979). Even though this pair of species is not representing the same species, they surely show a series of morphological similarities.

P. gladioli shows some affinities with the series *Corymbifera*. Both series are distinguished by the structure of their cultures. Species of the series *Corymbifera* are characterized by well-defined synnemata, whereas in *P. gladioli*, the conidiophores are usually mononematous, with only a few small fascicles occasionally present. Moreover the sclerotia which are typically present in *P. gladioli* are lacking in the *Corymbifera*. In both series the majority of species can cause a rot in below ground roots, bulbs, or onions.

Species of the series *Gladioli* show many affinities with those of the other series of the subgenus *Penicillium*, though especially with the series *Viridicata*. All of them agree in the structure of their conidiophores. The *Gladioli* are mainly distinguished from the other species of the present section by their hard, well-developed sclerotia and poor sporulation.

Section *Digitatum* Frisvad & Samson, **sect. nov.**
= Series *Digitata* Raper & Thom ex Stolk & Samson, Adv. Pen. Asp. Syst.: 183, 1985 = Series *P. digitatum* Raper & Thom, Man. Penicillia: 385, 1949 (nom. inval., arts 21,36)

Sectio generis *Penicillium* subgeneris *Penicillium*, penicillis biverticillatis vel raro terverticillatis, stipitibus levibus, metulis phialidibusque magnis, conidiis olivaceis, magnis, ellipsoideis vel cylindricis; coloniae in omnibus substratis velutinae; creatinum, nitritum et nitratum velut substratum nitrogeni parce assimilantur, in medio creatinum et sucrosium continente acidum haud formatur; 30ºC et supra non crescentes neque 5% NaCl addito; fructus citricos putrescens.
Typus *P. digitatum*.

P. digitatum (Pers.:Fr.) Sacc., Fung. Ital.: 894, 1881.
= *Monilia digitata* Pers. ex Fr., Syst. Mycol. 3: 411, 1832.
= *Monilia digitata* Pers., Syn. Meth. Fung.: 693, 1801.
= *Aspergillus albus tenuissimus, graminis dactyloidis facie, seminibus rotundis* Mich., Nova Pl. Gen.: 213, 1729.
= *Mucor caespitosus* L., Sp. Pl. 2: 1186, 1753.
= *Penicillium olivaceum* Wehmer, Beitr. Kennt. Einh. Pilze 2: 73, 1895.
= *P. olivaceum* Sopp, Skr. Vidensk. Selsk. Christiana 11: 176, 1912.
= *P. olivaceum* var. *norvegicum* Sopp, Skr. Vidensk. Selsk. Christiana 11: 177, 1912.
= *P. olivaceum* var. *italicum* Sopp, Skr. Vidensk. Selsk. Christiana 11: 179, 1912.
= *P. digitatoides* Peyronel, Germi Atmosferici Fung. Micel.: 22, 1913.
= *P. lanosogrisellum* Biourge, Cellule 33: 196, 1923.

= *P. terraconense* Ramírez & Martínez, Mycopathologia 72: 187, 1980.

Lectotype: icon in Saccardo, Fung. Ital.: tab. 894 Jul. 1881, Epitype proposed here: CBS 112082.
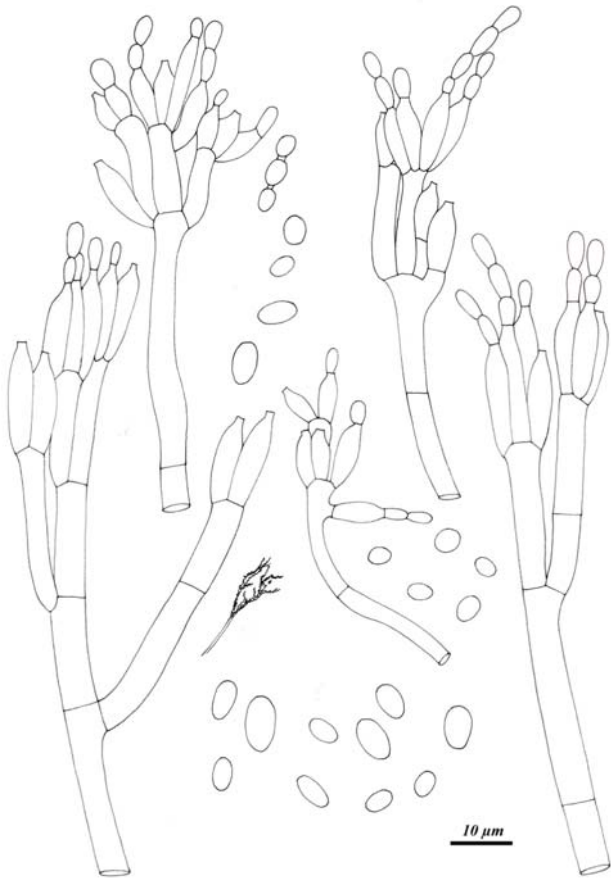
Fig. 20. Conidiophores and conidia of *P. digitatum*

This series comprises only the very distinct species *P. digitatum*. The olive coloured conidia and large irregular micromorpholopgical structures and cylindrical phialides and conidia are unique in *Penicillium*. No relatives are known although it shows some resemblance to the anamorph structures of Penicilliopsis and its placement in subgenus *Penicillium* has been questioned (Stolk and Samson, 1985). There are no extrolites in common between *P. digitatum* and the two members of *Italica*.

Of the synonyms of *P. digitatum*, only *P. terraconense* was available as an ex type culture (CBS 177.81). This was a typical *P. digitatum*.

Section *Viridicata* Frisvad & Samson, sect. nov.

Sectio generis *Penicillium* subgeneris *Penicillium*, penicillis terverticillatis, stipitibus levibus vel plerumque asperulatis vel rugosis, conidiis globosis vel subglobosis; coloniae 5% NaCl addito plerumque stimulantur, 37ºC haud crescentes, granulares vel modice fasciulatae; species praecipue in cerealibus, bulbis horticolis, *Allio cepa*, carne, nucibus mel productis e lacte derivatis inventae; patulinum, griseofulvinum vel deoxybrevianamidum E

haud producta, sed una vel complures substantiarum sequentium: verrucolonum, acidum penicillicum, xanthomegninum, rugulovasinum, acidum cyclopiazonicum, viridicatina, territrema.

Typus *P. viridicatum*.

Series *Viridicata* Raper & Thom ex Pitt, Gen. Penicil.: 334, 1979

= Series *P. viridicatum* Raper & Thom, Man. Penicillia: 481, 1949 (nom. inval., arts 21,36)

= Series *P. cyclopium* Raper & Thom, Man. Penicillia: 490, 1949 (nom. inval., arts 21,36)

= Series *P. ochraceum* Raper & Thom, Man. Penicillia: 475, 1949 (nom. inval., arts 21,36)

= *Ochracea* Fassatiová, Acta Univ. Carol. Biol. 12: 324, 1977
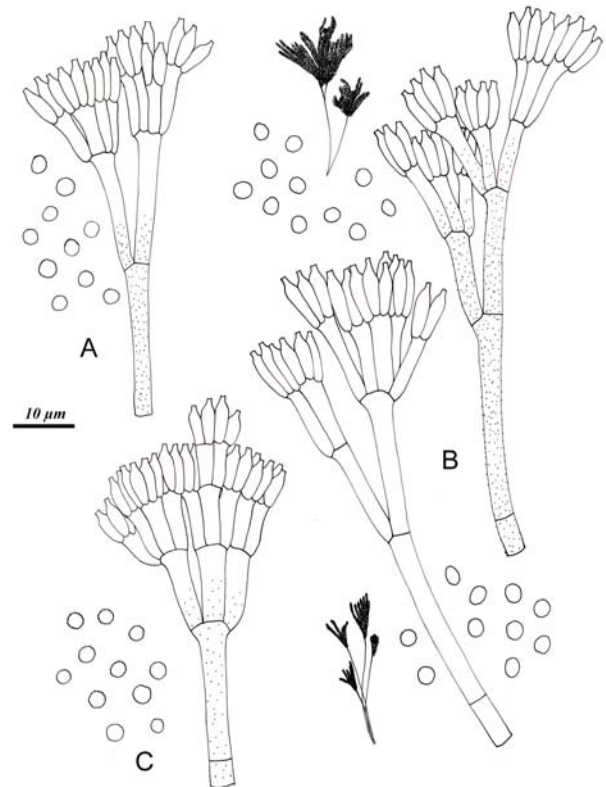
Fig. 21. Conidiophores and conidia of (A) *P. aurantiogriseum*, (B) *P. melanoconidium* and (C) *P. viridicatum*

Accepted species:

P. aurantiogriseum Dierckx, Ann. Soc. Scient. Brux. 25: 88, 1901

= *P. aurantiogriseum* var. *poznaniense* K.M. Zalesky, Bull. Int. Acad. Pol. Sci. Lett., Sér. B 1927: 444, 1927. Neotype: IMI 195050

P. cyclopium Westling, Ark. Bot. 11: 90, 1911.

= ?*P. aurantiocandidum* Dierckx, Ann. Soc. Scient. Brux. 25: 88, 1901.

= ? *P. puberulum* Bain., Bull. Trimest. Soc. Mycol. Fr. 23: 16, 1907.

= *P. porraceum* Biourge, Cellule 33: 188, 1923.

= *P. aurantiovirens* Biourge, Cellule 33: 119, 1923.

= ?*P. janthogenum* Biourge, Cellule 33: 143, 1923.

= *P. brunneoviolaceum* Biourge, Cellule 33: 145, 1923.

= *P. martensii* Biourge, Cellule 33: 152, 1923.

= *P. aurantio-albidum* Biourge, Cellule 33: 197, 1923.
= *P. johanniolii* K.M. Zalessky, Bull. Int. Acad. Pol. Sci. Lett., Sér. B 1927: 453,1927.
= *P. cyclopium* var. *aurantiovirens* (Biourge) Fassatiová, Acta Univ. Carol. Biol. 12:326, 1977.
= *P. cordubense* Ramírez & Martínez, Mycopathologia 74: 164, 1981
= *P. viridicyclopium* Abe, J. gen. Appl. Microbiol. 2: 107, 1956.
Neotype: IMI 089372

P. freii Frisvad & Samson, **sp. nov**.
A *Penicillio aurantiogriseo* coloniis crustosis et inertia 30ºC crescendi distinguitur; xanthomegninum, viomelleinum, vioxanthinum, cyclopeptinum, dehydrocyclopeptinum, cyclopeninum, cyclopenolum, viridicatolum, 3-methoxyviridicatinum formantur, neque auranthinum, anacinum, acidum terrestricum.
Typus: IMI 285513

P. melanoconidium (Frisvad) Frisvad & Samson, **comb. nov.**
Basionym: *P. aurantiogriseum* var. *melanoconidium* Frisvad, Mycologia 81:849, 1989.
Holotype: IMI 321503

P. neoechinulatum (Frisvad, Filt. & Wicklow) Frisvad & Samson, **comb. nov**.
Basionym: *P. aurantiogriseum* var. *neoechinulatum* Frisvad, Filt. & Wicklow, Can. J. Bot. 65: 767, 1987.
Holotype: IMI 296937

P. polonicum K. Zaleski, Bull. Int. Acad. Pol. Sci. Lett., Sér. B 1927: 445, 1927.
= *P. aurantiogriseum* var. *polonicum* (K.M. Zalessky) Frisvad, Mycologia 81: 850.
= *P. carneolutescens* G. Smith, Trans. Br. Mycol. Soc. 22: 252, 1939.
Neotype: CBS 222.28

P. tricolor Frisvad, Seifert, Samson & Mills, Can. J. Bot. 72: 937, 1997 (check).
Holotype: DAOM 216240

P. viridicatum Westling, Ark. Bot. 11: 88, 1911.
= *P. olivinoviride* Biourge, Cellule 33: 132, 1923.
= *P. blakesleei* K.M. Zalessky, Bull. Int. Acad. Pol. Sci. Lett., Sér. B, 1927: 441, 1927.
= *P. stephaniae* K.M. Zalessky, Bull. Int. Acad. Pol. Sci. Lett., Sér. B, 1927: 451, 1927.
= *P. ochraceum* Bain. apud Thom, Penicillia: 309, 1930.
= *P. verrucosum* var. *ochraceum* (Bain.) Samson, Stolk & Hadlok, Stud. Mycol. (Baarn) 11: 42, 1976.
= *P. olivicolor* Pitt, Gen. Penicil.: 368, 1979.
= *P. aurantiogriseum* var. *viridicatum* (Westling) Frisvad & Filt., Mycologia 81: 850, 1989.
Neotype: IMI 039758ii
All species included in the series *Viridicata* produce similar two-, occasionally three- stage-branched (terverticillate) conidiophores, as well as robust phialides. The conidia of most species range from ellipsoidal or subglobose to globose, with walls smooth or nearly so (with the exception of the strongly roughened conidia of *P. neoechinulatum*).

Species of the *Viridicata* are very important spoilage and mycotoxin producing fungi in cereals, and consequently a correct identification is very important. Unfortunately, the identification of these species is often problematic, because of the great morphological variation within the species and the presence of morphologically intergrading strains.

Raper & Thom (1949) considerably reduced the number of species by regarding many of them as synonymns. However, they still maintained a great number of closely related species, which they distinguished by minor differences, such as colony texture and colour. In an attempt to simplify species determination in this series, Samson *et al.* (1976) proposed to emend the description of *P. verrucosum*, thus creating a large, variable species, which they divided into six varieties, mainly based on conidial colour. Pitt (1979) used colony diameter at different temperatures and water conditions together with conidial and colony pigmentation as means to separate the species of *Penicillium*. Frisvad (1981, 1983, 1985) introduced the application of physiological criteria, such as the production of extrolites (for details see above) in an attempt to come to a more accurate delimitation of species. In the present paper the species are based on both morphological and physiological characters (especially on profiles of extrolites). Since the morphological differences between most species of the *Viridicata* are very slight, the taxonomical importance of the extrolites is emphasized. Species of the series *Viridicata* show close affinities with the series *Corymbifera*, since the structure of the conidiophores is identical. Both series are distinguished by morphological criteria. In the *Viridicata* the conidiophores range from strictly mononematous to very slightly synnematous, whereas in the *Corymbifera* many conidiophores are aggregated into well-defined synnemata, usually appearing as conspicuous or loosely branched coremia. The *Viridicata* are mainly separated from the *Expansa* by their globose to subglobose conidia and by their weak growth on creatine as sole N-source.

