

Integrated Transcriptomic Analysis Reveals mRNA and ncRNA Expression and Potential Co-Expression Networks in the Gonads of *Panulirus homarus*

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Abstract

Background

Sex-biased gonadal transcription is closely associated with the distinct reproductive functions of male and female crustaceans. However, coding and non-coding RNA expression profiles in lobster gonads remain poorly characterized. We therefore investigated sex-biased mRNA, lncRNA, and circRNA expression in the ovaries and testes of the scalloped spiny lobster, *Panulirus homarus*.

Results

Total RNA sequencing of three ovarian and three testicular samples identified 6,139 differentially expressed genes, 761 differentially expressed lncRNAs, and 238 differentially expressed circRNAs. Ovary-biased genes were enriched in pathways associated with the cell cycle, oocyte meiosis, progesterone-mediated oocyte maturation, and oxidative phosphorylation, whereas testis-biased genes were associated with PI3K–Akt signalling, ribosome-related processes, cytoskeletal organization, and extracellular matrix–receptor interaction. Several reproduction-associated genes, including *Dmrt1*, *Foxl2*, *Fem1B*, *Fem1C*, *Pxt*, *Mael*, *Vasa*, *Lhx9*, *Hpgds*, and *Yl*, exhibited pronounced gonad-biased expression. Exploratory correlation analyses further identified candidate lncRNA–mRNA and circRNA–mRNA expression associations involving selected reproduction-associated genes. RT–qPCR analysis supported the direction of the RNA-sequencing-derived expression differences for the selected transcripts.

Conclusions

This study delineates the sex-biased coding and non-coding RNA expression landscapes of *P. homarus* gonads and identifies candidate genes and RNA associations linked to distinct ovarian and testicular functions. These findings expand the transcriptomic resources available for spiny lobsters and provide a foundation for the functional investigation of crustacean gonadal biology and reproductive development.

Background

The scalloped spiny lobster, *Panulirus homarus*, is an economically important tropical crustacean widely distributed throughout the Indian Ocean and western Pacific regions, with considerable potential for aquaculture development in the South China Sea [1]. In recent years, increasing market demand has promoted the rapid expansion of *P. homarus* aquaculture. However, the lack of efficient artificial breeding technologies has resulted in continued reliance on wild-caught juveniles for seed production, thereby limiting the sustainable development of this emerging industry [2]. Notably, *P. homarus* exhibits pronounced sexual dimorphism, characterized by substantial differences in growth performance and reproductive strategies between males and females. For example, males generally exhibit faster growth than females under sex-separated culture conditions, whereas variations in sex ratio can significantly

affect reproductive output and population dynamics [3]. These characteristics underscore the importance of characterizing the molecular basis of sex-biased gonadal development, as such knowledge may contribute to a better understanding of reproductive biology and provide candidate molecular resources for future breeding-related research. However, despite its economic importance, the transcriptomic differences and candidate molecular associations between male and female gonads of *P. homarus* remain poorly characterized.

Characterizing the molecular factors associated with sex differentiation and gonadal function is an important objective in crustacean reproductive biology [4]. In crustaceans, these processes are closely linked to reproductive success, population sustainability, and aquaculture productivity [5, 6]. Unlike vertebrates, reproductive development in decapod crustaceans is governed by a complex neuroendocrine network involving the eyestalk, endocrine organs, gonads, and downstream signaling pathways. The X-organ–sinus gland (XO–SG) complex serves as a key neuroendocrine regulatory center [7], and members of the crustacean hyperglycemic hormone (CHH) family have been implicated in the regulation of vitellogenesis, gametogenesis, and gonadal maturation [8, 9]. These endocrine factors constitute important components of reproductive regulation in decapods, although the downstream transcriptional patterns associated with gonadal characteristics remain incompletely characterized in many lobster species.

In addition to neuroendocrine regulation, numerous genes and signaling pathways have been implicated in sex differentiation and gonadal function in decapod crustaceans [10–12]. The insulin-like androgenic gland hormone (IAG) pathway is an important component of male sexual differentiation [13], whereas genes such as *Dmrt*, *Tra-2*, *Fem-1*, and *Foxl2* have been associated with sex-biased gonadal expression, germ-cell development, and reproductive function [14–16]. Nevertheless, most previous studies have focused on individual genes or specific signaling pathways. Comprehensive transcriptional comparisons of ovarian and testicular tissues, particularly those integrating coding and non-coding RNAs, remain limited in lobster species [17].

Recently, non-coding RNAs (ncRNAs) have gained attention for their regulatory roles in reproductive biology. Long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) have been implicated in gonadal development and sex-related processes in several species through diverse transcriptional and post-transcriptional mechanisms. However, studies on lncRNA and circRNA regulatory networks in crustaceans, especially lobsters, are limited, and comprehensive analyses of coding and non-coding transcripts are rare.

In this study, we performed comparative total RNA sequencing of ovarian and testicular tissues from *P. homarus* to characterize sex-biased mRNA, lncRNA, and circRNA expression profiles. Differential expression and functional enrichment analyses were conducted to identify gonad-associated transcriptional signatures, whereas expression correlation analysis was used to construct candidate lncRNA–mRNA and circRNA–mRNA co-expression networks. This study provides a transcriptomic

resource for prioritizing reproduction-associated genes and ncRNA candidates for subsequent functional investigation in *P. homarus*.

Methods

Sample collection

P. homarus individuals used in this study were maintained at the Sanya Tropical Fisheries Research Institute, South China Sea Fisheries Research Institute, Sanya, China. The experimental lobsters were 15 months old and had an average body weight of 220.0 ± 11.7 g. Before sampling, the animals were acclimated for one week under controlled conditions at a salinity of 34.5 ± 0.5 ppt, temperature of $28 \pm 1^\circ\text{C}$, and pH of 7.8 ± 0.1 . Testicular tissues were collected from six males, and ovarian tissues were collected from six females. Each tissue sample was divided into two portions: one was immediately frozen in liquid nitrogen and stored at -80°C for RNA extraction, and the other was fixed for histological examination. Three males and three females were selected for RNA sequencing based on RNA integrity and histological confirmation of their respective gonadal conditions [18, 19]. The remaining samples, together with the RNA-seq samples, were included in the subsequent RT-qPCR analysis.

Gonadal histology

Gonadal histology was examined to characterize the reproductive condition of each sampled individual and to provide morphological context for the transcriptomic comparison. Gonadal tissues were fixed in 4% paraformaldehyde for 24 h, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections of 5 μm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin. Histological sections were examined using a light microscope (ECLIPSE 200, Nikon, Japan). Testicular condition was evaluated according to the organization of the seminiferous lobules and the presence of different spermatogenic cell types. Ovarian condition was assessed based on oocyte morphology, yolk deposition, nuclear visibility, and association with follicular cells.

RNA extraction, library construction, and sequencing

Total RNA was extracted from ovarian (O) and testicular (T) tissues using the mirVana™ miRNA Isolation Kit (Ambion, USA). To remove potential genomic DNA contamination, RNA samples were treated with DNase I. RNA integrity, purity, and concentration were assessed using agarose gel electrophoresis, NanoDrop spectrophotometry, Qubit fluorometric quantification, and an Agilent 2100 Bioanalyzer. Only RNA samples with OD_{260/280} values close to 2.0 and RNA integrity numbers (RINs) greater than 8.0 were used for library preparation. Strand-specific total RNA libraries were constructed after rRNA removal. The qualified libraries were sequenced on the DNBSEQ-T7 platform with a paired-end 150 bp strategy by OE Biotech Co., Ltd. (Shanghai, China).

Sequencing data analysis

Raw paired-end reads generated from the DNBSEQ-T7 platform were subjected to quality control and preprocessing using fastp (v0.19.5). Specifically, reads containing more than 5% ambiguous nucleotides (N), reads with more than 20% of bases having a Phred quality score ≤ 10 , and reads shorter than 50 bp after trimming were removed. The remaining high-quality reads were retained as clean reads for subsequent genome alignment and transcriptomic analyses [20]. The quality of clean reads was initially evaluated using FastQC (v0.11.5). Clean reads were subsequently aligned to the *P. homarus* reference genome (NCBI assembly accession: GCA_043589495.1) using HISAT2 (v2.2.1) with strand-specific parameters [21]. The resulting BAM files were analyzed using RSeQC (v2.6.4) to evaluate alignment quality, read distribution, and strand specificity, thereby ensuring reliable genome mapping.

