



Transcriptome and miRNA Profiles of Black Tiger Shrimp, *Penaeus monodon*, Under Different Salinity Conditions

Yundong Li, Falin Zhou, Jianhua Huang, Lishi Yang, Song Jiang, Qibin Yang and Shigui Jiang*

Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture and Rural Affairs, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Guangzhou, China

Keywords: transcriptome, miRNA, salinity, *Penaeus monodon*, penaeid shrimp

INTRODUCTION

The black tiger shrimp (*Penaeus monodon*) has high economic and nutritional value, and is the second widely cultured penaeid shrimp species worldwide (FAO, 2018). Extensive researches and development programs of the black tiger shrimp have focused on understanding its various biological processes, improving farming practices, and advancing selective breeding programs to achieve the sustainable shrimp farming industry. Through the genetic breeding research conducted by our team on *P. monodon* in China, we have cultivated two new varieties of *P. monodon*, “Nanhai No. 1” and “Nanhai No. 2,” which have solved problems related to their growth and survival rates. However, despite breakthroughs in shrimp breeding, salinity remains an impeding factor for the development of shrimp aquaculture.

Salinity is an important environmental factor related to the physiological function, growth, and survival of shrimp (Li et al., 2017). *P. monodon* is primarily cultured in coastal areas with estuarine and oceanic water, while the production of euryhaline shrimp has expanded to inland recently, where have the advantage of the economic development and clean environment with sufficient groundwater supply (Roy et al., 2010). *P. monodon* is to be a promising aquaculture species for low salinity farming in inland worldwide. However, long-term low salinity cultivation is associated with multiple problems, including slow growth and increased susceptibility to pathogens. These problems restrict the large-scale promotion of *P. monodon* cultures in low salinity areas.

Previous studies on the responses of shrimp to salinity stress have primarily been concentrated on several important genes (Shekhar et al., 2013; Zhao et al., 2015; Xu et al., 2016), but the molecular mechanisms underlying the responses of shrimp to salinity stress, in relation to other salinity-regulated genes, remains unclear. Recently, a transcriptome regulation mechanism of the rapid and reversible post-transcription, via the action of non-coding RNA molecules, has been reported to be involved in environmental stress survival responses (Ebert and Sharp, 2012; Biggar and Storey, 2015). Various studies have shown that miRNAs were involved in many biochemical and physiological processes and played important roles in stress responses in aquatic organisms (Burgos-Aceves et al., 2018; Wang et al., 2018, 2020). However, to the best of our knowledge, few studies focused on stress-responsive miRNAs in *P. monodon*.

Transcriptomic profiling using next-generation sequencing technologies addresses this gap by providing a new approach to study stress response mechanisms in shrimp. For example, the transcriptome analysis of three different salinities cultivation conditions for *Litopenaeus vannamei* revealed numerous potential genes related to the responses of osmose (Zhang et al., 2016). In a transcriptome analysis of *L. vannamei* under chronic low-salinity stress, 855 genes showed

OPEN ACCESS

Edited by:

Kit Yue Kwan,
Beibu Gulf University, China

Reviewed by:

Huan Wang,
Ningbo University, China
Jianguo He,
Sun Yat-sen University, China

*Correspondence:

Shigui Jiang
jiangsg@21cn.com

Specialty section:

This article was submitted to
Marine Fisheries, Aquaculture and
Living Resources,
a section of the journal
Frontiers in Marine Science

Received: 02 July 2020

Accepted: 07 September 2020

Published: 07 October 2020

Citation:

Li Y, Zhou F, Huang J, Yang L, Jiang S,
Yang Q and Jiang S (2020)
Transcriptome and miRNA Profiles of
Black Tiger Shrimp, *Penaeus
monodon*, Under Different Salinity
Conditions. *Front. Mar. Sci.* 7:579381.
doi: 10.3389/fmars.2020.579381

significant expression changes, and these were enriched in lipid metabolism, the phosphonate and phosphinate metabolism pathways, and steroid hormone biosynthesis (Chen et al., 2015). Transcriptomic profiling has also elucidated potentially novel miRNAs or stress-related miRNAs. There were 4,524 known miRNAs and 73 novel miRNAs reported to be significantly differentially expressed after copper exposure by small RNA sequencing analysis in shrimp under copper stress (Guo et al., 2018). A miRNA analysis of the hepatopancreas of *L. vannamei* using high-throughput sequencing, examined the potential regulatory mechanisms of cold adaptation and revealed 68 known mature miRNAs, 47 novel miRNAs, and 34 miRNAs that were related to cold adaptation (He et al., 2018). Overall, these transcriptomic and miRNA analyses of stress responses in shrimp allow us to understand their molecular mechanisms and identify novel genes or miRNAs related to environmental stress.

Here, we performed RNA sequencing analysis and detected differential expression genes and miRNAs in the gills of *P. monodon* at different groups with high, medium, and low salinity culture conditions, respectively. The quality control of sequencing data was conducted to present a high-quality dataset. We also performed an integrative comparative analysis of mRNA and miRNA at three salinities, to screen for key genes and miRNAs associated with salinity stress. These transcriptomic and miRNA data could provide a valuable resource for further shrimp studies on screening genes related to salinity stress responses and understanding the molecular mechanism of salinity stress.

METHODS

Ethics Statement

The study was approved by the Animal Care and Use Committee of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (No. SCSFRI96-253). Sampling and all experiments strictly complied with the guidelines and regulations established by the committee.

Shrimp Rearing and Stress Treatment

In the present study, healthy black tiger shrimp with 7.25 ± 0.26 g weights were collected from the experimental base of the South China Sea Fisheries Research Institute (SCSFRI, Shenzhen, China). All shrimp were cultured in laboratory tanks for 1 week and fed by shrimp diet for four times a day. The laboratory tanks were filled with filtered seawater (pH, 7.8–8.2; temperature, 27–29°C; salinity, 18 psu) and aerated constantly. To maintain water quality, we changed about 1/3 of the tanks' water daily. Then, all individuals were randomly divided into three groups which would be cultivated in three different environments for 30 days: high salinity [HC, 30 psu (practical salinity units)], medium salinity (MC, 18 psu), and low salinity (LC, 3 psu). Before the start of the experiment, the salinity of tanks' water was changed gradually (3 psu per day) to make the shrimp acclimate the 3 psu and 30 psu. A total of three tanks (500 L per tank) with 40 shrimp per tank for each group, and the gill tissues of the shrimp were collected from each tank and stored in liquid nitrogen at –80°C immediately until further RNA extraction. A total of nine samples (HC1, HC2, HC3; MC1, MC2, MC3; and LC1, LC2,

LC3) were used for the subsequent transcriptome and small RNA sequencing (Tables 1, 2). The experimental design and analysis pipeline was shown in Supplementary Figure 1.

RNA Extraction, Library Construction, and Sequencing

The TRIzol reagent (Invitrogen, Shanghai, China) was used to perform the total RNA extraction, according to the manufacturer's instructions. The 0.8% agarose gel electrophoresis and NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) were used to assess the RNA integrity and concentration, repetitively. There were three biological replicates for each group, and the construction of mRNA and small RNA were performed for all samples by Shanghai OE Biotech (Shanghai, China). Based on the protocol, the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina) and TruSeq Small RNA Library Prep Kit (Illumina) was used to construct mRNA and small RNA libraries, respectively. To construct the Small RNA sequencing libraries, the 3' end of the miRNA was specifically ligated with 3' SR Adaptor. The excess 3' SR Adaptor was hybridized with SR RT Primer, and the M-MuLV Reverse Transcriptase (Shanghai Sangon Biotech, China) was used to synthesis the first strand cDNA. It was used for PCR amplification with index (X) primer, SR Primer (Illumina) and LongAmp Taq 2× Master Mix. The DNA fragments within 140–150 bp of these amplification products were recovered by denaturing 12% polyacrylamide gel electrophoresis. To ensure the corresponding relationship between the mRNA and miRNA, we simultaneously constructed the transcriptome and mRNA libraries for each sample. All libraries were sequenced using Illumina HiSeq™ 2500 instruments (Illumina).

