

Rasamsonia pulvericola sp. nov., isolated from house dust

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Abstract: In the course of a global survey of the indoor mycobiota, we sampled and analysed settled dust from 87 buildings from 14 countries, using both a modified dilution-to-extinction method and 454-pyrosequencing. *Rasamsonia* is a recently established genus including thermotolerant or thermophilic species, five of which have been isolated from humans, including the emerging pathogen *R. argillacea*. A new species, *R. pulvericola*, was recovered from one residence in Songkhla, Thailand, and is morphologically characterised and compared phylogenetically with other members of the genus. *Rasamsonia pulvericola* forms a clade with *R. brevistipitata* and shares morphological characters such as usually biverticillate and never terverticillate conidiophores, and subglobose to ellipsoidal conidia. It has a lower maximum growth temperature and is the first mesophilic species added to the genus. The ITS sequence of *R. pulvericola* was not detected in the 454-pyrosequencing data for Thailand or other countries, but a similar ITS sequence was detected in Micronesia, probably representing another undescribed *Rasamsonia* species.

Key words:

environmental metagenomics
Eurotiales
Geosmithia
indoor moulds
Talaromyces
Trichocomaceae

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INTRODUCTION

Humans spend up to 90 % of their time indoors (Klepeis *et al.* 2001) and share their work and living environments with a multitude of microorganisms. The fungal biota may be associated with food, textiles, or building materials as agents of biodeterioration, house plants as phyloplane inhabitants or endophytes, or animals such as pets (Flannigan *et al.* 2011). In the north temperate societies of the western world that are most studied by conventional culturing methods, the indoor mycobiota is comprised of about 100 common fungi, and about 100 less frequently isolated species (Samson *et al.* 2010). Housing on other continents and in other climates is less intensively sampled, and deeper investigations of the fungi occurring in the built environment using next generation sequencing and alternative isolation techniques are only beginning. This paper reports on the isolation of a new fungal species in the recently recognised genus *Rasamsonia*, isolated from house dust samples that were also subjected to 454-pyrosequencing by Amend *et al.* (2010).

Rasamsonia (*Eurotiales*, *Trichocomaceae*) was established for a phylogenetically distinct clade of asexual and sexual species formerly classified in *Geosmithia* and *Talaromyces* (Houbraken *et al.* 2012). The genus currently consists of nine species: *R. aegroticola*, *R. argillacea* (syn. *Geosmithia argillacea*), *R. brevistipitata*, *R. byssochlamydoides* (syn. *Talaromyces byssochlamydoides*), *R. composticola*, *R. cylindrospora* (syn. *Geosmithia cylindrospora*), *R. eburnea* (syn. *Talaromyces eburneus*), *R. emersonii* (syn. *Talaromyces emersonii*), and *R. piperina* (Su & Cai 2012, Houbraken *et al.*

al. 2012, Houbraken *et al.* 2013). *Rasamsonia* species have roughened, paecilomyces-like conidiophores and olive-brown conidia, and ascomata with a scanty hyphal covering have been observed in four species. They have been detected in North America, Europe and Asia from substrates such as soil, compost, conifer wood chips, indoor air, human and canine patients (Houbraken *et al.* 2012).

All previously known *Rasamsonia* species are thermotolerant or thermophilic, with optimum growth temperatures above 30 °C and maximum growth temperatures above 45 °C (as defined by Cooney & Emerson 1964, Crisan 1964, Maheshwari *et al.* 2000). Thermotolerant and thermophilic fungi are of concern because of their potential as opportunistic pathogens (e.g. Robert & Casadevall 2009) and of industrial interest for their ability to produce thermostable enzymes (Maheshwari *et al.* 2000). Several *Rasamsonia* species have been isolated from human patients, including *R. aegroticola* (Barton *et al.* 2010), *R. argillacea* (Giraud *et al.* 2010), *R. cylindrospora* (Houbraken *et al.* 2013), *R. eburnea* (Houbraken *et al.* 2010), and *R. piperina* (Giraud *et al.* 2010). An increase in clinical reports suggests *R. argillacea* is an emerging pathogen, although its morphological similarity to *Paecilomyces* suggests it may have been misdiagnosed previously in clinical cases (Houbraken *et al.* 2010, De Ravin *et al.* 2011).

