



# 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 (Petruzzi 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.

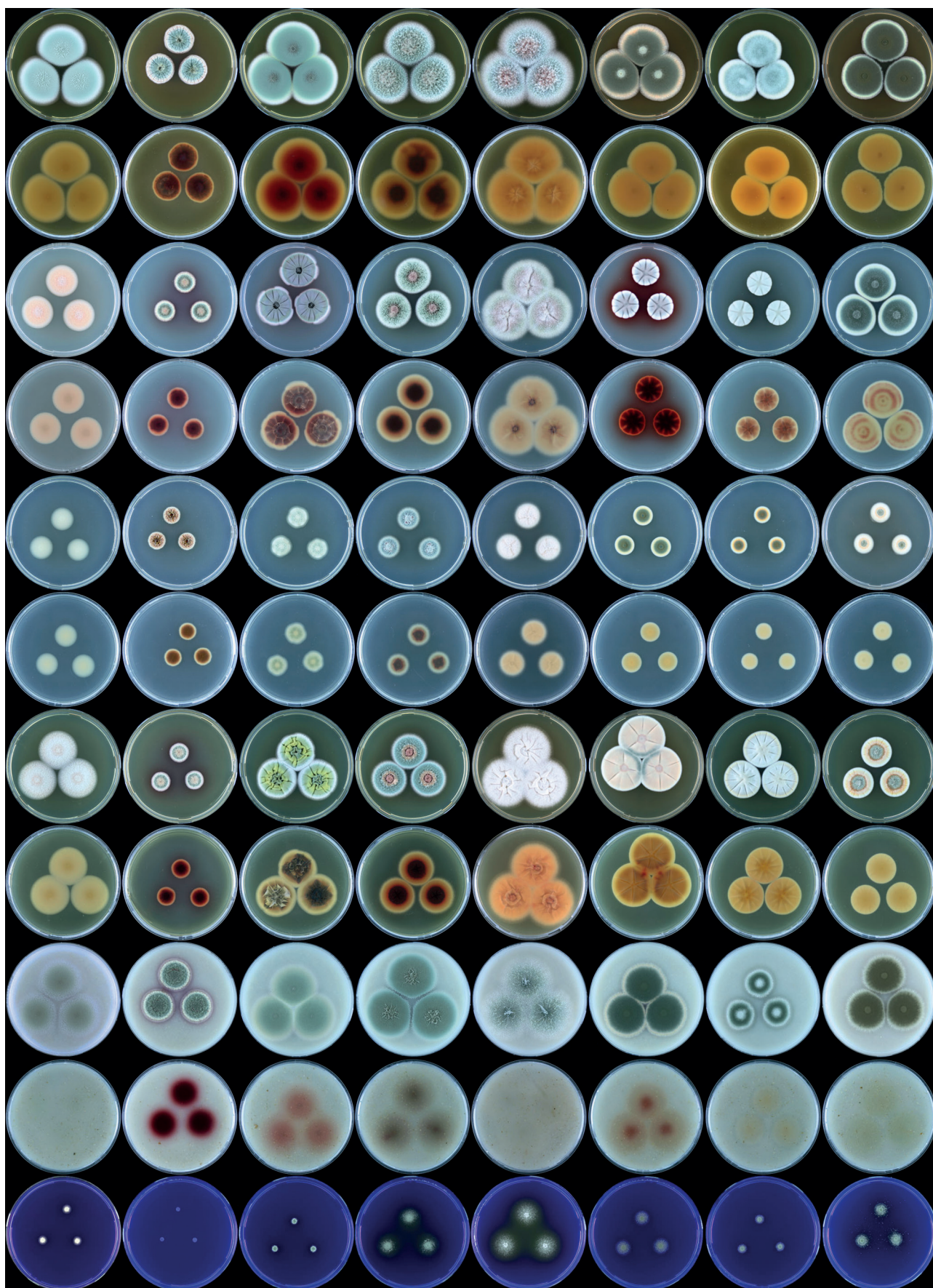
<sup>5</sup> Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

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                                                                       |          |                  |            |          |          |
|                        |                     | IMI 104624 = IBT 3968                                                                                                         | Plastic; UK, 1963                                                                          |          |                  |            |          |          |
|                        |                     | IMI 147406 = KCTC 6773 = IBT 21723                                                                                            | <i>Malus pumila</i> ; Belfast, North Ireland, UK, 1970                                     |          |                  |            |          |          |
|                        |                     | IBT 19715                                                                                                                     | Air, cake factory; Denmark                                                                 |          |                  |            |          |          |
|                        |                     | IBT 23821                                                                                                                     | Soli; Scafati, Italy                                                                       |          |                  |            |          |          |
|                        |                     | IBT 29986                                                                                                                     | Contaminant of agar plate; Denmark                                                         |          |                  |            |          |          |
|                        | 252.31              | NRRL 1034                                                                                                                     | <i>Narcissus</i> bulb; the Netherlands                                                     | JX315668 | JX315624         | JX315654   | JX315687 | JX315706 |
|                        | 263.93              |                                                                                                                               | Bronchoalveolar lavage of immunocompetent female patient with pneumonia by <i>Nocardia</i> | JX315669 | JX315625         | JX315653   | JX315688 | JX315707 |
