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Whole-genome sequencing and analysis of the symbiotic *Mycena* sp. L02 with *Gastrodia Elata*

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Abstract

Background *Mycena* is a widespread genus of mushroom capable of decomposing various dead plant substrates. *Gastrodia elata* is a fully mycoheterotrophic orchid whose seed germination depends on specific *Mycena* strains. However, only a few *Mycena* species are capable of inducing germination, necessitating the identification of novel germinating fungal resources.

Results The genome of *Mycena* sp. L02 was sequenced using the Illumina NovaSeq and Oxford Nanopore Technologies (ONT) platforms. The final assembly spanned 160.06 Mb, with 36,246 genes predicted and repetitive sequences accounting for 31.90% (46,496,154 bp) of the genome. A total of 1,339 CAZyme genes were annotated, along with 3,772 genes involved in host–pathogen interactions, 88.33% of which were associated with loss of pathogenicity, reduced virulence, or unaffected pathogenicity. Comparative genomic analysis between germinating and non-germinating *Mycena* strains revealed that their CAZymes and PHI gene characteristics represent common traits shared across the *Mycena* genus, with no distinctive features identified. Furthermore, pathways enriched in unique gene families of the germinating fungi—such as glutathione metabolism, sulfur metabolism, phosphatidylinositol signaling system, and inositol phosphate metabolism—may contribute to the germination of *G. elata* seeds.

Conclusions The abundance of CAZymes and low-virulence genes in L02 ensures sufficient nutrient acquisition and may facilitate hyphal penetration of the lignin-rich seed coat of *G. elata*, thereby enabling successful symbiosis. Additionally, KEGG pathways enriched in the unique gene families of the germinating fungi may contribute to the stimulation of seed germination in *G. elata*. Overall, this study provides a valuable genomic foundation for screening high-performance germinating fungi and further investigating the molecular mechanisms underlying the symbiosis with *G. elata*.

Keywords *Mycena*, Genome, Germination, *G. elata* seeds

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Introduction

Gastrodia elata Bl., as a rootless, leafless, achlorophyllous, and fully mycoheterotrophic orchid, relying on symbiotic fungi from *Mycena* spp. and *Armillaria* spp. to provide essential nutrients for seed germination and tuber development [1]. In November 2023, *G. elata* was officially listed by the Chinese government in the *Catalog of Substances Recognized as Both Food and Traditional Chinese Medicinal Materials* [2]. *G. elata* exhibits not only high nutritional value but also pharmacological properties, including neuroprotective, antioxidant, anti-inflammatory, and cardiovascular disease-alleviating activities [1]. Consequently, increasing demand for *G. elata* has driven overharvesting of wild populations, leading to its designation as a Vulnerable species by the International Union for Conservation of Nature [3] and inclusion as a Class II protected plant in China [4]. This underscores the urgent need for large-scale cultivation of *G. elata*.

G. elata can be cultivated both asexually and sexually [5]. However, multiple generations of asexual propagation often lead to cultivar degeneration of immature tuber, resulting in significant declines in both yield and quality [6]. In contrast, sexually propagated immature tuber displays greater vigor, stress resistance, and high-yield, high-quality mature tubers, offering an effective solution to cultivar degeneration [7]. Within the sexual reproduction process, seed germination is a critical step [8, 9]. Like other orchids, *G. elata* produces dust-like seeds lacking endosperm and nutrient reserves, relying on *Mycena* strains to develop into protocorms [10]. As such, these fungi that facilitate seed germination are referred to as germinating fungi, and their quality directly influences the yield and quality of cultivated *G. elata*.

The genus *Mycena* (Basidiomycota, Agaricales, Mycenaceae) are saprophytic fungi, comprising over 600 described species globally [11]. However, only a few species have been shown to facilitate *G. elata* seed germination, including *M. osmundicola* [12], *M. dendrobii* [13], *M. anoectochila* [14], *M. orchidicola* [15], *M. purpureofusca* [6], *M. abramsii*, *M. citrinomarginata*, *M. scorodonius* [5], *M. subpiligera* [16], and *Mycena* sp. WM (WM) [17]. This limited diversity is attributed to: (1) germinating fungi are weak saprotrophs that grow slowly and are difficult to culture, complicating their isolation and identification [18]; 2) temporal mismatches between fungal fruiting body and *G. elata* growth stages, hindering field collection [19]; 3) reduced survival rates after repeated subculturing, leading to diminished or absent germination efficacy [20]; and 4) geographic variability in *G. elata* seed ecotypes, necessitating region-specific fungal strains [5]. Thus, expanding the repository of high-quality germinating fungi is essential for improving the germination rate of *G. elata* seeds.

Current research on *G. elata* germinating fungi focuses on optimizing germination protocols, assessing fungal germination capacity, and elucidating symbiotic mechanisms. This includes identifying suitable nutrient conditions [21], developing methods to promote seed germination [22], screening for optimal germination strains [5, 19], and evaluating symbiotic compatibility [23]. However, relatively few studies have investigated the intrinsic characteristics of germinating fungi themselves as a means to understand their symbiotic compatibility with *G. elata* seeds. Among the reported germinating strains, only WM has a publicly available genome.

To identify genomic features enabling fungal symbiosis with *G. elata*, we initially collected five germination-promoting fungal isolates and conducted field trials (inoculating each fungal strain into a 2-hectare plot planted with *G. elata* seeds). Ultimately, we identified a superior strain, *Mycena* sp. L02, which achieved a high yield of approximately 45,000 t/ha. Here, we present the whole-genome sequencing of L02 to uncover genomic characteristics underlying its symbiotic compatibility with *G. elata* seeds.

