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RESEARCH ARTICLE

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New penicillin-producing *Penicillium* species and an overview of section *Chrysogena*

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Key words

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Abstract Species classified in *Penicillium* sect. *Chrysogena* are primary soil-borne and the most well-known members are *P. chrysogenum* and *P. nalgiovense*. *Penicillium chrysogenum* has received much attention because of its role in the production on penicillin and as a contaminant of indoor environments and various food and feedstuffs. Another biotechnologically important species is *P. nalgiovense*, which is used as a fungal starter culture for the production of fermented meat products. Previous taxonomic studies often had conflicting species circumscriptions. Here, we present a multigene analysis, combined with phenotypic characters and extrolite data, demonstrating that sect. *Chrysogena* consists of 18 species. Six of these are newly described here (*P. allii-sativi*, *P. desertorum*, *P. goetzii*, *P. halotolerans*, *P. tardochrysogenum*, *P. vanluykii*) and *P. lanoscoeruleum* was found to be an older name for *P. aethiopicum*. Each species produces a unique extrolite profile. The species share phenotypic characters, such as good growth on CYA supplemented with 5 % NaCl, ter- or quarterverticillate branched conidiophores and short, ampulliform phialides (< 9 µm). Conidial colours, production of ascomata and ascospores, shape and ornamentation of conidia and growth rates on other agar media are valuable for species identification. Eight species (*P. allii-sativi*, *P. chrysogenum*, *P. dipodomyis*, *P. flavigenum*, *P. nalgiovense*, *P. rubens*, *P. tardochrysogenum* and *P. vanluykii*) produce penicillin in culture.

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INTRODUCTION

Penicillium sect. *Chrysogena* was introduced by Frisvad & Samson (2004) for species having ter- or quarterverticillate branched conidiophores, relatively short phialides (< 10 µm) and smooth to finely roughened conidia. Four series and eight species (*P. aethiopicum*, *P. chrysogenum*, *P. confertum*, *P. dipodomyis*, *P. flavigenum*, *P. mononematosum*, *P. nalgiovense* and *P. persicinum*) were accepted in this section. Only species lacking a sexual state were included, but a close affinity with *Eupenicillium egyptiacum* was suggested. Recently, single name nomenclature was applied in *Penicillium* and both asexual and sexual reproducing species were included in the redefined genus (Houbraken & Samson 2011). Using a multigene approach, *Penicillium* was divided into 25 sections and sect. *Chrysogena* was expanded to include species with a sexual state (*P. egyptiacum*, *P. kewense*, *P. molle* and *P. sinaicum*), and the recently resurrected species *P. rubens*.

With the exception of *P. chrysogenum*, *P. nalgiovense* and *P. rubens*, the species of sect. *Chrysogena* are primary soil-borne (Frisvad & Samson 2004). *Penicillium chrysogenum* (and *P. rubens*) garner much research interest because of health ramifications that are a consequence of their occurrence in various food products (Pitt & Hocking 2009, Samson et al. 2010) and indoor environments, including damp building materials, indoor air and dust (Chang et al. 1995, Hunter & Lea 1995, Gravesen 1999, Scott et al. 2004, Bekker et al. 2012). Another biotechnologically important species of this section is *P. nalgiovense*, which is used as a fungal starter culture for the production of fermented meat products (Leistner 1990).

Penicillium chrysogenum is best known for the production of the antibiotic penicillin and for this reason its taxonomy has received much attention. Initially, Fleming's penicillin producing strain was identified as *P. rubrum* (Fleming 1929) but because of changing taxonomic schemes, it was often called *P. notatum* (Thom 1945, Raper & Thom 1949), *P. chrysogenum* (Samson et al. 1977), *P. griseoroseum* (Pitt 1980) or *P. rubens* (Houbraken et al. 2011a). When Charles Thom was about to finish his monograph in 1930, he received the Fleming strain (CBS 205.57 = NRRL 824 = IMI 015378), then identified as *P. rubrum*, and re-identified it as *P. notatum* (Thom 1945). In the subsequent monograph of Raper & Thom (1949), series *Chrysogena*, based on Thom's (1930) subsect. *Radiata*, was introduced and four species were accepted: *P. chrysogenum*, *P. cyaneofulvum*, *P. meleagrinum* and *P. notatum*. The name *P. notatum* was maintained for Fleming's strain but the strain still used for the industrial production of penicillin (the 'Wisconsin strain' = NRRL 1951 = CBS 307.48) was identified as *P. chrysogenum* (Raper & Thom 1949). Considerable variation was observed among strains of this series, making it difficult to designate distinct phenotypic differences because of intergrading strains. Therefore, Samson et al. (1977) placed *P. cyaneofulvum*, *P. meleagrinum*, *P. notatum* and six additional species and varieties into synonymy with *P. chrysogenum* and as a result, both Fleming's penicillin producing strains and the Wisconsin strain were classified as *P. chrysogenum*. Although various species similar to *P. chrysogenum* were examined by

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Samson et al. (1977), *P. griseoroseum*, *P. brunneorubrum* and *P. citreoroseum* were not included. These species were considered in the monograph of Pitt (1980), but the latter two species were synonymized with *P. griseoroseum*, Fleming's strain was identified as *P. griseoroseum* and the Wisconsin strain as *P. chrysogenum*. Following Pitt's monograph, various new approaches were applied to the taxonomy of *P. chrysogenum*. Physiological, extrolite and isozyme data suggested that *P. griseoroseum* and related synonyms were conspecific with *P. chrysogenum* (Frisvad & Filtenborg 1989, Banke et al. 1997), in which case the less commonly used name *P. griseoroseum* would have displaced the better known *P. chrysogenum*. To avoid a name change for penicillin producing strains, Kozakiewicz et al. (1992) proposed formal conservation of the name *P. chrysogenum* and rejection of the older *P. griseoroseum*, along with its synonyms *P. citreoroseum* and *P. brunneorubrum*. The proposal was accepted and the name *P. chrysogenum* is currently listed as a *nomen conservandum* (McNeill et al. 2006). More recently, the taxonomy of *P. chrysogenum* was subjected to multigene sequence and microsatellite analysis (Scott et al. 2004, Henk et al. 2011, Houbraken et al. 2011a). Both Scott et al. (2004) and Henk et al. (2011) show the presence of four clades within the species; however, the subdivisions are discordant. The studies agree on the existence of two main clades and based on a polyphasic approach, Houbraken et al. (2011a) named these clades *P. chrysogenum* and *P. rubens*. Interestingly, Fleming's strain and the Wisconsin strain both reside in a clade with *P. rubens* (Houbraken et al. 2011a).

The first aim of the present study was to elucidate the phylogenetic relationships among species belonging to sect. *Chrysogena* using partial RPB1, RPB2 (RNA polymerase II genes), β-tubulin and calmodulin gene sequences. A further objective was to describe the six new species identified as belonging to this section, using a combination of sequence data, phenotypic characteristics and extrolite data, including penicillin production. In addition, an overview of species belonging to sect. *Chrysogena* and their synonyms is presented. The taxonomy of *P. chrysogenum* s.str. has often been controversial and the ultimate goal of this manuscript is to obtain a robust, reproducible and stable species concepts for this economically important species. A network analysis based on eight genes (RPB1, RPB2, calmodulin, β-tubulin, ITS, acetyl-CoA ligase (FacA), phosphoadenosine-5-phosphosulfate reductase (ParA), anthranilate synthase multifunctional protein (TrpC)) is performed in order to get insight in the haplotype diversity among *P. chrysogenum*, *P. rubens* and closely related species.

MATERIAL AND METHODS

Strains

Ex-type and representative strains were obtained from the culture collections of the CBS-KNAW Fungal Biodiversity Centre (CBS), Technical University of Denmark (IBT), USDA-ARS, National Center for Agricultural Utilization Research (NRRL) and the working collection of the department of Applied and Industrial Mycology housed at CBS (DTO). An overview of the strains is given Table 1. More information can be found in the on-line database of CBS at www.cbs.knaw.nl/databases.

DNA extraction, PCR amplification, sequencing and data analysis

Total genomic DNA was extracted using the Ultraclean™ Microbial DNA isolation kit (MoBio, Solana Beach, USA) according to the manufacturer's instructions. To estimate phylogenetic relationships among species of sect. *Chrysogena*, parts of the RPB1 (RNA polymerase II largest subunit; regions E and F, according Matheny et al. 2002), RPB2 (polymerase II second largest subunit; regions 5–7), calmodulin (cmd) and β-tubulin genes (benA) were amplified and sequenced according the methods described previously (Houbraken & Samson 2011, Houbraken et al. 2012). To test the applicability of ITS sequencing for species identification, sequences were generated of the strains listed in Table 1 using primers V9G and LS266 (de Hoog & Gerrits van den Ende 1998).

Each individual dataset was aligned using the Muscle software as implemented in MEGA5 (Tamura et al. 2011). Prior to combining datasets, each individual dataset was analysed using Neighbour Joining (NJ) analysis in MEGA5. The number of bootstrap replicates was set to 1 000 and *P. griseofulvum* CBS 185.27^{NT} was used as outgroup. The combined RPB1, RPB2, benA and cmd dataset was used to study the phylogeny of sect. *Chrysogena*. Statistical support was measured by Bayesian tree inference (BI) analysis using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). To identify the most suitable substitution model for the Bayesian analyses, we used MrModeltest v. 2.3 (Nylander 2004), utilizing the Akaike information criterion (AIC). The Bayesian analysis was performed with two sets of four chains (one cold and three heated) and the STOPRULE option, stopping the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100; the first 25 % of trees were removed as burnin. Statistical support was also measured by Maximum Likelihood (ML) analysis using the RAxML (randomized axelerated maximum likelihood) software (Stamatakis 2008). The phylogram obtained with RAxML was used for presenting the data.

Morphological analysis and extrolite analysis

For macromorphological analysis, strains were inoculated at three points onto Czapek yeast agar (CYA), CYA supplemented with 5 % NaCl (CYAS), yeast extract sucrose agar (YES), malt extract agar (MEA), creatine agar (CREA), dichloran 18 % glycerol agar (DG18) and oatmeal agar (OA). Plates were incubated in the dark for 7 d at 25 °C. In addition, CYA plates were inoculated and incubated for 7 d at 15, 30 and 37 °C in darkness. After incubation, colony diameters were measured and the degree of sporulation, obverse and reverse colony colours and the production of soluble pigments were determined. Colony photographs were taken with a Canon 400D camera under incandescent light. Furthermore, isolates were examined for the production of alkaloids reacting with Ehrlich reagent, using the filter paper method described by Lund (1995). Microscopic observations were made using Olympus BH-2 or Zeiss Axioskop 2 Plus microscopes. Mounts were made in 85 % lactic acid and excess conidia were washed away with a drop of ethanol. Manual measurements were made for at least 20 conidia, ascospores, phialides, metulae, branches and ascomata. Detailed analysis of the ornamentation of the ascospores was performed using scanning electron microscopy (SEM) using the method described by Houbraken et al. (2011b).

For extrolite analyses, cultures were grown on CYA and YES for 7 d at 25 °C. After incubation, five plugs were taken from each agar medium, pooled and extracted according the method described by Smedsgaard (1997). The extracts were subsequently analysed according the HPLC-diode array detection method (Frisvad & Thrane 1987) as modified by Houbraken et al. (2012). Penicillin production was tested according the method described by Andersen & Frisvad (1994).

ITS barcoding

To assess the sequence diversity of the ITS locus of strains belonging to sect. *Chrysogena*, an UPGMA (unweighted pair group method with arithmetic mean) dendrogram based on Kimura 2-parameter distances (K2P, recommended by CBOL, www.barcoding.si.edu) was constructed in MEGA5.

Table 1 *Penicillium* strains used in this study.

Species	CBS no.[1]	Other collection numbers[2]	Substrate, locality and remarks	Haplotype
P. allii-sativi	131541	DTO 148-I4 = IBT 15987	Mixed pig feed; Stora, Zagora, Bulgaria	20
	131544	DTO 148-I8 = IBT 18101 = FRR 2818	Sorghum malt toxic to day-old ducklings; Potchefstroom, South Africa	21
	132071	DTO 149-A5 = IBT 26504 = LJC 384	*Allium sativum* (garlic); Anchoris, Lujan, Mendoza, Argentina	24
	132072	DTO 149-A6 = IBT 26505 = LJC 215	*Allium sativum* (garlic); La Holanda, Lavalle, Mendoza, Argentina	20
	132073	DTO 149-A7 = IBT 26506 = LJC 044	*Allium sativum* (garlic); Pocito, San Juan, Argentina	25
	132074[T]	DTO 149-A8 = IBT 26507 = LJC 206	Ex-type; *Allium sativum* (garlic); Lavalle, Mendoza, Argentina	20
	132075	DTO 149-A9 = IBT 26514 = LJC 481	*Allium sativum* (garlic); La Blanca, Maipu, Mendoza, Argentina	26
	132076	DTO 149-B1 = IBT 26515 = LJC 394	*Allium sativum* (garlic); Vistalba, Lujan, Mendoza, Argentina	27
	132077	DTO 149-B2 = IBT 26516 = LJC 317	*Allium sativum* (garlic); Andrade, Rivadavia, Mendoza, Argentina	25
	132198	DTO 149-B4 = IBT 26518 = LJC 128	*Allium sativum* (garlic); Lavalle, Mendoza, Argentina	28
	132207	DTO 149-F3 = IBT 24377 = EXF 633	Saltern; Secovlje Saltern, Slovenia	31
P. chrysogenum	259.29	DTO 071-G7 = MUCL 28649	Representative of *P. cyaneofulvum*; unrecorded source	4
	282.97	DTO 095-E6 = IBT 15162	Barley; South Africa	40
	289.53	DTO 148-I9 = IBT 19373 = IMI 089373	Gelatin, UK	22
	302.67	DTO 071-H6 = IBT 27042 = ATCC 18476 = IMI 129964	Ex-type of *P. aromaticum* f. *microsporum* nom. inval.; cheese (?); Leningrad region, Russia	12
	306.48[T]	DTO 012-I1 = IBT 5233 = NRRL 807 = IMI 24314	Ex-lectotype; cheese, Storrs, Connecticut, USA	6
	314.48	DTO 071-G8 = ATCC 10431 = IMI 039764 = MUCL 28658 = MUCL 29077 = MUCL 29143 = NRRL 837	Ex-type of *P. cyaneofulvum*; unrecorded source	4
	355.48	DTO 098-D4 = ATCC 10108 = IMI 039759 = IMI 039759ii = NRRL 821	Ex-type of *P. notatum*; decaying branches of *Hyssopus*, Norway	42
	412.69	DTO 071-H9 = IBT 30174 = IBT 23022 = IMI 140340	Ex-type of *P. harmonense*; soil; Syria	37
	776.95	DTO 095-F4 = IBT 14462	Lechuguilla cave; Carlsbad, New Mexico; USA	
	111215	DTO 071-I8 = IBT 21928	Mouldy leaves of *Salvia officinalis* (sage) plant; Farum, Denmark	11
	116046	DTO 001-C2 = IBT 30183	Water used in production process of cardboard; the Netherlands	13
	131516	DTO 064-E8 = IBT 29739 = IBT 30133	Air in cleanroom of vaccine production plant; the Netherlands	33
	131517	DTO 068-C3 = IBT 30182	Indoor environment; Denmark	34
	131518	DTO 068-C4 = IBT 30176	Indoor environment; Finland	35
	131519	DTO 068-C5 = IBT 30175	Indoor environment; Finland	36
	131520	DTO 078-E5 = IBT 29738	Indoor environment clean room; the Netherlands	2
	131521	DTO 087-I2	Swab sample from ceiling in archive; Utrecht, the Netherlands	11
	131522	DTO 091-D4	Indoor environment of pharmaceutical company; the Netherlands	2
	131524	DTO 098-E6 = IBT 30140 = NRRL 841	Ex-type of *P. brunneo-rubrum*; unrecorded source	12
	131525	DTO 098-E7 = IBT 30146 = NRRL 834	Ex-type of *P. citreoroseum*; unrecorded source	4
	131526	DTO 098-E9 = IBT 30136 = NRRL 889	Ex-type of *P. roseocitreum*; unrecorded source	4
	131527	DTO 098-F1 = IBT 30147 = NRRL 817	Ex-type of *P. chlorophaeum*; unrecorded source	12
	131529	DTO 100-G4 = IBT 30148 = NRRL 819	Distributed as *P. fluorescens* nom. inval.; unrecorded substrate; Czech Republic	4
	131530	DTO 100-G6 = IBT 30150 = NRRL 822	Sputum of a woman with a lung disease; unknown locality	6
	131531	DTO 100-G8 = IBT 30144 = NRRL 827	Unrecorded source. Capable of volatilizing potassium telluride	6
	131532	DTO 100-H3 = IBT 30138 = NRRL 2136	Representative of *P. meleagrinum* (Thom, 1930; Raper & Thom, 1949: 366); unrecorded source	8
	131533	DTO 102-B4 = IBT 26889 = C238	House dust; Wallaceburg, ON, Canada. Representative of group 2 in the study of Scott *et al.* (2004)	11
	131534	DTO 102-B5 = IBT 26890 = C71.1	House dust; Wallaceburg, ON, Canada. Representative of group 3 in the study of Scott *et al.* (2004)	12
	131535	DTO 102-B7 = IBT 26892 = C200	House dust; Wallaceburg, ON, Canada. Representative of group 3 in the study of Scott *et al.* (2004)	14
	131536	DTO 103-E7 = IBT 30084	Unknown substrate; Dry Valley, Antarctica	15
	131538	DTO 148-I1 = IBT 6041	Dust; China	17
	131545	DTO 149-A1 = IBT 22435	Bread; Italy	4
	132068	DTO 149-A2 = IBT 22435	Bread; Italy	4
	132199	DTO 149-B5 = IBT 29402	Damaged oil painting; Kharkov, Ukraine	4
	132201	DTO 149-C1 = IBT 30085	Soil; Dry Valley, Antarctica	15
	132202	DTO 149-C2 = IBT 30086	Soil; Dry Valley, Antarctica	15
	132203	DTO 149-C3 = IBT 30087	Soil; Dry Valley, Antarctica	15
	132205	DTO 149-C5 = IBT 30737	Bee; USA	11
	132208	DTO 100-H2 = IBT 30139 = NRRL 842	Representative of *P. brunneorubrum*; unrecorded source	7
	132209	DTO 100-G5 = IBT 30143 = NRRL 820	Ex-lectotype of *P. griseoroseum*; unrecorded source	5
	132211	DTO 100-F7 = DTO 086-I4 = IBT 30177	Surface of operating room; the Netherlands	
	132212	DTO 102-B9 = IBT 27840	Indoor environment; Wallaceburg, Ontario, Canada	13

