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Genome-wide identification and transcriptome analysis of the cytochrome P450 genes revealed its potential role in the growth of *Flammulina filiformis*

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Abstract

Background The CYP450 family members have been extensively studied in plants, where they play essential roles in metabolism, responses to biotic and abiotic stresses, and the regulation of growth and development. However, their functions in edible fungi remain largely unexplored. *Flammulina filiformis*, an economically important mushroom, lacks a comprehensive analysis of its CYP450 genes. Therefore, this study aims to identify and characterize the CYP450 gene family in *F. filiformis* at the genome-wide level, investigate their expression patterns, and explore their potential biological functions, providing valuable insights into their roles in fungal growth and adaptation.

Results In this study, 59 *CYP450* genes, categorizing into 6 distinct clades, were identified within the genome of *F. filiformis*. Subcellular localization predictions suggested that the majority of these *CYP450* genes are located in the endomembrane system. These 59 genes were distributed randomly across 12 chromosomes. Gene duplication analysis revealed the presence of 3 pairs of tandem repeats and 3 pairs of segmental repeat genes. Transcriptomic analysis revealed 861 differentially expressed genes (DEGs) in ML compared with M, and 3208 DEGs in P compared with ML. The 'oxidoreductase activity' category was significantly enriched in the ML vs. M and P vs. ML comparisons, with *CYP450* genes being predominantly represented among the DEGs. Transcriptional expression analysis demonstrated that 4 genes exhibited the highest expression levels in the M sample, 6 genes in the ML sample, and 10 genes in the primordium. Furthermore, quantitative real-time PCR (qRT-PCR) analysis revealed that 11 genes, including *HNY6_9861*, *HNY6_4590*, *HNY6_1561*, *HNY6_281*, *HNY6_12367*, *HNY6_8704*, *HNY6_9581*, *HNY6_8517*, *HNY6_11881*, *HNY6_9098* and *HNY6_5841*, exhibited an increasing trend in expression levels across the lower, middle and upper parts of the stipe in both white and yellow strains. This suggests that *CYP450* genes may involved in the elongation of the stipe of *F. filiformis*.

Conclusions These results provide a foundation for further exploration of the molecular evolution mechanism and potential functions of the *CYP450* genes of *F. filiformis* in the regulation of growth and development.

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Keywords Cytochrome P450, Gene family, Expression pattern, *Flammulina filiformis*

Background

Cytochrome P450 (CYP450) represents a large family of heme proteins capable of self-oxidation, and belongs to the class of monooxygenases. It is named for its specific absorption peak at 450 nm in the form of carbon monoxide binding [1]. The CYP450 family is widely present in various organisms, including animals, plants, and microorganisms. The function of *CYP450* genes is more reported in plants, with a clearer understanding of their mechanism. Research indicates that the majority of CYP450 proteins are membrane-bound, exhibiting a molecular weight range of 45–62 kDa, with an average molecular weight of approximately 55 kDa in plant species [2]. Structural analyses revealed that CYP450 enzymes typically possess four conserved domains: heme-binding domain (FxxGxRxCxG), I-helix, PERF domain, and K-helix [2].

According to the previous reports, the CYP450 family genes have multiple biological functions, participating in the biosynthesis of various substances including plant hormones, flavonoids, lignin, alkaloids, antioxidants, and phenolic compounds, etc [3]. Furthermore, these enzymes play a critical roles in plant resistance to biotic and abiotic stresses. In terms of metabolite synthesis, *CYP90* is involved in the biosynthesis of brassinosteroids [4], *CYP71A31* and *CYP71AU56* genes may be responsible for the biosynthesis of indole-3-acetic acid [5]. In *Arabidopsis thaliana*, *AtCYP88A3* and *AtCYP88A4* are involved in the biosynthesis of gibberellins, with mutations in homologous genes in barley and maize resulting in dwarf phenotypes [6]. The sweet potato protein IbCYP82D47 has been shown to interact with the carotenoid biosynthesis-related protein IbGGPPS12, thereby significantly increasing the carotenoid content in transgenic sweet potato [7]. Regarding plant stress resistance, the overexpression of the sweet potato gene *IbCYP73A1* improves the capacity of transgenic sweet potato plants to scavenge reactive oxygen species under abiotic stress conditions [8]. In *Arabidopsis*, AtCYP82C2 is capable of increasing defense-related gene expression and immunoglobulin content in response to jasmonic acid, thereby increasing resistance to *Botrytis cinerea*. Similarly, transgenic tobacco plants overexpressing soybean *GmCYP82A3* exhibit resistance to *B. cinerea*, *Phytophthora parasitica*, and salt and drought stress [9]. Previous report demonstrated that members of the cotton CYP450 family are involved in regulating salt tolerance in cotton through whole-genome identification and expression pattern analysis [10]. Bioinformatics analysis of the cucumber CYP450 family identified a gene, *CsCYP82D102*, which is up-regulated in response to powdery mildew, methyl jasmonate, and salicylic acid treatments. Transgenic cucumber plants overexpressing the *CsCYP82D102* gene showed enhanced resistance to powdery mildew [11]. Furthermore, the *CYP450* genes play crucial regulatory roles in the growth and development of organisms by regulating compound synthesis. The overexpression of the *CYP90D1* gene in grapes results in a substantial increase in brassinosteroid content, thereby promoting shoot elongation [4]. Although the functions of *CYP450* genes have been extensively investigated, research focusing on edible fungi remains limited. Previous studies have indicated that CYP450 proteins in basidiomycetes are involved in regulating the release of exogenous substances and the decomposition of lignin metabolites via catalase activity [12]. CYP66 is involved in the developmental processes of *Agaricus bisporus* [13], and the *CYP450* genes in *Hypsizygus marmoreus* may play a role in the regulation of the melanin synthesis pathway [14].

F. filiformis is considered as an independent species rather than a variety of *F. velutipes* [15], and is also called winter mushroom or golden needle mushroom. This species is an important edible and medicinal fungus within *Basidiomycota* that is extensively cultivated and marketed in China, Japan, and other Asian countries. *F. filiformis* holds an important position in the food and health product industries due to its high nutritional value (rich in polysaccharides, amino acids and trace elements) and unique flavor. In addition, the growth and development of *F. filiformis* is a complex regulatory process, and the research on the related regulatory genes will be of great significance. Commercial cultivation of *F. filiformis* requires low temperatures, typically below 10℃, to stimulate primordia formation and the growth of fruiting bodies. The growth and development of *F. filiformis* encompass both vegetative and reproductive phases, involving processes such as mycelial expansion, cold-induced primordia initiation, primordia differentiation, fruiting body development, and stipe elongation. These processes are governed by the interplay of multiple environmental factors and complex regulatory mechanisms mediated by various genes. Some genes related to the growth and development of *F. filiformis* have been identified. The expression of the jacalin-related lectin encoding gene *Fv-JRL1* was highest in the primordia, and *Fv-JRL1*-RNAi strains exhibiting a reduced number of primordia compared with the control strain H1123; conversely, strains with overexpressed *Fv-JRL1* presented accelerated growth rates and earlier fruiting body formation [16]. Additionally, the expression of the *pdd1* (Primordium development defect 1) gene in *F. velutipes* increased during primordium development, and its overexpression

facilitated primordium formation, resulting in significantly longer stipe length, more fruiting bodies, and higher yields than the wild-type strain F19 [17]. The overexpression of the hydrophobin gene *Hyd9* significantly increases the number of primordia and fruiting bodies [18]. The chromatin-modifying protein FfJmhy enhances the expression of genes related to cell wall enzymes by mediating demethylation at histone H3K9 sites, thereby regulating cell wall remodeling during stipe elongation in *F. filiformis*, and exerting a positive influence on stipe elongation [19]. Conversely, the transcription factor Ste12-like has been shown to negatively regulate stipe elongation in *F. filiformis* [20]. Despite these findings, the molecular regulatory mechanisms underlying the growth and development of *F. filiformis* remain largely unexplored. Investigating the genes and their functional mechanisms associated with the growth and development of *F. filiformis* through advanced genomics, transcriptomics, proteomics, metabolomics, and bioinformatics approaches will significantly advance molecular biology research in edible fungi, including *F. filiformis.*

The identification of gene families at the whole-genome level is essential for elucidating gene function. The assembly and publication of whole-genome sequences across numerous species have facilitated the identification of *CYP450* genes in various organisms, including rice [21], soybean [22], sweet potato [6], tea plant [23], peanut [24], cucumber [11], tomato [2], aphids [25], *Fusarium graminearum* [26] and others. Although the critical roles of *CYP450* genes in growth and development are well established, the genome identification, expression patterns, and biological functions of the CYP450 family in edible fungi remain unexplored. This work employed bioinformatics approaches to identify members of the *CYP450* gene family at the whole-genome level in *F. filiformis* and conducted a comprehensive analysis of their evolutionary relationships, chromosomal localization, and gene duplication events. The expression patterns of the *CYP450* genes in different tissues and developmental stages were examined via transcriptome sequencing and qRT-PCR. These analyses provide a foundation for the functional characterization of key candidate genes and have significant implications for further research on the growth and development mechanisms, as well as the molecular genetic breeding of *F. filiformis*.

