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Low temperature-induced diapause mechanisms of *Coridius chinensis* via transcriptomics sequencing

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Abstract

Coridius chinensis, a valued species used in traditional Chinese medicine, undergo overwintering diapause. To clarify the mechanisms, we examined how low temperature treatments can affect diapause and cold tolerance in *C. chinensis*. Using transcriptome analysis, we obtained differently expressed 3225 mRNAs (DEGs) and 177 long non-coding RNAs (lncRNAs). The more down-regulated DEGs and lncRNAs in diapause groups (DP) than low-temperature (LT) stress groups when compared with wild control (WC) groups. From co-expression network, 6 DEGs with 14 corresponding lncRNAs were finally identified and positively correlated to low temperature variation. Among them, 3 DEGs referred insulin signaling pathway as GAD and tdcB were up-regulated trends in WC-LT-DP, while JAK2 was decreased after low temperature stress but increased at diapause stage. The qPCR test of GAD and JAK2 were obtained the similar results as transcriptome. Additionally, the genes annotated in the juvenile hormone synthesis pathway were almost at down-expression in DP when compared with LT groups, which was consistent with changes in juvenile hormone contents. Our results indicated the differences between cold stress and diapause in *C. chinensis* were closely related with the insulin metabolism and juvenile hormone synthesis, as well as diversity regulation in DEGs mean the diapause occurrence refers various fields changing in cold stress.

Key words: RNA-seq; diapause; lncRNA-mRNA co-expression; *Coridius chinensis*; insulin pathway

Introduction

Coridius chinensis, also known as *Aspongopus chinensis* Dallas, is an endemic insect of the order Hemiptera from China. It is used as Traditional Chinese medicine (TCM) for the treatment of pain, erectile dysfunction, and other diseases [1]. It has been recorded in the books “Compendium of Materia Medica” and “Chinese Pharmacopoeia” for the treatment of impotence and enuresis caused by deficiencies within the spleen and kidney [2,3]. Studies in modern pharmacology have shown that *C. chinensis* insects have a variety of nutritional or otherwise active ingredients, including fatty acids, proteins, nucleosides, and dopamine [4,5]. These ingredients have a variety of pharmacological uses, which include the improvement of reproductive health, antibacterial properties, and tumor prevention [1,6,7]. The asponguanine A separated from *C. chinensis* predominantly induced the proliferation of neural stem cells [8] and anti-tumor effect by inducing apoptosis and cell cycle blockage [3,5,9]. Though useful in medicine, the annual production of wild *C. chinensis* is insufficient for pharmacological purposes due to the limited reproduction capacity caused by diapause [10]. Elucidating the diapause mechanisms of *C. chinensis* is an important step toward the improvement of reproduction techniques of *C. chinensis*.

Diapause is a biologically programmed deep resting phase that is widespread in small ectotherms, such as insects. It can be induced by photoperiod [11], temperature [12], or pollution [13] and is characterized by a cessation of reproductive and ontogenetic development, depressed metabolic rate, and increased stress tolerance [14,15]. There are multiple stages of diapause, including induction, preparation, initiation, maintenance, termination, and late developmental stages, which are affected by physiological and biochemical regulation [16,17]. Previous studies have asserted that the regulation of storage and membrane lipid profiles is affected by diapause [14], including a shift in chemical compositions (cuticular hydrocarbon) in *Drosophila montana* [18], and changes in sugar and polyol content in *Ostrinia nubilalis* [19]. This demonstrates that the strategies of insects in diapause are varied and complex.

Recently, RNA-seq technology has provided valuable insight into gene expression regulation mechanisms by demonstrating a broader range of characteristics such as

alternative splice forms and novel transcripts [20]. Previous studies of diapause using RNA-seq were completed using *Poecilocoris lewisi* [21], *Megachile rotundata* [22], and striped stem-borer *Chilo suppressalis* [23]. Long non-coding RNAs (lncRNAs) possess a wide range of functional activities, including chromatin remodeling, transcriptional control, and post-transcriptional processing [24,25,26.] Previous studies have documented lncRNA's role in insect development, including within the honey bee [27], and *Plutella xylostella* [28]. However, there is still little knowledge about the lncRNA and mRNA of *C. chinensis* with relation to diapause. In constructing the transcriptome and screening the key lncRNA and mRNA affecting diapause, we can discover important relationships that affect *C. chinensis* reproduction. In this study, we compared transcriptome profiles and constructed a co-expression network of DEGs and lncRNAs in diapause, using graduated cold stress in wild groups of *C. chinensis*. The juvenile III hormone with related gene expression in the juvenile III synthesis pathway was further analyzed. The purpose of this study was to elucidate the mechanism of diapause occurrence as a result of cold stress in order to facilitate *C. chinensis* production.

Materials and Methods

Animal collection and treatment

C. chinensis samples were collected between July and November of 2015, from Hexi Village, Gaoqiao Town, Huichuan District, Zunyi City, Guizhou Province, China (longitude: 27.72, latitude: 106.95, elevation: 1090 m). The samples of *C. chinensis* were divided into three groups: wild control (WC), low temperature (LT), and winter diapause groups (DP). Within each group, there were 3 sub-groups: adult males, adult females, and adult males and females combined. All collected samples of *C. chinensis* were bred in dark, ventilated insect cages with cobblestone bedding and 70% relative humidity. They were fed pumpkin seedlings. The study took place over 4 weeks. WC groups were kept at a constant environmental temperature of 26°C. LT groups were placed in an environment with gradually decreasing temperatures for 7 days at each temperature (26°C, 22°C, 15°C, and 11°C). DP groups were maintained at 10°C. The treatment temperatures simulated different life stages of *C. chinensis* in the wild. The

insects were rapidly frozen in liquid nitrogen following the 28 days of treatment for further RNA extraction.

Total RNA isolation, sequencing, and assembly

Total RNA was extracted from the three treatment groups of *C. chinensis* (WC, LT, DP). First, RNA was isolated using the TRNzol Reagent Kit (DP421, TIANGEN, Inc., China). The NanoDrop 2000 was further used to measure the RNA purity and concentration; agarose gel electrophoresis was also used to confirm the RNA quality. RNA passed the quality control was then randomly fragmented to serve as a template for first-strand cDNA synthesis using random primers. The second strand cDNA was then purified with QIAquick PCR purification kit (DP, Qiagen, Inc., USA) and enriched by PCR to construct the cDNA library. Finally, cDNA library sequencing was completed using the Illumina HiSeq 2000 platform of Shanghai Biotechnology Ltd. (Shanghai, China).