|                        | 264.93              |                                                                                                                               | Bronchoalveolar lavage of male AIDS-patient; New Caledonia                                 | JX965247 | JX965331         | JX965196   | JX965284 | JX965319 |
|                        | 274.95              |                                                                                                                               | Sculpture in castle Troja; Prague, Czech Republic                                          | JX965214 | JX965321         | JX965190   | JX965249 | JX965285 |
|                        | 277.95              | AK 128/94 = AK 188/94                                                                                                         | Soli; Chvalatice, Czech Republic                                                           | JX965215 | JX965322         | JX965191   | JX965250 | JX965286 |
|                        | 329.48 <sup>1</sup> | ATCC 10445 = ATCC 8725 = CCTM 3641<br>= CECT 2913 = DSM 2213 = IFO 5857 =<br>IHEM 4008 = IMI 034912 = NRRL 1032a<br>= QM 7562 | Air contaminant; Washington DC, USA                                                        | JX965216 | JX965323         | JX965192   | JX965251 | JX965287 |
|                        | 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<br>= QM 1960                                                                              | Unknown source; USA                                                                        | JX965217 | JX965324         | JX965193   | JX965252 | JX965288 |
|                        | 379.97              |                                                                                                                               | Sputum; Leiden, the Netherlands                                                            | JX965218 | JX965325         | JX965198   | JX965253 | JX965289 |
|                        | 390.96              |                                                                                                                               | Contaminant of <i>Coniothyrium minitans</i> ; Italy                                        | JX965219 | JX965326         | JX965194   | JX965254 | JX965290 |
|                        | 433.62              |                                                                                                                               | Ground domestic waste; Verona, Italy                                                       | JX965220 | JX965327         | JX965195   | JX965255 | JX965291 |
|                        | 436.62              |                                                                                                                               | Alum solution; unknown origin                                                              | JX965221 | JX965328         | JX965197   | JX965256 | JX965292 |