Methods

Strains, culture conditions and Preparation of samples

F. filiformis strains AW27 (cultivated strain, white) and HNY6 (wild strain, yellow) were used in this study. The cultivation substrate consisted of 69% cottonseed hulls, 20% rice bran, 10% wheat bran, and 1% lime, with a moisture content of 65%. After thorough mixing, 2 kg of the substrate was placed into polypropylene plastic bags. The bags were then sterilized at normal pressure and allowed to cool naturally to room temperature before use. Under sterile conditions, the well-cultured liquid strains of *F. filiformis* strains were inoculated into the substrate, with an inoculation volume of 10 mL per bag. The mycelium was incubated in a culture room maintained at a temperature of 25 °C. Once the mycelia fully colonized the substrate, the bags were opened and scratched, after which they were transferred to a culture room with an ambient temperature of 15 °C to stimulate bud formation. Upon emergence of the fruiting bodies, the temperature is further reduced to between 5 °C and 7 °C, with the air humidity level maintained at approximately 90%. Cultivation continued until the fruiting bodies reached a height of approximately 15 cm. Samples of mycelium cultivated at 25 °C (M), mycelium treated at 15 °C (ML), the primordium (P), the lower part of the stipe (Bottom), the middle part of the stipe (Middle), the upper part of the stipe (Top), and the pileus (Pileus) were collected in 50 mL centrifuge tubes, frozen in liquid nitrogen, and subsequently stored in an ultralow-temperature refrigerator (-80 °C) for transcriptome sequencing and expression pattern detection. Each group of samples contains 3 biological replicates. Among them, sample taken from different culture media at the same stage are defined as 1 biological replicate. The samples for transcriptome sequencing included mycelium cultivated at 25 °C (M), mycelium treated at 15 °C (ML), and the primordium (P), totaling 9 samples.

Identification of gene families and physicochemical characteristics analysis

A high-quality genome was constructed using a wild *F. filiformis* monokaryotic strain (accession number: PRJNA1110060), and the whole-genome sequence, coding sequence (CDS), amino acid sequence, and corresponding annotation information were obtained. The Hidden Markov Model (PF00067) of the protein structure of the typical CYP450 family was downloaded from the Pfam database (http://pfam.xfam.org/). The protein sequences containing the CYP450 characteristic domain in *F. filiformis* were retrieved using the TBtools software [27], and the incomplete reading frame sequences and redundant sequences were manually eliminated. The remaining candidate protein domains were further verified using the Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), and the sequences that did not contain the CYP450 conserved domain or had an incomplete domain were further excluded. The physicochemical parameters of the proteins were predicted using the ExPASy ProtParam tool (http://web.ExPASy.org/protparam/) [28]. The subcellular localization prediction was generated using BUSCA (http://busca.biocomp.unibo.it/) [29].

Phylogenetic analysis of CYP450 family members

The amino acid sequences of 59 members of the CYP450 family in *F. filiformis* were analyzed through multiple sequence alignments using Clustal X software. Subsequently, a phylogenetic tree was constructed using MEGA 7 software [30] with parameters set to the neighbor-joining (NJ) method, a bootstrap value of 1000, 'No. of differences' as the model, 'pairwise deletion' for gaps/missing data treatment. The iTOL online tool was utilized to enhance the visual presentation of the phylogenetic tree (https://itol.embl.de/).

Chromosome localization and gene replication analysis

Based on the genomic annotation data of *F. filiformis*, the distribution positions of 59 CYP450 family genes on the chromosomes and the total lengths of the 12 chromosomes of *F. filiformis* were obtained. Visualization and analysis of gene positions were performed using TBtools. Pairwise similarity comparisons of the CYP450 family genes were conducted. Gene duplication events are defined by two criteria: an alignment sequence length covering ≥ 80% of the longest genes and sequence similarity ≥ 70% within the alignment region [31]. Additionally, adjacent genes exhibiting more than 70% identity within 200 kb were classified as tandem duplication genes [32]. Different colored lines were employed to connect tandem and segmental duplicated genes.

Analysis of conserved motifs and domains of amino acids

The conserved motifs within the CYP450 protein sequences were identified utilizing the MEME online platform (http://meme-suite.org/tools/meme) [33]. The analysis was conducted with the number of motifs specified at 10, while other parameters were maintained at their default settings. The results were subsequently visualized using TBtools. Additionally, the conserved domains within the protein sequences were predicted using the CDD database to ascertain their locational information, and visualization was performed using TBtools based on the amino acid sequences and gene IDs. The logo of the conserved motifs was generated via an online tool (https://weblogo.berkeley.edu/logo.cgi) [34] based on the amino acid sequence.

De Novo assembly transcriptome analysis

Transcriptome sequencing was performed on samples M, ML, and P from white *F. filiformis* strains by Novogene Co., Ltd. (Beijing, China). Total RNA was extracted from the samples using the Spin Column Plant Total RNA Purification Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's protocol. The mRNA in the total RNA was enriched using magnetic beads with Oligo (dT), and approximately 4 mg mRNA was collected from each sample for library construction using the NEBNext® Ultra™ Directional RNA Library Prep Kit (NEB) following the manufacturer's protocol. The constructed libraries were sequenced on the Illumina sequencing platform (Illumina HiSeqTM 2500). Clean reads were obtained from raw data by removing adapter-containing reads, reads with N bases, and low-quality reads (Phred score Q ≤ 20) using fastp software (version 2.19) for filtering and quality control. Quality-controlled reads were mapped to the reference genome using Hisat2 (version 2.0.5). Finally, gene expression levels were quantified by counting the aligned BAM files against the gene annotation file using FeatureCounts software (version 1.5.0-p3). Gene expression levels were quantified using the fragments per kilobase of transcript per million fragments mapped (FPKM). Differentially expressed genes (DEGs) were analyzed using DESeq2 (version 1.20.0), with the significance threshold set at $padj < 0.05$ and $|\log2 \text{ fold change}| > 1$. Gene function was annotated via the following databases, including Nr (NCBI non-redundant protein sequences), Nt (NCBI non-redundant nucleotide sequences), Pfam (Protein family), KOG/COG (Clusters of Orthologous Groups of proteins), and Swiss-Prot (A manually annotated and reviewed protein sequence database) using Blastx software (version 2.2.23). Gene Ontology (GO) enrichment analysis of differentially expressed genes was performed using the cluster Profiler R package, with GO terms exhibiting $p < 0.05$ considered significantly enriched.

Quantitative real-time PCR (qRT-PCR) assay

Total RNA was isolated from mycelium and fruiting body of *F. filiformis* using MolPure® TRIeasy™ Plus Total RNA Kit (YEASEN) according to the protocol. Approximately 1.0 μg total RNA was used for cDNA synthesis using Hifair II 1st Strand cDNA Synthesis SuperMix Kit (YEASEN). qRT-PCR was performed on CFX96 real-time PCR detection system (Bio-Rad) using the 2 × Hieff qPCR SYBR Green Master Mix (YEASEN). The reaction system (20 μL) contained 10 μL qPCR SYBR Green Mix, 8 μL ddH_2O, 0.5 μL forward primer, 0.5 μL reverse primer and 1.0 μL cDNA. Reaction program: 94℃ for 3 min for pre-denaturation; denatured at 94℃ for 20 s, annealing at 60℃ for 15 s, extended for 10 s at 72℃, 40 cycles. Three independent biological replicates and three independent technical replicates were used for all the samples. The *GAPDH* gene was used as an internal control, and the relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method [35]. The primers used in this work are listed in Table S1.

Results

Identification of CYP450 family genes in *F. filiformis*

The typical protein domain of the CYP450 family (PF00067) was utilized to extract sequences from the genome of *F. filiformis*. Following validation using the

CDD conserved domain database and the removal of incomplete sequences, a total of 59 CYP450 family members were identified. These members are represented by gene IDs in the genomic dataset (Table 1), with detailed gene and protein sequences provided in Table S2 and Table S3. The encoded proteins exhibit varying lengths, from 344 to 656 amino acids, and molecular weights ranging from 38.94 kDa (HNY6_8704) to 74.75 kDa (HNY6_9098), with an average molecular weight of 57.12 kDa. The predicted isoelectric points of these proteins range from 5.68 (HNY6_9581) to 9.69 (HNY6_10552). Additionally, the CYP450 proteins of *F. filiformis* display diverse hydrophilicity, with values ranging from −0.339 (HNY6_11381) to 0.072 (HNY6_9888), and instability index ranging from 30.48 (HNY6_8704) to 52.57 (HNY6_12360). According to BUSCA subcellular localization predictions, the majority of CYP450 proteins are located in the endomembrane system, with the exceptions of HNY6_10552, which is found in the mitochondrial membrane, and HNY6_11869 and HNY6_1787, which are located in the organelle membrane.

Phylogenetic analysis of CYP450 proteins

To investigate the evolutionary relationships among *F. filiformis* CYP450 enzymes, a phylogenetic tree was constructed using MEGA software based on amino acid sequences. The results revealed that the 59 CYP450 members could be categorized into 6 clades, namely CYP64, CYP58, CYP67, CYP52, CYP503, and CYP_FUM15. Notably, the CYP64 clade comprised the largest number of members, totaling 25, followed by the CYP_FUM15 clade with 13 members, the CYP52 clade with 7 members, and both the CYP503 clade and CYP58 clade with 5 members each. The CYP67 clade contained the fewest members, with only 4 (Fig. 1).

