Comparative Transcriptome Analysis of the Ovary and Testis in Noble Scallop (Chlamys nobilis)

Fan Sigang1, Guo Yihui* and Xu Youhou2

1Key Laboratory of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, PR China
2Guangxi Key Laboratory of Beibu Gulf Marine Biodiversity Conservation, Beibu Gulf University, Qinzhou, PR China

ABSTRACT

Noble scallop (Chlamys nobilis) is an economically important cultured marine bivalve shellfish common in southern China. Investigation on the molecular regulatory mechanisms of gonadal maturation in scallop is critical in the aquacultural industry. Here, gonads in maturing stage were obtained from noble scallops and sequenced using an Illumina high-throughput sequencer, producing 6.68 and 6.70 Gb of data for the ovary and testis, respectively. Reproduction-related genes, including vasa, nanos, and vitellogenin, and sex-determining genes, such as FoxL2, Dmrt, and sox9, were detected. Transcriptome comparison revealed 2,842 differentially expressed genes (DEGs), of which 591 exhibited biased expression in the ovary and 2,251 exhibited biased expression in the testis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of the DEGs were conducted. Results showed that GO terms and KEGG pathways related to protein glycosylation, fatty acid biosynthetic processes, hydrolase activity, and AMPK were enriched in the ovary, whereas those related to male organ formation and spermiogenesis were enriched in the testis. The glycosphingolipid biosynthesis pathway was identified for the first time in a mollusc testis. The present study provides the first transcriptomic analysis of C. nobilis, which will help clarify the molecular mechanisms of gonadal maturation.

INTRODUCTION

Sex is one of the most fundamental features of life. Sex determination and reproductive regulation have been extensively studied in mammals, insects, birds, and fish. Both genetics and the environment can influence sex determination (Zhang et al., 2014; Morishita et al., 2010). Molluscs are good animal models for studying sex determination and reproductive regulation due to their diverse modes of reproduction, including dioecy, hermaphroditism (e.g., bay scallop), and sex reversal (e.g., Pacific oyster). Studies on mollusc reproduction can provide insights into the molecular mechanisms of sex determination and reproductive regulation. Previously isolated and characterized reproduction-related genes include soxE (Santerre et al., 2014), beta-catenin (Santerre et al., 2014), FoxL2 (Naimi et al., 2009), vitellogenin (Zheng et al., 2012b), Dmrt2 (Shi et al., 2014), Dmrt5 (Shi et al., 2014), and 5-hydroxytryptamine receptors (HTR) (Wang, 2014). In recent years, next generation sequencing technology has been widely used in genome and transcriptome analyses with low cost, high efficiency, and superior accuracy, with a growing number of reproduction-related genes found from such studies (Matsumoto et al., 2013; Zhang et al., 2014, 2019). More than 40 gene models were identified with high accuracy to encode reproduction-related genes reported for P. fucata and other molluscs (Matsumoto et al., 2013). Sex-related genes and pathways have also been identified in the transcriptomes of Crassostrea hongkongensis and Patinopecten yessoensis (Tong et al., 2015; Li et al., 2016b). In the Pacific oyster, certain genes, including SoxH, FoxL2, and Dsx, are thought to be involved in sex determination (Zhang et al., 2014). In P. yessoensis, FoxL2, Dmrt, and soxH are also considered to be key candidates for scallop sex determination/differentiation (Li et al., 2016b). Compared with model species, however, studies on bivalve sex determination genes and pathways are still limited. The noble scallop (Chlamys nobilis) is an economically important and edible marine bivalve, which has been cultured in the Southern Sea of China since the 1980s (Zheng et al., 2012a). Recent research on C. nobilis...
has focused on genes involved in immunity (Yang et al., 2016; Zhang et al., 2016a) and reproduction (Zheng et al., 2012b; Shi et al., 2014) and on transcriptome sequencing for identification of candidate genes associated with carotenoid-based coloration (Liu et al., 2015). In this study, we employed Illumina sequencing technology and de novo assembly to obtain the transcriptome of the maturing ovaries and testes of C. nobilis. Many genes associated with sex determination and maturation were determined, and various differentially expressed genes (DEGs) between the ovaries and testes were identified. In addition, GO and KEGG enrichment analyses of DEGs demonstrated significant differences between the ovaries and testes. This study will be useful to clarify the molecular mechanisms of gonadal maturation in C. nobilis.

MATERIALS AND METHODS

Tissue collection
Eight healthy specimens of C. nobilis (N = 4 for each sex), averaging 51 mm in shell height, were purchased from an aquaculture farm in Shenzhen, Guangdong Province, China. The ovaries and testes were dissected and freeze-dried in liquid nitrogen and stored at -80°C. A 5-mm thick section of gonad from each individual was cut and then fixed in 4% paraformaldehyde for 24 h.

Sample histology
Histological methodology followed that of Yan et al. (2010). After fixation in 4% paraformaldehyde, the gonad sections were dehydrated in graduated ethanol washes, and embedded in paraffin. Sections (6 µm thick) were prepared, and then stained with hematoxylin and eosin according to routine histological techniques. The sections were examined to assess the stage of gonadal development using a Nikon Eclipse CI microscope equipped with a Nikon DS-U3 image analyzing system (Nikon, Japan).

RNA extraction and cDNA library construction
Total RNA was isolated from each sample using Trizol Reagent (Invitrogen, Carlsbad, USA) following the manufacturer’s protocols. Quantity and integrity of the RNA samples were confirmed by 1% agarose gel and a 2100-Bioanalyzer (Agilent Technologies, USA). 1 µg of RNA from each ovary or testis were pooled, respectively.

Then cDNA was synthesized using the mRNA fragments as templates as usual and was sequenced by the oebiotech (Shanghai, China) using the Illumina HiSeq™ 2500 sequencing platform (Illumina, Inc, USA).