This series has been discussed by Frisvad and Lund (1993) and Lund and Frisvad (1994). It is characterized by the seed and cereal habitats (good amylase production) and production of several extrolites. It is clearly a polythetic series concerning extrolites, as no metabolites are common to all nine species (Svendsen and Frisvad, 1994; Larsen and Frisvad, 1995; Smedsgaard and Frisvad, 1997).

The ex type culture of *P. aurantiogriseum* var. *poznaniense* (NRRL 972) is a typical *P. aurantiogriseum* and not a *P. crustosum* as claimed by Pitt (1979).

Two names, *P. aurantiocandidum* and *P. puberu-lum* predate *P. cyclopium*. The ex type culture of *P. aurantiocandidum* is very degenerated and deteriorating and it identity is questionable. It is proposed to treat *P. aurantiocandidum* a *nomen dubium*. *P. puberulum* is based on neotype material consisting of a mixed culture. NRRL 1889 (also NRRL 2040) represents *P. cyclopium*, while NRRL 845 represents *P. commune*. This species is therefore also here regarded as a *nomen dubium*.

Ex type cultures of *P. aurantiovirens* (CBS 294.48), *P. brunneoviolaceum* (CBS 256.29), *P. aurantioalbidum* (NRRL 887), *P. johaniolii* (NRRL 956), *P. viridicyclopium* (CBS 349.59), *P. martensii* (authentic, CBS 111.43) and *P. cordubense* (CBS 162.81) were inseparable from *P. cyclopium*. The ex type culture of *P. carneolutescens* (CBS 278.39) proved to be a synonym of *P. polonicum*. Ex type cultures of *P. olivinoviride* (CBS 264.29), *P. ochraceum* = *P.olivicolor* (CBS 246.32) were found to be synonyms of *P. viridicatum*.
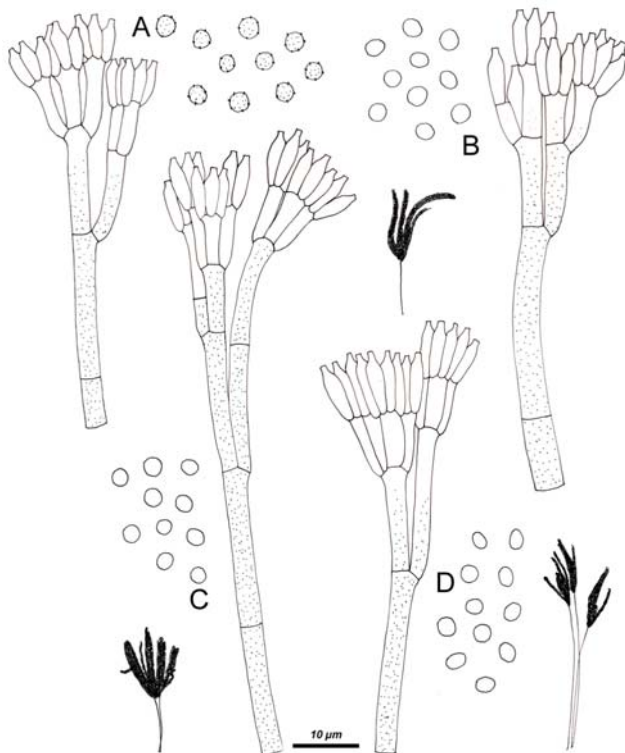
Fig. 22. Conidiophores and conidia of (A) *P. crustosum* (B) *P. neoechinulatum,* (C). *P. aethiopicum* and (D). *P. verrucosum.*

Series *Verrucosa* Frisvad, Int. Mod. Meth. Pen. Asp. Clas., 274, 2000.
Type species: *P. verrucosum*

Accepted species:

P. nordicum Dragoni & Cantoni ex Ramírez, Adv. Pen. Asp. Syst.: 139, 1985.

= *P. nordicum* Dragoni & Cantoni, Ind. Aliment 155: 283, 1979 (nom. inval., art 36).
= *P. mediolanense* Dragoni & Cantoni, Ind. Aliment 155: 281, 1979 (*nom. inval.*, art 36)
Type: ATCC 44219

P. thymicola Frisvad & Samson, **sp.nov**.
A *Penicillio nordico* conidiis asperulatis et reverso luteo-aurantio coloniae in agaris CYA et YES distinguitur; fumiquinazolinum F, daldininum C, alantrypinonum formantur, neque anacina et ochratoxinum A.
Typus: CBS 111225

P. verrucosum Dierckx, Ann. Soc. Scient. Brux. 25: 88, 1901.
= *P. casei* Staub, Zentralbl. Bakt. ParasitKde. Abt. II, 31: 454, 1911.
Neotype: IMI 200310

Micromorphologically these species are similar to series *Viridicata*, *Corymbifera*, *Camembertii* and *Solita*. A number of differences set these species apart from any other member of the subgenus , however. They are all slow growing species that can grow on nitrite as sole N-source, but only weakly on creatine as sole N-source. The production of verrucolone is common to all species in *Verrucosa* (Larsen *et al*., 2002). Some of the extrolites produced by *P. verrucosum* (the verrucins and a red brown pigment) are autapomorphic and only citrinin is shared with other *Penicillium* species. *P. verrucosum* has been found on cereals from temperate zones, whereas *P. nordicum* has been recovered from salted meat products and cheese from Northern and Southern Europe. *P. thymicola* is a less common species, but has been found on herbs from Sourthern Europe. These species have often been referred to species in *Viridicata*, but differs in a large number of features from those species, including growth on nitrite-sucrose agar and no acid production on creatine-sucrose agar.

P. casei (CBS 302.48) is a typical *P. verrucosum* in all aspects. *P. mediolanense* (ATCC 44220), an invalid name, is a synonym of *P. nordicum*.

Series *Corymbifera* Frisvad, Int. Mod. Meth. Pen. Asp. Clas., 275, 2000
Type species: *P. hirsutum*

Accepted species:

P. albocoremium (Frisvad) Frisvad, Int. Mod. Tax. Meth. Pen. Asp. Clas.: 275, 2000.
Basionym: *P. hirsutum* var. *albocoremium* Frisvad, Mycologia 81: 856, 1989.
Holotype: IMI 285511

P. allii Vincent & Pitt, Mycologia 81: 300, 1989.

= *P. hirsutum* var. *allii* (Vincent & Pitt) Frisvad, Mycologia 81: 856, 1989.
Holotype: MU Vincent 114

P. hirsutum Dierckx, Ann. Soc. Scient. Brux. 25: 89, 1901.
= *P. corymbiferum* Westling, Ark. Bot. 11: 92, 1911.
= *P. verrucosum* var. *corymbiferum* (Westling) Samson, Stolk & Hadlok, Stud. Mycol.(Baarn) 11: 36, 1976.
= ? *P. hispalense* Ramírez & Martínez, Mycopathologia 74: 169, 1981.
Neotype: IMI 040213
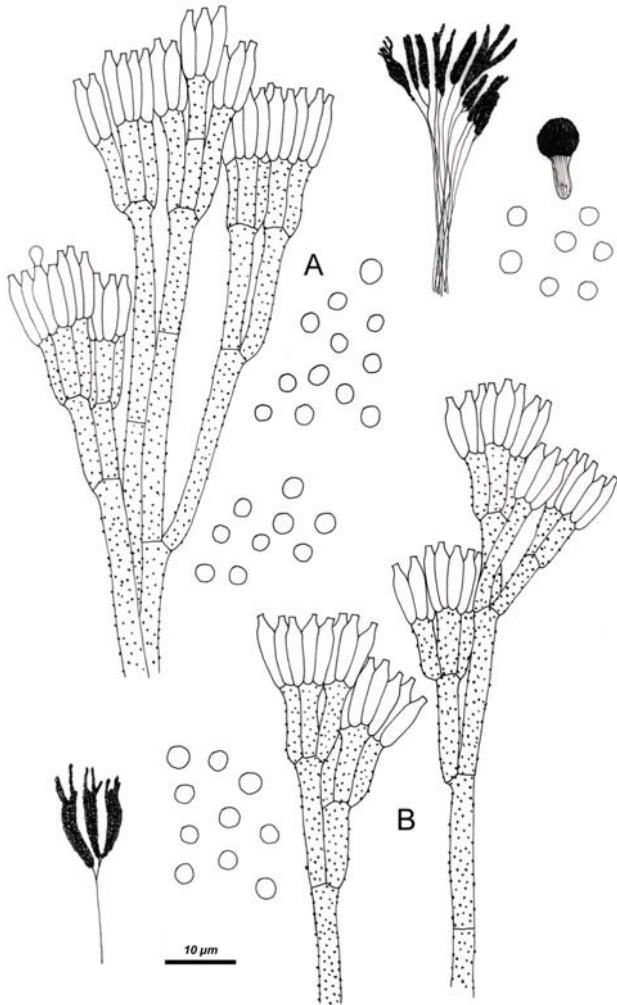
Fig. 23. Conidiophores and conidia of (A) *P. hirsutum* and (B) *P. allii*

P. hordei Stolk, Ant. van Leeuwenhoek 35: 270, 1969.
= *P. hirsutum* var. *hordei* (Stolk) Frisvad, Mycologia 81: 856, 1989.
Holotype: CBS 701.68
P. radicicola Overy & Frisvad, Syst. Appl. Microbiol.: 633, 2003.
Holotype: C 60161

P. tulipae Overy & Frisvad, Syst. Appl. Microbiol. 634, 2003.
Holotype: C 60162

P. venetum (Frisvad) Frisvad, Int. Mod. Tax. Meth. Pen. Asp. Clas.: 275, 2000.
= *P. hirsutum* var. *venetum* Frisvad, Mycologia 81: 856, 1989.
Holotype: IMI 321520
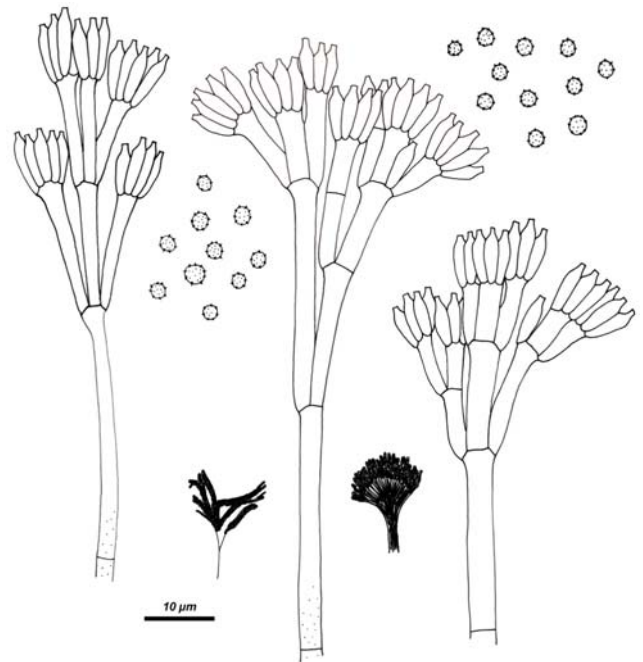
Fig. 24. Conidiophores and conidia of *P. hordei*

Section diagnosis: Conidiophores mononematous to strongly synnematous, usually with most conidiophores aggregated into small fascicles or well-developed coremia, but a variable number of them mononematous. Coremia commonly consisting of a stalk, comprised of the stipes of the conidiophores and an apical feather-like capitulum, comprised of separate, diverging conidiophores. Stipes variable in length, typically very long, with walls conspicuously or finely roughened, (occasionally smooth). Penicilli large, two- to three-stage-branched. Metulae in verticils of 2-6. Branches 1(2) per branching point. All elements of the penicillus appressed, the lower branch occasionally a little divergent. Phialides with a cylindrical base and a short, but distinct, slightly narrowed neck. Conidia globose to slightly subglobose, with walls smooth or roughened, adhering in parallel, occasionally tangled chains, sometimes forming diverging, loose columns. Teleomorph absent. Sclerotia not present.

Frisvad & Filtenborg (1989) recognized five varieties of *P. hirsutum*. It is preferred here to regard them as species because of their marked, morphological differences. Together seven species comprise the new series *Corymbifera*, which is characterized by deep fasciculate to coremiform colonies, conidiophores with walls strongly roughened (less pronounced in *P. hordei*, which produces finely roughened to smooth-walled stipes) globose, smooth-walled conidia (except the finely roughened conidia of *P. hordei*) and by the production of terrestric acid

(except *P. allii* and *P. albocoremium*) and roque-fortine C. The *Corymbifera* differ from the *Gladioli* in the absence of sclerotia, as well as in the pronounced fasciculation. Cultures of the *Viridicata* lack the production of well-developed coremia. The *Corymbifera* and *Claviformia* have the fasciculate to coremiform character of the colonies in common, though the coremia of the *Claviformia* are usually better developed. Both series differ mainly in the shape of the conidia.

These seven distinct fasciculate species are all associated with onions, tap root plants and flower bulbs, except *P. hordei* which is associated with barley and other cereals. *P. hordei* has, however, been found on lilaceous bulbs also. *P. albocoremium sensu lato* have recently been split into three species (Overy and Frisvad, 2003) in agreement with chemotaxonomic studies by Larsen and Frisvad (1995) and Smedsgaard and Frisvad (1997). In the extrolite study of Svendsen and Frisvad (1994) all these species clustered with *P. crustosum,* except *P. hordei*, which clustered with *P. aurantiogriseum*, another cereal-borne species. The seven species in this series seems to most closely related to *Viridicata* and *P. crustosum*.

