Polyphasic taxonomy of the heat resistant ascomycete genus
Byssochlamys and its Paecilomyces anamorphs

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Abstract
Byssochlamys and related Paecilomyces strains are often heat resistant and may produce mycotoxins in contaminated pasteurised foodstuffs. A comparative study of all Byssochlamys species was carried out using a polyphasic approach to find characters that differentiate species and to establish accurate data on potential mycotoxin production by each species. Phylogenetic analysis of the ITS region, parts of the β-tubulin and calmodulin genes, macro- and micromorphological examinations and analysis of extrolite profiles were applied. Phylogenetic analyses revealed that the genus Byssochlamys includes nine species, five of which form a teleomorph, i.e. B. fulva, B. lagunculariae, B. nivea, B. spectabilis and B. zollerniae, while four are asexual, namely P. brunneolus, P. divaricatus, P. formosus and P. saturatus. Among these, B. nivea produces the mycotoxins patulin and byssochlamic acid and the immunosuppressant mycophenolic acid. Byssochlamys lagunculariae produces byssochlamic acid and mycophenolic acid and thus chemically resembles B. nivea. Some strains of P. saturatus produce patulin and brefeldin A, while B. spectabilis (anamorph P. variotii s.s.) produces viriditoxin. Some micro- and macromorphological characters are valuable for identification purposes, including the shape and size of conidia and ascospores, presence and ornamentation of chlamydospores, growth rates on MEA and CYA and acid production on CREA. A dichotomous key is provided for species identification based on phenotypical characters.

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INTRODUCTION

Byssochlamys species produce ascospores which are heat-resistant, and survive considerable periods of heat above 85 °C (Beuchat & Rice 1979, Splitstoesser 1987). In addition to their heat resistance, Byssochlamys species can grow under very low oxygen tensions (Taniwaki 1995) and can form pectinolytic enzymes (Beuchat & Rice 1979, Splittstoesser 1987). In addition to their heat resistance, Byssochlamys species can grow under very low oxygen tensions (Taniwaki 1995) and can form pectinolytic enzymes. The combination of three of these physiological characteristics makes Byssochlamys species very important spoilage fungi in pasteurised and canned fruit. Byssochlamys has a Paecilomyces anamorph and the genus was revised by Stolk & Samson (1971). Samson (1974) accepted three Byssochlamys species: B. fulva, B. nivea and B. zollerniae with similar Paecilomyces anamorphs. Since then only B. verrucosa has been added to this genus (Samson & Tansey 1975).

Paecilomyces was erected by Bainier (1907) to accommodate a single species, P. variotii, but many species were added (Brown & Smith 1957, Samson 1974). Luangsa-ard et al. (2004) presented a phylogenetic analysis of the 18S rDNA, demonstrating that Paecilomyces is polyphyletic across the Sordariomycetidae and Eurotiales. The type species P. variotii is a morphologically variable taxon, and has been re-described under a variety of names broadening its circumscription. Thom (1930) and Samson (1974) mentioned the diversity in conidial shape and size, and Thom made a tentative division based on conidial size.

Paecilomyces variotii and anamorphs of Byssochlamys species share several micromorphological characters, including phialides with cylindrical bases that taper abruptly into long cylindrical necks and produce catenate conidia. Some characters are constant at the species level, but vary among species. Houbraken et al. (2006) demonstrated that Byssochlamys and its associated anamorph species can be separated into at least nine taxa by investigating the micro- and macroscopical characteristics of Byssochlamys and Paecilomyces variotii-like isolates. In this study, we have extended this to a polyphasic approach by adding molecular and extrolite data and present a revised taxonomy and nomenclature of the accepted taxa.

MATERIALS AND METHODS

Strains of Byssochlamys and Paecilomyces used in this study are listed in Table 1, and are preserved in the Fungal Biodiversity Centre (CBS), Utrecht, the Netherlands.

