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Publication Date

2012-02-04

The genome of the xerotolerant mold *Wallemia sebi* reveals adaptations to osmotic stress and suggests cryptic sexual reproduction

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March 2012

The work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231

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Elsevier Editorial System(tm) for Fungal Genetics and Biology
Manuscript Draft

Manuscript Number: FGB-11-136R1

Title: The genome of the xerotolerant mold Wallemia sebi reveals adaptations to osmotic stress and suggests cryptic sexual reproduction

Article Type: Regular Article

Keywords: Aqua(glycero)porins; electron microscopy; halophile; ion homeostasis; solute accumulation; xerophile

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Abstract: Wallemia (Wallemiales, Wallemiomycetes) is a genus of xerophilic Fungi of uncertain phylogenetic position within Basidiomycota. Most commonly found as food contaminants, species of Wallemia have also been isolated from hypersaline environments. The ability to tolerate environments with reduced water activity is rare in Basidiomycota. We sequenced the genome of Wallemia sebi in order to understand its adaptations for surviving in osmotically challenging environments, and we performed phylogenomic and ultrastructural analyses to address its systematic placement and reproductive biology. Wallemia sebi has a compact genome (9.8 Mb), with few repeats and the largest fraction of genes with functional domains compared with other Basidiomycota. We applied several approaches to searching for osmotic stress-related proteins. In silico analyses identified 93 putative osmotic stress proteins; homology searches showed the HOG (High Osmolarity Glycerol) pathway to be mostly conserved. Despite the seemingly reduced genome, several gene family expansions and a high number of transporters (549) were found that also provide clues to the ability of W. sebi to colonize harsh environments. Phylogenetic analyses of a 71-protein dataset support the position of Wallemia as the earliest diverging lineage of Agaricomycotina, which is confirmed by septal pore ultrastructure that shows the septal pore apparatus as a variant of the Tremella-type. Mating type gene homologs were identified although we found no evidence of meiosis during conidiogenesis, suggesting there may be aspects of the life cycle of W. sebi that remain cryptic.

18 January 2012

Dear Dr. Galagan,

Thank you for giving us the opportunity to revise our manuscript “The genome of the xerotolerant mold *Wallemia sebi* reveals adaptations to osmotic stress and suggests cryptic sexual reproduction” (FGB-11-136) for publication in *Fungal Genetics and Biology*.

We appreciate the points raised by the reviewers and hope that we have addressed their comments satisfactorily in our response to reviews.

Thank you for your time and your consideration.

Sincerely,

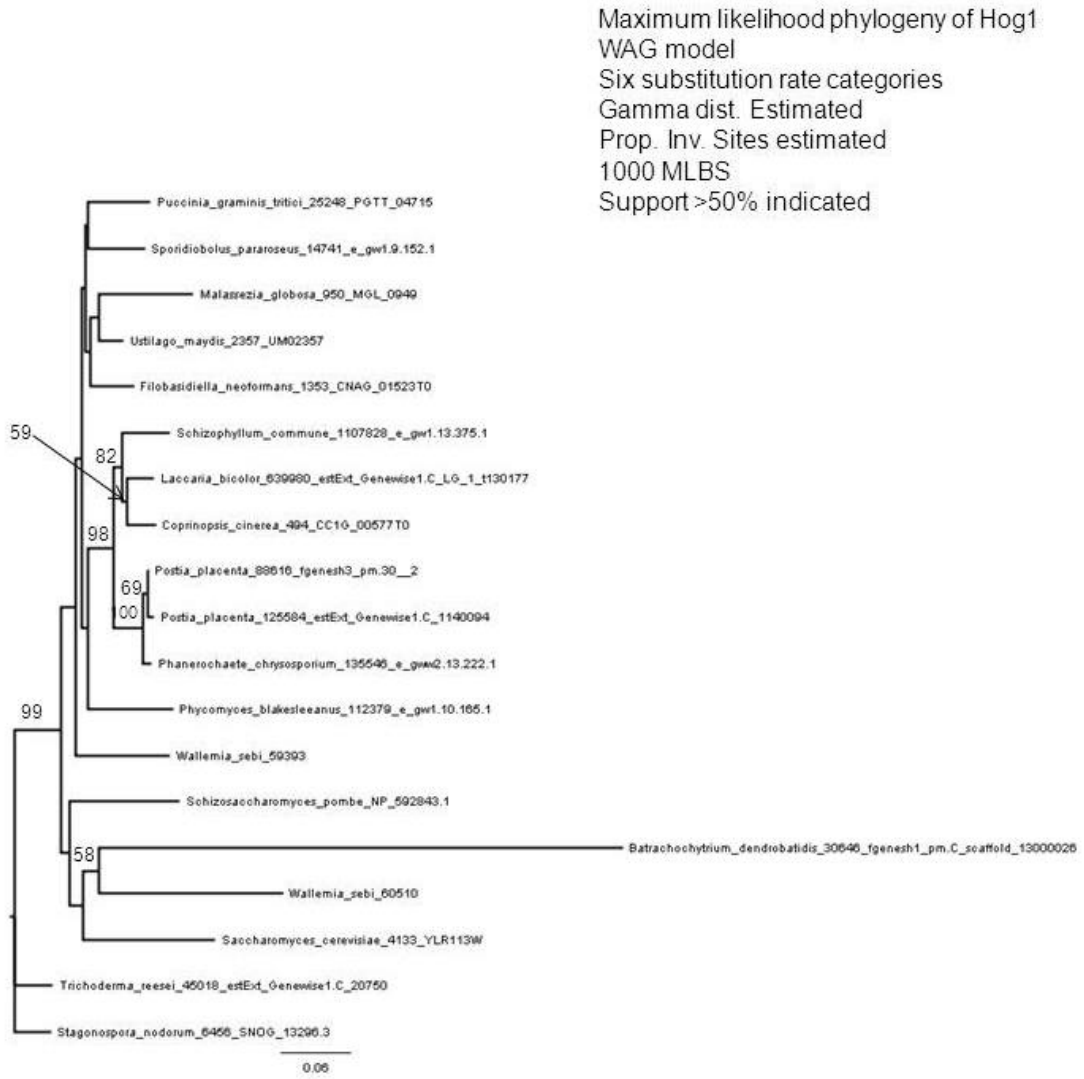
Mahajabeen

Response to reviewers' comments

Reviewer #1:
Suggestions for major improvements:

1) Conduct phylogenetic analysis to study the evolutionary origin of the duplicated genes. In a recently published *Verticillium* comparative genomics paper (Klosterman SJ. 2011 PlosPathogen), a duplication of Hog1 is also reported. The phylogenetic analysis from that study rejected recent duplication within the genome as the source of that additional Hog1 gene. It would be interesting to compare these two duplicates in the background of other orthologous copies from different fungal lineages.

Sequences of the two WsHog1 homologs were used in BLASTp searches to extract orthologous Hog1 sequences from all the taxa used in the phylogenetic and CAFE analyses. We conducted a maximum likelihood search using a WAG+G+I model to examine the evolutionary origin of the two WsHog1. The results of the phylogenetic analyses to examine the origin of the duplicated WsHog1 homologs suggest that the homologs are not the result of a recent duplication; however, the weak support values for the relationships in the phylogeny make it unclear when the duplication originated. See figure below:



2) Since RNA-seq data have been generated, the authors could use this data set to validate any functional important for the genes of interest. Suggest to add the statistic numbers as total annotated genes that have RNA-seq evidence, and describe the genes of interest with RNA-seq support.

Validation of the genes of interest using RNA-seq data would be an interesting exercise, but would perhaps be more useful if we had another species of *Wallemia* with which to compare functional importance. Also a thorough analyses of the transcriptome of *W. sebi* could be the basis for a future study.

Minor comments:

A) To increase clarity of the manuscript, details in methods such as how many computer nodes, specific computing commands can be moved to supplementary information.

We left the details in the methods to enable readers to easily obtain and follow the computing commands.

B) Add information of the sequenced strain: such as where, when the strain was isolated.

Information of the sequenced strain was added to the methods.

C) In line 310: MIP family of transporters that transport small solutes in response to an osmotic gradient. Providing evidence to support the statement.

We thank the reviewer for catching this error. The sentence (now Line 317) should have stated the “the Major Facilitator Superfamily of transporters”. It has been corrected and a reference added.

Reviewer #2: This manuscript is very well crafted and an excellent contribution to fungal research. This work shows the expanding scope of fungal genomics. It settles the phylogenetic placement of an enigmatic fungus, explains at least partially the molecular basis for the ability of Wallemia to invade low water potential niches, and establishes a solid starting point for studies of mating systems and sexuality in ancestral Agaricomycotina. I have only a few minor corrections (below), and I suggest that the manuscript be accepted with only these small changes.

Minor Corrections:

A) Line 148 : remove 'our in-house gap' replace with Gap

The change has been made.

B) Line 181 : remove 'over'

The change has been made.

C) Line 335 : use correct notation for expected values.

The correct notation is now used.

Suggestions and comments:

A) The genome size appears to be an estimate from sequencing alone. If this size has been corroborated by other means please state this.

The genome size is an estimate only from the sequencing data. A line has been added to this effect in the results section.

B) Please include another general statement about the homology between Wallemia and other fungi along the lines of 1000 out of the 5284 predicted genes had no homologs in any other fungi (or Basidiomycota, Ascomytoca, etc.). The traditional venn diagrams can be helpful. It will be useful to know how many of the genes in such a small genome are unique.

Thank you for the suggestion. We have added the following line to the results section "Approximately 30% (1689 proteins) in the genome have no homolog in any of the fungi used for the phylogenetic and CAFE analyses (for list of taxa see Fig. 1, Supplementary Fig. 1 A–E) in this study".

C) Does the 97.26% placement of (90% Id and 85% covered) ESTs reflect error in EST generation or assembly? This bit of the methods should be explained slightly more.

The sentence now reads "Newbler assembled consensus EST sequence data was used to assess the completeness of the final assembly using alignments of 90% identity and 85% coverage or higher. This resulted in 97% of the EST consensus sequences aligned to the genome assembly." Additional details have also been added to the Genome Annotation section (lines 178-181)

D) Similarly, why are there 6708 EST contigs but only 5284 genes?

There are a number of possible reasons for the 6708 contigs versus 5284 genes. For example, the model filtering process may have left out some real genes. Also, there could have been RNA contaminants, some of which were assembled into contigs. It is also possible that the contigs represent fragments of genes (a many-to-one relationship), which is sometimes noticed in the browser.

