BMC Genomics

RESEARCH Open Access

# DNA methylation regulates growth traits by influencing metabolic pathways in Pacific white shrimp (*Litopenaeus vannamei*)

Xin Zhang[1], Chaoqun Hu[1], Ting Chen[1], Pengying Li[2], Yehui Tan[1], Chunhua Ren[1], Yanhong Wang[1], Xiao Jiang[1], Bo Ma[1,3], Jiayue Yin[1,3], Yunyi Huang[1,3], Liyan Liu[4], Huo Li[5] and Peng Luo[1*]

## Abstract

**Background** DNA methylation is a critical epigenetic modification that dynamically regulates gene expression associated with economic traits. Pacific white shrimp (*Litopenaeus vannamei*) is one of the most important aquatic species for culturing, and growth trait is one of the most important economic traits for its production. However, research on DNA methylation regulation of growth traits is still at an early stage. This study explored DNA methylome dynamics and their associations with the regulatory mechanism behind growth traits using full-subfamily individuals with discrepant growth performance.

**Results** The DNA methylation-related genes in *L. vannamei* were identified, and the expression of DNA methylation genes showed significantly higher levels in the slow growth (SG) group compared to the fast-growing (FG) individuals. The Whole Genome Bisulfite Sequencing (WGBS) analysis revealed that the methylation levels in the muscles of shrimp were notably decreased in SG individuals compared to FG individuals. A total of 532 differentially methylated promoters and 2,067 differentially methylated regions were identified. Through integrative analysis of DNA methylation and transcriptomic data from SG and FG group shrimp, a total of 47 genes were screened out with differential methylation levels (DMGs) and expression levels (DEGs). Functional enrichment analysis revealed that the overlapping DEGs/DMGs were enriched mainly in metabolic pathways, starch and sucrose metabolism, linoleic acid metabolism, ascorbate and aldarate metabolism, pentose and glucuronate interconversions.

**Conclusions** DNA methylation plays a role in the regulation of growth traits in *L. vannamei*. The level of DNA methylation was found to be negatively correlated with growth traits. Through comprehensive analysis, it was discovered that DNA methylation predominantly affects growth performance by up-regulating the expression of genes involved in metabolic pathways, such as glucose metabolism and amino acid metabolism in *L. vannamei*. This suggests a higher metabolism activity in SG individuals derived DNA methylation to cope with some unknown internal stress or environmental stress rather than being allocated for growth.

**Keywords** *Litopenaeus vannamei*, Growth trait, DNA methylation, Gene expression

*Correspondence:
Peng Luo
luopeng@scsio.ac.cn
[1]Key Laboratory of Breeding Biotechnology and Sustainable Aquaculture (KLBBSA), Guangdong Provincial Key Laboratory of Applied Marine Biology (LAMB), South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China
[2]Shanghai Ocean University, Shanghai, China
[3]University of Chinese Academy of Sciences, Beijing, China
[4]Yuehai Feed Group Co., Ltd, Zhanjiang, China
[5]Guangdong Jinyang Biotechnology Co., Ltd, Maoming, China

## Introduction

Epigenetics encompasses the regulation of gene expression without changes to the DNA sequence [1]. Key mechanisms include DNA methylation, histone modifications, genomic imprinting, non-coding RNAs, gene silencing, nucleolar dominance, maternal effects, transposon activation, and RNA editing [2, 3]. DNA methylation, a prevalent and critical epigenetic modification, involves the attachment of a methyl group to the cytosine base in CpG dinucleotides. This typically occurs in promoter regions, regulating gene transcription [4]. DNA methylation variations were observed across a wide array of organisms, including viruses, prokaryotes, and eukaryotes [5, 6]. This modification not only influences gene expression, growth, and development but also enhances protection against environmental stresses and sustains genomic integrity [7, 8]. Typically, DNA methylation inhibits the binding of specific proteins to DNA, thereby impeding the transcription process [9].

Fish genomes generally show DNA methylation levels comparable to those in vertebrates, primarily in the form of CpG methylation [10]. In multiple studied fish species, approximately 70-80% of CpG sites were methylated, similar to the levels observed in the mouse genome [11–13]. In contrast, the CpG methylation levels in mollusks and crustaceans were significantly lower. For example, only approximately 15% of CpG sites were methylated in the Pacific oyster genome [14], whereas in *Procambarus virginalis*, only 2–3% of CpG sites were methylated [15]. In Daphnia, CpG methylation levels were less than 1% or slightly above 1% [16, 17]. Additionally, research has shown that gene body methylation plays a role in regulating gene family expansion and functional diversification, thereby influencing phenotypic variation [18].

DNA methylation plays a crucial role in regulating various aspects of aquatic animals, including growth, disease resistance, sex differentiation, and environmental adaptation [19]. Research has demonstrated that heterosis, a phenomenon of hybrid vigor, is associated with the downregulation of DNA methylation [20, 21]. Conversely, the upregulation of DNA methylation levels in growth-related genes can result in slower growth rates [22]. In grass carp, high methylation of the upstream GC island of the RIG-I gene has been found to reduce RIG-I gene expression, thereby increasing susceptibility to grass carp hemorrhagic necrosis virus [23]. Additionally, the methylation and demethylation of specific genes, such as *cyp19a*, can influence sex reversal and alter sex ratios [24]. Environmental stressors, including hypoxia and heat stimulation, have been shown to impact DNA methylation levels, increasing the adaptability of organisms to their environment [25, 26]. Overall, DNA methylation serves as a fundamental mechanism in the regulation and adaptation of aquatic animals to various biological and environmental conditions.

*Litopenaeus vannamei*, known for its wide salinity tolerance, rapid growth, and robust disease resistance, is the predominant shrimp species in aquaculture. Growth trait is critical criteria for the success of the animal farming industry. Numerous studies have identified genes and single-nucleotide polymorphisms (SNPs) associated with growth performance in *L. vannamei* [27–30]; however, the underlying growth regulatory mechanisms mediated by DNA methylation remain elusive. To address this gap, we conducted a systematic analysis of the genome-wide methylome and transcriptome variations in the muscle of *L. vannamei* via whole-genome bisulfite sequencing (WGBS) and RNA sequencing (RNA-seq). This comprehensive approach aims to elucidate the intricate regulatory mechanisms governing growth traits in marine shrimp and to improve strategies for the genetic breeding of *L. vannamei*.

## Results

### Discrepant growth performance and DNA methylation levels in the FG and SG

The size of the shrimps in the full-subfamily significantly differed (Fig. 1A), and different weight differences were

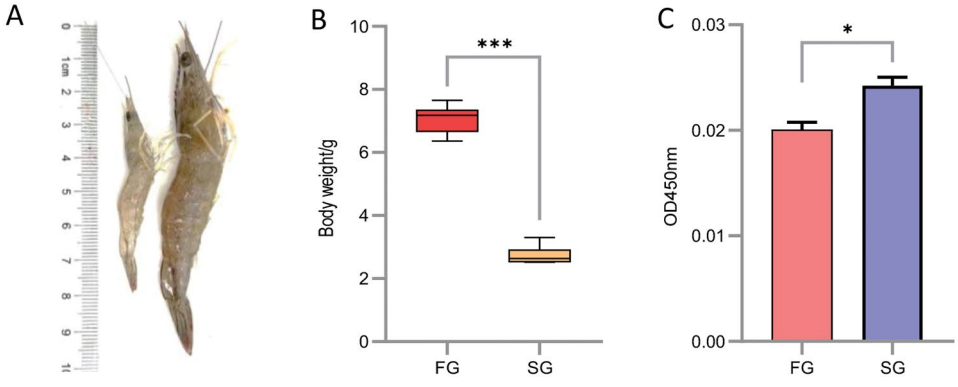


**Fig. 1** Differential growth performance of *L. vannamei* shrimp from a full-subfamily. **A**, Differential body sizes. **B**: Significant difference in body weight between 6 randomly selected FG individuals and 6 SG individuals. **C**: Genomic DNA methylation levels in the muscles of FG and SG shrimp individuals

observed among the 30 selected shrimp individuals, with the maximum weight of the shrimp being 9.22 g and the minimum weight being 2.53 g, with an average weight of 5.17 g. Six individuals in the FG and the SG groups were further selected for subsequent analysis, which revealed significant discrepancies in body weight ($p < 0.05$) (Fig. 1B). Furthermore, the genomic DNA methylation levels (5-mC) in the FG individuals (2.00%) were lower than those in the SG individuals (2.42%) ($p < 0.05$) (Fig. 1C).

#### Identification of methylation genes in *L. vannamei*

A total of 21 methylation-related genes were identified from the genomic database (CIBNOR_Pvan_1v2), and all the sequences were used to establish a phylogenetic tree from the six crustacean species (Fig. 2A(a)). Sequence analysis revealed that the methylation-related genes could be divided into four subgroups. There were five members in subgroup I; containing motifs 2, 3, 4 and 5. Similarly, six members were included in subgroup II, which contains motifs 3, 4 and 5; four members were included in subgroup III, which contains motif 3(Fig. 2A(b)) indicating that DCM superfamily, which is evolutionarily conserved among DNA methyltransferases. Six members were included in subgroup IV, which contains motifs 1–10. According to the domain information, the Dcm domain (or Dcm superfamily domain) is present in methyltransferases, except for the COG2263 domain (or COG2263 superfamily domain) presented in subgroup III (Fig. 2A(c)).

