De novo transcriptome assembly and comprehensive assessment provide insight into fruiting body formation of *Sparassis latifolia*

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The genes associated with fruiting body formation of *Sparassis latifolia* are valuable for improving mushroom breeding. To investigate this process, 4.8 × 10⁸ RNA-Seq reads were acquired from three stages: hyphal knot (SM), primordium (SP), and primordium differentiation (SPD). The de novo assembly generated a total of 48,549 unigenes, of which 71.53% (34,728) unigenes could be annotated by at least one of the KEGG (Kyoto Encyclopedia of Genes and Genomes), GO (Gene Ontology), and KOG (Eukaryotic Orthologous Group) databases. KEGG and KOG analyses respectively mapped 32,765 unigenes to 202 pathways and 19,408 unigenes to 25 categories. KEGG pathway enrichment analysis of DEGs (differentially expressed genes) indicated primordium initiation was significantly related to 66 pathways, such as “Ribosome”, “metabolism of xenobiotics by cytochrome P450”, and “glutathione metabolism” (among others). The MAPK and mTOR signal transduction pathways underwent significant adjustments during the SM to SP transition. Further, our research revealed the PI3K-Akt signaling pathway related to cell proliferation could play crucial functions during the development of SP and SPD. These findings provide crucial candidate genes and pathways related to primordium differentiation and development in *S. latifolia*, and advances our knowledge about mushroom morphogenesis.

Cauliflower mushroom (*Sparassis*) is widely known for its characteristic fruiting body that appears similar to cauliflower, and is recognized as an edible mushroom with numerous medicinal properties as well. Among members of this genus, the only species that current can be artificially cultivated is *S. latifolia*. In the last several years, however, there has been successful progress on the artificial breeding of *Sparassis* in Japan, China, and Korea. Prior to 2006, *S. latifolia* had been wrongly recognized as *S. crispa* until phylogenic evidence from three nuclear rDNA marker genes and mitochondrial gene confirmed *S. latifolia* as a new species. Furthermore, the *S. crispa* of Asia present significant differences from conspecific strains originating from Europe and North America, so the Asian isolates were re-classified as *S. latifolia* based on a morphological comparison and molecular phylogeny analysis.

Our current understanding of the process entailing fruiting body initiation and development is nascent, and how the mycelium forms the fruiting body is a prominent subject in the field of fungi molecular biology, especially concerning *Coprinopsis cinerea*, *Schizophyllum commune*, and certain non-model species, such as *Agaricus bisporus*, *Flammulina velutipes*, and *Boletus edulis*. The formation stage of fruiting body is the most complicated transformations in the cyclogeny of mushrooms, co-regulated by genetic, physiological, growth, as well as environmental factors. Due to the lack of knowledge about the key mechanism underpinning fruiting body formation, numerous edible mushrooms still cannot be readily cultivated via artificial techniques. For some valuable and rare mushroom species, particularly *S. latifolia*, little research had been conducted on how the developing fruiting body is controlled because suitable analytical tools and the prerequisite genetic information were unavailable. Yang et al. found that vitamin B6 metabolism, glycine metabolism, and cystathionine lyase, which might play an important role during light-induced primordia formation. Accordingly, a much better understanding of this process should enable researchers to realize the fruiting body formation of *S. latifolia*, potentially providing new opportunities to promote its industrial production.
Reverse and forward genetics tools such as RNAi and mutants analysis, have been widely applied to the research of gene function in many fungi10–12. Among the four successive stages of mushroom formation, that is the hyphal knot, initial primordium, primordium, and fruiting body13, the formation of initial primordium is the most critical step. For different stages, some genes have been cloned whose functional analysis has been carried out. A recent report showed that Nox functioned as signal in crosstalk between reactive oxygen species (ROS) and the CA2+ pathway, which regulated development of the mushroom fruiting body14. It has also been found that the dst gene is essential to transform primordia into mature fruiting bodies15,16. In C. cinerea, mutation of Ubv2 could result in a fruiting defect by seriously affecting clamp cell morphogenesis and nuclear migration17, and en2 has been shown to control the elongation of primordia18,19. Along with those genes, numerous transcription factors (TFs) might participate in the transition of asexual propagation to sexual propagation development, such as WC-120, FlbB31, and Pro122. Additionally, the MAPK and cAMP/PKA signaling pathways might be involved in regulating sexual development in fungi13,24.