Highlights:

- The genome of a xerophilic fungus *Wallemia sebi* was sequenced and found to be 9.8 Mb
- Phylogenomics and ultrastructure show that *Wallemia* belongs in Agaricomycotina
- 93 putative osmotic stress proteins are identified
- The High Osmolarity Glycerol pathway is found to be mostly conserved
- Meiosis is not found during conidiogenesis but mating-type homologs are identified

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Abstract

Wallemia (Wallemiales, Wallemiomycetes) is a genus of xerophilic Fungi of uncertain phylogenetic position within Basidiomycota. Most commonly found as food contaminants, species of *Wallemia* have also been isolated from hypersaline environments. The ability to tolerate environments with reduced water activity is rare in Basidiomycota. We sequenced the genome of *Wallemia sebi* in order to understand its adaptations for surviving in osmotically challenging environments, and we performed phylogenomic and ultrastructural analyses to address its systematic placement and reproductive biology. *Wallemia sebi* has a compact genome (9.8 Mb), with few repeats and the largest fraction of genes with functional domains compared with other Basidiomycota. We applied several approaches to searching for osmotic stress-related proteins. *In silico* analyses identified 93 putative osmotic stress proteins; homology searches showed the HOG (High Osmolarity Glycerol) pathway to be mostly conserved. Despite the seemingly reduced genome, several gene family expansions and a high number of transporters (549) were found that also provide clues to the ability of *W. sebi* to colonize harsh environments. Phylogenetic analyses of a 71-protein dataset support the position of *Wallemia* as the earliest diverging lineage of Agaricomycotina, which is confirmed by septal pore ultrastructure that shows the septal pore apparatus as a variant of the *Tremella*-type. Mating type gene homologs were identified although we found no evidence of meiosis during conidiogenesis, suggesting there may be aspects of the life cycle of *W. sebi* that remain cryptic.

Keywords: Aqua(glycero)porins, electron microscopy, halophile, ion homeostasis, solute accumulation, xerophile.

1. Introduction

Cell survival depends on an organism's ability to sense and respond to environmental stresses. In saline environments organisms respond to osmolarity changes through multiple signaling pathways (Hohmann, 2009; Lenassi et al., 2007). The yeast *Saccharomyces cerevisiae* is the model eukaryote for studying responses to osmotic stress (Gunde-Cimerman et al., 2009; Hohmann, 2009). However, the relatively recent discovery of fungi in hypersaline environments (Gunde-Cimerman et al., 2000) has also enabled the study of salt tolerance in other eukaryotic systems (Gunde-Cimerman et al., 2009). The genus *Wallemia* Johan-Olsen (Johan-Olsen, 1887) (Wallemiales, Wallemiomycetes, Basidiomycota) contains three species all of which are osmotolerant (Zalar et al., 2005). Of these, *W. sebi* (Fr.) v. Arx (1970), a common food-borne contaminant that has been isolated from environments with different levels of water activity (a_w), is considered cosmopolitan (Amend et al., 2010; Domsch et al., 1980; Gunde-Cimerman et al., 2009; Kralj Kunčič et al., 2010; Liu et al., 2010; Matheny et al., 2006; Pitt and Hocking, 2009; Samson et al., 2004; Zalar et al., 2005).

The diverse habitats from which strains of *W. sebi* have been isolated (e.g., jam, dried fish, marine sponges, and house dust) suggest that it can adjust its physiology to adapt to different environments, but the genes involved in controlling its responses have not been fully elucidated. Under high salinity conditions species of *Wallemia* have an altered cell morphology (compared with low salinity conditions), e.g., decreased hyphal compartment length, and increased cell wall thickness (Kralj Kunčič et al., 2010). The ability to grow at a low a_w has only been found in members of 10 unrelated fungal orders, mostly Ascomycota (De Hoog et al., 2005; Kralj Kunčič et al., 2010). All three representatives of Wallemiales are xerotolerant (Gunde-

85 Cimerman et al., 2009; Zalar et al., 2005) although only *W. sebi* can grow, albeit slowly, without
86 a solute in its growth medium (Kralj Kunčič et al., 2010).

87 The ability to survive osmotic stress requires several adaptations involving
88 osmoregulation, ion homeostasis, accumulation of solutes, as well as possible modifications of
89 cell morphology (Gunde-Cimerman et al., 2009; Hohmann et al., 2007; Kralj Kunčič et al.,
90 2010). Aqua(glycero)porins, members of the MIP (Major Intrinsic Protein) family, have been
91 demonstrated in *S. cerevisiae* and play a role in the osmoregulation of diverse organisms by
92 mediating the transport of small molecules such as glycerol across biological membranes
93 (Pettersson et al., 2005; Tanghe et al., 2006). The sensing of changes in the osmolarity of the
94 habitat is also essential for the survival of the cell. In Fungi, (e.g., *S. cerevisiae*) the HOG (High
95 Osmolarity Glycerol) response pathway facilitates the adaptation of cells to the increased
96 osmolarity of the environment (Hohmann, 2009; Hohmann et al., 2007; Krantz et al., 2006). The
97 osmotic stress response genes have been thoroughly investigated in *Aspergillus nidulans* by *in*
98 *silico* analyses (Miskei et al., 2009). Here, we investigate whether these physiological
99 mechanisms of osmotic stress tolerance described in Ascomycota are also conserved in the
100 basidiomycete *Wallemia*.

101 *Wallemia* was initially ascribed to the hyphomycetes (a catch-all term for Fungi that bear
102 conidia on hyphae) due to its mold-like growth habit and putatively asexual spore production
103 (Madelin and Dorabjee, 1974). Two subsequent ultrastructural studies of the septal pore
104 apparatus, while conflicting in their evidence for a septal pore cap, showed septal pore swellings
105 consistent with a placement in Basidiomycota (Moore, 1986; Terracina, 1974). Phylogenetic
106 analyses of different genes have confirmed the position of *Wallemia* as a member of
107 Basidiomycota (Samson et al. 2004, Matheny et al. 2006). However, different datasets and

analyses of up to six combined loci have suggested conflicting placements of Wallemiomycetes, ranging from the earliest diverging lineage of Basidiomycota to sister lineage to Ustilaginomycotina or Agaricomycotina (Matheny et al. 2006).

Nothing is known of the mating behavior of *W. sebi* and no teleomorphic stage or fruit body has ever been observed. Conidial ontogeny in *W. sebi* appears to be of a type of meristematic arthroconidium formation that is unique in Fungi but the exact stages in conidiogenesis are uncertain; however, it has been speculated that the "conidia" are produced by repeated meiosis and that the resulting spores thereby represent meiospores produced by a sexual teleomorph (Hashmi and Morgan-Jones, 1973; Moore, 1986). We sequenced the genome of *Wallemia sebi* and conducted genomic, phylogenetic, and ultrastructural studies to describe the components of the osmotic stress response pathways, resolve the phylogenetic position of the Wallemiomycetes, and assess evidence for sexual reproduction.

2. Materials and Methods

2.1 RNA isolation

Wallemia sebi strain CBS 633.66, obtained from Centraalbureau voor Schimmelcultures, Netherlands, was isolated from date honey by R.B. Kenneth in 1966 (http://www.cbs.knaw.nl/). The culture was grown for three days at 22C in 2% (w/v) malt extract (ME) plus 20% (w/v) dextrose broth. The liquid cultures were filtered using Whatman filter paper set inside a Buchner funnel under vacuum. DNA was extracted using a modified CTAB protocol (Murray and Thompson, 1980) and purified using a cesium chloride density gradient (Richards et al. 1994). For RNA isolation, the mycelium was carefully transferred to a 50 ml

tube placed in liquid nitrogen. RNA was isolated using a total RNA extraction protocol (Kramer, 2007) with slight modifications. DNA was removed from the extraction using TURBO DNase (Ambion Inc, Austin, Texas).

2.2.1 Genome sequencing and assembly.

The *Wallemia sebi* CBS 633.66 genome was sequenced using several platforms, assembled with Newbler v. 2.5 (Margulies et al., 2005), and annotated using the JGI Annotation pipeline (SOM). The genome was sequenced using a combination of 454 (Titanium unpaired, 9.4 kb and 11.2 kb Titanium paired end), and Illumina (2x76 bp, 0.3kb insert paired end) sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website (http://www.jgi.doe.gov/). A single lane of the Illumina data was assembled with the Velvet assembler version 0.7.55 (Zerbino and Birney, 2008) with a hash length of 61and the following options: -long_mult_cutoff 1 -ins_length 250 -exp_cov 127 -cov_cutoff 60 -scaffolding no, to produce an assembly with a final graph with 1580 nodes and n50 of 72958, max 401417, total 9761865, using 92035368/117161274 reads. Contigs ≥ 800bp in length were shredded into 1000bp chunks, if possible, with 800bp overlap for Newbler assembly. Reverse complemented shreds were also created at contig ends. After eliminating possible contaminant data, the combined set of velvet shredded fragments and 454-reads was assembled with Newbler and the following options: -fe reads2remove.MPA -sio -info -consed -finish -nrm –rip -a 50 -l 350 -g -ml 20 -mi 97 -e 39. This resulted in 56 scaffolds with an N/L50 of 9/337.4 Kb, and 89 contigs with an N/L50 of 14/259.7 Kb (Supplementary Table 1). One round of automated gap closure using Gap Resolution (Trong et al., 2009) resulted in a final assembly with 85 contigs with an N/L50 of 14/259.3 Kb. The final contig N/L50 is slightly less than the original assembly due to the expected gap size estimations being larger than observed. Newbler assembled

consensus EST sequence data was used to assess the completeness of the final assembly using alignments of 90% identity and 85% coverage or higher. This resulted in 97% of the EST consensus sequences aligned to the genome assembly.

2.2.2 cDNA library construction, sequencing and assembly.

Two separate RNA preparations of *W. sebi* RNA were used to construct two 454-cDNA libraries (CHBA and CHAW) using the cDNA Rapid Library Preparation Method as outlined in the Roche kit. Sequencing of libraries CHBA and CHAW resulted in 1,037,800 and 1,244,360 ESTs respectively. Ribosomal RNA, low quality and low complexity reads were filtered out, then the 98% remaining reads were assembled using Newbler v2.3 (Prerelease-6/30/2009) with default parameters, resulting in 6,708 contigs (>50 bp long) and mean length of 1575 bp.

2.3 Genome Annotation

The *W. sebi* CBS 633.66 genome was annotated using the JGI annotation pipeline, which takes multiple inputs (scaffolds, ESTs, and known genes) and runs several analytical tools for gene prediction and annotation, and deposits the results in the JGI Genome Portal (http://www.jgi.doe.gov/Wallemia) as part of the integrated fungal resource MycoCosm (http://jgi.doe.gov/fungi) for further analysis and manual curation.

Genomic assembly scaffolds were masked using RepeatMasker (Smit et al., 1996-2010) and the RepBase library of 234 fungal repeats (Jurka et al., 2005). tRNAs were predicted using tRNAscan-SE (Lowe and Eddy, 1997). Using the repeat-masked assembly, several gene prediction programs falling into three general categories were used: 1) *ab initio* - FGENESH (Salamov and Solovyev, 2000); GeneMark (Isono et al., 1994), 2) *homology-based* - FGENESH+; Genewise (Birney and Durbin, 2000) seeded by BLASTx alignments against GenBank's database of non-redundant proteins (NR: http://www.ncbi.nlm.nih.gov/BLAST/), and

3) *EST-based* - EST_map (http://www.softberry.com/) seeded by EST contigs. Genewise models were extended where possible using scaffold data to find start and stop codons. EST BLAT alignments (Kent, 2002) were used to extend, verify, and complete the predicted gene models. BLAT was run, with the options of 95% identity and 80% coverage of EST length, to map 2239146 raw ESTs to the genome assembly. Using BLAT, 2083886 (93%) ESTs were aligned to the genome assembly. Unaligned ESTs may reflect missing regions in the genome assembly, or contaminant RNA in EST sequencing. The resulting set of models was then filtered for the best models, based on EST and homology support, to produce a non-redundant representative set of 5,284 gene models with characteristics described. This representative set was subject to further analysis and manual curation. Measures of model quality included proportions of the models complete with start and stop codons (95% of models), consistent with ESTs (97% of models covered ≥75% of exon length), supported by similarity with proteins from the NCBI NR database (84% of models).

