

Basidioascus undulatus: genome, origins, and sexuality

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Abstract: *Basidioascus undulatus* is a soil basidiomycete belonging to the order *Geminibasidiales*. The taxonomic status of the order was unclear as originally it was only tentatively classified in the class *Wallemiomycetes*. The fungi in *Geminibasidiales* have an ambiguously defined sexual cycle. In this study, we sequenced the genome of *B. undulatus* to gain insights into its sexuality and evolutionary origins. The assembled genome draft was approximately 32 Mb in size, had a median nucleotide coverage of 24X, and contained 6123 predicted genes. Previous morphological descriptions of *B. undulatus* relied on interpretation of putative sexual structures. In this study, nuclear staining and confocal microscopy showed meiosis occurring in basidia and genome analysis confirmed the existence of genes involved in meiosis and mating. Using 35 protein-coding genes extracted from genomic information, phylogenomic and molecular dating analyses confirmed that *B. undulatus* indeed belongs to a lineage distantly related to *Wallemia* while retaining a basal position in *Agaricomycotina*. These results, combined with differences in septal pore morphology, led us to move the order *Geminibasidiales* out of the *Wallemiomycetes* and into the new class *Geminibasidiomycetes* cl. nov. Finally, the concept of *Agaricomycotina* is emended to include both *Wallemiomycetes* and *Geminibasidiomycetes*.

Key words:

Agaricomycotina

Basidiomycota

Geminibasidiomycetes

septal pore ultrastructure

Wallemiomycetes

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INTRODUCTION

Using morphological characters, Matsushima (2003) described *Basidioascus undulatus* as the only species in the new genus *Basidioascus* based on a single strain isolated from the tropical rainforest soil in Cape Tribulation National Park, Queensland, Australia. Matsushima (2003) photographed fertile structures of *B. undulatus*, which he interpreted as asci giving rise to ascospores, bearing hooks that could be either croziers or clamps. The genus was named *Basidioascus* after what he interpreted as “basidia-like asci” and the species epithet *undulatus* was given to recall “ascospores with a wavy wall” (Matsushima 2003). Also, *B. undulatus* produced a geotrichum-like asexual morph in culture, characterized by chains of aseptate arthroconidia. MycoBank (Robert *et al.* 2013) and *Ainsworth & Bisby's Dictionary of the Fungi* (Kirk *et al.* 2008) classified *B. undulatus* in *Saccharomycetes* because the asexual morph was assumed to be a *Geotrichum* (a genus typified by an asexual morph and usually associated with sexual morphs in *Galactomyces* or *Dipodascus*).

During a survey of heat resistant fungi in Canadian soils, Nguyen *et al.* (2013) isolated nine additional strains of *B.*

undulatus, and a second species of *Basidioascus*, named *B. magus*. A third yeast-like species, *B. persicus*, was recently described from soil in Iran (Nasr *et al.* 2014). Soil appears to be the main habitat for *Basidioascus* species and their distribution is probably broad. However, their ecological role is currently unknown, but they are presumably saprobic as are many soil inhabiting fungi (Domsch *et al.* 1980).

Surprisingly, phylogenetic analyses with rDNA sequences showed that *Basidioascus* was related to *Wallemiomycetes* (*Basidiomycota*) rather than *Saccharomycetes* (*Ascomycota*) (Nguyen *et al.* 2013). This finding initiated a revision of its taxonomy and a re-interpretation of its morphology as a basidiomycete. The structures identified as asci and ascospores by Matsushima (2003) are reinterpreted as thick-walled basidiospores, and the subtending cell as a basidium that usually produces a single basidiospore. Most unusual was that the basidia appeared to be forcibly discharged, leaving them collapsed with the basidiospore still attached by a long, cylindrical sterigma (Nguyen *et al.* 2013). The species of *Basidioascus*, and of its sister genus *Geminibasidium* (*G. donsium* and *G. hirsutum*), were classified in the new order *Geminibasidiales* (Nguyen *et al.* 2013). The *Geminibasidiales* are a phylogenetic sister

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group to *Wallemiales* and were placed tentatively under the class *Wallemiomycetes* (Nguyen *et al.* 2013). *Wallemiales* currently includes a single genus *Wallemia* with three species: *W. sebi*, *W. muriae*, and *W. ichthyophaga* (Zalar *et al.* 2005). The phylogenetic placement of *Wallemiomycetes* in the fungal kingdom was at first ambiguous (Matheny *et al.* 2006) because only a few protein coding genes were used in phylogenetic analyses and because ribosomal genes did not provide robustly supported conclusions. However, a few recent studies, through phylogenomic analyses with a large number of protein coding genes, demonstrate that this lineage is an early diverging one within *Agaricomycotina* (Padamsee *et al.* 2012, Zajc *et al.* 2013).

In this study, our first objective was to gain further insight into the sexuality of *B. undulatus* because the structures referred to as basidia and basidiospores were only putatively identified as such (Nguyen *et al.* 2013). For this purpose, we performed nuclear staining on these presumed sexual structures and observed them with laser confocal microscopy. Further, we sequenced the genome of *B. undulatus* and looked for genes involved in meiosis and mating to support our findings. Our second objective was to resolve the tentative placement of *Geminibasidiales* in *Wallemiomycetes*. We conducted phylogenomic analysis using 35 single copy protein-coding genes from the *B. undulatus* genome and we performed a molecular clock analysis to date the divergence of *B. undulatus* from *Wallemia* species and other fungi. The third objective was to investigate the septal pore morphology, which has proved significant in basidiomycete systematics, especially at class rank and particularly in lineages of *Agaricomycotina* (van Driel *et al.* 2009). We imaged the septal pore of *B. undulatus*, *G. donsium*, and *W. sebi* using transmission electron microscopy to support our interpretation of the higher classification of the *Geminibasidiales*.

MATERIALS AND METHODS

Growth, DNA extraction and sequencing

The ex-type strain of *Basidioascus undulatus* (DAOM 241956) was inoculated in 2 % malt extract broth in an Erlenmeyer flask on an orbital shaker at 25 °C for 2 wk. The broth culture was transferred to two 50 mL Falcon tubes and centrifuged at 10000 × g for 5 min. The liquid was decanted, leaving only the fungal tissue. The fungal tissue was frozen in liquid nitrogen and crushed with a sterile pestle. DNA was extracted with the OmniPrep kit (G-Biosciences, St Louis, MO) following the manufacturer's instructions. DNA quality and quantity were verified with Qbit (Life Technologies, Burlington, Canada). Whole-genome sequencing (101 base pairs (bp) paired-end) was performed on an Illumina HiSeq 2500 with TrueSeq V3 chemistry at the National Research Council Canada facility in Saskatoon (Saskatchewan).

Genome assembly and annotation

The quality of the reads was checked with the program FastQC v. 0.10.1 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Using fastx_trimmer (part of the FASTX-Toolkit v. 0.0.13; http://hannonlab.cshl.edu/fastx_toolkit/), eight bases from the 5' end were trimmed to yield reads of

93 bp in length of higher quality. *De novo* assembly was performed using SPAdes v. 3.0 (Bankevich *et al.* 2012) with the BayesHammer error correction (Nikolenko *et al.* 2013) and mismatch correction enabled (parameters: --careful and k=21, 31, 41, 51, 61, 71, 81, 91). Final contigs were assembled into scaffolds with SSPACE v2.0 (Boetzer *et al.* 2011) (parameters: -x 1 -m 45 -o 10 -t 0 -r 0.7 -k 5 -a 0.7 -n 10 -z 2000 -T 16 -p 1) and contigs shorter than 2000 bp were discarded. Assembly statistics were generated with QUAST v. 2.3 (Gurevich *et al.* 2013). The assembly was checked by aligning the corrected reads onto the scaffolds using Bowtie2 v2.1.0 (Langmead & Salzberg 2012). Alignments produced by Bowtie2 in SAM format were converted to sorted BAM format by SAMtools v. 0.1.19 (Li *et al.* 2009) and statistics for coverage were generated with Qualimap v. 0.8.1 (Garcia-Alcalde *et al.* 2012). To benchmark the completeness of our genome assembly, CEGMA v. 2.5 (Parra *et al.* 2007) was run on the scaffolds to detect the percentage of conserved eukaryotic genes (CEGs).

Genome annotation was performed following established guidelines (Haas *et al.* 2011). Repeats in scaffold sequences were masked with RepeatMasker v. 4.0.5 (<http://www.repeatmasker.org>) (parameters: -no_is -species fungi) using the Repbase libraries (<http://www.girinst.org/>). The masked scaffolds were used as input for the MAKER2 v. 2.10 (Holt & Yandell 2011) genome annotation pipeline. In the MAKER2 pipeline, the GeneMark-ES v. 2.3e (Borodovsky & Lomsadze 2011) *ab initio* gene prediction tool was enabled and the NCBI RefSeq protein sequences were aligned to the genome using exonerate v. 2.2.0 (Slater & Birney 2005). Predicted gene models exhibiting strong evidence by exon alignment were exported as protein sequences and coding nucleotide sequences (CDS). Predicted gene models lacking evidence from exon alignment were discarded in downstream analyses. To determine function, the protein sequences were used as input for InterProScan 5RC6 (Jones *et al.* 2014) (parameters: -dp -f -t p -iplookup -pa -goterms) and were also compared to the manually curated protein data set from UniProt/Swiss-Prot by blastp v. 2.2.28+. The results in XML format from blastp v. 2.2.28+ and InterProScan were loaded into Blast2GO v. 2.7.1 (Conesa *et al.* 2005) and merged to create an annotation table (available from the first author on request). The gene models with BLAST hits having e-value of less than 1.0E⁻¹⁰⁰ and mean similarity hit of ≥ 70% were assumed to be orthologs and they were given names following recommended conventions (<http://www.uniprot.org/docs/proknameprot>). Ribosomal RNA's were predicted by RNAmmer v. 1.2 (Lagesen *et al.* 2007). Data files are publicly available at NCBI (Genome Accession No. JTLS000000000 version JTLS010000000; BioProject Accession No. PRJNA247992) and JGI MycoCosm portal (Grigoriev *et al.* 2014).