Following genome alignment, uniquely mapped reads were assigned to annotated protein-coding genes using HTSeq-count (v0.9.1) with strand-specific settings to generate raw read count matrices for each sample. The resulting count matrix was used as input for downstream statistical analyses. Gene expression levels were calculated as fragments per kilobase of transcript per million mapped reads (FPKM) and were used for expression distribution analysis, sample correlation analysis, and hierarchical clustering.

Differential expression analysis of mRNAs

Differential expression analysis of mRNAs was performed using DESeq2 (v1.22.2) based on raw read count matrices. DESeq2 uses a generalized linear model based on the negative binomial distribution to estimate dispersion and identify differentially expressed transcripts between groups. Differences in sequencing depth and RNA composition among samples were normalized using the median-of-ratios normalization method implemented in DESeq2. Principal component analysis (PCA), Pearson correlation analysis, and hierarchical clustering were performed using normalized expression data to evaluate the consistency of biological replicates, sample reproducibility, and overall transcriptomic differences between the testis and ovary groups. mRNAs with $|\log_2(\text{fold change})| \geq 1$ and an FDR-adjusted P value < 0.05 were defined as differentially expressed genes.

Identification and co-expression analysis of lncRNAs

Transcripts were independently assembled for each sample using StringTie (v1.3.3b), and the sample-specific assemblies were subsequently merged to generate a non-redundant transcript set. Putative lncRNA transcripts were initially screened according to transcript length (> 200 nucleotides), exon number (≥ 2), and genomic relationships with annotated protein-coding genes. The coding potential of the filtered transcripts was evaluated using CPC2, CNCI, Pfam, and PLEK [22–24]. Only transcripts consistently classified as non-coding by all four approaches were retained as high-confidence lncRNA candidates. Clean reads were mapped to the retained lncRNA transcript set using Bowtie2 (v2.2.9), and lncRNA expression levels were quantified using eXpress (v1.5.1) with strand-specific parameters. The

resulting raw read counts were used for differential expression analysis with DESeq2 (v1.22.2). lncRNAs with $|\log_2(\text{fold change})| \geq 1$ and an FDR-adjusted P value < 0.05 were defined as DElncRNAs. To identify candidate lncRNA–mRNA co-expression associations, Pearson correlation coefficients were calculated between DElncRNAs and DEmRNAs based on their expression profiles across all RNA-seq samples using the `cor.test` function in R v4.0.5. DElncRNA–DEmRNA pairs with $|r| > 0.95$ and $P \leq 0.05$ were retained as candidate co-expression pairs for subsequent network analysis.

Identification and co-expression analysis of circRNAs

circRNAs were identified from the genome-aligned sequencing reads using CIRI (v2.0.6) based on the detection of back-splice junction reads. Candidate circRNAs supported by reliable back-splice junction signals were retained for subsequent analyses. circRNA expression levels were quantified based on back-splice junction read counts, and differential expression analysis was performed using DEGseq (v1.36.1) [25]. circRNAs with $|\log_2(\text{fold change})| \geq 1$ and an FDR-adjusted P value < 0.05 were defined as DEcircRNAs. To identify candidate circRNA–mRNA co-expression associations, Pearson correlation coefficients were calculated between DEcircRNAs and DEmRNAs based on their expression profiles across all RNA-seq samples using the `cor.test` function in R v4.0.5. DEcircRNA–DEmRNA pairs with $|r| > 0.95$ and $P \leq 0.05$ were retained as candidate co-expression pairs for subsequent network analysis. Three independent biological replicates were included in each group.

Functional annotation

Protein-coding transcripts were annotated using the *P. homarus* reference genome and sequence similarity searches in public databases. mRNA sequences were matched to the Swiss-Prot database for Gene Ontology (GO) annotations. GO enrichment analysis was conducted on differentially expressed genes, mRNAs co-expressed with DElncRNAs, and host genes of DEcircRNAs, using all annotated mRNAs as the background set. Enrichment significance was assessed with a hypergeometric test, and P -values were adjusted via the Benjamini–Hochberg method, considering GO terms with q -values < 0.05 as significant. GO terms were classified into biological process, molecular function, and cellular component categories. KEGG pathway annotation was done through the KEGG Automatic Annotation Server (KAAS; <http://www.genome.jp/tools/kaas/>). Based on the obtained KEGG annotations, pathway enrichment analysis was performed using KOBAS for DEGs, mRNAs co-expressed with DElncRNAs, and host genes of DEcircRNAs. Similar to GO enrichment analysis, the background set consisted of all KEGG-annotated transcripts. Pathway enrichment significance was assessed using a hypergeometric test followed by Benjamini–Hochberg correction. KEGG pathways with corrected p -values < 0.05 were considered significantly enriched [26]. GSEA was performed using GSEA v4.1.0 based on a ranked list of gene-level statistics from the ovary-versus-testis comparison. Gene sets with $|\text{NES}| > 1$, nominal $P < 0.05$, and FDR $q < 0.25$ were considered significantly enriched.

Quantitative real-time PCR (RT-qPCR)

To validate RNA-seq results, RT-qPCR was performed on eight DEGs, four DElncRNAs, and four DEcircRNAs. Testes from six males and ovaries from six females were used, treating each as an independent biological replicate. After removing genomic DNA, 1 µg of total RNA was reverse-transcribed into cDNA using the EasyScript® kit (TransGen Biotech). Primers for mRNAs and lncRNAs were based on *P. homarus* genome annotations, while divergent primers for circRNAs targeted back-splice junctions. Random primers facilitated cDNA synthesis from non-polyadenylated RNA. Primer sequences are in Supplementary Table S2.

RT-qPCR was performed using RealUniversal Color PreMix (SYBR Green) (TIANGEN Biotech, Beijing, China) on a qTOWER³ G real-time PCR system (Analytik Jena GmbH, Jena, Germany). Each reaction was performed in a total volume of 20 µL containing 10µL of RealUniversal Color PreMix, 0.6 µL each of forward and reverse primers, 2 µL of diluted cDNA, and nuclease-free water. The thermal cycling conditions consisted of an initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s. Product specificity was evaluated from the melting profiles generated at the end of the amplification procedure, with a single distinct peak considered indicative of specific amplification. Primer amplification efficiencies, determined from standard curves, ranged from 90% to 110%. No-template controls were included to monitor potential contamination.

β-Actin and elongation factor 1-alpha (EF1α) were used as reference genes based on their relatively stable expression across the gonadal samples. The mean Ct value of the three technical replicates was used for subsequent calculations. Relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, with the arithmetic mean Ct value of β-actin and EF1α used for normalization.

Statistical and bioinformatics analyses

Statistical analyses of the RT-qPCR data were performed using individual animals as biological replicates. Six ovarian and six testicular samples were included, and each biological sample was analyzed using three technical replicates. The mean Ct value of the three technical replicates was used to calculate the ΔCt value for each biological sample. Normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene’s tests. Differences between the two tissues were evaluated using an independent-samples t-test. Data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS v25.0, and $P < 0.05$ was considered statistically significant.

