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Rasamsonia, a new genus comprising thermotolerant and thermophilic *Talaromyces* and *Geosmithia* species

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Abstract The phylogenetic relationship among *Geosmithia argillacea*, *Talaromyces emersonii*, *Talaromyces byssochlamydoides* and other members of the Trichocomaceae was studied using partial *RPB2* (RNA polymerase II gene, encoding the second largest protein subunit), *Tsr1* (putative ribosome biogenesis protein) and *Cct8* (putative chaperonin complex component TCP-1) gene sequences. The results showed that these species form a distinct clade within the Trichocomaceae and *Trichocoma paradoxa* is phylogenetically most closely related. Based on phenotypic and physiological characters and molecular data, we propose *Rasamsonia* gen. nov. to accommodate these species. This new genus is distinct from other genera of the Trichocomaceae in being thermotolerant or thermophilic and having conidiophores with distinctly rough walled stipes, olive-brown conidia and ascomata, if present, with a scanty

covering. Species within the genus *Rasamsonia* were distinguished using a combination of phenotypic characters, extrolite patterns, ITS and partial calmodulin and β -tubulin sequences. *Rasamsonia brevistipitata* sp. nov. is described and five new combinations are proposed.

Keywords *Emersonii* · *Argillacea* · Taxonomy · Extrolites · Phylogeny · Trichocomaceae

Introduction

Thermophilia and thermotolerance have been variously defined (Apinis 1963; Cooney and Emerson 1964; Craveri et al. 1964; Crisan 1973). The commonly accepted definition is that of Cooney and Emerson (1964), who defined thermophilic species as those which can grow at or above 50°C, but not below 20°C. The definition of thermotolerance is similar except that the maximum growth temperature is at or above 45°C, having a maximum near 50°C, and the minimum growth temperature is below 20°C. Thermophilic fungi have gained much attention in biotechnology the last decades, primarily because of their capacity to produce thermostable enzymes, which are stable at room temperature and can be purified by heat treatment (Maheshwari et al. 2000; Turner et al. 2007). For example, the thermophilic species *Talaromyces emersonii* produces multiple β -glucosidase enzymes, a number of which are components of a potent

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cellulytic enzyme system and are used in commercial applications (Collins et al. 2007; Coughlan et al. 1993; McHale and Coughlan 1981; Murray et al. 2004).

Thermophilic fungi are not restricted to a specific taxonomic order and occur all over the fungal tree of life. Examples are *Rhizomucor* in the Mucorales, *Myceliophthora* in Sordariales and *Talaromyces*, *Thermomyces* and *Thermoascus* in the Eurotiales (Mouchacca 1997). The majority of *Talaromyces* species are mesophiles but exceptions are species within sections *Emersonii* and *Thermophila*. Section *Emersonii* includes *Talaromyces emersonii*, *T. byssochlamydoides*, *T. bacillisporus* and *T. leycettanus*, all of which grow well at 40°C. *Talaromyces bacillisporus* is thermotolerant, *T. leycettanus* is thermotolerant to thermophilic, and *T. emersonii* and *T. byssochlamydoides* are truly thermophilic (Stolk and Samson 1972). The sole member of *Talaromyces* section *Thermophila*, *T. thermophilus*, grows rapidly at 50°C (Evans and Stolk 1971; Evans 1971; Stolk and Samson 1972). The current classification of these thermophilic *Talaromyces* species is mainly based on phenotypic and physiological characters, such as their ability to grow above 40°C, ascospore color, the structure of ascomatal covering and the formation of a certain type of anamorph. Stolk and Samson (1972) stated that the members of the section *Emersonii* have anamorphs of either *Paecilomyces* (*T. byssochlamydoides* and *T. leycettanus*) or *Penicillium cylindrosporum* series (*T. emersonii* and *T. bacillisporus*). Later, Pitt (1979) transferred the species belonging to the *Penicillium cylindrosporum* series to the genus *Geosmithia*, based on various characters such as the formation of conidia from terminal pores instead of on collula (necks), a character of *Penicillium* and *Paecilomyces*. Within the genus *Geosmithia*, only *G. argillacea* is thermotolerant, and Stolk et al. (1969) and Evans (1971) proposed a connection with members of *Talaromyces* sect. *Emersonii*. The phylogenetic relationship of the thermophilic *Talaromyces* species within *Talaromyces* and the Trichocomaceae is unknown.

In this study, the phylogenetic relationship among *Geosmithia argillacea*, *T. emersonii*, *T. byssochlamydoides* and other thermotolerant/thermophilic members of the Trichocomaceae is studied using partial *RPB2* (RNA polymerase II gene, encoding the second largest protein subunit), *Tsr1* (putative ribosome biogenesis protein) and *Cct8* (putative chaperonin complex component

TCP-1) gene sequences. These data indicate that these species are closely related and phenotypic and physiological characters show that they represent a new genus. The taxonomy of this new genus is studied here using a combination of phenotypic characters, extrolite patterns, ITS and partial calmodulin and β -tubulin sequences. One new species is described and five new combinations are proposed.

Materials and methods

Strains

The strains used in the study on the phylogeny of the Trichocomaceae are listed in Supplementary Table 1. An overview of strains used for phenotypic analysis and phylogeny is shown in Table 1. All cultures are maintained in the culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

Morphological characterization

Cultures for morphological observation were inoculated in a three point position onto the following agar media: 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). All media were prepared as described by Samson et al. (2010). Two sets were inoculated per strain: one set was incubated at 25°C, the other at 37°C. Colony diameter and other macroscopical features were recorded after 7 days of incubation. Microscopical features were studied by light microscopy (Olympus BH2 and Zeiss Axioskop 2 Plus) and microscopic mounts were made from MEA or OA.

Molecular analysis

Strains were grown on MEA for 7–14 days prior to DNA extraction. Genomic DNA was extracted using the Ultraclean™ Microbial DNA isolation Kit (MoBio, Solana Beach, U.S.A.) according to the manufacturer's instructions. For the phylogenetic analysis of the Eurotialean taxa, parts of the *RPB2*, *Tsr1* and *Cct8* genes were sequenced. The latter two

Table 1 *Rasamsonia* isolates used in this study

CBS no.	Name	Other collections	Origin	GenBank accession numbers (ITS/BenA/Cmd)
CBS 101.69 ^T	<i>R. argillacea</i> (= <i>Geosm. argillacea</i>)	DTO 97E4 = IMI 156096 = IBT 31199	Mine tip with a very high surface temperature; Staffordshire, UK	JF417491; JF417456; JF417501
CBS 102.69	<i>R. argillacea</i> (= <i>Geosm. argillacea</i>)	DTO 97E5 = IBT 31200	Air; UK	JF417490; JF417457; JF417502
CBS 907.70	<i>R. argillacea</i> (= <i>Geosm. argillacea</i>)	DTO 97E7	Unknown source; Reading, UK	JF417474; JF417458; JF417503
CBS 128787	<i>R. argillacea</i> (= <i>Geosm. argillacea</i>)	DTO 73F3	Heat treated fruit concentrate; imported into the Netherlands	JF417475; JF417459; JF417504
CBS 128785 ^T	<i>R. brevisipitata</i>	DTO 25H2 = IBT 31187	Indoor environment of school, from cork with bitumen, Germany	JF417488; JF417454; JF417499
CBS 128786	<i>R. brevisipitata</i>	DTO 26B1 = IBT 31188	Indoor environment of school, from cork with bitumen, Germany	JF417489; JF417455; JF417500
CBS 413.71 ^T	<i>R. byssochlamydoidea</i> (= <i>Tal. byssochlamydoidea</i>)	DTO 149D6 = IBT 11604	Dry soil under Douglas fir; Oregon, USA	JF417476; JF417460; JF417512
CBS 533.71	<i>R. byssochlamydoidea</i> (= <i>Tal. byssochlamydoidea</i>)	DTO 138G6 = IBT 11601 = IBT 31184	Piles of peat; near Oldenburg, Germany	JF417477; JF417461; JF417513
CBS 275.58 ^{NT}	<i>R. cylindrospora</i> (= <i>Geosm. cylindrospora</i>)	DTO 138F8 = IBT 31202 = ATCC 18223 = IMI 071623	Culture contaminant; Berkshire, England, UK	JF417470; JF417448; JF417493
CBS 432.62	<i>R. cylindrospora</i> (= <i>Geosm. cylindrospora</i>)	DTO 138F7 = IBT 31201	Man, sputum; the Netherlands	JF417471; JF417449; JF417492
CBS 100538 ^T	<i>R. eburnea</i> (= <i>Tal. eburnea</i>)	DTO 105D6 = IBT 17519	Soil; Taipei, Taiwan	JF417483; JF417462; JF417494
CBS 124445	<i>R. eburnea</i> (= <i>Tal. eburnea</i>)	DTO 49D7 = IBT 31193	Blood culture, patient with peritonitis; UK	JF417472; JF417450; JF417495
CBS 102881	<i>R. eburnea</i> (= <i>Tal. eburnea</i>)	DTO 97E9 = IBT 31195 = UAMH 9714	Man, bronchial washing (apparently etiologic); Toronto, Ontario, Canada	JF417484; JF417451; JF417496
CBS 124447	<i>R. eburnea</i> (= <i>Tal. eburnea</i>)	DTO 45I3 = IBT 31191	Contaminant of blood culture (pseudo-outbreak); UK	JF417473; JF417452; JF417497
CBS 124446	<i>R. eburnea</i> (= <i>Tal. eburnea</i>)	DTO 49D9 = IBT 31192	Peritoneal dialysis fluid; UK	JF417487; JF417453; JF417498

