



# Delimitation and characterisation of *Talaromyces purpurogenus* and related species

N. Yilmaz<sup>1,5</sup>, J. Houbraken<sup>1</sup>, E.S. Hoekstra<sup>2</sup>, J.C. Frisvad<sup>3</sup>, C.M. Visagie<sup>1,4</sup>, R.A. Samson<sup>1</sup>

## Key words

*Penicillium purpurogenum*  
rubratoxin  
polyphasic taxonomy

**Abstract** Taxa of the *Talaromyces purpurogenus* complex were studied using a polyphasic approach. ITS barcodes were used to show relationships between species of the *T. purpurogenus* complex and other *Talaromyces* species. *RPB1*, *RPB2*,  $\beta$ -tubulin and calmodulin sequences were used to delimit phylogenetic species in the complex. These data, combined with phenotypic characters, showed that the complex contains four species: *T. purpurogenus*, *T. ruber* comb. nov. and two new species *T. amestolkiae* sp. nov. and *T. stollii* sp. nov. The latter three species belong to the same clade and *T. purpurogenus* is located in a phylogenetic distant clade. The four species all share similar conidiophore morphologies, but can be distinguished by macromorphological characters. *Talaromyces ruber* has a very distinct colony texture on malt extract agar (MEA), produces bright yellow and red mycelium on yeast extract sucrose agar (YES) and does not produce acid on creatine sucrose agar (CREA). In contrast, *T. amestolkiae* and *T. stollii* produce acid on CREA. These two species can be differentiated by the slower growth rate of *T. amestolkiae* on CYA incubated at 36 °C. Furthermore, *T. stollii* produces soft synnemata-like structures in the centre of colonies on most media. Extrolite analysis confirms the distinction of four species in the *T. purpurogenus* complex. The red diffusing pigment in *T. purpurogenus* is a mixture of the azaphilone extrolites also found in *Monascus* species, including N-glutaryl-rubropunctamine and rubropunctatin. *Talaromyces purpurogenus* produced four different kinds of mycotoxins: rubratoxins, luteoskyrin, spiculisporic acid and rugulovasins and these mycotoxins were not detected in the other three species.

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## INTRODUCTION

*Penicillium purpurogenum* was described by Stoll (1903–1904) and the type culture (CBS 286.36) was isolated as a culture contaminant of *Aspergillus oryzae* in Japan. This species was characterised by dark grey-green colonies with mycelium varying from pinkish to yellow and yellow red, as well as the production of red pigments on potato agar. In the same paper, Stoll (1903–1904) also described *P. rubrum* and this isolate was provided by Grassberger, who authorised Stoll to describe the species. It was characterised by dark-green colonies on sugar-gelatine agar. The culture Stoll used for his description is no longer available and therefore it was re-described by Raper & Thom (1949) based on strains NRRL 1062 (CBS 370.48) and NRRL 2120. According to Raper & Thom's concept, *P. purpurogenum* forms spreading dark yellow-green colonies with rough-walled conidia while *P. rubrum* produces more restricted grey-green colonies with smooth-walled conidia. Pitt (1980) used a broader species concept for *P. purpurogenum* and considered the differences proposed by Raper & Thom (1949) to distinguish *P. purpurogenum* from *P. rubrum* to be insignificant. He also considered *P. crateriforme* to be conspecific with *P. purpurogenum* based on the red pigments produced and its ability to grow at 37 °C, and based on the original descriptions

he also considered *P. sanguineum* and *P. vanilliae* synonyms (Pitt 1980).

Both *P. purpurogenum* and *P. rubrum* are claimed to produce rubratoxins (Wilson & Wilson 1962, Moss et al. 1968, Natori et al. 1970). Because *P. rubrum* was not accepted by Pitt (1980) and *P. purpurogenum* has been regarded as a producer of glauconic acid rather than rubratoxins, Frisvad (1989) considered *P. crateriforme* to be the correct name for the species producing rubratoxins. Rubratoxin B is mutagenic, hepatotoxic, nephrotoxic and splenotoxic to several animals (Burnside et al. 1957, Lockard et al. 1981, Surjono et al. 1985, Engelhardt et al. 1987, Kihara et al. 2001). The first human rubratoxicosis was reported by Richer et al. (1997). Three teens drinking homemade rhubarb wine, which had a high level of rubratoxin B became critically ill, with one requiring immediate liver transplant. Even though rubratoxin B has negative health effects, it has potential as an anti-tumor agent (Wang et al. 2007, Wada et al. 2010). *Penicillium crateriforme* has also been reported to produce the mouse mycotoxin spiculisporic acid (Oxford & Raistrick 1934, Fujimoto et al. 1988). Later, spiculisporic acid has been used as a commercially available biosurfactant (Ishigami et al. 2000). Isolates belonging to *P. crateriforme* also produces the clavine alkaloids rugulovasines A and B and chlororugulovasines A & B (Dorner et al. 1980, the producer ATCC 44445 was identified as *P. rubrum*) (see Table 2). *Penicillium purpurogenum* is an important species in biotechnology for its ability to produce enzymes such as xylanases and cellulases (Steiner et al. 1994, Belancic et al. 1995) and pigments, which are used as natural colorants and biosorption (Say et al. 2004, Mapari et al. 2009, Jeya et al. 2010, Zou et al. 2012). *Penicillium purpurogenum* inoculated oak chips are used in artificial aging of Italian wines (Petrucci et al. 2010, 2012).

<sup>1</sup> CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; corresponding author e-mail: r.samson@cbs.knaw.nl.

<sup>2</sup> Albert Schweitzerstraat 2, 6562 XA Groesbeek, The Netherlands.

<sup>3</sup> Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark.

<sup>4</sup> Department of Microbiology, University of Stellenbosch, Private Bag X1, Stellenbosch 7600, South Africa.

<sup>5</sup> Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Table 1 *Talaromyces* strains used in this study.

Species	CBS no.	Other numbers	Substrate and locality	ITS	$\beta$ -tubulin	calmodulin	RPB1	RPB2
<i>T. amestolkiae</i>		DTO 173F3	Soli; Indonesia	JX965223	JX965330	JX965189	JX965248	
		FRR 1097	Chicken feed suspected to be toxic; Victoria, Australia					
		IBT 20202	Greenhouse; Lyngby, Denmark					
		IMI 061385 = KCTC 6774 = IBT 4538	Paper pulp; UK, 1955	JX315668	JX315624	JX315654	JX315687	JX315706
		IMI 104624 = IBT 3968	Plastic; UK, 1963	JX315669	JX315625	JX315653	JX315688	JX315707
		IMI 147406 = KCTC 6773 = IBT 21723	<i>Melus pumila</i> ; Belfast, North Ireland, UK, 1970	JX965247	JX965331	JX965196	JX965284	JX965319
		IBT 19715	Air, cake factory; Denmark	JX965214	JX965321	JX965190	JX965249	JX965285
		IBT 23821	Soli; Scafati, Italy	JX965215	JX965322	JX965191	JX965250	JX965286
		IBT 29986	Contaminant of agar plate; Denmark	JX965216	JX965323	JX965192	JX965251	JX965287
	252.31	NRRL 1034	<i>Narcissus</i> bulb; the Netherlands					
	263.93		Bronchoalveolar lavage of immunocompetent female patient with pneumonia by <i>Mocardia</i>					
	264.93		Bronchoalveolar lavage of male AIDS-patient; New Caledonia					
	274.95		Sculpture in castle Troja; Prague, Czech Republic					
	277.95	AK 128/94 = AK 188/94	Soli; Chvaletice, Czech Republic					
	329.48 <sup>1</sup>	ATCC 10445 = ATCC 8725 = CCTM 3641 = CECT 2913 = DSM 2213 = IFO 5857 = IHEM 4008 = IMI 034912 = NRRL 1032a = QM 7562	Air contaminant; Washington DC, USA					
	353.93	DAOM 31954 = DSM 1184	Angiosperm wood; Ontario, Canada	JX315672	JX315626	JX315652	JX315691	JX315710
	365.48 <sup>2</sup>	ATCC 10486 = IMI 040035 = NRRL 1066 = QM 1960	Unknown source; USA					
	379.97		Sputum; Leiden, the Netherlands	JX965217	JX965324	JX965193	JX965252	JX965288
	390.96		Contaminant of <i>Coniothyrium minutans</i> ; Italy	JX965218	JX965325	JX965198	JX965253	JX965289
	433.62		Ground domestic waste; Verona, Italy	JX965219	JX965326	JX965194	JX965254	JX965290
436.62		Alum solution; unknown origin	JX965220	JX965327	JX965195	JX965255	JX965291	
626.93		<i>Ananas carnosus</i> cultivar; Martinique	JX965221	JX965328	JX965197	JX965256	JX965292	
884.72		Manure; France	JX315678	JX315622	JX315651	JX315697	JX315716	
101305		Soli; Hong Kong, China	JX965224	JX965332		JX965259	JX965293	
101349		Soli; Hong Kong, China	JX965225	JX965333		JX965260	JX965294	
102303		Raw coffee beans; unknown origin						
102689		Air; Japan	JX965226	JX965334		JX965261	JX965295	
113143	IMI 079195 = NRRL 1132	Contaminant of culture; Washington DC, USA, 1940						
132695	DTO 189C1 = IBT 23485	Wheat; Italy	JX965228	JX965338	JX965199	JX965262	JX965297	
132696 <sup>T</sup>	DTO 179F5	Ex-type strain of <i>Talaromyces amestolkiae</i> . House dust; South Africa	JX315660	JX315623	JX315650	JX315679	JX315698	
132697	DTO 189D1 = IBT 28795	Coffee cherries; Uganda	JX965227	JX965337			JX965296	
132698	DTO 189B5 = IBT 20197	Greenhouse; Lyngby, Denmark	JX965229	JX965336		JX965263	JX965298	
<i>T. purpurogenus</i>		DTO 173E6	Soli; Indonesia	JX965230	JX965339		JX965264	JX965299
		189B4 = IBT 18380; CCRC 32601	Dung of pig; Taipei City, Taiwan	JX965231	JX965340		JX965265	JX965300
		193H1 = IBT 12779	Oregano; imported to Denmark	JX965232	JX965342		JX965266	JX965302
		193H5 = IBT 3933		JX965233	JX965341		JX965267	JX965301
	184.27	FRR 1047 = IMI 094165 = LSHB P154 = MUCL 29224 = ATCC 52215 = NRRL 1057 = KCTC 6784 = Thom 4894.13	Ex-type of <i>Penicillium crateriforme</i> . Soli; Louisiana, USA	JX315665	JX315637	JX315658	JX315684	JX315703

