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Comparative genomics reveals unique wood-decay strategies and fruiting body development in the Schizophyllaceae

Running title: Wood-decay and development in *Schizophyllaceae*

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Summary

- Agaricomycetes produce the most efficient enzyme systems to degrade wood and the most complex morphological structures in the fungal kingdom. Despite decades-long interest in their genetics bases, the evolution and genetic repertoiresfunctional diversity of both wood-decay and fruiting body formation are incompletely known.

- Here, we perform comparative genomic and transcriptomic analyses of wood-decay and fruiting body development in the *Auriculariopsis ampla* and *Schizophyllum commune* (Schizophyllaceae), a familyspecies with secondarily simplified morphologies and an enigmatic wood-decay strategy and weak pathogenicity to woody plants.

- The plant cell wall degrading enzyme repertoires of Schizophyllaceae are transitional between those of white rot species and less efficient wood-degraders such as brown rot or mycorrhizal fungi. Rich repertoires of suberinase and tannase genes were found in both species, with tannases restricted to Agaricomycetes that preferentially colonize bark-covered wood, suggesting potential complementation of their weaker wood-decaying abilities and adaptations to wood colonization through the bark. Global fFruiting body transcriptomes in the twoof *A. ampla* and *S. commune* species revealed a high rate of divergence in developmental gene expression, but also several genes with conserved developmental expression, including novel transcription factors and small-secreted proteins, some of the latter we suggestmight represent fruiting body effectors.

- Taken together, our analyses highlighted novel aspects of wood-decay diversity and fruiting body development in a widely distributed family of mushroom-forming fungi.

Keywords: bark degradation, fruiting body development, genome, mushroom-forming fungi, RNA-Seq, small secreted proteins, transcription factors, wood decay

Introduction

Mushroom-forming fungi (Agaricomycetes) are of great interest for comparative genomics due to their importance as wood-degraders in global carbon cycling and as complex multicellular organisms that produce agriculturally or medicinally relevant fruiting bodies. Recent advances in genome sequencing has brought new light on several aspects of lignocellulose decomposition and the genetic repertoire of fruiting body development in mushroom-forming fungi (Ohm *et al.*, 2010, 2011; Sakamoto *et al.*, 2011; Sipos *et al.*, 2017; Krizsán *et al.*, 2019)~~(Ohm *et al.*, 2010, 2011; Sakamoto *et al.*, 2011; Sipos *et al.*, 2017; Krizsán *et al.*, 2019)~~.

The Agaricomycetes display diverse strategies to utilize lignocellulosic substrates. ~~While genomic analyses have helped to uncover the main patterns of duplication and loss of plant cell wall degrading enzyme (PCWDE) families, our understanding of the enzymatic repertoires of Agaricomycetes and how they use it to degrade various lignocellulosic components of plants is far from complete.~~ Fungi have traditionally been classified either as white rot (WR), in which all components of the plant cell wall are being degraded (Floudas *et al.*, 2012), or brown rot (BR), in which mostly cellulosic components are degraded, but lignin is left unmodified or only slightly modified (Martinez *et al.*, 2009). Comparative genomics has improved our understanding of the evolution of plant cell wall degrading enzyme (PCWDE) families significantly, yet our understanding of the enzymatic repertoires of Agaricomycetes is far from complete. Several species have been recalcitrant to ~~such~~ classification as WR or BR, which prompted a reconsideration of ~~the boundaries of the~~this classic ~~WR and BR~~ dichotomy (Riley *et al.*, 2014;

Floudas *et al.*, 2015). Such species are found across the fungal phylogeny, but seem to be particularly common among early-diverging Agaricales, including the Schizophyllaceae, Fistulinaceae and Physalacriaceae (Ohm *et al.*, 2010; Riley *et al.*, 2014; Floudas *et al.*, 2015). *Schizophyllum commune*, the only hitherto genome-sequenced species in the Schizophyllaceae, for example, produces white rot like symptoms, but lacks lignin-degrading peroxidases, one of the hallmark gene families ~~(e.g. lignin-degrading peroxidases)~~ of WR ~~fungi~~ (Ohm *et al.*, 2010; Riley *et al.*, 2014; Floudas *et al.*, 2015; Zhu *et al.*, 2016). ~~Accordingly, i~~It lacks the ability to degrade lignin and achieves weak degradation of wood (Schmidt & Liese, 1980; Padhiar & Albert, 2011; Floudas *et al.*, 2015), although this might be complemented by pathogenic potentials on living plants or the activity of other, more efficient degraders that co-inhabit the same substrate(Schmidt & Liese, 1980). Analyses of the secretome and wood-decay progression of *S. commune* revealed both WR and BR-like behaviors (Takemoto *et al.*, 2010; Zhu *et al.*, 2016)~~(Takemoto *et al.*, 2010; Zhu *et al.*, 2016)~~, although several questions on the biology of this species remain open. For example, whether the Schizophyllaceae shows specialization to decay certain types/parts of trees or how they compare with pathogenic Agaricomycetes are not known.

Fruiting body production, is a highly integrated developmental process triggered by a changing environment, such as a drop in temperature, nutrient depletion or shifts in light conditions (Kües & Liu, 2000; Kües & Navarro-González, 2015; Sakamoto *et al.*, 2018). It results from the concerted expression of structural and regulatory (Martin *et al.*, 2008; Stajich *et al.*, 2010; Ohm *et al.*, 2011; Muraguchi *et al.*, 2015; Nagy *et al.*, 2016; Lau *et al.*, 2018) genes as well as other processes, such as alternative splicing (Gehrmann *et al.*, 2016; Krizsán *et al.*, 2019), allele-specific gene expression (Gehrmann *et al.*, 2016) and probably selective protein modification (Pelkmans *et al.*, 2017; Krizsán *et al.*, 2019). Known structural genes include ones coding for hydrophobins (Lugones *et al.*, 1996; Wösten *et al.*, 1999;

Bayry *et al.*, 2012) , lectins (Cooper *et al.*, 1997; Boulianne *et al.*, 2000; Hassan *et al.*, 2015), several cell wall chitin and glucan-active CAZymes (Wessels, 1994; Fukuda *et al.*, 2008; Sakamoto *et al.*, 2011; Konno & Sakamoto, 2011), and include probably genes for cerato-platanins, expansin-like proteins (Sipos *et al.*, 2017; Krizsán *et al.*, 2019), among others~~and an array of other genes (Liu, 2005)~~. Regulators of fruiting body development have been characterized in several species, in particular in *Coprinopsis cinerea (Stajich et al.*, 2010; Cheng *et al.*, 2013; de Sena-Tomas *et al.*, 2013; Muraguchi *et al.*, 2015; Masuda *et al.*, 2016) and *S. commune (Ohm et al.*, 2010, 2011; Pelkmans *et al.*, 2017). Despite ~~much~~ advances in this field, several aspects of fruiting body development are ~~quite~~ poorly known, including, for example what genes have conserved developmental roles across fruiting body forming fungi or how cell-cell communication is orchestrated in developing fruiting bodies. *S. commune* has served as a model organism for fruiting body development for a long time (Kües & Liu, 2000; Ohm *et al.*, 2010; Kües & Navarro-González, 2015). This species, like the genus *Auriculariopsis* ~~other Schizophyllaceae (e.g. the genus *Auriculariopsis*)~~ produce secondary simplified, 'cyphelloid' fruiting bodies, which derived from more complex ancestors. Whether this simplification is correlated with a reduced repertoire of developmental (structural or regulatory) genes or what the streamlined development of the Schizophyllaceae can tell about the minimal genetic toolkit required for fruiting body development are not known, however. ~~are reduced morphologies derived from more complex ancestors. Cyphelloid fruiting bodies are inverted cup-like forms with unstructured (e.g. *A. ampla*) or slightly structured (e.g. *S. commune*) spore-bearing surfaces (hymenophore). Albeit the hymenophore structure of *S. commune* resembles gills (hence the common name 'split gill'), it is not homologous to real gills of mushrooms, rather, it results from the congregation of several individual cup-like fruiting bodies.~~