Materials and methods

DNA extraction and sequencing

L02 was obtained in 2023 from a *G. elata* cultivation site located in Xiaocaoba Township, Yiliang County, Zhaotong City, Yunnan Province, China. The strain was cultured on potato dextrose agar (PDA) plates at 28 °C for 5 days prior to DNA extraction. High-quality genomic DNA was isolated from fresh mycelia using the cetyltrimethylammonium bromide (CTAB) method [24]. The purity of the DNA was further enhanced using a Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA, USA). DNA concentration was measured at 76.9 ng/μL using a Qubit Fluorometer (Invitrogen, Carlsbad, CA, USA). Purity was assessed with a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA), yielding absorbance ratios of A260/A280 = 2.16 and A260/A230 = 2.3. DNA integrity was verified via 1% agarose gel electrophoresis. For molecular identification, the internal transcribed spacer (ITS) region of the strain [25] was amplified using primers ITS-F (3′-CCGTGTTTCAAGACGGG-5′) and ITS-R (3′-CTTGGTCATTTAGAGGAAGTAA-5′), followed by sequencing to confirm the identity of the strain as *Mycena* sp. L02. Sequencing libraries were constructed using the Illumina TruSeq Nano DNA LT kit (Illumina, San Diego, CA, USA) for short-read sequencing and the Oxford Nanopore Technologies (ONT) Ligation Sequencing Kit SQK-LSK109 (Oxford Nanopore Technologies, Oxford, UK) for long-read sequencing. Whole-genome sequencing was performed on an Illumina NovaSeq 6000 platform in paired-end mode (2 × 150 bp). Long-read sequencing was carried

out on a PromethION 48 device using FLO-PRO002 flow cells. Basecalling was performed using the Guppy basecaller integrated within the MinKNOW software.

Genome assembly, gene prediction, and annotation

The de novo genome assembly was performed using Flye (v2.9.1-b1781) [26], NextDenovo (v2.5.0) [27], and NECAT (v0.01) [28]. The NextDenovo assembly was selected for the final genome set, as it yielded the longest N50 and the smallest number of contigs. Assembly completeness was evaluated using Benchmarking Universal Single-Copy Orthologs (BUSCO, v5.4.5) [29] with the lineage dataset fungi_odb10 to assess the presence of conserved single-copy orthologs.

Repetitive sequences were identified using both de novo and homology-based approaches. For *de novo* repeat annotation, TRF (v4.10.0) [30] and RepeatModeler (v2.0.4) [31] were employed. RepeatModeler integrates two *de novo* repeat-finding algorithms, RECON (v1.0.8) [32] and RepeatScout (v1.0.5) [33], which use complementary approaches to identify repeat boundaries and classify repeat families. Homology-based repeat annotation was conducted using RepeatMasker (v4.1.4) [34] with a custom library combining Repbase (v20150807) [35] Brassicaceae repeats and *de novo* predictions from RepeatModeler.

Transfer RNAs (tRNAs) were predicted with tRNAscan-SE (v2.0) [36], ribosomal RNA (rRNA) genes with Barrnap (v0.9) [37], and other non-coding RNAs by alignment with the Rfam database [38]. For *de novo* gene prediction, Augustus (v2.5.5), GlimmerHMM (v3.0.4), and GeneMark-ES (v4.71) were use [39–41]. Homology-based gene prediction was carried out using Exonerate (v2.2.0) with protein sequences from phylogenetically related species [42]. Final gene models were generated by integrating both ab initio and homology-based predictions using EVidenceModeler (EVM, v2.0.0) [43].

Protein-coding genes were functionally annotated by performing sequence similarity searches against the following databases: Carbohydrate-Active Enzymes (CAZymes) [44], Gene Ontology (GO) [45], Kyoto Encyclopedia of Genes and Genomes (KEGG) [46], Pfam, NR, and the Pathogen–Host Interactions (PHI) Database, etc.

Table 1 Genome assembly parameters of L02

Parameter	Contig
Min sequence length	14,165
Max sequence length	3,091,502
Total sequence number	637
N50	291,997
N50 Number	106
Total sequence length	145,762,527
GC content %	50.8083

Comparative genomic analysis

Publicly available genome sequences of 32 *Mycena* strains were obtained from NCBI [17, 47–49]. *Armillaria gallica*, a previously reported symbiotic fungus of *G. elata* from our earlier study [50], was included as the outgroup (detailed information provided in Supplementary Table 1). For genomes lacking annotation, structural gene prediction and functional annotation were performed. All 34 strains were uniformly subjected to CAZyme and PHI annotation and analysis. Protein sequences from all strains were filtered to remove those shorter than 50 amino acids. The qualifying sequences were consolidated into a single file, which served as both the reference database and query set for an all-versus-all BLASTP analysis using an E-value cutoff of $1e^{-10}$. The resulting alignments were processed using OrthoMCL (v2.0.8) [51] with the alignment length coverage threshold set to 70%. Gene families were clustered using the MCL algorithm with an inflation value of 1.5. The clustering results were subsequently organized and statistically summarized using a Perl script. KEGG enrichment analysis was performed using the R package clusterProfiler, based on the unique genes of strains L02 and WM and their functional annotation results [52, 53]. A maximum likelihood phylogenomic tree was reconstructed based on a concatenated alignment of 105 single-copy orthologous genes (Table S2). The individual gene alignments were performed using MAFFT [54], followed by quality control with Gblocks (v 0.91b) [55] to remove unreliable alignment regions. The curated alignments for each gene were then concatenated into a supermatrix. To assess the robustness of the phylogenetic tree and to quantify gene tree discordance, we computed both gene concordance factors (gCF) and site concordance factors (sCF) using IQ-TREE (v2.2.2) (Table S3) [56]. The maximum likelihood tree was inferred from the concatenated supermatrix using FastTree [57], and the reliability of the tree topology was assessed through bootstrap analysis with 1000 replicates alongside the gCF and sCF values.

Results

Genome assembly and characteristics analysis

The genome size of L02 was 160.06 Mb, consisting of 637 contigs. The N50 was 291,997 bp, and the longest contig measured 3,091,502 bp, with a total contig length of 145,762,527 bp. The GC content was approximately 50.81% (Table 1). BUSCO assessment revealed that 96.0% (1692 BUSCOs) of the genes were complete, 0.6% (11 BUSCOs) were fragmented, and 3.4% (61 BUSCOs) were missing (Table 2).