Results

Gonadal histology

Histological features of all examined *P. homarus* specimens were consistent with gonads that had not attained full maturity. The testes displayed a lobular organization, with numerous seminiferous lobules separated by connective-tissue septa. Spermatogonia, primary spermatocytes, secondary

spermatocytes, and spermatids were concurrently present within individual lobules. The presence of meiotic spermatocytes and differentiating spermatids indicated ongoing spermatogenic activity. Despite active spermatogenesis, the absence of substantial accumulations of mature spermatozoa suggested that the testes had not yet attained a fully mature, sperm-filled condition. Ovarian development in *P. homarus* was classified into eight stages on the basis of oocyte morphology and size, the extent of yolk deposition, and progressive changes in nuclear and nucleolar architecture. These comprised the oogonial (< 15 µm), bouquet (15–17 µm), chromatin–nucleolus (17–45 µm), oil-globule (45–60 µm), pre-yolk-platelet formation (35–125 µm), yolk-platelet formation (105–150 µm), pre-maturation (150–300 µm), and maturation (300–400 µm) stages. Collectively, these stages delineate the progressive continuum of oocyte growth and maturation [27]. As shown in Fig. 1, the examined ovaries contained oocytes measuring < 120 µm in diameter that, together with their morphological characteristics, were consistent predominantly with the pre-yolk-platelet formation stage. The ovarian tissue was composed predominantly of early developing oocytes, with no substantial accumulation of large yolk granules. The ooplasm contained numerous lipid-like vacuoles, while the nuclei and nucleoli remained clearly discernible, features characteristic of early ovarian development. Collectively, these histological features indicated that the ovaries were at a developing, rather than fully mature, stage .

Sequencing data quality assessment

Following quality control procedures, high-throughput sequencing of testis and ovary samples demonstrated valid base rates exceeding 97.30%, Q30 values surpassing 98.10%, with no discernible base composition bias. These metrics collectively suggest a high level of sequencing quality. Subsequent alignment of clean reads to the *P. homarus* reference genome yielded overall mapping rates exceeding 90%, with some samples achieving rates greater than 98%, thereby indicating efficient read alignment to the reference genome (Table 1). In summary, the sequencing data obtained in this study are of adequate quality to support subsequent differential expression analysis and integrative transcriptomic investigations.

Table 1
Quality control statistics of transcriptome sequencing data

Sample	Raw Data (Gbp)	Clean Data (Gbp)	Q30 (%)	Valid bases (%)	Total mapped reads (%)
(Ovary1) O1	13.84	13.48	98.34	97.37	91.83
(Ovary2) O2	14.02	13.69	98.15	97.67	91.26
(Ovary3) O3	13.97	13.59	98.37	97.30	90.18
(Testis1) T1	14.03	13.67	98.27	97.42	97.47
(Testis2) T2	13.95	13.61	98.11	97.55	98.00
(Testis3) T3	13.85	13.54	98.31	97.77	98.23

Identification of differentially expressed mRNAs

PCA showed clear separation between ovarian and testicular samples, while samples within each tissue group clustered relatively closely, indicating distinct overall expression profiles between the two gonadal tissues. A total of 6,139 DEGs were identified, including 2,895 ovary-biased and 3,244 testis-biased genes, based on the ovary-versus-testis comparison. KEGG enrichment analysis revealed that genes with testis-biased expression were predominantly enriched in pathways related to PI3K–Akt signaling, cytoskeletal remodeling, and amino acid metabolism. In contrast, genes with ovary-biased expression were enriched in pathways associated with the cell cycle, oocyte meiosis, and progesterone-mediated oocyte maturation (Fig. 2). Integrating the transcriptomic results with histological observations, the enrichment of cell cycle- and meiosis-related pathways was consistent with the cellular characteristics of ovaries at the pre-yolk-platelet formation stage, whereas cytoskeleton- and metabolism-related pathways reflected transcriptional signatures associated with spermatogenic testes.

To further characterize pathway-level transcriptional differences between the ovary and testis, gene set enrichment analysis (GSEA) was performed using genes ranked according to the ovary-versus-testis comparison. The cell cycle (NES = 2.13, nominal P < 0.001, FDR q < 0.001), oocyte meiosis (NES = 1.93, nominal P < 0.001, FDR q = 0.001), and oxidative phosphorylation (NES = 2.08, nominal P < 0.001, FDR q < 0.001) pathways were enriched toward the ovarian side of the ranked gene list. By contrast, the ribosome (NES = - 2.95, nominal P < 0.001, FDR q < 0.001), ECM–receptor interaction (NES = - 2.05, nominal P < 0.001, FDR q = 0.004), and alanine, aspartate and glutamate metabolism (NES = - 1.66, nominal P = 0.008, FDR q = 0.050) pathways were enriched toward the testicular side (Fig. 3).

Identification and functional characterization of differentially expressed lncRNAs

A total of 761 DElncRNAs were identified, of which 494 showed ovary-biased expression and 267 showed testis-biased expression (Fig. 4). The identified lncRNAs exhibited considerable variation in transcript length, with relatively large proportions distributed within the 200–399 nt, 400–599 nt, and > 2,000 nt intervals. To examine potential expression-level relationships between DElncRNAs and protein-coding genes, Pearson correlation analysis was performed between DElncRNAs and DEmRNAs across the six RNA-seq samples. Pairs satisfying $|r| > 0.95$ and $P \leq 0.05$ were retained as candidate lncRNA–mRNA co-expression associations. KEGG enrichment analysis showed that the co-expressed mRNAs were overrepresented in pathways including oxidative phosphorylation, thermogenesis, nucleocytoplasmic transport, and PI3K–Akt signaling. GO analysis further assigned these genes to cellular components such as the cytoplasm, cytosol, nucleoplasm, and mitochondrion, as well as molecular functions including metal-ion binding, ATP binding, and oxidoreductase activity. These results link sex-biased lncRNA expression to mRNA modules associated with cellular organization, energy metabolism, and signal transduction, providing candidate molecular associations for further investigation of sex-biased gonadal transcription in *P. homarus*.

Identification and functional characterization of differentially expressed circRNAs

A total of 238 differentially expressed circRNAs were identified, including 143 ovary-biased and 95 testis-biased circRNAs (Fig. 5). Approximately 49% of the circRNAs originated from exonic regions of protein-coding genes. Functional enrichment analysis of their corresponding host genes was therefore performed to explore biological processes potentially associated with these circRNAs. Host genes of DEcircRNAs were enriched in pathways related to endocytosis, Hedgehog signaling, and amino acid metabolism, suggesting that circRNA-associated host genes may be involved in biological processes related to gonadal functions.

Integrated transcriptomic analysis identifies candidate molecular associations across coding and non-coding RNAs

To identify expression associations across coding and non-coding RNA classes, separate lncRNA–mRNA and circRNA–mRNA co-expression networks were constructed using pairs that satisfied $|r| > 0.95$ and $P \leq 0.05$. The networks comprised nine selected reproduction-associated mRNAs, 45 DElncRNAs, and 42 DEcircRNAs. The selected mRNAs included genes associated with sex-biased gonadal functions and germline biology, including *Yl*, *Foxl2*, *Fem1B*, *Fem1C*, *Pxt*, *Siwi*, *Mael*, *Hpgds* and *Vasa*. Within the resulting networks, *Yl*, *Foxl2*, and *Fem1B* were connected to multiple differentially expressed ncRNAs,

identifying them as highly connected nodes within the selected expression modules (Fig. 6). These network properties prioritize specific lncRNA–mRNA and circRNA–mRNA pairs for subsequent investigation.

RT-qPCR validation

RT-qPCR analysis was performed to assess the expression patterns of selected mRNAs, lncRNAs, and predicted circRNA candidates. The four selected DElncRNAs displayed expression directions consistent with those estimated from RNA-seq, although the magnitudes of fold change differed between the two methods. The selected predicted circRNA candidates also showed broadly consistent expression directions.

Among the eight selected mRNAs, *Yl*, *Fem1B*, *Mael*, and *Pxt* showed ovary-biased expression, whereas *Lhx9*, *Hpgds*, *Dmrt1*, and *Foxl2* showed testis-biased expression. Overall, the RT-qPCR results supported the direction of the expression differences detected by RNA-seq (Fig. 7).

Discussion

This study characterized sex-biased mRNA, lncRNA, and circRNA expression patterns in ovarian and testicular tissues of *P. homarus*. By integrating differential expression, functional enrichment, and cross-RNA correlation analyses, we resolved distinct gonadal transcriptional states and delineated candidate associations between non-coding RNAs and reproduction-associated mRNAs. These findings provide a transcriptomic resource for prioritizing reproduction-associated genes and ncRNA candidates.

