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Comparative transcriptomic analysis and validation of the ovary and testis in the ridgetail white prawn (*Exopalaemon carinicauda*)

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The ridgetail white prawn, *Exopalaemon carinicauda* is an important cultured seawater species in China. As female *E.carinicauda* grows faster than males, it is significant to search for the differentially expressed genes (DEGs) between males and females. However, there is no public available *E.carinicauda* genome data, and genes related to *E. carinicauda* sex differences are unclear. In this study, the transcriptome sequencing for ovary and testis tissues of *E. carinicauda* were conducted, and 20,891 DEGs were identified including 11,709 up-regulated DEGs and 9,182 downregulated DEGs. The functional categories related to meiosis and reproduction were enriched as well as the steroidogenesis KEGG pathway was clustered. Furthermore, the genes related to male reproduction and cell cycle were dug out which were verified by real-time PCR. In addition, two-color fluorescent *in situ* hybridization result showed that *foxj1b* might play roles during early stage of the ovary development. Therefore, our result provides clues for the study of genes related to reproduction and sex difference in *E.carinicauda*.

KEYWORDS

Exopalaemon carinicauda*, reproduction, gonad, transcriptome, *foxj1b

Abbreviations: DEGs, the differentially expressed genes; NR, Non-Redundant Protein Sequence Database; COG, Database of Clusters of Orthologous Genes; Pfam, The Pfam protein families database; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCA, Principal component analysis; FDR, False Discovery Rate; *EcF*, the ovary group of female *Exopalaemon carinicauda; EcM*, the testis group of male *Exopalaemon carinicauda*.

Introduction

The ridgetail white prawn, *Exopalaemon carinicauda* which belongs to long arm shrimp family and white shrimp genus, mainly distributed in the southeast coastal areas of China (Xu et al., 2010). As a kind of small and important economic shrimp, *E. carinicauda* processes delicious meat quality, with characteristics of high protein content and low-fat level, which is popular with majority of consumers (Wang et al., 2020). The high-resolution genetic linkage map of *E. carinicauda* was constructed by our laboratory and 52 sex-related QTL markers were identified, which is an important work for explicating the sex determination mechanism (Lv et al., 2020). However, genes related to *E. carinicauda* sex differences including spermatogenesis and oogenesis are still unknown. Therefore, it is necessary to excavate the DEGs between male and female in *E. carinicauda*.

Previous studies have shown that there are sexual dimorphisms in economic traits in many aquatic animals (Wan et al., 2021). As for growth characteristics, males grow faster than females in tilapia and yellow catfish (Huang et al., 2022), while females of half-smooth tongue sole showed a growth advantage, with females growing about 30% faster than males (Liao et al., 2014). Among crustaceans, male *Eriocheir sinensis* has a growth advantage over females, but female *E. sinensis* are favourite among consumers for their greater taste (Du et al., 2019); Female *Fenneropenaeus chinensis* are more popular than males with breeders since they are larger than males at the same period (Wang Q. et al., 2019). While *Procambarus clarkii* males are larger than female individuals in the same sexual morphotype (Hamasaki et al., 2020; Shen et al., 2022). Similar to most aquatic animals, the growth trait of *E. carinicauda* also shows dimorphism, female individuals are larger than concurrent males (Lv et al., 2020). Therefore, searching the differentially expressed genes related to valuable characters between males and females is significant for molecular breeding and superior seed cultivation.

Transcriptome sequencing is not only an important means of studying gene structure and expression patterns (Wang Q. et al., 2019), but a bridge to infer gene functions based on phenotype. Due to the particularity of species, it is challenging to conduct gene function research in economic aquatic animals compared to model organisms. Thus, transcriptome sequencing is essential in searching for genes related to some purpose for non-vertebrate animals. For example, Wang et al. obtained the differential expression factors of muscle and gonads of *E. sinensis* between females and males by transcriptome sequencing, which is important for the study of growth traits between the sexes (Wang B. et al., 2019). And Zhang et al. found the sex-determining gene in *Portunus trituberculatus* by transcriptome sequencing, which provided an important basis for the systematic study of sex determination mechanisms in *P. trituberculatus* (Zhang et al.,2022).

There was no high-quality genome data which had been published since *E. carinicauda* processed a large complex genome (Yuan et al., 2017; Li et al., 2019). In this study, we conducted the transcriptome sequencing of ovary and testis to identify the differentially expressed genes and significantly enriched pathways between male and female. Furthermore, we found some genes related to male reproduction, cell cycle and ovary development, which was verified by real-time PCR and two-color fluorescent *in situ* hybridization. Our study would be helpful for further investigating genes related to *E. carinicauda* reproduction and sex difference.

Materials and methods

Bioethics statement

The animal study was reviewed and approved by *Ethics Committee of Yellow Sea Fisheries Research Institute*. Written informed consent was obtained from the owners for the participation of their animals in this study.

Experiment animals and sample collection

The *E. carinicauda* used in this study was produced by RiZhao Haichen Aquaculture Co., Ltd and the samples including ovary and testis were taken from the live and healthy shrimp. The total RNA was extracted from the samples by TRIzol reagent according to the manufacturer's instructions. The genome DNA was removed by DNase I. The purity and concentration of RNA was determined and quantified by 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara CA, USA) and ND-2000 (NanoDrop Thermo Scientific, Wilmington, DE, USA). Quality-qualified RNA sample ($OD_{260/280}$ = 1.8~2.2, $OD_{260/230}$≥2.0, RIN≥8.0, 28S:*18S*≥1.0, Total>1μg) was used for subsequent constructing sequencing libraries.