Quality Validation, Data Cleaning, and Normalization

The quality of the raw sequencing data was validated by FastQC software (Andrews, 2010) (Table 1). Next, we used the paired-end splicing method in the Trinity (Grabherr et al., 2011) software to obtain the transcript sequence and designated the longest sequence as the unigene according to the sequence similarity and length. We then used the TGICL (Pertea et al., 2003) software to cluster and extend redundantly, to obtain a set of final unigenes, that served as the reference sequence for the subsequent analysis (Supplementary Figures 2C,D). For the small RNA sequencing data, the primer and adapter sequences were removed from the original data, and a sequenced fragment with reliable quality was selected after quality inspection and length screening of the bases. The adapter sequence was removed using cutadapt (version 1.14) software (Marcel, 2011), and the sequence with a length of 15–41 bp was retained. Reads containing N bases were filtered out using the NGSQCToolkit (version 2.3.3) software (Patel and Jain, 2012). The clean reads with high quality were finally obtained for the subsequent analysis (Table 2, Supplementary Figure 3A). The statistics summary of the sequencing data is presented in Table 2. The FPKM was

TABLE 1 | The statistics summary for the RNA-seq data of the nine samples.

Sample name	RNA-seq						
	Raw reads	Raw bases	Clean reads	Clean bases	Valid bases %	Q30 %	GC %
HC1	50738124	7610718600	49236624	7382338470	97.00	94.45	45.50
HC2	50070750	7510612500	48478994	7268894580	96.78	94.50	45.00
HC3	50471830	7570774500	48743030	7308267352	96.53	94.10	44.00
MC1	49304948	7395742200	47468446	7117119054	96.23	94.01	45.50
MC2	50386986	7558047900	48525278	7275715614	96.26	94.11	45.50
MC3	50217760	7532664000	48485970	7269993126	96.51	94.34	45.00
LC1	49842448	7476367200	48220504	7230152087	96.71	94.36	45.00
LC2	49281406	7392210900	47663910	7146623289	96.68	94.24	46.00
LC3	49409288	7411393200	47972088	7192992271	97.05	94.61	45.00

TABLE 2 | The statistics summary for the smallRNA-seq data of the nine samples.

Sample name	smallRNA-seq						
	Raw_reads	Raw_adaptor	Reads_length	Reads_Q20	Reads_N	Clean_reads	Reads_uniq
HC1	26632177	26480814	22634737	22609297	22604781	22604781	947640
HC2	28092509	27999363	18768214	18747278	18743733	18743733	1463712
HC3	22561374	22405977	18798221	18777805	18774099	18774099	981886
MC1	22489386	22170711	18483943	18465031	18461764	18461764	732662
MC2	19659523	19383377	16305671	16287547	16284314	16284314	772175
MC3	22483298	22105766	18566741	18548077	18544654	18544654	866788
LC1	25565542	25425400	22987325	22962409	22957776	22957776	1001894
LC2	25570719	25348867	21662368	21639070	21635076	21635076	972856
LC3	25364789	25226008	22699354	22675224	22670827	22670827	863254

(raw_adaptor, number of reads after removal of adaptor; reads_length, number of reads in the range of 15–41 bp; reads_Q20, number of reads with a Q20 percentage >80%; reads_N, number of reads after removing N from the sequence; clean_reads, reads used for analysis after sequence removal and quality control; reads_uniq, number of clean read types).

calculated to assess the expression levels of the unigenes, and the miRNA expression was normalized to transcripts per million.