Sex differentiation and gonadal development constitute essential biological processes within the life cycle of crustaceans, with their regulatory mechanisms exerting a direct impact on reproductive capacity and population dynamics [28, 29]. Nonetheless, in contrast to shrimp and crab species, the molecular mechanisms governing gonadal development in lobsters—particularly *P. homarus*—remain inadequately understood, and comprehensive analyses incorporating non-coding RNAs are conspicuously absent. This study addresses this knowledge gap by systematically characterizing the multi-layered transcriptomic features associated with gonadal differences through integrated mRNA, lncRNA, and circRNA analyses, thereby providing critical molecular insights.

Sex-biased transcriptomic characteristics associated with gonadal functions

The transcriptional differences between the ovaries and testes involved multiple biological pathways rather than being restricted to a single gene or process [30]. From a developmental first-principles perspective, the differentiation between testicular and ovarian structures is indicative not only of transcriptomic sexual dimorphism but also of two fundamentally distinct strategies of cellular resource allocation. The ovary is primarily involved in supporting oocyte growth, acquiring meiotic competence,

and making energetic investments. In contrast, the testis is focused on proliferative signaling, cytoskeletal remodeling, translational activity, and the coordination of the extracellular microenvironment [31, 32]. This interpretation is corroborated by pathway enrichment analyses, which demonstrate that ovary-biased genes are significantly enriched in pathways related to the cell cycle, oocyte meiosis, oxidative phosphorylation, and progesterone-mediated oocyte maturation [33]. Concurrently, testis-biased genes exhibit enrichment in the PI3K–Akt signaling pathway, ribosome-related processes, cytoskeletal regulation, and ECM–receptor interaction. In particular, ECM-associated transcription may be related to the organization of seminiferous lobules and interactions between germ cells and the surrounding somatic microenvironment [34].

Functional enrichment analysis indicated that the transcriptional differences between ovarian and testicular tissues involved multiple coordinated biological processes. GSEA showed that the cell cycle, oocyte meiosis, and oxidative phosphorylation pathways were enriched toward the ovarian side of the ranked gene list. The enrichment of cell-cycle and meiotic gene sets was consistent with the proliferation and meiotic development of oocytes, whereas oxidative-phosphorylation enrichment may reflect the energetic and biosynthetic requirements of oocyte growth. In contrast, the ribosome, ECM–receptor interaction, and alanine, aspartate and glutamate metabolism pathways were enriched toward the testicular side. These pathways may reflect differences in protein synthesis, cell–matrix interactions, amino-acid interconversion, and metabolic support associated with active spermatogenesis. In particular, ECM-associated transcription may be related to the organization of seminiferous lobules and interactions between germ cells and the surrounding somatic microenvironment

Testis-biased reproduction-associated candidate genes

In this study, multiple candidate regulatory genes with testis-biased expression, such as *Dmrt1*, *Lhx9*, *Hpgds*, and *Foxl2*, were identified in *P. homarus*. The expression profiles of these genes suggest their potential association with testis-biased transcriptional programs and spermatogenesis-related processes.

Among the genes examined, *Dmrt1* exhibited a marked testis-biased expression pattern, aligning with its conserved role in male gonadal differentiation, spermatogenesis, and reproductive maintenance across various taxa, such as *Macrobrachium nipponense* [35], *Penaeus monodon* [36], and zebrafish [37]. DM-domain transcription factors are widely acknowledged as important components associated with male reproductive development. Consequently, the testis-biased expression of *Dmrt1* in *P. homarus* suggests its potential involvement in a conserved male gonadal regulatory module within decapod crustaceans [38]. In addition to *Dmrt1*, the expression of *Lhx9* and *Hpgds*, which is biased towards the testis, is particularly noteworthy. *Lhx9*, a transcriptional regulator implicated in gonad formation and somatic cell development, may indicate the regulatory requirements necessary for the organization of testicular tissue, the support of gonadal somatic cells, and the maintenance of the germ-cell microenvironment [39]. *Foxl2* notably demonstrated a testis-biased expression, a pattern that deviates from its traditional role as a female-associated factor in most vertebrates [40, 41]. In vertebrate systems, *Foxl2* is typically

considered a crucial regulator of ovarian maintenance and female gonadal development. The testis-biased expression of *Foxl2* in *P. homarus* may thus suggest a lineage-specific reconfiguration of the sex-regulatory network in decapod crustaceans [42–44]. The testis-biased expression of *Foxl2* suggests a species-specific expression pattern in *P. homarus*, although its functional role requires further investigation [45, 46].

Ovary-biased reproduction-associated candidate genes

In this investigation, *Fem1B* and *Fem1C* demonstrated ovary-biased expression, indicating that members of the FEM family may play a role in maintaining the female gonadal state in *P. homarus*. Although *Fem1* was originally identified within the sex-determination pathway of *Caenorhabditis elegans* [47, 48], recent research in crustaceans suggests that this gene family may have functions extending beyond binary sex determination, potentially contributing to gonadal development, gametogenesis, and the maintenance of reproductive tissue identity [49–51]. Consequently, the heightened expression of *Fem1B* and *Fem1C* in the ovary of the scalloped spiny lobster may indicate their potential association with ovarian-biased transcriptional programs during early ovarian development at the pre-yolk-platelet formation stage [52].

Furthermore, the ovary-biased expression of *Mael*, *Vasa*, and *Siwi* was consistent with the prominent representation of germline-associated transcripts in ovarian tissue. These genes are typically linked to the maintenance of germ-cell identity, regulation of RNA metabolism, and activity within the piRNA pathway [53]. As a highly conserved defense system within the germline, the piRNA pathway plays a crucial role in maintaining genome stability, primarily by suppressing the activity of transposable elements [54, 55]. During the later stages of oocyte development, ovarian tissues experience heightened transcriptional activity, energy metabolism, and cytoplasmic remodeling. Consequently, the developing female germline may necessitate enhanced molecular protection to mitigate the risks associated with transposon activation, aberrant RNA dynamics, and genome instability [56]. The ovary-biased expression of these genes implies that oocyte maturation in *P. homarus* may involve more than just yolk accumulation and meiotic progression; it may instead represent a complex developmental process that integrates germ-cell identity maintenance, RNA homeostasis, and genome-protection mechanisms.

Candidate non-coding RNA associations underlying sex-biased gonadal transcription

A significant contribution of this study is the comprehensive profiling of mRNAs, lncRNAs, and circRNAs within the gonads of *P. homarus*. Prior transcriptomic research on crustacean gonads has predominantly concentrated on protein-coding genes and specific sex-related pathways. By incorporating the non-coding RNA dimension, this study offers a more extensive, multilayered framework for elucidating sex-biased gonadal development in the scalloped spiny lobster.

A total of 761 DElncRNAs and 238 DEcircRNAs were identified, indicating that transcriptional differences between the testis and ovary of *P. homarus* involve not only protein-coding genes but also extensive alterations in non-coding RNA expression patterns. Previous studies have demonstrated that lncRNAs

and circRNAs may participate in gene regulation through diverse mechanisms, including cis-, trans-, and post-transcriptional regulatory processes [57, 58]. Although the functional roles of these non-coding RNAs in *P. homarus* remain to be experimentally validated, their differential expression patterns and predicted interactions with reproduction-related genes suggest that they may represent candidate regulatory molecules associated with sex-biased gonadal characteristics [59, 60].

Further analyses of lncRNA–mRNA and circRNA–mRNA co-expression networks have unveiled significant expression correlations between various ncRNAs and core genes implicated in gonadal development. Notably, the genes *Yl*, *Foxl2*, and *Fem1B* demonstrated high connectivity within these networks, indicating their potential role as candidate convergence nodes where coding and non-coding regulatory signals may intersect [61]. The network developed in this study indicates that gonadal development in the scalloped spiny lobster is unlikely to be regulated by a single gene or pathway. Rather, it appears to be a complex, multilayered process involving the coordinated interaction of protein-coding genes, non-coding RNAs, and post-transcriptional regulatory networks [62, 63]. To substantiate the biological functions of the candidate genes and the regulatory axes mediated by non-coding RNAs identified in this study, future studies integrating expanded biological sampling, spatial expression analysis, RNA interference, overexpression assays, RNA pull-down, RNA immunoprecipitation, and in situ hybridization will be required to determine whether the candidate associations identified here have functional roles in gonadal biology.