Series *Solita* Frisvad, Int. Mod. Meth. Pen. Asp. Clas., 279, 2000
Type species: *P. solitum*

Accepted species:

P. discolor Frisvad & Samson, Ant. van Leeuwenhoek, 72: 120, 1997.
Holotype: IMI 285513

P. echinulatum Fassatiová, Acta Univ. Carol. Biol. 12: 326, 1977.
    = *P. cyclopium* var. *echinulatum* Raper & Thom, Man. Penicil.: 497, 1949.
    = *P. palitans* var. *echinoconidium* Abe, J. Gen. Appl. Microbiol. 2: 111, 1956.
Holotype: PRM 778523

P. solitum Westling, Ark. Bot. 11: 65: 1911.
    = *P. majusculum* Westling, Ark. Bot. 11: 60, 1911.
    = *P. conditaneum* Westling, Ark. Bot. 11: 63, 1911.
    = *P. paecilomyceforme* Szilvinyi, Zentralbl. Bakt. ParasitKde., Abt. II, 103: 156, 1941.
    = *P. casei* Staub var. *compactum* Abe, J. Gen. Appl. Microbiol. 2: 101, 1956.
    = *P. mali* Novobr., Biol. Nauki 10: 105, 1972 (nom. inval., art. 36, 37, 38)
    = *P. verrucosum* var. *melanochlorum* Samson, Stolk & Hadlok, Stud. Mycol. (Baarn) 11: 41, 1976.
    = *P. mali* Gorlenko & Novobr., Mikol. Fitopatol. 17: 464, 1983 (nom. inval., art. 64)

    = *P. melanochlorum* (Samson, Stolk & Hadlok) Frisvad, Adv. Pen. Asp. Syst.: 330, 1985.
Neotype: CBS 424.89

P. cavernicola Frisvad & Samson, **sp. nov.**
    = *P. crustosum* var. *spinulosporum* Sasaki (*nom. inval.*, Art. 36)
A *Penicillio echinulato* reverso violaceo-brunneo coloniae in agaro CYA distinguitur; asteltoxinum, glyanthrypinum, aurantiaminum, dipodazinum formantur neque cyclopeptinum, dehydrocyclopeptinum, cyclopeninum, cyclopenolum, viridicatinum, viridicatolum.
Typus: CBS 100540

Micromorphologically these species resemble series *Viridicata*. This series contains three closely related species with rough or thick walled dark green conidia and rough walled conidiophore stipes. All species grow well on creatine as sole N-source. They all produce the viridicatin biosynthetic family except *P. cavernicola*. The three species were distinct yet included in the same main cluster in the HPLC analysis based on extrolites reported by Svendsen and Frisvad (1994). In an electrospray mass spectrometric (ES-MS) study of extrolites of the terverticillate Penicillia, *P. solitum* clustered with *P. echinulatum* when grown on CYA agar and *P. echinulatum* clustered with *P. discolor* as its nearest neighbour when cultures were grown on YES agar (Smedsgaard and Frisvad, 1997). The reason these species also clustered with several members of *Viridicata* and *P. crustosum* was that the viridicatin biosynthetic family was present in all these species and these dominated the ES-MS profiles. Concerning volatile secondary metabolites the three species were distinct and not very similar (Larsen and Frisvad, 1995).

P. solitum has several synonyms. The ex type cultures of *P. majusculum* (CBS 423.89), *P. paecilomyceforme* (CBS 160.42), *P. mali* (CBS 500.73), *P. casei* var *compactum* (CBS 427.65) and *P. melanochlorum* (CBS 487.75) were examined and all were typical or deteriorated *P. solitum*.

P. palitans var. *echinoconidium* (CBS 337.59) was examined and is inseparable from *P. echinulatum*.

Series *Camemberti* Raper & Thom ex Pitt, Gen. Penicil.: 358, 1979
    = Series *P. camemberti*, Raper & Thom, Man. Penicillia: 421, 1949 (nom. inval., arts 21,36)
    = Series *P. commune*, Raper & Thom, Man. Penicillia: 429, 1949 (nom. inval., arts 21,36)
Type species: *P. camemberti*
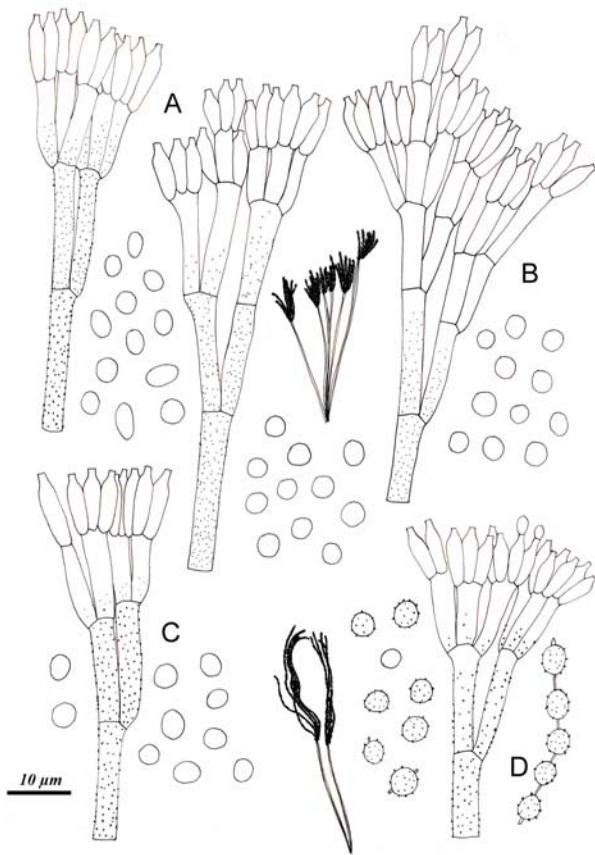
Fig. 25. Conidiophores and conidia of (A) *P. commune,* (B) *P. solitum,* (C) *P. camemberti* and (D) *P. echinulatum.*

Accepted species:

P. commune Thom, Bull. Bur. Anim. Ind. USDA 118: 56, 1910.
= *P. fuscoglaucum* Biourge, Cellule 33: 128, 1923.
= *P. flavoglaucum* Biourge, Cellule 33: 130, 1923.
= *P. lanosoviride* Thom, Penicillia: 314, 1930.
= *P. ochraceum* Thom var. *macrosporum* Thom, Penicillia: 310, 1930.
= *P. lanosoviride* Thom, Penicillia: 314, 1930.
= *P. lanosogriseum* Thom, Penicillia: 327, 1930.
= *P. psittacinum* Thom, Penicillia: 369, 1930.
= *P. australicum* Sopp ex van Beyma, Ant. van Leeuwenhoek 10: 53, 1944.
= *P. cyclopium* var. *album* G. Smith, Trans Brit. Mycol. Soc. 34: 18, 1951.
= *P. roqueforti* var. *punctatum* Abe, J. Gen. Appl. Microbiol. 2: 99, 1956.
= *P. caseiperdens* Frank, Beitr. Tax. Gat. Pen.: 91, 1966.
= *P. verrucosum* var. *album* (G. Smith) Samson, Stolk & Hadlok, Stud. Mycol. (Baarn) 11:35, 1976.
= *P. album* (G. Smith) Stolk & Samson, Adv. Pen. Asp. Syst.: 185, 1985.
Neotype: IMI 039812

P. camemberti Thom, Bull. Bur. Anim. Ind. USDA 82: 33, 1906.
= *P. album* Epstein, Ark. Hyg. Bakt. 45: 360, 1902.

= *P. epsteinii* Lindau, Rabenh. Krypt.-Fl. 1, Abt. 8: 166, 1904
= *P. rogeri* Wehmer apud Lafar, Handb. Tech. Mykol. 4: 226, 1906.
= *P. caseicola* Bain., Bull. Trimest. Soc. Mycol. Fr. 23: 94, 1907.
= *P. camemberti* var. *rogeri* Thom, Bull. Bur. Anim. Ind. US Dept. Agric. 118: 52, 1910.
= *P. biforme* Thom, Bull. Bur. Anim. Ind. US Dept. Agric. 118: 54, 1910.
= *P. camemberti* Sopp, Skr. Vidensk. Selsk. Christiana 11: 179, 1912.
= *P. candidum* Roger apud Biourge, Cellule 33: 193, 1923.
Lectotype: IMI 027831

P. caseifulvum Lund, Filt. & Frisvad, J. Food Mycol 1: 97, 1998.
Holotype: C 24999

P. palitans Westling, Ark. Bot. 11: 83, 1911.
Neotype: IMI 040215

P. crustosum Thom, Penicillia: 399, 1930.
= *P. pseudocasei* Abe, J. Gen. Appl. Microbiol. 2: 102, 1956.
= *P. pseudocasei* Abe ex G. Smith, Trans. Brit. Mycol. Soc. 46: 335, 1963.
= *P. terrestre* sensu Raper & Thom, Man. Penicil.: 450, 1949.
= *P. farinosum* Novobranova, Nov. Sist. Niz. Rast. 11: 232, 1974.
= *P. expansum* var. *crustosum* (Thom) Fassatiová, Acta Univ. Carol. Biol. 12: 329, 1977.
= *P. solitum* var. *crustosum* (Thom) Bridge, D. Hawksw., Kozak., Onions, R.R.M. Paterson, Sackin & Sneath, J. Gen. Microbiol. 135: 2957, 1989.
Neotype: IMI 091917

P. atramentosum Thom, Bull. Bur. Anim. Ind. US Dept. Agric. 118: 65, 1910.
Neotype: IMI 039752

These six species are united by their growth on creatine and nitrite as sole N-source and their association to proteinaceous and lipid-containing foods. Their micromorphology is similar to that of *Viridicata* (see above), except *P. atramentosum*, which has smooth stipes and a more divaricate penicillus structure similar to that of *P. chrysogenum*. Furthermore *P. atramentosum* is the most alkalitolerant of all terverticillate Penicillia. All species are particularly common on cheese, but may also be found on nuts. *P. camemberti* is the domesticated form of *P. commune* and is strictly associated to white mould cheese such as Camembert and Brie. *P. caseifulvum* is only found as a surface contaminant of blue cheeses, and may improve the flavour of these cheeses. *P. commune* and *P. atramentosum* were both described by Thom (1910) during his studies of cheese associated Penicillia and still these species are found as dominant

species on cheese (Lund *et al*., 1996). Polonelli *et al*. (1997) found that *P. palitans* was close to *P. commune*, but disitinct, and this was later confirmed by Lund (1995b). *P. crustosum* is also found on cheese, but is also common on nuts and in soil. Like *P. expansum* and *P. solitum*, *P. crustosum* can produce a rot in apples, albeit less severe than that of the two former species (Frisvad, 1981, Pitt *et al*., 1991).

Isolates in four of the species produce rugulovasine A and three of the species produce cyclopiazonic acid.

Ex type cultures of *P. fuscoglaucum* (CBS 261.29), *P. lanosoviride* (NRRL 930), *P. ochraceum* var. *macrosporum* (CBS 247.32), *P. lanosogriseum* (CBS 216.30), *P. psittacinum* (CBS 265.29), *P. australicum* (NRRL 935), *P. cyclopium* var. *album* = *P. verrucosum* var. *album* = *P. album* (CBS 343.51), and *P. roqueforti* var. *punctatum* (CBS 341.59) were all examined and found to be inseparable from *P. commune*.

Ex type cultures of *P. candidum* (NRRL 876), *P. rogeri* (CBS 123.08), *P. caseicola* (CBS 303.48) and *P. biforme* (CBS 297.48) were found to be inseparable from *P. camemberti*

Ex type or authentic cultures of *P. terrestre* (authentic, CBS 380.48), *P. pseudocasei* (CBS 340.59), and *P. farinosum* (CBS 499.73) were all typical *P. crustosum*.

ECOLOGY AND DISTRIBUTION OF SPECIES IN *PENICILLIUM* SUBGENUS *PENICILLIUM*

Most species in *Penicillium* subgenus *Penicillium* are associated to the foods and feeds of terrestrial animals or in some cases the dung of these animals (Frisvad *et al*., 2000). However few *Penicillium* species, if any, are generalists, and they are associated to particular habitats (Frisvad, 1988; Filtenborg *et al*., 1996). Some of these associations are so strong that they were recognized early on (Westerdijk, 1949), like the association of *P. italicum* and *P. digitatum* to citrus fruits, but most authors have regarded the Penicillia as ubiquitous "weed" organisms (Thom, 1930; Raper and Thom, 1949; Pitt, 1979). It is true that all Penicillia can grown on laboratory substrates, for example malt extract agar (made from barley) and oat meal agar, but this does not indicate that they are all associated to cereals, and even *P. digitatum* and *P. italicum* grow well on autoclaved cereal based laboratory media and do not require special media based on citrus peels. Actually malt extract and oat meal agar are excellent for inducing sclerotium and synnema production as

compared to chemically defined media or semi-synthetic media such as CYA and YES agar. On the other hand the latter media (containing the minerals, vitamins, amino acids etc. from yeast extract) are good for inducing the production of many extrolites (Frisvad and Filtenborg, 1989). The laboratory media have a strong influence on the phenotype of the Penicillia, but data from these substrates does not predict which habitats the fungi are associated to. The collection and examination of isolates used for this study has clearly shown that there are strong associations between the *Penicillium* species and their natural habitats. These associations are probably first of all based on chemical communications via extrolites and extracellular enzymes, but much more research is needed to explain these important association phenomena.

Abiotic factors also play a role, including the combination of temperature, redox potential, pH, pressure, water activity, and atmosphere (Andersen and Frisvad, 2001), but still none of these factors can explain the association of *P. italicum* to citrus fruits, *P. expansum* to pomaceous fruits, *P. commune* to cheeses and *P. aurantiogriseum* to cereals. Processing or treatment with chemicals may of course change the associated mycobiota of foods and feeds. There are examples of changed associations based on more extreme conditions. *P. italicum* and *P. digitatum* are strongly associated to citrus fruits, but if fungicide treatment is applied, a third species, *P. ulaiense*, associated to these non-climateric fruits will dominate (Holmes *et al*., 1994). *P. expansum* is the dominating *Penicillium* pathogenic to apples, but if fungicides are used, *P. solitum* will dominate (Pitt *et al*., 1991). The third known example of the influence of fungicides is natamycin treatment that will favour *P. discolor* at the expense of *P. commune*, the otherwise dominating species on cheese (Lund *et al*., 1995; Frisvad *et al*., 1997). Members of series *Roqueforti* are a special case, growing very well at low pH, often in conjunction with organic acids, and at high CO_2 content of the atmosphere. This has the consequence that *P. roqueforti*, *P. carneum* and *P. paneum* are the dominating species growing on rye bread, blue cheeses and silage, but the reason for these associations may very well be their co-evolution with lactic acid bacteria, which produce all the metabolic products, lactic acid, CO_2 etc., that members of series *Roqueforti* are easily tolerating. The reason *P. carneum* is more common on dried lactic acid fermented meat products (salami) than *P. roqueforti* may be that *P. carneum* produces patulin, which inhibits the growth of many bacteria.