286.36 <sup>†</sup>	IMI 091926 = CECT 20441 = KCTC 6821 = LSHB P.48 = NCTC 586 = Thom 17	Ex-type strain of <i>Talaromyces purpurogenus</i> . Parasitic on a culture of <i>Aspergillus oryzae</i> ; Japan	JX315671	JX315639	JX315655	JX315690	JX315709
108923	ATCC 20204 = IBT 4183 = IFO 5722	Sputum; Leiden, Netherlands	JX965236	JX965343	JX965200	JX965268	JX965303
113158	IBT 11628	Unknown source; Japan	JX965235	JX965344	JX965201	JX965269	JX965304
122411	IBT 17430 = DTO 49F6	Wheat, Winnipeg, Canada	JX965234	JX965345	JX965202	JX965270	JX965307
132707	IMI 136128 = MR 008 = IBT 3658 = IBT 5015 = DTO 189A1	Mould field corn; Wisconsin, USA	JX315661	JX315638	JX315642	JX315680	JX965305
101965 <sup>3</sup>	DTO 49F7 = DTO 189 A4 = IBT 10612 =	Unknown source	JX965237	JX965347	JX315659	JX315682	JX965306
122434 <sup>4</sup>	IBT 3560 = CCRC 31681 = BCRC 31681 = NCIM 762 = NRRL 1059 = ATCC 10064 = Thom 5694.11	Unknown source. Identified as <i>Penicillium purpurogenum</i> by Raper & Thom (1949); collected as <i>Penicillium sanguineum</i> by CBS	JX315663	JX315640	JX315659	JX315682	JX315701
	DTO 189A7 = IBT 13594 = DAOM 215356	Soil in forest; Canada	JX965238	JX965348	JX965203	JX965271	JX965308
	FRR 1503 = ATCC 48975 = IAM 13746	Weathered preserved wood stakes; North Queensland, Australia.					
	NRRL 1180 = IBT 3940	Unknown source; USA					
195.88	NRRL 1159 = IBT 4423	Chickens in cold storage; unknown	JX965240	JX965350	JX965204	JX965273	JX965310
196.88	FRR 1714 = IBT 3951	Unknown	JX315666	JX315627	JX315657	JX315685	JX315704
237.93	ACC 828-81	Unknown	JX315667	JX315628	JX315656	JX315686	JX315705
368.73		Unknown			JX965351	JX965274	
370.48 <sup>5</sup>	ATCC 10520 = IMI 040036 = NRRL 1062 = VKM F-345 = IBT 4431 = IBT 3927	Ex-neotype. Currency paper; Washington, USA	JX315673	JX315630	JX315649	JX315692	JX315711
868.96		Tracheal secretion; Heidelberg, Germany	JX315677	JX315631	JX315643	JX315696	JX315715
101144	IMI 178519 = IBT 10708	Ex experimental paint sample; Woolwich, UK	JX965239	JX965352	JX965205	JX965272	JX965309
113140	DTO 193 H7 = IBT 19712	Air cake factory; Denmark	JX965241	JX965353	JX965206	JX965275	JX965311
132699	DTO 189B7 = IBT 21772	Ex sandy soil; Marhaba Club Beach, Sousse, Tunisia	JX965242	JX965354	JX965207	JX965276	JX965312
132700	DTO 173G7	Soil; Indonesia	JX965243	JX965354	JX965207	JX965277	JX965313
132703	DTO 193I3 = IBT 10708 = IMI 170519	Ex experimental paint sample; Woolwich, UK					JX965314
132704 <sup>NT</sup>	DTO 193H6 = IBT 10703 = CBS 113137	Aircraft fuel tank; UK	JX315662	JX315629	JX315641	JX315681	JX315700
169.91 <sup>6</sup>	NRRL 1033	Unknown substrate; South Africa identified as <i>Penicillium funiculosum</i> by Raper & Thom (1949)	JX315664	JX315634	JX315647	JX315683	JX315702
265.93		Bronchoalveolar lavage of patient after lung transplantation (subclinical); France	JX315670	JX315635	JX315648	JX315689	JX315708
372.87 <sup>7</sup>		Faeces of a woman; Hamburg	JX965244	JX965355			
408.93 <sup>T</sup>		Ex-type strain of <i>Talaromyces stollii</i> ; AIDS patient; the Netherlands	JX315674	JX315633	JX315646	JX315693	JX315712
581.94		Unknown	JX315675	JX315632	JX315645	JX315694	JX315713
582.94		Unknown	JX965245	JX965356	JX965208	JX965278	
624.93		<i>Ananas carmosus</i> cultivar; Martinique	JX315676	JX315636	JX315644	JX315695	JX315714
625.93		<i>Ananas carmosus</i> cultivar; Martinique		JX965360	JX965211	JX965279	JX965316
100372		Pineapple; location unknown		JX965357	JX965210	JX965282	JX965317
132705	DTO 172F7	Soil; Indonesia		JX965358	JX965212	JX965280	JX965318
132706	DTO 28C1	Indoor air from bakery, Avenhorn, the Netherlands	JX965246	JX965359	JX965213	JX965283	JX965320

<sup>1</sup> NRRL 1032a was identified as *Penicillium funiculosum* by Raper & Thom (1949)

<sup>2</sup> Identified as *Penicillium purpurogenum* var. *rubisclerotiorum* by Raper & Thom (1949). It produced limited numbers of dark red sclerotia.

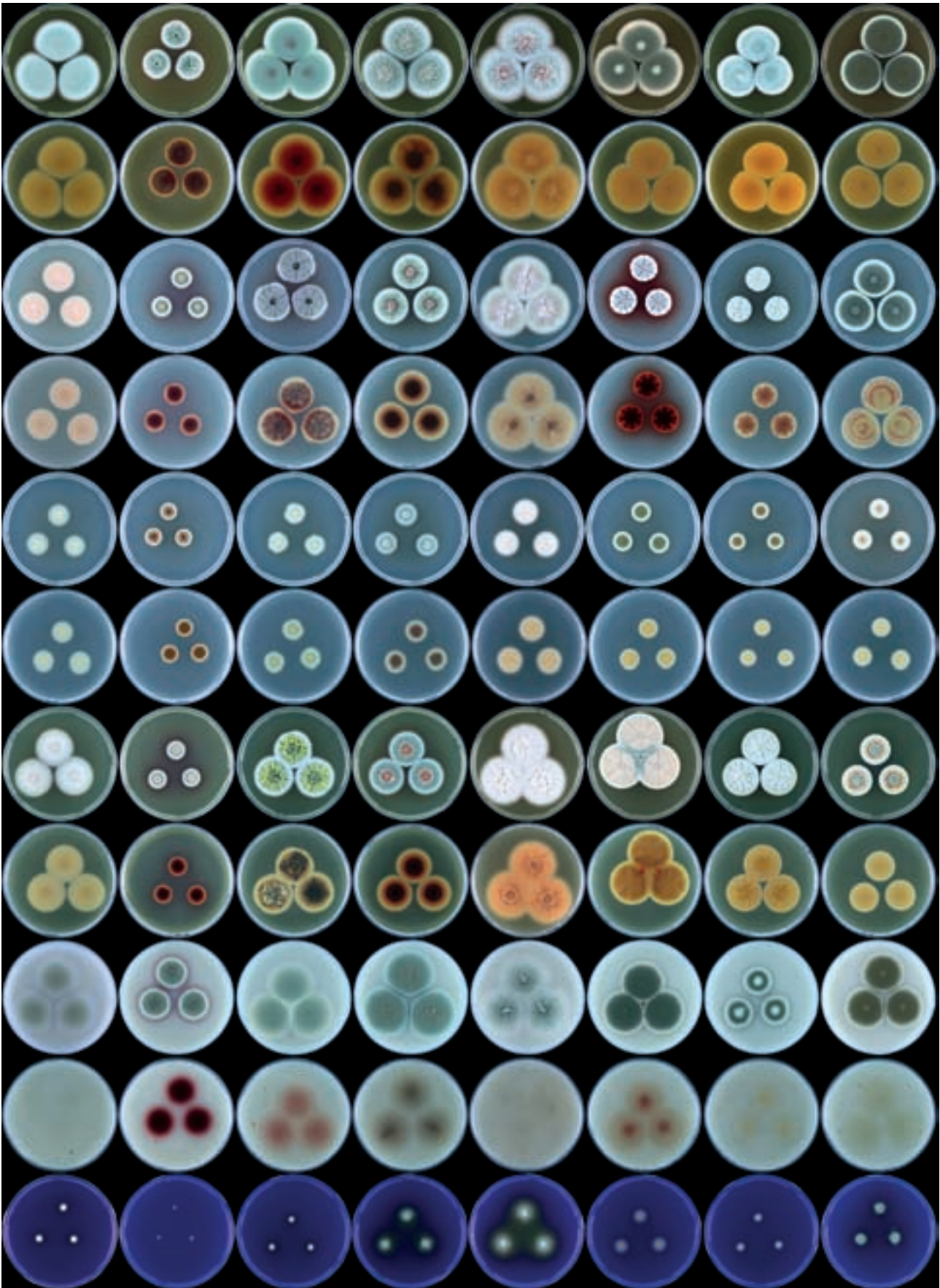
<sup>3</sup> The isolate was sent to CBS by S. Ochiai, Jonquil Consulting Inc., Tokyo, Japan.

<sup>4</sup> Raper & Thom (1949) reported faster growth and floccose margins for this strain. Pitt (1980) does not mention this strain.

<sup>5</sup> NRRL 1062 was used by Raper & Thom (1949) to describe *Penicillium rubrum*.

<sup>6</sup> NRRL 1033 was identified as *Penicillium funiculosum* by Raper & Thom (1949).

<sup>7</sup> Identified as *Penicillium dendriticum*. The isolate was received from Dr E. Dollefeld, Hamburg.



**Fig. 1** Agar colonies of species of the *Talaromyces purpurogenus* complex on different media. Columns, left to right: *T. ruber* (CBS 370.48), *Talaromyces* sp. (NRRL 2120), *T. ruber* (CBS 132704<sup>NT</sup>), *T. amestolkiae* (CBS 132696<sup>T</sup>), *T. stollii* (CBS 408.93<sup>T</sup>), *T. purpurogenus* (CBS 132707), *P. crateriforme* (CBS 184.27<sup>T</sup>), *P. sanguineum* (CBS 122434). Rows to bottom: MEA obverse, MEA reverse, CYA obverse, CYA reverse, DG18 obverse, DG18 reverse, YES obverse, YES reverse, OA obverse, OA obverse, CREA reverse incubated at 25 °C for 7 d.

Benjamin (1955) introduced the name *Talaromyces* as a sexual morph and this genus was characterised as producing soft yellow ascomata that consist of interwoven hyphae. Following the concept of single name nomenclature, 40 species from *Penicillium* subg. *Biverticillium* were transferred and combined into *Talaromyces* (Samson et al. 2011). The morphologically circumscribed species *Penicillium purpurogenum* sensu Pitt (1980) is one of several complexes of cryptic phylogenetic species that occur in the genus.

In the current study, the *T. purpurogenus* species complex was revised based on a polyphasic approach incorporating macro- and micro-morphology, extrolite production and multi-gene derived phylogeny. The phylogenetic relationships between species of the *T. purpurogenus* complex and other members of *Talaromyces* are studied using ITS barcodes. For the detailed delimitation of phylogenetic species, sequences of four alternative genes,  $\beta$ -tubulin, calmodulin, *RPB1* and *RPB2*, were used.

## MATERIALS AND METHODS

### Strains

Cultures used for comparisons in this study were obtained from the culture collections of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands, the IBT culture collection, Lyngby, Denmark and fresh isolates deposited in the working collection of the Department of Applied and Industrial Mycology (DTO), housed at CBS. Strains studied are listed in Table 1.