Sequence data analysis and assembly
Raw reads were processed by the NGS QC Toolkit (Pertea et al., 2012). Low quality bases which the percentage of low quality bases (base quality<20) and reads containing poly-N larger than 35bp were removed to obtain clean reads. De novo assembly of the clean reads was performed using the short reads assembling program Trinity (Grabherr et al., 2011). The TIGR Gene Indices Clustering (TGICL) tool was used to remove redundant sequences and perform further assembly (Pertea et al., 2003).

Sequence annotation
Unigenes were aligned with protein databases NR, Swiss-Prot, and KEGG using BLASTx alignment (e value < 10^-5). Gene names were assigned to each assembled sequence based on the best BLAST hit (highest score).

To annotate the assembled sequences with GO terms describing biological processes, molecular functions, and cellular components, the Nr BLAST results were imported into Blast2GO (Conesa et al., 2005; Götz et al., 2008). WEGO was used for GO functional classification of all unigenes (Ye et al., 2006). Additionally, pathway assignments were carried out based on the KEGG database.

Sequence mapping and differential expression analysis
Sequence reads were mapped to reference sequences using bowtie2 (Langmead and Salzberg, 2012) and eXpress. Expression levels for each unigene were measured with FPKM (Fragments Per Kb per Million Fragments) and input into the DEseq R package for differential expression analysis (Anders and Huber, 2013). Only genes with a false discovery rate (FDR) ≤ 0.05 were and log (fold change) values≥3 were selected as the thresholds, to identify differentially expressed genes (DEGs) between the testis (control) and ovary. GO and KEGG enrichment analyses of DEGs were performed using R based on hypergeometric distribution.

Quantitative RT-PCR validation
To validate the Illumina sequencing data, 11 DEGs between the ovary and testis were chosen for quantitative real-time PCR (qRT-PCR). All primers used were manufactured by Invitrogen (Shanghai, China) (Table 1). The RNA samples used for qRT-PCR amplifications were the same as those used to construct the RNA-Seq library mentioned above. For each sample, quantification was performed using gDNA Eraser and the Prime-Script™ Reverse Transcriptase Reagent Kit with gDNA Eraser (TaKaRa, Japan). The β-actin of C. nobilis was used as an internal reference. The qRT-PCR was performed in triplicate for each sample using the CFX96 real-time PCR Detection System (Eppendorf, Germany) in a 25 µl reaction system, with the following components: 12.5 µl of 2 × SYBR Green Real-time PCR Master Mix (Takara, Japan),
Table I. Primers used in real-time PCR confirmation.

| Genes         | Primer sequences (5’-3’) | Amplicon length (bp) | Source              |
|---------------|--------------------------|----------------------|---------------------|
| Sox11         | F:AGTTGGTTCGGAGACTGAGCG  | 218                  | This study          |
|               | R:TTTACCCACTGGTTTGGCCCTT |                      |                     |
| Sox2          | F:ATGAAAGGAGCATCCCGATACA| 81                   | This study          |
|               | R:CAATGGCGCATTCAGCGGTTAA |                      |                     |
| Dmrt-4        | F:GAGAGTTCAGACGACTATCC   | 94                   | This study          |
|               | R:GGGTGGAGATGAGGACAGAGTT |                      |                     |
| Vitellogenin  | F:AAAGGCTGATATGAAGACGAC  | 104                  | Zheng et al., 2012  |
|               | R:GCCTTTGCGATTTTGTTGC    |                      |                     |
| Nanos-like protein 1 | F:TCAGGGACGCACCAAGGGAG | 107                  | This study          |
|               | R:GAATGGACAGTGTCGGAGCTA  |                      |                     |
| 5-HT receptor | F:GTACAGGTAAAATGGAGGCGAACA| 76                  | This study          |
|               | R:GGCGACCCAAAGCCGATAA    |                      |                     |
| Sox9          | F:TCCTCCACCAAGATGACAAACA  | 227                  | This study          |
|               | R:CAATGGTGACAGGAAGGGCAGCGT|                     |                     |
| Sox2B         | F:TATTTGGTGCGCTGGTGCC    | 137                  | This study          |
|               | R:GGGTGGTGGATGAGCGAGTTTT |                      |                     |
| Vasa          | F:AGTTGGTTCGGAGATGCTTT   | 100                  | This study          |
|               | R:GGGTGGTGGATGAGCGAGTTTT |                      |                     |
| FoxL2         | F:GTGGGTGGATGCTGGTGCC    | 125                  | This study          |
|               | R:GGGTGGTGGATGAGCGAGTTTT |                      |                     |
| Estrogen receptor | F:CCCTGGAGCCTGGGATGATGGT | 81                   | This study          |
|               | R:TTATTCATGGCGACTGGTGGTGC|                      |                     |
| β-actin       | F:CAACACACTGGCTCCTGCGATC | 94                   | Zheng et al., 2012  |
|               | R:CTGGGACCCATGAACCTCTCGT |                      |                     |

0.5 μl of each primer (10 μM), 1.0 μl of cDNA, and 10.5 μl of RNase-free water. The PCR program was as follows: 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 30 s. To assess the specificity of the PCR amplification, a melting curve was obtained at the end of the reaction, and a single peak was observed. Data were quantified using the 2^-ΔΔCT method. The amplification efficiencies of the target and reference genes were verified and found to be approximately equal. All qRT-PCR data were analyzed using SPSS 19.0. Differences between means were considered significant at the 95% confidence level (P < 0.05). The results were expressed as means ± standard errors.

RESULTS

Histological analysis of gonads

Histology of the sampled C. nobilis gonads revealed that both the female ovaries and male testes were at the maturing stage. The ovaries were filled with oocytes and the testes were filled with sperm (Fig. 1).