The expression profiles of two DNA methyltransferase (DNMT), *dnmt1* (XP_027222128.1) and *dnmt3a* (XP_027231607.1) were quantified in the individuals from the FG and the SG groups via qRT-PCR. The results indicated that the expression of *dnmt3a* in the SG group was significantly greater than that in the FG group ($p < 0.001$) (Fig. 2B), the expression of *dnmt1* in the SG group was significantly greater than that in the FG group ($p < 0.05$) (Fig. 2C). Using previously published RNA-seq data [31], two DNA methylation-related genes (*dnmt3a*, *dnmt1*), represented by counts per million (CPM) values in different tissues, were identified (Fig. 2D, E). These genes were mainly expressed in the tissues of brain, testes, ovaries, and hepatopancreas.

#### Transcriptome dynamics in the muscles of shrimp with different growth traits

To investigate the transcriptomic dynamics of muscles exhibiting distinct growth traits, comparative transcriptomic analysis was conducted using three individuals each from the fast growth (FG) and slow growth (SG) groups. Following the filtration of low-quality reads, a total of 40.26 Gb of clean reads were obtained, characterized by a relatively high Q30 quality score, with a minimum of 94.44%. The mapping rates across all samples varied between 77.74% and 85.62%; detailed mapping data were presented in Table 1 (Supplementary Table S2). After transcript assembly, a total of 13,288 expressed genes were identified in each sample (Supplementary Table S3).

To elucidate the transcriptomic differences in muscles between the two groups, a total of 726 differentially expressed genes (DEGs) were identified. Among these genes, 645 were downregulated, and 210 were upregulated (Fig. 3A, B). GO enrichment analysis revealed that the up-regulated DEGs were significantly enriched in structural molecular activity, structural constituent of cuticle, extracellular region items (Fig. 3C, Supplementary Table S4), and the down-regulated DEGs were significantly enriched in adenyl nucleotide binding, adenyl ribonucleotide binding and ATP binding items (Fig. 3D, Supplementary Table S4). KEGG pathway analysis showed that the up-regulated DEGs were significantly enriched in starch and sucrose metabolism, MAPK signaling pathway, and carbon metabolism pathways (Fig. 3E, Supplementary Table S5); while the down-regulated DEGs were significantly enriched in Motor proteins, cell cycle and galactose metabolism pathways (Fig. 3F, Supplementary Table S5).

#### Methylome dynamics in the muscles of shrimp with different growth traits

For the methylome analysis, detailed information regarding the raw data quality control statistics is presented in Table 2. The FG group presented average methylation levels of 97.48% for CG, 0.5% for CHG, and 2.03% for CHH, whereas the SG group presented average methylation levels of 97.49% for CG, 0.49% for CHG, and 2.01% for CHH. Notably, the CG context presented the highest methylation levels compared with those of CHG and CHH (Fig. 4A, B, C). A comprehensive mapping analysis of methylation within functional genomic regions was conducted, examining C loci across various regions, including promoters, exons, introns, repeats, CGIs, CGI shores, CGI shelves, and open seas. Specifically, the genome-wide methylation landscape for the three sequence contexts (CG, CHG, CHH) is presented in Fig. 4D (double coordinate display), and their distributions on gene functional elements were depicted in Fig. 4E. Methylation levels within 2 kb upstream and downstream of genes were illustrated in Fig. 4F. The data indicated that CG context methylation levels were generally lower in the FG group than in the SG group. In total, 20432 CG differentially methylated regions (DMRs), 161 CHG DMRs, and 1655 CHH DMRs were identified in the DNA methylation dynamics between the FG and SG groups (Fig. 4G). The corresponding differentially methylated genes (DMGs) covering these DMRs were

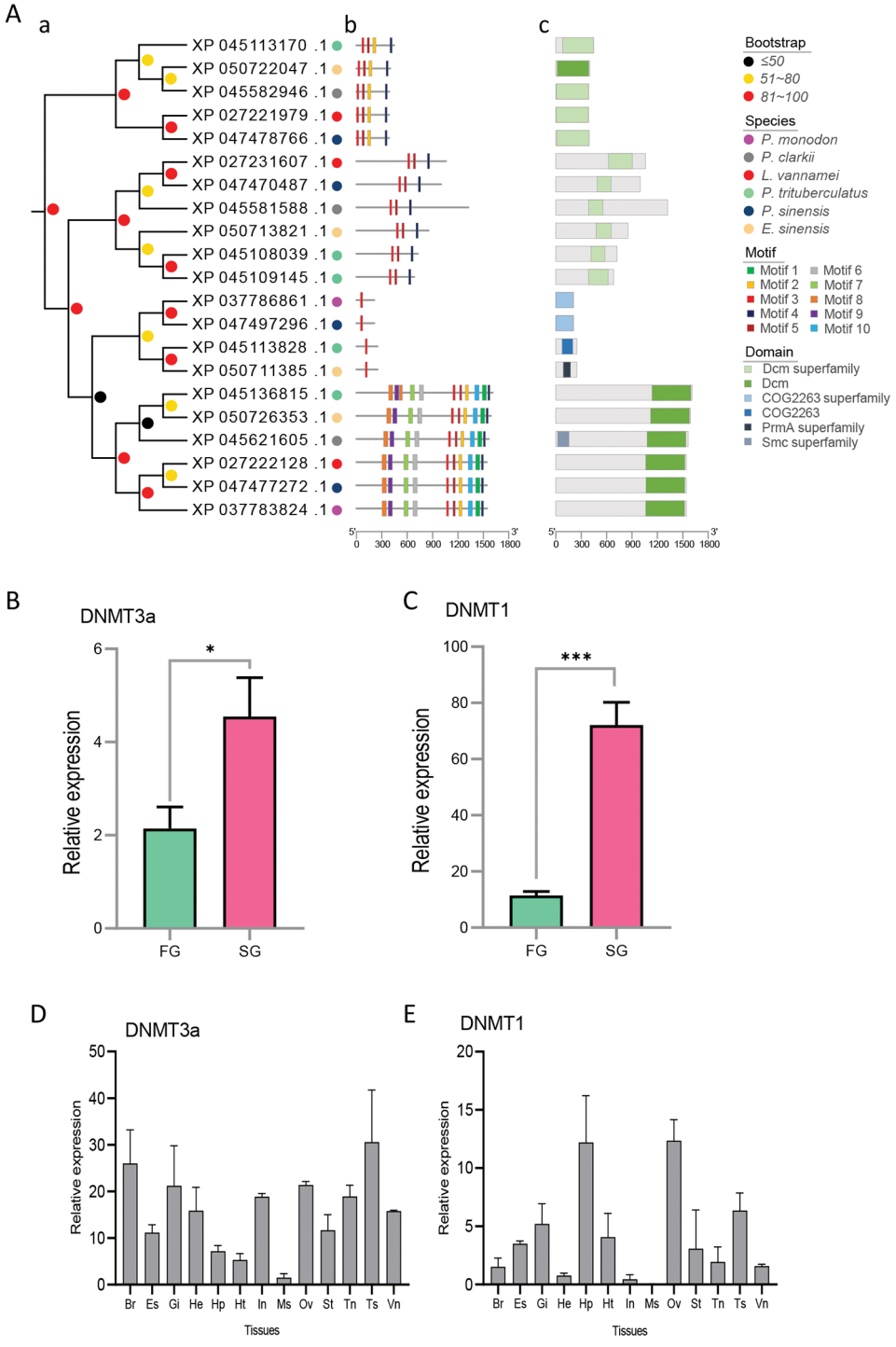


**Fig. 2** The gene structures and domain compositions of methylation-related genes. **A**: (**a**) Phylogenetic analysis of methylation-related genes in *L. vannamei* and other five arthropod species (*Procambarus clarkii*, *Penaeus monodon*, *Litopenaeus chinensis*, *Portunus trituberculatus*, *Eriocheir sinensis*). (**b**) The gene structures of methylation-related genes. The different boxes represented different motifs, respectively. (**c**) The domain compositions of methylation-related genes encoded proteins. The different boxes represented different motifs, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article). **B**: mRNA expression levels of two DNA methylation-related genes (*dnmt1* and *dnmt3a*) mRNA in the FG and the SG shrimp individuals ($n=3$). **C**: tissues distribution of two DNA methylation-related genes (*dnmt1*, *dnmt3a*) ($n=3$). Br: brine, Es: eyestalk, Gi: gill, He: hemocyte, Hp: hepatopancreas, Ht: heart, In: intestine, Ms: muscle, Ov: ovary, St: stomach, Tn, thoracic nerve, Ts: testy, Vn: ventral nerve. Figure **C** and **D** were the expression of dnmt3a, and dnmt1; and the Fig. **E** and **F** were the tissue distribution of dnmt3a, and dnmt1

**Table 1** Raw data quality control statistics for transcriptome sequencing

| Sample | Raw reads | Clean reads | Q30 | GC pct | Total map | Unique map | Multi map |
|---|---|---|---|---|---|---|---|
| FG1 | 7.3G | 7.08G | 96.3 | 50.34 | 79.65% | 72.49% | 7.16% |
| FG2 | 6.6G | 6.37G | 94.44 | 50.23 | 85.62% | 77.91% | 7.71% |
| FG3 | 6.98G | 6.74G | 96.2 | 50.88 | 77.74% | 70.36% | 7.38% |
| SG1 | 6.48G | 6.26G | 96.09 | 50.81 | 82.57% | 75.27% | 7.31% |
| SG2 | 6.94G | 6.69G | 95.96 | 49.5 | 79.67% | 74.82% | 4.85% |
| SG3 | 7.34G | 7.12G | 96.2 | 50.61 | 79.2% | 73.23% | 5.97% |