	132213	DTO 102-B2 = IBT 26887 = C317.2	Indoor environment; Wallaceburg, Ontario, Canada; representative of group 3 in the study of Scott et al. (2004)	9
	132214	DTO 102-B6 = IBT 26891 = C77.2	Indoor environment; Wallaceburg, Ontario, Canada; representative of group 3 in the study of Scott et al. (2004)	13
	132215	DTO 013-E6 = IBT 30181	Flour for production of tortillas; USA	16
	132216	DTO 068-B8 = IBT 30179	Industrial environment; Germany	8
	132217	DTO 102-B3 = IBT 26888 = C8.18	Indoor environment; Wallaceburg, Ontario, Canada; Scott et al. (2004)	10
		DTO 100-F7 = DTO 086-I4 = IBT 30177	Unrecorded source	2
		DTO 100-H1 = IBT 30149 = NRRL 839	Representative of *P. cyaneofulvum* (Raper & Thom 1949: 372); unrecorded source	4
		DTO 100-G9 = IBT 30141 = NRRL 837	Ex-type of *P. cyaneofulvum*; unrecorded source	4
			Contaminant in *Postia placenta* MAD 698R culture. No strain available, full genome sequenced	13
P. confertum	171.87^{T}	DTO 072-A9 = IBT 21515 = IBT 3098 = IBT 5672 = IMI 296930 = NRRL 13488 = NRRL A-26904	Ex-type; cheek pouch; Arizona, USA	
P. desertorum	129469	IBT 20395	A1 horizon soil; Utah, USA	
	130050	IBT 14084 = IMI 297544	Shrub land soil; Wyoming, USA	
	131229	IBT 14452	A1 horizon grass land soil; Wyoming, USA	
	131514	DTO 015-H9	Soil; Chubut, Argentina	
	131515	DTO 016-B5	Soil; Chubut, Argentina	
	131542	DTO 148-I5 = IBT 16313	Soil under *Artemisia tridentata*, cool desert; 16 km north of Rawlins, Wyoming, USA	
	131543^{T}	DTO 148-I6 = IBT 16321	Ex-type; soil under *Oryzopsis hymenoides*, cool desert; 20 km east of Little America, Wyoming, USA	
P. dipodomyis	170.87	DTO 217-B4 = IBT 21522	Cheek pouch; Arizona	
	110412^{T}	DTO 072-B6 = IBT 5333 = IMI 296926 = NRRL 13485 = NRRL A-26136	Ex-type; cheek pouch of kangaroo rat; Arizona, USA	
	110413	DTO 217-B5 = IBT 17759	Barley; Starr Valley, Wyoming, USA	
	110414	DTO 217-B6 = IBT 12700	Kangaroo rat; Socorro County, Seviletta Natl. Wildlife Refuge, New Mexico, USA	
	110415	DTO 217-B7 = IBT 11425	Saddle, mouldy leather, leather probably from Saudi Arabia	
	112570	DTO 217-B8 = IBT 3353	Soil; Walnut Crater, Arizona, USA	
P. egyptiacum	137.70	DTO 092-B7 = IBT 14685	Unknown source; Izmir, Bornova, Turkey	
	244.32^{NT}	DTO 088-F6 = IBT 14684 = ATCC 10441 = IMI 040580 = NRRL 2090	Neotype of *P. egyptiacum*; holotype of *P. nilense*; soil; Cairo, Egypt	
	457.72	DTO 088-G5 = NRRL 22307 = IBT 30195	Desert soil; Egypt	
	458.72	IBT 14687	Desert soil; Egypt	
	867.70	DTO 088-G2 = IBT 14686	Root; Israel	
	456.72	DTO 088-G4 = ATCC 24075 = IMI 084589 = IBT 14682	Ex-type of *E. molle* and *P. molle*; soil; Pakistan	
P. flavigenum	419.89^{T}	DTO 072-B4 = IBT 21526 = IBT 3091 = IMI 293207	Ex-type, wheat flour; Denmark	
	110406	IBT 16616	Soil under *Chrysothmnus nauseosus*; Table rock road/highway 80, Wyoming, USA	
	110407	DTO 217-C5 = IBT 14060	White beans; USA	
	110409	DTO 217-C6 = IBT 3230	Sand; Tunisia	
	110411	DTO 217-C7 = IBT 11693	Barley; Canada	
	132247	DTO 149-C7 = IBT 30948	Painting on canvas (lining); Provost church, Ljubljana, Slovenia	
P. goetzii	285.73^{T}	DTO 088-G6 = IBT 30199	Ex-type; soil; Calgary, Alberta	
	581.67	DTO 088-F8 = NRRL 3556 = IBT 4980 = IBT 4993	Soil; Lahore, Pakistan	
	635.70	DTO 088-F9 = IBT 30200	Soil; USA	
	812.70	DTO 088-G1 = IBT 30196	Culture contaminant, in *Spiromastix warcupii* CBS 576.63	
		DTO 055-H1 = IBT 30198	Endophyte from roots of *Pinus ponderosa* (ponderosa pine) and *Pseudotsuga menziesii* (Douglas-fir); Mission Creek watershed, Okanogan-Wenatchee National Forest, north-central Washington state, USA	
		DTO 055-H2	Endophyte from roots of *Pinus ponderosa* (ponderosa pine) and *Pseudotsuga menziesii* (Douglas-fir); Mission Creek watershed, Okanogan-Wenatchee National Forest, north-central Washington state, USA	
		DTO 055-H3	Endophyte from roots of *Pinus ponderosa* (ponderosa pine) and *Pseudotsuga menziesii* (Douglas-fir); Mission Creek watershed, Okanogan-Wenatchee National Forest, north-central Washington state, USA	
P. griseofulvum	185.27^{NT}	DTO 072-A5 = IBT 6740 = ATCC 11885 = IMI 075832 = IMI 075832ii = NRRL 2152 = NRRL 2300	Ex-neotype; unrecorded substrate; Belgium	
P. halotolerans	131537^{T}	DTO 148-H9 = IBT 4315	Ex-type; salt marsh; Egypt	
P. kewense	183.72	DTO 092-B8 = IBT 14680	Soil; the Netherlands	
	344.61^{IsoT}	DTO 088-F7 = ATCC 18240 = IMI 086561 = NRRL 3332 = IBT 24547	Isotype; culture contaminant of mineral oil CMI 1959; Surrey, Kew, England	
P. lanosocoeruleum	215.30^{T}	DTO 035-H4 = IBT 3545 = ATCC 10459 = CBS 334.48 = IMI 039818 = NRRL 888	Ex-type; culture contaminant of *P. cyclopium* culture; USA	
	484.84	DTO 072-A8 = IBT 21501 = IBT 5903 = IMI 285524	Ex-type of *P. aethiopicum; Hordeum vulgare* (barley); Addis Abeba, Ethiopia	

Table 1 (cont.)

Species	CBS no.[1]	Other collection numbers[2]	Substrate, locality and remarks	Haplotype
P. mononematosum	172.87^{T}	DTO 072-B2 = IBT 21535 = IMI 296925 = NRRL 13482	Ex-type; burrow system of *Dipodomys spectabilis* (banner-tailed kangaroo rat); Arizona, USA	
	109616	DTO 217-B9 = IBT 4309 = IBT 4310 = IBT 5509	Salt marsh soil; Egypt	
	112104	DTO 217-C1 = IBT 3073 = IBT 5521 = IBT 5522 = IBT 6071 = NRRL A-26910 = NRRL 13483	Kangaroo rat; 8 km east of Portal, Arizona, USA	
	112105	DTO 217-C2 = IBT 11891	Squash; France	
	112106	DTO 217-C3 = IBT 11682	Jerusalem artichoke; Denmark	
	112575	DTO 217-C4 = IBT 4308 = IBT 4391 = IBT 5507	Marsh soil; Egypt	
P. nalgiovense	318.92	IBT 12383	Sausage, imported from Italy; Denmark	
	352.48^{NT}	DTO 072-A6 = IBT 21536 = ATCC 10472 = IMI 039804 = NRRL 911	Neotype; Ellischauer cheese; Czech Republic	
	109610	DTO 217-C9 = IBT 11965 = FRR 3284	Salami; Germany	
	112438	DTO 217-D1 = IBT 23346	Ice; Svalbard, Norway	
P. persicinum	111235^{T}	DTO 072-B8 = IBT 24565	Ex-type; soil; Qinghai Province, China	
P. rubens	197.46	DTO 065-B3	Must contaminant, Belgium. The strain first used for producing penicillin in submerged culture (Raper & Thom 1949: 368–370)	1
	205.57	DTO 065-B1 = IBT 30143 = IMI 015378	Culture contaminant in bacterial culture, UK. Fleming's original penicillin producing strain	1
	307.48	DTO 065-B2 = IBT 5857 = NRRL 1951 = IMI 40233	Mouldy cantaloupe Peoria, Illinois, USA. 'Wisconsin strain', parent of most high yielding penicillin producing strains; full genome sequenced	1
	319.59	DTO 098-D2 = ATCC 18226 = IMI 068231	Ex-type of *P. chrysogenum* mut. fulvescens; soil, Japan; cinnamon-coloured conidia	39
	339.52	DTO 071-H2 = IBT 30130 = ATCC 22349 = IMI 041606 = IMI 041606ii	Ex-type of *P. camerunense* nom. inval.; root of *Elaeis guineensis*, together with *Chalara paradoxa*	41
	349.48	DTO 098-G1 = IBT 4350 = ATCC 10468 = IMI 039762 = NRRL 836	Unrecorded substrate; Scotland. Representative of *P. meleagrinum* (Thom 1930, Raper & Thom 1949: 366)	1
	401.92	DTO 001-C6	Gypsum, building materials; the Netherlands (used as model organism; e.g. Bekker et al. 2012)	19
	478.84	DTO 071-I2 = IBT 21511	Air in fruit store; Denmark	19
	111216	DTO 071-I9 = IBT 22809	Saltern; Slovenia	19
	129667^{T}	DTO 098-E8 = IBT 30129 = NRRL 792 = ATCC 9783	Ex-lectotype; unrecorded source	1
	131513	DTO 015-F3 = IBT 30659	Tattoo paint; the Netherlands	32
	131523	DTO 095-E9 = IBT 30661	Cap of PET bear bottle; Kaulile, Belgium	38
	131528	DTO 100-G3 = IBT 30145 = NRRL 812	Solution containing 4 percent iron-alum; USA	3
	131540	DTO 148-I3 = IBT 14508	Lechuguilla Cave; Carlsbad, New Mexico, USA	19
	132069	DTO 149-A3 = IBT 22703	Soil under *Larix*; 3 km west of Uthoss, Russia	1
	132204	DTO 149-C4 = IBT 30427	Unrecorded substrate; Germany	19
	132206	DTO 149-C6 = IBT 30738	Bee; USA	30
	132210	DTO 100-F6 = NRRL 843 = IBT 5303	Unrecorded source; approximated *P. baculatum* (Raper & Thom 1949: 363)	1
		DTO 100-G7 = NRRL 824 = IBT 30142	Culture contaminant in bacterial culture, UK; Fleming's original penicillin producing strain	1
		DAOM 234047	Indoor air; Saskatchewan, Canada	
		DAOM 234052	Pipe wrap in a house; Ontario, Canada	
		DAOM 234054	House dust; Alberta, Canada	
P. sinaicum	279.82^{T}	DTO 097-D3	Ex-type; marine sludge; Suez Canal, 30 km N of Port Said, Sinai Peninsula, Egypt	
P. tardochrysogenum	132200^{T}	DTO 149-B9 = IBT 30075	Ex-type; soil; McMurdo Dry Valley, Antarctica	29
P. vanluykii	131539^{T}	DTO 148-I2 = IBT 14505	Ex-type; Lechuguilla Cave; Carlsbad, New Mexico, USA	18
	132070	DTO 149-A4 = IBT 23469	Soil; Bose Jubony, Isla 25 da Mugo, Shetland del Sur, Antarctica	23
	132197	DTO 149-B3 = IBT 26517 = LJC 005	Garlic; Villa Aberastain, Pocito, San Juan, Argentina	18
Penicillium sp. (near *P. kewense*)	103.71		Soil of wheat field; Kiel, Germany	
	227.81	DTO 103-D7 = CBS 653.82 = NRRL 2094	Unknown source. Intermediate between *P. brefeldianum* and members of the *Carpenteles* series such as *P. egyptiacum* (Raper & Thom 1949: 146)	
	653.82	DTO 088-G7 = CBS 227.81 = NRRL 2094	Unknown source. Intermediate between *P. brefeldianum* and members of the *Carpenteles* series such as *P. egyptiacum* (Raper & Thom 1949: 146)	

[1] CBS: culture collection of the CBS-Fungal Biodiversity Centre, Utrecht, The Netherlands.

[2] ATCC: American Type Culture Collection, Manassas, VA, USA; DAOM: Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; DTO: internal culture collection of CBS-Fungal Biodiversity Centre; IBT: culture collection of Center for Microbial Biotechnology (CMB) at Department of Systems Biology, Technical University of Denmark; IHEM: culture collection of the Scientific Institute of Public Health – Mycology section, Brussels, Belgium; IMI: CABI Genetic Resources Collection, Surrey, UK; LJC: Coleccion de fitopatogenos de cultivos horticolas, Mendoza, Argentina; NRRL: Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois, USA.

Haplotype diversity

In order to study the haplotype diversity among *P. chrysogenum*, *P. rubens* and closely related species, the RPB1, RPB2, calmodulin and β-tubulin sequence datasets were expanded with ITS, FacA (acetyl-CoA ligase; (facA-F_Pc (TGGAAGTGGTACTTCGAG), facA-R_Pc (ACACGACCGCGGATCCAGTA))), ParA (3-phosphoadenosine-5-phosphosulfate reductase; (parA-F_Pc (CCCGAGATTGTTTTCACCAA), parA-R_Pc (ACCTTGGCCACCCAGTCGTA))) and TrpC (anthranilate synthase multifunctional protein; (trpC-F_Pc (GCAGTGGAGGGTGTTCAGTT), trpC-R_Pc (TTAACCTCGACCAGAGGCTCCAT))) gene sequences. These datasets were supplemented with sequences obtained from the two full genome initiatives (van den Berg et al. 2008, http://genome.jgi.doe.gov/). The software programme DnaSP v. 5.10 (Librado & Rozas 2009) was used to find the different haplotypes in the alignment. Gaps and missing data were not considered during this calculation. Network v. 4.6.1.0 (www.fluxus-engineering.com) was used to generate a haplotype network using the median-joining network algorithm. Sequences were deposited in the GenBank nucleotide database under accession numbers JX996198–JX997117.