|                        | 626.93              |                                                                                                                               | <i>Ananas comosus</i> cultivar; Martinique                                                 |          | JX965329         |            | JX965257 |          |
|                        | 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                                                           |          | JX965334         |            |          |          |
|                        | 102689              |                                                                                                                               | Air; Japan                                                                                 | JX965226 | JX965335         |            | 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 =<br>MUCL 29224 = ATCC 52215 = NRRL 1057<br>= 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 |
| 113161              | IBT 17430 = DTO 49F6                                                  | Wheat; Winnipeg, Canada                                                                                                                 | JX965234 | JX965345 | JX965202 | JX965270 | JX965307 |
| 122411              | IMI 136128 = MR 008 = IBT 3658 = IBT 5015 = DTO 189A1                 | Mould field corn; Wisconsin, USA                                                                                                        | JX315661 | JX315638 | JX315642 | JX315680 | JX315699 |
| 101965 <sup>3</sup> | DTO 49F7 = DTO 189A4 = IBT 10612 = IBT 3560 = CCRC 31681 = BCRC 31681 | Unknown source                                                                                                                          | JX965237 | JX965347 |          |          | JX965306 |
| 122434 <sup>4</sup> | = 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 |
| <i>T. ruber</i>     | 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                                                                                                                     |          |          |          |          |          |
|                     | NRRL 1159 = IBT 4423                                                  | Chickens in cold storage; unknown                                                                                                       | JX965240 | JX965350 | JX965204 | JX965273 | JX965310 |
|                     | FRR 1714 = IBT 3951                                                   | Unknown                                                                                                                                 | JX315666 | JX315627 | JX315657 | JX315685 | JX315704 |