### Morphological analysis

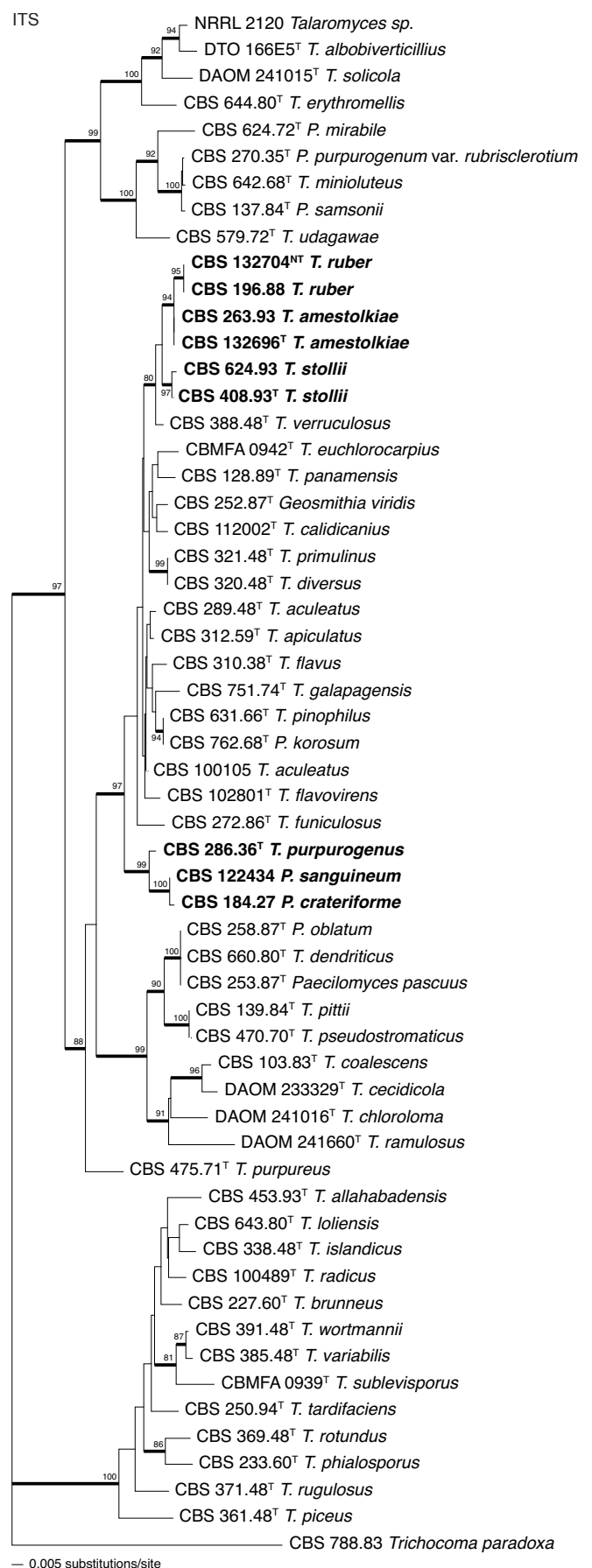
Macroscopic characters were studied on Czapek yeast extract agar (CYA), CYA supplemented with 5 % NaCl (CYAS), yeast extract sucrose agar (YES), creatine sucrose agar (CREA), dichloran 18 % glycerol agar (DG18), oatmeal agar (OA) and malt extract agar (Oxoid) (MEA). The strains were inoculated at three points on 90-mm Petri dishes and incubated for 7 d at 25 °C in darkness. All media were prepared as described by Samson et al. (2010). The temperature-growth response of strains was studied on CYA. Strains were inoculated at 3 points and incubated at 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36 and 40 °C for 7 d in darkness. After incubation, the colony diameter on the various agar media was measured. The degree of sporulation, obverse and reverse colony colours and the production of soluble pigments were also determined. Colony colours were described using Kornerup & Wanscher (1967). Colonies were photographed with a Canon EOS 400D. Species were characterised microscopically by preparing slides from MEA. Lactid acid was used as mounting fluid. Specimens were examined using a Zeiss AxioSkop2 plus microscope, and the NIS-Elements D software package from Nikon was used for making photographs and taking measurements.

### DNA extraction, PCR amplification and sequencing

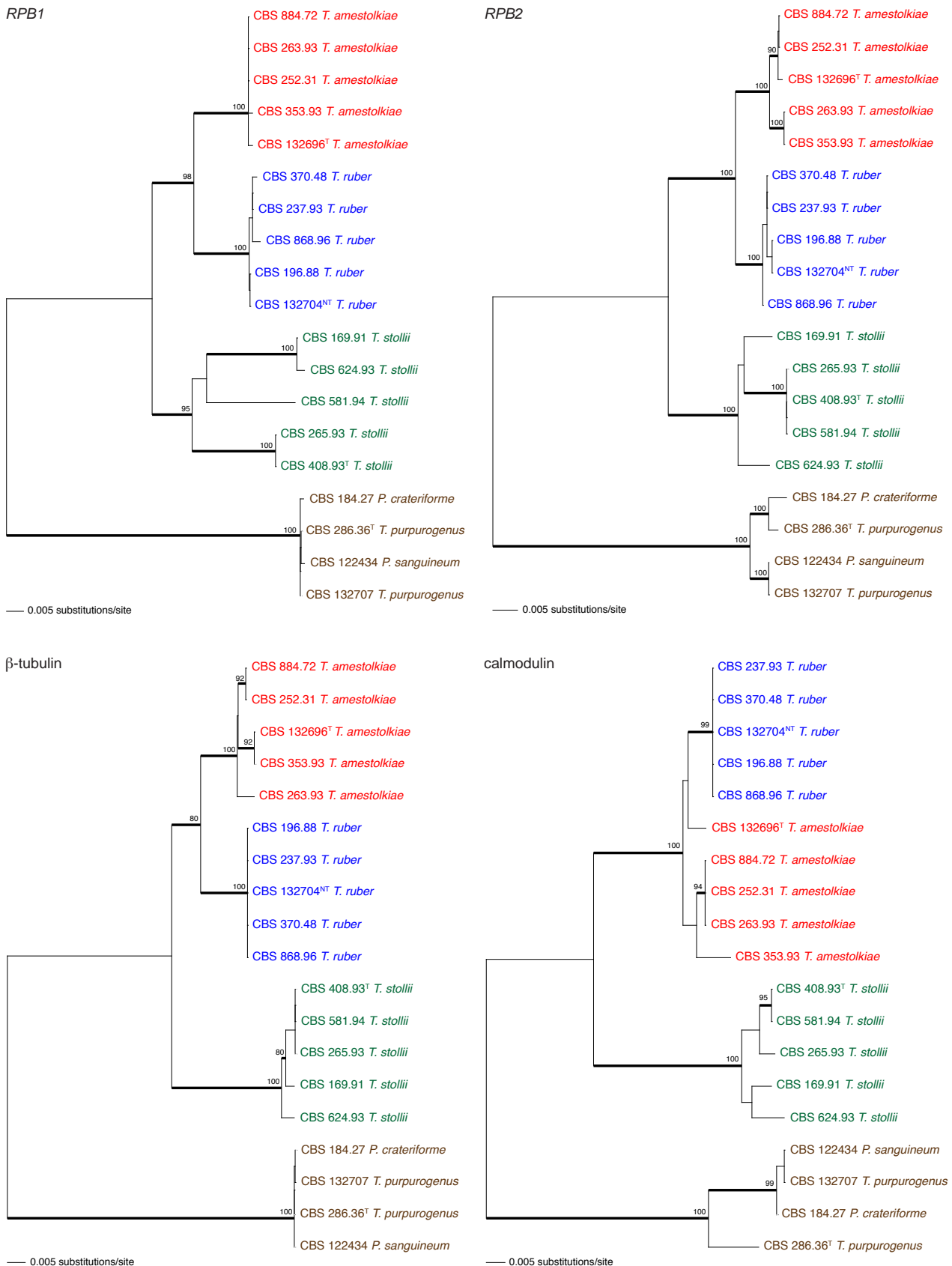
DNA extractions were prepared from strains grown for 7 to 14 d on MEA using the Ultraclean™ Microbial DNA isolation Kit (Mo-Bio, Solana Beach, USA). Extracted DNA was stored at -20 °C. The ITS regions and regions of the  $\beta$ -tubulin, calmodulin, *RPB1* and *RPB2* genes were amplified and sequenced according to previously described methods (Houbraken et al. 2007, 2011, 2012, Houbraken & Samson 2011, Samson et al. 2011).

### Data analysis

Sequence contigs were assembled using Seqman from DNA-Star Inc. Newly generated ITS sequences were included in a dataset obtained from the Samson et al. (2011) study. For the alternative genes, only isolates belonging to the *T. purpurogenus* species complex were included in the analysis. Datasets were



**Fig. 2** Neighbour-joining tree of the ITS1-5.8S-ITS2 rDNA region, showing placement of species described in this paper and other closely related *Talaromyces* species. Numbers at branching nodes represent bootstrap values (1 000 replicates), with **bold** branches indicating bootstrap values higher than 80 %. *Trichocoma paradoxa* was selected as outgroup. All strains in this phylogram are regarded as *Talaromyces*, although they are sometimes labelled as *Sagenoma*, *Penicillium* or *Erythrogythotheca*.



**Fig. 3** Neighbour-joining trees of *RPB1*, *RPB2*,  $\beta$ -tubulin and calmodulin showing phylogenetic placement of the newly described species. Numbers at branching nodes represent bootstrap values (1 000 replicates), with **bold** branches indicating bootstrap values higher than 80 %. *Talaromyces purpurogenus* was selected as outgroup. Species indicated in **bold** are treated in this paper.

aligned using the Muscle software within MEGA5 (Tamura et al. 2011). Neighbour-joining analyses on individual datasets were performed in MEGA5 and node confidence determined using bootstrap analysis with 1 000 replicates. *Trichocoma paradoxa* (CBS 788.83) was selected as outgroup for ITS analysis. For the alternative gene phylogenies, *T. purpurogenus* was selected as

outgroup. Unique, newly generated sequences were deposited in GenBank and their accession numbers are shown in Table 1.

**Extrolites**

Extrolites were extracted from fungal strains grown on CYA, YES, and some strains were additionally grown on MEA and

OA at 25 °C for 7 d for extrolite extraction. Three agar plugs of each medium were extracted as described in Nielsen et al. (2011) and Houbraken et al. (2012). The extracts were analysed by using high performance liquid chromatography with diode-array detection (HPLC-DAD) (Frisvad & Thrane 1987) for extracts made before 2011 and by UHPLC-DAD (Houbraken et al. 2012) for extracts made later. The compounds eluting and detected were identified by comparing retention time, retention index and UV spectra measured from 200–600 nm. The UV spectra were compared to a database of UV spectra (Nielsen et al. 2011), and to literature data (see for example the UV spectrum of pestalasin A shown in Nonaka et al. 2011).