All predicted gene models were functionally annotated using SignalP (Nielsen et al., 1997), TMHMM (Melen et al., 2003), InterProScan (Zdobnov and Apweiler, 2001), BLASTp (Altschul et al., 1990) against nr, and hardware-accelerated double-affine Smith-Waterman alignments (deCypherSW; http://www.timelogic.com/decypher_sw.html) against SwissProt (http://www.expasy.org/sprot/), KEGG (Kanehisa et al., 2008), and KOG (Koonin et al., 2004). KEGG hits were used to assign EC numbers (http://www.expasy.org/enzyme/), and Interpro and SwissProt hits were used to map GO terms (http://www.geneontology.org/). Multigene families were predicted with the Markov clustering algorithm (MCL (Enright et al., 2002)) to cluster the proteins, using BLASTp alignment scores between proteins as a similarity metric. MCL clustering of *W. sebi* protein sequences that assigned proteins to clusters based on sequence

200 similarity was performed (Enright et al., 2002). Each resulting cluster can then be thought of as a
201 protein family possibly sharing a biological function.

202 **2.4 CAFE analyses**

203 Analysis of protein family gain and loss was performed with the CAFE software (De Bie
204 et al., 2006). CAFE uses a stochastic model of gene birth and death in a phylogeny, and infers
205 the most likely gene family size at internal nodes of the tree. As input to CAFE, we used Pfam
206 domains, and the number of genes with a given domain from each organism. We considered a
207 Pfam domain expanded if (i) it was given a significant family-wide P-value (< 0.01); (ii) domain
208 counts in *W. sebi* were above the mean of the counts in all other organisms, (iii) validated by
209 manual inspection. The Viterbi assignments (De Bie et al., 2006) were examined on the branch
210 leading to *W. sebi*. CAFE results for 5 gene families are presented (Supplementary Fig. 1 A–E).

211 **2.5 Creating databases**

212 The databases generated included all the deduced amino acid sequences from genes that
213 may play a role in osmotic stress response in both *S. cerevisiae* and *A. nidulans*. The first
214 database (Database No. 1) included the translated open reading frames (ORFs) corresponding to
215 osmotic stress response genes from the *A. nidulans* genomic database (Broad Institute)
216 (http://www.broadinstitute.org/annotation/genome/aspergillus_nidulans/MultiHome.html). The
217 second database (Database No. 2) comprised all the deduced protein sequences of *W. sebi*
218 obtained from the *W. sebi* v1.0 database (http://genome.jgi-psf.org/Walse1/Walse1.home.html).
219 The third database (Database No. 3) comprised all the deduced protein sequences from the *A.*
220 *nidulans* genomic database (Broad Institute)
221 (http://www.broadinstitute.org/annotation/genome/aspergillus_nidulans/MultiHome.html). The
222 fourth database (Database No. 4) includes osmotic stress response proteins deduced from the *S.*

cerevisiae (http://yeastmine.yeastgenome.org/yeastmine/template.do?name=GOTerm_GeneOrganism&scope=all) and *S. pombe* AMIGO databases (http://old.genedb.org/amigo-cgi/search.cgi) using "response to osmotic stress" as keyword. The fifth database (Database No. 5) includes all protein sequences deduced from the *S. cerevisiae* SGD (*Saccharomyces* Genome Database) (http://www.yeastgenome.org/) and *S. pombe* (http://www.sanger.ac.uk/Projects/S_pombe/) genomic databases.

2.6 Homology search and annotation of *W. sebi* osmotic stress proteins

Wallemia sebi osmotic stress response homologs were identified using BLASTp search program (Altschul et al., 1990) by comparing stress response proteins from Database No. 1 to putative proteins in Database No. 2. Blastall and formatdb software were downloaded (http://www.ncbi.nlm.nih.gov/Ftp). The results were filtered according to the 1 E–40 expectation value (E) cut-off criteria (Miskei et al., 2009). Less stringent values recovered proteins that were not necessarily involved in osmotic stress response. To reduce the number of mis-annotated proteins, sequences of putative osmotic stress response proteins found in the *W. sebi* database were compared to putative proteins in Database No. 3 using BLASTp (E-value 1 E–40). Proteins that presented the highest homology in these results and were identical to the initial proteins used as query from Database 1 were selected. Other outcomes of the homology search were disregarded.

Similarly, additional *W. sebi* orthologs of osmotic stress response proteins were identified by comparing putative osmotic stress response proteins from Database No. 4 to putative proteins in Database No. 2, using E-value 1 E–40 as the cut-off value. *Wallemia sebi* putative osmotic stress response proteins were compared with the putative proteins in Database No. 5. Among

them, protein sequences with the highest homology also identical to the original query sequences (Database No. 4) were selected.

2.7 Phylogenetic analyses.

For molecular sampling, 71 single to low-copy proteins (Supplementary Table 2) that are orthologous across the Fungi were mined from 20 available fungal genomes including *W. sebi* using the HAL pipeline (Robbertse et al., 2011). The protein clusters were aligned using MUSCLE (Edgar, 2004) and processed through GBlocks (Castresana, 2000) and in Phylip format (Felsenstein, 1981). The individual protein trees were analyzed using 100 RAxML (Stamatakis, 2006) bootstraps. A super-alignment was created using the 71 proteins and analyzed using RAxML.

To visualize support for alternative topologies among the individual data partitions, we performed a consensus supernetwork analysis of the 71 single-protein trees using SplitsTree4 (Huson, 2005; Huson et al., 2004) (Supplementary Fig. 2). To assess the sensitivity of the placement of *W. sebi* to model selection, we performed Bayesian phylogenetic analyses of the concatenated dataset using the CAT Dirichlet process mixture model, implemented in PhyloBayes 2.3, which has been shown to overcome long-branch attraction in some phylogenomic analyses (Lartillot, 2004; Lartillot et al., 2007). We ran four independent chains using default parameters for a total of 21652 cycles (sampling ca. 732270 tree topologies), discarded 100 cycles as burn-in from each chain, and merged treefiles for calculation of posterior probabilities (PP). The consensus topology was compared with the phylogeny obtained with RAxML analyses of the full 71-protein, 29627-position alignment under the WAG model (Stamatakis et al. 2008).

To assess the impact of rapidly-evolving sites, we used the AIR applications of the

Bioportal server (Kumar et al., 2009) to construct datasets that exclude the fastest-evolving positions. We first used AIR-Identifier, which placed 3749, 5023, and 3856 sites in the sixth through eighth rate categories (respectively) of a discrete gamma distribution. We then used AIR-Remover to generate datasets that exclude positions in the seventh and eighth, or sixth through eighth rate categories, resulting in alignments of 20748 and 16999 positions (i.e., 57% to 70% of the original dataset). We analyzed both datasets with RAxML at the CIPRES portal (www.phylo.org/portal2), using the WAG+G+I model with 100 rapid bootstrap replicates and ML optimization (Stamatakis et al., 2008).

The dataset was pruned to include 16 taxa and was analyzed with RAxML. The resulting tree was used to create an ultrametric phylogram in TreeEdit (Rambaut and Charleston, 2001) using non-parametric rate smoothing. The chronogram was used in CAFE (De Bie et al., 2006) runs to detect significant gene family size changes between the lineages, specifically in gene families related to osmotic stress.

2.8 Electron Microscopy of Septal Pore Apparatus

The processing of one week old hyphal samples of *W. sebi* (grown on 2% ME and 20% dextrose agar) for septal ultrastructural studies followed Kumar et al. (Kumar et al., 2007), except that the samples were dehydrated in a graded ethanol series, and infiltrated using Spurr's low viscosity resin (Spurr, 1969) prepared using the modified formulation of Ellis (Ellis, 2006).

2.9 Conidiogenesis study using light microscopy

Wallemia sebi cultures were grown on no. 2 cover slips coated with a thin layer of 2% ME and 20% dextrose made with 1.5% Noble's agar. The liquid spore suspension used to inoculate the cover slip cultures was collected from 5-day-old slant cultures by flooding with 2mL of distilled water, hand mixing to release spores, vortexing the extracted suspension to

reduce spore clumping, and diluting the suspension to a final concentration of approximately 60,000 spores per mL. To an agar-coated cover slip 20 μL of spore suspension was added and incubated for 2-6 days at 21°C with a 12-hour day.

Cells were fixed for 1 hr on the cover slips by fume fixation using 2% glutaraldehyde in 0.1M sodium cacodylate buffer by suspending the cover slips above the fixative on a bent glass rod in a microscope slide staining dish sealed with Parafilm™. The cultures were then stained with 1-2 drops of 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) solution (2μg/mL H_2O) for 10 min in darkness, rinsed briefly with distilled H_2O and viewed under differential interference contrast and epifluorescence with the appropriate filters. Images were captured using a Zeiss Axioscope with a 1.4 NA condenser and Plan Neofluor 100× objective lens and a 2.0× Optivar on a Spot Insight™ video

3. Results

3.1 Genome sequencing and annotation

The *Wallemia sebi* (CBS 633.66) genome is estimated via sequencing to be 9.8 Mb and is assembled in 56 scaffolds, the nine largest of which contain half of the total sequence (Supplementary Table 1). The assembled genome coverage is estimated to be 71×. There are 5284 genes, predicted with extensive support from EST data and by homology to other Basidiomycota, which represents the smallest gene count in the second smallest Basidiomycota genome sequenced to date. Approximately 30% (1689 proteins) of the genome has no homolog in any of the fungi used for the phylogenetic and CAFE analyses (for list of taxa see Fig. 1 and Supplementary Fig. 1 A–E) in this study. Intergenic distances are much smaller than in other fungal genomes and repetitive elements compose only 0.8% of the genome. The annotated

315 genome is accessible from the Joint Genome Institute (JGI) portal
316 (http://www.jgi.doe.gov/Wallemia) and from GenBank (accession number AFQX00000000).
317 Median intergenic distances and intron lengths of *W. sebi* were the shortest compared to the 15
318 other fungal genomes examined (Supplementary Fig. 3 A–B). No segmental duplications and
319 very few tandem duplications were observed. The largest tandem duplication (scaffold_1:67784-
320 78144) includes five tandem genes that code for the Major Facilitator Superfamily of
321 transporters, which are capable of transporting small solutes in response to an osmotic gradient
322 (Pao et al., 1998). A search of the genome (http://genome.jgi-psf.org/Walse1/Walse1.home.html)
323 using the keyword "transport" recovered 549 proteins from filtered models. The largest gene
324 clusters (Supplementary Table 3) do not include *Wallemia*-specific hypothetical proteins and
325 also suggest a lack of significant gene family expansions in this genome.

