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Taxonomic studies of the *Penicillium glabrum* complex and the description of a new species *P. subericola*

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Abstract A mycological survey of fungi, present in several stages of the manufacturing of cork discs for champagne stoppers in Portugal, was made. Sixty-nine strains belonging to the *Glabra* series of the genus *Penicillium* were isolated and subsequently grouped according to their partial β-tubulin gene sequences. Six groups with different partial β-tubulin gene sequences were observed, and a selection of isolates of each group was made. These selected isolates and various related ex-type strains were subjected to a taxonomical study using a polyphasic approach. This approach included analysis of macro- and microscopic features, the comparison of extrolite profiles and sequencing a part of the β-tubulin and calmodulin gene. The six β-tubulin types were reduced to three different species. One group of isolates was centred on the ex-type strain of *P. glabrum*, a second group accommodated the type strain of *P. spinulosum* and a third group contained isolates which were unique in their β-tubulin and calmodulin sequences, extrolite profiles and growth characteristics. This group of isolates is described as the new species *Penicillium subericola*. The type strain of *P. subericola* CBS 125096^{T} was isolated from Portuguese raw cork, but additional isolates were found from soil, air and lumen.

Keywords Taxonomy · Phylogeny · Tubulin · Cork

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Introduction

Cork is the outer bark of the cork oak tree (*Quercus suber*). It is the most suitable material for cork stoppers, due to its unique properties, such as elasticity, compressibility and impermeability to gas or liquids (Lopes et al. 2001; Mano 2002). During a survey of the colonizing mycobiota of cork slabs along the industrial manufacture of cork stoppers, numerous *Penicillium* isolates were isolated and identified using morphological characters. More than half of the isolates belonged to the *Glabra* series, and were present in all production stages. However, identification of the different isolates up to species level appeared to be difficult due the high similarities in macro- and micromorphology.

Raper and Thom (1949) placed *P. glabrum* (as *P. frequentans*), *P. spinulosum* and *P. purpurescens* in the *P. frequentans* series, and later this series was synonymised with the *Glabra* series by Pitt (1979). The *Glabra* series was created to accommodate the fast growing Penicillia with monoverticillate conidiophores and contains eight species (*P. chermesinum*, *P. sclerotiorum*, *P. donkii*, *P.*

decumbens, *P. thomii*, *P. glabrum*, *P. spinulosum* and *P. purpurescens*). Among those species, *P. glabrum* and *P. spinulosum* were morphologically similar and could be best differentiated based on conidial ornamentation. However, the morphological resemblance has caused much confusion and isolates are often misidentified or not differentiated by taxonomists using morphological and physiological techniques (Pitt et al. 1990).

Sixty-nine strains originating from cork and belonging to the *Glabra* series were grouped according to their partial β-tubulin gene sequences. A subset of these strains was selected for macro- and microscopic analysis, extrolite profiling and sequencing a part of the β-tubulin and calmodulin gene. In addition, ex-type strains of various related species were included in the analysis. Our polyphasic taxonomic approach shows that a group of isolates share peculiar differences with other known species, and a new species is proposed for this group of isolates.

Materials and methods

Fungal strains

For our taxonomic study, a selection of these sixty-nine strains isolated from cork, was made and supplemented with related (ex-type) strains (Table 1). Spore suspensions of the cultures were maintained in 20% glycerol at −80°C.

Sequencing and data analysis

The strains were grown for 2–3 days at 25°C on malt peptone medium. Genomic DNA was isolated using the Ultraclean™ Microbial DNA Isolation Kit (MoBio, Solana Beach, U.S.A.) according the manufacturer's instructions. Fragments, containing a part of the β-tubulin or calmodulin gene, were amplified and subsequently sequenced according the procedure previously described (Houbraken et al. 2007). The alignments and analyses were preformed as described by Samson et al. (2009). Newly obtained sequences were deposited in Genbank nucleotide sequence database under GQ367499-369547, GU372883-GU372894 and GU991606-GU991609.

Phenotypic identification

All strains were grown on malt extract agar (MEA, Oxoid), Czapek Yeast autolysate agar (CYA), creatine agar (CREA) and Yeast Extract Sucrose agar (YES) (Samson et al. 2010). These media were inoculated in a three-point position and incubated at 25°C for 7 days. In addition, CYA plates were incubated at 30°C and 37°C. After incubation, the culture characteristics were recorded. Microscopic characters were determined on MEA and CYA.

Extrolite extraction and analysis

A selection of ten cork isolates was made based on the results of the β-tubulin analysis, and subjected to extrolite profiling. In addition, various related ex-type strains were examined. The extrolite extractions from the culture media were preformed according to the methods described by Frisvad and Thrane (1987) and Smedsgaard (1997), using 500 μL ethylacetate/methanol/dichloromethane 3:2:1 (vol./vol./vol.) with 1% formic acid. The mixture was ultrasonicated in a bath for 60 min. The organic solvent was transferred to a new vial and evaporated in a fume hood for 24 h. The extract was re-dissolved in 400 μL methanol, analysed by HPLC with diode array detection (DAD) and the extrolites were identified by their UV spectra and retention times.