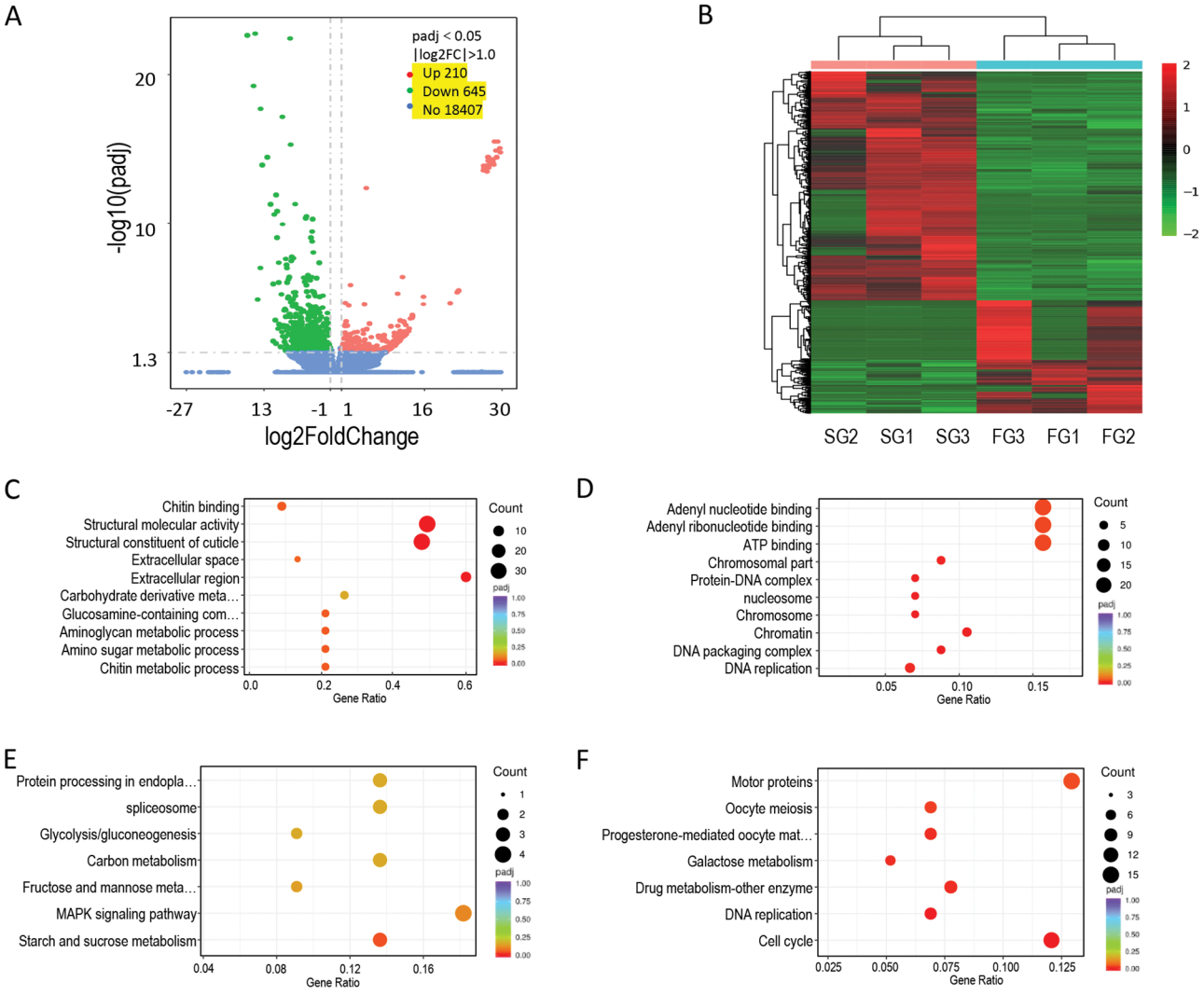


**Fig. 3** Functional enrichment analysis of DEGs in muscles of the FG and the SG. **A**, Volcano plot showing DEGs; **B**, heatmap of DEG hierarchical cluster analysis; **C**, GO enrichment of DEGs; **D**, KEGG pathway enrichment of DEGs. **E**, GO enrichment of upregulated genes between the two groups. **F**, KEGG enrichment of upregulated genes between the two groups

identified, resulting in the discovery of 1823 CG_DMR genes, 334 CHH_DMR genes, and 57 CHG_DMR genes across the three methylated environments (Fig. 4H). Subsequent DNA methylation analysis of the muscles from the two groups focused primarily on the CG context. Furthermore, hypomethylated DMRs (hypo) were more prevalent than hypermethylated (hyper) DMRs in regions such as CGIs, CGI shores, introns, and others, except for the 3' UTR regions (Fig. 4I). To elucidate the functions of the DMGs associated with the 20,432 CG DMRs, GO and KEGG enrichment analyses were performed. The top 10 GO categories were enriched primarily in metabolic processes, cellular macromolecule metabolic processes, organic substance metabolic processes, and primary metabolic processes (Fig. 4J), with detailed information provided in Supplementary Table S6. KEGG pathway

**Table 2** Raw data quality control statistics for whole-genome bisulfite sequencing

| Sample | Raw Bases (G) | Clean Bases (G) | Clean ratio (%) | Q20 (%) | Q30 (%) | GC Content (%) | BS Conversion Rate (%) |
|---|---|---|---|---|---|---|---|
| FG1 | 77.02 | 67.91 | 88.17 | 97.37 | 93.07 | 21.23 | 99.645 |
| FG2 | 77.14 | 68.09 | 88.27 | 97.56 | 93.61 | 21.08 | 99.639 |
| FG3 | 76.55 | 67.45 | 88.11 | 97.55 | 93.6 | 20.96 | 99.642 |
| SG1 | 76.44 | 67.38 | 88.15 | 97.43 | 93.24 | 21.05 | 99.639 |
| SG2 | 76.2 | 67.22 | 88.22 | 97.36 | 93.02 | 20.61 | 99.639 |
| SG3 | 77.27 | 68.39 | 88.51 | 97.58 | 93.66 | 21.25 | 99.657 |

analysis revealed that CG DMRs were significantly enriched in metabolic pathways, amino sugar and nucleotide sugar metabolism, fatty acid metabolism, and other pathways, which aligns with the GO enrichment results (Fig. 4K). Detailed information is presented in Supplementary Table S7.

### Integrative analysis of DNA methylation and transcriptomics

We integrated WGBS and RNA-seq data to study the correlation between DNA methylation and gene expression in muscle tissues. Figure 5A shows patterns of differentially expressed genes (DEGs) and differentially methylated regions (DMRs). We identified 532 differentially methylated promoters, 2067 DMRs, and 726 DEGs in the FG and SG groups. Notably, 47 genes were both DEGs and differentially methylated genes (DMGs) with DMRs, including 4 DMGs with differentially methylated positions (DMPs) (Fig. 5B). Gene body methylation positively correlated with gene expression, but promoter methylation negatively impacted gene expression (Fig. 5C). DMR analysis revealed 8 hypomethylated/upregulated DMGs, 19 hypermethylated/downregulated DMGs, 6 hypermethylated/upregulated DMGs, and 19 hypomethylated/downregulated DMGs (Fig. 5D). DMP analysis revealed 1 hypomethylated/upregulated DMG, 1 hypermethylated/downregulated DMG, 1 hypermethylated/upregulated DMG, and 3 hypomethylated/downregulated DMGs (Fig. 5E). Figure 6A shows that CG-type DMR-targeted DMGs were enriched in 15 GO subcategories ($p < 0.05$) and 13 KEGG pathways ($p < 0.05$) (Fig. 6B). Functional analysis revealed that these DMGs regulate metabolic pathways. Genes such as *AASS*, *4CL-like 1*, *UP1*, *RNR*, *UGDH*, and *PGM2* were upregulated in the SG group and enriched in pathways such as starch and sucrose metabolism, suggesting increased energy consumption in the SG group.

## Discussion

As mentioned, methylation levels were generally lower in crustaceans than in fish and terrestrial animals such as mice. In our study, the CpG methylation level in the whole genome of *L. vannamei* ranged from 2.67–2.89%, which is consistent with the levels reported in *P. virginalis* and *Daphnia* [32]. CG DMRs predominated, accounting for 97.49% of the methylation context, similar to findings in the large yellow croaker [27]. Overall, WGBS analysis revealed that the DNA methylation levels in the FG shrimp were lower compared to those in the SG shrimp. Additionally, RNA-seq identified numerous DEGs between the two groups. It is well-established that DNA methylation generally acts as a negative regulator of gene expression [33–35]. However, genebody methylation is more widespread and shows a positive correlation with gene expression [36]. In the current study, a large number of DEGs were upregulated in the SG group. Moreover, the DNA methylation levels in the gene body regions (including 3'-UTR, 5'-UTR, exons, and introns) of the SG shrimp were higher than those in the FG shrimp. We hypothesize that the relatively high genebody methylation levels in SG shrimp can regulate the expression of related genes, such as *UDP-α-D-glucose 6-dehydrogenase* (*UGDH*) and *Phosphoglucomutase 2* (*PGM2*), *UDP-glucose 6-dehydrogenase* (*UGDH*) and *cytochrome P450* (*Cyp6a13*). Subsequently, this enhanced gene expression impacts the growth performance of *L. vannamei*.