Identification of meiosis and mating genes

BLAST was used for finding the *Basidioascus undulatus* mating and meiosis genes. The protein sequences predicted from evidence-supported gene models, determined above, were formatted into a local BLAST database with makeblastdb v. 2.2.28+ (Camacho *et al.* 2009).

Genes previously determined to be involved in meiosis in *Saccharomyces cerevisiae* and *Cryptococcus neoformans*

(Halary *et al.* 2011) were chosen as input queries (e-value cut off < 1.0E⁻⁰⁵) for the *B. undulatus* protein BLAST database using blastp v. 2.2.28+. *Saccharomyces cerevisiae* and *C. neoformans* were chosen because they are well studied genetically and all meiosis-specific proteins are present (Halary *et al.* 2011).

Mating genes were located with the protein domains identified by InterProScan, and by blastp v. 2.2.28+ using known mating genes in *Saccharomyces cerevisiae* as blastp input queries (e-value cut off < 1.0E⁻⁰⁵).

Phylogenomics and molecular dating

Protein sequences of selected fungi (*Agaricus bisporus* var. *bisporus* (H97) v. 2.0 (Morin *et al.* 2012), *Alternaria brassicicola* (Ohm *et al.* 2012), *Arthrotrichum oligospora* ATCC 24927 (Yang *et al.* 2011), *Aureobasidium pullulans* var. *pullulans* EXF-150 (Gostincar *et al.* 2014), *Auricularia subglabra* v. 2.0 (Floudas *et al.* 2012), *Botrytis cinerea* v. 1.0 (Staats and van Kan 2012), *Coccidioides immitis* RS (Sharpton *et al.* 2009), *Coprinopsis cinerea* (Stajich *et al.* 2010), *Cryptococcus neoformans* var. *neoformans* JEC21 (Loftus *et al.* 2005), *Dacryopinax* sp. DJM 731 SSP1 v. 1.0 (Floudas *et al.* 2012), *Fomitiporia mediterranea* v. 1.0 (Floudas *et al.* 2012), *Fomitopsis pinicola* FP-58527 SS1 v. 3.0 (Floudas *et al.* 2012), *Malassezia globosa* (Xu *et al.* 2007), *Mixia osmundae* IAM 14324 v. 1.0 (Toome *et al.* 2014b), *Monacrosporium haptotylum* CBS 200.50 (Meerupati *et al.* 2013), *Neurospora crassa* OR74A v. 2.0 (Galagan *et al.* 2003), *Penicillium chrysogenum* Wisconsin 54-1255 (van den Berg *et al.* 2008), *Puccinia striiformis* f. sp. *tritici* PST-130 (Cantu *et al.* 2011), *Pyronema confluens* CBS 100304 (Traeger *et al.* 2013), *Rhizophagus irregularis* DAOM 181602 v. 1.0 (Tisserant *et al.* 2013), *Saccharomyces cerevisiae* S288C (Goffeau *et al.* 1996), *Sclerotinia sclerotiorum* v. 1.0 (Amselem *et al.* 2011), *Taphrina deformans* (Cisee *et al.* 2013), *Tilletiaria anomala* (Toome *et al.* 2014a), *Tremella mesenterica* Fries v. 1.0 (Floudas *et al.* 2012), *Trichoderma atroviride* v. 2.0 (Kubicek *et al.* 2011), *Tuber melanosporum* (Martin *et al.* 2010), *Ustilago maydis* (Kamper *et al.* 2006), *Wallemia ichthyophaga* EXF-994 (Zajc *et al.* 2013), and *W. sebi* v. 1.0 (Padamsee *et al.* 2012)) were downloaded from the JGI MycoCosm portal (Grigoriev *et al.* 2014) and formatted into separate BLAST databases with makeblastdb v. 2.2.28+. The 246 reliable single copy ortholog protein data set from FUNYbase (Marthey *et al.* 2008) was downloaded. Only amino acid sequences coming from nuclear genes yielding a topological score of > 90 % (Marthey *et al.* 2008) were considered for our phylogenomic analysis (information on exact genes chosen are available from the first author on request). These amino acid sequences were used as blastp v. 2.2.28+ search queries (e-value threshold < 1.0E⁻⁰⁵) against the protein databases built from data downloaded from JGI MycoCosm described above. Protein sequences were aligned with T-Coffee v10.00.r1613 (Notredame *et al.* 2000) (parameters: t_coffee sequence.fasta -output score_ascii,aln) and poorly aligned regions and columns containing gaps were automatically discarded (parameters: t_coffee -other_pg seq_reformat -in sequence.aln -struc_in sequence.score_ascii -struc_in_f number_aln -action +use_cons +keep '[8-9]' +rm_gap 1 > sequence.best.aln). The alignments were

concatenated and converted to PHYLIP format with SeaView v. 4.5.3 (Gouy *et al.* 2010). Three independent phylogenomic analyses were performed with the parallelized version of PhyloBayes 3 (pb_mpi v1.4) (Lartillot *et al.* 2013, Lartillot *et al.* 2009) using the CAT-GTR model (Lartillot & Philippe 2004). Analyses were stopped when convergence was attained (effective size > 100 and maxdiff < 0.1 determined with the programs bpcomp (parameters: -x 1000 50 run1 run2 run3) and tracecomp (parameters: -x 1000 run1 run2 run3), which are part of pb_mpi software package). A lognormal 'relaxed clock' molecular dating analysis was performed with the non-parallelized version of PhyloBayes 3 (pb v3.2e) with a birth-death prior using the tree topology generated from the converged Bayesian analysis above (parameters: -d combined.phy -T bpcomp.con.tre -r outgroup.txt -cal calib.txt -ln -bd md1). *Rhizophagus irregularis* (*Glomeromycota*) was specified as the outgroup. Date constraints previously determined were used as calibrations (Hibbett *et al.* 1997, Smith *et al.* 2004, Taylor & Berbee 2007, Berbee & Taylor 2010, Prieto & Wedin 2013); the exact calibrations are available from the first author on request. Chronograms and statistics were obtained with readdiv (parameters: -x 1000 50 md1), which is part of PhyloBayes 3.

Confocal laser scanning microscopy

To study nuclear behaviour and to look for indicators of meiosis, *Basidioascus undulatus* DAOM 241956 was grown on corn meal agar (CMA, Acumedia Manufacturers, Lansing, MI) for 1 wk and mounted in DNA stains: DAPI-Fluoromount-G™ mounting medium (EMS, Hatfield, PA) or aqueous SYTO 9 (25 µM) (Life Technologies, Burlington, ON). Samples were visualized under confocal laser scanning microscopy using an LSM 510 DUO (Carl Zeiss MicroImaging, Göttingen, Germany) with a Plan-Apochromat 40×/1.4 Oil DIC objective and electronic zoom 4. An excitation diode laser (405 nm) and emission light (420–700 nm) were used for DAPI. An excitation Argon laser (488 nm) and emission light (505–550 nm) were used for SYTO 9. Images were captured using ZEN 2009 Imaging Software (Carl Zeiss MicroImaging).

