

# *Aspergillus* subgenus *Polypaecilum* from the built environment

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**Abstract:** Xerophilic fungi, especially *Aspergillus* species, are prevalent in the built environment. In this study, we employed a combined culture-independent (454-pyrosequencing) and culture-dependent (dilution-to-extinction) approach to investigate the mycobiota of indoor dust collected from 93 buildings in 12 countries worldwide. High and low water activity ( $a_w$ ) media were used to capture mesophile and xerophile biodiversity, resulting in the isolation of approximately 9 000 strains. Among these, 340 strains representing seven putative species in *Aspergillus* subgenus *Polypaecilum* were isolated, mostly from lowered  $a_w$  media, and tentatively identified based on colony morphology and internal transcribed spacer rDNA region (ITS) barcodes. Further morphological study and phylogenetic analyses using sequences of ITS,  $\beta$ -tubulin (*BenA*), calmodulin (*CaM*), RNA polymerase II second largest subunit (*RPB2*), DNA topoisomerase 1 (*TOP1*), and a pre-mRNA processing protein homolog (*TSR1*) confirmed the isolation of seven species of subgenus *Polypaecilum*, including five novel species: *A. baarnensis*, *A. keratitidis*, *A. kalimae* sp. nov., *A. noonimiae* sp. nov., *A. thailandensis* sp. nov., *A. waynelawii* sp. nov., and *A. whitfieldii* sp. nov. Pyrosequencing detected six of the seven species isolated from house dust, as well as one additional species absent from the cultures isolated, and three clades representing potentially undescribed species. Species were typically found in house dust from subtropical and tropical climates, often in close proximity to the ocean or sea. The presence of subgenus *Polypaecilum*, a recently described clade of xerophilic/xerotolerant, halotolerant/halophilic, and potentially zoopathogenic species, within the built environment is noteworthy.

**Key words:** *Basipetospora*, Canine pathogens, Halophile, *Phialosimplex*, Xerophile.

**Taxonomic novelties:** *Aspergillus kalimae* Tanney, Visagie & Seifert, *A. noonimiae* Tanney, Visagie & Seifert, *A. thailandensis* Tanney, Visagie & Seifert, *A. waynelawii* Tanney, Visagie & Seifert, *A. whitfieldii* Tanney, Visagie & Seifert.

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## INTRODUCTION

Research on the mycobiota of the built environment traditionally focused on mesophilic fungi, especially in the context of mouldy buildings and human health. However, the incorporation of low water activity ( $a_w$ ) media in some culture-dependent studies revealed an abundance of xerophilic fungi indoors (Udagawa 1994, Beguin 1995, Takahashi 1997, Takatori *et al.* 2001, Michulez *et al.* 2015, Hirooka *et al.* 2016, Rocchi *et al.* 2017, Visagie *et al.* 2017). Although scant, culture-independent studies incorporating species-rank resolution also corroborate observations on the abundance of known xerophiles in the built environment (Amend *et al.* 2010, Nonnenmann *et al.* 2012, Adams *et al.* 2013a). The selection and proliferation of xerophilic fungi indoors is a result of temperature and humidity control, especially in North America, and the presence of low  $a_w$  niches, such as gypsum wallboard, carpets, mattresses, and associated dust (Summerbell *et al.* 1992, Miller *et al.* 2008).

While xerophilic species recovered from the built environment may be saprotrophic on skin and nails causing superficial infections (Summerbell *et al.* 2005, Hubka *et al.* 2012, de Hoog *et al.* 2014), reports implicating them as causal agents of mycoses in immunocompetent patients are infrequent (de Hoog *et al.* 2005, 2014, Guarro *et al.* 2008). However, common xerophiles such as species of *Aspergillus* subgenus *Aspergillus* and *Wallemia sebi* are potential sources of allergenic compounds (Van Bronswijk *et al.* 1986, Green *et al.* 2006, Itabashi *et al.*

2007, Slack *et al.* 2009). Additionally, the colonization of human dander and mattress dust by xerophilic *Aspergillus* spp. stimulates the development and proliferation of house dust mites (*Pyroglyphidae*) by improving substrate quality, potentially increasing associated allergen concentrations (Van Bronswijk & Sinha 1973, Lustgraaf 1978a, b). Xerophilic fungi are also important agents of food spoilage, especially in stored grains and cereals and other foods preserved by dehydration or high salt or sugar content (Pitt & Hocking 2009). Xerophiles can cause biodeterioration of cultural heritage artifacts, for example on library materials or the foxing of the Leonardo da Vinci self-portrait (Cavka *et al.* 2010, Piñar *et al.* 2013, Micheluz *et al.* 2015, Piñar *et al.* 2015, Polo *et al.* 2017).

Common indoor xerophiles include species of *Aspergillus*, *Cladosporium*, *Penicillium*, and *Wallemia* (Amend *et al.* 2010, Nonnenmann *et al.* 2012, Haas *et al.* 2014, Visagie *et al.* 2014, Jančić *et al.* 2015, Nguyen *et al.* 2015, Segers *et al.* 2016, Balolong *et al.* 2017). During a house dust survey specifically focused on these fungi from Canada and Hawaii, Visagie *et al.* (2017) isolated 1 039 strains, including 296 *Aspergillus* representing 37 species, predominantly classified in sections *Aspergillus*, *Nidulantes* (*A. versicolor* clade), and *Restricti*. Overall, species of section *Aspergillus* and *Restricti* are particularly abundant in the built environment and some exhibit profound tolerance to low  $a_w$  substrates. For example, Stevenson *et al.* (2017) recently showed that *A. penicillioides* could develop mycelia and sporulate at 0.654  $a_w$  and conidia could germinate at

0.585  $a_w$ , exceeding the previous fungal record of germination at 0.61  $a_w$  by *Xeromyces bisporus* (Pitt & Christian 1968).

Emerging evidence suggests the abundance of a little known xerotolerant/xerophilic and halotolerant/halophilic *Aspergillus* clade in the built environment, *Aspergillus* subgenus *Polypaecilum*. Kocsubé *et al.* (2016) established the subgenus for a clade of species formerly classified in *Basipetospora*, *Phialosimplex*, and *Polypaecilum*. Prior to the integration of anamorphic genera into a unified taxonomic and nomenclatural system, the morphology of the species classified in these genera distinguished them from each other, and obscured their phylogenetic relationship with *Aspergillus*. *Polypaecilum*, with *P. insolitum* as its type, was described for species with sparingly branched conidiophores and polyphialides with only a few conidiogenous apertures (Smith 1961). As the genus grew, species with more elaborate polyphialides were described, associated with sexual stages in *Thermoascus* (Apinis 1967). *Phialosimplex* (Sigler *et al.* 2010) was one of several genera with solitary phialides and basipetal chains of asexual conidia extracted from the form-concept of *Paecilomyces* (Samson 1974) and *Acremonium* (Gams 1971), including some *Sagenomella* (Gams 1978) species now classified in *Talaromyces* (Yilmaz *et al.* 2014), and *Taifanglania* (Liang *et al.* 2009) now considered a synonym of *Acrophialophora* (Zhang *et al.* 2015). *Basipetospora* was described for the chain-forming chlamydospores of *Monascus* (Cole & Kendrick 1968), but other species unrelated to *Monascus* were included based on morphological and ontogenetic similarities (Wheeler *et al.* 1988). With molecular phylogenetic sampling, it became clear that the type species of *Polypaecilum* and *Phialosimplex* belonged to the same clade (Kocsubé *et al.* 2016), along with some of the secondary species of *Basipetospora*, and that *Polypaecilum* was the oldest name for this clade.

Species of *Aspergillus* subgenus *Polypaecilum* lack vesiculate conidiophores, instead having reduced structures producing conidia from phialides, sometimes polyphialides, characterized by long and thin collula. Species occur in or on a variety of substrates, including hypersaline or arid habitats to preserved foods, and are usually isolated using growth media amended with high salt, sugar, or other osmolyte concentrations. The presence of species of subgenus *Polypaecilum* in indoor environments is of concern, with *A. caninus* and *A. chlamydosporus* associated with mycoses in canines (Sigler *et al.* 2010), a case of human keratitis attributed to *A. keratitidis* (Hsieh *et al.* 2009), and cases of otomycosis attributed to *A. insolitum* (Smith 1961, Yamashita 1972, Yamashita & Yamashita 1972, Howard 2002).

An ongoing study investigating the mycobiota of dust from the built environment in 12 countries using culture-dependent and culture-independent methods resulted in the frequent isolation and detection of strains belonging to *Aspergillus* subgenus *Polypaecilum*. In this paper, we report on the diversity of species of *Aspergillus* subgenus *Polypaecilum* detected from dust originating from Hawaii, Indonesia, Mexico, Micronesia, Thailand, and Uruguay. We describe five novel species based on morphological and phylogenetic distinctions.

## MATERIALS AND METHODS

### Sampling and isolation

Settled dust was collected in 2009 from 93 buildings in 12 countries using sterilized Duststream<sup>®</sup> collectors (Indoor

Biotechnologies Inc, Charlottesville, VA) attached to vacuum cleaners. Fungal isolations from the dust were made using a modified dilution-to-extinction method (D2E; Collado *et al.* 2007) as described in Visagie *et al.* (2014). A high water activity medium, 2 % malt extract agar (2 % MEA; 20 g Bacto malt extract, Difco Laboratories, Sparks, USA; 15 g agar, EMD Chemicals Inc., Gibbstown, USA; 1.5 g Bacto yeast extract, Difco Laboratories, Sparks, USA; 1 L distilled water), and a lower water activity medium, 20 % sucrose 2 % MEA (MEA20S; same as MEA amended with 200 g EMD sucrose), were used in an attempt to capture more biodiversity in the samples. During a subsequent survey focused on xerophilic fungi from Canada and Hawaii, a similar isolation method was used, replacing the 2 % MEA and MEA20S with dichloran 18 % glycerol agar (DG18; Hocking & Pitt 1980), malt extract yeast extract 10 % glucose 12 % NaCl agar (MY10-12), and malt extract yeast extract 50 % glucose agar (MY50G) (Samson *et al.* 2010).

Reference strains were obtained from the UAMH Centre for Global Microfungal Biodiversity (UAMH) and Westerdijk Fungal Biodiversity Institute (CBS). Strains newly isolated during this study were deposited into the Canadian Collection of Fungal Cultures (DAOMC), CBS and UAMH, and specimens into the Canadian National Mycological Herbarium (DAOM).

### Morphology and characterization of strains

Strains were grown on Czapek yeast autolysate agar (CYA; Pitt 1973), CYA with 15 % NaCl (CYA15NaCl), CYA with 50 % sucrose (CYA50S), Blakeslee's malt extract agar (MEA; Blakeslee 1915), oatmeal agar (OA), DG18, MY10-12, yeast extract sucrose agar (YES; Frisvad 1981), as well as MEA with a NaCl gradient of 5, 10, 15 and 20 % (MEA5NaCl–MEA20NaCl), and MEA with a glucose gradient of 25, 30, 35 and 40 % (MEA25G–MEA40G). Colony characters were recorded from 45 mm Petri dishes after 7 d of incubation at 25 °C, upside down, in the dark with plates kept in ventilated bags. Additional MEA plates were incubated at 37 °C. Colour names and codes used in descriptions follow those of Kornerup & Wanscher (1967). Colours are only described for colonies as they are perceived in total by the naked eye (including conidia and mycelia), rather than separately for conidial masses and other parts of the colony as is typically done for *Aspergillus*. Microscopic preparations were made from 8–14 d old MEA cultures, or from MEA15NaCl in some cases as indicated in descriptions. Observations were made using Olympus SZX12 dissecting and Olympus BX50 compound microscopes. Images were captured with attached Infinity3 and InfinityX cameras using Infinity Analyze v. 6.5.1 software (Lumenera Corp., Ottawa, Canada). To evaluate variations, at least 50 conidia were measured and presented as mean ± standard deviation in the descriptions.

Scanning electron microscopy was used to observe conidiophores and sclerotia of DAOMC 251740 (*Aspergillus keratitidis*). Individual sclerotia or ca. 2 × 2 mm blocks of agar containing mycelia were aseptically transferred to glass vials containing a fixative solution (2.5 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.0) and incubated overnight at 4 °C. Samples were then rinsed in Millipore<sup>®</sup> water for 15 min and dehydrated through a series of ethanol (30, 50, 70, 80, 90, 95, 100 % twice) for 15 min per step. Sclerotium samples immersed in 100 % ethanol were cooled in liquid nitrogen and sectioned using a scalpel. Samples were critical point dried using CO<sub>2</sub> in a Biodynamics Research

Corp. Critical Point Dryer (Rockville, MD, USA) then mounted on an aluminum stub using carbon tape. Samples were gold coated with an Emitech K550V Gold Sputter Coater (EM Technologies Ltd., Ashford, Kent, UK) and observed and imaged with the SEM Quanta 600 (FEI Company, Brno, Czech Republic) at 20 kV, with a working distance of ca. 6 mm, spot size 2.5, and secondary electron detector. Photographic plates were prepared in Affinity Photo v. 1.5.2 (Serif Europe Ltd, UK). For aesthetic purposes, micrographs were edited using the inpainting brush tool without altering scientifically significant areas.

## DNA extraction, sequencing, and phylogenetic analyses

DNA was extracted from 14 d old colonies grown on MEA or MEA15NaCl using the Ultraclean™ Microbial DNA isolation Kit (MoBio Laboratories Inc., Solana Beach, USA). PCR amplifications were done in 10 µl volumes containing 1 µl Titanium Taq buffer (Takara Bio USA, Mountain View, USA), 0.5 µl (2 µM) dNTPs, 0.04 µl (3.2 µM) of each primer, 0.1 µl 10 × Titanium Taq polymerase (Takara Bio USA, Mountain View, USA), 0.5 µl template DNA, and 7.82 µl MilliQ water. Loci chosen for amplification were the internal transcribed spacer rDNA region (ITS), β-tubulin (*BenA*), calmodulin (*CaM*), RNA polymerase II second largest subunit (*RPB2*), DNA topoisomerase 1 (*TOP1*), and a pre-mRNA processing protein homolog (*TSR1*). Primers used for PCR included V9G and LS266 for ITS (Masclaux *et al.* 1995, de Hoog & Gerrits van den Ende 1998), Bt2a and Bt2b for *BenA* (Glass & Donaldson 1995), CF1 and CF4 for *CaM* (Peterson *et al.* 2005, Hong *et al.* 2006), RPB2-5FEur and RPB2-7CrEur for *RPB2* (Houbraken *et al.* 2012), TOP1-2708F and TOP1-3498R for *TOP1* (Stielow *et al.* 2015), and TSR1-F1526Pc and TSR1-R2434 for *TSR1* (Houbraken & Samson 2011). An annealing temperature of 56 °C was used for amplification of ITS, *BenA*, and *CaM*, 50 °C for *TSR1*, 58 °C for *TOP1*, and a stepdown PCR (5 cycles 60 °C, 5 cycles 58 °C followed by 30 cycles 54 °C) for *RPB2*. Subsequent sequencing reactions were set up using the BigDye Terminator Cycle Premix Kit (Applied Biosystems, Waltham, USA) and the same primer pairs used for PCR amplification. The internal sequencing primers RPB2-F311 and RPB2-R310 were also added for obtaining cleaner results for *RPB2* (Houbraken & Samson 2011). Sequence contigs were assembled in Geneious v. 10.0.2 (Biomatters Ltd., Auckland, New Zealand). Newly generated sequences were deposited in GenBank and included in a dataset containing publicly available sequences for other species of subgenus *Polypaecilum* (see Table 1).

To compare our strains with known species, multiple phylogenetic analyses were done. Data partitions were aligned using MAFFT v. 7.305b (Katoh & Standley 2013) with the G-INS-i option (L-INS-i for ITS) and afterwards manually adjusted in Geneious as needed. An appropriate substitution model for each aligned dataset was selected based on the Akaike information criteria values (Akaike 1974) calculated in MrModeltest v. 2.3 (Nylander 2004). Phylogenies were calculated for each gene, followed by a concatenated dataset of the six genes (genes treated as separate partitions), using Maximum Likelihood (ML) in RAxML v. 8.0.0 (Stamatakis 2014), and Bayesian Inference (BI) in MrBayes v 3.2. (Ronquist *et al.* 2012). For ML, support in nodes were calculated with a bootstrap analysis of 1 000 replicates. Trees were visualised in Figtree v. 1.4.2 (<http://tree.bio.ed>

[ac.uk/software/figtree](http://www.ac.uk/software/figtree)) and visually edited in Adobe® Illustrator® CC. Aligned datasets were uploaded to TreeBase ([www.treebase.org](http://www.treebase.org)) with submission ID 20938.

To compare the diversity discovered from D2E isolations with 454-pyrosequencing results obtained from the same house dust samples (Amend *et al.* 2010; raw sequences deposited in NCBI's sequence read archive SRS010093 and SRS010094), type strains of subgenus *Polypaecilum* were first BLASTed against the ITS 454-sequence dataset. All sequences with more than ~94 % similarity to an ITS ex-type sequence were extracted. Additional sequences obtained from mainly culture-independent studies, e.g. cloning or next generation sequencing (NGS) were harvested from GenBank (search performed on 19 June 2017). All 1 081 sequences obtained were compiled and aligned in MAFFT using the FFT-NS-2 option. The alignment was trimmed and manually adjusted in Geneious. A neighbour-joining tree was calculated in PAUP\* v 4.0b10 using the generalized time-reversible model with the equal rates of variation option selected. The tree was visualized in Figtree and edited for publication with Adobe® Illustrator® CC.