|                     | ACC 828-81                                                            | Unknown                                                                                                                                 | JX315667 | JX315628 | JX315656 | JX315686 | JX315705 |
|                     | ATCC 10520 = IMI 040036 = NRRL 1062 = VKM F-345 = IBT 4431 = IBT 3927 | Unknown                                                                                                                                 |          |          | JX965351 | JX965274 |          |
|                     |                                                                       | Ex-neotype. Currency paper; Washington, USA                                                                                             | JX315673 | JX315630 | JX315649 | JX315692 | JX315711 |
|                     | 888.96                                                                | Tracheal secretion; Heidelberg, Germany                                                                                                 |          |          |          |          |          |
|                     | 101144                                                                | Ex experimental paint sample; Woolwich, UK                                                                                              | JX315677 | JX315631 | JX315643 | JX315696 | JX315715 |
|                     | 113140                                                                | Air cake factory; Denmark                                                                                                               | JX965239 |          |          | JX965272 | JX965309 |
|                     | 132699                                                                | Ex sandy soil; Marhaba Club Beach, Sousse, Tunisia                                                                                      | JX965241 | JX965352 | JX965205 | JX965275 | JX965311 |
|                     | 132700                                                                | Soil; Indonesia                                                                                                                         | JX965242 | JX965353 | JX965206 | JX965276 | JX965312 |
|                     | 132703                                                                | Ex experimental paint sample; Woolwich, UK                                                                                              | JX965243 | JX965354 | JX965207 | JX965277 | JX965313 |
|                     | 132704 <sup>NT</sup>                                                  | Aircraft fuel tank; UK                                                                                                                  |          |          |          |          | JX965314 |