Transmission electron microscopy

Actively growing hyphae of *Basidioascus undulatus* DAOM 241956, *Geminibasidium donsium* DAOM 241966, and *Wallemia sebi* CBS 633.66 were prepared for transmission electron microscopy using cryo-preparation methods. Hyphae were grown on thin, sterile, deionized dialysis membrane segments overlaying appropriate media at 23 °C. The leading edge of growing mycelia and supporting membranes were trimmed with a sharp razor blade to approximately 5 × 5 mm and after 30–40 min (time to recover from trimming) were removed from the agar surface and immediately cryo-fixed by rapid plunging into liquid propane cooled to –186 °C with liquid nitrogen (Hoch 1986, Roberson & Fuller 1988, McDaniel & Roberson 2000). After rapid freezing, the samples were freeze-substituted in 1 % glutaraldehyde (w/v) and 1 % tannic acid (w/v) in anhydrous acetone at –85 °C for 72 h. After washing in cold acetone (–85 °C), the samples were warmed slowly to room temperature in 1 % OsO₄ (w/v) in acetone, washed in acetone, and infiltrated and flat embedded on glass slides in Spurr's resin (Spurr 1969). Using

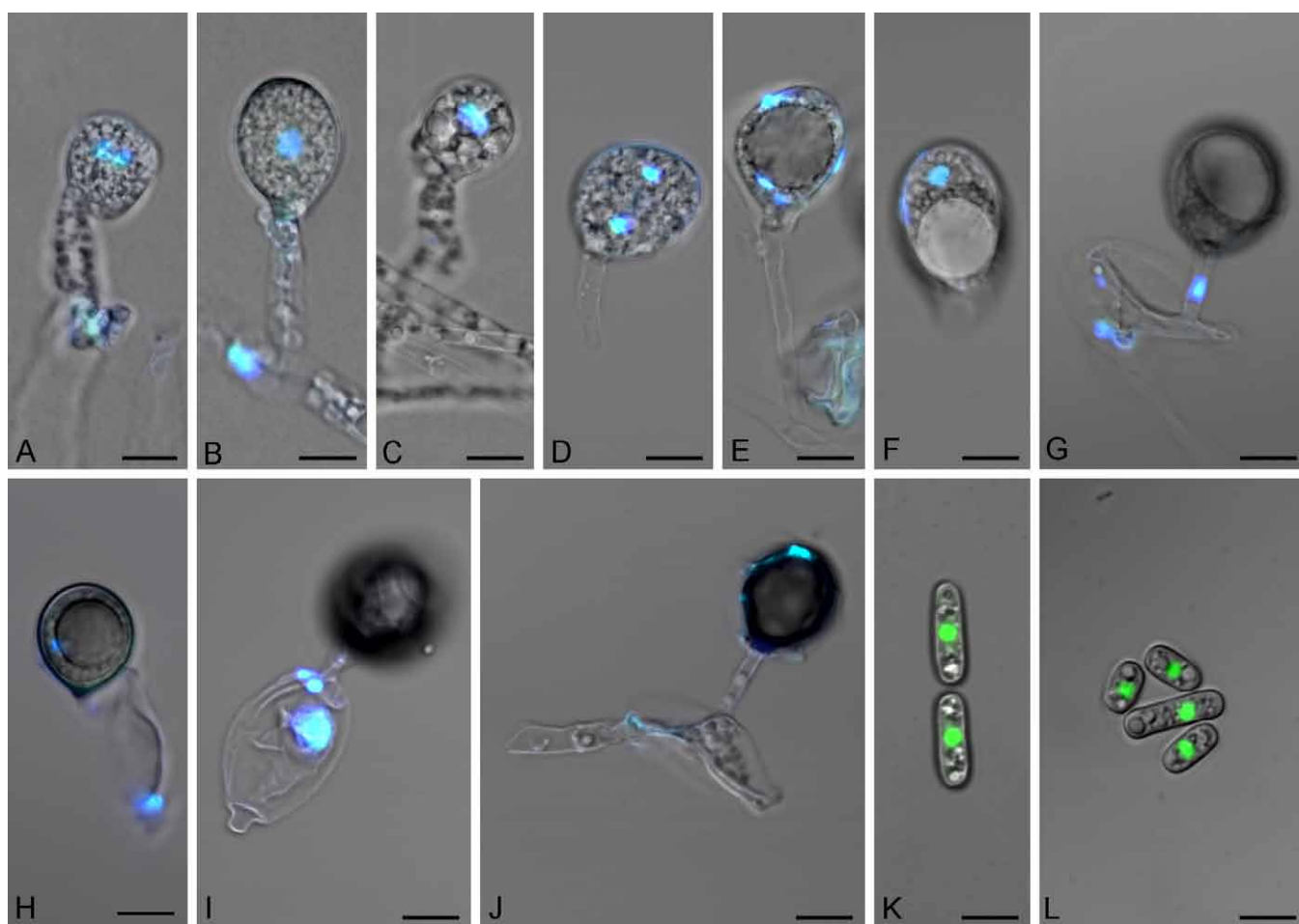


Fig. 1. *Basidioascus undulatus* (DAOM 241956) sexual and asexual structures stained with DAPI (A–J) and SYTO 9 (K–L) and imaged with confocal microscopy. **A.** Dikaryotic basidium (2 nuclei). **B.** Karyogamy. **C.** Anaphase I. **D.** Telophase I and collapsed basal lateral projection. **E.** Telophase II (4 nuclei). **F.** Ejected basidium (4 nuclei). **G–H.** Maturation of a basidiospore on a basidium and the migration of a nucleus through the sterigma and into the basidiospore. **I.** Collapsing basidium with the three remaining nuclei. **J.** A totally collapsed basidium and probably one nucleus inside the mature basidiospore. **K–L.** Single nuclei inside arthroconidia. Bar = 5 μ m.

phase contrast optics (100 \times), we examined the slides for well-preserved hyphae and mounted the selected cells on resin blocks (Howard & O'Donnell 1987) then hand-trimmed them. Selected hyphae were sectioned using a Leica Ultracut R ultramicrotome (Leica Microsystems, Bannockburn, IL), collected on copper grids, and post-stained for 10 min in 2 % uranyl acetate in 50 % ethanol and for 5 min in Sato's lead citrate (Hanaichi *et al.* 1986). Sections were then examined using a JEOL 1200EX (JEOL, Tokyo,) transmission electron microscope equipped with a SIA L3C CCD camera (SIA, Duluth, GA). Measurements from captured images were made with ImageJ (Schneider *et al.* 2012).

RESULTS

Genome sequencing, assembly and annotation

Short-read Illumina sequencing generated approximately 12 million paired end reads (six million reads in the forward (R1) direction, and six million reads in the reverse (R2) direction) of 101 bp in length each. After trimming to 93 bp, about 1.1 Gb of data was assembled *de novo* to yield a genome assembly size of about 32 Mb. The GC content in *Basidioascus undulatus*

was 58 %. The final assembly contained 2992 scaffolds and the longest scaffold was 97 Kb. The nucleotide coverage varied for each scaffold but it was 28X on average and the median nucleotide coverage was 24X. The N50 statistic was 15 Kb. According to CEGMA, 81 % and 88 % of complete and partial CEGs, were detected respectively.

A total of 13935 gene models were detected *ab initio* using GeneMark-ES, but only 6123 gene models were supported by evidence from protein alignment to the NCBI RefSeq fungal protein data. We kept the set of 6123 gene models with evidence for further manual annotation and used it in downstream analyses. Only 3681 of these (60 %) were considered complete because they contained a start and stop codon while the remaining 2442 (40 %) lacked either a start codon, a stop codon, or both.

Meiosis and meiosis specific genes

To determine whether the putative sexual structures identified by Nguyen *et al.* (2013) truly represented basidia, we followed the fungus's ontogeny and performed nuclear staining by laser confocal microscopy (Fig. 1). Three nuclear divisions normally occur during basidiospore maturation: meiosis

I, meiosis II, followed by four different patterns of post-meiotic mitosis (Duncan & Galbraith 1972). We observed dikaryotic nuclei in the basidia (Fig. 1A), karyogamy (Fig. 1B), anaphase I (Fig. 1C), and the telophase I stage where the basal lateral projection was collapsed (Fig. 1D). Four nuclei from telophase II (Fig. 1E) were seen in both non-discharged and discharged basidia (Fig. 1F). The migration of a nucleus through the sterigma (Fig. 1G) into the basidiospore (Fig. 1H) was observed as the basidiospore matured. There were three remaining nuclei in the collapsing basidium (Fig. 1I) and they eventually degenerated at a later stage when the basidium completely collapsed (Fig. 1J). Whole arthroconidia became stained instead of just their nuclei when DAPI was used. A single nucleus could be spotted in each arthroconidium when stained with SYTO 9 (Fig. 1K–L).

Our microscopic observations correlated with our analysis of the genome to detect meiotic genes (Table 1). All meiosis-specific genes, as defined in Malik *et al.* (2008) and Halary *et al.* (2011), were found in the genome.

Mating genes

We looked for mating genes such as those encoding for homeodomain proteins, G-protein coupled pheromone receptor, high mobility group (HMG) DNA binding proteins, mitogen-activated protein kinases (MAPK, MAPKK, MAPKKK) and the subunits of the trimeric GTPase protein ($G\alpha$, $G\beta$, $G\gamma$) (James *et al.* 2013). We followed the *Saccharomyces cerevisiae* standard for gene names. Most of the mating response genes and the pheromone processing genes (Table 2) were detected in the *B. undulatus* genome. All of the mating genes detected were located on different scaffolds, except for *KSS1* and *FUS3*, which co-occurred on scaffold 2837. The presence of most mating genes suggests that a mating type locus exists, but its structure and gene order could not be determined because our assembled genome was too fragmented.