## RESULTS

### Sampling, isolations, and identification

D2E experiments using 2 % MEA and MEA20S resulted in the isolation of ±8 000 house dust strains. Strains were initially grouped based on colony morphology and ITS sequences, resulting in the identification of 340 strains representing six putative species of *Aspergillus* subgenus *Polypaecilum*. Strains were recovered from MEA20S and 2 % MEA, with a strong isolation bias for *A. keratitidis*. Strains originated from Indonesia (1 of 6 houses), Mexico (4 of 8 houses), Micronesia (5 of 9 houses), and Thailand (7 of 10 houses). Subgenus *Polypaecilum* strains were not recovered from house dust collected in Australia, Canada, the Netherlands, New Zealand, South Africa, UK, Uruguay, or USA. New isolations targeting xerophiles resulted in the isolation of four strains, representing two species belonging to subgenus *Polypaecilum*, among the ±1 000 isolates. These strains originated from Hawaii and were isolated using DG18 and MY10-12.

### Morphology

Strains isolated from house dust conformed to the morphology typical of accepted species of *Aspergillus* subgenus *Polypaecilum*, that is, solitary phialides instead of aspergilli and conidia occurring in chains, heads, or singly. Sporulation was mostly less dense than is usually observed in *Aspergillus*. Species of *Aspergillus* subgenus *Polypaecilum* were grown under a variety of conditions and observed using light microscopy. Observed species' growth rates are summarised in Table 2 and morphological characters in Figs 1–5.

Intraspecific variation was conspicuous among *A. keratitidis* colonies grown on CYA, YES, and MY10-12; for example, CYA colonies in some strains (e.g.: ex-type, BCRC 34221, DAOMC 251738, DAOMC 251743, DAOMC 251744) were white to orange grey (6B6), but white to dull green (25C4–D4) in others (e.g.: DAOMC 251745, DAOMC 251747, DAOMC 251748, DAOMC 251740). Sclerotia were also observed in *A. keratitidis*,

**Table 1.** Strains used for phylogenetic comparisons in *Aspergillus* subgenus *Polypaecilum*.

Species	Strains	Country	Location	Source	GenBank accession nr.						
					ITS	<i>BenA</i>	<i>CaM</i>	<i>RPB2</i>	<i>TOP1</i>	<i>TSR1</i>	
<i>A. atacamensis</i>	EXF:6659	Chile	Coastal Range hills of the Atacama Desert, south of Iquique	Cave wall	KX900618						
	EXF:6660, CBS:142046 (ex-type)	Chile	Coastal Range hills of the Atacama Desert, south of Iquique	Cave wall	KX900619						
	EXF:6661	Chile	Coastal Range hills of the Atacama Desert, south of Iquique	Cave soil	KX900620						
<i>A. baamensis</i>	CBS:380.74, IFO:9650 (ex-type)	Japan	Osaka	Wakame ( <i>Undaria pinnatifida</i> )	KY980621	KY980549	KY980585	JN121509	KY980482	JN121815	
	DAOMC:251735, KAS:7918	USA	Hawaii, Kailua	House dust	KY980623	KY980551	KY980587	KY980448	KY980484	KY980516	
	DAOMC:251736, KAS:7919	USA	Hawaii, Kailua	House dust	KY980624	KY980552	KY980588	KY980449	KY980485	KY980517	
	DAOMC:251737, KAS:7920	USA	Hawaii, Kailua	House dust	KY980625	KY980553	KY980589	KY980450	KY980486	KY980518	
<i>A. caninus</i>	CBS:128032, UAMH:10337 (ex-type)	USA	Texas, San Antonio	Bone marrow aspirate from canine	KY980618	KY980546	KY980582	JN121445	KY980478	KY980514	
<i>A. chlamydosporus</i>	CBS:109945, FMR:7371, IMI:387422, UAMH:10961 (ex-type)	Spain		Disseminated infection in dog	KY980617	KY980545	KY980581	KY980444	KY980477	KY980513	
<i>A. insolitus</i>	CBS:181.90, UAMH:11255	Germany	Hamburg	Human onychomycosis pedis	KY980620	KY980548	KY980584	KY980446	KY980480	KY980515	
	CBS:384.61, ATCC:18164, IFO:8788, IMI:075202, LSHB:BB414, MUCL:3078, QM:7961, UAMH:11254 (ex-type)	United Kingdom	Yorkshire, Leeds	Human ear	KY980622	KY980550	KY980586	KY980447	KY980483	JN121816	
<i>A. kalimae</i>	DAOMC:251762, CBS:143506, UAMH:11837, KAS:8135, SLOAN:4181, PN08TH-526 (ex-type)	Thailand	Chumphon	House dust	KY980650	KY980578	KY980614	KY980475	KY980511	KY980543	
<i>A. keratitidis</i>	BCRC:34221, DTO:198-E8 (ex-type)	Taiwan		Human keratitis	KY980616	KY980544	KY980580	KY980443	KY980476	KY980512	
	DAOMC:251750, KAS:7927	USA	Hawaii, Kailua	House dust	KY980626	KY980554	KY980590	KY980451	KY980487	KY980519	
	DAOMC:251738, UAMH:11847, KAS:8109, SLOAN:7944, R11D-47	Indonesia	Yogyakarta	House dust	KY980627	KY980555	KY980591	KY980452	KY980488	KY980520	
	DAOMC:251743, UAMH:11835, KAS:8110, SLOAN:7957, PN10TH-16	Thailand	Surat Thani	House dust	KY980628	KY980556	KY980592	KY980453	KY980489	KY980521	
	DAOMC:251744, KAS:8111, SLOAN:4480, PN10TH-686	Thailand	Surat Thani	House dust	KY980629	KY980557	KY980593	KY980454	KY980490	KY980522	

Table 1. (Continued).

Species	Strains	Country	Location	Source	GenBank accession nr.					
					ITS	<i>BenA</i>	<i>CaM</i>	<i>RPB2</i>	<i>TOP1</i>	<i>TSR1</i>
	DAOMC:251745, KAS:8112, SLOAN:4487, PN10TH-692	Thailand	Surat Thani	House dust	KY980630	KY980558	KY980594	KY980455	KY980491	KY980523
	DAOMC:251746, KAS:8113, SLOAN:4533, PN10TH-732	Thailand	Surat Thani	House dust	KY980631	KY980559	KY980595	KY980456	KY980492	KY980524
	DAOMC:251747, KAS:8114, SLOAN:4562, PN10TH-751	Thailand	Surat Thani	House dust	KY980632	KY980560	KY980596	KY980457	KY980493	KY980525
	DAOMC:251739, UAMH 11839; KAS:8116, SLOAN:7948, WL03MI-221	Micronesia	Malem	House dust	KY980633	KY980561	KY980597	KY980458	KY980494	KY980526
	DAOMC:251748, KAS:8117, SLOAN:4080, PN07TH-365	Thailand	Songkla	House dust	KY980634	KY980562	KY980598	KY980459	KY980495	KY980527
	DAOMC:251749, KAS:8118, SLOAN:4448, PN10TH-658	Thailand	Surat Thani	House dust	KY980635	KY980563	KY980599	KY980460	KY980496	KY980528
	DAOMC:251740, UAMH:11845, KAS:8119, SLOAN:7956, WL03MI-184	Micronesia	Malem	House dust	KY980636	KY980564	KY980600	KY980461	KY980497	KY980529
	DAOMC:251741, KAS:8120, SLOAN:7946, WL04MI-442	Micronesia	Lelu	House dust	KY980637	KY980565	KY980601	KY980462	KY980498	KY980530
	DAOMC:251742, KAS:8131, SLOAN:7960, WL03MI-215	Micronesia	Malem	House dust	KY980647	KY980575	KY980611	KY980472	KY980508	KY980540
<i>A. noonimiae</i>	DAOMC:251754, UAMH:11836, CBS:143382, KAS:8125, SLOAN:7955, PN06TH-370 (ex-type)	Thailand	Songkla	House dust	KY980641	KY980569	KY980605	KY980466	KY980502	KY980534
<i>A. pisci</i>	CBS:101166 (ex-type)	Netherlands		Yeast extract	MF362690	MF362691		JN121415		JN121722
<i>A. salinarus</i>	CBS:138583, DSM:27530 (ex-type)	Germany	Bavaria, Berchtesgaden salt mine	Salt water	KY980619	KY980547	KY980583	KY980445	KY980479	KJ855526
	EXF:10245	Austria	Hallstadt	Salt mine	KX900624					
	EXF:10248	Austria	Hallstadt	Salt mine	KX900625					
<i>A. salisburgensis</i>	CBS:142047, EXF:10247 (ex-type)	Austria	Hallstadt	Salt mine	KX900623					
	EXF:10244	Austria	Hallstadt	Salt mine	KX900621					
	EXF:10246	Austria	Hallstadt	Salt mine	KX900622					
<i>A. sclerotialis</i>	CBS:366.77, IAM:14794 (ex-type)	France		Fodder of ray-grass and lucerne	KF267869	KY980579	KY980615	JN121505	KY980481	JN121811

(continued on next page)

Table 1. (Continued).

Species	Strains	Country	Location	Source	GenBank accession nr.					
					ITS	BenA	CaM	RPB2	TOP1	TSR1
<i>A. thailandensis</i>	DAOMC:251755, UAMH:11840, CBS:143383, KAS:8126, SLOAN:4554, PN10TH-749 (ex-type)	Thailand	Surat Thani	House dust	KY980642	KY980570	KY980606	KY980467	KY980503	KY980535
	DAOMC:251756, UAMH:11841, KAS:8127, SLOAN:4626, PN10TH-797	Thailand	Surat Thani	House dust	KY980643	KY980571	KY980607	KY980468	KY980504	KY980536
<i>A. waynelawii</i>	DAOMC:251753, UAMH:11843, KAS:8122, SLOAN:7943, PN03TH-136	Thailand	Bangkok	House dust	KY980638	KY980566	KY980602	KY980463	KY980499	KY980531
	DAOMC:251751, UAMH:11926, CBS:143384, KAS:8123, SLOAN:7951a, WL03MI-231 (ex-type)	Micronesia	Malem	House dust	KY980639	KY980567	KY980603	KY980464	KY980500	KY980532
	DAOMC:251752, KAS:8124, SLOAN:7951b	Micronesia	Malem	House dust	KY980640	KY980568	KY980604	KY980465	KY980501	KY980533
<i>A. wentii</i>	CBS:104.07, ATCC:1023, DSM:3701, IMI:017295, IMI:017295ii, NRRL:1269, NRRL:375 (ex-type)	Indonesia	Java	Soybeans	EF652151	EF652106	EF652131	EF652092	KV878210 <sup>1</sup>	JN121725
<i>A. whitfieldii</i>	DAOMC:251759, KAS:8128, SLOAN:4098, PN07TH-381	Thailand	Songkla	House dust	KY980644	KY980572	KY980608	KY980469	KY980505	KY980537
	DAOMC:251760, UAMH:11842, CBS:143385, KAS:8129, SLOAN:4178, PN08TH-523 (ex-type)	Thailand	Chumphon	House dust	KY980645	KY980573	KY980609	KY980470	KY980506	KY980538
	DAOMC:251761, KAS:8130, SLOAN:7602, PN10TH-761	Thailand	Surat Thani	House dust	KY980646	KY980574	KY980610	KY980471	KY980507	KY980539
	DAOMC:251757, KAS:8132, SLOAN:7953, WL03MI-246	Micronesia	Malem	House dust	KY980648	KY980576	KY980612	KY980473	KY980509	KY980541
	DAOMC:251758, UAMH:11846, KAS:8134, SLOAN:7961, WL05MI-555	Micronesia	Lelu	House dust	KY980649	KY980577	KY980613	KY980474	KY980510	KY980542

<sup>1</sup> Extracted from whole genome sequence obtained from *Aspergillus wentii* strain DTO 134E9.

**Table 2.** Growth rates of *Aspergillus* subgenus *Polypaecilum* species examined during this study.

	CYA	CYA15NaCl	CYA50S	MEA 37 °C	MEA	MEA25G	MEA30G	MEA35G	MEA40G	MEA5NaCl	MEA10 NaCl	MEA15NaCl	MEA20NaCl	DG18	YES	MY10-12
<i>A. baarnensis</i>	no growth to germination	germination to 3	germination to 3	germination to microcolonies	no growth	no growth	no growth to microcolonies	no growth to microcolonies	no growth to microcolonies	germination to 6	7–10	8–11	5–7	germination to 5	no growth to microcolonies	6–11
<i>A. caninus</i>	11–12	microcolonies	7–10	27–28	15–17	15–17	15–17	13–16	12–13	no growth	no growth	no growth	no growth	germination	7–9	germination
<i>A. chlamydosporus</i>	10–11	6–8	12–15	10–13	10–12	11–13	11–12	10–11	10–11	4–5	no growth	no growth	no growth	5–6	11–17	germination
<i>A. insolitus</i>	6–13	5–15	10–20	microcolonies to 7	6–8	6–9	9–11	8–11	11–13	10–11	5–9	no growth to 5	no growth to microcolonies	8–10	7–16	3–10
<i>A. kalimae</i>	germination	germination	germination	no growth	no growth	no growth	germination	germination	microcolonies	3–4	7	7	4	microcolonies	germination	6
<i>A. keratitidis</i>	3–7	5–8	6–8	no growth to 4	3–6	4–8	5–8	6–10	7–12	5–8	5–10	microcolonies to 7	germination to microcolonies	microcolonies to 8	6–9	microcolonies to 9
<i>A. noonimiae</i>	6–7	7	9–10	10–11	6	6	8	9–10	10	11	8	microcolonies	germination	7–8	9–10	6–7
<i>A. pisci</i> <sup>1</sup> *	15–20	n.a.	n.a.	n.a.	15–20	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
<i>A. salinarus</i>	germination	germination	germination	germination	no growth	no growth	no growth	no growth	no growth	microcolonies	5-6	6-7	4-5	germination	germination	6–7
<i>A. thailandensis</i>	11–12	10–11	13–15	germination to 4	13–14	14	15–16	9–18	17–19	15	10–11	5	germination	14–15	14–16	9–10
<i>A. waynelawii</i>	5–6	5–8	6–9	microcolonies to 6	4–6	5–6	6–7	6–8	7–9	8	6–7	microcolonies to 4	germination	6–7	5–8	3–5
<i>A. whitfieldii</i>	3–4	1–5	4–6	no growth	4–5	4–5	5–6	6	6–8	6–8	4–5	microcolonies	no growth (microcolonies in KAS8128)	6–8	6–8	4–6

\* incubated at 25 °C for 2 wk.

<sup>1</sup> Data from Pitt & Hocking (2009).

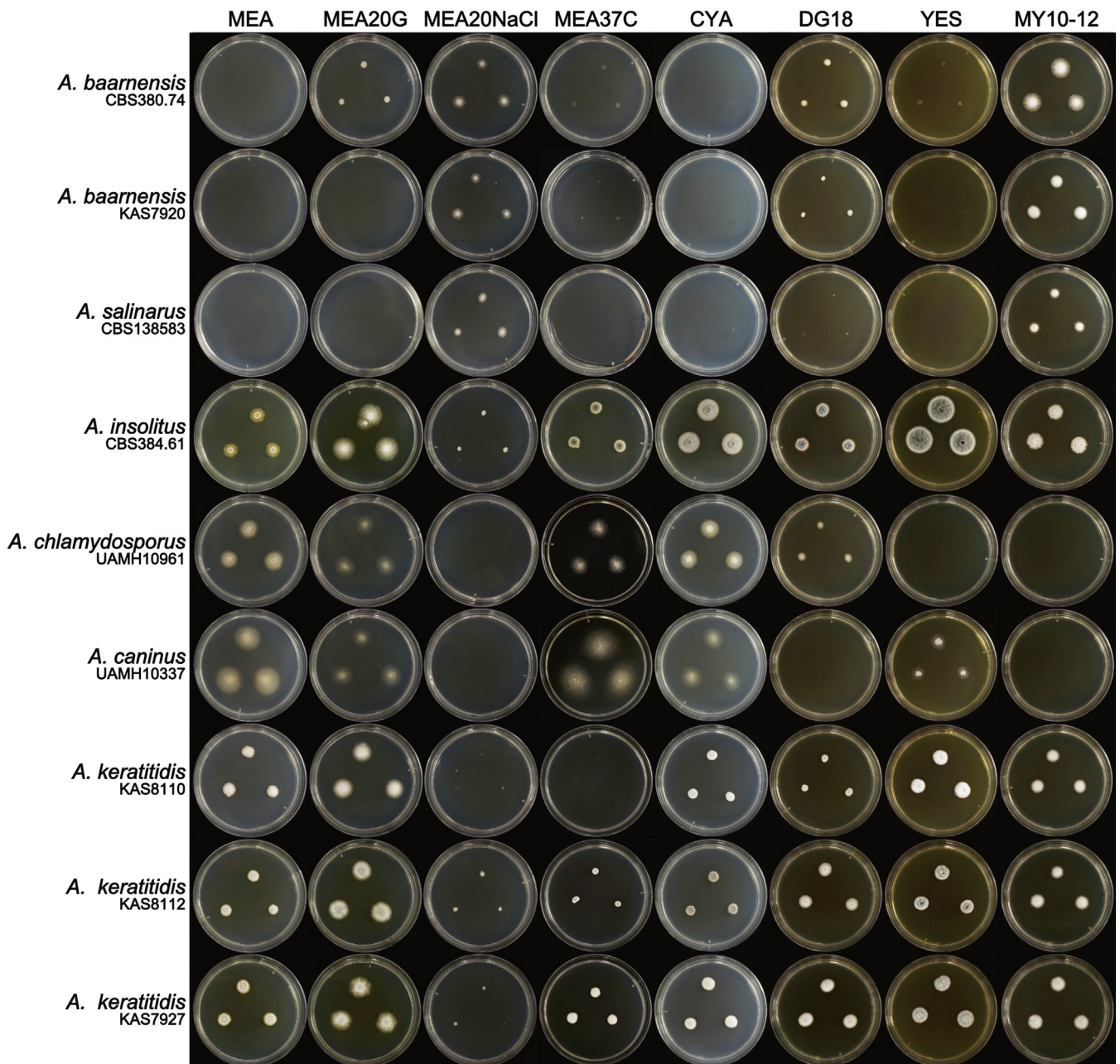


Fig. 1. Overview of colony characters in species of *Aspergillus* subgenus *Polypaecilum* treated in this paper.

not previously reported in this species: they were cream, orange to brownish, 150–450  $\mu\text{m}$  diam, composed of angular to isodiametric cells surrounded by a layer of thick-walled, compressed isodiametric cells, and produced abundantly on MEA20S and oatmeal agar (OA; Samson *et al.* 2010) after prolonged incubation (>8 wk). Evidence of asci or ascospores was not found using light or scanning electron microscopy (Figs 2, 3).