DNA extraction, sequencing and analysis
Total fungal genomic DNA was isolated using FastDNA® Kit (Bio 101, Carlsbad, USA) according to the manufacturer’s instructions. Amplification and sequencing of the ITS region (including internal transcribed spacer regions 1 and 2, and the 5.8S rRNA regions of the nuclear ribosomal RNA gene cluster), and parts of the β-tubulin and calmodulin genes were performances as described by Houbraken et al. (2007). Contigs were assembled from the forward and reverse sequences with the software package SeqMan from the Lasergene package (DNASTAR Inc., Madison, WI). The alignments of the sequence datasets were performed using Clustal W in MEGA 3.1 (Thompson et al. 1994, Kumar et al. 2004) and were, when necessary, adjusted by eye. Phylogenetic analyses of alignments were done using PAUP v. 4.0b10 (Swofford 2000). Alignment gaps were treated as fifth characters, and gaps were removed when necessary. Phylogenetic analyses were performed using Clustal W in MEGA 3.1 (Thompson et al. 1994, Kumar et al. 2004) and were, when necessary, adjusted by eye. Phylogenetic analyses of alignments were done using PAUP v. 4.0b10 (Swofford 2000). Alignment gaps were treated as fifth characters, and gaps were removed when necessary.

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The resistance to propionic acid are described by Frisvad. The presence of chlamydospores. For the analyses of the features of the sexual Paecilomyces state, the media oatmeal agar (OA) and potato-dextrose agar (PDA) were used (for media formulations, see Samson et al. 2004). The surfaces of conidia, chlamydospores and ascospores were examined after prolonged incubation (up to 70 d). Conditions and media for measuring the microaerophily, growth on 0.5 % acetic acid and the resistance to propionic acid are described by Frisvad & Samson (2004).

### Table 1  
Byssochlamys and Paecilomyces isolates examined in this study.

| Species               | Accession No. | Source and notes                                      |
|-----------------------|---------------|------------------------------------------------------|
| B. fulva              | CBS 132.33    | Bottled fruit, UK; ex-type of Paecilomyces fulvus     |
| CBS 146.48            |               | Bottled fruit, UK                                     |
| CBS 135.62            |               | Fruit juice, Switzerland; ex-type of Paecilomyces toduc |
| CBS 604.71            |               | Unknown source                                        |
| CBS 113954            |               | Unknown source, patulin producer acc. to Rice et al. (1977) |
| B. lagunculariae      | CBS 373.70T   | Wood of Laguncularia racemosa (Mangue), Brazil        |
| CBS 696.95            |               | Pasteurized strawberries, the Netherlands             |
| CBS 110378            |               | Unknown source, France                                |
| B. nivea              | CBS 100.11T   | Unknown source                                        |
| CBS 133.37            |               | Milk of cow, USA; ex-type of Arachniotus trisporus    |
| CBS 271.95            |               | Mushroom bed, China                                   |
| CBS 102119            |               | Pasteurized drink yoghurt, Belgium                    |
| CBS 113245            |               | Pasteurized fruit juice, Switzerland                  |
| B. spectabilis        | CBS 338.51    | Fruit juice, Switzerland                               |
| CBS 102.74            |               | Unknown source; ex-type of Paecilomyces variotii      |
| CBS 101079            |               | Heat processed fruit beverage, Japan                   |
| CBS 121581            |               | Spoiled sweetened tea, USA                            |
| B. verrucosa          | CBS 605.74T   | Nesting material of Leptota ocelata, Australia         |
| B. zollerniae         | CBS 374.70T   | Wood of Zolleria Illicifolia and Protum heptaphyllum, Brazil |
| P. brunneolus         | CBS 370.70T   | Non fat dry milk, Canada                              |
| P. divaricatus        | CBS 284.45T   | Muclage bottle with library paste, USA                 |
| CBS 110429            |               | Lecithis unsitata (Lecthidaceae), wood, Brazil; ex-type strain of P. lecythidis |
| P. formosus           | CBS 628.66    | Quebracho-tanned sheep leather, France                |
| CBS 371.70            |               | Anona squamosa, Brazil; ex-type of Paecilomyces maximus |
| CBS 990.739           |               | Unknown source                                        |
| CBS 296.93            |               | Man, bone marrow of patient, Uzbekistan               |
| CBS 113247            |               | Soil, Thailand                                        |
| CBS 372.70            |               | Lecithis unsitata (Lecthidaceae), wood, Brazil; ex-type strain of P. lecythidis |
| P. saturatus          | CBS 323.34T   | Unknown source; ex-type of Paecilomyces mandshuricus var. saturatus |
| CBS 368.70            |               | Medicine containing quinine, UK                       |
| CBS 251.55T           |               | Acetic acid, Brazil; ex-type of P. dactylomorphus     |
| CBS 990.73A           |               | Unknown source; ex-type of Penicillium viniferum      |
| CBS 492.84            |               | Lepidium sativum, Denmark                             |
| Talaromyces byssochlamydoides | CBS 413.71T | Dry soil under Pseudotsuga menziesi, USA              |
| Talaromyces emeritoni  | CBS 393.64T   | Compost, Italy                                        |
| Thermosascus crustaceus| CBS 181.67T  | Parthenium argentatum (Compostae), decaying plant, USA |