Thermostable enzymes offer several advantages for biotechnology applications, including a reduction in the risk of microbial contamination, prolonged storage viability, and an increase in rates and yields enabled by high temperature processes (Haki & Rakshit 2003, Turner *et al.* 2007).

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Table 1. Strains used for phylogenetic analyses of *Rasamsonia pulvericola* (T = ex-type).

Name	Strain no.	Origin	GenBank accession no.		
			ITS	BenA	Cmd
<i>R. aegroticola</i>	DTO 137A8 ^T	Respiratory secretions from cystic fibrosis patient; France	JX272988.1	JX273020.1	JX272956.1
	DTO 137B7	Respiratory secretions from cystic fibrosis patient; France	JX272980.1	JX273012.1	JX272948.1
<i>R. argillacea</i>	CBS 101.69 ^T	Soil from coal spoil tip; Staffordshire, UK	JF417491.1	JF417456.1	JF417501.1
	CBS 907.70	Unknown source; Reading, UK	JF417474.1	JF417458.1	JF417503.1
<i>R. brevistipitata</i>	CBS 128785 ^T	Indoor environment of school, from cork with bitumen; Germany	JF417488	JF417454	JF417499
	CBS 128786	Indoor environment of school, from cork with bitumen; Germany	JF417489.1	JF417455.1	JF417500.1
<i>R. byssochlamydoides</i>	CBS 413.71 ^T	Dry soil under <i>Pseudotsuga menziesii</i> ; Oregon, USA	JF417476	JF417460	JF417512
	CBS 533.71	Piles of peat; near Oldenburg, Germany	JF417477.1	JF417461.1	JF417513.1
<i>R. composticola</i>	CGMCC 3.13669 ^T	Rice straw and cow dung compost; Yunnan, China	JF970184	JF970183	JQ729688
	CGMCC 3.14946	Rice straw and cow dung compost; Yunnan, China	JQ178360	JQ060951	JQ729689
<i>R. cylindrospora</i>	CBS 275.58 ^T	Culture contaminant; Berkshire, UK	JF417470	JF417448	JF417493
	CBS 432.62	Human sputum; the Netherlands	JF417471.1	JF417449.1	JF417492.1
<i>R. eburnea</i>	CBS 100538 ^T	Soil; Taipei, Taiwan	JF417483	JF417462	JF417494
	CBS 124447	Contaminant of blood culture; UK	JF417473.1	JF417452.1	JF417497.1
<i>R. emersonii</i>	CBS 393.64 ^T	Compost; Italy	JF417478	JF417463	JF417510
	CBS 397.64	Soil; near Nottingham, UK	JF417480.1	JF417466.1	JF417509.1
<i>R. piperina</i>	CBS 104.69	Wood chips of <i>Picea abies</i> and <i>Pinus sylvestris</i> ; Sweden	JX272994.1	JX273026.1	JX272962.1
	CBS 408.73 ^T	Seed of <i>Piper nigrum</i> ; Spain	JX272968.1	JX273000.1	JX272936.1
<i>R. pulvericola</i>	DAOM 242435 ^T	House dust; Songkhla, Thailand	KF242514	KF242520	KF242522
	DAOM 242436	House dust; Songkhla, Thailand	KF242515	KF242521	KF242523
<i>Talaromyces flavus</i>	NRRL 2098 ^T	Unknown; New Zealand	EU021596.1	EU021663.1	EU021694.1
<i>Trichocoma paradoxa</i>	CBS 103.73	Unknown; Japan	JF417486	JF417469	JF417506

Rasamsonia emersonii produces an array of thermostable, lignocellulolytic enzyme cocktails that function at temperatures 10–20 °C higher than commercially available enzymes from *Trichoderma* species (Viikari *et al.* 2007). Cellulase (Moloney *et al.* 1983), xylanase (Tuohy *et al.* 1993), amylase (Bunni *et al.* 1989), β -glucanase (Murray *et al.* 2001), and β -glucosidase (Murray *et al.* 2004) systems have been characterised in *R. emersonii*. Potential applications of thermostable enzymes derived from *R. emersonii* include food processing, biofuel, and bioconversion processes (Fernandes *et al.* 2008, Waters *et al.* 2010).