## RESULTS

Morphological examination of strains previously identified as *T. purpurogenus* showed the presence of four distinguishable morphological groups and these are treated here as distinct species (Fig. 1): *T. purpurogenus*, *T. ruber* comb. nov., *T. stollii* sp.

nov. and *T. amestolkiae* sp. nov. *Talaromyces purpurogenus* is distinct from the other three species by its inability to grow below 18 °C, slow growth on the agar media CYA, the production of a bright red diffusing pigment on CYA at 25 °C and bright yellow and orange mycelium on DG18 at 25 °C. *Talaromyces ruber* has a velvety texture on both CYA and MEA at 25 °C and produces bright yellow and red mycelium on YES. It also produces a very distinct colony texture on MEA, where bundles of hyphae are produced underneath the velvety texture. *Talaromyces amestolkiae* and *T. stollii* are distinguished from *T. ruber* and *T. purpurogenus* by the production of acid on CREA. *Talaromyces stollii*, however, does grow faster on CYA at 36 °C than *T. amestolkiae* and some of the studied *T. amestolkiae* strains produced sclerotia after 2 wk incubation at 25 °C. Furthermore, *T. stollii* has soft synnemata-like or tufted structures at the centre of colonies on most media. Morphological data is supported by phylogenetic results, as discussed below (Fig. 2, 3).

Barcodes of the ITS locus were used to study the phylogenetic relationship between strains previously identified as *T. purpuro-*

**Table 2** Strains of *Talaromyces purpurogenus* previously identified as *P. crateriforme*, *P. rubrum* or *P. purpurogenum* and their production of mycotoxins.

Original number	Other collection numbers	Toxin reported	Reference	Isolate data
P-13	NRRL 3290 = NRRL A-11785 = ATCC 26940 = KCTC 6825 = BRCC 31680 = IBT 3936	Rubratroxin A and B*	Wilson & Wilson (1962)	From Dennis N. Cox, Georgia, USA
1968-10-28a	IMI 136126 = MR 006 = IBT 10710	Rubratroxin A and B	Moss & Hill (1970)	Mould field corn, Wisconsin, EB Smalley
1968-10-28b	IMI 136127 = MR 007 = IBT 5016	Rubratroxin A and B	Moss & Hill (1970)	Mould field corn, Wisconsin, EB Smalley
1968-10-28c	IMI 136128 = MR 008 = IBT 3658 = IBT 5015 = DTO 189 A1	Rubratroxin A and B	Moss & Hill (1970)	Mould field corn, Wisconsin, EB Smalley
	IMI 112715 = MR 185 = IBT 10712	Rubratroxin A and B*	Moss & Hill (1970)	Rhizosphere of <i>Trifolium alexandrinum</i> , Egypt, A. El Esaily
	IMI 129717 = MR 043/RC	Rubratroxin A and B	Moss & Hill (1970)	PKC Austwick
	IMI 129718 = MR 043/OB6	Rubratroxin A and B	Moss & Hill (1970)	PKC Austwick
	IMI 129719 = MR 043/OA	Rubratroxin A and B	Moss & Hill (1970)	PKC Austwick
	IMI 129716 = MR 180	Rubratroxin B	Moss & Hill (1970)	Van der Walt, South Africa
	NRRL 2019 = IBT 3549	Rubratroxin B	Data reported here	Unknown source
FAT 1141	ATCC 20204 = IBT 4183 = IFO 5722 = CBS 113158	Rubratroxin B*	Data reported here	Japan, S. Abe
CP 187	ATCC 44445 = IBT 4433 = IBT 10711 = KCTC 16067 = CBS 113159	Rugulovasine A* and B*, chlororugulovasine A and B	Dorner et al. (1980)	Field corn kernel, Georgia, RA Hill
	ATCC 44445	Rubratroxin B*	Data reported here	Field corn kernel, Georgia, RA Hill
	CBS 286.36 = IMI 091926 = CECT 20441 = KCTC 6821 = LSHB P.48 = NCTC 586 = NCTC Ad 36 = Thom 17		Data reported here	Kral, Czech Republic (ex-type)
	NRRL 1057 = CBS 124.27 = MUCL 29224 = LSHB P154 = ATCC 52215 = IMI 094165 = KCTC 6784 = Thom 4894.13 = FRR 1047	Rubratroxin B*		Soil, Louisiana, Gilman and Abbott (ex-type of <i>P. crateriforme</i> )
	NRRL 1059 = IBT 10612 = IBT 3560 = CCRC 31681 = BCRC 31681 = Thom 5694.11 = NCIM 762 = ATCC 10064			C.W. Emmons ( <i>P. sanguineum</i> )
FA 184-WZ-15	IBT 11628 = CBS 113161	Rubratroxin B*	Data reported here	Wheat, Winnipeg, Canada, JT Mills
FA 158-B1-1X	IBT 11632	Rubratroxin B*	Data reported here	Barley, Winnipeg Canada, JT Mills
FA 156-B1-1	IBT 11694	Rubratroxin B*	Data reported here	Barley, Winnipeg Canada, JT Mills
U-92-10 MB nr. 4	IBT 12779			Oregano imported to Denmark
U-92-5-6	IBT 13014			Oregano imported to Denmark
DANL 451(20)	IBT 17318 = CBS 113162			Air in cake factory, Denmark
KELS 9a	IBT 17326			Air in cake factory, Denmark
UAMH 8046	IBT 17340, IBT 17341, IBT 17342 = CBS 113160, IBT 17343	Rubratroxin B*	Richer et al. (1997)	Mouldy home-made rhubarb wine, Canada, L. Sigler
F1150 (B)	IBT 17540			Unknown origin
CCRC 32601	IBT 18380			Dung of pig, Taipei City, Taiwan, S.S. Tzean
Pr	IBT 20484			Rye flour, Denmark
Det 287/98 nr. 146	IBT 21742			Agricultural soil, Canada, Keith Seifert
Lee no. 3	IBT 23074			Soil, South Korea, H.B. Lee
Lucab 201_LAB01	IBT 30226			Soil, Serro de Cip, Brazil, Lucas Abreau

\* Confirmed chemically in this study.

**Table 3** Extrolite production by *Talaromyces amestolkiae*, *T. purpurogenus*, *T. ruber* and *T. stollii* as detected by HPLC-DAD.

Species	Extrolite	Strains producing the extrolite
<i>T. amestolkiae</i>	Berkelic acid	CBS 329.48, CBS 365.48, CBS 433.62, CBS 436.62, CBS 884.72, CBS 353.93, CBS 277.95, CBS 113143, CBS 132695, CBS 132697, FRR 1095, IBT 20202, IBT 23821, IMI 061385, IMI 104624, IMI 147406
	N-Glutarylubropunctamine	CBS 365.48, CBS 436.62, IMI 147406
	Mitorubrinic acid	CBS 433.62, CBS 436.62, CBS 132695, FRR 1095, IBT 20202, IBT 23821
	Pestalasin A	CBS 252.31, CBS 365.48, CBS 433.62, CBS 436.62, CBS 884.72, CBS 113143, CBS 132695, FRR 1095, IBT 19175, IBT 23821, IMI 061385, IMI 147406
	A purpactin	CBS 433.62, CBS 436.62
	Vermicellin	CBS 433.62, CBS 132695, FRR 1095
	'm328' (= berkeleyacetal)	CBS 252.31, CBS 433.62, CBS 353.96, CBS 263.93, CBS 264.93, CBS 277.95, CBS 390.96, CBS 113143, CBS 132695, FRR 1095, IBT 20202, IBT 23821, IBT 29986, IMI 061385, IMI 147406
	'HHH' (blue fluorescing)	CBS 232.31, CBS 329.48, CBS 365.48, CBS 433.32, CBS 436.32, CBS 884.72, CBS 263.93, CBS 264.93, CBS 353.93, CBS 274.95, CBS 277.95, CBS 390.96, CBS 113143, CBS 132695, CBS 132697, FRR 1095, IBT 19175, IBT 20202, IBT 23821, IBT 29986, IMI 061385, IMI 104624, IMI 147406
	'm334'	CBS 465.48, CBS 433.62, CBS 884.72, FRR 1095, IBT 19175, IBT 20202, IBT 23821, IMI 147406
	<i>T. purpurogenus</i> <sup>1</sup>	N-Glutarylubropunctamine
Luteoskyrin		ATCC 20204 (weak), CBS 113160, IMI 136127, IMI 136128, NRRL 1749, NRRL 3290
Mitorubrin, mitorubrinol, mitorubrinic acid		ATCC 20204, ATCC 44445, CBS 184.27, CBS 286.36, CBS 113160, IBT 11632, IBT 12779, IBT 17540, IBT 31167, IMI 112715, IMI 136126, IMI 136127, IMI 136128, NRRL 1749, NRRL 3290
Purpactins		ATCC 20204, ATCC 44445, CBS 286.36, CBS 113160, IBT 11632, IBT 12779, IBT 17540, IBT 31167, IMI 112715, IMI 136126, IMI 136127, NRRL 1749, NRRL 3290
Rubratoxin A & B		ATCC 20204, ATCC 44445, CBS 184.27, CBS 286.36, CBS 113160, IBT 11632, IBT 12779, IBT 17540, IBT 31167, IMI 112715, IMI 136126, IMI 136127, IMI 136128, NRRL 1749, NRRL 2019, NRRL 3290
Rugulovasine A and B		ATCC 44445 <sup>2</sup> , CBS 184.27, IBT 12779, IBT 31167, IMI 136127, IMI 136128, NRRL 3290
<i>T. ruber</i>	Austin and austinol	CBS 370.48, CBS 368.73, CBS 195.88, CBS 196.88, CBS 237.93, CBS 113140, FRR 1503, IMI 113729, IMI 139462, IMI 178519, NRRL 1180
	N-Glutarylubropunctamine	CBS 196.88, IBT 22364
	Mitorubrin	CBS 368.73, CBS 237.93, CBS 132699, FRR 1503, NRRL 1180
	Pestalasin A	CBS 196.88, CBS 237.93, CBS 113140, FRR 1503, IMI 113729, IMI 139462, NRRL 1180
	A purpactin	CBS 237.93, CBS 132699, FRR 1503
	Vermicellin	CBS 368.73, CBS 196.88, CBS 237.93, CBS 132699, FRR 1503, IMI 139462, NRRL 1180
	'DDD'	CBS 368.73, CBS 195.88, CBS 196.88, CBS 237.93, CBS 868.96, CBS 113140, FRR 1503, IBT 22364, IMI 113729, IMI 139462, NRRL 1180
	'm334'	CBS 368.73, CBS 195.88, CBS 196.88, CBS 237.93, CBS 868.96, CBS 113140, CBS 132699, FRR 1503, IBT 22364, IMI 113729, IMI 139462, IMI 178519, NRRL 1180
<i>T. stollii</i>	austins	CBS 132706, CBS 100372
	'HHH'	CBS 408.93, CBS 132706, DTO 60-D5, CBS 265.93, CBS 582.94

<sup>1</sup> Spiculisporic acid was found in CBS 184.27 (Oxford & Raistrick 1934), but could not be detected by us using HPLC-DAD, as it has UV end-absorption below 200 nm.