Septal pore morphology

We imaged the septal pores of *Wallemia sebi*, *Basidioascus undulatus*, and *Gemmibasidium donsium*, all currently classified in *Wallemiomycetes*, using identical fixation methods to strengthen comparisons (Fig. 2). *Basidioascus undulatus*, *G. donsium* and *W. sebi* all have a dolipore septum characteristic of *Agaricomycotina*. The pore swelling of *B. undulatus* and *G. donsium* is electron dense, but is not in *W. sebi*. The adseptal tubular extensions that arise from sheets of endoplasmic reticulum in *W. sebi* were obvious (Fig. 2E–F) but they were either absent or unclear in *B. undulatus* (Fig. 2A–B) and *G. donsium* (Fig. 2C–D). Electron-dense regions were evident near the septal pore in all three fungi, but this area was denser in *W. sebi* than in *B. undulatus* and *G. donsium*. An electron-dense septal pore occlusion extending across the septal pore was observed in all three fungi. This occlusion was non-membranous in *W. sebi*, which agrees with previously published findings (Terracina 1974, Padamsee *et al.* 2012) and the *W. sebi* data on the AFTOL Structural and Biochemical Database (Celio *et al.* 2007). The septal pore occlusion in *B. undulatus* and *G. donsium* had a membrane, making it more clearly defined compared to that of *W. sebi*. Furthermore, this

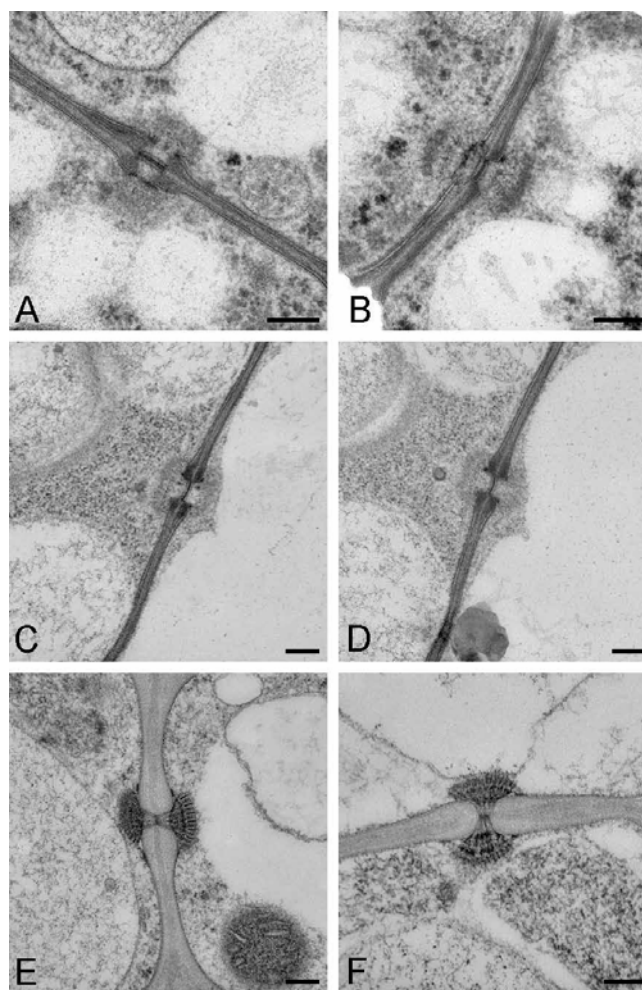


Fig. 2. Transmission electron micrographs showing septal pore morphology. **A–B.** *Basidioascus undulatus* (DAOM 241956 ex-type). **C–D.** *Gemmibasidium donsium* (DAOM 241966 ex-type). **E–F.** *Wallemia sebi* (CBS 633.66). Bar = 20 μm .

occlusion was almost twice as wide in *B. undulatus* and *G. donsium* ($\sim 0.12 \mu\text{m}$) compared with *W. sebi* ($\sim 0.06 \mu\text{m}$). Also, the septal pore of *W. sebi* had striations that appeared to be fine fibrils vertically arranged at the pore opening, but these were absent in *B. undulatus* and *G. donsium*. We did not see a septal pore cap in our sections of *W. sebi*, *B. undulatus* or *G. donsium*.

Phylogenomic analysis and molecular dating

Because *Basidioascus* is currently classified in *Wallemiomycetes*, and the phylogenetic position of the fungi classified in this class has been unstable in different analyses as a result of sparse taxon sampling, low gene sampling and conflicts in certain genealogies (Zalar *et al.* 2005, Matheny *et al.* 2006), we performed a phylogenomic analysis using protein sequences from 35 single copy protein-coding genes to study its phylogenetic position in a more robust fashion (Fig. 3A). Our results were similar to those obtained from the rDNA phylogenetic analysis in Nguyen *et al.* (2013), showing *Basidioascus* as a distant lineage to *Wallemia*, with a posterior probability of 1.00. The results were also similar to those of Padamsee *et al.* (2012) and Zajc *et al.* (2013), where

Table 1. List of known and putative meiosis genes in the genome of *Saccharomyces cerevisiae*, *Cryptococcus neoformans* and *Basidiobasus undulatus*. Meiosis specific proteins are highlighted in red.

Process	Gene name	<i>Saccharomyces cerevisiae</i> ^a		<i>Cryptococcus neoformans</i> ^b		<i>Basidiobasus undulatus</i> vs. <i>Saccharomyces cerevisiae</i>				<i>Basidiobasus undulatus</i> vs. <i>Cryptococcus neoformans</i>				<i>Basidiobasus undulatus</i>					
		Locus ID	Score	E-value	% identity	% positive	Locus ID	Score	E-value	% identity	% positive	Locus ID	Score		E-value	% identity	% positive	Probable Locus ID	
DSB generation	SPO11	YHL022C	XM_767420.1	Not detected	72.8	0.0	25.46	43.54	102794F55C	191	0.0	32.92	51.49	102794F55C	189	0.0	32.92	51.49	102794F55C
	REC107/MEI2	YJR021C	Absent	Not detected					NA					NA					Absent or not detected
	MEI4	YER044C	Absent	Not detected					NA					NA					Absent or not detected
	REC102	YLR329W	Absent	Not detected					NA					NA					Absent or not detected
	REC104	YHR157W	Absent	Not detected					NA					NA					Absent or not detected
	REC114	YMR133W	Absent	Not detected					NA					NA					Absent or not detected
	SKI8	YGL213C	XP_567964.1	Not detected					A5C17B7D33	79.3	0.0	40	54.29	A5C17B7D33					A5C17B7D33
	MER1	YNL210W	Absent	Not detected					NA					NA					Absent or not detected
	HFM1/MER3	YGL251C	XP_774045.1	286	0.0	30.69	50.14	50.14	268E1B92EF	285	0.0	29.55	48.66	268E1B92EF					268E1B92EF
	NAM8/MRE2	YHR086W	XP_568215.1	88.6	0.0	27.24	46.24	46.24	C3284824DB	211	0.0	75.57	83.21	C3284824DB					Conflict
Removal of	MRE11	YMR224C	XP_571170.1	365	0.0	40.59	58.02	58.02	550A163039	629	0	56.55	74.34	550A163039					550A163039
	RAD50	YNL250W	XP_771929.1	543	0.0	30.88	52.16	52.16	1CAAD52FF0	869	0	38.91	61.16	1CAAD52FF0					1CAAD52FF0
Spo11	XRS2/NBS1	YDR369C	Absent	Not detected					NA					NA					Absent or not detected
	SAE2/COM1	YGL175C	Absent	Not detected					NA					NA					Absent or not detected
Strand invasion	RAD51	YER095W	XP_567016.1	481	0.0	69.28	85.58	85.58	D36F2DAE01	533	0	77.71	84.46	D36F2DAE01					D36F2DAE01
	DMC1	YER179W	XP_772121.1	376	0.0	58.13	75.94	75.94	1B6A6FD704	388	0.0	67.13	80.28	1B6A6FD704					1B6A6FD704
	RAD52	YML032C	XP_569087.1	186	0.0	51.9	74.05	74.05	EA2B481C23	237	0.0	60.57	78.29	EA2B481C23					EA2B481C23
	RAD54	YGL163C	XP_570462.1	525	0.0	61.26	78.21	78.21	4E6C655AE5	599	0	71.22	82.93	4E6C655AE5					4E6C655AE5
	RDH54	YBR073W	Absent	ED83E37023	476	0.0	42.43	60.41	60.41	NA					NA				ED83E37023
	RFA1	YAR007C	XP_775959.1	350	0.0	41.02	62.08	62.08	1512E55FF2	489	0.0	51.93	70.39	1512E55FF2					1512E55FF2
	RFA2	YNL312W	XP_776149.1	95.9	0.0	37.78	55.56	55.56	C06D199EC7	174	0.0	40.51	61.18	C06D199EC7					C06D199EC7
	RFA3	YJL173C	Absent	Not detected					NA					NA					Absent or not detected
	SAE3	YHR079C-A	Absent	Not detected					NA					NA					Absent or not detected
	RAD55	YDR076W	Absent	Not detected					NA					NA					Absent or not detected
DNA damage checkpoint	PCH2	YBR186W	XP_567632.1	177	0.0	36.88	53.49	53.49	1586D154C9	418	0.0	47.79	66.81	1586D154C9					1586D154C9
	MEC1	YBR136W	XP_568889.1	483	0.0	24.05	43.61	43.61	87D911C03D	713	0	61.72	76.24	87D911C03D					Conflict
	RAD17	YOR368W	XP_569244.1	Not detected					Not detected					D5120AB0E3					D5120AB0E3
	RAD24	YER173W	Absent	D5120AB0E3	54.3	0.000	27.48	43.51	43.51	NA				NA					D5120AB0E3
	DDC1	YPL194W	Absent	Not detected					NA					NA					Absent or not detected
Regulation of crossover frequency	MLH1	YMR167W	XP_571158.1	361	0.0	53.03	70.03	70.03	F0BB260AD1	642	0	51.99	66.51	F0BB260AD1					F0BB260AD1
	MLH3	YPL164C	XP_570272.1	115	0.0	23.47	44.13	44.13	BC2D40152B	178	0.0	37.67	57.88	BC2D40152B					BC2D40152B
	MSH4	YFL003C	XP_773414.1	347	0.0	31.07	50.64	50.64	CAE76A5567	546	0	39.76	59.04	CAE76A5567					CAE76A5567
	MSH5	YDL154W	XP_566842.1	304	0.0	30.46	52.12	52.12	A5F9B81D55	436	0.0	39.91	58.36	A5F9B81D55					A5F9B81D55

Table 1. (Continued).