Results

Grouping of members of the Glabra series isolated from cork

The genetic variation within the strains isolated from cork was investigated using the partial β-tubulin sequences. The strains isolated from cork and four ex-type strains (*P. glabrum*, *P. frequentans*, *P. paczoskii* and *P. spinulosum*) were added to the dataset, and subjected to an UPGMA analysis (Sneath and Sokal 1973). The sum of branch length of the optimal tree was 0.1301 and the dendrogram is shown in Fig. 1. In total, 422 positions were present in the final dataset. Six groups could be identified among the cork isolates belonging to the *Glabra* series. The largest group (50 isolates) shared the same partial β-tubulin sequence with the type of *P. glabrum*, CBS 125543 (Group 1). One cork isolate (CBS 127703) appeared to have a unique partial β-tubulin sequence differing from other isolates in this clade (group 2). Group 4 was the second largest group and consisted of 14 isolates. This group was closely related with group 3 (3 isolates) and these two groups only differed by one base pair. Group 5 and 6 were deviating from the other groups and the β-tubulin data shows that members of group 6 share sequences with the type of *P. spinulosum*. Group 5 contained one isolate and this strain will be described here as a new species *P. subericola*. Each unique sequence type was compared by a BLAST search in the NCBI database with the *P. glabrum* strains identified by Serra et al. (2008). In total three *P. glabrum* sequences were deposited by Serra et al. (2008) and NRRL 35621 appeared to have identical sequences as "group 2", while the other two sequences (NRRL 35626 and NRRL 35684) were unique and not

Table 1 List of isolates belonging to Series *Glabra* and related Penicillia

CBS no.	Other no.	Name	Remarks
CBS 235.60	ATCC 18483=FRR 634	*E. pinetorum*	Ex-type of *P. silvaticum*; forest soil, USSR
CBS 295.62	ATCC 14770=CCRC 31517=DSM 2438= IFO 7743=IMI 094209=MUCL 31196= NRRL 3008	*E. pinetorum*	Ex-type; soil, conifer and hardwood forest, Wisconsin, USA
CBS 260.29	IMI 092242=NRRL 774=Thom4733.60	*P. glabrum*	Ex-type of *P. flavidorsum*; unrecorded source
CBS 213.28	FRR 770=IMI 092265=IMI 092265ii=NRRL 770	*P. glabrum*	Ex-type of *P. oledzskii*; soil under conifer, Poland
CBS 344.59	ATCC 18486=IFO 5359=IMI 068617=NRRL 3460	*P. glabrum*	Ex-type *P. spinuloramigenum*; butter, Japan
CBS 228.28	FRR 752=IMI 092232=MUCL 29114=NRRL 752	*P. glabrum*	Ex-type of *P. terlikowskii*; soil under conifer, Poland
CBS 229.28	FRR 751=IMI 092231=MUCL 29111=NRRL 751	*P. glabrum*	Ex type of *P. paczowskii*; soil under conifer, Poland
CBS 105.11		*P. glabrum*	Ex-type of *P. frequentans*; unknown substrate, Germany
CBS 127700		*P. glabrum*	Non-boiled cork
CBS 127701		*P. glabrum*	Cork, after the 1st boiling process
CBS 126333		*P. glabrum*	Cork discs
CBS 127702		*P. glabrum*	Non-boiled cork
CBS 127703		*P. glabrum*	Non-boiled cork
CBS 127704		*P. glabrum*	Non-boiled cork
CBS 127705		*P. glabrum*	Non-boiled cork
CBS 126336		*P. glabrum*	Non-boiled cork
CBS 125543	IBT 22658	*P. glabrum*	Ex-type; unrecorded source
CBS 687.77	IJFM 3745=IMI 253783	*P. grancanariae*	Ex-type of *P. grancanariae*; air, Gran Canaria, Spain
CBS 336.79	ATCC 38669=IJFM 3840=VKM F-2181	*P. palmense*	Ex-type; air, Gran Canaria, Spain
CBS 126.64		*P. purpurescens*	Soil, Erzurum, Turkey
CBS 366.48	ATCC 10485=IMI 039745=NRRL 720=QM 1959	*P. purpurescens*	Neotype; soil, Canada
CBS 328.48	ATCC 10444=IMI 040234=NRRL 1915	*P. spinulosum*	Ex-type of *P. trzebinskii*; forest soil, Poland
CBS 269.35	IMI 190574	*P. spinulosum*	Ex-type of *P. mucosum*; soil, beech forest; Germany
CBS 268.35	IMI 189582	*P. spinulosum*	Ex-type of *P. mediocre*; soil, pine forest; Germany
CBS 289.36	IMI 190573	*P. spinulosum*	Ex-type of *P. tannophagum*; tannin solution, Germany
CBS 271.35	IMI 190675	*P. spinulosum*	Ex-type of *P. tannophilum*; leaf litter, Germany
CBS 374.48	ATCC 10498=IMI 024316=MUCL 13910=MUCL 13911=NRRL 1750	*P. spinulosum*	Ex-type; culture contaminant, Germany
CBS 223.28		*P. spinulosum*	Unknown source
CBS 127698		*P. spinulosum*	Non-boiled cork
CBS 127699		*P. spinulosum*	Non-boiled cork
CBS 125096		*P. subericola*	Non-boiled cork, Portugal
CBS 127706	KAS 1289=IBT 22618	*P. subericola*	Lumber, Vancouver, BC, Canada
CBS 125097	IBT 23009	*P. subericola*	Air, margarine factory, Vejle, Denmark
CBS 125100	FRR 4914=IBT 30068	*P. subericola*	From dried grapes (sultanas, *Vitis vinifera*), Mildura, Vic, Australia
CBS 125099	IBT 20217	*P. subericola*	Acidified lake, Butte, Montana, USA
CBS 125098	IBT 20218	*P. subericola*	Acidified lake, Butte, Montana, USA
CBS 347.59	FAT 340=IFO 6031=IMI 068221	*P. thomii*	Ex-type of *P. thomii* var. *flavescens*; unrecorded substrate, Japan
CBS 350.59	ATCC 18333=FRR 3395=IFO 5362= IMI 068615	*P. thomii*	Ex-type of *P. yezoense*; butter, Japan