RESULTS

Phylogeny

The phylogenetic relationship among members of sect. *Chrysogena* was studied by combining the RPB1, RPB2, cmd and

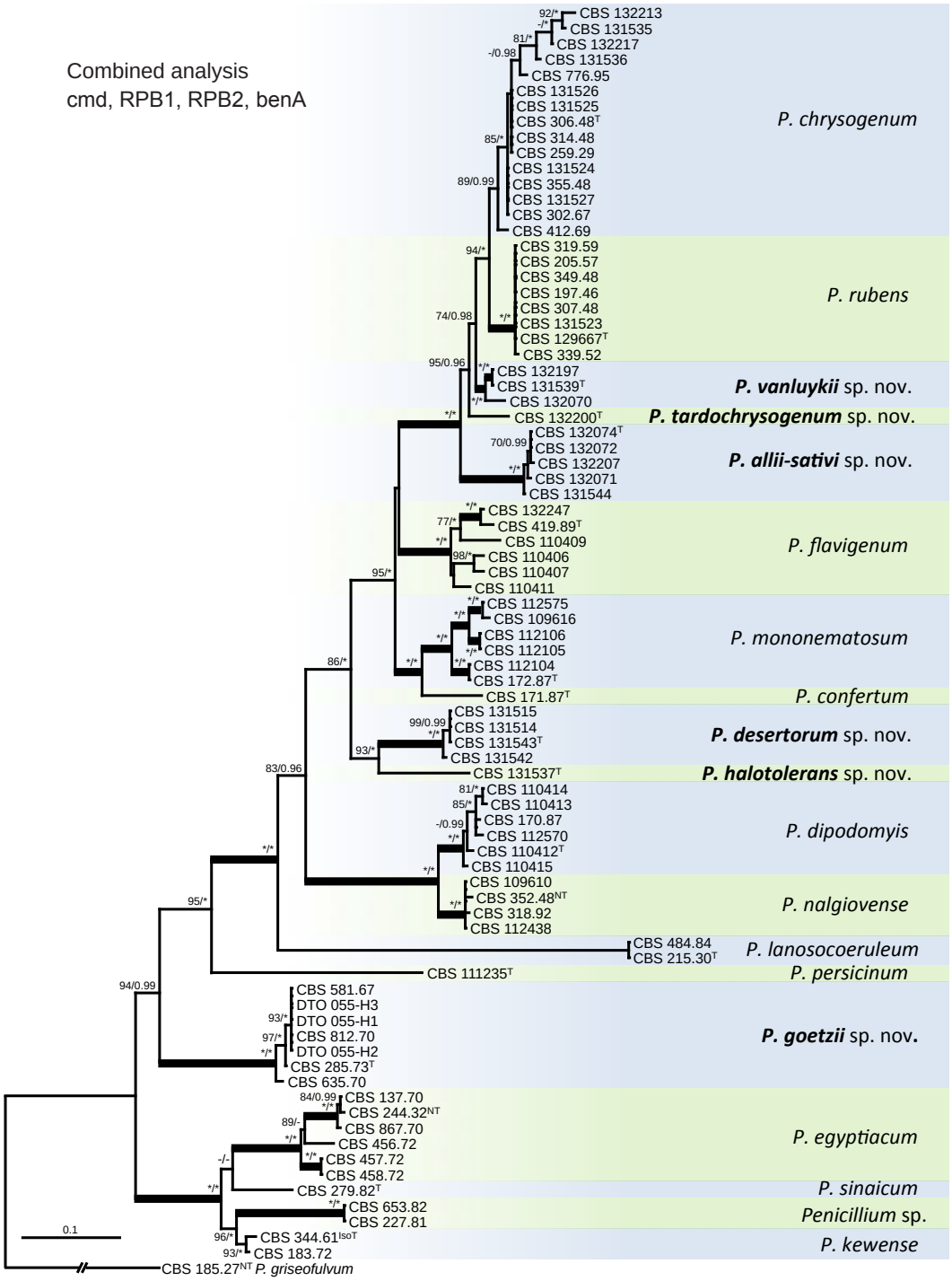

Fig. 1 Best-scoring Maximum Likelihood (ML) tree using RAxML based on a combination of partial calmodulin, β-tubulin, RPB1 and RPB2 sequences, showing the relationship among members of *Penicillium* section *Chrysogena*. The bootstrap (bs) values of the ML analysis and the BI posterior probabilities (pp) values are presented at the nodes (bs/pp). Values less than 70 % supported in the ML analysis or less than 0.95 in the BI analysis are omitted, whereas asterisks indicate full support (100 % bs, 1.00 pp). The branches with more than 95 % bootstrap support and 1.00 pp values are thickened. The phylogram is rooted with *Penicillium griseofulvum* CBS 185.27^{NT}.

benA datasets. Prior to this analysis, NJ analysis was performed on each individual dataset to determine incongruences. The individual RPB1, RPB2, cmd and benA datasets were 722, 958, 505 and 446 bp positions long, respectively. The optimal model was determined using MrModeltest and the SYM+G model was optimal for the calmodulin and RPB2 dataset, the model SYM+I+G for the RPB1 and HKY+I+G model for the BenA partition.

Eighteen lineages were observed among isolates assigned to sect. *Chrysogena* (Fig. 1) (Houbraken & Samson 2011) and six represent new species, named here as *P. allii-sativi*, *P. desertorum*, *P. goetzii*, *P. halotolerans*, *P. tardochrysogenum* and *P. vanluykii*. *Penicillium allii-sativi*, *P. chrysogenum*, *P. rubens*, *P. tardochrysogenum* and *P. vanluykii* together formed a well-supported clade in each analysis of the individual genes (> 87 % bootstrap support), except for calmodulin (Fig. 2). The clustering within this clade was generally poorly supported and varied among the examined datasets; however, analysis of the combined dataset generated highly supported clades (Fig. 1). Analysis of the combined dataset shows that *P. allii-sativi* is basal to the other species of this clade, and that *P. chrysogenum* and *P. rubens* are sister species with *P. vanluykii* basal to them. The position of isolates CBS 412.69 (ex-type of *P. harmonense*), DTO 102-B2 and DTO 102-B7 were in conflict between the cmd dataset and the phylogram based on combined nucleotide data. This set of isolates resides in the *P. rubens* clade in the cmd dataset with statistical support (bootstrap value 84 %), while they were positioned in the *P. chrysogenum* clade in the combined phylogram.

All *P. dipodomyis*, *P. flavigenum* and *P. nalgiovense* isolates formed distinct well-supported clades in all datasets, and all *P. desertorum* strains clustered in a well-supported clade except in the β-tubulin gene tree. *Penicillium dipodomyis* and *P. nalgiovense* are sister species in all individual datasets and some variation was observed within the *P. dipodomyis* clade; however, this pattern was incongruent among datasets. Fig. 1 (combined analysis) shows that *P. confertum* is basal to *P. mononematosum*, but it renders the *P. mononematosum* clade paraphyletic in the β-tubulin dataset. Three subclades are consistently formed in *P. mononematosum* phylograms. One clade includes two strains from a salt marsh in Egypt (CBS 109616, CBS 112575) while CBS 112105 and CBS 112106 also form a separate clade, as do CBS 172.87^{T} and CBS 112104. The ex-type of *P. lanoscoeruleum* CBS 215.30^{T} and *P. aethiopicum* CBS 484.84^{T} resolve in a single clade and priority is given to the former (oldest) name.

Four species are known to form ascospores and three of these, *P. egyptiacum*, *P. kewense* and *P. sinaicum*, group in a clade with full support (100 % bootstrap support, 1.00 pp). Three lineages were observed within the *P. egyptiacum* clade. One lineage was centred on the neotype of *P. egyptiacum* (CBS 244.32^{NT}), another on CBS 457.72 and CBS 458.72 and the third consisted of CBS 456.72^{T}, the ex-type strain of both *Penicillium molle* and *Eupenicillium molle*. These lineages were present in all

Table 2 Overview of extrolites produced by species belonging to *Penicillium* section *Chrysogena*.

Species	Extrolites
P. allii-sativi	1) atlantinone A; 2) chrysogenamide; 3) 2-(4-hydroxyphenyl)-2-oxo acetaldehyde oxim; 4) a naptho-γ-pyrone; 5) penicillins; 6) 2-pyruvoylaminobenzamide; 7) roquefortine C, D & meleagrin; 8) verrucosidin, normethylverrucosidin, deoxyverrucosidin & verrucosidinol; 9) 'ALKONA'; 10) 'AURCH'; 11) 'CRYPT'; 12) 'DERH', 'GULLA' & 'KUTZ' (atromentins?); 13) 'OTOF'; 14) 'SENGAX'; 15) 'SNORL'; 16) 'SPOFI'; 17) 'VERNX'
P. chrysogenum	1) andrastrin A & B; 2) chrysogine, 2-pyruvoylaminobenzamide, 2-acetyl-quinazolin-4(3H)-one & 2-(2-hydroxypropionylamino)-benzamide; 3) citreoisocoumarin; 4) penicillins; 5) roquefortine C, D & meleagrin; 6) secalonic acid D & F; 7) sorbicillins; 8) xanthocillins; 9) 'met Ø'; 10) 'DOLDO'
P. confertum	1) asteltoxin; 2) roquefortine C, D & meleagrin; 3) secalonic acid D
P. desertorum	1) austalides?; 2) 2-(4-hydroxyphenyl)-2-oxo acetaldehyde oxim; 3) Raistrick phenols; 4) 'FOL'
P. dipodomyis	1) diaporthins (citreoisocoumarin, diaportinic acid, diaportinol, dichlorodiaporthin & 6-methyl-citreoisocoumarin); 2) dipodazin; 3) penicillins; 4) 'CD' 1-5 & 'CRYPT'; 5) 'CDU'; 6) 'DI' (an indol-alkaloid); 7) 'DIOR'; 8) 'DIPA'; 9) 'FCD'; 10) 'GNALDI'; 11) 'met Ø'; 12) 'TOLO'; 13) 'VIK'
P. egyptiacum (= incl. *E. molle*)	1) 10,23-dihydro-24,25-dehydroaflavinine (Wang et al. 1995, also seen in this study); 2) macrophorin H (Wang et al. 1995); 3) mollenines A and B (Wang et al. 1998); 4) penicillic acid; 5) Raistrick phenols; 6) secalonic acid D & F; 7) tetronic acids; 8) xanthocillin X (Vesonder 1979, NRRL 1022, not seen in this study)
P. flavigenum	1) penicillins; 2) penitrem A; 3) roquefortine C & meleagrin; 4) sorbicillins
P. goetzii	1) andrastin A; 2) citreoisocoumarin; 3) fumitremorgin A, verruculogen; 4) isoepoxydon; 5) 10,23-dihydro-24,25-dehydroaflavinine & 10,23,24,25-tetrahydro-24-hydroxyaflavinine; 6) 'GLAD'
P. halotolerans	1) andrastin A; 2) Raistrick phenols, roquefortine C, D and meleagrin; 3) 'CUCU' and other polar polyketides; 4) 'PLIL'
P. kewense	1) andrastin A; 2) fumitremorgin A & verruculogen; 3) 10,23-dihydro-24,25-dehydroaflavinine & 10,23,24,25-tetrahydro-24-hydroxyaflavinine; 4) isoepoxydon; 5) 4'-oxomacrophorin A & D; 6) roquefortine C; 7) 'KEWS' 1-3
P. cf. *kewense*	1) andrastin A; 2) isoepoxydon; 3) 10,23-dihydro-24,25-dehydroaflavinine
P. lanosocoeruleum (=*P. aethiopicum*)	1) griseofulvins (dechlorogriseofulvin, dehydrogriseofulvin griseofulvin, griseophenone C etc.), de; 2) isoepoxydon; 3) tryptoquialanins & tryptoquialanons; 4) viridicatumtoxin; 5) 'BR'; 6) 'met U'; 7) 'PRU'; 8) 'RAIS'; 9) 'SNOK'; 10) 'VERNX'
P. mononematosum	1) andrastin A & B; 2) citreoisocoumarin; 3) cyclopaldic acid & derived chromanols; 4) fumitremorgin A, B, C, TR-2 & verruculogen; 5) isochromantoxins; 6) viriditoxin; 7) 'ASTYL'; 8) 'GULLA'; 9) 'MER'; 10) 'MONTI'; 11) 'PJIM'; 12) 'PLOT'; 13) 'OKA' 1 & 2 (okaramins?); 14) 'PAEL'; 15) 'PYTO'; 16) 'SNAT'; 17) 'TRYP' (= dehydrocurvularin?); 18) 'VERNX'
P. nalgiovense	1) chrysogine, 2-pyruvoylaminobenzamide, 2-acetyl-quinazolin-4(3H)-one & 2-(2-hydroxypropionylamino)-benzamide; 2) citreoisocoumarin; 3) diaporthins (citreoisocoumarin, diaportinic acid, diaportinol, dichlorodiaporthin & 6-methyl-citreoisocoumarin); 4) dipodazin; 5) nalgiovensin, nalgiolaxin and bisanthron-derivatives of those; 6) penicillins
P. persicinum	1) andrastin A & B; 2) chrysogine, 2-pyrovoylaminobenzamide, 2-acetyl-quinazolin-4(3H)-one & 2-(2-hydroxypropionylamino)-benzamide; 3) griseofulvins; 4) roquefortine C & D; 5) 'AURIN'; 6) 'DOLDO'; 7) 'MURA'; 8) 'XYLA'
P. rubens	1) andrastin A & B; 2) chrysogine, 2-pyruvoylaminobenzamide, 2-acetyl-quinazolin-4(3H)-one & 2-(2-hydroxypropionylamino)-benzamide; 3) citreoisocoumarin; 4) 7-deacetoxyyanuthone; 5) penicillins; 6) roquefortine C, D & meleagrin; 7) sorbicillins (including bisorbibutenolide, bisorbicillinol, bisvertinoquinol, bisvertinolone,2',3'-dihydrosorbicillin, oxosorbicillinol tautomer, sorhinones A, B, & C, rezishanones A, B, C & D, sorbicillin); 8) xanthocillins; 9) PR-toxin; 10) quinazolone X (based on UV spectrum, not yet structure elucidated); 11) 'DOLDO'
P. sinaicum	1) 10,23-dihydro-24,25-dehydroaflavinine; 2) isoepoxydon or similar compound; 3) ML-236A; 4) pseurotin A; 5) indolalkaloids; 6) HO6; 7) 'FOPT'; 8) 'FORN' 1, 2 & 3
P. tardochrysogenum	1) asperentins; 2) penicillins; 3) secalonic acid D & F; 4) 'met Ø'
P. vanluykii	1) andrastin A; 2) chrysogine; 3) penicillins; 4) roquefortine C, D and meleagrin, and the uncharacterized extrolites 'CRYPT' (4 compounds), 'POO', 'KNOLF', 'TBRE', 'FJOR' (2 compounds).

analysed individual datasets. The combined analysis placed *P. goetzii* basal to the asexual *Penicillium* species; however, this is not the case for individual datasets. In the RPB1 dataset, this species was grouped together with other ascospore producing species (89 % bootstrap support).

Morphology, physiology and extrolites

Penicillium chrysogenum, *P. rubens*, *P. tardochrysogenum*, *P. vanluykii* and *P. allii-sativi* are phenotypically similar and share characters such as a fast growth rate on YES with dense sporulation (except *P. tardochrysogenum*), a CYAS : CYA ratio greater than 1, ter- or quarterverticillate divergently branched conidiophores, and relatively short phialides (< 9 µm). Penicillin is produced by all species and roquefortine C, D and meleagrin by all except *P. tardochrysogenum*. There are also differences among the species of this section. *Penicillium vanluykii* produces dark green conidia on MEA and CYA, yellow soluble pigments on CYA incubated at 30 °C and a series of characteristic unidentified extrolites. In common with *P. vanluykii*, *P. allii-sativi* also produces conidia in shades of dark green on CYA; however, there is no yellow soluble pigment production on CYA incubated at 30 °C or in insignificant amounts. This species also produces a diagnostic array of extrolites (Table 2) including the potent mycotoxin verrucosidin. *Penicillium tardochrysogenum* is represented by one strain (CBS 132200^{T}). It is unique in this clade for its more restricted and floccose colonies on MEA, a lack of sporulation on YES and the production of finely roughened conidia. This species does not produce yellow soluble pigments on CYA when incubated at 30 °C and produces the asperentins, a series of compounds not produced by other members of series *Chrysogena*.

Phylogenetic analyses show that *P. halotolerans* and *P. desertorum* are sister species (Fig. 1) and phenotypic characters support their classification in sect. *Chrysogena* (CYAS : CYA ratio > 1; velvety colonies and production of short, ampulliform phialides). *Penicillium halotolerans* can be differentiated from *P. desertorum* by the production of yellow soluble pigments on CYA incubated at 30 °C. Furthermore, the conidiophores of *P. desertorum* have various short, divaricate branches at various levels along the stipe, while *P. halotolerans* has ter- or quarterverticillate branched conidiophores like other species of sect. *Chrysogena*. Strains of *P. desertorum* consistently produce species-specific profiles of extrolites (Table 2). Some of these extrolites are partially characterised and details on retention time, retention index and UV maxima (nm) are given in Table 3. *Penicillium halotolerans* is only known from its ex-type strain (CBS 131537^{T}) and this isolate produces a unique combination of extrolites, namely andrastin A, roquefortine C & D, meleagrin and Raistrick phenols.

Four of the 18 species (*P. egyptiacum*, *P. goetzii*, *P. kewense* and *P. sinaicum*) are capable of forming a sexual state. These species are characterised by the production of creamish, avellaneous or ochraceous ascomata, ter- to quarterverticillate branched conidiophores and globose to subglobose conidia. Isolates grown on CYA for 7 d at 25 °C typically produce brown or red-brown soluble pigments. However, they differ from each other by various characters. *Penicillium egyptiacum* is a good acid producer on CREA, while the other species do not or produce limited amounts of acidic compounds. These species also differ in ascospore size and ornamentation (Fig. 3). The ascospores of *P. egyptiacum* measure 2–3 × 2.5–3.5 µm, but vary in their ornamentation. CBS 244.32^{NT} and CBS 137.70 have inconspicuous ridges and smooth-walled valves, while ascospores of CBS 457.72 have closely separated equatorial ridges, with prominent secondary ridges and roughened valves. In contrast, the ascospores of *P. goetzii* are larger, 3–4.5 × 2.5–4 µm, with two distinct equatorial ridges and often two secondary ridges that are connected by transverse ribs and valves ornamented with a reticulate pattern. The ascospores of *P. kewense* take an intermediate position between those of *P. egyptiacum* and *P. goetzii*, and *P. sinaicum* is unique in having ascospores without a distinct equatorial ridge and reticulate valves (Fig. 3). *Penicillium egyptiacum*, *P. goetzii*, *P. kewense* and *P. sinaicum* also produce species-specific patterns of extrolites. Penicillic acid, Raistrick phenols and secalonic acids D & F are produced by *P. egyptiacum* but not by the other ascospore producing species. On the other hand, andrastin A, fumitremorgin A and verruculogen are produced by *P. goetzii* and *P. kewense*, and the uncharacterised compound 'GLAD' is only produced by *P. goetzii*.