Library preparation and Illumina Hiseq NovaSeq 6000 sequencing

RNA purification, reverse transcription, library construction and sequencing were performed at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China). The construction of the sequencing library used the Illumina TruSeq™ RNA sample preparation Kit (Illumina, San Diego, CA). The purification of mRNA was by oligo-dT-attached magnetic beads and then fragmented by fragmentation buffer. Taking these short fragments as templates, double-stranded cDNA was synthesized using a SuperScript double-stranded

cDNA synthesis kit (Invitrogen, CA) with random hexamer primers (Illumina). Then the synthesized cDNA was subjected to end-repair, phosphorylation and 'A' base addition according to Illumina's library construction protocol. Libraries were size selected for cDNA target fragments of 200–300 bp on 2% Low Range Ultra Agarose followed by PCR amplified using Phusion DNA polymerase (New England Biolabs, Boston, MA) for 15 PCR cycles. After quantified by TBS380, two RNAseq libraries were sequenced in single lane on an Illumina Hiseq xten/ NovaSeq 6000 sequencer (Illumina, San Diego, CA) for 2×150bp paired-end reads.

De novo assembly and annotation

The raw paired end reads were trimmed by SeqPrep (https://github.com/jstjohn/SeqPrep) and quality was controlled by Sickle (https://github.com/najoshi/sickle) with default parameters. Then clean data from the gonads including ovary and testis were assembled denovo with Trinity (http://trinityrnaseq.sourceforge.net/). All the assembled transcripts would be aligned by six databases including the NCBI protein nonredundant (NR), COG, Swiss-Prot, Pfam, COG, GO and KEGG according to the sequence similarity. The function of the transcript was annotated by BLAST2GO (http://www.blast2go.com/b2ghome) including biological processes, molecular functions and cellular components. And the pathway analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/).

Differential expression analysis and functional enrichment

The expression level of each transcript was calculated according to the transcripts per million reads (TPM) method in order to identify DEGs between ovary and testis. The software RSEM (http://deweylab.biostat.wisc.edu/rsem/) was used to quantify gene abundances for further analysis of the gene expression level. Essentially, differential expression analysis was performed using the DESeq2. Genes satisfied with Q value ≤ 0.05, DEGs with $|\log_2 FC|>1$ and Q value<=0.05 DESeq2/Qvalue<=0.001(DEGseq) were considered to be significantly different expressed genes. The software Goatools (https://github.com/tanghaibao/Goatools) were used to identify DEGs were significantly enriched in GO terms including biological processes, molecular functions and cellular components. And the software KOBAS (http://kobas.cbi.pku.edu.cn/home.do) was used to do KEGG pathway enrichment analysis with metabolic pathways at Bonferroni-corrected P-value ≤ 0.05 compared with the whole-transcriptome background.

Checking the expression level by real-time PCR

In order to check the accuracy of the transcriptome data, the DEGs were verified by real-time fluorescent quantitative PCR (real-time PCR). Total RNA from ovary and testis of *E.carinicauda* was extracted with TRIzol (Invitrogen, 15596026) according to the manufacturer's instructions. The quality and concentration of the obtained RNA were analyzed by agarose gel electrophoresis and spectrophotometer (Thermo, USA) respectively. Then, the genome DNA was removed by DNase I and cDNA were obtained using the ReverTra Ace reverse transcriptase kit (Toyobo, Japan) and random primers. The fluorescence quantitative PCR assay was performed using the SYBR green mix (Toyobo, Japan) according to the manufacturer's instructions: 2×SYBR Green real-time master mix, 10 μL; cDNA, 2μL; F, 0.4μL; R, 0.4μL; H_2O, 7.2μL. The PCR amplification conditions were 95°C for 3min; 45cycles × (95°C for 15s; 55°C for 20s; 72°C for 30s); 65°C for 0.06s; 95°C, 0.5s. The primers used in this study was designed by Primer5 and the sequences were listed in Figure S1.

Two-color fluorescent *in situ* hybridization

We examined the expression of *foxj1b* in the gonads by two-color fluorescent *in situ* hybridization. Gonads including testis and ovary with different stages were fixed in 4%PFA overnight and dehydrated by 30% sucrose. After being embedded with O.C.T. compound (SAKURA, 4583), the gonad was sectioned by a freezing microtome (Leica, CM1950). Probes were designed and synthesized according to the cDNA sequence of the *foxj1b* and *vasa*, and labeled with digoxin (Roche Diagnostics, 11277073910) and fluorescein labeling (Roche Diagnostics, 11685619910) respectively. And they were purified by the clean-up purification kit (Sigma, S5059-70EA). Two-color fluorescent *in situ* hybridization experiments were then carried out as described (Lauter et al., 2011), and the sections was dealt with prehybridization, permeabilization, hybridization, antibody incubation, staining reaction and stop reaction. The nucleus was stained with DAPI. The section was photographed by laser confocal microscope (Leica, Sp8). Primer sequences used for synthetic probes were listed in Figure S1.