The adaptor sequences, low-quality reads (Q value ≤ 20), and ambiguous nucleotides (N percentage $\geq 10\%$) from the raw reads were filtered to obtain clean reads, which were used for genome assembly using Trinity 2.4.0. The quality of the assembly was evaluated using TransRate and BUSCO[29]. By performing pair-end joining and gap filling, contigs were assembled into transcripts and subsequently clustered to obtain unigenes.

DEGs screening and functional annotation

The expression level of unigenes in fragments per kilobases per million reads (FPKM) was calculated using Bowtie (V2.0). The differential gene expression analysis was performed using edgeR (V2.20.9). Gene expression differences (fold change) among the WC groups, LT groups, and DP groups were further calculated. Genes showing a false discovery rate (FDR value) < 0.05 with $|\log_2FC| > 1$ were defined as differentially expressed genes (DEGs). In addition, all assembled unigenes were annotated in Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) by Goatools, and significantly enriched terms were filtered by $P \leq 0.05$ in Fisher's exact test.

Construction of lncRNA-mRNA co-expression network

Potential lncRNAs were obtained by filtering the data for annotated transcripts, overlapped sequences, and transcripts with potential coding ability or specific length of open reading frame (ORF) (> 225 bp). The lncRNAs with significant differential expressions (DElncRNAs) between the WC groups, LT groups, and DP groups were calculated with edgeR.

The DEGs and DElncRNAs related to diapause were obtained from WC, LT, and DP groups using the WGCNA package (V1.61). The power value of the adjacency matrix parameter was selected when the squared value of the correlation coefficient reached 0.9. The average connection degree of the overall network node was equal to 1, which was in line with the characteristics of the scale-free small world network. Under the condition of this parameter, the coefficient of dissimilarity between genes was calculated, and the system clustering tree was obtained. According to the hybrid dynamic tree cutting standards, the minimum number of genes in each gene network and the pruning height were set. Each module that significantly correlated with the distinct state of samples was then analyzed, and a lncRNA-mRNA co-expression network was constructed based on the correlation between the expression level of the module and the DEGs and lncRNA contained in the module.

Quantitative real-time PCR (qRT-PCR) analysis

To verify the accuracy of transcriptome data, seven genes were selected for qRT-PCR verification. The 20 µL reaction system of qRT-PCR consisted of 1 µL of cDNA, 0.4 µL each of forward and reverse primers, 10 µL of 2× SYBR Green qPCR Master Mix (MCE, Beijing, China), and 8.8 µL of sterile water. The PCR reaction parameters were as follows: 95°C for 30 seconds; 35 cycles of 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 30 seconds. The melt curve was 1 cycle of 95°C for 15 seconds, 60°C for 60 seconds, and 95°C for 15 seconds. Three technical replicates of the qRT-PCR assay were used with three biological replicates. Gene expression was calculated via the $2^{-\Delta\Delta C_t}$ method. The primers used for qRT-PCR are listed in Table S1.

Juvenile III determination

The samples of treated groups were selected and transferred into electropolished pipes. After adding 4 ml of PBS solution, the body of *C. chinensis* was powdered and

measured into two 1.5 ml pipes. The supernatants were obtained by centrifugating at 5000 r/min at 4°C and used to test the contents of juvenile III with an ELISA kit (JM-0004102, JINGMEI, Inc, China). Following the protocol, using a blank well as zero, the absorbance was read at 450 nm. Finally, basing the linear regression equation of the standard curve on standard concentration and OD value, the sample concentrations of Juvenile III were obtained.

Statistical analysis

Data were analyzed and visualized using R (version 4.0.5). Adobe Illustrator CS6 software (V20219) was used for picture assembly. Significant differences were assessed using one-way ANOVA, as appropriate, followed by Duncan's test. A P-value of < 0.05 was identified as a statistically significant difference.

Results

Illumina sequencing

Through transcriptome sequencing, a total of 402,128,042 reads were obtained from the 3 groups of *C. chinensis* (Table s2). The GC ratio of DP groups was higher than other groups, with GC ratios for LT and WC groups ranging between 41.14-43.02%. After filtering the low-quality sequences, 385,548,643 high-quality clean reads were retained. Over 75% of clean reads in each sample were mapped to the hexapod reference genes, except WC_1, which was removed in the subsequent analysis.

Following clustering and redundancy removal, 319,101 transcript sequences were retained. Unigene lengths primarily ranged from 501-1000 bp, followed by 0-500 bp and 1001-1500 bp, respectively (Figure 1A). Homologous species of *C. chinensis* were predicted by sequence alignment using the NR database. Around 44.26% of sequences aligned with *Halyomorpha halys*, followed by *Lasius niger* (3.75%) and *Diaphorina citri* (3.13%) (Figure 1B). A total of 94,705 transcripts were annotated, of which 91,540 were annotated in NR, 47,593 in Swiss-Prot, 43,918 in Pfam, 6,682 in COG, 14,927 in GO, and 30,884 in KEGG databases (Figure 1C). The Pfam annotation indicated the greatest number of annotated protein family were Ank_3 and Ank (Figure 1D).

Comparative transcriptomics between low temperature stress and diapause

DEGs identified in each comparison group were illustrated using a Venn diagram (Figure 2A). In comparing the number of up- and down-regulated DEGs, we found that

more genes were significantly down-regulated after cold stress (**Figure 2B**). In comparison with LT groups, DP samples exhibited 790 down-regulated and 430 up-regulated DEGs, demonstrating that the gene expression response was more clustered in down-regulated genes.

GO and KEGG pathway enrichment analysis was performed in WC vs. LT, WC vs. DP, and LT vs. DP comparison groups. Our analysis showed that 27, 30, and 19 GO biological processes and 14, 7, and 13 KEGG signaling pathways were obtained, respectively (**Figure S1**). These DEGs were significantly associated with nucleic acid-protein covalent cross-linking, the oxidation-reduction process, and small molecule metabolic processes. KEGG analyses demonstrated the DEGs between LT vs. DP groups were significantly enriched in antigen processing, apoptosis, and lysosome pathways.

A total of 73,578 potential lncRNAs were identified, with 239 DElncRNAs obtained from comparison groups. 121, 87, and 31 DElncRNAs were obtained, respectively from WC vs. LT, WC vs. DP, and LT vs. DP comparison groups (**Figure 2C**). The Venn diagram of DElncRNA for each comparison group showed similar trends to that of DEGs, except the number of up-regulated DElncRNA in LT vs. DP groups was much higher (**Figure 2D**).

Construction and analysis of lncRNA-mRNA co-expression network

After removing redundant sequences, 177 DElncRNA sequences with 3,225 unique DEGs were used for co-expression network construction (**Figure 3**). Our results showed a total of 10 related modules except gray modules. A topological overlap matrix (TOM) graph was constructed by combining partitioned module information and gene tree graph results (**Figure 3C, D**). In a comparison of color modules, there was a higher overlap between genes of same color module. The purple and turquoise modules in this graph had a significant positive correlation with the low temperature induced characterization of sample. The turquoise module had 641 elements (including 21 lncRNA and 620 mRNA) and had much higher positive correlation (0.74) with the low temperature stress characterization than other modules. Therefore, the elements contained in this module were used to complete further analysis.