Table 4. *Penicillium* species and their specific habitats

Typical habitat*	Species
Indoor air	*P. brevicompactum, P. chrysogenum, P. commune, P. polonicum. P. expansum, P. olsonii,*
pomaceous fruit	*P. expansum, P. crustosum, P. solitum*
citrus	*P. italicaum, P. digitatum, P. ulaiense*
nuts	*P. discolor*
onions	*P. radicicola, P. albocoremium, P. allii*
bulbs and roots	*P. tulipae, P. hirsutum, P. venetum , P. gladioli*
silage	*P. paneum, P. carneum*
mushrooms	*P. brevicompactum*
cheese hard	*P. commune, P. discolor*
cheese soft	*P. camemberti, P. roqueforti, P. caseifulvum,*
bread	*P. roqueforti P. paneum, P. carneum*
salami & ham	*P. nalgiovense, P. nordicum*
cereal grains	*P. hordei, P. verrucosum, P. cyclopium, P. freii, P. tricolor,*
oak	*P. glandicola*
dung	*P. coprophilum, P. coprobium, P. concentricum, P. vulpinum, P. formosanum, P. clavigerum*
soil	*P. persicinum,*

* Listed are the typical habitats of the species. For a more detailed description of occurrence see the text.

Strong associations

P. italicum and *P. ulaiense* in series *Italica* and *P. digitatum* in series *Digitata* are strongly associated to citrus fruits. There is no strong evidence they are associated to any other plant products, although they are occasionally reported from soil and plant roots or stems. This may be caused by cross contamination from *P. digitatum* spoiling citrus fruits. When these fruits are spoiled a large number of air-borne conidia are produced. Fungi isolated directly from citrus fruits rots are most probably one of the three species listed above, and the same species are unlikely to thrive on any other substrate.

P. sclerotigenum has only been found in association with yam tubers and yam flour (*Discorea batatas* and *D. cayenensis*). It appears to be strictly associated to this particular habitat.

P. gladioli has only been found on *Gladiolus* bulbs, but has not been reported since 1970. We were unsuccesful to isolate this species in Europe in spite our extensive efforts. Perhaps new ways of protecting flower bulbs from diseases have eradicated the species.

P. tulipae is primarily associated to *Tulipa*, but it has also been found on other flower bulbs such as *Chrysanthemum* and *Lilium*. It is also occasionally associated to other plant roots. *P. hirsutum* has been found on some of the same bulbs, but appears to be less strictly associated to plant roots, because it has also been found on butter and apples. However cross-contamination from plant roots cannot be excluded. *P. venetum* is most often found on roots of *Iris*, *Hyacinthus* and *Ornithogalum*, but has also been found on *Asparagus*.

P. allii is a very common garlic rotting organism while *P. albocoremium* and *P. radicicola* are commonly found on other *Allium* spp., especially *Allium cepa*. However the latter two species are also common on other plant roots often used for human food.

P. verrucosum, *P. hordei*, and all members of series *Viridicata* are all typically associated to stored cereal grains. There may be a tendency of *P. viridicatum*, *P. melanoconidium*, *P. aurantiogriseum* and *P. polonicum* to grow on corn and wheat in warmer climates and for *P. cyclopium*, *P. freii*, *P. verrucosum*, *P. tricolor* and *P. hordei* to thrive in colder climates especially on barley and wheat. *P. neoechinulatum* from Series *Viridicata* however, has until now only been found on seeds gathered by kangaroo rats in the Sonoran desert. *P. polonicum*, *P. aurantiogriseum* and *P. viridicatum* may also cross-contaminate dried meat products, such as salami. The contamination source may be wheat flour.

P. glandicola have only been reliably been recorded from oak trees, acorns and cork and appears to be strictly associated to *Quercus* spp. The species name is thus well chosen. Its occasional occurrence in soil and in guts of deer may easily be explained by its association to oak trees.

P. roqueforti, *P. carneum* and *P. paneum* have only been found on substrates in which lactic acid bacteria have grown, or substrates that have the same characteristics. The substrates may have been preserved with organic acids that are quite similar to lactic acid, including sorbic acid, benzoic acid, propionic acid and acetic acid, or they may contain some alcohol and/or they may have high CO_2 or low O_2 content. Examples are rye bread, yoghurt, cheese or silage (Boysen *et al*., 1996; Lund *et al*., 1996)

P. camemberti, *P. caseifulvum*, *P. commune*, *P. palitans* and *P. atramentosum* appear to be mostly associated to cheese and other milk products (Lund *et al*., 1995). *P. commune* and *P. palitans* have also been reported from dried meat products and nuts, but are much less common on such substrates. *P. atramentosum* has been found on Norwegian gamalost and on other cheeses, but is much more prevalent in alkaline soils (Kubatová, 1990). We have found it to be domi-

nating in limestone quarry soils in Denmark together with *P. scabrosum* (Banke and Frisvad, unpublished).

P. nordicum and *P. nalgiovense* are very salt tolerant and are mostly associated to dried or salted meat products, such as salami and dried hams. Both species also occur on cheese, but are less common there (Lund *et al.*, 1995)

P. marinum is probably associated to marine algae, but too few isolates have been found to substantiate this. The two extant isolates are from coastal sands.

P. coprophilum, P. coprobium, P. concentricum, P. vulpinum, P. formosanum and *P. clavigerum* all appear to be strongly associated to animal dung. This association is supported by several common features in these fungi, including production of ellipsoidal conidia, synnema, and patulin or griseofulvin in combination with roquefortine C. The same combination of features is also found in series *Urticicolae*, *P. expansum* and *P. sclerotigenum* and it is likely that the dung Penicillia, *Urticicolae* and *P. expansum* all share a common dung-borne ancestor.

Strong associations to several different habitats

P. echinulatum and *P. cavernicola* are often found on lipid rich substrates such as butter, margarine and cheese. *P. echinulatum* has also been found on wood shavings and in wet mechanical pulp. *P. cavernicola* has been found in caves with bats. There are too few isolates of these species reported to conclude what their primary habitats might be.

P. discolor has been found on natamycin treated cheeses, but was originally isolated from vegetable roots and weevil-damaged pecans (Frisvad *et al.*, 1997). It has often been isolated from walnuts, black walnuts, chestnuts and pecans, which may be its primary habitat.

Plurivorous species

The following species grow very well at low water activities and at a wide range of temperatures.

P. expansum, *P. solitum* and *P. crustosum* are all known for their rot of pomaceous fruits (Raper and Thom, 1949; Frisvad, 1981; Pitt *et al.*, 1991), but they also occur in other quite different habitats. *P. expansum* and *P. crustosum* have been found repeatedly on nuts and oilseeds, and all three apple rotting organisms have also been found on dried meat. *P. crustosum* has also been found occasionally in corn and rice and on cheese, so this species appear to be more of a generalist among these Penicillia. *P. expansum* has been found commonly on wood in Canadian buildings, while *P. crustosum* can be common on cardboard, leather, textiles and wood (K.A. Seifert, personal com.)

P. aethiopicum has been found on several tropical plant products and in tropical soil and no particular group of foods seems to be particularly prone to infection with this fungus.

P. chrysogenum, P. dipodomyis, P. flavigenum, P. nalgiovense P. mononematosum and *P. confertum* are common in dry habitats and may originally have inhabited desert habitats and salty soils. They are able to grow on foods at low water activities. *P. chrysogenum* has been found on dried cereals, salted meat and many other low water activity foods, but is also common in indoor air environments together with *Aspergillus versicolor* (Samson *et al.*, 2004).

P. thymicola has been found on dried herbs and sorghum. *P. griseofulvum* and *P. dipodomyicola* are common on grasses and their seeds including dry barley and wheat. The former species may also be found on pasta and white bread.

P. brevicompactum, P. bialowiezense and *P. olsonii* are very common in soil from tropical rain forests and soil in green-houses in other areas of the world. These species have been found on mouldy mushroom, tomatoes, green coffee, in processed foods, and many other substrates. The two former species are also common in temperate forest soil (Zaleski, 1927), maybe because of their growth on basidiocarps.

P. percicinum has only been found in soil, so its primary habitat is unknown.

Abiotic and nutritional factors

All species in *Penicillium* subgenus *Penicillium* are able to grow at 25ºC, so they are not psychrophiles according to the most common definitions. Most species are, however, capable of growing at 5ºC and some are growing faster at 15 than 25ºC. Species not able to grow at 30ºC include *P. bialowiezense, P. marinum, P. formosanum, P. ulaiense, P. gladioli, P. digitatum, P. solitum, P. cavernicola, P. nordicum, P. thymicola, P. verrucosum, P, freii* and *P. tricolor*. Further species growing very poorly at 30ºC include *P. brevicompactum, P. olsonii, P. expansum, P. coprobium, P. glandicola, P. vulpinum, P. italicum, P. camemberti, P. caseifulvum, P.commune, P. palitans, P. echinulatum*, and *P. hirsutum*. Species growing faster at 15ºC than 25ºC include *P. bialowiezense, P. marinum, P. thymicola, P. verrucosum* and *P. radicicola*.

Most species in subgenus *Penicillium* tolerate 5% NaCl very well. Species that grow faster on media with 5% NaCl than without NaCl, include *P. bialowiezense, P. brevicompactum, P. olsonii, P. chrysogenum, P. dipodomyis, P. flavigenum, P. nalgiovense, P. confertum, (P. mononematosum), (P. marinum), (P. glandicola), P. gladioli, (P. commune), P. palitans, P. discolor, P. solitum, P. cavernicola, P. echinulatum, P. nordicum, P. thymicola, P. verrucosum, P. aurantiogriseum, P. cyclopium, P. freii, P. melanoconidium (P. neoechinulatum), P. polonicum, P. viridicatum, P. albocoiremium, P. allii, P. hirsutum, P. hordei, P. radicicola*, and *P. venetum*. Series

Roqueforti and *Italica, P. clavigerum, P. formosanum* and *P. digitatum* are strongly inhibited by 5% NaCl.

Most species can tolerate low pH, but members of series *Roqueforti* can grow in the presence of 1% propionic acid and 0.5% acetic acid. 50 ppm sorbic acid and benzoic acid inhibits most species, but series *Roqueforti* members actually grow faster on such media, maybe using the acids as further carbon sources. The species most strongly inhibited by these fungicidal preservatives are *P. atramentosum* and *P. digitatum*.

Creatine positive species are concentrated in series *Roqueforti, Expansa* (except *P. sclerotigenum*), *Claviformia* (except *P. clavigerum* and *P. formosanum*), *Camemberti*, and *Solita*.

Only one species in subgenus *Penicillium* cannot use nitrate as N-source, namely *P. digitatum.*

Mycotoxins produced by Penicillium subgenus Penicillium (for a more detailed list of all extrolites see Frisvad *et al.*, 2004)

The terverticillate Penicillia are well known for their mycotoxin production (Frisvad and Filtenborg, 1983; 1989). Some of the first terverticillate Penicillia shown to be toxigenic were *P. cyclopium* and *P. viridicatum* (Purchase, 1974). Unfortunely nearly all these reports were based on misidentified strains. For example *P. cyclopium* was reported to produce penitrem A, but the producing organism was *P. crustosum* (Pitt, 1979b, Frisvad, 1989). Penitrem A was reported to be produced by strains were identified as *P. commune, P. lanosocoeruleum, P. palitans, P. martensii, P. meleagrinum, P. piceum, P. verrucosum* var. *melanochlorum* and *P. verrucosum* var. *cyclopium*, but they all proved to be *P. crustosum* (Pitt, 1979b, Frisvad, 1989). Cyclopiazonic acid, cyclopiamine and cyclopiamide were named after a strain identified as *P. cyclopium*, but that strain proved to be *P. griseofulvum* (Frisvad, 1989). Other strains of *P. cyclopium* (Leistner and Pitt, 1977) were also claimed to be cyclopiazonic acid producers, but the strains proved to be *P. commune* (Frisvad, 1989). Other strains identified as *P. cyclopium* and *P. cyclopium* var. *album* were reported to produce cyclopaldic acid and cyclopolic acid (Birch and Kocor, 1960), but the producing strains were *P. commune* again (Frisvad, 1989). *P. viridicatum,* claimed responsible for producing viridicatumtoxin, was later reidentified to *P. expansum* (de Jesus *et al.*, 1982), but the producer was a *P. aethiopicum* (Frisvad, 1989). Strains identified as *P. viridicatum* (Walbeek *et al.*, 1969; Ciegler *et al.*, 1973) were reported to produce ochratoxin and often also citrinin, but all these strains proved to be *P. verrucosum* or *P. nordicum* (Frisvad and Filtenborg, 1983, Frisvad, 1985; Larsen *et al.*, 2002a). The strains producing citrinin and ochratoxin A were all *P. verrucosum* (Frisvad, 1985, 1989). *P. viridicatum* has also

been claimed to produce viridicatin (Cunningham and Freeman, 1953) and viridicatic acid (Birkinshaw and Samant, 1960); however, the first isolate was *P. solitum* and the second was *P. crustosum* (Frisvad, 1989).

Aflatoxins or sterigmatocystin have not been reliably reported from any *Penicillium* species. The report of aflatoxin production in *Penicillium puberulum* (Hodges *et al.*, 1964) could not be confirmed. The strain, *P. polonicum* ATCC 15683 = NRRL A-12539 = IBT 14609 did not produce any aflatoxin in our experiments.

Ochratoxin A has been reported from many Penicillia, but actually it is only produced by *P. verrucosum* and *P. nordicum* (Frisvad, 1985; Land and Hult, 1987; Pitt, 1987; Larsen *et al.*, 2002a). *P. verrucosum* is the species reponsible for producing ochratoxin A in cereals, while *P. nordicum* can produce ochratoxin A in meat products and cheese.

The nephrotoxin citrinin has been found in *P. radicicola, P. expansum*, and *P. verrucosum*. It may thus potentially occur in carrots and potatoes (*P. radicicola*), cereals (*P. verrucosum*) and pomaceous fruits and nuts (*P. expansum*).