Conclusions

This study provides an integrated characterization of sex-biased mRNA, lncRNA, and circRNA expression in ovarian and testicular tissues of *P. homarus*. Distinct transcriptional profiles were identified between the two gonadal tissues, including differential expression of several reproduction-associated candidate genes, such as *Dmrt1*, *Foxl2*, *Fem1*, *Pxt*, *Mael*, *Lhx9*, *Hpgds* and *Yl*. Functional enrichment analyses further identified distinct pathway-level transcriptional signatures associated with ovarian and testicular tissues. Expression correlation analyses delineated candidate lncRNA–mRNA and circRNA–mRNA networks and prioritized highly connected coding and non-coding RNA pairs for subsequent investigation. The resulting dataset provides a focused transcriptomic framework for investigating sex-biased gonadal biology in *P. homarus*.

Abbreviations

ncRNAs

Non-coding RNAs

lncRNAs

Long non-coding RNAs

circRNAs

Circular RNAs

DEGs

Differentially expressed genes
DElncRNAs
Differentially expressed long non-coding RNAs
DEcircRNAs
Differentially expressed circular RNAs
RT-qPCR
Reverse transcription quantitative real-time PCR
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes

Declarations

Funding

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Authors' contributions

L.Z., R.Z., and Y.L. designed the research framework. L.Z., F.Z., Z.J., and E.L. carried out the experiments. L.Z. and F.Z. drafted the manuscript. F.Z., Z.J., R.Z., and H.L. performed the bioinformatics analyses. Q.Y., S.J. and Y.D. performed the validation. F.Z. and Z.J. performed the formal analysis. Q.Y., H.L., and F.Z. conducted the investigation. J.H. and Y.Y. performed the data curation. E.L. and Y.L. reviewed and edited the manuscript. L.Z. and Y.L. supervised the study. Y.L. acquired the funding. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The animal study protocol was approved by the Animal Ethics Committee of South China Sea Fisheries Research Institute (protocol code No. nhdf2026-05; 6 April 2026).

Availability of data and materials

The raw sequencing data generated in this study are available in the NCBI Sequence Read Archive (SRA) database under BioProject accession number PRJNA1507272.

Consent for publication

Not applicable

Conflicts of Interest

The authors declare no conflicts of interest.

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Figures

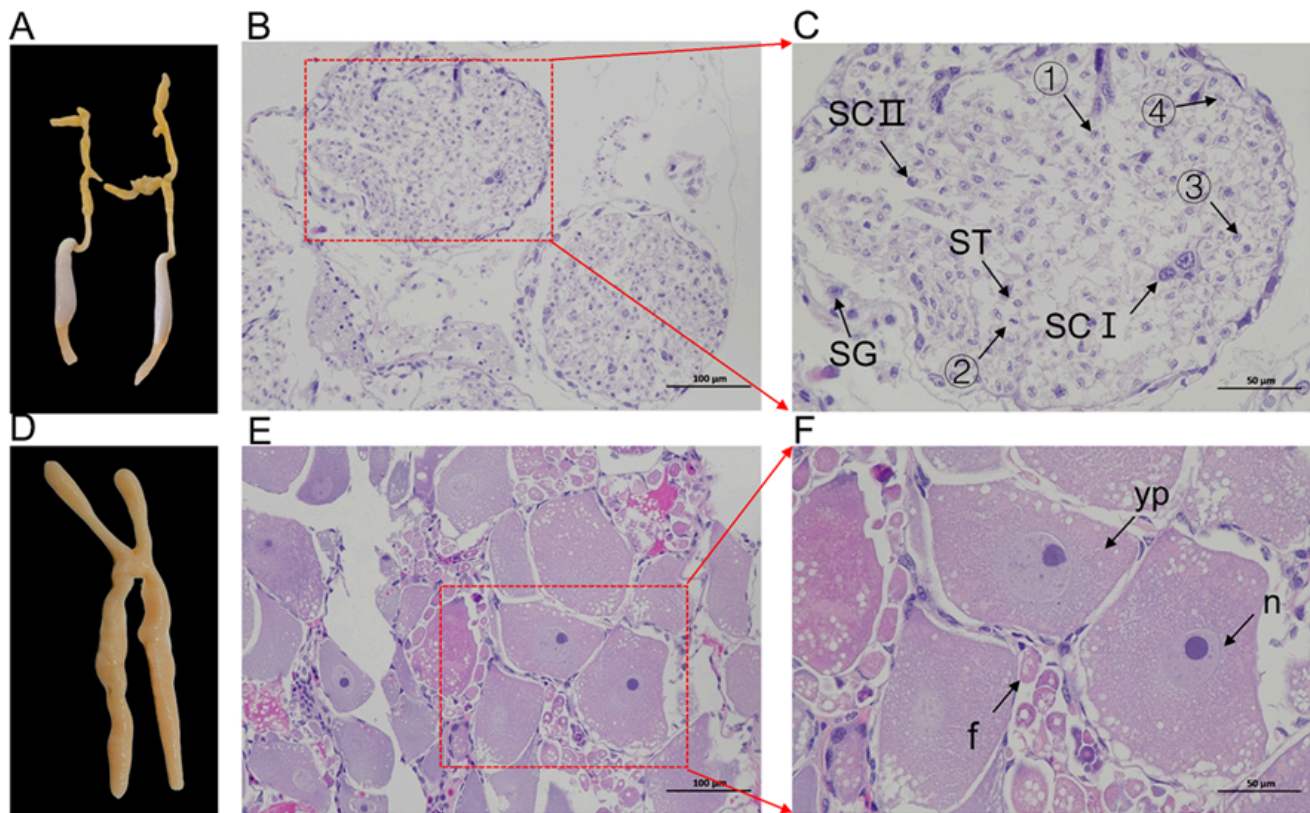


Figure 1

Histological structure of the testis and ovary in *P. homarus*. (A, D) Gross morphology of the testis and ovary, respectively. (B, E) Low-magnification transverse sections of the testis and ovary, showing the general organization of gonadal tissues. (C, F) High-magnification views of the corresponding regions indicated in B and E. B and E are shown at 200× magnification, whereas C and F are shown at 400× magnification. SG, spermatogonia; SCI, primary spermatocytes; SCII, secondary spermatocyte; ST, spermatids; ♂, meiotic prophase; ♀, meiotic metaphase; ♂, meiotic anaphase; ♀, meiotic telophase; yp, yolk particle; fc, follicle cell; n, nucleus.

