Comparative transcriptome sequencing analysis of female and male *Decapterus macrosoma*

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**ABSTRACT**

Sexual growth dimorphism is a common phenomenon in teleost fish and has led to many reproductive strategies. Growth- and sex-related gene research in teleost fish would broaden our understanding of the process. In this study, transcriptome sequencing of shortfin scad *Decapterus macrosoma* was performed for the first time, and a high-quality reference transcriptome was constructed. After identification and assembly, a total of 58,475 nonredundant unigenes were obtained with an N50 length of 2,266 bp, and 28,174 unigenes were successfully annotated with multiple public databases. BUSCO analysis determined a level of 92.9% completeness for the assembled transcriptome. Gene expression analysis revealed 2,345 differentially expressed genes (DEGs) in the female and male *D. macrosoma*, 1,150 of which were female-biased DEGs, and 1,195 unigenes were male-biased DEGs. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses showed that the DEGs were mainly involved in biological processes including protein synthesis, growth, rhythmic processes, immune defense, and vitellogenesis. Then, we identified many growth- and sex-related genes, including *Igf*, *Fabps*, EF-hand family genes, *Zp3*, *Zp4* and *Vg*. In addition, a total of 19,573 simple sequence repeats (SSRs) were screened and identified from the transcriptome sequences. The results of this study can provide valuable information on growth- and sex-related genes and facilitate further exploration of the molecular mechanism of sexual growth dimorphism.

**INTRODUCTION**

Sexual growth dimorphism, which refers to growth rate differing between the sexes, is a common phenomenon in teleost fish. In certain fish species, such as tilapia, males grow faster and larger than females (*Toguyeni et al., 1997*), while in chinook salmon...
(Oncorhynchus tshawytscha), common carp (Cyprinus carpio), and half-smooth tongue sole (Cynoglossus semilaevis), females grow significantly faster and larger than males (Ma et al., 2011). And the mechanism of sexual growth dimorphism is complex (Rennie et al., 2008).

Fish growth is a complex polygenic trait that is regulated by many factors, including nutrition, environment, reproductive activity, and energy metabolism (Wang et al., 2018). Fortunately, sexually dimorphic growth can be exploited to explore candidate networks and genes that enhance body size or growth speed, which may lead to rapid and significant economic gains (Dutney, Elizur & Lee, 2017). Based on analyses of genes that are differentially expressed between females and males, an increasing number of growth-related genes have been identified (Wang et al., 2018; Lou et al., 2018; Ma et al., 2016). Sex is one of the most intriguing characteristics in the life sciences. Associated mechanisms are highly conserved in many animals (e.g., mammals and birds) (Yang et al., 2015; Chen et al., 2017). Many of the genes involved in sexual differentiation are identical in distantly related species, indicating that the gene regulatory mechanisms of sex differentiation and determination may be similar (Ottolenghi et al., 2007; Smith et al., 2009). However, the sex determination mechanisms in teleost fish are quite different (Devlin & Nagahama, 2002; Ribas et al., 2016). Among animals, teleost fish exhibit the most diverse sex determination system, leading to many reproductive strategies (Kobayashi, Nagahama & Nakamura, 2013; Casas et al., 2016). Unlike sex in birds, humans, and other higher vertebrates, which is relatively stable, sex in teleost fish is unstable or variable (Barske & Capel, 2008). The study of sex-related genes in teleost fish has attracted wide interest from an increasing number of researchers. As teleost fish are important protein resources for humans, understanding the growth and sex of teleost fish is important for predicting potential impacts and for effective management.

Decapterus macrosoma (Fig. 1) Bleeker, 1851 (Actinopterygii, Perciformes, Carangidae, Decapterus) usually swims in schools and generally inhabits open water at depths of 20–170 meters. It feeds on planktonic invertebrates and is mainly distributed in the East China Sea, the South China Sea, the waters around Taiwan, the south of Tokyo Bay in Japan, and the warm-water areas of the Indian-Pacific Ocean (Institute of Zoology, Chinese Academy of Sciences, Institute of Oceanology, Chinese Academy of Sciences & Shanghai Ocean University, 1962). D. macrosoma is widely distributed in the South China Sea, is the main target of light seining fisheries there, and has high economic value (Yang et al., 2009; Zhang et al., 2016a, 2016b, 2016c). Carangidae fish play a significant role in global marine fisheries, and annual Decapterus production is second only to that of Trachurus (Zhang et al., 2020). D. macrosoma exhibits sexual growth dimorphism; similar to various teleost fish; the female fish tend to be stronger and larger than the male fish (Shan et al., 2021a). These biological features render D. macrosoma an ideal non-model marine fish species for growth- and sex-related gene research. However, the National Center for Biotechnology Information (NCBI) database has very little genome assembly data (only 14 data points) for Carangidae fishes and no genome assembly data for Decapterus.