The co-expression network contained 229 nodes (20 lncRNAs and 209 DEGs) and 689 connected edges (**Figure S2**). A total of 9 DEGs with GO function annotations and 11 DEGs with KEGG signaling pathways were obtained. Moreover, lncRNA-DEGs-GO / KEGG signaling pathway was independently constructed based on the DEGs involvement in GO function and KEGG pathways, as well as the relevant co-expression lncRNAs. The network contained 6 DEGs that participate in the GO node with KEGG signaling pathway. The network also contained 14 lncRNAs which referred to 7 KEGG signal pathways, including the primary metabolic process, hydrolase activity GO node, immune system, nervous system, and amino acid metabolism (**Figure S3**). The following DEGs were clustered alone within hydrolase activity and primary metabolic processes: DN128286_c0_g1, DN135849_c2_g2, DN141355_c2_g1, and DN146935_c1_g1, annotated respectively in genes of JAK2 (Janus Kinase 2), tdcB (threonine dehydratase), glutamic acid decarboxylase (GAD), and DEF(defensin) , which referred with insulin pathway. Two additional DEGs (DN144125_c1_g3 and DN108165_c1_g2) were clustered alone in relation to hydrolase activity and primary metabolic processes. The expression levels of the 6 DEGs were clustered in two types, except DN128286_c0_g1, and appeared in the up-regulating trends in WC- LT (**Figure S4**). However, all the 6 DEGs were up-regulated in LT- DP.

Comparison of gene expression patterns between low temperature stress and diapause in juvenile hormone synthesis

The unigenes annotated in the juvenile synthesis pathway were screened, and subsequently compared for expression level. A total of 40 DEGs were identified, including those encoding the juvenile hormone esterase methyl, farnesoate epoxidase, and aldehyde dehydrogenase (Figure 4A). Overall, the unigenes showed down-expression in DP when compared with LT. 6 unigenes showed higher expression levels in DP than in WC. In addition, 5 unigenes (DN109327_c0_g1, DN115166_c7_g5, DN146573_c5_g1, DN134628_c3_g5, and DN138878_c4_g1) coding for juvenile hormone esterase, farnesol dehydrogenase and juvenile hormone epoxide hydrolase were found differentially expressed between the WC and DP groups, but not significantly expressed between the LT and DP groups (Table S3). The concentration

of juvenile III was shown to gradually decline in the WC, LT and DP groups. The JXC group was significantly different from the other groups (Figure 4B).

Validation of DEGs with qPCR

To validate the reliability of the transcriptome data, 6 DEGs were selected to verify their expression levels by qPCR. The *Actb* gene of *C. chinensis* was used as a reference control. All qPCR results correlated with the data obtained from RNA sequencing, showing similar trends in the up- or down-regulation of *C. chinensis*, thereby verifying the robustness of our transcriptome sequencing analysis (Figure 5).

Discussion

Insect diapause usually occurs after a certain period of cold at a specific temperature. Low temperatures are associated with the slow decline of metabolic rate to facilitate cold tolerance [30] but do not always result in diapause. The relationship between low temperature stress and diapause is important to assess the mechanisms behind diapause. *C. chinensis* is a valuable medical insect, exhibiting adult reproductive diapause during winter that begins in October or November every year depending on environmental temperature [11]. In this study, we compared the gene profile of *C. chinensis* under 3 temperature regimes: constant temperature, gradually reduced temperature, and diapause temperature. From the three comparison groups (WC vs. LT, WC vs. DP, and LT vs. DP), we identified 1145, 1673, and 1220 DEGs, respectively. LT and DP treatments showed a higher number of DEGs at down-regulated levels when compared with WC groups (Figure 1). The DEGs of the three comparison groups showed significant clustering in different biological processing and signal pathways (Figure S1). The DEGs in the LT vs. DP group also showed significant enrichment in the sulfur compound biosynthetic process and sulfur compound metabolic process. Sulfur compounds, such as GSH, GSSG, GSH-px, and GST, are important members of the antioxidant enzyme, which was related to diapause in silkworm [31] and European Corn Borer [32]. Therefore, sulfur compounds may play a role in diapause of *C. chinensis*.

Some hormones with related signal pathways were present with diapause,

including juvenile hormone (JH) and insulin signal pathway [33]. JH can control abnormal insect growth [34], maintain a high level of growth in the early years of insects, and decrease growth until adult morphology [35]. Insulin was an important component of the upstream regulation of JH. By regulating JH, the adult diapause of *Drosophila*, *Culex pipiens pallens* [36], and *Pieris brassicae* ceased [37]. The fork head transcription factor (FOX) is located downstream of the insulin signaling pathway and has an "antagonistic effect" with insulin. In the presence of insulin, FOXO is inhibited. When insulin was deficient, FOXO was activated and migrated into the nucleus, which played an important role in the accumulation of nutrients in diapause insects [38,39]. JAK2 is the upstream gene of FOXO, a downstream factor of the insulin signaling pathway, and was a protein in the immune-related signaling pathway JAK/STAT. Immunity also protects the insect from microbial infection during diapause [38].

In this paper, we found that 21 DEGs related to low temperature induced were involved in antigen processing and presentation, apoptosis, and autophagy pathways. Among them, unigene DN128286-c0-g1 screened and annotated JAK2, which was significantly related with diapause in *C. chinensis* by WGCNA analysis. Further expression analysis showed JAK2 was at down-regulated levels after low temperature treatments and up-regulated levels at diapause when compared with the WC group. This indicated the opposite expression regulated by JAK2, likely indicating different strategies used to endure cold stress and diapause. The unigene TRINITY_DN141355-c2-g1, annotated as *gadA* and *gadB*, belongs to glutamic acid decarboxylase (GAD) and was the rate-limiting enzyme that converts glutamate into the inhibitory neurotransmitter, γ -aminobutyric acid (GABA). This unigene was present in GABA-secreting neurons and non-neural cells such as islets B cells, thyroid, and thymus [41]. GABA can cause islet β cell damage and reduce insulin secretion. Additionally, we found that the gene expression level of FOXO6 (DN140179_c3_g1) showed increasing at low temperatures than diapause stages (Figure 5). In summary, the different expression regulated of member in insulin signaling pathway likely played a key role in the diapause of *C. chinensis*. Previous studies have documented JH III in relation to diapause in *C. chinensis* [11]. In this paper, we found that the concentration of JH III

was reduced gradually with a reduction in temperature and that the 34 unigenes referenced in JH III synthesis showed a similar trend with down-expression levels following WC, LT, and DP.