*genus* and other *Talaromyces* species. The ITS alignment included eight strains and was 469 bp characters long. The results showed that strains belonging to *T. amestolkiae*, *T. ruber* and *T. stollii* form a phylogenetically distinct clade, separate from the distinctly related *T. purpurogenus* clade. ITS gave low bootstrap support within the clade where *T. amestolkiae*, *T. ruber* and *T. stollii* are located and thus detailed analysis was performed using four more variable protein-coding genes. For *RPB1*, *RPB2*,  $\beta$ -tubulin and calmodulin the alignments were, respectively, 850, 1050, 450 and 466 bp long and contained 19 taxa, five representative strains of each studied species. Because the clade containing *T. purpurogenus* and its synonyms are distinct from the other species discussed in this paper, *T. purpurogenus* was used as the outgroup for the multi-gene analysis. Except for calmodulin, which could not distinguish between *T. amestolkiae* and *T. ruber*, all gene sequences supported consistent and coherent clades with high bootstrap support. Strain CBS 196.88, designated as neotype of *Penicillium minioluteum* by Pitt (1980), is distinct from *T. minioluteus* (CBS 642.68<sup>†</sup>) and resolved in the *T. ruber* clade (Fig. 2). Many strains previously identified as *Penicillium purpurogenum* var. *rubrisclerotium* were resolved in a clade with *T. amestolkiae*. However, the ex-type strain of *P. purpurogenum* var. *rubrisclerotium* (CBS

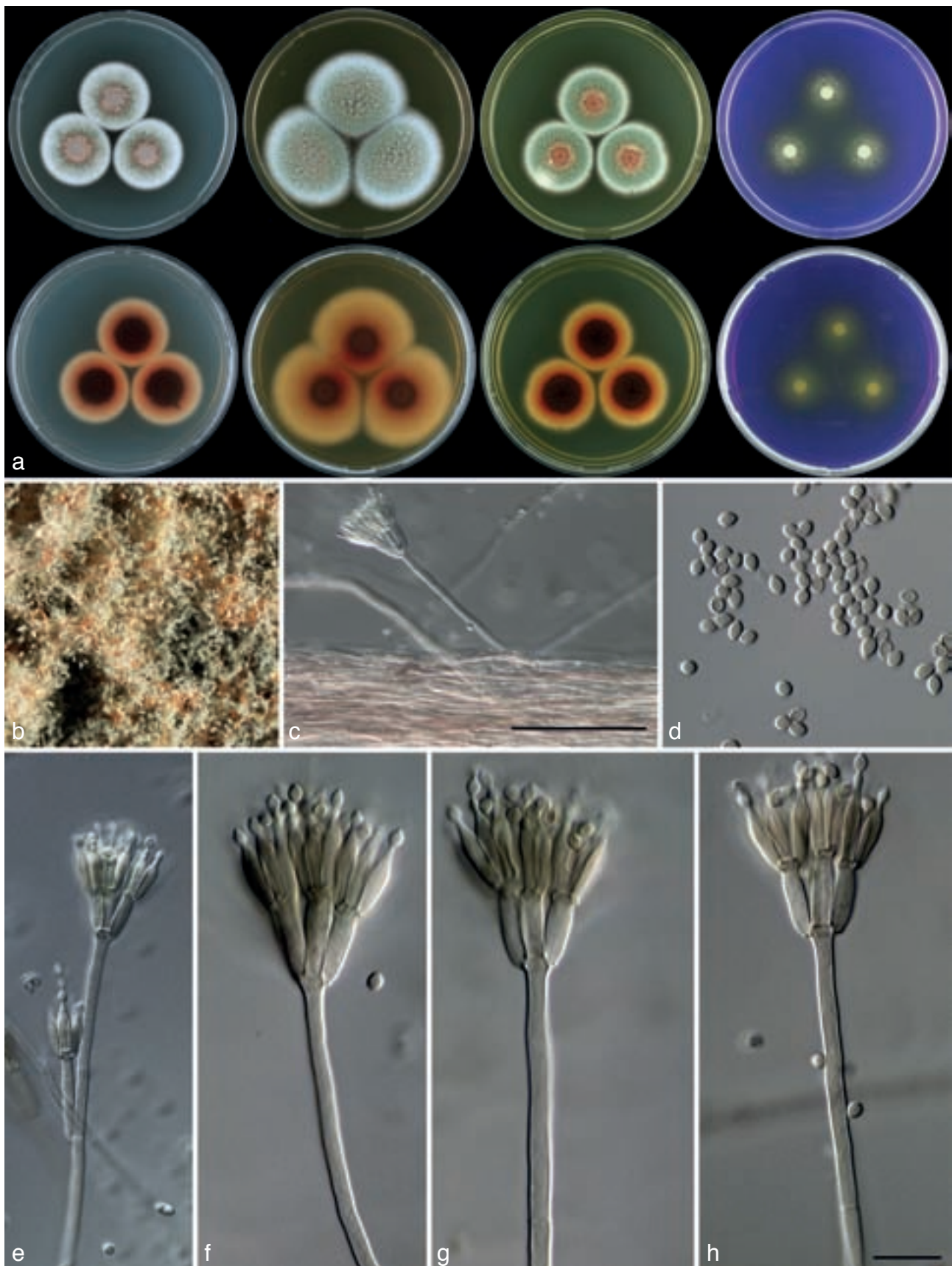
270.35<sup>†</sup>) is resolved in a distinct clade closely related to *T. minioluteus* (Fig. 2).

#### Extrolite data

The four species treated here produce many extrolites. *Talaromyces purpurogenus* isolates can produce four different mycotoxins: rubratoxins (A & B) (Moss et al. 1968, 1971, Moss & Hill 1970), rugulovasines (A and B) and chlororugulovasins A and B (Cole et al. 1976, Dorner et al. 1980, Mapari et al. 2009), luteoskyrin (reported here) and spiculisporic acid (Oxford & Raistrick 1934) (Table 2, 3) (see Frisvad 1989, as *P. crateriforme*), in addition to mitorubrins (mitorubrin, mitorubrinol, mitorubrinol acetate, mitorubrinic acid) (Büchi et al. 1965, Chong et al. 1971), N-glutarylubropunctamine, PP-R, monascin and monascorubramine (Mapari et al. 2009, as *P. crateriforme*) and purpactins (Nishida et al. 1991, Tomoda et al. 1991). We could confirm the production of rubratoxins, rugulovasines, luteoskyrin, mitorubrins, 'Monascus red pigments' and purpactins in *T. purpurogenus* (Table 3). The red azaphilone 'Monascus pigments' are diffusible in *T. purpurogenus*, but not in the other three species (Fig. 1).

*Talaromyces ruber* isolates produced austins, mitorubrins, *Monascus* pigments, pestalasin A, a purpactin, and chromo-





**Fig. 4** Morphological characters of *Talaromyces amestolkiae* (CBS 132696<sup>T</sup>). a. Colonies incubated on CYA, MEA, YES, CREA, from left to right (top row = obverse, bottom row = reverse); b. colony texture on MEA; c–g. conidiophores produced on MEA; h. conidia. — Scale bars: c = 50  $\mu$ m; g = 10  $\mu$ m and applies to d–h.

phore groups 'DDD' and 'm334' and the antibiotic vermicellin (Fuska et al. 1979). *Talaromyces stollii* isolates produced austins and chromophore group 'HHH'. *Talaromyces amestolkiae* produced berkelic acid, mitorubrinic acid, red 'Monascus pigments', a purpactin and vermicellin, and the chromophore

groups 'HHH', 'm328' and 'm334'. A strain identified as *Penicillium rubrum* was isolated from the acid and metal polluted Berkeley Pit Lake in Montana (Stierle et al. 2006), and this strain is probably *T. amestolkiae*. Of the extrolites extracted from this strain, berkelic acid was one of them. In addition to

these extrolites, all species produce other extrolites that were unique to one of the species or in common between several of the four species.

### Taxonomy

***Talaromyces amestolkiae*** Yilmaz, Houbraken, Frisvad & Samson, *sp. nov.* — MycoBank MB801358; Fig. 4

*Etymology.* Latin, *amestolkiae*: named in honour of Amelia C. Stolk, who pioneered taxonomic studies on *Penicillium* and *Aspergillus* at CBS from 1940–1976.

*Typus.* Herbarium CBS H-21050 (dried specimen), also maintained under CBS 132696, isolated from house dust from South Africa.

*Conidiophores* biverticillate, subterminal branches present, have a greenish to brownish pigmentation; *stipes* smooth walled, 93–164 × 2.5–3 µm; *branches* 2–3 when present, 15–49 × 2–3 µm; *metulae* in verticils of 3–5, 11–13 µm across apex, 9.5–14 × 3–4 (av. ± stdev = 11.9 ± 1.2 × 3.4 ± 0.2) µm; *phialides* acerose, 3–6 per metula, 9.5–12 × 2.5–3 (av. ± stdev = 11.9 ± 1.0 × 2.6 ± 0.2) µm; *conidia* smooth, some rough, ellipsoidal, 2–3 × 1.5–2.5 (av. ± stdev = 2.6 ± 0.2 × 1.9 ± 0.2) µm.

Colony morphology — CYA, 7 d: 12 °C 5–7 mm, 15 °C 7–10 mm, 18 °C 10–14 mm, 21 °C 13–20 mm, 24 °C 21–30 mm, 27 °C 24–35 mm, 30 °C 30–35 mm, 33 °C 28–31 mm, 36 °C 8–14 mm, 40 °C no growth. CYA, 25 °C, 7 d: Colonies 29–30 mm, low, raised at centre, margins wide (2–3 mm), entire; mycelium white and yellow, red in centre; texture floccose with overlaying funicles and tufts; sporulation moderately dense to dense; conidia *en masse* greyish green (26E6–26E7); exudate absent; soluble pigment very weak, with inconspicuous red pigment in some strains, reverse coloration dark brownish red (11F8–12F8). MEA, 25 °C, 7 d: Colonies 33–42 mm, low, plane; margins very wide (3–5 mm), entire; mycelium white, red at centre; texture tufted at centre, elsewhere floccose with overlaying funicles, floccose at margins; sporulation moderately dense to dense; conidia *en masse* greyish to dull green (25D4–26D4); exudate absent; soluble pigment absent; reverse coloration dark brownish red (9F8) at centre, greyish yellow to greyish orange (3C5–5C5) at margins. OA, 25 °C, 7 d: Colonies 50–52 mm, low, plane; margins very wide (5–6 mm), entire; mycelium white and yellow, red at centre; texture floccose with overlaying funicles; sporulation dense; conidia *en masse* greyish green (25D4–25D6); exudate present in some strains, clear; soluble pigment absent; reverse coloration red (11E4) at centre, red pigmentation absent in some strains. DG18, 25 °C, 7 d: Colonies 17–18 mm, low, slightly raised at centre; margins narrow (1 mm), entire; mycelium white; texture velvety with overlaying funicles; sporulation moderately dense; conidia *en masse* similar to CYA; exudate present in some strains, clear; reverse coloration dark brown (8F8). YES, 25 °C, 7 d: Colonies 27–28 mm, low, sulcate; margins narrow (1–2 mm), entire; mycelium white, red at centre; texture floccose with tufts present; sporulation moderately dense, conidia *en masse* similar to CYA; exudate absent; soluble pigment absent; reverse coloration brownish red (11F8–12F8). CREA, 25 °C, 7 d: Colonies 15–24 mm, poor acid production, only within colony periphery. CYAS, 25 °C, 7 d: Typically no growth, some strains restricted growth, 6–8 mm.

Distinguishing characteristics — *Talaromyces amestolkiae* belongs to the same clade as *T. ruber* and *T. stollii*. It is distinguished from *T. ruber* and *T. purpurogenus* by acid production on CREA, and floccose and funiculose texture on MEA. It is distinguished from *T. stollii* by its slower growth at 37 °C.

***Talaromyces purpurogenus*** (Stoll) Samson, Yilmaz, Frisvad & Seifert — MycoBank MB560667; Fig. 5

*Basionym.* *Penicillium purpurogenum* Stoll, Beitr. Morph. Biol. Char. Penicill.: 32. 1904.