326 **3.2 Osmoregulation genes**

327 We examined *W. sebi* proteins involved in osmoregulation using comparative genomics
328 and CAFE (De Bie et al., 2006) analyses (Supplementary Fig. 1 A–E). The presence and
329 conservation of the *W. sebi* aqua(glycero)porin genes were compared with Fps1-like (defined by
330 a conserved regulatory region in the N-terminus), Yfl054-like (having a very long N-terminal
331 extension including a conserved stretch), and other (not falling into either of the previous two
332 categories) aquaporin proteins (Pettersson et al., 2005) using CLUSTAL W (Thompson et al.,
333 1994). *Wallemia sebi* has three Yfl054-like aquaglyceroporin genes (31249, 32912, and 59835)
334 (Supplementary Table 4); no aquaporin groups were identified and are presumed to be absent.
335 CAFE analyses inferred the likely MIP family size at the node uniting *W. sebi* with
336 Agaricomycotina to be three, which corresponds with the three aquaglyceroporins found
337 (Supplementary Fig. 1A).

Other genes involved in the osmoregulatory system include those incorporated in the HOG signaling system (Supplementary Fig. 4), which is a mitogen-activated protein kinase (MAPK) cascade (Hohmann et al., 2007; Lenassi et al., 2007). In general, *Wallemia sebi* has the third lowest number of protein kinase (Pkinase) domains from among the 16 Fungi that were examined (Supplementary Fig. 1B), which suggests a significant contraction in Pkinase domains (p-value = 0.015) according to the Viterbi assignments on the branch leading to *W. sebi*. *Wallemia sebi* has two Hog1 homologs (59393 and 60510) that belong to the Pkinase family, which may function in osmotolerance. Genes involved in the ability to live with osmotic stress were also investigated using *in silico* analyses identifying (at $E \leq 1$ E–40) 93 putative osmotic stress proteins (Supplementary Table 4) including the two Hog1-like genes, noted earlier.

Wallemia sebi has many, but not all, of the genes of the HOG pathway including putative homologs of genes encoding various proteins (e.g., Ste11p, Cla4p) involved in activating the upstream HOG pathway (Supplementary Fig. 4; Supplementary Table 4). Downstream targets include orthologs to Rck2p (Teige et al., 2001) and Sgd1p (Lin et al., 2002).

CAFE analyses also suggested gene family size expansions (Supplementary Table 5) compared with the inferred ancestral gene family sizes in the heat shock protein (HSP20) family (PF00011, p-value < 0.001, Supplementary Fig. 1C) and amino acid transporter (AA_trans, PF01490, p-value = 0.017, Supplementary Fig. 1D) family. In addition, the stress responsive A/B barrel domain (Dabb, PF07876, Supplementary Fig. 1E) had an equal or higher copy number compared to other Fungi analyzed.

3.3 Phylogenetic position of Wallemiomycetes

Phylogenetic analyses of 71 proteins (Supplementary Table 2) support *Wallemia* as the earliest diverging lineage of Agaricomycotina (Fig. 1). In RAxML (Stamatakis, 2006) analyses

all branches receive 100% bootstrap support except for the branch uniting *Wallemia* and the remaining Agaricomycotina (96%) and the branch placing Pucciniomycotina as sister to the rest of Basidiomycota (84%) (Fig. 1). SplitsTree4 (Huson and Bryant, 2006) supernetwork analyses show a clear resolution of the Agaricomycetes, Ustilaginomycotina, and Pucciniomycotina, but suggests that there may be conflicting phylogenetic signal among the different genes regarding resolution of the deepest divergences within Basidiomycota, including *Wallemia* and *Filobasidiella* (Tremellomycetes) (Supplementary Fig. 2). In PhyloBayes (Lartillot, 2004) analyses the branch uniting *Wallemia* and the remaining Agaricomycotina is supported with a posterior probability (PP) of 1.0, while the branch placing Pucciniomycotina as sister group to the rest of the Basidiomycota received a PP of 0.96 (Fig. 1). Analyses of the two datasets with varying proportions of rapidly-evolving sites excluded resulted in identical topologies that supported the placement of *Wallemia* as sister group to the rest of Agaricomycotina (96% to 99%), while support for the monophyly of Ustilaginomycotina and Agaricomycotina increased to 96% and 100% (compared to 84% in the full dataset).

3.4 The Septal Pore Apparatus of *Wallemia sebi*

Transmission electron microscopy (TEM) of the septal pore apparatus of *W. sebi* shows hyphal septa with dolipore-like pore swellings (Figs. 2 and 3) characteristic of Agaricomycotina and some Ustilaginomycotina. Sections through the septal pore apparatus revealed adseptal finger-like processes that arise from sheets of endoplasmic reticulum (ER) that form the septal pore cap. These finger-like extensions were concentrated at and directed toward the pore and descend from a cap of ER. Serial sections showed that the processes were extensions of the ER sheets around the septal pore. The membrane of the adseptal process is covered on the cytoplasmic side with electron-dense material. Electron-dense regions were observed at the

384 septal pore. Each region has a substructural pattern that consists of three electron-dense bands
385 alternating with two electron-light bands. The electron-dense bands are consistently found
386 associated with septal pores. An additional electron-dense band at the middle of the septal pore,
387 and striations that appear to be fine fibrils oriented vertically through the septal pore, were
388 apparent in many median sections. The septal pore cap was absent from some septa. Searches for
389 Spc14 or Spc33, which are genes involved in septal pore cap formation that are unique to
390 Agaricomycotina that have perforated septal pore caps (Van Peer et al., 2010), recovered no
391 homologs in the genome of *W. sebi*.

392 **3.5 Mating and meiosis genes**

393 The genome sequence of *W. sebi* was investigated for mating-type gene homologues
394 using protein sequences from *Filobasidiella neoformans*, *Coprinopsis cinerea*, *Ustilago maydis*,
395 and *Malassezia globosa* (Basidiomycota) as queries. Five genes encoding putative
396 homeodomain-motif transcription factors were identified. Comparison of the *W. sebi*
397 homeodomain (HD) proteins against GenBank showed that only one of these, Ws-Sxi1 (PID
398 67157) (Fig. 4), was similar to the HD genes involved in mating in basidiomycetes and was most
399 similar to Sxi1 from *Cryptococcus gattii*, yet highly divergent ($1e^{-5}$). A single *STE3* pheromone
400 receptor (P/R) homologue was identified (PID 59221). Both of the putative mating type genes
401 (Sxi1 and *STE3*) are located near each other (~20 kb apart) at the end of scaffold 2. Inspection of
402 the region in between the two genes identified two other putative mating type genes. Adjacent to
403 the *STE3* gene is a putative pheromone, with a sequence for C-terminal farnesylation. A putative
404 transcription factor encoding a High Mobility Group (HMG) DNA binding motif was also
405 observed as seen in many Ascomycota mating type loci (Lee et al., 2010). However, this gene
406 shows no clear homology to the other mating type-specific HMG genes found in other Fungi by

comparison to GenBank. Comparison of the location of neighboring genes to the genomic location of genes near *STE3* in other Basidiomycota (*F. neoformans*, *M. globosa*, *C. cinerea*, and *U. maydis*) identified conserved synteny suggesting this is the putative mating type region of *W. sebi* (Fig. 4). The arrangement of HD genes as a pair of divergently transcribed HD genes, found in all Basidiomycota except *F. neoformans* is also absent in the putative *MAT* locus of *W. sebi* (Lee et al., 2010). Overall, more conserved synteny was observed between the *STE3* region of *W. sebi* and *M. globosa* (14/34 genes) than with the other three species.

We searched the *W. sebi* genome for eight genes known to be specific to meiosis (e.g., Spo11, Rec8) (Malik et al., 2008) using protein homologs from *S. cerevisiae* and *C. cinerea* (Burns et al., 2010). Candidate *W. sebi* homologs were identified for seven of the eight meiosis-specific genes with the Hop1 homolog apparently absent (Table S6).

3.6 Mode of Conidiogenesis

Conidium development was examined in fixed cells of *W. sebi* using fluorescence and differential interference contrast microscopy (Fig. 5). The conidia develop basipetally with no evidence of meiosis. A phialide-like conidiophore with a single nucleus inside the conidiogenous region develops on the fertile branches of uninucleate hyphae. The nucleus within the conidiogenous region divides and a septum forms between nuclei. The apical nucleus divides to form a pair of conidia, while the basal nucleus apparently migrates back into the base of the conidiophore. It subsequently migrates into the apex of the conidiogenous cell and divides to initiate another round of conidia formation basipetal to the first pair of conidia. Subsequent nuclear divisions and septum formation result in 12 or more conidia from the series of basipetal nuclear divisions.

4. Discussion

Wallemia sebi has one of the smallest genomes reported to date in Basidiomycota, and the smallest in Agaricomycotina; only *M. globosa* (Ustilaginomycotina) has a smaller genome at 9 Mbp (Xu et al., 2007). In contrast, most Basidiomycota genomes are >30 Mbp, and some are the largest known in Fungi. For example, *Uromyces vignae* (Pucciniomycotina) is estimated at 402 Mbp (Kullman et al., 2005). Despite the small genome size, some gene family expansions were observed in *W. sebi* (Supplementary Table 5; Supplementary Fig. 1 A–E). The three most significant gene family expansions, HSP20, Dabb, and AA_trans, may represent adaptations to an osmotically challenging environment. Dabb in particular is known to be upregulated in response to salt stress in the plant *Populus balsamifera* (Finn et al., 2010). The expansion in the AA_trans family could be involved in transporting small solutes across a membrane (Finn et al., 2010), which in turn may also enable *W. sebi* to survive osmotic stress. The ability to live under high osmotic stress may also be correlated with a relatively high number of transporters according to KEGG (Kanehisa et al., 2008), KOG (Koonin et al., 2004), and Pfam domain analyses (Supplementary Table 3; Supplementary Fig. 5 A–B).

Other proteins that have been shown to play a role in adapting to hyperosmotic environments (Duran et al., 2010), include the two Hog1 homologs found in *W. sebi*. Little is known about the function of the three Yfl054-like proteins (Supplementary Table 4); however, the absence of *YFL054* enhances the passive diffusion of ethanol, which in turn suggests the ability to change membrane composition (Pettersson et al., 2005). The absence of orthodox aquaporin genes (*AQY1* and *AQY2*) in *W. sebi* also suggests an osmotolerant lifestyle; for example, it is known that the absence of these aquaporins have resulted in a growth advantage in *S. cerevisiae* after recurring high and low osmolarity conditions (Furukawa et al., 2009). The *in*

453 *silico* analyses identified 93 putative osmotic stress proteins (Supplementary Table 4); however,
454 these did not include all the genes that were found by homology searches of the HOG pathway
455 components (Supplementary Table 4), which demonstrates the desirability of applying several
456 approaches to determine gene identities.

457 Previous analyses (Matheny et al., 2006; Zalar et al., 2005) have alternatively placed
458 Wallemiomycetes at the base of Basidiomycota, as sister to Ustilaginomycotina, or as sister to
459 Agaricomycotina, and the class is currently accepted as *incertae sedis* within Basidiomycota
460 (Hibbett et al., 2007). Our data show that even within a limited taxon sampling, expansion of
461 character sampling to include 71 protein datasets (Supplementary Table 2) have resolved
462 Wallemiomycetes as the earliest diverging lineage of Agaricomycotina (Fig. 1). Relationships
463 between the three subphyla of Basidiomycota have likewise been difficult to definitely resolve,
464 especially in regards to whether Ustilaginomycotina or Pucciniomycotina represent the earliest
465 diverging lineage (Hibbett, 2006). Our results support Ustilaginomycotina as sister to the branch
466 uniting Wallemiomycetes and the remaining Agaricomycotina, and Pucciniomycotina as sister to
467 the rest of Basidiomycota (Fig. 1).

