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ARTICLE

A new family and genus in *Dothideales* for *Aureobasidium*-like species isolated from house dust

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Abstract: An international survey of house dust collected from eleven countries using a modified dilution-to-extinction method yielded 7904 isolates. Of these, six strains morphologically resembled the asexual morphs of *Aureobasidium* and *Hormonema* (sexual morphs ?*Sydowia),* but were phylogenetically distinct. A 28S rDNA phylogeny resolved strains as a distinct clade in *Dothideales* with families *Aureobasidiaceae* and *Dothideaceae* their closest relatives. Further analyses based on the ITS rDNA region, β-tubulin, 28S rDNA, and RNA polymerase II second largest subunit confirmed the distinct status of this clade and divided strains among two consistent subclades. As a result, we introduce a new genus and two new species as *Zalaria alba* and *Z. obscura*, and a new family to accommodate them in *Dothideales*. *Zalaria* is a black yeast-like fungus, grows restrictedly and produces conidiogenous cells with holoblastic synchronous or percurrent conidiation. *Zalaria* microscopically closely resembles *Hormonema* by having only one to two loci per conidiogenous cell, but species of our new genus generally has more restricted growth. Comparing the two species, *Z. obscura* grows faster on lower water activity (a_w) media and produces much darker colonies than *Z. alba* after 7 d. Their sexual states, if extant, are unknown.

Key words:
18S
28S
BenA
black yeast
Dothidiomycetes
ITS
RPB2
xerotolerant fungi
Zalaria

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INTRODUCTION

The average person in industrialized countries spends approximately 90 % of their time indoors (Höppe & Martinac 1998). This makes the indoor environment one of the most important human-fungal interfaces. We are constantly exposed to fungal spores, fragments, and metabolites and their impact ranges from human health (Piecková & Jesenská 1999) as pathogens (De Hoog *et al*. 2014, Garber 2001) or allergens (Aimanianda *et al*. 2009, Karvala *et al*. 2011, Tanno *et al*. 2016) to food spoilage (Pitt & Hocking 2009, Samson *et al*. 2010) or damage to building materials (Flannigan & Miller 2011). Although indoor environments are not generally recognized as extreme environments, microclimates such as dishwashers contain relatively high water activity (a_w) coupled with high temperatures (Zalar *et al.* 2011), and building materials like plaster, drywall, and cement have very low a_w (Flannigan & Miller 2011), while plastics like polyvinyl chloride (PVC, used in construction) offer little in the way of available carbon but still support oligotrophic fungi (Webb *et al.* 2000). Studying the indoor mycobiota is therefore important to better understand these interactions and how they may affect us.

The black yeast *Aureobasidium pullulans* (*Dothideales*) is recorded from a wide variety of sources, including environments with significant osmotic stress, such as hypersaline waters in salterns (Gunde-Cimerman *et al*. 2000), bathrooms, food, and feeds (Samson *et al*. 2010), water-damaged wood (Andersen *et al.* 2011), and polythermal glaciers (Zalar *et al*. 2008). In surveys of the indoor environment, *A. pullulans* is one of the most abundant and widespread fungi reported (Adams *et al*. 2013, Amend *et al*. 2010, Nonneman *et al.* 2012, Van Nieuwenhuijzen *et al.* 2016). The morphospecies exhibits a high degree of phenotypic plasticity (Slepecky & Starmer 2009) and strains can have significantly different pigmentation (Yurlova *et al*. 1995, Zalar *et al*. 2008). While this high degree of variation may contribute to its unique adaptability (Gostinčar *et al*. 2014), it also makes definitive morphological identification challenging, and the many ITS variants identified as this species in GenBank are unlikely to represent one species. Reports of *A. pullulans* being one of the most abundant members in fungal communities based on near-neighbour analyses of next-generation sequencing (NGS) data may be skewed to some extent.

The class *Dothideomycetes* is the largest in *Ascomycota* and was recently examined and re-defined using multigene phylogenetics (Hyde *et al.* 2013, Schoch *et al.* 2009, Thambugala *et al*. 2014). These studies showed the order *Dothideales* to be a monophyletic sister to *Myriangiales*.

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Thambugala *et al*. (2014) reviewed and re-evaluated the morphologically-based taxonomy of *Dothideales* known from culture, informed by a combined phylogeny of 28S rDNA, 18S rDNA and ITS. They accepted two families, synonymising the often-accepted *Dothioraceae* (e.g. Barr 2001) with *Dothideaceae,* as first proposed by Von Arx & Müller (1975), and introducing *Aureobasidiaceae* for *Aureobasidium* and closely related genera. The polythetic morphological definitions provided by Thambugala *et al.* (2014) did not identify unique diagnostic characters among the sexual or asexual morphs in either family. Both families include a poorly integrated mixture of genera known from fungarium specimens with others that are mostly known from culture. For both families, sexually and asexually typified genera were keyed out separately. Sexually typified genera were separated by characters of the stromata, number of ascospores per ascus, and ascospore characters such as septation and pigmentation. In *Dothideales*, the characters normally used to classify asexual morphs were clearly phylogenetically uninformative, and mixtures of black yeast, hyphomycetous, coelomycetous, and intermediate asexual morphs are scattered over the various clades of *Aureobasidiaceae* and *Dothideaceae.* Both families include a variety of asexual morphs, including sporodochial hyphomycete forms usually observed in nature (e.g. *Kabatiella, Kabatina*), coelomycete (e.g. *Endoconidioma, Neocylindroseptoria, Rhizosphaera*), and black yeast-like forms usually observed in culture.

Black yeasts have a confused taxonomic history that may eventually be clarified with the single name classification system, but presently remains difficult to navigate. Black yeasts are a phylogenetically diverse morphogroup of asexual morphs, mostly in *Dothideales* or *Chaetothyriales,* which produce dark, slimy colonies and at least some budding yeast-like cells in culture. Many species have one or more hyphal asexual morphs in addition to the yeast-like forms. This pleiomorphy complicates their identification and taxonomic interpretation (De Hoog & Hermanides-Nijhof 1977). Two of the most frequently reported black yeast genera are *Aureobasidium* (*Aureobasidiaceae*) and *Hormonema* (*Dothideaceae*). *Aureobasidium* was associated with several sexually typified genera in *Dothideales,* and although no sexual morph is definitively known for the most frequently reported species *A. pullulans*, an ascospore-derived strain identified as *Columnosphaeria fagi* (CBS 171.93), has identical ITS and *RPB2* sequences to the clade including the ex-type culture of *A. pullulans*. The similar asexually typified genus *Hormonema,* (*H. dematioides* type), generally considered the asexual morph of *Sydowia polyspora*, is also frequently reported. A single name solution for this clade is not yet proposed and we use the name *Hormonema* for comparisons of asexual morphotypes. Despite the differences in associated sexual morphs, *Aureobasidium* and *Hormonema* are difficult to distinguish morphologically when grown in culture. For example, neither Hermanides-Nijhof (1977) nor De Hoog & Yurlova (1994) could find morphological differences among asexual morphs in cultures of the sexually typified genera *Pringsheimia, Dothidea, Dothiora* or *Sydowia*, all of which they attributed to *Hormonema*. *Aureobasidium* and *Hormonema* were considered distinct in the dual nomenclature era, because of the different sexual morphs. Hermanides-Nijhof (1977) defined *Aureobasidium* by the production of synchronous blastoconidia from undifferentiated, hyaline cells, whereas *Hormonema* was said to produce conidia in basipetal succession from hyaline or dark hyphae. Wang & Zabel (1990) suggested that at least some conidiogenous cells of *H. dematioides* were phialidic or percurrent. In a later review, *Aureobasidium* was distinguished based on its conidiogenous cells having multiple loci (synchronous conidiogenesis), in contrast to one or two loci in *Hormonema* (De Hoog & Yurlova 1994). These characters are difficult to detect, and are best observed along hyphae at the growing margin of the colony. Colonies of both morphs often begin as palely pigmented growths, which become slimy and almost black as the colonies mature. The different patterns and apparent plasticity of conidiogenesis, including yeast-like cells, young blastospores, swollen blastospores, chlamydospores, and septation and constrictions of hyphae confound interpretations of homologous characters (Guterman & Shabtai 1996, Zalar *et al.* 2008).