Table 1 continued

CBS no.	Name	Other collections	Origin	GenBank accession numbers (ITS/BenA/Cmd)
CBS 393.64 ^T	<i>R. emersonii</i> (= <i>Tal. emersonii</i>)	DTO 4811 = ATCC 16479 = IMI 116815 = IMI 116815ii = IBT 31218 = IBT 21695	Compost; Italy	JF417478; JF417463; JF417510
CBS 396.64	<i>R. emersonii</i> (= <i>Tal. emersonii</i>)	DTO 108B2 = IBT 31180	Soil; Baarn, the Netherlands	JF417479; JF417467; JF417511
CBS 397.64	<i>R. emersonii</i> (= <i>Tal. emersonii</i>)	DTO 108B3 = IMI 105413 = IBT 31179	Soil; near Nottingham, UK	JF417480; JF417466; JF417509
CBS 355.92	<i>R. emersonii</i> (= <i>Tal. emersonii</i>)	DTO 138G4	Young mushroom compost; Beitem-Roeselare, Belgium	JF417482; JF417465; JF417508
CBS 549.92	<i>R. emersonii</i> (= <i>Tal. emersonii</i>)	DTO 138G5 = CBS 814.70 = IMI 154228 = IBT 31203 = IBT 31181 = IBT 24759	Sugar cane bagasse; Reading, UK	JF417481; JF417464; JF417507
CBS 103.73	<i>Trichocoma paradoxo</i>	IBT 31160	Unknown; Japan	JF417486; JF417469; JF417506
CBS 247.57	<i>Trichocoma paradoxo</i>	IBT 31159	Unknown; Hachijō, Japan	JF417485; JF417468; JF417505

CBS culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands; DTO internal culture collection of CBS-KNAW Fungal Biodiversity Centre; IMI CABI Genetic Resources Collection, Surrey, UK; IBT culture collection of Center for Microbial Biotechnology (CMB) at Department of Systems Biology, Technical University of Denmark; ATCC American Type Culture Collection, Manassas, VA, USA

genes were chosen, since they were among the best-performing genes for recovering robust phylogenies in the study by Aguileta et al. (2008). The partial *RPB2*, *Tsr1* and *Cct8* sequences consists of exon data only and the alignment of the data set was performed using the Muscle software on the translated amino acid data. The ITS (incl. 5.8S rDNA), β -tubulin and calmodulin loci were sequenced in order to study the phylogenetic relationship among *T. emersonii*, *T. byssochlamydoidea*, *T. eburneus*, *G. argillacea* and *G. cylindrospora*. The obtained sequences were aligned using the software Muscle on the EMBL-EBI website and subsequently combined. Primers used for amplification and sequencing and annealing temperatures are shown in Table 2. Maximum likelihood analysis was performed on the two data sets using RAxML version 7.2.8. Each locus was treated as a separate partition. The number of bootstrap replicates was set on 1,000

replicates (Stamatakis et al. 2008). A second measure of branch support was conducted using a Bayesian tree inference (BI) analysis. Prior to analysis, the best nucleotide substitution models were determined for each partition with MrModeltest v2.2 (Nylander 2004). A BI analysis was performed with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). One tree was saved per 100 generations, and the run was automatically ended when the standard deviation of split frequencies was below 0.01. To avoid suboptimal trees being taken into account for the consensus tree, a burn-in of 25% of the saved trees was used. *Coccidioides immitis* sequences were downloaded from the full genome data set (Sharpton et al. 2009) and used as an outgroup in the study of the phylogeny of selected Trichocomaceae. *Trichocoma paradoxa* CBS 247.57 and CBS 103.73 were used as an outgroup in the phylogenetic analysis.

Table 2 Primers used in this study for amplification and sequencing

Locus	Primer	Sequence (5′–3′)	Annealing temperature (°C)	Fragment size (bp)	References
<i>RPB2</i>	5F_Eur	(Fwd) GAYGAYCGKGGAYCAYTTCGG	48–51	ca. 1220	Modified after RPB2-5F; Liu et al. (1999)
	7CR_Eur	(Rev) CCCATRGCTGYTTRCCCAT			
<i>Tsr1</i>	F1526Pc	(Fwd) GARTAYCCBCARTCNGAGATGT	48–50	ca. 820	This study
	R2434	(Rev) ASAGYTGVARDCCTTRAACCA			
<i>Cct8</i>	F94	(Fwd) CGCAAC AAGATYGTBATYAACCA	50–52	F94–R1595: 1400–1450	This study
	F660	(Fwd) GIGTKGTBAAGATCATGGGWGG		F660–R1595: 850–890	This study
	R1595	(Rev) RTCMACRCCNGTIGTCCAGTA		This study	
<i>BenA</i>	T10	(Fwd) ACGATAGGTTACCTCCAGAC	55	T10–Bt2b: 570–620	O’Donnell and Cigelnik (1997)
	Bt2a	(Fwd) GGTAACCAAAATCGGTGCTGCTTTC		Bt2a–Bt2b: 520–550	
	Bt2b	(Rev) ACCCTCAGTGTAGTGACCCTTGGC		Glass and Donaldson (1995)	
ITS	V9G	(Fwd) TTACGTCCCTGCCCTTTGTA	55	ca. 1000	de Hoog and Gerrits van den Ende (1998)
	LS266	GCATTCCCAAACAACCTCGACTC			Masclaux et al. (1995)
<i>Cmd</i>	Cmd5	(Fwd) CCGAGTACAAGGARGCCTTC	55	ca. 630	Hong et al. (2006)
	Cmd6	(Rev) CCGATRGAGGTCATRACGTGG			Hong et al. (2006)

Extrolite analysis

All strains, except *Trichocoma paradoxa* isolates, (Table 1) were grown for 7 and 14 days at 37°C on the solid media YES, CYA, OA, malt extract agar (MEA-B) (Blakeslee formula, Difco malt extract) (Raper and Thom 1949, p. 67), MEA, Wickerham's Antibiotic Test Medium (WATM) (Raper and Thom 1949, pp. 69–70) and Raulin-Thom (Smith 1960, p. 259) with added oat meal (RTO agar) (using 2.67 g ammonium tartrate and 30 g oatmeal/l) prior to extrolite extraction. *Trichocoma paradoxa* was grown on the above mentioned agar media for 4 weeks at 25°C. All media contained a trace metal mixture of 5 ppm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 10 ppm $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Smith 1949). Five agar plugs taken along a diameter of the fungal colony were used for extraction. The agar plugs were transferred in ethyl acetate/dichloromethane/methanol (3:2:1, v/v/v) with 1% (v/v) formic acid and ultrasonicated for 50 min. The extracts were transferred to a 1.5 ml autosampler screw-cap vials, evaporated to dryness and re-dissolved in 400 μl methanol by ultrasonication in 10 min. Subsequently, the extracts were filtered through 0.45 μm filter (Minisart RC4, Sartorius, Germany) and kept at -18°C prior to analysis. The extracts were analyzed by ultra high performance liquid chromatography (U-HPLC) using alkylphenone retention indices and diode array UV–VIS detection as described by Frisvad and Thrane (1987). The U-HPLC used was Dionex Ultimate 3000 with two pumps. The extrolites were separated on a Kinetex C_{18} (150 \times 2.1 mm) column with 2.6 μm particles. The column temperature was held at 60°C. The extract injection volume was 1 μl . A gradient based on water with 50 ppm trifluoroacetic acid (TFA) and acetonitrile (AcCN) with 50 ppm TFA, at a flow rate of 0.8 ml/min, was used: Start conditions 85% H_2O with TFA and 15% AcCN with TFA. Gradient: 15–25% AcCN in 1/2 min, from 25 to 65% AcCN in 5 1/2 min, from 60 to 100% AcCN in 1 min, at 100% AcCN in 1 min, back to 15% AcCN in 1 min and then 1 min constant at 15% AcCN. The run-time was therefore 10 min. UV chromatograms were recorded at 210 and 280 nm, but diode array detection was carried out in a range from 190 to 600 nm. Identification of extrolites was performed by comparison of the UV–Visible spectra and retention times of the extrolites with those present in the collection at Department of Systems Biology, Kgs. Lyngby, Denmark.