Table II. Summary statistics of C. nobilis gonad transcriptome sequencing.

|               | Female          | Male            |
|---------------|-----------------|-----------------|
| Raw reads     | 46,404,820      | 46,527,700      |
| Raw bases     | 5,800,602,500   | 5,815,962,500   |
| Clean reads   | 46,404,820      | 46,527,700      |
| Clean bases   | 5,800,127,180   | 5,815,485,557   |
| Valid ratio (base) | 99.99%       | 99.99%          |
| Q30 (%)       | 94.86%          | 95.00%          |
| GC content (%)| 42.00%          | 43.00%          |

Sequence analysis and assembly

As shown in Table II, a total of 92,932,520 raw sequence reads were generated (46,404,820 and 46,527,700 reads from the ovary and testis, respectively). After stringent quality checking and data clean-up, the two sequence datasets consisted of clean reads only. The number of clean read is same as that of raw read, but with a different number of bases. The valid data rate was 99.99%, which proved that high-quality bases were obtain
Table III. Assembly statistics of *C. nobilis* gonad transcriptome.

|              | All   | >=500 bp | >=1000 bp | N50 | Total length | Max length | Min length | Average length |
|--------------|-------|----------|-----------|-----|--------------|------------|------------|----------------|
| Unigene      | 73812 | 41944    | 21830     | 1806| 7917655      | 50665      | 301        | 1072           |

Table IV. Top 10 genes with the greatest difference in expression in *C. nobilis* male and female gonads.

| ID            | Genes                                      | M/F (FPKM) | P-value (ovary-testis) |
|---------------|--------------------------------------------|------------|------------------------|
| **Male up-regulated genes** | | | |
| comp62793_c0_seq2 | Sperm motility kinase X-like | 13424.20 | 0.000877499 |
| comp60994_c0_seq1 | Testis-specific serine/threonine-protein kinase 1 | 9304.39 | 0.000289122 |
| comp68313_c0_seq1 | Kelch-like protein 10 | 7501.98 | 0.000164084 |
| comp60179_c0_seq1 | E3 ubiquitin-protein ligase MARCH3 | 5508 | 0.000702734 |
| comp60764_c0_seq1 | Potassium voltage-gated channel protein Shaw | 3429.44 | 0.000026587 |
| comp69860_c0_seq1 | Hemicentin-1 | 2574.88 | 0.000973017 |
| comp40556_c0_seq1 | Sperm protein | 1518.80 | 0.000973017 |
| comp64884_c0_seq1 | Testis-specific serine/threonine-protein kinase 1 | 9304.39 | 0.000289122 |
| comp68313_c0_seq1 | Kelch-like protein 10 | 7501.98 | 0.000164084 |
| comp60179_c0_seq1 | E3 ubiquitin-protein ligase MARCH3 | 5508 | 0.000702734 |
| comp60764_c0_seq1 | Potassium voltage-gated channel protein Shaw | 3429.44 | 0.000026587 |
| comp69860_c0_seq1 | Hemicentin-1 | 2574.88 | 0.000973017 |
| comp40556_c0_seq1 | Sperm protein | 1518.80 | 0.000973017 |
| **Female up-regulated genes** | | | |
| comp60048_c0_seq1 | Sarcoplasmic calcium-binding proteins II | 1157.04 | 0.094002299 |
| comp62792_c1_seq1 | Deleted in malignant brain tumors 1 protein-like | 807.1372549 | 0.093875806 |
| comp74276_c0_seq3 | Titin | 320.6800826 | 0.329993152 |
| comp60317_c0_seq1 | Caveolin-1-like | 236.754902 | 0.136813623 |
| comp73049_c2_seq1 | MAM and LDL-receptor class A domain-containing protein 1-like | 206.6078431 | 0.063965965 |
| comp54366_c0_seq1 | Fatty acid synthase-like | 180.1588235 | 0.234334085 |
| comp53233_c0_seq1 | GTP-binding protein Rhes-like | 137.6715686 | 0.077940042 |
| comp71473_c0_seq1 | MAM and LDL-receptor class A domain-containing protein 1-like | 124.9294118 | 0.084507302 |
| comp71132_c0_seq1 | Low-density lipoprotein receptor-related protein 2-like | 123.143986 | 0.80498201 |
| comp63224_c0_seq1 | Alkyl/aryl-sulfatase BDS1-like | 102.2399077 | 0.180469613 |

Genes above and below the line are up-regulated in males and females, respectively.

during sequencing. Detailed sequencing and assembly results are shown in Table III. All reads were deposited in the Short Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) with the accession number SRX3049257 and SRX3055267.

A total of 73,812 unigenes were assembled from the ovary and testis transcriptomes (Table III). The N50 length was 1,806 bp and the average length was 1,072 bp. The number of contigs above 500 bp was 41,944, accounting for 57%. In addition, 21,830 unigenes (30%) were greater than 1,000 bp. The assembly program produced a substantial number of high-quality long sequences.
Table V. Top 10 genes specifically expressed in *C. nobilis* male and female gonads.