Results

De novo assembly of *E. carinicauda* gonad transcriptome

We obtained 48.22Gb raw data from 6 samples transcriptome sequencing including ovary and testis and the raw data were upload into the NCBI SRA database with the accession number

PRJNA856985. After RNA sequencing based on the Illumina platform and sequence assembly, a total of 47.22 Gb clean data were generated. And the average of each sample was over 6.71Gb with the percentage of Q30 over 94.4% (Supplementary Table 1). The clean data was done *de novo* assembly with Trinity and the optimized evaluation of the assembly results showed that there were 105,440 unigenes and 191,647 transcripts with the average length of N50 was 1,719bp. The clean reads were aligned to the reference sequence obtained from the trinity assembly, and the alignment rate for this analysis ranged from 75.25% to 76.62%.

Identification of DEGs in ovary and testis as well as GO and KEGG analysis

Through the expression quantity matrix analysis, the ovary samples were significantly clustered together, so were the testis samples, which indicates a good reproducibility between the parallel samples (Figure 1A). The PCA plot showed clearly that the principal components were similar between different replicates in the same sample (Figure 1B). By analyzing the expression of the samples, we can see that there were 33,495 transcripts expressed in the *EcF* groups and 48,719 transcripts expressed in the *EcM* groups as well as 17,532 transcripts were shared in both kinds of groups (Figure 1C).

Analysis of DEGs in *E. carinicauda* ovary and testis by transcriptome sequencing helped us to have a better understanding of the gonad development. We screened the DEGs by the principle of FDR<=0.05 and $|\log_2 FC|>1$. There were 20,891 differentially expressed genes including 11,709 upregulated genes and 9,182 down-regulated genes (*EcM-vs-EcF*) (Figure 1D). The heatmap of the 25 genes with the top expression level in testis was shown in Figure 2A, while the other

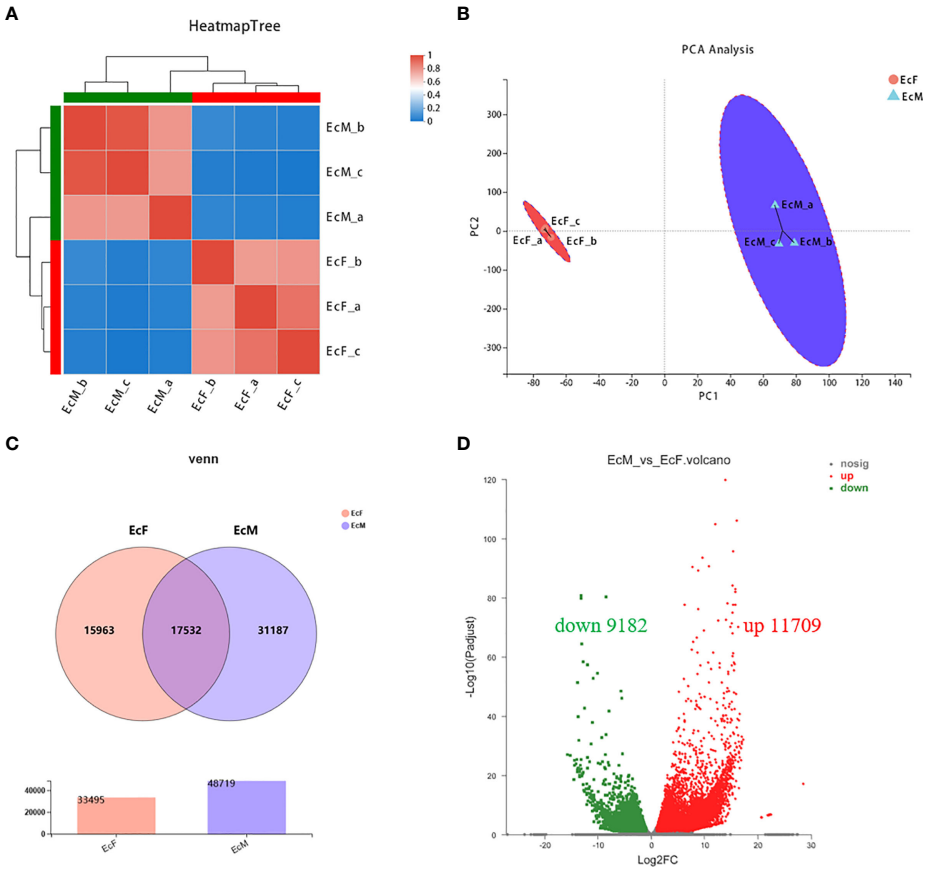

FIGURE 1
The profile of transcriptome sequence between ovary and testis in *E carinicauda*. **(A)** Heatmap Tree of the 3 ovary and 3 testis samples. The right and lower sides are sample names while the left and upper sides are sample clusters. **(B)** Principal component analysis (PCA) of the 3 replicated ovary samples and 3 replicated testis samples. The distance of each sample point represents the similarity of the sample, the closer distance indicates the higher similarity between the samples. **(C)** Venn diagram of DEGs in comparison between *EcF* and *EcM* group. **(D)** Volcano plot of DEGs identified from ovary and testis. Red dots indicate the significantly upregulated transcript. Green dots mean the significantly downregulated transcript. Black dots are the non-significant difference of transcript.