*Aspergillus baarmensis* strains isolated from Hawaiian dust were almost identical to the ex-type strain (CBS 380.74; Fig. 4), except the Hawaiian strains did not grow on CYA15NaCl or YES, whereas CBS 380.74 exhibited sparse growth on these media. Some Hawaiian *A. baarmensis* strains did not grow on DG18.

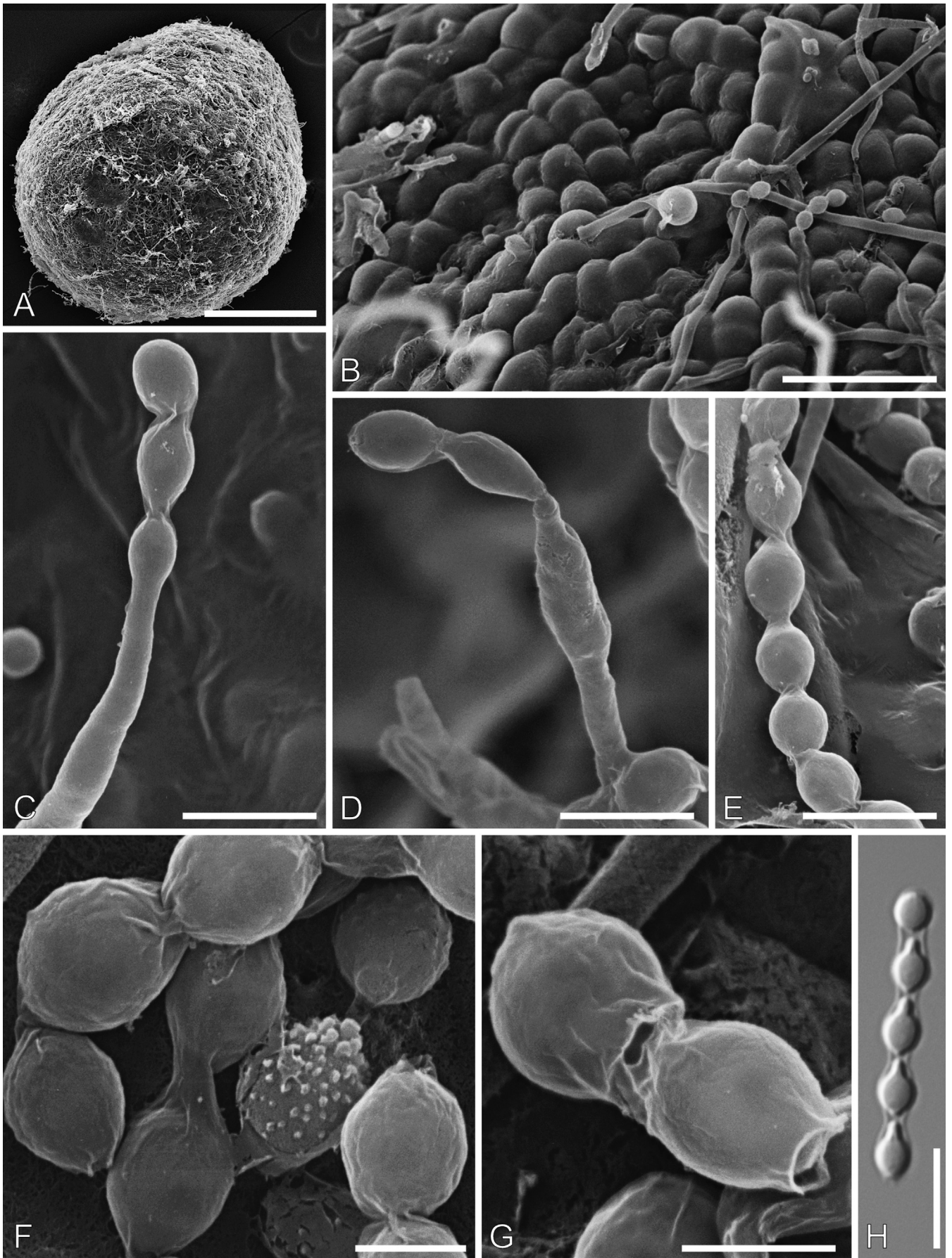
## Phylogeny

Phylogenetic relationships among species in subgenus *Polypaecilum* were assessed using a concatenated alignment of *BenA*, *CaM*, *ITS*, *RPB2*, *TOP1*, and *TSR1* (Fig. 6). Fifteen species comprising subgenus *Polypaecilum* were delineated with strong

support based on ML bootstrap and BI posterior probability values, including five novel species isolated from house dust. The halophilic species *A. baarmensis*, *A. salinarus*, and *A. salisburgensis* formed a strongly supported clade sister to *A. caninus* and *A. chlamydosporus*, species implicated in canine mycoses. These clades were in turn sister to *A. atacamensis* and *A. kalimae*, with *A. whitfieldii* forming a weakly to moderately supported basal clade. Remaining species of subgenus *Polypaecilum* formed a strongly supported clade: *A. insolitus*, *A. keratitidis*, *A. pisci*, *A. sclerotialis*, *A. waynelawii*, *A. noonimiae*, and *A. thailandensis*. *Aspergillus insolitus* and *A. pisci*, species previously classified in *Polypaecilum* and distinguished by complex polyphialides, comprised a strongly supported basal clade. *Aspergillus waynelawii* belongs to a clade including the halotolerant, sclerotia-forming species *A. keratitidis* and *A. sclerotialis*.

Individual gene phylogenies were generated to assess genealogical concordance of the novel species of subgenus *Polypaecilum* described herein: *A. kalimae*, *A. noonimiae*, *A. thailandensis*, *A. waynelawii*, and *A. whitfieldii* (Fig. 7; Table 1).





**Fig. 2.** Scanning electron microscope images showing characters observed for *Aspergillus keratitis* (DAOMC 251740). **A, B.** Sclerotia. **C, D.** Conidiophores. **E–H.** Conidia. Note membranous sheath enveloping conidia in C–H. Scale bars: A = 200  $\mu$ m, B = 20  $\mu$ m, C–E = 5  $\mu$ m, F, G = 2  $\mu$ m, H = 10  $\mu$ m.

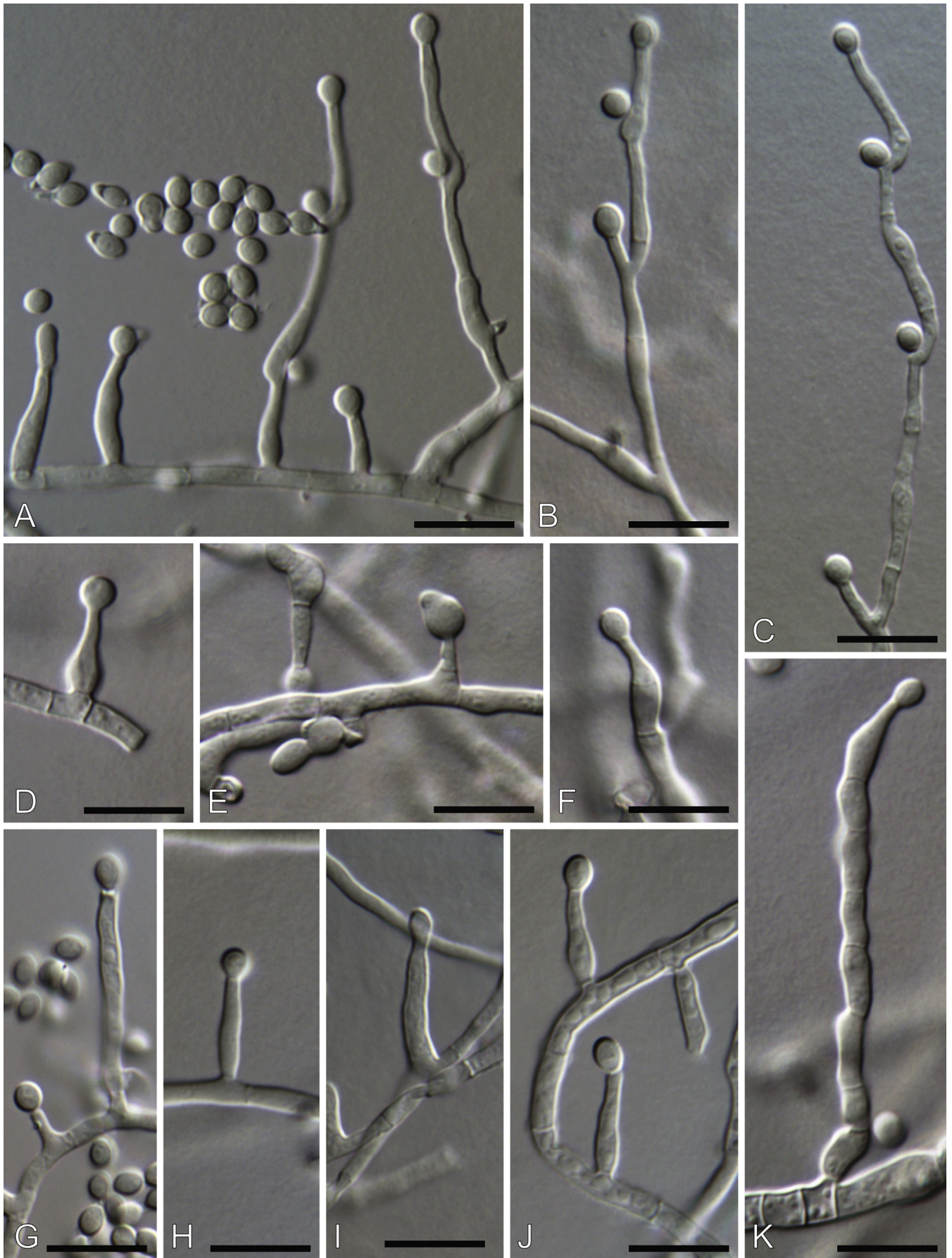
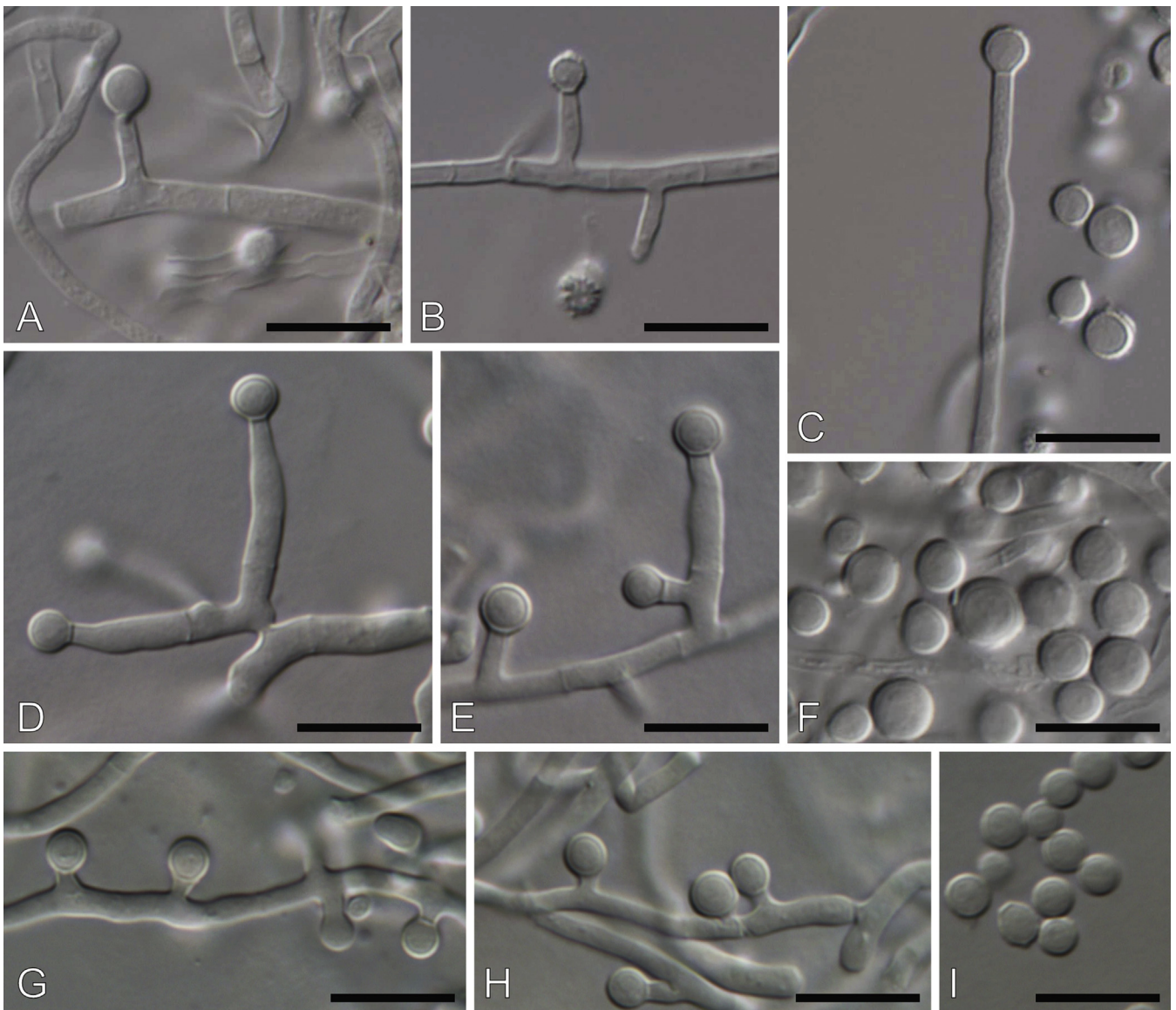


Fig. 3. Overview of conidiophores and conidia in species of *Aspergillus* subgenus *Polypaecilum*: A–K. *Aspergillus keratitidis* (BCRC 34221 in A–C; DAOMC 251743 in D–F, K; DAOMC 251739 in G, H; DAOMC 251748 in I, J). Scale bars = 10  $\mu$ m.



**Fig. 4.** Overview of conidiophores and conidia in species of *Aspergillus* subgenus *Polypaecilum*: **A–F.** *Aspergillus baarnensis* (CBS 380.74 in A–C, F; DAOMC 251737 in D, E). **G–I.** *Aspergillus salinaris* (CBS 138583). Scale bars = 10  $\mu$ m.