A large-scale survey of the mycobiota of the built environment from 87 buildings in 14 countries (Amend *et al.* 2010) resulted in the discovery of a new *Rasamsonia* species, described here as *R. pulvericola*. This novel species is described and its phylogenetic delimitation and classification in *Rasamsonia* is supported using the internal transcribed spacer (ITS), partial calmodulin (*Cmd*), and β -tubulin sequences (*BenA*).

MATERIALS AND METHODS

Isolation, cultural, and morphological characterisation

Settled dust was collected from nine buildings in Thailand in April 2009, using sterilised collectors attached to vacuum cleaners. A dilution-to-extinction method modified from Collado *et al.* (2007) (Seifert *et al.*, unpubl.) was used to isolate and culture fungi on both a high water activity medium, malt extract agar (MEA; 20 g Bacto malt extract, Difco Laboratories, Sparks, MD; 15 g agar, EMD Chemicals, Gibbstown, NJ; 1 L distilled water) and a reduced water activity medium, 20 % sucrose MEA (20SMEA; same recipe as MEA with 200 g EMD sucrose added) dispensed in 96-tube microplates.

Isolates were characterised using morphological characters and ITS barcode sequences, and maintained on 2 % Malt Extract Agar (MEA). For culture descriptions, three-point inoculations using conidia suspended in 0.1 % agar from selected isolates (DAOM 242435, 242436) were made on Czapek yeast autolysate agar (CYA), MEA,

Oatmeal Agar (OA), creatine sucrose agar (CREA), 20 % sucrose CYA (CY20S), yeast extract-sucrose agar (YES), potato dextrose agar (PDA), and Blakelee's malt extract agar (BLK) (Frisvad & Samson 2004). Colony colours were described using the alphanumeric codes of Kornerup & Wanscher (1978). To determine cardinal temperatures, two representative isolates (DAOM 242435, 242436) were inoculated at three points on MEA and incubated at 5 °C intervals from 5–40 °C. Each isolate was grown in triplicate on each medium and temperature treatment in the dark. Colony diameters and morphological and colony features were recorded every 7 d for 1 mo. Microscopic observations were made using material mounted in 85 % lactic acid and an Olympus BX50 light microscope, with micrographs captured using an Evolution MP Colour Camera (Media Cybernetics, Silver Spring, MD) and Image-Pro Plus 6.0 (Media Cybernetics) software. Microscopic measurements are presented as ranges calculated from the mean ± standard deviation of each measured value, with outliers in brackets. Colony photographs were taken with a Nikon Coolpix P5000 (Nikon, Tokyo) and photographic plates were assembled using Adobe Photoshop v. 5.5 (Adobe Systems, San Jose, CA).

DNA extraction, sequencing, and phylogenetic analysis

Genomic DNA was extracted from 2-wk-old *Rasamsonia pulvericola* strains using the Ultraclean Microbial DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's protocol. The ITS (including 5.8s rDNA) for all strains was amplified using the primers ITS4–ITS5 (White *et al.* 1990). Two of the six isolates, DAOM 242435 and 242436, were selected as representative strains for the amplification of additional genes. Partial β -tubulin and calmodulin loci were sequenced to further study the phylogenetic relationship of *R. pulvericola* with other *Rasamsonia* spp. Genes were amplified using primers and PCR conditions described by Houbraken *et al.* (2012). Newly generated sequences were deposited in GenBank (Table 1).

The sequences of the putative new species, along with reference sequences obtained from GenBank (Table 1), were aligned by Muscle (Edgar 2004) and manually edited and combined using Geneious Pro v. 5.6.5 (Biomatters, Auckland). The best-fit nucleotide substitution model was selected using MrModelTest v. 2.2 (Nylander 2004). Phylogenetic analysis was conducted using a Bayesian inference (BI) analysis with MrBayes v. 3.2 (Ronquist *et al.* 2012). Four simultaneous Markov chains were run for 1 000 000 generations (standard deviation of split frequencies <0.01) with a burn-in time of 250 000 generations and a sampling frequency of 1 in 100 generations. *Talaromyces flavus* (NRRL 2098) was used as outgroup for the analysis.