Process	Gene name	Saccharomyces cerevisiae ^a	Cryptococcus neoformans ^b	Basidioascus undulatus vs. Saccharomyces cerevisiae				Basidioascus undulatus vs. Cryptococcus neoformans				Basidioascus undulatus		
				Locus ID	Score	E-value	% identity	% positive	Locus ID	Score	E-value		% identity	% positive
Synaptonemal complex	SGS1	YMR190C	XP_776787.1	FAB5DF8B5F	526	0.0	45.14	64.24	FAB5DF8B5F	597	0	43.47	60.52	FAB5DF8B5F
	MEI5	YPL121C	Absent	Not detected					NA					Absent or not detected
	MUM2	YBR057C	Absent	Not detected					NA					Absent or not detected
	NDJ1	YOL104C	Absent	Not detected					NA					Absent or not detected
	RAD1	YPL022W	XP_772313.1	29F5BE17B9	258	0.0	33.14	50.95	29F5BE17B9	374	0.0	40.1	56.48	29F5BE17B9
	Rad2	YGR258C	XP_566738.1	41955FBDBD	248	0.0	44.04	67.51	41955FBDBD	355	0.0	54.75	70.82	41955FBDBD
	HOP1	YIL072W	50255507	D659917BB2	141	0.0	31.54	56.54	D659917BB2	170	0.0	34.16	50	D659917BB2
	HOP2	YGL033W	50255433	Not detected					Not detected					Absent or not detected
	MND1	YGL183C	XP_772550.1	5756414F12	62	0.0	20.94	43.59	5756414F12	115	0.0	37.13	51.05	5756414F12
	ZIP1	YDR285W	Absent	2C075CC8D6	204	0.0	79.7	86.47	NA					2C075CC8D6
ZIP2	YGL249W	Absent	Not detected					NA					Absent or not detected	
ZIP3	YLR394W	Absent	Not detected					NA					Absent or not detected	
Zip4/Spo22	YIL073C	134115038	Not detected					Not detected					Absent or not detected	
DNA Repair	HTA1	YDR225W	XP_567962.1	2C075CC8D6	204	0.0	79.7	86.47	2C075CC8D6	130	0.0	57.98	73.11	2C075CC8D6
	HTA2	YBL003C	XP_569065.1	2C075CC8D6	206	0.0	80.45	87.22	F84A4F188E	205	0.0	79.39	87.79	Conflict
	RED1	YLR263W	Absent	Not detected					NA					Absent or not detected
	SMC5	YOL034W	XP_570071.1	8388BCCBA3	362	0.0	27.24	48.17	8388BCCBA3	529	0.0	32.52	51.9	8388BCCBA3
	SMC6	YLR383W	XP_775824.1	13E0CFCD07	340	0.0	27.58	47.32	13E0CFCD07	476	0.0	29.98	50.65	13E0CFCD07
	EXO1	YOR033C	XP_777034.1	36DFC1EC56	276	0.0	43.67	65.33	36DFC1EC56	389	0.0	54.49	71.07	36DFC1EC56
	HRR25	YPL204W	XP_570121.1	22D0CDA5D9	464	0.0	65.33	83.28	22D0CDA5D9	594	0	95.22	98.63	22D0CDA5D9
	RAD23	YEL037C	XP_777611.1	0FBB054DC3	54.7	0.000	40.54	60.81	0FBB054DC3	91.3	0.0	60.81	78.38	0FBB054DC3
	MSH2	YOL090W	XP_567098.1	E449052477	746	0	43.93	63.77	E449052477	1087	0	58.26	73.21	E449052477
	MSH3	YCR092C	XP_569494.1	7F6ED5B406	354	0.0	29.13	49.57	7F6ED5B406	790	0	45.31	65.61	7F6ED5B406
Mismatch repair	MSH6	YDR097C	XP_772722.1	122F99619E	672	0	36.44	56.87	122F99619E	1026	0	51.24	66.83	122F99619E
	MLH2	YLR035C	Absent	8C03A290CE	86.7	0.0	31.93	53.61	NA					8C03A290CE
	PMS1	YNL082W	57225775	8C03A290CE	176	0.0	48.65	67.03	8C03A290CE	236	0.0	60.51	75.38	8C03A290CE
	MMS4	YBR098W	58260752	Not detected					Not detected					Not detected or absent
	MUS81	YDR386W	XP_777360.1	E55E7AB0BD	84	0.0	30.49	49.78	E55E7AB0BD	101	0.0	32.39	50.7	E55E7AB0BD
	SLX1	YBR228W	XP_567159.1	A74C037F01	83.2	0.0	52.44	68.29	A74C037F01	108	0.0	61.54	74.36	A74C037F01
	TOP1	YOL006C	XP_572925.1	DB366D3081	439	0.0	49.78	66.89	DB366D3081	518	0.0	56.71	70.56	DB366D3081
	TOP2	YNL088W	XP_566700.1	4AE1080675	1197	0	52.97	71.47	4AE1080675	1491	0	66.48	79.24	4AE1080675
	TOP3	YLR234W	XP_773035.1	BCF17A4E2A	266	0.0	43.67	60.54	BCF17A4E2A	518	0.0	57.95	70.91	BCF17A4E2A
	SLX4	YLR135W	Absent	Not detected					Not detected					Not detected or absent

Table 1. (Continued).

Process	Gene name	Saccharomyces cerevisiae ^a		Cryptococcus neoformans ^b		Basidiobolus undulatus vs. Saccharomyces cerevisiae			Basidiobolus undulatus vs. Cryptococcus neoformans			Basidiobolus undulatus Probable Locus ID				
		Locus ID	Score	E-value	% identity	% positive	Locus ID	Score	E-value	% identity	% positive		Locus ID	Score	E-value	% identity
	SLX5	YDL013W		Not detected		Not detected										Not detected or absent
	SLX8	YER116C		Absent	52.4	0.000	35.9	48.72	52369A1C42							52369A1C42
Nonhomologous end joining	YKUJ70	YMR284W	XP_573016.1	AD4E738F04	115	0.0	24.21	42.14	AD4E738F04	228	0.0	32.28	47.76			AD4E738F04
	YKU80	YMR106C	XP_568810.1	1FC448F440	60.8	0.000	30.29	48.57	1FC448F440	241	0.0	28.83	46.23			1FC448F440
	DNL4	YOR005C	XP_572602.1	9206958189	223	0.0	27.5	43.38	E8D29BB570	902	0	71.5	83.49			Conflict
	LIF1	YGL090W	Absent	Not detected					NA							Absent or not detected
Other	MSC1	YML128C	XP_570348.1	6453C9DC91	53.1	0.000	22.02	45.83	6453C9DC91	168	0.0	34.33	51.12			6453C9DC91
	MSC7	YHR039C	XP_773481.1	AE68F812D8	179	0.0	26.57	46.65	AE68F812D8	185	0.0	28.6	45.59			AE68F812D8
	MSC3	YLR219W	Absent	Not detected					NA							Absent or not detected
	MSC6	YOR354C	Absent	Not detected					NA							Absent or not detected
	SRS2	YJL092W	Absent	C8820A864B	273	0.0	28.95	44.65	NA							C8820A864B
	MPS3	YJL019W	Absent	Not detected					NA							Absent or not detected
	REC8	YPR007C	134090540	Not detected					130D4D9937	65.1	0.0	38.04	59.78			130D4D9937
	Mcd1/Rad21	6320201 ^b	58266400	130D4D9937	68.6	0.0	35.05	57.73	130D4D9937	142	0.0	32.61	46.65			130D4D9937
	SMC1	BAA09230.1 ^b	XP_568851.1	82DC381A41	534	0.0	31.89	54.54	82DC381A41	894	0	44.85	64.74			82DC381A41
	SMC2	P38989.1 ^b	XP_572171.1	68E0241B20	516	0.0	42.44	63.29	68E0241B20	720	0	58.48	70.98			68E0241B20
	SMC3	CAA74655.1 ^b	XP_570201.1	10D8B5FBE4	248	0.0	41.53	60.17	10D8B5FBE4	404	0.0	57.95	72.56			10D8B5FBE4
	SMC4*	EDV09389.1 ^b	XP_571168.1	C71F05C621	895	0	41.41	60.31	C71F05C621	1367	0	56.6	73.61			C71F05C621
	SCC3	P40541.1 ^b	XP_567136.1	Not detected					Not detected							Absent or not detected
	PDS5	Q04264.1 ^b	XP_567466.1	062A151CC3	326	0.0	24.85	45.12	062A151CC3	681	0	35.27	55.63			062A151CC3

^a These LocusID's are from Saccharomyces Genome Database (<http://www.yeastgenome.org/>).