Lately, rapid progress in sequencing technology, especially RNA sequencing, had undoubtedly advanced research in life sciences and substantially augmented our capacity for gene mining and functional analysis25–28. For example, the well-studied transcriptome of C. cinerea from its mycelium to primordium phase showed that transcriptomic changes lead the transformation from an undifferentiated structure to fruiting body structure25. Similar research was also performed in A. bisporus, Agrocybe aegerita, and Hypsizygus marmoreus, and much knowledge about mycelium–fruiting transition has been gained25–31. Yet, the mechanism responsible for the initiation of primordium and following developmental stages of S. latifolia remains understudied, hindering a comprehensive understanding of its fruiting development.

To fill this knowledge gap, here the transcriptome of three key developmental stages of S. latifolia was comparatively studied, to produce comprehensive sets of gene transcripts and glean critical information about transcriptome-level changes that occur as the fruiting body forms. This study can improve our understanding of dynamic changes characterizing the formation of the fruiting body, and point to some molecular mechanisms underlying S. latifolia’s development. Moreover, these first comprehensive transcripts sets of S. latifolia could serve as important resources for investigating other aspects of fruiting body formation more generally.

Methods

Sample preparation and RNA extraction. The S. latifolia strain (No. CCMJ 1100) was provided by the Culture Collection Center of Mycology of Jilin Agriculture University. Liquid spawn of the strain were inoculated into pine sawdust medium that had been sterilized at 121 °C for 2.5 h, and incubated in the dark at 24 °C. The pine sawdust medium contained 76% pine sawdust, 18% bran, 2% corn flour, 1.5% sucrose, 1.5% gypsum and 1% calcium superphosphate. The mycelia spread throughout the cultivation bags at 10 days post-inoculation (dpi) and began to form the hyphal knot, which could be visible at 70 dpi. The hyphal knots were collected and denoted as SM. The cultivation bags were then placed under controlled conditions (18–20 °C, 10 h/14 h light–dark cycle, light intensity maintained at 800–1000 Lux). After 100 dpi, a little primordium could be observed and this was sampled and denoted as SP. Later, at about the 115 dpi, the young fruiting body had formed and this was collected and denoted as SPD. For three distinct developmental stages (SM, SP, SPD), every treatment group consisted of three replicates. All samples were isolated carefully and promptly frozen in liquid nitrogen, then stored at − 80 °C.

Total RNA extraction of each sample was done using the TRIzol kit (Invitrogen, CA, USA), by following to its manual’s descriptions. Then, we checked the quality of RNA using agarose gel electrophoresis and a BioAnalyzer (Agilent, CA, USA), and measured the quantity of RNA using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Ma, USA). Eventually, a more than adequate amount of good quality RNA samples were obtained and these utilized for the RNA-Seq library construction and the quantitative real-time PCR analysis.

Library construction and Illumina Sequencing. To construct the cDNA library and perform deep sequencing, the Illumina’s protocols were followed (Illumina Inc., San Diego, CA, USA). Briefly, DNA was degraded with DNase I, and the mRNA enriched with magnetic Oligo (dT). The mRNA was sheared into short fragments (280 bp) by a fragmentation buffer and these ensuing fragments served as templates to synthesize the first-strand cDNA using random hexamer primers synthesis; the second-strand was synthesized with DNA polymerase I and RNaseH (Thermo Fisher Scientific). Then deep sequencing was conducted on the Illumina HiSeq 2500 platform with the PE150 approach, by the Shanghai Realgene Biotechnology Corporation. Eventually, a more than adequate amount of good quality RNA samples were obtained and these utilized for the RNA-Seq library construction and the qualitative real-time PCR analysis.

Transcriptome assembly of protein coding genes and their annotation. The raw paired-end reads were checked using the FastQC Package (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Clean reads were obtained after first removing their adapter, any low quality bases, and the short reads (< 20 bp), then we de novo assembled the transcriptome by using Trinity software (version Trinityrnaseq_r2014-04–13, http://trinityrnaseq.sourceforge.net/) under its default parameters32. A clustering analysis was implemented with CD-HIT software (version 4.8.1, https://github.com/weizhongli/cdhit)33, with a 95% identity threshold applied to minimize redundancy. The contigs were overlapped and aligned and these defined as the final sets of unigenes.