Fungal ITS was amplified from house dust samples and analysed using 454 GS FLX titanium technology (454 Life Sciences, Branford, CT) by Amend *et al.* (2010). The data were reformatted into a local BLAST database and queried for sequences of our putative new species using BioEdit v. 7.1.3.0 (Hall 1999).

RESULTS

Six *Rasamsonia* strains were isolated on 20SMEA among 907 fungal cultures originating from the Thailand house dust samples. All isolates were recovered from pooled samples from one house in Saba Yoi, Songkhla. Morphologically, the fungus is most similar to *R. brevistipitata*, and is described as *R. pulvericola* below, using the standardised format established by Houbraken *et al.* (2012).

ITS barcodes and partial *Cmd* and *BenA* sequences were combined from 22 strains, including all other known species of *Rasamsonia* (Table 1), to investigate the phylogenetic relationship of *R. pulvericola* in the genus. The data set comprised 1 619 nucleotides after alignment of the partitions for the ITS (559 bases), *BenA* (497), and *Cmd* (563), including alignment gaps. The best-fit model selected by MrModelTest v. 2.3 was GTR+I+G.

The multilocus phylogenetic analysis confirmed the distinctiveness of nine previously described species, and confirmed the delineation of our new species (Fig. 1). It supports the classification of *R. pulvericola* in *Rasamsonia*, with *R. brevistipitata* as the closest sister species.

A total of 40 892 sequences were generated from the Thailand house dust samples by 454-pyrosequencing (Amend *et al.* 2010). No ITS sequences identical with that of the isolated strains of *R. pulvericola* were detected in Thailand or other sampled countries. One OTU (FX2FH6V01AM9Y3), closely related to *R. pulvericola*, sharing 447 of 458 bp, with one pairwise alignment gap, was represented by three ITS sequences in one Micronesian house dust sample.

TAXONOMY

Rasamsonia pulvericola Tanney & Seifert, sp. nov.

Mycobank MB804677

(Figs 2–3)

Etymology: From the Latin *pulveris*, of dust, *-cola*, living in, named for the substrate in which it was discovered, house dust.

Diagnosis: Penicilli biverticillate, sometimes monoverticillate; stipes verrucose and short (10–)14.5–27 (–38) × (1.5–)2–2.5 (–3) μ m. Metulae, when present, 7.5–10.5(–13) × 2–2.5(–3) μ m. Phialides (7–)7.5–10.0(–12) × 2.0(–2.5) μ m, in verticils of 3–9. Conidia subglobose, smooth walled, pale brown, 2.0–2.5 × 2.0–2.5 μ m.

Type: **Thailand**: Songkhla, Saba Yoi, isolated from settled dust from a single residence, April 2009, *P. Noonim* (DAOM 242435 – holotype, a dried culture; DAOM 242531 – ex-type culture).

Description: *Cultures*: Colony diameters in mm, 7 d, 30 °C: PDA 4–5; YES 3–4; OA 2–3; MEA 2–3; CY20S 2–3; BLK 1; CYA; 1; CREA >1, poor growth, no acid or base production; 14 d, PDA 14–16; BLK 11–13; MEA 10–12; OA 9; CY20S 6–8; YES 7–8; CYA 2–4; CREA 1, poor growth, no acid or base production. Growth temperature range 20–35 °C,