|                     |                                                                       |                                                                                                                                         | JX315662 | JX315629 | JX315641 | JX315681 | JX315700 |
|                     | 169.91 <sup>6</sup>                                                   | Unknown substrate; South Africa identified as <i>Penicillium funiculosum</i> by Raper & Thom (1949)                                     | JX315664 | JX315634 | JX315647 | JX315683 | JX315702 |
| <i>T. stollii</i>   | 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 carnosus</i> cultivar; Martinique                                                                                             | JX315676 | JX315636 | JX315644 | JX315695 | JX315714 |
|                     | 625.93                                                                | <i>Ananas carnosus</i> cultivar; Martinique                                                                                             |          | JX965360 | JX965211 | JX965279 | JX965316 |
|                     | 100372                                                                | Pineapple; location unknown                                                                                                             |          | JX965357 |          | JX965282 | JX965317 |
|                     | 132705                                                                | Soil; Indonesia                                                                                                                         |          | JX965358 | JX965212 | JX965280 | JX965318 |
|                     | 132706                                                                | Indoor air from bakery, Avenhorn, the Netherlands                                                                                       | JX965246 | JX965359 | JX965213 | JX965283 | JX965320 |
|                     |                                                                       |                                                                                                                                         |          |          |          |          |          |
|                     |                                                                       |                                                                                                                                         |          |          |          |          |          |
|                     |                                                                       |                                                                                                                                         |          |          |          |          |          |