Gene prediction

A total of 36,246 protein-coding genes were predicted in the L02 genome, with an average gene length

Table 2 Completeness assessment of the genome assembly

Property	Number	Percent (%)
Complete BUSCOs	1,692	96.0%
Complete and single-copy BUSCOs	1,631	92.5%
Complete and duplicated BUSCOs	61	3.5%
Fragmented BUSCOss	11	0.6%
Missing BUSCOs	61	3.4%
Total BUSCO groups searched	1,764	100%

Table 3 Gene prediction of the L02 genome

Parameter	Value
Total Genes length	56,410,437
Genes Percentage of genome	38.7002%
Total Genes Number	36,246
Average gene length	1,556.3
Total Exons Number	181,580
Average Exons Per Gene	5
Total Exons length	44,431,269
Exons Percentage Of genome	30.4819%
Average Exons length	244.6
Average Introns length	82.4
Total CDSs length	44,431,269
CDSs Percentage of genome	30.4819%
Average CDS length	1,225.8

of 1,556.3 bp. The total length of these genes was 56,410,437 bp, accounting for 38.70% of the L02 genome (Table 3). Repetitive elements in the L02 genome were classified into interspersed repeats and tandem repeats based on their sequence characteristics. Approximately 31.90% (46,496,154 bp) of the genome was composed of repetitive sequences, of which 31.83% (46,400,981 bp) were interspersed repeats. These interspersed repeats include SINEs, LINEs, LTR elements, DNA transposons, rolling-circle, and unclassified repeats. Notably, the unclassified category accounts for 29.27% (42,671,475 bp) of the total (Table 4). A total of 37,700 tandem repeats were identified, including 34,580 microsatellites, 918 minisatellites, and 5 satellite DNAs. Additionally, the genome contained predictions for 324 tRNAs, 51 non-coding RNAs (ncRNAs), and one 18 S rRNA gene (Table 5).

Gene annotation

Functional annotation of protein-coding genes in *Mycena* sp. L02 was performed (Fig. 1). In total, 13,029 genes were annotated with 5,317 GO terms, including 3,074 BP terms, 618 CC terms, and 1,625 MF terms (Fig. S1). To further elucidate the biological functions of these protein-coding genes in *Mycena* sp. L02, we annotated 4,239 genes using the KEGG database (Fig. S2). Among these, the highest number of genes (3,891) were assigned to Brite Hierarchies. Pfam annotations were assigned to 15,955 genes (Fig. S3). A total of 30,610 genes were functionally annotated through the NR database. 93.10% of these were annotated as *Mycena* (28,497 genes), with 39.43% annotated as *M. venus* (12,068), 34.3% annotated as *M. galopus* ATCC 62,051 (10,490), 15.7% annotated as *M. sanguinolenta* (4,808), and 2.0% annotated as *M. chlorophos*, among others (Fig. S4).

Comparative genomic analysis

General genomic features of Mycena

Currently, a total of 36 *Mycena* genomes spanning 31 species are publicly available on NCBI. We selected 32 (including two distinct strains of *M. sanguinolenta*) genomes for comparative analysis with L02. Except for WM (46%), *M. belliarum*, and *M. citricol*or (for which no genomic data is available), all other genomes displayed BUSCO completeness scores exceeding 90%, demonstrating that the assemblies successfully captured most conserved single-copy genes universal in fungi (Fig. 2). The quality of the *Mycena* genome assemblies varied considerably, with scaffold numbers ranging from 30 in *M. indigotica* to 63,394 in WM, averaging 2,630.97 scaffolds per genome.

Genome sizes within *Mycena* varied widely, spanning from 43.6 to 501.2 Mb. The genomes of L02 and WM, both associated with *G. elata* seed germination, were similar in size—approximately 160 Mb—ranking 22nd and 25th smallest. respectively, among the 33 strains examined. Predicted gene counts ranged from 13,505 in *M. chlorophos* to 96,999 in *M. olivaceomarginata*. *Mycena* sp. L02 and WM were predicted to encode 36,246 and 33,701 genes, respectively. Both values are close to the average of 34,338 genes, with these strains having the 20th and 15th smallest genomes in the set.

Phylogenetic analysis revealed that L02 and WM clustered together on a single branch with 100% bootstrap support, indicating a high degree of genetic similarity. Furthermore, L02 was grouped within a larger clade containing *M. albidollacea*, *M. olivaceomarginata*, *M. galopus*, *M. haematopus*, and *M. sanguinolenta*, suggesting a close evolutionary relationship among these taxa (Fig. 3).

CAZymes and PHI genes in Mycena

To evaluate genomic traits underlying symbiosis with *G. elata* seeds, we focused on CAZymes and PHI gene profiles across the 33 strains. A positive correlation was observed between the number of CAZymes/PHI genes and genome size (Fig. 4).

A total of 1,339 and 711 CAZymes were annotated in the L02 and WM genomes, respectively. These included 146 and 77 Glycosyl Transferases (GTs) (comprising 10.90% and 10.83% of total CAZymes), 42 and 20 Polysaccharide Lyases (PLs) (3.14% and 2.81%), 244 and 114 Carbohydrate Esterases (CEs) (18.22% and 16.03%), 292 and 144 Auxiliary Activities (AAs) (21.81% and 20.25%),

Table 4 Detailed repeat element length statistics by class/subclass in L02

Class	Subclass	Number of elements	Length occupied (bp)	Percentage of sequence (%)
Retroelements		5,699	3,383,441	2.32
SINEs		10	400	0
	Penelope	74	6,681	0
LINEs		1,034	103,180	0.07
	CRE/SLACS	8	628	0
	L2/CR1/Rex	110	6,284	0
	R1/LOA/Jockey	158	13,649	0.01
	R2/R4/NeSL	31	1,762	0
	RTE/Bov-B	98	17,869	0.01
	L1/CIN4	216	18,473	0.01
LTR		4,655	3,279,861	2.25
	BEL/Pao	183	13,103	0.01
	Ty1/Copia	1,247	713,201	0.49
	Gypsy/DIRS1	2,933	2,514,493	1.73
	Retroviral	184	10,301	0.01
DNA	transposons	1943	346,065	0.24
	hobo-Activator	317	23,098	0.02
	Tc1-IS630-Pogo	505	112,345	0.08
	En-Spm	0	0	0
	MULE-MuDR	182	16,500	0.01
	PiggyBac	20	1,058	0
	Tourist/Harbinger	306	95,158	0.07
	Other	8	597	0.24
Rolling-circles		286	76,229	0.05
Unclassified		113,999	42,671,475	29.27
Total interspersed repeats			46,400,981	31.83
Satellites		86	18,905	0.01
Simple repeats		0	0	0
Low complexity		0	0	0
Small RNA		0	0	0

Table 5 NcRNA prediction statistics

Type	Copy	Average Length (bp)	Total Length (bp)	% of Genome
ncRNA	51	154.2156	7,865	0.0053
5 S rRNA	0	0	0	0
5.8 S rRNA	0	0	0	0
18 S rRNA	1	498.0	498	0.0003
28 S rRNA	0	0	0	0
tRNA	324	90.4166	29,295	0.0200