= *Penicillium sanguineum* Sopp, Skr. Vidensk.-Selsk. Christiania, Math.-Naturvidensk. Kl. 11: 175. 1912.

= *Penicillium crateriforme* J.C. Gilman & E.V. Abbott, Iowa State Coll. J. Sci. 1: 293. 1927.

*Typus.* CBS 286.36<sup>T</sup> (the ex-type strain is deteriorated, CBS 132707 can be regarded as typical for the species).

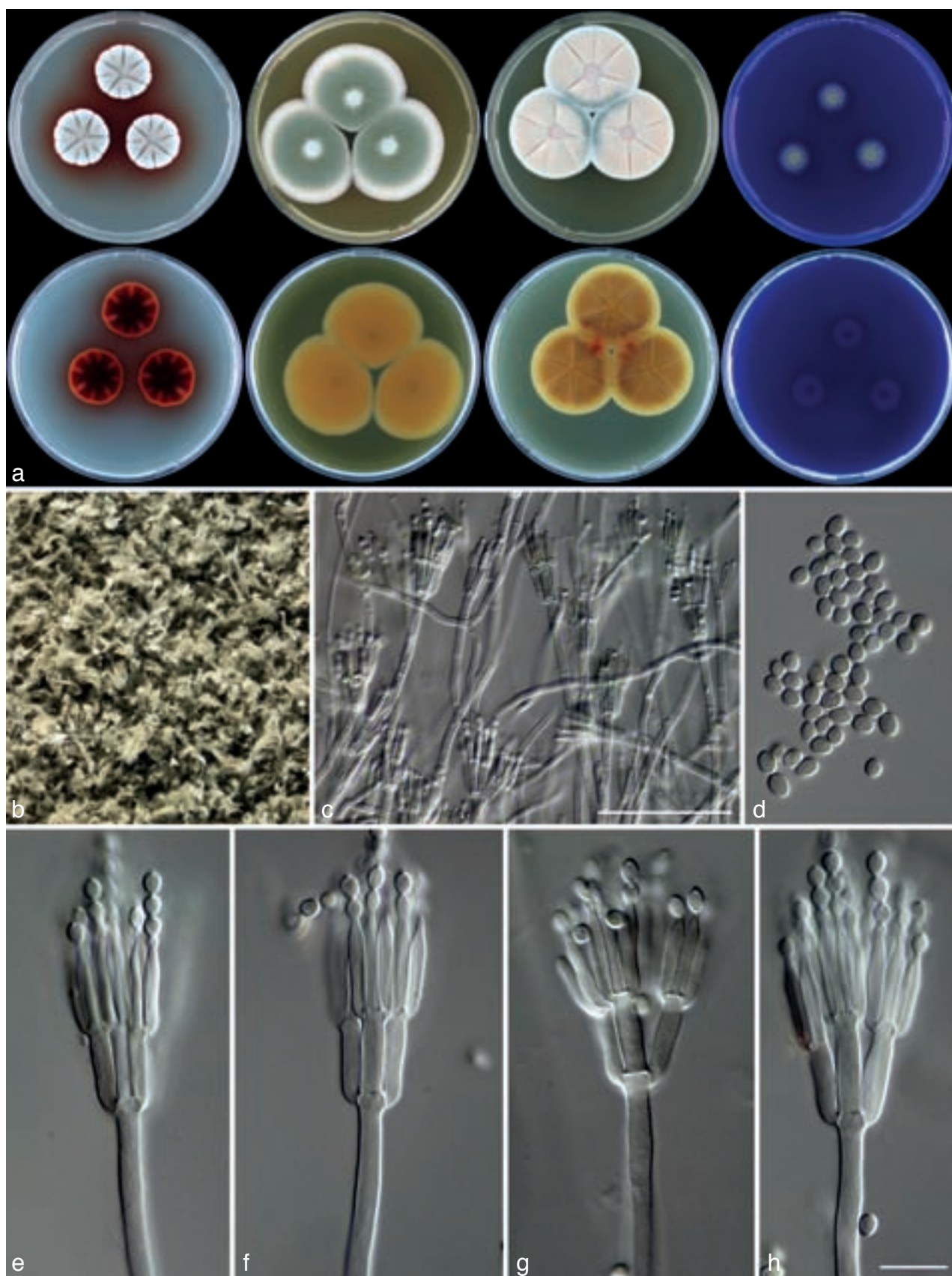
*Conidiophores* strictly biverticillate, subterminal branches absent; *stipes* smooth walled, 150–250 × 2.5–3.5 µm; *metulae* in verticils of 3–5, 9–13 µm across apex, 12–14.5 × 2.5–4 (av. ± stdev = 13.2 ± 0.8 × 3.2 ± 0.5) µm; *phialides* acerose, 3–6 per metula, 12–13.5 × 2–3 (av. ± stdev = 12.8 ± 0.5 × 2.4 ± 0.3) µm; *conidia* smooth, ellipsoidal, 3–3.5 × 2–2.5 (av. ± stdev = 3.1 ± 0.2 × 2.3 ± 0.1) µm.

Colony morphology — CYA, 7 d: 12 °C no growth, 15 °C no growth, 18 °C no growth, 21 °C 6–15 mm, 24 °C 11–20 mm, 27 °C 18–27 mm, 30 °C 18–27 mm, 33 °C 18–25 mm, 36 °C 14–25 mm, 40 °C no growth. CYA, 25 °C, 7 d: Colonies 20–25 mm, moderately deep, sulcate; margins very narrow (0.5–1 mm); mycelium white and red; texture floccose; sporulation sparse to moderately dense; conidia *en masse* dull green (27D3–28D3); exudate absent, soluble pigment typically bright red, absent in some isolates; reverse coloration dark brown to violet brown (9F8–11F8) fading to reddish brown (9D8), in non-soluble pigment producers pale and light red. MEA, 25 °C, 7 d: Colonies 33–41 mm, low slightly at point of inoculation; margins wide (3–4 mm), entire; mycelium orange and white; texture floccose, with some velvety areas, some strains covered by white sterile mycelium; sporulation moderately dense, in some strains absent, conidia *en masse* dull green (26E4–26E5); exudate absent, sometimes clear droplets; soluble pigment absent; reverse coloration brownish yellow to brownish orange (5C7–6C7). OA, 25 °C, 7 d: Colonies 28–35 mm, low, plane; margins wide (2–3 mm), entire; mycelium white and orange; texture velvety and floccose; sporulation moderately dense to dense, conidia *en masse* dull green (26E4–26E5); exudate absent; soluble pigment absent; reverse coloration dull red (9C4), colour lacking in some. Colonies produce an apple-like fruity odour. DG18, 25 °C, 7 d: Colonies 11–15 mm, low, plane; margins wide (1–2 mm), entire; mycelium white and bright orange; texture velvety, some floccose mycelium present; sporulation sparse to moderately dense, conidia *en masse* dark green (27F5); exudate absent; soluble pigment absent; reverse coloration light to brownish orange (5A4–5C4). YES, 25 °C, 7 d: Colonies 25–35 mm, low, sulcate; margins wide (1–2 mm), entire; mycelium white and orange, yellow in strains; texture floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (26E4–26E5); exudate absent; soluble pigment absent; reverse coloration light yellow to brown (4A5–6D7), some strains dark red to dark brown (8F4). CREA, 25 °C, 7 d: Colonies 7–11 mm. Typically no acid production; strain CBS 122434 has poor acid production. CYAS, 25 °C, 7 d: No growth to microcolonies of up to 5 mm.

Distinguishing characteristics — *Talaromyces purpurogenus* is distinct from the other three very similar species. It is not able to grow at temperatures below 18 °C, grows slower and produces a bright red diffusing pigment on CYA at 25 °C and has bright yellow and orange mycelium on DG18 at 25 °C.

***Talaromyces ruber*** (Stoll) Yilmaz, Houbraken, Frisvad & Samson, *comb. nov.* — MycoBank MB801360; Fig. 6

*Basionym.* *Penicillium rubrum* Stoll, Beitr. Morph. Biol. Char. Penicill.: 35. 1904.



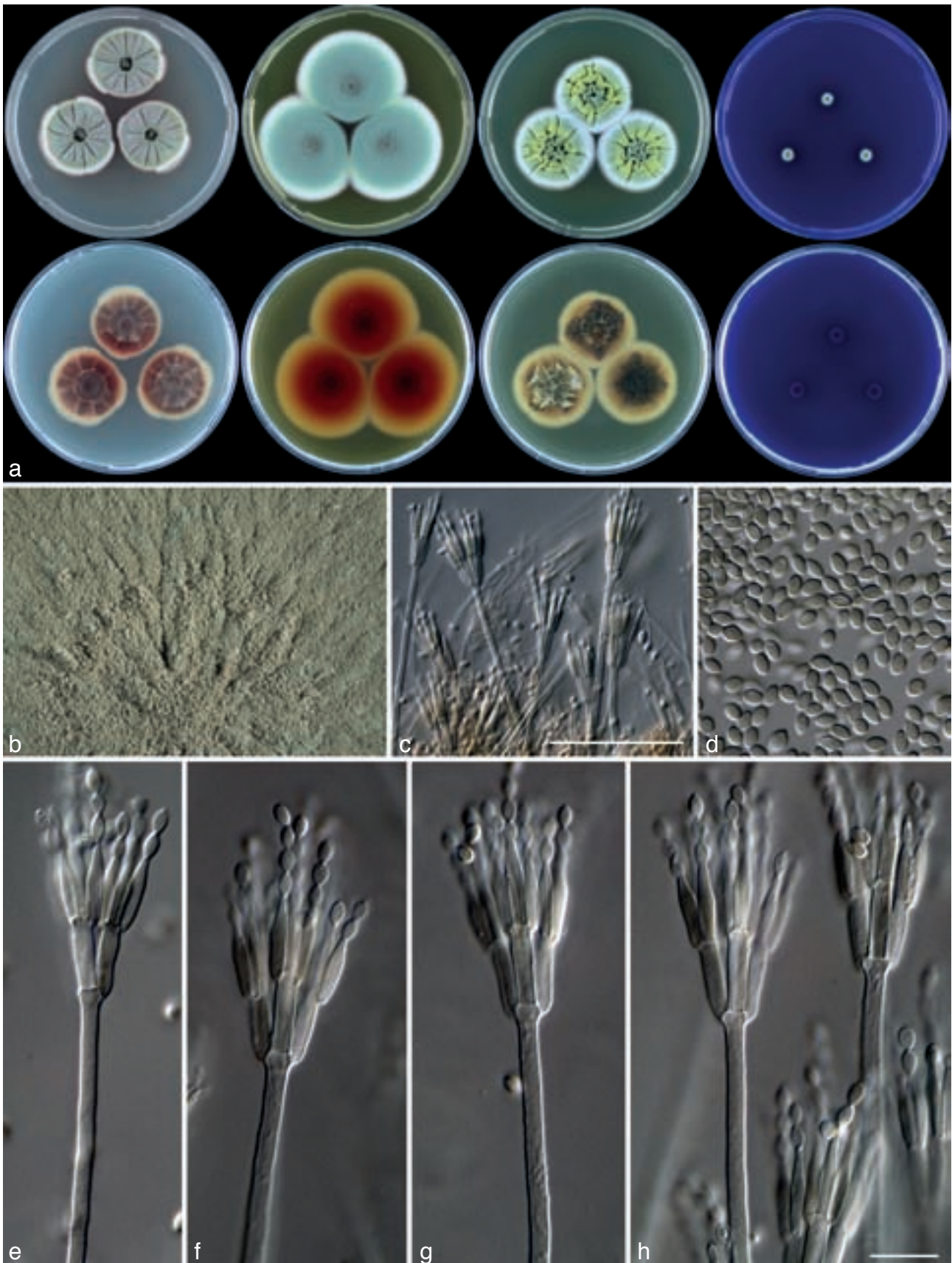
**Fig. 5** Morphological characters of *Talaromyces purpurogenus* (CBS 132707). a. Colonies incubated on CYA, MEA, YES, CREA, from left to right (top row = obverse, bottom row = reverse); b. colony texture on MEA; c–g. conidiophores produced on MEA; h. conidia. — Scale bars: c = 50  $\mu\text{m}$ ; g = 10  $\mu\text{m}$  and applies to d–h.