During our survey of fungi isolated from house dust using a dilution-to-extinction approach, many isolates morphologically resembled *Aureobasidium* and *Hormonema*, but six were phylogenetically distinct. Here we introduce these as two new species, in a new genus and family in *Dothideales*. We present a 28S rDNA (nuclear large ribosomal subunit) phylogeny of *Dothidiomycetes* to determine the strains' phylogenetic position and subsequently present phylogenies of *Dothideales* based on *BenA* (β-tubulin), ITS rDNA, 28S rDNA, 18S rDNA (nuclear small ribosomal subunit), and *RPB2* (RNA polymerase II second largest subunit), to determine the relationships within the order. Strains were characterized morphologically and compared to morphologically similar species and genera. This work follows previous reports of new taxa of indoor fungi discovered by dilution-to-extinction, including species of *Rasamsonia* (Tanney & Seifert 2013), *Aspergillus*, *Penicillium* and *Talaromyces* (Visagie *et al.* 2014, 2017, Sklenář *et al*. 2017), *Wallemia* (Jancic *et al*. 2015, Nguyen *et al*. 2015), *Spiromastix, Pseudospiromastix, Sigleria* (Hirooka *et al*. 2016), and *Myrmecridium* (Crous *et al.* 2016).

MATERIALS AND METHODS

Isolations

Settled house dust was collected from twelve countries (Australia, Canada, Federated States of Micronesia, Indonesia, Mexico, The Netherlands, New Zealand, South Africa, Thailand, the United Kingdom, Uruguay, and USA) using sterilized Duststream® collectors (Indoor Biotechnologies, Charlottesville, VA) attached to vacuum cleaners. Isolations were made from malt extract agar (MEA) and MEA with 20 % sucrose using a dilution-to-extinction method modified from Collado *et al*. (2007) as described in Visagie *et al.* (2014). More recent isolations targeting xerophilic fungi from Canadian and Hawaiian house dust were made as described in Visagie *et al.* (2017).

Living strains of new species are deposited in the Canadian Collection of Fungal Cultures (DAOMC, Ottawa, Canada), the Westerdijk Fungal Biodiversity Institute (CBS, Utrecht, The Netherlands) and dried specimens are accessioned in the

Table 1. Information on house dust isolates included in this study.

Species	Strain number	Isolation medium	Origin	Collector[a]	Date collected	Isolator[b]
Aureobasidium melanogenum	SLOAN 1260 = AA07MX-884	MEA	Mexico, Nayarit, Sayulita	A. Amend	31 Jan. 2009	E. Whitfield & K. Mwange
Aureobasidium melanogenum	SLOAN 1606 = BH02AU-110	MEA	Australia, Tasmania, Hobart	B. Horton	10 Feb. 2009	E. Whitfield & K. Mwange
Aureobasidium melanogenum	SLOAN 5623 = TA10NZ-214a	MEA	New Zealand, Wellington, Wellington	T. Atkinson	3 May 2009	E. Whitfield & K. Mwange
Aureobasidium melanogenum	KAS 5840	MY50G	Canada, Ontario, Stittsville	K.A. Seifert	20 Dec. 2014	C.M. Visagie
Aureobasidium melanogenum	KAS 7917	MY1012	USA, Hawaii, Kailua	A. Amend	11 May 2015	C.M. Visagie
Aureobasidium melanogenum	KAS 7956	DG18	USA, Hawaii, Kailua	A. Amend	11 May 2015	C.M. Visagie
Aureobasidium melanogenum	SLOAN 7256 = AA04US-587	20%S-MEA	USA	A. Amend	unknown	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 7261 = AA02US-306	20%S-MEA	USA, California, Berkeley	A. Amend	31 Mar. 2005	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 203 = 7050035.79-65	n.a.	Canada, Saskatchewan, Regina	Health Canada	12 Mar. 2007	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 207 = 7050035.79-71	n.a.	Canada, Saskatchewan, Regina	Health Canada	12 Mar. 2007	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 48 = 7050035.79-127	20%S-MEA	Canada, Saskatchewan, Regina	Health Canada	12 Mar. 2007	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 62 = 7050035.79-148	20%S-MEA	Canada, Saskatchewan, Regina	Health Canada	12 Mar. 2007	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 349 = 7330009.33-45	20%S-MEA	Canada, Saskatchewan, Regina	Health Canada	21 Aug. 2007	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 359 = 7330009.33-60	20%S-MEA	Canada, Saskatchewan, Regina	Health Canada	21 Aug. 2007	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 7249 = 7330009.34-925	n.a.	Canada, Saskatchewan, Regina	Health Canada	21 Aug. 2007	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 1652 = BH02AU-154a	20%S-MEA	Australia, Tasmania, Hobart	B. Horton	10 Feb. 2009	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 1653 = BH02AU-154b	20%S-MEA	Australia, Tasmania, Hobart	B. Horton	10 Feb. 2009	E. Whitfield & K. Mwange
Aureobasidium pullulans	SLOAN 3214 = KJ09SA-65	MEA	South Africa, Western Cape, Kuilsrivier	K. Jacobs	24 Jul. 2009	E. Whitfield & K. Mwange
Aureobasidium pullulans	KAS 5951	DG18	Canada, British Columbia, Victoria	B. Kendrick	27 Jan. 2015	C.M. Visagie
Aureobasidium species	SLOAN 41 = 7050035.79-119	MEA	Canada, Saskatchewan, Regina	Health Canada	12 Mar. 2007	E. Whitfield & K. Mwange
Aureobasidium subglaciale	SLOAN 7263 = AA02US-332	20%S-MEA	USA, California, Berkeley	A. Amend	31 Mar. 2005	E. Whitfield & K. Mwange
Hortaea werneckii	KAS 7942	MY50G	USA, Hawaii, Kailua	A. Amend	11 May 2015	C.M. Visagie
Hortaea werneckii	KAS 7947	MY50G	USA, Hawaii, Kailua	A. Amend	11 May 2015	C.M. Visagie
Hortaea werneckii	KAS 7949	MY1012	USA, Hawaii, Kailua	A. Amend	11 May 2015	C.M. Visagie
Hortaea werneckii	KAS 7953	MY1012	USA, Hawaii, Kailua	A. Amend	11 May 2015	C.M. Visagie
Rhizosphaera pini	DAOMC 251499 = KAS 6309	MY50G	Canada, New Brusnwick, Little Lepreau	A. Walker	29 Jan. 2015	C.M. Visagie
Sydowia polyspora	DAOMC 251470 = KAS 5918	DG18	Canada, British Columbia, Victoria	B. Kendrick	27 Jan. 2015	C.M. Visagie
Sydowia polyspora	DAOMC 251471 = KAS 5919	DG18	Canada, British Columbia, Victoria	B. Kendrick	27 Jan. 2015	C.M. Visagie
Sydowia polyspora	DAOMC 251469 = KAS 5999	DG18	Canada, New Brusnwick, Little Lepreau	A. Walker	29 Jan. 2015	C.M. Visagie
Zalaria alba	DAOMC 250847 = SLOAN 52 = 7050035-79-132	20%S-MEA	Canada, Saskatchewan, Regina	Health Canada	12 Mar. 2007	E. Whitfield & K. Mwange

Table 1. (Continued).

Species	Strain number	Isolation medium	Origin	Collector[a]	Date collected	Isolator[b]
Zalaria alba	DAOMC 250848 = SLOAN 352 = 7330009.33-5	MEA	Canada, Saskatchewan, Regina	Health Canada	21 Aug. 2007	E. Whitfield & K. Mwange
Zalaria obscurum	DAOMC 250851 = SLOAN 7266 = AA02US-340	20%S-MEA	USA, California, Berkeley	A. Amend	31 Mar. 2005	E. Whitfield & K. Mwange
Zalaria obscurum	DAOMC 250852 = SLOAN 7674 = AA02US-351	20%S-MEA	USA, California, Berkeley	A. Amend	31 Mar. 2005	E. Whitfield & K. Mwange
Zalaria obscurum	DAOMC 250849 = SLOAN 7244 = 7330009.34-884	n.a.	Canada, Saskatchewan, Regina	Health Canada	21 Aug. 2007	E. Whitfield & K. Mwange
Zalaria obscurum	DAOMC 250850 = SLOAN 7246 = 7330009.34-921	n.a.	Canada, Saskatchewan, Regina	Health Canada	21 Aug. 2007	E. Whitfield & K. Mwange

[a] Collector of house dust sample; [b] isolator of strain using the modified dilution to exctinction method.

Canadian National Mycological Herbarium (DAOM, Ottawa, Canada). Strains used in this study are summarized in Table 1.