### Extrolite analysis
Strains studied (Table 1) were three-point inoculated on MEA, YES, PDA, OA and CYA agars. All isolates were analysed for extrolite metabolites after 2 wk growth at 30 °C. The cultures were extracted according the method of Smedsgaard (1997) and analysed with high performance liquid chromatography (HPLC) with diode array detection (DAD) (Frisvad & Thrane 1987, 1993). The metabolites found were compared with a spectral UV library derived from authentic standards, including patulin, viriditoxin, mycophenolic acid, byssochlamic acid and physcion, using the same conditions (the maximum similarity is a match of 1 000). The retention indices were compared with those of standards.

### RESULTS

#### DNA sequencing
The trees constructed by maximum parsimony analyses of the datasets of the protein coding genes β-tubulin and calmodulin and the ITS region exhibited similar topologies (Fig. 1–3). Molecular analyses revealed that in the genus Byssochlamys nine taxa can be recognised. Five taxa form a teleomorph, namely B. fulva, B. lagunculariae, B. nivea, B. spectabilis and B. zollerniae, while four are strictly anamorphic, i.e. P. brunneolus, P. divaricatus, P. formosus and P. saturatus. Analyses of the ITS showed that B. verrucosa is not a member of the genus Byssochlamys and is related to Thermosascus (Fig. 1). This was also confirmed by analyses of the partial β-tubulin and calmodulin sequence data (data not shown).

The basal nodes were also similar for all three parsimony trees (Fig. 1–3). Byssochlamys nivea and B. fulva were sister
species, as were *B. lagunculariae* and *P. saturatus*. These four species form a distinct clade (group I). *Byssochlamys* spectabilis and *P. brunneolus* also clustered together and form, together with *P. formosus*, another distinct clade in *Byssochlamys* (group II). *Paecilomyces divaricatus* was basal to these groups in all analyses, while the position of the single *chlamys* on these trees (Fig. 1, 2).

**Fig. 1** One of three equally parsimonious trees of the analysed ITS region (55 of the 629 characters were parsimony informative; tree length = 294, CI = 0.738, RI = 0.855, RC = 0.631, HI = 0.262).

**Fig. 2** One of eight equally parsimonious trees of the analysed partial calmodulin gene sequences (204 of the 607 characters were parsimony informative; tree length = 328, CI = 0.708, RI = 0.889, RC = 0.630, HI = 0.292). Luangs-ard et al. (2005), other species previously classified in *Paecilomyces* sect. *Isarioidea* are now mainly accommodated in *Isaria* or newly described genera.

Various microscopic characters are valuable for identification purposes, including the shape and sizes of conidia and ascospores, and presence and ornamentation of chlamydoospores. Useful macroscopical features are growth rates on MEA and CYA, and acid production on CREA. An overview of various macro- and microscopical features is presented in Table 2. The ratio between the colony diameters on MEA incubated at 30 °C or 37 °C is also diagnostic.