468 Despite two previous studies of the septal pore apparatus of *W. sebi* (Moore, 1986;
469 Terracina, 1974), differences in interpretations of their structure contributed to uncertainty in
470 placement of *Wallemia* within Basidiomycota. Terracina (Terracina, 1974) reported the absence
471 of parenthesomes/septal pore caps in *W. sebi* (similar to members of Entorrhizomycetidae) and
472 an abundance of consistently folded endoplasmic reticulum with dense staining material near the
473 septal pore. Moore (Moore, 1986) interpreted this radiating endoplasmic reticulum as vesiculate
474 parenthesomes similar to those of members of Tremellales and Filobasidiales (Tremellomycetes,
475 Agaricomycotina). Our data (Figs. 2 and 3) show that the septal pore apparatus resembles the

476 *Tremella*-type (Berbee and Wells, 1988), providing further support of its phylogenetic
477 placement, with two notable differences. First, in *Tremella*, the almost uniformly cylindrical
478 saccules open directly into the cytoplasm but in *W. sebi*, the finger-like extensions are not
479 uniformly cylindrical and lack openings on the abseptal side of the pore cap, i.e., saccules are
480 absent, contrary to the report by Moore (Moore, 1986). Second, in *Tremella*, an electron dense
481 layer covers the cytoplasmic face of the inner surface of the saccular membrane (Berbee and
482 Wells, 1988); however, in *W. sebi* the coating is on the outside of the adseptal processes. The
483 septal pore cap is not present at all septa in *W. sebi*, a condition reported in another member of
484 Tremellomycetes (Müller et al., 1988), and that helps to explain the differences in previous
485 interpretations. *Wallemia sebi* is the first species with elaborated septal pore caps, i.e., those with
486 cupulate, reticulate or cylindrical extensions, to be sequenced. The absence of Spc14 and Spc33
487 (Van Peer et al., 2010) suggests that other genes may be involved in the development of septal
488 pore caps with cupulate, reticulate or cylindrical extensions.
489 Molecular evidence, facilitated by detecting homologs of mating and meiosis in genome
490 sequences, can be used to test for sex in organisms that lack any outward evidence of this
491 capacity (Schurko et al., 2009). Mating genes in Basidiomycota fall into two classes of
492 molecules, the HD transcription factors and the pheromones and their cognate G protein-coupled
493 receptors (P/R) (Lee et al., 2010). In bipolar species of Basidiomycota, one or both of these gene
494 classes are found in a single locus, whereas in tetrapolar species, the two gene classes are found
495 in separate, unlinked regions of the genome. The discovery of a single putative mating-type locus
496 (Fig. 4) and a near-complete set of meiosis genes (Supplementary Table 6) leads to the prediction
497 that *W. sebi* is capable of outcrossing and has a bipolar mating system with two mating types
498 analogous to some other Basidiomycota such as *Ustilago hordei* and *Filobasidiella neoformans*,

499 which also have HD and P/R genes linked together in the genome (Lee et al., 2010). However,
500 although Moore (1986) hypothesized that what were interpreted by other authors as asexually
501 produced conidia were, in fact, meiospores, our study of conidiogenesis shows a developmental
502 pattern that makes it extremely unlikely that meiosis occurs during conidia formation (Moore,
503 1986). Thus, the identity of the putative teleomorph of *W. sebi* remains a mystery.
504
505
506 **Acknowledgements:**
507 We thank Francis Martin and Daniel Eastwood for their permission to use unpublished data from
508 the genomes of *Agaricus bisporus* and *Serpula lacrymans*, respectively for the KEGG/KOG
509 analyses. This study was supported by National Science Foundation Assembling the Tree of Life
510 grants: (NSF DEB 0732968) to DSH & MCA and (NSF DEB 0732550) to DJM. The *Wallemia*
511 *sebi* genome project was conducted by the U.S. Department of Energy Joint Genome Institute
512 and supported by the Office of Science of the U.S. Department of Energy under Contract No.
513 DE-AC02-05CH11231. G. Celio and the Imaging Center, College of Biological Sciences,
514 University of Minnesota, provided assistance with the ultrastructural work. We thank the
515 Minnesota Supercomputer Institute for support of the AFTOL Structural and Biochemical
516 Database development. Support was received from the Minnesota Agricultural Experiment
517 Station for the University of Minnesota Mycological Culture Collection. R. Kaur, W.R. Pilcher,
518 K. Stephenson, A. Rodriguez, and the Z. Chen Lab at LSU AgCenter provided valuable lab
519 assistance. We thank C. Clark, K. Damann, and R. Schneider at LSU AgCenter for use of their
520 equipment.
521

References

Altschul, S., Gish, W., Miller, W., Myers, E., Lipman D., 1990. Basic local alignment search tool. J. Mol. Biol. 215**,** 403–410.

Amend, A.S., Seifert, K.A., Samson, R., Bruns, T.D., 2010. Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. Proc. Natl. Acad. Sci. 107**,** 13748–13753.

Berbee, M., Wells, K., 1988. Ultrastructural studies of mitosis and the septal pore apparatus in *Tremella globospora*. Mycologia. 80**,** 479–492.

Birney, E., Durbin, R., 2000. Using GeneWise in the *Drosophila* annotation experiment. *Genome Res*. 10, 547–548.

Burns, C., Stajich, J.E., Rechtsteiner, A., Casselton, L., Hanlon, S.E., Wilke, S.K., Savytskyy, O.P., Gathman, A.C., Lilly, W.W., Lieb, J.D., Zolan, M.E., Pukkila, P.J., 2010. Analysis of the basidiomycete *Coprinopsis cinerea* reveals conservation of the core meiotic expression program over half a billion years of evolution. PLoS Genet. 6**,** e1001135.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17**,** 540–552.

De Bie, T., Cristianini, N., Demuth, J., Hahn, M., 2006. CAFE: a computational tool for the study of gene family evolution. Bioinformatics. 22**,** 1269–1271.

De Hoog, S., Zalar, P., van den Ende, B.G., Gunde-Cimerman, N., Relation of Halotolerance to Human-Pathogenicity in the Fungal Tree of Life: An Overview of Ecology and Evolution under Stress, in: N. Gunde-Cimerman, Oren, A., Plemenitas, A., (Eds.), Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya. Springer, Dordrecht, Netherlands, 2005, pp. 371–395.

545 Domsch, K.H., Gams, W., Anderson, T.H., 1980. Compendium of soil fungi. Academic Press
546 Ltd., London.
547 Duran, R., Cary, J.W., Calvo, A.M., 2010. Role of the Osmotic Stress Regulatory Pathway in
548 Morphogenesis and Secondary Metabolism in Filamentous Fungi. Toxins. 2**,** 367–381.
549 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high
550 throughput. Nucleic Acids Res. 32**,** 1792–1797.
551 Ellis, E., 2006. Solutions to the problem of substitution of ERL 4221 for vinyl cyclohexene
552 dioxide in Spurr low viscosity embedding formulations. Microsc. Today. 14**,** 32–33.
553 Enright, A., Van Dongen, S., Ouzounis, C., 2002. An efficient algorithm for large-scale detection
554 of protein families. Nucleic Acids Res. 30**,** 1575–1584.
555 Felsenstein, J., 1981. PHYLIP: Phylogeny inference package (version 3.2). Cladistics. 5**,** 164–
556 166.
557 Finn, R., Mistry, J., Tate, J., Coggill, P., Heger, A., Pollington, J., Gavin, O., Gunesekaran, P.,
558 Ceric, G., Forslund, K., Eddy, S.R., Sonnhammer, E.L., Bateman, A., 2010. The Pfam
559 protein families database. Nucleic Acids Res. 38**,** D211–222.
560 Furukawa, K., Sidoux-Walter, F., Hohmann, S., 2009. Expression of the yeast aquaporin Aqy2
561 affects cell surface properties under the control of osmoregulatory and morphogenic
562 signalling pathways. Mol. Microbiol. 74**,** 1272–1286.
563 Gunde-Cimerman, N., Ramos, J., Plemenitas, A., 2009. Halotolerant and halophilic fungi.
564 Mycol. Res. 113**,** 1231–1241.
565 Gunde-Cimerman, N., Zalar, P., de Hoog, S., Plemenitas, A., 2000. Hypersaline waters in
566 salterns - natural ecological niches for halophilic black yeasts. FEMS Microbiol. Ecol.
567 32**,** 235–240.

568 Hashmi, M.H., Morgan-Jones, G., 1973. Conidium ontogeny in hyphomycetes. The meristem
569 arthrospores of *Wallemia sebi*. Can. J. Bot. 51**,** 1669–1671.
570 Hibbett, D.S., 2006. A phylogenetic overview of Agaricomycotina. Mycologia. 98**,** 917–925.
571 Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf,
572 S., James, T., Kirk, P.M., Lücking, R., Thorsten Lumbsch, H., Lutzoni, F., Matheny,
573 P.B., McLaughlin, D.J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W.,
574 Stalpers, J.A, Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny,
575 G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W.,
576 Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D.,
577 Ironside, J.E., Kõljalg, U., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J.,
578 Miadlikowska, J., Miller, A., Moncalvo, J-M., Mozley-Standridge, S., Oberwinkler, F.,
579 Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schüssler,
580 A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir,
581 A., Weiss, M., White, M.M., Winka, K., Yao, Y.J., Zhang, N. 2007. A higher-level
582 phylogenetic classification of the Fungi. Mycol. Res. 111**,** 509–547.
583 Hohmann, S., 2009. Control of high osmolarity signalling in the yeast *Saccharomyces cerevisiae*.
584 FEBS Letters. 583**,** 4025–4029.
585 Hohmann, S., Krantz, M., Nordlander, B., 2007. Yeast osmoregulation. Methods Enzymol. 428**,**
586 29–45.
587 Huson, D.H., Bryant, D., 2006. Application of Phylogenetic Networks in Evolutionary Studies.
588 Mol. Biol. Evol. 23**,** 254–267.
589 Huson, D.H., Dezulian, T., Klopper, T., Steel, M.K., 2004. Phylogenetic super-networks from
590 partial trees. IEEE/ACM T. Comput. Bi. 1**,** 151–158.