Morphology

The strains considered here were suspected to be xerophilic and were thus characterized from colonies grown on a wide range of media, including MEA, potato-dextrose agar (PDA; Oxoid CM139), oatmeal agar (OA), dichloran 18 % glycerol agar (DG18; Hocking & Pitt 1980), starch-nitrate agar (SNA; Dodman & Reinke 1982), malt extract yeast extract with 50 % glucose agar (MY50G), malt extract yeast extract 10 % glucose 12 % NaCl agar (MY10-12; Pitt & Hocking 2009), and MEA with the addition of 5–24 % NaCl (MEA5NaCl, MEA10NaCl, MEA15NaCl, MEA24NaCl). The malt extract used for media was always BD Bacto™ (Mississauga, ON). Plates were incubated for 7 and 14 d in the dark at 25 °C. Additional MEA plates were incubated at 10 and 30 °C. Colour names and codes used in descriptions refer to Kornerup & Wanscher (1967). Microscope preparations were made from colonies grown on MEA and DG18, using lactic acid as mounting fluid. An Olympus BX50 compound microscope attached with an InfinityX camera powered by Infinity Analyze v. 6.5.1 software (Lumenera, Ottawa, ON) was used for microscopic observations, capturing images and making measurements. Photographic plates were prepared in Affinity Photo v. 1.5.2 (https://affinity.serif.com).

DNA extraction, sequencing, and analysis

DNA was extracted from 7–10-day-old cultures grown on Blakeslee's malt extract agar (MEA; (Blakeslee 1915)) using the UltraClean™ Microbial DNA isolation Kit (MoBio Laboratories, Solano Beach, CA) with extracts stored at -20 °C. Loci were amplified using the following primer pairs: 28S rDNA with LR0R & LR5 (Vilgalys & Hester 1990); ITS with V9G/LS266 (Gerrits van den Ende & De Hoog 1999, Masclaux *et al.* 1995); *RPB2* with fRPB2-5F/ fRPB2-7cR (Liu *et al*. 1999); 18S rDNA with NS1/NS4 (White *et al*. 1990); and *BenA* with T10/Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997). An annealing temperature of 55 °C was used for all reactions. PCR amplification was performed in 10 µL volume reactions, containing 0.5 µL template DNA, 1 µL Titanium *Taq* buffer (Takara Bio USA, Mountain View, CA), 0.5 µL (2 mM) dNTP's, 0.04 µL (3.2 mM) of each primer, 0.1 µL Titanium *Taq* polymerase (Takara Bio USA), and 7.82 µL MilliQ water.

Sequencing reactions were set up using the BigDye™ Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA) and the same primer pairs used for PCR amplification, with additional sequence reactions set up for 28S rDNA with primers LR3/LR3R (Vilgalys & Hester 1990). Sequence contigs were assembled in Geneious v. 8.1.8 (BioMatters, Auckland, NZ) and are deposited in GenBank (KX579092–KX579121, KY654326, KY659498, KY659500–KY659528). Accession numbers are also displayed on phylogenetic trees. BLAST searches were performed using NCBI to determine closest sequence matches and whether our species were detected in previous studies.

Phylogenetic analyses

The phylogenetic position of our strains within *Dothideomycetes* was determined using a 28S rDNA phylogeny compared to reference sequences obtained from Schoch *et al*. (2009) and Hyde *et al*. (2013). Secondly, phylogenies of *BenA*, ITS, 28S rDNA, *RPB2* and 18S rDNA were used to determine the relationships among our strains within the new genus, and the relationship of the genus and family with close relatives in *Dothideales* and *Myriangiales*. Newly generated sequences obtained from dust isolates belonging to *Aureobasidium, Hortaea, Rhizosphaera,* and *Sydowia* are also included in the phylogenies. Reference sequences for comparisons were obtained from GenBank and accession numbers are included on trees.

Table 2. BLAST results showing origin of strains or clones belonging to *Zalaria*.

Gene	GenBank nr	Original identification	Current identification	Strain/clone	Origin and source	Publication
ITS	KM103983	Uncultured fungus	*Zalaria cf sp2*	Rcw 47	USA; Red-cockaded woodpecker excavation in living pine	Unpublished
ITS	KJ130063	*Aureobasidium* sp.	*Zalaria cf sp2*	C 21	China, Henan, Nanyang City, Baotianman Natural Reserve Area; forest soil	Unpublished
ITS	KF800358	Uncultured fungus	*Zalaria cf sp2*	CMH 267	USA, Missouri, Kansas City; House dust	Rittenour *et al.* (2014)
ITS	KC253970	*Aureobasidium pullulans*	*Zalaria cf sp2*	UOA/HCPF ENV57A	Greece; hospital incubator	Unpublished
ITS	FJ235939	Fungal sp.	*Zalaria sp1*	AB 6	Antarctica; wood	Unpublished
ITS	AF121282	*Aureobasidium pullulans*	*Zalaria sp2*	ATCC 16628	The Netherlands; Soil	Unpublished
LSU	AB456556	*Aureobasidium* sp.	*Zalaria cf sp2*	WB 27	Thailand, Prachuap Khiri Khan, Pran Buri Forest Park; sediment in mangrove forest	Unpublished
LSU	AY167611	*Aureobasidium* sp.	*Zalaria cf sp2*	CECT 11965	Spain?; cork samples	Álvarez-Rodríguez *et al.* (2003)
LSU	KJ130062	*Aureobasidium* sp.	*Zalaria cf sp2*	C 21	China, Henan, Nanyang City, Baotianman Natural Reserve Area; forest soil	Unpublished
LSU	JN004186	*Aureobasidium* sp.	*Zalaria cf sp2*	SP 9	Portugal; Sound grapes	Unpublished
LSU	KC433818	*Aureobasidium* sp.	*Zalaria cf sp2*	DBVPG 5996	Italy, Mont Blanc, Miage glacier; supraglacial sediments	Turchetti *et al.* (2013)

Datasets were aligned using MAFFT v. 7.017 (Katoh & Standley 2013) with the L-INS-i algorithm. For the *BenA* dataset, G-INS-i was used. Alignments were manually trimmed in Geneious. The *Dothideomycetes* phylogeny was calculated using RAxML v. 8.0.0 (Stamatakis 2014) and support at nodes calculated using a bootstrap analysis of 1000 replicates. Additional phylogenies were calculated based on Maximum Likelihood done in RAxML and Bayesian tree interference (BI) using MrBayes v. 3.2 (Ronquist *et al*. 2012). For BI, the most suitable model for each dataset was determined using MrModeltest v. 2.3 (Nylander 2004) based on the lowest Akaike information criteria (Akaike 1974) value. Trees were visualized in Figtree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) and prepared for publication in Adobe® Illustrator® CS6. Aligned datasets and trees were uploaded to TreeBASE (www.treebase.org) with submission ID 19764.

RESULTS

Phylogeny

Dothideomycetes 28S rDNA phylogeny (Fig. 1): The aligned 28S rDNA dataset contained 515 taxa and was 2448 bp long. *Schismatomma decolorans* was selected as outgroup based on Schoch *et al.* (2009). In general, our phylogeny shared similar topologies to those observed in Schoch *et al.* (2009) and Hyde *et al.* (2013). The phylogeny placed our isolates within the monophyletic order *Dothideales*, although there was poor support on the branch separating it from *Myriangiales*. Furthermore, our isolates resolved in a clade distinct from the two families currently recognized in *Dothideales*, i.e. *Aureobasidiaceae* and *Dothideaceae*. Therefore, we introduce the new genus *Zalaria* and classify it in a new family named *Zalariaceae* below.

Dothideales phylogenies (Figs 2–3): To examine relationships and the phylogenetic species concept within *Zalaria* and its relationship with related families more closely, we calculated focused phylogenies based on 18S rDNA, 28S rDNA, *BenA*, ITS, and *RPB2*, including only closely related species and genera from *Dothideaceae* and *Aureobasidiaceae,* as well as species in the order *Myriangiales*. All loci consistently resolved the *Zalaria* strains in clades distinct from *Aureobasidiaceae* and *Dothideaceae*. In the ITS phylogeny, they resolved as a close relative of *Myriangiales*, but this tree had poor backbone support. All loci, except for the highly conserved 18S rDNA, resolved strains into two clades, which were strongly supported in 28S rDNA, *BenA*, ITS, and *RPB2*. They are described below as *Zalaria alba* and *Z. obscura* spp. nov. Furthermore, the 28S rDNA phylogeny revealed several strains that we consider identical to *Z. obscura*. These strains (CBS 122350; CBS 122359; EXF-922; EXF-1934; EXF-1936) originated from Norwegian arctic ice and were published in Zalar *et al.* (2008), but were never given a formal name. Remaining house dust isolates were identified here as *Aureobasidium pullulans*, *A. melanogenum*, *A. subglaciale*, an undescribed *Aureobasidium* species (DTO 285-D8), *Rhizosphaera pini*, *Sydowia polyspora*, and *Hortaea werneckii* (*Capnodiales*).