To predict their function, every unigene was assessed using Transdecoder software (version 5.5.0, http://transdecoder.github.io/), and then these were submitted as BLASTP queries, with an E-value < 1e−5, against four databases: GeneBank NR, Swiss-Prot, KOG, and KEGG34. GO annotation was done using Blast2GO35. Goatools (https://github.com/tanghaibao/Goatools) and KOBAS were utilized to perform the enrichment analysis for GO terms and KEGG pathways, respectively36.
Identification and annotation of differentially expressed genes (DEGs). The DEGs between two samples were isolated using the RSEM package and NOIseq (non-parametric approach for the differential expression analysis of RNA-Seq data in R) package. RSEM was used to count the reads of every gene, whose level of gene expression quantified according to fragments per kilobase of exon per million mapped reads (FPKM). We used the $|\log_2(\text{fold change})| \geq 1$ and probability $\geq 0.8$ as the cut-off criteria to designate the DEGs. The GO and KEGG analyses of functional enrichment were performed against the whole-transcriptome background, with a Bonferroni corrected P-value set at 0.01.

qRT-PCR analysis. The M-MLV reverse transcriptase (NEB, MA, USA), with oligo (dT), was used as the primer to reverse transcribe each sample of total RNA (1 μg). The genes of interest were respectively analyzed by qRT-PCR. Three biological replicates with three technical replicates were performed with 18S rRNA gene as the internal control. Each cultivation sample was then placed under a lit condition, and their hyphal knots gradually increased in both volume and density, accompanied by a burgeoning primordium after 30 DMKA (Fig. 1B). During primordium differentiation, many irregular acanthine bulges appear on the surface of each primordium during the 40–45 DMKA period (Fig. 1C).

Results
Developmental stages of S. latifolia. The development of S. latifolia could be divided into four successive and overlapping stages: the hyphal knot (SM), primordium (SP), primordium differentiation (SPD) (Fig. 1), and the final fruiting body stages. The transitional transformations from SM to SP, and from SP to SPD, are the most critical steps in cauliflower mushroom cultivation. Under the standard culture condition, the mature mycelium turned into hyphal knots (Fig. 1A), and the day of this event marked as 0 days after hyphal knots' formation was clearly visible (DMKA); from this moment onward, the mycelium come into sexual reproduction. Each cultivation sample was then placed under a lit condition, and their hyphal knots gradually increased in both volume and density, accompanied by a burgeoning primordium after 30 DMKA (Fig. 1B). During primordium differentiation, many irregular acanthine bulges appear on the surface of each primordium during the 40–45 DMKA period (Fig. 1C).

RNA sequencing and transcriptome assembly. Before the assembly, raw data was subjected to quality control to remove bad and low quality reads. The three samples (SM, SP and SPD) generated more than 60 Gb of clean data from paired-end (PE) reads, each sample comprised of three biological replicates (Table S2). Unigenes of different stages were assembled and integrated together by utilizing the software Trinity and CD-hit, which generated 48,549 unigenes (Table 1); their respective length mainly ranged from 300 to 4500 bp, and averaged 2488 bp (Fig. S1). When the clean reads were mapped back to the unigenes' sets, most positions of the expressed genes were covered by more than 107 reads (Fig. S2).

Functional annotation of the S. latifolia transcriptome. By making use of TransDecoder, overall, 40,876 (84.19%) protein coding unigenes were obtained. These protein coding unigenes were annotated using BLASTP against the Nr protein database, of which 16.08% did not have any hits, and might instead be non-coding RNA transcripts. After counting the results for the Nr database search (Fig. S3, Dataset 1), we found that the top seven-hit fungi species were all basidiomycetes (Fig. S3), whose sets of unigenes matched well with the genes of Trametes versicolor, implying their close phylogenetic relationship. KOG classifications put 19,408 unigenes (46.6% of total protein coding unigenes) into 25 functional groups (Fig. 2 and Dataset 2). The largest cluster was “General function prediction only”, this followed by “Carbohydrate transport and metabolism”, “Transcription”, and “Amino acid transport and metabolism.” Overall, 23,226 protein coding unigenes could be classified into 58 GO groups (Fig. 3 and Dataset 3). Among them, there were 23, 19, and 16 groups involved in the biological process, cellular component, and molecular
function categories, respectively. The GO terms “cellular process”, “metabolic process”, and “signal-organism process” accounted for the largest proportion subsumed under biological process. Gene numbers involved in “cell”, “cell part”, “organelle”, and “membrane”, were the most abundant under cellular component. The GO terms “binding”, “catalytic activity”, and “transporter activity” encompassed most of the genes under molecular function. In addition, there were 32,765 unigenes that could be mapped to 297 reference canonical KEGG pathways (Dataset 2).