Downstream Analysis

The differential expression analysis was conducted by the DESeq2 package (Anders and Huber, 2010), and the genes (or miRNA) with fold changes (FC) ≥ 2 ($|\log_2 \text{ratio}| \geq 1$) and adjusted $p < 0.05$ were considered as differentially expressed genes (DEGs) or differentially expressed miRNAs (DEMs). Bowtie software was used to perform a mismatch-free alignment with the mature sequences of the miRNAs in miRBase (version 21.0) (Griffiths-Jones et al., 2006), and the sequences on the alignment were considered to be known miRNAs (Supplementary Figure 3B). Novel miRNAs with a sequence length of at least 18 bp were identified using Mirdeep2 software and were mapped onto the reference genome (Friedlaender et al., 2012). The secondary structure of the novel miRNAs was predicted by RNAfold software (Denman, 1993), and the target genes of the identified miRNAs were performed by MiRanda with the parameters $S \geq 150$ and $\Delta G \leq -30$ kcal/mol (John et al., 2004). The analysis workflow was shown in Supplementary Figure 1.

Analysis of Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Twelve DEGs and six DEMs were randomly selected from the HC and LC groups, and the qRT-PCR was performed using the extracted total RNA to validate the selected DEGs and DEMs. The LightCycler[®] 480 II Real-time PCR Instrument (Roche, Basel, Switzerland) was used for the qRT-PCR reactions. The miRNA expression level was determined by a 10- μ L PCR mixture which was mixed of 1 μ L of cDNA, 0.2 μ L of miRNA-specific primer, 0.2 μ L of universal primer (Qiagen), 5 μ L of 2 \times QuantiFast[®] SYBR[®] Green PCR Master Mix (Qiagen, Hilden, Germany), and 3.6 μ L of nuclease-free water. The mixture was incubated in a 384-well optical plate (Roche) at 95°C for 5 min, and then performed 95°C for 10 s with 40 cycles, followed by at 60°C for 30 s. For mRNA expression, the PCR mixtures, including 2 mL of real-time PCR-ready cDNA, 0.5 mL of specific primer (10 mM), 6.3 mL of SYBR Premix Ex Taq (2 \times), and 2.7 mL of ddH₂O, for a total volume of 12 mL, were performed 30 s denaturation at 95°C, followed by 95°C for 5 s with 40 cycles, and 62°C for 20 s. The Supplementary Table 1 shown the primers used for these genes and miRNAs. The internal control for normalization was defined by Elongation factor 1a (EF-1a), and the $2^{-\Delta\Delta CT}$ method was performed to calculated the expression levels of relative genes (Livak and Schmittgen, 2001).

Technical Validation

For the importance of the total RNA quality in the libraries construction and the downstream analyses, the 260:280 ratios of all extracted RNA samples used in our study were ≥ 2 , and the RNA integrity number (RIN) ≥ 7.0 . We totally constructed nine mRNA and nine miRNA libraries for three groups (HC, MC, and LC) in which three biological replicates for each group (**Supplementary Figure 1**).