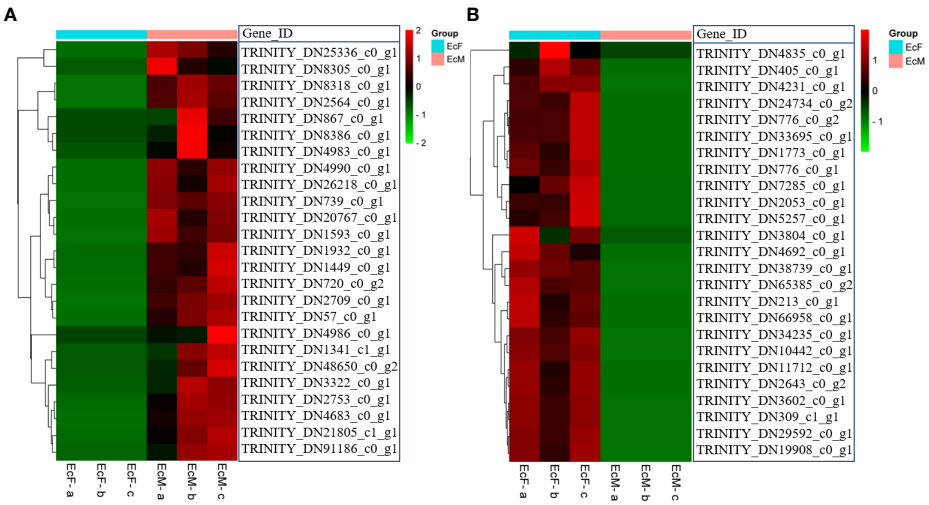

FIGURE 2
Heatmap of the top 25 highly expressed genes in ovary and testis. **(A)** The top 25 highly expressed genes in the testis; **(B)** The top 25 highly expressed genes in the ovary; Each column represents one sample and each row represents one transcript. The color in the figure represents the expression level of the transcript in this sample. The red color represents the high expression level of the transcript in the sample; The green color represents the lower expression level.

25 genes with the highest expression level in ovary were listed in Figure 2B.

The top 20 GO terms enrichment analysis of the DEGs were listed in Figure 3 which included metabolic process, cellular metabolic process and organic cyclic compound metabolic process. And the functional categories were mainly related to meiosis and reproduction including DNA recombination and cellular aromatic compound metabolic process. The KEGG pathway analysis of the top 20 pathways was shown in Figure 4 which was mainly involved in steroidogenesis including ubiquinone and other terpenoid-quinone biosynthesis, fatty acid elongation and fatty acid degradation.

Differentially expressed genes related to male reproduction

Combined with the annotation of the NR database, we found a number of genes specifically expressed in testis (Figure 5A). Then, we checked the results by real-time PCR and the expression level of these genes was in accord with the transcriptome data (Figure 5B) which indicated the accuracy of the transcriptome sequencing. These genes showed a significantly higher expression level in males than in females (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001). The annotations of these genes are mainly related to spermatogenesis and male reproduction including some proteins which involved in this process.

Differentially expressed genes related to cell cycle

As is known to us, spermatogenesis and oogenesis show different characteristics and they contain various events. We identified some DEGs related to cell cycle and the functions related to anaphase promoting factors, spindle assembly checkpoints and cell division. Some of them are highly expressed in testis while others show a high level in the ovaries and they present their own characteristics (Figure 6A). And this result was further examined by real-time PCR (Figure 6B). Just as meiosis in other species, the complete of ovary meiosis need more time since oogenesis need to accumulate much vitellogenin while the spermatogenesis process doesn't need (Tsukimura, 2001; Wei et al., 2021). Consequently, the cell proliferation and differentiation in testis and ovary of *E. carinicauda* showed a sexual dimorphism.

Excavation and identification of the transcription factor *foxj1b* associated with ovarian differentiation

Transcription factors are proteins that can bind to specific DNA sequences and generally process one or more functional domains (Lambert et al., 2018). Usually, the transcription factors play functions by two ways. Besides binding to promoter upstream regions of the gene, the transcription factor could also form

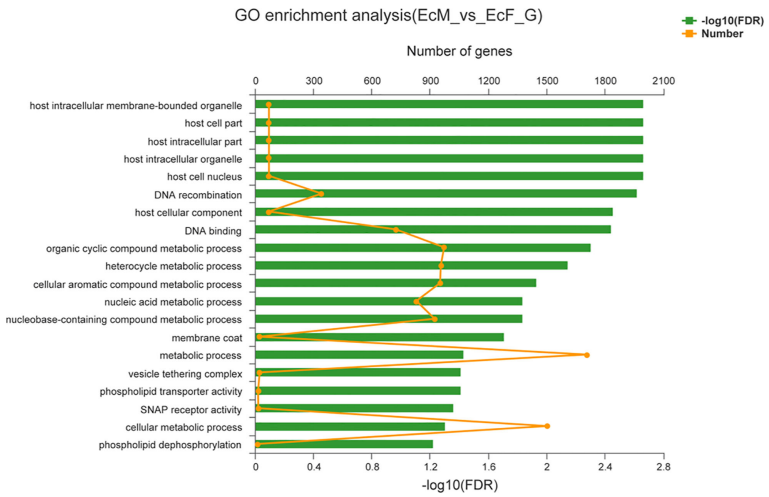

FIGURE 3
Column diagrams of top 20 enriched biological processes of GO classifications. The ordinate represents the GO term and the upper abscissa indicates the number of transcripts of the GO term on the alignment, corresponding to different points on the broken line; the lower abscissa indicates the significance level of enrichment, the lower abscissa indicates the height of the column, where the smaller the FDR, the greater the-log10 (FDR) value, the more significantly enriched the GO term is.

complexes with other transcription factors to affect gene transcription (Pope & Medzhitov, 2018). There are 23 families including 567 transcription factor members (Figure 7A). And we found *foxj1b* which comes from the fork head family might be associated with ovary differentiation. We did a preliminary validation by two-color fluorescent *in situ* hybridization using ovary section samples in various stage (Figure 7B). The results showed that *foxj1b* is mainly expressed in early development of the ovary (Ovary I) and late ovulation stage (Ovary III). There were little signals in testis and mature ovary (Ovary II).