Analysis of DEGs in the three developmental stages. To determine the candidate genes associated with fruiting body formation, the transcriptome of the SM, SP, and SPD stages were compared for pairs of adjacent developmental stages. Correlation analysis revealed that all sample replicates for each stage clustered together into a single clade (Fig. 4). A total of 8,822 DEGs between SM and SP were identified, these consisting of 5,195 up-regulated and 3,627 down-regulated genes (Fig. 4 and Dataset 4). There were 568, 134, and 21 unigenes respectively assigned to the “Oxidation–reduction process”, “Chromatin binding”, and “MAP kinase tyrosine phosphatase activity”; the expression of these DEGs were all up-regulated during the transition from SM to SP (Fig. S4). The main GO terms of the DEG sets were enriched in “poly(A) RNA binding”, “viral transcription”, “nuclear-transcribed mRNA catabolic process”, and “SRP-dependent co-translational protein targeting to membrane” (Table S3; Dataset 5). KEGG enrichment analysis indicated these DEGs were markedly enriched in 40 pathways (FDR( false discovery rate) < 0.01), including “ribosome”, “systemic lupus erythematosus”, “metabolism of xenobiotics by cytochrome P450”, and “ubiquitin mediated proteolysis”, among others (Table S4, Dataset 6).

From the SP to SPD phase, a total of 1,347 DEGs were identified, these comprising 690 up-regulated as well as 657 down-regulated genes (Dataset 7). These DEGs were considerably enriched in 251 GO terms, including “protein binding”, “extracellular region”, “extracellular space”, “transport”, and “cell surface” (Dataset 8), and also

Table 1. The assembled results for *S. latifolia* transcriptome.

| Unigenes | Number | Mean length (bp) | N50 (bp) | Reads mapped (%) |
|----------|--------|------------------|----------|------------------|
| SM 1     | 23,415 | 1372             | 1904     | 99.4             |
| SM 2     | 24,013 | 1415             | 1993     | 98.8             |
| SM 3     | 23,921 | 1448             | 2,030    | 99.4             |
| SP 1     | 25,101 | 2013             | 2930     | 99.2             |
| SP 2     | 25,226 | 1936             | 2789     | 99.1             |
| SP 3     | 24,807 | 1933             | 2755     | 98.9             |
| SPD 1    | 24,456 | 2098             | 3035     | 99.2             |
| SPD 2    | 23,704 | 1883             | 2757     | 98.4             |
| SPD 3    | 24,460 | 2056             | 2978     | 99.2             |

Total 48,549 2488 3629 99.1

Figure 2. The KOG functional categories of *S. latifolia* unigenes.

A: RNA processing and modification
B: Chromatin structure and dynamics
C: Energy production and conversion
D: Cell cycle control, cell division, chromosome partitioning
E: Amino acid transport and metabolism
F: Nucleotide transport and metabolism
G: Carbohydrate transport and metabolism
H: Coenzyme transport and metabolism
I: Lipid transport and metabolism
J: Translation, ribosomal structure and biogenesis
K: Transcription
L: Replication, recombination and repair
M: Cell wall/membrane/envelope biogenesis
N: Cell motility
O: Posttranslational modification, protein turnover, chaperones
P: Inorganic ion transport and metabolism
Q: Secondary metabolites biosynthesis, transport and catabolism
R: General function prediction only
S: Function unknown
T: Signal transduction mechanisms
U: Intracellular trafficking, secretion, and vesicular transport
V: Defense mechanisms
W: Extracellular structures
Y: Nuclear structure
Z: Cytoskeleton
enriched in 44 KEGG pathways (FDR < 0.01), such as “Complement and coagulation cascades”, “Glutathione metabolism”, “Tyrosine metabolism” (Dataset 9).

**Expression changes of genes and pathways involved in fruiting body formation.** The RNA-Seq data was further accessed by examining the expression levels of those genes involved in fruiting body formation that had been identified earlier. The eln2 gene functions as a positive regulator in the elongation of primordium18. Here, there were 83 eln2-like transcripts we identified in the S. latifolia transcriptome by using the blast tools with the E-value < 1e⁻⁵ and shared sequence identity more than 60%. For most of these genes, their expression was up-regulated in the SP stage (Fig. 5A), and some of them were up-regulated in the SM phase, which suggested the functional diversification of eln2 genes. Many important fruiting body formation-related...
genes, such as NADPH, PKAC, PRO, UBC2, and WC-I, were significantly changed during the transformation of different stages (Fig. 5).