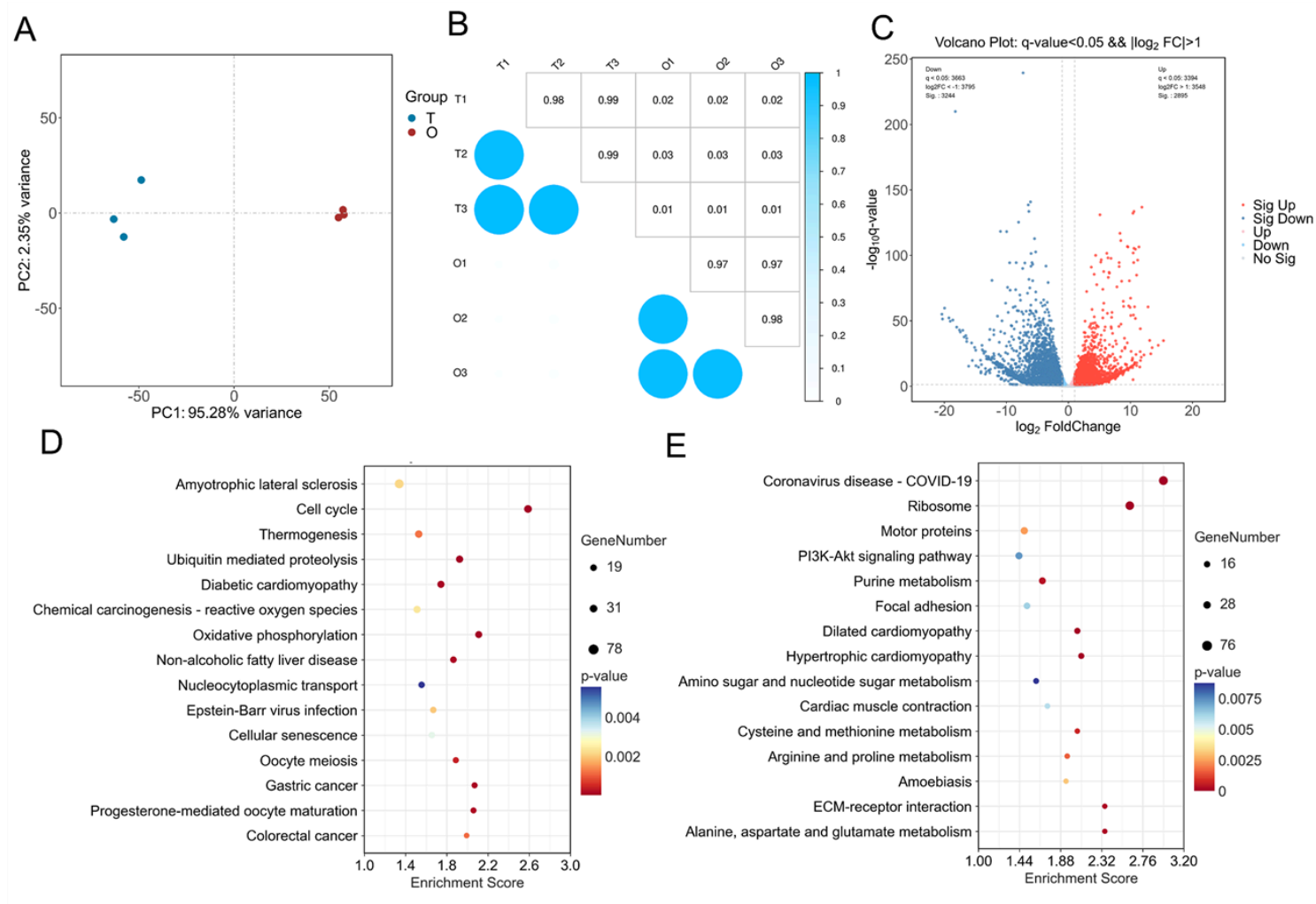


Figure 2

Differentially expressed gene analysis between testis and ovary tissues of *P. homarus*. (A) Principal component analysis (PCA) of gene expression profiles. (B) Pearson correlation heatmap of sample relationships. Circle sizes and color intensity represent the correlation coefficients. (C) Volcano plot of differentially expressed genes (DEGs). (D) KEGG enrichment analysis of ovary-biased DEGs. (E) KEGG enrichment analysis of testis-biased DEGs.

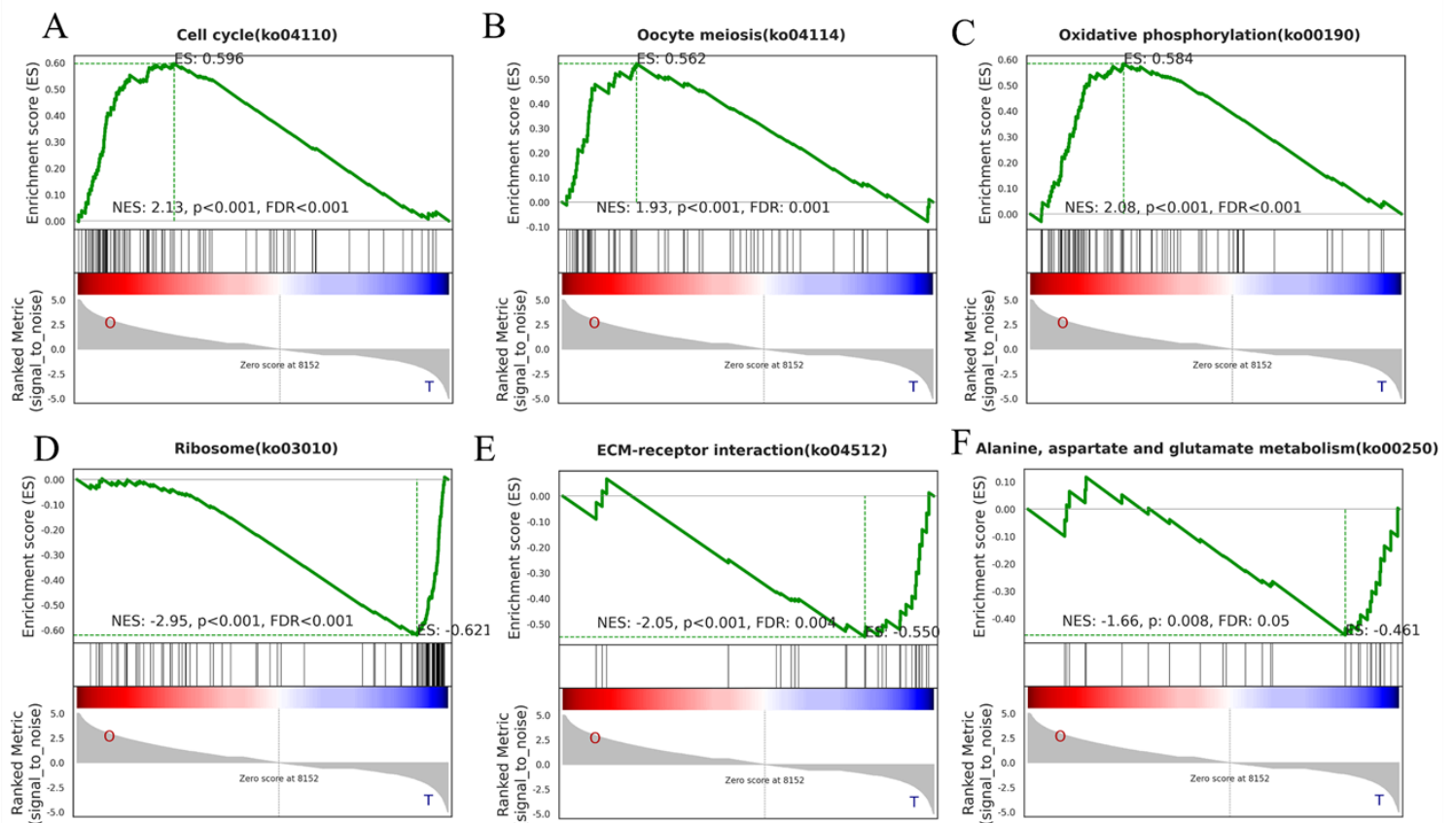


Figure 3

Gene set enrichment analysis (GSEA) of sex-biased pathways. (A) Cell cycle pathway positively enriched in the ovary. (B) Oocyte meiosis pathway positively enriched in the ovary. (C) Oxidative phosphorylation pathway positively enriched in the ovary. (D) Ribosome pathway positively enriched in the testis. (E) ECM-receptor interaction pathway positively enriched in the testis. (F) Alanine, aspartate and glutamate metabolism pathway positively enriched in the testis.