Results

Generic delimitation

Macro- and microscopical analysis of *Talaromyces emersonii*, *T. byssochlamydoides*, *T. eburneus*, *Geosmithia argillacea* and *G. cylindrospora* showed that these species share various phenotypic and physiological characters. Shared phenotypic characters include conidiophores with distinct rough-walled stipes and ellipsoidal, ovoid or cylindrical conidia, which are in shades of olive-brown. The ascomata of *T. emersonii*, *T. byssochlamydoides*, *T. eburneus* have a scanty covering and the ascospores are smooth walled, globose, subglobose or ovoidal. Furthermore, these species are thermophilic or thermotolerant and have optimal growth at 37°C or higher (see Supplementary Fig. 1).

The phylogenetic position of *T. emersonii*, *T. byssochlamydoides*, *G. argillacea* and *G. cylindrospora* was studied using partial *RPB2*, *Cct8* and *Tsr1* sequences. The total length of the combined data sets was 2343 bp (exon data only). The structure of the partitions was as follows: the *Cct8* partition was 726 bp long and had 368 variable sites; the *Tsr1* data set included 639 characters, and 438 of those were variable and the *RPB2* gene partition was 978 bp long, 502 were found to be variable. The SYM+I+G model was found to be optimal for the *Tsr1* partition, whereas the best substitution model for the *RPB2* and *Cct8* sequence matrix was determined to be GTR+I+G. The thermophilic species *T. emersonii* and *T. byssochlamydoides* from section *Emersonii* and the thermotolerant species *G. argillacea* and *G. cylindrospora* group together, and the mesophile *Trichocoma paradoxa* is basal to these species. The two other species of section *Emersonii*, *T. bacillisporus* and *T. leycettanus*, are accommodated in other clades. *Talaromyces bacillisporus* belongs to the genus *Talaromyces*, and *T. leycettanus* groups with *Hamigera avellanea* and *Warcupiella spinulosa*. The Eurotialean *Geosmithia* species and related teleomorphs are polyphyletic (Fig. 1). *G. argillacea* and *G. cylindrospora* are closely related to *T. emersonii* and *T. byssochlamydoides*, *G. namyslowskii* is placed within *Penicillium* and *Eupenicillium*, and *G. viridis* and *T. bacillisporus* (anamorph *G. swiftii*) are accommodated in a clade comprising species mainly belonging to *Penicillium* subgenus

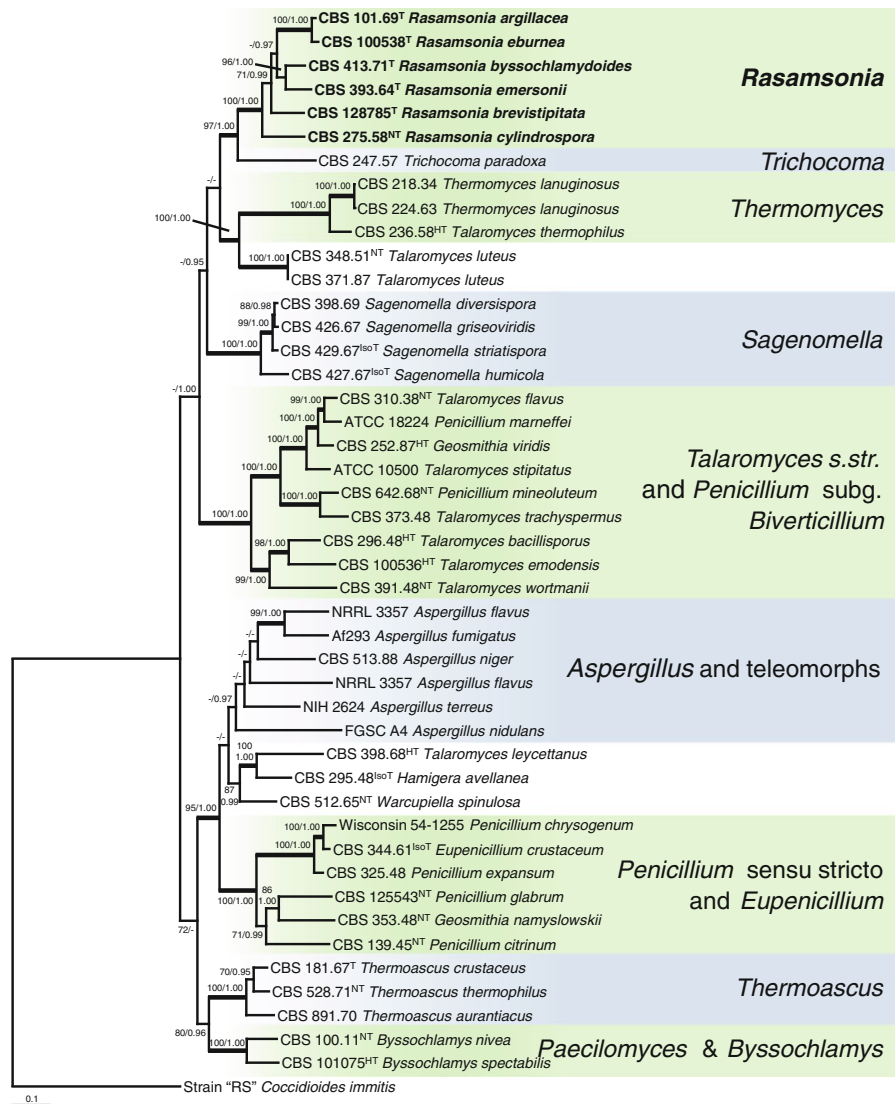


Fig. 1 Best-scoring Maximum Likelihood tree using RAxML based on combined data set of partial *RPB2*, *Cct8* and *Tsr1* sequences. The thermophiles are accommodated in three different clades: *Rasamsonia*, *Thermomyces* and *Thermoascus*. *Rasamsonia* species form a distinct clade in the Trichocomaceae and are closely related to *Trichocoma*. The bootstrap percentages of the RAxML analysis (first number) and BI Posterior

Probabilities (second number) analysis are given at the nodes. Only bootstrap values above 70% or posterior probability values higher than 0.9 are shown. Branches supported less than 70% bootstrap value or 0.9 posterior probability are indicated with a hyphen and branches with 95% bootstrap or more are thickened. The tree is rooted with *Coccidioides immitis* (strain "RS", Sharpton et al. 2009)

Biverticillium and *Talaromyces*. Based on phenotypic, physiological and molecular data, we propose to transfer the species *T. emersonii*, *T. byssochlamydoidea*, *T. eburneus*, *G. argillacea* and *G. cylindrospora* to *Rasamsonia* gen. nov.