NCBI-BLAST (Table 2): BLAST searches resulted in several hits similar to *Zalaria* ITS and 28S rDNA sequences. These sequences were from a diverse range of studies and originate from the USA, China, Greece, The Netherlands, Portugal, Spain, Thailand, and

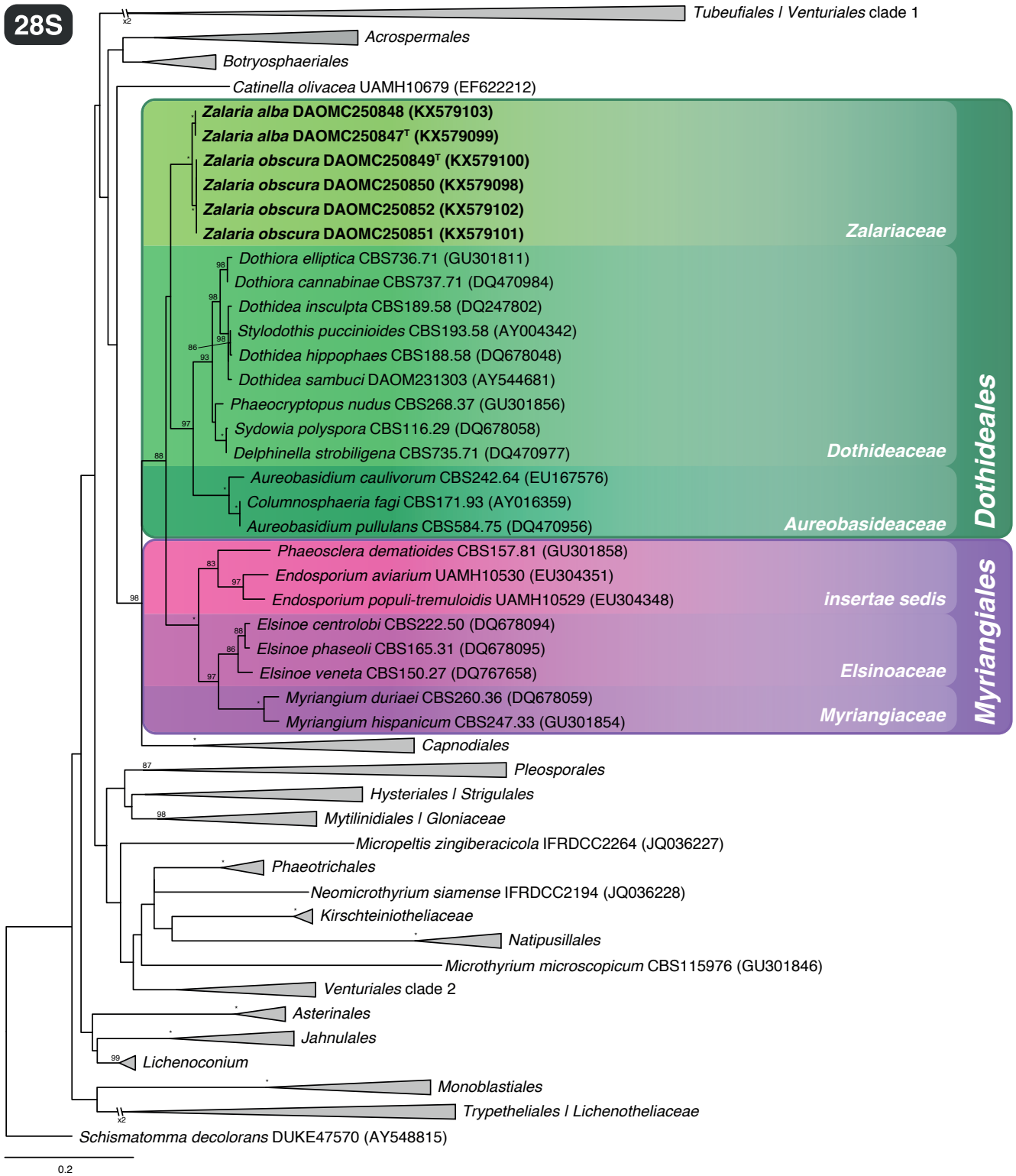

Fig.1. Phylogenetic tree of *Dothideomycetes* based on 28S rDNA showing the distinct nature of the new family *Zalariaceae* within *Dothideales*. *Schismatomma decolorans* was selected as outgroup. Bootstrap support values higher than 80 % are indicated above branches (* indicates 100 % bootstrap support). House dust isolates are shown in bold text.

Antarctica and from habitats including house dust, cork samples, grapes, a hospital incubator, sediment, soil, wood, and woodpecker excavations. Our *Zalaria* isolates were obtained from house dust collected in the USA (CA) and Canada (Regina, SK).

Morphology

Strains were characterized morphologically on several agar media and shared several characters with species of *Aureobasidium* and *Hormonema*. As noted in the Introduction, the only way to reliably distinguish between these groups

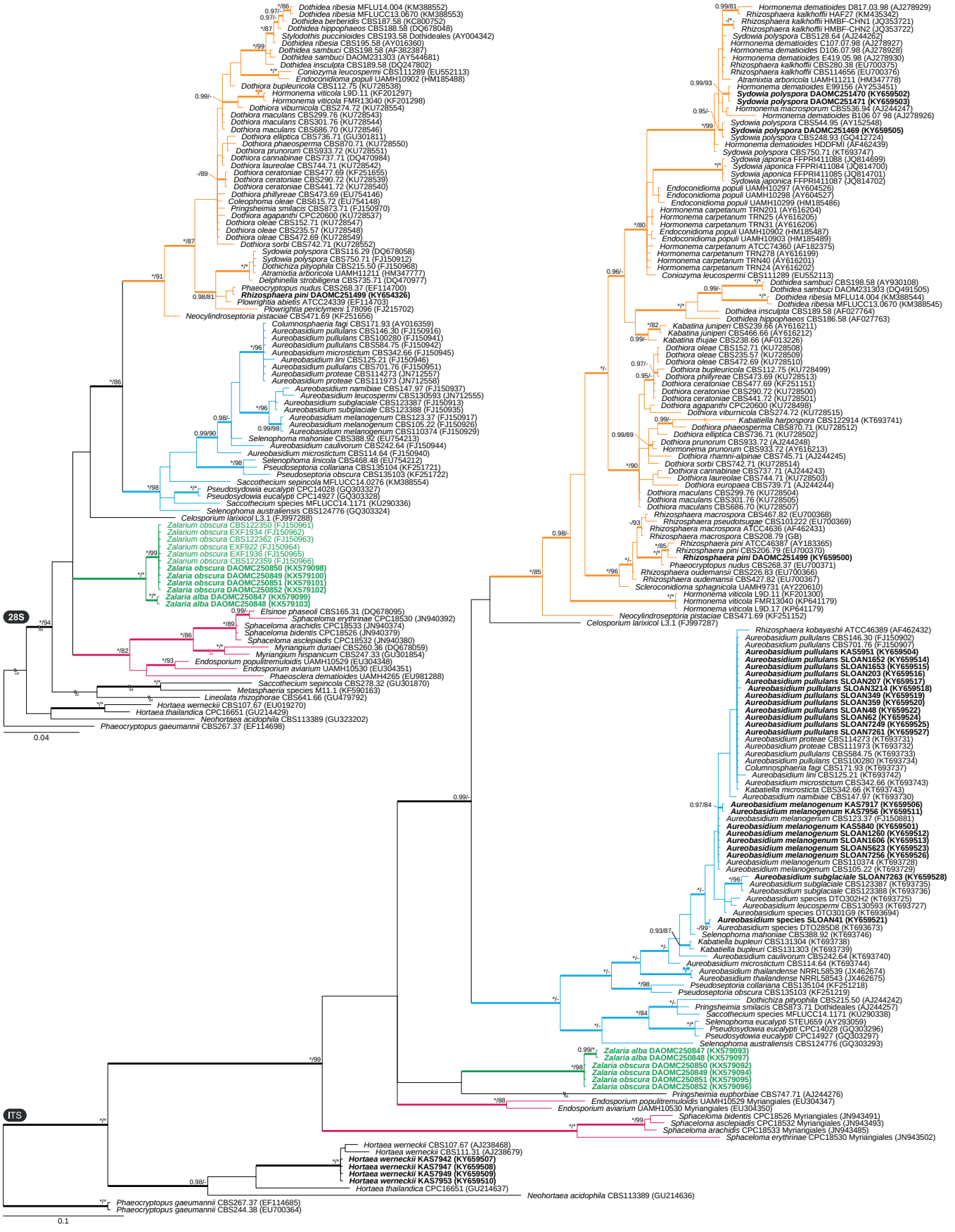

Fig. 2. Phylogenies based on 28S rDNA and ITS showing the relationship of *Zalaria* (green text and branches) with other closely related genera from families *Dothideaceae* (orange branches), *Aureobasidiaceae* (blue branches) and order *Myriangiales* (maroon branches). Bootstrap support values or Bayesian posterior probabilities higher than 79 % or 0.94 are indicated above thickened branches (* indicates 100 % or 1.00; – indicates lack of support). House dust isolates from this study are indicated by bold text. GenBank accession numbers are provided between brackets.