| ID          | Genes                                                                 | Male (FPKM) | P-value     |
|-------------|------------------------------------------------------------------------|-------------|-------------|
| **Male**    |                                                                        |             |             |
| comp47962_c0_seq1 | Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit C-like | 1396.36     | 4.82E-06    |
| comp57762_c0_seq1 | GTPase IMAP family member 7-like                                     | 872.17      | 4.91E-06    |
| comp70989_c1_seq5 | DC-STAMP domain-containing protein 1-like isoform X1             | 856.55      | 4.97E-06    |
| comp56505_c0_seq4 | Von Willebrand factor D and EGF domain-containing protein         | 590.00      | 7.95E-06    |
| comp71598_c0_seq3 | Transient receptor potential cation channel subfamily M member 8-like | 234.21      | 0.000101621 |
| comp63167_c0_seq1 | Glutathione S-transferase omega                                     | 217.48      | 0.000132248 |
| comp54517_c0_seq1 | 43 kDa receptor-associated protein of the synapse-like isoform X1  | 118.22      | 0.00134454454 |
| comp20654_c0_seq1 | Radial spoke head protein 3 homolog B-like                           | 110.42      | 0.001736294 |
| comp50734_c0_seq1 | Sodium- and chloride-dependent taurine transporter-like            | 107.07      | 0.001945348 |
| **Female**  |                                                                        |             |             |
| comp76189_c0_seq1 | Calmodulin-like                                                      | 440.24      | 2.43E-05    |
| comp64460_c0_seq11 | Gamma-butyrobetaine dioxygenase-like isoform X2                 | 313.82      | 6.20E-05    |
| comp63277_c0_seq1 | B1 bradykinin receptor-like                                         | 305.75      | 6.72E-05    |
| comp22346_c0_seq1 | Vitellogenin                                                          | 303.95      | 6.84E-05    |
| comp61921_c0_seq1 | DBH-like monoxygenase protein 1                                     | 232.22      | 0.000165541 |
| comp55524_c0_seq3 | Transposon Ty3-1 Gag-Pol polyprotein                               | 220.57      | 0.000197763 |
| comp60101_c0_seq1 | Serine/threonine-protein kinase mos-like                            | 208.02      | 0.000242767 |
| comp71662_c0_seq5 | Solute carrier family 22 member 21-like                            | 184.70      | 0.000371409 |
| comp59103_c1_seq1 | Sulphotransferase 1A2-like                                          | 167.67      | 0.000530388 |
| comp51636_c0_seq1 | Long-chain fatty acid CoA ligase 1                                  | 164.08      | 0.000574806 |

Genes above and below the line are specifically expressed in males and females, respectively.

Table VI. Real-time PCR confirmation of DEGs between the ovary and testis.

| Sequence ID   | Genes     | Fold-change Ovary/Testis | Real-time PCR Ovary/Testis |
|---------------|-----------|--------------------------|----------------------------|
| comp31527_c0_seq1 | sox11     | 3.30                     | 4.54±1.27                  |
| comp44628_c0_seq2 | sox2      | 12.32                    | 8.23±2.58                  |
| comp5019_c0_seq1 | dme-4     | 0.40                     | 0.42±0.12                  |
| comp52532_c1_seq1 | vitellogenin | 2392.10              | 12126.21±5034.37           |
| comp52668_c0_seq2 | nanos-like protein 1 | 2.87                  | 3.00±0.95                  |
| comp60205_c0_seq1 | 5-HT receptor | 36.18                  | 11.39±2.58                 |
| comp63638_c0_seq1 | sox9      | 2.17                     | 2.11±0.87                  |
| comp67199_c0_seq1 | soxB2     | 4.96                     | 8.35±4.92                  |
| comp67225_c0_seq1 | vasa      | 2.25                     | 3.53±1.48                  |
| comp73569_c0_seq3 | foxJ2     | 57.15                    | 58.08±15.35                |
| comp82439_c0_seq1 | estrogen receptor | Inf                     | Inf                        |

Functional annotation

For validation and annotation of assembled unigenes, BLASTx alignment between unigenes and protein databases, including Nr, Swiss-Prot and KEGG, was conducted, with an E value threshold of 10^{-5} (Supplementary Table S1). The best alignment results were used to annotate protein function and determine the sequence direction of the unigenes. The results indicated...
that 23,535 unigenes (31.89%) were annotated to known proteins in the nr database. Furthermore, 59.95% of the unigenes were matched with C. gigas. In addition, 17,220 unigenes (23.33%) were similar to known proteins in the Swiss-Prot database.

We found that 15,714 annotated unigenes (66.77%) were assigned to one or more sub-categories of GO terms. Among the 160,580 GO terms, 76,442 (47.60%), 63,203 (39.36%), and 20,935 (13.04%) were involved in biological processes, cellular components, and molecular functions, respectively. For biological processes, genes involved in cellular processes (GO: 0009987) and single-organism processes (GO: 0044699) were highly represented. Regarding molecular functions, binding (GO: 0005488) was the most represented GO term, followed by catalytic activity (GO: 0003824). Cells (GO: 0005623) and cell parts (GO: 0044464) were the most represented categories for cellular components.

Unigene KEGG pathway analysis was carried out as an alternative approach for functional categorization and annotation. In total, 6,483 unigenes were divided into six major categories covering metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, and human diseases. Among these annotated unigenes, 4,521 were classified into metabolism, including carbohydrate metabolism (808 sequences) and amino acid metabolism (686 sequences); 1,910 were grouped into genetic information processing, including translation (783 sequences), folding, sorting, and degradation (640 sequences), transcription (236 sequences), and replication and repair (251 sequences); 3,491 were assigned to environmental information processing; 2,208 were assigned to cellular processes; and 5,823 were classified into organismal systems.

**Differential expression analysis**

Differential expression analysis revealed that 2,842 sex-biased unigenes (including up-regulated genes and specifically expressed unigenes) were significantly differentially expressed between the ovaries and testes (FDR ≤ 0.05, Supplementary Table S2). Among them, 591 unigenes exhibited biased expression in the ovary and 173 unigenes were expressed specifically in the ovary. A total of 2,251 testis-biased unigenes were identified from the transcriptome and 965 unigenes were exclusively expressed in the testis. Due to the lack of genomic information for *C. nobilis*, a large fraction of DEGs (63.69%) could not be annotated, although they might contain novel genes important for gonadal differentiation and development. However, some well-known reproduction-related DEGs were detected. For example, dmrt2 and TSSK1 were isolated in testis-biased unigenes, and HTR, Fox2L, nanos,

---

Fig. 2. Gene ontology (GO) annotation of ovary-biased genes (A) and testis-biased genes (B).
vitellogenin, and sox2 were detected in ovary-biased unigenes (Supplementary Table S2). Tables IV and V show the top 10 up-regulated and specifically expressed genes in the testis and ovary of C. nobilis, respectively.