In summary, the diapause state of *C. chinensis* is further induced by low temperature as demonstrated by changes in related hormones, signal pathways, and biosynthetic compounds. Through transcriptome analysis, genes related to diapause showed up- or down-regulation trends and different in multi-process. The genes referred in insulin signaling pathway were identified positively with cold temperature. The hormone JH III exhibited lower concentrations in diapause samples than in samples subjected to low temperature stress. This could mean that the regulation in insulin and JH signal pathway facilitated the diapause transition.

Conclusion

In the study, the gene profiles of *C. chinensis* in low-temperature induced diapause were first studied using Illumina sequencing technology. Gene expression during diapause was reduced when compared with low temperature treatments. Positively related lncRNA-mRNA showed different expression levels, while the genes referred in insulin signaling pathway were identified. And the lowest JH III concentration was found in diapause samples. This data not only provides genomic information for *C. chinensis*, but also serves as a valuable resource for further research on diapause and its molecular mechanisms.

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Competing interests

The authors declare that they have no conflict of interest.

Authors' Contributions

Lin Li and Xiao-Hui Hou conceived the idea and designed the study; Chao bo Liu, Tian

Xu and Keyi Xiong analyzed the transcriptome data to write the originally manuscript;
while Lin Li and Xiao-Hui Hou edited the manuscript.

Data availability

Raw data of sequence was deposited in NCBI's SRA database, BioProject
PRJNA648717, as 9 Biosamples, three for each treatment group and collection data,
with accession SAMN16220144- SAMN16220152.

References

1. Liu Q, Cen C, Fu H, Wang F, Wang Y, Xu T, Hou X. Antioxidant activity of *Coridius chinensis* extracts on manganese-induced testicular damage in rats. *Environ. Toxicol.* 2019; 34:1067-1073.
2. Fan Q, Zhu GL, Sun XL, Cai HB, Lian ZM. AFLP Analysis on Genetic Diversity of Physic-edible *Aspongopus chinensis* Dallas. *Medicinal Plant.* 2010; 1: 42-44.
3. Li S, Li L, Peng HB, Ma XJ, Huang LQ, Li J. Advances in studies on chemical constituents, pharmacological effects and clinical application of *Aspongopus chinensis*. *Zhongguo Zhong Yao Za Zhi.* 2020; 45:303-311.
4. Shi YN, Tu ZC, Wang XL, Yan YM, Fang P, Zuo ZL, Hou B, Yang TH, Cheng YX. Bioactive compounds from the insect *Aspongopus chinensis*. *Bioorg Med Chem Lett.* 2014; 24:5164-9.
5. Tan, J, Tian, Y, Cai, R, Luo, R, Guo, J. Chemical Composition and Antiproliferative Effects of a Methanol Extract of *Aspongopus chinensis* Dallas. *Evid Based Complement Alternat Med.* 2019, 2019, 2607086.
6. Yu, S, DS, Li Hy, Huang Fp. Study on the effect of *Coridius chinensis* of water extract on two kinds of cancer cells. *Journal of Guangxi Normal University: Nature Science Edition.* 2015; 33: 104-107.
7. Huang H, Du J, Li SW, Gong T. Identification and functional analysis of a lysozyme gene from *Coridius chinensis* (Hemiptera: Dinidoridae). *Biology (Basel).* 2021; 10: 330.
8. Di Lei, Shi YN, Yan YM, Jiang LP, Hou B, Wang XL, Zuo LZ, Chen YB, Yang CP, Chen YX. Nonpeptide small molecules from the insect *Aspongopus chinensis* and their neural stem cell proliferation stimulating properties. *Rsc Advances.* 2015; 5: 70985-70991.
9. Fan Q, Wei H, Cai HB, Sui XG, Diao JX, Wang QR. Effects of the serum containing *Aspongopus chinensis* Dallas on the expression of SW480 apoptosis-associated factors, FADD and p53. *Journal of Anhui Agri. Sci.* 2011; 39: 7828-7831.
10. Zhou WZ, Wu YF, Yin ZY, Guo JJ, Li HY. Juvenile Hormone is an important factor in regulating *Aspongopus chinensis* Dallas diapause. *Front Physiol.* 2022; 13:873580.
11. Wu Y, Tian Y, Tan J, Zhao S, Zhou W, Luo R, Guo J. Differential metabolism of juvenile hormone III between diapause and non-diapause of *Aspongopus chinensis* Dallas (Hemiptera: Dinidoridae) revealed by transcriptome sequencing. *Journal of Asia-Pacific Entomology.* 2021; 24: 199-204
12. Kostal V, Shimada K, Hayakawa Y. Induction and development of winter larval diapause in a drosophilid fly, *Chymomyza costata*. *J. Insect Physiol.* 2000; 46: 417-428.