**Extrolite analysis** The extrolite profiles of the taxa are listed in Table 3. Most strains of *B. fulva* produced byssochlamic acid, but *B. fulva* could be subdivided into two chemically different groups: one group of isolates produced meriditin (a compound produced by *Eupenicillium meridianum*) and a peptide with a cycloaspeptide

**Morphological characterisation** The genus *Byssochlamys* is morphologically well-defined and characterised by almost naked ascomata in which croziers and globose asci are formed with ellipsoidal ascospores. The ascosomal initials consist of swollen antheridia and coiled ascogonia. All *Byssochlamys* species have a *Paecilomyces* anamorph. The *Byssochlamys* anamorphs belong to *Paecilomyces* sect. *Paecilomyces*, containing mesophilic, thermotolerant and thermophilic species (Samson 1974). As discussed by
which was earlier reported from an isolate identified as \textit{P. divaricatus} (Jiu & Mizuba 1974). Other unique extrolites were \textit{Cornexistin} and/or \textit{byssochloramic acid} and \textit{viridicatumtoxin}. ‘\textit{OLK}’ and ‘\textit{URT}’ are apolar indole-terpene compounds, but the chemical structure is as yet unknown. ‘\textit{OLK}’ has a palisade UV spectrum.

All strains of \textit{P. divaricatus} produced anthraquinones including emodin. However CBS 110429 only produced another type of anthraquinone, CBS 110428 and CBS 110430 produced ascofuranone. Only the ex-type culture of \textit{B. zollerniae} was available for study and some unique unknown extrolites were found in this isolate. In this study, we could not confirm viriditoxin production in \textit{P. divaricatus} and assume that the strain studied by Jiu & Mizuba (1974) was probably a \textit{B. spectabilis} isolate.

Our phylogenetic analysis showed that \textit{B. verrucosa} is related to \textit{Thermoascus}. \textit{Byssochlamys verrucosa} produced cornexistin and/or \textit{byssochloramic acid}, and also some specific unidentified extrolites only seen in this species. \textit{Thermoascus aurantiacus} also produces compounds with chromophores (UV spectra) consistent with cornexistin, \textit{byssochloramic acid} or similar tropolones (Frisvad unpubl. data). The production of cornexistin and \textit{byssochloramic acid} demonstrates that the species chemically resemble \textit{B. fulva}, \textit{laguncularia}, \textit{B. nivea} and \textit{P. divaricatus}.

\textit{Paecilomyces saturatus} is chemically somewhat diverse. Group I produced at least one of the following extrolites: patulin, mycophenolic acid and a compound which chemically resembles aspergillaric acid (CBS 251.55\textsuperscript{T}, CBS 223.52, CBS 492.84 and IBT 21716), while group II produced brefeldin A (CBS 323.34\textsuperscript{T} and CBS 990.73A) and may fit with \textit{P. mandshuricus} var. \textit{saturatus}. \textit{Paecilomyces formosus} consistently produced variotin and related compounds, except CBS 296.93, which may be chemically close to \textit{P. saturatus} (group I).

\textbf{DISCUSSION}

\textit{Byssochlamys} isolates were included in this study to verify the connection between the anamorphic \textit{P. variotii} complex and holomorphic \textit{Byssochlamys} species. The genus \textit{Paecilomyces} was monographed by Samson (1974) who recognised 31 species divided into two sections, \textit{Paecilomyces} and \textit{Isariaoidae}. However, the phylogenetic analysis of the 18S rDNA demonstrates that \textit{Paecilomyces} is polyphyletic across two subclasses, \textit{Sordariomycetidae} and \textit{Eurotiomycetidae} (Luangsa-ard et al. 2004).
Therefore, Paecilomyces is only monophyletic within the order Eurotiales and characterised by a Byssochlamys teleomorph. In the present study the P. variotii complex could be divided into four species, P. divaricatus, P. formosus, P. saturatus and P. variotii.

Udagawa & Suzuki (1994) described Talaromyces spectabilis, but phylogenetic analysis of the 18S rDNA clarified that this species belongs to the genus Byssochlamys (Luangsa-ard et al. 2004). Recently, Houbraken et al. (2008) showed that this species is heterothallic and that it is linked to the anamorphic species P. variotii. This was confirmed in the present study by morphological features and extrolite data. The presence of a teleomorph with heat-resistant ascospores for P. variotii explains the ability of this fungus to spoil heat treated fruit juices (Piecková & Samson 2000).