591 Isono, K., McIninch, J., Borodovsky, M., 1994. Characteristic features of the nucleotide
592 sequences of yeast mitochondrial ribosomal protein genes as analyzed by computer
593 program GeneMark. DNA Res. 1**,** 263–9.
594 Johan-Olsen, O., 1887. Op sop på klipfisk den såkaldte mid. Christiania Videnkabs-Selskab
595 Forhandl. 12**,** 5.
596 Jurka, J., Kapitonov, V., Pavlicek, A., Klonowski, P., Kohany, O., Walichiewicz, J., 2005.
597 Repbase Update, a database of eukaryotic repetitive elements. Cytogenet. Genome Res.
598 110**,** 462–467.
599 Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayama, T.,
600 Kawashima, S., Okuda, S., Tokimatsu, T. Yamanishi, Y., 2008. KEGG for linking
601 genomes to life and the environment. Nucleic Acids Res. 36**,** D480-D484.
602 Kent, W., 2002. BLAT--the BLAST-like alignment tool. Genome Res. 12**,** 656–664.
603 Koonin, E., Fedorova, N., Jackson, J., Jacobs, A., Krylov, D., Makarova, K., Mazumder, R.,
604 Mekhedov, S., Nikolskaya, A., Rao, B.*,* Rogozin, I.B., Smirnov, S., Sorokin, A.V.,
605 Sverdlov, A.V., Vasudevan, S., Wolf, Y.I., Yin, J.J., Natale, D.A., 2004. A
606 comprehensive evolutionary classification of proteins encoded in complete eukaryotic
607 genomes. Genome Biol. 5**,** R7.
608 Kralj Kunčič, M., Kogej, T., Drobne, D., Gunde-Cimerman, N., 2010. Morphological Response
609 of the Halophilic Fungal Genus *Wallemia* to High Salinity. Appl. Environ. Microbiol. 76**,**
610 329–337.
611 Kramer, C., Isolation of total RNA from *Neurospora* mycelium, in: E. Rosato, (Ed.), Circadian
612 Rhythms: Methods and protocols. Humana Press Inc., Totowa, New Jersey, 2007, pp.
613 291–303.

614 Krantz, M., Becit, E., Hohmann, S., 2006. Comparative genomics of the HOG-signalling system
615 in fungi. Curr. Genet. 49**,** 137–151.
616 Kullman, B., Tamm, H., Kullman, K., Fungal Genome Size Database.
617 <http://www.zbi.ee/fungal-genomesize/>. 2005.
618 Kumar, S., Skjaeveland, A., Orr, R.J.S., Enger, P., Ruden, T., Mevik, B-H,. Burki, F., Botnen,
619 A., Shalchian-Tabrizi, K., 2009. AIR: A batch-oriented web program package for
620 construction of supermatrices ready for phylogenomic analyses. BMC Bioinformatics.
621 10**,** 357.
622 Kumar, T., Celio, G., Matheny, P.B., McLaughlin, D.J., Hibbett, D.S., Manimohan, P., 2007.
623 Phylogenetic relationships of *Auriculoscypha* based on ultrastructural and molecular
624 studies. Mycol. Res. 111**,** 268–274.
625 Lartillot, N., 2004. A Bayesian Mixture Model for Across-Site Heterogeneities in the Amino-
626 Acid Replacement Process. Mol. Biol. Evol. 21**,** 1095–1109.
627 Lartillot, N., Brinkmann, H., Philippe, H., 2007. Suppression of long-branch attraction artifacts
628 in the animal phylogeny using a site-heterogeneous model. BMC Evol. Biol. 7**,** S4.
629 Lee, S. C., Ni, M., Li, W., Shertz, C., Heitman, J., 2010. The Evolution of Sex: a Perspective
630 from the Fungal Kingdom. Microbiol. Mol. Biol. Rev. 74**,** 298–340.
631 Lenassi, M., Vaupotic, T., Gunde-Cimerman, N., Plemenitas, A., 2007. The MAP kinase
632 HwHog1 from the halophilic black yeast *Hortaea werneckii*: coping with stresses in solar
633 salterns. Saline Systems. 3**,** 3.
634 Lin, H., Nguyen, P., Vancura, A., 2002. Phospholipase C interacts with Sgd1p and is required for
635 expression of *GPD1* and osmoresistance in *Saccharomyces cerevisiae* Mol. Genet.
636 Genomics. 267**,** 313–320.

637 Liu, W.C., Li, C.Q., Zhu, P., Yang, J.L., Cheng, K.D., 2010. Phylogenetic diversity of culturable
638 fungi associated with two marine sponges: *Haliclona simulans* and *Gelliodes carnosa*,
639 collected from the Hainan Island coastal waters of the South China Sea. Fungal Divers.
640 42**,** 1–15.
641 Lowe, T., Eddy, S., 1997. tRNAscan-SE: a program for improved detection of transfer RNA
642 genes in genomic sequence. Nucleic Acids Res. 25**,** 955–964.
643 Madelin, M.F., Dorabjee, S., 1974. Conidium ontogeny in *Wallemia sebi*. T. Brit. Mycol. Soc.
644 63**,** 121–130, IN14–IN15.
645 Malik, S.-B., Pightling, A.W., Stefaniak, L.M., Schurko, A.M., Logsdon, J.M .Jr., 2008. An
646 Expanded Inventory of Conserved Meiotic Genes Provides Evidence for Sex in
647 *Trichomonas vaginalis*. PLoS ONE. 3**,** e2879.
648 Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J.,
649 Braverman, M.S., Chen, Y.J., Chen, Z., Dewell, S.B., Du, L., Fierro, J.M., Gomes, X.V.,
650 Godwin, B.C., He, W., Helgesen, S., Ho, C.H., Irzyk, G.P., Jando, S.C., Alenquer, M.L.,
651 Jarvie, T.P., Jirage, K.B., Kim, J.B., Knight, J.R., Lanza, J.R., Leamon, J.H., Lefkowitz,
652 S.M., Lei, M., Li, J., Lohman, K.L., Lu, H., Makhijani, V.B., McDade, K.E., McKenna,
653 M.P., Myers, E.W., Nickerson, E., Nobile, J.R., Plant, R., Puc, B.P., Ronan, M.T., Roth,
654 G.T., Sarkis, G.J., Simons, J.F., Simpson, J.W., Srinivasan, M., Tartaro, K.R., Tomasz,
655 A., Vogt, K.A., Volkmer, G.A., Wang, S.H., Wang, Y., Weiner, M.P., Yu, P., Begley,
656 R.F., Rothberg, J.M., 2005. Genome sequencing in microfabricated high-density picolitre
657 reactors. Nature. 437**,** 376–380.

Matheny, P.B., Gossmann, J.A., Zalar, P., Kumar, T.K.A., Hibbett, D.S., 2006. Resolving the phylogenetic position of the Wallemiomycetes: an enigmatic major lineage of Basidiomycota. Can. J. Bot. 84**,** 1794–1805.

Melen, K., Krogh, A., von Heijne, G., 2003. Reliability measures for membrane protein topology prediction algorithms. J. Mol. Biol. 327**,** 735–744.

Miskei, M., Karányi, Z,. Pócsi, I., 2009. Annotation of stress-response proteins in the aspergilli. Fungal Genet. Biol. 46**,** S105–S120.

Moore, R., 1986. A note on *Wallemia sebi*. Anton. Leeuw. 52**,** 183–187.

Müller, W., Montijn, R., Humbel, B., van Aelst, A., Boon, E., van der Krift, T., Boekhout, T., 1988. Structural differences between two types of basidiomycete septal pore caps. Microbiol. 144**,** 1721–1730.

Murray, H.G., Thompson, W.F., 1980. Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 8, 4321–4325

Nielsen, H., Engelbrecht, J., Brunak, S., von Heijne, G., 1997. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng. 10**,** 1–6.

Pao, S.S., Paulsen, I.T., Saier, M.H.Jr., 1998. Major Facilitator Superfamily. Microbiol. Mol. Biol. Rev. 62, 1–34.

Pettersson, N., Filipsson, C., Becit, E., Brive, L., Hohmann, S., 2005. Aquaporins in yeasts and filamentous fungi. Biol. Cell. 97**,** 487–500.

Pitt, J.I., Hocking, A.D., 2009. Xerophiles, in: Fungi and Food Spoilage. Springer US, pp. 339–355.

Rambaut, A., Charleston, M., TreeEdit: phylogenetic tree editor version 1. Univ. of Oxford, Oxford, UK, 2001.

681 Richards, E., Reichardt, M., Rogers, S., 1994. Preparation of Genomic DNA from Plant Tissue.
682 Curr. Protoc. Mol. Biol. 27, 2.3.1–2.3.7.
683 Robbertse, B., Yoder, R.J., Boyd, A., Reeves, J., Spatafora, J.W., 2011. Hal: an Automated
684 Pipeline for Phylogenetic Analyses of Genomic Data. PLoS Curr. 3**,** RRN1213.
685 Salamov, A., Solovyev, V., 2000. *Ab initio* gene finding in *Drosophila* genomic DNA. Genome
686 Res 10**,** 516–522.
687 Samson, R. A., Hoekstra, E.S., Frisvad, J.C., 2004. Introduction to food- and airborne fungi.
688 Centraalbureau voor Schimmelcultures (CBS), Utrecht.
689 Schurko, A. M., Neiman, M., Logsdon Jr, J.M., 2009. Signs of sex: what we know and how we
690 know it. Trends Ecol. Evol. 24**,** 208–217.
691 Smit, A., Hubley, R., Green, P., RepeatMasker Open-3.0 <www.repeatmasker.org>. 1996-2010.
692 Spurr, A., 1969. A low viscosity resin embedding medium for electron microscopy. J. Ultra.
693 Res. 26**,** 31–43.
694 Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with
695 thousands of taxa and mixed models. Bioinformatics. 22**,** 2688–2690.
696 Stamatakis, A., Hoover, P., Rougemont, J., 2008. A Rapid Bootstrap Algorithm for the RAxML
697 Web Servers. Syst. Biol. 57**,** 758–771.
698 Tanghe, A., Van Dijck, P., Thevelein, J.M., 2006. Why do microorganisms have aquaporins?
699 Trends Microbiol. 14**,** 78–85.
700 Teige, M., Scheikl, E., Reiser, V., Ruis, H., Ammerer, G., 2001. Rck2, a member of the
701 calmodulin-protein kinase family, links protein synthesis to high osmolarity MAP kinase
702 signaling in budding yeast. Proc. Natl. Acad. Sci. 98**,** 5625–5630.
703 Terracina, F.C., 1974. Fine structure of the septum in *Wallemia sebi*. Can. J. Bot. 52**,** 2587–2590.

Thompson, J., Higgins, D., Gibson, T., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22**,** 4673–4680.

Trong, S., LaButti, K., Foster, B., Han, C., Brettin, T., Lapidus, A., Gap Resolution: A Software Package for Improving Newbler Genome Assemblies. Proceedings of the 4th Annual Meeting on Sequencing Finishing, Analysis in the Future, 2009, pp. 35.

Van Peer, A.F., Wang, F., Van Driel, K.G.A., De Jong, J.F., Van Donselaar, E.G., Müller, W.H., Boekhout, T., Lugones, L.G., Wösten, H.A.B., 2010. The septal pore cap is an organelle that functions in vegetative growth and mushroom formation of the wood-rot fungus *Schizophyllum commune*. Environ. Microbiol. 12**,** 833–844.

Xu, J., Saunders, C.W., Hu, P., Grant, R.A., Boekhout, T., Kuramae, E.E., Kronstad, J.W., DeAngelis, Y.M., Reeder, N.L., Johnstone, K.R., Leland, M., Fieno, A.M., Begley, W.M., Sun, Y., Lacey, M.P., Chaudhary, T., Keough, T., Chu, L., Sears, R., Yuan, B., Dawson, T.L. Jr., 2007. Dandruff-associated *Malassezia* genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. Proc. Natl. Acad. Sci. 104**,** 18730–18735.