^b These LocusID's are from NCBI Protein database (<http://www.ncbi.nlm.nih.gov/protein/>).

Table 2. List of mating genes.

Process	Gene name	Saccharomyces cerevisiae ^a	Basidioascus undulatus vs. Saccharomyces cerevisiae				
		Locus ID	Locus ID	Score	E-value	% identity	% positive
Mating response	STE3	YKL178C	601070A992	84.7	0.0	25.17	44.37
	KSS1	YGR040W	03DD35A234	341	0.0	58.5	72.45
	FUS3	YBL016W	03DD35A234	355	0.0	57.48	74.83
	STE7	YDL159W	C56EA11F93	209	0.0	49.32	66.06
	STE11	YLR362W	A726A87E58	319	0.0	52.6	67.86
	GPA1	YHR005C	00D128FF38	243	0.0	55	75.5
	STE4	YOR212W	51579617AB	195	0.0	43.67	61.22
	STE18	YJR086W	Not detected				
	STE20	YHL007C	ABF41DDAEF	375	0.0	59.26	76.09
	STE12	YHR084W	Not detected				
	FAR1	YJL157C	Not detected				
	STE5	YDR103W	Not detected				
Pheromone processing	KEX1	YGL203C	C1EB8324D8	249	0.0	34.79	51.2
	KEX2	YNL238W	1DAB65F8AC	480	0.0	47.45	63.53
	STE13	YOR219C	C51BE5DE56	131	0.0	42.11	63.16
	RAM1	YDL090C	B544DBFB4B	169	0.0	37.09	53.31
	RAM2	YKL019W	B2B303A077	82.4	0.0	30.73	45.31
	RCE1	YMR274C	Not detected				
	STE24	YJR117W	Not detected				
	STE14	YDR410C	CC90F4A946	99.8	0.0	45.45	58.68
	AXL1	YPR122W	98D2221B58	129	0.0	30.38	51.9
	STE6	YKL209C	ED8249ED54	359	0.0	25.6	43.36

a: These LocusID's are from Saccharomyces Genome Database (<http://www.yeastgenome.org/>).

the *Wallemiomycetes* were shown to be an early diverging lineage of *Agaricomycotina*.

We dated the divergence of *Basidioascus* from other lineages using some reliable fossil data of basidiomycetes (Hibbett *et al.* 1997, Smith *et al.* 2004) and the latest calculated calibration points (Berbee & Taylor 2010, Prieto & Wedin 2013) (Fig. 3B). Under these assumptions, the split between *B. undulatus* and *Wallemia* was estimated at 250±29 Mya, and that between *W. sebi* and *W. ichthyophaga* was about 33±6 Mya.

DISCUSSION

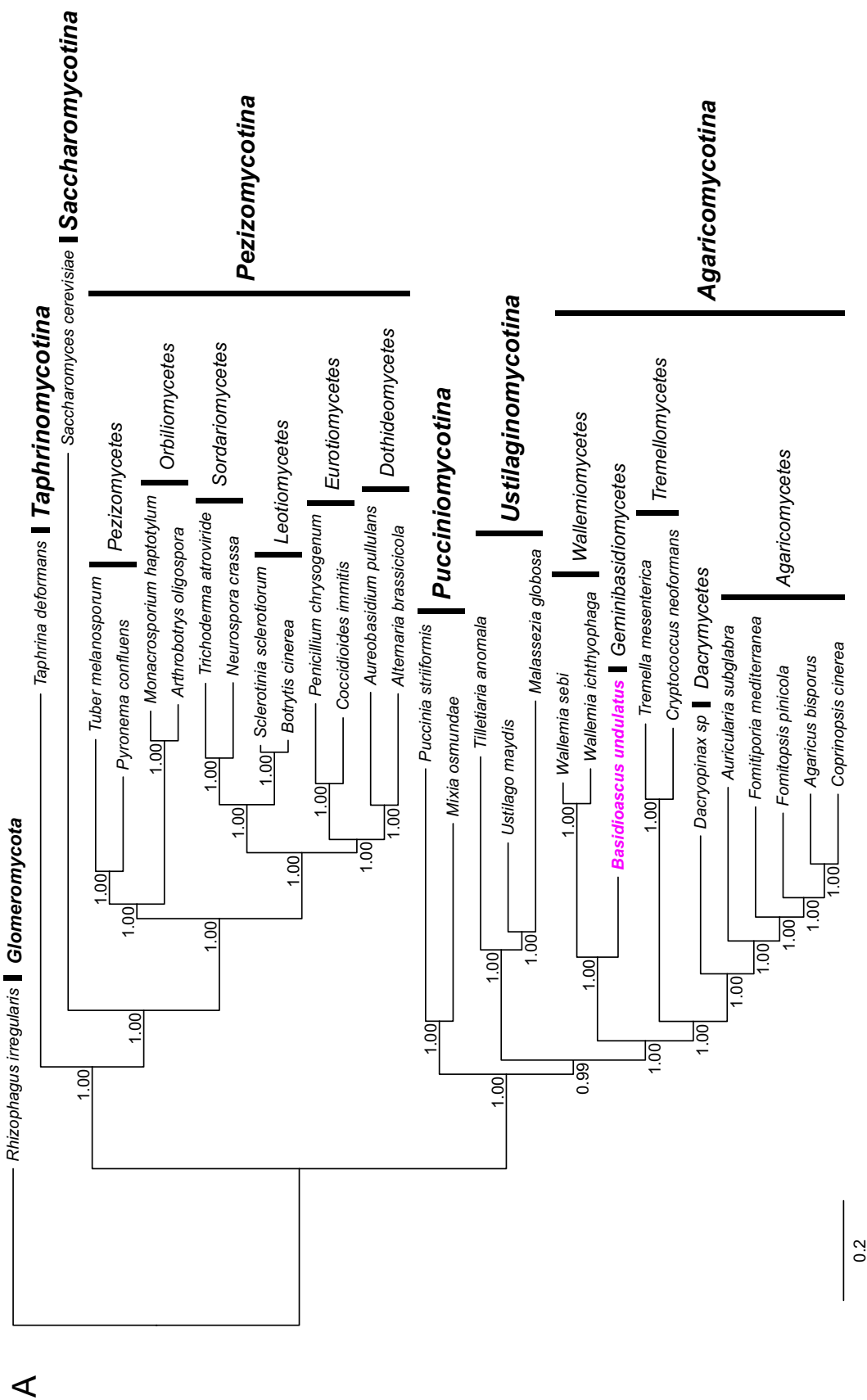
Genome sequencing and annotation

Basidioascus undulatus is the first fungus in *Geminibasidiales* with a sequenced genome. The 1.1 Gb of sequence data gave a median nucleotide coverage of 24X and a final assembly that contained 2992 scaffolds, which is similar to what has been produced in many recent whole genome sequencing studies (e.g. Sims *et al.* 2014). *Wallemia sebi* and *W. ichthyophaga* were the closest known relatives to *B. undulatus*. Despite being considered close relatives, the genome assembly size of *B. undulatus* (32 Mb) was larger than *W. sebi* (9.8 Mb) (Padamsee *et al.* 2012) and *W. ichthyophaga* (9.6 Mb) (Zajc *et al.* 2013). Also, the GC content of *B. undulatus* was higher (58 %) compared to *W.*

sebi (40 %) and *W. ichthyophaga* (45 %) (Table 3). Based on these results, the fungi of these two genera are more distantly related than initially suspected.

We were able to detect 88 % of partial core eukaryotic genes (CEGs). We assumed that downstream annotation with this genome assembly should, in theory, reveal close to the same proportion of the total number of genes contained in *B. undulatus*.

Many gene prediction tools are available, but GeneMark-ES was selected because it was shown previously to be accurate at detecting genes in fungal genomes (Ter-Hovhannisyan *et al.* 2008). GeneMark-ES considers branch point sequences in the intron model, which guides lariat formation during splicing, providing greater accuracy at locating intron boundaries. Because RNA data were not acquired in our study, we chose to use GeneMark-ES, which does not require RNA data for algorithm training. Nevertheless, genome annotation based on genome sequences alone has limitations. First, without RNA data, CDS predictions cannot be validated and genes from splice sites that use donor and acceptor sequences other than the canonical GT-AG introns cannot be predicted. The frequencies of the non-canonical GC-AG introns are 1.0–1.2 % in some ascomycetes (Rep *et al.* 2006) and could be as high as 3 % in basidiomycetes (Misiek & Hoffmeister 2008). To compensate for the absence of RNA data, the fungal NCBI RefSeq protein sequence data set (13 March 2014 release), which contains curated