Genome sequencing in researched species can be classified based on the availability of reference genome sequences for the species, de novo sequencing (species without reference
genomic sequences), and whole-genome resequencing (species with reference genomic sequences) (Dong, 2018). The latter is not applicable to D. macrosoma since it has no available genome assembly data in the public database. Thus, de novo sequencing was adopted in this study. Despite the lack of genomic information on D. macrosoma, transcriptome sequencing could reveal the relationships among genes, the biological characteristics of this species and the external environment, contributing to the future development of many effective gene resources (Sánchez et al., 2011).

To date, studies on D. macrosoma in China and elsewhere have mainly focused on growth and reproduction and on resource assessment (Kong et al., 2019; Hou et al., 2020); few studies on the D. macrosoma transcriptome have been reported. In this study, the gonads, livers, hearts, kidneys, and muscle tissues of females and males were used for transcriptome analysis. Then, gene expression profiles of different D. macrosoma individuals were generated, and differentially expressed genes (DEGs) between D. macrosoma males and females were detected. Subsequently, important candidate genes and pathways involved in growth-related and sex-related genes were identified. In addition, abundant simple sequence repeats (SSRs) were detected in the reads of deep coverage sequence regions. The aim of this study was to detect growth-related and sex-related genes in teleost fish and to lay the foundation for revealing the sexual growth dimorphism mechanisms of teleost fish. In addition, the transcriptomic resource in this study can provide support for future studies on molecular regulatory mechanisms in teleost fish.

**MATERIALS AND METHODS**

**Ethics approval and sample collection**

All the experimental procedures were approved by the ethics committee of Laboratory Animal Welfare and Ethics of South China Sea Fisheries Research Institute (accession no. nhdf 2022-06). The procedures involving animals in this study were conducted in
accordance with the Laboratory Animal Management Principles of China. The
*D. macrosoma* specimens sampled in this study were captured in the South China Sea
(10°N, 110°30′E) in July 2019 using light seine nets. The ovaries of female *D. macrosoma*
are reddish-yellow, and the spermathecae of *D. macrosoma* are milky white and
flattened in bands. Three healthy female and three healthy male fish (female fork length:
213–226 mm; male fork length: 208–218 mm) at stage IV sexual maturity were selected
and adaptively raised in aerated, circularly flowing seawater for 6 d. During the adaptation
period, the fish were not fed to minimize contaminating sequences from food. Then, all
specimens were anaesthetized and killed by severing the spinal cord. Then, the gonads,
livers, hearts, kidneys, and muscle tissues were rapidly removed, stored temporarily in
liquid nitrogen, and then preserved in a −80 °C freezer for later RNA extraction. Before
RNA extraction, the gonads, livers, hearts, kidneys, and muscle tissues were separately
ground with liquid nitrogen, and all the tissues were blended in equal amounts to generate
mixed samples.

**RNA extraction and illumina sequencing**

Total RNA was extracted from the six samples using TRIzol reagent. Then, RNA integrity
was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA,
USA), and samples with an RNA Integrity Number (RIN) ≥7 were used for subsequent
cDNA library preparation (*Zhang, Lou & Han, 2019*). The RNA of tissues from every
individual was pooled in equal amounts. The statistical power of this experimental design,
calculated in RNASeqPower, is 0.80 (*Hart et al., 2013*).

Then, mRNA (RNA with a poly-A tail) was extracted from the total RNA using
magnetic beads with Oligo (dT) probes and purified. Fragmentation buffer was applied to
lyse the mRNA into fragments with a suitable size, and the fragmented mRNA was
reverse-transcribed into double-stranded cDNA by the N6 random primer. Then, the
cDNA fragments were repaired with phosphate at the 5′ end, and an “A” base was added to
the 3′ end, after which adaptors were ligated to the cDNA fragments. PCR was performed
to amplify the ligation products. After thermal denaturation, the single-stranded DNA was
cyclized using splint oligonucleotides and DNA ligase (*Shan et al., 2021b*). Finally, the
cDNA libraries were sequenced on the Illumina NovaSeq high-throughput sequencing
platform based on sequencing-by-synthesis (SBS) technology, thus generating many
high-quality reads. The reads or bases generated by the sequencing platform were the raw
data and were saved in FASTQ format, and the quality score of most bases reached or
exceeded Q30. The raw data from each sequenced sample included two FASTQ files, each
containing the reads determined at both ends of all cDNA fragments.