116 and 63 Carbohydrate-Binding Modules (CBMs) (8.66% and 8.86%), and 499 and 293 Glycoside Hydrolases (GHs) (37.27% and 41.21%) (Fig. 4). Within the GH families, GH16 (45, 9%; 21, 7.2%), GH18 (30, 6%; 21, 7.2%), and GH28 (29, 6%; 17, 5.8%) were the most abundant in L02 and WM, respectively. For AAs, the most prevalent families were AA3_2 (71, 24%; 37, 25.7%), AA7 (58, 20%; 20, 13.9%), and AA2 (52, 18%; 22, 15.3%). Among CEs, CE10 (127, 52%; 47, 41.2%), CE1 (33, 14%; 17, 14.9%), and CE4 (26, 11%; 14, 12.3%) were the most common. The total number of CAZymes in L02 ranked 14th among the 33 strains and was nearly double that of WM. On average, the *Mycena* strains possessed 1249.61 ± 431.58 CAZymes genes. GHs were the most abundant category (467.52 ± 150.63), accounting for 37.91 ± 3.07% of total CAZymes, followed by AAs (282.61 ± 121.33, 21.96 ± 3.26%), CEs (227.64 ± 75.24, 18.33 ± 1.56%), GTs (143.42 ± 39.30, 11.87 ± 1.94%), CBMs (102.73 ± 54.63, 7.88 ± 1.93%), and PLs (25.70 ± 11.30, 2.06 ± 0.53%). Notably, the number of PLs in L02 (42, 3.14%) was significantly higher than the average across all strains.

A search against the PHI database identified 3,772 and 3,102 PHI genes in the L02 and WM genomes, respectively. In both strains, the largest functional category was "reduced virulence" (2,029 genes, 48.73% of total PHI genes in L02; 1,544 genes, 49.78% in WM), followed by

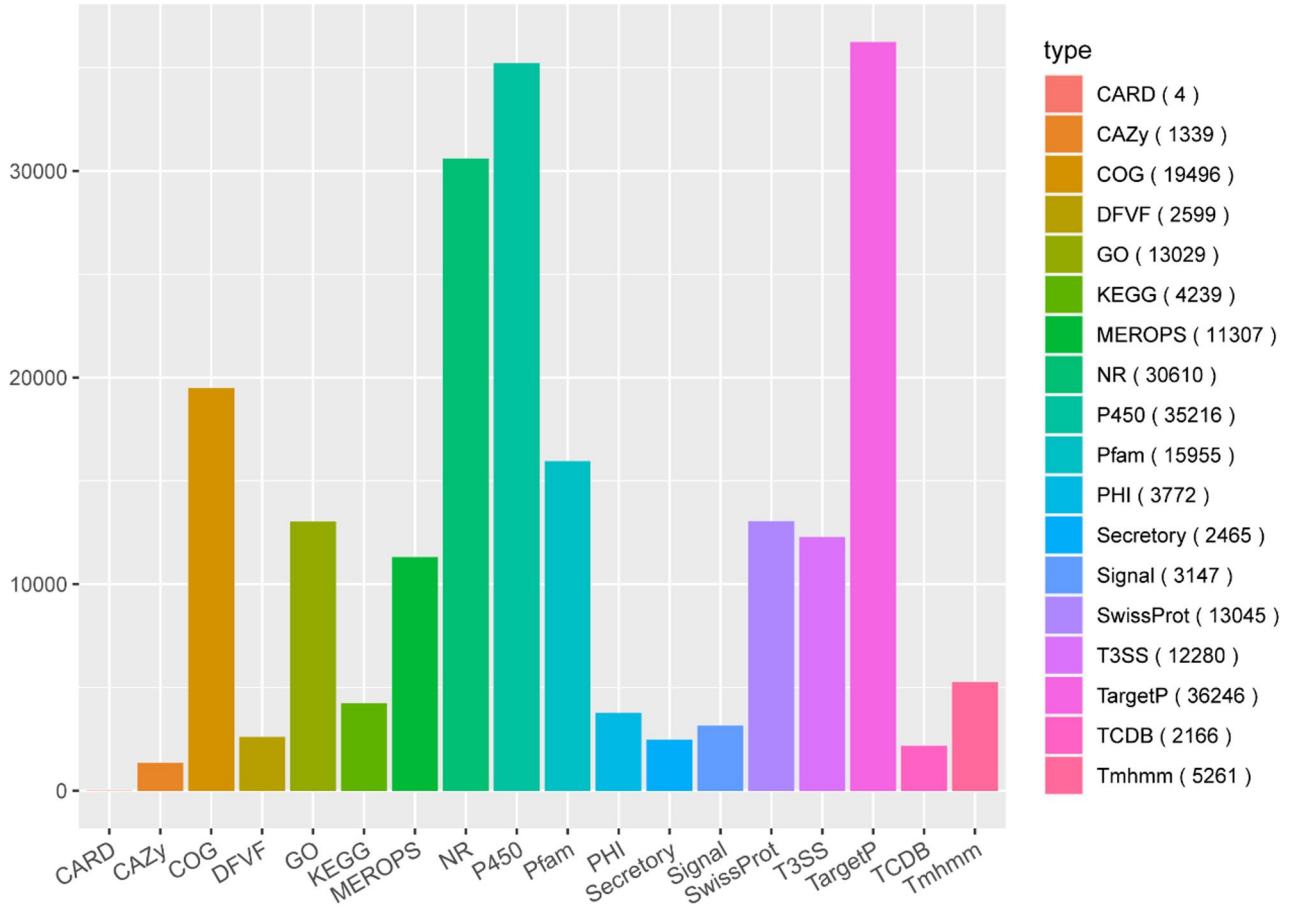

Fig. 1 Functional annotation of protein-coding genes in L02

"unaffected pathogenicity" (1,222 genes, 29.35%; 888 genes, 28.63%) and "loss of pathogenicity" (427 genes, 10.25%; 324 genes, 10.44%). Collectively, over half of the annotated PHI genes (58.98% in L02, 60.22% in WM) were associated with either non-pathogenicity or attenuated virulence.

On average, the *Mycena* strains contained 4276.18 ± 1186.54 PHI genes. The "reduced virulence" category was the most prevalent (2094.55 ± 611.03 genes, 48.82 ± 1.00% of total PHI genes), followed by "unaffected pathogenicity" (1267.49 ± 320.48 genes, 29.81 ± 1.08%) and "loss of pathogenicity" (441.70 ± 136.90 genes, 10.30 ± 0.64%). This indicates that genes associated with attenuated virulence dominate the PHI repertoire across the *Mycena* genus. Interestingly, while the number of "unaffected pathogenicity" genes in L02 (1,222) ranked 16th among the strains, it was significantly higher than that of WM (888), which ranked 4th.