RESULTS

The obtained data were tested for contamination. For comparison, the most suitable result with coverage $>80\%$ and an e -value $<1e-10$ was obtained. The top 10 species in each sample were either the same species or related species between samples, and it was preliminarily judged that the samples were contamination-free. The top five species were *P. monodon* (50,946 reads; 62.14%), *L. vannamei* (8,870 reads; 10.82%), *Cyprinus carpio* (3,759 reads; 4.59%), *Fenneropenaeus chinensis* (3,032 reads; 3.7%), and *Marsupenaeus japonicus* (1,569 reads; 1.91%) (**Supplementary Figure 2A**). By applying FastQC to the entire dataset, we successfully obtained high-quality clean data with 94% of the bases scoring Q30 and above (**Table 1**). Then, a total of 64,475 unigenes were assembled. The range of these unigenes was from 301 bp to 38,289 bp with 1,275 bp of the average length. The length of most transcripts was between 300 to 600 bp, and 11,771 transcripts were larger than 2,000 bp (**Supplementary Figure 2D**). We identified 20,990 (32.56%), 16,505 (25.60%), 15,488 (24.02%) 14,284 (22.15%), and 7,508 (11.64%) genes homologous to the sequences in the Nr, Swiss-Prot, GO, KOG, and KEGG databases, respectively. All unigenes were functionally annotated, with 6,281 (9.74%) unigenes could match to all databases, and 21,997 (34.12%) could match to at least one database (**Supplementary Figure 2B**). The distribution of GC content frequency of the assembled unigenes was shown in **Supplementary Figure 2C**. We identified 2,671, 1,317, and 2,062 DEGs and 50, 69, and 55 DEMs, respectively, in three pairwise comparisons (HC vs. LC, MC vs. LC, and HC vs. MC) (**Supplementary Figures 4A,C**). These DEGs were mainly involved in energy metabolism, immune system, transport and signal transduction, and genetic information process. We totally identified 1,740 known miRNAs and predicted 111 novel miRNAs. These miRNAs greatly enriched the miRNA database of *P. monodon*, and more research are needed to verify and explore its functions. The data on these miRNAs were deposited in figshare. Although the \log_2 fold change values of the sequencing data analysis were different from the qPCR analysis, the expression levels of these selected DEGs and DEMs were highly consistent between these two analyses (**Supplementary Figures 4B,D**). These DEGs and DEMs were valuable for understanding the molecular mechanism of shrimp response to salinity stress.

In conclusion, our findings provide a high-quality transcriptome and miRNA dataset characterizing how *P. monodon* responds to long-term salinity stress. Additionally, we

screened multiple genes and miRNAs related to the salinity stress response. The dataset provides a valuable resource for screening genes related to salinity stress responses, which will contribute to improve our understanding of the molecular mechanisms of salinity stress in shrimp.

CODE AVAILABILITY

The software utilized in this study, including the specific versions used, were described in the part of methods and the following list:

1. FastQC version 0.11.6 was used for the quality assessment of the FASTQ data: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>
2. Trinity software version r20131110 was used to assemble the transcript sequence: <https://sourceforge.net/projects/trinityrnaseq/>
3. TGICL17 software version 2.1 was used to cluster and extend redundantly in order to obtain a set of final unigenes: <https://sourceforge.net/projects/tgicl/files/>
4. Cutadapt version 1.14 was used to remove the adapter sequence: <http://code.google.com/p/cutadapt/>
5. NGSQCToolkit version 2.3.3 was used to filter out reads containing N bases: <http://www.nipgr.res.in/ngsqctoolkit.html>
6. Mirdeep2 software was used to identify novel miRNAs: <https://github.com/rajewsky-lab/mirdeep2>
7. MiRanda software was used to predict the target genes of the identified miRNAs: <http://www.microna.org/microna/getDownloads.do>
8. DESeq2 package v1.8.2 was used for the differential expression analysis: <https://www.rdocumentation.org/packages/DESeq2>.

DATA AVAILABILITY STATEMENT

The raw FASTQ files for this study can be found in the NCBI Sequence Read Archive (SRA) [<http://identifiers.org/ncbi/insdc.sra:SRP262105>] with accession number SRP262105 (Bioproject ID: PRJNA631809) (NCBI Sequence Read Archive., 2020). The annotation information of the transcripts in this study was deposited in figshare (Li, 2020a). In addition, the quality assessment data of raw reads and differentially expressed genes were deposited in figshare (Li, 2020b,c). The differentially expressed miRNAs, predicted novel miRNAs, and corresponding heatmaps were deposited in figshare (Li, 2020d,e). The assembled unigenes, novel miRNA structures, and predicted target genes were also deposited in figshare (Li, 2020f).

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Care and Use Committee of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (No. SCSFRI96-253).

AUTHOR CONTRIBUTIONS

ShJ conceived the project and supervised the work. YL performed the bioinformatics analysis and prepared the manuscript. FZ and JH conducted the experiment. LY, SoJ, and QY collected the samples and performed sequencing. All authors read and approved the final manuscript.