Zalar, P., Sybren de Hoog, G., Schroers, H-J., Frank, J.M., Gunde-Cimerman, N., 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, cl. et ord. nov.). Anton. Leeuw. 87**,** 311–328.

Zdobnov, E., Apweiler, R., 2001. InterProScan--an integration platform for the signature-recognition methods in InterPro. Bioinformatics. 17**,** 847–848.

Zerbino, D., Birney, E., 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res. 18**,** 821–829.

Figure legends:

Figure 1. Consensus topology and branch lengths from PhyloBayes analyses of 71 protein datasets. Support values are shown as PhyloBayes posterior probability/RAxML bootstrap frequencies from analyses of the full dataset using the CAT model implemented in PhyloBayes/RAxML analyses using the WAG+G+I model excluding the 7th and 8th rate categories/analyses excluding 6th, 7th, and 8th rate categories. Branches for which support values are not depicted were supported by maximal values in all four analyses. Details of analyses are provided in supplementary material. *Batrachochytrium dendrobatidis* was selected as an outgroup.

Figure 2. Transmission electron micrographs of septal pore organization in *Wallemia sebi* (CBS 633.66). A. Median longitudinal section through a septal pore apparatus showing septal pore swelling (se), septal pore (P), and associated adseptal finger-like processes (AP). B. Section through the septal pore apparatus of another septum showing adseptal processes. C–F. Serial sections tracing finger-like processes from near the pore towards the margin of the septal pore cap. Finger-like processes, arising from sheets of endoplasmic reticulum (ER) that form the septal pore cap, were found concentrated at and directed towards the pore. G. Longitudinal section through finger-like processes that extend from the ER sheets around the septal pore. The membrane (M) of the adseptal process is covered on the cytoplasmic side with electron-dense material (Dm). H. Finger-like processes descending from the septal pore cap. The membrane of the finger-like processes is continuous (arrowhead) with that of the pore cap. I. Cross section of adseptal processes. J. Septal pore apparatus in which septal pore caps were not observed. K.

Electron dense region (Edr) at the septal pore. The region has a substructural pattern consisting of three electron-dense bands alternating with two electron-light bands. An additional electron dense band (b) at the middle of the septal pore, and striations that appear to be fine fibrils (f) oriented vertically through the septal pore are visible. Figures 2 A–K: copyright Regents of the University of Minnesota and David McLaughlin. Bars = 0.1 μm.

Figure 3. Three-dimensional reconstruction of the septal pore apparatus in *Wallemia sebi*. AP, adseptal finger-like processes; Dm, electron-dense material; Edr, electron dense region; ER, endoplasmic reticulum; M, membrane; P, septal pore; se, septal pore swelling. Figure 3: copyright Regents of the University of Minnesota and David McLaughlin.

Figure 4. Mating type locus in *Wallemia sebi*. Genes shown in red are mating type homologs, genes shown in light blue are syntenic in at least two of the following Basidiomycota—*Filobasidiella neoformans*, *Coprinopsis cinerea*, *Ustilago maydis*, and *Malassezia globosa*—genes in orange are syntenic in all four species, and genes in white are not syntenic and/or are unique to *W. sebi*.

Figure 5. Fluorescence and differential interference contrast (DIC) micrographs of fixed cells of *Wallemia sebi* (CBS 633.66) stained with DAPI showing stages of conidiophore development, conidia formation and associated nuclear behavior. Each pair of figures illustrates DAPI stained cells (left) with nuclei (N) fluorescing bright blue and DIC image of the same cells (right). A–B. A young developing conidiophore with an apical swelling and a constriction (thick arrow) below the swollen apex. Note the single nucleus situated below the apical portion. C–D. Conidiophore

with slightly elongated apical region and constriction (arrow) with the nucleus now in the apical area. E–F. A phialide-like conidiophore with a single nucleus inside the conidiogenous region or zone and another nucleus below the constriction (arrow). G–H. The single nucleus within the conidiogenous region has divided and each nucleus has been separated by septa (thin arrows) between them. Another nucleus is migrating into the base of the conidiogenous region through the constriction. I–J. A conidiophore with a nucleus at the base of the conidiogenous region and two uninucleate (young) conidia. K–L. Two binucleate conidiogenous cells, the lower of which appears to be the result of a recent mitotic division. Septa separating the two conidiogenous cells are clear from the DIC images (arrows). M–N. Septal development results in the formation of four uninucleate conidia borne on the phialide-like conidiophore. O–P. A new cell (nc), which is a meristem-like extension of the conidiophore apex, is formed basipetally. Q–T. Stages leading to the formation of the six conidia-cell stage by nuclear division and subsequent septal delimitation in the newly formed basal cell. U–X. Eight and 10 conidia-cell stages resulting from basipetal nuclear division and wall development. Note the persistent nucleus at the base of the conidiophore in Fig. 5U. Bars = 3 μm.

Supplementary Figure 1. Results of the CAFE analyses showing gene family expansions/extractions for five protein families. Gene family size is inferred at each node and terminal branch. A. Major Intrinsic Protein family; B. Protein kinase domains; C. Heat shock protein (HSP20) family; D. Amino acid transporter family; E. Stress response A/B barrel domain proteins (Dabb).

795 Supplementary Figure 2. Consensus supernetwork of 71 single-protein trees constructed with
796 SplitsTree4.
797
798 Supplementary Figure 3. Comparison of *Wallemia sebi* genome with 15 other fungal genomes.
799 A. Median intron lengths; B. Median intergenic distances (in blue) and median coding DNA
800 sequences (CDS) (in red).
801
802 Supplementary Figure 4. Reconstruction of the HOG pathway with *Wallemia sebi* genes based
803 on the model pathway of *Saccharomyces cerevisiae*. Note apparent reduction in some SHO1
804 branch components and loss of some homologous transcription factors.
805
806 Supplementary Figure 5. KEGG and KOG analyses comparing numbers of predicted genes
807 found in *W. sebi*, *Agaricus bisporus* H97, and *Serpula lacrymans* S7.9. A. KEGG annotation of
808 predicted genes; B. KOG classification of predicted genes.
809

Figure 1

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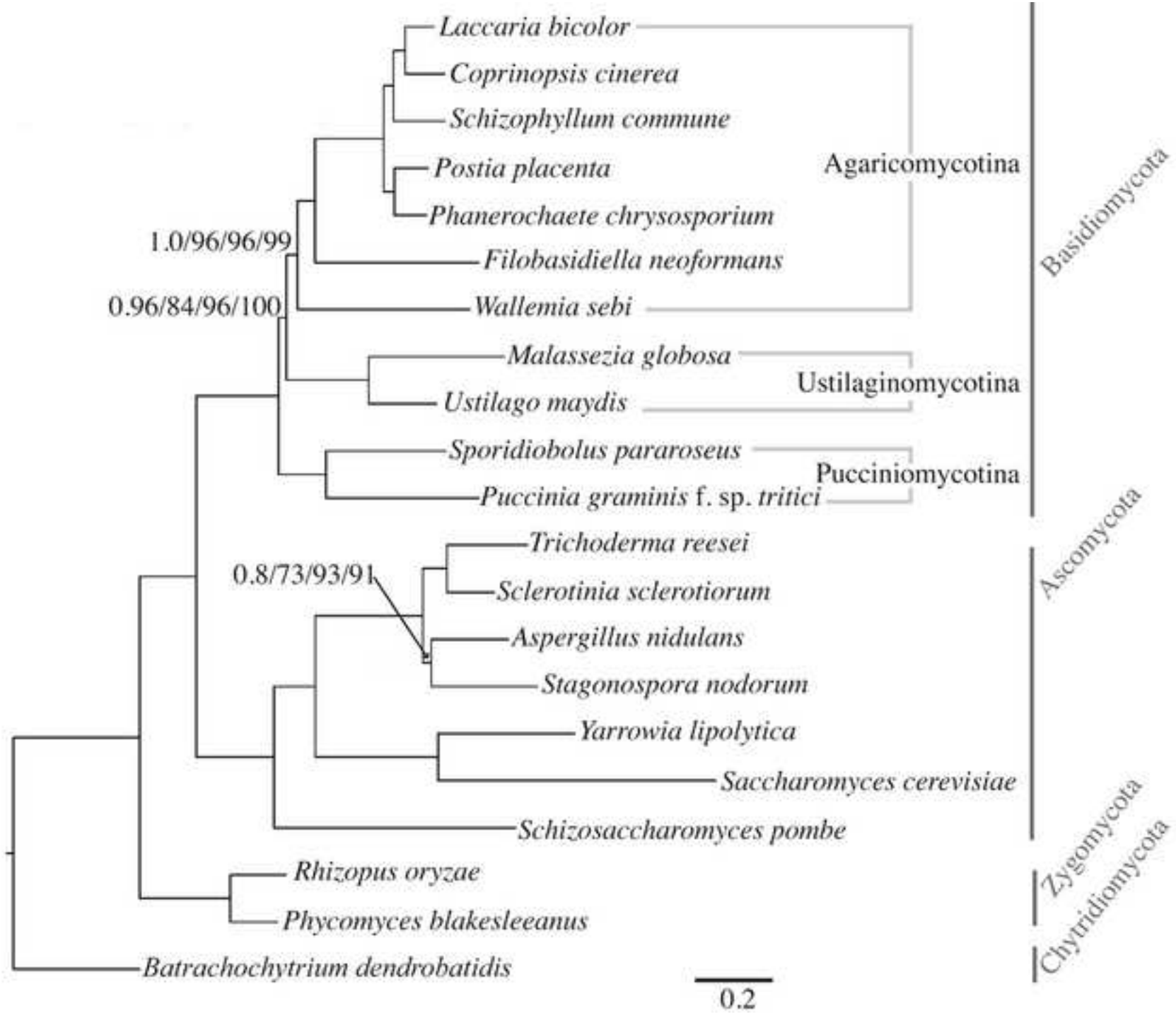