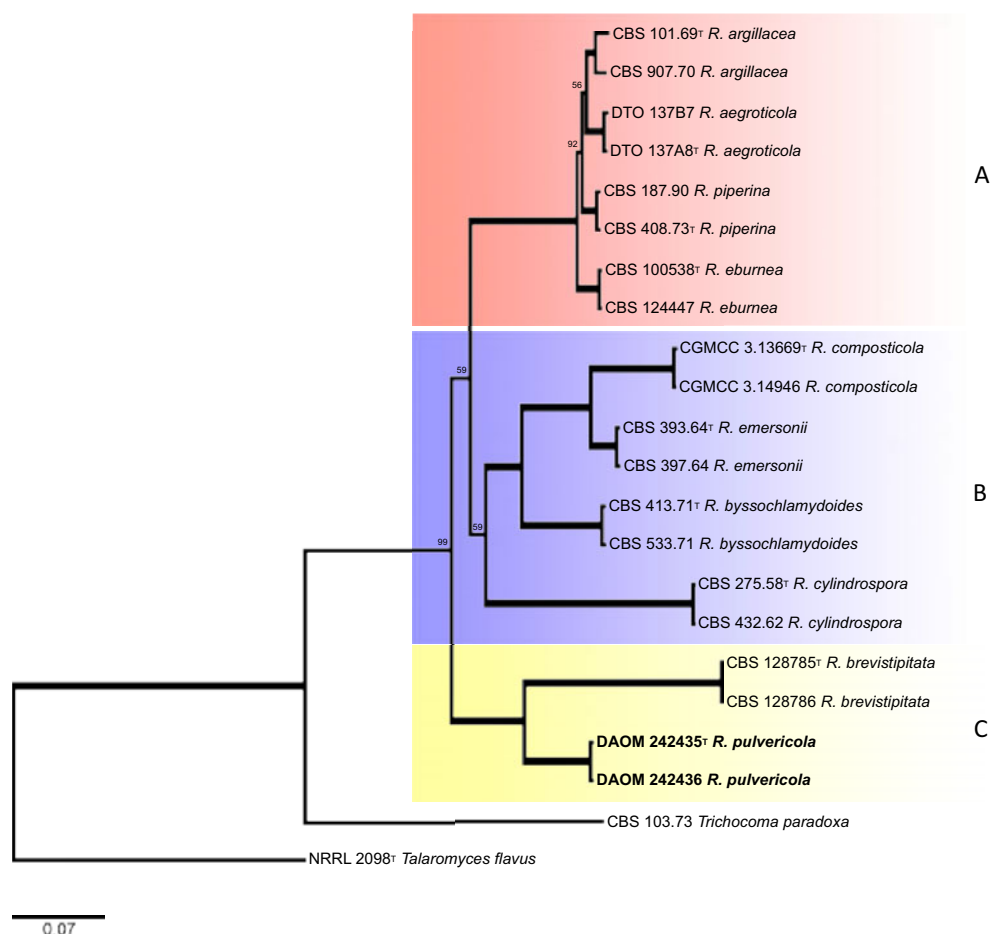


Fig. 1. Consensus phylogram of 15 002 trees resulting from a Bayesian analysis of the combined ITS, calmodulin, and β -tubulin sequence alignment. Strain accession numbers are followed by the species name (^T = ex-type). Thick branches indicate a posterior probability value of 1.0; values lower than 1.0 are presented at nodes. The tree was rooted to *Talaromyces flavus* (NRRL 2098). Clades referred to in the text are labelled on the right.

optimum growth at 30 °C, minimum slightly below 20 °C, and maximum slightly about 35 °C.

Colonies at 30 °C on OA flat, slightly undulate margin, with prolific sporulation, greenish yellow (1A6: Primrose Yellow) with dense yellowish brown (5D6: Yellow Brown) conidia in centre, reverse yellowish white (2A2: Pale, Yellowish White); colonies on CYA irregular with raised aerial mycelium in centre, lobate margin, no or poor sporulation, white to yellowish white (1A2: Yellowish White), reverse white to orangish white (5A2: Orange White); on YES colonies with abundant woolly aerial mycelium, lobate margin, moderate sporulation, yellowish to pale yellow (1A2–1A3: Pale Yellow), reverse brownish orange (5C5: Topaz); colonies on CY20S morphologically similar to those on YES but more restricted diameter growth and reverse yellowish green (3B4: Straw Yellow); colonies on BLK velvety, centre slightly convex, smooth entire margin, moderate to good sporulation, greenish yellow (1A6–1B6: Greenish Yellow), reverse moderately concentric with white, light yellow (3A5: Light Yellow), and olive (3D7: Olive) outer to inner rings; colonies on MEA velvety, flat, not sulcate, smooth entire margin, moderate to good sporulation, greenish yellow (1A6–1B6: Greenish Yellow), reverse olive yellow (3C5: Olive Yellow);

colonies on PDA velvety, convex, slightly sulcate in centre, smooth entire margin, moderate sporulation, greenish yellow (1A6–1B6: Greenish Yellow), reverse grayish green (1C3–1C5: Grayish Green) with sulcate centre; colonies on CREA irregular, flat, filiform margin, no or poor sporulation, light yellow (1A4: Light Yellow), reverse white. Exudates and soluble pigments were absent for all media.