ITS barcoding

ITS sequences were generated to assess the suitability of this locus for species identification in sect. *Chrysogena* and 44 % of the species can unequivocally be identified with this locus. *Penicillium confertum*, *P. goetzii*, *P. halotolerans*, *P. lanoscoeruleum*, *P. mononematosum*, *P. nalgiovense* and *P. percisinum* can be reliably identified by ITS sequencing. Five ITS sequence variants are present in our revised concept of *P. chrysogenum*. A total of 61 % of the *P. chrysogenum* strains have identical ITS sequences and this sequence is also present in *P. tardochrysogenum* (100 %) and *P. allii-sativi* (100 %). A different *P. chrysogenum* sequence was observed in 15 % of the examined strains (e.g. CBS 776.95, CBS 131522, CBS 132211). This sequence is shared with *P. rubens*, all strains of which have identical ITS sequences. The three other unique *P. chrysogenum* sequences were represented by CBS 131538 (2 %), CBS 131516 (10 %) and CBS 111215 (12 %). The three investigated *P. vanluykii* isolates have a ITS sequence that is shared with NRRL 3710, a strain identified as *P. chrysogenum* by Henk et al. (2011). ITS sequences could not distinguish *P. dipodomyis* and *P. flavigenum*, while *P. kewense* and *P. sinaicum* share sequences with *P. egyptiacum* CBS 456.72, CBS 457.72 and CBS 458.72.

Haplotype diversity

A detailed analysis was performed on 88 *P. allii-sativi*, *P. chrysogenum*, *P. rubens*, *P. tardochrysogenum* and *P. vanluykii* isolates, including ex-type and authentic strains, supplemented with isolates used in other taxonomic studies (Samson et al. 1977, Scott et al. 2004, Houbraken et al. 2011a) and other representative strains from culture collections. Haplotypic groups were defined based on the combined sequence alignment of eight loci (cmd, RPB1, RPB2, benA, TrpC, parA, FacA, ITS). Forty-three haplotype groups were detected, most containing only one strain. The haplotype network is shown in Fig. 4 and the haplotype assignment of each strain is included in Table 1. This data demonstrates that haplotype diversity among *P. chrysogenum* strains is higher than among *P. rubens* strains. The full genome sequenced *P. rubens* strain Wisconsin 54-1255 belongs to haplotype 1. This haplotype includes most of the other *P. rubens* strains, including the ex-type strain CBS 129667^{T} (9/20 *P. rubens* isolates). Serendipitously, a strain of *P. chrysogenum* for which no culture is available, had its full genome sequenced unexpectedly as a contaminant of a *Postia placenta* MAD 698R culture (http://genome.jgi.doe.gov/Pench1/Pench1.info.html). Our haplotype analysis shows that this strain belongs to haplotype group 13, together with strains CBS 132214, CBS 132212 and CBS 116046; perhaps one of these strains could be selected as 'epitype' kind of voucher to represent this genome strain. However, CBS 116046 is a good penicillin producer, but no penicillin production was observed in CBS 132214 and CBS 132212. In contrast, both CBS 132214 and CBS 132212 produce roquefortine C, but CBS 116046

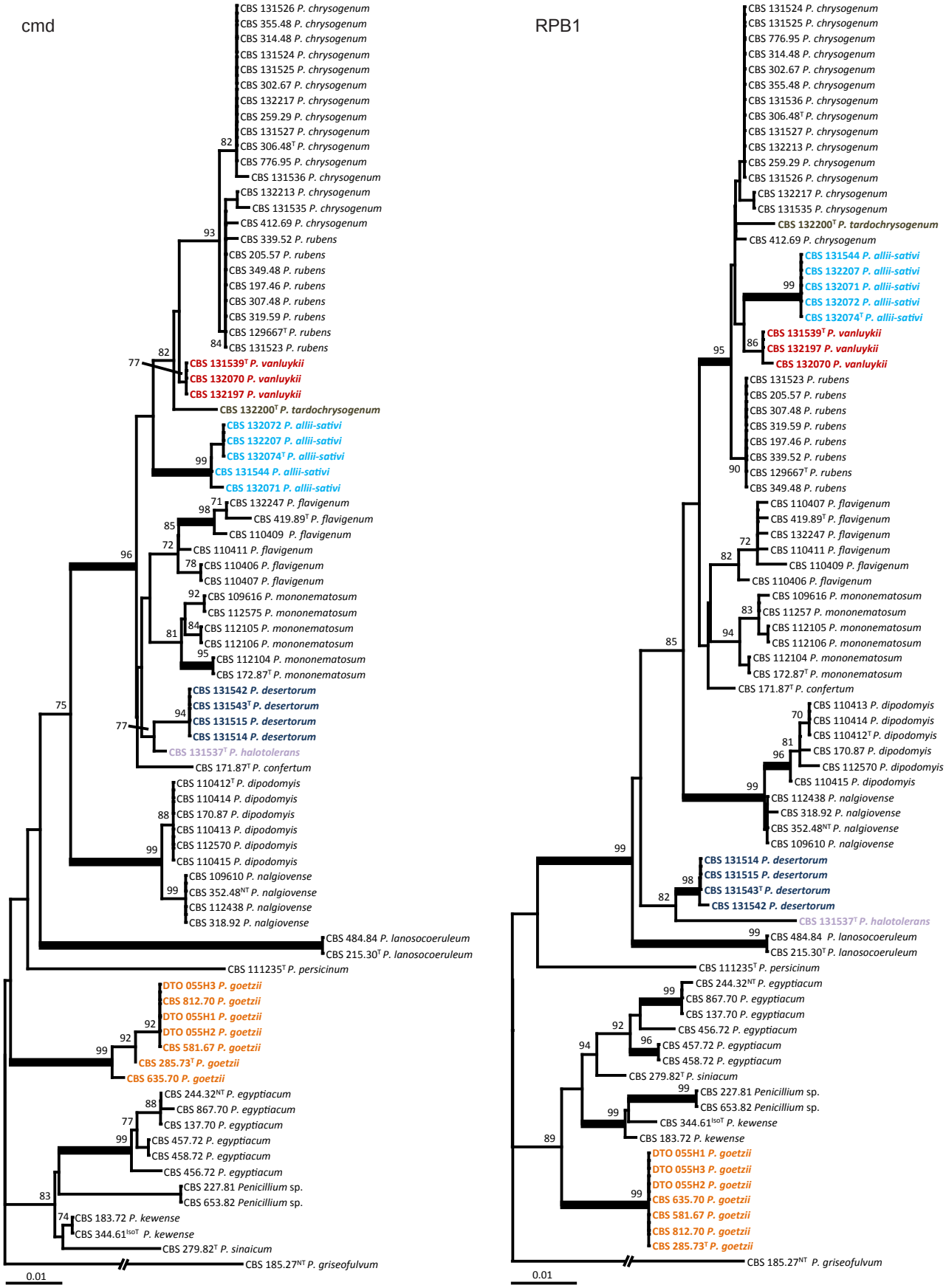

Fig. 2 Best-scoring Neighbour Joining (NJ) phylograms based on calmodulin, RPB1, RPB2 and β-tubulin datasets using MEGA5. Well-supported branches (> 95 % bootstrap supported) are in **bold**, values less than 70 % bootstrap support are not shown. *Penicillium griseofulvum* CBS 185.27^{NT} was used as outgroup.

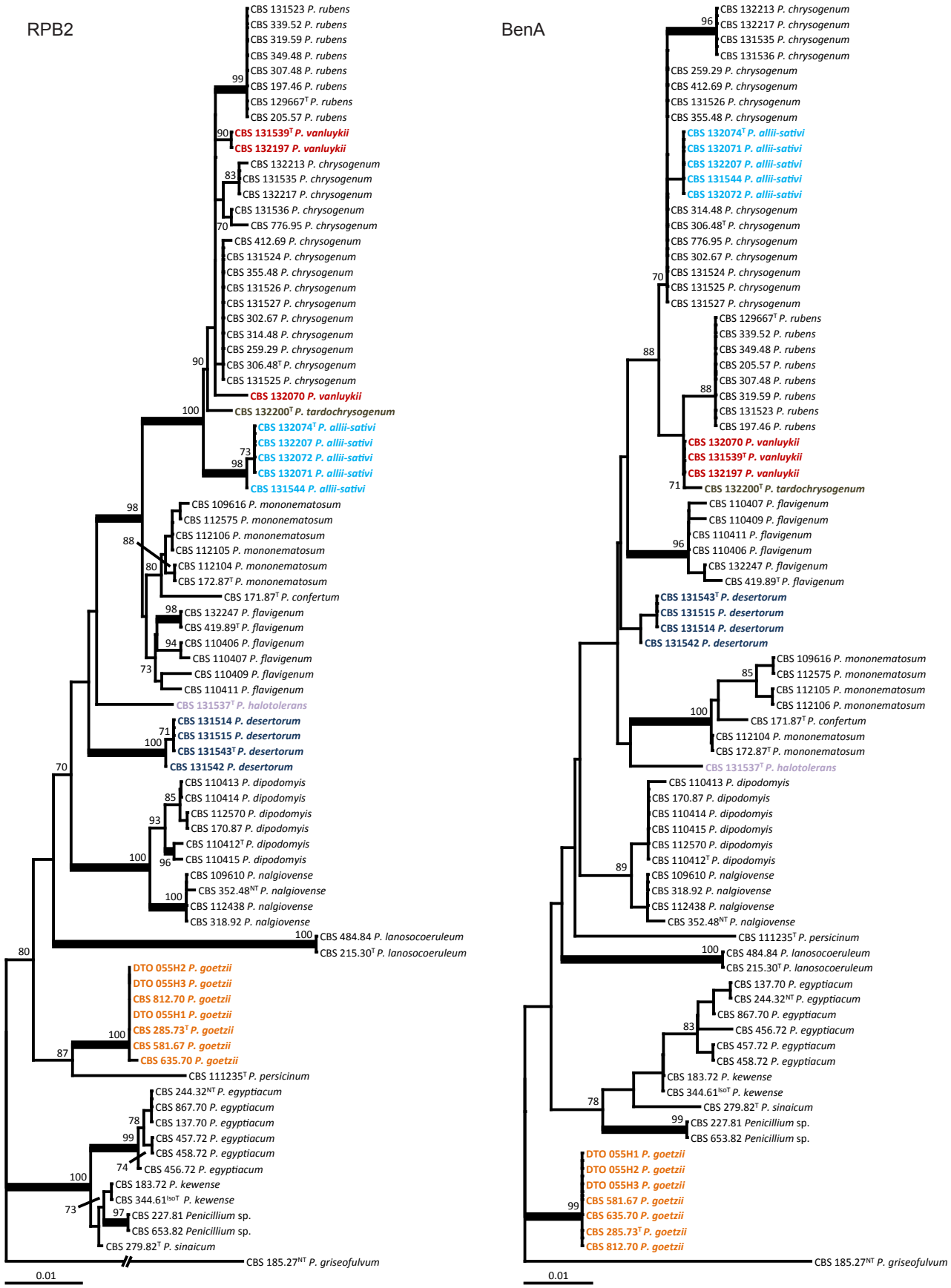
RPB2
CBS 131523 P. rubens
CBS 339.52 P. rubens
CBS 319.59 P. rubens
CBS 349.48 P. rubens
CBS 307.48 P. rubens
CBS 197.46 P. rubens
CBS 129667T P. rubens
CBS 205.57 P. rubens
CBS 131539T P. vanluykii
CBS 132197 P. vanluykii
CBS 132213 P. chrysogenum
CBS 131535 P. chrysogenum
CBS 132217 P. chrysogenum
CBS 131536 P. chrysogenum
CBS 776.95 P. chrysogenum
CBS 412.69 P. chrysogenum
CBS 131524 P. chrysogenum
CBS 355.48 P. chrysogenum
CBS 131526 P. chrysogenum
CBS 131527 P. chrysogenum
CBS 302.67 P. chrysogenum
CBS 314.48 P. chrysogenum
CBS 259.29 P. chrysogenum
CBS 306.48T P. chrysogenum
CBS 131525 P. chrysogenum
CBS 132070 P. vanluykii
CBS 132200T P. tardochrysogenum
CBS 132074T P. allii-sativi
CBS 132207 P. allii-sativi
CBS 132072 P. allii-sativi
CBS 132071 P. allii-sativi
CBS 131544 P. allii-sativi
CBS 109616 P. mononematosum
CBS 112575 P. mononematosum
CBS 112106 P. mononematosum
CBS 112105 P. mononematosum
CBS 112104 P. mononematosum
CBS 172.87T P. mononematosum
CBS 171.87T P. confertum
CBS 132247 P. flavigenum
CBS 419.89T P. flavigenum
CBS 110406 P. flavigenum
CBS 110407 P. flavigenum
CBS 110409 P. flavigenum
CBS 110411 P. flavigenum
CBS 131537T P. halotolerans
CBS 131514 P. desertorum
CBS 131515 P. desertorum
CBS 131543T P. desertorum
CBS 131542 P. desertorum
CBS 110413 P. dipodomyis
CBS 110414 P. dipodomyis
CBS 112570 P. dipodomyis
CBS 170.87 P. dipodomyis
CBS 110412T P. dipodomyis
CBS 110415 P. dipodomyis
CBS 109610 P. nalgiovense
CBS 352.48NT P. nalgiovense
CBS 112438 P. nalgiovense
CBS 318.92 P. nalgiovense
CBS 484.84 P. lanosocoeruleum
CBS 215.30T P. lanosocoeruleum
DTO 055H2 P. goetzii
DTO 055H3 P. goetzii
CBS 812.70 P. goetzii
DTO 055H1 P. goetzii
CBS 285.73T P. goetzii
CBS 581.67 P. goetzii
CBS 635.70 P. goetzii
CBS 111235T P. persicinum
CBS 244.32NT P. egyptiacum
CBS 867.70 P. egyptiacum
CBS 137.70 P. egyptiacum
CBS 457.72 P. egyptiacum
CBS 458.72 P. egyptiacum
CBS 456.72 P. egyptiacum
CBS 183.72 P. kewense
CBS 344.61ISOT P. kewense
CBS 227.81 Penicillium sp.
CBS 653.82 Penicillium sp.
CBS 279.82T P. sinaicum
CBS 185.27NT P. griseofulvum
0.01
BenA
CBS 132213 P. chrysogenum
CBS 132217 P. chrysogenum
CBS 131535 P. chrysogenum
CBS 131536 P. chrysogenum
CBS 259.29 P. chrysogenum
CBS 412.69 P. chrysogenum
CBS 131526 P. chrysogenum
CBS 355.48 P. chrysogenum
CBS 132074T P. allii-sativi
CBS 132071 P. allii-sativi
CBS 132207 P. allii-sativi
CBS 131544 P. allii-sativi
CBS 132072 P. allii-sativi
CBS 314.48 P. chrysogenum
CBS 306.48T P. chrysogenum
CBS 776.95 P. chrysogenum
CBS 302.67 P. chrysogenum
CBS 131524 P. chrysogenum
CBS 131525 P. chrysogenum
CBS 131527 P. chrysogenum
CBS 129667T P. rubens
CBS 339.52 P. rubens
CBS 349.48 P. rubens
CBS 205.57 P. rubens
CBS 307.48 P. rubens
CBS 319.59 P. rubens
CBS 131523 P. rubens
CBS 197.46 P. rubens
CBS 132070 P. vanluykii
CBS 131539T P. vanluykii
CBS 132197 P. vanluykii
CBS 132200T P. tardochrysogenum
CBS 110407 P. flavigenum
CBS 110409 P. flavigenum
CBS 110411 P. flavigenum
CBS 110406 P. flavigenum
CBS 132247 P. flavigenum
CBS 419.89T P. flavigenum
CBS 131543T P. desertorum
CBS 131515 P. desertorum
CBS 131514 P. desertorum
CBS 131542 P. desertorum
CBS 109616 P. mononematosum
CBS 112575 P. mononematosum
CBS 112105 P. mononematosum
CBS 112106 P. mononematosum
CBS 171.87T P. confertum
CBS 112104 P. mononematosum
CBS 172.87T P. mononematosum
CBS 131537T P. halotolerans
CBS 110413 P. dipodomyis
CBS 170.87 P. dipodomyis
CBS 110414 P. dipodomyis
CBS 110415 P. dipodomyis
CBS 112570 P. dipodomyis
CBS 110412T P. dipodomyis
CBS 109610 P. nalgiovense
CBS 318.92 P. nalgiovense
CBS 112438 P. nalgiovense
CBS 352.48NT P. nalgiovense
CBS 111235T P. persicinum
CBS 484.84 P. lanosocoeruleum
CBS 215.30T P. lanosocoeruleum
CBS 137.70 P. egyptiacum
CBS 244.32NT P. egyptiacum
CBS 867.70 P. egyptiacum
CBS 456.72 P. egyptiacum
CBS 457.72 P. egyptiacum
CBS 458.72 P. egyptiacum
CBS 183.72 P. kewense
CBS 344.61ISOT P. kewense
CBS 279.82T P. sinaicum
CBS 227.81 Penicillium sp.
CBS 653.82 Penicillium sp.
DTO 055H1 P. goetzii
DTO 055H2 P. goetzii
DTO 055H3 P. goetzii
CBS 581.67 P. goetzii
CBS 635.70 P. goetzii
CBS 285.73T P. goetzii
CBS 812.70 P. goetzii
CBS 185.27NT P. griseofulvum
0.01

Fig. 2 (cont.)