Short descriptions of B. lagunculariae, B. spectabilis, P. brunneolus, P. divaricatus, P. formosus and P. saturatus are presented. The concepts of B. fulva, B. nivea and B. zollerniae are unchanged since the descriptions by Stolk & Samson (1971), with the remark that B. nivea var. langunculariae is elevated to species level. Byssochlamys verrucosa is not discussed here, because the taxon probably belongs to Thermoascus; it was fully described by Samson & Tansey (1975). Microscopic dimensions and cultural characters are summarised in Table 3.

**Discussion of accepted taxa**

**Byssochlamys lagunculariae** (C. Ram) Samson, Houbraken & Frisvad, comb. nov. — MycoBank MB512557; Fig. 4a–f

Basionym. Byssochlamys nivea Westling var. lagunculariae C. Ram, Nova Hedwigia 16: 311. 1968.

Byssochlamys lagunculariae strains grow fast on MEA, covering the dish within 7 d at 30 °C. Depending on the isolate, it predominantly forms conidia (CBS 373.70T) or ascomata (CBS 696.95). Colonies 25–55 mm on MEA at 37 °C after 7 d of incubation. Growth occurs under microaerophilic conditions, in the presence of 0.5 % acetic acid or 1 000 ppm propionic acid (pH 3.8). No growth is observed on CYA with 5 % NaCl. Poor growth and no acid production on CREA.

Morphologically, B. lagunculariae is similar to B. nivea and shares various characters such as fast growth rate on MEA at 30 °C and globose (to ellipsoidal) conidia with a flattened base. Chlamydospores are present, uncoloured and smooth-walled. Though similar in shape to those of B. nivea, the conidia and ascospores of B. lagunculariae are generally smaller in size. Another difference is that B. lagunculariae grows well on CYA while B. nivea grows rather poorly.

Our molecular studies and morphological examinations both revealed that B. lagunculariae is clearly distinct from B. nivea. In the original description by Ram (1968), B. lagunculariae was described as a variety of B. nivea, distinguished by its smaller conidia and ascospores. Stolk & Samson (1971) synonymised it with B. nivea, but the present study showed that the growth rate on CYA, and smaller conidial and ascospore sizes are constant characters that can be used to differentiate between these species.

This species produces a similar range of extrolites to B. nivea, but patulin has not been detected in B. lagunculariae. The ex-type culture was isolated from wood of Laguncularia racemosa (mangue) in Brazil and other strains identified as this species were found in soil, and pasteurised strawberries and aloe juice. The occurrence of this species in heat treated products makes it an important food spoilage organism.
Fig. 4 Byssochlamys lagunculariae. a–d. Conidiophores; e. conidia; f. asci and ascospores. — Byssochlamys zollerniae. g. h. Phialides; i. chlamydospores; j. conidia. — Scale bars = 10 µm.
Fig. 5  *Byssochlamys spectabilis*. a–e. Conidiophores; f. conidia; g. ascomata; h, i. asci and ascospores. — Scale bars = 10 µm.
**Byssochlamys spectabilis** (Udagawa & Shoji Suzuki) Houbraken & Samson, Appl. Environm. Microbiol. 74: 1618. 2008. — Fig. 5

Basionym. *Talarratomyces spectabilis* Udagawa & Shoji Suzuki, Mycotaxon 50: 82. 1994.

Anamorph. *Paecilomyces variotii* Bainier, Bull. Trimestriel Soc. Mycol. France 23: 26. 1967.

= *Penicillium variotii* (Bainier) Sacc., Syll. Fung. 22: 1273. 1913.

Colonies spreading rapidly on MEA at 30 °C and covering the dish within 7 d. Similar or higher growth rate at 37 °C than at 30 °C. Good growth under microaerophilic conditions, on MEA with 0.5 % acetic acid and on CYA with 1 000 ppm propionic acid (pH 3.8). No or weak growth on CYA with 5 % NaCl (0–10 mm). Since *B. spectabilis* forms its ascospores in a heterothallic manner, only the anamorph is usually produced. The conidiophores are irregularly branched and ellipsoidal and/or cylindrical; truncate conidia are formed, which are often pale yellow brown. Chlamydospores present, smooth-walled, in some isolates finely roughened. Often broad, thick-walled hyphae are present in fresh isolates. Ascospores are formed when strains of opposite mating types are grown together. Our recent study (Houbraken et al. 2008) showed that strains originating from treated products are mostly frequently capable of producing fertile progeny. The ascospores are ellipsoidal, smooth to finely roughened, 5.5–6.5 × 3.5–4.5 μm. Colonies on MEA agar growing rapidly, attaining a diameter of 7 cm within 7 d at 30 °C. Poor growth and no acid production on CREA.