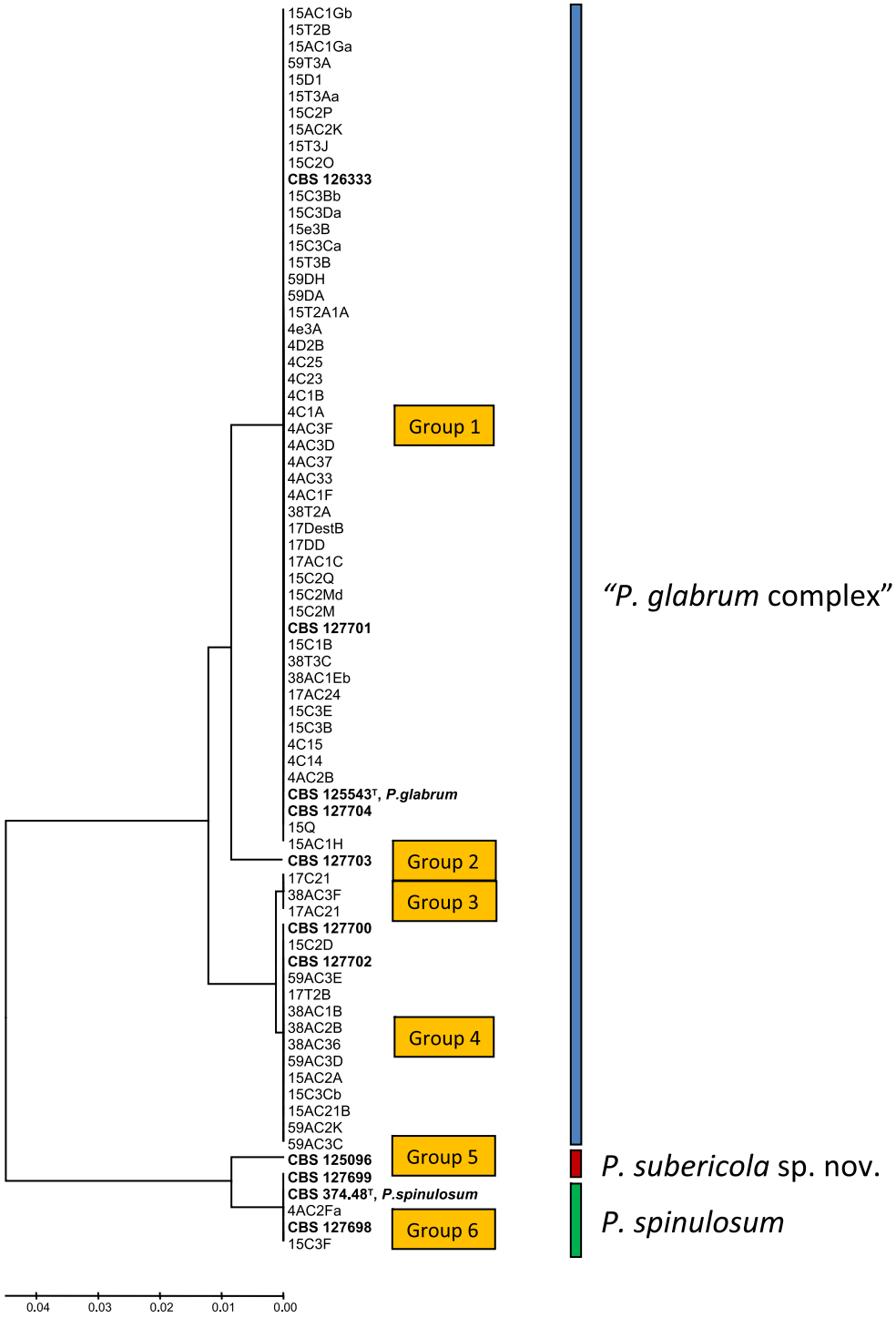

Fig. 1 Cladogram showing the results of the UPGMA analysis of the isolated cork strains belonging to *Penicillium* series *Glabra*. The strains presented in bold are used in the detailed phylogenetic analysis

assignable to any of our groups. A selection of strains was made and the isolates presented in bold in Fig. 1 were used for a detailed polyphasic study.