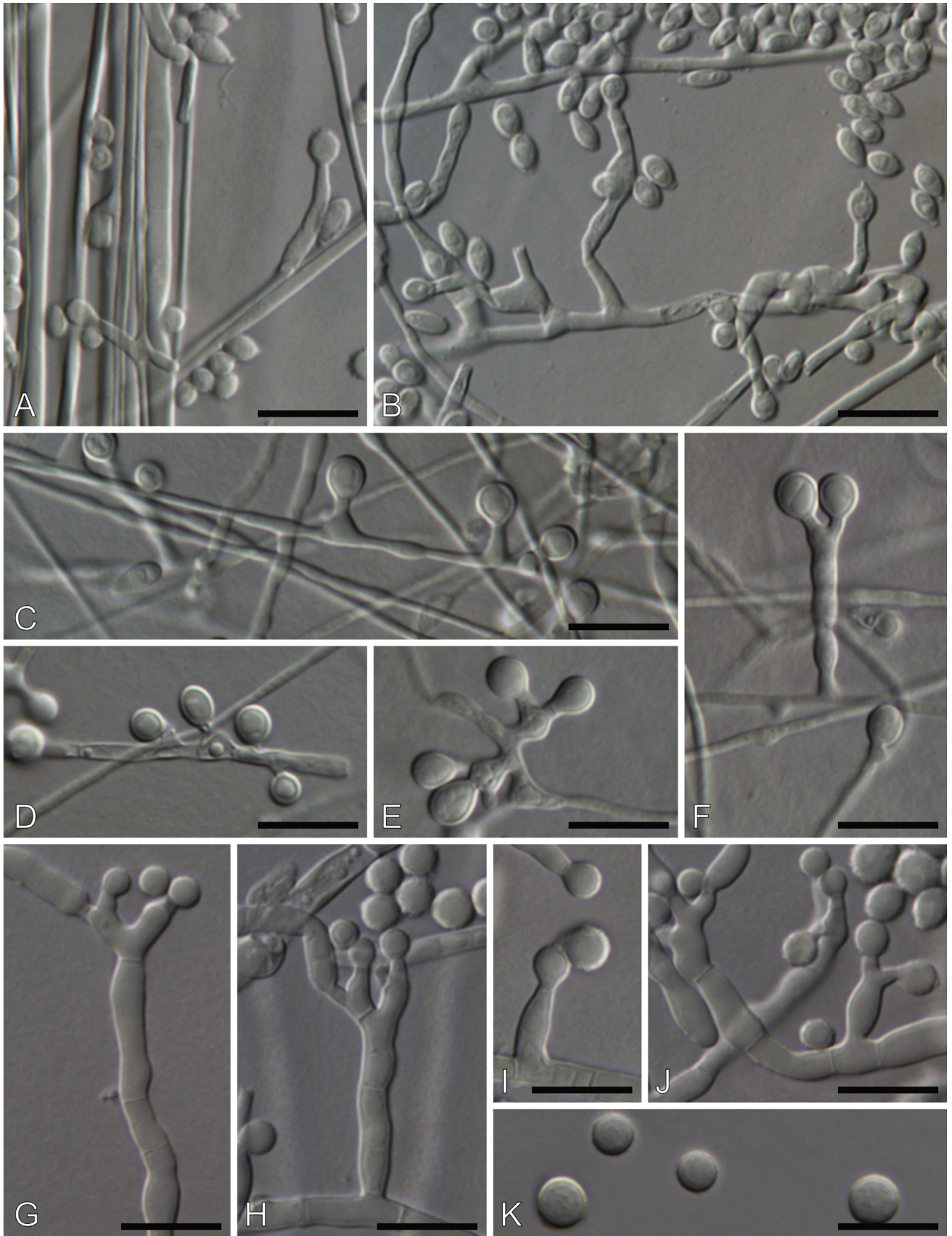
Genealogical concordance was subsequently demonstrated for all species of subgenus *Polypaecilum* and confirmed the distinctiveness of the five novel species recovered from house dust. Individual gene phylogenies were generally congruent; however, discordance was observed in the ITS phylogeny, which also contained species for which sequences of other genes were unavailable, e.g., *A. atacamensis* and *A. salisburgensis*. In the ITS phylogeny, *A. baarnensis*, *A. salinarus*, and *A. salisburgensis* formed a strongly supported clade, with *A. chlamydosporus* as a basal species. The positions of *A. whitfieldii* and *A. kalimae* were inconsistent between different gene phylogenies. The ITS phylogeny placed *A. kalimae* with strong support sister to *A. atacamensis* in a weakly supported clade with *A. caninus* and *A. chlamydosporus* as basal species, while the *RPB2* phylogeny placed *A. kalimae* basal to the subgenus *Polypaecilum* lineage (with weak support) or variously basal to the *A. baarnensis*–*A. caninus* clade (with weak support and long branch; *BenA*, *CaM*, and *TSR1*). The placement of *A. whitfieldii* was similarly discordant among single gene phylogenies.

Representative strains of the most commonly isolated species, *A. keratitidis*, showed considerable colony morphology and sequence variation. However, gene genealogies and colony

morphologies did not clearly identify coherent clades within *A. keratitidis*.

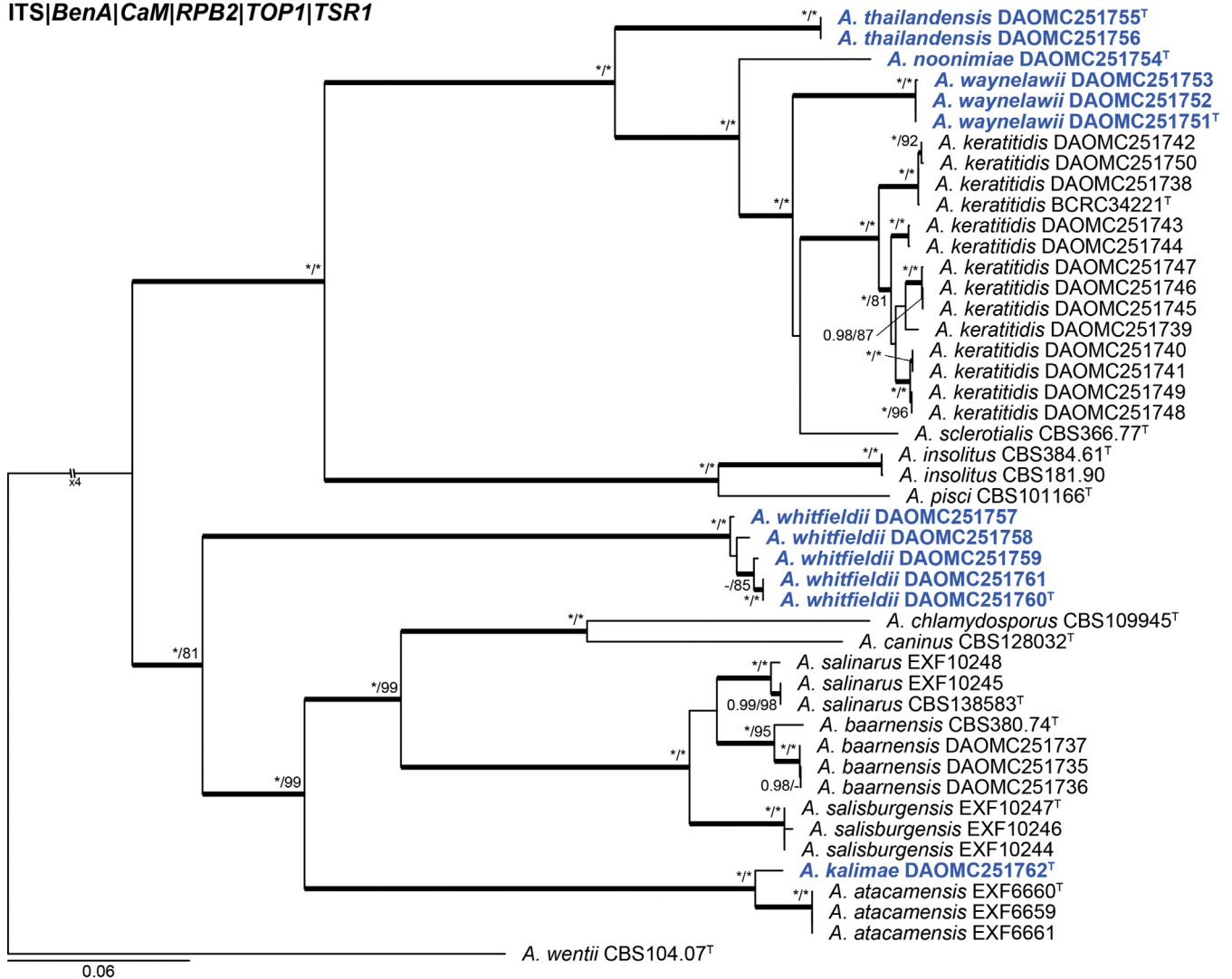
Phylogenetic analyses summarized in Fig. 7 showed that all genes sufficiently resolved species in subgenus *Polypaecilum*. Notably, the ITS DNA barcode adequately distinguished between all species of subgenus *Polypaecilum*.

Sequences attributable to subgenus *Polypaecilum* accounted for 980 of 194 240 454-pyrosequencing reads (Fig. 8). Reads originating from pyrosequencing detected five of the six isolated species, failing to detect *Aspergillus noonimiae*, a species represented by only a single strain from Thailand. Species detected by pyrosequencing and not cultured in their country of origin included *A. atacamensis*, *A. baarnensis*, *A. insolitus*, *A. pisci*, *A. salinarus*, and *A. sclerotialis*. The dust from which *A. baarnensis* was isolated was not analysed using pyrosequencing. According to pyrosequencing results (Table 3), *A. keratitidis* and *A. waynelawii* are abundant in Mexican house dust; however, no strains of subgenus *Polypaecilum* were isolated from Mexico. Similarly, *A. whitfieldii* was not isolated but was detected by pyrosequencing in Indonesia, Mexico, and Uruguay. The ITS phylogeny including pyrosequencing reads also revealed three clades representing putatively undescribed species that were not



**Fig. 5.** Overview of conidiophores and conidia in species of *Aspergillus* subgenus *Polypaecilum*: **A, B.** *Aspergillus caninus* (CBS 128032). **C–F.** *Aspergillus chlamydosporus* (UAMH 10961). **G–K.** *Aspergillus insolitus* (CBS 384.61). Scale bars = 10  $\mu$ m.

ITS|BenA|CaM|RPB2|TOP1|TSR1



**Fig. 6.** Phylogenetic tree based on a concatenated dataset of ITS, *BenA*, *CaM*, *RPB2*, *TOP1* and *TSR1*, showing the relationships between species from subgenus *Polypaecilum*. *Aspergillus wentii* was selected as outgroup. Posterior probabilities (>0.95) / Bootstrap values (>80) are indicated above thickened branches (\* = 1.0/100). New species are shown in blue bold text. <sup>T</sup> = ex-type.

captured by the D2E method. No subgenus *Polypaecilum* strains were isolated from New Zealand, however four reads from two homes in New Zealand represent an unknown species ("unknown clade 3").

## TAXONOMY

***Aspergillus kalimae*** Tanney, Visagie & Seifert **sp. nov.**  
Mycobank MB822732. [Fig. 9.](#)

**Etymology:** Latin, *kalimae*, named after Dr. Kalima Mwange, Socio-Environmental Expert-Advisor for the Economic and Social Council of the United Nations, who conducted the original isolations for our indoor house dust project.

**Diagnosis:** Conidiophores solitary phialides, mono- to polyphialidic, sclerotia absent, no growth at 37 °C on MY10-12, negligible growth on most media, better growth observed on low  $a_w$  media.

**Typus:** **Thailand**, Chumphon, from house dust, 2009, collected by Paramee Noonim, isolated by Kalima Mwange and Ed

Whitfield (holotype DAOM 745800, culture ex-type DAOMC 251762 = UAMH 11837 = CBS 143506 = KAS 8135 = SLOAN 4181 = PN08TH-526).

**ITS barcode:** KY980650 (alternative markers: *BenA* = KY980578; *CaM* = KY980614; *RPB2* = KY980475; *TOP1* = KY980511; *TSR1* = KY980543).

**Colony diam (7 d, in mm):** CYA germination; CYA15NaCl germination; CYA50S germination; MEA 37 °C (on MY10-12) no growth; MEA no growth; MEA25G no growth; MEA30G germination; MEA35G germination; MEA40G microcolonies; MEA5NaCl 3–4; MEA10NaCl 7; MEA15NaCl 7; MEA20NaCl 4; DG18 microcolonies; YES germination; MY10-12 6.

**Colony characters (25 °C, 7 d):** **CYA15NaCl:** Colonies sparse, low, colourless; sporulation absent; exudate absent; soluble pigment absent; reverse color yellowish (2A2). **MEA15NaCl:** Colonies moderately dense, low, plain, white; sporulation moderately dense; exudate absent; soluble pigment absent; reverse color yellowish white (2A2–3A2). **DG18:** Colonies sparse, low, colourless; sporulation absent; exudate absent; soluble pigment absent; reverse color yellowish (2A2). **YES:** Colonies germinating. **MY10-12:** Colonies moderately dense,

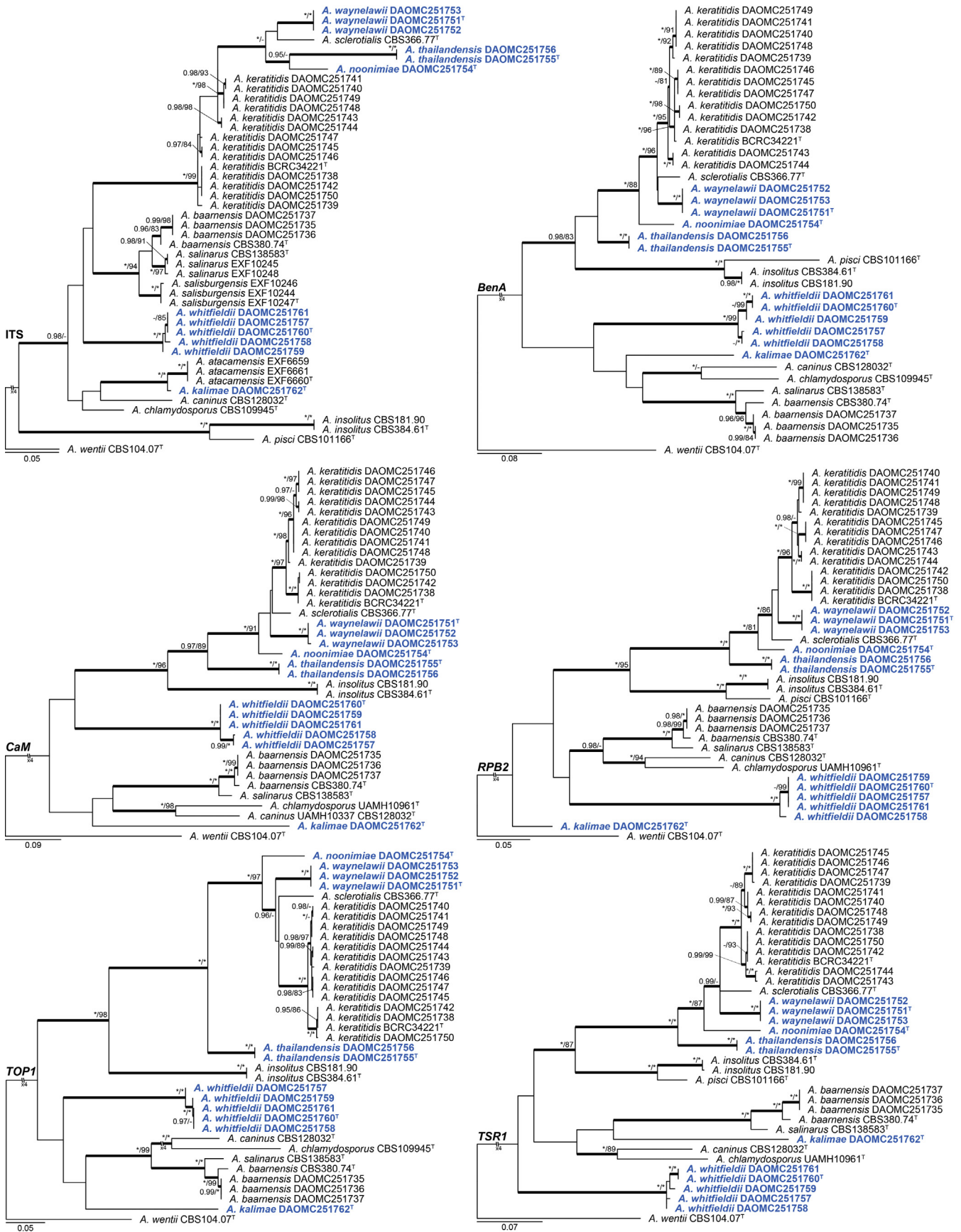
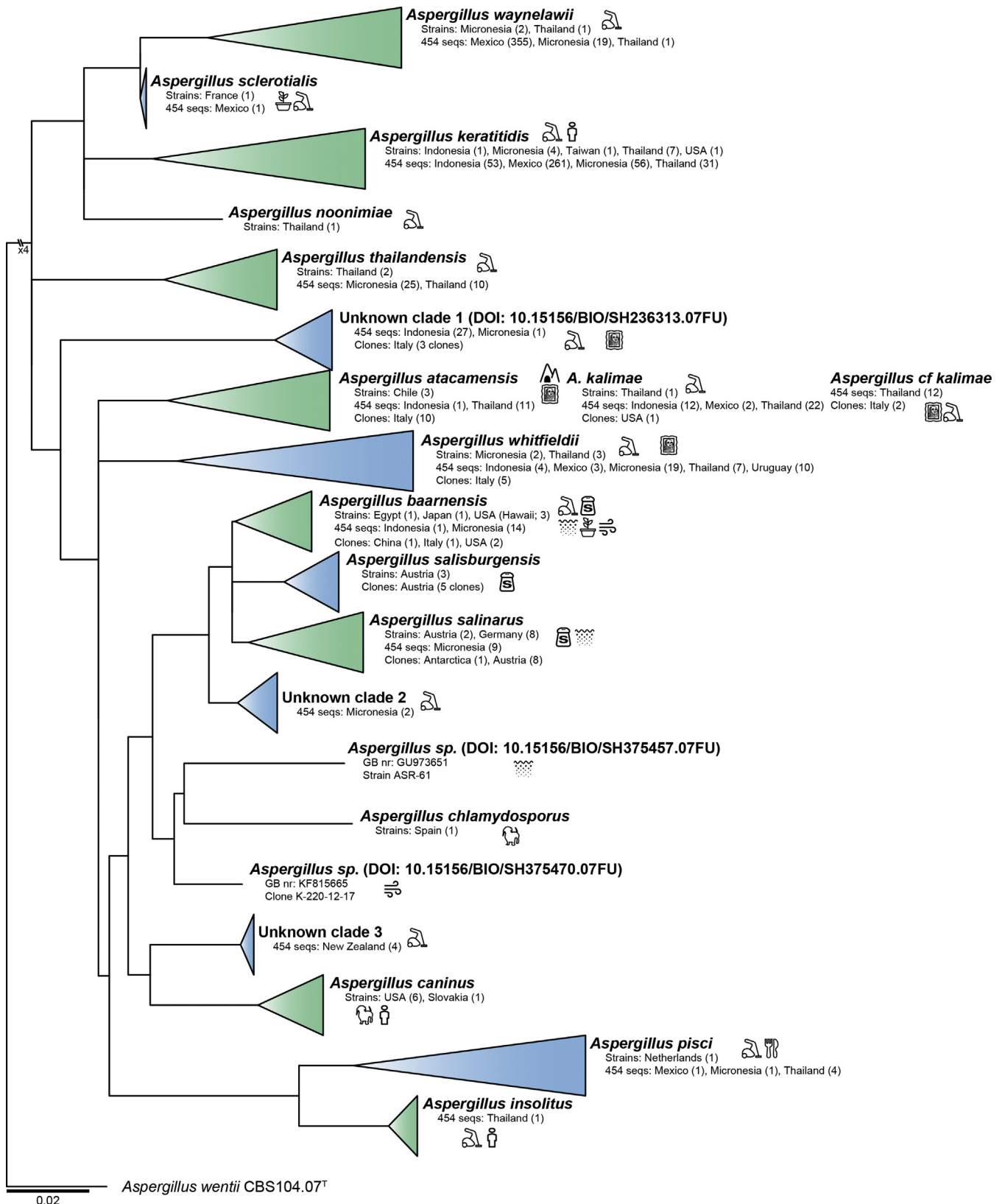


Fig. 7. Phylogenetic trees based on ITS, BenA, CaM, RPB2, TOP1 and TSR1, showing the relationships between species from subgenus *Polypaecilum*. *Aspergillus wentii* was selected as outgroup. Posterior probabilities (>0.95) / Bootstrap values (>80) are indicated above thickened branches (\* = 1.0/100). New species are shown in blue bold text. <sup>T</sup> = ex-type.