**De novo assembly and functional annotation**

Reads containing sequencing adaptors and primer sequences were excluded from all the
original sequences via Trimmomatic (v 0.35) (*Bolger, Marc & Bjoern, 2014*), and
low-quality data were filtered out to ensure that we obtained high-quality clean reads
(*Wang, Lou & Shui, 2019*). Afterward, the Trinity (v 2.11.0) software package was applied
to assemble the high-quality clean reads *de novo* (*Grabherr et al., 2013*). First, the
sequencing reads were broken into short fragments (K-mers), which were then extended into long fragments (contigs). Next, the long fragments were overlapped to generate fragment sets. Finally, transcript sequences were separately identified from each fragment set based on de Bruijn graphs and sequencing read information. Based on the identification outcomes, different contigs from the same transcript were connected using double-end information for further sequence splicing, yielding a transcript. The lead transcript in each transcript clustering unit was selected as the unigene sequence, and cluster analysis and further elimination of redundancy in the unigene data were conducted via the TIGR Gene Indices clustering tools (TGICL); nonredundant unigenes were ultimately obtained (Chen et al., 2016). To perform the quantitative assessment of assembly and completeness, BUSCO software version 5.4.3 (Benchmarking Universal Single-Copy Orthologs) was applied using default setting (Simão et al., 2015). Finally, to obtain information on the unigenes, comparison software, including Diamond (Buchfink, Xie & Huson, 2015) and BLAST, was used to compare all the unigenes with those in databases, including Cluster of Orthologous Groups of Proteins (COG), Gene Ontology (GO), the Kyoto Encyclopedia of Genes and Genomes (KEGG), euKaryotic Orthologous Groups (KOG), protein family (Pfam), the Swiss-Prot protein (Swiss-Prot) database, and the nonredundant protein (Nr) database.

**Gene expression and differential expression analysis**

The transcriptome data obtained by Trinity splicing were used as reference sequences to estimate the gene expression level of each sample by RSEM (Dewey & Li, 2011). Next, we used the short reads alignment tool Bowtie2 to map all unigenes to the assembled transcriptome, which served as a reference (Langmead, 2009). The fragments per kilobase of exon model per million mapped fragments (FPKM) value was used to denote the expression abundance of the corresponding unigenes; this value can eliminate the influence of gene length and sequencing quality differences on the calculation of gene expression (Trapnell et al., 2010). To show the relationships among the six samples, we used the FactoMineR in R to perform a principal component analysis (PCA). To detect DEGs, the clean data were aligned with the assembled reference sequences, and the number of reads per gene was obtained based on the results. Then, the read count data were standardized using the trimmed mean of M-values (TMM), followed by differential expression analysis via DEGseq (Love, Huber & Anders, 2014). In this process, the well-established Benjamini-Hochberg method was used to correct the significance (Q-value) obtained from testing the original hypothesis. Moreover, the Q-value, i.e., the false discovery rate (FDR), was used as the key index for screening DEGs to reduce the number of false-positive results induced by the independent statistical hypothesis testing of the expression levels of many genes. The thresholds for screening were Q-value ≤ 0.01 and |log₂ fold change| ≥ 1 (Wang et al., 2010).

**Functional enrichment analysis of DEGs**

The obtained DEGs were subjected to GO enrichment analysis. Using the GO annotation results, significant enrichment analysis was conducted on the DEGs from the
transcriptomes of female and male *D. macrosoma* using GOseq in R. According to the results of the GO analysis combined with biological significance, the genes for subsequent studies were selected to analyze the biological functions of DEGs (*Shan et al., 2021a*).

A pathway analysis was performed on the DEGs using KEGG annotations. The significance of the DEG enrichment in each pathway was calculated using the hyper geometric distribution test, and a *p*-value ≤0.05 indicated significantly enriched terms. Next, the DEG enrichment in KEGG pathways was determined via KOBAS (v 2.0) (*Xie et al., 2011*). The functional enrichment and pathway enrichment results for the DEG unigenes from *D. macrosoma* were visualized using R software (*Dong et al., 2020*).

**Simple sequence repeat marker detection**

The MicroSATellite identification tool (MISA, v 2.1) was applied to identify the SSR loci in the assembled *D. macrosoma* transcript reference (*Sebastian et al., 2017*). The thresholds of the minimum repeat number for various unit types were set as 1-10, 2-6, 3-5, 4-5, 5-5, and 6-5. Taking the threshold 1-10 as an example, this setting indicated that a single nucleotide repeat type was repeated at least 10 times before it was considered a microsatellite.

**RESULTS**

**Sequencing and assembly of the *D. macrosoma* transcriptome**

Transcriptome sequencing was performed on the six *D. macrosoma* samples using Illumina NovaSeq high-throughput sequencing. After quality control, a total of 42.61 Gb of clean data were acquired, and the Q30 base percentage in each sample was at least 93.43%. The sequencing data (clean data) from the six *D. macrosoma* samples are summarized in Table S1. Transcriptome assembly was completed after clustering by removing redundancy with Trinity and TGICL, and the detailed results for the assembled unigenes of each sample are presented in Table S2. The length distribution of unigenes in the *D. macrosoma* transcriptome are displayed in Fig. S1. The results of the BUSCO analysis showed that 92.9% were completed (237 BUSCOs), 2.7% were fragmented (seven BUSCOs), and 4.4% were missing (11 BUSCOs).

The raw reads in this study are archived in the NCBI Short Read Archive (SRA) databases under BioProject PRJNA825736, with accession numbers SRR18748694–SRR18748699. This Transcriptome Shotgun Assembly project was deposited at DDBJ/EMBL/GenBank under the accession GJWF0000000. The version described in this article is the first version, GJWF01000000.