The MAPK pathway was significantly changed in the transition from SM to SP (Fig. 5B, Fig. S8). The Pkc (CL5150_Contig2_All), Mkk1_2 (CL4664_Contig1_All), Pbs2 (CL2325_Contig1_All), and as well as others, were all up-regulated in the transition from SM to SP. Both the PI3K-Akt signaling pathway and mechanistic target of rapamycin (mTOR) signaling pathway were found significantly up-regulated during the development of SP to SPD (Figs. S9 and S10). Further, those genes encoding 3-phosphoinositide-dependent protein kinase 1 (PDKs, CL4393_Contig11_All, Unigene2284_All, CL4393_Contig13_All, CL4393_Contig16_All, CL4393_Contig5_All), mTOR (Unigene6719_All, CL458_Contig8_All, Unigene6718_All), serum and glucocorticoid-regulated kinase (SGKs, CL1207_Contig2_All, CL1207_Contig1_All), were also up-regulated during the development of the SP and SPD stages. Lighting is perhaps important for inducing primordium initiation and fruiting body maturation39, and indeed both photosynthesis and phototransduction pathways were detected in our annotation.
Yet there was no significant enrichment for light-related pathways, which suggested that photo stimulation may less important for S. latifolia's fruiting body formation that that of other mushroom fungi.

**Validation of DEGs by qRT-PCR.** A total of 27 genes, including those associated with fruiting body formation, the MAPK pathway, the PI3K-Akt and mTOR signaling pathways, were validated by the qRT-PCR analyses and the expression (Figs. S5–S7). Our results from the qRT-PCR and RNA-Seq data matched up well with each other, which indicated the good quality of the RNA-seq results.

**Discussion**

_Sparassis latifolia_ is a precious highly-prized mushroom type, mostly because of its medicinal properties. Previous research on _S. latifolia_ has mainly focused on ways to improve its medical applications and mushroom production. Surprisingly little is actually understood of its fruiting body's growth process, as well as the production of medicinally relevant bioactive metabolites, chiefly because genomic information for _S. latifolia_ is unavailable. Our study provided comprehensive cDNA sets that will certainly be useful in further investigations of this species, especially for identifying the candidate genes associated with the fruiting body formation in _S. latifolia_ or other edible mushroom species. Moreover, this data can benefit the phylogenetic, diversity, and functional research of other species of this genus and their affinity.

Fruiting body development is often induced by accompanying its living condition, which is substantially changed. The transfer of environmental stress signals to cells via the MAPK pathways plays an essential role in diverse intracellular signaling processes in both plants and fungi. There are many orthologs of MAP kinases gene families which are involved in hyphal and sporocarp growth in fungi. In the fruiting body formation of _S. latifolia_, this pathway showed significantly up-regulation during both SP and SPD development (Figs. S4 and S8), and this was supported by the GO and KEGG enrichments (Datasets 5, 6 and 8, 9), which agreed with previous findings reported for _C. cinerea_ as well as _H. marmoreus_.

Not only physiological conditions but also nutritional conditions could substantially influence the process of fruiting body formation in fungi. On the hand, a nitrogen-deficient condition could sustain the basic growth and development of fungi; on the other, it could avoid microbial competition. The nitrate reductase gene (Unigene6931_All) was up-regulated in SM and SP by more than tenfold that in SP (Fig. 5), which suggests development of SM and SPD in _S. latifolia_ was promoted by the nitrate starvation condition. In _C. cinerea_, the mTOR signaling pathway was considered to function as a necessary regulator throughout primordium formation. Generally, the mTOR pathway and its associated regulated pathway PI3K-Akt were expressed more during SM, SP, and SPD of _S. latifolia_. These results suggested that continual perception of environmental change might be more important for the later stages of fruiting body formation than those that occur in its early developmental stages. Lastly, other pathways, such as those of "Ribosome", "Systemic lupus erythematosus", and "Metabolism of xenobiotics by cytochrome P450" (among others), also varied continuously as the above key single pathways underwent changes in their activity.