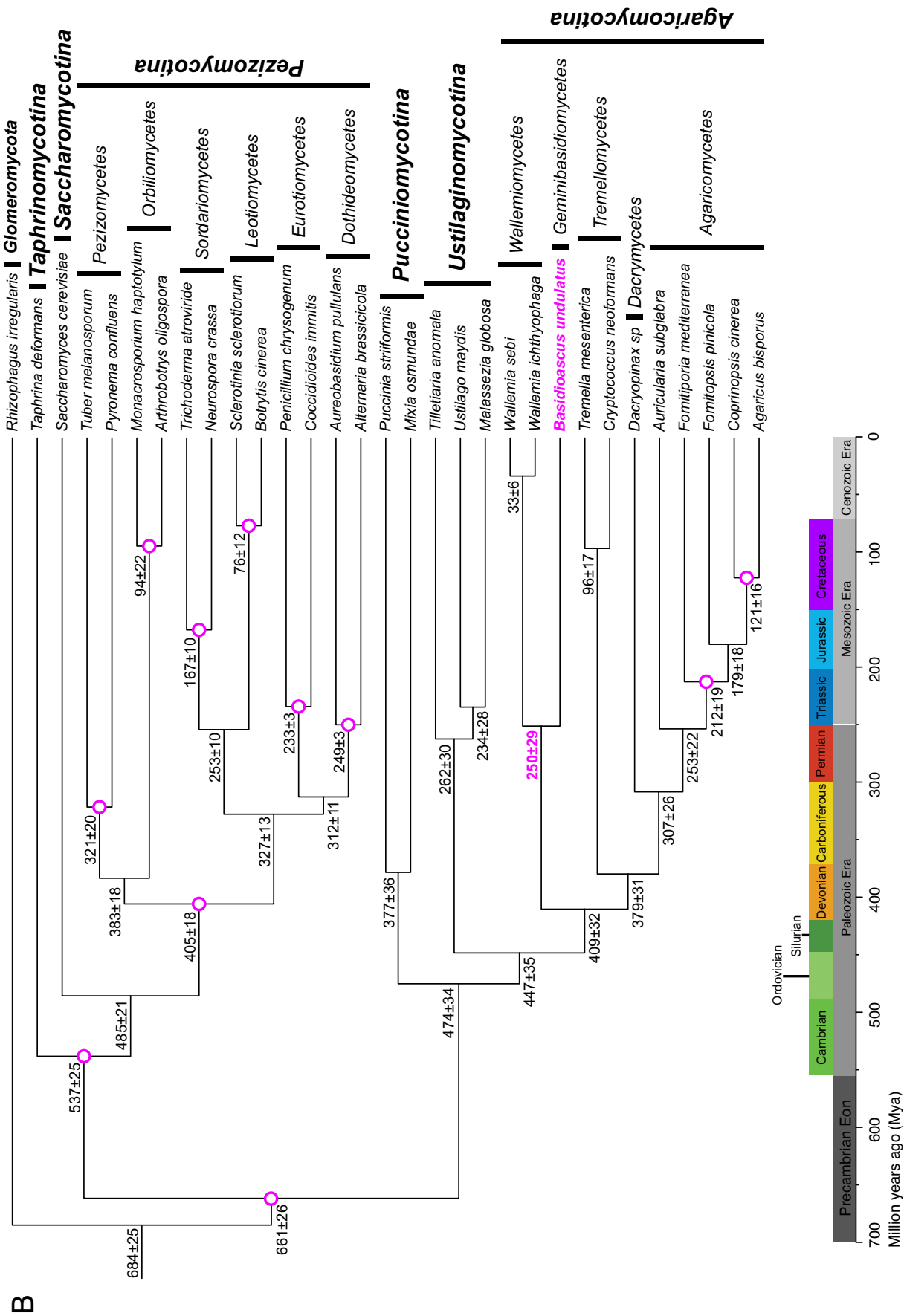


Fig. 3. Phylogenetic trees resulting from phylogenomic analysis and molecular dating. **A.** Consensus topology and branch lengths from analyses of concatenated amino acid sequences from 35 single copy genes with a total of 10129 data columns. Gapped and poorly aligned sites were removed. Analyses were performed with the CAT-GTR model. Posterior probabilities are shown at the nodes of the tree. Scale bar indicates expected changes per site. **B.** Chronogram resulting from a lognormal relaxed molecular clock analysis with the birth-death prior. The mean divergence time with standard error are shown at each node. Circled nodes were pre-calibrated before the analysis. The paleontological periods, in million years ago (Mya), are shown as a scale at the bottom. *Rhizophagus irregularis* (*Glomeromycota*) was used as the outgroup.

Table 3. Genome assembly and annotation statistics of *Basidioascus undulatus* compared to *Wallemia sebi* and *W. Ichthyophaga*.

Descriptive statistic	<i>B. undulatus</i>	<i>W. sebi</i>	<i>W. ichthyophaga</i>
Genome assembly			
Sequencing platform	Illumina Hi-Seq	Illumina Hi-Seq & 454	Illumina Hi-Seq
Total number of reads	12 Million ^a	—	—
Read length after trimming	93 bp	—	—
Data size	1.1 Gb	—	3.7 Gb
Assembly size	32 Mb ^c	9.8 Mb	9.6 Mb
Estimated Percent GC	58% ^c	40%	45%
Nucleotide coverage	24X ^b	71X	>270X
Number of contigs	3058	114	95
Number of scaffolds	2992	56	82
Longest scaffold size	97 Kb ^c	900 Kb	790 Kb
N50	15 Kb ^c	340 Kb	440 Kb
Genome annotation			
Percent core eukaryotic genes detected	88% ^d	—	—
All predicted gene models	13935 ^e	5284	4863
Evidence supported gene models	6123 ^e	—	—
Evidence supported complete gene models	3681	—	—

Statistics were found by the following programs: (a) FastQC, (b) qualimap, (c) QUAST, (d) CEGMA, and (e) MAKER.

protein sequences from completed fungal genome projects, was aligned to our *B. undulatus* genome using the program “exonerate”. In the MAKER2 pipeline, aligning protein sequences to predicted genes on the genome generates ‘evidence’ expressed as the Annotation Evidence Distance (AED) score for exons (eAED) (Eilbeck *et al.* 2009). The eAED score is a metric that measures how a predicted gene determined from GeneMark-ES agrees with the protein alignment evidence from exonerate while accounting for protein reading frames shifts. This score is helpful for assessing annotation quality of a predicted gene (Eilbeck *et al.* 2009). Overall, 13 935 genes were predicted by GeneMark-ES but only 6123 of those genes were supported by protein alignment evidence. For each of the 6123 predicted genes, we made further functional annotation with a BLAST search against the UniProt/Swiss-Prot manually curated data set and an InterProScan analysis. Using this information, 452 genes were manually annotated with confidence. Future releases of the *B. undulatus* genome with RNA sequence data would increase the number of annotated genes and allow discovery and validation of completely novel genes or splice variants.

Meiosis

We used genomic information in combination with confocal microscopy to gain further understanding of sexuality in *Basidioascus undulatus*. Fungal taxonomists infer function of meiosporangia in newly discovered fungi based on morphological similarities to proven meiosporangia in similar or related fungi. Proof of meiosis by nuclear staining is not usually required. In our case, *B. undulatus* is different morphologically, ontogenetically, and phylogenetically from known basidiomycetes and it was difficult to assess whether previous morphological interpretations by Nguyen *et al.* (2013) were correct without nuclear staining experiments. We suggested previously that the clavate structures found

on somatic hyphae were basidia (Nguyen *et al.* 2013). These basidia were deciduous, forcibly discharged, and had a basal lateral projection that eventually collapsed. Sterigmata grew on the basidium, and a basidiospore developed at the tip of each sterigma. To verify these interpretations, we visualized these putative sexual structures with nuclear staining and confocal microscopy. Our observations suggested that meiosis occurred in the clavate structures (Fig. 1) providing the evidence for previous interpretation of them as basidia. Post-meiotic mitosis in the basidium was not seen but it was difficult to visualize nuclei in the mature basidiospores. Although we saw evidence of meiosis with microscopy, we also confirmed the presence of meiotic genes in the genome (Table 1). We did not detect *HOP2* among the 10 meiosis specific genes. The *HOP2* gene product is involved in preventing synapsis between non-homologous chromosomes during meiotic double-strand break repair (Malik *et al.* 2008). The function of *HOP2* could be served by another unknown gene or gene product in *B. undulatus*, or perhaps our genome assembly was not complete enough to detect it. With 88 % of CEGs detected, about 1 out of every 10 genes would not be detected during the annotation procedure.

Basidioascus undulatus produces cells attached in chains, which are presumed to be arthroconidia (Nguyen *et al.* 2013). These arthroconidia were indeed single-nucleated (Fig. 1K–L) and therefore represent an asexual morph of *B. undulatus*. Given these results, *B. undulatus* indeed exhibits both sexual and asexual morphs in culture.

Mating

The genes located at the MAT locus orchestrate the fungal mating process and determine the sex of individuals (Lee *et al.* 2010). In the fungal kingdom, mating is most comprehensively studied in *Saccharomyces cerevisiae*. Therefore, we used what is known about mating in *S. cerevisiae* as a model to

guide our interpretations of mating genes in the *B. undulatus* genome.

Homeodomain (HD) transcription factors control the expression of pheromone and pheromone receptor genes. Normally, dimerization occurs between two paralogous HD transcription factors, HD1 and HD2, to form HD1-HD2 complex. In *Ustilago maydis*, this is termed the bE/bW heterodimer, and in *S. cerevisiae* the a1/a2 complex (Lee *et al.* 2010). We identified the HD1 (8946F71C4C) and HD2 (AF24C22F02) proteins in *B. undulatus*.

Pheromone production and processing are also important in mating. All orthologs for pheromone processing (*KEX1*, *KEX2*, *STE13*, *RAM1*, *RAM2*, *STE14*, *AXL11*, *STE6*) were detected in *B. undulatus* except for the *RCE1/STE24* genes responsible for cleavage of the pheromone peptide (Table 2).