FUNDING

This work was supported by grants from the National Key R&D Program of China (2018YFD0900303), Central

Public-interest Scientific Institution Basal Research Fund, CAFS (2020TD30), China Agriculture Research System-48 (CARS-48), and Central Public-interest Scientific Institution Basal Research Fund, South China Sea Fisheries Research Institute, CAFS (Grant Nos. 2019TS25 and 2020ZD01).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.579381/full#supplementary-material>

REFERENCES

- Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biol.* 11:R106. doi: 10.1186/gb-2010-11-10-r106
- Andrews, S. (2010). *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Available online at: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/> (accessed November 20, 2018).
- Biggar, K. K., and Storey, K. B. (2015). Insight into post-transcriptional gene regulation: stress-responsive microRNAs and their role in the environmental stress survival of tolerant animals. *J. Exp. Biol.* 218, 1281–1289. doi: 10.1242/jeb.104828
- Burgos-Aceves, M. A., Cohen, A., Smith, Y., and Faggio, C. (2018). MicroRNAs and their role on fish oxidative stress during xenobiotic environmental exposures. *Ecotox. Environ. Safe.* 148, 995–1000. doi: 10.1016/j.ecoenv.2017.12.001
- Chen, K., Li, E., Li, T., Xu, C., Wang, X., Lin, H., et al. (2015). Transcriptome and molecular pathway analysis of the hepatopancreas in the Pacific white shrimp *Litopenaeus vannamei* under chronic low-salinity stress. *PLoS ONE* 10:e0131503. doi: 10.1371/journal.pone.0131503
- Denman, R. B. (1993). Using RNAFOLD to predict the activity of small catalytic RNAs. *BioTechniques* 15, 1090–1095.
- Ebert, M. S., and Sharp, P. A. (2012). Roles for microRNAs in conferring robustness to biological processes. *Cell* 149, 515–524. doi: 10.1016/j.cell.2012.04.005
- FAO (2018) *The State of World Fisheries and Aquaculture 2018*. Rome: Food and Agriculture Organization of the United Nations (FAO) Press.
- Friedlaender, M. R., Mackowiak, S. D., Li, N., Chen, W., and Rajewsky, N. (2012). miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Res.* 40, 37–52. doi: 10.1093/nar/gkj112
- Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* 29, 644–52. doi: 10.1038/nb.1883
- Griffiths-Jones, S., Grocock, R. J., van Dongen, S., Bateman, A., and Enright, A. J. (2006). miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res.* 34, D140–D144. doi: 10.1093/nar/gkj112
- Guo, H., Lu, Z., Zhu, X., Zhu, C., Wang, C., Shen, Y., et al. (2018). Differential expression of microRNAs in hemocytes from white shrimp *Litopenaeus vannamei* under copper stress. *Fish Shellfish Immun.* 74, 152–161. doi: 10.1016/j.fsi.2017.12.053
- He, P., Wei, P., Zhang, B., Zhao, Y., Li, Q., Chen, X., et al. (2018). Identification of microRNAs involved in cold adaptation of *Litopenaeus vannamei* by high-throughput sequencing. *Gene* 677, 24–31. doi: 10.1016/j.gene.2018.07.042
- John, B., Enright, A. J., Aravin, A., Tuschl, T., Sander, C., and Marks, D. S. (2004). Human microRNA targets. *PLoS Biol.* 2, 1862–1879. doi: 10.1371/journal.pbio.0020363
- Li, E., Wang, X., Chen, K., Xu, C., Qin, J. G., and Chen, L. (2017). Physiological change and nutritional requirement of Pacific white shrimp *Litopenaeus vannamei* at low salinity. *Rev. Aquacult.* 9, 57–75. doi: 10.1111/raq.12104
- Li, Y. (2020a). *Data From: Transcriptome and miRNA Profiles of Black Tiger Shrimp, Penaeus Monodon, Under Different Salinity Conditions*. Annotation. Lasi: Figshare.