Although the absolute numbers of CAZymes and PHI genes varied considerably among the 33 *Mycena* strains, their relative proportions within the total CAZymes and PHI repertoires were remarkably consistent. The overall characteristics of the CAZymes and PHI gene complements in the germinating fungi L02 and WM are thus in line with the general patterns observed across the *Mycena* genus.

Gene family analysis in Mycena

Gene family analysis revealed a total of 399 gene families shared across all 33 *Mycena* strains (Table S6). Strain L02 possessed 1,370 unique gene families, while strain WM possessed 3,481 unique gene families. Annotations of the gene families common to both germinating fungi (L02 and WM) showed that the most predominant functional categories included hypothetical proteins, integrase catalytic domain-containing proteins, and P-loop containing nucleoside triphosphate hydrolase proteins (Table S7). To further investigate the characteristics of the germinating fungi, an enrichment analysis was performed on their unique gene families, which revealed significant enrichment in 29 KEGG pathways, including Meiosis - yeast, Cell cycle - yeast, MAPK signaling pathway - yeast, Cell cycle, and Oocyte meiosis, etc. (Fig. 5).

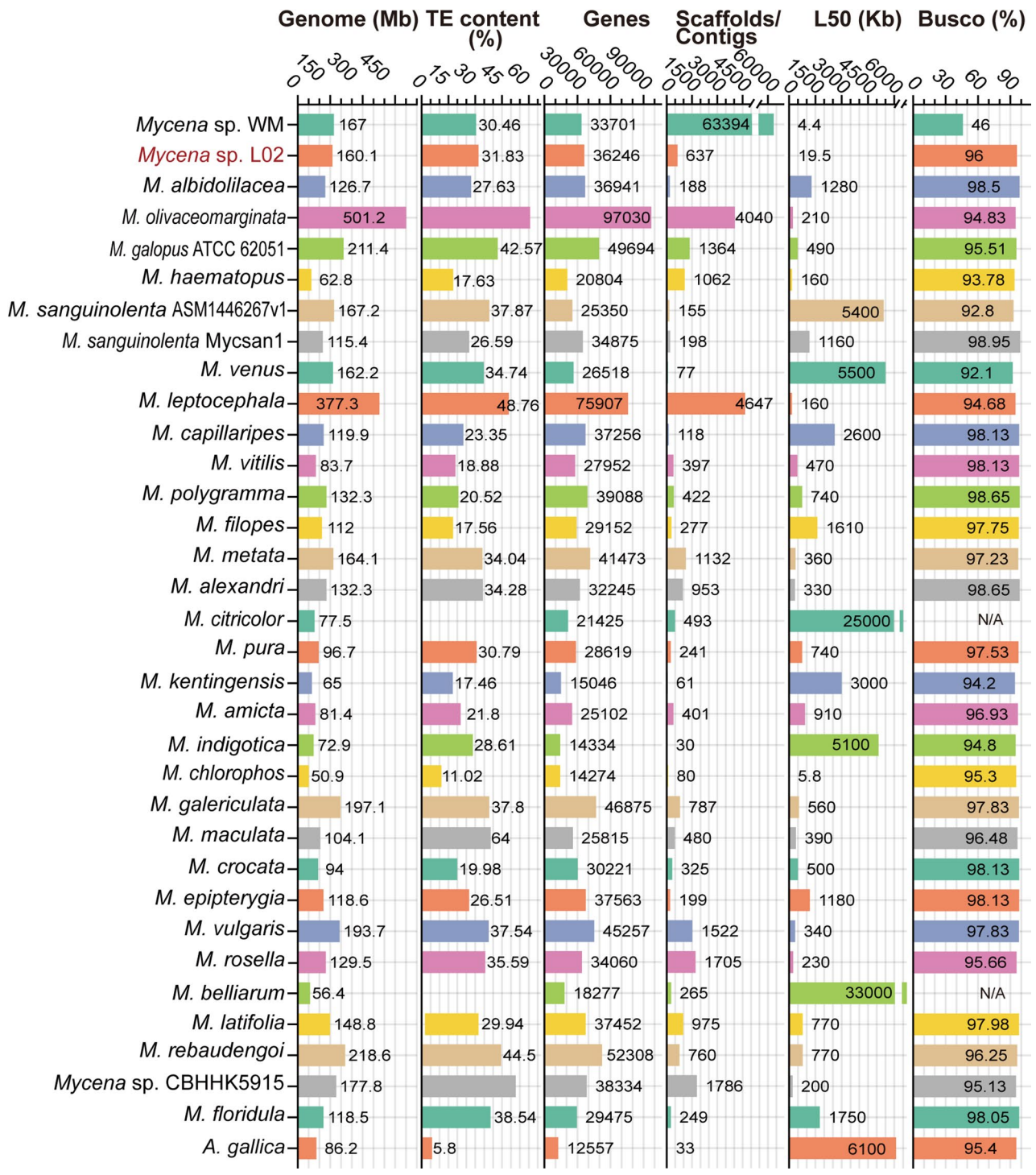

Fig. 2 General features analysis of 33 *Mycena* genomes. Genome, genome size; TE content, coverage of TE in the genome; genes, number of genes; Scaffolds/Contigs, number of scaffolds/contigs; L50, N50 length; BUSCO, genome completeness. The scores for *M. belliarum* and *M. citricolor* are indicated as 'N/A' (Not Available) as their genomic data could not be obtained for this assessment. Further details are provided in Table S4

Discussion

The genome expansion of L02 represents a shared derived character (synapomorphy) within the *Mycena* genus. The nuclear genome size of typical fungi lies between that of prokaryotes and other eukaryotes [58–60]. Compared to most other Agaricomycetes, such as *Armillaria spp.*, *Amanita spp.*, *Pluteus spp.*, etc., the *Mycena* genomes are notably larger [47, 48]. This large-scale genome expansion in *Mycena* is driven by the emergence of new gene families, gene duplication, expansion of the secretome