**Functional annotation of unigenes**

*Calculation of the success rates of gene function annotation*

The unigenes were further functionally characterized based on the description of their similar sequences. The number and percentage of unigenes matched to various databases are shown in Table S3. All unigenes were compared against the databases listed. The results indicated that 28,174 (48.18%) unigenes matched homologous sequences from at least one database.
Analysis of gene function annotation

The unigenes in the transcriptome were compared against the NCBI protein database Nr to acquire *D. macrosoma* gene sequences and their functional information and to further determine the gene sequence similarity between *D. macrosoma* and its sister species. The *D. macrosoma* transcriptome contained many unigenes that were highly similar to gene sequences from *Stegastes partitus* (7,552), *Larimichthys crocea* (6,312), *Oreochromis niloticus* (1,916), *Notothenia coriiceps* (1,108), *Maylandia zebra* (785), *Cynoglossus semilaevis* (725), *Astatotilapia burtoni* (646), *Neolamprologus brichardi* (562), *Haplochromis nyererei* (515), and *Danio rerio* (461). The other 5,932 unigenes showed similarities to gene sequences from 394 other species (Fig. S2).

A total of 23,633 unigenes were grouped into 42 functional classes across the three major GO categories (cellular component, biological process, and molecular function). Among the 23 functional classes under biological process, “cellular process” (14,321 unigenes) and “biological regulation” (9,126 unigenes) contained the most genes; among the six functional classes under cellular component, “cellular anatomical entity” (14,940 unigenes) and “intracellular” (8,916 unigenes) contained the most genes; and among the 13 functional classes under molecular function, “binding” (13,234 unigenes) and “catalytic activity” (7,120 unigenes) contained the most genes.

KEGG pathway enrichment indicated that 22,890 unigenes significantly matched to 343 pathways. Among them, “mitogen-activated protein kinase (MAPK) signaling pathway” (569) contained the most unigenes, followed by “endocytosis” (544) and “calcium signaling pathway” (502).

DEG expression and enrichment analysis

**DEG screening**

The relationships among the six samples are shown in Fig. 2. Unigenes with Q-values ≤ 0.01 and |log₂ fold change| ≥ 1 were identified as DEGs between female *D. macrosoma* and male *D. macrosoma* using DESeq2. After filtration, a total of 2,345 DEGs (Table S4) were obtained between male and female *D. macrosoma*, 1,150 of which were female-biased DEGs, while 1,195 unigenes were male-biased DEGs (Fig. 3).

**DEG annotation**

To explore possible differences in gene function between female and male *D. macrosoma*, GO and KEGG enrichment analyses were performed on the 2,345 DEGs identified between male and female *D. macrosoma* (Table S5). Visualization of the GO enrichment results (Fig. 4) showed that 1,903 unigenes were assigned to 60 GO terms (Table S6), and female and male *D. macrosoma* clearly differed in their biological processes. The top 10 enriched GO terms are listed in Table S7.

The KEGG analysis (Fig. S3) revealed that the DEGs were significantly enriched for 225 KEGG pathways (Table S8). The top 20 significantly enriched metabolic pathways (Fig. 5) included “tight junction” (ko04530), “regulation of actin cytoskeleton” (ko04810), and “ribosome biogenesis in eukaryotes” (ko03008).
**Figure 2** PCA plot of the six samples. The horizontal and vertical coordinates indicate the first and second principal components respectively, and the contribution of each principal component is shown in parentheses. The two triangles are the mean coordinates (centroids) of the male and female samples respectively.

**Figure 3** MA plot of DEGs. Each dot represents a gene. The blue and orange dots in the graph represent genes with significant expression differences. The blue dots are female-biased DEGs, the orange dots are male-biased DEGs and the black dots represent genes with no significant expression differences. The names of sex- and growth-related genes are showed in the figure.
According to the sequence annotation results, many DEGs were sex-controlled and gonad-development-associated genes, including the zona pellucida sperm-binding protein (Zps), sperm acrosome membrane-associated (Spacas), vitellogenin (Vg), cytochrome p450 enzyme (Cyps), stAR-related lipid transfer protein (Start), and sperm-associated antigen 17 (Spag17), as well as other potential candidate protein-encoding genes (Table 1).

Growth, a complex trait, is jointly controlled by many genes. In this study, we identified multiple genes involved in growth regulation, such as genes related to fibroblast growth factor receptors, which control growth at the muscle tissue level and others (Table 2).
SSR analysis
To explore genetic diversity, the SSRs of *D. macrosoma* were identified using MISA software. A total of 19,573 SSRs were located in 11,433 unigenes, 8,140 of which contained multiple SSRs. The frequency with which different SSRs appeared varied (Table S9), and mononucleotide SSRs had the highest frequency, accounting for 51.79% of total SSRs, followed by trinucleotide SSRs (22.63%). In addition, the relative SSR abundance varied greatly. For example, A/T was the most common mononucleotide SSR, while AC/GT had the highest frequency among dinucleotide SSRs.