The extrolite viriditoxin was produced by all investigated isolates. Whether this extrolite is also produced in foodstuffs is unknown.

*Byssochlamys spectabilis* commonly occurs in air, compost, infected humans and various foodstuffs (including pasteurised fruit juices, rye bread). It is frequently found in heat treated products, although after isolation only the anamorph is produced.

**Paecilomyces brunneolus** (N. Inagaki) Samson & Houbraken, comb. nov. — MycoBank MB512559; Fig. 6g–i

Basionym. *Paecilomyces variotii* Bainvar. *brunneolus* N. Inagaki, Trans. Mycol. Soc. Japan 4: 4. 1962.

The type strain of *P. brunneolus* on MEA in 7 d forms colonies of 35–45 mm diam (30 °C) with well-defined margins. Colonies on MEA 20–30 mm diam in 7 d at 37 °C. Growth occurs under microaerophilic conditions and in the presence of 0.5 % acetic acid or 1 000 ppm propionic acid (pH 3.8). No growth is observed on CYA with 5 % NaCl. Poor growth and no acid production on CREA. Microscopical examination showed short, irregular branched conidiophores (2–3 × 15–25 μm). Conidia ellipsoidal to broadly cylindrical with truncate ends (1.5–)2–3(–3.5) × (3.5–)4–5(–5.5) μm. Chlamydospores present, smooth and hyaline.

**Paecilomyces brunneolus** is only known from its type culture, CBS 370.70. Analyses of a part of the 1.5–(2–3(–3.5) μm. Chlamydospores present, smooth and hyaline.

Fast growth on MEA at 30 °C and covering the dish within 7 d. Ratio between growth rates at 30 and 37 °C variable; most strains have slower or similar growth rates (< 1), though CBS 371.70 (ex-type strain of *P. maximus*) and CBS 113247 are exceptions and have a faster growth rate at 37 °C. Growth on MEA with 0.5 % acetic acid varying from absence of growth to more than 80 mm after 1 wk of incubation. Variable growth patterns on CYA with 5 % NaCl, 0–25 mm. All investigated strains have poor growth on CREA and acid production under colonies. Conidiophores irregularly branched, with olive-brown conidia. Chlamydospores present (often on small stalks), smooth walled, globose and (weakly) pigmented. The conidia of this species are variable, varying from ellipsoidal to cylindrical; all with truncate ends. In some isolates conidial shape might vary from ellipsoidal to cylindrical.
Fig. 6  
*Paecilomyces divaricatus.*  
a–c. Conidiophores;  
d. conidia;  
e. ascoma initials;  
f. ascospores.  
—  
*Paecilomyces brunneolus.*  
g, h. Conidiophores;  
i. conidia.  
— Scale bars = 10 µm.
Fig. 7 Paecilomyces saturatus. a–e. Conidiophores; f. conidia. — Paecilomyces formosus. g, h. Conidiophores; i. conidia. — Scale bars = 10 µm.
Ram (1968) described two Paecilomyces species, *P. lecythidis* and *P. maximus*, based on their cultural characters and their large-sized conidia.

The results of the sequencing of the ITS region, and parts of the protein coding genes β-tubulin and calmodulin, showed that *P. formosus* may consist of three taxa, *P. formosus*, *P. lecythidis* and *P. maximus*. However, the three species could not be distinguished by microscopical examination. One difference between strains belonging to the ' *P. maximus* -clade' and the other members of this diverse group, is the faster growth rate of this species at 37 °C than at 30 °C. The sequence and morphological diversity was not detected by the extrolite analyses: the ex-type cultures of *P. lecythidis* and *P. maximus* produced similar extrolite profiles, while the ex-type culture of *P. formosus* is degenerated and is a weak producer of extrolites.