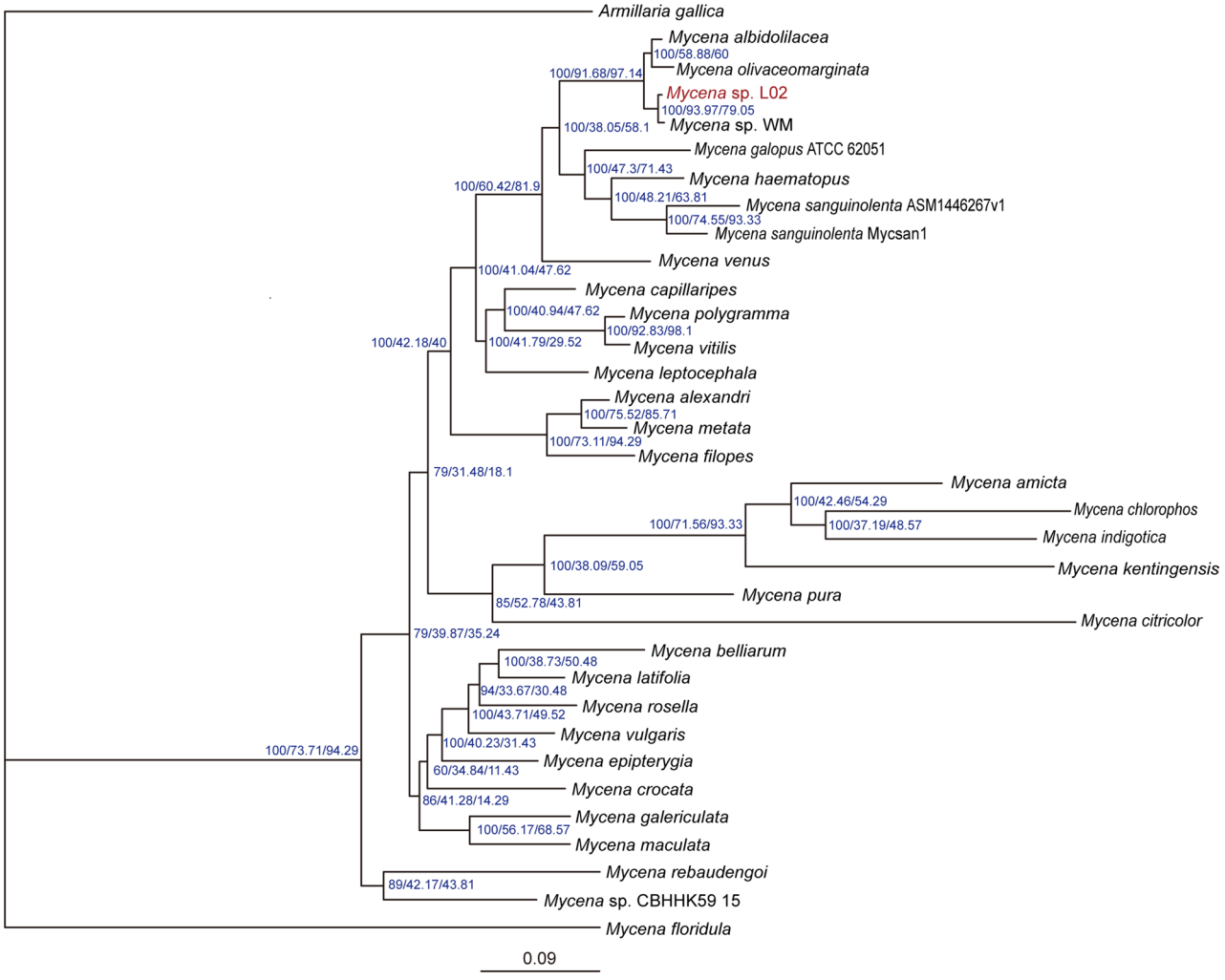

Fig. 3 General phylogeny of 33 *Mycena* genomes. The values displayed at the tree nodes correspond to the bootstrap support/sCF/gCF

encoding polysaccharide-degrading enzymes, TE, and horizontal gene transfer [47]. Repetitive sequences influence genome structure, size, and dynamics in various ways [61]. Differences in genome sizes among *Mycena* species are likely due to varying levels of repetitive sequence expansion [49]. Among these, TE proliferation and gene duplication are common drivers of fungal genome expansion [48, 49, 62, 63], and they are the main quantitative contributors to *Mycena* spp. genome expansion, as they not only promote gene duplication and the emergence of new gene families, but also enhance transcriptional activity [47]. Furthermore, *Mycena* genome size correlates strongly with CAZymes copy numbers [47]. In this study, repetitive elements accounted for approximately 31.90% of the L02 genome, and the number of CAZymes genes was also relatively high, consistent with typical *Mycena* genomic features.

Fungi produce a variety of CAZymes to degrade plant polysaccharides, facilitating infection and/or nutrient acquisition [64]. *Mycena* spp. harbor extensive CAZymes arsenals targeting all lignocellulosic components of plant cell walls [65], enabling saprotrophic or symbiotic lifestyles. Symbiotic *Mycena* strains colonize and decompose decaying plant matter, channeling nutrients to *G. elata* [66] and acting as metabolic bridges between plant debris and *G. elata* [1]. The abundance of CAZymes in L02 may provide ample nutritional capacity for its own growth and for promoting the germination of *G. elata* seeds. Notably, symbiotic *Armillaria* strains associated with *G. elata* also display elevated GH, CBM, and GT abundances compared to non-symbiotic counterparts [67, 68]. These enzymes facilitate cell wall degradation, fungal colonization, and secondary metabolite synthesis [69, 70], critical for symbiotic establishment. Similarly, CAZymes in *Mycena* sp. L02, like those in symbiotic *Armillaria*, may mediate its interaction with *G. elata* seeds. The AA family enzymes catalyze reactions involving non-carbohydrate components such as lignin [71]. Since the seed coat of *G. elata* is composed solely of lignin [72], lignin-degrading capacity is likely key to seed germination. The