Species delimitation in *Rasamsonia*

Species belonging to the genus *Rasamsonia* can be distinguished by phenotype (Table 3). The optimum and maximum growth temperature on MEA, the

Table 3 Differential characteristics of *Rasamsonia* species

	<i>R. argillacea</i>	<i>R. brevisipectata</i>	<i>R. byssochlamydoidea</i>	<i>R. cylindrospora</i>	<i>R. eburnea</i>	<i>R. emersonii</i>
Growth on CYA (7 days, 25°C)	15–25 mm	7–13 mm	No growth	3–8 mm	14–20 mm	No growth
Growth on CYA (7 days, 37°C)	30–40 mm	11–17 mm	19–27 mm	5–10 mm	30–40 mm	18–30 mm
Optimum and maximum growth temperature on MEA	Optimum 36°C; maximum above 50°C	Optimum 33°C; maximum slightly above 45°C	Optimum around 45°C; maximum between 50 and 55°C	Optimum 36°C; maximum at or slightly above 50°C	Optimum 36°C; maximum slightly above 50°C	Optimum between 45 and 50°C; maximum around 55°C
Shape and size conidia	Cylindrical or ovoid, (3–) 3.5–4.5 (–5.0) × 1.5–2.0 (–2.3) μm	Ellipsoidal or ovoid, (2.0–) 2.5–3.0 (3.5) × 1.7–2.1 μm	Cylindrical, 4–8 × 1–2.5 μm	Cylindrical, 4.0–5.0 × 1.6–2.1 μm	Cylindrical at first, becoming ellipsoidal or ovoid, 2.5–3.5 (–4) × 1.8–2.5 μm	Cylindrical, 3.5–4.5 (–5.0) × 1.5–3.0 μm
Ascospores/ascospores	Absent	Absent	Present, usually maturing within 7 days, 3.7–4.5 × 3.5–4 μm	Absent	Occasionally present, maturing slowly, 4–5 × 4–4.5 μm	Present, usually maturing within 7 days, 3.5–4 × 2.7–3.5 μm
Branching pattern conidiophores	Regularly branched, predominantly biverticillate, occasionally monoverticillate	Regularly branched, monoverticillate or biverticillate	Irregularly branched (<i>Paecilomyces</i> -type), terverticillate or quarterverticillate	Regularly branched, predominantly biverticillate, occasionally terverticillate or simple	Regularly branched, mono-, bi and terverticillate	Regularly branched, bi- or terverticillate
Extrolites ^a	“DOK”, “FLJR”, “FLOX”, “FXU”, “QOQ”, “RAI”, “SCOT”	Brefeldin A ^b	“BYB”, “BYS”, A corymbiferan lactone, emodin, emodin anthrone ^b , emodin bisanthrones ^b , “INDOLI”, “RUI”	Atrovenetin ^b , funalenone ^b , naphthalic anhydride ^b , “POLVE”	“CORT”, “FLJR”, “FLOX”, “MÆCO”, “RAI”	“ALKI”, atrovenetin ^b , funalenone ^b , “GUAX”, an isocoumarin, “JAJA”, naphthalic anhydride ^b , “PULSO”, secalonic acid D, “VIOL”

^a All extrolites with “capitals” have characteristic UV spectra and retention times, but could not be identified

^b Tentatively identified based on UV spectra

growth rate on CYA and YES and the reverse color on MEA at 36°C proved to be valuable characters for differentiation. Supplementary Fig. 1 compares temperature growth relations among *Rasamsonia* species and with the phylogenetically related species *Trichocoma paradoxa*. *Rasamsonia emersonii* and *R. byssochlamydoides* are thermophilic and have an optimum growth temperature around 45°C. The other *Rasamsonia* species are thermotolerant and have optimal growth around 33°C (*R. brevistipitata*) or 36°C (*R. argillacea*, *R. eburnea* and *R. cylindrospora*). *Trichocoma paradoxa* is mesophilic and has optimum between 21 and 24°C and a maximum between 30 and 33°C. The grouping of thermotolerant and thermophilic *Rasamsonia* species, although not supported by high bootstrap values, is also reflected in the phylogeny: *R. emersonii* and *R. byssochlamydoides* form a clade, and the other four species form another cluster. Growth of *Rasamsonia* species on CYA is poor with no or weak sporulation except for *R. argillacea* and *R. eburnea*, which grow well producing colony diameters larger than 30 mm. *Rasamsonia* species also differ in micro-morphology. The size and shape of the conidia, branching pattern of the conidiophores and the formation of ascomata are useful characters for differentiation. *Rasamsonia byssochlamydoides* deviates from the other species in having irregularly branched conidiophores (*Paecilomyces*-type). Ascospores were observed only in cultures of *R. emersonii* and *R. byssochlamydoides*. Although ascospores were reported in *R. eburnea* (Yaguchi et al. 1994), these were not detected in the present study after prolonged incubation of 8 weeks on OA and MEA at 33, 36 and 40°C. *Rasamsonia argillacea* and *R. eburnea* are phenotypically and phylogenetically closely related. The major phenotypic difference between these species is the blackish brown reverse of *R. eburnea* when grown on MEA at 36°C.

The results of the chemical analyses of the extracts of the different *Rasamsonia* species are listed in Table 3. Chemotaxonomically, the species of *Rasamsonia* can be divided into four groups: (1) the naphthalenone producers, *R. emersonii* and *R. cylindrospora*, which produce most extrolites on RTO agar, (2) the emodin, emodin anthron, and emodin bisanthron producer *R. byssochlamydoides*, which also produce most extrolites on RTO, (3) the producers of what was tentatively identified as Raistrick phenols and other small polar polyketides *R. eburnea* and

R. argillacea, best produced on YES agar, and (4) *R. brevistipitata*, a producer of only a few extrolites including a compound tentatively identified as brefeldin A. Media with nitrate, CYA and WATM, were highly inhibitory to extrolite production. *Trichocoma paradoxa* IFM H-53524 (= DTO 78H2 = CBS 788.83) and CBS 103.73 (= IBT 31160) were also analyzed for extrolites and found to produce rugulovasine A, corymbiferone (Overy et al. 2005a, b), corymbiferane lactones (Overy and Blunt 2004), and compounds related to naphthalic anhydride, atrovenetin and funalenone (Turner 1971), in addition to several unique extrolites. The latter three chromophore families were also detected in *R. cylindrospora* and *R. emersonii*. One of the corymbiferan lactones was also found in *R. byssochlamydoides*.

In order to study the phylogenetic relationship among *Rasamsonia* species, the ITS regions and partial calmodulin and tubulin sequences were combined and the total length of this data set was 1,766 nucleotides. The length of each partition was 706, 553 and 507 nucleotides for the ITS regions, and the partial calmodulin and tubulin gene regions, respectively, and number of variable sites were 158, 240 and 206 nucleotides, respectively. The SYM+G model was found to be optimal for the calmodulin partition, GTR+I+G was the best model for ITS, whereas the best substitution model for the tubulin matrix was determined to be K80 + I. Multilocus sequence analysis of the genus *Rasamsonia* revealed the presence of six species (Fig. 2). Five new combinations are proposed: *R. eburnea*, *R. argillacea*, *R. cylindrospora*, *R. emersonii* and *R. byssochlamydoides*, and one new species is described. We propose the name *R. brevistipitata* sp. nov. for this species. The phylogram supports the sibling relationship of *R. eburnea* and *R. argillacea*, however, the relationships between the other species are poorly or not supported (<95% bs).

Taxonomy

Rasamsonia Houbraken & Frisvad gen. nov. MycoBank MB 519868.

Latin diagnosis: Habitatione thermophila et thermotoleranti, culturis circa vel ultra 37°C optime crescentibus. Ascomatibus parce obtectis, ascosporis laevibus, globosis vel ovoideis, stipitibus conidiophorum et/vel hyphis sustinentibus distincte exasperates.

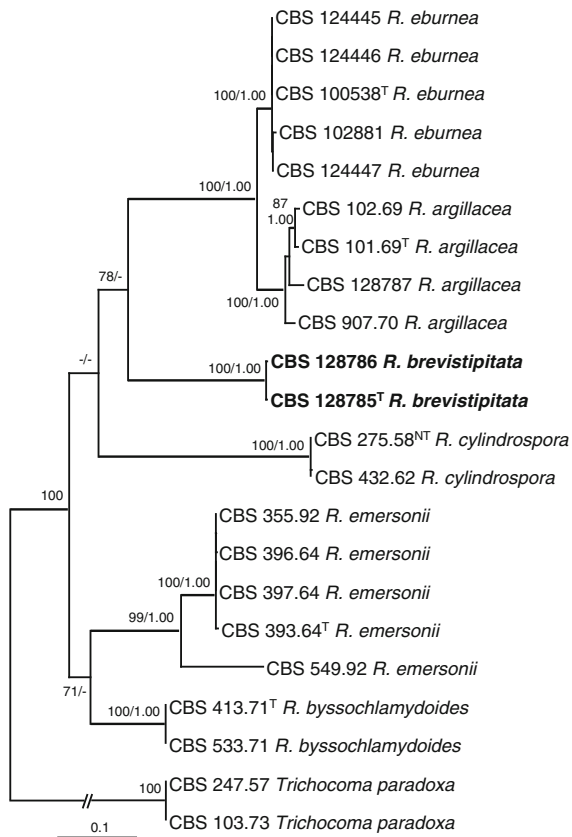


Fig. 2 Best-scoring Maximum Likelihood tree using RAxML based on combined data set of ITS, and partial β -tubulin and calmodulin sequences. Both *Rasamsonia brevistipitata* strains are on a single branch and positioned within the *Rasamsonia* clade. The bootstrap percentages of the RAxML analysis (first number) and BI Posterior Probabilities (second number) analysis are given at the nodes. Only bootstrap values above 70% or posterior probability values higher than 0.9 are shown

Conidiis ellipsoideis, ovoideis vel cylindraceis, diverse olivaceo-brunneis.

