Genome-Wide Characterization and Comparative Analysis of MYB Transcription Factors in Ganoderma Species

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ABSTRACT Numerous studies in plants have shown the vital roles of MYB transcription factors in signal transduction, developmental regulation, biotic/abiotic stress responses and secondary metabolism regulation. However, less is known about the functions of MYBs in Ganoderma. In this study, five medicinal macrofungi of genus Ganoderma were subjected to a genome-wide comparative analysis of MYB genes. A total of 75 MYB genes were identified and classified into four types: 1R-MYBs (52), 2R-MYBs (19), 3R-MYBs (2) and 4R-MYBs (2). Gene structure analysis revealed varying exon numbers (3-14) and intron lengths (7-1058 bp), and noncanonical GC-AG introns were detected in G. lucidum and G. sinense. In a phylogenetic analysis, 69 out of 75 MYB genes were clustered into 15 subgroups, and both single-copy orthologous genes and duplicated genes were identified. The promoters of the MYB genes harbored multiple cis-elements, and specific genes were co-expressed with the G. lucidum MYB genes, indicating the potential roles of these MYB genes in stress response, development and metabolism. This comprehensive and systematic study of MYB family members provides a reference and solid foundation for further functional analysis of MYB genes in Ganoderma species.

KEYWORDS Ganoderma MYB gene family genome-wide analysis comparative analysis

Ganoderma species, which are widely distributed around the world, are important macrofungi to Ganodermataceae (Polyporales, Basidiomycota). The most common pharmacological activities of Ganoderma species are antitumour, antioxidant, and antimicrobial activities, and at least 19 Ganoderma species have been studied for their medicinal functions in China to date (Dai et al. 2009; Yi et al. 2015; Basnet et al. 2017; Wu et al. 2019a). Polysaccharides (Cór et al. 2018; Ma et al. 2018) and triterpenes (Liang et al. 2019; Wahba et al. 2019) with vital medicinal functions have drawn the attention of researchers. In addition, great progress has been made in the classification/identification (Liao et al. 2015), genetic diversity (Midot et al. 2019), and fruiting body development (Sudheer et al. 2018) of Ganoderma species.

The MYB (myeloblastosis) transcription factor family is one of the largest transcription factor families and is widely distributed in eukaryotic organisms (Riechmann et al. 2000). The MYB domain contains 1 to 4 imperfect repeats that consist of approximately 50 amino acids (aa), and the repeat is characterized by conserved and regularly spaced tryptophans, which form a hydrophobic core to maintain the helix-turn-helix (HTH) secondary structure of the repeat (Sakura et al. 1989; Ogata et al. 1994). Accordingly, the MYB gene family is divided into four subfamilies, including 1R-MYB, 2R-MYB, 3R-MYB, and 4R-MYB (Ogata et al. 1994). Since the first identification of MYB genes from avian myeloblastosis virus (Klempnauer et al. 1982) and from plants (Paz-Ares et al. 1987), the function of MYB genes has been widely studied. Abundant evidence in plants has confirmed the diverse roles of MYB genes in signal...
transduction, developmental regulation, biotic and abiotic stress responses, disease resistance, and secondary metabolism regulation (An et al. 2019; Lee & Seo 2019; Qing et al. 2019; Wu et al. 2019b; He et al. 2020; Ohno et al. 2020). Similarly, a few studies have shown that MYB genes participate in stress response and developmental regulation in fungi (Valsecchi et al. 2017; Wang et al. 2018; Li et al. 2019).

Taking advantage of the numerous available genomes, comprehensive information regarding number, classification, gene structure, evolution, and expression pattern of MYB genes has been widely analyzed, especially in plants and fungi, such as Arabidopsis thaliana (Chen et al. 2006), Gossypium hirsutum (Salih et al. 2016), Phyllostachys edulis (Yang et al. 2019), Pleurotus ostreatus (Wang et al. 2018), and Ophiocordyceps sinensis (Li et al. 2019). Several genomes of Ganoderma species are currently available (Chen et al. 2012; Zhu et al. 2015); nevertheless, little is known about the MYB genes of Ganoderma species. Given the critical roles of MYB genes in eukaryotic organisms, exploring MYB genes among Ganoderma species will contribute to a better understanding of the specific structural and functional characteristics of macrofungi.