**Legend:**  

 House dust Food Mummy related Human Canine Air Soil Saline environments Historic parchment/paper Cave Plant material

**Fig. 8.** Neighbour joining tree based on ITS obtained from cultures used during this study, 454-pyrosequences and sequences obtained from GenBank (mostly from uncultured fungi) showing the relationships between species from subgenus *Polypaecilum*. *Aspergillus wentii* was selected as outgroup. Clades were collapsed to unproportional (with regards to sequence number) triangles. The number of sequences and their origins are indicated below species names.

**Table 3.** Species distribution in house dust detected in 454-pyrosequencing data collected during this study.

	<i>Aspergillus atacamensis</i>	<i>Aspergillus baarmensis</i>	<i>Aspergillus cf kalimae</i>	<i>Aspergillus insolitus</i>	<i>Aspergillus keratitidis</i>	<i>Aspergillus pisci</i>	<i>Aspergillus salinarum</i>	<i>Aspergillus sclerotiales</i>	<i>Aspergillus waynelawii</i>	<i>Aspergillus thailandensis</i>	<i>Aspergillus whitfieldii</i>	<i>Aspergillus kalimae</i>	Unknown clade 1	Unknown clade 2	Unknown clade 3
Total	24	30	24	2	802	12	18	2	750	70	86	72	56	4	8
Indonesia	1	1			53						4	12	27		
Indonesia House 2	1				23						4	7	27		
Indonesia House 3		1			17							5			
Indonesia House 4					8										
Indonesia House 5					5										
Mexico					261	1		1	355		3	2			
Mexico house 1					1						3				
Mexico house 2												1			
Mexico house 3					17										
Mexico house 4					32			1	354			1			
Mexico house 5					211	1			1						
Micronesia		14			56	1	9		19	25	19		1	2	
Micronesia 1					22						4				
Micronesia 2					10				1						
Micronesia 3					13				14		4				
Micronesia 4					7	1			1	24					
Micronesia 5					1				1						
Micronesia 7					1										
Micronesia 8		14			2		9		2	1	11		1	2	
New Zealand															4
New Zealand House 4															1
New Zealand House 6															3
Thailand	11		12	1	31	4			1	10	7	22			
Thailand House 1			1	1	7					4	4	2			
Thailand House 2	9		7			4				1		13			
Thailand House 3					4				1		1				
Thailand House 4					13							2			
Thailand House 6	1				5							5			
Thailand House 7	1		2		1					1	2				
Thailand House 9			2		1					4					
Uruguay												10			
Uruguay House 1												10			

moderately deep, plain, white; sporulation sparse; exudate absent; soluble pigment absent; reverse color pale to light yellow (3A3–4).

*Conidiophores* solitary phialides borne laterally or terminally on vegetative hyphae; *phialides* mono- to polyphialidic, hyaline, narrow, cylindrical, often peg-like, 2–21.5 × 1–3 µm; *conidia* borne solitary or commonly in small heads, hyaline, smooth or sometimes roughened, subglobose pyriform, obovoid or globose, sometimes with truncate base, 3–6 × 2.5–5 µm ( $\bar{x}$  = 3.8 ± 0.5 × 3.5 ± 0.5 µm), average width/length = 0.92, n = 91.

**Notes:** *Aspergillus kalimae* is closely related to *A. atacamensis* based on the ITS phylogeny. Like *A. atacamensis*, *A. kalimae* does not grow at 37 °C on MY10-12, exhibits optimal growth on

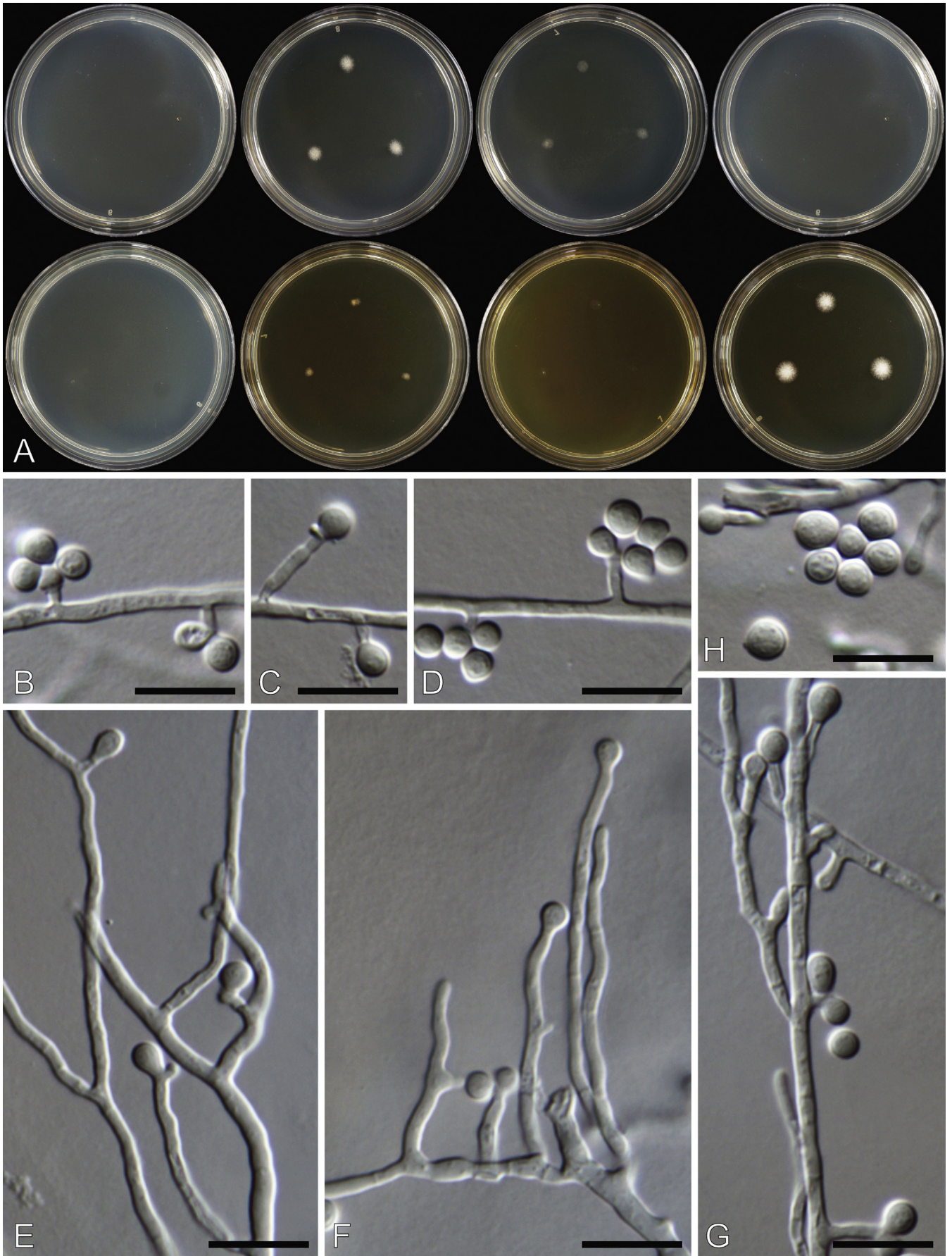
media amended with 10–15 % NaCl, and displays negligible growth (at most germination or microcolonies) on media lacking NaCl. *Aspergillus kalimae* is morphologically distinguished from *A. atacamensis* by its larger conidia (3.5–4.5 µm diam; [Martinelli et al. 2017](#)).

***Aspergillus noonimiae*** Tanney, Visagie & Seifert **sp. nov.** MycoBank MB822733. [Fig. 10](#).

**Etymology:** Latin, *noonimiae*, named after Dr. Paramee Noonim, a lecturer at Prince of Songkla University, Thailand who collected the dust samples from which the types of two species of subgenus *Polypaecilum* were isolated.

**Diagnosis:** Conidiophores solitary phialides, sometimes occurring in complex hyphal networks resembling branching





**Fig. 9.** *Aspergillus kalimae* (DAOMC 251762). **A.** Colonies from left to right: top row, MEA, MEA20NaCl, MEA40G, MEA 37 °C; bottom row, CYA, DG18, YES, MY10-12. **B–G.** Conidiophores. **H.** Conidia. Scale bars: B–H = 10 µm.



**Fig. 10.** *Aspergillus noonimiae* (DAOMC 251754). **A.** Colonies from left to right: top row, MEA, MEA20NaCl, MEA40G, MEA 37 °C; bottom row, CYA, DG18, YES, MY10-12. **B–H.** Conidiophores (polyphialides in F). **I.** Conidia. Scale bars: B–I = 10  $\mu$ m.

conidiophores, mono- to polyphialidic, sclerotia absent, growth at 37 °C, colonies produce a red soluble pigment.

*Typus: Thailand*, Sonkla, from house dust, 2009, collected by Paramee Noonim, isolated by Kalima Mwange and Ed Whitfield, (holotype DAOM 745797, culture ex-type DAOMC 251754 = UAMH 11836 = CBS 143382 = KAS 8125 = SLOAN 7955 = PN06TH-370).

*ITS barcode*: KY980641 (alternative markers: *BenA* = KY980569; *CaM* = KY980605; *RPB2* = KY980466; *TOP1* = KY980502; *TSR1* = KY980534).

*Colony diam (7 d, in mm)*: CYA 6–7; CYA15NaCl 7; CYA50S 9–10; MEA 37 °C 10–11; MEA 6; MEA25G 6; MEA30G 8; MEA35G 9–10; MEA40G 10; MEA5NaCl 11; MEA10NaCl 8; MEA15NaCl microcolonies; MEA20NaCl germination; DG18 7–8; YES 9–10; MY10-12 6–7.

*Colony characters (25 °C, 7 d)*: *CYA*: Colonies dense, moderately deep, lightly sulcate, brownish orange (5C3–6C3); sporulation dense; exudate clear; soluble pigment absent; reverse color brownish orange to brown (6C5–E6). *MEA*: Colonies dense, moderately deep, lightly sulcate, brownish orange (5C3–6C3), areas with reddish pink mycelia; sporulation dense; exudate clear; soluble pigment red to brownish red; reverse color dark brown to violet brown (8F8–10F8). *DG18*: Colonies moderately dense, low, lightly sulcate, white to orange to reddish grey (6B2–7B2); sporulation dense; exudate clear; soluble pigment brownish red, inconspicuous; reverse color dark brown to violet brown (8F8–10F8). *YES*: Colonies dense, moderately deep, sulcate, grey to orange grey to reddish grey (6B1–2–7B2); sporulation dense; exudate absent; soluble pigment brownish red, inconspicuous; reverse color dark brown to violet brown (8F8–10F8). *MY10-12*: Colonies dense, low, sulcate, white to orange to reddish grey (6B2–7B2); sporulation sparse; exudate absent; soluble pigment absent; reverse color dark brown to violet brown (8F8–10F8).

*Conidiophores* solitary phialides borne laterally or terminally on vegetative hyphae, sometimes occurring in complex hyphal networks resembling branched conidiophores; *phialides* mono- to polyphialidic, hyaline, cylindrical to ampulliform, sometimes peg-like, often curved irregularly, center swollen toward base or below midsection, neck broadly tapering towards apex, 3–21 × 1–4 µm; *conidia* borne solitary, in chains with pronounced connectors, or sometimes as small heads, often still attached to phialides by a membranous sheath, hyaline, smooth or roughened, subglobose, pyriform, obovoid or ovoid, sometimes with truncate base, young conidia sometimes appearing limoniform from connectives, 2.5–4.5 × 2.5–3.5 µm ( $\bar{x}$  = 3.1 ± 0.3 × 2.9 ± 0.3 µm), average width/length = 0.91, n = 110.

*Notes*: *Aspergillus noonimiae* is basal to a clade comprising *A. keratitidis*, *A. sclerotialis*, and *A. waynelawii*. It differs from species in this clade by more complexly-branched hyphal networks and by the production of a brownish red soluble pigment that becomes more conspicuous over time on especially on MEA and MEA20G. *Aspergillus noonimiae* grows faster on MEA at 37 °C versus 25 °C, is xerophilic, and exhibits good growth on MEA10NaCl.

***Aspergillus thailandensis* Tanney, Visagie & Seifert sp. nov.** MycoBank MB822734. Fig. 11.

*Etymology*: Latin, *thailandensis*, in reference to the origin of the house dust from which this species originates.

*Diagnosis*: Conidiophores solitary phialides, sometimes occurring in complex hyphal networks resembling branching conidiophores, mono- to polyphialidic, sclerotia absent, growth at 37 °C, fast growth on DG18 and YES.

*Typus: Thailand*, Surat, from house dust, 2009, collected by Paramee Noonim, isolated by Kalima Mwange and Ed Whitfield (holotype DAOM 745798, culture ex-type DAOMC 251755 = UAMH 11840 = CBS 143383 = KAS 8126 = SLOAN 4554 = PN10TH-749).

*Additional materials examined: Thailand*, Surat, from house dust, 2009, collected by Paramee Noonim, isolated by Kalima Mwange and Ed Whitfield, DAOMC 251756 = UAMH 11841 = KAS 8127 = SLOAN 4626 = PN10TH-797.

*ITS barcode*: KY980642 (alternative markers: *BenA* = KY980570; *CaM* = KY980606; *RPB2* = KY980467; *TOP1* = KY980503; *TSR1* = KY980535).

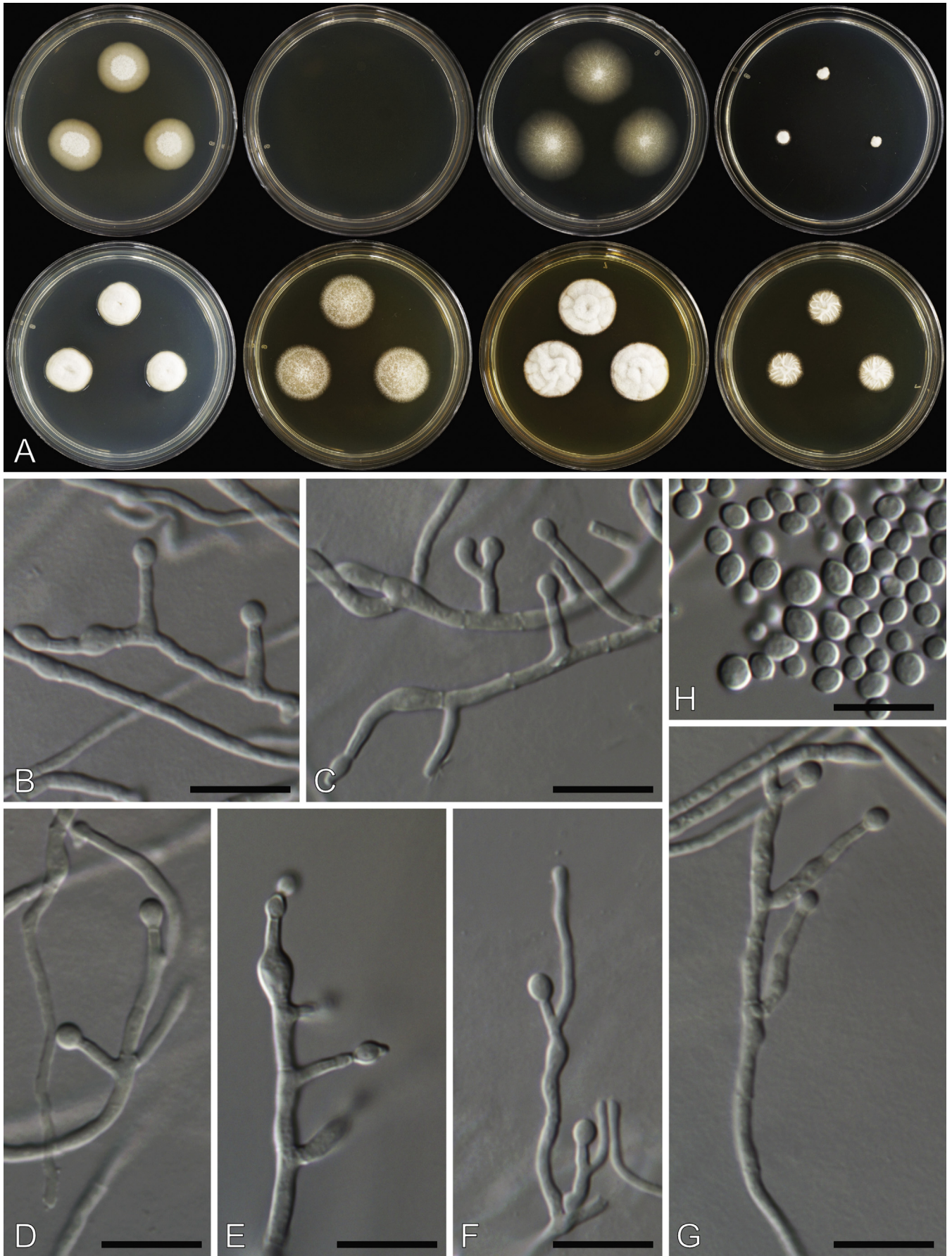
*Colony diam (7 d, in mm)*: CYA 11–12; CYA15NaCl 10–11; CYA50S 13–15; MEA 37 °C germination to 4; MEA 13–14; MEA25G 14; MEA30G 15–16; MEA35G 9–18; MEA40G 17–19; MEA5NaCl 15; MEA10NaCl 10–11; MEA15NaCl 5; MEA20NaCl germination; DG18 14–15; YES 14–16; MY10-12 9–10.