We detected 600 DEGs in several GO terms. Among them, 369 were detected in 1,195 testis-biased terms and 231 were detected in 964 ovary-biased terms (Supplementary Tables S3, S4). For the ovary-biased genes, GO terms related to protein glycosylation, fatty acid biosynthetic processes, hydrolase activity, Golgi cisternae membrane, and cell cortex were significantly enriched (Fig. 2a, Supplementary Table S4). Most testis-biased GO enrichment terms were related to male organ formation and spermiogenesis, such as fertilization, male genitalia morphogenesis, spermiogenesis, motile cilium, axoneme, cGMP activity, cAMP binding, and ATPase activity (Fig. 2b, Supplementary Table S4).

KEGG pathways related to metabolism of fatty acid, glycans, amino acids, hormones, and mineral absorption were enriched in the ovary-biased genes (Fig. 3a), whereas those related to meiosis, insulin resistance, glycosphingolipid biosynthesis, and adherens junction were enriched in the testis-biased genes (Fig. 3b).

Quantitative RT-PCR validation
To validate the expression profiles obtained from Illumina sequencing analysis, 11 genes were chosen for quantitative RT-PCR. Of these, seven matched the Illumina sequencing results (Table VI). Although the other four genes did not perfectly match the sequencing data, they still showed the same differential expression tendencies, as revealed by RNA-Seq. In general, the qRT-PCR results confirmed that the Illumina data were credible.

DISCUSSION
In this study, the transcriptome of the C. nobilis testis and ovary in the maturing stage were sequenced by RNA-Seq technology. Based on the transcriptome data, genes involved in germline development, gonad development, oocyte maturation, and fertilization were identified. Vasa, nanos, and piwi play important roles in germline development. Vasa is a key determinant in germline formation and in gonad differentiation in eukaryotes, and can be used to track germ cell specification, migration, and differentiation (Gallardo et al., 2007; Wessel et al., 2014; Xu et al., 2014). Nanos plays an important role in primordial germ cell development (Tsuda et al., 2003), whereas piwi deficiency can result in complete depletion of germline stem cells and sterility in both males and females (Lin and Spradling, 1997). Vitellogenin (Vtg), vitellogenin receptor (Vtgr), and estrogen receptor are involved in gonad development. In most oviparous animals, Vtg is a large multidomain apolipoprotein that serves as a precursor of the major egg yolk protein vitellin (Matsumoto et al., 2013). In Argopecten purpuratus, vitellogenin mRNA expression is specifically expressed in the mature ovary (Boutet et al., 2008), which is in accordance with our results (Tables IV and VI). In addition, VgR is responsible for binding circulating Vg and transporting it into oocytes through endocytosis (Tufail and Takeda, 2009). Estrogen receptor regulates the transactivation of estrogen target genes, such as teleost Vtg (Matsumoto et al., 2013). HTR is involved in the induction of oocyte maturation (Wang and He, 2014). 17-beta-hydroxysteroid dehydrogenases (17-beta-HSDs) are important enzyme in sex steroid metabolism (Moeller and Adamski, 2009). Some 17-beta-HSDs including 17-beta-HSD6, 17-beta-HSD8, 17-beta-HSD10, 17-beta-HSD11, 17-beta-HSD12 and 17-beta-HSDs14 were found in this study. The substrate of these HSDs are androgens and estrogens. Zona pellucida sperm-binding protein is useful in fertilization (Aagaard et al., 2010).

In this study, 591 unigenes exhibited biased expression in the ovary, of which 173 were expressed specifically in the ovary. A total of 2,251 testis-biased
unigenes were identified from the transcriptome, with 965 unigenes exclusively expressed in the testis. Our results showing more testis-biased than ovary-biased unigenes agree with previous findings for *P. yessoensis* (Li *et al.*, 2016b) and *P. olivaceus* (Zhang *et al.*, 2016b), but differ from that of *C. gigas*, in which more genes were found in the ovary (Zhang *et al.*, 2014).

Genes that exhibited the greatest differential or specific expression in the gonads are listed in Tables IV and V, respectively. Potassium voltage-gated channel protein was highly expressed in the testis, which is the first time this has been found in a mollusc. In mammals, potassium voltage-gated ion channel is present in the testis and spermatooza (*Jacob *et al.*, 2000). K⁺ channel activation is a primary event leading to acrosome reactions in mammalian spermatooza (Benoff, 2009). TSSK1 is one member of Tssk family. The serine/threonine protein kinase catalytic (S-TKc) domain of TSSK1 is highly conserved in animals. TSSK1 is expressed when spermatids differentiate into morphologically mature spermatooza during spermiogenesis, which highlights its important role in normal male reproduction (*Xu *et al.*, 2008). TSSK1 is only transcribed in the male gonads of *Atrina pectinata* and is highest in the mature testis (*Li *et al.*, 2016a), as found in our results (Table IV).