Chromosomal locations and duplication analysis of CYP450 genes

The 59 CYP450 family genes were randomly distributed on 12 chromosomes. Chromosome Chr6 harbored the highest number of genes, with a total of 11, followed by Chr10 with 9 genes, while Chr4 and Chr12 each contained only 1 gene. Furthermore, based on the criteria for gene duplication events, we identified 3 pairs of tandem duplicate genes in the CYP450 gene family of *F. filiformis*, namely *HNY6_12360* and *HNY6_12366*, *HNY6_1784* and *HNY6_1786*, *HNY6_2891* and *HNY6_2892*. Additionally, 3 pairs of segmental duplicate genes were identified, namely *HNY6_1362* and *HNY6_2710*, *HNY6_4969* and *HNY6_10906*, *HNY6_10740*, and *HNY6_11381* (Fig. 2, Table S4). These findings suggest that both tandem and segmental duplications contributed to the expansion of CYP450 genes in *F. filiformis*.

Motif compositions and conserved domains

The conserved domains within the protein sequences of *F. filiformis* CYP450 were examined using the CDD database, which revealed that all the sequences contained the typical conserved domain of the CYP450 family, namely the CypX superfamily. This observation confirms that the 59 identified members belong to the cytochrome P450 family. Further analysis of the CYP450 amino acid sequences was conducted using the MEME online tool, which predicted the presence of 10 conserved motifs (Fig. 3). The number and distribution of these motifs on different CYP450 protein sequences vary, ranging from 4 (e.g., HNY6_83, HNY6_8704) to 10 (e.g., HNY6_1362, HNY6_ HNY6_6485, HNY6_9082, HNY6_2915, HNY6_4365, HNY6_2159, HNY6_10186, HNY6_10194, HNY6_10740, HNY6_6085, HNY6_10206). Moreover, the number and distribution of conserved motifs among family members in different clades are similar. The identified conserved motifs included the typical conserved motifs of the CYP450 family: K-helix regin (motif1), PERF motif (motif4), and Heme-binding region (motif2). All 59 CYP450 members of *F. filiformis* contain the K-helix region, 53 members (89.8%) possess the conserved PERF motif, and 47 members (79.7%) contain the conserved Heme-binding region (Fig. 4).

Transcriptome analysis at different developmental stages

Due to the current lack of comprehensive annotation information for the genes of *F. filiformis*, some genes within the genome assembled in our laboratory remain functionally uncharacterized. Therefore, to obtain more gene function annotations, this work employed de novo transcriptome sequencing on the mycelia (M), low-temperature treated mycelia (ML), and primordia (P) of the white strain. This approach aimed to investigate gene-level alterations during the transition from mycelia to primordia. Principal component analysis (PCA) demonstrated significant overall differences among the three groups, whereas the variability among the three biological replicates within each group was minimal, suggesting that there were obvious distinctions in the transcriptional profiles at different developmental stages of *F. filiformis* (Fig. 5A). Comparative analysis of significantly differentially expressed genes (DEGs) between the ML and M samples revealed 431 up-regulated and 430 down-regulated genes; when comparing P to ML, 1392 genes were up-regulated and 1816 down-regulated (Fig. 5B). Therefore, the gene expression profiles exhibited more pronounced changes following the development of mycelia into fruiting bodies. The GO functional enrichment analysis of DEGs in the comparison groups ML vs. M and P vs. ML revealed a significant enrichment of the 'oxidoreductase activity' GO term (Fig. 5C). In the "oxidoreductase activity" GO term of ML vs. M, 135 genes were

Table 1 Identification of CYP450 genes and analysis of physicochemical properties of proteins in *Flammulina filiformis*

Gene ID	Chromosome	Start	End	Size(AA)	Molecular weight(kDa)	pI	Instability index	Grand average of hydropathicity (GRAVY)	Predicted location
HNY6_10186	Chr6	1,740,764	1,742,946	539	60.75	5.71	33.24	-0.175	endomembrane system
HNY6_10194	Chr6	1,760,615	1,762,411	517	57.79	9.22	42.32	-0.19	endomembrane system
HNY6_10195	Chr6	1,762,797	1,764,961	559	63.01	6.89	41.33	-0.127	endomembrane system
HNY6_10205	Chr6	1,790,012	1,792,351	451	51.25	6015	42.05	-0.294	endomembrane system
HNY6_10206	Chr6	1,792,615	1,794,667	546	60.85	6.57	43.62	-0.186	endomembrane system
HNY6_10552	Chr6	2,707,654	2,708,257	452	51.54	9.69	38.62	-0.17	mitochondrial membrane
HNY6_10740	Chr7	217,860	219,697	512	57.87	6.68	37.49	-0.263	endomembrane system
HNY6_10742	Chr7	228,944	230,858	534	60.48	7.68	43.62	-0.295	endomembrane system
HNY6_10858	Chr7	558,125	560,743	540	59.89	8.44	35.2	-0.119	endomembrane system
HNY6_10906	Chr7	684,824	687,839	595	67.55	6.64	42.68	-0.096	endomembrane system
HNY6_11175	Chr7	1,550,586	1,552,736	470	53.07	7.76	41.67	-0.073	endomembrane system
HNY6_11381	Chr7	2,161,141	2,165,335	468	53.28	8.79	39.45	-0.339	endomembrane system
HNY6_11869	Chr8	695,540	697,097	457	51.15	5.87	46.99	-0.162	organelle membrane
HNY6_11881	Chr8	741,160	741,402	468	51.59	7.22	37.41	0.013	endomembrane system
HNY6_1216	Chr1	3,007,617	3,009,747	511	57.32	7.89	41.17	-0.148	endomembrane system
HNY6_12360	Chr8	2,035,894	2,036,491	581	66.45	7.95	52.57	-0.144	endomembrane system
HNY6_12367	Chr8	2,051,513	2,053,961	607	68.67	8.06	42.09	-0.146	endomembrane system
HNY6_1362	Chr1	3,362,380	3,364,366	509	56.35	6.89	38.22	-0.048	endomembrane system
HNY6_1561	Chr1	3,859,169	3,861,552	523	58.58	7.62	41.27	0.028	endomembrane system
HNY6_1777	Chr10	326,411	327,055	507	57.48	8.51	47.76	-0.105	endomembrane system
HNY6_1784	Chr10	348,358	349,316	525	59.74	7.18	44.82	-0.085	endomembrane system
HNY6_1786	Chr10	352,850	355,175	454	51.28	7.05	46.86	-0.063	endomembrane system
HNY6_1787	Chr10	357,015	357,995	413	46.92	8.44	46.3	-0.118	organelle membrane
HNY6_1887	Chr10	631,591	633,983	386	43.41	7.86	46.12	-0.023	endomembrane system
HNY6_1909	Chr10	707,428	709,050	506	56.01	7.22	45.75	0.053	endomembrane system
HNY6_1915	Chr10	721,768	723,566	533	59.31	7.32	44.15	-0.139	endomembrane system
HNY6_2159	Chr10	1,338,431	1,340,994	493	54.99	7.29	35.12	-0.114	endomembrane system
HNY6_2187	Chr10	1,405,473	1,407,395	511	57.65	7.14	49.72	-0.115	endomembrane system
HNY6_2710	Chr11	771,679	773,377	510	57.1	6.58	43.58	-0.201	endomembrane system
HNY6_281	Chr1	729,664	731,634	532	59.43	7.62	45.56	0.031	endomembrane system
HNY6_2891	Chr11	1,298,915	1,301,225	533	58.78	7.16	31.05	-0.119	endomembrane system
HNY6_2892	Chr11	1,302,810	1,304,766	466	51.57	8.24	35.68	-0.125	endomembrane system
HNY6_2915	Chr11	1,362,630	1,364,812	529	59.9	6.7	51.51	-0.22	endomembrane system
HNY6_3733	Chr12	1,574,173	1,574,715	579	66.39	7.05	48.99	-0.144	endomembrane system
HNY6_4003	Chr2	725,670	727,816	491	55.68	8.12	46.12	-0.009	endomembrane system
HNY6_4033	Chr2	807,723	809,648	500	56.15	8.23	46.31	-0.06	endomembrane system
HNY6_4365	Chr2	1,655,774	1,657,225	510	57.32	6.89	43.84	-0.123	endomembrane system
HNY6_4590	Chr2	2,204,492	2,206,852	472	52.89	9.28	42.48	-0.037	endomembrane system
HNY6_4620	Chr2	2,275,563	2,276,151	583	66.68	6.61	36.57	-0.109	endomembrane system
HNY6_4845	Chr2	2,849,217	2,851,907	535	60.19	8.05	46.92	-0.181	endomembrane system
HNY6_4969	Chr2	3,188,477	3,189,373	595	68.1	7.39	45.44	-0.249	endomembrane system
HNY6_5840	Chr3	1,333,413	1,336,321	511	57.22	6.8	42.47	-0.111	endomembrane system
HNY6_5841	Chr3	1,336,357	1,338,315	540	61.06	8.8	40.04	-0.141	endomembrane system
HNY6_6084	Chr3	1,915,181	1,916,382	496	56.43	9.19	43.03	-0.259	endomembrane system
HNY6_6085	Chr3	1,917,999	1,921,105	507	56.46	7.29	43.83	-0.29	endomembrane system
HNY6_6485	Chr3	2,862,408	2,863,938	499	56.33	6.12	43.62	-0.131	endomembrane system
HNY6_6881	Chr3	3,899,450	3,901,638	530	59.29	6.59	42.02	-0.004	endomembrane system
HNY6_7262	Chr4	929,168	932,787	451	50.74	6.84	34.22	-0.126	endomembrane system
HNY6_83	Chr1	192,144	193,461	430	47.92	7.32	38.6	-0.045	endomembrane system
HNY6_8517	Chr5	573,012	573,489	494	55.28	8.3	48.31	-0.061	endomembrane system
HNY6_8571	Chr5	723,422	725,357	495	55.78	5.95	51.92	-0.206	endomembrane system