We here analyze PCWDE repertoires and fruiting body development in *Auriculariopsis ampla* and its close relative, *S. commune*. ~~both~~ Both species produce simple, cup-shaped fruiting bodies and can inhabit the bark of dead logs, with a preference for bark, albeit *S. commune* is also observed on decorticated or sapwood.~~of which primarily inhabit the bark of dead logs and produces simple, cup-shaped fruiting bodies.~~ We sequenced the genome of *A. ampla* using PacBio ~~platform~~ and generated RNA-Seq data for a time series of fruiting body development. Through analyses of gene repertoires for plant cell wall degradation in *A. ampla, S. commune* and 29 other Agaricomycetes, we detect signatures of adaptation to wood colonization through the bark and suggest that these two species have unusual plant cell wall degrading enzyme repertoires. Using the~~By sequencing~~ developmental transcriptomes of *A. ampla* and ~~comparing it to that of~~ *S. commune*, we identify genes with a conserved pattern during ~~developmental genes that might be linked to~~ fruiting body development, including small secreted proteins, some of which show extreme expression dynamics in fruiting bodies.

Methods

Genome sequencing

The sequenced strain of *Auriculariopsis ampla* was collected in Szeged, Hungary and cultured in liquid malt-extract medium (deposited in SZMC, under NL-1724). DNA was extracted using the DNeasy Blood & Tissue Culture kit (Qiagen), following the manufacturer's protocol. The genome was sequenced using Pacific Biosciences RS II platform. Unamplified libraries were generated using Pacific Biosciences standard template preparation protocol for creating >10kb libraries. 5 µ~~u~~g of gDNA was used for each library and sheared using Covaris g-Tubes (TM) to generate >10kb fragments. The sheared DNA fragments were then prepared using the Pacific

Biosciences SMRTbell template preparation kit, ~~by~~ treated~~ing~~ with DNA damage repair, had their ends repaired and 5’ phosphorylated. PacBio hairpin adapters were then ligated to create SMRTbell template, which were ~~then~~ size-selected using AMPure PB beads. SMRTbell libraries were sequenced on a Pacific Biosciences RSII sequencer using Version C4 chemistry and 4-hour sequencing movie run times. Filtered subread data was assembled with Falcon version 0.4.2 (https://github.com/PacificBiosciences/FALCON) and polished with Quiver version smrtanalysis_2.3.0.140936.p5 (https://github.com/PacificBiosciences/GenomicConsensus).

For the transcriptome, Stranded cDNA libraries were generated using the Illumina Truseq Stranded RNA LT kit. mRNA was purified from 1 μ~~u~~g of total RNA using magnetic beads containing poly-T oligos. mRNA was fragmented and reversed transcribed using random hexamers and SSII (Invitrogen) followed by second strand synthesis. The fragmented cDNA was ~~treated with~~ end-paired, A-tailed~~ing~~, adapters ligated to them~~ion~~, and subjected 8 cycles of PCR. The library was then quantified ~~library was then~~ and sequenced on ~~the~~ an Illumina HiSeq2500 sequencer using HiSeq TruSeq SBS sequencing kits, v4, following a 2x150 indexed run recipe. Illumina fastq files were QC filtered for artifact/process contamination and de novo assembled with Trinity v2.1.1 (Grabherr *et al.*, 2011).

The genome was annotated using the JGI Annotation pipeline (Grigoriev *et al.*, 2014) and made available via JGI MycoCosm (jgi.doe.gov/fungi;(Grigoriev *et al.*, 2014)). The data also deposited at DDBJ/EMBL/GenBank under the accession *(TO BE PROVIDED UPON PUBLICATION).*

Fruiting protocol, RNA extraction and transcriptome sequencing

Fruiting and RNA extraction

Auriculariopsis ampla was grown on solid sterilized poplar (*Populus alba*) bark and solid wood pieces plugged into MEA in 250 ml glass beakers. Cultures were incubated for 14 days in the dark at 30°C, then transferred to room temperature 60 cm under a light panel of 6 Sylvania Activa 172 Daylight tubes, with a 12 hr light/dark cycle and >90% relative humidity. Primordia started to develop 7 days after the transfer to light.

Vegetative mycelium, Stage 1 and 2 primordia, young and mature fruiting bodies were collected, flash-frozen in liquid nitrogen and stored at -80°C. Stage 1 and 2 primordia were defined as 0.1-1 mm closed, globular structures and 1-2 mm long initials with a central externally visible pit, respectively. Total RNA was extracted using the Quick-RNA Miniprep kit (Zymo Research), following the manufacturer's protocol. Three biological replicates were processed.

RNA-Seq

Transcriptome sequencing was performed using the TrueSeq RNA Library Preparation Kit v2 (Illumina) according to the manufacturer's instructions. RNA quality and quantity were assessed using RNA ScreenTape and Reagents on TapeStation (all from Agilent); only high quality (RIN >8.0) total RNA samples were processed. Next, RNA was DNaseI (ThermoFisher) treated and the mRNA was purified based on PolyA selection and fragmented. First strand cDNA synthesis was performed using SuperScript II (ThermoFisher) followed by second strand cDNA synthesis, end repair, 3'-end adenylation, adapter ligation and PCR amplification. Purification was done using AmPureXP Beads (Beackman Coulter). DNA cConcentration of each library was determined using the KAPA Library Quantification Kit for Illumina (KAPA Biosystems). Sequencing was performed on Illumina instruments using the HiSeq SBS Kit v4 250 cycles kit (Illumina) generating >20 million clusters for each sample.