*Colony characters (25 °C, 7 d)*: *CYA*: Colonies dense, deep, sulcate, white; sporulation moderately dense; exudate absent; soluble pigment absent; reverse color yellowish white to pale yellow (3A2–3). *MEA*: Colonies moderately dense, moderately deep, plain, white; sporulation moderately dense; exudate absent; soluble pigment absent; reverse color yellowish white to pale yellow (3A2–3). *DG18*: Colonies moderately dense, low, plain, orange grey to greyish orange to brownish orange (5B2–3–C3); sporulation absent; exudate absent; soluble pigment absent; reverse color yellowish white to pale yellow (3A2–3A3). *YES*: Colonies dense, deep, sulcate, white to cream; sporulation sparse; exudate absent; soluble pigment absent; reverse color greyish orange (5B5–6), light yellow (4A4). *MY10-12*: Colonies dense, low, sulcate, white to brownish orange (5C3); sporulation sparse; exudate absent; soluble pigment absent; reverse color yellowish white to pale yellow (3A2–3).

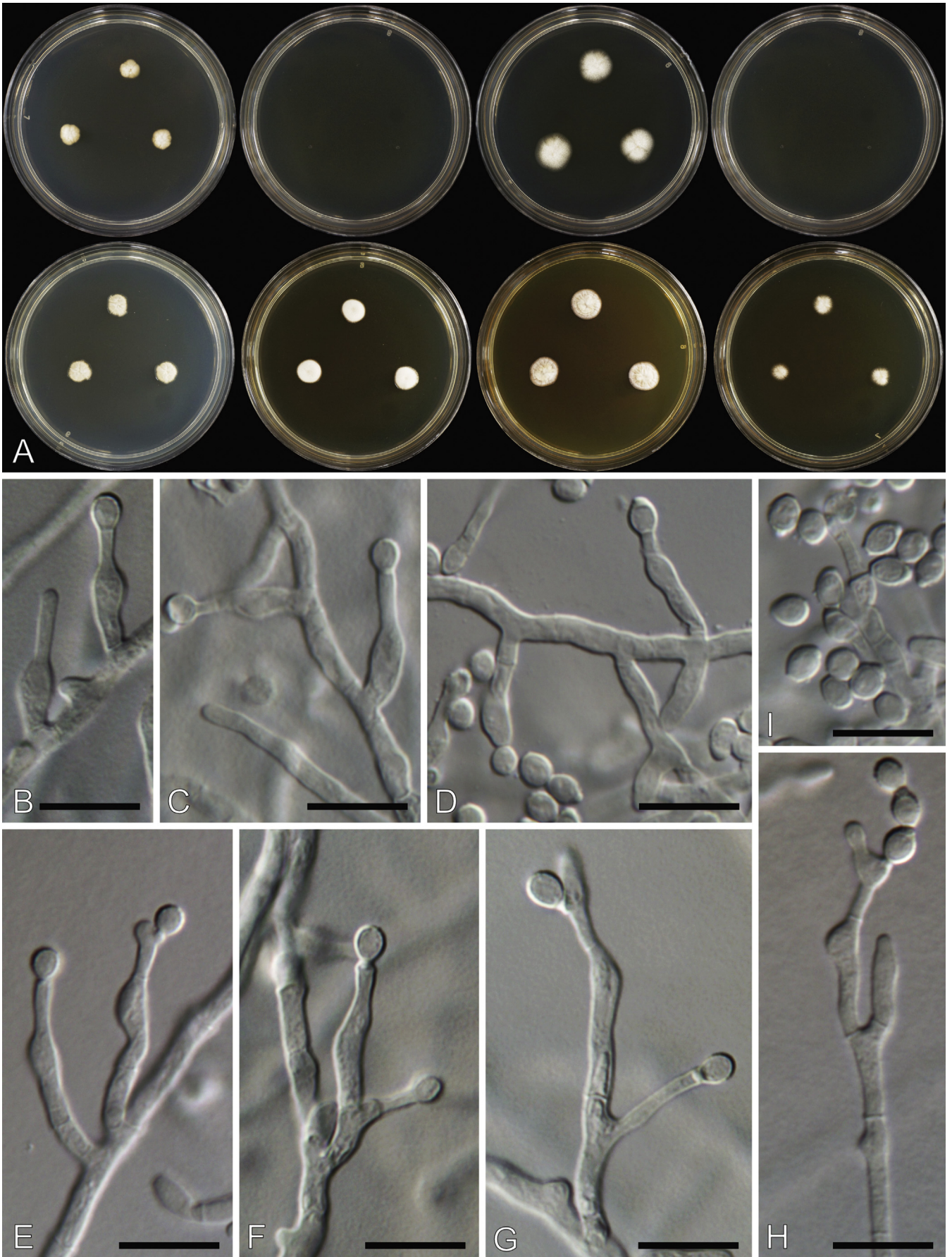
*Conidiophores* solitary phialides borne laterally or terminally on vegetative hyphae, sometimes occurring in hyphal networks resembling branched conidiophores; *phialides* mono- to polyphialidic, hyaline, cylindrical to lageniform, swollen at base or below midsection, neck cylindrical or broadly tapering towards apex, sometimes proliferating sympodially from necks, 2.5–12.5 × 1–2 µm; *conidia* borne in chains with pronounced connectors, hyaline, smooth or sometimes roughened, subglobose, pyriform, obovoid or ovoid with truncate base, 2–3 × 2–3 µm ( $\bar{x}$  = 2.6 ± 0.2 × 2.5 ± 0.2 µm), average width/length = 0.95, n = 91.

*Notes*: *Aspergillus thailandensis* is a basal lineage to the *A. keratitidis*–*A. noonimiae* clade and sister to the *A. insolitus*–*A. pisci* clade. The new species exhibits strong growth on DG18 and YES, some growth at 37 °C on MEA, is moderately halotolerant, and shows optimal growth on MEA35G and MEA40G.

***Aspergillus waynelawii* Tanney, Visagie & Seifert sp. nov.** MycoBank MB822735. Fig. 12.



**Fig. 11.** *Aspergillus thailandensis* (DAOMC 251755). **A.** Colonies from left to right: top row, MEA, MEA20NaCl, MEA20S, MEA 37 °C; bottom row, CYA, DG18, YES, MY10-12. **B–G.** Conidiophores (polyphialides in C, G). **H.** Conidia. Scale bars: B–H = 10 μm.



**Fig. 12.** *Aspergillus waynelawii* (DAOMC 251751 in A, H; DAOMC 251752 in B–G, I). **A.** Colonies from left to right: top row, MEA, MEA20NaCl, MEA40G, MEA 37 °C; bottom row, CYA, DG18, YES, MY10-12. **B–H.** Conidiophores (polyphialides in E, H). **I.** Conidia. Scale bars: B–I = 10 μm.

**Etymology:** Latin, *waynelawii*, named after Dr. Wayne Law, New York Botanic Garden, who collected the dust sample from which the ex-type culture was isolated.

**Diagnosis:** Conidiophores solitary phialides, sometimes occurring in hyphal networks resembling branching conidiophores, mono- to polyphialidic, conidia  $2.5\text{--}5.5 \times 2.5\text{--}4.5 \mu\text{m}$ , sclerotia absent, growth at  $37^\circ\text{C}$ .

**Typus:** **Micronesia**, Malem, from house dust, 2009, collected by Wayne Law, isolated by Kalima Mwange and Ed Whitfield (holotype DAOM 745796, culture ex-type DAOMC 251751 = UAMH 11926 = CBS 143384 = KAS 8123 = SLOAN 7951a = WL03MI-231).

**Additional materials examined:** **Micronesia**, Malem, from house dust, 2009, collected by Wayne Law, isolated by Kalima Mwange and Ed Whitfield, DAOMC 251752 = KAS 8124 = SLOAN 7951b = WL03MI-231b. **Thailand**, Bangkok, from house dust, 2009, collected by Paramee Noonim, isolated by Kalima Mwange and Ed Whitfield, DAOMC 251753 = UAMH 11843 = KAS 8122 = SLOAN 7943 = PN03TH-136.

**ITS barcode:** KY980639 (alternative markers: *BenA* = KY980567; *CaM* = KY980603; *RPB2* = KY980464; *TOP1* = KY980500; *TSR1* = KY980532).

**Colony diam (7 d, in mm):** CYA 5–6; CYA15NaCl 5–8; CYA50S 6–9; MEA  $37^\circ\text{C}$  microcolonies to 6; MEA 4–6; MEA25G 5–6; MEA30G 6–7; MEA35G 6–8; MEA40G 7–9; MEA5NaCl 8; MEA10NaCl 6–7; MEA15NaCl microcolonies to 4; MEA20NaCl germination; DG18 6–7; YES 5–8; MY10-12 3–5.

**Colony characters (25 °C, 7 d):** **CYA:** Colonies dense, moderately deep, sulcate, white; sporulation moderately dense; exudate absent; soluble pigment absent; reverse color brownish orange (5C5). **MEA:** Colonies dense, moderately deep, sulcate, white; sporulation moderately dense; exudate absent; soluble pigment absent; reverse color brownish orange (5C5). **DG18:** Colonies dense, moderately deep, sulcate, white; sporulation sparse; exudate absent; soluble pigment absent; reverse color light to greyish orange (5A5–B5). **YES:** Colonies dense, moderately deep, sulcate, white to brownish orange (5C3); sporulation dense; exudate absent; soluble pigment absent; reverse color brownish orange (5C5). **MY10-12:** Colonies dense, low, plain, white to brownish orange (5C3); sporulation moderately dense; exudate absent; soluble pigment absent; reverse color yellowish white to pale yellow (3A2–3).

**Conidiophores** solitary phialides borne laterally or terminally on vegetative hyphae, sometimes occurring in branched hyphal networks resembling branched conidiophores; **phialides** mono- to sometimes polyphialidic, cylindrical to ampuliform, sometimes curved irregularly, swollen toward base or above midsection, neck cylindrical or broadly tapering, sometimes extending sympodially from necks,  $3.5\text{--}23 \times 1.5\text{--}4 \mu\text{m}$ ; conidia borne solitary or in chains with pronounced connectors, hyaline, smooth or roughened, subglobose, pyriform, obovoid or ovoid with truncate base,  $2.5\text{--}5.5 \times 2.5\text{--}4.5 \mu\text{m}$  ( $\bar{x} = 3.4 \pm 0.5 \times 3 \pm 0.4 \mu\text{m}$ ), average width/length = 0.88,  $n = 76$ .

**Notes:** *Aspergillus waynelawii* is a moderately halotolerant species closely related to *A. keratitidis* and *A. sclerotialis*, distinguished by the absence of sclerotia and larger conidia than *A. keratitidis* ( $2.5\text{--}3.5 \mu\text{m}$ ; Hsieh et al. 2009) and wider, more globose conidia than *A. sclerotialis* ( $3\text{--}4.5 \times 1.5\text{--}2 \mu\text{m}$ ; Gams 1978). All species within this strongly supported clade grow at  $37^\circ\text{C}$ .

***Aspergillus whitfieldii* Tanney, Visagie & Seifert sp. nov.** MycoBank MB822736. Fig. 13.

**Etymology:** Latin, *whitfieldii*, named after Mr. Ed Whitfield, now an officer of the Toronto Police Service, who isolated thousands of strains of indoor moulds by dilution to extinction for this project.

**Diagnosis:** Conidiophores solitary phialides, sometimes occurring in complex hyphal networks resembling branching conidiophores, mono- to polyphialidic, sclerotia absent, no growth at  $37^\circ\text{C}$ , restricted growth on most media.

**Typus:** **Thailand**, Chumphon, from house dust, 2009, collected by Paramee Noonim, isolated by Kalima Mwange and Ed Whitfield (holotype DAOM 745799, culture ex-type DAOMC 251760 = UAMH 11842 = CBS 143385 = KAS 8129 = SLOAN 4178 = PN08TH-523).

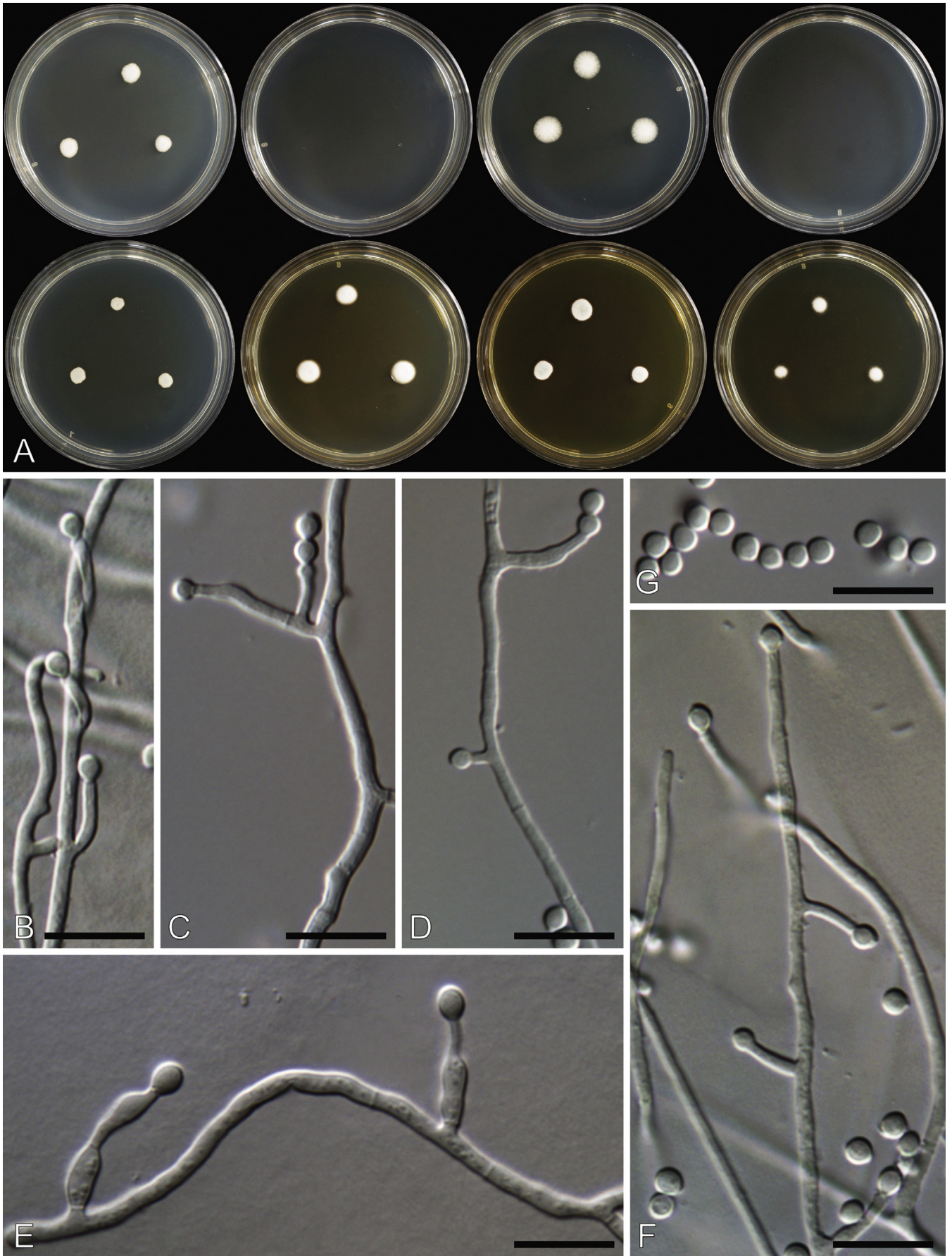
**Additional materials examined:** **Micronesia**, Malem, from house dust, 2009, collected by Wayne Law, isolated by Kalima Mwange and Ed Whitfield, DAOMC 251757 = KAS 8132 = SLOAN 7953 = WL03MI-246. **Micronesia**, Lelu, from house dust, 2009, collected by Wayne Law, isolated by Kalima Mwange and Ed Whitfield, DAOMC 251758 = UAMH 11846 = KAS 8134 = SLOAN 7961 = WL05MI-555. **Thailand**, Songkla, from house dust, 2009, collected by Paramee Noonim, isolated by Kalima Mwange and Ed Whitfield, DAOMC 251759 = KAS 8128 = SLOAN 4098 = PN07TH-381. **Thailand**, Surat Thani, from house dust, 2009, collected by Paramee Noonim, isolated by Kalima Mwange and Ed Whitfield, DAOMC 251761 = KAS 8130 = SLOAN 7602 = PN10TH-761.