Table 1 (continued)

Gene ID	Chromosome	Start	End	Size(AA)	Molecular weight(kDa)	pI	Instability index	Grand average of hydropathicity (GRAVY)	Predicted location
HNY6_8704	Chr5	1,102,904	1,104,280	344	38.94	6.87	30.48	-0.239	endomembrane system
HNY6_9082	Chr5	2,060,496	2,061,690	507	56.9	6.74	39.69	-0.11	endomembrane system
HNY6_9098	Chr5	2,101,803	2,102,310	656	74.75	6.97	36.87	-0.247	endomembrane system
HNY6_9581	Chr6	198,931	202,175	476	52.78	5.68	33.56	-0.066	endomembrane system
HNY6_9638	Chr6	358,908	361,211	582	65.21	8.84	33.31	-0.067	endomembrane system
HNY6_9861	Chr6	936,158	938,867	391	44.6	6.19	44.04	-0.183	endomembrane system
HNY6_9888	Chr6	992,676	994,642	522	57.64	6.8	35.2	0.072	endomembrane system
HNY6_9992	Chr6	1,250,235	1,252,002	521	58.25	9.62	49.06	-0.059	endomembrane system

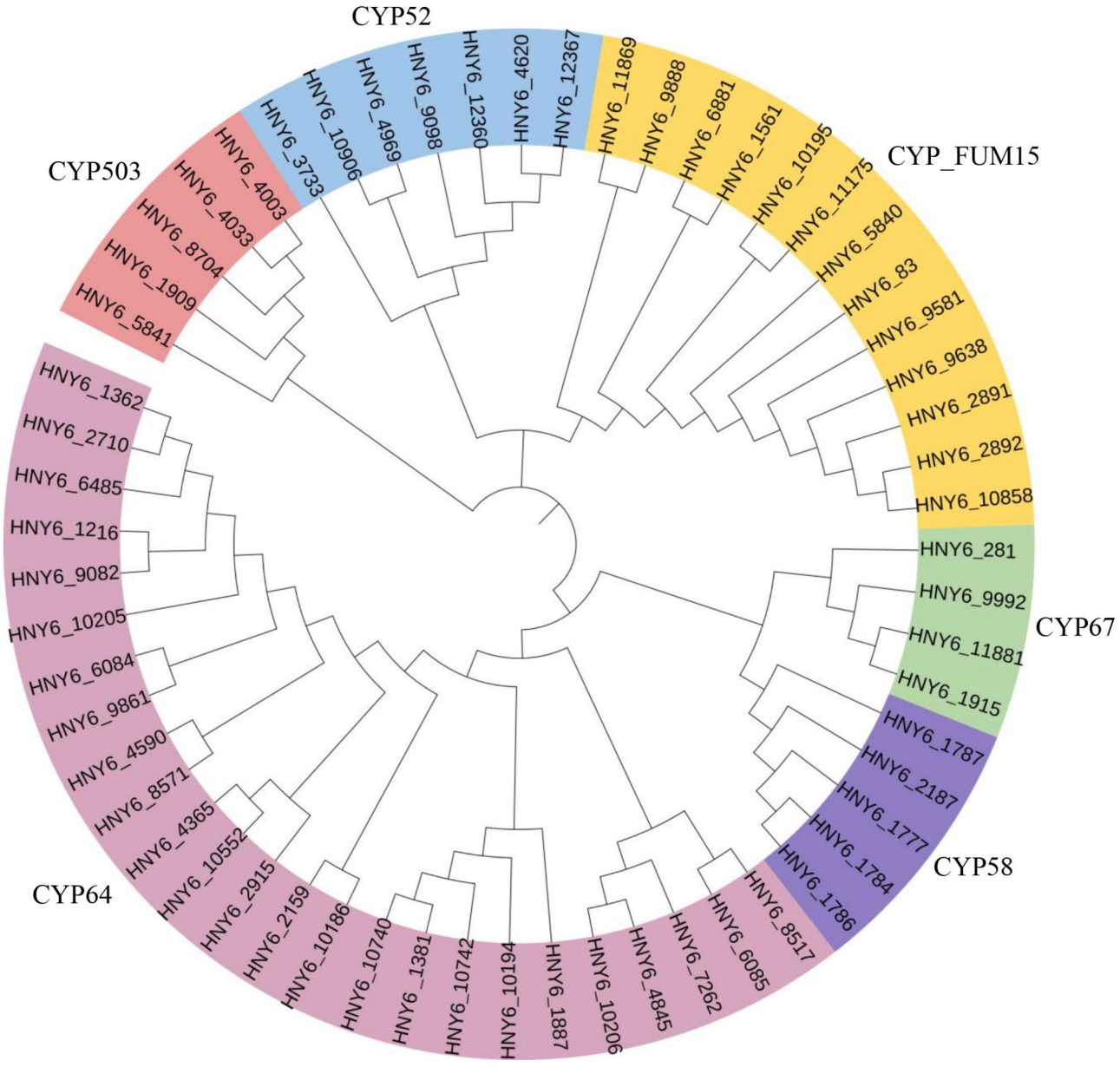

Fig. 1 Phylogenetic tree of CYP450 proteins of *Flammulina filiformis*. Phylogenetic tree was constructed by using Neighbor-joining method with 1000 bootstrap replications. Different clans are represented by different background colors, including CYP64, CYP58, CYP67, CYP_FUM15, CYP52, and CYP503

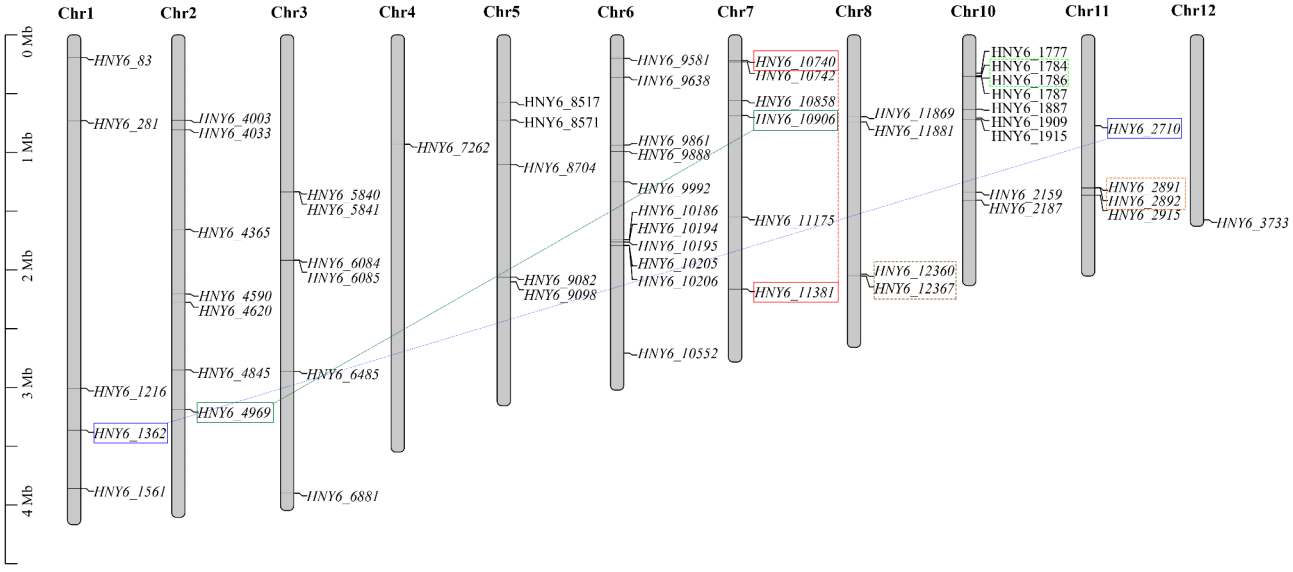

Fig. 2 Locations of CYP450 genes on chromosomes. Different colored lines between genes indicate gene duplication events, the ruler presents chromosome size

encompassed, among which the members of the CYP450 family were the most numerous, accounting for 16 (12 up-regulated and 4 down-regulated). Similarly, in the 'Oxidoreductase activity' GO term of P vs. ML, 390 genes were included, and the members of the CYP450 family were also the most predominant, with a total of 26 (15 up-regulated and 11 down-regulated) (Table S5). These results further demonstrated that the CYP450 family genes may play crucial roles in the mycelial growth and primordium formation of *F. filiformis*.