DNA methyltransferase (DNMT) enzymes act as crucial regulators in the DNA methylation process. Specifically, Dnmt1 is responsible for maintaining the existing DNA methylation patterns during DNA replication, while Dnmt3a initiates de-novo DNA methylation [27, 37]. In this study, *Dnmt1* and *Dnmt3a* were identified from the shrimp genome, and these genes exhibit evolutionary conservation. Tissue distribution analysis demonstrated distinct expression patterns of *Dnmt1* and *Dnmt3a* in shrimp tissues. High expression levels of these genes were detected in the ovary and testis, where they play vital roles in germ cell development and species-specific epigenetic reprogramming [38]. Moreover, relatively high expression levels of *Dnmt1* and *Dnmt3a* were observed in the intestine and hepatopancreas of shrimp. To date, there has been relatively limited research on DNA methylation in aquatic animals. However, studies in human diseases have shown that DNA methyltransferase regulates intestinal epithelial barrier function and regeneration [39, 40]. The intestine and hepatopancreas of shrimp are rich in epithelial cells, which are involved in digestion and nutrient absorption. These two tissues

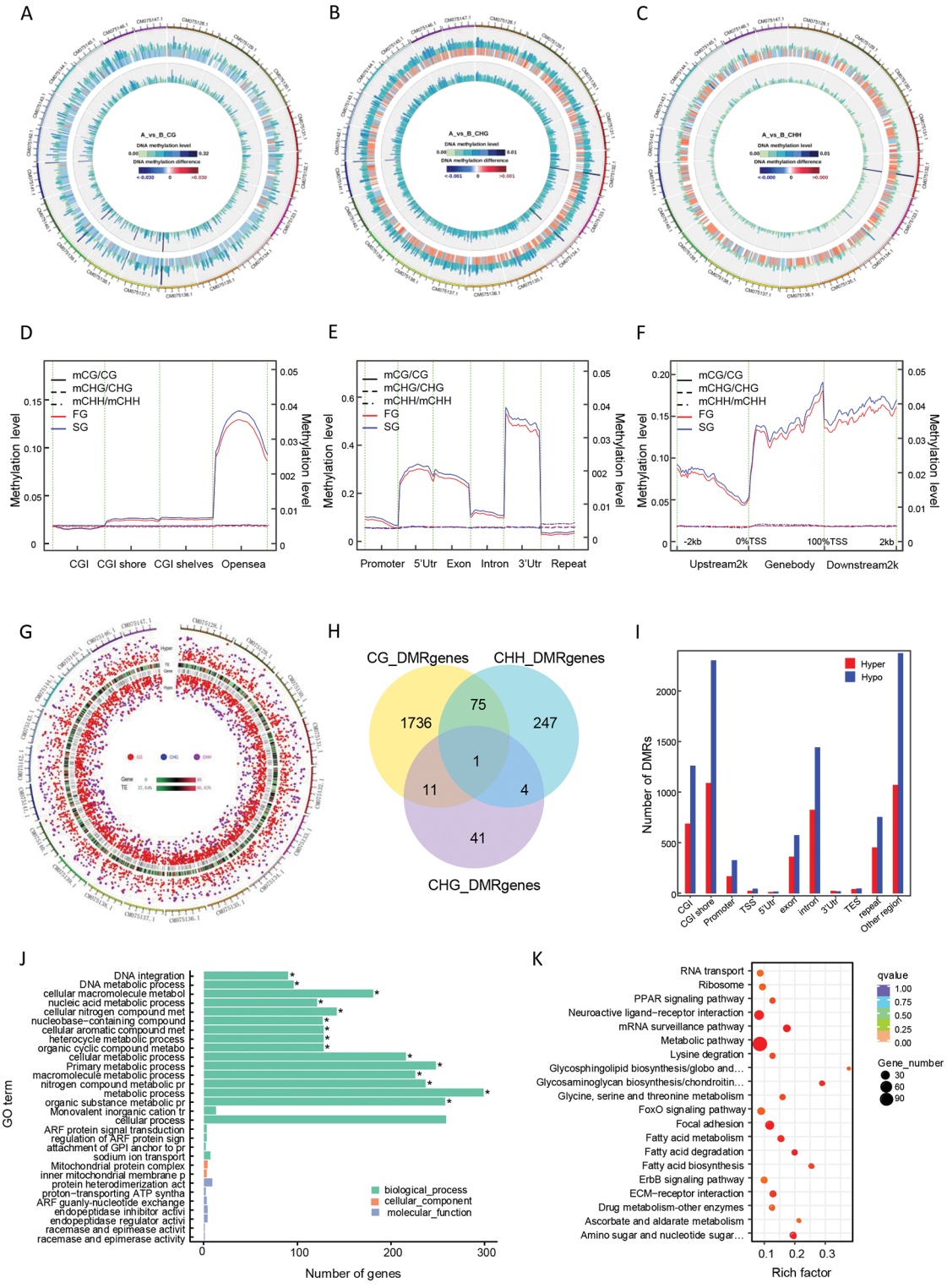


**Fig. 4** Comparative methylome analysis of the muscles of the FG and the SG. **A**, **B**, and **C** represent the Circos plots of the methylation levels of the three types (CG/CHG/CHH), among which the outer circle represents the FG group (designated A), the inner circle represents the SG group (designated B), and the central circle represents the differential methylation levels between the two groups. **D**, The muscle genome-wide methylation landscape of the three sequence contexts (mCG, mCHG, mCHH); **E**, density plot of methylation levels in different functional gene elements; **F**, density plot of methylation levels on different functional gene elements; **G**, landscape of three cytosine sequence contexts between the FG group and the SG group; **H**, Venn diagram of the DMR genes; **I**, distribution of hypomethylated and hypermethylated DMR numbers between the FG and the SG group in different functional gene elements; **J**, GO analysis of DMR target DMGs; **K**, KEGG analysis of DMR target DMGs. Note: DNA methylation is found in three different sequence contexts: CG (or CpG), CHG or CHH (where H correspond to A, T or C). The mCG, mCGH, and mCHH means the methylated CG (or CpG), CHG or CHH

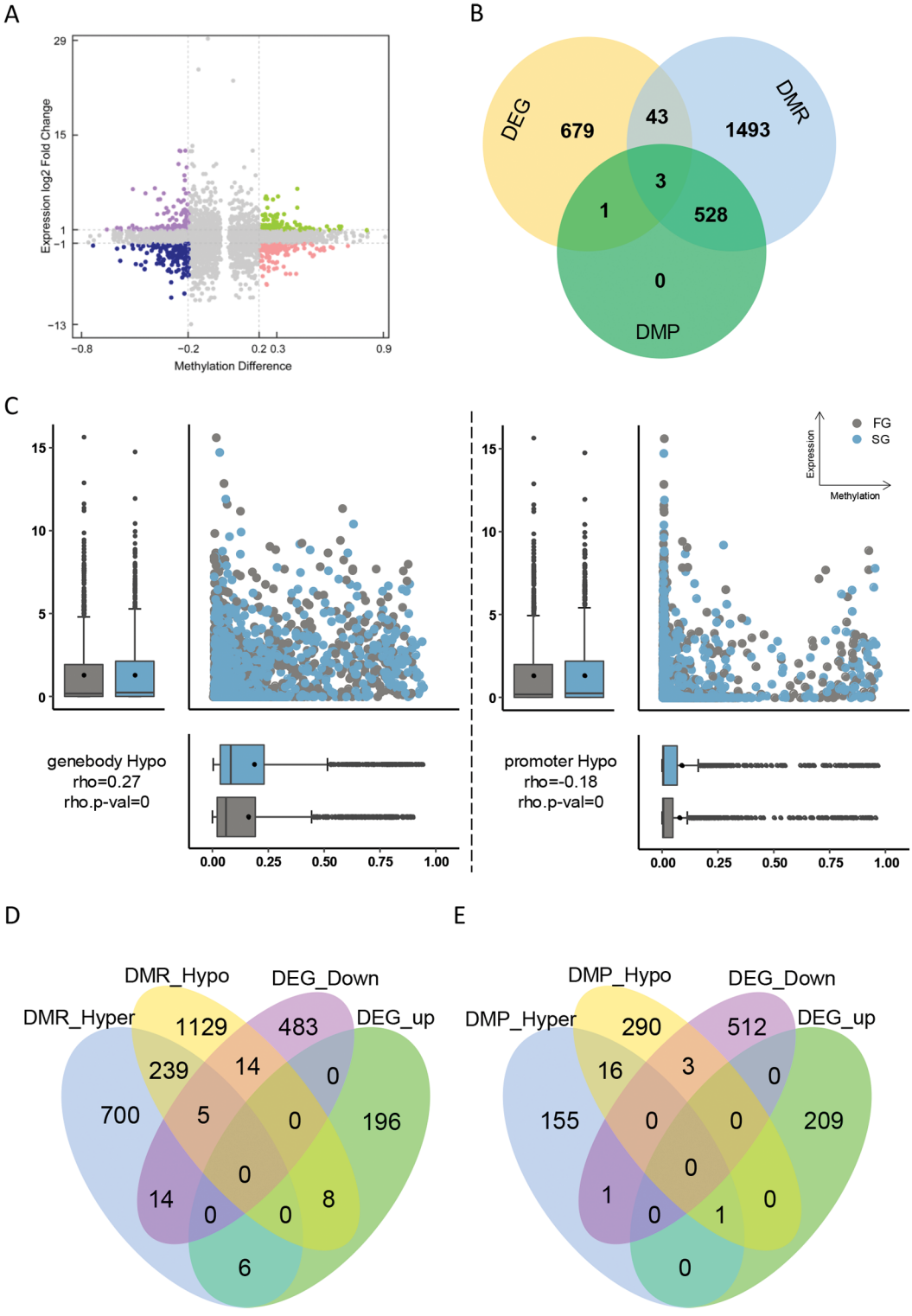


**Fig. 5** Integrative analysis of WGBS and transcriptomics in the muscles of the FG and SG groups. **A**, Volcano plot of DEG expression levels and DMR methylation levels. Venn diagram of the DEGs, DMRs and DMPs in the muscles of the two groups. **B**, Venn diagram of DEG expression and DMR target DMGs in muscles of the FG group and SG group. **C**: Relationships between the methylation levels and expression levels of methylation-related genes. **D**: Venn diagram of DEG expression and DMP target DMGs in muscles between the FG group and SG group