Typus: *Rasamsonia emersonii* (Stolk) Houbraken & Frisvad comb. nov.

Etymology: Named in honor of Robert A. Samson, for his excellent work in fungal taxonomy, and marking his 65th birthday and 40 years of service at the CBS-KNAW Fungal Biodiversity Centre.

Diagnostic features: Ascomata, if present, with a scanty covering, ascospores smooth walled, globose, subglobose or ovoidal. Stipes of conidiophores often distinctly rough walled, conidia ellipsoidal, ovoid or cylindrical, in shades of olive-brown. Thermophilic or thermotolerant, optimum around 37°C or higher.

Notes: *Rasamsonia* phenotypically resembles *Paecilomyces* and its teleomorph *Byssochlamys* and is placed within the order Eurotiales, family Trichocomaceae.

Byssochlamys/Paecilomyces and *Rasamsonia* both contain thermotolerant species, produce olive-brown conidia and form ascomata with no or scarce ascomatal covering. *Rasamsonia* differs in having regularly branched conidiophores with distinct rough walled structures and asci are borne in chains, while the conidiophores of *Paecilomyces* are irregularly branched, smooth walled and asci of *Byssochlamys* are born from croziers. Furthermore, the phialides of most *Rasamsonia* species (except *R. byssochlamydoidea*) are cylindrical and taper gradually towards the apices, while the phialides of *Paecilomyces* have a broad base tapering into a long narrow neck (*Paecilomyces*-type). The monotypic genus *Trichocoma* is phylogenetically closely related to *Rasamsonia* (Peterson 2008, this study). *Trichocoma* is characterized by asci borne in hyphal masses or tufts, which measure up to 10–20 mm in length (Kominami et al. 1952; Malloch 1985), while the covering of the ascomata of *Rasamsonia* are scanty. Furthermore, *Trichocoma* is a mesophile and has a maximum growth temperature of 33°C, while members of *Rasamsonia* have a maximum above 45°C (see Supplementary Fig. 1). There are also similarities between both genera: both share formation of bi- and terverticillate *Penicillium*-like structures, conidia are in shades of brown *en masse*, and both form rough walled hyphae.

Recently, the proposal to revise article 59 of the International Code of Botanical Nomenclature (ICBN) was accepted at the 2011 IBC Nomenclature Section at Melbourne and the principle of ‘one fungus : one name’ was established (Norvell 2011). In light of this decision, we propose a single name nomenclature for *Rasamsonia*.

Rasamsonia argillacea (Stolk, Evans & Nilsson) Houbraken & Frisvad **comb. nov.** Mycobank MB 519878. Supplementary Fig. 2.

Basionym: *Penicillium argillaceum* Stolk, Evans and Nilsson, Trans. Br. Mycol. Soc. 53: 307. 1969.

= *Geosmithia argillacea* (Stolk, Evans and Nilsson) Pitt, Can. J. Bot. 57: 2026. 1979.

Description: Colony diameters, 7 days, in mm., 25°C: CYA 15–25; CYAS 0–2; MEA 15–25; YES 15–24; DG18 6–12; OA 18–27; CREA: no growth. Colony diameters, 37°C: CYA 30–40; CYAS 9–10;

MEA 30–40; YES 35–46; DG18 13–22; OA 36–47; CREA: 14–21, poor growth, no acid and no base production. Optimum growth temperature 36°C, maximum above 50°C.

Good sporulation on CYA at 25 and 37°C, velvety, olive brown or crème-brown conidia, exudates and soluble pigments absent, colonies not or weakly sulcate, reverse pale brown with brown centre at 25°C and crème-brown at 37°C. Good sporulation on YES at 25°C, weak sporulation at 37°C, soluble pigments absent, mycelium inconspicuous, white, olive brown conidia, reverse brown. Colonies on MEA velvety to floccose, olive brown conidia, reverse light brown, occasionally with darker brown centre.

Stipes terminating in bi- or terverticillate structures, 60–350 × 2.0–3.5 µm, verrucose; branches subterminally present and at lower levels of the stipe, monoverticillate and short, suggesting the presence of monoverticillate conidiophores. Metulae in terminal whorls of 2–5, unequal in length, 10–15 (–30) × 1.5–2.5 µm. Phialides, 5–8 (–12) per metula, cylindrical base tapering more or less abruptly to a narrowed collula, 10–14 (–18) × 1.5–2.5 µm. Conidia smooth walled, cylindrical or ovoid, (3–) 3.5–4.5 (–5.0) × 1.5–2.0 (–2.3) µm.

Extrolites: Production better on YES agar than RTO, poor on other media. No extrolites could be identified, but among the chromophore families were several unidentified compounds with characteristic UV spectra (Table 3). Strain IBT 31197 (= DTO 137A4) was the best producer of extrolites, while CBS 102.69 and IBT 31196 (= DTO 137C1) produced few and CBS 101.69 produced none.

Diagnostic features: Thermotolerant, good growth on CYA (30–40 mm) and YES (35–46 mm) at 37°C, reverse on MEA uncolored or light brown, conidia cylindrical or ovoid and measuring (3–) 3.5–4.5 (–5.0) × 1.5–2.0 (–2.3) µm.

Distribution and ecology: *Rasamsonia argillacea* is a commonly occurring species and was isolated from hot environments, such as mine tips with a very high surface temperature, (indoor) air, clinical specimens and heat treated fruit concentrates. The presence of this species in heat treated food products suggests the potential presence of a teleomorph. Recently, this species was described as the cause of invasive mycosis in patients with chronic granulomatous disease (CGD, De Ravin et al. 2011; Machouart et al. 2011). Furthermore, this emerging pathogen was reported as

a chronic colonist of airways of cystic fibrosis patients (Giraud et al. 2010; Barton et al. 2010). *Rasamsonia argillacea* might be more common than reported in literature. Retrospective identification showed that all stored *P. variotii* strains from patient with CGD were *R. argillacea* and similar misidentifications also occur from non-CGD patients (Houbraken et al. 2010; De Ravin et al. 2011).

Notes: After comparison of the D1/D2 region of the 28S rDNA, Yaguchi et al. (2005) concluded that *R. eburnea* (as *T. eburneus*) is the teleomorph of *R. argillacea* (as *P. argillacea*). However, both species are distinct based on phenotype and sequences (see “Results” section).

Rasamsonia brevistipitata Weidner, Houbraken & Frisvad **sp. nov.** Mycobank MB 519870 (Fig. 3).

Latin diagnosis: Habitatione thermotoleranti, culturis restricte crescentibus in CYA (11–17 mm) et YES (22–30 mm) ad 37°C, coloniis reversis incoloratis vel dilute brunneis in agar MEA, stipitibus brevibus, conidiis ellipsoideis vel ovoideis, (2.0–) 2.5–3.0 (3.5) × 1.7–2.1 µm.

Etymology: *Rasamsonia brevistipitata* is referring to short stipes formed by this species.

Typus: Germany, Balingen: isolated from water damaged floor composed of bitumen and cork in a school, 2006, U. Weidner (holotype: CBS H-20546; culture ex type—CBS 128785 = DTO 25H2 = IBT 31187).

Description: Colony diameters, 7 days, in mm, 25°C: CYA 7–13; CYAS 0.5–2; MEA 17–25; YES 11–18; DG18 5–10; OA 20–28; CREA: 2–7, poor growth, no acid and no base production. Colony diameters at 37°C: CYA 11–17; CYAS 0; MEA 24–30; YES 22–30; DG18 8–14; OA 28–32; CREA: 2–7, poor growth, no acid and no base production. Optimum growth temperature 33°C; maximum slightly above 45°C.