Fig. 3. Phylogenies based on 18S rDNA, *RPB2* and *BenA* showing the relationship of *Zalaria* (green text and branches) with other closely related genera from families *Dothideaceae* (orange branches), *Aureobasidiaceae* (blue branches) and order *Myriangiales* (maroon branches). Bootstrap support values or Bayesian posterior probabilities higher than 79 % or 0.94 are indicated above thickened branches (* indicates 100 % or 1.00; – indicates lack of support). House dust isolates from this study are indicated by bold text. GenBank accession numbers are provided between brackets.

are the 1–2 conidiogenous loci per cell in *Hormonema* (and asexual morphs of many other *Dothideaceae* species), and up to 14 loci in *Aureobasidium* (De Hoog & Yurlova 1994, Yurlova *et al.* 1999). The new genus is more similar in this regard to *Hormonema,* with only 1–2 loci per conidiogenous cell. In general, growth of *Zalaria* species is more restricted than in any of these other genera.

Three types of conidiogenesis were observed in the *Zalaria* strains. Their yeast forms are very common, especially in younger colonies when cells reproduce by budding. After prolonged incubation, these yeast cells are often covered by melanized hyphal growth; microscopic observations suggest that hyphae from germinating yeast cells eventually give rise to this dark melanized growth (Figs 4C–D, 5C–D). With age, these hyphae may develop into dark brown, thick-walled chlamydospores (Figs 4E, I, 5E, I). Further, intercalary conidiogenous cells develop mostly at margins of young colonies.

The strains resolved as distinct clades observed in the phylogenies were also distinct morphologically, with *Z. alba* compared to *Z. obscura* growing more restrictedly on most media. *Zalaria obscura* is also capable of growth at low a_w media such as MEA-10%-NaCl, MY-1012 and MY50G, with *Z. alba* not growing on these media. Generally, *Z. alba* colonies are also more yeast-like and take up to 3 wk to darken, whereas *Z. obscura* colonies darken in 7 d and are often covered by a leathery layer within 14 d. This character was originally used for distinguishing the two varieties of *A. pullulans* var. *pullulans* and var. *melanogenum,* later recognized as two distinct species (Gostinčar *et al* 2014). Based on both phylogenetic and morphological results we introduce a new family, genus and two new species to accommodate our unique black yeast-like fungi.

Morphological examinations confirmed sequence-based identifications of the remaining house dust isolates (Figs 6–7). *Aureobasidium* strains produced the typical conidiogenous cells with multiple loci (Fig. 6A–G), while only 1–2 conidiogenous loci were observed in strains identified as *Sydowia polyspora* (Fig. 6H–K). *Hortaea werneckii* was common in Hawaiian dust samples and was only isolated from the halophilic MY10-12 medium. The typical pigmented hyphae, yeast-like growth, sympodial and percurrent conidiogenesis were observed in newly isolated strains (Fig. 7A–E). The strain identified as *Rhizosphaera pini* produced colonies with pycnidium-like structures and a *Hormonema*-like morph producing very large conidia, all characteristic of that species (Fig. 7F–I).

Taxonomy

Zalariaceae Visagie, Z. Humphries & Seifert, **fam. nov.**
MycoBank MB821627

Type genus: *Zalaria* Visagie *et al.* 2017.

*Diagnosis***:** Distinguished from other families classified in *Dothideales* and *Myriangiales* based on a short unique 28S rDNA sequence flanked by two conserved regions. The section defining *Zalariaceae* in our alignment (Treebase ID 19764) are found between nucleotide positions 39 to 62 and is indicated in bold text (5'-AGCTCAAATTTGAAA**TCTGGCC-CTTTC-AGGGTCCGAG**TTGTAATTTGTAGAGG-3').

Zalaria Visagie, Z. Humphries & Seifert, **gen. nov**.
MycoBank MB821628

Etymology: Named in honour of Polona Zalar, mycologist at the University of Ljubljana, Slovenia, in recognition of her studies on extremophilic fungi, including her 2008 study that included strains from Norwegian arctic regions that belong to this genus.

Diagnosis: Differs from *Aureobasidium* by blastic conidiogenesis occurring from one to two loci per conidiogenous cell. Morphologically *Zalaria* is indistinguishable from *Hormonema* (often reported as asexual morphs of *Sydowia*), leaving DNA sequences the only diagnostic character (see Diagnosis for family *Zalariaceae* above).

Type species: *Zalaria obscura* Visagie *et al.* 2017

Description: *Sexual morph* unknown. *Colonies* often covered in slimy masses of conidia or yeast-like cells, becoming dark and often leathery with time, occasionally with sparse aerial mycelium; cream-colored, red-brown, olive-brown, dark brown, or black; margins entire or fimbriate. *Hyphae* transversely and longitudinally septate, hyaline and thin-walled when young, frequently becoming melanized and thick-walled with age, may develop into chlamydospores. *Conidiogenous cells* undifferentiated, intercalary, terminal uncommon, cylindrical, with blastic conidiogenesis occurring from one to two loci per cell. *Chlamydospores* dark brown, smooth to lightly rough-walled, globose to ellipsoidal, septate. *Conidia* often yeast-like, hyaline, aseptate, smooth-walled, ellipsoidal to lemon-shaped, variable in size, indistinct hilum, budding common, polar, bipolar and multilateral.

Zalaria alba Visagie, Z. Humphries & Seifert, **sp. nov.**
MycoBank MB821629
(Fig. 4)

Etymology: Latin, *alba,* meaning white, in reference to colony appearance after 7 d of growth.

Diagnosis: Differs from *Z. obscura* in the inability to grow at lowered a_w. Colonies remain yellowish to orange-white until it darkens after about 3 wk. Conidia appearing more slender than *Z. obscura*. *ITS barcode*: KX579093. Alternative identification markers: 28S rDNA: KX579099, *RPB2*: KX579105, 18S rDNA: KX579111, *BenA*: KX579117.

Type: **Canada**: *Saskatchewan*: Regina, isol. ex house dust, 12 Mar. 2007, *E. Whitfield & K. Mwange* (DAOM 734001 – holotype; DAOMC 250847 – ex-type culture).

Description: *Colony diameters* (mm after 7 d (14 d at 25 °C)): MEA 5–6 (8–9); MEA 5 °C microcolonies, 10 °C microcolonies, 30 °C 2–5, 35 °C 1–2, 40 °C microcolonies; MEA-5 %-NaCl no growth, MEA-10 %-NaCl no growth, MEA-15 %-NaCl no growth, MEA-24 %-NaCl no growth; OA 6–7 (11–14), PDA