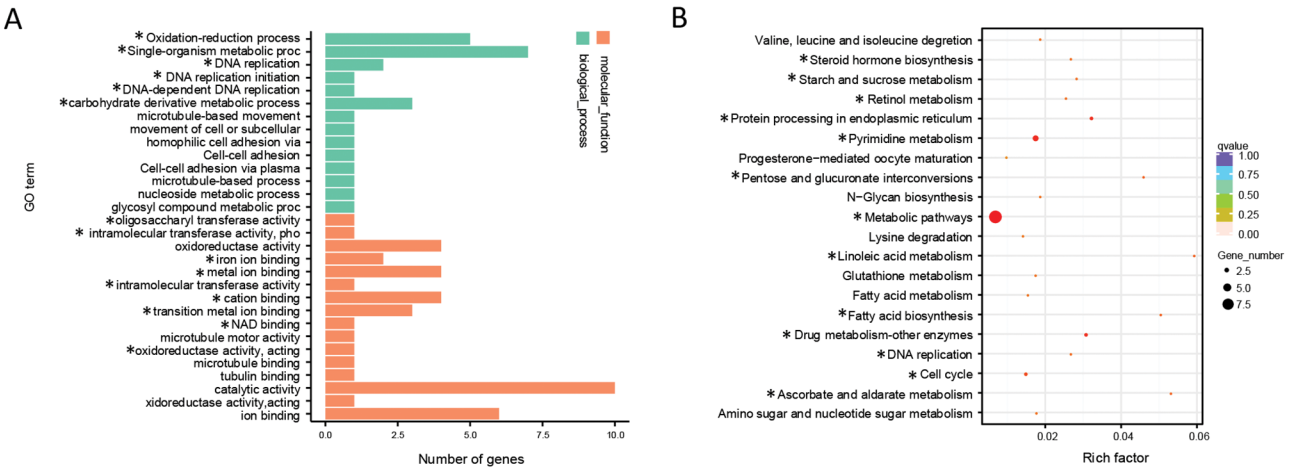


**Fig. 6** **A**, GO enrichment of the overlapping DMR-targeted DMGs with CG types; **B**, KEGG analysis of the overlapping DMR-targeted DMGs with CG types. The items and pathways at a significance of $p < 0.05$ were highlighted with a asterisk

are also of great significance for shrimp growth [41]. Collectively, it is hypothesized that DNA methylation may regulate shrimp growth by influencing gene expression in the hepatopancreas and intestine tissues. Although the expression levels of Dnmt1 and Dnmt3a were the lowest in the muscle tissue of shrimp, a significant difference in the expression of methylation-related genes were observed in the muscles of *L. vannamei* between two groups with extreme growth performance. This finding suggests the existence of a DNA methylation mechanism involved in regulating shrimp growth.

Growth is a critical trait in aquaculture production [42]. Discrepant growth traits in farmed animals pose significant challenges, potentially extending farming cycles and reducing commercial value due to size variations [43]. Numerous studies have highlighted the crucial role of epigenetic modifications in regulating growth traits [21, 44, 45]. Global DNA methylation (5-mC) assays further revealed that the DNA methylation levels in fast-growing (FG) shrimp were lower than those in slow-growing (SG) shrimp. These findings suggest a strong correlation between growth trait performance and DNA methylation in shrimp. On the basis of our results and previous studies demonstrating the impact of DNA methylation on growth traits in various animals(e.g., allotriploid fish [20], Pacific oyster [45], and half-smooth tongue sole [46]), we hypothesize that DNA methylation likely plays a regulatory role in shrimp growth traits. Generally, DNA methylation negatively regulates gene expression [47]. To identify methylation events genome-wide and explore the regulatory mechanisms underlying the discrepant growth traits of *L. vannamei*, we conducted an integrative analysis involving WGBS and transcriptomics. We hypothesize that DNA methylation downregulates the expression of growth-related genes, thereby influencing phenotype development.

In *L. vannamei* shrimp, the analysis of genes shared by DEGs and DMGs showed that promoter methylation levels were negatively associated with DEG expression, whereas genebody methylation levels were positively associated with DEG expression, which were similar to previous findings in other aquatic animals [27, 48, 49], which supporting the idea that the impact of DNA methylation on gene expression levels can be altered at methylated sites in entire gene regions [4]. Metabolic pathways, which were catalyzed by enzymes, sustain cell growth and proliferation through the generation of metabolites [50, 51]. The overlapped DEGs were primarily enriched in metabolic pathways, endoplasmic reticulum protein processing, ascorbate and aldarate metabolism, starch and sucrose metabolism, and the degradation of valine, leucine, and isoleucine. Additionally, they were involved in amino sugar and nucleotide sugar metabolism. Notably, *UGDH* and *UP1*, which were pivotal in various biological processes, were more highly expressed and have lower methylation in FG shrimp than in SG shrimp, suggesting a regulatory role in carbohydrate metabolism [52, 53]. Previous study revealed that as the growth rate of *L. vannamei* increased, the gene expression of *UGDH* involved in the starch and sucrose metabolic pathway was up-regulated for the degradation of the carbohydrate intake for body growth [54], indicating that DNA methylation could regulate the shrimp growth by regulating the expression of *UGDH* for enhancing the carbohydrate metabolism pathway. The expression of *UP1* was positively correlated with the metabolic activity of uridine [55]. Research revealed that uridine can promote the glycogen synthesis in *Nile tilapia* [56]. In addition, it has been found that dietary nucleotide supplementation could improve the growth performance [57, 58]. Taken together, the *UP1* is upregulated in FG shrimp accelerate the carbohydrate metabolism for enhance the growth

performance in a DNA methylation manner. Additionally, *PGM2* and *Cyp6a13*, which were more highly expressed in SG individuals, indicate increased glycogenolysis and fatty acid oxidation for energy. This finding appears contradictory to previous research on *Macrobrachium rosenbergii*, which reported significantly reduced *PGM2* expression in growth-retarded groups [59]. Subsequent studies have revealed that *PGM2* is primarily involved in stress response mechanisms in *L. vannamei*. Specifically, the expression of *PGM2* is up-regulated in response to *Vibrio* invasion [60] and cold stress [61], with similar patterns observed for *Cyp6a13* [62] which indicating that the energy of metabolism were likely used to cope with environment stress rather than growth in a DNA methylation regulation manner [63–65].

## Conclusions

The methylation levels in muscles were notably lower in the FG group than in the SG group. Integrative of WGBS and transcriptomics revealed that the overlapping DEGs/DMGs were enriched mainly in metabolic pathways, including ascorbate and aldarate metabolism, as well as starch and sucrose metabolism. It may be advisable to select appropriate feed additives (by supplementing relevant cofactors) to balance these metabolic pathways to enhance the growth of shrimp. Moreover, this study offers novel perspectives for the development of DNA methylation markers in the genetic breeding of the Pacific white shrimp, *L. vannamei*.

## Materials and methods

### Animal experiments and sample collection

All the shrimp used in this study were obtained from Guangdong Jinyang Biotechnology Co., Ltd., Maoming, Guangdong, China (N21°39′57.81″, E110°55′14.62). A full-sib shrimp family was produced by crossing an inner species. The full-sib progenies were first nursed in the tank for 15 days, and the full-sib shrimp were then moved to an indoor culture pond for further rearing (30 parts per thousand (ppt) and pH 8.2) at 28 ℃ under a 12-h dark: 12-h light photoperiod. After being reared in the culture pond for 90 days, 30 shrimp individuals were firstly selected by quick observation of their body size and then divided into two groups according to their body weight: the fast growth (FG) group and the slow growth (SG) group. Each group contained 15 FG shrimp and 15 SG shrimp. The shrimp were anesthetized on ice and killed by decapitation. Six individuals in each group were randomly selected for subsequent muscle sampling, and the muscle samples were immediately frozen in liquid nitrogen. The DNA and RNA of the 12 shrimp were extracted separately. Subsequently, the DNA and RNA from every two shrimp in each group were pooled to form a single sample for sequencing and analysis. Furthermore, the DNA methylation level in shrimps with discrepant growth trait were detected by using the Global DNA Methylation (5-mC) ELISA Easy Kit (Catalog#P-1030, EpigenTek). All experiments should be performed according to the corresponding kit instructions.