Some as yet only partially characterized neprotoxic glycopeptides have been isolated from *P. polonicum* and *P. aurantiogriseum*, both species common in cereals and to a certain extent in meat products (Yeulet *et al.* 1988, Mantle, 1993; Frisvad, 1995)

The hepatotoxic and nephrotoxic extrolites xanthomegnin, viomellein, and vioxanthin have been recovered from *P. cyclopium*, *P. freii*, *P. melanoconidium*, *P. tricolor* and *P. viridicatum*, (Lund and Frisvad, 1994; Frisvad, 1995). All these species are very common in cereals

Penicillic acid has been found in *P. aurantiogriseum, P. aurantiocandidum, P. cyclopium, P. freii, P. melanoconidium, P. neoechinulatum, P. polonicum, P. tricolor* and *P. viridicatum*. It probably increase the nephrotoxicity of ochratoxin A as this has been shown experimentally in pigs (Stoev *et al.*, 2001). All the members of series *Viridicata* can produce penicillic acid and occur in cereals together with *P. verrucosum* and thus ochratoxin A and penicillic acid often co-occur. However, *P. verrucosum* has never been found in warm habitats.

The neurotoxin verrucosidin is produced by *P. aurantiogriseum* and *P. polonicum* and could therefore occur in cereals and possibly meat products.

The neurotoxin roquefortine C is produced by 26 species in subgenus *Penicillium*: *P. albocoremium, P. allii, P. atramentosum, P. carneum, P. chrysogenum, P. clavigerum, P. concentricum, P. coprobium, P. coprophilum, P. crustosum, P. expansum, P. flavigenum, P. glandicola, P. griseofulvum, P. hirsutum, P. hordei, P. marinum, P. melanoconidium, P. paneum, P. persicinum, P. radicicola, P. roqueforti, P. sclerotigenum, P. tulipae, P. venetum*, and *P.*

vulpinum. It is probably most likely to be produced in cheeses, silage and meat products.

The highly toxic tremorgen penitrem A is produced by *P. carneum*, *P. crustosum, P. clavigerum, P. glandicola, P. melanoconidium,* and *P. radicicola*. Of these species, *P. crustosum* is the most important, already implicated in many mycotoxicoses. This species is a very common spoilage organism of cheese, nuts, meat and many other products. *P. carneum* may also be involved in silage intoxications of cattle and it can grow at very acidic or microaerophilic conditions.

The generally toxic patulin has been found in 14 species in subgenus *Penicillium*: *P. carneum*, *P. clavigerum*, *P. concentricum*, *P. coprobium*, *P. dipodomyicola*, *P. expansum*, *P. formosanum*, *P. gladioli*, *P. glandicola*, *P. griseofulvum*, *P. marinum*, *P. paneum*, *P. sclerotigenum*, and *P. vulpinum*. The most probable problems with this mycotoxin may be in applejuice and other juices (producer *P. expansum*) and in very acidic products, such as silage (producers *P. carneum* and *P. paneum*). *P. griseofulvum* may potentially produce patulin in cereals and pasta and *P. sclerotigenum* may potentially produce patulin in yams. All these Patulin producers also produce other mycotoxins, at least in pure culture, and thus may be more toxic than expected based on patulin alone.

The ergot-like rugulovasins have been found in *P. atramentosum* and *P. commune*. These alkaloids may thus be present in cheese samples with growth of these two fungi.

The ergot-like alkaloids fumigaclavine A and B have been found in *P. palitans* and may thus be produced in cheese.

The similar isofumigaclavines are produced by *P. roqueforti* and *P. carneum* and may be a problem in silage and cheese.

The mycotoxin cyclopiazonic acid is produced by *P. camemberti*, *P. clavigerum*, *P. commune*, *P. griseofulvum*, and *P. palitans*. Production in cheese by *P. camemberti*, *P. commune* and *P. palitans* is not unlikely, whereas *P. griseofulvum* may produce it in cereals and pasta.

The mycotoxin botryodiploidin has been found in *P. brevicompactum* and in *P. paneum*. The frequency of producing isolates in each species and the significance of this mycotoxin is unknown.

The chaetoglobosins have been reported as mycotoxins (Cole and Cox, 1981) and are among the major extrolites of *P. expansum* and *P. discolor*.

The cytotoxic communesin B is produced by *P. marinum* and *P. expansum*. Its significance as a mycotoxin is also still unknown.

PR-toxin has been found in *P. roqueforti* and *P. chrysogenum*. Despite several reports of PR-toxin production by *P. chrysogenum* (Frisvad and Filtenborg, 1983; Hohn *et al*., 1991; Dai *et al*., 1993; Möller *et al*., 1997), this has been difficult to reproduce as it

appears that the production of this toxin is strongly influenced by the type of yeast extract used in the media. PR-toxin has been found naturally occurring in maize causing mycotoxicosis (Vesely *et al.*, 1981).

The tripeptide mycotoxin viridic acid has been found in *P. viridicatum* (Holzapfel *et al*., 1986) and *P. nordicum* (Larsen *et al*., 2002b) and may be a problem in cereals and meat products. Viridicatumtoxin is produced by *P. aethiopicum*, *P. clavigerum* and by *P. brasilianum* in subgenus *Furcatum* (Frisvad and Filtenborg, 1990b).

The strongly acidic cardiotoxin terrestric acid is produced by *P. aurantiogriseum, P. crustosum, P. hirsutum P. hordei, P. radicicola, P. tricolor*, *P. tulipae*, and *P. venetum*. The significance of this metabolite is unknown.

The tryptoquialanins are chemically similar to the toxic tryptoquivalins (Cole and Cox, 1981). They are produced by *P. digitatum* and *P. aethiopicum* (Ariza *et al*., 2002), but their potential natural occurrence in citrus fruits or tropical cereals, respectively, is unknown.

The tremorgenic territrems are produced by *P. echinulatum* and *P. cavernicola* (Smedsgaard *et al*., in preparation). These species, especially the first, have been recovered from butter, margarine, liver pate, cheese and similar products.

Pharmaceuticals produced by Penicillium subgenus Penicillium

Interest in extrolites from species included in *Penicillium* subgenus *Penicillium* started early with the isolation of the antibiotic mycophenolic acid by Gosio (1889). This compound was later shown to be a potent pharmaceutical used as an immunosuppressing agent in organ transplantations (Bentley, 2000). Of species in subgenus *Penicillium*, mycophenolic acid is produced by *P. bialowiezense*, *P. brevicompactum*, *P. carneum* and *P. roqueforti*.

Of particular interest is penicillin, which was first discovered in a strain firstly identified as *P. rubrum* by Fleming (1928) and later reidentified as *P. notatum* (a synonym of *P. chrysogenum*) and later again isolated from a strain of *P. chrysogenum* from a canteloupe. Subsequently later research has shown that all strains examined of *P. chrysogenum* produce penicillin (Andersen and Frisvad, 1994) and furthermore that the other closely related species in series *Chrysogena* also produce penicillin, i.e. *P. dipodomyis*, *P. nalgiovense* and *P. flavigenum* (Frisvad *et al*., 1987; Banke *et al*., 1997). Another penicillin producer in subgenus *Penicillium* is *P. griseofulvum* (Laich *et al*., 2002). The latter authors also found parts of the penicillin genes in *P. nordicum* (incorrectly identified as *P. verrucosum*).

The important anticholerolemic agent compactin was first reported from a strain of *P. brevicompactum* (Brown *et al*., 1986), albeit via its antifungal activity.

Later other producers of compactin were reported as *P. citrinum* (Endo *et al*., 1976; Lam *et al*., 1981), *P. cyclopium* (Doss *et al*., 1986; Basaraa *et al*., 1998), *Paecilomyces viridis* (Murakawa *et al*., 1994) and *P. aurantiogriseum* (Wagschal *et al*., 1996). All these reports were based on misidentified strains, and the correct name of producer of compactin was shown to be *P. solitum* (Frisvad and Filtenborg, 1989). Further producers in subgenus *Penicillium* include *P. hirsutum* (Frisvad and Filtenborg, 1989).

The benzodiazepine-like alkaloids cyclopeptin, dehydrocyclopeptin, cyclopenin and cyclopenol were named after *P. cyclopium*, but the original was later re-identified as *P. solitum* (Frisvad and Filtenborg, 1989). *P. cyclopium* actually produces these alkaloids consistently (Lund and Frisvad, 1995). 3-methoxyviridicatin is of special interest, because it is active against HIV (Heguy *et al*., 1998). 3-methoxyviridicatin is produced by *P. albocoremium, P. allii, P. aurantiocandidum, P. commune, P. crustosum, P. cyclopium, P. discolor, P. echinulatum, P. freii, P. hirsutum, P. neoechinulatum, P. palitans, P. polonicum, P. radicicola, P. solitum, P. venetum* and *P. vulpinum*. Much more viridicatin than 3-methoxyviridicatin is produced by the creatine positive species *P. commune, P. crustosum, P. discolor, P. echinulatum, P. palitans, P. solitum* and *P. vulpinum*, however.

The antifungal pharmaceutical griseofulvin is produced by *P. aethiopicum*, *P. coprophilum*, *P. dipodomyicola*, *P. griseofulvum*, *P. persicinum*, and *P. sclerotigenum*.

The anticholerolemic agents, the pyripyropens, are produced by *P. coprobium* and *P. concentricum*.

The acetylcholinesterase inhibiting arisugacins are produced by the territrem producing species *P. echinulatum* and *P. cavernicola* (Smedsgaard, Svendsen and Frisvad, in prepraration).

Many other extrolites have been found in the terverticillate Penicillia as lead compounds, and time will show if any of these have a future as important pharmaceuticals. Many of these are listed in the synoptic key below.

List of accepted species in subgenus *Penicillium*

1. *P aethiopicum* Frisvad, Mycologia **81**: 848, 1989
2. *P. albocoremium* (Frisvad) Frisvad, Int. Mod. Tax. Meth. Pen. Asp. Clas.: 275, 2000
3. *P. allii* Vincent and Pitt, Mycologia **81**: 300, 1989
4. *P. atramentosum* Thom, Bull. Bur. Anim. Ind. US Dept. Agric. **118**: 65, 1910
5. *P. aurantiogriseum* Dierckx, Ann. Soc. Scient. Brux. **25**: 88, 1901
6. *P. bialowiezense* K. Zaleski, Bull. Int. Acad. Pol. Sci. Lett., Sér. B **1927**: 462, 1927
7. *P. brevicompactum* Dierckx, Ann. Soc. Scient. Brux. **25**: 88, 1901
8. *P. camemberti* Thom, Bull. Bur. Anim. Ind. US Dept. Agric. **82**: 33, 1906
9. *P. carneum* (Frisvad) Frisvad, Microbiology, UK, **142**: 546, 1996
10. *P. caseifulvum* Lund, Filt. & Frisvad, J. Food Mycol. **1**: 97, 1998
11. *P. cavernicola* Frisvad & Samson sp. nov.
12. *P. chrysogenum* Thom, Bull. Bur. Anim. Ind. US Dept. Agric. **118**: 58, 1910
13. *P. clavigerum* Demelius, Verh. Zool.-Bot. Ges. Wien **72**: 74, 1922
14. *P. commune* Thom, Bull. Bur. Anim. Ind. US Dept. Agric. **118**: 56, 1910
15. *P. concentricum* Samson, Stolk and Hadlok, Stud. Mycol. (Baarn) **11**: 17, 1976.
16. *P. confertum* (Frisvad, Filt. & Wicklow) Frisvad, Mycologia **81**: 852, 1989.
17. *P. coprobium* Frisvad, Mycologia **81**: 853, 1989.
18. *P. coprophilum* (Berk. & Curt.) Seifert & Samson, Adv. Pen. Asp. Syst.: 145, 1985.
19. *P. crustosum* Thom, Penicillia: 399, 1930.
20. *P. cyclopium* Westling, Ark. Bot. **11**: 90, 1911.
21. *P. digitatum* (Pers.:Fr.) Sacc., Fung. Ital.: 894, 1881.
22. *P. dipodomyicola* (Frisvad, Filt. & Wicklow) Frisvad, Int. Mod. Tax. Meth. Pen. Asp. Clas.: 275, 2000.
23. *P. dipodomyis* (Frisvad, Filt. & Wicklow) Banke, Frisvad and S. Rosendahl, Int. Mod. Tax. Meth. Pen. Asp. Clas.: 271, 2000.
24. *P. discolor* Frisvad & Samson, Ant. van Leeuwenhoek **72**: 120, 1997.
25. *P. echinulatum* Fassatiová, Acta Univ. Carol. Biol. **12**: 326, 1977.
26. *P. expansum* Link, Obs. Mycol. **1**: 16, 1809
27. *P. flavigenum* Frisvad & Samson, Mycol. Res. **101**: 620, 1997
28. *P. formosanum* Hsieh, Su & Tzean, Trans. Mycol. Soc. R.O.C. **2**: 159, 1987
29. *P. freii* Frisvad & Samson, sp. nov.
30. *P. gladioli* McCulloch & Thom, Science, N.Y. **67**: 217, 1928
31. *P. glandicola* (Oud.) Seifert & Samson, Adv. Pen. Asp. Syst.: 147, 1985.
32. *P. griseofulvum* Dierckx, Ann. Soc. Scient. Brux. **25**: 88, 1901
33. *P. hirsutum* Dierckx, Ann. Soc. Scient. Brux. **25**: 89, 1901
34. *P. hordei* Stolk, Ant. van Leeuwenhoek **35**: 270, 1969
35. *P. italicum* Wehmer, Hedwigia **33**: 211, 1894
36. *P. marinum* Frisvad & Samson sp. nov.
37. *P. melanoconidium* (Frisvad) Frisvad & Samson, comb. nov.
38. *P. mononematosum* (Frisvad, Filt. & Wicklow) Frisvad, Mycologia **81**: 857, 1989
39. *P. nalgiovense* Laxa, Zentbl. Bakt. ParasitKde., Abt. II **86**: 162, 1932
40. *P. neoechinulatum* (Frisvad, Filt. & Wicklow) Frisvad & Samson, comb. nov.
41. *P. nordicum* Dragoni & Cantoni ex Ramírez, Adv. Pen. Asp. Syst.: 139, 1985
42. *P. olsonii* Bain. & Sartory, Ann. Mycol. **10**: 398, 1912
43. *P. palitans* Westling, Ark. Bot **11**: 83, 1911
44. *P. paneum* Frisvad, Microbiology, UK **142**: 546, 1996
45. *P. persicinum* L. Wang, H. Zhou, Frisvad & Samson, Ant. van Leeuwenhoek 86: 177, 2004
46. *P. polonicum* K. Zaleski, Bull. Int. Acad. Pol. Sci. Lett., Sér. B **1927**: 445, 1927
47. *P. radicicola* Overy and Frisvad, Syst. Appl. Microbiol. **26**: 633, 2003
48. *P. roqueforti* Thom, Bull. Bur. Anim. Ind. US Dept. Agric. **82**: 35, 1906
49. *P. sclerotigenum* Yamamoto, Scient. Rep. Hyogo Univ. Agric., Agric. Biol. Ser. 2, **1**: 69, 1955
50. *P. solitum* Westling, Ark. Bot. **11**: 65, 1911
51. *P. thymicola* Frisvad & Samson sp. nov.
52. *P. tricolor* Frisvad, Seifert, Samson & Mills, Can. J. Bot. **72**: 937, 1994
53. *P. tulipae* Overy & Frisvad, Syst. Appl. Microbiol. **26**: 634, 2003
54. *P. ulaiense* Hsieh, Su & Tzean, Trans. Mycol. Soc. R.O.C. **2**: 161, 1987
55. *P. venetum* (Frisvad) Frisvad, Int. Mod. Tax. Meth. Pen. Asp. Clas.: 275, 2000
56. *P. verrucosum* Dierckx, Ann. Soc. Scient. Brux. **25**: 88, 1901
57. *P. viridicatum* Westling, Ark. Bot. **11**: 88, 1911
58. *P. vulpinum* (Cooke & Massee) Seifert & Samson, Adv. Pen. Asp. Syst.: 144, 198

Keys to species (see also http://www.cbs.knaw.nl/penicillium.htm)

The identification of the 58 taxa of subgenus *Penicillium* is difficult, because the micromorphology of the strains is very similar. In adition of the structures and branching of the conidiphores, the shape and ornamentation of conidia it is often helpful to use colony characters including growth rate, colour of the conidia and reverse. In a few species a diffusing pigment is characteristic. For most species the sisolates should be inoculated on four media: CYA, MEA, CREA and YES (for formulations see page 3-4) at 25°C (37°C on CYA).