For a more detailed conclusion and delimitation of these three groups more strains should be studied, particularly emphasising conidial shape and extrolites production. For the time being we propose to place *P. lecythidis* and *P. maximus* in synonymy with *P. formosus*.

This species is morphologically similar to *P. variotii* and the main difference is the consistent acid production on CREA.

*Paecilomyces formosus* has been isolated from tropical and subtropical soils, wood, sponge, man (bone marrow, blood), air in a bedroom (Denmark) and pot plant soil of *Sensevieria trifasciata* (Denmark).

*Paecilomyces saturatus* (Nakaz., Y. Takeda & Suematsu) Samson & Houbraken, *comb. nov.* — MycoBank MB512560; Fig. 7a–f

*Basionym. Paecilomyces mandshuricus* (Saito) Thom var. *saturatus* Nakaz., Y. Takeda & Suematsu, J. Agric. Chem. Soc. Japan 10: 102. 1934. = *Penicillium viniferum* Sakag., May. Inoue & Tada, Zentralbl. Bakteriol., 2. Abt. 100: 303. 1939. = *Paecilomyces dactylethromorphus* Bat. & H. Maia, Anais Soc. Biol. Pernambuco 15: 152. 1957.

The oldest basionym available for this taxon is *P. mandshuricus* var. *saturatus* and therefore the name of this taxon is derived from this varietal name.

Isolates growing on MEA at 30 °C cover the Petri dish within 7 d, with strong olive-brown sporulation. Good growth at 37 °C, though slower at 30 °C. Good growth on MEA with 0.5 % acetic acid and CYA with 1 000 ppm propionic acid (pH 3.8). No growth observed on CYA with 5 % NaCl. Poor growth and no acid production observed on CREA.

Morphological examination of various strains showed that this species forms fairly regularly branched, penicillium-like conidiophores, with ellipsoidal and/or cylindrical conidia without a distinct truncation. Chlamydoospores present, hyaline and smooth walled. No teleomorph observed.

The production of extrolites depends very much on the growth medium; patulin or brefeldin A can be produced.

*Paecilomyces saturatus* is an easily recognisable species with its ellipsoidal and/or cylindrical conidia and its fairly regularly branched, penicillium-like conidiophores, with the presence of chlamydoospores, as an intermediate form between *Penicillium* and *Paecilomyces*. Molecular studies now show that this species belongs to the *Byssochlamys* clade and is different from other olive-brown coloured species such as *Hamigera avellanea* (Luangsa-ard et al. 2004), *Penicillium digitatum* and *P. cylindrosporum* (unpubl. data). This species has been isolated from a variety of substrates, e.g. acetic acid, leather, medicine containing quinine, a dispersion of fenylacetate and dibutylmaleinate and *Lepidium sativum*.

**Notes on the ecology and extrolites production**

*Byssochlamys* and *Paecilomyces* species are often found in acidic habitats such as silage (Scurti et al. 1973, Escoula 1975a, b, Anderson et al. 1979), and in common with *Penicillium* series *Roqueforti* species (Frøsvad & Samson 2004) can also tolerate microaerophilic conditions (Escoula 1975a, b, Taniwaki 1995). *Byssochlamys nivea* was initially reported to produce patulin under the name *Gymnosascus* sp. (Karow & Forster 1944, Kuehn 1958), later confirmed by Kis et al. (1969), Scurti et al. (1973), Rice et al. (1977) and Draughon & Ayres (1980). *Byssochlamys fulva* was also reported to produce patulin, albeit by few strains (Escoula 1975a, b, Percebois et al. 1975, Rice et al. 1977). Besides their presence in pasteurised fruit, *B. fulva* and *B. nivea* also form toxic extrolites, such as byssotoxin A and byssolchlamic acid (Kramer et al. 1976, Rice et al. 1977). Besides mycotoxins, also an antitumor metabolite, byssochlamysol, a steroid against IGF-1 dependent cancer cells, is produced by *B. nivea* (Mori et al. 2003).

*Paecilomyces variotii* s.l. also produces mycotoxins (Scott 1965), such as patulin (Escoula 1975a, b), spingofungin E and F (Frommer et al. 1992) and viriditoxin, reported originally from an isolate named *Spicaria divaricata* (Jiu & Mizuba 1974).