Nutrients and energy reserves are key factors in supporting oocyte development. Glycogens, lipids, and proteins play important roles in the reproductive processes of many bivalves. Glycogens are an important energy source for gonad development in clam (*Li *et al.*, 2011; *Yan *et al.*, 2010) and oyster (*Zeng *et al.*, 2010). Lipids and proteins are accumulated as yitellin, which is a major organic component of oocytes in female gonads. In male gonads, lipids and proteins are energy sources during spermiogenesis after glycogen breakdown (*Li *et al.*, 2011; *Bi *et al.*, 2016). These above results were further shown in the transcriptome level findings in our study. GO terms and KEGG pathways related to protein glycosylation, fatty acid biosynthetic processes, and hydrolase activity were enriched in the *C. nobilis* ovary (Figs. 2 and 3). Our results are agreement with previous transcriptome research in ovary. For example, in the *Halitrus rufescens* ovary, a high percentage of transcripts are associated with metabolism and catalytic and enzymatic activity (*Valenzuela-Muñoz *et al.*, 2014); In *P. yessoensis*, ovary-biased genes are enriched in the glycan-related pathway, which suggests active glycan biosynthesis and metabolism (*Li *et al.*, 2016b); Here, the AMPK signaling pathway was identified in the *C. nobilis* ovary, which was reported in a mollusc ovary for the first time. The AMPK pathway is involved in controlling cellular energy homeostasis and, at the whole animal level, in regulating energy balance and food intake (Proszkowiec-Weglzarz *et al.*, 2016). Furthermore, AMPK plays a vital role in ovary development through mediating glucose, insulin-like growth factor-1 (IGF-1), follicle stimulating hormone (FSH), and adiponectin (*Ratchford *et al.*, 2007; *Tosca *et al.*, 2007; *Kayampilpy *et al.*, 2009; *Lu *et al.*, 2008; *Chen *et al.*, 2006).

In the *C. nobilis* testis, GO terms were enriched in male organ formation and spermiogenesis. Some KEGG pathways were similar to those of other molluscs. For example, meiosis is significantly enriched in *C. gigas* and *P. yessoensis* testes (Zhang *et al.*, 2014; *Li *et al.*, 2016b), and the insulin signaling pathway has been identified in *C. hongkongensis* (*Tong *et al.*, 2015). The glycosphingolipid biosynthesis pathway was first identified in a mollusc testis in this study. Glycosphingolipsids are ubiquitous molecules composed of a lipid and carbohydrate moiety. Glycosphingolipids have been isolated and identified in other animal sperm, including that of humans and sea urchins (*Ritter*, 1987; *Liu *et al.*, 1996), and are critical for sperm activation in *C. elegans* (*Dou *et al.*, 2012). Further studies on DEGs associated with GO terms and pathways are needed to reveal the different molecular mechanisms that exist between ovarian and testicular maturation processes.

**ACKNOWLEDGMENTS**

Funding for this research was provided by the Special Fund for Central Public-Interest Scientific Institution Basal Research Fund, South China Sea Fisheries Research Institute, CAFS (NO.2017YB10), Guangxi Key Laboratory of Beibu Gulf Marine Biodiversity Conservation, Beibu Gulf University (2019KB02), Marine Fisheries Research and Extension of Guangdong Province (B201601-Z01).

**Supplementary material**

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20190125080146

**Statement of conflict of interest**

The authors have declared no conflict of interest.

**REFERENCES**

Aagaard, J.E., Vacquier, V.D., Maccoss, M.J. and Swanson, W.J., 2010. Zp domain proteins in the abalone egg coat include a paralog of verl under positive selection that binds lysin and 18-kda sperm proteins. *Mol. Biol. Evol.*, 27: 193-203. https://doi.org/10.1093/molbev/msp221

Anders, S. and Huber, W., 2013. *Differential expression...
of RNA-seq data at the gene level—the DESeq package. European Molecular Biology Laboratory. https://www.semanticscholar.org/paper/Differential-expression-of-RNA-Seq-data-at-the-gene-Anders-Huber/81c42bf29edd0035b237375e2270f5a31762147

Benoff, S., 2009. Receptors and channels regulating acrosome reactions. *Hum. Fertil.*, 2: 42-55. https://doi.org/10.1080/1464727992000198311

Bi, J., Li, Q., Zhang, X., Zhang Z., Tian J., Xu Y. and Liu W., 2016. Seasonal variation of biochemical components in clam (*Saxidomus purpuratus* Sowerby 1852) in relation to its reproductive cycle and the environmental condition of Sanggou Bay, China. *J. Ocean Univ. China*, 15: 341-350. https://doi.org/10.1007/s11802-016-2855-6

Bouret, I., Moraga, D., Marinovic, L., Obreque, J. and Chavez-Crooker, P., 2008. Characterization of reproduction-specific genes in a marine bivalve mollusc: Influence of maturation stage and sex on mRNA expression. *Gene*, 407: 130-138. https://doi.org/10.1016/j.gene.2007.10.005

Chen, J., Hudson, E., Chi, M.M., Chang, A.S., Moley, K.H., Hardie, D.G. and Downs, S.M., 2006. AMPK regulation of mouse oocyte meiotic resumption in vitro. *Dev. Biol.*, 291: 227-238. https://doi.org/10.1016/j.ydbio.2005.11.039

Conesa, A., Götz, S., García-Gómez, J.M., Terol, J., Talón, M. and Robles, M., 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, 21: 3674-3676. https://doi.org/10.1093/bioinformatics/bti610

Dou, J., Chen, L., Hu, Y. and Miao, L., 2012. Cholesterol and the biosynthesis of glycosphingolipids are required for sperm activation in caenorhabditis elegans. *Biochim. biophys. Acta*, 1821: 934-942. https://doi.org/10.1016/j.bbadis.2012.03.005

Gallardo, T., Shirley, L., John, G.B. and Castrillon, D.H., 2007. Generation of a germ cell-specific mouse transgenic cre line, vasa-cre. *Genesis*, 45: 413-417. https://doi.org/10.1002/dvg.20310

Gaitánspitia, J.D., Sánchez, R., Bruning, P. and Cárdenas, L., 2016. Functional insights into the testis transcriptome of the edible sea urchin *Loxechinus albus*. *Sci. Rep.*, 6: 36516. https://doi.org/10.1038/srep36516

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A. and Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceci, E., Hacohen, N., Gnirke, A., Rhind, N., Palma, F., Bruce, W., Friedman N. and Regev A., 2011. Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nat. Biotechnol.*, 29: 644-642. https://doi.org/10.1038/nbt.1883