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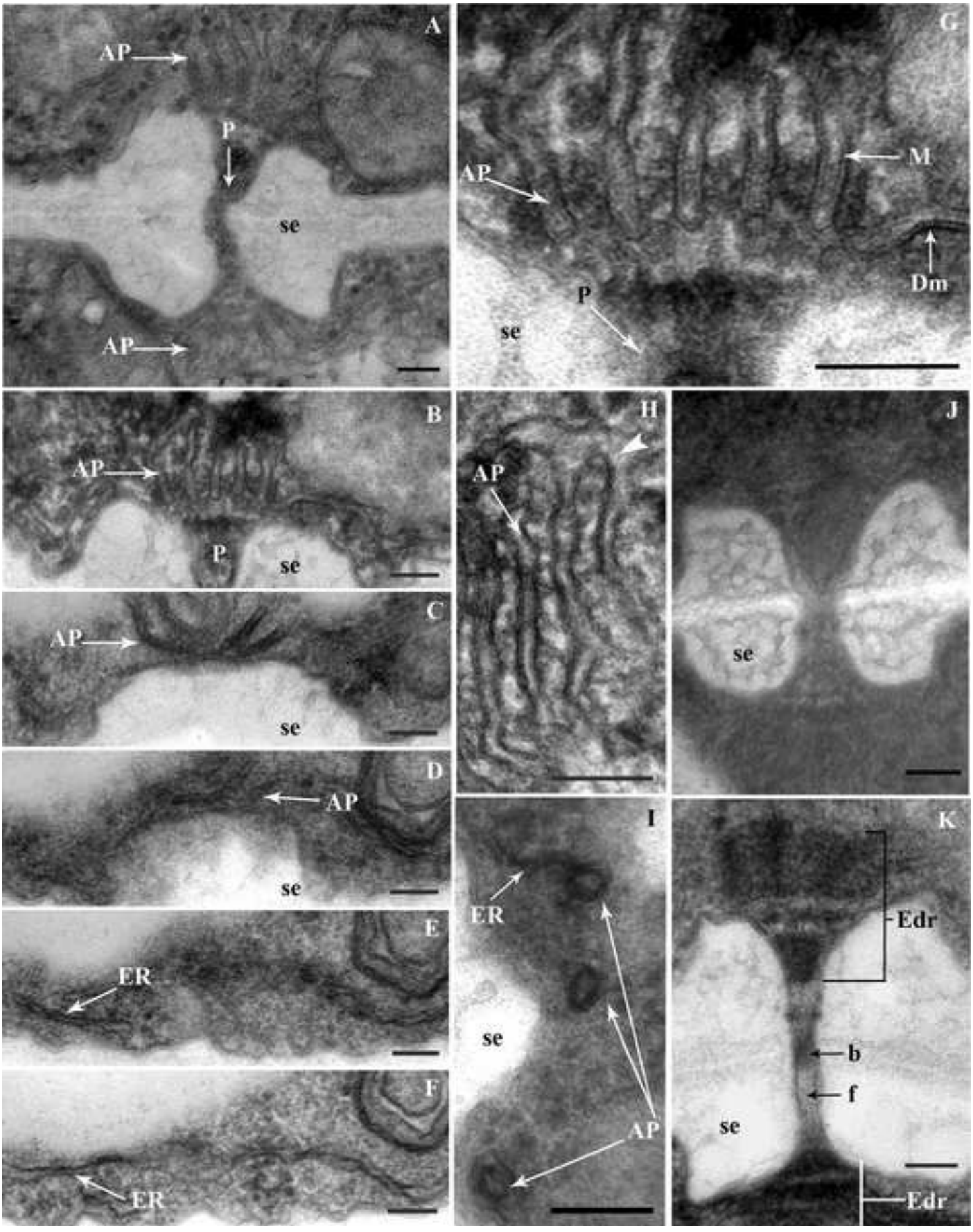

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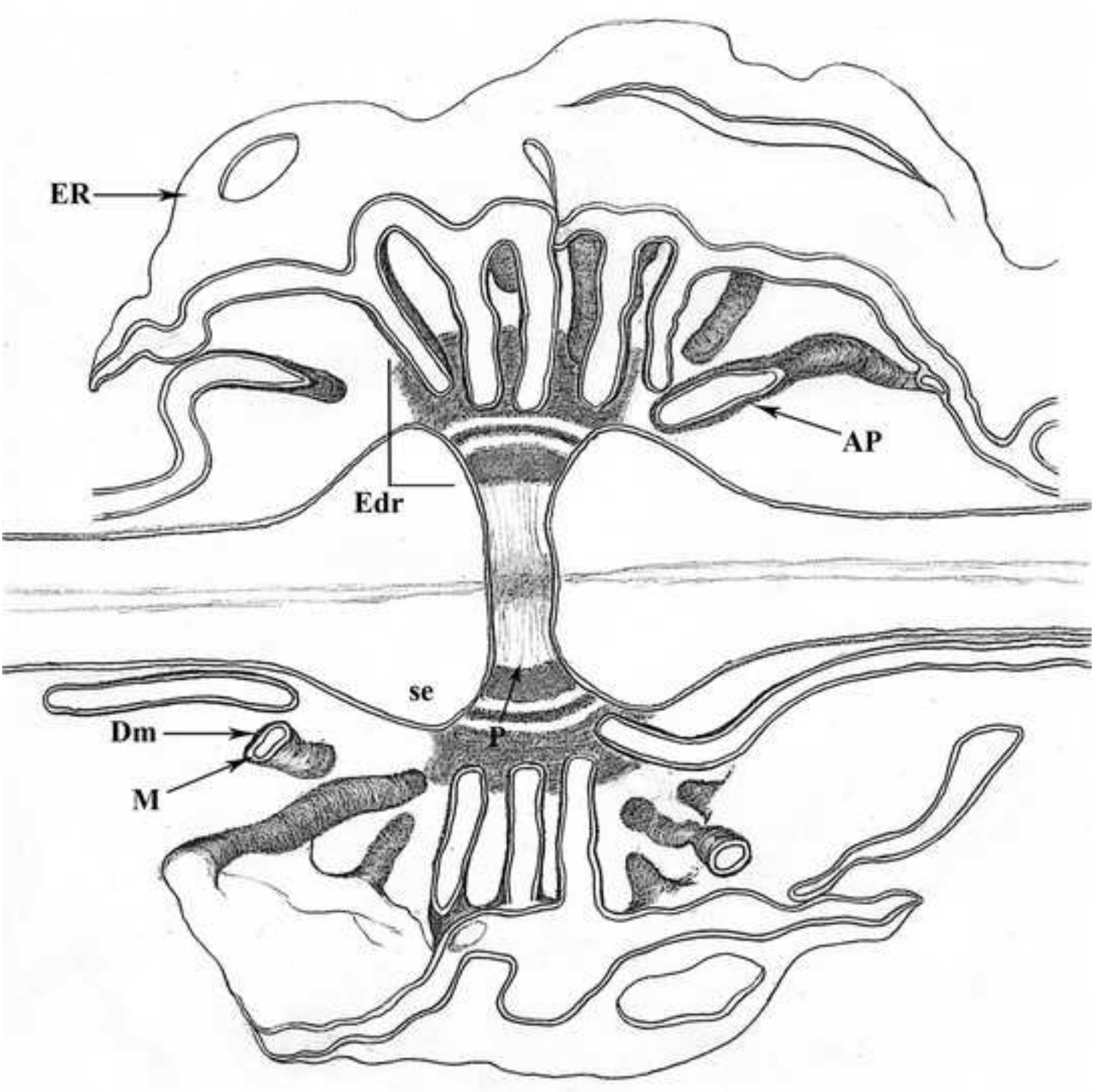

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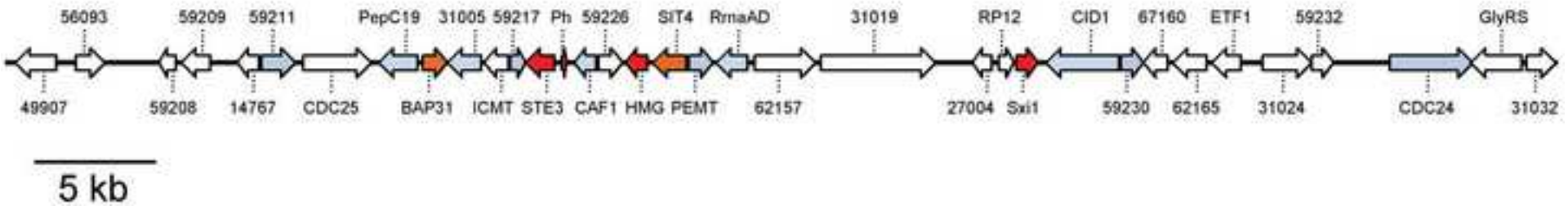

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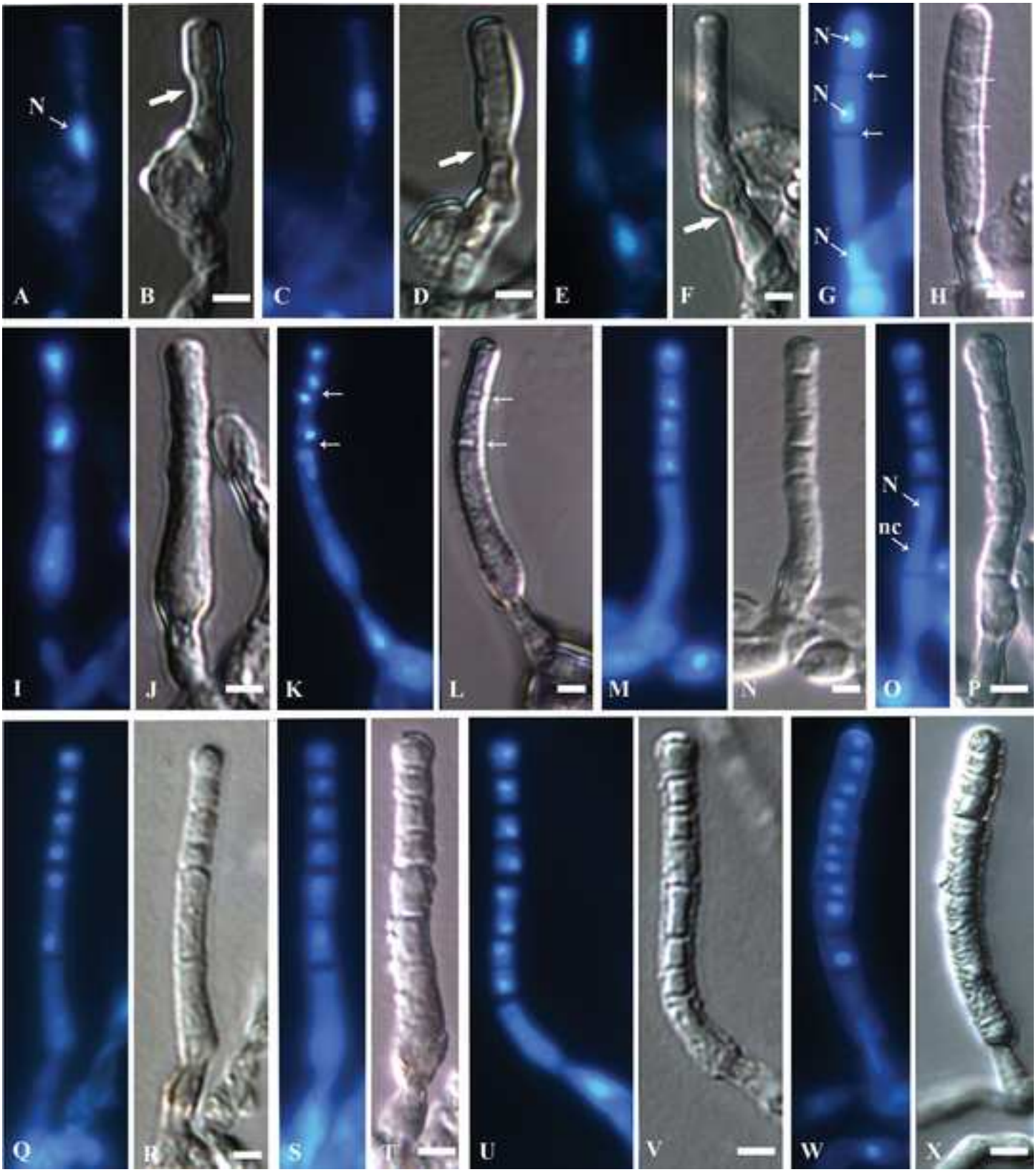

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