- 370 13. Nazari, P, Poorjavad, N, Lzadi, H. Simultaneous occurrence of diapause and cold Hardiness in
371 overwintering eggs of the apple oystershell scale, *Lepidosaphes Malicola* Borchsenius (Hem.:
372 Diaspididae). Zool Stud. 2020; 59: e25.
- 373 14. Hand SC, Denlinger DL, Podrabsky JE, Roy R. Mechanisms of animal diapause: recent
374 developments from nematodes, crustaceans, insects, and fish. Am J Physiol Regul. Integr
375 Comp Physiol. 2015; 310: R1193-R1211.
- 376 15. Lehmann P, Westberg M, Tang P, Lindström L, Käckelä R. The diapause lipidomes of three
377 closely related beetle species reveal mechanisms for tolerating energetic and cold stress in high-
378 latitude seasonal environments. Front Physiol. 2020; 11: 576617.
- 379 16. Tougeron, K. Diapause research in insects: historical review and recent work perspectives.
380 Entomologia Experimentalis et Applicata. 2019; 167: 27-36.
- 381 17. Kostal V. Eco-physiological phases of insect diapause. J Insect Physiol. 2006; 52: 113-27.
- 382 18. Diniz DFA, de Albuquerque CMR, Oliva LO, de Melo-Santos MAV, Ayres CFJ. Diapause and
383 quiescence: dormancy mechanisms that contribute to the geographical expansion of mosquitoes
384 and their evolutionary success. Parasit Vectors. 2017; 10: 310.
- 385 19. Ala-Honkola O, Kauranen H, Tyukmaeva V, Boetzi FA, Hoikkala A, Schmitt T. Diapause
386 affects cuticular hydrocarbon composition and mating behavior of both sexes in *Drosophila*
387 *montana*. Insect Sci. 2020; 27:304-316.
- 388 20. Kojić D, Popović ŽD, Orčić D, Purać J, Orčić S, Vukašinović EL, Nikolić TV, Blagojević DP.
389 The influence of low temperature and diapause phase on sugar and polyol content in the
390 European corn borer *Ostrinia nubilalis* (Hbn.). J Insect Physiol. 2018; 109:107-113.
- 391 21. Poelchau M.F, Reynolds JA, Elsik CG, Denlinger DL, Armbruster PA. RNA-Seq reveals early
392 distinctions and late convergence of gene expression between diapause and quiescence in the
393 Asian tiger mosquito, *Aedes albopictus*. J Exp Biol. 2013; 216:4082-90.
- 394 22. Lee S, Lee JH, Lee RH, Shin YP, Kim IW, Seo M, Kim MA, Hwang JS, Shin D, Lee HK. De
395 novo assembly and functional annotation of the Red-striped golden stink bug (*Poecilocoris*
396 *lewisi*) transcriptome. Gene. 2021; 767:145188.
- 397 23. Yocum GD, Childers AK, Rinehart JP, Rajamohan A, Pitts-Singer TL, Greenlee KJ, Bowsher
398 JH. Environmental history impacts gene expression during diapause development in the alfalfa
399 leafcutting bee, *Megachile rotundata*. J Exp Biol. 2018; 221: 173443.
- 400 24. Zhou Y, Sun D, Qian WL, Ding N, Liu W, Ma WH, Wang XP. Divergence in larval diapause
401 induction between the rice and water-oat populations of the striped stem borer, *Chilo*
402 *suppressalis* (Walker) (Lepidoptera: Crambidae). Environ Sci Pollut Res Int. 2018; 25:29715-
403 29724.
- 404 25. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and
405 development. Nat Rev Genet. 2014; 15:7–21.
- 406 26. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, Barrette TR, Prensner JR, Evans
407 JR, Zhao S, Poliakov A, Cao XH, Dhanasekaran SM, Wu YM, Robinson DR, Beer DG, Feng
408 FY, Lyer HK, Chinnaiyan AM. The landscape of long noncoding RNAs in the human
409 transcriptome. Nat. Genet. 2015; 47:199–208.
- 410 27. Humann FC, Tiberio GJ, Hartfelder K. Sequence and expression characteristics of long
411 noncoding RNAs in honey bee caste development–Potential Novel Regulators for
412 Transgressive Ovary Size. PLoS One. 2013; 8: e78915.
- 413 28. Wang Y, Xu T, He W, Shen X, Zhao Q, Bai J, You M. Genome-wide identification and

- characterization of putative lncRNAs in the diamondback moth, *Plutella xylostella* (L.). Genomics. 2018; 110: 35–42.
29. Theissinger K, Falckenhayn C, Blande D, Toljamo A, Gutekunst J, Makkonen J, Jussila J, Lyko F, Schrimpf A, Schulz R, Kokko H. De Novo assembly and annotation of the freshwater crayfish *Astacus astacus* transcriptome. Marine genomics. 2016; 28: 7–10.
 30. Toxopeus J, Gadey L, Andaloori L, Sanaei M, Ragland GJ. Costs of averting or prematurely terminating diapause associated with slow decline of metabolic rates at low temperature. *Comp. Biochem. Physiol. A Mol Integr Physiol.* 2021; 255: 110920.
 31. Sahoo A, Dutta A, Dandapat J, Samanta L. Low H₂O₂ and enhanced oxidative resistance in the diapause-destined pupa of silkworm, *Antheraea mylitta* (Lepidoptera: Saturniidae) suggest their possible involvement in dormancy and lifespan extension. BMC Zoology. 2018; 3:1-9.
 32. Jovanović-Galović A, Blagojević DP, Grubor-Lajsić G, Worland MR, Spasić MB. Antioxidant defense in mitochondria during diapause and postdiapause development of European corn borer (*Ostrinia nubilalis*, Hubn.). Arch Insect Biochem Physiol. 2007; 64:111-9.
 33. Chen H, Wu G, Zhou H, Dai X, Steeghs NWF, Dong X, Zheng L, Zhai Y. Hormonal regulation of reproductive diapause that occurs in the year-round mass rearing of *Bombus terrestris* queens. J Proteome Res. 2021; 20:2240-2250.
 34. Mirth CK, Tang HY, Makohon-Moore SC, Salhadar S, Gokhale RH, Warner RD, Koyama T, Riddiford LM, Shingleton AW. Juvenile hormone regulates body size and perturbs insulin signaling in *Drosophila*. Proc Natl Acad Sci U S A. 2014;111:7018-23.
 35. Jindra M, Palli SR, Riddiford LM. The juvenile hormone signaling pathway in insect development. Annu Rev Entomol. 2013; 58:181-204
 36. Brown MR, Sieglaff DH, Rees HH. Gonadal ecdysteroidogenesis in arthropoda: occurrence and regulation. Annu Rev Entomol. 2019; 54:105-25.
 37. Arpagaus M. Vertebrate insulin induces diapause termination in *Pieris brassicae* pupae. Roux's Arch Dev Biol. 1987; 196: 527-530.
 38. Jian T, Shen XJ, Tang SM, Qian P. Advances in research on diapause physiology and molecular regulation mechanism of silkworm. Acta Sericologica Sinica. 2017; 43: 1031-1038.
 39. Sim C, Denlinger DL. Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. Proc Natl Acad Sci U S A. 2008;105: 6777-81.

Supplementary information

Figure S1: GO biological processes and KEGG pathway that were significantly enriched with DEGs among 3 groups, Figure S2: lncRNA-mRNA co-expression network map, Figure S3: lncRNA-DEGs-GO node/KEGG pathway network diagram, Figure S4: 6 DEGs expression levels compared under different treatments.

Table S1: Primers for qPCR polymerase chain reaction.

Table S2: Sequencing data information of *Coridius chinensis*.

Table S3: The statistics of genes expression in juvenile synthesis pathway

454

455 **Figure legends**

456 **Figure 1.** The analysis of *C. chinensis* transcriptome unigenes. Sequence length
457 distribution and number (A), Homology search in the NR database (B), Functional
458 classification (C), Pfam annotation (D).

459 **Figure 2.** The statistics of different expression levels of mRNA (A, B) and lncRNAs
460 (C, D) in comparison groups.

461 **Figure 3.** WGCNA analysis of the DEGs and DElncRNAs. (A) Silhouette coefficient
462 with different K-value. (B) Clustering of DEGs on K-means. (C) Module partitioning
463 tree diagram. (D) Module TOM cluster heat map. (E) Sample characterization state and
464 module correlation heat map.

465 **Figure 4.** Juvenile hormone III synthesis pathway of *C. chinensis* transcriptome. (A)
466 The identified genes expression level comparison. (B) The contents of JH-III in 3 tested
467 groups.