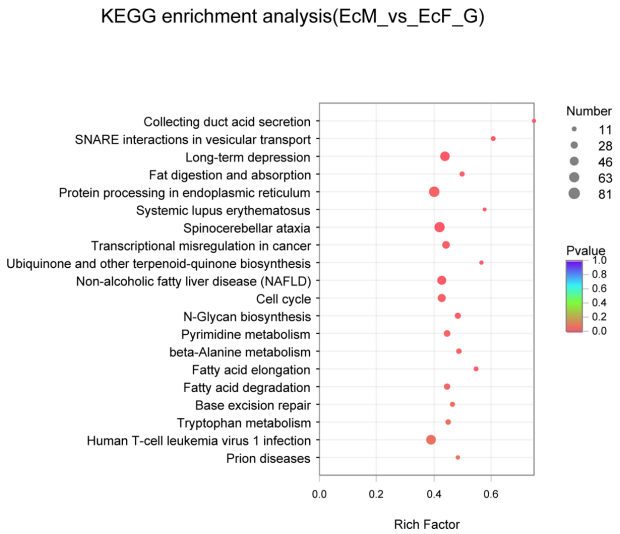

FIGURE 4
Scatter plots of KEGG pathway enrichment analysis about the top 20 significant pathways of DEGs in *EcF-vs-EcM*. The ordinate represents the pathway and the abscissa indicates the rich factor, where the bigger the rich factor, the more significantly enriched the KEGG pathway is.

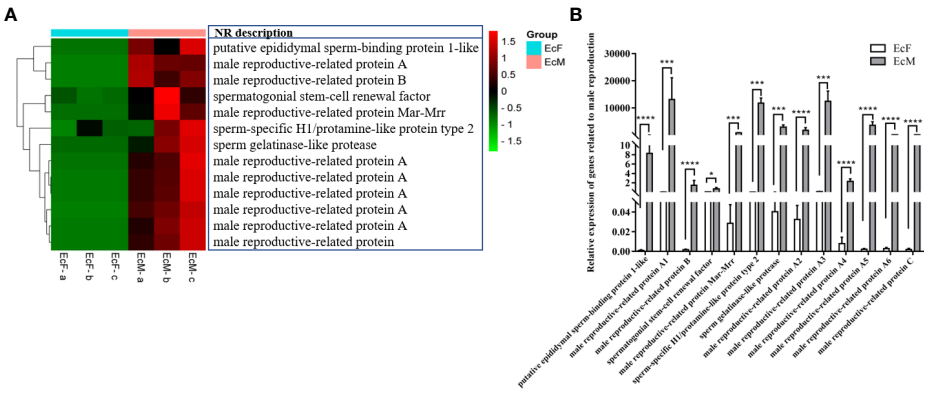

FIGURE 5
Differentially expressed genes (DEGs) related to male reproduction. **(A)** Heatmap of DEGs related to male reproduction. Each column in the figure represents one sample and each row represents one transcript, the color in the figure indicates the high or low expression of transcript in that sample, the red color represents the higher expression of the transcript in the sample, the green color indicates the lower expression. **(B)** The real-time PCR validation of gene expression related to male reproduction (*P<0.05, ***P<0.001, ****P<0.0001). The data analysis was conducted by $2^{-\Delta CT}$ and *18S* was chosen as reference gene.

Discussion

There are several characteristics in *E. carinicauda* including small body size which is fit to feed under laboratory conditions and whole transparent body as well as high fecundity which are suitable to genetic operation (Yuan et al., 2017). Therefore, *E. carinicauda* is a popular species in genetic study. So far, *E. carinicauda* is the only reported decapoda crustacean species which is suitable for gene editing (Gui et al., 2016). According to the result of the genome survey, the structure of the *E. carinicauda* genome was big and complicated (Yuan et al., 2017; Li et al., 2019). And there were no published *E. carinicauda* genome data at present. Therefore, it is essential to enrich and re-annotate the *E. carinicauda* genome by diverse omics data, especially for important tissues with significant differences between males and females. As for the study of gene function, the accurate annotation of the genome is essential and significant. However, there is still a long way to