In this study, the MYB gene family members in Ganoderma species, including G. australe, G. boninense, G. lucidum, G. sinense and G. tsugae, were extensively characterized. Gene numbers, sequence features, gene structures, genetic relationships and promoter cis-elements were analyzed and compared among these five species. An additional co-expression analysis of MYB genes was performed in G. lucidum. These investigations provide fundamental information about the Ganoderma MYB gene family and will facilitate gene function elucidation and molecular assisted breeding of Ganoderma.

**MATERIALS AND METHODS**

**Genome sequences and gene sets acquisition**

The genome sequences of five Ganoderma species downloaded from NCBI BioProjects were used in this study: G. australe (PRJNA476322), G. boninense (PRJNA421251), G. lucidum (PRJNA71455), G. sinense (PRJNA42807) and G. tsugae (PRJNA445345). Gene annotation results of G. sinense were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/assembly/GCA_002760635.1). For other genomes, gene models were predicted using Maker pipeline (Campbell et al. 2014) with G. sinense CDS sequences as EST evidence and UniProt protein sequences as protein evidence.

**Identification and classification of MYB genes in Ganoderma species**

All the annotated proteins in the five Ganoderma genomes were searched against to PFAM database (Pfam 32.0) with PfamScan (evalue \( \leq 1e^{-5} \)) (http://www.ebi.ac.uk/Tools/pf/pfamscan). Genes with hits to PFAM ID PF00449.30, PF08914.10, PF11831.7, PF12776.6, PF13921.5, PF15963.4 and PF16282.4, and with out PF04433.16 and PF16495.4 (SWIRM/SWIRM-associated) were considered as candidate MYB genes. Then, MYB genes were viewed and corrected on Apollo (Dunn et al. 2019) browser: 1) each of the candidate MYB genes of G. lucidum, G. sinense and G. boninense was manually corrected based on the alignments between genome sequences and corresponding transcriptionome data (PRJNA269646, PRJNA514399, PRJNA71455, PRJNA374969, PRJNA574544, PRJNA42807) with HISAT2 (Pertea et al. 2016); 2) the candidate MYB genes of G. australe and G. tsugae were corrected based on the alignments with their homologous MYB genes in the other three species with BLASTP (protein sequences) and BLASTN (coding sequences) (Camacho et al. 2009). All the above corrected sequences were searched against the five Ganoderma genomes and gene sets using both BLASTN and TBLASTN (Camacho et al. 2009) (query length coverage \( \geq 60\% \), sequence identity \( \geq 60\% \)) to avoid missing false-negative identification. Finally, all the MYB genes were reconfirmed by PfamScan and the MYB genes were classified into 1R-, 2R-, 3R- and 4R-MYB groups. A synten analysis of the MYB genes was conducted between the two most complete genomes, G. lucidum and G. sinense, using MCSCAN (Tang et al. 2008).

**Sequence information, gene structure, and phylogenetic analysis of MYB genes**

The theoretical isoelectric point (pI) and molecular weight (MW) were computed using the online Compute pi/Mw tool (http://web.expasy.org/compute_pi/). The subcellular localization of each MYB protein was predicted by the online WoLF PSORT tool (https://wolfpsort.hgc.jp/). The gene structures of the MYB genes were investigated using TBtools based on the genomic features defined in the gff file (Chen et al. 2018). The distribution of intron length was drawn by ggplot2 (Wickham 2016) in R.

A multiple sequence alignment of the Ganoderma MYB genes was performed using ClustaOmega based on full-length protein sequences (Sievers et al. 2011), and a maximal likelihood (ML) phylogenetic tree was constructed using RAxML (Stamatakis 2014) with 1000 bootstrap replicates and plotted by Interactive Tree Of Life (iTOl) (Lefunic & Bork 2019). In addition, an ML tree containing an additional 124 MYB protein sequences (including fungi, plants and animals) was built using the same method (Supplementary Table S1). The orthologous relationships of MYB genes in Ganoderma were determined using OrthoMCL v2.0.9 (Li et al. 2003). The single copy orthologous MYB genes were defined as follows: clustered in phylogenetic tree and in ortho-groups from OrthoMCL, and only one gene in each species.

**Analysis of promoter regions of Ganoderma species**

The promoter regions of the MYB genes, which were defined as the 2000 bp upstream of the transcript start, were extracted and used in the following analysis. GIMMY14 was not included in this analysis, as this gene starts at the beginning of the genome sequence. The cis-elements in the promoter regions were detected using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). A hierarchical clustering analysis of cis-elements in Ganoderma MYB genes was performed using the heatmap package (https://cran.r-project.org/web/packages/heatmap/) in R.