468 **Figure 5.** qRT-PCR analysis of 6 DEGs in *C. chinensis*.

469

Figures

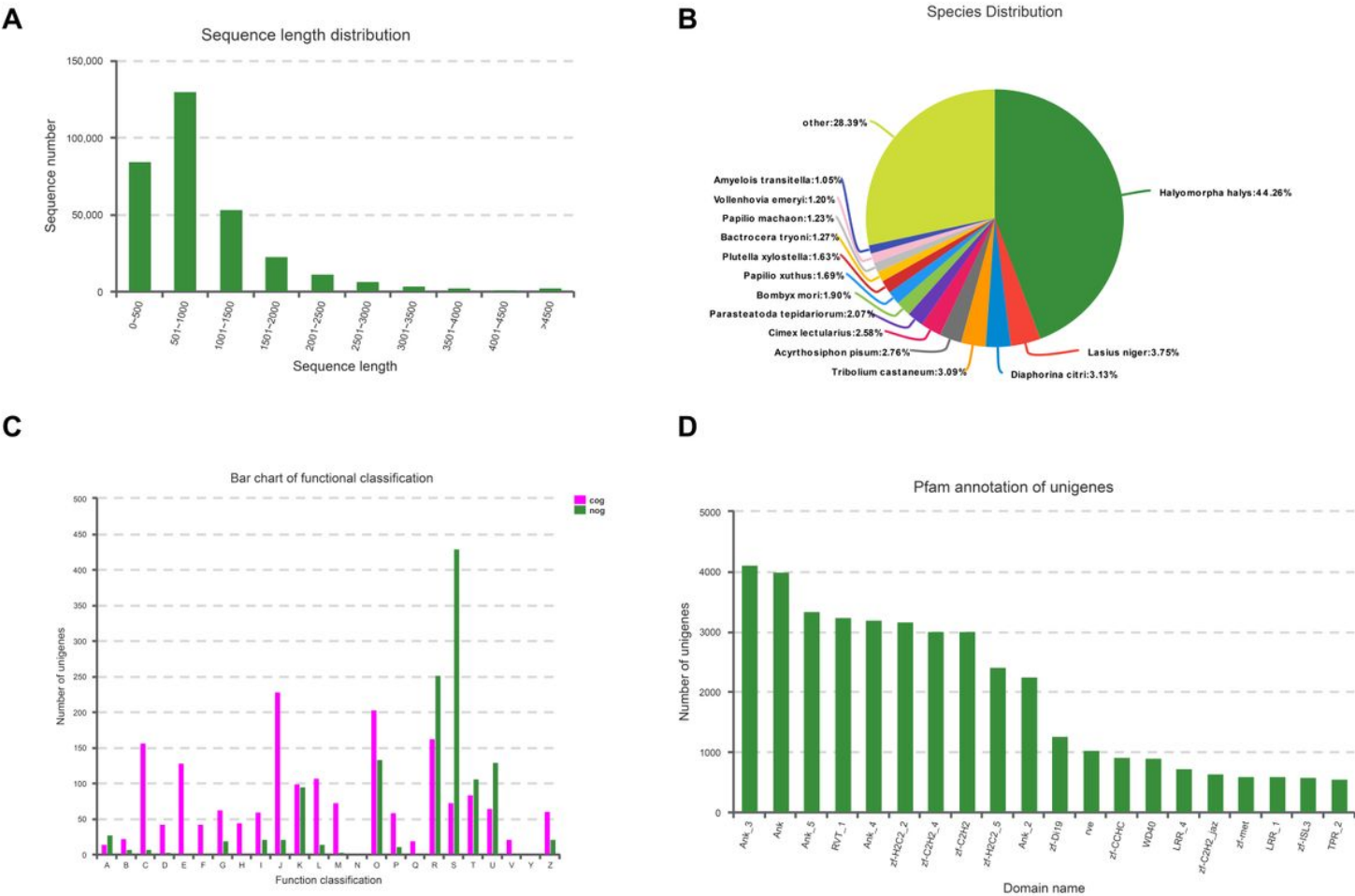


Figure 1

The analysis of *C. chinensis* transcriptome unigenes. Sequence length distribution and number (A), Homology search in the NR database (B), Functional classification (C), Pfam annotation (D).