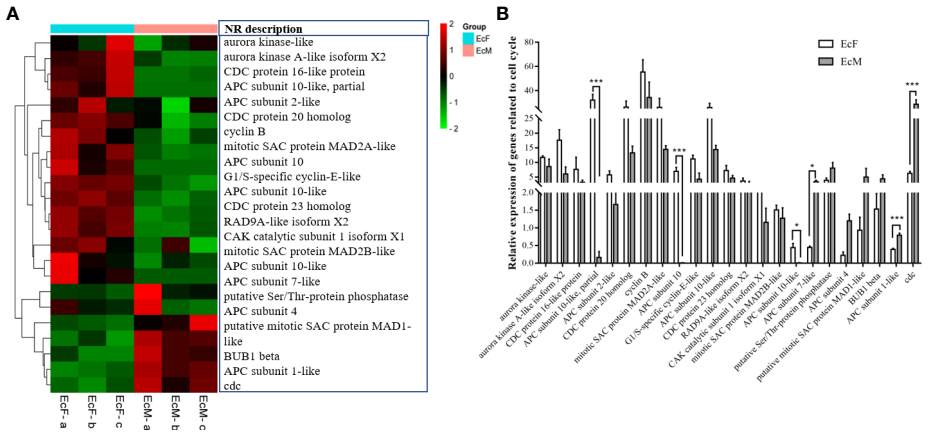

FIGURE 6
Differentially expressed genes (DEGs) related to cell cycle. **(A)** Heatmap of DEGs related to cell cycle. Each column in the figure represents one sample and each row represents one transcript, the color in the figure indicates the high or low expression of transcript in that sample, the red color represents the higher expression of the transcript in the sample, the green color indicates the lower expression level transcripts.
(B) Validation of gene expression related to cell cycle by real-time PCR (*P<0.05, ***P<0.001). The data analysis was conducted by $2^{-\Delta CT}$ and *18S* was chosen as reference gene.

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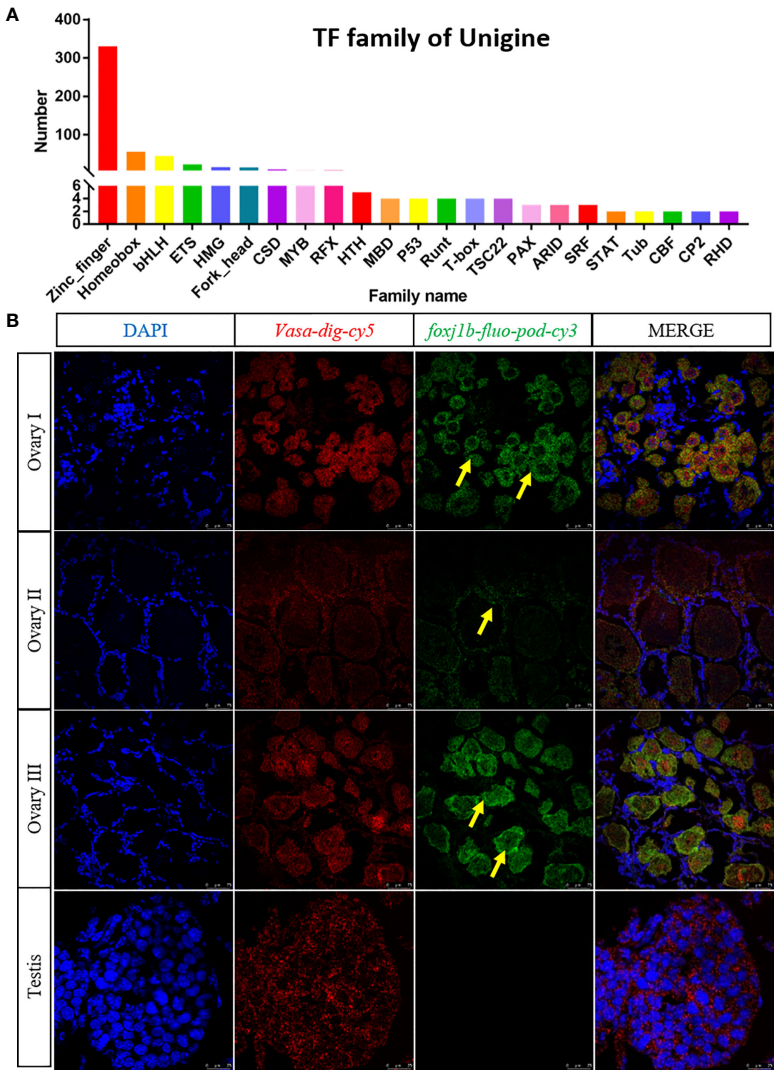

FIGURE 7
checking the expression profile of the transcription factor *foxj1b* by two-color fluorescent *in situ* hybridization. **(A)** Statistics of transcription factors in *E.carinicauda*. The abscissa stands for different families of transcription factors; the ordinate means the number of transcripts falling to the family. **(B)** Mapping the expression of *foxj1b* and *vasa* by two-color fluorescent *in situ* hybridization in gonads. The blue signal: DAPI; the red signal: *vasa*; the green signal: *foxj1b*.

go on the study of *E. carinicauda* functional genomics research. In this study, we obtained DEGs between ovary and testis by transcriptome sequencing, including 11,709 up-regulated genes and 9,182 down-regulated genes, which could provide clue for the study of *E. carinicauda* reproduction development.

Spermiogenesis is an important process in male reproduction (Staub & Johnson, 2018). The first stage of spermiogenesis is the spermatogonia division and proliferation, which could produce spermatocytes. And the second stage is the spermatocyte meiosis that could form a haploid sperm cell. The third stage is that the sperm cell metamorphoses into sperm. We identified several genes related to spermiogenesis (Figure S2) although their functions need to be further confirmed. Compared with spermiogenesis, the distinctive events in oogenesis are the synthesis of VTG and the process of oocyte maturation (Subramoniam, 2010). There are several transcripts which might be related to the synthesis of VTG in our transcriptome result (Figure S3). These findings offered clues for the study of *E. carinicauda* spermiogenesis and oogenesis.