Bioinformatic analyses of RNA-Seq data

RNA-Seq analyses and mapping of raw data were carried out as reported earlier (Sipos *et al.*, 2017; Krizsán *et al.*, 2019). “Total gene read” RNA-Seq count data was imported from CLC Genomic Workbench (ver. 9.5.2, CLC bio/ Qiagen) into R 3.0.2 (R Core Team, 2018). Only genes that were detected by at least five mapped reads in at least 25% of the samples were included in the study. Subsequently, “calcNormFactors” from “edgeR” 3.4.2 (Robinson *et al.*, 2010) was used to perform data scaling based on the “trimmed mean of M-values” (TMM) method73. Log transformation was carried out by the “voom” function of the “limma” package 3.18.13 (Ritchie *et al.*, 2015). Linear modeling, empirical Bayes moderation and the calculation of differentially expressed genes were done using “limma”. Genes showing at least four-fold gene expression change with an FDR value below 0.05 were considered as significantly differentially expressed. Multi-dimensional scaling (“plotMDS” function in edgeR) was applied to visually summarize gene expression profiles revealing similarities between samples.

Developmentally regulated genes were defined as genes showing an >4-fold change in expression through development. In comparisons of vegetative mycelia and stage 1 primordia, we only considered genes upregulated in primordia, to exclude genes that showed a highest expression in vegetative mycelium because those might be related to processes not relevant for fruiting body development (e.g. nutrient acquisition).

Phylogenetic analysis

Single-copy orthogroups were identified in MCL clusters of the 31 Agaricomycetes and were aligned by the l-ins-i algorithm of MAFFT (Katoh & Standley, 2013). Ambiguously aligned regions were removed using the 'strict' settings of Trim-Al. Trimmed alignments >100 amino acids were concatenated into a supermatrix. Maximum likelihood inference was performed in RAxML 8.2.11 under the PROTGAMMALG model, with a gamma-

distributed rate heterogeneity and a partitioned model. A bootstrap analysis in 100 replicates was performed.

Identification of orthologous groups

Orthogroups have been identified using OrthoFinder v 1.1.8 (Emms & Kelly, 2015). Two analyses were performed, one to delimit orthogroups across 31 Agaricomycetes species and the second to find co-orthologs shared by *A. ampla* and *S. commune*. Functional annotation done using InterProscan version 5.28-67.0.

Analyses of Carbohydrate Active Enzymes (CAZymes)

CAZymes were annotated using the CAZy annotation pipeline (Lombard *et al.*, 2014). Of all the families found in the dataset, we retained ones with a putative role in plant cell wall (PCW) degradation (Floudas *et al.*, 2012, 2015; Riley *et al.*, 2014; Nagy *et al.*, 2016)(Table S1) and analyzed their copy numbers across the 31 species. We also assessed genes encoding proteins with putative roles in suberin and tannin degradation. We extracted the best BLAST hits (BLAST 2.7.1+, e-value<0.001) from the 31 species for proteins implicated in suberin (Kontkanen *et al.*, 2009; Martins *et al.*, 2014) and tannin degradation (Gonçalves *et al.*, 2012; Nieter *et al.*, 2016). We then identified the ~~ortho~~MCL clusters of the 31 species containing the best hits. These clusters were used for further analysis as putative suberinases or tannases (Table S1).

We compared copy numbers of *A. ampla* and *S. commune* to that of 29 Agaricomycetes species, including brown rotters (BR), ectomycorrhizal (ECM), saprotrophs/litter decomposers/organic matter degraders (S/L/O), white rotters (WR) and uncertain. Phylogenetic PCA was performed on CAZyme copy numbers using the phyl.pca (Revell, 2009) function from phytools (Revell, 2012). A copy number matrix normalized by proteome size (Table S1), and the ML species tree, were used as input. Independent

contrasts were calculated under the Brownian motion model and the parameter mode="cov".

Analyses of transcriptome similarity and fruiting body genes

Pairwise comparisons of *A. ampla* and *S. commune* gene expression based on Pearson correlation coefficient among all replicates and developmental stages of orthogroups containing >1 developmentally regulated genes were performed using custom Python scripts (pandas v 0.18.1 and Matplotlib v. 1.1.1 libraries). The same analysis was performed for 252 co-orthologous transcription factors (TFs). The matrix of Pearson correlation coefficients was plotted as a heatmap using the Matplotlib 1.1.1 pyplot framework.

Expression heatmaps were created using the heatmap.2 function of R 'gplots' package. Hierarchical clustering with Euclidean distance and averaged-linkage clustering was carried out on FPKM values using 'hclust' function in R.

We identified TF genes in the 31 species based on InterPro annotations. Only proteins containing domains with sequence-specific DNA binding ability were considered as TFs (Krizsán *et al.*, 2019).

Small Secreted Proteins (SSPs) were defined as proteins shorter than 300 amino acids, having a signal peptide, an extracellular localization and no transmembrane domain. Proteins shorter than 300 amino acids were subjected to signal peptide prediction through SignalP 4.1(Petersen *et al.*, 2011) with the option "eukaryotic". Proteins having extracellular signal peptide were checked for their extracellular localization using WoLF PSORT 0.2 (Horton *et al.*, 2007) with the option "fungi" and were checked for the absence of transmembrane helices, using TMHMM 2.0 (Krogh *et al.*, 2001).

Results and Discussion

The genome of *Auriculariopsis* is typical for the Agaricales

To obtain a second representative genome from the Schizophyllaceae, we sequenced that of *Auriculariopsis* using PacBio, and assembled it to 49.8 Mb of DNA sequence in 351 scaffolds (mean coverage: 54.38x, 343 scaffolds >2 kpb, N50: 19, L50: 0.53 Mb). We predicted 15 576 protein coding genes, for which BUSCO analysis showed a 98.6% completeness (273 complete, 28 duplicated, 2 fragmented, 2 missing). We included *A. ampla* and *S. commune* in a comparative analysis with 29 other Agaricomycetes. A species phylogeny was reconstructed from 362 single-copy orthologs (142 436 amino acid characters) for the 31 taxa; the inferred topology resembles published genome-scale trees of Agaricomycetes very closely and received strong (>85%) bootstrap support for all but two nodes (Fig 1a). Across the phylogeny, the gene repertoire of *A. ampla* (15 576 genes) is very similar to that of *S. commune(Ohm et al.*, 2010) (16 319 genes) and the average gene count in the analyzed Agaricales species (17 655), but more than that of *F. ~~-~~ istulina hepatica (Floudas et al.*, 2015) (11 244 genes), the sister species of the Schizophyllaceae. We found 8 significantly overrepresented (***p***-value<=0.05) and 16 underrepresented (***p***-value<=0.05) InterPro domains in both species, relative to the other 29 species (Table S2).