Through sequence alignment, 59 CYP450 family genes identified in this study were selected from the transcriptome data, and the expression patterns of these genes in the three stages of M, ML, and P were constructed using FPKM values. The analysis demonstrated significant differences in the expression patterns of these 59 *CYP450* genes in different tissues (Fig. 6A). Specifically, the expression levels of 4 genes were the highest in sample M, namely, *HNY6_9888*, *HNY6_4969*, *HNY6_7262*, and *HNY6_1362*; the expression levels of 6 genes were the highest in sample ML, namely, *HNY6_1216*, *HNY6_4003*, *HNY6_6881*, *HNY6_10906*, *HNY6_4033*, and *HNY6_2891*; the expression levels of 10 genes were the highest in the primordium, namely, *HNY6_9581*, *HNY6_12367*, *HNY6_9861*, *HNY6_4590*, *HNY6_11881*, *HNY6_4365*, *HNY6_9098*, *HNY6_8704*, *HNY6_9992*, and *HNY6_9638*. Additionally, 5 CYP450 genes were randomly chosen for qRT-PCR detection. The results demonstrated that the gene expression trends in the qRT-PCR results were completely consistent with the transcriptome data (Fig. 6B), verifying the stability and repeatability of the transcriptome data.

Quantitative analysis of *CYP450* gene expression in fruiting bodies of different strains

To further investigate the function of the *CYP450* genes in *F. filiformis* during fruiting body development, we analyzed the expression patterns of 15 up-regulated genes in the P vs. ML group in different parts of the white strain fruiting body, primordium (P), lower part of the stipe (Bot), middle part of the stipe (Mid), upper part of the stipe (Top) and pileus (Fig. S1). The results revealed that *HNY6_9638* and *HNY6_9992* exhibited the highest expression levels in the middle part of the stipe, while *HNY6_2159* showed peak expression in both the middle and upper parts of the stipe. Notably, *HNY6_4365* demonstrated relatively high expression exclusively in the upper part, with reduced expression in other regions. Additionally, 11 genes including *HNY6_9861*, *HNY6_4590*, *HNY6_1561*, *HNY6_281*, *HNY6_12367*, *HNY6_8704*, *HNY6_9581*, *HNY6_8517*, *HNY6_11881*, *HNY6_9098* and *HNY6_5841* showed an increasing trend in the expression levels in the lower, middle and upper parts of the stipe, and the highest relative expression level was detected in the upper part of the stipe (Fig. 7). This expression pattern positively correlates with the elongation characteristics of the stipe in *F. filiformis*, where the elongation zone is situated in the upper part of the stipe [36], suggesting that the *CYP450* genes play a regulatory role in the elongation process of the stipe.

The expression patterns of the aforementioned 15 genes in various regions of the yellow *F. filiformis* fruiting bodies were further analyzed. The results revealed that the expression level of *HNY6_9992* was highest in the middle part of the stipe, while *HNY6_8517* exhibited a relatively high expression levels in the primordium and

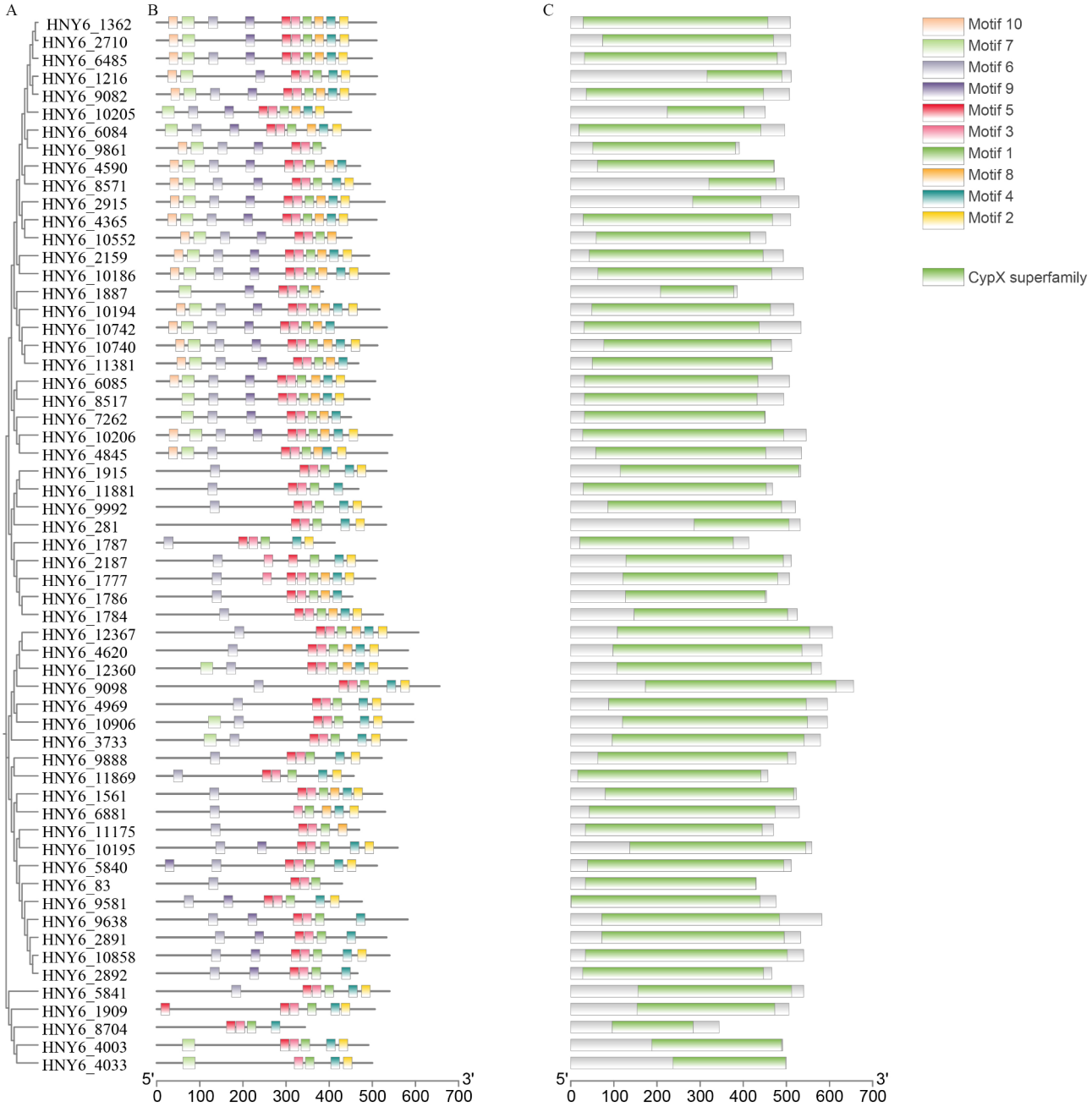

Fig. 3 Distributions of conserved motifs and domain of CYP450 family members in *Flammulina filiformis*. **A**, Phylogenetic tree of CYP450 family members. **B**, Conserved motifs of CYP450 proteins. **C**, Conserved domain of CYP450 proteins

the upper part of the stipe. The expression levels of the remaining genes showed an increasing trend in the lower, middle and upper parts of the stipe, similar to the pattern observed in the white strain. However, certain genes displayed expression patterns that differed from those in the white strain. For instance, *HNY6_281*, *HNY6_12367*, *HNY6_9581*, *HNY6_4365*, and *HNY6_11881* also presented relatively high expression levels in the pileus of the yellow strain (Fig. 8). These results demonstrated that the *CYP450* genes of *F. filiformis* may possess conserved biological functions in different varieties, although some variations exist.

Discussion

Gene family is defined as a group of genes that share similar sequences or functions within the genome. These proteins are typically structurally similar, although their expression patterns and biological functions may vary. Through the precise and comprehensive identification of gene family members and the analysis of their