Phylogenetic analysis

A combined dataset with partial β-tubulin and calmodulin gene sequences was analysed using RAxML (Fig. 2). The alignment had 230 distinct patterns and the proportion of gaps and completely undetermined characters in the alignment was 0.0302. The phylogenetic analysis showed that there were two main well supported clades. In one clade *P. spinulosum*, *P. palmense* and *P. subericola* were present and in the other clade *P. glabrum*, and *P. purpurescens* were located. *Penicillium purpurescens* was basal to *P. glabrum* and the *P. glabrum* isolates were divided in two groups. In one group the majority of the cork isolates were located, together with the type strain of

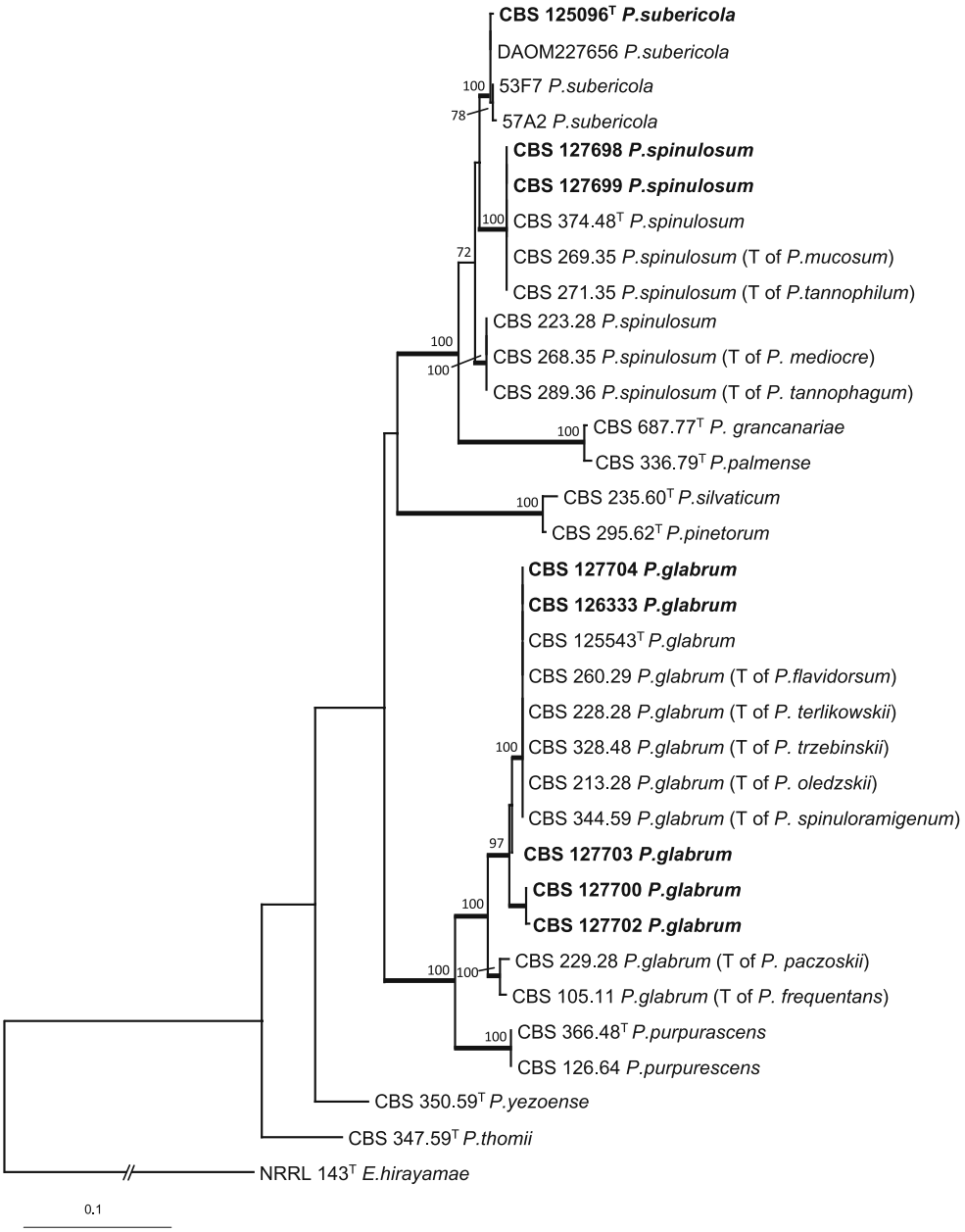

Fig. 2 Phylogram based on the combined dataset of partial β-tubulin and calmodulin gene sequences and analysed using RAxML. The strains in bold are isolated from cork

P. glabrum and the ex-type strains of *P. flavidorsum*, *P. spinuloramigenum*, *P. terlikowskii*, *P. trzebinskii* and *P. oledzskii*. The other group consisted of the type strains of *P. frequentans* and *P. paczowskii*. In the other clade, *P. palmense* was basal to *P. spinulosum* and *P. subericola*. The ex type of *P. palmense* clustered together with *P. grancanariae* CBS 687.77^{T}.

Penicillium spinulosum and *P. subericola* were on a branch with a fair bootstrap support (72%). Three groups were detected within this clade, but none of the phylogenetic relations between those groups were well supported. The isolates of *P. subericola* were on one branch. Interestingly, *P. spinulosum* was divided in two groups. One group compasses the type culture of this species and the type strains of *P. mucosum* CBS 269.35 and *P. tannophilum* CBS 271.35; the other group contained the type strains of *P. mediocre* CBS 268.35 and *P. tannophagum* CBS 289.36.