Because of the difficulty to use morphological characters a dichomotomous key is here only provided to the Sections and series, while the characters of the taxa in the individual series are described in the box keys.
A more electronic key is provided at http://www.cbs.knaw.nl/penicillium.htm. The following data sheet can be used the collect colony and morphological characters of the isolates.

Data sheet for identification of taxa of subgenus *Penicillium*.

CYA – Incubation 7 days at 25°C	Colony diameter in mm: …………
Colour obverse*	blue green / dark green / dull green / grey green / olive green / yellow; pure green / white
Colour reverse*	pale / yellow / orange / red / beige-light brown / brown / dark brown; blackish green
Ehrlich reaction	no reaction / violet / red-brown / yellow
CYA – Incubation 7 days at 30°C	Colony diameter in mm: …………
MEA – Incubation 7 days at 25°C	Colony diameter in mm: …………
Colony texture	velvety / granulate / weak fasciculate / fasciculate / weakly floccose / floccose
Colour colony obverse*	blue green / dark green / dull green / grey green / olive green / yellow; pure green / white
Prepare microscopic slide	One slide at the colony edge (for conidiophore) and one in the colony (for conidia)
CREA – Incubation 7–10 (14) days at 25°C	Colony diameter in mm: …………
Degree of growth	No or very weak / Weak to moderate / Moderate / Moderate to good / good
Acid production	None / Weak / Moderate / Good / High
Base production: after 7 days	Absent / Present
after 10-14 days	Absent / Present
YES – Incubation 7 days at 25°C	Colony diameter in mm: …………
Degree of sporulation	None / Weak / Moderate / Strong
Colour colony reverse*	pale / yellow / orange / red / beige-light brown / brown / dark brown; blackish green
Microscopic characters	
Conidia Length/width in μm	………….μm
Ornamentation	Smooth / fine roughened / rough-walled / echinulate
Phialide length in μm	………….μm
Metulae length in μm	………….μm
Stipe width in μm	………….μm
Ornamentation	Smooth / finely roughened / rough-walled / warted
Conidiophore adpressednes	Strongly adpressed / adpressed / neither adpressed nor divergent / divergent / strongly divergent
Conidiophore branching pattern*	Monoverticillate / biverticillate / terverticillate / quaterverticillate

* More than one character is possible to enter into the database

Synoptic key to series

1. *Aethiopica* Frisvad & Samson
2. *Camemberti* Raper & Thom ex Pitt
3. *Chrysogena* Raper & Thom ex Stolk & Samson
4. *Claviformia* Raper & Thom ex Stolk, Samson & Frisvad
5. *Corymbifera* Frisvad
6. *Digitata* Raper & Thom ex Stolk & Samson
7. *Expansa* Raper & Thom ex Fassatiova
8. *Gladioli* Raper & Thom ex Stolk & Samson
9. *Italica* Raper & Thom ex Pitt
10. *Mononematosa* Frisvad
11. *Olsonii* Pitt
12. *Persicina* Frisvad & Samson
13. *Roqueforti* Raper & Thom ex Frisvad
14. *Solita* Frisvad
15. *Urticicolae* Fassatiová
16. *Verrucosa* Frisvad
17. *Viridicata* Raper & Thom ex Pitt

Conidia cylindrical and ellipsoidal: 6, 9, 12

Conidia ellipsoidal: 1, ((2)), ((3)), 4, 6, 7 (except *P. marinum*), 9, 11, 12, 15

Conidia globose to subglobose: 2, 3, 5, (*P. marinum* in 7), 8, 10, 13, 14, 16, 17

Conidia rough-walled: *P. echinulatum, P. cavernicola* & *P. discolor* (in 14), *P. neoechinulatum* (in 17)

Conidia finely or clearly rough-walled: *P. hordei* (in 5), 11, 14, *P. thymicola* (in 16), *P. viricatum* & *P. neochinulatum* (in 17)

Conidia more than 6 μm: 6

Conidia olive coloured: 6

Conidia dark green: *P. palitans* & *P. atramentosum* (in 2), *P. dipodomyis* (in 3), 13, 14, *P. melanoconidium* (in 17)

Weak conidium production: *P. camemberti* and *P. caseifulvum* (in 2), 8

Stipes more than 500 μm long: 11

Stipes clearly rough walled: (1), (2), *P. glandicola* (in 4), 5, (8), 13, 14, 16, (17)

Stipes finely or strongly rough-walled: (1), (2), (*P. dipodomyis* (in 3)), (4), 5, ((*P. expansum* in 7)), 8, 13, 14, 16, (17)

Stipes smooth-walled: (1), *P. atramentosum* and *P. camemberti* (in 2), 3, 4 (except *P. glandicola*), 6, 7, (8), 9, 10, 11, 12, ((14, only stipes in agar)), 15, (17)

Phialides less than 6 μm: 15

Multiramulate: (11)

Biramulate: (3)

Divaricate rami: *P. atramentosum* (in 2), 3, 6, *P. marinum* (in 7), (4), (8), 10

Sinous stipes: (4), 10

Penicillus a long as it is broad: 11

Sclerotia: ((*P. coprobium* in 4)), *P. sclerotigenum* (in 7), 8, ((*P. olsonii* (in 11))), ((*P. roqueforti* (in 13)))

Acicular synnemata: *P. clavigerum* (in 4)

Capitulate synnemata: 4, (7), 9

Extracellular red colour on CYA: 12

Box keys to the individual series:

Section *Coronata* - Series *Olsonii*

Extrolites	*P. brevicompactum*	*P. olsonii*	*P. bialowiezense*
Mycophenolic acid	+	-	+
Brevianamide A	+	-	-
Raistrick phenols	+	-	+
Pebrolides	+	-	-
Silvatin derivatives	+	+?	-?
Asperphenamate	+	+	+
Botryodiploidin	+/-	-	-?
Breviones	-	+	+/-
Verrucolone	-	+	-
2-(4-hydroxyphenyl)-2-oxo acetaldehyde oxime	-	+	-
Phenotypic characters			
Ehrlich reaction	-	-	w
Growth rate on CYA	8-30 mm	26-40 mm	11-25 mm
Growth rate on YES	14-36 mm	35-56 mm	18-30 mm
Reverse colour, YES	Cream to beige	Yellow, yellow/cream	Cream to beige
Conidium colour	Dull green	Greyish green	Dull green
Sclerotia	-	-/+	-
Multiramulate	-/(+)	+	-
Plants, vegetables etc.	+	+	+
Ornamentals, green houses	-/+	+	-
Mushrooms	+	-	-
Fruits	(+)	-	+

Section *Roqueforti* - Series *Roqueforti*

Extrolites	*P. roqueforti*	*P. carneum*	*P. paneum*
Roquefortine C	+	+	+
Isofumigaclavine A & B	+	+	-
Marcfortines	-	-	+
PR-toxin & eremofortines	+	-	-
Mycophenolic acid	+/-	+	-
Patulin	-	+	+
Penicillic acid	-	-/+	-
Cyclopaldic acid	-	+	-
Penitrem A	-	+	-
Botryodiploidin	-	-	+/-
Geosmin	-	+	-
Phenotypic characters			
Ehrlich reaction	+/++	+/++	-
Blackish green reverse, CYA	+	-	-
Reverse colour, YES	Blackish green	Cream beige	Cream yellow/beige#
Growth rate on CYA 30ºC	(0-)6-11 mm	4-11(-26) mm	(10-)17-39 mm
Growth on CzP	12-25 mm	22-34 mm	5-34 mm
Small sclerotia	-/(+)	-	-
Habitat/substrate			
Blue cheese	+	-	-
Meat	-	+	-
Rye bread	+	(+)	+
Preserved foods	+	+?	+
Beverages	+	+	-
Mouldy bakers yeast	+	+	-
Silage	+	(+)	+
Soil	+	-	-

Often turn to strawberry red in age, with colour diffusing into the medium

Section *Chrysogena* Series *Chrysogena – including P. atramentosum* in series *Camemberti*

Extrolites	chrysogenum	flavigenum	dipodomyis	nalgiovense	nalgiovense II	atramentosum
Penicillin	+	+	+	weak	+	-
Roquefortine C	+	+	-	-	-	+
Meleagrin	+	+	-	-	-	+
Oxaline	-	-	-	-	-	+
Chrysogine	+	+	-	-	+	-
Rugulovasine	-	-	-	-	-	+
Nalgiovensin	-	-	-	+	+	-
Nalzovins	-	-	-	-	+	-
Dipodazin	-	-	+	-	+/-	-
Xanthocillin X	+	+	-	-	-	-
Secalonic acids	+/-	+/-	-	-	-	-
Penitrem A	-	+	-	-	-	-
Phenotypic character						
Ehrlich reaction	-	-	-	-	-	-
Creatine, growth	weak	Weak	weak	weak	Weak	++
Creatine, acid	-/+?	?	-	-	-	-
Reverse, CYA	Cream, beige, yellow	Yellow	Creamish brown	Dark red brown	Yellowish	Dark brown
Reverse, YES	Yellow	Yellow	Orange to orange yellow	Orange brown	Orange	Dark brown
Dark green conidia	-/(+)	-	+	-	+	+
Blue green conidia	+	+	-	-	-	-
Col. Diam. CYA						
Exudate	Yellow	Yellow	Clear	Brown	Clear	Brown
Stipe on MEA	Smooth	Smooth / rough	Finely rough	Smooth	Smooth	Smooth
Conidia on MEA	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Branching	Ter to quart	Ter	Ter to quar	Bi to ter	Ter to quarter	Ter
Conidial shape	El/sg	El/sg	Sg	Sg/g	El/sg	El
Odour	Fruity	Mouldy	None	None	None	Corn
Habitat/substrate						
On cheese	-/(+)	-	-	+	-/+	-/+
On salami	+/-	-	-	-	+	-
In desert soil	+	+	+	-	-/(+)	-
In basic soil	-	-	-	-	-	+
In other soil	-	-	-	-	-	+
In indoor air	+	-	-	-	-	-?

Bi = biverticillate; ter = terverticillate; quarter = quaterverticillate ; El = ellipsoid ; g = globose ; sg = subglobose

Series *Mononematosa* Box key to species in *Mononematosa*

Extrolites	P. mononematosum	P. confertum
Cyclopaldic acid	+	-
Fumitremorgins and verrucologen	+	-
Isochromantoxin	+	-
Viriditoxin	+	-
Asteltoxin	-	+
Meleagrin	-	+
Phenotypic character		
Ehrlich reaction	-	-
Diameter, 7 days, CYA 30ºC	26-33 mm	17-21 mm
Reverse colour, YES	Yellow cream to curry	Cream to brown yellow

Series *Aethiopica* and series *Persicina*

Extrolites	*P. aethiopicum*	*P. persicinum*
Griseofulvin	+	+
Chrysogine	-	+
Roquefortine C	-	+
Tryptoquialanins	+	-
Viridicatumtoxin	+	-
Phenotypic character		
Ehrlich reaction	-	-
Ellipsoidal conidia	+	+
Cylindrical conidia	-	+
Rough stipes	+/-	-
Fasciculate	+	-
Red diffusible pigment	-	+
Diameter, 7 days, CYA, 30ºC	29-35 mm	18-22 mm
Reverse colour, YES	Yellow to curry yellow	Strawberry red

Section *Expansa* - Series *Expansa* - including *P. crustosum* from series *Camemberti*

Extrolites	*P. expansum*	*P. marinum*	*P. crustosum*	*P. sclerotigenum*
Patulin	+	+	-	+
Roquefortine C	+	+	+	+
Communesins	+	+	-	-
Chaetoglobosins	+	+?	-	-
Penostatins	-	+	-	-
Expansolide	+	+	-	-
Geosmin	+	-	+?	-
Penitrem A	-	-	+	-
Cyclopenol etc.	-	-	+	-
Terrestric acid	-	-	+	-
Citrinin	+/-	-	-	-
Sclerotigenin	-	-	-	+
Griseofulvin	-	-	-	+
Gregatins	-	-	-	+
Phenotypic character				
Ehrlich reaction	++	++	-	-
Stipe	Smooth (to rough)	Smooth	Rough	Smooth
Conidium form	Ellipsoidal	Sg	Sg	Ellipsoidal
Synnemata	Fasciculate, synnemata occasionally	None	Not distinct	None
Sclerotia	-	-	-	+
Crustose	-	-	+	-
Colony diam., 7 d., CYA, 25ºC	26-50 mm	17-27 mm	32-46 mm	40-62 mm
Colony diam., 7d., CYA, 30ºC	0-3 mm	0 mm	15-26 mm	16-29 mm
Reverse colour, CYA	Brown to orange brown	Brown	Cream	Brown
Reverse colour, YES	Cream yellow to orange brown	Cream yellow	Yellow	Cream yellow
Apple rot	Pronounced	None	Restricted	None
Growth on CREA	++	++	++	w