Colonies on CYA at 25 and 37°C restricted, no or poor sporulation, velvety, pale olive brown conidia, crème colored mycelium, exudates and soluble pigments absent, colonies not sulcate, reverse crème or light brown. Moderate to good sporulation on YES at 25°C and 37°C, obverse pale olive brown, reverse light brown with dark brown centre. Colonies on MEA velvety, conidia olive brown, reverse uncolored or light brown.

Conidiophores predominantly arising from aerial mycelium, occasionally arising from basal mycelium;

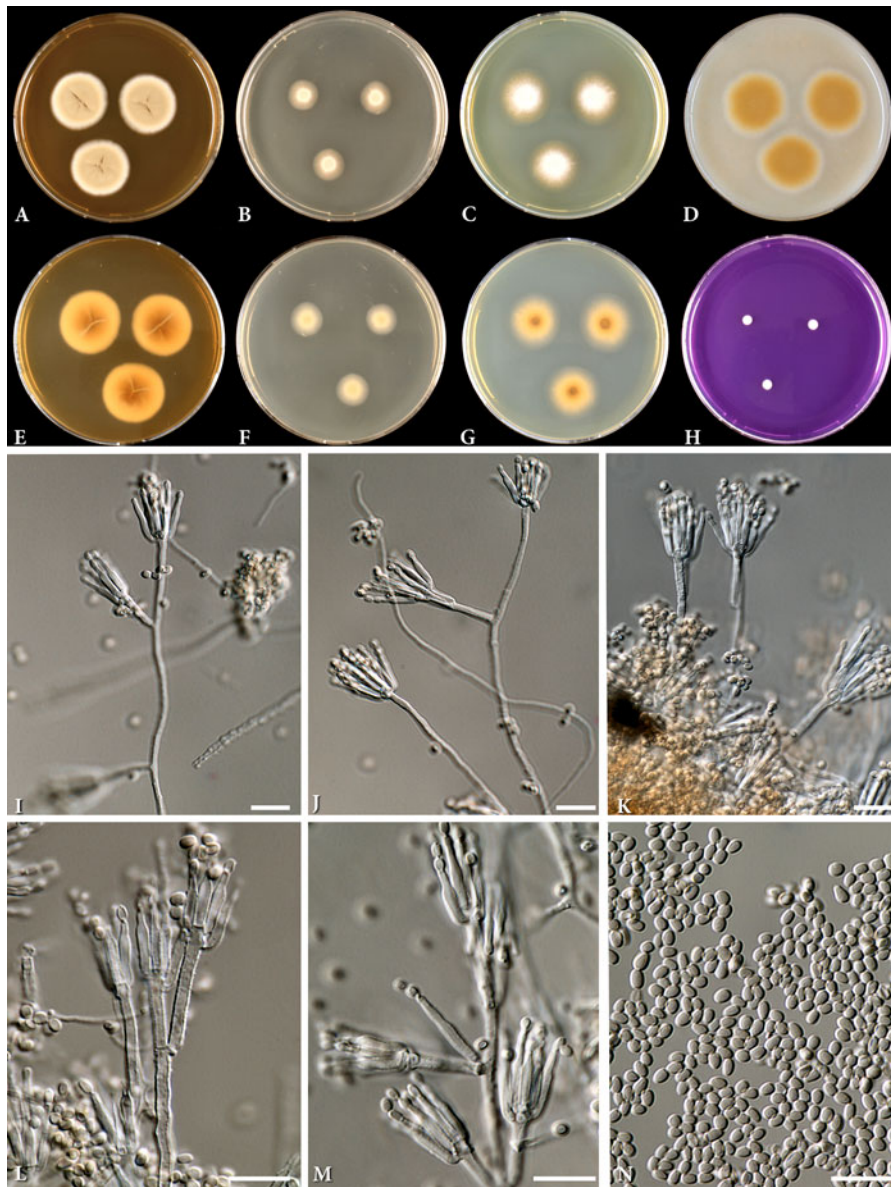


Fig. 3 *Rasamsonia brevistipitata*. **a–h** Colonies 7 days old 37°C: **a** MEA, **b** CYA, **c** YES, **d** OA, **e** MEA reverse, **f** CYA reverse, **g** YES reverse, **h** CREA; **i–m** conidiophores; **n** conidia. Scale bar 10 μ m

stipes terminating in monoverticillate structures when young and becoming biverticillate in older parts of the colony, (50–) 100–250 \times 2.0–3.5 μ m, branches on subterminally and intercalary positions, suggesting separate conidiophores, short (10–50 (–70) \times 2.0–3.5 μ m) and often monoverticillate, verrucose. Metulae, when present, in terminal whorls of 2–3 (–5), vesiculate, 12–17 (–20) μ m. Phialides *Paecilomyces*-type, in appressed verticils of 4–10, outer phialides

subapically placed on metula or stipe, cylindrical base slowly tapering in a long neck, 10–14 μ m. Conidia smooth walled, ellipsoidal or ovoid, (2.0–) 2.5–3.0 (3.5) \times 1.7–2.1 μ m. Ascospores and sclerotia absent.

Extrolites: Production best on YES and MEA; both isolates produced an extrolite tentatively identified as brefeldin A.

Diagnostic features: Thermotolerant, restricted growth on CYA (11–17 mm) and YES (22–30 mm)

at 37°C, reverse on MEA uncolored or light brown, conidia ellipsoidal or ovoid and measuring (2.0–) 2.5–3.0 (3.5) × 1.7–2.1 µm.

Distribution and ecology: This species was isolated from indoor environment in Germany. A BLAST search on GenBank with the ITS sequence of CBS 128785 showed a 100% homology with GenBank accession number FJ820827, a sequence generated during DNA analysis of outdoor air in Mainz, Germany (Fröhlich-Nowoisky et al. 2009).

Rasamsonia byssochlamydoides (Stolk & Samson) Houbraken & Frisvad **comb. nov.** MB 519877. Supplementary Fig. 3.

Basionym: *Talaromyces byssochlamydoides* Stolk & Samson, Stud. Mycol. 2: 45. 1972.

= *Paecilomyces byssochlamydoides* Stolk & Samson, Stud. Mycol. 2: 45. 1972.

Description: Colony diameters, 7 days, in mm, no growth at 25°C; 37°C: CYA 19–27; CYAS 0; MEA >60; YES 22–52; DG18 20–30; OA >60; CREA: 0–7, no or poor growth, no acid and no base production. Optimum growth temperature between 40 and 45°C, maximum above 50°C.

Poor growth on CYA at 37°C, sporulation, exudate and soluble pigments absent, colonies not sulcate, mycelium inconspicuous and white, reverse pale or pale with a brown centre. Sporulation on YES at 37°C weak or absent, soluble pigments absent, mycelium inconspicuous, obverse olive-brown when sporulation is present, reverse light brown and not influencing the medium color, occasionally with dark brown centre. Colonies on MEA spreading, velvety or with floccose mycelial overgrowth, olive brown conidia, thin layer of ascomata formed near the agar, white when young, orange or pink-reddish in age, reverse uncolored or brown.

Ascomata globose, up to 200 µm in diameter, covering very scanty. Asci eight-spored, subglobose to broadly ellipsoidal, 9–12.5 × 6.5–7.5 µm. Ascospores globose to subglobose, smooth, 3.5–4.5 × 4.0–5.5 µm. Conidiophores arising directly from the agar or in the floccose mycelium, irregularly branched (*Paecilomyces*-type), terverticillate or quarterverticillate, up to 300 µm in length; stipes broad, 3–6 µm, smooth to verrucose. Metulae in terminal whorls of 2–8, broad, unequal in length, (7) 10–15 × 2.0–5.0 µm. Phialides, cylindrical base tapering in a long narrowed collula, 10–15 (–20) × 2.5–3.5 µm. Conidia smooth walled, cylindrical, 4–8 × 1–2.5 µm.

Chlamydospores are rarely produced, globose or subglobose, about 4 µm in diameter (Stolk and Samson 1972).