*Typus.* Since no holotype is known herbarium CBS-H-21052 (dried specimen) is here designated as neotype. It is derived from CBS 132704, isolated from aircraft fuel tank from United Kingdom. CBS 370.48 was used by Raper & Thom to describe *Penicillium rubrum*, but it no longer displays all diagnostic characters.

*Conidiophores* biverticillate; *stipes* smooth walled, 110–232  $\times$  2.5–3  $\mu\text{m}$ ; *metulae* in verticils of 3–5, 7.5–11  $\mu\text{m}$  across apex, 7.5–10.5  $\times$  2.0–3 (av.  $\pm$  stdev = 9.6  $\pm$  1.0  $\times$  2.3  $\pm$  0.3)  $\mu\text{m}$ ; *phialides* acerose, 3–6 per metula, 9–12  $\times$  2–2.5 (av.  $\pm$  stdev = 9.8  $\pm$  2.8  $\times$  2.1  $\pm$  0.2)  $\mu\text{m}$ ; *conidia* smooth, ellipsoidal, 2.5–3.5  $\times$  1.5–2 (av.  $\pm$  stdev = 2.9  $\pm$  0.2  $\times$  1.8  $\pm$  0.1)  $\mu\text{m}$ .

Colony morphology — CYA, 7 d: 12 °C 3–5 mm, 15 °C 5–10 mm, 18 °C 9–13 mm, 21 °C 15–20 mm, 24 °C 17–25 mm, 27 °C 20–30 mm, 30 °C 24–30 mm, 33 °C 20–26 mm, 36 °C 14–17 mm, 40 °C no growth. CYA, 25 °C, 7 d: Colonies 22–30 mm, low, radially sulcate, in CBS 370.48<sup>T</sup> colonies are pink with no sporulation; margins low, wide (2–3 mm), entire;

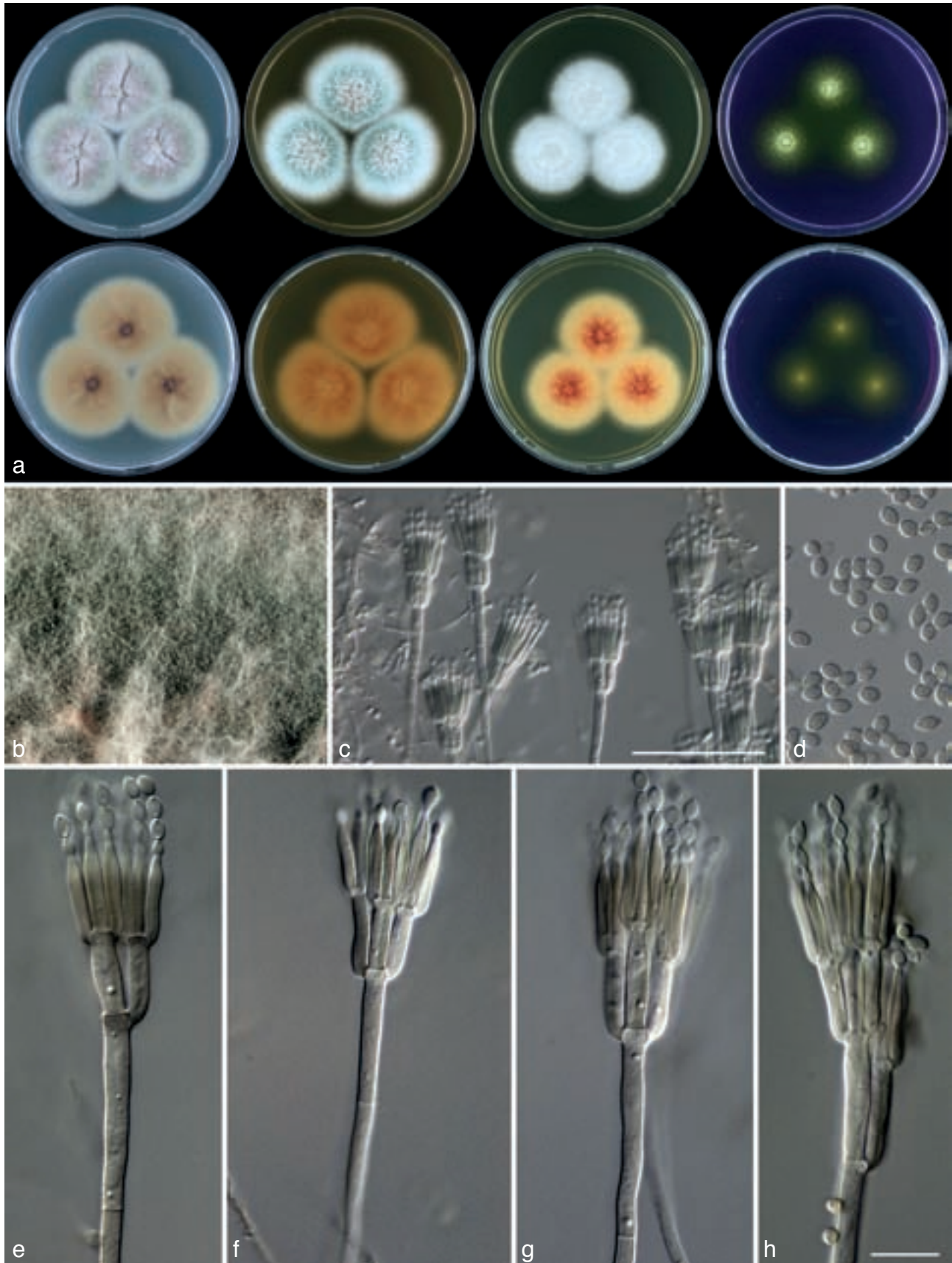
mycelium white, yellow and red; texture velvety, sometimes with funicles near margins; sporulation moderately dense, conidia *en masse* bright olive green to greyish green (26D4–27D4); exudate present in some strains, small clear and red droplets; soluble reddish pigment typically present, absent in some strains; reverse coloration brownish red (8E8–8F8). MEA,



**Fig. 6** Morphological characters of *Talaromyces ruber* (CBS 132704<sup>T</sup>). a. Colonies incubated on CYA, MEA, YES, CREA, from left to right (top row = obverse, bottom row = reverse); b. colony texture on MEA; c–g. conidiophores produced on MEA; h. conidia. — Scale bars: c = 50 µm; g = 10 µm and applies to d–h.

25 °C, 7 d: Colonies 35–38 mm, low, plane; margins low, very wide (5–6 mm), entire; mycelium, white and yellow; texture velvety, ropes of mycelium produced very close to media and sometimes inside the medium (Fig. 6b) sporulation dense, conidia *en masse* greyish green (26D4–26E4), some strains a lighter greyish green (26B3); exudate absent; soluble pigment

absent; reverse coloration brownish red to dark brown (8F8–8C8) at centre, elsewhere greyish yellow to greyish orange (4B4–4C4–5B4). OA, 25 °C, 7 d: Colonies 40–42 mm, low, plane; margins very wide (4–5 mm), entire, low; mycelium white and yellow; texture velvety and floccose; sporulation moderately dense, conidia *en masse* dull to dark green (27D4–27F8);



**Fig. 7** Morphological characters of *Talaromyces stollii* (CBS 408.93<sup>T</sup>). a. Colonies incubated on CYA, MEA, YES, CREA, from left to right (top row = obverse, bottom row = reverse); b. colony texture on MEA; c–g. conidiophores produced on MEA; h. conidia. — Scale bars: c = 50 µm; g = 10 µm and applies to d–h.

exudate absent, in some strains clear; soluble pigment absent; reverse coloration reddish brown (8D7). DG18, 25 °C, 7 d: Colonies 14–16 mm, plane, low, with a brownish orange colour; margins narrow (2–3 mm), entire; mycelium white; texture floccose; sporulation sparse, conidia *en masse* similar to CYA; exudate small clear droplets; soluble pigment absent; reverse coloration greyish green (30D6–30E6) at centre, elsewhere greenish white (30A2). YES, 25 °C, 7 d: Colonies 22–30 mm, low, raised at centre, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelium white and yellow, red in some strains, e.g. CBS 868.96; texture floccose; sporulation sparse to moderate dense, conidia *en masse* greyish green (27C5–27E6–27E7); exudate clear small droplets; reverse coloration greyish brown to brown (5F8–5F3) near centre, at margins brownish orange to light brown (5C4–5D4). CREA, 25 °C, 7 d: Colonies 10–14 mm, restricted growth, no acid production. CYAS, 25 °C, 7 d: Typically no growth, sometimes microcolonies up to 4 mm.

Distinguishing characteristics — *Talaromyces ruber* can be distinguished from *T. purpurogenus* by growth at lower temperatures, having a velvety texture on MEA, yellow mycelia and bright green conidia on YES after 7 d incubation at 25 °C. *Talaromyces ruber* can be distinguished from *T. stollii* and *T. amestolkiae* by absence of acid production on CREA. *Talaromyces ruber* has a velvety structure on both CYA and MEA at 25 °C, produces a very distinct colony texture on MEA and produces bright yellow and red mycelia on YES.

***Talaromyces stollii*** Yilmaz, Houbraken, Frisvad & Samson, *sp. nov.* — MycoBank MB801359; Fig. 7

*Etymology.* Latin, *stollii*: named in honour of Otto Stoll, a pharmacist who first described *P. rubrum* and *P. purpurogenum* for his PhD thesis at the K. Bayr Julius Maximilians University in Würzburg, Germany in 1905.

*Typus.* Herbarium: CBS H-21053 (dried specimen), derived from CBS 408.93, isolated from an AIDS patient, the Netherlands.

*Conidiophores* biverticillate, subterminal branches present, have a greenish to brownish pigmentation; *stipes* smooth walled, 94–247 × 3–4.5 µm; *metulae* in verticils of 3–5, 9.5–10 µm across apex, 11.5–14.5 × 2–3.5 (av. ± stdev = 12.5 ± 0.9 × 2.9 ± 0.4) µm; *phialides* acerose, 3–6 per metula, 13–17 × 2–2.5 (av. ± stdev = 14.2 ± 1.2 × 2.1 ± 0.2) µm; *conidia* smooth to lightly roughed, ellipsoidal, 2.5–4 × 2–2.5 (av. ± stdev = 3.2 ± 0.3 × 2.1 ± 0.2) µm.