**Conclusion**

Despite _S. latifolia_ now being a globally important edible and medical mushroom species, the molecular or genetic basis of its fruiting body formation and medical value remains largely uncharacterized, especially given the enormous difficulties inherent to the cultivation of cauliflower mushroom. The present work can inform future research work seeking to investigate in-depth the initiation of the fruiting body. In the transition from SM to SP, and then to SPD, environmental signals' sensing might be involved in primordium initiation as well as fruiting body development. Further functional analysis of the important candidate genes may lead to an enhanced understanding of the network regulating how the fruiting body develops. The crucial candidate genes mapped here to the MAPK, PI3K-Akt, and mTOR signaling pathways offers a convenient starting point for elucidating the molecular or genetic mechanism of fruiting body formation in _S. latifolia_.

**Data availability**

We have deposited the primary data underlying these analyses as follows: SubmissionID: SUB10980832; Bio-Project ID: PRJNA799466; BioSample accessions: SAMN25174015; [https://submit.ncbi.nlm.nih.gov/sra/SUB10980832/overview](https://submit.ncbi.nlm.nih.gov/sra/SUB10980832/overview).

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**References**

1. Kimura, T. Natural products and biological activity of the pharmacologically active cauliflower mushroom _Sparassis crispa_. Biomed. Res. Int. 2013, 982317. [https://doi.org/10.1155/2013/982317](https://doi.org/10.1155/2013/982317) (2013).
2. Ryoo, R., Sou, H. D., Ka, K. H. & Park, H. Phylogenetic relationships of Korean _Sparassis latifolia_ based on morphological and ITS rDNA characteristics. J. Microbiol. 51, 43–48. [https://doi.org/10.1007/s12275-013-2503-4](https://doi.org/10.1007/s12275-013-2503-4) (2013).
3. Dai, Y. C., Wang, Z., Binder, M. & Hibbett, D. S. Phylogeny and a new species of _Sparassis_ (Polyporales, Basidiomycota); evidence from mitochondrial _atp6_, nuclear rDNA and _rpl2_ genes. Mycologia 98, 584–592. [https://doi.org/10.3852/mycologia.98.4.584](https://doi.org/10.3852/mycologia.98.4.584) (2006).
4. Desjardin, D. E., Wang, Z., Binder, M. & Hibbett, D. S. _Sparassis cystidiosa_ sp. Nov. from Thailand is described using morphological and molecular data. Mycologia 96, 1010–1014. [https://doi.org/10.2307/3762085](https://doi.org/10.2307/3762085) (2004).
5. Lu, M. Y. et al. Genomic and transcriptomic analyses of the medicinal fungus _X. eriodictyon cinnamomea_ for its metabolite biosynthesis and sexual development. Proc. Natl. Acad. Sci. USA 111, E4743–E4752. [https://doi.org/10.1073/pnas.1417570111](https://doi.org/10.1073/pnas.1417570111) (2014).
6. Kues, U. & Liu, Y. Fruiting body production in basidiomycetes. Appl. microbiol. biot. 54, 141–152. [https://doi.org/10.1007/s0025 30000396](https://doi.org/10.1007/s002530000396) (2000).
1. Wessels, J. Wall growth, protein excretion and morphogenesis in fungi. New Phytol. 123, 397–413 (1993).
2. Umar, M. H. & Leo, J. L. D. The role of morphogenetic cell death in the histogenesis of the mycelial cord of Agaricus bisporus and in the development of macrofungi. Fungal Biol. 102, 719–735. https://doi.org/10.1016/S0937-5756(297005893) (1998).
3. Yang, C. et al. Integration of ATAC-Seq and RNA-Seq identifies key genes in light-induced primordia formation of Sparassis latitola. Int J Mol Sci. 21, 185 (2019).
4. Tirney, M. B. & Lamour, K. H. An introduction to reverse genetic tools for investigating gene function. The Plant Health Instructor. https://doi.org/10.1094/PHI-A-2005-1025-01 (2005).
5. Adams, M. D. & Sekelsky, J. J. From sequence to phenotype: reverse genetics in Arabidopsis. Nat. Rev. Genet. 3, 189–198. https://doi.org/10.1038/nrg572 (2002).