Conidiophores predominantly arising from aerial mycelium; penicilli biverticillate, sometimes monoverticillate, and often asymmetrical; stipes verrucose and short (10–) 14.5–27(–38) \times (1.5–)2–2.5 (–3) μm ($n = 35$). *Metulae*, when present, 7.5–10.5(–13) \times 2–2.5(–3) μm ($n = 30$), in terminal whorls of 2–3(–7), vesiculate (2.5–3.5 μm) and verrucose. *Phialides* (7–)7.5–10.0(–12) \times 2.0(–2.5) μm ($n = 55$), in verticils of 3–9, cylindrical base gradually tapering to a long collulum (2–4 μm). *Conidia* subglobose, smooth walled, pale brown, 2.0–2.5 \times 2.0–2.5 μm ($n = 60$), and borne in chains. *Ascospores* or *sclerotia* not observed.

Notes: *Rasamsonia pulvericola* is phylogenetically closest to *R. brevistipitata*. These two species share distinguishing characters, including subglobose or ellipsoidal conidia, conidiophores that are usually biverticillate and never terverticillate, and lower maximum growth temperatures

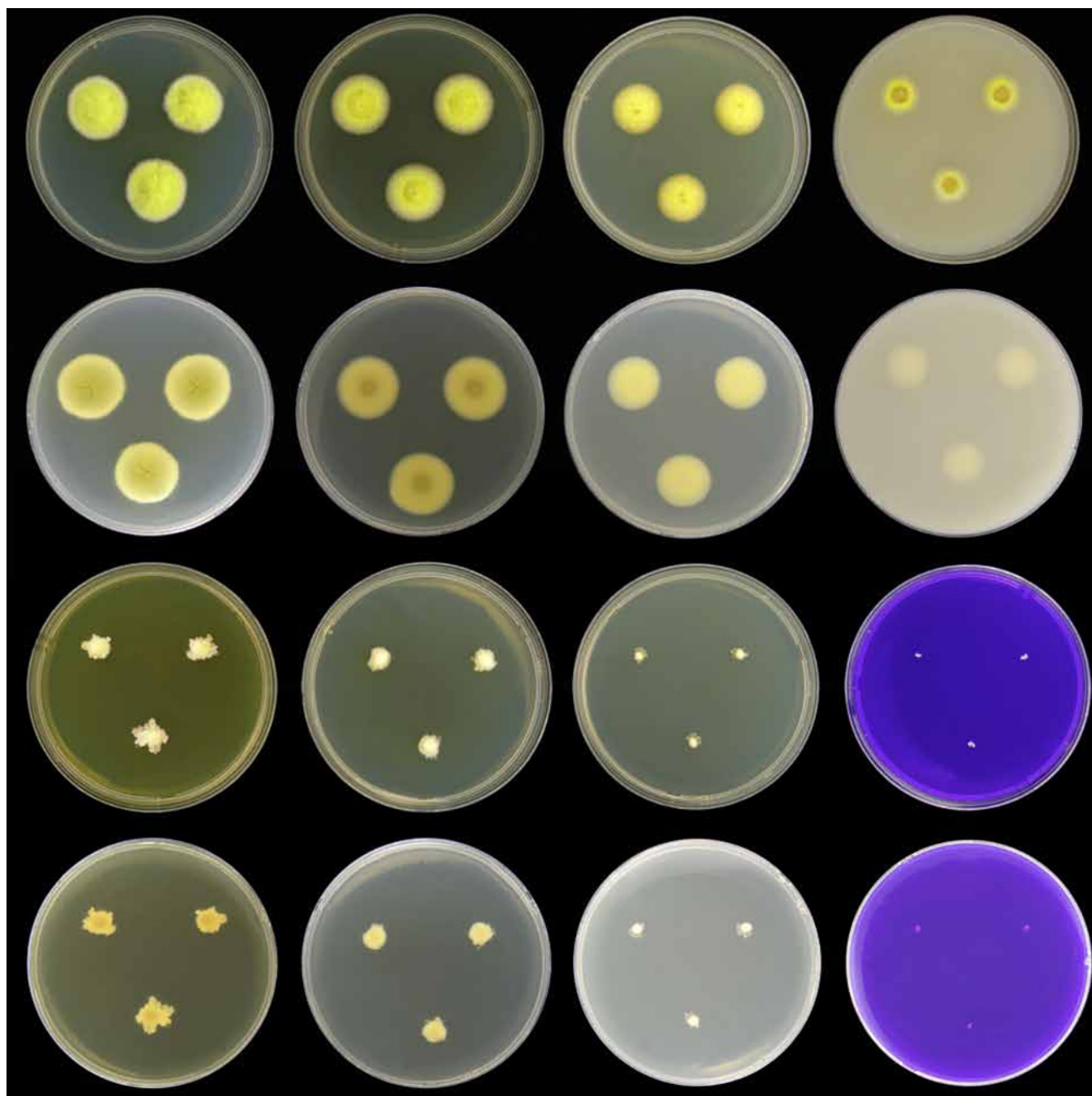


Fig. 2. Colonies of *Rasamsonia pulvericola* (DAOM 242435) after 14 d in the dark at 25 °C. From left to right: top row obverse on PDA, BLK, MEA, OA; second row reverse on PDA, BLK, MEA, OA; third row obverse on CY20S, YES, CYA, and CREA; bottom row reverse on CY20S, YES, CYA, and CREA.

compared with other species. The inability of *R. pulvericola* to grow at 40 °C further differentiates it from all other *Rasamsonia* species.

DISCUSSION

The recognition of *Rasamsonia pulvericola* as a novel *Rasamsonia* species was strongly supported by morphological characters and molecular phylogenetic analyses. *Rasamsonia pulvericola* and *R. brevistipitata* form a small, distinct clade sharing distinctive morphological and

growth characters. Both species have lower optimum and maximum growth temperatures than other *Rasamsonia* species; the optimum and maximum growth temperatures for *R. pulvericola* are 30 °C and 35 °C, respectively, and 33 °C and slightly above 45 °C for *R. brevistipitata*. Neither *R. pulvericola* nor *R. brevistipitata* produce the cylindrical conidia typical of other species of the genus. *Rasamsonia pulvericola* has a relatively narrow temperature growth range between 20–35 °C, perhaps reflecting its tropical origin. The inability to grow at 40 °C and restricted growth at 35 °C establishes *R. pulvericola* as the first mesophilic *Rasamsonia* species.