Extrolites: Production best on RTO, followed by MEA-B, MEA and OAT. Poor on remaining media, except the production of an indole-alkaloid on YES agar by CBS 533.71. All examined strains (CBS 533.71 and CBS 413.71) produced two chromophore families of extrolites (Table 3) each with 4–7 different extrolites. In addition, CBS 533.71 produced extrolites with UV spectra similar to Raistrick phenols, and emodin, emodin anthrone and emodin bisanthrone and emodin bisanthron. This series of anthraquinones and anthrones has also been found to co-occur in *Aspergillus wentii* (Rabie et al. 1986).

Diagnostic features: Thermophilic, irregularly branched conidiophores (*Paecilomyces*-type), ascomata produced and ripening within 7–10 days at 40°C, poor to moderate growth on CYA and YES agar.

Distribution and ecology: *Rasamsonia byssochlamydoides* was isolated from dry soil under Douglas fir (Oregon, USA) and piles of peat (Oldenburg, Germany).

Rasamsonia cylindrospora (G. Smith) Houbraken & Frisvad **comb. nov.** Mycobank MB 519876. Supplementary Fig. 4.

Basionym: *Penicillium cylindrosporum* G. Smith—Trans. Br. Mycol. Soc. 40: 483, 1957

= *Geosmithia cylindrospora* (G. Smith) Pitt—Can. J. Bot. 57(19): 2024, 1979

Description: Colony diameters, 7 days, in mm. 25°C: CYA 3–8; CYAS 0; MEA 10–20; YES 5–15; DG18 5–15; OA 9–17; CREA: no growth. Colony diameters at 37°C: CYA 5–10; CYAS 0; MEA 27–38; YES 10–19; DG18 15–26; OA 24–34; CREA: 1–5, poor growth, no acid and no base production. Optimum growth temperature 36°C, maximum around 50°C.

Colonies on CYA at 25 and 37°C restricted, velvety, olive brown conidia, exudates and soluble pigments absent, no sulcations in colony, reverse in shades of brown. Good sporulation on YES, soluble pigments absent, mycelium inconspicuous, white, olive brown conidia, reverse brown with dark brown centre. Colonies on MEA velvety to floccose, olive brown conidia, distinct blackish brown reverse at 37°C.

Conidiophores predominantly arising from basal mycelium, predominantly biverticillate, occasionally terverticillate or simple, additional branch both direct

under terminal whorl and further down the stipe, (50–) 100–200 × 2.0–3.5 µm, stipes verrucose, Metulae in terminal whorls of 2–4, unequal in length, 10–17 (–25) × 2.5–3.5 µm. Phialides, 5–8 (–12) per metula, cylindrical, with distinct neck, 10–14 × 2.0–2.5 µm. Conidia smooth walled, cylindrical, 4.0–5.0 × 1.6–2.1 µm.

Extrolites: Production best on RTO, followed by MEA, MEA-B and OA; production on remaining media poor. CBS 275.58 produced only one extrolite on MEA, which was not found in CBS 433.62. CBS 433.62 produced a large number (14) of naphthalenes, including some with UV spectra similar to “naphthalic anhydride”, atrovnetin and funalenone (Turner 1971, pp. 150–153; Inokoshi et al. 1999).

Diagnostic features: Thermotolerant, restricted growth on CYA (5–10 mm) and YES (10–19 mm) at 37°C, blackish brown reverse on MEA, conidia cylindrical and measuring 4.0–5.0 × 1.6–2.1 µm.

Distribution and ecology: Two *R. cylindrospora* isolates are currently present in the CBS culture collection. One strain was isolated from sputum in the Netherlands; the other as a culture contaminant in England, UK.

Notes: *Rasamsonia cylindrospora* was originally described as *Penicillium cylindrosporum* by Smith (1957) and this species was later transferred to the genus *Geosmithia* (Pitt 1979).

Rasamsonia eburnea (Yaguchi, Someya & Udagawa) Houbraken & Frisvad **comb. nov.** Mycobank MB 519875. Supplementary Fig. 5.

Basionym: *Talaromyces eburneus* Yaguchi, Someya & Udagawa Mycoscience 35: 249, 1994

= *Geosmithia eburnea* Yaguchi, Someya & Udagawa Mycoscience 35: 249, 1994

Description: Colony diameters, 7 days, in mm. 25°C: CYA 14–20; CYAS 1–3; MEA 10–20; YES 14–20; DG18 5–10; OA 23–30; CREA: 5–10, poor growth, no acid and base production. Colony diameters at 37°C: CYA 30–40; CYAS1–6; MEA 34–42; YES 48–55; DG18 10–20; OA 36–46; CREA: 12–18, poor growth, no acid and no base production. Optimum growth temperature 36°C, maximum above 50°C.

Colonies on CYA at 37°C spreading, velvety, olive-brown conidia, mycelium cream crème colored, exudates and soluble pigments absent, colonies not sulcate at 25°C, and distinct sulcate at 37°C, reverse (pale) brown. Good sporulation on YES at 37°C,

obverse pale olive-brown, mycelium inconspicuous, reverse pale brown and becoming blackish brown in age. Colonies on MEA velvety, olive-brown conidia, reverse distinct dark brown at 37°C (at 25°C often paler brown).

Conidiophores predominantly arising from basal mycelium, young structures monoverticillate, biverticillate in older parts, (50–) 100–350 × 2.0–3.5 µm, occasionally additional branches formed subterminally or at lower levels, suggesting presence of separate conidiophores and these structures are shorter (20–70 µm), stipes verrucose. Metulae, when present, in terminal whorls of 2–3 (–5), unequal in length, 10–20 × 2.5–3.5 µm. Phialides, in appressed verticils of 2–6, cylindrical, 10–14 (–17) µm. Conidia smooth walled, cylindrical at first and later becoming ellipsoidal or ovoid, 2.5–3.5 (–4) × 1.8–2.5 µm. Ascospores not detected in this study, but reported in literature (Yaguchi et al. 1994) as discrete, pale yellow, maturing slowly within 28–35 days, globose to subglobose, (30–) 70–125 µm, soft, scarcely covered by hyaline, delicate mycelium. Asci eight-spored, borne singly, subglobose to ovoid or pyriform, 10.5–13 (15) × 8–9.5 (–11) µm. Ascospores pale yellow, subglobose to more or less ovoid, 4–5 × 4–4.5 µm, thick walled, smooth but occasionally with foveolations, with an equatorial thickening under SEM.

Extrolites: Production best on YES, followed by RTO and MEA, none on other media. CBS 102881 and CBS 124445 (= IBT 31193) were the best producers of extrolites (Table 3).

Diagnostic features: Thermotolerant, good growth on CYA (30–40 mm) and YES (48–55 mm) at 37°C, blackish brown reverse on MEA at 37°C, conidia cylindrical at first and becoming ellipsoidal or ovoid.

Distribution and ecology: The type strain of *Rasamsonia eburnea* was isolated from Taiwanese soil. Other strains are isolated from clinical specimens, including blood culture, bronchial washing and dialysis fluid. The studies of Houbraken et al. (2010) and Barton et al. (2010) mainly used the same *Rasamsonia* strains (as *Geosmithia*). Strains NCPF 7594 and 7596 were isolated from the same patient (respectively from blood culture and peritoneal dialysis fluid) and re-identified here, based on deposited ITS sequences in GenBank, as *R. eburnea*. An isolate originating from a blood culture (CBS 124447; pseudo-outbreak, UK) was also re-identified as *R. eburnea* (Houbraken et al. 2010).

Notes: De Ravin et al. (2011) mention that *R. eburnea* is heterothallic and fails to form a teleomorph without mating appropriate strains. However, no additional details are given for this observation in their publication.

Rasamsonia emersonii (Stolk) Houbraken & Frisvad **comb. nov.** Mycobank MB 519874. Supplementary Fig. 6.

Basionym: *Talaromyces emersonii* Stolk, Antonie van Leeuwenhoek 31: 262. 1965.

= *Penicillium emersonii* Stolk, Antonie van Leeuwenhoek 31: 262. 1965.

Description: Colony diameters, 7 days, in mm., no growth at 25°C; 37°C: CYA 18–30; CYAS 0; MEA >50; YES 16–45; DG18 10–22; OA >60; CREA: 0–5, no or poor growth, no acid and no base production. Optimum growth temperature between 45 and 50°C, maximum above 50°C.