6. Taddei, M., Riet, P. & Myers, K. S. Insertional mutagenesis: a Swiss Army knife for functional genomics of Medicago truncatula. Trends Plant Sci. 10, 229–235. https://doi.org/10.1016/j.tplants.2005.03.009 (2005).
7. Kues, U. Life history and developmental processes in the basidiomycete Coprinus cinereus. Microbiol. Mol. Biol. Rev. 64, 316–353. https://doi.org/10.1128/MMBR.64.2.316-353.2000 (2000).
8. Mu, D. S. et al. Functions of the nicotinamide adenine dinucleotide phosphate oxidase family in Ganoderma lucidum: an essential role in ganoderic acid biosynthesis regulation, hyphal branching, fruiting body development, and oxidative-stress resistance. Environ. Microbiol. 16, 1709–1728. https://doi.org/10.1111/1462-9902.12326 (2014).
9. Terashima, K., Yuki, K., Muraguchi, H., Akiyama, M. & Kamada, T. The Dhm1 gene involved in mushroom photomorphogenesis of Coprinus cinereus encodes a putative photoreceptor for blue light. Genetics 171, 101–108. https://doi.org/10.1534/genetics.104.040048 (2005).
10. Kuratani, M. et al. The dst2 gene essential for photomorphogenesis of Coprinopsis cinerea encodes a protein with a putative FAD-binding domain. Fungal Genet. Biol. 47, 152–158. https://doi.org/10.1016/j.fgb.2009.01.006 (2010).
11. Nakazawa, T., Kondo, H., Nakahori, K. & Kamada, T. A mutation in the Ccub2 gene affects clamp cell morphogenesis as well as nuclear migration for dikaryosis in. Fungal Genet. Biol. 48, 519–525. https://doi.org/10.1016/j.fgb.2011.01.010 (2011).
12. Muraguchi, H. & Kamada. T. A mutation in the cln2 gene encoding a cytochrome P450 of Coprinus cinereus affects mushroom morphogenesis. Fungal Genet. Biol. 29, 49–59. https://doi.org/10.1016/S0963-2823(99)00118-0 (2000).
13. Hsu, K., Lee, Y., Lin, Y. & Chu, F. Cytochrome P450 genes in medicinal mushroom Antrodia cinnamomea T.T. Chang et W. N. Chang. Mol. Biotechnol. 35, 775–789. https://doi.org/10.1016/j.molbiotech.2009.08.009 (2009).
14. Masloff, S., Poggieler, S. & Kuck, U. The prol+ gene from Sordaria macrospora encodes a C-6 zinc finger transcription factor required for fruiting body development. Genetics 152, 191–199. https://doi.org/10.1093/genetics/152.1.191 (1999).
15. Palmer, G. E. & Horton, J. S. Mushrooms by magic: making connections between signal transduction and fruiting body development in the basidiomycete fungus Schizophyllum commune. FEMS Microbiol. Lett. 262, 1–8. https://doi.org/10.1016/j.femsle.2011.1574-9688.2008.00341.x (2006).
16. Lengeler, K. B. et al. Signal transduction cascades regulating fungal development and virulence. Microbiol. Mol. Biol. Rev. 64, 746–785. https://doi.org/10.1128/MMBR.64.4.746-785.2000 (2000).
17. Cheng, C. K. et al. 5′-Serial Analysis of Gene Expression studies reveal a transcriptomic switch during fruiting body development in Coprinopsis cinerea. BMC Genomics 14, 195. https://doi.org/10.1186/1471-2164-19-153 (2015).
18. Yu, G. I. et al. Deep insight into the Ganoderma lucidum by comprehensive analysis of its transcriptome. PLoS ONE 7, e40431. https://doi.org/10.1371/journal.pone.0040431 (2012).
19. Muraguchi, H. et al. Strand-specific RNA-Seq analyses of fruiting body development in Coprinopsis cinerea. PLoS ONE 10, e0141586. https://doi.org/10.1371/journal.pone.0141586 (2015).
20. Zhang, J. J. et al. Transcriptome analysis and its application in identifying genes associated with fruiting body development in basidiomycete Hypomyces marmoreus. PLoS ONE 10, e0123025. https://doi.org/10.1371/journal.pone.0123025 (2015).
21. Eastwood, D. C. et al. Environmental regulation of reproductive phase change in Agaricus bisporus by 1-octen-3-ol, temperature and CO2. Fungal Genet. Biol. 55, 54–66. https://doi.org/10.1016/j.fgb.2013.01.001 (2013).
22. Li, P., Deng, W., Li, T. H., Song, B. & Shen, Y. H. Illumina-based de novo transcriptome sequencing and analysis of Amanita exitialis basidicarps. Gene 532, 63–71. https://doi.org/10.1016/j.gene.2013.09.014 (2013).