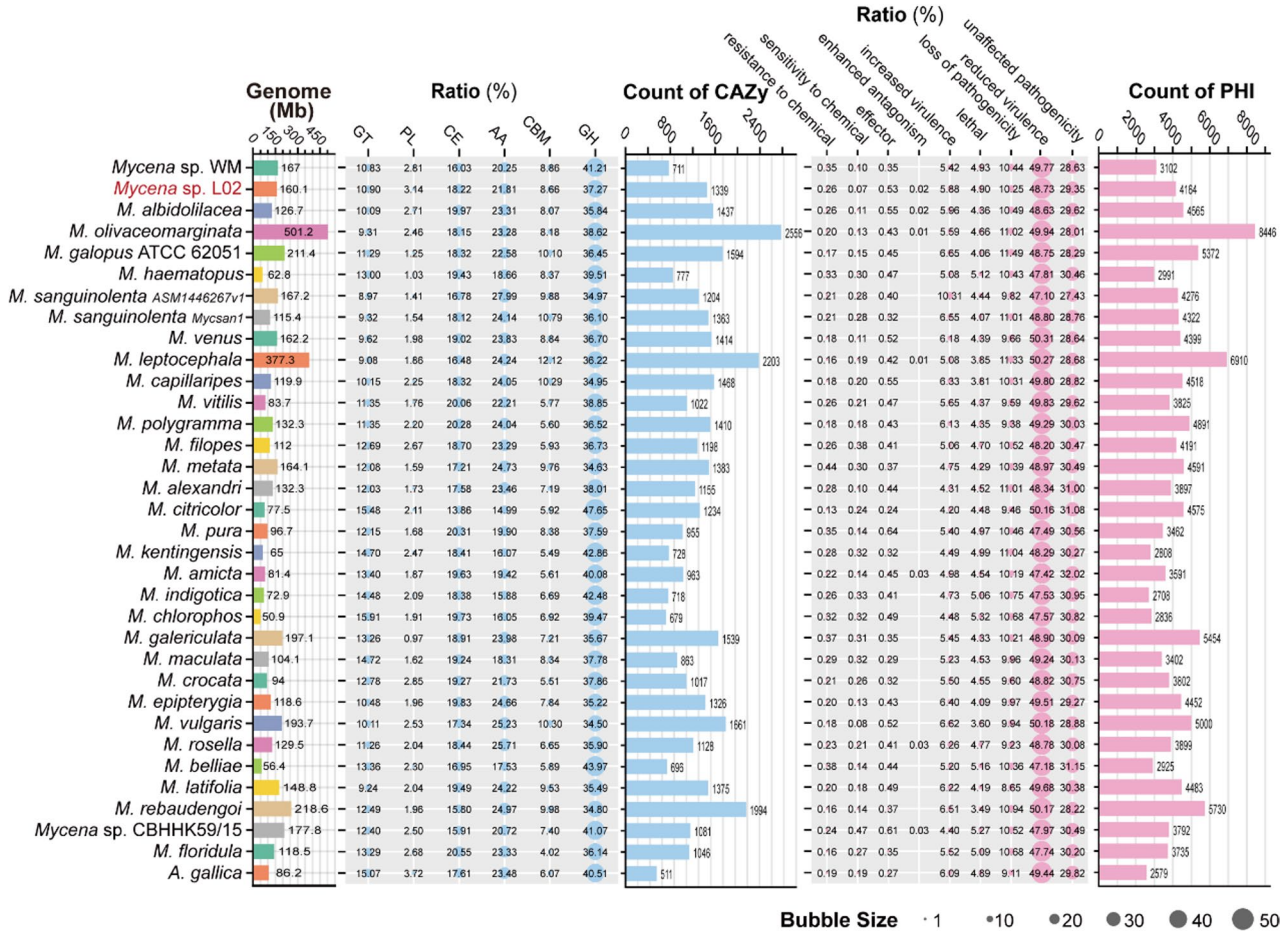

Fig. 4 Characteristics of CAZymes and PHI genes in 33 *Mycena* strains. Further details are provided in Table S5

extensive AA family in L02 may help its hyphae penetrate the lignified seed coat, facilitating a successful symbiotic relationship. However, the CAZymes profiles differ between the two germinating fungi, L02 and WM. Therefore, it cannot be conclusively determined whether CAZymes serve as a decisive factor in promoting the germination of *G. elata* seeds. A definitive conclusion would require further analysis based on a broader collection of genomic characteristics from diverse germinating fungi.

Weak virulence is associated with improved fungal adaptation to *G. elata* [1]. Once symbiosis is established, *G. elata* begins to grow and exhibit increased biological activity, while fungal activity becomes restricted. Highly virulent fungi may over-infect *G. elata*, inhibiting its growth or even leading to death, making them unsuitable for cultivation [1]. This has been demonstrated by comparative transcriptomic analyses of WM grown symbiotically with *G. elata* seeds and in pure culture [17]. Genome analysis of WM revealed that over half of its PHI genes are associated with reduced virulence and loss of pathogenicity, facilitating successful symbiosis with *G. elata* seeds [17]. L02 exhibits similar characteristics, with 3,772 genes annotated in the PHI database, of which 58.98% are related to reduced virulence and loss of pathogenicity.

Our comparative genomic analysis sought to identify genomic features of germinating fungi by focusing on CAZyme and PHI gene profiles. However, we found that many CAZymes, as well as genes associated with reduced virulence and loss of pathogenicity, are common across the 33 examined *Mycena* genomes. Consequently, these elements do not appear to be distinctive hallmarks of the germinating fungi when compared to their saprotrophic relatives, and their definitive role in establishing symbiosis with *G. elata* seeds remains unclear. We must also consider a key limitation in our dataset: the genome assembly for the WM strain is notably incomplete, as evidenced by its low BUSCO score (46%). This incompleteness likely led to an underestimation of its CAZyme and PHI gene counts. This provides a plausible explanation for the considerable discrepancy in gene numbers between the two germination fungi, L02 and WM, despite their shared ecological function. This assembly issue underscores a limitation in our current approach