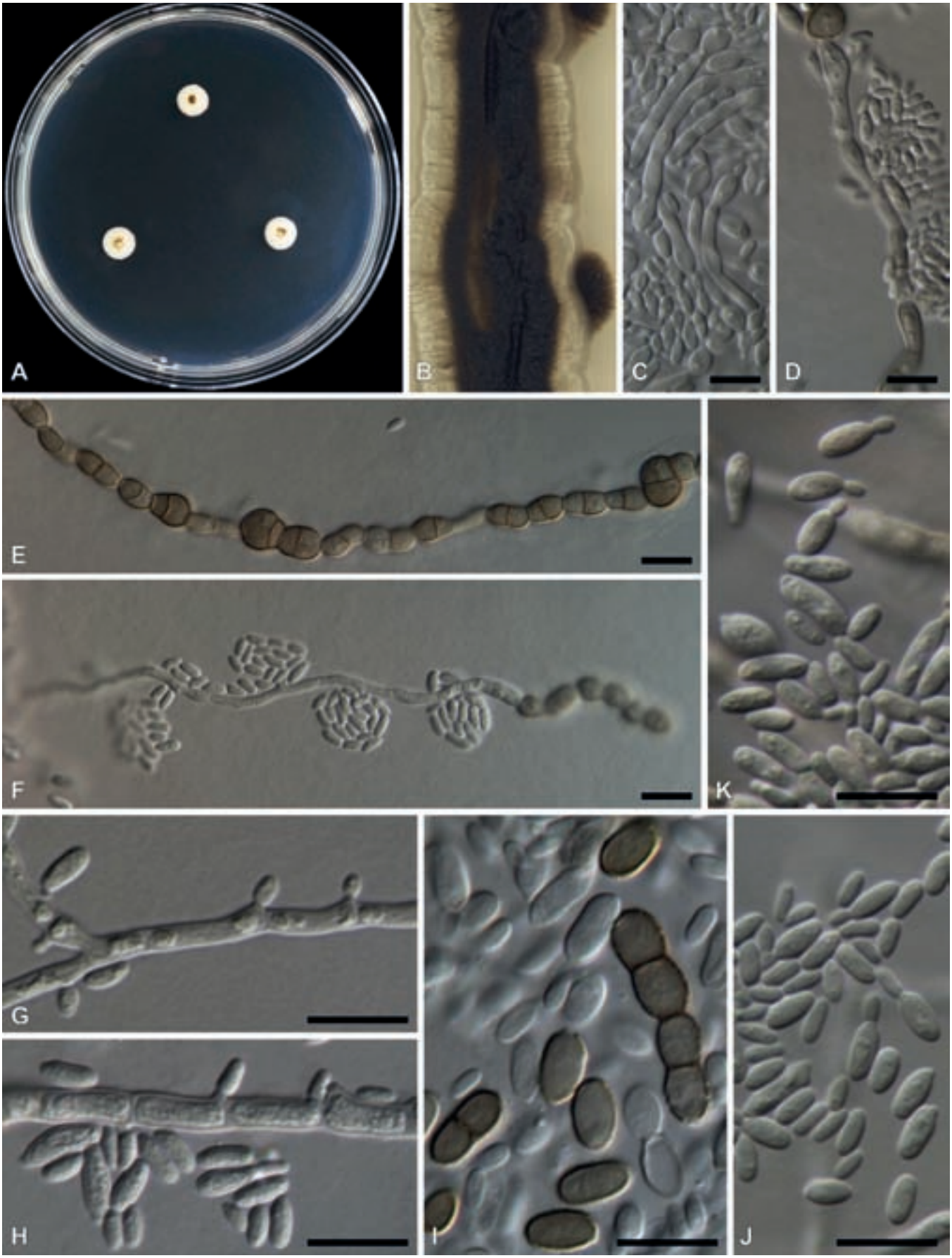

Fig. 4. Morphological characters of *Zalaria alba* (DAOMC 250847 in A–C, E, F; DAOMC 250848 in D, G–K). **A.** Colonies on MEA after 2 wk. **B.** Close-up colony on MEA after 4 wk. **C.** Germinating conidia. **D.** Germinating conidia with age. **E.** Melanized hyphae developing into arthrospores and chlamydospores. **F–H.** Intercalary conidiogenous cells. **I.** Chlamydospores. **J–K.** Conidia with some yeast-like budding occurring. Bars = 10 µm.

7–8 (10–11), DG18 3–4 (5–7), MY1012 no growth, MY50G no growth, SNA 3–5 (7–8).

Cultural characters: Colonies on MEA at 25 °C after 7 d yeast-like, smooth and slimy, obverse yellowish white to orange white (4A2–5A2), reverse greenish grey to pale orange (1B2–5A3), generally becoming dark within 3 wk, with dark areas sometimes present after 7 d, olive-yellow to dark brown (3D6-7F5), some aerial mycelium developing after longer incubation.

Microscopic characters: Young somatic hyphae at colony periphery mostly hyaline, smooth, thin-walled, branched, transversely septate, 1.5–5 µm diam; older hyphae towards colony centre melanized, dark brown, smooth to lightly roughened, thick-walled, branched, transversely and longi-septate, 2–6.5 µm diam, often developing into chlamydospores. *Conidiogenous cells* undifferentiated, intercalary, rarely terminal, mostly on hyaline hyphae, producing conidia percurrently from short lateral denticles not exceeding 2 µm long. *Chlamydospores* dark brown, smooth to lightly rough-walled, globose to ellipsoidal, septate to aseptate, one-septate spores sometimes constricted at septum, 5.5–10 × 3–7.5 µm ($\bar{x}$ = 8 ± 0.99 µm; 5.5 ± 0.71 µm). *Conidia* often yeast-like, hyaline, aseptate, smooth walled, ellipsoidal to lemon-shaped, variable in size, 2.5–10 × 1.5–5 µm ($\bar{x}$ = 5.5 ± 1.53 µm, 3 ± 0.73 µm), with an indistinct hilum, budding common, polar, bipolar and multilateral.

Notes: Growth on media with lowered a_w distinguishes between the two *Zalaria* species. *Zalaria alba* does not grow on MEA-5 %-NaCl, MY10-12 or MY50G after 14 d. In contrast, *Z. obscura* produces at least microcolonies on these media. Colony size varies significantly after 14 d on MEA at 25 °C, with *Z. alba* colonies (8–9 mm) more restricted than those of *Z. obscura* (12–14 mm). Also, *Z. obscura* colonies darken much faster than those of *Z. alba*. Microscopically these species are very similar. In general, however, spores of *Z. alba* seem more slender.

Additional material examined: **Canada**: *Saskatchewan*: Regina, isol. Ex house dust, 21 Aug. 2007, *E. Whitfield & K. Mwange* **(**DAOMC 250848 – culture).

Zalaria obscura Visagie, Z. Humphries & Seifert, **sp. nov.**
MycoBank MB821630
(Fig. 5)

Etymology: Latin *obscura,* dark, in reference to colony appearance after 7 d of growth.

Diagnosis: Differs from *Z. alba* in the ability to grow at lowered a_w. Colonies dark brown to black after about 7 d. Conidia appearing less slender than *Z. alba*. *ITS barcode*: KX579094. Alternative identification markers: 28S rDNA: KX579100, *RPB2*: KX579106, 18S rDNA: KX579112, *BenA*: KX579118

Type: **Canada**: *Saskatchewan*: Regina, isol. ex house dust, 21 Aug. 2007, *E. Whitfield & K. Mwange* (DAOM 734002 – holotype; DAOMC 250849 – ex-type culture).

Description: *Colony diameters* (mm after 7 d, (14 d) *at 25* °C)): MEA 25 °C 7–10 (12–14); MEA 5 °C microcolonies, 10 °C microcolonies, 30 °C 3–9, 35 °C 3–8, 40 °C 1–2 mm; MEA-5 %-NaCl 2–4 (3–5), MEA-10 %-NaCl microcolonies, MEA-15 %-NaCl no growth, MEA-24 %-NaCl no growth; OA 7–8 (15–16), PDA 7–9 (10–14), DG18 3–5 (6–8), MY1012 no growth, sometimes microcolonies after prolonged incubation, MY50G no growth (microcolonies), SNA 4–5 (8–9).

Cultural characteristics: Colonies on MEA at 25 °C after 7 d yeast-like, smooth and slimy, obverse dark brown (7F5) to black with some yellowish white to orange-white (4A2–5A2), olive-yellow (3D6), and olive (1E5) areas, surface leathery after 14 d; some aerial mycelium developing after prolonged incubation.

Microscopic characters: Young somatic hyphae at colony periphery mostly hyaline, smooth, thin-walled, branched, transversely septate, 1.5–4.5 µm diam; older hyphae towards colony centre melanized, dark brown, smooth to lightly roughened, thick-walled, branched, transversely and longi-septate, 2–11 µm diam, often developing into chlamydospores. *Conidiogenous cells* undifferentiated, intercalary, rarely terminal, mostly on hyaline hyphae, producing conidia percurrently from short lateral denticles not exceeding 2 µm long. *Chlamydospores* dark brown, smooth to lightly rough walled, globose to ellipsoidal, septate to aseptate, one-septate spores sometimes constricted at septum, 5–17 µm × 3.5–8 µm ($\bar{x}$ = 8 ± 2.11 µm; 6 ± 0.89 µm). *Conidia* often yeast-like, hyaline, aseptate, smooth-walled, ellipsoidal to lemon-shaped, variable in size, 2.5–10 × 1.5–5.5 µm ($\bar{x}$ = 5 ± 1.4 µm; 3.5 ± 0.77 µm), with an indistinct hilum, budding common, polar, bipolar and multilateral.

Notes: See notes under *Z. alba.*

Additional material examined: **Canada**: *Saskatchewan*: Regina, isol. exhouse dust, 21 Aug. 2007, *E. Whitfield & K. Mwange* (DAOMC 250850). **– USA**: *California*: Berkeley, isol. ex house dust, 31 Mar. 2005, *A. Amend, E. Whitfield & K. Mwange* (DAOMC 250851, 250852).

DISCUSSION

In this paper, we describe a novel lineage comprising six black yeast-like strains isolated from house dust collected in Canada and the USA as a new genus of *Dothideales*, *Zalaria.* Mature agar colonies become dark and leathery, but are covered with slimy masses of conidia or yeast-like cells. The conidiogenous cells are undifferentiated and usually intercalary, with blastic conidiogenesis occurring on 1–2 loci per cell, giving rise to aseptate, smooth-walled, ellipsoidal to lemon-shaped conidia, that commonly bud in a polar, bipolar or multilateral pattern; dark brown, rough-walled chlamydospores are often seen. Strains were resolved at the species level into two clades, described as *Z. alba* and *Z. obscura*, with strains of the latter growing faster and with colonies darkening within 7 d. No sexual morph is known for either species.