**Co-expression of MYB genes in G. lucidum**

Sixteen RNA-seq datasets of G. lucidum were downloaded from NCBI: SRR364264, SRR10199514, SRR10199515, SRR10199516, SRR10199517, SRR10199518, SRR10199523, SRR10199520, SRR10199519, SRR10199522, SRR364265, SRR364266, SRR5261648, SRR5261647, SRR5261649. A co-expression network was built for the MYB genes of G. lucidum (GIMYBs) by the Weighted Gene Co-expression Network Analysis (WGCNA) package (Langfelder & Horvath 2008) in R. The genes in modules containing GIMYBs were extracted and subjected to GO classification using egnog-mapper (Huerta-Cepas et al. 2019).

**Data availability**

The necessary information of public data used in this study are present within the article. Data S1 shows sequences of 75 Ganoderma
MYB genes. Data S2 shows GO classifications of genes related with GIMYBs. Table S1 shows accession numbers of 124 MYB proteins of other organisms used in the phylogenetic study. Table S2 shows comprehensive information of MYB genes identified in five Ganoderma species. Table S3 shows orthologous groups identified by OrthoMCL. Table S4 shows distribution of intron lengths. Table S5 shows length of MYB domains. Table S6 shows PfamScan results of 75 MYB genes in Ganoderma species. Figure S1 shows protein sequence alignment between GaMYB10 and GaMYB12. Figure S2 shows protein sequence alignment between GaMYB05 and GaMYB11. Figure S3 shows protein sequence alignment among GtMYB07, GtMYB10, GtMYB11 and GtMYB09. Figure S4 shows phylogenetic relationships of MYB genes among Ganoderma species and other organisms. Figure S5 shows sequence logos of MYB domains in different subgroups. Figure S6 shows co-expression modules in G. lucidum. Supplemental material available at figshare: https://doi.org/10.25387/g3.12286277.

RESULTS

Identification, classification and sequence information of MYB genes in Ganoderma species

Overall, 75 MYB genes were identified in G. australe (GaMYB01-12), G. boninense (GbMYB01-23), G. lucidum (GIMYB01-12), G. sinense (GsMYB01-13) and G. tsugae (GtMYB01-15) (Supplementary Data S1). G. boninense clearly had more MYB genes than the other four species; this may be explained by its genome sequencing method, which used diploid material [https://www.ncbi.nlm.nih.gov/biosample?LinkName=bioproject_biosample_all&from_uid=421251]. In G. lucidum, the 12 MYB genes were situated on seven chromosomes, with chromosome 3 containing the maximal number of three. Syntenic analysis showed that 12 of the 13 GsMYBs distributed on the 9 scaffolds of G. sinense had a strong linear relationship with GIMYB01-12 (Figure 1A). According to their numbers of repeat units, the 75 Ganoderma MYB genes were classified into four types: 1R-MYBs (52), 2R-MYBs (19), 3R-MYBs (2) and 4R-MYBs (2) (Supplementary Table S2). 1R-MYBs and 2R-MYBs existed in all five species, while 3R-MYBs and 4R-MYBs were present in only G. australe (GaMYB01) and G. lucidum (GIMYB07), and 4R-MYBs were found in only G. sinense (GsMYB01) and G. tsugae (GtMYB13) (Figure 1B).

The lengths of the proteins encoded by these 75 MYB genes ranged from 192 (GbMYB20) to 1597 aa (GaMYB07) with an average of 705.24 aa. The calculated pI ranged from 4.59 (GbMYB20) to 11.01 (GaMYB12) with an average of 7.27, and the MW ranged from 21.42 (GbMYB20) to 171.92 (GbMYB16) kDa with an average of 77.00 kD. The GC content of the coding sequences ranged from 50.59 to 67.26%, with an average of 59.17%. A total of 56 MYB genes were localized to the nucleus, 11 to the cytosol and 19 to the mitochondria (Supplementary Table S2).