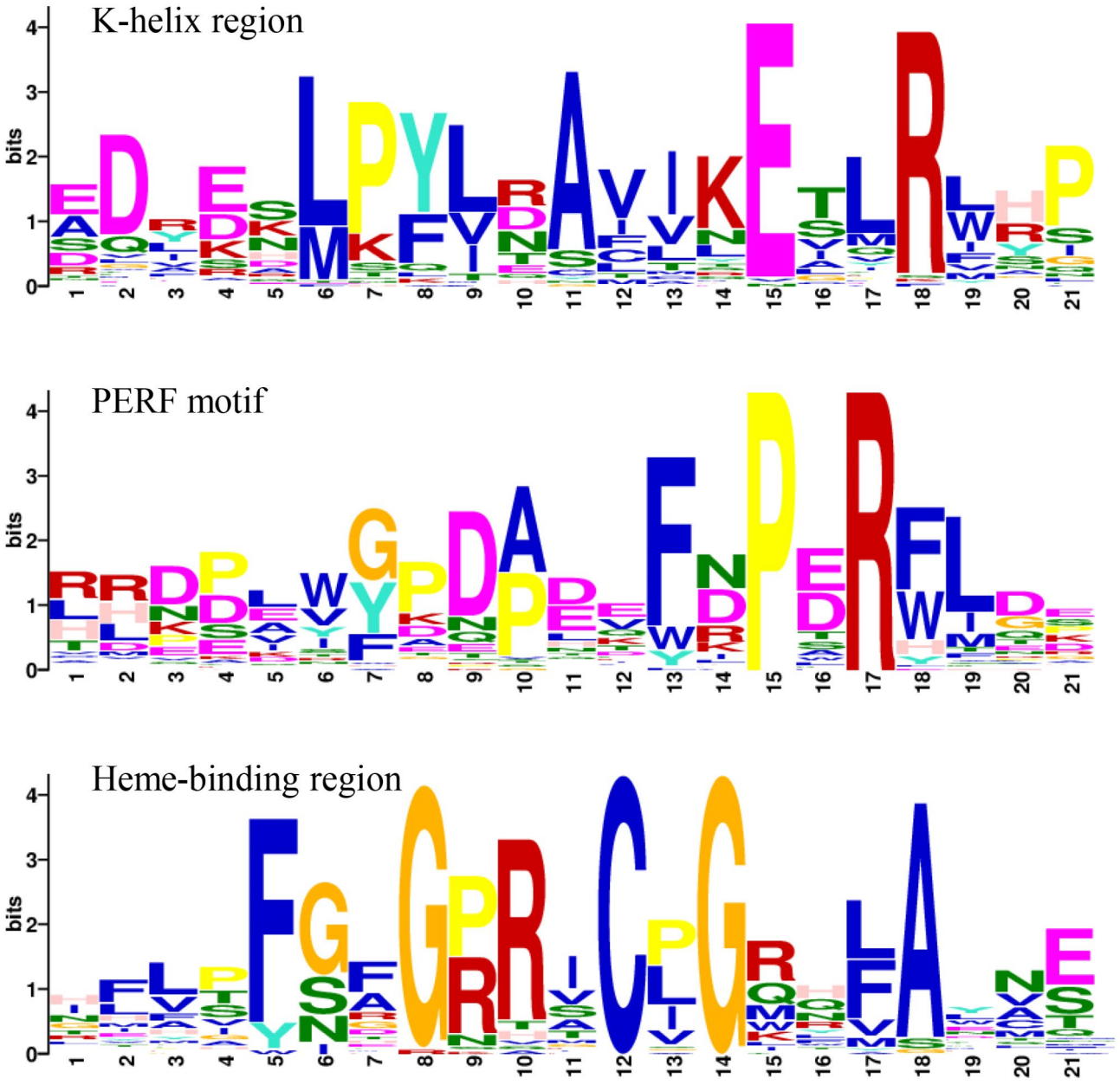

Fig. 4 WebLogo diagram of conserved motifs of CYP450 proteins. 3 conserved domains of CYP450 protein: K-helix, PERF domain, and heme-binding domain (FxxGxRxCxG). Letter size in this diagram is proportional to the degree of conservation of amino acid residues

spatiotemporal expression patterns, combined with functional annotations from databases, it is of vital importance for uncovering gene functions and evolution [37]. Genome-wide identification of gene families has been extensively conducted in numerous plant and animal species, which facilitates more efficient inference of the functions of candidate genes for subsequent experimental validation [38]. The number of CYP450 family members varies among species. For example, in plants, there are 326 in rice [21], 332 in soybean [22], 95 in sweet potato [6], and 233 in tomato [2]; in fungi, there are 20 in *Tilletia horrida* [39], and 83 in *Auricularia heimuer* [40]. A comparison of the genome size of these species and the number of identified *CYP450* genes revealed that the number of gene family members does not completely depend on the genome size. In this study, 59 CYP450 family members of *F. filiformis* (36.3 Mb) were randomly distributed on 12 chromosomes. At present, the CYP450 family members identified in fungi can be classified into approximately 400 subfamilies, namely CYP51-CYP69, CYP501-CYP699 and CYP5001-CYP6999 [41]. The CYP450 members of *F. filiformis* can be divided into the following 6 clades, namely, CYP64, CYP58, CYP67, CYP52, CYP503 and CYP_FUM15, among which the

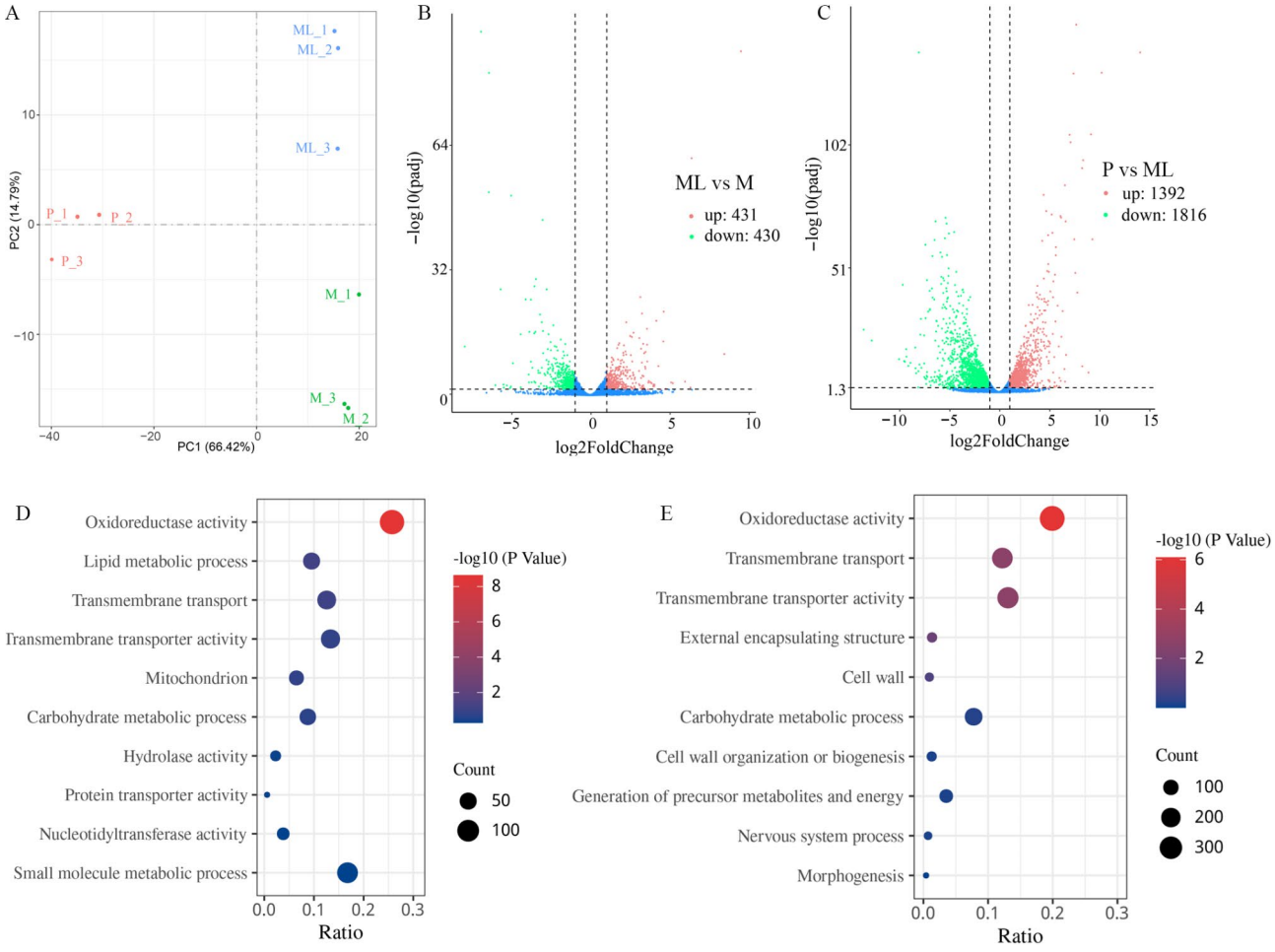

Fig. 5 Transcriptome analysis of *Flammulina filiformis* at different developmental stages. **A**, Principal component analysis of the three samples based on the gene expression profiles, M represents the mycelium cultured at 25℃, ML represents the mycelium transferred from 25℃ to 10℃ treatment for 3 d, P represents primordium. **B-C**, Volcano plots displaying the DEGs between ML and M or P and ML. **D-E**, Top 10 enriched GO terms of among different groups, including ML vs. M and P vs. ML

CYP64 branch contains the largest number of members (25). Similarly, 83 CYP450s of *Auricularia heimuer* were classified into 21 subfamilies, and the members of the CYP64 family were also the most. The CYP64 family is considered to be involved in the "Metabolism-Biosynthesis of other secondary metabolites" pathway [40]. The large number of the CYP64 subfamily in edible fungi may be related to the abundance of bioactive substances in edible fungi and may be involved in the metabolic regulation of various substances. In addition, CYP64 enzymes may also play a role in substrate degradation, utilization of bioactive components, detoxification, or other processes [24], thereby enhancing their environmental adaptability and ecological advantage in competitive microbial communities.