**DISCUSSION**

**Transcriptome sequencing analysis of *D. macrosoma***
To identify the functions of genes expressed in the *D. macrosoma* and the biological processes in which they were involved, cDNA libraries were built from sexually mature female and male *D. macrosoma*. RNA-seq was performed on an Illumina platform, which yielded 42.61 Gb of clean data. N50 is an important parameter used to evaluate RNA-seq assembly quality. In this study, the N50 length of the transcriptome from *D. macrosoma*...
was 2,266 bp, and the BUSCO analysis showed that 92.9% of transcriptomes were complete. We concluded that the transcriptome of *D. macrosoma* was sufficiently complete and well assembled; therefore, it may be valuable for gene function analyses.

### Table 1 Sex-related genes in *D. macrosoma*.

| Gene   | Annotation                                              | Log2 (♀/♂) | Qvalue     |
|--------|---------------------------------------------------------|------------|------------|
| 42sp43 | P43 5S RNA-binding protein-like isoform X1              | 6.85       | 1.40E–15   |
| Cts    | Cathepsin S-like                                        | 9.9        | 1.48E–30   |
| Ctsz   | Cathepsin Z                                             | 5.14       | 1.09E–04   |
| Cyp26a1| Cytochrome P450 26A1                                     | 4.11       | 3.84E–07   |
| Foxi1  | Forkhead box protein 11                                 | −4.17      | 0          |
| Gtf3a  | Transcription factor IIIa                               | 9.22       | 5.35E–32   |
| Mnd1   | Meiotic nuclear division protein 1 homolog isoform X1   | −3.42      | 9.22E–03   |
| Profilin2| Prolin-2-like isoform X1                                | 1.94       | 4.39E–06   |
| Spaca4 | Sperm acrosome membrane-associated protein 4-like isoform X1 | 9.62       | 2.16E–29   |
| Spag17 | Sperm-associated antigen 17                             | −5.74      | 4.39E–06   |
| Spata22| Spermatogenesis-associated protein 22 isoform X2        | −4.6       | 5.04E–04   |
| Spata6 | Spermatogenesis-associated protein 6-like isoform X2    | −3.63      | 2.64E–03   |
| Spata7 | Spermatogenesis-associated protein 7                     | −3.75      | 3.53E–04   |
| Start-7| STAR-related lipid transfer protein 7                    | 1.55       | 0          |
| Start-9| STAR-related lipid transfer protein 9                    | −3.38      | 7.03E–05   |
| Vg     | Vitellogenin-like                                        | 10.47      | 1.63E–65   |
| Wee2   | Weel-like protein kinase 2                               | 6.05       | 1.33E–12   |
| Zar1   | Zygote arrest protein 1                                  | 6.94       | 0          |
| Zp3    | Zona pellucida sperm-binding protein 3-like              | 8.85       | 1.32E–07   |
| Zp4    | Zona pellucida sperm-binding protein 4-like              | 9.34       | 1.82E–11   |

### Table 2 Growth-related genes in *D. macrosoma*.

| Gene   | Annotation                                              | Log2 (♀/♂) | Qvalue     |
|--------|---------------------------------------------------------|------------|------------|
| Efcb7  | EF-hand calcium-binding domain-containing protein 7      | −3.62      | 0          |
| Efcb8  | EF-hand calcium-binding domain-containing protein 8      | −5.75      | 1.49E–07   |
| Efhb   | EF-hand domain-containing family member B isoform X1    | −4.05      | 9.56E–05   |
| Efhc1  | EF-hand domain-containing protein 1                     | −7.27      | 1.43E–10   |
| Efhc2  | EF-hand domain-containing family member C2              | −6.44      | 2.75E–08   |
| Fabp   | Fatty acid-binding protein                              | 8.47       | 9.34E–35   |
| Fgfr1l | Fibroblast growth factor receptor-like 1                 | −3.44      | 1.74E–03   |
| Gdf3   | Growth differentiation factor 3                         | 4.66       | 6.18E–06   |
| Gdf9   | Growth differentiation factor 9-like                    | 5.91       | 4.97E–09   |
| Igf2bp3| Insulin-like growth factor 2 mRNA-binding protein 3 isoform X3 | 3.59       | 7.53E–06   |
| Ltpbp2 | Latent-transforming growth factor β-binding protein 2-like | −1.91     | 4.01E–03   |
| Mylc   | Myosin light chain                                      | −3.8       | 8.72E–03   |
| Pdgfa  | Platelet-derived growth factor subunit A-like isoform X2 | −2.86      | 7.63E–08   |
Functional annotation

Nearly half of *D. macrosoma* unigenes were not annotated to any sequences in the reference databases. The low annotation ratio seems unsurprising in non-model organisms without published genomes (Jung et al., 2011b; Robledo et al., 2014; Ma et al., 2016). Previous studies on transcriptome analyses indicate that unannotated sequences mainly represent transcripts spanning only untranslated mRNA regions, chimeric sequences derived from assembly errors (Wang et al., 2004), and sequences containing non-conserved protein regions (Mittapalli et al., 2010). Some may also be components of novel genes specific to this species, which are likely to be matched to certain genome sequences in the near future (Ma et al., 2016). A comparison against the Nr database showed that 29% (7,552 of 26,514 unigenes) of the comparable sequences were homologous to *S. partitus* sequences, and 24% (6,312 of 26,514 unigenes) were homologous to *L. crocea* sequences (Fig. S2), indicating that *D. macrosoma* was closely related to *S. partitus* and *L. crocea*.