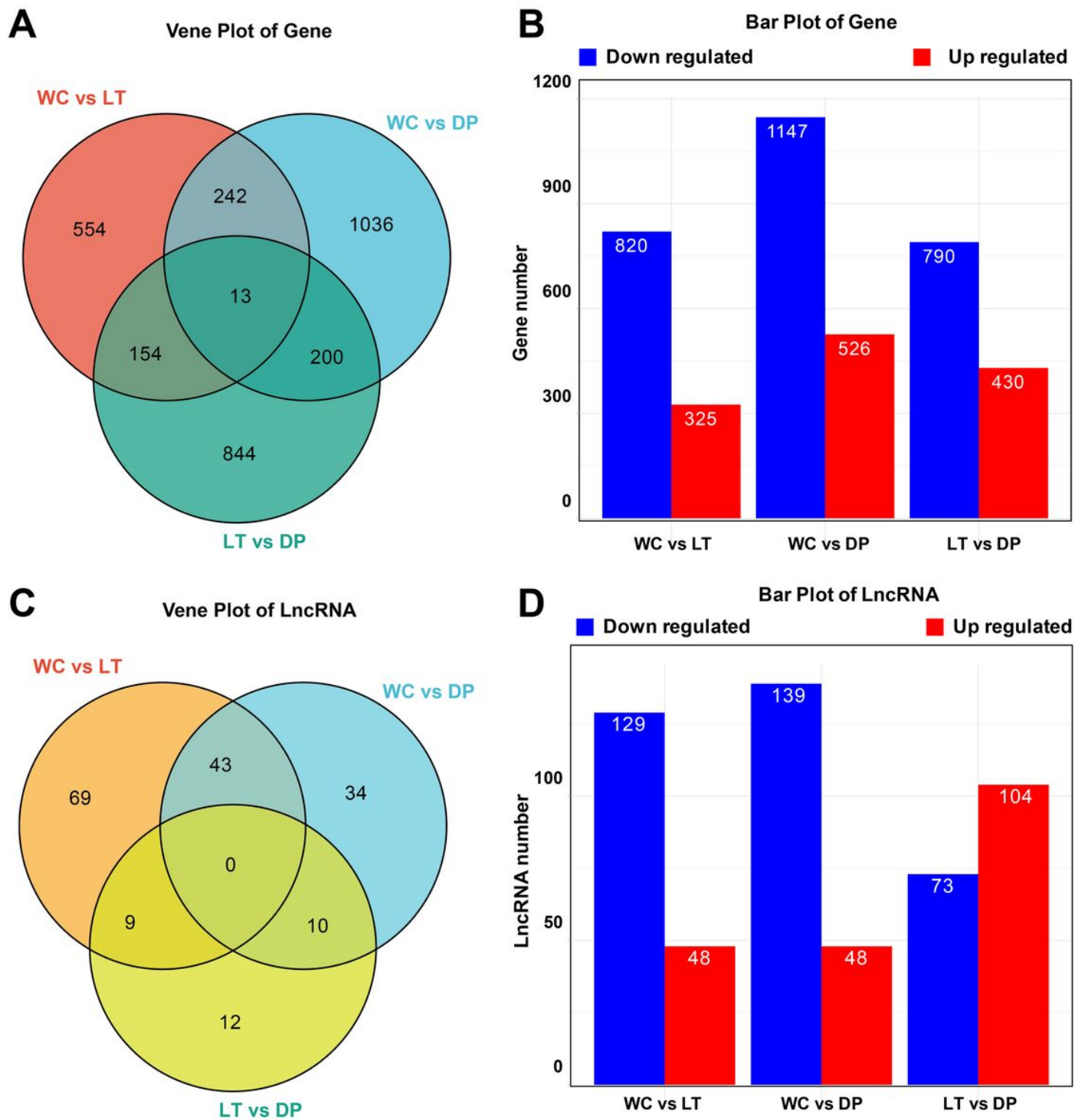


Figure 2

The statistics of different expression levels of mRNA (A, B) and lncRNAs (C, D) in comparison groups.

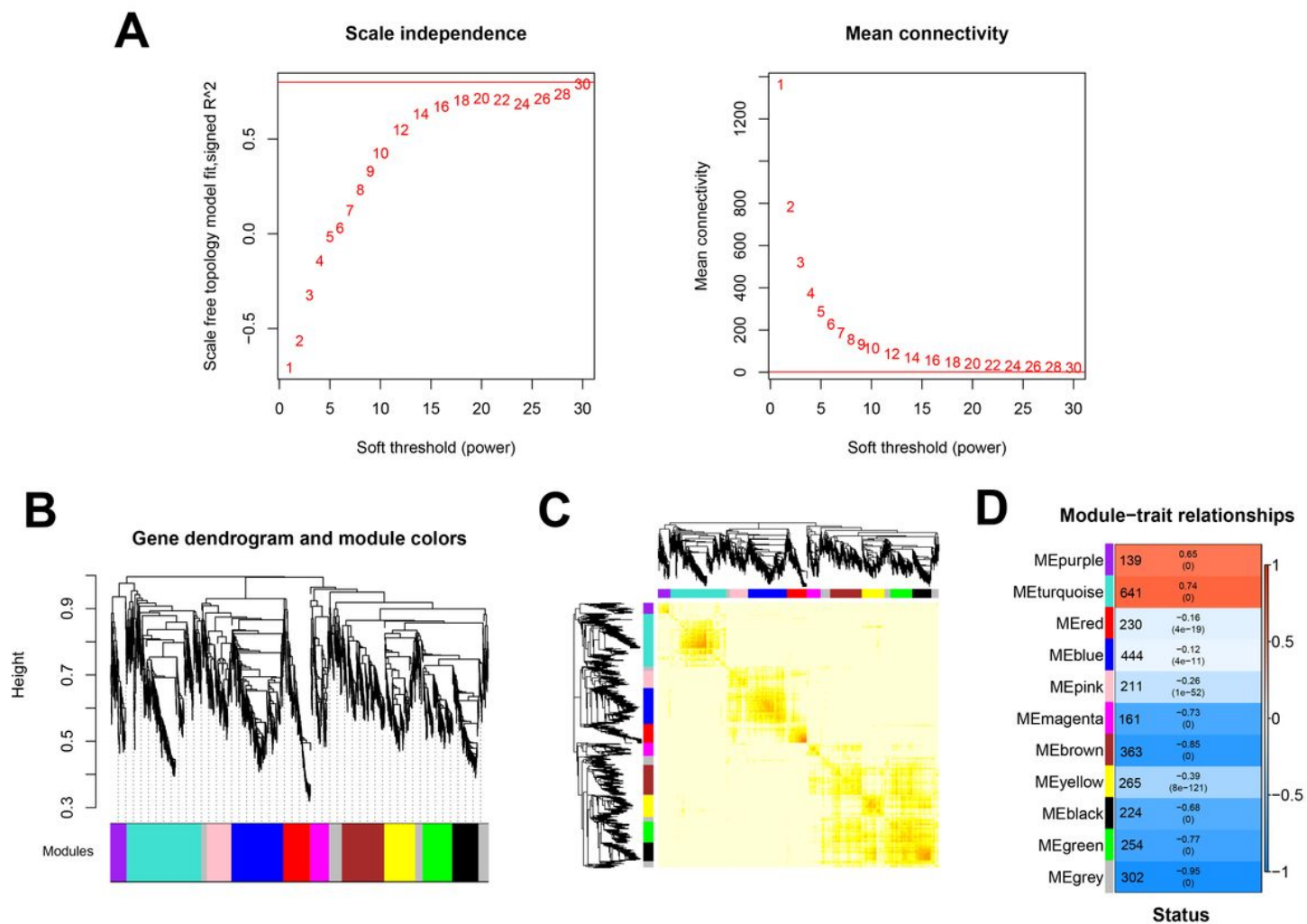
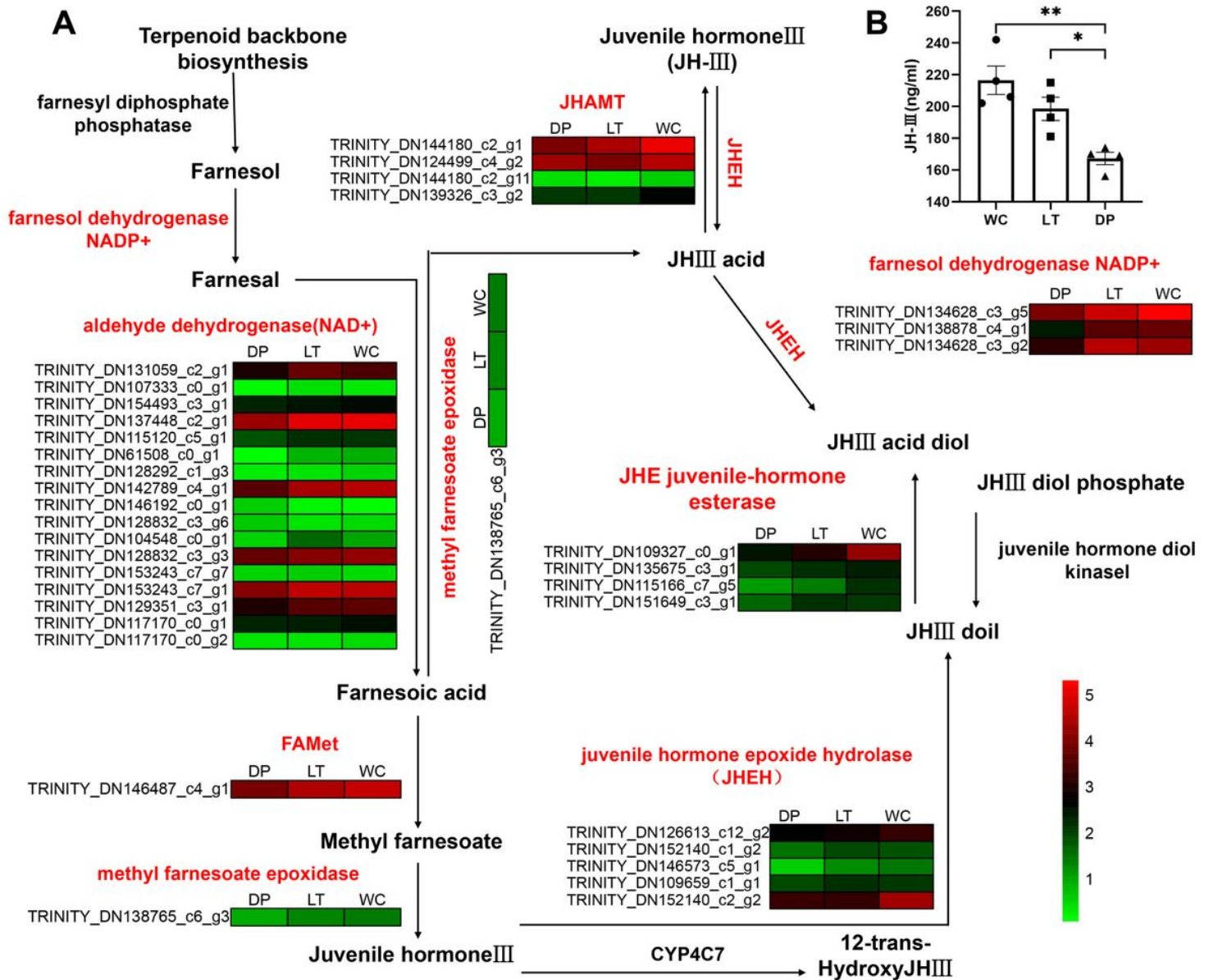