**ITS barcode:** KY980645 (alternative markers: *BenA* = KY980573; *CaM* = KY980609; *RPB2* = KY980470; *TOP1* = KY980506; *TSR1* = KY980538).

**Colony diam (7 d, in mm):** CYA 3–4; CYA15NaCl 1–5; CYA50S 4–6; MEA  $37^\circ\text{C}$  no growth; MEA 4–5; MEA25G 4–5; MEA30G 5–6; MEA35G 6; MEA40G 6–8; MEA5NaCl 6–8; MEA10NaCl 4–5; MEA15NaCl microcolonies; MEA20NaCl no growth (microcolonies in KAS8128); DG18 6–8; YES 6–8; MY10-12 4–6.

**Colony characters (25 °C, 7 d):** **CYA:** Colonies dense, low, plain, white; sporulation sparse; exudate absent; soluble pigment absent; reverse color yellowish white (2A2). **MEA:** Colonies moderately dense, low, plain, white to greenish grey (28B2); sporulation moderately dense; exudate absent; soluble pigment absent; reverse color yellowish white (2A2). **DG18:** Colonies dense, moderately deep, plain, white; sporulation sparse; exudate absent; soluble pigment absent; reverse color yellowish white (2A2). **YES:** Colonies dense, moderately deep, plain, white to greenish grey (28B2); sporulation sparse; exudate absent; soluble pigment absent; reverse color greyish to brownish orange (5B6–C6). **MY10-12:** Colonies dense, low, plain, white; sporulation sparse; exudate absent; soluble pigment absent; reverse color yellowish white (2A2).

**Conidiophores** solitary phialides borne laterally or terminally on vegetative hyphae, sometimes occurring in hyphal networks resembling branched conidiophores; **phialides** mono- to polyphialidic, hyaline, cylindrical to lageniform, swollen toward base or below midsection, neck cylindrical or tapering towards apex, sometimes peg-like,  $4\text{--}36 \times 1\text{--}3 \mu\text{m}$ ; conidia borne solitary or in chains with pronounced connectors, hyaline, smooth or sometimes roughened, subglobose, pyriform, obovoid or ovoid with truncate base,  $2\text{--}3 \times 2\text{--}3 \mu\text{m}$  ( $\bar{x} = 2.6 \pm 0.2 \times 2.4 \pm 0.2 \mu\text{m}$ ), average width/length = 0.92,  $n = 138$ .



**Fig. 13.** *Aspergillus whitfieldii* (DAOMC 251760). **A.** Colonies from left to right: top row, MEA, MEA20NaCl, MEA40G, MEA 37 °C; bottom row, CYA, DG18, YES, MY10-12. **B–F.** Conidiophores. **G.** Conidia. Scale bars: B–G = 10  $\mu$ m.

Notes: The phylogenetic position of *A. whitfieldii* is inconclusive based on present phylogenies, which place it basal to either of the two major subgenus *Polypaecilum* clades. *Aspergillus whitfieldii* exhibits optimal growth on MEA amended with 5 % NaCl or 20–25 % glucose and does not grow at 37 °C.

## Accepted species in *Aspergillus* subgenus *Polypaecilum*

***Aspergillus atacamensis*** Zalar, Azúa-Bustos, Gunde-Cimerman, *Extremophiles* 21: 766, 2017. [MB818565]. — type: CBS H-23062. ex-type: EXF-6660 = CBS 142046. ITS barcode: KX900619 (alternative markers: *BenA* = n.a.; *CaM* = n.a.; *RPB2* = n.a.).

***Aspergillus baarnensis*** Samson, Visagie & Houbraken, *Stud. Mycol.* 78: 154, 2014 ≡ *Oospora halophila* J.F.H. Beyma, *ZentBl. Bakt. ParasitKde*, 2 88: 134, 1933 ≡ *Basipetospora halophile* (J.F.H. Beyma) Pitt & A.D. Hocking, *Mycotaxon* 22: 198, 1985 = *Scopulariopsis halophilica* Tubaki, *Trans. Mycol. Soc. Japan* 14: 367, 1973. [MB809579]. — type: CBS 232.32. ex-type: CBS 232.32 = VKM F-204. ITS barcode: KY980621 (alternative markers: *BenA* = KY980549; *CaM* = KY980585; *RPB2* = JN121509).

***Aspergillus caninus*** (Sigler *et al.*) Houbraken *et al.*, *Stud. Mycol.* 78: 154, 2014 ≡ *Phialosimplex caninus* Sigler *et al.*, *Med. Mycol.* 48: 338, 2010. [MB809580]. — type: UAMH 10337. ex-type: CBS 128032 = UAMH 10337. ITS barcode: GQ169315 (alternative markers: *BenA* = KY980546; *CaM* = KY980582; *RPB2* = JN121445).

***Aspergillus chlamydosporus*** (Gené & Guarro) Houbraken *et al.*, *Stud. Mycol.* 78: 155, 2014 ≡ *Sagenomella chlamydospora* Gené & Guarro, *J. Clin. Microbiol.* 41: 1723, 2003 ≡ *Phialosimplex chlamydosporus* (Gené & Guarro) Sigler, *Med. Mycol.* 48: 341, 2010. [MB809584]. — type: IMI 387422. ex-type: CBS 109945 = IMI 387422 = FMR 7371. ITS barcode: AJ519984 (alternative markers: *BenA* = KY980545; *CaM* = KY980581; *RPB2* = JN121425).

***Aspergillus insolitus*** (G. Sm.) Houbraken, Visagie & Samson, *Stud. Mycol.* 78: 155, 2014 ≡ *Polypaecilum insolitum* G. Sm., *Trans. Brit. Mycol. Soc.* 44: 437, 1961. [MB809591]. — type: CBS 384.61. ex-type: CBS 384.61 = ATCC 18164 = IFO 8788 = IMI 75202 = LSHB BB414 = MUCL 3078 = QM 7961. ITS barcode: KY980622 (alternative markers: *BenA* = KY980550; *CaM* = KY980586; *RPB2* = KY980447).

***Aspergillus kalimae*** Tanney, Visagie & Seifert (published here). [MB822732]. — type: DAOM 745800. ex-type: DAOMC 251762 = CBS 143506 = UAMH 11837 = KAS 8135 = SLOAN 4181 = PN08TH-526. ITS barcode: KY980650 (alternative markers: *BenA* = KY980578; *CaM* = KY980614; *RPB2* = KY980475).

***Aspergillus keratitidis*** (W.-L. Chen, Y.-M. Ju, H.-M. Hsieh, H.-Y. Lin & F.-R. Hu) Zalar & W.-L. Chen, *Extremophiles* 21: 762, 2017 ≡ *Sagenomella keratitidis* W.-L. Chen, Y.-M. Ju, H.-M. Hsieh, H.-Y. Lin & F.-R. Hu, *Botanical Studies* 50: 332, 2009 [MB818566]. — type: BCRC 34221. ex-type: BCRC 34221 = DTO 198-E8. ITS barcode: KY980616 (alternative markers: *BenA* = KY980544; *CaM* = KY980580; *RPB2* = KY980443).

***Aspergillus noonimiae*** Tanney, Visagie & Seifert (published here). [MB822733]. — type: DAOM 745797. ex-type:

DAOMC 251754 = CBS 143382 = UAMH 11836 = KAS 8125 = SLOAN 7955 = PN06TH-370. ITS barcode: KY980641 (alternative markers: *BenA* = KY980569; *CaM* = KY980605; *RPB2* = KY980466).

***Aspergillus pisci*** (A.D. Hocking & Pitt) Houbraken, Visagie & Samson, *Stud. Mycol.* 78: 155, 2014 ≡ *Polypaecilum pisci* A.D. Hocking & Pitt [as '*pisce*'], *Mycotaxon* 22: 200, 1985. [MB809594]. — type: FRR 2732. ex-type: FRR 2732 = ATCC 56982 = IMI 288726. ITS barcode: n.a (alternative markers: *BenA* = n.a.; *CaM* = n.a.; *RPB2* = JN121415).

***Aspergillus salinarus*** (Greiner, Peršoh, Weig & Rambold) Zalar & Greiner, *Extremophiles* 21: 762, 2017 ≡ *Phialosimplex salinarum* Greiner, Peršoh, Weig & Rambold, *IMA Fungus* 5: 166, 2014 [MB818567]. — type: CBS H-23061. ex-type: CBS142047 = EXF-10247. ITS barcode: KY980619 (alternative markers: *BenA* = KY980547; *CaM* = KY980583; *RPB2* = KY980445).

***Aspergillus salisburgensis*** Zalar, Martinelli & Piñar, *Extremophiles* 21: 762, 2017. [MB818564]. — type: CBS H-23061. ex-type: EXF-10247 = CBS 142047. ITS barcode: KX900623 (alternative markers: *BenA* = n.a.; *CaM* = n.a.; *RPB2* = n.a.).

***Aspergillus sclerotialis*** (W. Gams & Breton) Houbraken *et al.*, *Stud. Mycol.* 78: 157, 2014 ≡ *Sagenomella sclerotialis* W. Gams & Breton, *Persoonia* 10: 109, 1978 ≡ *Phialosimplex sclerotialis* (W. Gams & Breton) Sigler, *Med. Mycol.* 48: 341, 2010. [MB809596]. — type: CBS 366.77. ex-type: CBS 366.77 = IAM 14794. ITS barcode: KF267869 (alternative markers: *BenA* = KY980579; *CaM* = KY980615; *RPB2* = JN121505).

***Aspergillus thailandensis*** Tanney, Visagie & Seifert (published here). [MB822734]. — type: DAOM 745798. ex-type: DAOMC 251755 = CBS 143383 = UAMH 11840 = KAS 8126 = SLOAN 4554 = PN10TH-749. ITS barcode: KY980642 (alternative markers: *BenA* = KY980570; *CaM* = KY980606; *RPB2* = KY980467).

***Aspergillus waynelawii*** Tanney, Visagie & Seifert (published here). [MB822735]. — type: DAOM 745796. ex-type: DAOMC 251751 = CBS 143384 = UAMH 11926 = KAS 8123 = SLOAN 7951a = WL03MI-231. ITS barcode: KY980639 (alternative markers: *BenA* = KY980567; *CaM* = KY980603; *RPB2* = KY980464).

***Aspergillus whitfieldii*** Tanney, Visagie & Seifert (published here). [MB822736]. — type: DAOM 745799. ex-type: DAOMC 251760 = CBS 143385 = UAMH 11842 = KAS 8129 = SLOAN 4178 = PN08TH-523. ITS barcode: KY980645 (alternative markers: *BenA* = KY980573; *CaM* = KY980609; *RPB2* = KY980470).

## DISCUSSION

Pitt (1975) defined xerophilic fungi as those able to grow under at least one set of environmental conditions at  $a_w$  lower than 0.85. As a result, these fungi often require special media for culturing. Xerophilic *Aspergillus* species are common in the built environment, especially members of sections *Aspergillus* and *Restricti* (Samson *et al.* 2010, Chen *et al.* 2017, Sklenář *et al.* 2017, Visagie *et al.* 2017). One of our two primary isolation media was MEA20S, a reduced  $a_w$  medium that favors the isolation of



moderately xerotolerant species (Visagie et al. 2014, Hirooka et al. 2016), some of which conform with Pitt's (1975) definition as true xerophiles. The results presented demonstrate the presence of xerotolerant and halotolerant species of subgenus *Polypaecilum* from indoor dust, with seven species isolated. Additional species were also detected by pyrosequencing that were missed during culturing or were non-viable, most significantly from Mexican samples from which no subgenus *Polypaecilum* strains were isolated.

*Aspergillus* subgenus *Polypaecilum* is a morphologically-cohesive clade of species exhibiting halotolerance/halophily and xerotolerance/xerophily (Kocsubé et al. 2016, Martinelli et al. 2017). The phylogenetic position of subgenus *Polypaecilum* is not conclusively proven, but phylogenies typically resolve it as a close relative of subgenera *Aspergillus* and *Cremeri* with variable branch support (Houbraken & Samson 2011, Greiner et al. 2014, Houbraken et al. 2014, Samson et al. 2014, Kocsubé et al. 2016, Taylor et al. 2016). Recent phylogenetic analyses by Kocsubé et al. (2016) using six protein-coding gene sequences (*BenA*, *CaM*, *Cct8*, *RPB1*, *RPB2*, and *Tsr1*) placed subgenus *Polypaecilum* sister to subgenus *Aspergillus* with weak to moderate support. The inclusion of rDNA loci (ITS and the more conserved 18S and 28S ribosomal rDNA) to this dataset resulted in the more strongly supported placement of subgenus *Polypaecilum* sister to subgenus *Cremeri*, although the ML bootstrap support values were more conservative than the Bayesian posterior probabilities. Overall, the current data support the placement of subgenus *Polypaecilum* within a basal *Aspergillus* clade including subgenera *Aspergillus* and *Cremeri*. This basal clade is characterized by species that exhibit good growth at low  $a_w$  and halotolerance or halophily (Peterson 1995, Hubka et al. 2013, Kocsubé et al. 2016). An eventual whole genome-based phylogenetic approach might provide stronger evidence supporting the definitive placement of subgenus *Polypaecilum* within or outside of *Aspergillus*.

In contrast to the vesiculate conidiophores more typical of the classical generic concept of *Aspergillus*, conidiophores of species of subgenus *Polypaecilum* are typically reduced to solitary phialides or are sparsely branched. Reduced or aberrant conidiophores are also seen in related subgenus *Aspergillus*, including section *Restricti*, some species of subgenus *Cremeri* such as *A. brunneo-uniseriatus* and *A. inflatus*, and more distantly related sections *Candidi* and *Versicolores*; in all these cases, characteristic vesiculate conidiophores co-occur. No asexual state is reported for *A. arxii* (subgenus *Cremeri*), which is only known by its cleistothecial sexual state (Fort & Guarro 1984). Prior to the phylogenetic relationship with *Aspergillus* revealed by DNA sequencing, the reduced conidiophore morphologies of many species now included in subgenus *Polypaecilum* resulted in their classification as species in genera such as *Basipetospora*, *Phialosimplex*, *Polypaecilum* and *Sagenomella*. This deviating conidiophore morphology and uncertain phylogenetic placement as one of the basal lineages in *Aspergillus* added to the controversy of including these general species in *Aspergillus* (Samson et al. 2014, Pitt & Taylor 2014, Taylor et al. 2016). However, similarly reduced, monophialidic asexual morphs also occur in other genera of *Eurotiomycetes*, such as *Penicillium* and *Talaromyces* (Visagie et al. 2014, Visagie et al. 2016, Yilmaz et al. 2014). Similarly, in *Sordariomycetes*, *Hypocreales*, monophialidic species ascribed to the traditional morphological concept of *Acremonium* are widely dispersed among more differentiated asexual morphs

(Summerbell et al. 2011). Thus, the occurrence of asexual morphs with solitary or reduced phialides is a frequent phylogenetic pattern, and not helpful for classification in the absence of supporting characters.

*Aspergillus* subgenus *Polypaecilum* is also sometimes distinguished by polyphialides, most striking in *A. insolitus* and *A. pisci* but also observed in *A. caninus*, *A. chlamydosporus*, *A. keratitidis*, *A. noonimiae*, *A. thailandensis*, *A. waynelawii*, and *A. whitfieldii*. *Aspergillus insolitus* and *A. pisci* form a strongly supported clade and are distinguished from other species of subgenus *Polypaecilum* by their large and more complexly branched polyphialides. Polyphialides are rare in *Aspergillus*; only *A. cejpai* ( $\equiv$  *Talaromyces cejpai*  $\equiv$  *Dichotomomyces cejpai* with an asexual morph very similar to *A. insolitus*), classified in subgenus *Fumigati*, exhibits polyphialides outside of the subgenus *Polypaecilum*.

Conidia of subgenus *Polypaecilum* species are pyriform to globose, smooth to roughened, and borne singly, in heads, or in chains. Gams (1978) described a conspicuous refringent cone present on conidia of *A. sclerotialis*, resulting from the collapse of the apical connector. This refringent cone was obvious in conidia of *A. baarnensis*, *A. keratitidis*, *A. noonimiae*, *A. thailandensis*, *A. waynelawii*, and *A. whitfieldii*. Conidial chains of some species of subgenus *Polypaecilum* also adhered within a persistent membranous sheath visible by SEM and light microscopy. This sheath was conspicuous and commonly observed in *A. keratitidis* strains (Fig. 2), but was also observed less frequently for *A. noonimiae*, *A. sclerotialis*, *A. waynelawii*, and *A. whitfieldii*. The sheath appeared continuous from the phialide to the apical conidium, and often tapered toward connectors. SEM showed the electron-opaque sheath enveloping roughened conidia, suggesting ornamentation is not a result of the tearing and adherence of the sheath to the conidia. This is an unusual character. Persistent conidial chains of *Ceratocystis adiposa* (Ceratocystidaceae, Microascales) are also enveloped in membranous sheaths, which extend into the neck of the conidiogenous cell between the outer conidial wall and the wall of the conidiogenous cell (Hawes & Beckett 1977a, b). However, the sheath of *A. keratitidis* appears to be continuous with the outer wall of the phialide, although additional investigation is required to determine its origin and nature. The presence of a conidial chain sheath appears to be unreported in *Aspergillus* (but compare with the prominent connectors and apical cones of *A. brunneo-uniseriatus*; Singh & Bakshi 1961).