In the *D. macrosoma* transcriptome, “cellular anatomical entity”, “cellular process”, and “binding” were the most enriched under the three major GO categories (Fig. 4). The results are similar to RNA-seq results for other aquatic animals, suggesting that the genes in these functional classes are conserved among species (Wang, Lou & Shui, 2019; Sun & Han, 2019). As indicated by the KEGG annotation results, the unigenes in the *D. macrosoma* transcriptome mainly participated in the “MAPK signaling pathway” (569), “endocytosis” (544), and “calcium signaling pathway” (502), indicating that cellular physiological pathways may largely rely on the regulation of functional gene expression, since functional genes are important specific mediators and effectors of the cell physiological activities of living organisms (DeMeester, Buchman & Cobb, 2001).

DEGs in female and male *D. macrosoma*

In this study, a total of 2,345 DEGs were detected in the transcriptomes of female and male *D. macrosoma*, 1,150 of which were highly expressed in female *D. macrosoma*, while 1,195 were highly expressed in male *D. macrosoma*. Compared with males, female *D. macrosoma* have a significantly faster growth rate. These DEGs might be associated with sex determination or correlated with growth. Our findings lay a foundation for further explorations of the molecular mechanisms underlying biological processes in *D. macrosoma*.

In this study, many growth- and sex-related genes were detected in the *D. macrosoma* transcriptome data. A large number of studies have shown that genes encoding growth axis components, including insulin-like growth factors (*Igf*), somatostatin, and their carrier proteins and receptors (De-Santis & Jerry, 2007), played crucial roles in regulating the formation of skeletal muscles in finfish.

The GO enrichment analysis of the DEGs revealed clearly different biological processes between female and male *D. macrosoma*, including growth, rhythmic processes, and immune defense. The GO enrichment analysis of unigenes indicated that the significantly enriched GO terms were mainly correlated with cilia and axonemes, including cilium assembly, cilium tissue, axoneme assembly, and the axonemal dynein complex. Cilia, which extend from the top of the matrix (from the centriole) and extrude from
microtubule-based hairlike organelles on the cell surface, function in motility and signal transduction and can regulate animal reproduction, development, and perception (Wei et al., 2016). The axonemal dynein complex not only constitutes the dynein arms of peripheral doublets in cilia or flagella but also powers the mutual sliding of peripheral doublets (Zhao et al., 2020). These GO terms were significantly more highly expressed in males than in females, indicating that cilia and axonemes may be more critical in *D. macrosoma* males than in females.

The KEGG enrichment analysis revealed that some sex-related pathways, including “MAPK signaling pathway”, “neuroactive ligand-receptor interaction”, “GnRH signaling pathway”, “TGF-beta signaling pathway”, and “p53 signaling pathway”. The MAPK signaling pathway is present in all eukaryotes. The MAPK signaling pathway is mainly involved in the regulation of gonadotropin subunit gene expression and the movement of primary spermatocytes across the blood-testis barrier (Di et al., 2002). It has a wide range of cellular roles in growth, differentiation, and stress responses (Jung et al., 2011a). In the study, the MAPK signaling pathway was significantly enriched in male *D. macrosoma* and was also enriched in female *D. macrosoma*, although without statistical significance, which might indicate that the MAPK signaling pathway plays different roles in the gonadal development of *D. macrosoma*. The neuroactive ligand-receptor interaction pathway is related to lactation performance in mice. In addition, genes related to this pathway have been found to be upregulated (Wei et al., 2013). Previous studies have shown that the neuroactive ligand-receptor interaction pathway plays an important role in teleost reproduction and gonadal development (Du et al., 2017; Li et al., 2017). Therefore, the neuroactive ligand-receptor interaction pathway may have an effect on gonadal development and sexual maturity in *D. macrosoma*.