- Li, Y. (2020b). *Data From: Transcriptome and miRNA Profiles of Black Tiger Shrimp, Penaeus Monodon, Under Different Salinity Conditions*. 1.Fast,QC, zip. Lasi: Figshare.
- Li, Y. (2020c). *Data From: Transcriptome and miRNA Profiles of Black Tiger Shrimp, Penaeus Monodon, Under Different Salinity Conditions*. 3.Gene_diff.zip. Lasi: Figshare.
- Li, Y. (2020d). *Data From: Transcriptome and miRNA Profiles of Black Tiger Shrimp, Penaeus Monodon, Under Different Salinity Conditions*. different_expressed_miRNA. Lasi: Figshare.
- Li, Y. (2020e). *Data From: Transcriptome and miRNA Profiles of Black Tiger Shrimp, Penaeus Monodon, Under Different Salinity Conditions*. Novel miRNA. Lasi: Figshare.
- Li, Y. (2020f). *Data From: Assembled unigenes, Novel miRNA, Structure and Target Gene Predicted*. Lasi: Figshare.
- Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262
- Marcel, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* 17, 10–12. doi: 10.14806/ej.17.1.200
- NCBI Sequence Read Archive. (2020). Available online at: <http://identifiers.org/ncbi/insdc.sra:SRP262105> (accessed May 11, 2020).
- Patel, R. K., and Jain, M. (2012). NGS QC toolkit: a toolkit for quality control of next generation sequencing data. *PLoS ONE* 7:e30619. doi: 10.1371/journal.pone.0030619
- Peratea, G., Huang, X., Liang, F., Antonescu, V., Sultana, R., Karamycheva, S., et al. (2003). TIGR gene indices clustering tools (TGICL): a software system for fast clustering of large EST datasets. *Bioinformatics* 19, 651–652. doi: 10.1093/bioinformatics/btg034
- Roy, L. A., Davis, D. A., Saoud, I. P., Boyd, C. A., Pine, H. J., and Boyd, C. E. (2010). Shrimp culture in inland low salinity waters. *Rev. Aquacult.* 2, 191–208. doi: 10.1111/j.1753-5131.2010.01036.x
- Shekhar, M. S., Kiruthika, J., and Ponniah, A. G. (2013). Identification and expression analysis of differentially expressed genes from shrimp (*Penaeus monodon*) in response to low salinity stress. *Fish Shellfish Immun.* 35, 1957–1968. doi: 10.1016/j.fsi.2013.09.038
- Wang, H., Wei, H., Tang, L., Lu, J., Mu, C., and Wang, C. (2018). Identification and characterization of miRNAs in the gills of the mud crab (*Scylla paramamosain*) in response to a sudden drop in salinity. *BMC Gen.* 19:609. doi: 10.1186/s12864-018-4981-6
- Wang, Z., Feng, Y., Li, J., Zou, J., and Fan, L. (2020). Integrative microRNA and mRNA analysis reveals regulation of ER stress in the Pacific white shrimp *Litopenaeus vannamei* under acute cold stress. *Comp. Biochem. Phys. D Gen. Proteom.* 33:100645. doi: 10.1016/j.cbd.2019.100645
- Xu, C., Li, E., Xu, Z., Wang, S., Chen, K., Wang, X., et al. (2016). Molecular characterization and expression of AMP-activated protein kinase in response to low-salinity stress in the Pacific white shrimp *Litopenaeus*

- vannamei*. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 198, 79–90. doi: 10.1016/j.cbpb.2016.04.005
- Zhang, D., Wang, F., Dong, S., and Lu, Y. (2016). *De novo* assembly and transcriptome analysis of osmoregulation in *Litopenaeus vannamei* under three cultivated conditions with different salinities. *Gene* 578, 185–193. doi: 10.1016/j.gene.2015.12.026
- Zhao, Q., Pan, L., Ren, Q., and Hu, D. (2015). Digital gene expression analysis in hemocytes of the white shrimp *Litopenaeus vannamei* in response to low salinity stress. *Fish Shellfish Immun.* 42, 400–407. doi: 10.1016/j.fsi.2014.11.020

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Li, Zhou, Huang, Yang, Jiang, Yang and Jiang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.