Table 3 A partial characterisation of extrolites from *Penicillium* section *Chrysogena* which have not yet been fully structure elucidated based on HPLC-DAD.

Extrolite	Retention time	Retention index	UV maxima (nm) (sh: shoulder)	Ref.
'CRYPT1'	10.62	769	200, 271	A[1]
'CRYPT2'	10.87	774	200, 271	A
'CRYPT3'	12.66	812	200, 271	A
'CRYPT4'	14.66	855	200, 271	A
'FJOR1'	6.43	799	200, 335	A
'FJOR2'	6.67	810	200, 228, 270, 330	A
'KNOLF'	16.76	900	202, 235sh, 270, 337	A
'POO'	15.07	864	202, 266, 319	A
'TBRE'	7.60	705	221, 267, 331	A
'KEWS1'	2.67	692	220, 275, 297, 380, 400sh	A
'KEWS2'	6.96	802	200, 225, 250, 275, 311sh, 378, 400sh	A
'KEWS3'	13.11	967	226, 250sh, 260, 276sh, 355sh, 376, 385	A
'AURIN'	6.48	814	200, 235, 311	A
'DOLDOX'	2.86	710	265	A
'MURA'	25.39	1479	201, 212sh, 265, 310	A
'XYLA'	5.03	773	201, 231, 281, 320	A
'FOPT'	12.54	976	200, 240sh, 319	A
'FORN1'	20.84	1280	204, 239, 292	A
'FORN2'	25.57	1494	204, 239, 292	A
'FORN3'	16.58	1111	204, 239, 292	A
'HO6'	8.22	847	200, 225, 242, 274sh, 323	A
'DOLDO'	4.63	710	280	B[2]
'met Ø'	7.95	852	210, 255, 275sh	B
'ALKONA'	11.60	1974	200, 215sh, 265, 287sh	B
'AURCH'	6.89	796	200, 228, 310	B
a naphtho-γ-pyrone	6.77	814	202, 232, 280, 328, 338, 405	B
chrysogenamide	9.87	963	221, 273, 280sh	B
'DERH'	8.02	869	223, 280, 359, 440sh	B
'GULLA'	8.15	875	220, 272, 359, 481sh	B
'KUTZ'	12.70	1057	220, 269, 320, 412	B
'OTOF'	12.48	1038	217, 271, 315	B
'SENGAX'	15.48	1360	220, 277, 330	B
'SNORL'	15.84	1380	210, 225, 264, 323	B
'SPOFI'	12.21	1106	200, 227sh	B
'CD1'	11.460	892	200, 273	C[3]
'CD2'	12.782	919	200, 273	C
'CD3'	13.612	935	200, 273	C
'CD4'	13.972	942	200, 273	C
'CD5'	15.960	981	200, 273	C
'CDU'	9.854	858	200, 220, 275	C
'CRYPT'	10.769	877	200, 269	C
'DI'	11.891	886	200, 240, 270, 325	C
'DIOR'	14.143	947	200, 261, 425	C
'DIPA'	17.554	995	200, 213, 236, 259, 295, 331	C
'FCD'	7.359	808	200, 215, 280, 341	C
'GNALDI'	10.174	865	200, 224, 335	C
'TOLO'	16.828	990	207, 250, 281, 376	C
'VIK'	25.092	1163	200, 210sh, 280sh, 330-375	C
Tetronic acid, *P. egyptiacum* 1	1.390	697[4]	227, 261, 322sh	C
Tetronic acid, *P. egyptiacum* 1	1.623	701[4]	200, 223, 270sh, 303	C
Tetronic acid, *P. egyptiacum* 1	1.918	708[4]	200, 225sh, 275	C
'BR'	3.846	733	200, 225, 271, 320sh, 421	C
'met U'	2.679	711	200, 230+, 263, 364	C
'PRU'	1.892	697	200, 235, 280	C
'RAIS'	3.51	716	214, 222sh, 270, 310	C
'SNOK'	14.15	911	(200), 275	C
'VERNX'	2.285	704	202, 285	C
'ASTYL'	16.819	994	263, 359	C
'GULLA'	15.283	964	220, 272, 359, 431sh	C
'MER'	7.351	798	222, 225sh, 263, 318	C
'MONTI'	17.416	992	200, 210sh, 266, 280sh, 372, 440sh	C
'PJIM'	13.656	993	200, 218, 270	C
'PLOT'	17.571	987	202, 265, 281. 360	C
'PAEL'	29.533	1291	230	C
'PYTO'	4.384	741	200, 276, 370	C
'SNAT'	19.394	1043	200, 224sh, 275	C
'TRYP'	12.304	893	202, 225, 279, 300sh	C
'VERNX2'	1.877	694	202, 285	C
'CUCU'	1.519	683	202, 222sh, 277, 300sh	C
'PLIL'	23.405	1218	200, 223sh, 299	C

[1] A: Nielsen & Smedsgaard 2003; [2] B: Nielsen et al. 2011; [3] C: Frisvad & Thrane 1987; [4] Inaccurate RI values, as chromatographic peaks were broad.

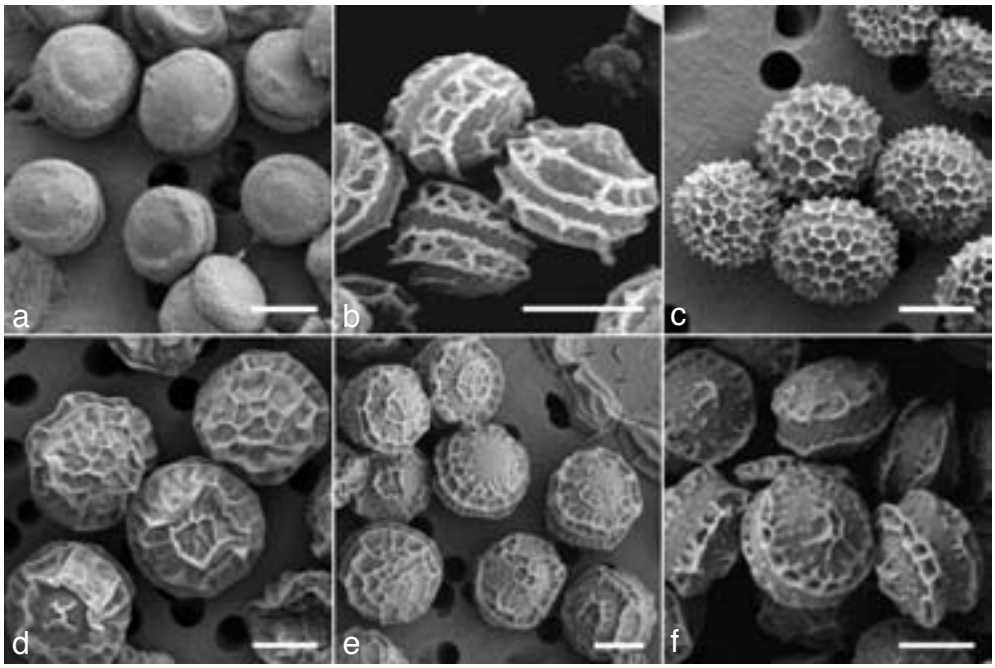

Fig. 3 Scanning Electron Micrographs of ascospores of species belonging to *Penicillium* section *Chrysogena*. a. *P. egyptiacum* CBS 244.32; b. *P. egyptiacum* CBS 457.72; c. *P. sinaicum* CBS 279.82; d. *P. goetzii* CBS 285.73; e. *P. kewense* CBS 344.61; f. *Penicillium* sp. CBS 103.71 — Scale bars = 2 µm.

does not. CBS 132214 was the only strain producing the uncharacterised compound 'met Ø'. These results suggest that even with 8 loci, the resulting haplotype assignments may not be precise enough to correlate with a precise genome.

TAXONOMY

Penicillium allii-sativi Frisvad, Houbraken & Samson, *sp. nov*. — MycoBank MB801873; Fig. 5

Typus. Argentina, Mendoza, Lavalle, Col 3 de Mayo, on bulbs of *Allium sativum* (garlic), *M. Makuch & J. Valdez* (CBS H-21058 holotype, cultures ex-type CBS 132074 = IBT 26507 = DTO 149-A8 = LJC 206).

Etymology. Referring to *Allium sativum* (garlic), the substrate where the type strain was isolated from.

Sporulation on CYA dense; colonies slightly polygonal in outline, velvety; mycelium white, sporulation in shades of dark green, exudate droplets large, clear, pale yellow or light brown; soluble pigments absent or occasionally present, light brown; colony reverse pale brown. Soluble pigments on YES absent; mycelium white; sporulation dense; sporulation dark green; exudate absent, reverse beige. Sporulation on DG18 dense; sporulation grey-green or dull green; reverse pale. Colonies on MEA velvety or slightly floccose; sporulation variable, grey-green, dark green or dull green; exudate droplets large, clear, pale yellow or light brown, reverse yellow-brown. No violet reaction with Ehrlich reagents. *Sclerotia* absent. *Conidiophores* borne from the agar surface, ter- or quarterverticillate, divaricate. *Stipes* 200–400 × 3–4 µm, smooth walled. *Branches* 15–25(–35) × 3–4 µm. *Metulae* unequal in length, in verticils of 3–8, 10–12(–16) × 2.5–3.5 µm. *Phialides* ampulliform, in verticils of 4–10, closely packed, 7.5–8.5 × 2–5.5 µm. *Conidia* globose to subglobose, smooth, 2.5–3.5 µm.

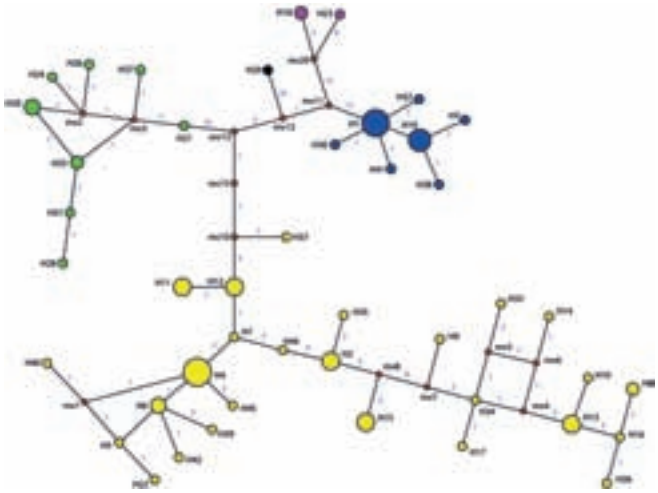

Fig. 4 Haplotype network of *P. chrysogenum* (yellow), *P. rubens* (blue), *P. vanluykii* (purple), *P. allii-sativi* (green) and *P. tardochrysogenum* (black) strains based on cmd, benA, RPB1, RPB2, TrpC, ParA, FacA and ITS sequences. In total, 43 different haplotypes were detected and a detailed list is given in Table 1. Red coloured circles represent median vectors. The lines between the groups connecting the haplotypes show the number of nucleotides differing.

Diagnosis — *Penicillium allii-sativi* is phenotypically similar to *P. chrysogenum* and *P. vanluykii*. Isolates of this species produce conidia in shades of dark green on CYA and yellow soluble pigment usually absent on CYA incubated at 30 °C.

Colony morphology — Colony diam, 7 d, in mm: CYA 26–38; CYA 15 °C 18–25; CYA 30 °C 22–32; CYA 37 °C: no growth–4; MEA 31–42; YES 45–58; DG18 26–40; CYAS 37–45(–60); creatine agar 18–30, weak or moderate growth, weak acid production.

Extrolites — Penicillins, Atlantinone A, chrysogenamide, 2-(4-hydroxyphenyl)-2-oxo acetaldehydeoxim, a naptho-γ-pyrone, 2-pyruvoylaminobenzamide, roquefortine C, D, meleagrin, verrucosidin, normethylverrucosidin, deoxyverrucosidin, verrucosidinol and the uncharacterised compounds 'ALKONA', 'AURCH', 'CRYPT', 'DERH', 'GULLA', 'KUTZ' (atromentins?), 'OTOF', 'SENGAX', 'SNORL', 'SPOFI', 'VERNX'.

Distribution & Ecology — This species has a broad distribution (Argentina, Bulgaria, France, Portugal, South Africa, UK) and has been isolated from garlic, soil, salterns, sorghum malt and mixed pig feed (Henk et al. 2011, this study). This species is not a pathogen on garlic like *P. allii* (Valdez et al. 2009).

Barcode & Molecular based ID — ITS sequencing is imprecise for species identification because all investigated strains of *P. allii-sativi* and *P. chrysogenum* CBS 306.48^T share the same ITS sequence (GenBank JX997021).

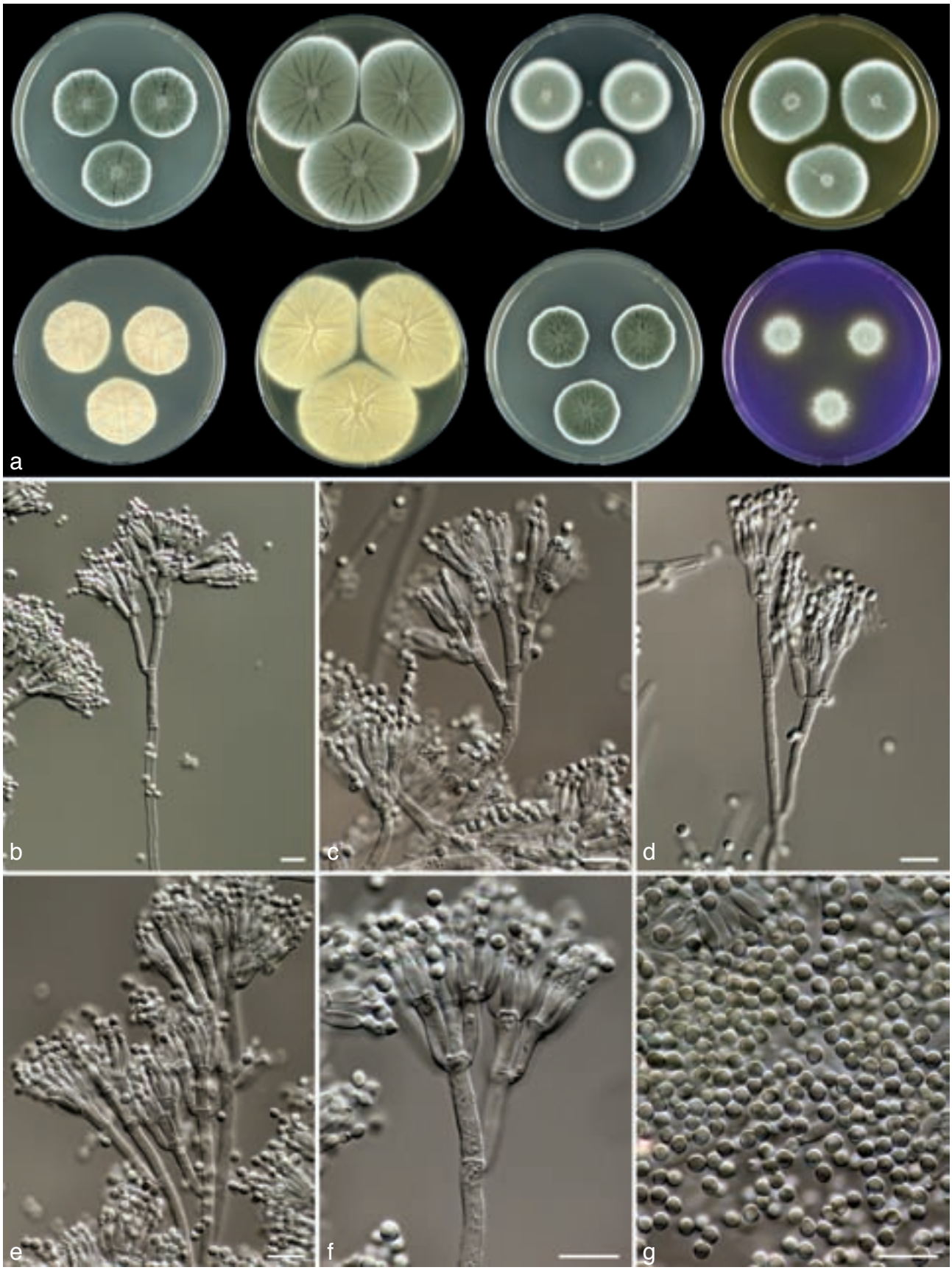

Fig. 5 *Penicillium allii-sativi*, CBS 132198. a. 7 d old cultures at 25 °C unless stated otherwise, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, CYA incubated at 30 °C obverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

Penicillium desertorum Frisvad, Houbraken & Samson, *sp. nov*. — MycoBank MB801874; Fig. 6

Typus. USA, Wyoming, 20 km east of Little America, ex cool desert soil under *Oryzopsis hymenoides*, *J.C. Frisvad* (CBS H-21056 holotype, cultures ex-type CBS 131543 = IBT 16321 = DTO 148-I6).

Etymology. Referring to desert; because this species is common in desert soil.