Some *A. keratitidis* strains produced abundant sclerotia submerged within or on the surface of agar, which was not reported by Hsieh et al. (2009). Sclerotia consisted of isodiametric cells surrounded by a layer or rind of thick-walled, compressed isodiametric cells. Similar sclerotia were described for *A. sclerotialis* by Gams (1978). The lack of observed sexual reproduction in subgenus *Polypaecilum* and its relationship with the homothallic subgenus *Aspergillus* suggests that these sclerotia might be immature ascomata that eventually may bear ascospores. Evidence of asci or ascospores was not detected using light or scanning electron microscopy, even in very old cultures. However, some *Aspergillus* species in section *Flavi* produce what were once considered strictly sclerotia, and were eventually coaxed to develop into mature ascomata after successful mating type crosses and manipulation of growth conditions (e.g.: Horn et al. 2009, 2011, 2013, 2014). Characterizing mating types and subsequent crosses on various media may eventually induce the formation of ascomata by species in

subgenus *Polypaecilum*. Alternatively, these structures may represent aborted or neotenous ascospores that function as sclerotia and do not mature into sexual reproductive structures. The production of sclerotia probably assists in the long-term persistence and dispersal of species of subgenus *Polypaecilum* in adverse environments and might explain why *A. keratitidis* was commonly isolated from the dust samples.

Species of subgenus *Polypaecilum* are associated with mycoses in humans and other animals. *Aspergillus chlamydosporus* was first discovered when an unknown fungus was isolated as the causal agent of a fatal disseminated mycosis in a five-year-old German shepherd mixed-breed dog (García & Blanco 2000). The fungus, initially considered a *Paecilomyces* species, was later described as *Sagenomella chlamydospora*, based on morphology (Gené et al. 2003). A later phylogenetic investigation using ITS and 18S sequences of the genus *Sagenomella* demonstrated that it was polyphyletic within *Trichocomaceae*, resulting in the introduction of the genus *Phialosimplex* to accommodate a clade including the canine pathogens now known as *A. chlamydosporus* and *A. caninus*. The ex-type strain of *A. insolitus* (CBS 384.61), originally described as *Polypaecilum insolitum*, was isolated from a human ear (Smith 1961). *Aspergillus insolitus* is implicated in additional cases of otomycosis and onychomycosis, as well as a report of an isolate from a female patient's lung tumour (Smith 1961, Yamashita 1972, Yamashita & Yamashita 1972, Piontelli et al. 1989, Listemann & Samson 1994). Based on a culture-independent study of sputum samples, subgenus *Polypaecilum* (as "*Phialosimplex*") was a dominant component of the lung microbial community in patients with severe chronic obstructive pulmonary disease (Su et al. 2015).

*Aspergillus keratitidis* was originally described from strains isolated on potato dextrose yeast agar from corneal scrapings, contact lenses, and contact lens storage solution associated with a case of bilateral keratitis (Hsieh et al. 2009). To our knowledge, *A. keratitidis* was known only from this original case, yet in the current study it was the most frequently isolated species and was also detected at high levels by pyrosequencing in Indonesia, Mexico, Micronesia, and Thailand. The prevalence of species of subgenus *Polypaecilum* in the built environment is of some concern as house dust may serve as an inoculum source for opportunistic infections. This is probably not a recent phenomenon and despite their apparent predominance in human habitats, they have not been implicated in widespread mycoses. However, the reduced conidiophore morphology of species of subgenus *Polypaecilum* could have led to the misidentification of past clinical isolates as *Paecilomyces*, *Sagenomella*, or *Scopulariopsis*. It is therefore conceivable that species of subgenus *Polypaecilum* may have been the causal agents of mycoses previously attributed to other taxa, for example *Monocillium indicum* by Mackie et al. (2004, Sigler et al. 2010).

Some species of subgenus *Polypaecilum* seem to be associated with birds; for example, the isolations of *A. atacamensis* from cave soil beneath an ancient guano deposit in the Chilean Atacama Desert and *A. insolitus* from chicken litter (Riegel et al. 1996, Martinelli et al. 2017). Chen et al. (2015) reported an unidentified species of subgenus *Polypaecilum* ("*Phialosimplex* sp.") isolated from edible bird nests in Malaysia using Sabouraud dextrose agar. A culture-independent study found that *A. sclerotialis* accounted for 37 % of the fungi present in a bio-aerosol sample of a broiler chicken confinement facility, at an estimated concentration of 698 cells/m<sup>3</sup> (Nonnenmann et al.

2010). De Hoog et al. (2005) observed that orders including both xerophilic and opportunistic pathogenic species strongly coincided, yet at the species level xerophily and pathogenicity seemed to be mutually exclusive. Many mammalian fungal pathogens, such as *Cryptococcus neoformans*, *A. fumigatus*, and especially species of *Onygenales* (e.g.: *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*), are associated with guano (McDonough et al. 1961, Ajello 1964, Wicklow 1968, Sarosi & Serstock 1976, Krutzsch & Watson 1978), an ammonia-rich and high-salt substrate (Hogg & Morton 1983, Sims & Wolf 1994). Baumgardner (2009) suggested that the ability of *Blastomyces dermatitidis* to grow in ammonia-rich, organic carbon-deficient microenvironments may be important to its competitive success; some degree of halotolerance is probably intrinsic for survival in ammonia-rich microenvironments high in nitrogen salts. The association and occurrence of species of subgenus *Polypaecilum* with birds and ammonia-rich microenvironments could therefore be investigated by isolating from respective substrates with selective media amended with salt or ammonia. Molecular diagnostic methods combined with better species concepts and associated DNA barcodes will provide a better understanding of how widespread and common subgenus *Polypaecilum* is in natural and built environments and its significance and occurrence as a pathogen.

Despite being completely absent from our isolates obtained using D2E, pyrosequencing yielded four species of subgenus *Polypaecilum* in varying abundance from Mexican indoor dust: *Aspergillus waynelawii* (355 reads), *A. keratitidis* (261 reads), *A. whitfieldii* (3 reads), and *A. sclerotialis* (1 read). Discrepancies between culture-dependent and culture-independent methods can be attributed to inherent biases for each method, for example, media selection and likelihood of isolating rare or recalcitrant species, sample storage and handling, primer bias, and selective amplification (Bridge & Spooner 2001, Allen et al. 2003, Anderson & Cairney 2004, Tedersoo et al. 2010, U'Ren et al. 2014). The labor-intensive nature of culture-dependent methods also makes reaching the asymptote of the species accumulation curve prohibitive. Culture-dependent methods select for viable cells while most studies employing culture-independent methods, such as the current one, do not discriminate between DNA of living or dead cells (but see Nocker et al. 2007, Vesper et al. 2008). The absence of strains of subgenus *Polypaecilum* from Mexican house dust is puzzling, yet probably a result of inadequate culture numbers, a suboptimal isolation technique, or the occurrence of dead versus living propagules in our samples at the time of collection, or some unknown variable occurring between the time the samples were processed for pyrosequencing and when they were processed for D2E.

ITS sufficiently delineates all known species of subgenus *Polypaecilum*, in contrast to the insufficient interspecific ITS variation typically observed in for instance subgenus *Aspergillus*, sections *Flavi* and *Nigri* (Samson et al. 2014). The exception was in the *A. kalimae* and *A. atacamensis* clade (Fig. 8), where clades became difficult to interpret once 454-pyrosequences were added. Therefore, ITS barcodes derived from environmental nucleic acid sequences (ENAS) are probably sufficient for delineating and identifying species of subgenus *Polypaecilum* based on currently understood species concepts and boundaries. Pyrosequencing detected four clades representing putative novel species (Fig. 8). "Unknown clade 1" comprises sequences from this study (Indonesia and Micronesia) and clones from two other studies (KC535132, Piñar et al. 2013; KP828179 and

KP828193, Piñar *et al.* 2015). Some authors propose the description of novel species based solely on ENAS (Hibbett & Taylor 2013, Hibbett *et al.* 2016), a controversial practice recently tested by the description of *Hawksworthiomyces sequentia* with a sequence designated as the holotype (De Beer *et al.* 2016). The description of novel taxa known only from ENAS is currently prohibited by the International Code of Nomenclature for algae, fungi and plants, but could provide a way to meaningfully incorporate the vast number of ENAS into taxonomic treatments and communicate species concepts, if implemented carefully and practiced with discretion. According to the example presented by De Beer *et al.* (2016), the description of “unknown clade 1” as a novel *Aspergillus* species is warranted because this clade is represented by sequences derived from separate studies and is phylogenetically distinct from known species of subgenus *Polypaecilum*. Given the robust taxonomic framework and sequence coverage of *Aspergillus* in general, and for subgenus *Polypaecilum* in particular, the argument can be confidently made that unknown clade 1 is a novel species based on its phylogenetic distance from neighbouring species. Given the issues with the legitimacy of such a description, we chose not to formally describe this clade but are confident this species could soon be isolated, especially given the recent description of species of subgenus *Polypaecilum* from hypersaline environments using high-salt media (Greiner *et al.* 2014, Martinelli *et al.* 2017). For example, an unknown subgenus species of *Polypaecilum* detected from mummies (Piñar *et al.* 2013), old parchment, and da Vinci's self portrait (Piñar *et al.* 2015) by uncultured clones was soon described as *A. atacamensis* from cave soil isolates by Martinelli *et al.* (2017). Isolation methods using media amended with high concentrations of salt and other osmolytes will invariably capture more species diversity in subgenus *Polypaecilum* and lead to the description of novel species.

Propagules of fungi with narrow host, substrate, or environmental requirements, such as obligate plant biotrophs (e.g.: rusts, powdery mildews, ectomycorrhiza), are effectively stranded indoors. The detection of a fungus in the built environment does not necessarily indicate its ability to grow in this niche, i.e.: indoor dust may serve as a bank of dormant, transient propagules derived from the phylloplane, pedosphere, and the dermatoplane (Scott *et al.* 2011). DNA-based surveys also detect dead cells. Therefore, although the detected indoor and outdoor fungal biodiversity might appear to overlap when present or absent taxa are compared (e.g.: Adams *et al.* 2015), fungi capable of sustained growth and reproduction are more significant for occupant health and indoor ecology than transient or dead spores. In this study, indoor dust samples were not assessed microscopically for active fungal growth before processing. However, we hypothesize that species of subgenus *Polypaecilum* are capable of growth and reproduction on dust substrates, and do not just represent transient propagules originating outdoors.

Indoor dust is a heterogeneous mixture of organic and inorganic particles composed from outdoor sources (e.g.: fungi, pollen, minerals, metal oxides, salt), occupants and occupant activities (e.g.: hair, skin flakes, nail clippings, textiles, associated microbiome), and building materials and contents (e.g.: glass, cellulose, plant and insect parts) (Macher 2001, Rintala *et al.* 2012); it is a unique substrate distinct from outdoor dust (Rasmussen *et al.* 2001). Indoor dust can provide a source of nutrients to fungi, house dust mites, and other microorganisms and may also provide a favourable microclimate to xerophiles,

e.g.: the hygroscopic nature of indoor dust and the elevated relative humidity (RH) of floors versus ambient air (e.g.: 20 % higher; Jo & Sohn 2007). As a substrate, indoor dust is amenable to growth and reproduction by fungi capable of exploiting such a resource, for example xerophilic *Aspergillus* and *Wallemia* species above 80 % equilibrium relative humidity (ERH) levels (Korpi *et al.* 1997, Pasanen *et al.* 1997, Dannemiller *et al.* 2017). Xerophiles are prevalent and undoubtedly amplifying within the built environment, as evinced by the constant association of xerophiles with some indoor substrates, e.g.: dust, mattresses, and carpets, and indicators suggesting frequent occupant exposure, e.g.: 36 % of patient sera showing sensitization to *Wallemia sebi* allergens (Beguin & Nolard 1994, Beguin 1995, Desroches *et al.* 2014).

Thus, the abundance of xerophilic species of subgenus *Polypaecilum* in indoor dust is not unexpected. Most species in the subgenus are described from strains isolated from xeric or hypersaline habitats, such as a cave in the Atacama Desert (*A. atacamensis*), dried salted fish (*A. pisci*), salt mine brine (*A. salinarus*), and even salt crystals (*A. baamensis*). Species of subgenus *Polypaecilum* typically exhibit relatively slow growth and a range of xerophily or halophily. Hence the absence of species of subgenus *Polypaecilum* isolated from the built environment in previous studies is probably a consequence of the common use of high  $a_w$  media or media lacking high salt concentrations, which select against xerophilic or halophilic species. Sequences of species of subgenus *Polypaecilum* were also detected in the built environment by recent studies using culture-independent methods, for example from decaying conifer wood in a Japanese home (Horisawa *et al.* 2017), house air and dust in Kansas City (KF800155, KF800467, KF800547; Rittenour *et al.* 2014), and mummies in the Capuchin Catacombs (Piñar *et al.* 2013). Degradation and enzymatic profile studies show differing amylolytic, cellulolytic, chitinolytic, keratinolytic, lypolytic, and proteolytic capabilities by various species of subgenus *Polypaecilum* (Pangallo *et al.* 2013, Greiner *et al.* 2014, Martinelli *et al.* 2017). Therefore, species of subgenus *Polypaecilum* can variously utilize major components of indoor dust, suggesting potential niche partitioning.

We isolated or detected species of subgenus *Polypaecilum* from buildings predominantly in subtropical or tropical climates (e.g. Hawaii, Indonesia, Mexico, Micronesia, Thailand, and Uruguay). It is noteworthy that all five novel species described here were isolated from Thailand. Rijckaert *et al.* (1981) reported a statistically significant increase in xerophilic *A. penicillioides*, *A. restrictus*, and *Wallemia sebi* spores in house dust from tropical climates versus maritime temperate and Mediterranean climates. Subgenus *Polypaecilum* strains were also associated with buildings in close proximity to oceans or seas, for example, the abundance of strains isolated from buildings within <100–700 m from the coast in Kosrae, Micronesia, the detection of >200 *Polypaecilum* reads from a shop within 400 m from the coast in Sayulita, Mexico, and isolation of *A. baamensis* from Hawaii. An unknown species (“unknown clade 3”, Fig. 8) was also detected by pyrosequencing from two residential buildings within 250–500 m from the coast in Dunedin, New Zealand. Other halotolerant and xerophilic species were found co-occurring with subgenus *Polypaecilum* in the same samples investigated in this present study. *Wallemia mellicola*, isolated from the indoor dust samples from Uruguay, Indonesia, and Micronesia, and *W. tropicalis*, isolated from the Micronesian dust, are halophilic and xerophilic species showing growth on a minimum  $a_w$  of 0.78 (28 % NaCl, 13 % MgCl<sub>2</sub>) (Jančić *et al.* 2015).

The genus *Sigleria* was described by Hirooka *et al.* (2016) for two moderately xerotolerant species, *S. amend* and *S. carmichaelii*, isolated from the Mexican, Micronesian, and Thai dust samples. They also described the moderately xerotolerant species *Spiromastix frutex*, *S. kosraensis*, and *S. minimus* from the dust samples originating from Mexico, Micronesia, and New Zealand. Tanney *et al.* (2015) reported the isolation of the obscure xerophile *Diploöspora rosea* from Micronesian house dust, a species capable of growth on MEA amended with 60 % sucrose (ca.  $a_w = 0.78$ ), which was also associated with dust mites, historical artifacts in an archival environment, and marine environments (Pinzari *et al.* 2012). Halotolerance is indicative of subgenus *Polypaecilum*, for example the isolation of species from hypersaline habitats (*A. atacamensis*, *A. baarnensis*, *A. insolitus*, *A. pisci*, *A. salinarus*, and *A. salisburgensis*) and varying halotolerance exhibited by all other species. Indoor fungal communities are strongly influenced by local outdoor fungal biodiversity (Adams *et al.* 2013b); therefore, halotolerant species occurring indoors probably originate from local habitats with elevated salt concentration environments (e.g.: maritime habitats). It is conceivable that salt content of indoor dust is higher in buildings close to oceans (Fergusson & Kim 1991), thus providing a habitat for endemic halotolerant fungi capable of exploiting this niche, such as species of subgenus *Polypaecilum*. Alternatively, propagules of halotolerant fungi might be transient or at a dead end in the built environment, instead originating strictly from outdoor sources. For example, recalcitrant sclerotia produced by *A. keratitidis* growing outdoors might be passively transported indoors, where they viably persist for an extended period of time, resulting in its frequent detection by D2E.

The built environment is an important human-microbe interface, where human activities knowingly or inadvertently select for the establishment and proliferation of some fungi over others (e.g.: Andersen *et al.* 2017). While the presence of xerophiles indoors is well established, their ecology and biodiversity is not sufficiently characterized. A better understanding of fungal ecology, biodiversity and species concepts, and improved barcoding abilities (e.g.: Abarenkov *et al.* 2016), will ameliorate the current resolution of ENAS studies, facilitate source tracking of organisms in the built environment, and progress our knowledge of interactions between occupants and fungi. In this study, we provide additional evidence showing the abundance of xerophiles indoors, notably a clade containing potentially halotolerant and zoopathogenic species. In this study, we expanded the number of known species in the recently described *Aspergillus* subgenus *Polypaecilum* from 10 to 15: *A. atacamensis*, *A. baarnensis*, *A. caninus*, *A. chlamydosporus*, *A. insolitus*, *A. kalimae*, *A. keratitidis*, *A. noonimiae*, *A. pisci*, *A. salinarus*, *A. salisburgensis*, *A. sclerotialis*, *A. thailandensis*, *A. waynelawii* and *A. whitfieldii*, and also increased the number of extant strains from about 20 to several hundred. Evidence of undescribed species within this clade provides compelling incentive to accelerate culture-based efforts from hypersaline, xeric, and built environments using appropriate growth media and techniques such as D2E.

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