Phenotypic analysis

The strains isolated from cork were inoculated on the agar media MEA, CYA 25°C, CYA30°C, CYA 37°C, CREA and YES and were compared with the type strains of *P. glabrum*, *P. spinulosum*, *P. frequentans* and *P. paczoskii.* None of the examined strains were able to grow on CYA incubated at 37°C. In Fig. 3 an overview is shown of growth patterns on various agar media. There was a large variation in macromorphology among the *Glabra* strains.

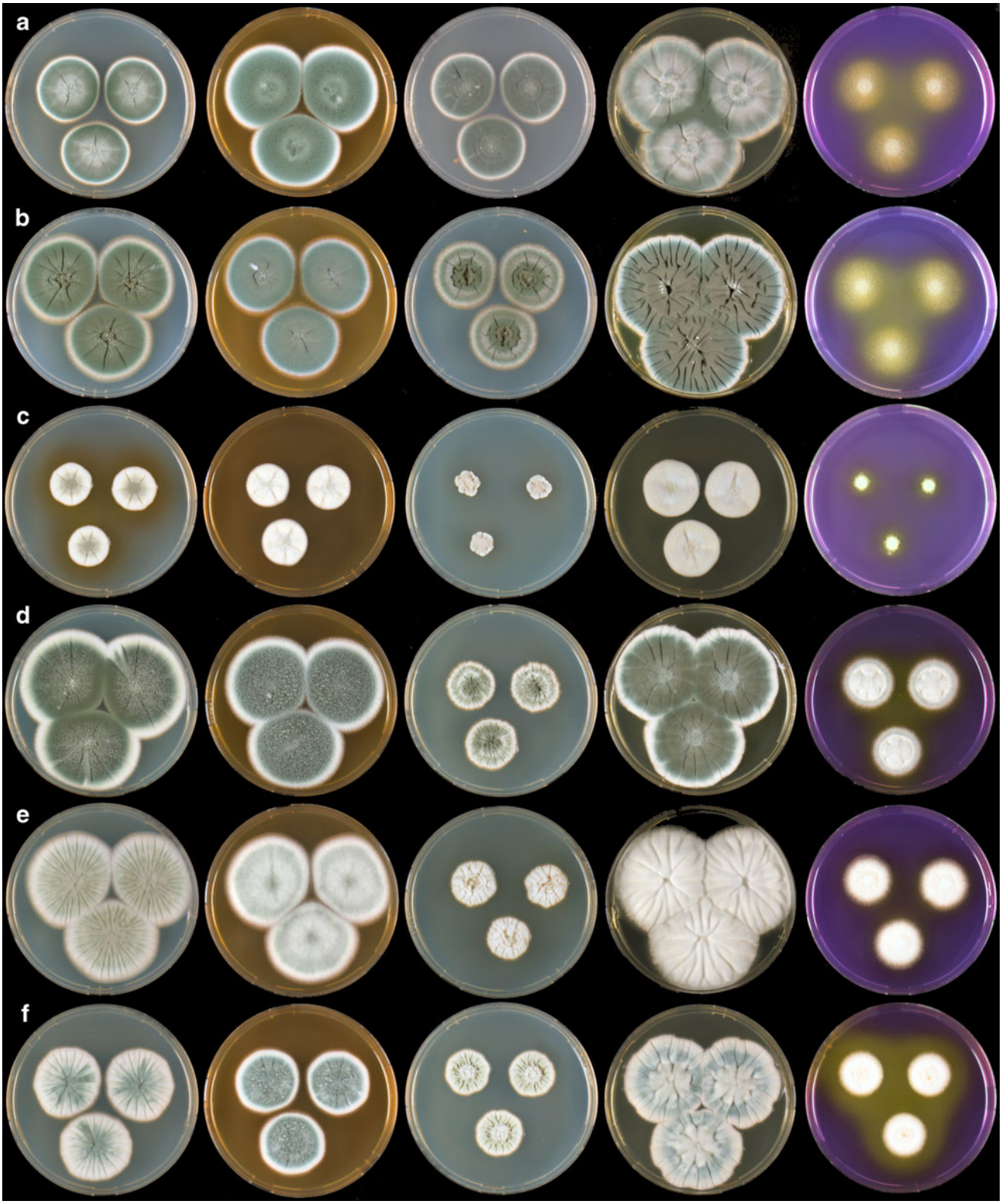

Fig. 3 Colonies incubated for 7 days. Columns, from left to right CYA at 25°C, MEA, CYA at 30°C, YES, creatine agar; rows, top to bottom, *Penicillium glabrum* CBS 127701, *P. glabrum* CBS 127702, *P. glabrum* CBS 125543^{T}, *P. spinulosum* CBS 127699, *P. spinulosum* CBS 374.48^{T}, *P. subericola* CBS 125096^{T}

The type strain of *P. glabrum* and *P. spinulosum* were deviating and showed reduced growth rates and weak sporulation. The reverse colours on CYA of the *Glabra* members were in shades of orange or orange brown, and occasionally in crème colours. The intensity of these colours varied per isolate and ranged from pale orange-brown to vivid orange or red-orange (in *P. spinulosum*). The variation observed among the *Glabra* cork isolates could not clearly be correlated to any of the six groups previously assigned with the partial β-tubulin data. No clear distinctive characters to differentiate between *P. glabrum*, *P. spinulosum* and the new species could be observed on CYA, MEA and YES. However, there was a striking difference on creatine agar. Isolates of *P. spinulosum* and the new species *P. subericola* grew moderate to good on this medium and the majority of both species produced base compounds after prolonged incubation. The colony diameter was generally larger than 25 mm, while *P. glabrum* isolates grew more restricted (often less than 25 mm) Fig. 3.