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|                     |                                                                       |                                                                                                                                         |          |          |          |          |          |
|                     |                                                                       |                                                                                                                                         |          |          |          |          |          |
|                     |                                                                       |                                                                                                                                         |          |          |          |          |          |

<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. Dolléfeld, 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

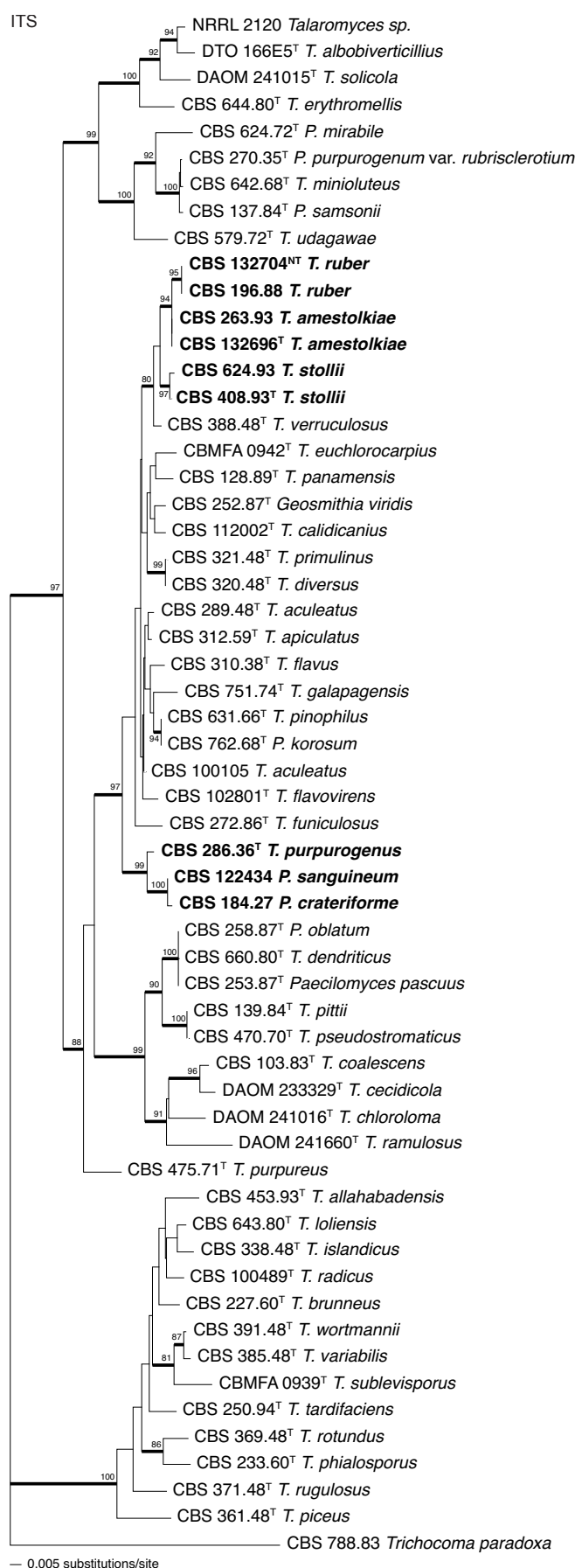
Macroscopic characters were studied on Czapek yeast extract agar (CYA), CYA supplemented with 5 % NaCl (CYAS), yeast extract sucrose agar (YES), creatine sucrose agar (CREA), dichloran 18 % glycerol agar (DG18), oatmeal agar (OA) and malt extract agar (Oxoid) (MEA). The strains were inoculated at three 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

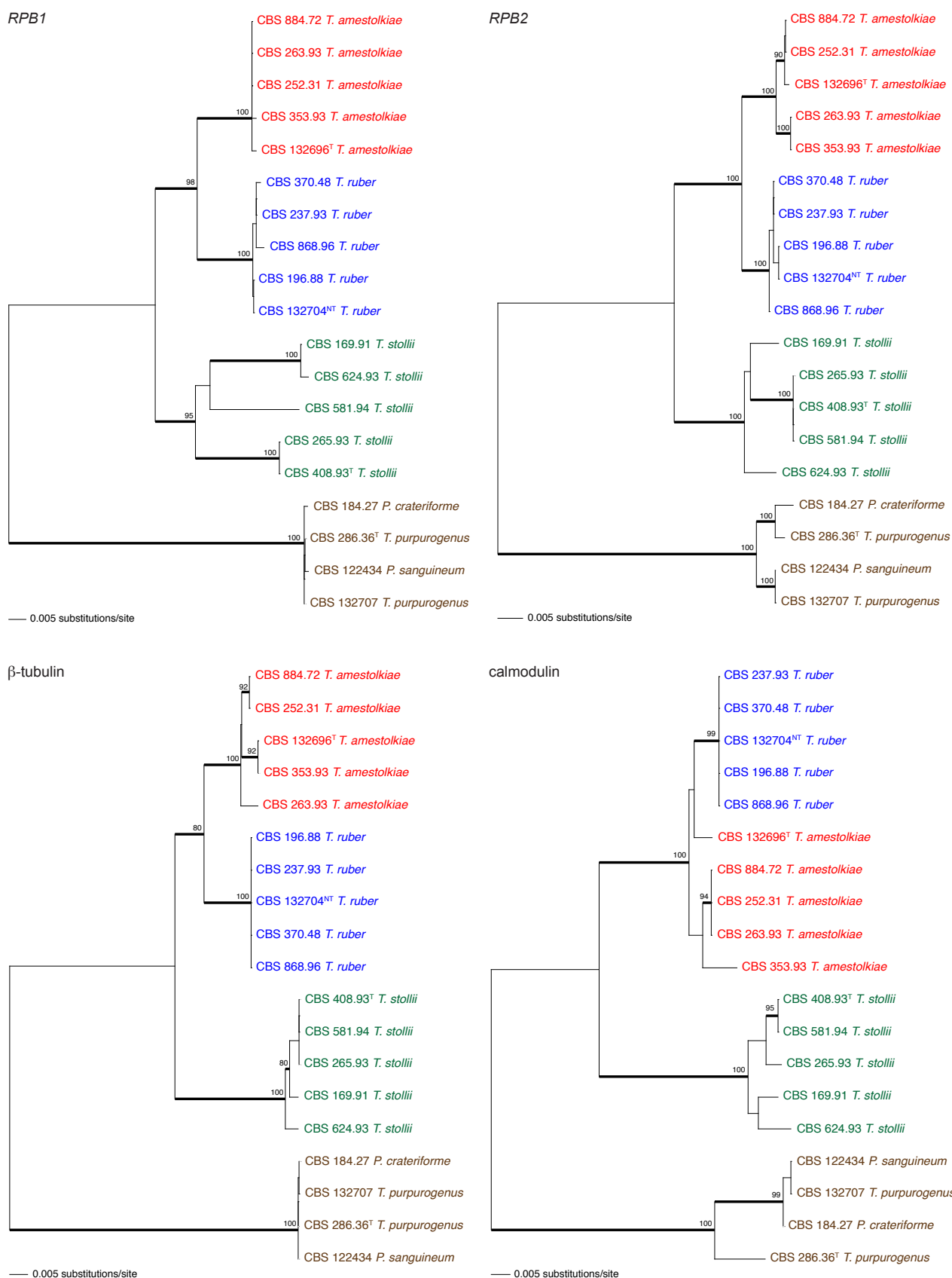
DNA extractions were prepared from strains grown for 7 to 14 d on MEA using the Ultraclean™ Microbial DNA isolation Kit (Mo-Bio, Solana Beach, USA). Extracted DNA was stored at -20 °C. The ITS regions and regions of the  $\beta$ -tubulin, calmodulin, *RPB1* and *RPB2* genes were amplified and sequenced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



**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 Houbraeken 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 (Houbraeken 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                                       | Rubratxin A and B*                                 | Wilson & Wilson (1962) | From Dennis N. Cox, Georgia, USA                                        |
| 1968-10-28a        | IMI 136126 = MR 006 = IBT 10710                                                                                 | Rubratxin A and B                                  | Moss & Hill (1970)     | Mould field corn, Wisconsin, EB Smalley                                 |
| 1968-10-28b        | IMI 136127 = MR 007 = IBT 5016                                                                                  | Rubratxin 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                                                          | Rubratxin A and B                                  | Moss & Hill (1970)     | Mould field corn, Wisconsin, EB Smalley                                 |