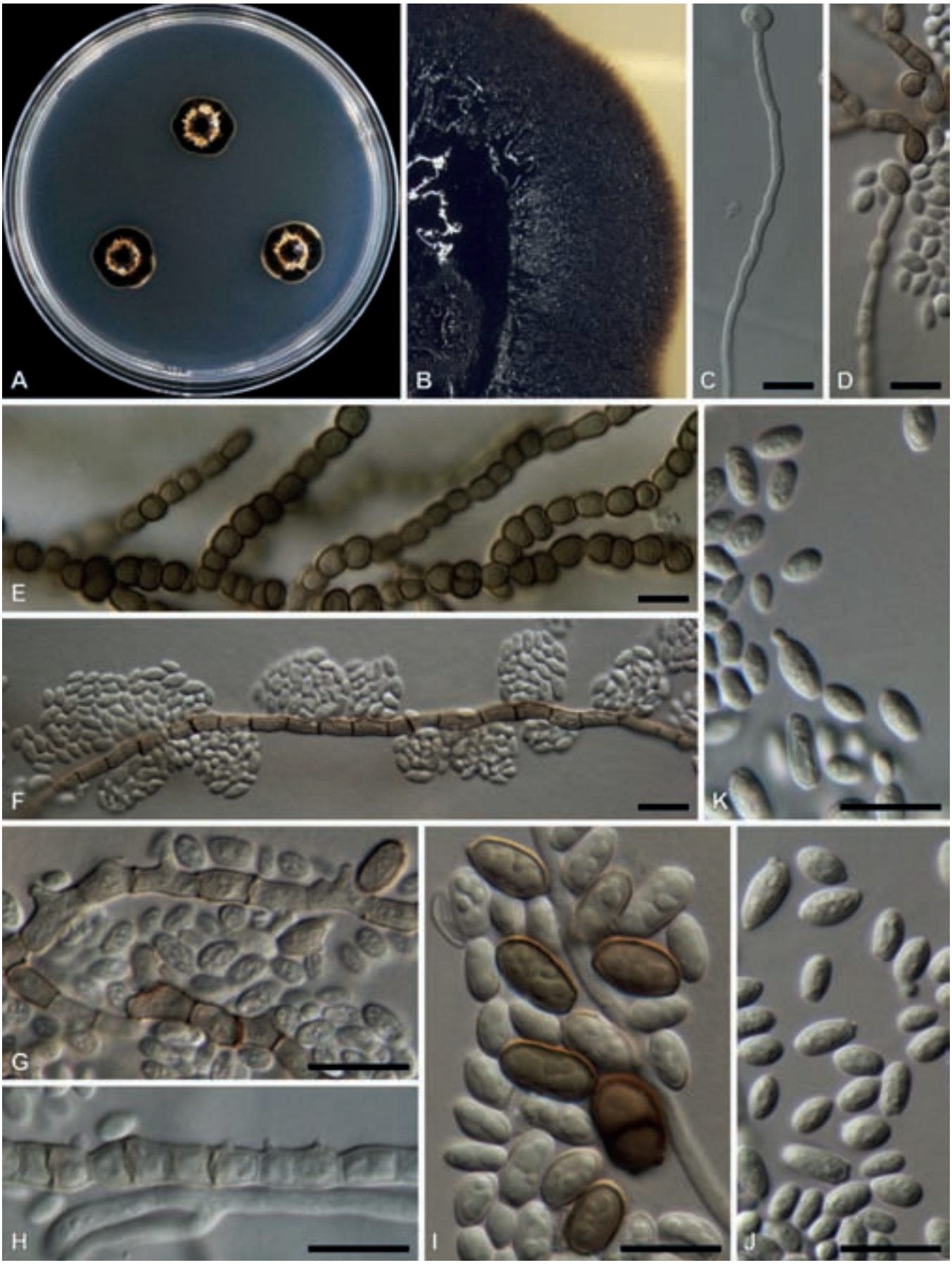

Fig. 5. Morphological characters of *Zalaria obscura* (DAOMC 250849 in A, B, D, F, G, J, K; DAOMC 250852 in C, H, I; DAOMC 250850 in E). **A.** Colonies on MEA after 2 wk. **B.** Close-up colony on MEA after 4 wk. **C.** Germinating conidia. **D.** Germinating conidia with age. **E.** Melanized hyphae developing into chlamydospores. **F–H.** Intercalary conidiogenous cells. **I.** Chlamydospores. **J–K.** Conidia with some yeast-like budding occurring. Bars = 10 µm.

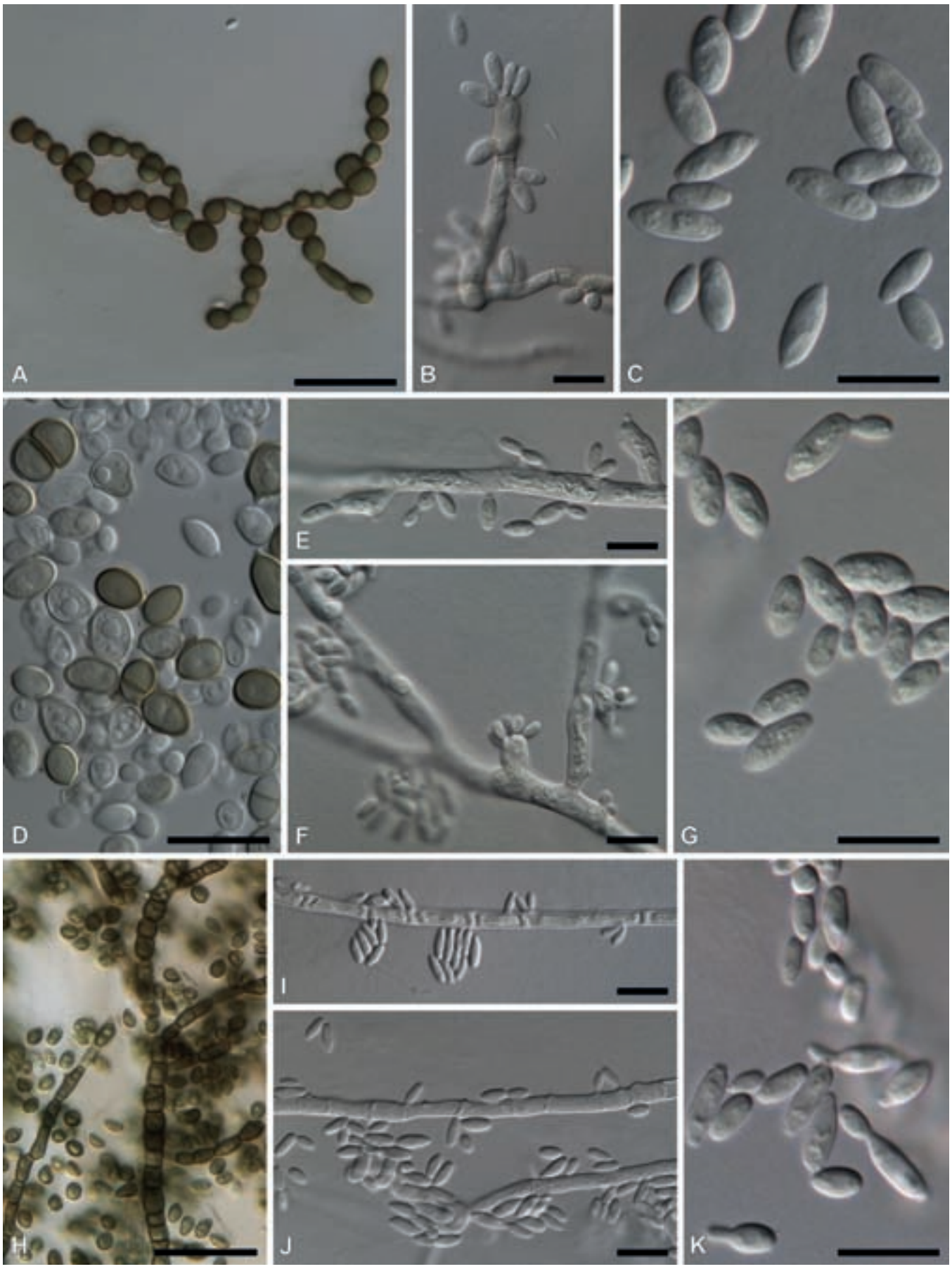

Fig. 6. **A–C.** *Aureobasidium pullulans* (KAS 5840). **A.** Melanized hyphae/chlamydospores. **B.** Conidiogenous cells with multiple loci. **C.** Conidia. **D–G.** *Aureobasidium melanogenum* (KAS 1951). **D.** Dark brown conidia. **E–F.** Conidiogenous cells with multiple loci. **G.** Conidia. **H–K.** *Sydowia polyspora* (DAOMC 251471). **H.** Melanized hyphae/chlamydospores. **I–J.** Hormonema-like conidiogenous cells with 1–2 loci. **K.** Conidia. Bars = 10 µm except A and H = 50 µm.