Phylogenetic analysis of Ganoderma MYB genes

To determine the phylogenetic and orthologous relationships among the MYB proteins, an ML phylogenetic tree was constructed using the full-length MYB protein sequences of the Ganoderma species, and orthologous groups were determined by OrthoMCL analysis (Supplementary Table S3). Sixty-nine MYB genes were divided into 15 subgroups (designated as subgroup 1 to subgroup 15), while the other 6 MYB genes (GbMYB11, GbMYB14, GsMYB11, GsMYB04, GIMYB12 and GtMYB14) were designated as outer clade (Figure 2A). Different types of MYB genes tended to be classified into separate subgroups. Most subgroups (subgroups 1, 3, 4, 5, 6, 7, 10, 11, 12 and 14) contained 1R-MYBs only, subgroups 8 and 15 contained 2R-MYBs only, and subgroup 9 contained 2R-MYBs as well as all the 3R-MYBs and 4R-MYBs.

The MYB genes in subgroups 1, 4, 9, 10 and 15 were single-copy orthologous genes, because each of the MYB genes in a given subgroup had only one copy in each species. These MYB genes represented conserved sequences in Ganoderma, and may have originated from a common ancestor. On the other hand, intraspecific duplication events were found in G. australe and G. tsugae. In G. australe, the whole protein sequences of GaMYB10 and GaMYB12 showed an identity of 64.5%, and both the N-terminals and C-terminals had identical protein sequence patterns (Supplementary Figure S1); GaMYB05 and GaMYB11 showed a higher identity of 88.0%, and the difference was mainly in the extended N-terminus of 48 aa in GaMYB11 (Supplementary Figure S2). In G. tsugae, GtMYB10, GtMYB09, GtMYB11 and GtMYB07 showed high mutual similarity and were grouped into

Figure 1 Synteny analysis of MYB genes in G. lucidum and G. sinense (A) and distribution of MYB genes in five Ganoderma species (B). Numbers 1-20 represent the 20 largest scaffolds of G. sinense, and I-XIII represent the 13 chromosomes of G. lucidum. The gray lines in the background represent collinear blocks between G. lucidum and G. sinense, while the red lines represent syntenic MYB gene pairs. Ga, G. australe; Gb, G. boninense; Gl, G. lucidum; Gs, G. sinense; Gt, G. tsugae.
subgroup 7, accompanied by a homolog, GlMYB01 (Figure 2; Supplementary Figure S3).

In the phylogenetic analysis including Ganoderma and other organisms, the 75 Ganoderma MYB genes and 124 MYB genes from the other organisms formed multiple clades (Supplementary Table S1; Supplementary Figure S4). Clades consisting of MYB genes from different taxa may have evolved from the same ancestor and may have similar biological functions. Clades unique to Ganoderma also existed.

Gene structure and MYB domain of Ganoderma MYB genes

A rich diversity of MYB gene structure was observed in Ganoderma. Varying numbers of exons, from 3 to 14, and introns, from 2 to 13, were detected in the 75 MYB genes; intronless MYB genes were not found. In total, 489 exons and 414 introns were identified, and all the MYB genes started with 0-phase-introns. On average, the 1R-MYBs contained 5.48 introns, the 2R-MYBs contained 4.96 introns, the 3R-MYBs contained 9 introns, and the 4R-MYBs contained 8 introns. Two MYB genes had intron lengths shorter than 10 bp (GaMYB02, 7 bp; GaMYB01, 8 bp), while three had intron lengths of approximately 1000 bp (GsMYB04, 1058 bp; GbMYB04, 921 bp; GMYB111, 944 bp). The average intron length was 74 bp across all five species, 71 bp in G. australis, 75 bp in G. boninense, 76 bp in G. lucidum, 81 bp in G. sinense, and 65 bp in G. tsugae. In general, most introns (90%) were 48-112 bp in length, and G. tsugae had most narrowest length distribution pattern, with all intron lengths between 46 and 280 bp (Figure 3; Supplementary Table S4).

Five GC-AG introns (noncanonical introns) in G. lucidum and six in G. sinense were detected, while most introns were classical GT-AG introns (Figure 2B). In G. lucidum, GlMYB05 (1R-MYB), GlMYB06 (1R-MYB), GlMYB07 (3R-MYB), GlMYB08 (1R-MYB) and GlMYB09 (1R-MYB) each contained a single GC-AG intron (Figure 2B). In G. sinense, GsMYB12 (1R-MYB) contained two GC-AG introns; GsMYB01 (4R-MYB), GsMYB02 (1R-MYB), GsMYB03 (1R-MYB) and GsMYB13 (2R-MYB) each contained one GC-AG intron (Figure 2B). The four gene pairs GlMYB05/GsMYB03, GlMYB07/GsMYB01, GlMYB08/GsMYB12 and GIMYB09/GsMYB13 each had identical gene structures (including exon number and intron phase) and the same GC-AG intron location, suggesting that the MYB genes containing GC-AG introns were conserved in G. lucidum and G. sinense.