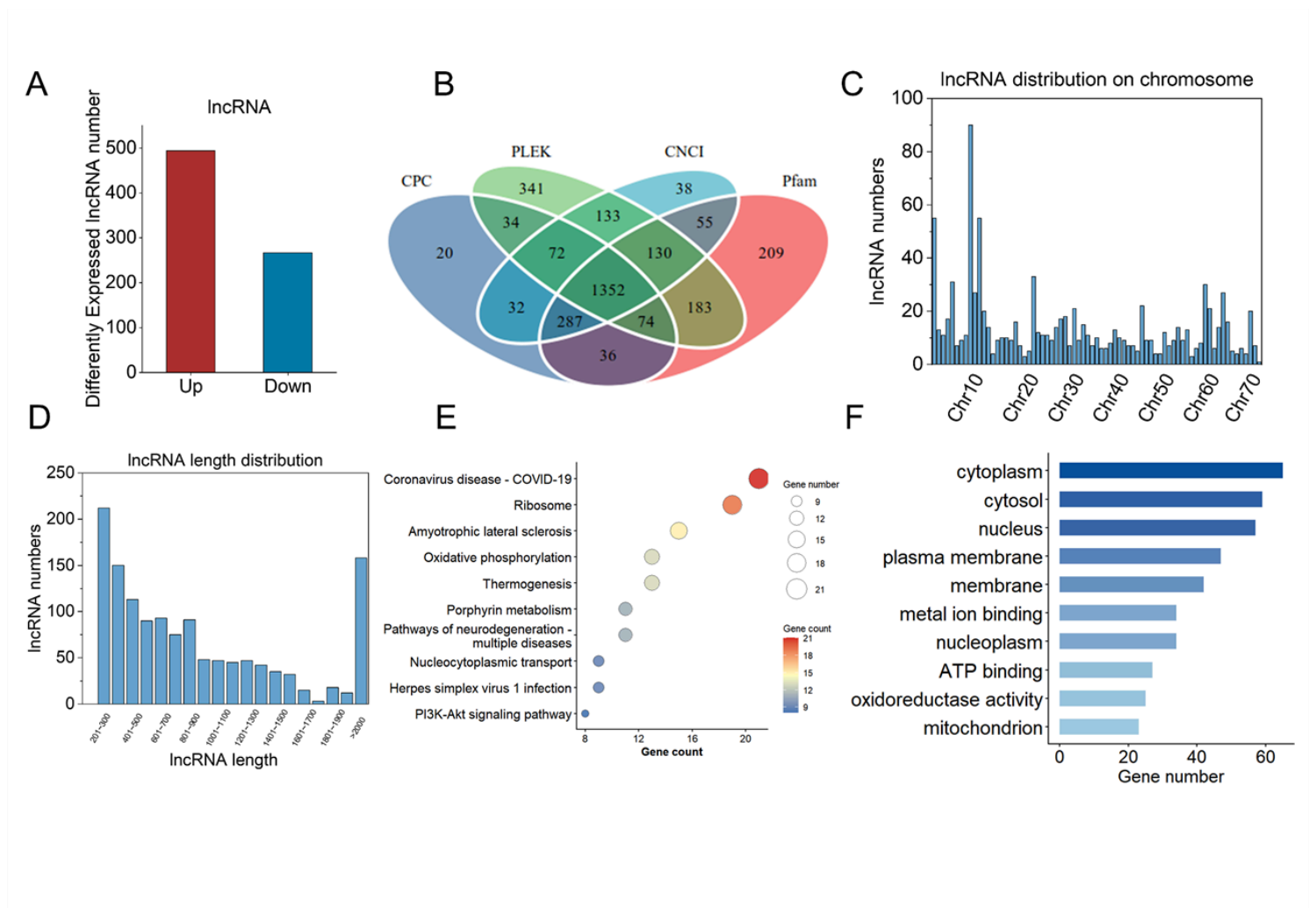


Figure 4

IncRNA analysis in the gonads of *P. homarus*. (A) Bar plot of DEIncRNAs. “Up” indicates IncRNAs upregulated in the ovary relative to the testis, whereas “Down” indicates IncRNAs downregulated in the ovary relative to the testis. (B) Venn diagram of IncRNAs predicted by four software tools. (C) Chromosomal distribution of IncRNAs; the x-axis represents chromosome numbers. (D) Length distribution of IncRNAs. (E) KEGG pathway enrichment analysis of mRNAs co-expressed with DEIncRNAs. (F) GO enrichment analysis of mRNAs co-expressed with DEIncRNAs.

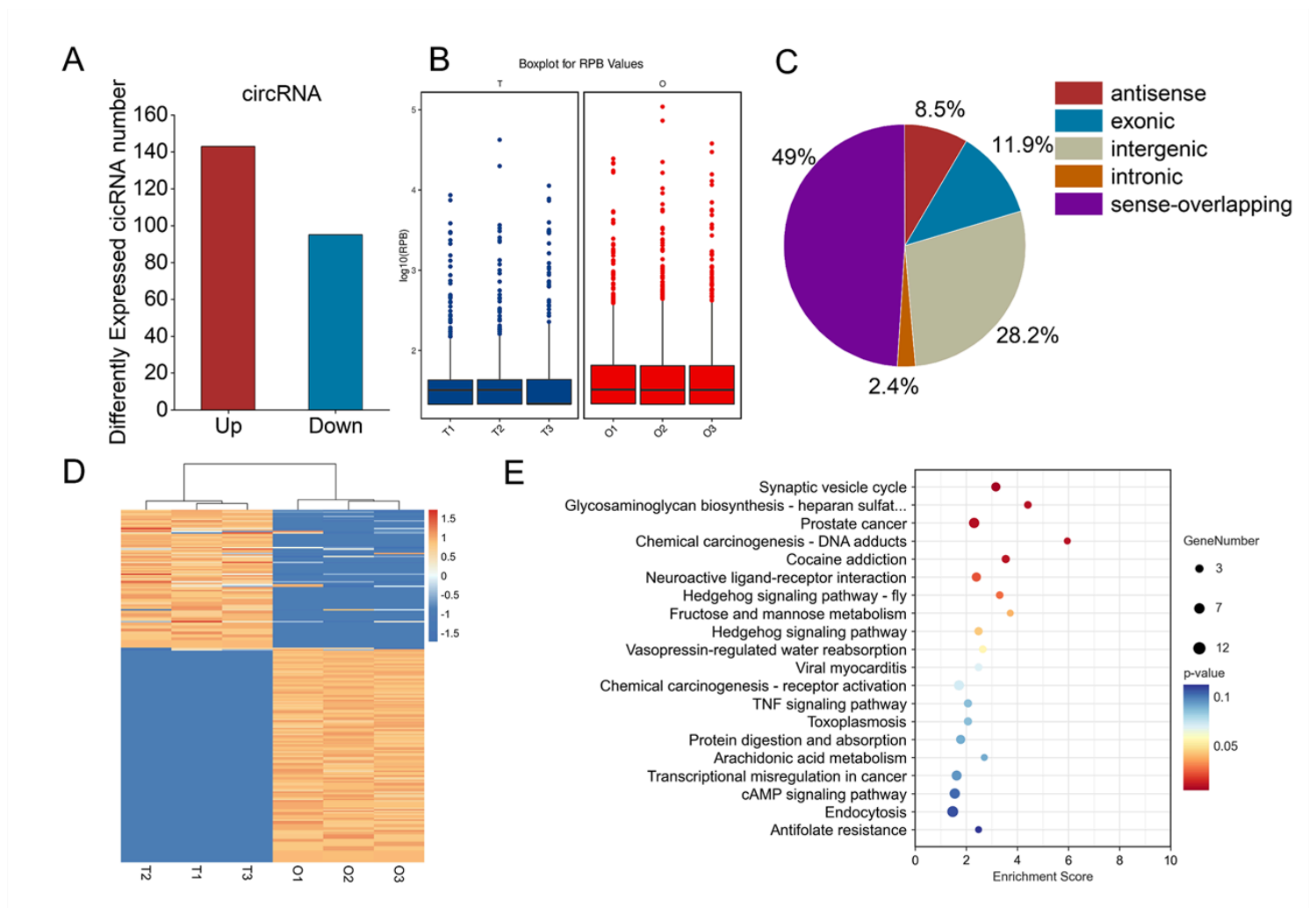
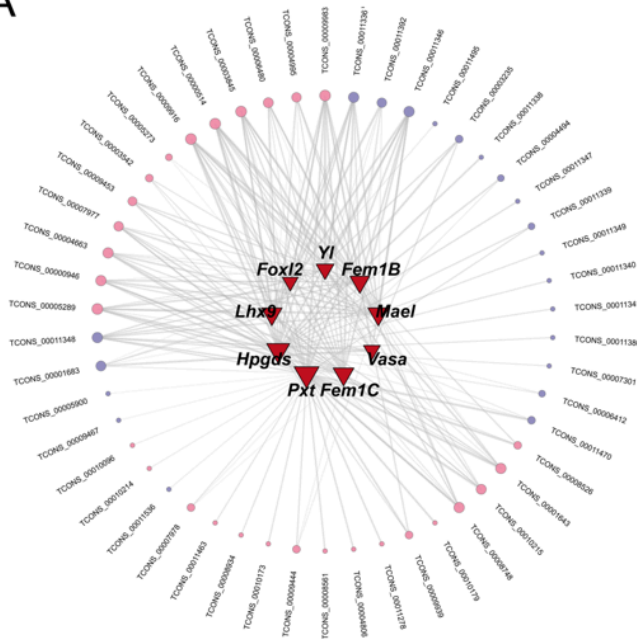