The majority of CYP450 proteins in *F. filiformis* possess 3 typical conserved motif; however, some members are deficient in either the PERF motif or the Heme-binding region, which might have been lost during the long-term evolution process. The variations in gene length, protein size, and physicochemical properties may contribute to the diversity of CYP450 family members, facilitating organismal adaptation to complex environments. Gene duplication events are a primary driver of genome amplification and evolution in plants, contributing to the expansion of gene families. In the present study, 3 pairs of tandem duplication genes and 3 pairs of segmental duplication genes in the CYP450 family of *F. filiformis* were identified. *F. filiformis*, as a saprophytic fungi, is chronically exposed to complex lignocellulosic substrates and competitive microbial communities. The expansion of CYP450 gene may enhance its environmental adaptability through detoxification function, participation in secondary metabolism or other biological processes [24]. CYP450 monooxygenase, an important oxidoreductase, regulates both oxidation processes and highly diversified and complex structural modifications and participates in numerous significant cellular processes. Extensive

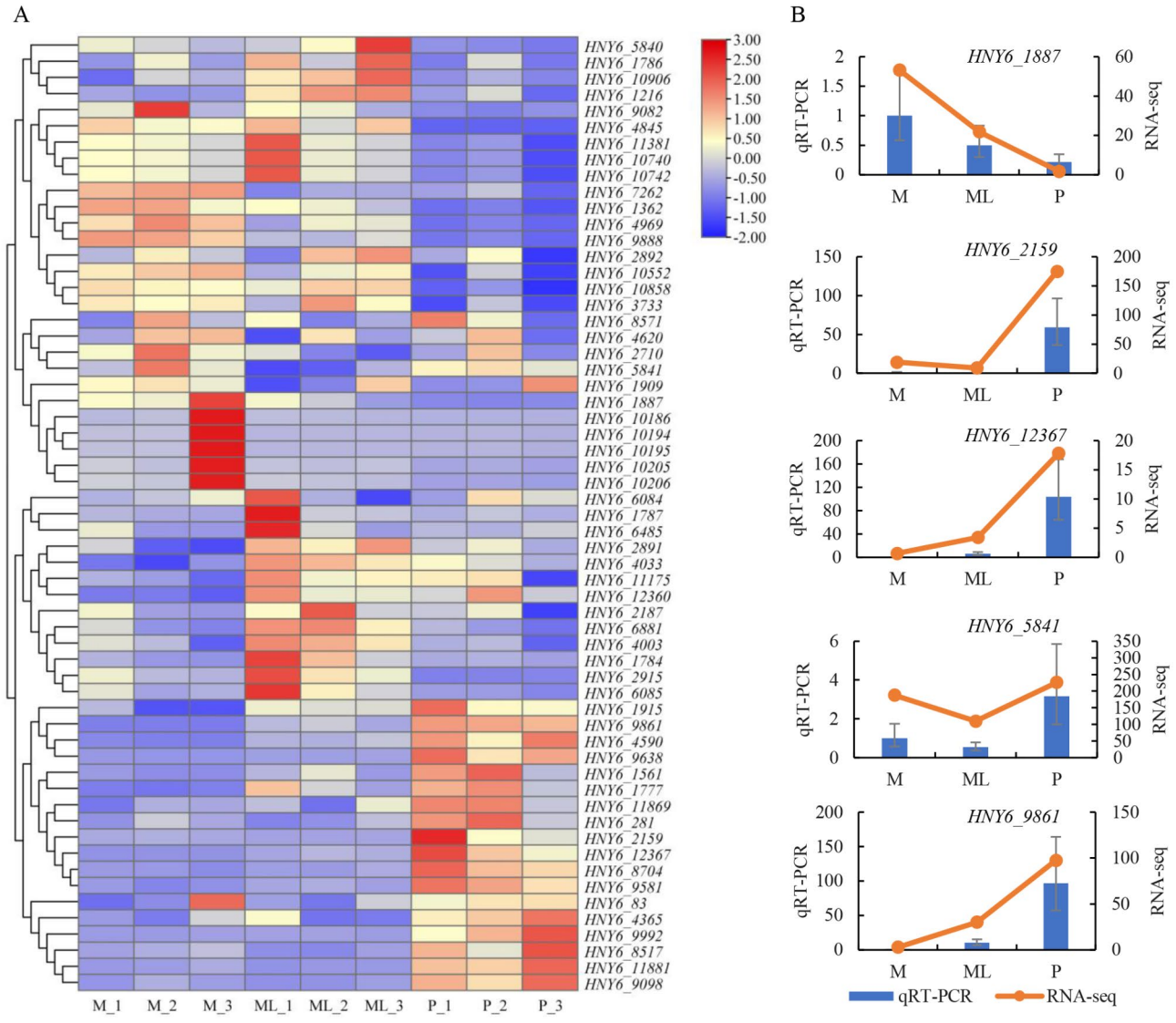

Fig. 6 Expression heatmap of *CYP450* genes in different tissue and qRT-PCR validation of transcriptome data. **A**, The expression of 59 *CYP450* genes in the different tissue using transcriptome data. **B**, qRT-PCR validation of transcriptome data was performed using 5 randomly selected genes

research has been conducted on the functions of *CYP450* genes in plants, highlighting their crucial roles in metabolism, responses to biotic and abiotic stresses, and the regulation of plant growth and development [3, 4, 23]. Nevertheless, homologous genes in different species may exhibit functional variations. Previous report indicated that the *CYP450* gene *eln2* is involved in regulating stipe elongation in *Coprinus cinereus* [42], and *CYP450* genes have also been associated with the development process of fruiting bodies in *Lentinula edodes* [43].

Transcriptome analysis of *F. filiformis* revealed that the DEGs in the ML vs. M and P vs. ML comparison groups were significantly enriched in the 'oxidoreductase activity' category. Among them, the members of the CYP450 family were the most numerous, indicating that the related genes are involved in the regulation of the morphogenesis and growth and development processes of *F. filiformis*. The expression patterns of genes offer valuable insights into their biological functions. Therefore, 59 *CYP450* genes in different tissues of *F. filiformis* were analyzed. Our results revealed that 4 genes exhibited the highest expression levels in mycelium, 6 genes showed peak expression in mycelium subjected to low temperature treatment, and 10 genes were most highly expressed in primordia. Consistent with previous studies, which have demonstrated the tissue-specific expression of numerous *CYP450* genes in other species, our findings underscore this phenomenon. For instance, the CYP71 subfamily in sweet potato exhibits specific expression in multiple tissues, while *SiCYP78A1* and *SiCYP94D9* in foxtail millet are predominantly expressed in leaves, and the expression level of *SiCYP78A6* is relatively high

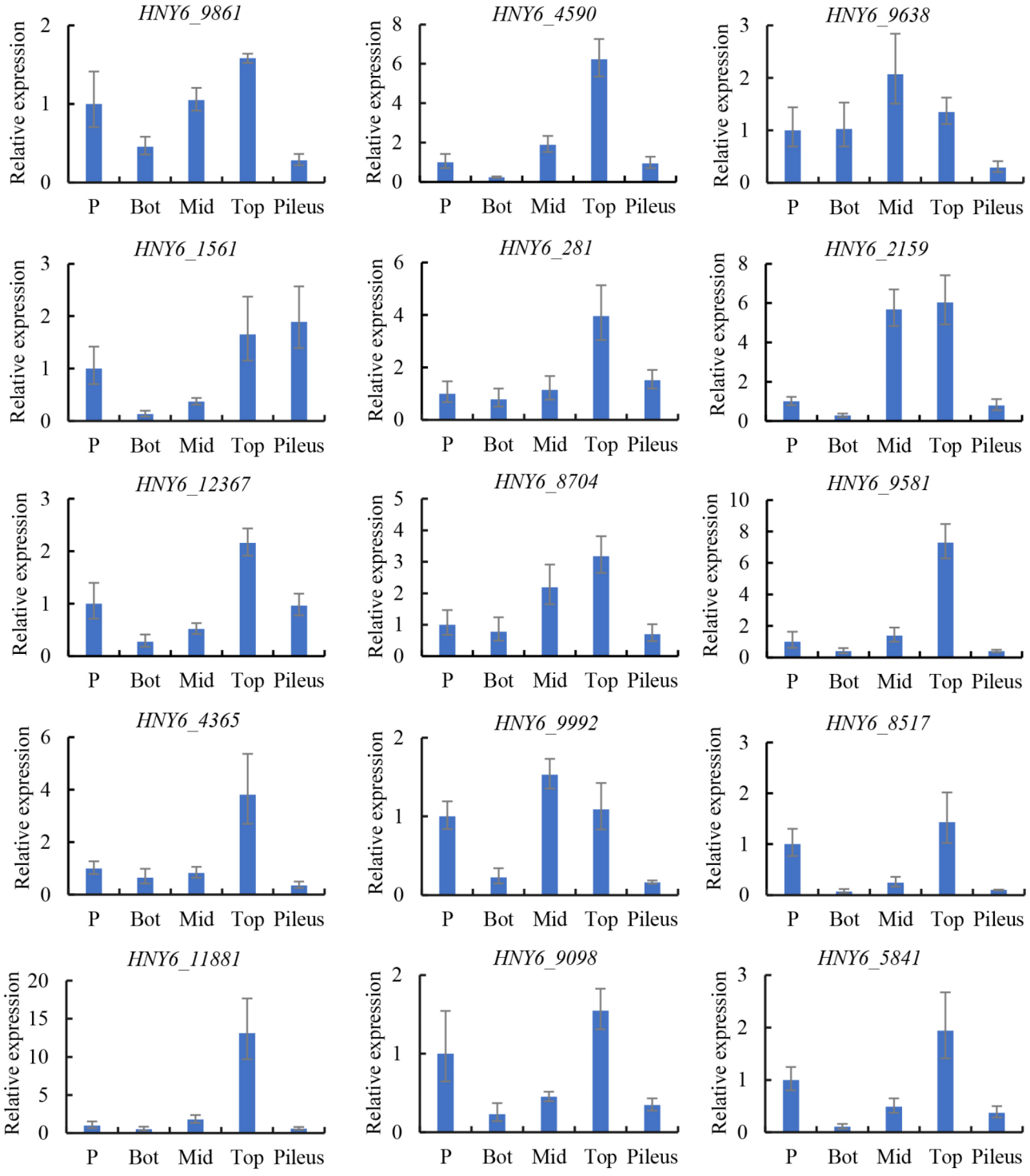

Fig. 7 Expression of the 15 selected candidate CYP450 genes in different tissue of *Flammulina filiformis* white variety. P represents primordium, Bot represents bottom part of stipe, Mid represents middle part of stipe, Top represents top part of stipe, Pileus represents pileus of *Flammulina filiformis* white variety