Götz, S., Garcia-gómez, J.M., Terol, J., Williams, T.D., Nagaraj, S.H., Nueda, M.J., Robles, M., Talón, M., Dopazo, J. and Conesa, A., 2008. High-throughput functional annotation and data mining with the blast2go suite. *Nucl. Acids Res.*, 36: 3420-3435. https://doi.org/10.1093/nar/gkn176

Ijuin, T., Kitajima, K., Song, Y., Kitazume, S., Inoue, S., Haslam, S. M., Morris, H.R., Dell, A. and Inoue, Y., 1996. Isolation and identification of novel sulfated and nonsulfated oligosialyl glycosphingolipids from sea urchin sperm. *Glycoconjugate J.*, 13: 401-413. https://doi.org/10.1007/BF00731473

Jacob, A., Hurley, I.R., Goodwin, L.O., Cooper, G.W. and Benoff, S., 2000. Molecular characterization of a voltage-gated potassium channel expressed in rat testis. *Mol. Hum. Reprod.*, 6: 303-313. https://doi.org/10.1093/molehr/6.4.303

Kayampilly, P.P. and Menon, K.M., 2009. Follicle-stimulating hormone inhibits adenosine 5’-monophosphate-activated protein kinase activation and promotes cell proliferation of primary granulosa cells in culture through an akt-dependent pathway. *Endocrinology*, 150: 929-935. https://doi.org/10.1210/en.2008-1032

Langmead, B., Salzberg, S.L., 2012. Langmead b, salzberg sl. fast gapped-read alignment with bowtie 2. *Nat. Methods*, 9: 357-359. https://doi.org/10.1038/nmeth.1923

Li, H.H., Kong, L.F., Yu, R., Yu, H. and Li, Q., 2016a. Characterization, expression, and functional analysis of testis-specific serine/threonine kinase 1 (tssk1) in the pen shell *Arinapectinata*. *Inverteb. Reprod. Dev.*, 2: 1-8. https://doi.org/10.1080/07924259.2016.116167

Li, Y., Zhang, L., Sun, Y., Ma, X., Wang, J., Li, R., Zhang, M., Wang, S., Hu, X. and Bao, Z., 2016b. Transcriptome sequencing and comparative analysis of ovary and testis identifies potential key sex-related genes and pathways in scallop *P Hindoimetopsis yessoensis*. *Mar. Biotechnol.*, 4: 1-13. https://doi.org/10.1007/s10126-016-9706-8

Li, Q., Lin, Y., Ke, Q. and Kong L., 2011. Gametogenic cycle and biochemical composition of the clam *Mactra chinensis* (Mollusca: Bivalvia): Implications for aquaculture and wild stock management. *Mar. Biol. Res.*, 7: 407-415. https://doi.org/10.1080/17451000.2010.515686
Lin, H. and Spradling, A.C., 1997. A novel group of pumilio mutations affects the asymmetric division of germline stem cells in the Drosophila ovary. Development, 124: 2463–2476.

Liu, H., Zheng, H., Zhang, H., Deng, L., Liu, W., Wang, S., Meng, F., Wang, Y., Guo, Z., Li, S. and Zhang G., 2015. A de novo transcriptome of the noble scallop, Chlamys nobilis, focusing on mining transcripts for carotenoid-based coloration. BMC Genom., 16: 44. https://doi.org/10.1186/s12864-015-1241-x

Lu, M., Tang, Q., Olefsky, J.M., Mellon, P.L. and Webster, N.J., 2008. Adiponectin activates adenosine monophosphate-activated protein kinase and decreases luteinizing hormone secretion in LbetaT2 gonadotropes. Mol. Endocrinol., 22: 760-771. https://doi.org/10.1210/me.2007-0330

Ma, H. and Yu, Z., 2009. Development of twenty-two polymorphic microsatellite loci in the noble scallop, Chlamys nobilis. Conserv. Genet., 10: 1587-1590. https://doi.org/10.1007/s10592-008-9800-1

Matsumoto, T., Masaoka, T., Fujitaya, A., Nakamura, Y., Satoh, N. and Awaji, M., 2013. Reproduction-related genes in the pearl oyster genome. Zool. Sci., 30: 826-850. https://doi.org/10.2108/zsj.30.826

Moeller, G. and Adamski, J., 2009. Integrated view on 17beta-hydroxysteroid dehydrogenases. Mol. Cell Endocrinol., 301: 7-19. https://doi.org/10.1016/j.mce.2008.10.040

Morishita, F., Furukawa, Y., Matsushima, O. and Minakata, H., 2010. Regulatory actions of neuropeptides and peptide hormones on the reproduction of mollusces. Can. J. Zool., 88: 825-845. https://doi.org/10.1139/Z10-041

Naimi, A., Martinez, A.S., Specq, M.L., Diss, B., Mathieu, M. and Sourdaine, P., 2009. Molecular cloning and gene expression of Cg-Foxl2 during the development and the adult gametogenic cycle in the oyster Crassostrea gigas. Comp. Biochem. Physiol. B, 154: 134-142. https://doi.org/10.1016/j.cbpb.2009.05.011

Patel, R.K. and Jain, M., 2012. NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. PLoS One, 7: e30619. https://doi.org/10.1371/journal.pone.0030619

Pertea, G., Huang, X., Liang, F., Antonescu, V., Sultana, R., Karamycheva, S., Lee, Y., White, J., Cheung, F., Parvizi, B., Tsai, J. and Quackenbush, J., 2003. TIGR Gene Indices clustering tools (TGICL): a software system for fast clustering of large EST datasets. Bioinformatics, 19: 651-652. https://doi.org/10.1093/bioinformatics/btg034

Ritter, G., Krause, W., Geyer, R., Stirm, S. and Wiegandt, H., 1987. Glycosphingolipid composition of human semen. Arch. Biochem. Biophys., 257: 370-378. https://doi.org/10.1016/0003-9861(87)90579-0