Regarding calcium ion regulation, many biological reactions are triggered or regulated through subtle changes in the intracellular calcium ion concentration. The precise sensing of intracellular calcium ion concentrations within different ranges by EF-hand proteins (as intracellular sensors) is the key to this regulatory process. The regulatory function of signaling via EF-hand proteins is mainly ascribed to their calcium ion-induced conformational changes, which consequently expose many hydrophobic target protein binding sites. Moreover, the functions of buffer proteins, which regulate calcium ion concentration, are affected by the selectivity of calcium ions, the dynamics of binding calcium ions, etc. Calmodulin (CaM) and related protein-coding (EF-hand superfamily) genes participate in several cellular physiological processes, including development, growth, and cell differentiation, and play significant roles in the nervous system, immune system, reproductive system, motor system, etc. (Qi et al., 2015). Past studies have shown that the EF-hand superfamily was significantly expressed in *C. myriaster* (Chen, 2019). EF-hand proteins are involved in regulating almost all cellular functions. For instance, CaM-dependent protein kinases have important effects on the regulation of the immune and nervous systems and on sperm formation (Zhu, 2008). Hence, the EF-hand protein family may directly or indirectly regulate cell growth in *D. macrosoma*. In this study, five EF-hand proteins (*i.e.*, EF-hand domain-containing protein 1, EF-hand domain-containing family member C2, EF-hand calcium-binding domain-containing protein 7,
EF-hand calcium-binding domain-containing protein 8, and EF-hand domain-containing family member B isoform X1) were screened from the *D. macrosoma* transcriptome. They all showed higher expression in males than in females, indicating that CaM and related protein-coding genes may have vital functions in male *D. macrosoma* growth and reproduction.

Fatty acid-binding proteins (FABPs) are a group of proteins that coordinate lipid trafficking and signaling in cells. The proteins play an important role in facilitating cellular uptake and transfer of fatty acids (FAs), targeting FAs to specific metabolic pathways, and participating in the regulation of gene expression and cell growth (*Haunerland & Spener, 2004*). As shown in Table 2, Fabp showed significantly higher expression in female *D. macrosoma* than in males, indicating that the Fabp gene may play critical roles in sex differentiation in *D. macrosoma*. Thus far, information on the progress of the fish Fabps gene is limited to the expression patterns in Atlantic salmon (*Torstensen et al., 2009*) and promoter function in zebrafish (*Her, Chiang & Wu, 2004*). Therefore, a broad area of interest remains regarding exploration of the functions of the FABP family in teleost fish (*Ma et al., 2016*).

Insulin-like growth factors (IGFs) and relevant carrier proteins are some of the most important components of the somatotropic axis. They are widely recognized to play a significant role in regulating the formation of skeletal muscles in finfish (*De-Santis & Jerry, 2007*). In this study, compared to the male *D. macrosoma*, female *D. macrosoma* showed significantly higher Igf2bp3 expression levels, suggesting that Igf2bp3 may play an important role in the reproduction and growth of *D. macrosoma*.

The zona pellucida is the extracellular matrix around oocytes, protecting them and playing an important role in their fusion with sperm (*Dumont & Brummett, 1985*). The zona pellucida comprises four glycoproteins (ZP1-4), which bind to each other to form long filaments (*Wassarman, 1988; Litscher & Wassarman, 2018*). Zona pellucida proteins were first discovered in mammalian egg membranes. They were later discovered in the inner layer of fish chorions (*Litscher & Wassarman, 2007*). Studies have found that ZP3 was a primary female-specific reproductive molecule (*Liu, Wang & Gong, 2006*). Recently, a number of copies of the Zp genes have been identified in some teleost species, such as carp and medaka (*Chang et al., 1997; Kanamori et al., 2003*). Zp3 and Zp4 function as major sperm receptors and induce acrosome reaction (*Litscher & Wassarman, 2007*). In this study, the expression levels of Zp3 and Zp4 in females were higher than those in males, showing that these genes may play vital roles in ovarian development in fish and implicating their roles in *D. macrosoma* genesis and reproduction.

Vitellogenin (Vg), a protein found in mature females of nonmammary oviparous animals, functions as the precursor for vitellin in nearly all oviparous animals (*Liu et al., 2016*). Synthesized by hypothalamic-pituitary-gonadal (HPG) axis-activated estrogen receptors (ERs), Vg is a critical substance in yolk synthesis and an important reproductive protein in fishes. Vg also plays an important role in the reproduction and development of oviparous animals (*Wu, 2018*). In oviparous animals, Vg not only provides saccharides,
fats, proteins, and other nutrients for the development of mature oocytes into embryos but also binds and transports metal ions (Zn$^{2+}$, Fe$^{3+}$, Cu$^{2+}$, Mg$^{2+}$ and Ca$^{2+}$), carotenoids, thyroxines, retinols, and riboflavin into oocytes (Valle, 1993; Taborsky, 1980; Babin, 1992; Azuma, Irie & Seki, 1993; Mac, Nimpf & Schneider, 1994). The vast majority of studies suggest that although the Vg gene exists in larvae and males, it can be expressed only by adult females. Although Vg is a female-specific protein (including in fishes), estrogen-induced anomalies can result in its production in male fishes. Hence, Vg has been widely investigated as a sensitive biomarker for monitoring environmental estrogen-like substances (Wallace, 1985; Specker & Sullivan, 1994). Unuma et al. (1998) detected the expression of the Vg gene in the gonads of both female and male Pseudocentrotus depressus, and its expression gradually decreased with testis development. We inferred that Vg could be utilized as a nutrient substance for sperm development. Studies on Halocynthia roretzi (Akasaka, Harada & Sawada, 2010; Akasaka et al., 2013) have shown that the vWFD and CT structural domains in the C-terminus of the Vg protein may be positioned in the vitellin coat and bind to two enzymes in sperm (HrProacrosin and HrSpermosin), thereby participating in the fertilization process once sperm has entered eggs (Liu et al., 2016). In this study, Vg was significantly upregulated in females compared with males. Therefore, its expression in the testes may serve as a nutrient substance for spermatogenesis or may assist in fertilization, indicating that Vg may be especially important in the reproduction and development of both female and male D. macrosoma.