The Schizophyllaceae may be adapted to ~~decaying barked wood~~early colonization of wood

We analyzed copy numbers of 45 PCWDE families (Table S1) ~~as well as~~ putative suberin- and tannin-degrading families as well as pathogenicity-related genes. Phylogenetically corrected principal component analyses portray a ~~clear~~ separation of the Schizophyllaceae from species with most nutritional modes~~the other 29 Agaricomycetes~~, but patterns of separation differ based on the main substrate of the PCWDE families. Based on cellulose repertoires, *A. ampla* and *S. commune* cluster together with WRs and S/L/O,

suggesting a similar arsenal of CAZymes for cellulose degradation (Fig 1b). Enzyme families acting on crystalline cellulose (cellobiohydrolases - GH6, GH7) were present in lower numbers than in WRs and S/L/O species, similar to ectomycorrhizal ones. The pattern was mostly identical for hemicellulases and pectinases (Fig 1b) where CAZyme copy numbers were similar to that of WRs and litter decomposers. However, some CAZymes with xylanase and pectinase activities, including xylosidases, pectate lyases, pectin acetylesterases, and acetyl xylan esterases (AA8, GH30, GH43, GH95, CE12, PL1, PL3, PL4), have higher copy numbers in the two species than in most WRs. This could imply their ability to degrade hemicellulose and pectin, as reported previously (Zhu *et al.*, 2016). However, ligninolytic CAZymes revealed a clear difference from WR species. Here, both Schizophyllaceae clustered towards ectomycorrhizal and BR species, which lack the ability to effectively attack lignin polymers. We find that this pattern was primarily driven by the absence of class II peroxidases (PODs, AA9) and reductions in copper radical oxidases (CROs) in the Schizophyllaceae. The absence of PODs has been already shown before (Martinez *et al.*, 2009; Floudas *et al.*, 2012; Riley *et al.*, 2014) , ~~whereas~~ CROs, which supply hydrogen peroxide in lignin degradation, were found to have very low numbers of CROs (AA5, 2-3 genes) in *Auriculariopsis* and *Schizophyllum* as compared to ECM, S/L/O and WR species. In this regard, the Schizophyllaceae resembles BR species, which usually have reduced CRO repertoires ~~and are usually underrepresented in BRs~~(Floudas *et al.*, 2015)., ~~were found to have very low numbers of CROs (AA5) in *Auriculariopsis* and *Schizophyllum* as compared to ECM, S/L/O and WR species.~~

Because *A. ampla* and *S. commune* often occur on ~~bark~~ dead logs as first colonizers, we also examined protein families that putatively degrade important bark compounds. Suberin, lignin and tannins represent the major components of bark (Kontkanen *et al.*, 2009; Gonçalves *et al.*, 2012; Martins *et al.*, 2014). We built on previous datasets to obtain putative suberinase

(Kontkanen *et al.*, 2009; Martins *et al.*, 2014) and tannase (Gonçalves *et al.*, 2012; Nieter *et al.*, 2016) copy numbers for 31 species in our dataset. In general, suberin comprises aromatic compounds cross linked by poly-aliphatic and fatty-acid like components which requires extracellular esterases and lipases for their breakdown (Kontkanen *et al.*, 2009). Based on phylogenetic PCA of putative suberinases *A. ampla* and *S. commune* were transitional between typical WR and ECM, BR (*Fistulina*), uncertain (*Cylindrobasidium, Pluteus)* or tentative WR (*Fibulorhizoctonia*) species. This separation is most pronounced along the first axis (PC1), the main contributor of which is the AA3 family. *A. ampla* and *S. commune* had few genes in this family, similar to most ECM species. In terms of most other families, *A. ampla* and *S. commune* resembled WR species. The cutinase (CE5) repertoires of the two species are similar to those of litter decomposers and certain WR taxa (e.g. *Galerina, Dendrothele, Fibulorhizoctonia,* and *Peniophora*), although this family was missing from several WR species. Tannin acyl hydrolases (tannase, EC 3.1.1.20) are responsible for the degradation of tannins, polyphenolic plant secondary metabolites characteristic to the bark and wood tissues. Tannases were found in 10 out of 31 species, mostly in those that occur preferentially on bark, such as *Auriculariopsis, Schizophyllum, Peniophora*, *Dendrothele* and *Plicaturopsis,* and a few others *(Gymnopus, Pterula, Fibulorhizoctonia, Omphalotus* and *Fistulina*). This could indicate a specialization of these species to substrates with high tannin content, such as bark, suggesting adaptations to the early colonization of bark-covered wood. Notably, *Pluteus*, a species with an uncertain nutritional mode, groups closely together with *A. ampla* and *S. commune* on the suberinase PCA, although it had low pectinase, hemicellulase and cellulase copy numbers, leading to a position close to ECM species and some litter decomposers in other PCA analyses (Fig S1).

As *S. commune* has been reported as a weak pathogen of shrubs and trees (Takemoto *et al.*, 2010), we examined the copy numbers of 22 gene families previously reported to be linked to pathogenicity in Agaricomycetes (Mondego *et al.*, 2008; Olson *et al.*, 2012; Sipos *et al.*, 2017) ~~by Agaricomycetes~~, including ~~CBM50s,~~ salicylate hydroxylases, secondary metabolism-related genes, homologs of pathogenesis-related 1 protein, among others. Both species have rich repertoires of ~~the~~pathogenicity genes (Fig S5, Table S3), similar to other WR and pathogen species (e.g. *Armillaria* spp.), although none of the families stand out as enriched. This is in line with the reported weak pathogenic ability that has been reported for *S. commune* (but not yet for *A. ampla*) and may reflect a recent acquisition of the potential or that other processes (e.g. gene expression regulation) underlie its evolution.

Taken together, the CAZyme composition of *A. ampla* and *S. commune* shows similarity to that of WR species when concerned with cellulases, hemicellulases and pectinolytic gene families. What sets them apart from most WRs is the absence of class II peroxidases, which is also the case for BRs and ectomycorrhizal fungi. However, they have several putative suberinases and tannases that might depolymerize important bark components. This might indicate an adaptation to degrading bark components, which, although needs to be verified by additional studies, would provide a framework for interpreting the odd CAZyme composition of the Schizophyllaceae (Riley *et al.*, 2014; Floudas *et al.*, 2015) and would expand our understanding of the nutritional diversity of wood-decay fungi.

Transcriptomics reveals a high rate of developmental evolution

Auriculariopsis ampla and *S. commune* have a similar developmental progression (Fig 2a-2e), permitting a comparison of their transcriptional programs. Fruiting body development starts in both species with the

appearance of minute globose primordia (stage 1 primordia), in which a cavity develops (Stage 2 primordia). This cavity further expands in *A. ampla* to produce an open, pendant fruiting body (Young fruiting body and fruiting body stages), whereas in *S. commune* radial slits emerge from within the cup to form pseudolamellae~~several such units form a multi-lobed assemblage~~.