Sporulation on CYA dense; colonies entire or slightly polygonal in outline, velvety, radially sulcate; mycelium white, conidia dull green or greyish dull green, exudate absent or sparsely pro-

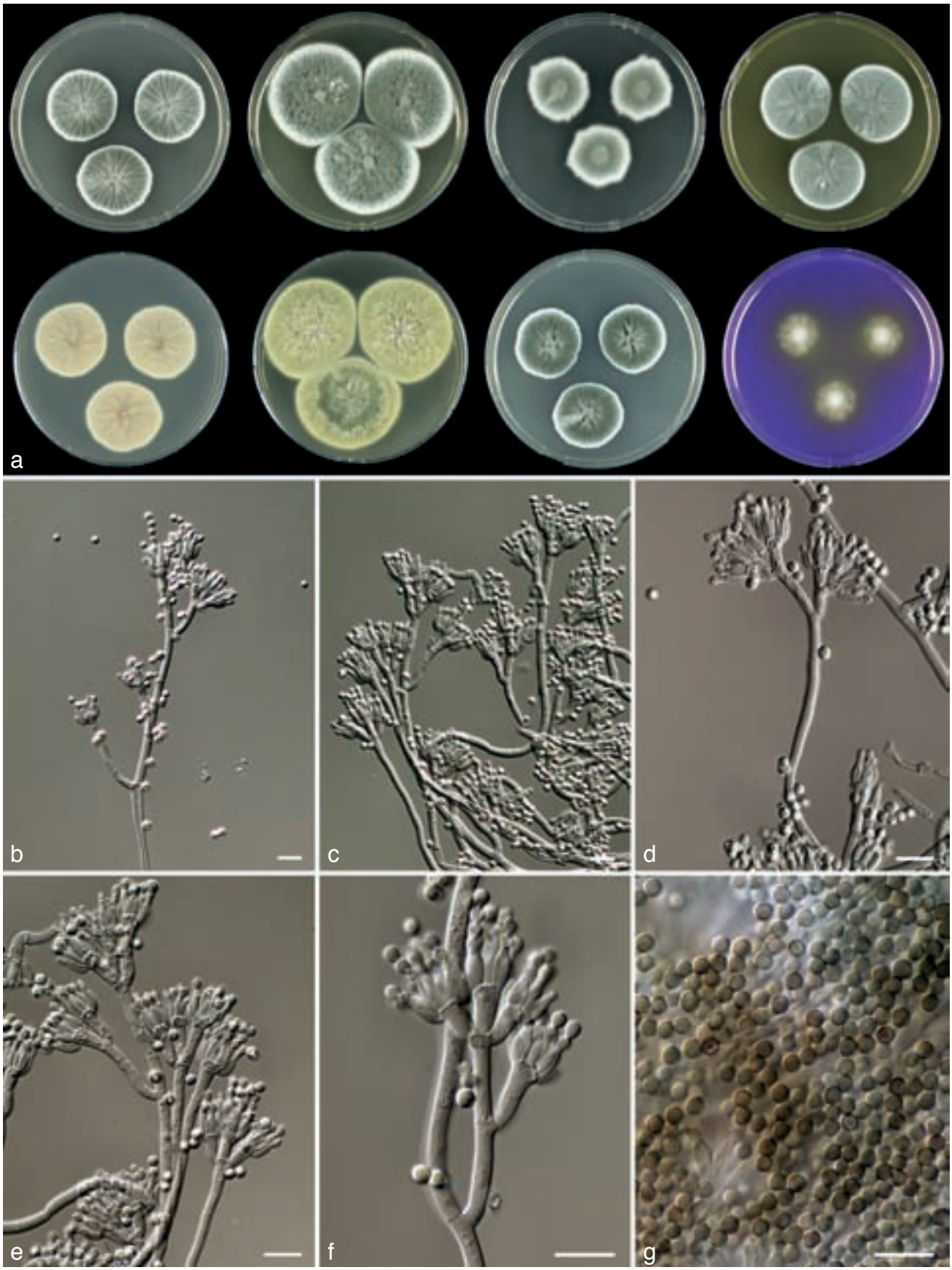

Fig. 6 *Penicillium desertorum*, CBS 131543^{T}. a. 7 d old cultures at 25 °C unless stated otherwise, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, CYA incubated at 30 °C obverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

duced; soluble pigments absent; colony reverse brown. Soluble pigments on YES absent; mycelium white; sporulation dense; sporulation dark green; exudate absent, reverse beige with a brown centre or brown, with cerebriform sulcations. Sporulation on DG18 dense; sporulation grey *en masse*; reverse pale, transparent. Colonies on MEA velvety or slightly floccose; sporulation dense, conidia grey-green with a blue shade; exudate droplets absent, reverse unaffected or becoming brown. No violet reaction with Ehrlich reagent. *Sclerotia* absent. *Conidiophores* borne from surface, with (short) divaricate branches at various levels along the stipe. *Stipes* long, 200–400 × 2.5–3.5 µm, smooth walled and occasionally very finely roughened. *Branches* 8–15

(–25) × 2.5–3.5 µm. *Metulae* equal in length, occasionally inflated, densely packed, 3–8, 8–10(–15) × 2.5–3.5 µm. *Phialides* ampulliform, in verticils of 4–10, closely packed, 6–7.5 × 2–3 µm. *Conidia* globose, smooth, 2.5–3(–3.5) µm.

Diagnosis — Isolates of *P. desertorum* do not produce yellow pigments on CYA incubated at 25 °C and 30 °C and colonies on YES have a beige-brown or brown, cerebriform, sulcate reverse. This species is unique in sect. *Chrysogena* by the production of conidiophores that have several short, divaricate branches at various levels along the stipe.

Colony morphology — Colony diam, 7 d, in mm: CYA (20–) 24–37; CYA 15 °C 17–25; CYA 30 °C (15–)20–32; CYA 37 °C: no growth–4; MEA 20–37; YES 37–55; DG18 20–30; CYAS 24–38; creatine agar 10–23, weak growth, weak to moderate acid production.

Extrolites — 2-(4-hydroxyphenyl)-2-oxo acetaldehyde oxim, Raistrick phenols, austalides?, 'FOL'.

Distribution & Ecology — This species has a world-wide distribution and has been found in Argentina, Iran, USA (Wyoming, New Mexico), Canada (British Columbia), Puerto Rica and Costa Rica. Arid or desert soil seems to be the primary substrate of this species. Only a selected number of strains are included in Table 1.

Barcode & Molecular based ID — Two ITS sequence types are detected in *P. desertorum*. DTO 016-B5, DTO 148-I5 and DTO 148-I6 share the same ITS sequence and this type is species specific (GenBank JX997010). DTO 015-H9 shares its ITS sequence with the type of *P. chrysogenum* CBS 306.48^T (GenBank JX997038) and therefore ITS sequencing is imprecise for identification of *P. desertorum*. Partial β-tubulin, calmodulin, RPB1 or RPB2 sequences are recommended for species identification.

Penicillium goetzii J. Rogers, Frisvad, Houbraken & Samson, *sp. nov.* — MycoBank MB801876; Fig. 7

Typus. Canada, Calgary, ex soil, *J. Bissett* (CBS H-21061 holotype, cultures ex-type CBS 285.73 = DTO 088-G6).

Etymology. Named after John Richard Goetz III, a student of Jack Rogers who isolated this species (isolates DTO 055-H1, DTO 055-H2 and DTO 055-H3) and performed experiments with it.

Sporulation on CYA variable, absent to dense; velvety or slightly floccose, colonies with a feathery edge, radially sulcate; mycelium white and occasionally pinkish, conidia grey-green, exudate sparsely produced, clear, light brown or reddish brown; soluble pigments brown or reddish brown; colony reverse beige, sometimes with a reddish brown centre. Soluble pigments not produced on YES; mycelium white; sporulation often absent, occasionally present and dense, grey green *en masse*; exudate absent, reverse yellow, sometimes with a yellow-orange centre. Sporulation on DG18 absent or poor; sporulation grey *en masse*; mycelium white, reverse pale or bright yellow. Colonies on MEA floccose; sporulation variable, absent to dense, conidia grey-green *en masse*; mycelium white, exudate droplets absent or produced as clear or light brown droplets, reverse unaffected or becoming yellow. No violet reaction with Ehrlich reagent. *Ascomata* white when young, becoming creamish brown in time, maturing within 3–6 wk, 150–350 µm. *Asci* 6.5–11 × 5.5–8 µm. *Ascospores* ellipsoidal, with two distinct equatorial ridges and often two secondary ridges which are connected by transverse ribs, valves ornamented with a reticulate pattern, 3–5 × 3–4.5 µm. *Conidiophores* borne from surface and aerial mycelium, ter- to quarterverticillate, 200–400 × 2.5–3.5 µm, smooth walled. *Branches* 12–20 × 2.5–3.5 µm. *Metulae* equal in length, slightly inflated, 2–6, 8–12(–15) × 2.5–3.5 µm. *Phialides* ampulliform, in verticils of 4–10, closely packed, 7–9(–10) × 2–3 µm. *Conidia* broadly ellipsoidal, smooth, 2–2.5 × 2–3 µm.

Diagnosis — *Penicillium goetzii* is characterised by fast growth rate on CYA, production of brown soluble pigments on CYA and ascospores measuring 3–4.5 × 2.5–4 µm. It forms larger colonies on DG18 after 7 d of incubation at 25 °C (22–30 mm) than *P. kewense* (12–19 mm) and differs from *P. egyptiacum* by ascospore size and ornamentation.

Colony morphology — Colony diam, 7 d, in mm: CYA (30–) 33–42; CYA 15 °C 18–28; CYA 30 °C (10–)15–27; CYA 37 °C: no growth; MEA 33–42; YES 40–55; DG18 22–30; CYAS 30–40; creatine agar 15–30, weak growth, acid production absent or weak.

Extrolites — Andrastin A, citreoisocoumarin, fumitremorgin A, verruculogen, isoepoxydon, 10,23-dihydro-24,25-dehydroaflavinine & 10,23,24,25-tetrahydro-24-hydroxyaflavinine and the uncharacterised compound 'GLAD'.

Distribution & Ecology — The primary substrate seems to be soil, but this species was also isolated as an endophyte in coniferous roots (Goetz 2006) and culture contaminant of a *Spiromastix warcupii* culture. The species has a broad distribution and has been isolated from Canada (Alberta, British Colombia), Pakistan and the USA.

Barcode & Molecular based ID — This species can be identified reliably by ITS sequencing. Two ITS sequence types were detected. CBS 581.67, 812.70, 285.73^T and DTO 055-H1, DTO 055-H2 and DTO 055-H3 share the same ITS sequence (e.g. GenBank JX997042) and CBS 635.70 has a unique ITS sequence type (GenBank JX997112).

Penicillium halotolerans Frisvad, Houbraken & Samson, *sp. nov.* — MycoBank MB801875; Fig. 8

Typus. Egypt, ex salt marsh, *A.H. Moubasher* (CBS H-21060 holotype, cultures ex-type CBS 131537 = IBT 4315 = DTO 148-H9 = MOUS S42).

Etymology. Named after its ability to grow well in the presence of 5 % NaCl.

Sporulation on CYA dense; colonies entire, velvety; mycelium white, sporulation dull green with a blue tinge; exudate droplets clear, small; soluble pigments absent; colony reverse light brown. Soluble pigments on YES absent; mycelium white; sporulation moderate to dense; sporulation dull green to blue-green; exudate absent, reverse cream. Sporulation on DG18 moderate dense; sporulation blue-green; reverse pale. Colonies on MEA velvety, slightly floccose in centre; sporulation green to grey-green, reverse yellow-brown. No violet reaction with Ehrlich reagent. *Sclerotia* absent. *Conidiophores* borne from surface; stipes (100–)200–300(–500) × 2–3.5 µm, smooth walled, ter- to quarterverticillate, bearing terminal verticils of 2–4 metulae. *Branches* divaricate, 10–20(–40) × 2–3.5 µm. *Metulae* unequal in length, (8–)10–15 × 2–3 µm. *Phialides* ampulliform to cylindrical, in verticils of 2–6, 7.5–9 × 2–2.5 µm. *Conidia* globose, smooth, 2–3 µm.

Diagnosis — *Penicillium halotolerans* can be distinguished from *P. desertorum* by the production of yellow soluble pigments on CYA when incubated at 30 °C, slightly smaller conidia and the production of the extrolites andrastin A, roquefortine C & D, meleagrin and Raistrick phenols.

Colony morphology — Colony diam, 7 d, in mm: CYA 27–35; CYA 15 °C 19–23; CYA 30 °C 20–25; CYA 37 °C: germination (0–2); MEA 31–39; YES 41–51; DG18 26–32; CYAS 32–38; creatine agar 16–22, weak growth, no acid production.

Extrolites — Andrastin A, roquefortine C & D, meleagrin, Raistrick phenols and the uncharacterised compounds such as 'CUCU' and 'PLIL'.

Distribution & Ecology — This species is known only from its type, isolated from a salt march in Egypt. An ITS sequence

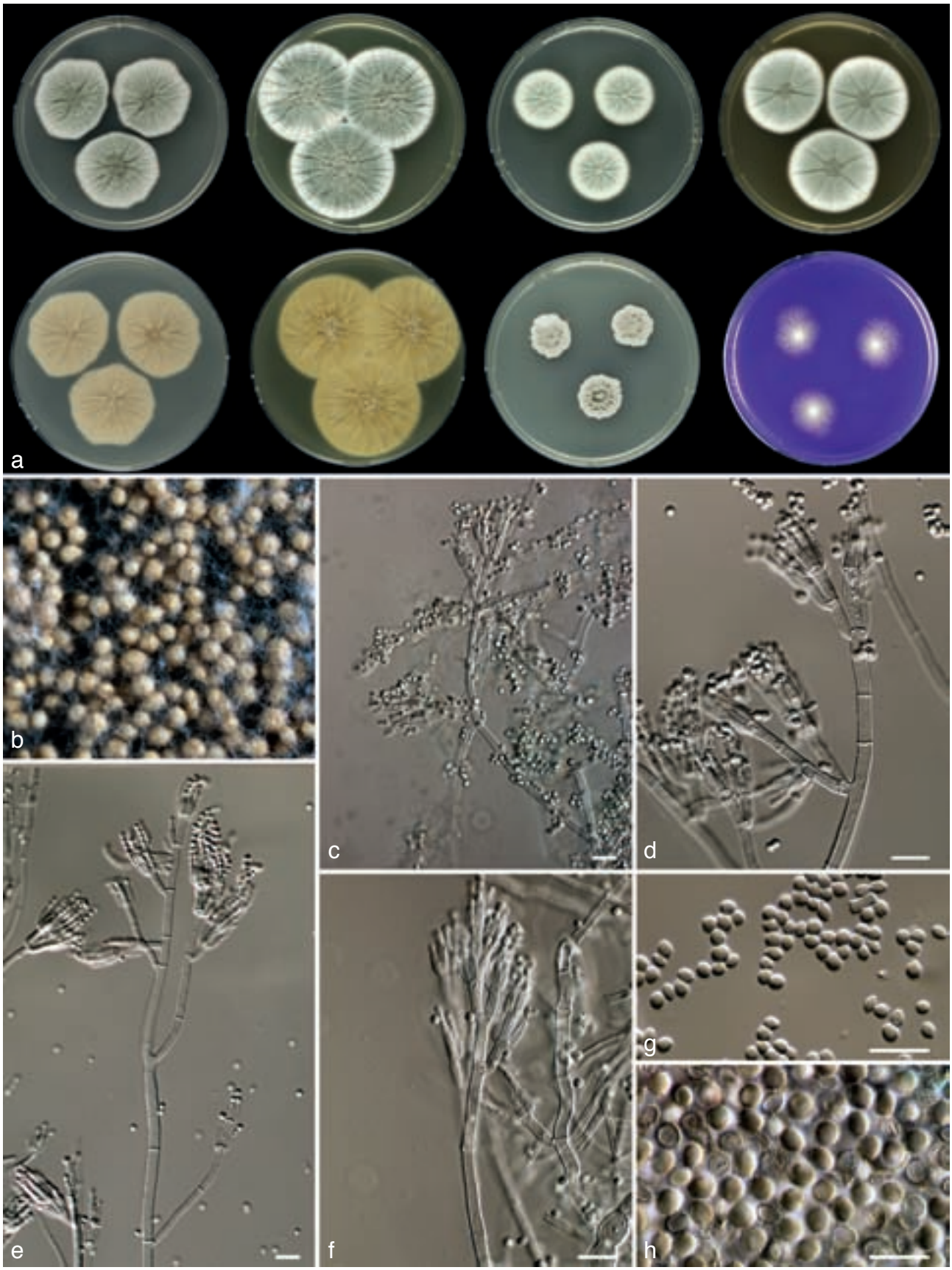

Fig. 7 *Penicillium goetzii*, CBS 581.67. a. 7 d old cultures at 25 °C unless stated otherwise, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, CYA incubated at 30 °C obverse, CREA obverse; b. ascomata; c–f. conidiophores; g. conidia; h. ascospores. — Scale bars = 10 µm.

deposited in GenBank (HQ607840) and obtained from a strain (ATT111) isolated from a nest of the ant *Atta texana* in Texas, USA, was identical to that generated from CBS 131537^{T}.

Barcode & Molecular based ID — This species can be reliably identified using ITS barcoding (GenBank JX997005).

Penicillium tardochrysogenum Frisvad, Houbraken & Samson, *sp. nov.* — MycoBank MB801877; Fig. 9

Typus. Antarctica, McMurdo Dry Valley, *S. Onofri* (CBS H-21057 holotype, cultures ex-type CBS 132200 = IBT 30075 = DTO 149-B9.