Microscopic analysis of the strains showed that *P. glabrum*, *P. spinulosum* and *P. subericola* sp. nov. were very similar to each other. All species were predominantly monoverticillate, with vesiculate conidiophores and 6–12 ampulliform phialides. The main microscopical difference was the conidia ornamentation, which was smooth to slightly rugose in *P. glabrum* and *P. subericola* sp. nov., and distinctly rugose in *P. spinulosum*. Moreover, the conidia of *P. subericola* tended to be more rugose than in *P. glabrum* and the conidiophores of this species occasionally were branched, a character not observed in *P. glabrum* and *P. spinulosum*.

Extrolites analysis

The majority of the strains assigned to *P. glabrum*, *P. spinulosum* and *P. subericola* produced a pattern of extrolites typical for each species (see Table 2). The *P. glabrum* isolates had a typical extrolites profile containing asterric acid, bisdechlorogeodin, sulochrin or citromycetin, while isolates of *P. spinulosum* produce asperfuran, palitantin and frequentin. Asperfuran, deoxybrevianamide E and unidentified compounds which were tentatively named AMF were found in the *P. subericola*. These AMF compounds are indols with an extended chromophore similar to penitremone. Two cork isolates which phylogenetically clearly belong to *P. glabrum* (CBS 126333 and 127701) were chemically weak and show no detectable extrolite production.

Discussion

The majority of cork isolates were identified as *P. glabrum* using the current taxonomical schemes. Four different sequence types of β-tubulin within *P. glabrum* could be detected. BLAST searches on the NCBI database and local databases of the CBS-Fungal Biodiversity Centre showed that many more sequence types are present in *P. glabrum*. This intra-species β-tubulin variation is in contrast with species in subgenus *Penicillium*, where various species share the same tubulin sequence (Samson et al. 2004). The large variability among *P. glabrum* isolates originating from cork is also observed using microsatellite primers (Basílio et al. 2006). Our analysis show that *P. flavidorsum*, *P. spinuloramigenum*, *P. terlikowskii*, *P. trzebinskii* and *P. oledzskii* are synonyms of *P. glabrum.*

Raper and Thom (1949) placed *P. glabrum* (*P. frequentans*), *P. spinulosum* and *P. purpurescens* in the *P. frequentans* series. Our data show that these three species are phylogenetic related. Pitt (1979) named this the *Glabra* series and expanded it with Penicillia, which have monoverticillate penicilli and a colony diameter on CYA larger than 30 mm after 7 days at 25°C. *Penicillium chermesinum*, *P. sclerotiorum, P. donkii, P. decumbens, P. thomii, P. glabrum, P. spinulosum* and *P. purpurescens* were included, but the phylogenetic analysis of the genus *Penicillium* by Peterson (2000) showed that the former four species were not closely related to *P. glabrum*. Furthermore, Peterson (2000) named this monophyletic clade “Group 2”, and showed that the species *E. pinetorum*, *P. asperosporum*, *P. lividum* and *E. lapidosum* were related to *P. glabrum*. These findings in a large extent supported in our study, but there are some differences. The taxonomic position of *E. lapidosum* warrants further attention. This species was not included in our phylogenetic study because the type strain of this species (CBS 343.48) is phylogenetically unrelated to the *Glabra* group (J. Houbraken, unpublished data). This is in contrast with the observation made by Peterson (2000), which stated that *E. lapidosum* was conspecific with *P. thomii*.

Our data show that *P. palmense* and *P. grancanariae,* both isolated from air in Gran Canaria, Spain (Ramirez et al. 1978), are synonymous. The type strains of *P. frequentans* and *P. paczowskii* were considered to be synonyms of *P. glabrum* and *P. spinulosum* respectively (Pitt, 1979). However, based on calmodulin, tubulin and RPB2 data (data not shown) both type strains are placed in a separate clade related to *P. glabrum,* suggesting that *P. frequentans*/*P. paczowskii* and *P. glabrum* are two distinct species. This evidence is also supported by the extrolites profiles of these species (Frisvad, unpublished data).

Phenotypical differences were observed between the type strains and the cultures isolated from the cork. This is probably due to the fact that the type strains are maintained in cultures collections for a considerable period. Gradual degeneration of various traits due to long-term maintenance and sub culturing are reported. Also degeneration could be due to the lyophili-

Table 2 Extrolite profile of the cork isolates and type or authentic isolates belonging to *Glabra* series on CYA, YES and OA after 7 days of incubation