Poor to moderate growth on CYA at 37°C, no or poor sporulation, exudates and soluble pigments absent, colonies not sulcate, mycelium inconspicuous and white, reverse pale to light brown. Sporulation on YES at 37°C weak or absent, soluble pigments absent, mycelium inconspicuous, if sporulation is present, then colony observe pale olive-brown, reverse light brown and not influencing the medium color. Colonies on MEA spreading, velvety to floccose, olive brown conidia; ascospores present and often covered under layer of conidiophores, white when young, becoming yellow-brown or orange-brown within 7–10 days, reverse light brown, occasionally dark brown (CBS 549.92).

Ascospores reddish to orange brown, globose to subglobose, up to 300 µm in diameter; covering scanty. Asci eight-spored, subglobose or broadly ellipsoidal, 8–10.5 × 6.5–8 µm. Ascospores smooth, subglobose to broadly ellipsoidal, 3.5–4 × 2.5–3.5 µm. Conidiophores arising directly from the agar, regularly branched, bi- or terverticillate, 50–150 µm, stipes verrucose, width 2.0–3.5 µm. Metulae in terminal whorls of 3–5, equal in length, 10–15 (–20) × 2.0–3.0 µm. Phialides, 5–10 per metula, cylindrical with a short collula, 8.5–10 × 2–2.5 µm. Conidia smooth walled, cylindrical, 3.5–4.5 (–5.0) × 1.5–3.0 µm.

Extrolites: Production best on OAT, followed by RTO, MEA-B and MEA; no production on other media. CBS 549.92 is the best extrolite-producer followed by CBS 396.64 and CBS 397.64. Weak

extrolite production by the ex-type strain CBS 393.64, which produced one naphthalenone and secalonic acid D. CBS 549.92 produced a large number (10) of naphthalenones, including some with UV spectra similar to naphthalic anhydride, atrovnetin and funalenone (Turner 1971, pp. 150–153; Inokoshi et al. 1999) and other as yet unknown extrolites (Table 3). Strain CBS 549.92 differed from the other strains in additionally producing an indole alkaloid, and an isocoumarin and two partially characterized extrolites (Table 3). It also produced more naphthalenones than the other strains.

Diagnostic features: Thermophilic, regularly branched conidiophores with olive-brown colored conidia, ascospores present and ripening within 7–10 days at 37°C, poor to moderate growth on CYA and YES agar.

Distribution and ecology: *Rasamsonia emersonii* is a relatively common fungus, but is probably overlooked since it is not able to grow at 25°C. This species was isolated from hot environments such as compost, but also from soil and outdoor air (Fröhlich-Nowoisky et al. 2009; Hultman et al. 2009). *Rasamsonia emersonii* was also reported to be the causal agent of a chronic airway colonization. Reexamination of the deposited ITS sequences showed that these strains are more close to *R. argillacea* (Cimon et al. 1999; Giraud et al. 2010).

Notes: The results of the multigene analysis shows that CBS 549.92 (= CBS 814.70) deviates from other strains of *R. emersonii*. This strain is also phenotypically different and has intensely bright yellow colored mycelium, but this could not be related to any extrolites in the extract of the fungus. The isolate may represent a new species, and more strains should be collected and examined to determine its taxonomic status.

Discussion

The genus *Rasamsonia* encompasses one new species, three species formally accommodated in *Talaromyces* and two species previously classified in *Geosmithia*. Phylogenetic analyses showed that the genus *Geosmithia* is polyphyletic and forms lineages in the Hypocreales and Eurotiales (Ogawa et al. 1997; Ogawa and Sugiyama 2000; this study). *Geosmithia lavendula*, the type species of *Geosmithia*, is

accommodated in the Hypocreales and is related to *Acremonium alternatum*, the type species of *Acremonium* (Ogawa et al. 1997; Rossman and Seifert 2011). Several new *Geosmithia* species belonging to the Hypocreales were described in the last decade and the majority of these species are associated with galleries of phloem feeding bark beetles (Kolařík et al. 2004, 2005, 2010). Four of eight *Geosmithia* species belonging to the Eurotiales are reclassified in *Rasamsonia*. *Geosmithia swiftii* (teleomorph *Talaromyces bacillisporus*) and *G. viridis* belong to a clade comprising mainly *Talaromyces* species and members of *Penicillium* subgenus *Biverticillium* (Fig. 1), and *G. namyslowskii* and *G. malachitea* (teleomorph *Chromocleista malachitea*) belong to *Penicillium* s. str. (Ogawa et al. 1997). The transfer of these species to other genera and making *Geosmithia* a monophyletic genus will be performed in another study. Our results show that the members of the *Talaromyces* section *Emersonii*, as defined by Stolk and Samson (1972), belong to different genera. Two species are disposed in *Rasamsonia*, but *T. bacillisporus* and *T. leycettanus* are retained in *Talaromyces*. *Talaromyces bacillisporus* is related to *Talaromyces flavus*, which is the type species of *Talaromyces*. Stolk and Samson (1972), who showed that the ascematal coverings of *T. bacillisporus* are much better developed than those of *R. emersonii* and *R. byssochlamydoides*, already had doubts about the relationship between *T. bacillisporus* and *R. emersonii*. They suggest that *T. bacillisporus* resembles an intermediate form between the section *Emersonii* and section *Talaromyces*. Pitt (1979) placed the anamorph of *T. bacillisporus* in *Geosmithia* (as *G. swiftii*), suggesting a close relationship with *R. argillacea* and *R. cylindrospora*. However, the conidia of *T. bacillisporus* are cream coloured not olive-brown. Phylogenetic analysis shows that *T. leycettanus* is not related to *Talaromyces* but rather to *Hamigera* and *Warcupiella* and this confirms earlier findings (Peterson 2008).

Extrolite analysis showed that *R. emersonii*, *R. cylindrospora* and *R. byssochlamydoides* produced naphthalenones or anthraquinones. Many species in *Talaromyces sensu stricto* also produce naphthalenones and anthraquinones (Frisvad et al. 1990), and these metabolites could act ecologically as antioxidants and thermoprotectants. It seems that these type of compounds have been present in a common ancestor of *Talaromyces* and *Rasamsonia*.

Rasamsonia eburnea and *R. argillacea* produced extrolites not seen in any other fungal species yet. *Trichocoma paradoxa* is phylogenetically related to *Rasamsonia* and this species produces species specific extrolites, but also naphthalenones, which indicates a relationship with *R. cylindrospora* and *R. emersonii*. However, naphthalenones are found in *T. macrosporus* and *P. duclauxii* (Frisvad et al. 1990) and also in the phylogenetically more distant species *Penicillium atrovenerum*, *P. raistrickii*, *P. hordei* and *P. hirsutum* (Turner 1971; Overly et al. 2005a, b) and so these antioxidant metabolites may have evolved more than once.

The Eurotialean genera *Talaromyces*, *Thermoascus*, *Thermomyces*, *Coonemeria* and *Dactylomyces* contain thermophilic species (Apinis 1967; Mouchacca 1997; Stolk and Samson 1972; Stolk 1965). The current study shows that no thermophiles are accommodated in *Talaromyces*. *Talaromyces emersonii* and *T. byssochlamydoides* are combined in *Rasamsonia* and *Talaromyces thermophilus* is phylogenetically closely related to the type species of *Thermomyces*, *T. lanuginosus*, and not to the type of *Talaromyces*, *T. flavus*. *Talaromyces thermophilus* and *T. lanuginosus* share similar features including thermophilicity, thick-walled chlamydospores or chlamydospore-like conidia. Further research is needed to clarify the taxonomy of *Thermomyces* and the relationship between *T. thermophilus* and the other species in *Thermomyces*. Various taxonomical studies of the thermophilic genus *Thermoascus* were performed (Apinis 1967; Mouchacca 1997; Pitt et al. 2000; Stolk 1965). Apinis (1967) split this genus in two: *Thermoascus* was retained for its type species *T. aurantiacus*, and *T. thermophilus* and *T. crustaceus* were transferred to *Dactylomyces*. Based on anamorph differences, Mouchacca (1997) divided *Dactylomyces* further in two, creating the genus *Coonemeria* for *T. crustaceus*. Although these species have different anamorphs (*Paecilomyces/Polypaecilum*), our phylogenetic study (Fig. 1) shows that these three species are related and should be retained in the genus *Thermoascus*. In conclusion, our data shows that eurotialian thermophiles are not present in *Talaromyces* s. str. but only in *Thermomyces*, *Thermoascus* and *Rasamsonia*.

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