To compare their development, we generated RNA-Seq data from 5 developmental stages of *A. ampla* (vegetative mycelium, stage 1 and stage 2 primordia, young and mature fruiting bodies, see Fig 2a-b,d) in biological triplicates, >30 million (30-78M, mean: 46M) paired-end 150 base reads for each sample on Illumina platform (mean read mapping: 83%, Table S43). Corresponding data for the same developmental stages (Fig 2c, e) ~~for~~ from *S. commune* were taken from (Krizsán *et al.*, 2019). Based on global transcriptome similarity, fruiting body samples grouped together, away from vegetative mycelium (Fig 3a), consistent with the complex multicellular nature of fruiting bodies as opposed to a simpler cellularity level of vegetative mycelia. ~~Among the fruiting body samples, s~~Stage 1 and stage 2 primordia were similar to each other in both species, whereas young fruiting bodies and mature fruiting bodies formed distinct groups. We identified 1466 developmentally regulated genes in *A. ampla*, which is similar in magnitude to that we reported for *S. commune* (2000), but less than that for more complex species (e.g. 7583 and 4425 in *Coprinopsis* and~~,~~ *Armillaria*; taken from (Krizsán *et al.*, 2019). Of the developmentally regulated genes, 967 showed a significant (≥4) fold change in the transition from vegetative mycelium to stage 1 primordia. In terms of significantly differentially expressed genes (DEGs), the highest numbers of up and downregulated were also found between vegetative mycelium and stage 1 primordia (11656~~6~~ and 8412~~2~~ genes, Fig 3b), with much fewer DEGs found in comparisons of subsequent stages. ~~which is consistent with the position of samples on the MDS plot (Fig 3a). Much fewer genes were differentially expressed between stage 1 and 2 primordia and between stage 2 primordia and young fruiting~~

bodies. In fruiting bodies, we found 10910 and 37 significantly up- and downregulated genes, respectively, a comparatively higher number that is potentially related to sporulation.

We assessed the similarity between the 2 species' developmental transcriptomes by analyzing the expression of one-to-one orthologous gene pairs, hereafter referred to as co-orthologs. To identify co-orthologs, proteomes of *A. ampla* and *S. commune* were clustered into 18,804 orthogroups using MCLOrthoFinder, of which 7463 represented co-orthologs. Of these, 7369 co-orthologs were expressed under our experimental conditions in both species (Table S54). Pairwise similarity across the 7369 co-orthologs was the highest within speciescomparison between developmental stages showed highest similarity within species across all 7369 co-orthologs (Fig 4a). This pattern was more pronouncedstronger in an analysis of developmentally regulated co-orthologs (Fig 4b, see Methods), indicating that developmental gene expression in *A. ampla* and *S. commune* has diverged since their last common ancestor so that similarity between homologous similar fruiting body stages of the two species is lower than that between different stages of the same species. Vegetative mycelia of both species differed most from all other stages of the same species but were showed some similaritysimilar across species. Similarly, we observed a strong similarity between young fruiting bodies and fruiting bodies of *A. ampla* and *S. commune*, indicating that late stages of fruiting body development share more similarity across species than do early stages. Similar patterns were observed when the analyses were restricted to co-orthologous transcription factors (Fig 4c) and its developmentally regulated subset (Fig 4d). Similarity between late developmental stages of the two species was more pronounced in the analysis of developmentally regulated genes. Given that *A. ampla* and *S. commune* are each other's closest relatives, the higher within-species than among-species low overall similarity of gene expression among their fruiting bodies (Fig 4) indicates

that developmental gene expression has been diverging rapidly since their common ancestored at a high speed since their divergence. This is surprising in comparison to similar analyses of animals, where gene expression patterns could be predicted from tissue identities across the entire mammalian clade (Breschi *et al.*, 2017). This suggests that fruiting body development evolved at a high rate in the Schizophyllaceae, erasing identities of similar developmental stages across species. Nevertheless, these data revealed some conserved patterns of gene expression during fruiting body maturation between phylogenetically closely related species, indicating that there should be genes with similar expression profiles in *A. ampla* and *S. commune*.

Despite the low global similarity, several genes with conserved expression patterns could be identified. The most highly upregulated co-ortholog in *A. ampla* and *S. commune* was a heat shock protein 9/12 family member, that is homologous to *Aspergillus nidulans* awh11 and *S. cerevisiae* hsp12, two farnesol-responsive heat shock proteins. These genes showed 254- and 855- fold had a significant upregulation in stage 1 primordia of both *A. ampla* and *S. commune*, (fold change 254x and 855x, respectively) and had high expression values in all fruiting body tissues (>5,000 FPKM, maximum fold change within fruiting bodies 3.4-3.7), suggesting an important role of heat shock proteins during fruiting body development. In further support of this hypothesis, homologs of these genes were found developmentally regulated or differentially expressed also in *Laccaria bicolor* fruiting bodies *(Martin et al.*, 2008), *Lentinula edodes (Song et al.*, 2018), *Armillaria ostoyae, Coprinopsis cinerea, Lentinus tigrinus,* and *Rickenella mellea (Krizsán et al.*, 2019). Another co-ortholog with significant upregulation in stage 1 primordia included A1 aspartic proteases, although the expression dynamics were somewhat different in the two species. We observed an induction in stage 1 primordia in both, but, while upregulation in *A. ampla* was >200x compared to VM, it was only 14x in *S. commune*.

Aspartic proteases of the ~~diverse~~ A1 family have been reported as highly induced in fruiting bodies in several previous studies (Martin *et al.*, 2008; Sabotic *et al.*, 2009; Rahmad *et al.*, 2014; Song *et al.*, 2018; Krizsán *et al.*, 2019), ~~although~~ but no mechanistic hypothesis for their role in fruiting bodies~~y~~ ~~development~~ has been proposed yet.

Putative fruiting body genes show developmentally dynamic expression

We further examined the expression patterns of previously reported fruiting body genes in *A. ampla* and *S. commune* ~~of fruiting body genes reported from other species~~. Of the fungal cell wall (FCW) associated genes, hydrophobins were mostly developmentally regulated (8 out of 11 genes) in *A. ampla* (Fig S2), often with significantly increased expression coincident with the transition from vegetative mycelium to stage 1 primordia (in six genes), as observed previously (van Wetter *et al.*, 1996, 2000; Banerjee *et al.*, 2008; Ohm *et al.*, 2011; Song *et al.*, 2018). Several members of two functionally similar families, cerato-platanins (4 of 5 genes dev. reg.) and expansin-like genes (10 of 21 genes) were likewise developmentally regulated in both species. Although both families were mostly ~~cerato-platanins and expansins were often~~ associated with the plant cell wall (Baccelli, 2014; Tovar-Herrera *et al.*, 2015), their dynamic expression in fruiting bodies observed here and previously (Sipos *et al.*, 2017; Krizsán *et al.*, 2019) suggest potential FCW-related roles. S~~Functional annotations uncovered s~~everal putatively FCW-active CAZymes (Fig S3), were developmentally regulated, including chitin- and glucan- active GH and GT families, carbohydrate-binding modules, carbohydrate esterases, AA1 multicopper oxidases, AA9 lytic polysaccharide monooxygenases, reinforcing the view that cell wall remodeling is a fundamental mechanism in fruiting body development (Sakamoto *et al.*, 2006, 2011, 2017; Busch & Braus, 2007; Martin *et al.*, 2008; Ohm *et al.*, 2010; Buser *et al.*, 2010; Konno & Sakamoto, 2011; Krizsán *et al.*, 2019) ~~but also starch cleaving glycosyl hyrolases (e.g.~~