Upon release into the environment, pheromones must bind to pheromone receptors to initiate a signaling cascade involving the mitogen-activated protein kinase (MAPK) pathway to turn on mating genes (Jones & Bennett 2011). MAPK pathways regulate the activity of high mobility group (HMG) DNA binding proteins, which are transcription factors regulating pheromone responsive genes in the MAT loci (Hartmann *et al.* 1996). Most of the mating response genes were detected (*STE3*, *KSS1*, *FUS3*, *STE7*, *STE11*, *GPA1*, *STE4*, *STE20*) (Table 2) except the G-protein γ subunit (*STE18*), the transcription factor *STE12*, the coordinator of MAPK pathway *STE5*, and the cell cycle control *FAR1* protein. We identified four HMG DNA binding proteins (B013112742, C8B77D01D8, C1AD40EA22, and 9CC40B0A26).

We were unable to isolate single arthroconidia or successfully germinate basidiospores so mating tests were not possible. Thus, it is unknown whether *B. undulatus* is homothallic or heterothallic. *Wallemia sebi* apparently has a bipolar mating system (Padamsee *et al.* 2012) but no discernable mating type locus could be identified in the genome of *W. ichthyophaga* (Zajc *et al.* 2013). Our genome assembly was too fragmented to determine the structure of the mating type locus conclusively and therefore the mating system in *B. undulatus* remains uncharacterized until more sequencing is done. The detection of a near complete cellular machinery for the mating response, pheromone processing, and meiosis suggests that *B. undulatus* is capable of outcrossing. However, future experiments with gene knockout mutants will be needed to validate these identified genes.

Higher classification and divergence of *Geminibasidiales*

Basidioascus undulatus and *Geminibasidium donsium* have been classified in *Geminibasidiales*, a sister order to *Wallemiales*. *Geminibasidiales* was placed tentatively in the class *Wallemiomycetes* (Nguyen *et al.* 2013). The main reason for this initial inconclusive placement was the absence of septal pore ultrastructure data and that only ribosomal genes could be used to make a phylogenetic analysis with enough taxon sampling to reach a conclusion. More genomes are available now and phylogenomic analysis with protein coding genes is more feasible and informative.

Our phylogenomic analysis shows the same relationships previously determined with rDNA, where *Wallemia* and

Basidioascus are distantly related sister groups that occupy a basal phylogenetic position in *Agaricomycotina* (Fig. 3A). The distance of this relationship is also reflected in differences in GC content and genome size between *B. undulatus* and *Wallemia* discussed above. According to the sampling and assumptions made in our analyses, molecular dating suggests that the split between *B. undulatus* and *W. sebi* occurred 250 Mya (Fig. 3B), which is around the same time that *Sordariomycetes* split from *Leotiomycetes* (253 Mya) and *Exobasidiomycetes* from the *Ustilaginomycetes* and *Malasseziomycetes* (262 Mya), but we also note that not all nodes corresponding to class ranks in our analysis represent divergences at around 250 Mya. If our dates are accurate, it means *Wallemia* and *B. undulatus* shared a common ancestor until a divergence event occurred between the Permian and Triassic period. Interestingly, it was during this period that Earth experienced its most severe extinction event (Sahney & Benton 2008). The split between *W. sebi* and *W. ichthyophaga* was dated at 33±6 Mya, an estimate that differs from previous analyses; a 11.9 Mya split was proposed by Zajc *et al.* (2013), but with different methods, taxon sampling, and calibrations used for the molecular dating analysis. These estimates should all be considered approximate because the fungal fossil record is rather limited and many studies generate radically different age estimates for the same divergence events (Lücking *et al.* 2009).

Before sequence data began to dominate fungal systematics, morphological characters of the septa and septal pore caps were considered important for classifying fungi at the class or higher taxonomic levels (Khan & Kimbrough 1982, Oberwinkler & Bandoni 1982, Müller *et al.* 2000, van Driel *et al.* 2009) and septal pore morphology is still considered essential for delimiting classes in *Basidiomycota*. However, the overall septal pore morphologies of *B. undulatus* and *G. donsium* are similar and there are significant differences when compared to *W. sebi* (see p. 219 above; Fig. 2). The septal pore cap is probably not a reliable or practical feature for the classification of *Wallemia*, *Basidioascus* and *Geminibasidium* because it is sometimes present or absent in *Wallemia* (Padamsee *et al.* 2012, D.J. McLaughlin pers. comm.) and was not seen in *B. undulatus* or *G. donsium*.

Given the results obtained from phylogenomics analysis, molecular dating, and septal pore ultrastructure, we propose to classify *Geminibasidiales* in a new class *Geminibasidiomycetes*. Although *Geminibasidium* could not be included in the phylogenomic analysis and molecular dating because its genome is not yet sequenced, we are confident from previous rDNA phylogenetic analysis (Nguyen *et al.* 2013), morphological characters, and similarities in the septal pore morphology (Fig. 2) that *Geminibasidium* will remain a close sister group to *Basidioascus*. Previous phylogenies position *Wallemiomycetes* at the base of *Basidiomycota* (Matheny *et al.* 2006, Zalar *et al.* 2005), as a sister group to or as the earliest diverging lineage of *Agaricomycotina* (Padamsee *et al.* 2012, Nguyen *et al.* 2013, Zajc *et al.* 2013). The classification for the *Wallemiomycetes* is shown as *incertae sedis* in the *Basidiomycota* in the NCBI taxonomy (Federhen 2012), MycoBank (Robert

et al. 2013), and the Encyclopedia of Life (<http://www.eol.org>). In the taxonomic section below, we amend the description of the subphylum *Agaricomycotina* to include the classes *Geminibasidiomycetes* and *Wallemiomycetes* along with the *Agaricomycetes*, *Tremellomycetes*, and *Dacrymycetes*, which are well delimited classes of *Agaricomycotina* (Hibbett 2007). By formally connecting the basal *Geminibasidiomycetes* and *Wallemiomycetes* to the *Agaricomycotina*, we eliminate the uncertain status of these taxa. The subphylum name *Wallemiomycotina* (Mycobank no. MB 550364) was published recently to accommodate *Wallemia* (Doweld 2014). There was no discussion of the rationale behind this proposal and we consider it unnecessary to include *Wallemiomycetes* (with or without the *Geminibasidiomycetes*) in its own subphylum.

TAXONOMY

Agaricomycotina Doweld, *Prosyllabus*: lxxxvii (2001).
Mycobank MB560553

Homonym: *Agaricomycotina* R. Bauer *et al.*, *Mycol. Prog.* **5**: 45 (2006).

Description: Members of the *Basidiomycota* that have a cell wall carbohydrate composition with a dominance of glucose and presence of xylose and having a type B secondary structure of the 5S RNA (Bauer *et al.* 2006). Fungi that belong to this subphylum are classified in the classes *Agaricomycetes*, *Dacrymycetes*, *Tremellomycetes*, *Wallemiomycetes* or *Geminibasidiomycetes*.

Notes: Although *Agaricomycotina* Dowell (2001) is the oldest name, it is the concept of proposed by Bauer *et al.* (2006) (i.e. *Hymenomycetes sensu* Swann & Taylor 1995) that is widely used today. We modified it above only to explicitly state all of the accepted classes within *Agaricomycotina*. The cell wall of *B. persicus* was composed mostly of glucose and xylose (Nasr *et al.* 2014), and assuming that this is the same for other species of *Basidioascus* and *Geminibasidium*, this placement for the class *Geminibasidiomycetes* is appropriate. The cell wall carbohydrate composition of *Wallemia* species is unknown.

Wallemiomycetes Zalar *et al.*, *Antonie van Leeuwenhoek* **87**: 322 (2005).
Mycobank MB501496

Description: Class of xerophilic basidiomycetes belonging to the subphylum *Agaricomycotina*. These fungi produce basauxic anamorphs and do not produce basidiomata in culture. Species have dolipore septa with adseptal tubular extensions that arise from sheets of endoplasmic reticulum that form the septal pore cap. The septal pore cap is sometimes absent. The septal pore has an electron-dense non-membranous septal pore occlusion and striations that are oriented vertically.

Type order: *Wallemiales*.

Notes: This description is altered to exclude characters of *Geminibasidiales* previously added to the concept of *Wallemiomycetes* by Nguyen *et al.* (2013). Information about the septal pore morphology was added, as detailed in Padamsee *et al.* (2012).

Geminibasidiomycetes H.D.T. Nguyen & Seifert, **cl. nov.**

Mycobank MB811680

Description: Class of xerotolerant basidiomycetes belonging to the subphylum *Agaricomycotina*. *Basidiomata* not produced in culture. *Basidia* arising from somatic hyphae or from swollen basidium-bearing cells (primary cells) with a basal lateral projection occurring either on the basidium or the swollen primary cell. *Basidiospores* symmetrical on sterigma, not forcibly discharged, and brown at maturity. *Arthroconidial* and/or yeast-like asexual morphs sometimes produced. Species have a dolipore septum that is electron-dense at the pore swelling with an electron-dense membranous septal pore occlusion. Some species are heat resistant.

Type order: *Geminibasidiales*.

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