In total, 104 MYB domains were identified in the 75 MYB genes, and most MYB domains were distributed in or near the N-terminus (Figure 2B). The MYB domains had a broad range of lengths, from 37 to 226 aa, with an average of 64.3 (Supplementary Table S5). The MYB genes in subgroups 12, 13 and 14 had clearly shorter MYB domains than those of other subgroups. Most MYB domains spanned the intron regions, indicating that they might be affected by alternative splicing. The regularly spaced tryptophans of the MYB domain were conserved within each subgroup yet diverse among subgroups (Supplementary Figure S5). In addition, each of the four MYB genes in subgroup 12 contained an HSA domain and GbMYB15 contained a FAD_binding and a FAD-oxidase_C domain (Supplementary Table S6).

MYB genes in the same subgroup shared similar gene structures, including gene length, intron phase, number/location of exons and number/location/length of MYB domains. However, MYB genes in

Figure 2 Phylogenetic relationships and gene structures of Ganoderma MYB genes. (A) The ML phylogenetic tree was based on full-length protein sequences with 1000 bootstrap resamplings; the dots on the nodes represent bootstrap values; Ga, G. australis; Gb, G. boninense; Gl, G. lucidum; Gs, G. sinense; Gt, G. tsugae; and the numbers behind the MYB genes represent the different subgroups. (B) Red rectangles and black lines represent exons and introns, respectively; the number above the black line represent the intron phase; the yellow rectangles represent MYB domains; and the black rectangles represent GC-AG introns.
different subgroups showed significant differences. Subgroups 2, 12 and 13 contained obviously longer genes than the other groups, while subgroup 8 had an obviously longer MYB domain. These results show that the MYB gene structures were consistent with the phylogenetic topology.

Promoter analysis of Ganoderma MYB genes
The promoter regions of the Ganoderma MYB genes had GC contents of 34.35–63.4%, with an average of 55.03% (Supplementary Table S2). The CTCC motif, CAAT box, G box and TATA box were the most frequently occurring cis-elements in the promoter regions. The CTCC motif and CAAT box occurred in all the promoters, with the CTCC motif having an average occurrence of 25.2 and the CAAT box having an average occurrence of 17.6 (Figure 4). Cis-elements related to light response (SP1, GATA motif, TCCC motif, GT1, and ATCT motif), hormone response (CGTCA motif, TGA, TCA element, and GARE motif) and developmental process (CAT box) (Skriver et al. 1991; Terzaghi & Cashmore 1995; Treger et al. 1998; Laloum et al. 2013; Zhang et al. 2016) existed in the promoters of some MYB genes, suggesting that these MYB genes may participate in multiple biological processes. Similar cis-element patterns were detected in the promoter regions of some duplicated gene pairs, such as GaMYB10/12 and GtMYB09/10 (Figure 4). However, different patterns were observed in other gene pairs, such as GaMYB05/11 and GtMYB07/11, implying a diversity of potential functions among the duplicated MYB genes. Notably, MYB binding sites were identified in the promoters of all the detected MYB genes except GtMYB07, indicating extensive cooperation among MYB genes.

Co-expression analysis of G. lucidum MYB genes
Based on the WGCNA analysis using 16 G. lucidum RNA-seq datasets from the NCBI database, 35 modules were identified, and 12 GIMYBs were clustered into 4 modules (Supplementary Figure S6). The genes that had the most similar expression patterns to the GIMYBs in the target module were classified into three main categories: biological process, cellular component and molecular function (Supplementary Data S2). The most enriched biological process (GO: 0006960) was metabolism (GO: 0019782). In the cellular component category (GO: 0043291), intracellular (GO: 0005775) was enriched.