### Identification and expression analysis of DNA methylation genes

PF00145 (C-5 cytosine methyltransferase conserved domain) was used as a query to search for DNA methylation genes against genomic resources to acquire candidate genes via HMMER (https://www.ebi.ac.uk/Tools/HMMER/) and Pfam (http://pfam.xfam.org/) [66]. Then, the putative DNA methylation genes sequences were further blasted against the NCBI nonredundant protein database via the BLASTP (https://blast.ncbi.nlm.nih.gov/) method [66]. The conserved motifs of the amino acid sequences were predicted via MEME software [67]. The NCBI Conserved Domain Database was used to search the conserved domains of the amino acid sequences with an E value threshold of 0.01. The phylogenetic trees of DNA methylation genes from six crustacean species (*L. vannamei*, *Penaeus monodon*, *Procambarus clarkii*, *Portunus trituberculatus*, *Penaeus chinensis* and *Eriocheir sinensis*) were constructed on the phyloSuite platform. The divergent tree was obtained from Timetree5 (http://timetree.org/) [66]. Furthermore, a phylogenetic tree of DNA methylation enzymes from six crustaceans was constructed via the TBtools platform [68].

The expression levels of DNA methylation enzyme related genes (*dnmt1*, *dnmt3a*) in the muscles of shrimp from the two groups were assessed by RT-qPCR. Briefly, the total RNA was extracted with TRIzol reagent and reverse transcribed with PrimeScriptTM RT reagent Kit containing gDNA eraser (Takara). The cDNA samples obtained were then subjected to a Thermal Cycler Dice©R Real Time System III (Takara) for quantitative analysis with the TB GreenTM Premix EX TaqTM II Kit (Takara). The PCR primers used were listed in Tab. S1. Gene expression was normalized to that of *GAPDH*, which was used as a reference [69]. The relative expression levels of DNA methylation enzyme related genes were calculated using the comparative Ct method with the formula $2^{-\Delta\Delta Ct}$.

The expression profiles of DNA methylation genes in different healthy *L. vannamei* tissues, including eyestalk (Es), brain (Br), abdominal nerve (AN), thoracic nerve (TN), gill (Gi), heart (Ht), hepatopancreas (Hp), hemolymph (He), muscle (Ms), stomach (St), intestine (In), were analyzed according to RNA-seq data [31].

### RNA-seq and data analysis

The Agilent 2100 bioanalyzer were employed to evaluate the integrity and total quantity of RNA. The RNA with an OD260/OD280 ratio ranging from 1.8 to 2.4, along with an RNA integrity number (RIN) that satisfies the condition $7 \leq RIN < 10$, is considered suitable for RNA-seq. Following successful quality assessment, the sequencing library were constructed and quality control processes were employed. Oligo dT magnetic beads were utilized to enrich mRNA from total RNA. The mRNA was subsequently subjected to end repair, A-tailing, adapter ligation, size selection, amplification, and purification, culminating in the preparation of the library. Upon passing quality control, Illumina sequencing was conducted to obtain paired-end reads of 150 bp. The raw sequencing data (raw reads) underwent processing through fastp software to eliminate reads containing adapters, poly-N reads, and low-quality reads, resulting in clean reads. The clean reads were aligned to the reference genome (CIBNOR_Pvan_1v2) by using the HISAT2 v2.0.5, and the count reads for each gene were calculated via FeatureCounts (version 1.5.0-p3), and the FPKM value for each gene were calculated. DESeq2 software (version 1.20.0) were employed to analysis the differential expression genes between the two groups. The DEGs were selected for Gene ontology (GO) enrichment analysis via the clusterProfiler (version 3.8.1) software. Additionally, clusterProfiler (version 3.8.1) software was used to analyze the statistical enrichment of DEGs in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [27].

### WGBS analyses

The DNA samples underwent bisulfite conversion following the EZ DNA Methylation-Gold Kit protocol. The converted DNA was then fragmented into random lengths of 200–400 bp using an ultrasonic processor. Single-stranded DNA (ssDNA) segments, after bisulfite treatment, were converted by using the EZ DNA Methylation-GoldTM Kit (zymo Research). Next, a methylation sequencing adapter was ligated to the DNA fragment, which was then subjected to size selection and PCR amplification. Library preparation was performed using the Accel-NGS MethylSEQ DNA Library Kit (Swift, USA, catalog number: 30096). The resulting libraries were evaluated for quality on the Agilent 5400 system (Agilent, USA) and quantified via QPCR. Double-ended sequencing of the samples was carried out on the Illumina platform (Illumina, USA). Following quality control and data filtering using FastQC software, the bisulfite-treated reads were aligned to a reference genome set to -X 700 -dovetail using Bismark software (version 0.24.0). Differential methylation regions (DMRs) and differential methylation promoters (DMPs) were identified using DSS software (version 2.12.0). Based on the distribution of DMRs across the genome, genes were defined as those overlapping with DMRs within the genebody region (from the transcription start site (TSS) to the transcription end site (TES)) or promoter region (2 kb upstream of the TSS). The DMRs associated genes were identified for the GO enrichment analysis and KEGG enrichment via the GOseq R package and KOBAS software (version 3.0) [27].

### Integrative analysis of WGBS and RNA-seq data

To investigate the relationship between DNA methylation and gene expression in the muscles of the two groups, we conducted a correlation analysis to identify mutually DMGs, including DMRs and DMPs, from WGBS data and DEGs from RNA-seq data. The correlation between DMR methylation levels and corresponding differential gene expression levels in the transcriptome was visualized via a combination of scatter plots [70] and boxplots [71], as previously described. The MethExpress software were used for integrated analysis of methylation and transcriptome data. The threshold for differential methylation level is set to 0.2, and the threshold for differential expression log2(Fold change) is set to 1. Visualization of the results is performed using the plotting tools in R (ggplot2). The GO and KEGG pathway enrichment analyses of the overlapping DMGs were further conducted by using the clusterProfiler (default parameter) as described previously [27].

### Statistical analysis

The data were presented as means ± standard deviations ($n = 3$). For the statistical analysis, parametric tests were chosen and carried out. Specifically, unpaired t-tests were employed to analyze the data. Statistical analysis and the diagram display were conducted via GraphPad Software. Statistical significance is indicated by a $p$ value of less than 0.05.

#### Abbreviations

| | |
|---|---|
| WGBS | Whole-genome bisulfite sequencing |
| DMP | Differentially methylated promoters |
| DMRs | Differentially methylated regions |
| DMGs | Differential methylated genes |
| TSS | Transcription start site |
| TES | Transcription end site |
| CpG islands | CGIs |
| RNA-seq | RNA sequencing |
| DEGs | Differential expression genes |
| CPM | Counts per million |
| ssDNA | Single-stranded DNA |
| AASS | Aminoadipate-Semialdehyde Synthase |
| 4CL-like 1 | 4-Coumarate: CoA ligase 4 |
| UP1 | Uridine Phosphorylase 1 |
| RNR | Ribonucleotide reductase |
| UGDH | UDP-Glucose 6-Dehydrogenase |
| PGM2 | Phosphoglucomutase 2 |
| UTR | Untranslated Regions |
| Cyp6a13 | Cytochrome P450 family 6 subfamily A member 13 |
| cyp19a | Cytochrome P450 family 19 subfamily A |

| | |
|---|---|
| RIG-I gene | Retinoic acid inducible gene-I |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |
| DNMT | DNA methyltransferase |
| Trmt | TRNA Methyltransferase |
| qRT-PCR | Real-Time Quantitative Reverse Transcription PCR |
| SNPs | Single-nucleotide polymorphisms |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11688-6.

Supplementary Material 1: Figure S1 Sequences alignment of DNA (cytosine-5)-methyltransferase 3A. Figure S2 Sequences alignment of DNA (cytosine-5)-methyltransferase 1. Figure S3 Sequences alignment of tRNA (cytosine(38)-C(5))-methyltransferase.

Supplementary Material 2: Supplementary Table S1 Primers used in the present study.

Supplementary Material 3: Supplementary Table S2 Overview of the transcriptome data.

Supplementary Material 4: Supplementary Table S3 Expressed genes assembled in detail.

Supplementary Material 5: Supplementary Table S4 GO enrichment of the DEGs in the FG and the SG.

Supplementary Material 6: Supplementary Table S5 KEGG enrichment of the DEGs in the FG and the SG.

Supplementary Material 7: Supplementary Table S6 GO enrichment of FG vs SG. CG DMR genes.

Supplementary Material 8: Supplementary Table S7 KEGG enrichment of FG vs SG. CG DMR genes.

Supplementary Material 9: The PCR primers' efficiency validation.

**Acknowledgements**
We also thank the data archive support from the National Earth System Data Center, National Science & Technology Infrastructure of China (http://www.geodata.cn).

**Author contributions**
X. Zh.: Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing-Original Draft, Writing-Review & Editing; C. H: Project administration, Supervision, Funding acquisition; T. Ch., Y. T., C. R.: Supervision; P. L., Y. W., Y. H., B. M., J. Y.: Investigation; X. J.: Validation; L. L., H. L.: Formal analysis; P. L.: Conceptualization, Supervision, Project administration, Funding acquisition.