Series *Urticicolae*

Extrolites	P. griseofulvum	P. dipodomyicola
Patulin	+	+
Griseofulvin	+	+
Roquefortine C	+	-
Cyclopiazonic acid	+	+
Cyclopiamine	+	-
Cyclopiamide	+	-
Mycelianamide	+/-	-
Fulvic acid	+	-
Penicillin	+	?
Phenotypic character		
Ehrlich reaction	+	++
Phialides ≤6.5 µm	+	+
Conidium colour	Grey green	Dull green
Reverse colour CYA	Creamish yellow	Dark brown
Branching	Ter to quarter	Biv to ter
Synnemata MEA	Distinct when fresh	Not distinct
Reverse colour, YES	Cream yellow to beige	Yellow olive to dark olive
CYAS, diam., 7 d., 25ºC	17-23 mm	24-28 mm
CYA, diam., 30ºC, 7 d.	17-22 mm	17-21 mm
Ehrlich reaction	++, violet	++, violet
Habitat/substrate		
Soil	+	+
Cereals	+	-
Desert seeds	+	(+)

Series *Claviformia*

Extrolites	coprophilum	concentricum	glandicola	coprobium	clavigerum	formosanum	vulpinum
Patulin	-	+	+	+	+	+	+
Griseofulvin	+	-	-	-	-	-	-
Roquefortine C	-	+	+	+	+?	-	+
Meleagrin	-	+	+	+	-	-	+
Pyripyropens	-	-	-	+	-	-	-
Xanthomegnin	-	-	-	-	+/-	-	-
Cyclopiazonic acid	-	-	-	-	+/-	-	-
Geosmin	-	-	-	-	+	+	-
Patulonide	-	+	+	-	-	-	-
Penitrem A	-	-	+	-	+	-	-
Ehrlich reaction	-	-	-	-	Yellow	-	-
Phenotypic characters							
CYA >> CYAS	-	-	-	-	+	+	-
CYA, diam., 7 d., 30ºC	7-20 mm	0 mm	0-6 mm	0-4 mm	7-13 mm	0 mm	0-4 mm
Growth on UNO	++	++	++	++	w	w	+/++
Growth on CREA	++	++	++	++	w	w	++
Synnemata	Capitulate	Capitulate	Feathery	Capitulate	Acicular	Thin, feathery	Capitulate
Concentric rings	-	+	+	+	-	-	+
Conidium colour	Greenish grey	Blue green	Dull green	Dark dull green	Dull to grey green	Greenish white	Greenish grey
Conidiophore stipe	Sm/ro	Sm	Ro	Sm	Sm/ro	Sm	Sm
Colony reverse MEA	Brown	Orange	Orange	Cream	Cream/brown	Cream	Brown
Habitat/substrate							
Animal fur	-	-	-	-	+	-	-
Insects	-	-	-	-	-	-	+
Dung & dungy soil	+	+	+/-	+	+/-	+	+
Oak, acorn, cork	-	-	+	-	-	-	-

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Series *Italica* - including *P. digitatum* from *Digitata*.

Extrolites	*P. italicum*	*P. ulaiense*	*P. digitatum*
Deoxybrevianamide E	+	+	-
Italinic acid	+	-	-
Formylxanthocillin X	+	-	-
PI-3	+	-	-
Tryptoquialanins	-	-	+
Ehrlich reaction	-	-	-
Biphenyl Resistant	-	+	-

Phenotypic characters			
Synnemata	Occasional	Distinct	None
Conidia	Cylindrical to ellipsoidal	Long cylindrical	Large cylindrical
Conidium colour	Greenish grey	Greenish grey	Olive
Reverse colour CYA	Red brown	Cream	Beige
CYA, diam., 7 d., 25ºC	26-50 mm	13-26 mm	15-55 mm
CYAS, diam., 7 d., 25ºC	3-17 mm	0(-13) mm	0 mm

Series *Gladioli* including *P. sclerotigenum* from *Expansa*:

Extrolites	*P. gladioli*	*P. sclerotigenum*
Patulin	+/-	+
Atrovenetins	+	-
Gladiolic acid	+	-
Glyanthrypine	+	-
Gregatins	-	+
Sclerotigenin	-	+
Griseofulvin	-	+
Roquefortine C	-	+
Phenotypic characters		
Ehrlich reaction	-	-
Sclerotia	Avellaneous, pale brown	Buff, avellaneous, orange brown
Conidia	Subglobose	Ellipsoidal
CYAS > CYA	+	-
CYA, 7 d., diam., 30ºC	0 mm	16-29 mm

Section *Viridicata* Series *Viridicata*, including *P. verrucosum* in *Verrucosa*

Extrolites	AG	AC	CY	FR	ME	NE	PO	TR	VI	VE
Penicillic acid	+	+	+	-/+	+	+	+	-	-/+	-
Xanthomegnin	-	-	+	+	+/-	-	-	+	+	-
Asteltoxin	-	-	-	-	-	-	-	+	-	-
Cyclopenol etc.	-	+	+	+	-	+	+	-	-	-
Verrucofortine	-	+	+	-	-	-	+	+	-	-
Penitrem A	-	-	-	-	+	-	-	-	-	-
Terrestric acid	+	-	-	-	-	-	-	+	-	-
Brevianamide A	-	-	-	-	-	-	-	-	+/-	-
Viridamine	-	-	-	-	-	-	-	-	+	-
Aurantiamine	+	-	-	+	-	+	-	-	-	-
Auranthine	+	-	-	-	-	-	-	-	-	-
Anacine	+	-	-	-	-	-	+	-	-	-
Puberulonic acid	-	+	-	-	-	-	-	-	-	-
Verrucosidin	+	-	-	-	+	-	+	-	-	-
Ochratoxin A	-	-	-	-	-	-	-	-	-	+
Citrinin	-	-	-	-	-	-	-	-	-	+/-
Verruculone	-	-	-	-	-	-	-	-	-	+
Verrucin	-	-	-	-	-	-	-	-	-	+
Pseurotin	+	+	+	-	-	-	+	-	-	-

Oxaline	-	-	-	-	+	-	-	-	-	-
Sclerotigenin	-	-	-	-	+	-	-	-	-	-
Viridic acid	-	-	-	-	-	-	-	-	+	-
Phenotypic characters										
Ehrlich reaction	p	+	P+	p	p	+	+	-	P+	-
Reverse on YES	y	y	Y	y	y	y	y	br	y	Red brown
CREA: acid	+	+	+	+	+	+	+	+	+	-
Growth on nitrite	-	-	-	-	-	-	-	-	-	+
RT reaction	+	+	+	+	-	+	+	-	+	-
Exudate	+	++	+	++	(+)	++	+	++	+	++
Conidium ornam.	sm	sm	Sm	sm	Sm	ro	sm	sm	fr	sm
Stipe ornam.	Ro/sm	Ro/sm	Ro/sm	Ro/sm	Ro/sm	ro	Ro/sm	Ro!!	ro	Ro!
Yellow green conidia	-	-	+	-	+	-	-	-	+	+
Blue green conidia	+	+	(+)	+	-	+	+	-	-	-
Grey green conidia	-	-	-	-	-	-	-	+	-	-
Dark green conidia	-	-	-	-	+	-	-	-	-	-
Good sporulation YES	+/-	-	-	-	+	-	+	+	+/-	-
Habitat/substrate										
Cereals	+	+	+	+	+	-	+	+	+	+
Desert seeds	-	-	-	-	-	+	-	-	+	-
Salami	(+)	-	-	-	(+)	-	+	-	-	+
Indoor air	-	-	-	-	-	-	+	-	-	-

AG: *P. auarantiogriseum*, AC: *P. cyclopium* II (formerly called *P. aurantiocandidum* or *P. aurantiovirens*), CY: *P. cyclopium*, FR: *P. freii*, ME: *P. melanoconidium*, NE: *P. neoechinulatum*, PO: *P. polonicum*, TR: *P. tricolor*, VI: *P. viridicatum*, VE: *P. verrucosum*, y: yellow, br: brown, sm: smooth, ro: rough, fr: finely roughened, p: pink reaction in Ehrlich test, P+: pink reaction and yellow and brown colours developing.

Series *Corymbifera*

Extrolites	*hirsutum*	*albocoremium*	*allii*	*hordei*	*venetum*	*radicicola*	*tulipae*
Roquefortine C	+	+	+	+	+	+	+
Penitrem A	-	-	-	-	-	-	+
Meleagrin	-	+	+	-	+?	+	+
Cyclopenol, cyclopenin etc.	+/-	+	+	-	+	+	-
Fulvic acid	-	-	+	-	-	-	-
Compactin	+	-	-	-	-	-	-
Hordein	-	-	-	+	-	-	-
Terrestric acid	+	-	-	+	+	+	+
Atrovenetins	+	+	+	+	+		
Citrinin	-	-	-	-	-	+	-
Phenotypic character							
Ehrlich reaction	+	+	p	+	-	+	+
Conidium ornam.	Smooth	Smooth	Smooth	Rough	Smooth	Smooth	Smooth
Conidium colour	Green	Greyish green	Green	Green	Blue green	Greyish green	Greysih green
Mycelium colour	Yellow	White	White	Yellow	White	White	White
Reverse colour, CYA	Yellow brown	Brownish yellow to brownish orange	Dark brown	Yellow	Dark yellow brown	Deep orange	Orange to light orange
Synnemata	Yellow	White feathery	None	Yellow, loose	None	White feathery	White feathery
Exudate	Dark brown	Clear	Dark brown	Yellow?	Dark brown		
Habitat/substrate							
Flower bulbs	-/+?	+	-	-	+		
Onions, garlic	+?	+?	+	-	-		
Vegetables	-??	+	?	-	-		
Cereals	-	-	-	+	-		
Agricultural soil	+/-?	+	+	+	+		

P: pink reaction in Ehrlich test

Series *Verrucosa* including *P. viridicatum* from *Viridicata*

Extrolites	*P. verrucosum*	*P. nordicum*	*P. nordicum* II	*P. thymicola*	*P. viridicatum*
Ochratoxin A	+	+	+?	-	-
Citrinin	+/-	-	-	-	-
Daldinin D	-	-	-	+	-
Verrucolone, PC-2	+	+	+	+	-
Anacin	-	+	+	+	-
Verrucin	+	-	-	-	-
Lumpidin	-	-	+	-	-
Fumiquinazoline F	-	-	-	+	-
Alantrypinone	-	-	-	+	-
Penigequinolone	-	-	-	+/-	-
Dipodazin	-	-	-	+/-	-
Sclerotigenin	-	+	-	-	-
2-methylisoborneol	+	+	+	+	-
Xanthomegnin	-	-	-	-	+
Brevianamide A	-	-	-	-	+
Viridic acid	-	+	-	-	+
Viridamine	-	-	-	-	+
Phenotypic character					
Ehrlich reaction	-	yellow	pink	yellow	Yellow, pink, brown
Growth on nitrite	+	+	+	+	-
Reverse YES	Red brown	Cream	Cream	Yellow	Yellow
Habitat/substrate					
Cereals	+	-	-	-	+
Meat	-	+	+	-	-
Fish, salted	-	-	+	-	-
Herbs, sorghum	-	-	-	+	-

Series *Camemberti*

Extrolites	*P. commune*	*P. camemberti*	*P. palitans*	*P. caseifulvum*	*P. crustosum*	*P. atramentosum*
Cyclopiazonic acid	+	+	+	-	-	-
Rugulovasine	+	-	-	+	-	+
Cyclopenin	-/+	-	+	+	+	-
Roquefortine C	-	-	-	-	+	+
Oxaline	-	-	-	-	-	+
Cyclopaldic acid	+/-	-	-	-	-	-
Fumigaclavine	-	-	+	-	-	-
Palitantin	+	-	+	-	-	-
Terrestric acid	-	-	-	-	+	-
Penitrem A	-	-	-	-	+	-
Phenotypic character						
Ehrlich reaction	++	+/-	++	+	-	-
CYA, diam. 7 d., 30ºC	0-4 mm	0-3 mm	0-7 mm	0 mm	15-26 mm	0 mm
Dark green conidia	-	-	+	-	-	+
Grey green conidia	+	+	-	+	(+)	-
Acid on CREA	+	+	+	+	++	-
Floccose colonies	-	+	-	+	-	-
Crustose colonies	-	-	-	-	+	-
Habitat/substrate						
Cheese	+	+	+	+	+	+
Meat	+	-	+	-	+	-
Nuts	+	-	-	-	+	-
Alkaline habitats	-	-	-	-	-	+

Similarities between *P. crustosum* and other species claimed to be identical or closely related to it

Extrolites	*P.crustosum*	*P. expansum*	*P. solitum*	*P. aurantiogriseum*
Terrestric acid	+	-	-	+
Penitrem A	+	-	-	-
Roquefortine C	+	+	-	-
Cyclopenol	+	-	+	-
Compactin	-	-	+	-
Chaetoglobosins	-	+	-	-
Communesins	-	+	-	-
Patulin	-	+	-	-
Expansolide	-	+	-	-
Penicillic acid	-	-	-	+
Verrucosidin	-	-	-	+
Auranthine	-	-	-	+
Aurantiamine	-	-	-	+
Phenotypic character				
Ehrlich reaction	+	+	-	-
Ellipsoidal conidia	-	+	-	-
Rough stipes	+	- (+)	+	+
Conidial crusts	+	-	-	-
High growth rate	+	+	-	-
Growth on creatine	+	+	+	-
Habitat/substrate				
Apple rot	+	+	+	-
Cereal associated	-	-	-	+

Series *Solita*, including *P. neoechinulatum* from Series *Viridicata*.