DISCUSSION
A genome-wide comprehensive analysis of Ganoderma MYB genes revealed both similarities and differences among Ganoderma species. The similarities are mainly reflected in three aspects: (1) 1R-MYB and 1R-MYB2 types are general MYB types in Ganoderma species; (2) MYB orthologs that showed high similarity in the phylogenetic analysis might possess similar functions; and (3) CTCC motifs and CAAT boxes occur in all promoter regions with high frequency, indicating their conserved functions in the regulation of MYB genes. The differences are mainly reflected in four aspects: (1) the MYB number varies among different species, indicating gene gain and loss during speciation; (2) 2R-MYB exist exclusively in G. australe and G. lucidum, and 2R-MYB exist exclusively in G. sinense and G. tsugae; (3) GC-AG introns are observed in only G. lucidum and G. sinense; and (4) only some of the five species underwent MYB gene duplications. The divergence of MYB genes among species might contribute to species evolution and adaptability. Hence, we speculated that some MYB genes may be developed as markers for species identification.
The number of MYB gene family members varies greatly among species. The MYB gene number is dramatically expanded to 100-500 in plants (Chen et al. 2006; Salih et al. 2016; Yang et al. 2019; Li et al. 2020), with a reduced number of 4-5 in animals (Davidson et al. 2005) and a moderate number of 10-40 in fungi (Verma et al. 2016; Wang et al. 2018; Li et al. 2019). In this study, the Ganoderma species had 12 to 23 MYB genes, similar to other fungi. In addition, the dominant MYB type differs between fungi and plants. In plants, 2R-MYBs (R2R3) are the dominant MYB type, especially in P. edulis, which contains 96.47% 2R-MYBs (Yang et al. 2019). In addition, 2R-MYBs in plants tend to have conserved splicing patterns. For example, A. thaliana and rice have a 2R-MYB gene structure of three exons and two introns (Katiyar et al. 2012). However, in this study, the Ganoderma species had mostly 1R-MYBs, with no clearly conserved gene structure found. As the dominant 2R-MYBs play central roles in plant-specific processes (Lee & Seo 2019; He et al. 2020), it is likely that the dominant 1R-MYBs might play core roles in relevant processes in Ganoderma species.

Fungal introns are typically short, with mean intron lengths ranging from 69 bp in Cryptococcus neoformans to 256 bp in Saccharomyces cerevisiae (Kupfer et al. 2004). The average intron lengths in the whole gene sets of G. lucidum and G. sinense are 87 and 82 bp, respectively (Chen et al. 2012; Zhu et al. 2015). In this study, the average intron length of the Ganoderma MYB genes was 74 bp, consistent with the typical short introns of fungal species. However, three long introns with lengths of approximately 1000 bp were also identified. Studies have shown that gene expression can be impacted by intron length due to the increased time required to transcribe genes containing long introns (Swinburne et al. 2008). Intron length also determines the effect of the exon junction complex (Ashton-Beaucage et al. 2010), and genes containing long introns tend to have alternative splicing (Roy et al. 2008). However, the possible functions of the long introns in the Ganoderma MYB genes need to be elucidated further.

In total, 10 of the 75 MYB genes were found to contain GC-AG introns, and these MYB genes may recruit different splicing mechanisms during mRNA maturation. Studies have shown that 98% or more of introns are canonically spliced (GT-AG) in fungi, and GC-AG introns usually have an occurrence of approximately 1% (Kupfer et al. 2004). In G. lucidum and G. sinense, 41.7% and 38.5% of the MYB genes contained GC-AG introns, indicating a high proportion of noncanonical introns in Ganoderma MYB genes. More sophisticated splicing mechanisms are active in G. lucidum and G. sinense, and the significance of these GC-AG introns needs further study. Moreover, this reminds us that the traditional gene prediction method could be unconvinced without consideration of noncanonical splicing.

The analysis of the cis-elements in the promoter regions of Ganoderma MYB genes and the co-expressed target genes of the GMYBs help us understand the potential functions of MYB genes in Ganoderma. Similar to the roles MYB genes play in plants, Ganoderma MYB genes are widely involved in various biological processes, including stress response, development, and metabolism. Given their important regulatory roles, MYB genes should be included in future studies of macrofungal biological processes.

CONCLUSION
In this study, a total of 75 MYB genes were identified in five Ganoderma species, of which 69 were clustered into 15 subgroups. Both single-copy orthologous genes and duplicated genes were identified in subgroups. Varying sequence characteristics were observed among subgroups. Multiple regulatory cis-elements existed in the promoters of Ganoderma MYB genes, and some genes related to stress response, development and metabolism co-expressed with GMYBs. Our results suggest that MYB genes participate in multiple biological processes in Ganoderma. However, further function validation is required in the future. Given the increasing interest in Ganoderma species, the fundamental information of MYB genes from this study will facilitate the biological studies in this genus.

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