GH15, CBM20), which might be related to the mobilization of glycogen reserves during development. Two out of 10 members of the Kre9/Knh1 family were developmentally regulated. This family is involved in *β*-1,6-glucan synthesis and remodeling in *Aspergillus fumigatus (Costachel et al.*, 2005), *Candida albicans (Lussier et al.*, 1998), *Saccharomyces cerevisiae (Brown & Bussey, 1993)* and *Ustilago maydis* (Robledo-Briones & Ruiz-Herrera, 2013) and has been shown to be developmentally expressed in Agaricomycetes fruiting bodies (Szeto *et al.*, 2007; Krizsán *et al.*, 2019). Its widespread FCW-associated role in both Asco- and Basidiomycota suggests a plesiomorphic role in *β*-glucan assembly and co-option for in the cell wall and suggests that this family has been co-opted for fruiting body development in Agaricomycetes. Several other previously reported putatively FCW-active CAZyme families (Martin *et al.*, 2008; Wang *et al.*, 2013; Park *et al.*, 2014; Zhang *et al.*, 2015; Sakamoto *et al.*, 2017; Krizsan *et al.*, 2018; Song *et al.*, 2018) (e.g GH5, GH142 (Sakamoto *et al.*, 2005, 2011; Hurtado-Guerrero *et al.*, 2009; Ene *et al.*, 2015), Fig S2), also showed developmental expression in *A. ampla*, reinforcing the view that cell wall remodeling is a fundamental mechanism in fruiting body development (Sakamoto *et al.*, 2006, 2011, 2017; Busch & Braus, 2007; Martin *et al.*, 2008; Ohm *et al.*, 2010; Buser *et al.*, 2010; Konno & Sakamoto, 2011; Krizsan *et al.*, 2018).

W*A. ampla* and *S. commune* have reduced repertoires of defense-related genes compared to *Coprinopsis*e found developmental regulation of a diverse array of putative defense-related genes by searching for homologs of *Coprinopsis* defense genes (Plaza *et al.*, 2014) (Fig S2) consistent with their simplified fruiting body morphologies. *A. ampla* and *S. commune* have reduced repertoires of defense-related genes compared to *Coprinopsis* (Fig S2). For example, no homologs of aegerolysins or the ETX/MTX2 pore-forming toxin genes (Lakkireddy *et al.*, 2011), and have s exist in their genomes, whereas lectins are represented by 14 lectin genes as opposed to 39 and 25 in *C. cinerea* and *A. ostoyae*. The Schizophyllaceae have several

thaumatin genes, which ~~has~~ have been associated with defense in both fungi (Plaza *et al.*, 2014) and plants (Rajam *et al.*, 2007; Zhang *et al.*, 2017), fungal pathogenicity (Zhang *et al.*, 2018) but also with FCW remodeling (Grenier *et al.*, 2000; Sakamoto *et al.*, 2006). Thaumatins possess endo-*β*-1,3-glucanase activity, and ~~are~~ can degrade cell wall components of ~~*Lentinula* *L.*~~ *edodes (Sakamoto et al.*, 2006) and *Saccharomyces (Grenier et al.*, 2000). These properties and their developmental expression in axenic fruiting bodies suggest a role in FCW remodeling, although antimicrobial activities have also been predicted for certain members (Krizsán *et al.*, 2019). Cerato-platanins represent a similar case (see also above); they are widely expressed in pathogenic fungal-plant interactions (Chen *et al.*, 2013; Gaderer *et al.*, 2014), fruiting bodies (Gaderer *et al.*, 2014; Sipos *et al.*, 2017; Krizsán *et al.*, 2019) and defense assays (Plaza *et al.*, 2014) and are significantly enriched in Agaricomycetes genomes (Chen *et al.*, 2013; Krizsán *et al.*, 2019). Thaumatins and cerato-platanins provide examples for families traditionally associated with plant pathogenicity but for which deeper analyses reveal morphogenetic functions, suggesting a link between morphogenesis and pathogenicity.~~We detected four developmentally regulated cerato-platanin genes in *A. ampla*, three of which showed an induction in stage 1 primordia (Fig S2). *S. commune* had three developmentally regulated cerato-platanins, with non-matching expression profiles. Further, in *A. ampla* we found three developmentally regulated lectin genes (Fig S2), as opposed to *S. commune*, which had eight (Krizsan *et al.*, 2018). All three genes belong to the ricin-B lectin family and harbor a CBM13 domain, which has mannose, N-acetylgalactosamine and xylane binding activities (Boraston *et al.*, 2000; Fujimoto, 2013). Ricin-B lectins have been reported as developmentally expressed in fruiting bodies of all Agaricomycetes tested so far (Liu *et al.*, 2000; Wang *et al.*, 2013; Plaza *et al.*, 2014; Sipos *et al.*, 2017; Krizsan *et al.*, 2018; Song *et al.*, 2018), although their functions are unclear. It is the largest family of basidiomycete lectins (Krizsan *et al.*, 2018) and was shown to be toxic to nematodes (Schubert *et*

~~*al.*, 2012; Hassan *et al.*, 2015), although their diverse carbohydrate-binding abilities (mannose and N-acetylgalactosamine) could confer additional or other functions as well.~~

F-box and BTB/POZ proteins have recently been reported as an interesting family related to fruiting body development (Krizsán *et al.*, 2019). *Auriculariopsis* has 246 F-box protein encoding genes, of which 12 were developmentally regulated in our dataset. Of the 96 BTB/POZ domain-containing proteins 26 were developmentally regulated, including some genes with remarkable expression dynamics during development (e.g. ~~fold change~~ 526~~- fold change×~~, Auramp1_515369). This is similar to figures reported for *S. commune (Krizsán et al.*, 2019). These domains are involved in protein-protein interactions and have been reported to act as transcriptional repressors (Collins *et al.*, 2001), members of selective proteolysis pathways, and include homologs of yeast *Skp1 (Connelly & Hieter, 1996)* too. Although very little functional information on these families is available in fungi, their expression dynamics in development and previously reported regulatory roles suggest they could be important players in fruiting body development.

Conserved patterns of transcription factor~~s~~ expression

~~We examined expression patterns of transcription factors (TFs) and their similarity between the two species.~~ We identified 433 and 437 TFs in the genomes of *A. ampla* and *S. commune* respectively, of which 252 were co-orthologs. These were distributed across 28 TF families, with C2H2-type Zinc finger and Zn (2)-C6 fungal-type TFs being the most dominant (Table S6~~5~~). Individually, 14.5% and 16% of the *Auriculariopsis* and *Schizophyllum* and 17% of the co-orthologous TFs were developmentally regulated, respectively. Of the