|                    | IMI 112715 = MR 185 = IBT 10712                                                                                 | Rubratxin A and B*                                 | Moss & Hill (1970)     | Rhizosphere of <i>Trifolium alexandrinum</i> , Egypt, A. El Esaily      |
|                    | IMI 129717 = MR 043/RC                                                                                          | Rubratxin A and B                                  | Moss & Hill (1970)     | PKC Austwick                                                            |
|                    | IMI 129718 = MR 043/OB6                                                                                         | Rubratxin A and B                                  | Moss & Hill (1970)     | PKC Austwick                                                            |
|                    | IMI 129719 = MR 043/OA                                                                                          | Rubratxin A and B                                  | Moss & Hill (1970)     | PKC Austwick                                                            |
|                    | IMI 129716 = MR 180                                                                                             | Rubratxin B                                        | Moss & Hill (1970)     | Van der Walt, South Africa                                              |
|                    | NRRL 2019 = IBT 3549                                                                                            | Rubratxin B                                        | Data reported here     | Unknown source                                                          |
| FAT 1141           | ATCC 20204 = IBT 4183 = IFO 5722 = CBS 113158                                                                   | Rubratxin 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                                                                                                      | Rubratxin 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 | Rubratxin 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                                                                                          | Rubratxin B*                                       | Data reported here     | Wheat, Winnipeg, Canada, JT Mills                                       |
| FA 158-B1-1X       | IBT 11632                                                                                                       | Rubratxin B*                                       | Data reported here     | Barley, Winnipeg Canada, JT Mills                                       |