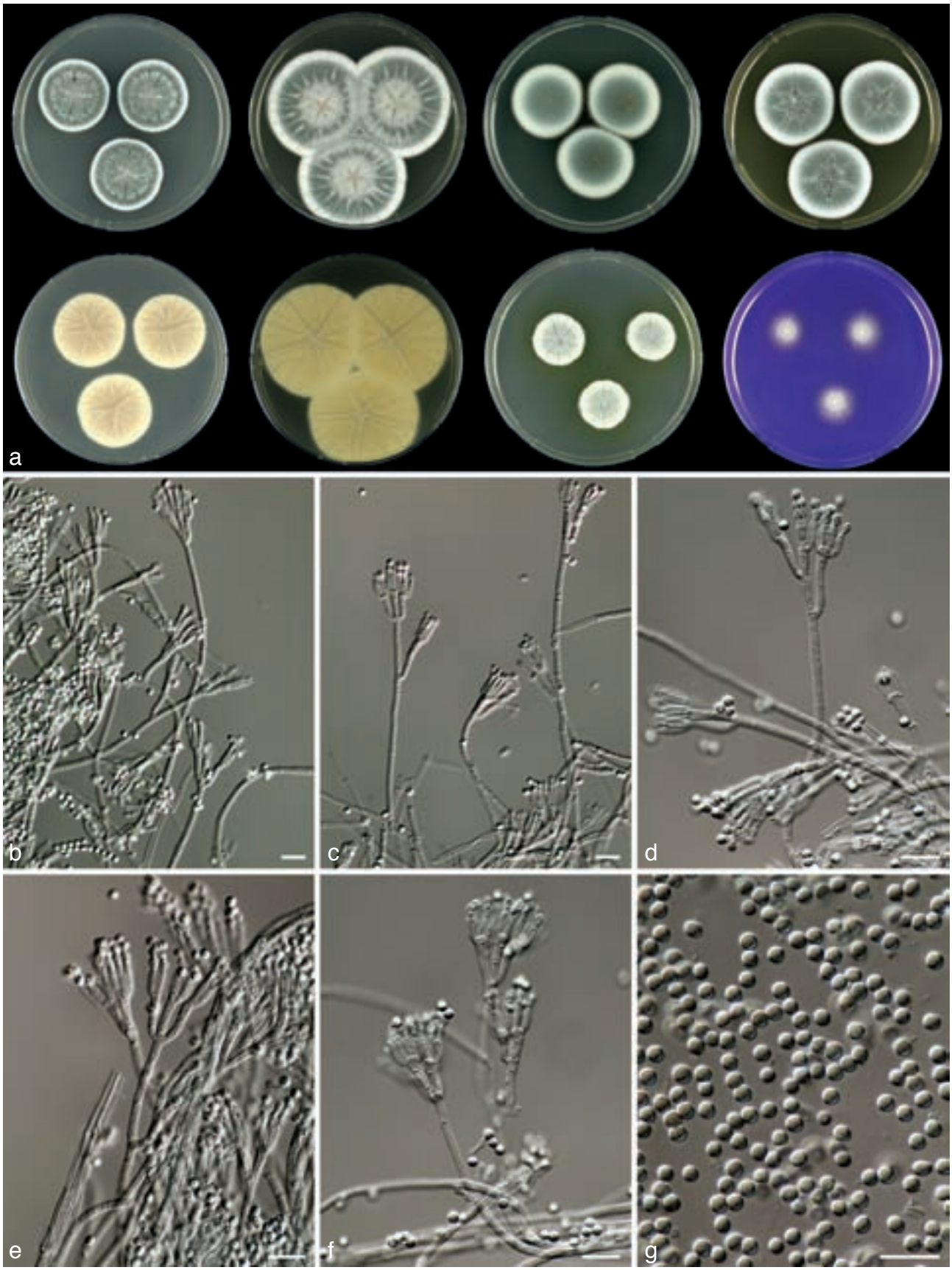

Fig. 8 *Penicillium halotolerans*, CBS 131537^{T}. a. 7 d old cultures at 25 °C unless stated otherwise, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, CYA incubated at 30 °C obverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

Etymology. Named after its resemblance to *P. chrysogenum* and its relative slow growth rate.

Sporulation on CYA dense; colonies entire, velvety to slightly floccose, distinctly radially sulcate; mycelium white, sporulation grey green; exudate droplets clear or pale brown, large; soluble pigments absent; colony reverse brown. Soluble pigments on YES absent; mycelium white; sporulation absent; exudate absent, reverse yellow-brown. Sporulation on DG18 dense; conidia grey green *en masse*; reverse pale. Colonies on MEA floccose with a wide, non-sporulating edge (4–8 mm); exudate droplets large in centre, smaller towards the rim of colony, hyaline; sporulation bluish grey green, reverse

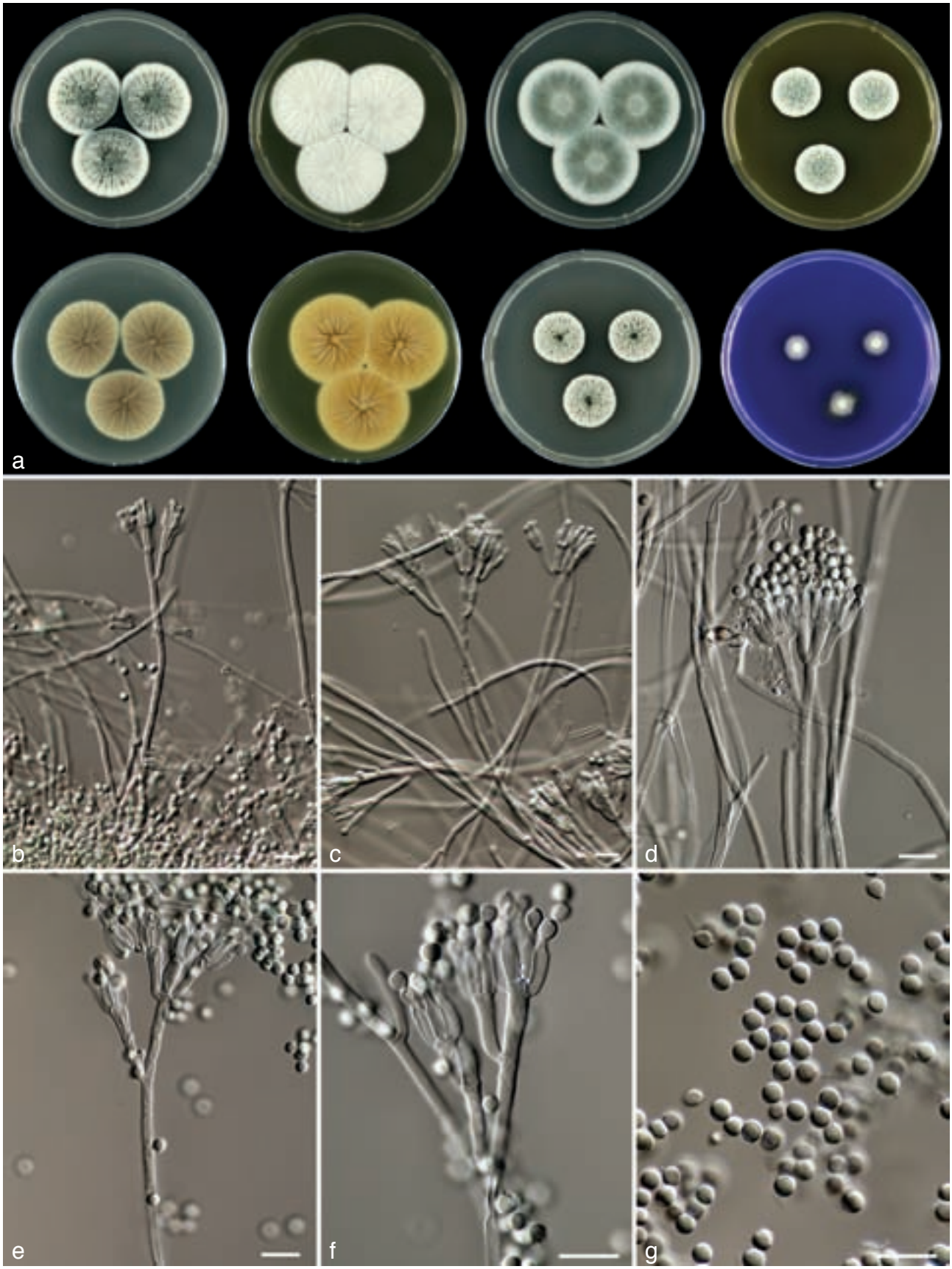

Fig. 9 *Penicillium tardochrysogenum*, CBS 132200^{T}. a. 7 d old cultures at 25 °C unless stated otherwise, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, CYA incubated at 30 °C obverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

brown and in yellow-brown in valves of sulcations. No violet reaction with Ehrlich reagent. *Sclerotia* absent. *Conidiophores* mainly borne from aerial mycelium, sometimes direct from agar surface, ter- to quarterverticillate; stipes 150–400 × 2–3 µm, smooth walled. *Branches* divaricate, 10–20(–25) × 2–3 µm. *Metulae* equal in length, occasionally unequal, in verticils of 2–4, 10–13(–18) × 2.5–3.5 µm. *Phialides* ampulliform, in verticils of 3–8, closely packed, short, 7–9 × 2–3 µm. *Conidia* globose, finely roughened, 2.7–3.5 µm.

Diagnosis — *Penicillium tardochrysogenum* differs from other members of series *Chrysogena* by more restricted and floccose colonies on MEA, lack of sporulation on YES and

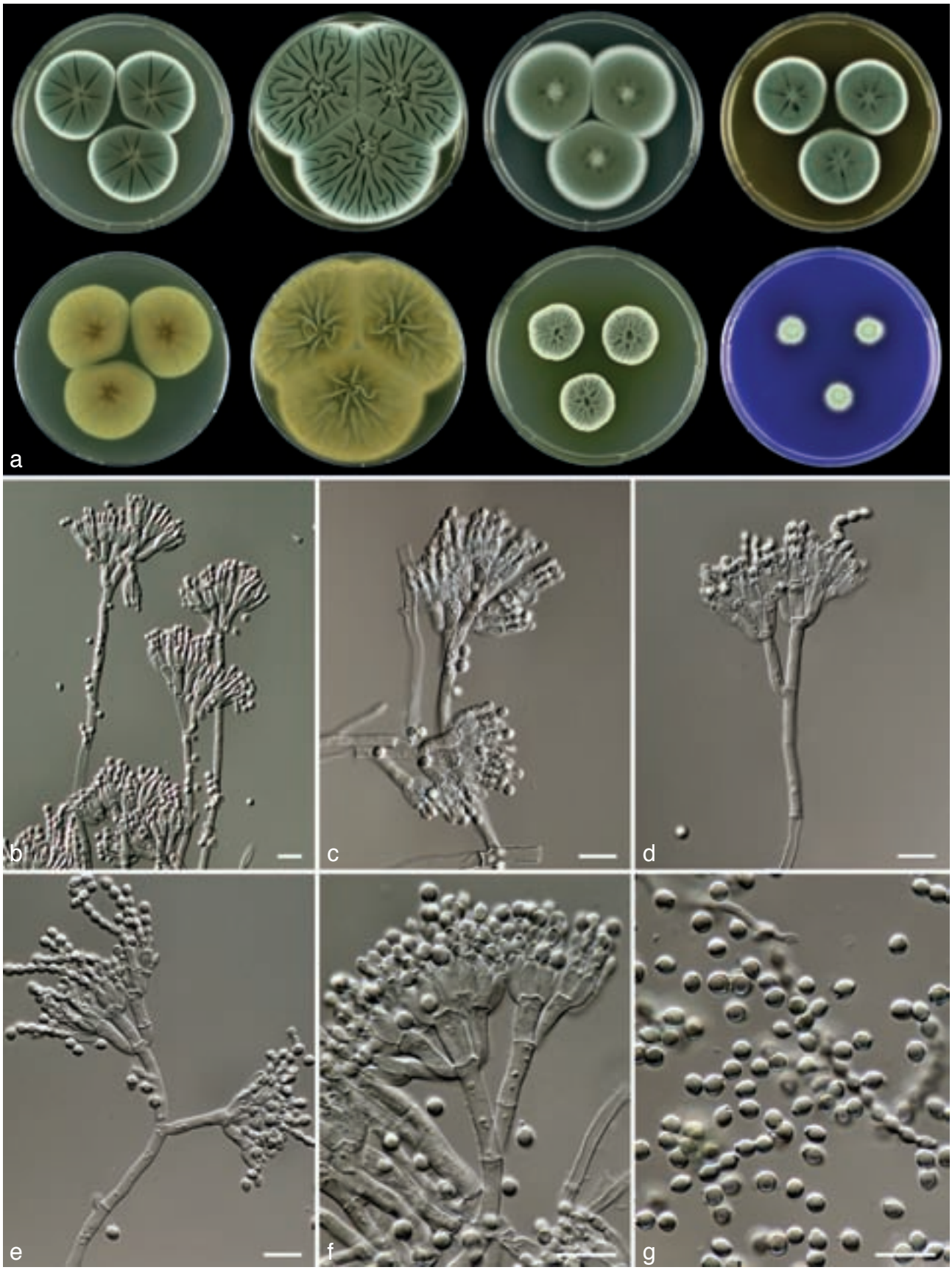

Fig. 10 *Penicillium vanluykii*, CBS 132070. a. 7 d old cultures at 25 °C unless stated otherwise, left to right, first row, all obverse, CYA, YES, DG18, MEA; second row, CYA reverse, YES reverse, CYA incubated at 30 °C obverse, CREA obverse; b–f. conidiophores; g. conidia. — Scale bars = 10 µm.

finely roughened conidia. It does not produce yellow soluble pigments on CYA incubated at 30 °C. The species produces the asperentins, a series of compounds not produced by other members of series *Chrysogena*.

Colony morphology — Colony diam, 7 d, in mm: CYA 29–37; CYA 15 °C 16–20; CYA 30 °C 20–25; CYA 37 °C: germination (0–2); MEA 18–24; YES 35–45; DG18 34–40; CYAS 36–44; creatine agar 8–12, weak growth, no or poor acid production.

Extrolites — Penicillins, secalonic acids D & F, asperentins and the uncharacterised extrolite met Ø.

Distribution & Ecology — This species is only known from its type, which was isolated from the McMurdo Dry Valley, Antarctica.

Barcode & Molecular based ID — This species shares ITS sequences with the type of *P. chrysogenum* CBS 306.48^T (GenBank JX997093). Partial β-tubulin, calmodulin, RPB1 or RPB2 can be used for species identification.

Penicillium vanluykii Frisvad, Houbraken & Samson, *sp. nov.* — MycoBank MB801878; Fig. 10

Typus. USA, New Mexico, Carlsbad, ex Lechuguilla Cave, *D. Northup* (CBS H-21059 holotype, cultures ex-type CBS 131539 = IBT 14505 = DTO 148-I2).

Etymology. Named after Abraham van Luyk, a CBS mycologist who worked on the antibiotic activity of *Penicillium* in the 1940s.

Sporulation on CYA dense; colonies entire, velvety, sulcate radially; mycelium white, sporulation dark green to green; exudate droplets hyaline, light brown or absent, small; soluble pigments absent, in 149A4 yellow soluble pigments produced; colony reverse pale brown or yellow-brown. Soluble pigments on YES absent; mycelium white; sporulation dense; conidia dark green or green *en masse*; exudate absent, reverse greenish brown in centre with pale brown edge. Sporulation on DG18 moderate dense; conidia green or dull green *en masse*; reverse unaffected or pale brown. Colonies on MEA velvety; exudate droplets absent; sporulation green to dark green, reverse unaffected, sometimes with dark brown centre. No violet reaction with Ehrlich reagent. *Sclerotia* absent. *Conidiophores* borne from surface; quarterverticillate. *Stipes* 100–300 × 2.5–3.5 µm, smooth walled. *Branches* divaricate, 15–25 × 2.5–3.5 µm. *Metulae* equal in length, 3–8, 8–12 × 3–3.5(–4) µm. *Phialides* ampulliform, in verticils of 4–10, closely packed, short, 6.5–8.5 × 2–3 µm. *Conidia* globose to subglobose, smooth, with distinct connectives, 2.5–3.5 µm.

Diagnosis — *Penicillium vanluykii* is phenotypically similar to *P. allii-sativi* and *P. chrysogenum*. This species is characterised by the production of dark green conidia on MEA and CYA, yellow soluble pigment production on CYA incubated at 30 °C and a series of incompletely characterised extrolites.

Colony morphology — Colony diam, 7 d, in mm: CYA 30–45; CYA 15 °C 18–25; CYA 30 °C 18–27; CYA 37 °C: germination–4; MEA 30–40; YES 50–65; DG18 35–47; CYAS 40–55; creatine agar 15–20, weak to moderate growth, weak acid production.

Extrolites — Penicillins, chrysogine, roquefortine C and meleagrin, and andrastin A and the uncharacterised extrolites 'CRYPT' (4 compounds), 'POO', 'KNOLF', 'TBRE', 'FJOR' (2 compounds).

Distribution & Ecology — This species has a world-wide distribution and is found in the USA (Florida, New Mexico, Ohio), South Shetland Islands, Antarctica, Argentina (San Juan), the UK (Henk et al. 2011, this study).

Barcode & Molecular based ID — DTO 148-I2, DTO 149-A4 and DTO 149-B3 share the same ITS sequence, which can be used for precise species identification (GenBank JX997025).

LIST OF SPECIES CURRENTLY ACCEPTED IN PENICILLIUM SECTION CHRYSOGENA

The following list includes accepted species in sect. *Chrysogena* and their presently accepted synonyms. Our data indicate that more species might exist in this section. For example, three phylogenetic species are present in *P. mononematosum* (according the PSC) and also *P. egyptiacum* might represent three taxa.