Colony morphology — CYA, 7 d: 12 °C 4–6 mm, 15 °C 5–10 mm, 18 °C 13–18 mm, 21 °C 19–25 mm, 24 °C 30–35 mm, 27 °C 36–43 mm, 30 °C 38–44 mm, 33 °C 35–44 mm, 36 °C 24–35 mm, 40 °C no growth. CYA, 25 °C, 7 d: Colonies 38–42 mm, low, raised at centre, lightly radially sulcate; margins wide (2–3 mm), entire; mycelium white and red; texture floccose; sporulation sparse, conidia *en masse* greyish to dull green (27C4–27D4); exudate present, small pinkish or yellowish droplets; soluble pigment absent; reverse coloration dark brown (8F8) at point of inoculation, elsewhere greyish red (7B3). MEA, 25 °C, 7 d: Colonies 45–50 mm, low, plane; margins wide (3–4 mm), entire; mycelium white, at centre sometimes red, sometimes yellow; texture floccose and funiculose, white sterile tufts covering colonies; sporulation moderately dense, conidia *en masse* greyish to dull green (27C4–27D4); exudate absent; soluble pigment absent; reverse coloration brownish orange to brownish yellow (5C6–6C7). OA, 25 °C, 7 d: Colonies 44–48 mm, low, plane; margins very wide (4–7 mm), entire; mycelium white; texture floccose, with funiculose that rise from colony centre similar to synnemata; sporulation sparse, conidia *en masse* similar to CYA; exudate present, clear; soluble pigment absent; reverse coloration reddish at centre, green elsewhere, some strains yellowish. DG18, 25 °C, 7 d: Colonies 18–25 mm,

low, plane; margins low, wide (2–3 mm), entire; mycelium white; texture floccose; sporulation absent; exudates absent, sometimes yellow droplets; soluble pigment absent; reverse coloration pale, some strains brownish orange (5C6) at centre, fading into pale yellow (4A3) at margins. YES, 25 °C, 7 d: Colonies 33–38 mm, low, lightly sulcate; margins wide (3–4 mm), entire; mycelium white; texture floccose; sporulation very sparse; exudate absent; soluble pigment absent; reverse coloration similar to CYA. CREA, 25 °C, 7 d: Colonies 20–30 mm; sparse sporulation, poor acid production, only within colony periphery. CYAS, 25 °C, 7 d: No growth to microcolonies of up to 5 mm.

Distinguishing characteristics — *Talaromyces stollii* is distinguished from *T. ruber* and *T. purpurogenus* by acid production on CREA. *Talaromyces stollii* does, however, grow faster on CYA at 36 °C than *T. amestolkiae*. In addition, *T. stollii* has unique soft synnemata-like or tufted structures in the centre of colonies on most media.

## DISCUSSION

Cultures that previously were identified as *P. purpurogenum* or *P. rubrum* were analysed in this study and phylogenetic, morphological and extrolite results show that the *T. purpurogenus* complex consists of four distinct species. The species described below are quite common on textiles, paper, soil, dung, plant debris, coffee-berries, corn, indoor air and dust, and are distributed worldwide. *Talaromyces purpurogenus* has been implicated in the biodeterioration of cellulose materials such as textiles, paper and adhesives, while it also has the ability to grow on plant material such as corn, where it may produce mycotoxins (Moss et al. 1971). *Talaromyces purpurogenus* produces four types of mycotoxins: rubratoxin A & B, rugulovasins, spiculisporic acid and luteoskyrin, and none of the other three species treated have been found to produce mycotoxins. The newly described species *T. amestolkiae* and *T. stollii* grow well at 37 °C and some strains were isolated from AIDS patients and might be opportunistic pathogens. It is not yet known if any other species in this group can be opportunistic pathogens. *Talaromyces purpurogenus* was reported as the causal agent of a disseminated mycosis in a German shepherd dog (Zanatta et al. 2006), but it remains unknown if this species identification is correct using the newly proposed taxonomy. This group is also biotechnologically important, because of their production of enzymes (Carvalho et al. 2003, Jeya et al. 2010) and extrolites. For example, the mycotoxin rubratoxin A & B produced by *T. purpurogenus* has been shown to act as cancer metastasis suppressors (Wada et al. 2010) and spiculisporic acid can be used as a detergent (Ishigami et al. 2000). From a biotechnological point of view we would recommend using *T. ruber* for enzyme production, because *T. purpurogenus* produces four types of mycotoxins and *T. amestolkiae* and *T. stollii* are potentially pathogenic to immuno-compromised persons. However, it is not known whether the enzymes reported from *T. purpurogenus* (Steiner et al. 1994, Belancic et al. 1995) are indeed from this species or one of the other three taxa treated here or even any of them.

Most of the isolates produced the extrolites characteristic of the species (Table 3), but some isolates should be grown on other media to examine whether they can also produce the remaining extrolites found in productive strains. Most extrolites supported the phylogram in Table 3. Production of purpactin, pestalasin A, vermicellin and 'm334' supported that *T. ruber* and *T. amestolkiae* are closely related. On the other hand common production of 'HHH' indicated that *T. amestolkiae* and *T. stollii* are closely related, while common production of austin indicates that *T. ruber* and *T. stollii* are closely related. Purpactin was pro-

duced by the outgroup *T. purpurogenus* but also by *T. ruber* and *T. amestolkiae*. Rubratoxins, spiculisporic acid, rugulovasins, chlororugulovasins and luteoskyrin were autapomorphic for *T. purpurogenus*, while berkelic acid and 'm328' were autapomorphic for *T. amestolkiae*. Metabolite 'DDD' was autapomorphic for *T. ruber* and a larger number of derivatives of 'HHH' were autapomorphic for *T. stollii*. It should be noted that some of these extrolites are also found outside the *T. purpurogenus* complex. For example, luteoskyrin is produced by *T. islandicus* (Uraguchi et al. 1961) and spiculisporic acid is produced by *T. trachyspermus* (Clutterbuck et al. 1931) and *T. ucrainicus* (Fujimoto et al. 1988).

The species in this complex generally produce yellow, orange and red pigments in the mycelium or as diffusing pigments. Extrolites responsible for these colours are two groups of azaphilone polyketide pigments the mitorubins (mitorubrin, mitorubrinol, mitorubrinol acetate and mitorubrinic acid) (Büchi et al. 1965) and the *Monascus* red pigments (N-glutaryl monascorubramin, N-glutaryl rubropunctamin, monascorubramine, monascin, PP-R and others (Mapari et al. 2009)). These azaphilone polyketides are produced by all the species treated in this paper and several other species in *Talaromyces*, but they appear to be produced in different ratios and amounts in different isolates and species (Frisvad et al. 1990, van Reenen-Hoekstra et al. 1990, Samson et al. 2011). Also, especially on MEA, we observed that when a strain that produced red pigment was transferred to another MEA plate, the strain sometimes lost the ability to produce the red pigment. However, red pigment production was consistent on CYA. Apart from the medium employed for extrolite production, the age of the strain may also play a role: older strains of *T. purpurogenus*, such as isolates formerly called *P. crateriforme* and *P. sanguineum*, have lost their ability to produce high amounts of diffusible red pigments. The red pigments have resulted in some confusion, especially in the concept of *T. purpurogenus* and *T. ruber*. *Talaromyces purpurogenus* and *T. ruber* were described by Stoll (1903–1904). Raper & Thom (1949) considered the species as distinct. *Talaromyces purpurogenus* was distinguished from *T. ruber* by the production of spreading dark yellow green colonies and smooth-walled conidia in the latter species. This is in comparison to the sometimes more restricted dark green colonies and rough-walled conidia they observed in *T. purpurogenus*. Although Pitt (1980) synonymised *T. ruber* with *T. purpurogenus*, our data indicate that these two species are distinct and they are re-described below. *Talaromyces ruber* can be distinguished from *T. purpurogenus* by growth at lower temperatures, its velvety texture on MEA, yellow mycelium and bright green conidia on YES after 7 d incubation at 25 °C. With regards to conidia ornamentation, all strains examined of both these species produced smooth-walled conidia and this character is thus not diagnostic for species recognition. No type material was designated for *T. ruber*, therefore Raper & Thom (1949) centred their description of *T. ruber* on NRRL 1062 and NRRL 2120. Our analysis shows these two strains belong to different species. NRRL 1062 (= CBS 370.48) is designated here as the neotype of *T. ruber*, while NRRL 2120 represents a new phylogenetically unrelated species (Fig. 2).

*Penicillium sanguineum* and *P. crateriforme* are considered synonyms of *T. purpurogenus*. In Sopp's description of *P. sanguineum*, he states that this species produces bright red pigments, which colours the entire gelatine medium, as well as producing yellow coloured mycelium (Sopp 1912). Although no type material exist for this species, the description by Sopp (1912) indicates that it belongs to the *T. purpurogenus* complex. *Penicillium crateriforme* (CBS 184.27<sup>T</sup>) is resolved in a clade together with the ex-type cultures for *T. purpurogenus* (CBS 286.36<sup>T</sup>) and is considered a synonym of *T. purpurogenus*.

Pitt (1980) neotypified *P. minioluteum* using strain IMI 89377ii (CBS 196.88). CBS 642.68<sup>T</sup> is a subculture of the same strain obtained from the IMI in 1968, but it morphologically fits Biourge's description of *P. minioluteum*. It was therefore considered the correct neotype of the species as discussed in earlier studies (van Reenen-Hoekstra et al. 1990). Our phylogenetic data show that *T. minioluteus* (CBS 642.68) remains in a clade distantly related to *T. ruber* (CBS 196.88).

This study resulted in the delimitation of *T. amestolkiae* and *T. stollii*, two new species closely related to *T. ruber*. *Talaromyces amestolkiae* and *T. stollii* are distinguished from *T. purpurogenus* and *T. ruber* by their acid production on CREA and floccose to funiculose texture of MEA. Compared to *T. amestolkiae*, *T. stollii* grows faster on CYA at 36 °C, as well as producing unique synnemata/tufted mycelium on most media. *Talaromyces amestolkiae* and *T. stollii* share the production of the 'HHH' family of extrolites. Although these species are resolved amongst known sexual species, we did not observe cleistothecia for strains studied. Future studies that aim to induce sexual reproduction would be interesting, especially for explaining the morphological and genetic variation observed between *T. stollii* strains. Also, sclerotia were produced by *T. amestolkiae* strains, but these never matured into cleistothecia. Many strains previously identified as *P. purpurogenum* var. *rubrisclerotium* were resolved in a clade with *T. amestolkiae*. However, the ex-type strain of *P. purpurogenum* var. *rubrisclerotium* (CBS 270.35<sup>T</sup>) is resolved in a distinct clade closely related to *T. minioluteus* (Samson et al. 2011).

This paper addressed the taxonomic difficulties experienced in the *T. purpurogenus* complex. Results showed that this complex contains four distinct species and that they can be identified using morphological characters, extrolites and/or genetic data. The ITS barcodes could reliably separate the four species within this complex. However there is only one base pair difference between *T. ruber* and *T. amestolkiae*, and thus the alternative genes were needed for taxon identification. Although calmodulin could not resolve *T. amestolkiae* from *T. ruber*, *RPB1*, *RPB2* and  $\beta$ -tubulin gave a clear species delineation and can be used for identifying species within this clade.

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