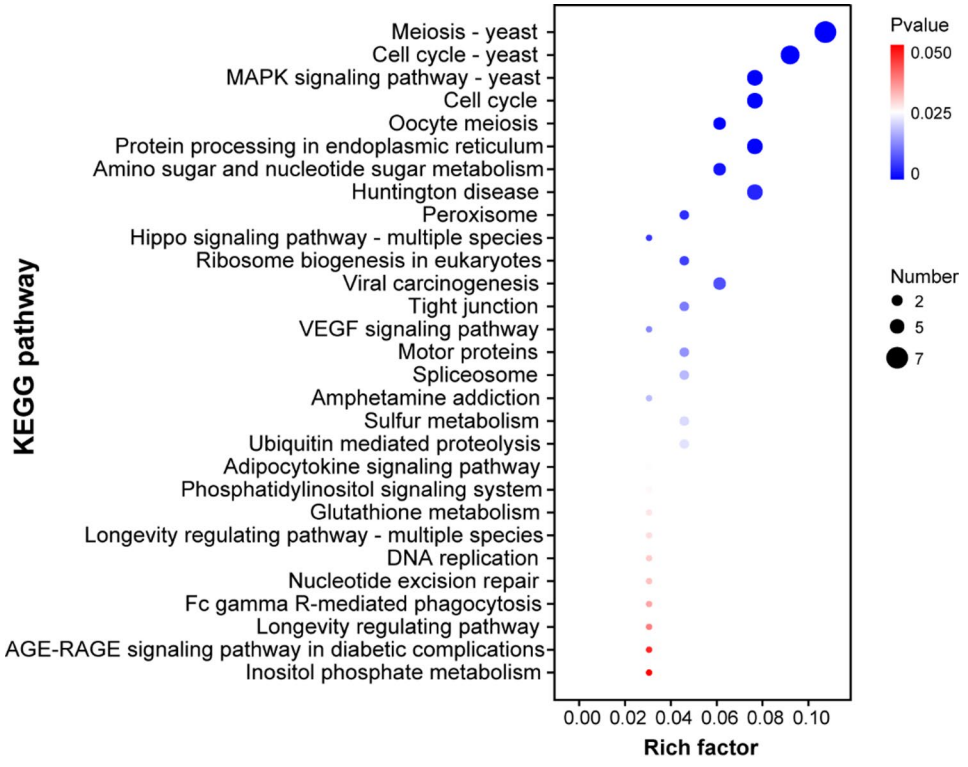

Fig. 5 KEGG enrichment analysis of unique gene families in germinating Fungi

and highlights that the true genetic repertoire of the WM strain may be underrepresented in this comparative analysis.

As symbiotic fungi, the pathways enriched in the unique gene families of the germinating fungi may contribute to the germination of *G. elata* seeds. In natural environments, the seeds of *G. elata* are dispersed, and the surrounding germinating fungi need to grow and proliferate rapidly to establish contact with the seeds. Pathways such as Cell Cycle, Cell cycle - yeast, DNA Replication, and Ribosome biogenesis in eukaryotes may support rapid cell division, protein synthesis, and DNA replication in the fungus, providing the foundation for vigorous hyphal growth and subsequent invasion of the seeds. There is increasing evidence that ROS act as regulatory participants in seed germination, though maintaining their homeostasis during seed imbibition is critical [73, 74]. The Glutathione metabolism of the germinating fungi may not only help maintain its own redox balance but could also assist *G. elata* seeds in modulating ROS levels during the early stages of germination. Sulfur is an essential element for all living organisms. Sulfur deficiency affects plant growth, disease resistance, and performance, significantly influencing the nutritional quality of crops [75]. The Sulfur metabolism of the germinating fungi may supply sulfur to support *G. elata* seed germination. Phosphatidylinositol and inositol phosphates play important roles in plant stress responses and development [76–78]. The Phosphatidylinositol signaling system and Inositol phosphate metabolism in the germinating fungi may help *G. elata* seeds coordinate stress adaptation and developmental processes.

This study has certain limitations. Primarily, the conclusions are based on a limited number of germination fungus genomes and should therefore be considered preliminary. The availability of only a few genomic samples constrains the generalizability of our findings. Furthermore, the potential incompleteness of the published WM genome assembly may affect comparative analyses. Despite these limitations, the high-quality genome of strain L02 reported here provides a valuable and reliable resource for future research into the mechanisms underlying seed germination. Future work involving a larger-scale comparative genomic analysis of a greater number of germinating and non-germinating *Mycena* strains will be essential to more definitively elucidate the genomic basis of this symbiotic relationship.

Conclusion

In this study, we sequenced and analyzed the genome of *Mycena* sp. L02, a fungal strain promoting *G. elata* seed germination. The 160.06 Mb genome harbors 36,246 protein-coding genes and 31.90% repetitive sequences. Functional annotation revealed 1,339 CAZymes genes in L02, which may facilitate nutrient acquisition to support its symbiotic relationship with *G. elata* seeds. Additionally,

3,772 genes in L02 were associated with pathogen–host interactions, and 58.98% of them were annotated with loss of pathogenicity, and reduced virulence, while only 4.90% were associated with lethal effects. This suggests that L02 is a low-virulence strain suitable for symbiosis with *G. elata*. Comparative genomic analysis between germinating and non-germinating *Mycena* strains revealed that the CAZymes and PHI gene characteristics of the germinating fungi align with general traits common across the *Mycena* genus, with no distinctive genomic features identified. We also found that, as symbiotic fungi, the pathways enriched in the unique gene families of the germinating fungi—such as glutathione metabolism, sulfur metabolism, phosphatidylinositol signaling system, and inositol phosphate metabolism, etc.—that may contribute to the germination of *G. elata* seeds. In conclusion, this study enhances our understanding of the diversity within the *Mycena* genus and provides a valuable resource for exploring the molecular mechanisms underlying the symbiosis between *Mycena* and *G. elata*.

Abbreviations

WM	*Mycena* sp. WM
L02	*Mycena* sp. L02
PDA	Potato dextrose agar
CTAB	Cetyltrimethylammonium bromide
ONT	Oxford Nanopore Technologies platforms
BUSCO	Benchmarking Universal Single-Copy Orthologs
tRNA	Transfer RNA
rRNA	ribosomal RNA
CAZyme	Carbohydrate-Active Enzyme
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
PHI	Pathogen–Host Interactions
ITS	Internal transcribed spacer
ncRNAs	non-coding RNAs
BP	Biological process
CC	Cellular component
MF	Molecular function
RNase H	Ribonuclease H
GTs	Glycosyltransferases
PLs	Polysaccharide lyases
CEs	Carbohydrate esterases
CBMs	Carbohydrate-binding modules
GHs	Glycoside hydrolases
AAs	Auxiliary activity enzymes

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

JJL analyzed the data, and prepared the manuscript. BS participated in partial data analysis. JYM and SBL provided the ***Mycena*** sp. L02. QLZ and FW guided data analysis. YCL and LBL conducted critical review of the manuscript. All listed authors have made substantial, direct, and intellectual contributions to this study, and have approved its publication.

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Data availability

The genome sequence data from this study are available under NCBI BioProject accession PRJNA1262138, with the corresponding BioSample accession SAMN48449941 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1262138).

Declarations

Ethics approval and consent to participate

We collected *Mycena* sp. L02 from Yunnan Senhao Fungi Industry Co., Ltd. We collected Mycena sp. L02 in accordance with relevant institutional, national and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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