Species	Isolates	Extrolites
P. glabrum	CBS 213.28	Asterric acid, bisdechlorogeodin, questin, sulochrin
	CBS 328.48=FRR 1915	Asterric acid, bisdechlorogeodin, citromycetin, PI-3, PI-4
	ATCC 42228=IBT 13946	Asterric acid, bisdechlorogeodin, sulochrin
	CBS 127703	Asterric acid, bisdechlorogeodin, PI-4, sulochrin
	CBS 127700	Asterric acid, bisdechlorogeodin, PI-4, sulochrin
	CBS 126336	Asterric acid, citromycetin, bisdechlorogeodin, PI-4, questin, questinol,sulochrin
	CBS 127702	Asterric acid, citromycetin, bisdechlorogeodin, PI-4, questin, questinol, sulochrin
	CBS 127704	Asterric acid, bisdechlorogeodin, PI-4, questinol, sulochrin
	CBS 126333	No metabolites expressed
	CBS 127701	No metabolites expressed
P. palmense	CBS 336.79=IBT 4912	4 chromophore types in common with *P. subericola*, and 4 chromophore types only found in this species
	ATCC 38669=IBT 16227	4 chromophore types in common with *P. subericola*, and 4 chromophore types only found in this species
P. spinulosum	NRRL 1750	Asperfuran
	DAOM 215366=IBT 22621	Asperfuran, palitantin, frequentin
	DAOM 227655=IBT 22622	Asperfuran, palitantin
	CBS 127698	2 chromophore types found in this isolate and CBS 127699
	CBS 127699	2 chromophore types found in this isolate and CBS 127698
P. subericola	CBS 125096	AMF[a], deoxybrevianamide E
	CBS 125100=FRR 4914=IBT 30068	AMF, deoxybrevianamide E
	IBT 23009 & IBT 23010	AMF
	DAOM 227656=IBT 22618	AMF, asperfuran, deoxybrevianamide E
	CBS 125099=IBT 20217	AMF, asperfuran
	CBS 125098=IBT 20218	AMF
	IBT 23016	AMF
E. pinetorum	WSF 15-c=IBT 22704	Asperfuran and 4 chromophore types on seen in this species
	RMF 9252=IBT 22795	Asperfuran and 4 chromophore types on seen in this species
	CBS 311.63=IBT 22192	Asperfuran and 4 chromophore types on seen in this species
P. purpurescens	CBS 366.48	5 chromophore types only seen in this species

[a] AMF compounds are not fully chemically identified indols with an extended chromophore similar to penitremone

zation process, and colony characteristics could be affected due to a lower survival of spores in lyophilised cultures, compared to the fresh cultures (Okuda et al. 1990). The main distinction between *P. glabrum* and *P. spinulosum* was the conidia wall texture, which was smooth to finely rugose in *P. glabrum* and finely roughened to distinctly spinose in *P. spinulosum.* Some isolates belonging to the *Glabra* series were difficult to identify correctly even by skilled taxonomists (Pitt et al. 1990). However, to overcome this problem molecular and chemical techniques combined with classical taxonomy were analysed together here, giving a more accurate answer to the taxonomic position of these closely related species. In this study we show that *P. glabrum* can be differentiated from *P. spinulosum* and *P. subericola* by its weak growth on creatine agar.

The concept of exo-metabolome was introduced by Thrane et al. (2007) to enclose all the metabolites produced by fungi in interaction with the environment. The cork isolates belonging to the *Glabra* series could be grouped in three different extrolite profiles. One similar to the type strain of *P. glabrum*, a second group produced extrolites in common with the type strain of *P. spinulosum* and a third one characteristic of *P. subericola*. Two isolates were chemically weak and did not produce any extrolites. This might be due to degeneration by long-term maintenance, sub-culturing or lack of selection pressure from the environment. The non-production of expected metabolites could also be due to some (point) mutations on the regulatory gene (Larsen et al. 2005). Moreover, *P. spinulosum* cork isolates produced also some metabolites that were not characteristic of the species, although some of them were described in some *P. spinulosum* isolates. Since the production of secondary metabolites is more or less genus or species specific (Frisvad et al. 1998,

2008) the existence of *P. glabrum* cork isolates that produced two different extrolite profiles indicated the existence of intraspecific variability.

The species concept, based not only on DNA sequences, but also in ecological, phenotypic characters and exometabolome profiles provide a more accurate and real classification, as verified by studies on *Penicillium* subgenus *Penicillium* (Samson and Frisvad 2004) and black Aspergilli (Samson et al. 2007). Applying this polyphasic approach, *P. spinulosum* and *P. subericola* can be regarded as two separate species. Hoff et al. (2008) suggested in their study of *P. chrysogenum* that closely related species could be mating types of the same biological species. However, no differences in extrolite patterns and phenotype could be observed in isolates of different mating types of *Paecilomyces variotii* (Houbraken et al. 2008, Samson et al. 2009). Furthermore, our studies showed that the two mating types discovered in *Aspergillus fumigatus* (O'Gorman et al. 2009) and *Penicillium chrysogenum* (Hoff et al. 2008) produced the same pattern of extrolites and are identical in their phenotype (Houbraken, Samson and Frisvad, unpublished data). In case of *P. subericola* we have observed differences in both growth patterns and extrolite production and hence the description of a new species is warranted.

The cork isolates now classified as *P. glabrum* species showed a high intraspecific variability. The macro- and micromorphologies, extrolites profiles and results of the sequencing of partial regions of the β-tubulin and calmodulin genes supported that variability. If the results were analyzed separately (e.g. the extrolite profile and β-tubulin sequencing) probably some of them could indicate the existence of at least two different species. The analysis of more isolates of this species isolated from different sources and from different geographic locations is needed to determine species boundaries in *P. glabrum* and related species.