23. Wang, M. et al. Transcriptome and proteome exploration to provide a resource for the study of Agrocybe aegerita. PLoS ONE 8, e56686. https://doi.org/10.1371/journal.pone.0056686 (2013).
24. Grabherr, M. G. et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–652. https://doi.org/10.1038/nbt.1883 (2011).
25. Li, W. & Godzik, A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22, 1658–1659. https://doi.org/10.1093/bioinformatics/btl158 (2006).
26. Ogata, H., Goto, S., Sato, K., Fujibuchi, W. & Kanehisa, M. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30. https://doi.org/10.1093/nar/28.1.27 (2000).
27. Conesa, A. & Gotz, S. Blast2GO: A comprehensive suite for functional analysis in plant genomics. Int J. Plant Genom. 2008, 619832. https://doi.org/10.1155/2008/619832 (2008).
28. Xie, C. et al. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res. 39, 316–322 (2011).
29. Li, B. & Dewey, C. N. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12, 323. https://doi.org/10.1186/1471-2105-12-323 (2011).
30. Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L. & Wold, B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods 5, 621–628. https://doi.org/10.1038/nmeth.1226 (2008).
31. Morimoto, N. & Oda, Y. Effects of light on fruit-body formation in a basidiomycete Coprinus macrorhizus. Plant Cell Physiol. 14, 217–225. https://doi.org/10.1093/cercor/bhs050 (1993).
32. Chandra, B. et al. Vegetative development of Sparassis crispa in various growth conditions and effect of electric pulse simulation on its fruit body production. Ai Mag. 4, 267–274. https://doi.org/10.4236/aim.2014.45033 (2014).
45. Ma, L., Lin, Y. Q., Yang, C., Ying, Z. H. & Jiang, X. L. Production of liquid spawn of an edible mushroom, *Sparassis latifolia* by submerged fermentation and mycelial growth on pine wood sawdust. *Sci. Hortic.* **209**, 22–30. https://doi.org/10.1016/j.scienta.2016.06.001 (2016).
46. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30. https://doi.org/10.1093/nar/28.1.27 (2000).
47. Alonsomonge, R., Roman, E. S., Arana, D. M., Pla, J. & Nombela, C. Fungi sensing environmental stress. *Clin. Microbiol. Infect.* **15**, 17–19. https://doi.org/10.1111/j.1469-0691.2008.02690.x (2009).
48. Xu, J. R. MAP kinases in fungal pathogens. *Fungal Genet. Biol.* **31**, 137–152. https://doi.org/10.1016/j.fgb.2000.1237 (2000).
49. Dixon, K. P., Xu, J., Smirnoff, N. & Talbot, N. J. Independent signaling pathways regulate cellular turgor during hyperosmotic stress and appressorium-mediated plant infection by *Magnaporthe grisea*. *Plant Cell* **11**, 2045–2058. https://doi.org/10.1105/tpc.11.10.2045 (1999).
50. Cano-Dominguez, N., Alvarez-Delfín, K., Hansberg, W. & Aguirre, J. NADPH oxidases NOX-1 and NOX-2 require the regulatory subunit NOR-1 to control cell differentiation and growth in *Neurospora crassa*. *Eukaryot. Cell* **7**, 1352–1361. https://doi.org/10.1128/ec.00137-08 (2008).
51. Lilleskov, E. A., Fahey, T. J., Horton, T. R. & Lovett, G. M. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology* **83**, 104–115. https://doi.org/10.1890/02-680124 (2002).
52. Bidartondo, M. I., Ek, H., Wallander, H. & Soderstrom, B. Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi? *New Phytol.* **151**, 543–550. https://doi.org/10.1046/j.1469-8137.2001.00180.x (2001).

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Conceptualization, S.L.L.; methodology, Q.Z.H. and W.M.Y.; software, W.M.Y.; validation, Q.Z.H.; investigation, L.T.L.; resources, L.T.L.; data curation, L.T.L.; writing-original draft preparation, S.L.L and Q.Z.H.; writing-review and editing, L.T.L.; funding acquisition, S.L.L. All authors have read and agreed to the published version of the manuscript.

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**Competing interests**

The authors declare no conflict interests.

**Additional information**

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