Proszkowicz-Weglarz, M., Richards, M.P., Ramachandran, R. and Memmery, J.P., 2006. Characterization of the amp-activated protein kinase pathway in chickens. Comp. Biochem. Physiol. B, 143: 92-106. https://doi.org/10.1016/j.cbpb.2005.10.009

Ratchford, A.M., Chang, A.S., Chi, M.M., Sheridan, R. and Moley, K.H., 2007. Maternal diabetes adversely affects AMP-activated protein kinase activity and cellular metabolism in murine oocytes. Am. J. Physiol. End. Metab., 293: E1198-206. https://doi.org/10.1152/ajpendo.00503.2007

Roberts, A. and Pachter, L., 2013. Streaming fragment assignment for real-time analysis of sequencing experiments. Nat. Methods, 10: 71-73. https://doi.org/10.1038/nmeth.2251

Santerre, C., Sourdain, P., Adeline, B. and Martinez, A.S., 2014. Cg-SoxE and Cg-β-catenin, two new potential actors of the sex-determining pathway in a hermaphrodite lophotrochozoan, the Pacific oyster Crassostrea gigas. Comp. Biochem. Physiol. A. Mol. Integr. Physiol., 167: 68-76. https://doi.org/10.1016/j.cbpa.2013.09.018

Shi, Y., Wang, Q. and He, M., 2014. Molecular identification of dmrt2, and dmrt5, and effect of sex steroids on their expressions in Chlamys nobilis. Aquaculture, 426-427: 21-30. https://doi.org/10.1016/j.aquaculture.2014.01.021

Sappington, T.W. and Raikhel, A.S., 1998. Molecular characteristics of insect vitellogenins and vitellogenin receptors. Insect. Biochem. Mol. Biol., 28: 277. https://doi.org/10.1016/S0965-1748(97)00110-0

Tsuda, M., Sasaoka, Y., Kiso, M., Abe, K., Haraguchi, S., Kobayashi, S. and Saqa, Y., 2003. Conserved role of nanos proteins in germ cell development. Science, 301: 1239-1241. https://doi.org/10.1126/science.1085222

Tosca, L., Chabrolle, C., Uzbekova, S. and Dupont, J., 2007. Effects of metformin on bovine granulosa cells steroidogenesis: possible involvement of adenosine 5’ monophosphate-activated protein kinase (AMPK). Biol. Reprod., 76: 368-378. https://doi.org/10.1095/biolreprod.106.055749

Tong, Y., Zhang, Y., Huang, J., Xiao, S., Zhang, Y., Li, J., Chen, J. and Yu, Z., 2015. Transcriptomics Analysis of Crassostrea hongkongensis for the discovery of reproduction-related genes. PLoS One, 10: e0134280. https://doi.org/10.1371/
Comparative Transcriptome Analysis of the Ovary and Testis

Ye, J., Fang, L., Zheng, H., Zhang, Y., Chen, J., Zhang, Z., Wang, J., Li, S., Li, R., Bolund, L. and Wang, J., 2006. WEGO: a web tool for plotting GO annotations. *Nucl. Acids Res.*, **34**(Web Server issue), W293-297. https://doi.org/10.1093/nar/gkl031

Zeng, Z., Ni, J. and Ke, C., 2013. Expression of glycogen synthase (GYS) and glycogen synthase kinase 3β (GSK3β) of the Fujian oyster, *Crassostrea angula*, in relation to glycogen content in gonad development. *Comp. Biochem. Phys. B.*, **166**: 203-214. https://doi.org/10.1016/j.cbpb.2013.09.003

Zhang, N., Xu, F. and Guo, X., 2014. Genomic analysis of the Pacific oyster (*Crassostrea gigas*) reveals possible conservation of vertebrate sex determination in a mollusc. *G3 (Bethesda)*, **6**: 1417-1427. https://doi.org/10.1534/g3.114.013904

Zhang, Q., Lu, Y., Zheng, H., Liu, H. and Li, S., 2016a. Differential immune response of vitellogenin gene to vibrio anguillarum in noble scallop chlamys nobilis and its correlation with total carotenoid content. *Fish Shellf. Immunol.*, **50**: 11-15. https://doi.org/10.1016/j.fsi.2016.01.001

Zhang, W., Liu, Y., Yu, H., Du, X., Zhang, Q., Wang, X. and He, Y., 2016b. Transcriptome analysis of the gonads of olive flounder (*Paralichthys olivaceus*). *Fish Physiol. Bioche.*, **42**: 1-14. https://doi.org/10.1007/s10695-016-0242-2

Zhang, S., Gong, Y., Xu, J., Hu, M., Xu, P. and Jiang, Y., 2019. The genetic basis of skin color and body shape of domesticated purse red carp revealed by comparative transcriptome analysis. *Pakistan J. Zool.*, **51**: 1675-1684. http://dx.doi.org/10.17582/journal.pij/2019.51.5.1675.1684

Zheng, H., Zhang, Q., Liu, H., Liu, W., Sun, Z., Li, S. and Tao Z., 2012b. Cloning and expression of the Pacific oyster (*Crassostrea gigas*) vitellogenin (vg) gene and its correlations with total carotenoid content and total antioxidant capacity in noble scallop *Chlamys nobilis* (bivalve: Pectinidae). *Aquaculture*, **366–367**: 46-53. https://doi.org/10.1016/j.aquaculture.2012.08.031

Zheng, H., Liu, H., Liu, W., Sun, Z. and Zhang, Q., 2012a. Changes of total carotenoid and lipid content in scallop tissues of *Chlamys nobilis* (Bivalve: Pectinidae) during gonad maturation. *Aquaculture*, **342–343**: 7-12. https://doi.org/10.1016/j.aquaculture.2012.01.037