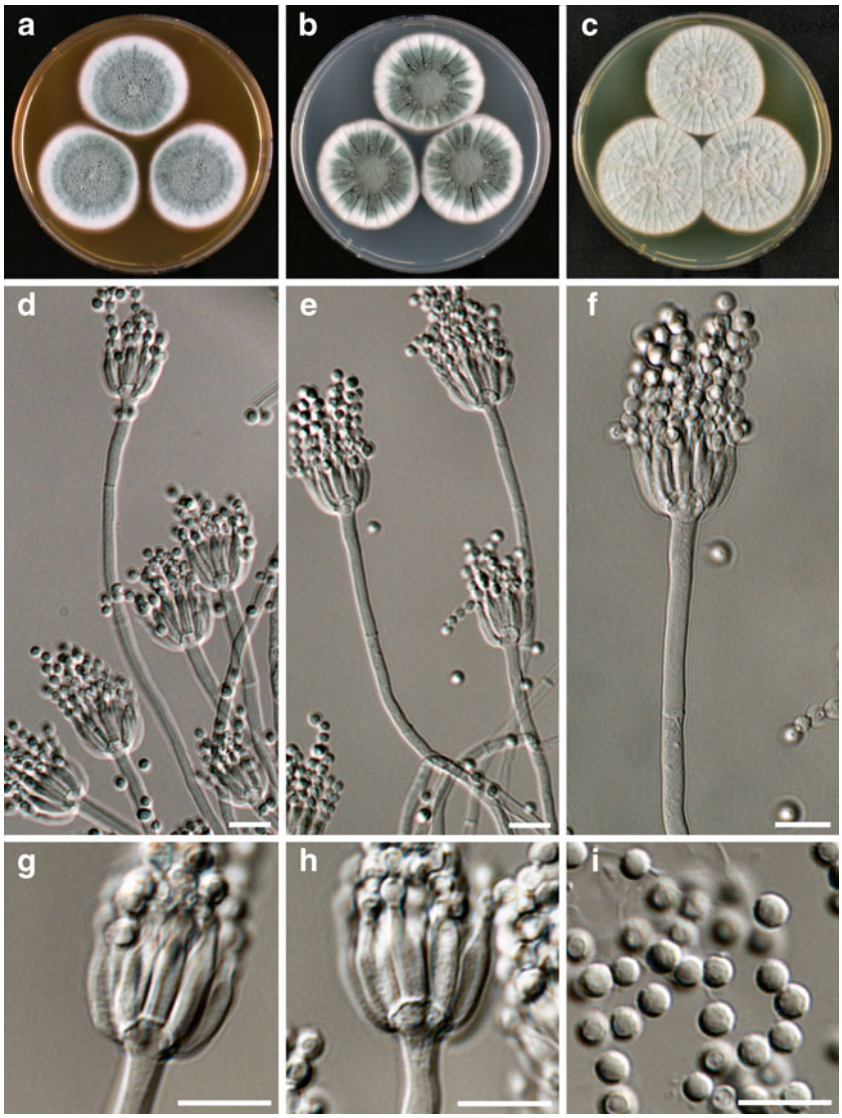

Fig. 4 *Penicillium subericola*, cultures incubated for 7 days at 25°C, A. MEA, B. CYA, C. YES. D-I. Conidiophores, phialides and conidia. Scale bar= 10 μm

Penicillium subericola Baretto, Frisvad & Samson, sp. nov.—Mycobank MB 517383 - Fig. 4.

Penicillio glabro simile, sed bene crescenti in agaro creatino et formatione mixtionis chemicae obscurae (sed in P. glabro non producenti) distinguitur.

Culture ex type: CBS 125096, ex raw cork, Portugal

Colony diameters at 7 days in mm: CYA at 25º C: 37–44; CYA at 30°C: 16–34; CYA at 37°C: no growth; MEA 35–42; YES 39–46; CREA 14–26, moderate to good growth with moderate to good acid production, base production after prolonged incubation (14 days).

Good sporulation on CYA, grey-green, velvety and floccose in centre, non sporulating margins 1–6 mm, few small hyaline exudates droplets present, reverse colour cream to brownish. Colonies on MEA grey-green, good sporulation, floccose some isolates with velvety colonies and/or velvety with floccose in the centre, exudate absent, reverse is orange brown. Colonies on YES in various shades of green-grey, none or weak sporulation, mycelium inconspicuous, white margins with 1–2 mm, exudates absent, reverse orange-brown to yellow-brown, strongly sulcated (wrinkled).

Conidiophores strictly monoverticillate, stipes vesiculate up to 6 μm, smooth, occasionally short 40 μm, majority longer, width 3.0–4.0, vesicles 4.5–7.0 μm, phialides flask shaped, 10–14×2.0–3.0 μm, conidia globose, finely roughened, 3–3.5 μm.

Extrolites: asperfuran, deoxybrevianamide E and unidentified compounds which are indols with an extended chromophore similar to penitremone.

Other isolates examined: CBS 127706 ex-lumber, Vancouver, BC, Canada; CBS 125100=IBT 30068, from dried grapes (sultanas, *Vitis vinifera*), Mildura, Vic, Australia; CBS 125099=IBT 20218 and CBS 125098=IBT 20217, both from acidified lake, Butte, Montana.

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