Transcription factors regulate the target gene expression by their specific functional domains. Currently, they play increasingly important roles in sex determination and differentiation, embryo development, and organ formation.

Sry is the first transcription factor identified in humans for sex determination, which could regulate the expression of Sox9 (Sinclair et al., 1990; Vining et al., 2021). Then transcription factors such as *amh*, *dmy* and *foxl2* were successively found to play roles in sex differentiation and ovary development respectively (Matsuda et al., 2002; Georges et al., 2014; Song et al., 2021). For instance, *amh* is a sex-determining gene in a chicken and *dmy* could determine the sex medaka (Matsuda et al., 2002; Smith & Sinclair, 2004). While *foxl2* belongs to the fork head family which could play roles in ovary differentiation in mammals and zebrafish (Yang et al., 2017; Tucker, 2021). However, there were no genes which were reported related to ovary differentiation in *E. carinicauda*. We found 567 transcription factors by transcriptome sequencing (Figure 7A). After a comparison of the expression level between males and females, we focused on the transcription factor *foxj1b*. The two-color fluorescent *in situ* hybridization results showed that the *foxj1b* was mainly expressed in early development and late ovulation stage of the ovary but the expression level was very low in mature ovary and no signal could be detected in testis (Figure 7B). Our results suggested that *foxj1b* might play roles in ovary early development in *E. carinicauda* by two-color fluorescent *in situ* hybridization for the first time. However, the specific function of *foxj1b* in *E. carinicauda* still needs to be further studied by gene editing or overexpression.

In conclusion, we discovered 105,440 differentially expressed genes in testis and ovary by transcriptome sequencing, including genes related to reproduction and cell cycle as well as GO terms enrichment and KEGG pathway analysis. Furthermore, the results were verified by real-time PCR and two-color fluorescent *in situ* hybridization. Our results would lay the foundation for subsequent studies of gene functions related to male reproduction and ovary development in *E. carinicauda*.

Data availability statement

The datasets presented in this study can be found in online repositories. The data presented in the study are deposited in the NCBI SRA repository, accession number PRJNA856985.

Ethics statement

The animal study was reviewed and approved by Ethics Committee of Yellow Sea Fisheries Research Institute.

Author contributions

JL designed the study. SJ and LJ performed the experiments and analyzed the data. SJ wrote the manuscript. JJL, JW and JTL helped in manuscript revising. JL and PL checked the experiment design and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.995790/full#supplementary-material

References

Du, J., Liu, Y., Song, C., and Cui, Z. (2019). Discovery of sex-related genes from embryonic development stage based on transcriptome analysis in *Eriocheir sinensis*. *Gene* 710, 1–8. doi: 10.1016/j.gene.2019.05.021

Georges, A., Auguste, A., Bessiere, L., Vanet, A., Todeschini, A. L., and Veitia, R. A. (2014). FOXL2: a central transcription factor of the ovary. *J. Mol. Endocrinol.* 52 (1), R17–R33. doi: 10.1530/JME-13-0159

Gui, T., Zhang, J., Song, F., Sun, Y., Xie, S., Yu, K., et al. (2016). CRISPR/Cas9-mediated genome editing and mutagenesis of EcChi4 in *Exopalaemon carinicauda*. *G3 (Bethesda)* 6 (11), 3757–3764. doi: 10.1534/g3.116.034082

Hamasaki, K., Osabe, N., Nishimoto, S., Dan, S., and Kitada, S. (2020). Sexual dimorphism and reproductive status of the red swamp crayfish *Procambarus clarkii*. *Zool Stud.* 59, e7. doi: 10.6620/ZS.2020.59-07

Huang, P., Guo, W., Wang, Y., Xiong, Y., Ge, S., Gong, G., et al. (2022). Genome-wide association study reveals the genetic basis of growth trait in yellow catfish with sexual size dimorphism. *Genomics* 114 (3), 110380. doi: 10.1016/j.ygeno.2022.110380

Lambert, S. A., Jolma, A., Campitelli, L. F., Das, P. K., Yin, Y., Albu, M., et al. (2018). The human transcription factors. *Cell* 172 (4), 650–665. doi: 10.1016/j.cell.2018.01.029

Lauter, G., Söll, I., and Hauptmann, G. (2011). Multicolor fluorescent *in situ* hybridization to define abutting and overlapping gene expression in the embryonic zebrafish brain. *Neural Dev.* 6, 10. doi: 10.1186/1749-8104-6-10

Liao, X., Xu, G., and Chen, S. L. (2014). Molecular method for sex identification of half-smooth tongue sole (*Cynoglossus semilaevis*) using a novel sex-linked microsatellite marker. *Int. J. Mol. Sci.* 15 (7), 12952–12958. doi: 10.3390/ijms150712952

Li, J., Lv, J., Liu, P., Chen, P., Wang, J., and Li, J. (2019). Genome survey and high-resolution backcross genetic linkage map construction of the ridgetail white prawn *Exopalaemon carinicauda* applications to QTL mapping of growth traits. *BMC Genomics* 20, 598. doi: 10.1186/s12864-019-5981-x