**Funding**
This research was supported by the National Key Research and Development Program of China (2023YFD2401701), the Seed Industry Revitalization Project of Provincial Rural Revitalization Strategy Special Funds (2022-SPY-00-001), the Research on Breeding Technology of Candidate Species for Guangdong Modern Marine Ranching (2024-MRB-00-001), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB07302004), the Molecular Mechanism of Rapid Growth and Stress Resistance Traits in ***Penaeus vannamei*** (E4DQ2B01) and the Science and Technology Planning Project of Guangdong Province, China (2023B12060047).

**Data availability**
Data for this manuscript are available at Scientific Data Center, South China Sea Institute of Oceanology, CAS https://data.scsio.ac.cn/metaData-detail/1871893753677438976.

## Declarations

**Ethics approval and consent to participate**
The animal experiments were conducted in accordance with the guidelines and approval of the Animal Ethics Committee of South China Sea Institute of Oceanology, Chinese Academy of Sciences.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

Received: 16 December 2024 / Accepted: 9 May 2025
Published online: 20 May 2025

**References**

1. Ilango S, Paital B, Jayachandran P, Padma PR, Nirmaladevi R. Epigenetic alterations in cancer. FBL. 2020;25(6):1058–109.
2. Zhu W, Yang C, Liu Q, Peng M, Li Q, Wang H, Chen X, Zhang B, Feng P, Chen T et al. Integrated analysis of DNA methylome and transcriptome reveals epigenetic regulation of cold tolerance in *Litopenaeus vannamei*. Int J Mol Sci. 2023;24(14).
3. Vanyushin BF. Epigenetics today and tomorrow. Russian J Genetics: Appl Res. 2014;4(3):168–88.
4. Kumar S, Mohapatra T. Dynamics of DNA methylation and its functions in plant growth and development. Front Plant Sci. 2021;12.
5. Bonasio R, Li Q, Lian J, Mutti NS, Jin L, Zhao H, Zhang P, Wen P, Xiang H, Ding Y, et al. Genome-wide and caste-specific DNA methylomes of the ants *Camponotus floridanus* and *Harpegnathos saltator*. Curr Biology: CB. 2012;22(19):1755–64.
6. Schübeler D. Function and information content of DNA methylation. Nature. 2015;517(7534):321–6.
7. Liu Z, Zhou T, Gao D. Genetic and epigenetic regulation of growth, reproduction, disease resistance and stress responses in aquaculture. Front Genet. 2022;13.
8. Fan Y, Sun C, Yan K, Li P, Hein I, Gilroy EM, Kear P, Bi Z, Yao P, Liu Z et al. Recent Advances in Studies of Genomic DNA Methylation and Its Involvement in Regulating Drought Stress Response in Crops. Plants (Basel). 2024;13(10).
9. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology. 2013;38(1):23–38.
10. Klughammer J, Romanovskaia D, Nemc A, Posautz A, Seid CA, Schuster LC, Keinath MC, Lugo Ramos JS, Kosack L, Evankow A, et al. Comparative analysis of genome-scale, base-resolution DNA methylation profiles across 580 animal species. Nat Commun. 2023;14(1):232.
11. Feng S, Cokus SJ, Zhang X, Chen P-Y, Bostick M, Goll MG, Hetzel J, Jain J, Strauss SH, Halpern ME. Conservation and divergence of methylation patterning in plants and animals. Proc Natl Acad Sci U S A. 2010;107(19):8689–8694.
12. Yang Y, Zhou T, Liu Y, Tian C, Bao L, Wang W, Zhang Y, Liu S, Shi H, Tan S. Identification of an epigenetically marked locus within the sex determination region of channel catfish. Int J Mol Sci. 2022;23(10):5471.
13. Wan ZY, Xia JH, Lin G, Wang L, Lin VC, Yue GH. Genome-wide methylation analysis identified sexually dimorphic methylated regions in hybrid tilapia. Sci Rep. 2016;6(1):35903.
14. Gavery MR, Roberts SB. A context dependent role for DNA methylation in bivalves. Brief Funct Genomics. 2014;13(3):217–22.
15. Vogt G. Studying phenotypic variation and DNA methylation across development, ecology and evolution in the clonal marbled crayfish: A paradigm for investigating epigenotype-phenotype relationships in macro-invertebrates. Sci Nat. 2022;109(1):16.
16. Kvist J, Gonçalves Athanàsio C, Shams Solari O, Brown JB, Colbourne JK, Pfrender ME, Mirbahai L. Pattern of DNA methylation in *Daphnia*: evolutionary perspective. Genome Biol Evol. 2018;10(8):1988–2007.
17. Hearn J, Plenderleith F, Little TJ. DNA methylation differs extensively between strains of the same geographical origin and changes with age in *Daphnia magna*. Epigenetics Chromatin. 2021;14:1–14.
18. Asselman J, De Coninck DI, Pfrender ME, De Schamphelaere KA. Gene body methylation patterns in Daphnia are associated with gene family size. Genome Biol Evol. 2016;8(4):1185–96.