~~These included 5 of the~~ eight previously characterized TF genes of *S. commune (Ohm et al.*, 2011)~~:~~ *c2h2, gat1, hom1, tea1* and *fst4* showed

significant changes in expression, in most cases at the initiation of fruiting body development, whereas *fst3, bri1* and *hom2* showed more or less flat expression profiles (Fig S6~~5~~). Th~~is~~ is ~~ese expression profiles are~~ consistent with previous RNA-Seq based reports (Pelkmans *et al.*, 2017) in *Schizophyllum* and other species (Morin *et al.*, 2012; Plaza *et al.*, 2014; Zhou *et al.*, 2014; Pelkmans *et al.*, 2016), except in *hom1* and *gat1*, which, in our data behaved differently, probably due to the different resolution of developmental stage data. The expression profiles of all eight genes were very similar between *A. ampla* and *S. commune*. Homologs of ~~*Lentinula* *L. edodes PriB*~~ (Endo *et al.*, 1994; Miyazaki *et al.*, 1997) (Auramp1_518770, Schco3_2525437) were also developmentally regulated, with an expression peak in stage 1 primordia. Homologues of *Coprinopsis exp1*, which was reported to be involved in cap expansion (Muraguchi *et al.*, 2008), were present in both species (Auramp1_481073, Schco3_2623333) and had a matching expression profile, but ~~were not developmentally regulated in our data. In our experiments, *exp1* homologs (Auramp1_481073, Schco3_2623333)~~ showed highest expression in vegetative mycelia and lower expression afterwards, which might be related to the lack of proper caps in *A. ampla* and *S. commune*.

Of the 252 co-orthologous TFs, 42 were developmentally regulated in both species, 27 of which had similar expression profiles between *A. ampla* and *S. commune*. Nine of the most interesting of these TFs are shown on Fig ~~5~~S6. Six of these genes showed ~~Three of these genes showed highest expression in vegetative mycelia and are probably not relevant to fruiting body development. For the other six genes an~~ upregulation ~~was observed~~ at the transition from vegetative mycelia to stage 1 primordia, which is compatible with potential roles in the initiation of fruiting body development or accompanying morphogenetic changes. Such TFs, with conserved, developmentally dynamic expression might be related to sculpting the ~~specialized,~~ cyphelloid~~s~~ fruiting bod~~y morpholog~~ies of *Auriculariopsis* and

Schizophyllum or more widely conserved fruiting body functions. This also shows the power of comparative transcriptomics to identify genes with conserved expression patterns ~~during fruiting body development~~ (Trail *et al.*, 2017) and to generate hypotheses that are testable by gene knockouts or functional assays.

Small secreted proteins show dynamic expression in fruiting bodies

We detected ~~several genes encoding SSPs in the fruiting body transcriptomes. In *A. ampla* and *S. commune*~~ 316 and 354 genes encoding SSPs in the fruiting body transcriptomes of *A. ampla* and *S. commune*~~were detected~~, respectively, half of which contained no known InterPro domains (Fig 5~~6~~a, Table S7~~6~~, Fig S4). The SSPs ~~in the two species~~ belonged to 283 orthogroups in *A. ampla*, and 315 in *S. commune*, of which~~.~~ 133 ~~orthogroups~~ were shared by the two species, whereas 150 and 182 were specific to *A. ampla* and *S. commune,* respectively (Fig 5~~6~~b). Twenty un-annotated ~~In the 133 shared orthogroups 39 and 54 genes were developmentally regulated in *A. ampla* and *S. commune,* respectively. From these, 20~~ co-orthologs were developmentally regulated in both species (Fig 5~~6~~d) and had a similar expression profiles. Annotated SSPs in the two species (Fig 5~~6~~c) had similar expression dynamics and mainly comprised hydrophobins, ceratoplatanins, CFEM domain containing proteins, concanavalin-type lectins, and glycosyl hydrolases (Fig 5c).

We detected several developmentally regulated SSP-s with no annotations and a higher than average cysteine content (mean 5.79-6.23% as opposed to 1.67-1.63% for the proteomes of *A. ampla* and *S. commune* respectively), some of which showed high expression dynamics (FC>50, Fig 5~~6~~e). We found 8, 15 and 2, *Auriculariopsis*-specific, *Schizophyllum*-specific and shared SSPs, respectively, with no known domains but an >50-fold ~~high~~ expression dynamics (Fig S4, Table S7~~6~~). For example, one of the

orthogroups (Auramp1_494084, Auramp1_549528, Schco3_2664662) showed a considerable upregulation in stage 1 primordia in both species (FC=11656 - 1870), suggesting a role in the transition from vegetative mycelium to fruiting body initials. Given the role ofSuch SSPs resemble effector proteins involved in cell-to-cell communication in ectomycorrhizal and pathogenic interactions between fungi and plants (Pellegrin *et al.*, 2015; de Freitas Pereira *et al.*, 2018) our observations. Their expression in fruiting bodies raises the possibility that they play signaling roles and may be responsible for sculpting the fruiting bodies of these fungi. SSPs with an upregulation in during morphogenetic processes (ECM root tips and/or fruiting bodies) have been reported in *Laccaria (Martin et al.*, 2008; Pellegrin *et al.*, 2015, 2017) and *Pleurotus (Feldman et al.*, 2017) suggesting a role in tissue differentiation and that some of the SSPs initially found in ECM root tips may actually be morphogenetic in nature. Whether morphogenesis-related SSPs occur ubiquitously among mushroom-forming fungi and what is the mechanistic basis of their role, needs further research. Neverthless, a morphogenetic role would provide an explaination for the rich SSPs complement of fruiting-body forming Agaricomycetes that are neither ectomycorrhizal or pathogenic (Pellegrin *et al.*, 2015; Krizsán *et al.*, 2019).

~~Discussion~~Conclusions

In this study we performed comparative genomic and transcriptomic analyses of *Auriculariopsis ampla, Schizophyllum commune* and 29 other Agaricomycetes, to understand their peculiar role in forest ecosystems and the development of their specialized fruiting body morphologies. The two analyzed members of the Schizophyllaceae proved a rich source of information for shaping our understanding of Agaricomycete biology and their interaction with plants. The Schizophyllaceae are pioneer colonizers of dead plant materials with suggested weak pathogenic potentials for

Schizophyllum (Takemoto *et al.*, 2010), probably necessitating strategies for invading through and/or feeding on the bark. We observed a preponderance of tannase and suberinase genes in these and other bark-specialized fungal species, which might degrade biopolymers enriched in bark tissues. On the other hand, developmental transcriptomes have highlighted several interesting genes potentially related to fruiting body development, including transcription factors (including 9 new conserved TFs), carbohydrate-active enzymes, heat shock proteins, aspartic proteases, as well as small secreted proteins. Taken together, the comparative genomics approach we used here ~~has led to the consideration of~~revealed some novel aspects of well-known and important processes, such as a putative strategy to decay bark through tannases and suberinases, or the role of small secreted proteins in f-ungal morphogenesis.~~identify conserved genes related to fruiting body development and their peculiar wood-decay strategy. The two analyzed members of the Schizophyllaceae proved a rich source of information for shaping our understanding of Agaricomycete biology. The CAZyme composition of *A. ampla* and *S. commune* suggests that the wood-degrading strategies of the two species show similarity to WRs when concerned with cellulases, hemicellulases, and pectinolytic gene families. What sets them apart from WRs is the absence of class II PODs, which is also the case for BRs and ectomycorrhizal fungi. However, the reduction in ligninolytic genes is compensated by the presence of suberinases and tannases required to depolymerize important components bark, to which these species might be adapted. Such an adaptation, although needs to be verified by additional studies would provide a framework for interpreting the odd CAZyme composition of the Schizophyllaceae (Riley *et al.*, 2014; Floudas *et al.*, 2015) and would expand our understanding of the nutritional diversity of wood-decay fungi.~~