Lv, J., Lu, X., Ti, X., Liu, P., Li, J., and Li, J. (2020). QTL mapping and marker identification for sex determination in the ridgetail white prawn, *Exopalaemon carinicauda*. *Genomics* 112 (6), 5240–5247. doi: 10.1016/j.ygeno.2020.09.037

Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., et al. (2002). DMY is a y-specific DM-domain gene required for male development in the medaka fish. *Nature* 417 (6888), 559–563. doi: 10.1038/nature751

Pope, S. D., and Medzhitov, R. (2018). Emerging principles of gene expression programs and their regulation. *Mol. Cell* 71 (3), 389–397. doi: 10.1016/j.molcel.2018.07.017

Shen, Y., Wang, Q., Wang, W., and Li, Y. (2022). Exploration of an XX/XY sex determination system and development of PCR-based sex-specific markers in *Procambarus clarkii* based on next-generation sequencing data. *Front. Genet.* 13, 850983. doi: 10.3389/fgene.2022.850983

Sinclair, A. H., Berta, P., Palmer, M. S., Hawkins, J. R., Griffiths, B. L., Smith, M. J., et al. (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* 346 (6281), 240–244. doi: 10.1038/346240a0

Smith, C. A., and Sinclair, A. H. (2004). Sex determination: insights from the chicken. *Bioessays* 26 (2), 120–132. doi: 10.1002/bies.10400

Song, W., Xie, Y., Sun, M., Li, X., Fitzpatrick, C. K., Vaux, F., et al. (2021). A duplicated amh is the master sex-determining gene for sebastes rockfish in the Northwest pacific. *Open Biol.* 11 (7), 210063. doi: 10.1098/rsob.210063

Staub, C., and Johnson, L. (2018). Review: Spermatogenesis in the bull. *Animal* 12 (s1), s27–s35. doi: 10.1017/S1751731118000435

Subramoniam, T. (2010). Mechanisms and control of vitellogenesis in crustaceans. *Fisheries Sci.* 77 (1), 1–21. doi: 10.1007/s12562-010-0301-z

Tsukimura, B. J. A. Z. (2001). Crustacean vitellogenesis: Its role in oocyte development. *Amer. Zool.* 41, 465–476. doi: 10.1093/icb/41.3.465

Tucker, E. J. (2021). The genetics and biology of FOXL2. *Sex Dev.* 1–10. doi: 10.1159/000519836

Vining, B., Ming, Z., Bagheri-Fam, S., and Harley, V. (2021). Diverse regulation but conserved function: SOX9 in vertebrate sex determination. *Genes (Basel)* 12 (4), 486. doi: 10.3390/genes12040486

Wang, Q., He, Y., and Li, J. (2019). Conjoint analysis of SMRT- and illumina-based RNA-sequencing data of *Fenneropenaeus chinensis* provides insight into sex-biased expression genes involved in sexual dimorphism. *Front. Genet* 10, 1175. doi: 10.3389/fgene.2019.01175

Wang, B., Kumar, V., Olson, A., and Ware, D. (2019). Reviving the transcriptome studies: An insight into the emergence of single-molecule transcriptome sequencing. *Front. Genet.* 10, 384. doi: 10.3389/fgene.2019.00384

Wang, J., Li, J., Ge, Q., Chen, Z., and Li, J. (2020). Effects of inbreeding on genetic characteristic, growth, survival rates, and immune responses of a new inbred line of exopalaemon carinicauda. *Int. J. Genomics* 2020, 1–11. doi: 10.1155/2020/5735968

Wan, Z. Y., Lin, V. C. L., and Hua, Y. G. (2021). Pomc plays an important role in sexual size dimorphism in tilapia. *Mar. Biotechnol. (NY)* 23 (2), 201–214. doi: 10.1007/s10126-020-10015-2

Wei, L. L., Chen, T. T., Luo, B. Y., and Qiu, G. F. (2021). Evidences for red pigment concentrating hormone (RPCH) and beta-pigment dispersing hormone (beta-PDH) inducing oocyte meiotic maturation in the Chinese mitten crab, *Eriocheir sinensis*. *Front. Endocrinol. (Lausanne)* 12, 802768. doi: 10.3389/fendo.2021.802768

Xu, W., Xie, J., Shi, H., and Li, C. (2010). Hematodinium infections in cultured ridgetail white prawns, *Exopalaemon carinicauda*, in eastern China. *Aquaculture* 300 (1-4), 25–31. doi: 10.1016/j.aquaculture.2009.12.024

Yang, Y. J., Wang, Y., Li, Z., Zhou, L., and Gui, J. F. (2017). Sequential, divergent, and cooperative requirements of Foxl2a and Foxl2b in ovary development and maintenance of zebrafish. *Genetics* 205 (4), 1551–1572. doi: 10.1534/genetics.116.199133

Yuan, J., Gao, Y., Zhang, X., Wei, J., Liu, C., Li, F., et al. (2017). Genome sequences of marine shrimp exopalaemon carinicauda holthuis provide insights into genome size evolution of caridea. *Mar. Drugs* 15 (7), 213. doi: 10.3390/md15070213

Zhang, W., Lv, J., Lan, W., Gao, B., and Liu, P. (2022). Discovery of sex-determining genes in *Portunus trituberculatus*: A comparison of Male and female transcriptomes during early developmental stages. *Front. Mar. Sci.* 8, 811052. doi: 10.3389/fmars.2021.811052