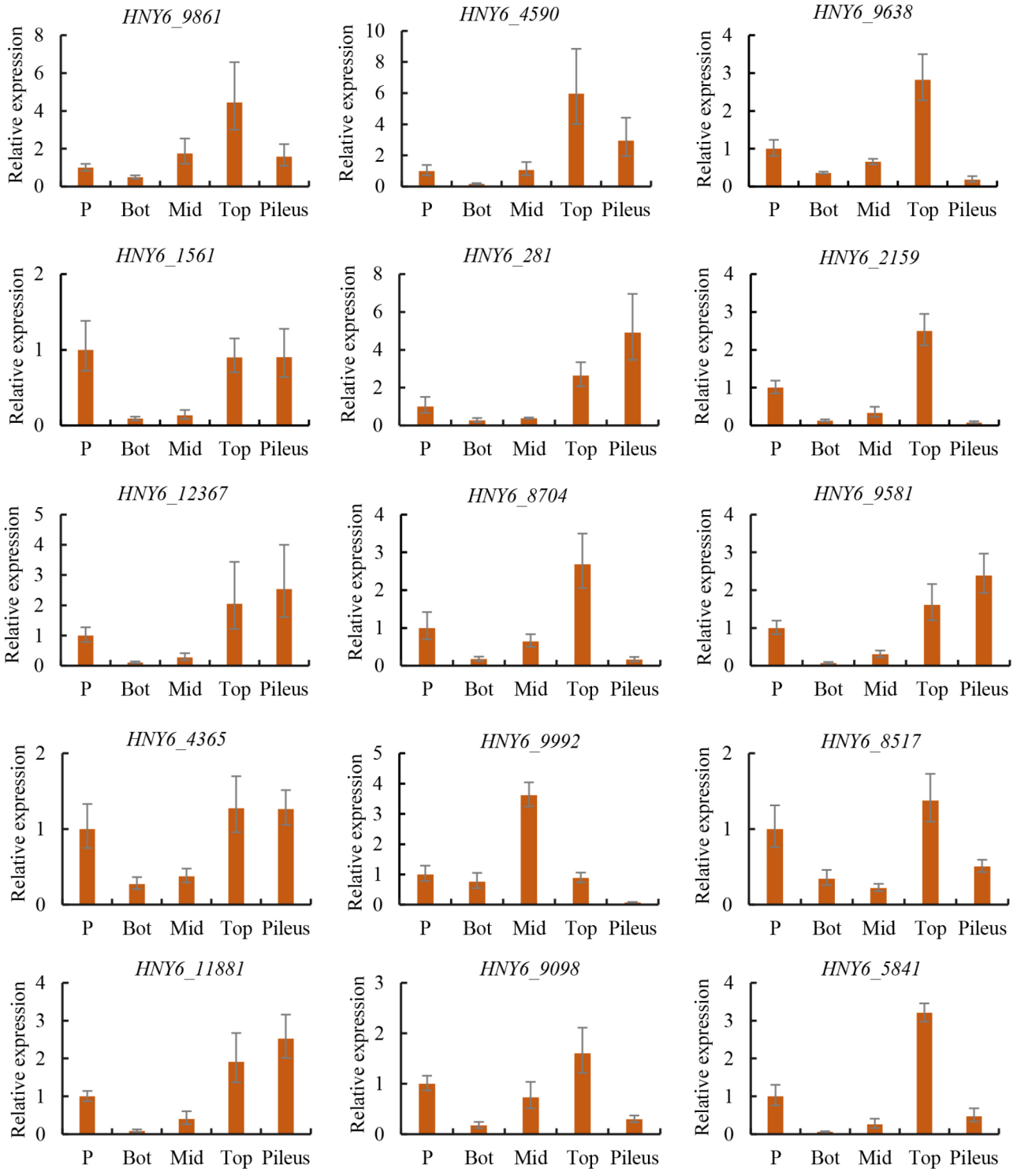

Fig. 8 Expression of the 15 selected candidate CYP450 genes in different tissue of *Flammulina filiformis* yellow variety. P represents primordium, Bot represents bottom part of stipe, Mid represents middle part of stipe, Top represents top part of stipe, Pileus represents pileus of *Flammulina filiformis* yellow variety

in panicles [44]. Additionally, *Arabidopsis CYP77A6* is specifically expressed in flowers, and *CYP708A2* is expressed only in roots [45]. These observations suggest that *CYP450* genes may play significant roles in different species and tissues, which urges us to explore their functional mechanisms in processes such as growth and development and organ formation.

In terms of the research on gene families of other species, *PeUGDH* gene of bamboo showed the highest expression level in the stem, followed by that in the leaf, and the lowest in the root. The expression pattern of the *PeUGDH* gene was consistent with the trend of lignin content in bamboo, indicating that the *PeUGDH* gene is of great significance for the synthesis of lignin [46]. We found that the expression levels of 11 *CYP450* genes, namely *HNY6_9861*, *HNY6_4590*, *HNY6_1561*, *HNY6_281*, *HNY6_12367*, *HNY6_8704*, *HNY6_9581*, *HNY6_8517*, *HNY6_11881*, *HNY6_9098*, and *HNY6_5841*, showed highest relative expression level in the upper part of the stipe, which was positively correlated with the elongation characteristics of the stipe, where the elongation zone is located [18]. Similarly, CYP450 genes *Le.cyp1* and *Le.cyp2* were shown to be more actively transcribed at the beginning stage of fruiting-body formation of *L. edodes*, the stipe of the premature fruiting body contained larger amounts of both transcripts. *Le.cyp1* and *Le.cyp2* were believed to be involved in the regulation of the growth of the stipe [43]. Expression analyses in yellow *F. filiformis* also revealed that the majority of these genes exhibited expression patterns consistent with those in the white strain, indicating that the expression patterns were conserved and may play a regulatory role in the elongation process of the stipe. Despite these findings, the regulatory functions and mechanisms of these candidate genes in growth and development require further investigation. Nevertheless, the results of this work provide a foundation for further exploration of the molecular evolution and potential functions of the *CYP450* gene family in *F. filiformis*. Further exploration of the function and molecular mechanism of the CYP450 gene of *F. filiformis* may accelerate the breeding process of new varieties (shorter growth period and better environmental adaptability), and provide references for molecular breeding of other edible fungi.

Conclusion

We identified 59 *CYP450* genes in the genome of *F. filiformis*, which were classified into 6 clades in the phylogenetic tree. Gene duplication analysis revealed that both tandem and segmental duplications contributed to the expansion of CYP450 genes in *F. filiformis*. Transcriptomic analysis revealed that the 'oxidoreductase activity' category was significantly enriched in the ML vs. M and P vs. ML comparisons, with *CYP450* genes being predominant represented among the DEGs. Expression analysis revealed an increasing trend in the expression levels of 11 *CYP450* genes in the lower, middle and upper parts of the stipe in both white and yellow strains. The results of this work provide a foundation for further exploration of the molecular evolution mechanism and potential functions in the regulation of the growth and development of the *CYP450* genes of *F. filiformis*.

Abbreviations

CYP450	Cytochrome P450
DEGs	Differentially Expressed Genes
qRT-PCR	Quantitative real-time Polymerase Chain Reaction
RNAi	RNA interference
CDS	Coding Sequence
CDD	Conserved Domain Database
FPKM	Fragments Per Kilobase of Transcript Per Million Fragments Mapped
GO	Gene Ontology
PCA	Principal Component Analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11555-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Not applicable.

Author contributions

X. L. designed and performed the experiments and wrote the paper. J. H., X. L. and Y. C. performed some experiments, and M. L., B. W., and D. J. analyzed the data. W. P. revised the paper. X. H. conceived the experiment and revised the paper. All authors have read and approved the manuscript.

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Data availability

All datasets supporting the results of this study are included in this article and its Supplementary data files.The genome data of F. filiformis used in this study can be accessed through the NCBI accession number: PRJNA1110060.The transcriptomic data used in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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