~~Our analyses revealed a large number of genes with developmentally dynamic expression in fruiting bodies of both *A. ampla* and *S. commune*.~~,

including transcription factors (including 9 new conserved TFs), carbohydrate-active enzymes, heat shock proteins, aspartic proteases, as well as small secreted proteins. Particularly interesting are SSP-s with a highly dynamic expression through development, because SSPs have been described as key components of intercellular communication in pathogenic and ectomycorrhizal associations (Pellegrin *et al.*, 2015; de Freitas Pereira *et al.*, 2018). Although mechanistic evidence is still lacking, it is conceivable that SSP-s with fruiting body specific expression might be involved in intercellular communication in fruiting bodies and thus contribute to sculpting their morphologies, similarly to their mycorrhiza and pathogenicity-related counterparts. This hypothesis would provide an explanation for the rich SSPs content of fruiting-body forming Agaricomycetes that are neither ectomycorrhizal or pathogenic (Pellegrin *et al.*, 2015; Krizsan *et al.*, 2018).

Our data also suggest that despite the close phylogenetic relatedness of *Auriculariopsis* and *Schizophyllum*, their developmental transcriptomes have diverged significantly since their common ancestors, indicating a high rate of developmental gene expression in these taxa. Such divergence might be related to morphogenetic differences between the two species: while *A. ampla* produces simple cyphelloid (cup-shaped) fruiting bodies, those of *S. commune* consist of several congregated cyphelloid modules. Despite this divergence, several genes with a matching expression profile could be identified, highlighting conserved roles that await further characterization. These data have the potential to highlight not only the genes involved in the development of cyphelloid fruiting bodies, but also that of other agaricomycete fruiting body types and as such, should be immensely useful to understanding the general principles and shared properties of fruiting body development in mushroom-forming fungi.

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Author Contributions

LGN and GMK designed research; EA and BK cultured and fruited ***A. ampla***, EA, JC, IN, BB performed RNA-Seq; MC, CA, KL, AL, RR and IVG sequenced, assembled and annotated the genome of A. ampla; BH and ED predicted CAZyme genes in the analyzed genomes; EA, NS, KK, BB, GMK and LGN performed comparative analyses and bioinformatics. LGN, EA and NS wrote the paper. All authors have read and commented on the manuscript.

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Figure Legends

Fig 1. Phylogenetic relationships and lignocellulose degrading gene repertoire of *A. ampla* compared to *S. commune* and 29 other Agaricomycetes. A, species tree showing the phylogenetic affinities of the Schizophyllaceae (bold, left panel) and copy number distribution of cellulose, hemicellulose, pectin, lignin degrading gene families as well as those of putative suberinases and tannases. B, phylogenetic principal component analyses of cellulose, lignin and suberin degrading enzymes. Species names colored based on nutritional mode (WR - white rot, BR - brown rot, ECM - ectomycorrhizal, S/L/O - soil and litter decomposer, Uncertain - nutritional mode not known with certainty). For better visibility, a few species have been moved slightly on the plots (information in Table S1) See also Fig S1 for original plots.

Fig 2. Fruiting bodies and developmental stages of *A. ampla* and *S. commune*. Developmental stages are indicated on each panel. A, fruiting bodies of *A. ampla* produced in vitro, on sections of barked poplar logs plugged into malt-extract agar. B and C, fruiting bodies of *A. ampla* and *S.*

commune in their natural habitat. D, cross sections of developmental stages of *A. ampla*: left - stage 1 primordium (left), stage 2 primordium (middle) and mature fruiting body (right). E, Cross section of a mature fruiting body of *S. commune*, showing congregated single fruiting bodies.

Fig 3. Overview of the developmental transcriptome of *A. ampla*. A, Multi-dimensional scaling for RNA-Seq replicates from 5 developmental stages of *Auriculariopsis ampla*. Biological replicates belonging to similar tissue type group together. The replicates for P1 and P2 cluster together and remaining developmental stages keep apart. B, Graphical representation of number of significantly upregulated (green) and downregulated (red) genes among developmental stages and tissue types in *A. ampla*.

Abbreviations: VM - vegetative mycelium, P1 - stage 1 primordium, P2 - stage 2 primordium, YFB - young fruiting body, FB - mature fruiting body.

Fig 4. Global transcriptome similarity between developmental transcriptomes of *A. ampla* and *S. commune*. Pearson correlation coefficient-based heatmaps show similarity among developmental stages of the two species for all 7369 co-orthologs (A), for 1182 developmentally regulated co-orthologs (B), for 252 co-orthologous transcription factor pairs (C) and 42 developmentally regulated co-orthologous TF pairs (D). Warmer color indicates higher similarity. Biological replicates are indicated next to the heatmap (R1-R3). E, paired heatmap of gene expression (FPKM) for 7369 co-orthologous gene pairs between *A. ampla* and *S. commune*. Developmental stages for both species are as follows: VM - vegetative mycelium, P1 - stage 1 primordium, P2 - stage 2 primordium, YFB - young fruiting body, FB - mature fruiting body.

~~**Fig 5.** The expression patterns of developmentally regulated co-orthologous transcription factors and their similarity across the two species. A, Expression patterns for 8 previously characterized TFs in *S. commune* and *A. ampla*. B, developmentally regulated co-orthologous TFs in the two species~~

~~with high expression dynamics during fruiting body development. *S. commune* and *A. ampla* genes are shown by blue and orange lines respectively.~~

Fig ~~6~~5. Small secreted proteins in fruiting body transcriptomes. A, Repertoires of annotated vs unannotated and developmentally regulated (DR) vs. non-developmentally regulated (NOT-DR) SSPs in fruiting body transcriptomes of *Auriculariopsis ampla* and *Schizophyllum commune*. B, Venn-diagram depicting orthology relationships among SSPs of the two species. Number in each cell represent the number of shared or species-specific orthogroups. C, functional annotation terms (InterPro domains) present in SSPs of both *A. ampla* and *S. commune*. Terms specific to either species are not shown. D, expression heatmaps of co-orthologous SSPs in the two species. Orthogroup IDs are shown next to rows. Blue and red correspond to low and high expression, respectively. Greyed-out rows denote missing genes in orthogroup in which the 2 species did not have the same number of genes. Color coded bar next to heatmap shows functional annotations of the orthogroups. See Fig S4 for heatmaps of species-specific genes. E, expression profiles of genes in four of the orthogroups through development, including two orthogroups of unannotated genes. Blue and orange denote *Auriculariopsis* and *Schizophyllum* genes, respectively. Variances across the three biological replicates are shown at corresponding developmental stages. See Table S7~~6~~ for protein IDs.