Figure 5

circRNA analysis in the gonads of *P. homarus*. (A) Summary of DEcircRNAs. “Up” indicates circRNAs upregulated in the ovary relative to the testis, whereas “Down” indicates circRNAs downregulated in the ovary relative to the testis. (B) Box plot of circRNA expression levels. (C) Distribution of circRNA types. (D) Clustering heatmap of DEcircRNAs. (E) KEGG enrichment analysis of host genes of DEcircRNAs.

A



B

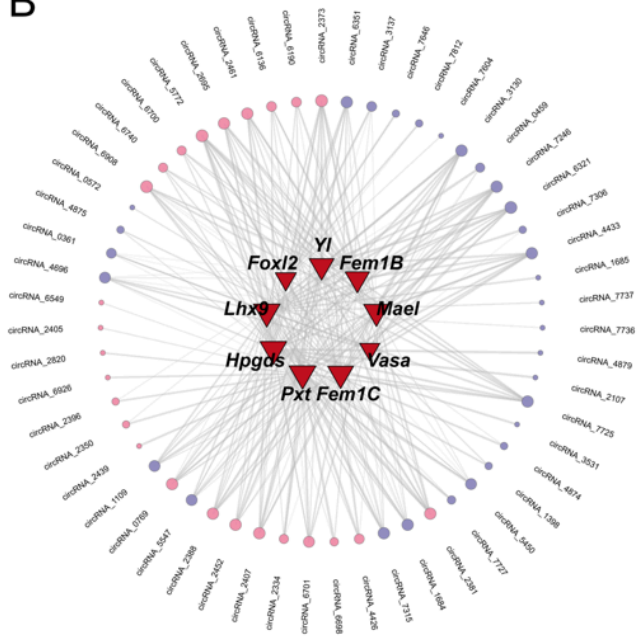


Figure 6

Candidate coding–non-coding RNA co-expression networks associated with sex-biased gonadal transcription in *P. homarus*. (A) lncRNA–mRNA co-expression network. (B) circRNA–mRNA co-expression network. In the co-expression networks (A and B), red triangles represent highly connected mRNA nodes identified based on connectivity degree within the network. The size of each node indicates its connectivity degree. Outer circular nodes represent associated lncRNAs or circRNAs, with pink and blue indicating upregulated and downregulated non-coding RNAs, respectively. Gray edges represent co-expression relationships, and edge intensity reflects the strength of correlation coefficients ($|r| = 0.95–1.00$).

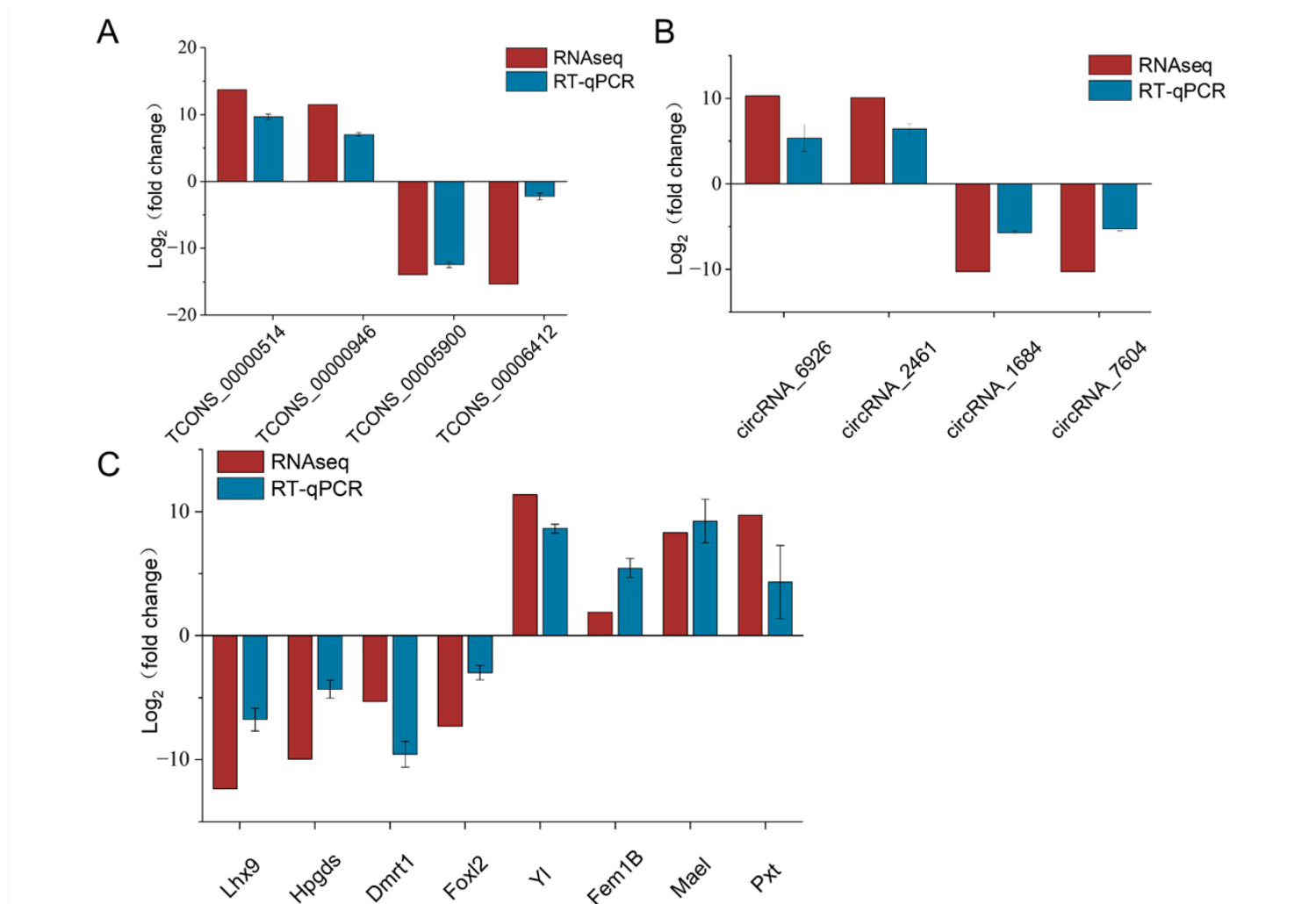


Figure 7

RT-qPCR validation of RNA-seq results. (A) RT-qPCR validation of selected differentially expressed lncRNAs. (B) RT-qPCR analysis of selected predicted circRNA candidates. (C) RT-qPCR validation of eight gonadal development-related differentially expressed mRNAs, including testis-biased genes (*Lhx9*, *Hpgds*, *Dmrt1*, and *Foxl2*) and ovary-biased genes (*Yl*, *Fem1B*, *Mael*, and *Pxt*). Red bars represent RNA-seq data, and blue bars represent RT-qPCR results. The y-axis indicates log₂ fold change. Positive values indicate ovary-biased expression, whereas negative values indicate testis-biased expression.

Supplementary Files

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