Apart from being reported as being a potential mycotoxin, viriditoxin has also been reported to be a candidate for treatment of antibiotic resistant bacteria (Wang et al. 2003). Among the known extrolites are the antifungal drug variotin (Takeuchi et al. 1959, 1964, Suzuki et al. 1990, Omolo et al. 2000), and other drug candidates such as conenstaxins (Nakajima et al. 1991, Fields et al. 1996), SCH 643432 (Hegde et al. 2003) and a penicillin-like compound (Burton 1949). The sideramins ferriburin and fusigen (Diekmann 1967, Domsch et al. 1980) and the organic acids 3-indole-acetic acid (Bakalinerov 1968, Voinova-Raikova et al. 1969), citric acid (Loesecge 1945), ethyleneoxide-α, β-dicarboxylic acid (Sakaguchi et al. 1993), (32,5E)-octa-3,5-diene-1,3,4-tricarboxylic acid 3,4-anhydride (Aldridge et al. 1980) have also been reported. The possible mycotoxins and/or potential drugs byssotoxin A, spingofungin E and F, SCH 643432 and byssolchlamysol were not available to us as standards. Given the taxonomic revision presented here, it remains to be seen which species produce these extrolites. Some connections between species and bioactive extrolites were confirmed or established here and several species had a high consistent extrolite profile. However, the chemotaxonomy of *P. saturatus* is unresolved, because there appear to be two chemotypes, which may or may not indicate that there are two species rather than one. Likewise, *B. fulva* appears to have two chemotypes, with only byssolchlamic acid as a common extrolite in all isolates examined.

**KEY TO BYSSOCYLAMYS AND RELATED PAECILOMYCES ANAMORPHS**

1. Conidia with conspicuously truncate ends or a flattened base ........................................... 2
2. Conidia ellipsoidal or cylindrical with inconspicuously truncated ends ................................ 2
3. Conidia predominantly ellipsoidal and/or cylindrical chlamydo-spores absent or present; *Byssochlamys* teleomorph absent or present ................................................ 3
4. Conidia predominantly globose to subglobose, chlamydo-spores present; *Byssochlamys* teleomorph present ........................ 8
Fig. 8  Byssochlamys fulva. a. Conidiophores; b. conidia; c. asci and ascospores. — Byssochlamys nivea. d. Conidiophores; e. conidia; f. ascospores. — Byssochlamys verrucosa. g. Conidiophores and conidia; h. asci and ascospores. — Scale bars = 10 µm.
3. Conidia cylindrical and/or ellipsoidal; measuring 3.4–4.2 × 1.7–2.1 μm, colonies on MEA restricted, attaining a diameter of less than 45 mm in 7 d at 30 °C .......................... 4
4. Conidia larger 2.3–8(–13) × 1.5–4.5 μm, cylindrical or ellipsoidal, colonies on MEA larger than 45 mm after 7 d at 30 °C .......................... 5
5. Chlamydomyces absent, ratio between diameters on MEA at 37:30 °C between 0.60 and 0.75, colonies with more or less feathery margins ........................................ P. divaricatus**
6. Conidia measuring 3.7–5.6 × 2.4–3.6 μm, no acid production on CREA; Byssochlamys teleomorph produced in a heterothallic manner .................. B. spectabilis
7. Conidia measuring 3.2–5.7(–10) × 2–2.9(–3.4) μm, acid production under colony on CREA; Byssochlamys teleomorph absent ............................... P. formosus
8. Chlamydomyces distinctly rough-walled ........................................ B. zollerianna (Fig. 4g–j)
9. Conidia measuring 3.1–3.8 × 2.5–3.2 μm, good growth on CYA ......... B. lagunculariae
10. Conidia measuring 3.1–4.3 × 2.6–3.4 μm, moderate growth on CYA .......... B. nivea (Fig. 8d–f)

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* Byssochlamys vernicosa is phylogenetically unrelated to the true Byssochlamys spp., but the taxon is included in the key because of its striking morphological similarity to other Byssochlamys species.
** Smooth elliptoidal ascospores once observed in a fresh isolate (5.3–7 × 3.8–4.9 μm).

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