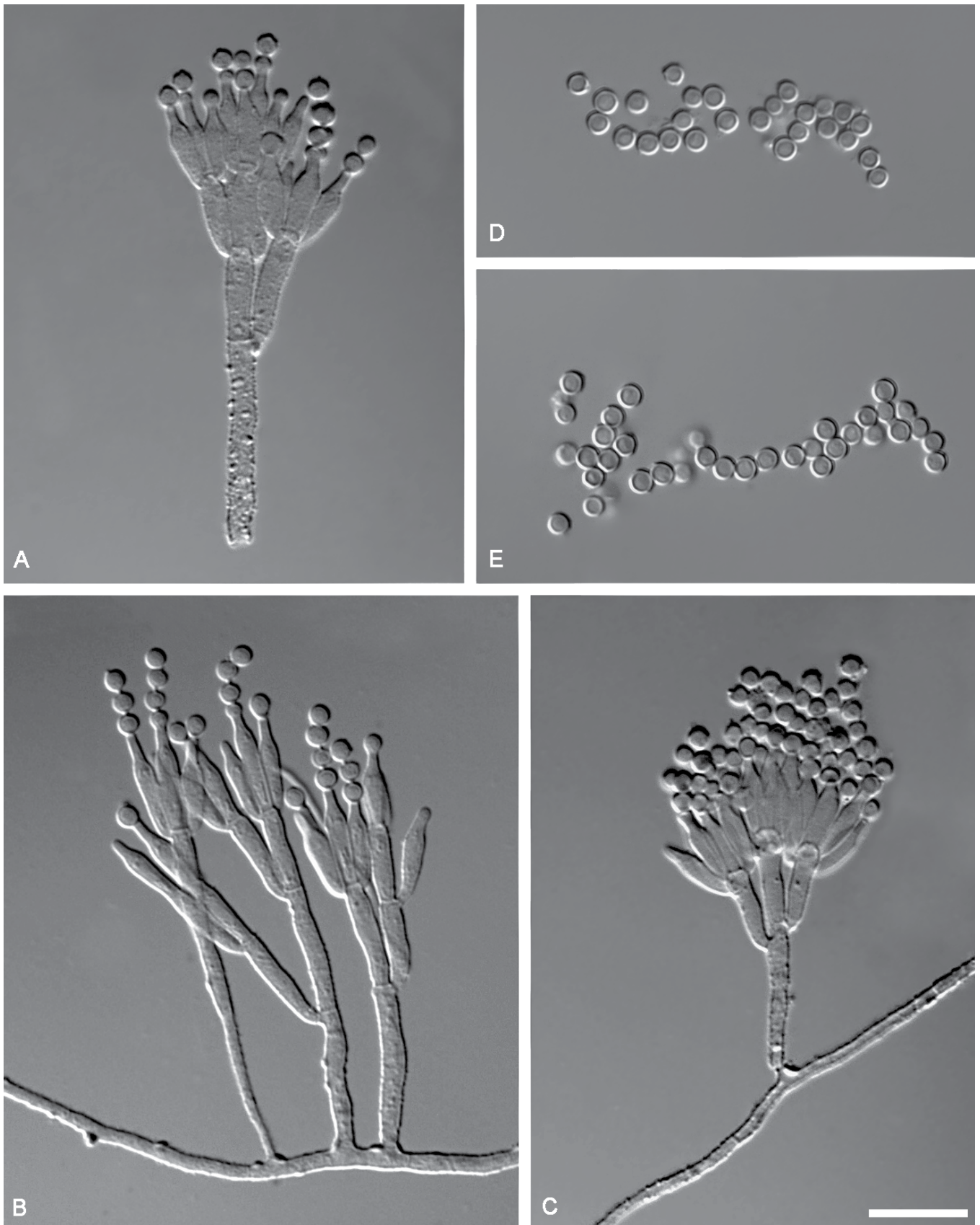


Fig. 3. Conidiophores (A–C) and conidia (D–E) of *Rasamsonia pulvericola* (DAOM 242435) after 14 d in the dark at 25 °C on MEA. Bar = 10 µm.

There are minor differences in the phylogram topology presented here from previous analyses. Su & Cai (2012) found, with low posterior probability, that *R. cylindrospora*

was sister to the remaining species in the genus. In contrast, the present analysis supports the recognition of three clades within *Rasamsonia*, with *R. cylindrospora* occurring in clade

B (Fig. 1). *Rasamsonia pulvericola* and *R. brevistipitata* form the well supported (PPV = 0.99) clade C that is the basal to the other two clades.

Rasamsonia pulvericola was not detected in the 454-pyrosequencing data from Thailand or other countries sampled (Amend *et al.* 2010). However, one OTU was closely related to *R. pulvericola* (97 %), and probably represents an additional novel *Rasamsonia* species in the *R. pulvericola*/*R. brevistipitata* clade. This OTU was found in settled dust from an office in Tofol, Kosrae, Micronesia, represented by 3 of 7 696 sequences recovered from that country. Cultures representing this OTU were not found among the 805 strains derived from Micronesian dust (Tanney, unpubl. data).

The dilution-to-extinction method allowed the isolation of this rare fungus from the Thailand house dust sample, despite being undetected using the 454-pyrosequencing method. This emphasises the complementarity of using both culture-dependent and culture-independent methods when profiling microbial biodiversity in environmental samples. The culturing method used to obtain these cultures is labour-intensive and time-consuming. However, cultures are necessary for describing and naming novel taxa, facilitate multi-gene phylogenetic analyses, and allow morphological, metabolic, and enzymatic characterisation. In the absence of cultures, one might assume *R. pulvericola* was thermotolerant or thermophilic, like other members of the genus. Its mesophilic nature may be surprising for a fungus isolated from a tropical habitat, but also suggests that comparison of the structure and function of the enzymes of *R. pulvericola* with those of its siblings may provide valuable information. The need to populate sequence databases, such as GenBank, with sequences of unrepresented but described species is apparent. Such efforts will strengthen the taxonomic utility of environmental sequence data by allowing users to associate sequences with meaningful names and corresponding biological information.

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