| FA 156-B1-1        | IBT 11694                                                                                                       | Rubratxin 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                                                         | Rubratxin 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                    | CBS 184.27, CBS 286.36, CBS 113160, IBT 11632, IBT 12779, IMI 112715, IMI 136126, IMI 136127, IMI 136128, NRRL 3290                                                                                                                                                          |
|                                     | 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.

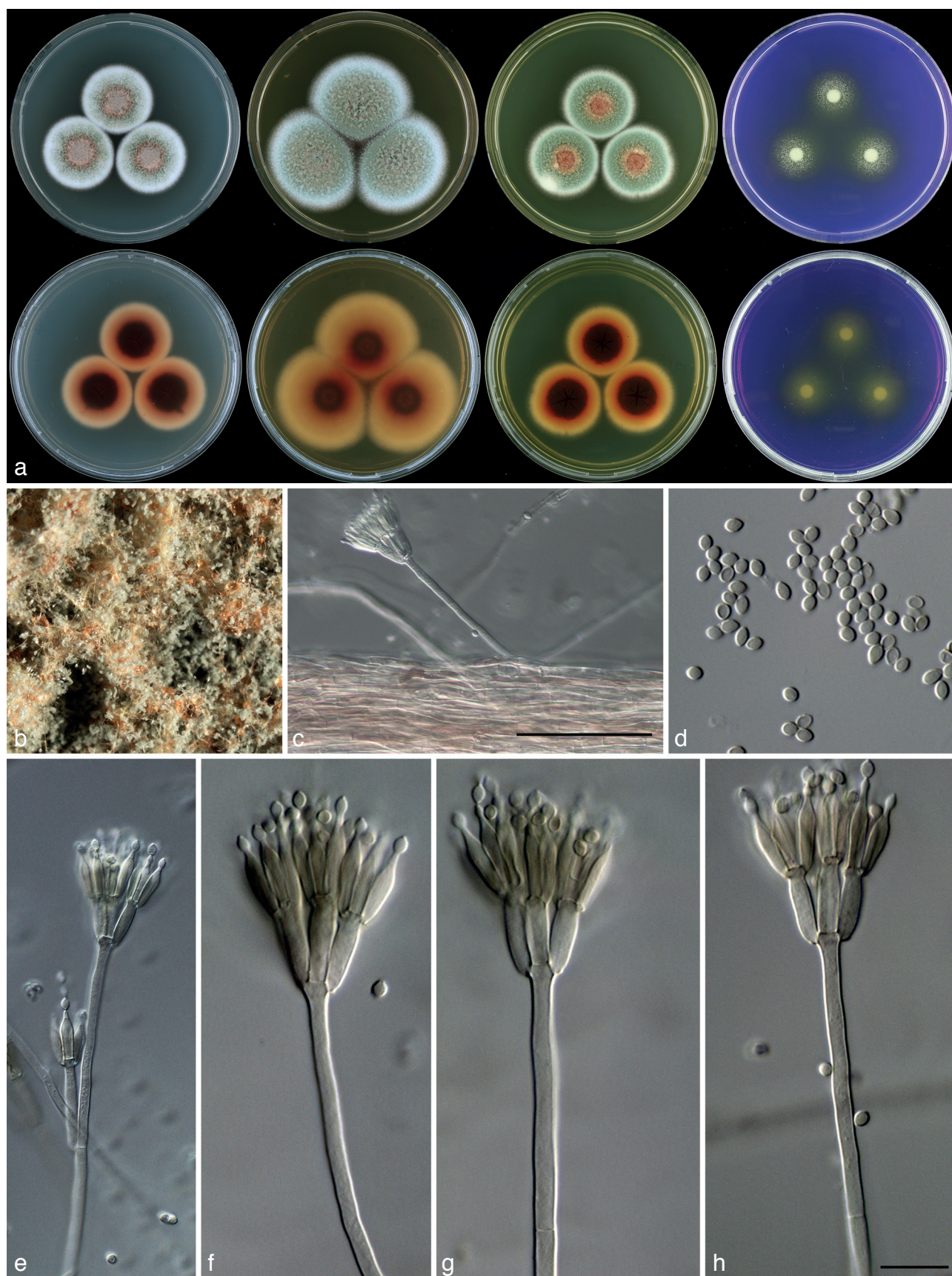
*genus* and other *Talaromyces* species. The ITS alignment included eight strains and was 469 bp characters long. The results showed that strains belonging to *T. amestolkiae*, *T. ruber* and *T. stollii* form a phylogenetically distinct clade, separate from the distinctly related *T. purpurogenus* clade. 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>1</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>1</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 mitorubrin (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, mitorubrin, '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, mitorubrin, 'Monascus pigments', pestalasin A, a purpactin, and chromo-

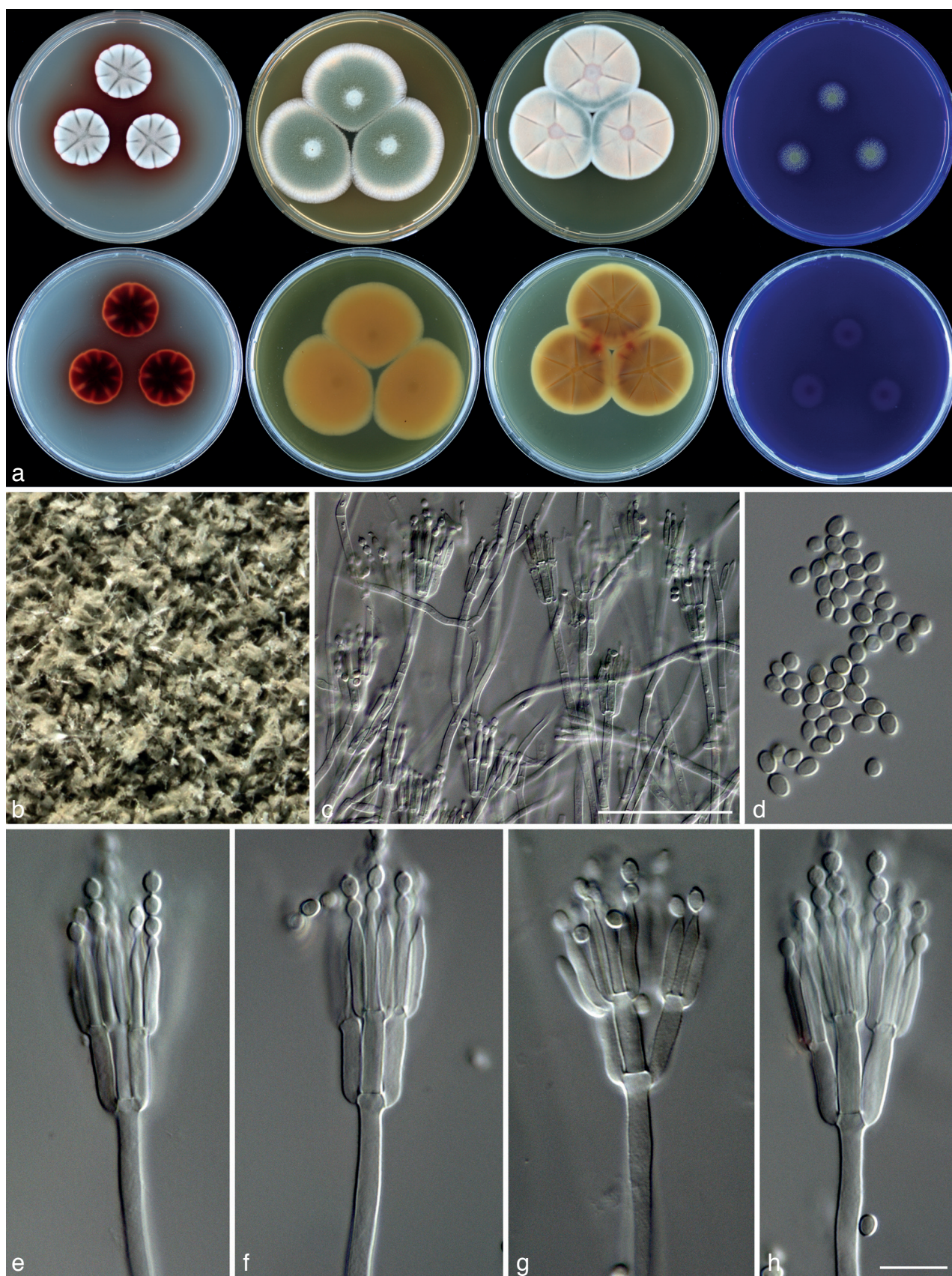


**Fig. 4** Morphological characters of *Talaromyces amestolkiae* (CBS 132696<sup>T</sup>). a. Colonies incubated on CYA, MEA, YES, CREA, from left to right (top row = obverse, bottom row = reverse); b. colony texture on MEA; c–g. conidiophores produced on MEA; h. conidia. — Scale bars: c = 50  $\mu$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





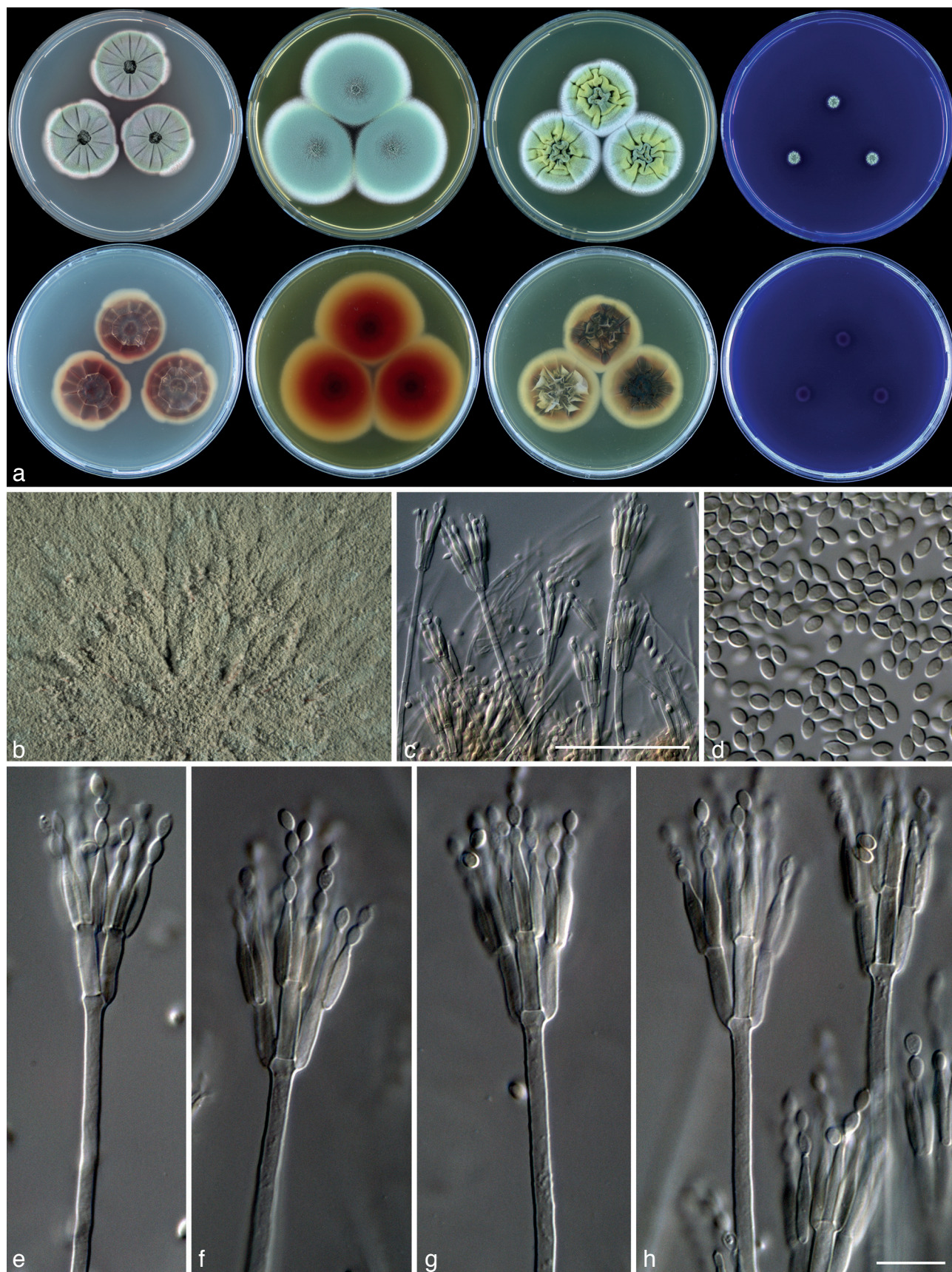
**Fig. 5** Morphological characters of *Talaromyces purpurogenus* (CBS 132707). a. Colonies incubated on CYA, MEA, YES, CREA, from left to right (top row = obverse, bottom row = reverse); b. colony texture on MEA; c–g. conidiophores produced on MEA; h. conidia. — Scale bars: c = 50  $\mu$ m; g = 10  $\mu$ 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  $\mu$ m; **metulae** in verticils of 3–5, 7.5–11  $\mu$ 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)  $\mu$ 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)  $\mu$ 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)  $\mu$ 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;

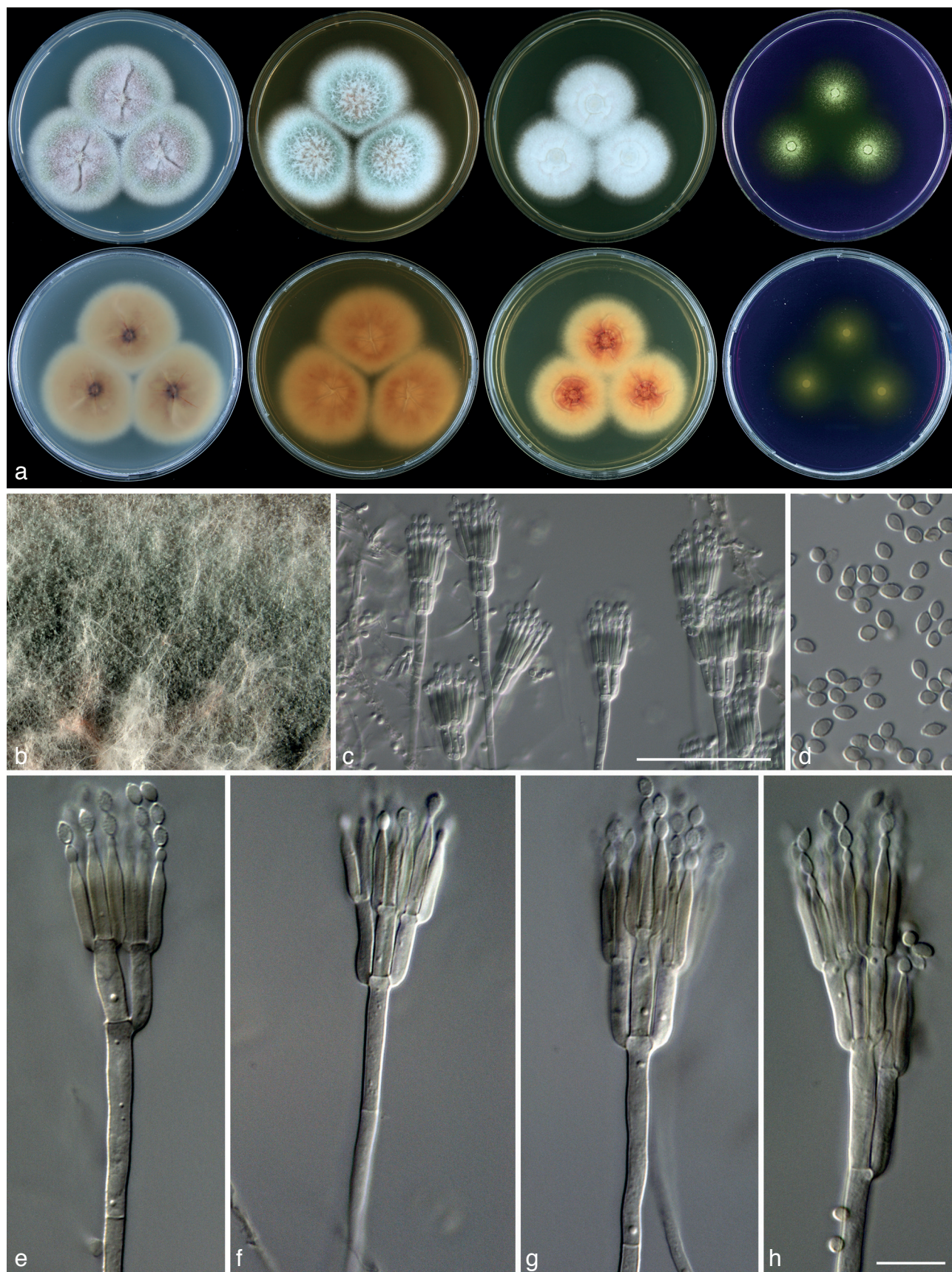
mycelium white, yellow and red; texture velvety, sometimes with funicles near margins; sporulation moderately dense, conidia *en masse* bright olive green to greyish green (26D4–27D4); exudate present in some strains, small clear and red droplets; soluble reddish pigment typically present, absent in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