19. Liu Z, Zhou T, Gao D. Genetic and epigenetic regulation of growth, reproduction, disease resistance and stress responses in aquaculture. Front Genet. 2022;13:994471.
20. Ren L, Zhang H, Luo M, Gao X, Cui J, Zhang X, Liu S. Heterosis of growth trait regulated by DNA methylation and MiRNA in allotriploid fish. Epigenetics Chromatin. 2022;15(1):19.
21. Ou M, Mao H, Luo Q, Zhao J, Liu H, Zhu X, Chen K, Xu H. The DNA methylation level is associated with the superior growth of the hybrid fry in Snakehead fish (*Channa Argus* × *Channa maculata*). Gene. 2019;703:125–33.
22. Huang Y, Wen H, Zhang M, Hu N, Si Y, Li S, He F. The DNA methylation status of myod and IGF-I genes are correlated with muscle growth during different developmental stages of Japanese flounder (*Paralichthys olivaceus*). Comp Biochem Physiol B: Biochem Mol Biol. 2018;219:33–43.
23. Shang X, Wan Q, Su J, Su J. DNA methylation of CiRIG-I gene notably relates to the resistance against GCRV and negatively-regulates mRNA expression in grass carp, *Ctenopharyngodon idella*. Immunobiology. 2016;221(1):23–30.
24. Wen A, You F, Sun P, Li J, Xu D, Wu Z, Ma D, Zhang P. CpG methylation of dmrt1 and cyp19a promoters in relation to their sexual dimorphic expression in the Japanese flounder *Paralichthys olivaceus*. J Fish Biol. 2014;84(1):193–205.
25. Beemelmanns A, Ribas L, Anastasiadi D, Moraleda-Prados J, Zanuzzo FS, Rise ML, Gamperl AK. DNA methylation dynamics in Atlantic salmon (*Salmo salar*) challenged with high temperature and moderate hypoxia. Front Mar Sci. 2021;7:604878.
26. Pierron F, Lorioux S, Héroin D, Daffe G, Etcheverria B, Cachot J, Morin B, Dufour S, Gonzalez P. Transgenerational epigenetic sex determination: environment experienced by female fish affects offspring sex ratio. Environ Pollut. 2021;277:116864.
27. Yang J, Liu M, Zhou T, Li Q, Lin Z. Genome-wide methylome and transcriptome dynamics provide insights into epigenetic regulation of kidney functioning of large yellow croaker (*Larimichthys crocea*) during low-salinity adaption. Aquaculture. 2023;571:739410.
28. Yu Y, Wang Q, Zhang Q, Luo Z, Wang Y, Zhang X, Huang H, Xiang J, Li F. Genome scan for genomic regions and genes associated with growth trait in Pacific white shrimp *Litopeneaus vannamei*. Mar Biotechnol. 2019;21:374–83.
29. Huang W, Cheng C, Liu J, Zhang X, Ren C, Jiang X, Chen T, Cheng K, Li H, Hu C. Fine mapping of the high-pH tolerance and growth trait-related quantitative trait loci (QTLs) and identification of the candidate genes in Pacific white shrimp (*Litopenaeus vannamei*). Mar Biotechnol. 2020;22:1–18.
30. Shin HS, Chimborazo MEM, Rivas JME, Lorenzo-Felipe Á, Soler MM, Serrano MJZ, Martín JF, Artiles JSR, Sánchez AP, Navarro JL. Genetic parameters for growth and morphological traits of the Pacific white shrimp *Penaeus vannamei* from a selective breeding programme in the industrial sector of Ecuador. Aquaculture Rep. 2023;31:101649.
31. Li J, Zhao M, Zhang X, Zheng Z, Yao D, Yang S, Chen T, Zhang Y, Aweya JJ. The evolutionary adaptation of shrimp Hemocyanin subtypes and the consequences on their structure and functions. Fish Shellfish Immunol. 2024;145:109347.
32. Wikumpriya GC, Prabhatha MWS, Lee J, Kim C-H. Epigenetic modulations for prevention of infectious diseases in shrimp aquaculture. Genes. 2023;14(9):1682.
33. Dowen RH, Pelizzola M, Schmitz RJ, Lister R, Dowen JM, Nery JR, Dixon JE, Ecker JR. Widespread dynamic DNA methylation in response to biotic stress. Proc Natl Acad Sci. 2012;109(32):E2183–91.
34. Katirtzoglou A, Hansen SB, Sveier H, Martin MD, Brealey JC, Limborg MT. Genomic context determines the effect of DNA methylation on gene expression in the gut epithelium of Atlantic salmon (*Salmo salar*). Epigenetics. 2024;19(1):2392049.
35. Qin W, Scicluna BP, van der Poll T. The role of host cell DNA methylation in the immune response to bacterial infection. Front Immunol. 2021;12:696280.
36. Jjingo D, Conley AB, Yi SV, Lunyak VV, Jordan IK. On the presence and role of human gene-body DNA methylation. Oncotarget. 2012;3(4):462–74.
37. Cui D, Xu X. DNA methyltransferases, DNA methylation, and age-associated cognitive function. Int J Mol Sci. 2018;19(5):1315.
38. Shah P, Hill R, Dion C, Clark SJ, Abakir A, Willems J, Arends MJ, Garaycoechea JI, Leitch HG, Reik W, et al. Primordial germ cell DNA demethylation and development require DNA translesion synthesis. Nat Commun. 2024;15(1):3734.
39. Fazio A, Bordoni D, Kuiper JWP, Weber-Stiehl S, Stengel ST, Arnold P, Ellinghaus D, Ito G, Tran F, Messner B, et al. DNA methyltransferase 3A controls intestinal epithelial barrier function and regeneration in the colon. Nat Commun. 2022;13(1):6266.
40. Yu D-H, Gadkari M, Zhou Q, Yu S, Gao N, Guan Y, Schady D, Roshan TN, Chen M-H, Laritsky E, et al. Postnatal epigenetic regulation of intestinal stem cells requires DNA methylation and is guided by the Microbiome. Genome Biol. 2015;16(1):211.
41. Li S, Liu H, Huang W, Yang S, Xie M, Zhou M, Lu B, Li B, Tan B, Yang Y et al. Effect of three polychaetes on growth and reproductive performance, biochemical indices and histology of different tissues in the female Pacific white shrimp, *Litopenaeus vannamei* broodstock. Animal Nutrition 2025.
42. Froehlich HE, Gentry RR, Halpern BS. Synthesis and comparative analysis of physiological tolerance and life-history growth traits of marine aquaculture species. Aquaculture. 2016;460:75–82.
43. Ma B, Liu Y, Pan W, Li Z, Ren C, Hu C, Luo P. Integrative application of transcriptomics and metabolomics provides insights into unsynchronized growth in sea cucumber (*Stichopus monotuberculatus*). Int J Mol Sci. 2022;23(24):15478.
44. Jiang C, Deng C, Xiong Y. Differences of cytosine methylation in parental lines and F1 hybrids of large White× Meishan crosses and their effects on F1 performance. J Agric Biotechnol. 2007;2:195–200.
45. Yang H, Li Q. The DNA methylation level is associated with the superior growth of the hybrid crosses in the Pacific oyster *Crassostrea gigas*. Aquaculture. 2022;547:737421.
46. Zhao JL, Si YF, He F, Wen HS, Li JF, Ren YY, Zhao ML, Huang ZJ, Chen SL. Polymorphisms and DNA methylation level in the CpG site of the GHR1 gene associated with mRNA expression, growth traits and hormone level of half-smooth tongue sole (*Cynoglossus semilaevis*). Fish Physiol Biochem. 2015;41(4):853–65.
47. Bommarito PA, Fry RC. The role of DNA methylation in gene regulation. In: *Toxicoepigenetics*. Elsevier; 2019: 127–151.
48. Tai Z, Guan P, Zhang T, Liu W, Li L, Wu Y, Li G, Liu J-X. Effects of parental environmental copper stress on offspring development: DNA methylation modification and responses of differentially methylated region-related genes in transcriptional expression. J Hazard Mater. 2022;424:127600.
49. Hu Q, Ao Q, Tan Y, Gan X, Luo Y, Zhu J. Genome-Wide DNA methylation and RNA analysis reveal potential mechanism of resistance to *Streptococcus agalactiae* in GIFT strain of nile Tilapia (*Oreochromis niloticus*). J Immunol. 2020;204(12):3182–90.
50. Hamdalla MA, Rajasekaran S, Grant DF, Măndoiu II. Metabolic pathway predictions for metabolomics: a molecular structure matching approach. J Chem Inf Model. 2015;55(3):709–18.
51. Schiliro C, Firestein BL. Mechanisms of metabolic reprogramming in Cancer cells supporting enhanced growth and proliferation. Cells. 2021;10(5):1056.
52. Warfield BM, Reigan P. Multifunctional role of thymidine phosphorylase in cancer. Trends Cancer. 2022;8(6):482–93.
53. Yang Y, Ye Y, Deng Y, Gao L. Uridine and its role in metabolic diseases, tumors, and neurodegenerative diseases. Front Physiol. 2024;15.
54. Wang Y, Wang B, Liu M, Jiang K, Wang M, Wang L. Comparative transcriptome analysis reveals the potential influencing mechanism of dietary Astaxanthin on growth and metabolism in *Litopenaeus vannamei*. Aquaculture Rep. 2020;16:100259.
55. Nwosu ZC, Ward MH, Sajjakulnukit P, Poudel P, Ragulan C, Kasperek S, Radyk M, Sutton D, Menjivar RE, Andren A. Uridine-derived ribose fuels glucose-restricted pancreatic cancer. Nature. 2023;618(7963):151–8.
56. Zhou N-N, Wang T, Lin Y-X, Xu R, Wu H-X, Ding F-F, Qiao F, Du Z-Y, Zhang M-L. Uridine alleviates high-carbohydrate diet-induced metabolic syndromes by activating sirt1/AMPK signaling pathway and promoting glycogen synthesis in nile tilapia (*Oreochromis niloticus*). Anim Nutr. 2023;14:56–66.
57. LIN Y-H, WANG H, SHIAU S-Y. Dietary nucleotide supplementation enhances growth and immune responses of Grouper, *Epinephelus malabaricus*. Aquacult Nutr. 2009;15(2):117–22.
58. Tahmasebi-Kohyani A, Keyvanshokooh S, Nematollahi A, Mahmoudi N, Pasha-Zanoosi H. Dietary administration of nucleotides to enhance growth, humoral immune responses, and disease resistance of the rainbow trout (*Oncorhynchus mykiss*) fingerlings. Fish Shellfish Immunol. 2011;30(1):189–93.
59. Li X, Li Y, Dai X. Transcriptomics-based analysis of *Macrobrachium rosenbergii* growth retardation. Comp Biochem Physiol D: Genomics Proteomics. 2024;52:101298.
60. Sun M, Yu Y, Li S, Liu Y, Zhang X, Li F. Integrated application of transcriptomics and metabolomics provides insights into acute hepatopancreatic necrosis disease resistance of Pacific white shrimp Litopenaeus vannamei. mSystems. 2023;8(4):e00067–00023.
61. Al-Bakheit Aa, Traka M, Saha S, Mithen R, Melchini AJTP. Accumulation of palmitoylcarnitine and its effect on pro-inflammatory pathways and calcium influx in prostate cancer. The Prostate. 2016;76(14):1326–37.

62. Marie AD, Smith S, Green AJ, Rico C, Lejeusne C. Transcriptomic response to thermal and salinity stress in introduced and native sympatric *Palaemon* Caridean shrimps. Sci Rep. 2017;7(1):13980.
63. Eixelsberger T, Brecker L, Nidetzky B. Catalytic mechanism of human UDP-glucose 6-dehydrogenase: in situ proton NMR studies reveal that the C-5 hydrogen of UDP-glucose is not exchanged with bulk water during the enzymatic reaction. Carbohydr Res. 2012;356:209–14.
64. Dai P, Luan S, Lu X, Luo K, Kong J. Comparative transcriptome analysis of the Pacific white shrimp (*Litopenaeus vannamei*) muscle reveals the molecular basis of residual feed intake. Sci Rep. 2017;7(1):10483.
65. Hardwick JP. Cytochrome P450 Omega hydroxylase (CYP4) function in fatty acid metabolism and metabolic diseases. Biochem Pharmacol. 2008;75(12):2263–75.
66. Zhu B, Zhang M, Li M, Pan K, Yao P, Zhang X, Wu X. Genome-wide identification and gene expression pattern reveal dominant digestive enzyme genes contributing to major enzyme activities during the molting cycle of the Chinese mitten crab, *Eriocheir sinensis*. Aquaculture. 2025;597:741944.
67. Bailey TL, Johnson J, Grant CE, Noble WS. The MEME suite. Nucleic Acids Res. 2015;43(W1):W39–49.
68. Chen Cj, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–202.
69. Ruan Y, Wong N-K, Zhang X, Zhu C, Wu X, Ren C, Luo P, Jiang X, Ji J, Wu X et al. Vitellogenin receptor (VgR) mediates oocyte maturation and ovarian development in the Pacific white shrimp (*Litopenaeus vannamei*). Front Physiol. 2020;485(11).
70. Yang IV, Pedersen BS, Rabinovich E, Hennessy CE, Davidson EJ, Murphy E, Guardela BJ, Tedrow JR, Zhang Y, Singh MK, et al. Relationship of DNA methylation and gene expression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2014;190(11):1263–72.
71. Kretzmer H, Bernhart SH, Wang W, Haake A, Weniger MA, Bergmann AK, Betts MJ, Carrillo-de-Santa-Pau E, Doose G, Gutwein J, et al. DNA methylome analysis in Burkitt and follicular lymphomas identifies differentially methylated regions linked to somatic mutation and transcriptional control. Nat Genet. 2015;47(11):1316–25.

## Publisher's note