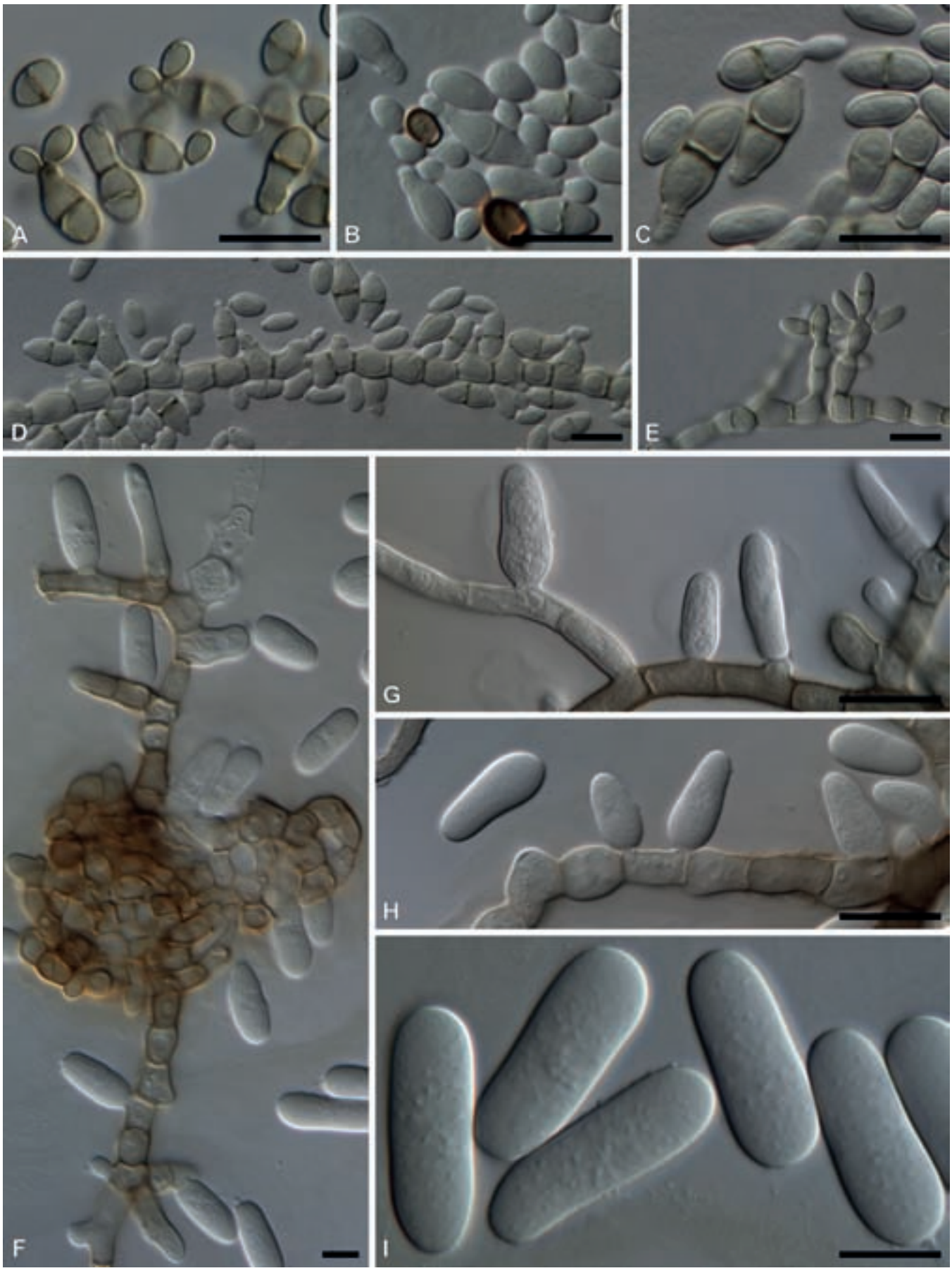

Fig. 7. A–E. *Hortaea werneckii* (DAOMC 251499). **A.** Yeast-like cells with sympodial producing daughter cells. **B–C.** Yeast-like cell with annellations. **D–E.** Conidiogenous apparatus. **F–I.** *Rhizosphaera pini* (DAOMC 251499). **F–H.** *Hormonema*-like conidiogenous cells. **I.** Conidia. Bars = 10 µm.

Because of the black yeast asexual morphology, *Zalaria* is difficult to distinguish from *Aureobasidium* and *Hormonema*. Conidiogenesis has been used to differentiate the latter two genera, but does not consistently provide accurate identification unless combined with growth rates and physiological characters such as carbohydrate assimilation (De Hoog & Yurlova 1994, Loncaric *et al*. 2009, Yurlova *et al.* 1996). The most reliable morphological character for distinguishing these genera is the number of loci present on conidiogenous cells. Species of *Zalaria* and *Hormonema* have only 1–2 loci per cell, whereas *Aureobasidium* has up to 14 (De Hoog &Yurlova 1994, Yurlova *et al.* 1999). Distinguishing *Zalaria* and *Hormonema* based only on conidiogenesis, however, is nearly impossible. Sexual morph characters are used to distinguish *Dothidea*, *Pringsheimia* and *Sydowia* (Thambugala *et al*. 2014) with similar asexual morphs, but no sexual morph has been observed in *Zalaria*.

Although the two species described here can be confidently interpreted as representing a new genus, our decision to propose a new family *Zalariaceae* is less satisfying. Our phylogenetic analyses (Figs 1–3) consistently resolved *Zalaria* strains as distinct from *Aureobasidiaceae* and *Dothideaceae*. Working within the framework and concepts adopted for the order (Thambugala *et al.* 2014), we could either synonymise all current families or introduce a new family; we chose the latter approach. Our proposal of a new family on primarily phylogenetic grounds, in the absence of presumably more informative characters of so-far unknown sexual morphs, perpetuates but does not add to the vagueness of phenotypic characters underlying the phylogeny. However, we hope that increased sampling, especially of sexually reproducing species across *Dothideomycetes*, of unsequenced but known and unknown taxa, will reveal morphological or other phenotypic characters that are predictive of phylogeny, resulting in stable family and genus concepts in *Dothideales*.

BLAST results with *Zalaria* strains (Table 2) recovered 11 sequences that we consider belong to *Zalaria*, but that were originally identified as *Aureobasidium* sp. or *A. pullulans*. These strains or clones originated from the USA, China, Thailand, Greece, The Netherlands, Portugal, Spain, and Antarctica. Strains placed by Zalar *et al*. (2008) in their group 5 are identified here as *Z. obscura* and originate from the Norwegian arctic region. Furthermore, a BLAST search of 454 pyrosequencing data generated during our house dust project (Amend *et al.* 2010), revealed 21 sequences belonging to *Zalaria* in dust collected from Australia, Canada, New Zealand, South Africa, and the USA. *Zalaria* seems to have a truly worldwide distribution, and occurs on many substrates, from wood, soil, dust, sediments, cork, and subglacial ice. Understanding its ecology will be very challenging. Zalar *et al.* (2008) suggested that, given the highly selective conditions of the environment, their then new group 5 might be restricted to areas like Kongsfjorden in Norway. Arctic environments possess low a_w because ice formation removes most of the available water, while a_w is lowered further as solute ions are expelled during the freezing process (Gunde-Cimerman *et al*. 2003). This lack of available water is a dominating factor in microbial life in arctic regions (Gunde-Cimerman *et al.* 2003) and favours the growth of xerotolerant and xerophilic fungi. Given the extreme environment of a polythermal glacier, it could be hard to imagine how an organism so specifically adapted could out-compete other life-forms in more hospitable climates. However, arctic fungal species seem to have very effective dispersal strategies over long distances (Geml 2011). Combined with *Zalaria*'s phenotypic plasticity, melanisation and the halotolerance of *Z. obscura* (similar to that in *Aureobasidium*), these species may be capable of widespread dispersal and also survive in or on many substrates.

Before our study, the only way to identify and communicate information on strains, clones or *Zalaria* OTU's was by means of UNITE's species hypotheses (Kõljalg *et al.* 2013), i.e. based on 0 % threshold, SH377734.07FU represents *Zalaria alba* and SH377739.07FU represents *Z. obscurum*. With formal names now available to these species hypotheses, communicating information about these fungi and studying the extent of their distribution, habitats and possible ecological roles will be much easier.

ACKNOWLEDGEMENTS

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