Extrolites	*P. solitum*	*P. echinulatum*	*P. discolor*	*P. cavernicola*	*P. neoechinulatum*
Cyclopenol etc.	+	+	+	-	+
Territrems	-	+	-	+	-
Chaetoglobosins	-	-	+	-	-
Aurantiamin	-	-	-	+	+
Compactin	+	-	-	-	-
Palitantin	+	+	+	-	-
Penicillic acid	-	-	-	-	+
Phenotypic character					
Ehrlich reaction	-	-	+	-	+
Creatine, growth	+	+	+	+	-
Conidium colour	Dark green	Dark green	Dark green	Dark green	Blue green
Conidium ornam.	Smooth/rough	Rough	Rough/(sm)	Rough	Rough
Reverse on YES	Orange yellow	Yellow	Orange red*	Yellow	Yellow, diffusing
Habitat/substrate					
Cured meat products	+	+	-	-	-
Cheese	+	+	+	-	-
Butter, margarine	-	+	-	+	-
Vegetables	+	-	+	-	-
Seeds	-	-	-	-	+
Natamycin resistant	-	-	+/-	-	-
Indoor air	(+)	(+)	-	-	-

DISCUSSION

Species in subgenus *Penicillium* are among the most frequently encountered filamentous fungi, They occur on mouldy foods, feeds and other substrata and in building environments, yet these species have been called some of the most difficult to identify of all fungi (Thom, 1930). In this paper we present a new taxonomy of those important and ubiquitous species, which we believe will be stable for many years. Both cladification and classification of the species point to the same species and species series (Samson *et al.*, 2004; Frisvad *et al.*, 2004). Most other studies of filamentous fungi are based on a morphological treatment occasionally backed up with sequence data from one gene, usually rDNA ITS data. We believe that a polyphasic approach is necessary in any taxonomic study and have included other ecologically important facets of fungal biology in addition to morphology and nucleotide sequences.

The most useful phenotypic characters were extrolites, which are such an important part of the biology of these fungi that it is impressive that previous taxonomic schemes have been at all successful without them. Extrolites are an integral part of classification and identification and are often called "morphological" characters. For example flavour compounds, odours, basidiocarp colours and toxins are regularly used in Basidiomycete taxonomy, and these composite features are all mixtures of extrolites, although they are never mentioned as such. Modern separation and spectrometric methods have enabled identification of all these components and structural elucidation has made it possible to elucidate their biosynthesis.

It is possible to identify the species based on micro- and macro-morphology, colours and physiological features, but identification of extrolites using either TLC, HPLC-DAD, GC-MS, HPLC-MS or MS make identification much easier. Simple tests that detect specific extrolites are the Ehrlich test, colony reverse colours and the strong halo formation by some species in series *Viridicata*. More of these simple tests should be developed to aid identification, as chromatographic and spectrometric equipment is not available in most mycological laboratories.

Despite successful application in bacterial and yeast taxonomy, nutritional features have rarely been used in the systematics of filamentous fungi. Few characters have been used with good results; however, especially isolate reaction on creatine sucrose agar.

Earlier attempts to classify *Penicillium* subgenus *Penicillium* have been successful for some very distinct species, but isolates of the most widespread species have often been placed incorrectly when too few taxonomic features have been used (Raper and Thom, 1949; Samson *et al.* 1976; Pitt, 1979). Attempts to use a large number of features (Bridge *et al.*, 1989 a,b; Paterson *et al.*,1989; Ahmad and Malloch, 1999,

2000) on the other hand has also resulted in many equivocal results. For example experience using the key based on the system of Bridge *et al.* (1992) resulted in a high number of isolates being identified as *P. solitum*, even though those isolates often represented other species. 35 species accepted in the present study were not accepted by Bridge *at al*. (1989a; 1992), even though typical isolates of some of them were included in their study such as *P. aethiopicum, P. carneum, P. concentricum, P. confertum, P. coprobium, P. coprophilum, P. dipodomyicola, P. dipodomyis, P. discolor, P. flavigenum, P. italicum, P. digitatum, P. nalgiovense, P. palitans, P. polonicum* and *P. venetum*. The extended medium regime of Ahmad and Malloch (1999, 2000) looked promising as aid for classification and identification of *Penicillium* subgenus *Penicillium*, but our experience has shown that the reactions on the new chemically well defined media are difficult to read accurately. The diagnostic tests were only effective for very distinct species such as *P. atramentosum* in our attempts to use the system of Ahmad and Malloch (1999, 2000). The limited numbers of nutritional and physiological tests we have used in our study were easier to read and gave clear results. In addition our use of YES agar (degree of conidium production, reverse colours) was of great help in recognizing the 58 species accepted by us.

Two features of our system will be a further help in identification of new isolates of *Penicillium* subgenus *Penicillium*. The isolates marked **Y** (current typical culture selected by us) in the following descriptions can be used as a current reference standard equivalent to the use of standards of chemical compounds for compound identification. Extrolites are a very efficient help in identifying species in *Penicillium*, but even without those we believe that accurate identification is possible. In order to allow the use of extrolites without having access to expensive analytical chemical equipment, we have advocated the use of the Ehrlich test to indicate production of indol alkaloids. We think that more of this kind of tests are needed in order to facilitate identification.

Reactions to abiotic factors (ecophysiology), especially temperature, water activity, pH and atmosphere are very significant, but have been underemphasized in most taxonomies of filamentous fungi. In agreement with Pitt (1979), we believe that these features will play a greater role in future taxonomy. Apart from being functionally and ecologically relevant, these features are often easy to record. We have selected a few of these characters (growth at 15, 25, 30 and 37 °C, 5 % NaCl) in order to keep the number of diagnostic media to a minimum, but other conditions such as high pH, low pH, low redox potential, and high carbon dioxide could have been employed. All species in series *Roqueforti* grow well at low oxygen tensions and high carbon dioxide tensions and *P. atramentosum* prefers an alkaline habitat. Media and conditions

reflecting such unique features have not been included in general in this study. Pitt (1979) used growth at 5, 25 and 37°C and growth on G25N (reduced water activity), but we believe that our combination of media reflects the most important abiotic factors and works better for the terverticillate Penicillia.

It is interesting that the classification proposed by Raper and Thom (1949) is closer to a phylogenetically sound taxonomy than that of Pitt (1979), perhaps because the latter work had an emphasis on the identification process. Our *Penicillium* series are much closer to those of Raper and Thom (1949) than those of Pitt (1979). It is paradoxical that Raper and Thom (1949) had a physiologically more correct classification without using physiological characters, whereas Pitt (1979) in introducing the use of temperature and water relations ended up with a classification that was less physiologically sound.

The number of species we accept (58) is quite high compared to those accepted by Samson *et al*. (1976, 1977a & b) or Pitt (1979) or Pitt and Cruickshank (1990). Many new species have been discovered, however, and they are all unique in the suite of diagnostic features that characterize them.

The section and series classification suggested here appears to be natural both in an ecological and phylogenetic sense. Most series suggested are not very different from those suggested by Raper and Thom (1949), including *Olsonii* (*P. brevicompactum* series), *Roqueforti*, *Chrysogena*, *Camemberti*, *Gladioli*, *Viridicata* (*P. ochraceum*, *P. viridicatum* and *P. cyclopium* series), *Corymbifera*, *Expansa*, *Claviformia*, *Digitata*, *Italica* and *Urticicola*. However, within some of these series, several changes were made: Concerning the series *Olsonii*, *P. olsonii* was placed by Raper and Thom (1949) in their *Biverticillata-Symmetrica*. This was later corrected by Pitt (1979). We now include *P. brevicompactum* and *P. bialowiezense* in this series, already suggested by Stolk and Samson (1985) and Frisvad *et al*., 1990b, and supported by isozyme data (Cruickshank and Pitt, 1987). In *Roqueforti*, not recognized by Pitt (1979), Raper and Thom included *P. casei*, now placed in *Verrucosa* as a synonym of *P. verrucosum*. The current *Roqueforti* series is strongly supported by ITS sequence data (Boysen *et al*., 1996; Skouboe *et al*.,

1999) and by the unique physiological and morphological characters by the three species included in it. Series *Chrysogena* was not recognized by Pitt (1979), but its members have a series of features supporting it, including production of penicillin by all species and growth at low water activities. The four species accepted by Raper and Thom (1949) have all been synonymized (Samson *et al*., 1977a), but two new species have since been discovered and *P. nalgiovense* transferred to the series (Banke *et al*., 1997). Several species in the Raper and Thom (1949) series *P. camemberti* and *P. commune* are now placed in series *Camemberti*, while some other floocose form are placed in *Viridicata* now. Series *Viridicata* now contains nine closely related species. The Raper and Thom (1949) concept of their three series *P. cyclopium*, *P. viridicatum* and *P. ochraceum* in addition to the *P. camemberti, P. commune* and *P. terrestre* series have been merged and has then been subdivided into four series here: *Viridicata*, *Verrucosa* (both containing species growing poorly on creatine as sole nitrogen source), and *Camemberti* and *Solita* (both containing species growing well on creatine as sole nitrogen source). Series *Corymbifera* no longer contains *P. glandicola*. It has been transferred to the series containing synnematous dung fungi *Claviformia*. Several new species have been added to *Corymbifera*, most of which are pathogenic to bulbs and onions. Series *Expansa* now contains three species, but not *P. crustosum*, which has been transferred to series *Camemberti*.

Raper and Thom (1949) placed many of the species according to colony texture, a feature that often is a result of fungal deterioration rather than taxonomic placement (Pitt, 1979). Pitt (1979) on the other hand emphasised colony diameters and had unrelated species in the same series because of their growth rate. His approach was based on a pragmatic approach to identify Penicillia. Although we strongly agree that identification should be a practical issue, simple keys for *Penicillium* identification are unrealistic. The keys provided in this paper will be helpful but only with a combination of DNA sequences, extrolite production and other phenotypical characters it will be possible to identify the taxa using electronic databases.

P. aethiopicum Frisvad, *Mycologia* 81: 848, 1989.

In *Penicillium* subgenus *Penicillium* section *Chrysogena* series *Aethiopica*

Type: Herb. IMI 285524
Culture ex type: CBS 484.84 = IBT 21501 = IBT 5903 = IMI 285524 = FRR 2942 (**T, Y**), ex *Hordeum vulgare*, Addis-Abeba, Ethiopia

Diagnostic features: Griseofulvin, viridicatumtoxin, tryptoquialanins, geosmin, ellipsoidal smooth-walled conidia, markedly sulcate colonies with a golden yellow reverse, growth at 37°C.

Similar species: Isolates of this species have earlier been identified as *P. expansum* (CSIR 1039, CSIR 1375, IMI 246656, IMI 279025), *P. crustosum*, *P. verrucosum* var. *cyclopium* or *P. aurantiogriseum* (CCM F-389), *P. verrucosum* var. *corymbiferum* (Leistner Sp. 1448 = CBS 109575), *P. viridicatum* (CSIR 1039), but differs from all those taxa by its growth at 37ºC. It differs by all species in section *Chrysogena* by being fasciculate.

Description:
Conidia: Smooth-walled, ellipsoidal, 2.8-3.2 x 3.3-3.8 μm, in long columns
Phialides: 7-9 μm, short collula
Metulae: 12-17 μm
Rami: 15-25 μm
Stipes: 200-350 μm, smooth to rough-walled
Synnemata or fasciculation: Weakly fasciculate
Sclerotia: None
Colony texture: Sulcate on CYA
Conidium colour CYA: Dull green.
Exudate droplets on CYA: Copious, clear
Reverse colour on CYA: Golden yellow
Reverse colour on YES: Yellow to curry yellow
Diffusible colour: Occasional; pale orange
Ehrlich reaction: No reaction
Odour and volatile metabolites: Geosmin, ethylacetate, 2-methyl-3-butene-2-ol, 2-pentanone, ethyl isobutanoate, isobutyl acetate, ethyl 2-methyl-butanoate, ethyl isopentanoate, and isopentyl acetate (Larsen and Frisvad, 1995)
Extrolites: 1) Griseofulvin, dechlorogriseofulvin, lichexanthone, 2) Viridicatumtoxin, 3) Tryptoquialanine A & B
Growth on creatine: weak to moderate growth
Acid and base production on creatine: Weak to moderate
Growth on UNO: Very good
Growth on nitrite: Weak

Abiotic factors:
Diam., 1 week, 25°C: CYA: 26-38 mm; MEA: 25-40 mm; YES: 34-57 mm; CREA: 15-34 mm; Cz: 17-28 mm, OAT: 20-37 mm: CYAS: 23-32 mm; CzBS: 17-23 mm; CzP: 0 mm; UNO: 11-16 mm; DG18: 31-38 mm
Diam., 1 week: 15°C: 20–25 mm; 30°C: 29-34 mm; 37°C: 3-9 mm
CYA/CYAS: 1.4 [1.1-1.7]
CYA15°C/CYA 25°C: 0.6 [0.6-0.7]
CYA30°C/CYA 25°C: 0.9 [0.8-0-9]
CZBS/CZ: 0.6 [0.5-0.7]
CZP/CZ: 0

Distribution: Pantropical (South America, Africa, India, Southeast Asia), but also in greenhouses in subtropical and temperate climates.

Ecology and habitats: Maize, sorghum, wheat, barley, kemiri nuts, cow peas, soybeans, mung beans, peanuts, cashews (Frisvad and Filtenborg, 1989, Pitt and Hocking 1998)

Biotechnological applications: Viridicatumtoxin is a weak antitumor agent (Raju *et al.*, 82) and griseofulvin is an important antifungal agent (Cole and Cox, 1981). The species is not used commercially.

Biodeterioration & phytopathology: No data

Mycotoxicoses and mycotoxins: Viridicatumtoxin is a nephrotoxin (Hutchison *et al.*, 1973); the toxicity of the tryptoquialanins is unknown, but the closely related tryptoquivalins are regarded as tremorgens (Cole and Cox, 1981). No reported mycotoxicosis.

Typical cultures: IBT 11191 = CBS 270.97, locust bean gum flour, imported to Denmark; IBT 16873 = CBS 287.97, tropical room Victoria, Vancouver Island, BC, Canada; IBT 5753 = IBT 3352 = IBT 3916 = IBT 3906 = IBT 4706 = CBS 109575, salami, Germany; IBT 5906 = CBS 109577 = IMI 279025, *Vitus* sp. fruit, India; IBT 5750 = CBS 109574 = IMI 246656, maize, Bhagalpur, India; IBT 5905 = CBS 109576, pearl millet, Zimbabwe; IBT 21721 = CBS 109602, cassava chips, Africa; CSIR 1029; CSIR 1375 = NRRL 5880, maize, South Africa; IBT 4701 = IMI 293194 = ATCC 58633, cucumber in greenhouse, Denmark; CCM F-389, tropical soil; IBT 5752, soil, Amazonas, Brazil.