Figure 3

WGCNA analysis of the DEGs and DElncRNAs. (A) Silhouette coefficient with different K-value. (B) Clustering of DEGs on K-means. (C) Module partitioning tree diagram. (D) Module TOM cluster heat map. (E) Sample characterization state and module correlation heat map.



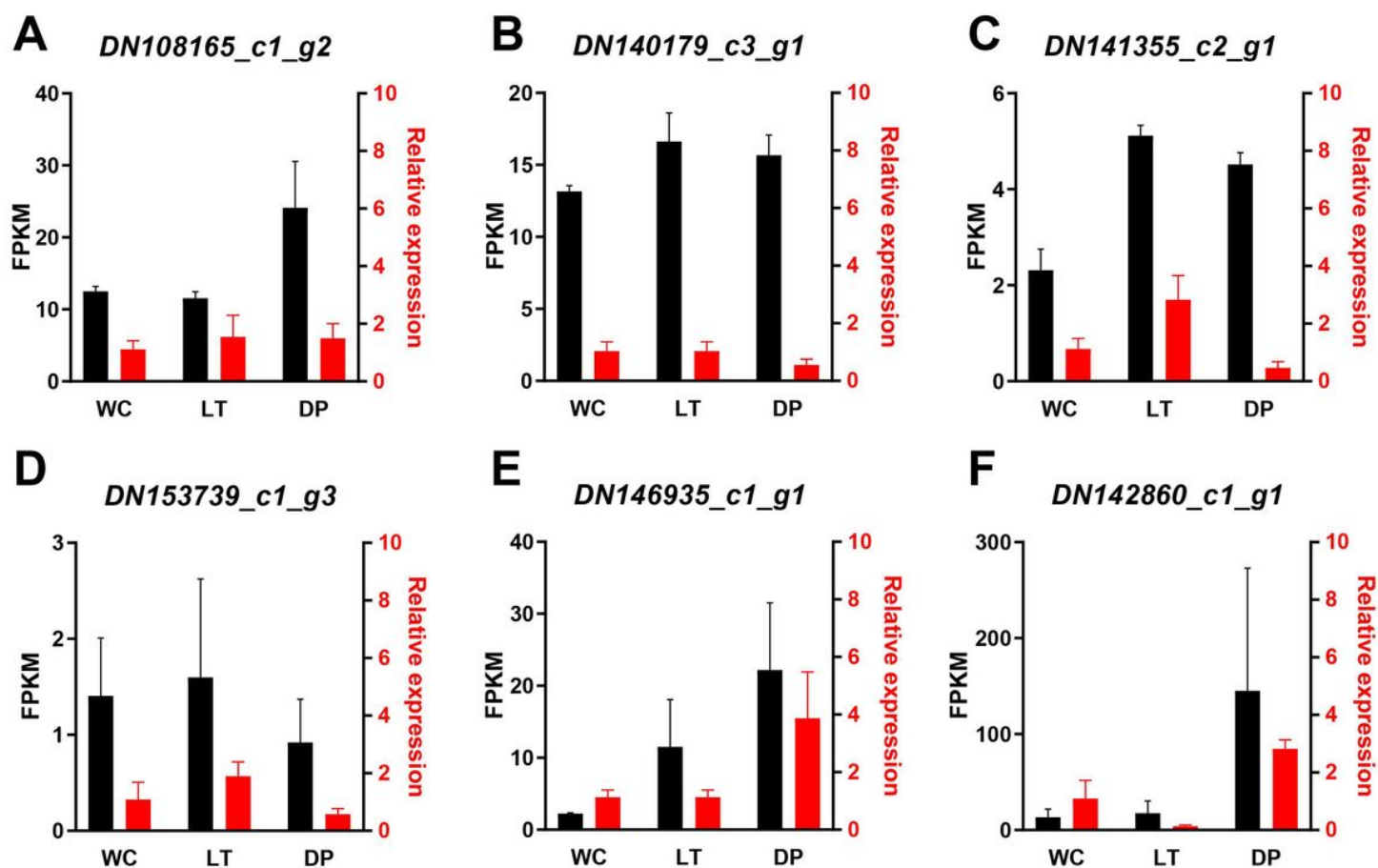


Figure 5

qRT-PCR analysis of 6 DEGs in *C. chinensis*.

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