Penicillium allii-sativi Frisvad, Houbraken & Samson, this study.

Typus. Argentina, Mendoza, Lavalle, Col 3 de Mayo, garlic, *M. Makuch & J. Valdez* (CBS H-21058).

Penicillium chrysogenum Thom, Bull. Bur. Anim. Ind. USDA 118: 58, 1910; nom. cons.

Typus. USA, Connecticut, Storrs, ex cheese, 1904, *C. Thom* (IMI 24314 typ. cons.).

= *Penicillium citreoroseum* Dierckx, Ann. Soc. Sci. Bruxelles 25: 86. 1901; nom. rej.
= *Penicillium griseoroseum* Dierckx, Ann. Soc. Sci. Bruxelles 25: 86. 1901; nom. rej.
= *Penicillium brunneorubrum* Dierckx, Ann. Soc. Sci. Bruxelles 25: 88. 1901; nom. rej.
= *Penicillium notatum* Westling, Ark. Bot. 11, 1: 95. 1911.
= *Penicillium cyaneofulvum* Biourge, Cellule 33: 171. 1923.
= *Penicillium roseocitreum* Biourge, Cellule 33: 184. 1923.
= *Penicillium chlorophaeum* Biourge, Cellule 33: 249. 1923.
? = *Penicillium chrysogenum* var. *brevisterigma* Forster, Brit. Pat. 691: 242. 1953; (nom. inval. Art. 36.1; without Latin diagnosis).
= *Penicillium aromaticum* f. *microsporum* Romankova, Uchen. Zap. Lenin. Univ. Zhdanov 191: 102. 1955; (nom. inval. Art. 36.1; without Latin diagnosis).
= *Penicillium harmonense* Baghdadi, Novosti Sist. Nizsh. Rast. 5: 102. 1968.

Notes — *Penicillium brunneorubrum*, *P. citreoroseum* and *P. griseoroseum* predate *P. chrysogenum* but these names are formally *nomina rejicienda* (McNeill et al. 2006). No (ex-type) material was available of *P. chrysogenum* var. *brevisterigma* and this invalidly described species is tentatively placed in synonymy with *P. chrysogenum.*

Penicillium confertum (Frisvad et al.) Frisvad, Mycologia 81: 852. 1989.

Typus: USA, Arizona, 6 km east of Portal, cheek pouch of *Dipodomys spectabilis* (IMI 296930).

= *Penicillium glandicola* var. *confertum* Frisvad, Filt. & Wicklow, Canad. J. Bot. 65: 769. 1987.

Penicillium desertorum Frisvad, Houbraken & Samson, this study.

Typus: USA, Wyoming, 20 km east of Little America, cool desert soil under *Oryzopsis hymenoides*, *J.C. Frisvad* (CBS H-21056).

Penicillium dipodomyis (Frisvad, Filt. & Wicklow) Banke, Frisvad & S. Rosend., Int. Mod. Meth. Pen. Asp. Clas.: 270. 2000.

Typus. USA, Arizona, 6 km east of Portal, cheek pouch of *Dipodomys spectabilis* (IMI 296926).

= *Penicillium chrysogenum* var. *dipodomyis* Frisvad, Filt. & Wicklow, Canad. J. Bot. 65: 766. 1987.
= *Penicillium dipodomyis* (Frisvad, Filt. & Wicklow) Banke, Frisvad & S. Rosend., Mycol. Res. 101: 622. 1997 (nom. inval. Art. 33.3, basionym not cited).

Penicillium egyptiacum J.F.H. Beyma, Zentralbl. Bakteriol., 2. Abt., 88: 137. 1933.

Typus. Egypt, Cairo, soil, *Y.S. Sabet* (CBS 344.32).

= *Eupenicillium egyptiacum* (J.F.H. Beyma) Stolk & D.B. Scott, Persoonia 4: 401. 1967.
= *Eupenicillium molle* Malloch & Cain, Canad. J. Bot. 50: 62. 1972.
= *Penicillium nilense* Pitt, The genus *Penicillium*: 145. 1980, '1979'.
= *Penicillium molle* Pitt, The genus *Penicillium*: 148. 1980, '1979'.

Penicillium flavigenum Frisvad & Samson, Mycol. Res. 101: 620. 1997.

Typus. Denmark, wheat flour, *J.C. Frisvad*, 1985 (CBS 419.89).

Penicillium goetzii J. Rogers, Frisvad, Houbraken & Samson, this study.

Typus. Canada, Calgary, soil, *J. Bissett* (CBS H-21061).

Penicillium halotolerans Frisvad, Houbraken & Samson, this study.

Typus. Egypt, salt marsh, *A.H. Moubasher* (CBS H-21060).

Penicillium kewense G. Sm., Trans. Brit. Mycol. Soc. 44: 42. 1961.

Typus. Contaminant of a culture stored under mineral oil, *G. Smith* (LSHTM BB400).

= *Eupenicillium crustaceum* F. Ludw., Lehrb. Nied. Krypt.: 263. 1892.

Notes — *Penicillium crustaceum* was described by Fries (1829: 407). Crusts of conidia are formed by several species in *Penicillium* and Fries' description of this species is not informative enough for to characterise it in modern terms. Although its exact identity cannot be established, Raper & Thom (1949: 515) indicated that this species could be the same as *P. expansum*. Brefeld (1874) described the formation of sclerotioid cleistothecia in detail, in a species he identified as "*Penicillium crustaceum* Fries, *Penicillum glaucum* Link". It is unlikely that Brefeld's fungus represented the species described by Link and Fries. The illustrations of the conidial state strongly suggest that Brefeld dealt with mixed cultures (Stolk & Scott 1967). Winter (1887) included Brefeld's fungus in his work on ascomycetes (as *P. crustaceum*) and later, Ludwig (1892) introduced the generic name *Eupenicillium* based on the name used by Winter and named this species *Eupenicillium crustaceum*. *Penicillium kewense* most closely resembles the species described by Brefeld (Scott & Stolk 1967) and therefore, applying single name nomenclature, we use this epithet for strains formerly identified as *E. crustaceum*.

Penicillium lanosocoeruleum Thom, the Penicillia: 322. 1930.

Typus. USA, culture contaminant of *P. cyclopium* culture, *C. Thom* (NRRL 888).

= *Penicillium aethiopicum* Frisvad, Mycologia 81: 848. 1990.

Notes — *Penicillium aethiopicum* CBS 484.84^{T} and *P. lanosocoeruleum* CBS 215.30^{T} are conspecific. This is supported by molecular data, phenotypic characteristics and extrolite data. Both species form ellipsoidal conidia (Raper & Thom 1949: 436) and produce the extrolites griseofulvin, tryptoquialanins and viridicatumtoxin (Frisvad et al. 2004, Chooi et al. 2010, Gao et al. 2011). Strain IBT 5753 is fully genome sequenced (Chooi et al. 2010).

Penicillium mononematosum (Frisvad et al.) Frisvad, Mycologia 81: 857. 1990.

Typus. USA, Arizona, 6 km east of Portal, burrow system of *Dipodomys spectabilis* (IMI 296925).

= *Penicillium glandicola* var. *mononematosa* Frisvad, Filt. & Wicklow, Canad. J. Bot. 65: 767. 1987.
= *Penicillium granulatum* var. *mononematosa* (Frisvad, Filt. & Wicklow) Bridge, Kozak. & R.R.M. Paterson, Mycol. Pap. 165: 38. 1992.

Notes — Our phylogenetic analyses (Fig. 1, 2) reveal three distinct clades within *P. mononematosum*. The occurrence of two types (I and II) was described by Frisvad & Samson (2004: 126). Both 'type II' isolates (CBS 112575, CBS 10916) were isolated from salt marsh soil in Egypt and cluster together in our phylogenetic analysis.

Penicillium nalgiovense Laxa, Zentralbl. Bakteriol., 2. Abt., 86: 160. 1932.

Typus. Czech Republic, Ellischauer cheese (CBS 352.48 neotype).

Penicillium persicinum L. Wang, H.B. Zhou, Frisvad & Samson, Antonie van Leeuwenhoek 86: 177. 2004.

Typus. China, Qinghai: soil (HMAS 80638-1-4).

Penicillium rubens Biourge, Cellule 33: 265. 1923.

Typus. Unrecorded source, *P. Biourge*, CBS H-20595 (NRRL 792 = IBT 30129 = ATCC 9783 = CBS 129667).

? = *Penicillium baculatum* Westling, Svensk Bot. Tidskr. 14: 139. 1910.
? = *Penicillium meleagrinum* Biourge, Cellule 33: 184. 1923.
= *Penicillium camerunense* R. Heim, Nouvel & Saccas, Bull. Acad. Belg. C1. Sci., Ser. 5, 35: 52. 1949 (nom. inval. Art. 36, without Latin diagnosis).
= *Penicillium chrysogenum* mut. fulvescens Takash., Arima & S. Abe, J. Gen. Appl. Microbiol. 2, 1-2: 92. 1956 (nom. inval. Art. 36, without Latin diagnosis).
= *Penicillium chrysogenum* mut. fulvescens Takash., Arima & S. Abe ex C. Ramírez , Man. Atlas Penicil.: 364. 1982.

Notes — Raper & Thom (1949: 363) stated that NRRL 843 (= CBS 132210 = DTO 100-F6 = IBT 5303) was similar to *P. baculatum*, but no ex-type of *P. baculatum* has been saved in culture collections. Therefore, we decided to place this species in synonymy with *P. rubens*. Similarly, no type material of *P. meleagrinum* is available. Raper & Thom (1949) based their description of *P. meleagrinum* on NRRL 836 (= CBS 349.48 = DTO 098-G1 = IBT 4350) and NRRL 2136 (= CBS 131532 = DTO 100-H3 = IBT 30138 = NRRL 2136). The former strain is re-identified here as *P. rubens* and the latter as *P. chrysogenum*. The exact position of *P. meleagrinum* is uncertain.

Penicillium sinaicum Udagawa & S. Ueda, Mycotaxon 14: 266. 1982.

Typus. Egypt, Sinai Peninsula, Suez Canal, 30 km north from Port Said, marine sludge, *H. Komatsu* (NHL 2894).

= *Eupenicillium sinaicum* Udagawa & S. Ueda, Mycotaxon 14: 266. 1982.

Penicillium tardochrysogenum Frisvad, Houbraken & Samson, this study.

Typus. Antarctica, Dry Valley, *S. Onofri* (CBS H-21057).

Penicillium vanluykii Frisvad, Houbraken & Samson, this study.

Typus. USA, New Mexico, Carlsbad, Lechuguilla Cave, *D. Northup* (CBS H-21059).

DISCUSSION

With this revision, *Penicillium* sect. *Chrysogena* now consists of 18 phylogenetic and phenotypic species, most of which are also diagnosable morphologically. Compared with the classification of Houbraken & Samson (2011), six new species are added to this section and *P. molle* is synonymised with *P. egyptiacum* and *P. aethiopicum* with *P. lanosocoeruleum*. Recent taxonomic studies on *P. chrysogenum* determined the presence of four lineages within this species (Scott et al. 2004, Henk et al. 2011, Houbraken et al. 2011a). Our results confirm those of Houbraken et al. (2011a), demonstrating that one lineage is

centred on the ex-type strain of *P. chrysogenum* CBS 306.48 (= 'clade 1' in Scott et al. (2004)) and another on *P. rubens* CBS 129667 (= 'Fleming species' fide Henk et al. (2011); 'clade 4' in Scott et al. (2004)). The other two lineages found by Scott et al. (2004) and Henk et al. (2011) do not correspond with each other. Our data support those of Henk et al. (2011) and show that the two other lineages recognised by Scott et al. (2004; 'clade 2' and 'clade 3') still represent *P. chrysogenum*. A comparison of sequences deposited in GenBank show that the two groups of isolates listed as 'species A' and 'species B' by Henk et al. (2011) correspond with the newly described species *P. vanluykii* and *P. allii-sativi*. A large number of species resembling *P. chrysogenum* have been described historically (Samson et al. 1977, Pitt 1980) and all are placed here in synonymy with *P. chrysogenum* or *P. rubens*. Houbraken et al. (2011a) focused on penicillin producing strains and also included the ex-type strains of *P. griseoroseum*, *P. notatum* and *P. rubens*. Various synonyms of *P. chrysogenum* were included in the study of Henk et al. (2011) and their species designations largely correspond with the current study. The only exception is the placement of *P. camerunense* CBS 339.52^{T} in synonymy with *P. rubens*, whereas Henk et al. (2011) treated this species as *P. chrysogenum*. Our multigene phylogeny (Fig. 1) and the haplotype network analysis (Fig. 4) demonstrate that *P. aromaticum* f. *microsporum* (nom. inval.), *P. brunneorubrum*, *P. chlorophaeum*, *P. citreorosum*, *P. cyaneofulvum*, *P. griseoroseum*, *P. harmonense*, *P. notatum* and *P. roseocitreum*, are all synonyms of *P. chrysogenum*. Additionally, *P. camerunense* (nom. inval.) and *P. chrysogenum* mut. fulvescens should be placed in synonymy with *P. rubens*. An overview of accepted species and their synonyms is given in the Taxonomy part of this paper.

Pitt (1974, 1980) treated *E. egyptiacum*, *P. kewense* (as *E. crustaceum*) and *E. molle* as distinct species based on the ornamentation and size of the ascospores. In contrast, Stolk & Samson (1983) defined *P. kewense* (as *E. crustaceum*) as one variable species. Although Stolk & Samson (1983) included five ascospore patterns in their circumscription of *P. kewense*, they treated *E. molle* and *E. egyptiacum* as small-spored strains of *P. kewense*. They also observed the same ornamentation, but the ribs and ridges on ascospores of *E. egyptiacum* were less pronounced. Our results show that *P. kewense* sensu Stolk & Samson (1983) can be divided into at least three species: *P. egyptiacum*, *P. goetzii* and *P. kewense*, while *P. molle* is placed in synonymy with *P. egyptiacum*. Our study also shows that the isolates with large ascospores represent a separate species, here named *P. goetzii*. Phylogenetic analyses indicate that this group of related species probably contains additional new species. For example, three lineages occur in *P. egyptiacum*, which might represent distinct species. Also, CBS 653.82 (= CBS 227.81 = NRRL 2094) forms a single strain lineage and Raper & Thom (1949: 146) noted that this strain is intermediate between *P. brefeldianum* and *P. egyptiacum*. The description of this species is deferred until more strains of this tentative new species are collected.

Polyphasic characterisation of *Penicillium* species allows identification using several different types of data, including colony characters and micromorphology (morphological species concept), extrolite profiles (phenotypic species concept) and correlations among multigene phylogenies (phylogenetic species concept). The new species described here meet all of these criteria as distinct species, although their morphological characters are similar to other species of *Penicillium* sect. *Chrysogena*, which are notoriously difficult to identify using classical taxonomic techniques. In common with other species level studies of *Penicillium* subgenus *Penicillium*, sequences of the ITS region have minimal resolution for distinguishing closely related species in sect. *Chrysogena* (Skouboe et al. 1999, Houbraken et al. 2011b). The individual gene trees based on RPB1 and β-tubulin sequence data generated the best clustering of species, and these genes are therefore promising loci for barcoding within this genus. Neither gene tree correlates well with the series proposed within sect. *Chrysogena* by Frisvad & Samson (2004), as already noted by Samson et al. (2004).

Both sexual and asexual species are accommodated in the currently defined sect. *Chrysogena*. The sexually competent members (*P. kewense*, *P. goetzii*, *P. egyptiacum*, *P. sinaicum*) are all homothallic and there are indications that *P. chrysogenum*, *P. dipodomyis* and *P. rubens* may reproduce in a heterothallic manner (Hoff et al. 2008, Henk et al. 2011, Henk & Fischer 2011). Repeated attempts to induce a sexual state in *P. chrysogenum* and related species were unsuccessful (Hoff et al. 2008, Eagle 2009, Henk & Fischer 2011, Henk et al. 2011, J. Houbraken unpubl. res., K.A. Seifert unpubl. res.). However, some unpublished crossing experiments with *P. chrysogenum* isolates have apparently resulted in the production of cleistothecia and ascospores, similar to those described recently for *P. kewense* (Böhm et al. 2012). The limited number of successful mating experiments in *P. chrysogenum* might be explained by the strains used in these experiments. Perhaps strains maintained for long periods in culture collections lose their fertility. For example, the heterothallic *Histoplasma capsulatum* lost fertility rapidly during laboratory passage, leading to speculation that selective pressures might serve to maintain fertility in the environment (Kwon-Chung et al. 1974, Fraser et al. 2007). For the heterothallic and heat resistant *Byssochlamys spectabilis* (syn. *Paecilomyces variotii*), it was shown that only strains derived from pasteurised products were fertile (Houbraken et al. 2008). It will therefore be promising to repeat the mating experiments with *Penicillium* strains freshly isolated from nature. Another possible reason for unsuccessful crossing experiments may be stringent conditions required for successful mating. Various growth factors induce the formation of cleistothecia, such as temperature, light, nutrient and oxygen levels (Han et al. 2003). Recently, Houbraken et al. (2010) showed that *P. psychrosexualis*, a species related to *P. roqueforti*, produces abundant cleistothecia at low temperatures (9–15 °C). The production of a sexual stage at low temperatures might be more widespread in *Penicillium*, and mating experiments at this temperature might result in the discovery of a sexual stage in other species.

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