SSR markers

SSRs, a significant class of molecular markers, are important resources in population genetics studies. In this study, 19,573 SSRs were acquired from 11,433 unigenes. Among the identified SSRs, mononucleotide SSRs were the most abundant, while fewer SSRs had more repetitive nucleotides (Table S9), which was consistent with the results of the SSR analysis in Oplegnathus punctatus (Du et al., 2017), Charybdis feriata (Zhang et al., 2017), and Sillago sihama (Tian et al., 2019). The D. macrosoma SSRs acquired in this study provide data that can be used to explore genetic diversity and aid in constructing genetic linkage maps and developing genetic resources for this species.

CONCLUSIONS

This study is the first to generate transcriptome data from female and male D. macrosoma. A total of 58,475 unigenes were obtained through assembly, and then 28,174 unigenes were functionally annotated, thereby enriching the functional genetic resources for this species. Many candidate genes related to growth and sex differentiation were detected by comparing transcriptomes from female and male D. macrosoma. A total of 19,573 SSR sites were obtained from 11,433 D. macrosoma unigenes, which yielded data to aid in studying the genetic diversity of this species. There are extremely limited genome assembly data for Carangidae fishes in public databases, and no genome assembly data exist for Decapterus. This study supplies valuable information about the growth- and sex-related genes of the Osteichthyes, fills the gap in public genome assembly data for Decapterus, lays a
foundation for further study of Carangidae fishes, and facilitates further investigations of fish transcriptomes.

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Author Contributions
- Zizi Cai performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Shigang Liu performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Wei Wang analyzed the data, prepared figures and/or tables, and approved the final draft.
- Rui Wang analyzed the data, prepared figures and/or tables, and approved the final draft.
- Xing Miao analyzed the data, prepared figures and/or tables, and approved the final draft.
- Puing Song conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
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- Yuan Li conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Longshan Lin conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
Animal Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

   The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethics committee of Laboratory Animal Welfare and Ethics of South China Sea Fisheries Research Institute (code: nhdf 2022-06 and date of approval: June 2022).

Data Availability
The following information was supplied regarding data availability:

   The data is available at NCBI Sequence Read Archive (SRA): PRJNA825736, SRR18748694–SRR18748699.

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REFERENCES
Akasaka M, Harada Y, Sawada H. 2010. Vitellogenin C-terminal fragments participate in fertilization as egg-coat binding partners of sperm trypsin-like proteases in the ascidian Halocynthia roretzi. Biochemical and Biophysical Research Communications 392(4):479–484 DOI 10.1016/j.bbrc.2010.01.006.

Akasaka M, Kato KH, Kitajima K, Sawada H. 2013. Identification of novel isoforms of vitellogenin expressed in ascidian eggs. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 320(2):118–128 DOI 10.1002/jez.b.22488.

Azuma M, Irie T, Seki T. 1993. Retinals and retinols induced by estrogen in the blood plasma of Xenopus laevis. Journal of Experimental Biology 178:89 DOI 10.1242/jeb.178.1.89.

Babin PJ. 1992. Binding of thyroxine and 3,5,3′-triiodothyronine to trout plasma lipoproteins. American Journal of Physiology 262(5):712–720 DOI 10.1152/ajpendo.1992.262.5.E712.

Barske LA, Capel B. 2008. Blurring the edges in vertebrate sex determination. Current Opinion in Genetics & Development 18(6):499–505 DOI 10.1016/j.gde.2008.11.004.

Bolger AM, Marc L, Bjoern U. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15):2114–2120 DOI 10.1093/bioinformatics/btu170.

Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. Nature Methods 12:59–60 DOI 10.1038/nmeth.3176.

Casas L, Saborido-Rey F, Ryu T, Michell C, Ravasi T, Irigoien X. 2016. Sex change in clownfish: molecular insights from transcriptome analysis. Scientific Reports 6(1):35461 DOI 10.1038/srep35461.

Chang YS, Hsu CC, Wang SC, Tsao CC, Huang FL. 1997. Molecular cloning, structural analysis, and expression of carp ZP2 gene. Molecular Reproduction and Development 46(3):258–267 DOI 10.1002/(SICI)1098-2795(199703)46:3<258::AID-MRD4>3.0.CO;2-O.

Chen JC. 2019. Transcriptomics of 5 economic fishes in Zhoushan sea area. MA thesis, Zhejiang Ocean University.
Chen YP, Cao Y, Hu SL, Huang Y, Lu XQ, Xu G, Long ZJ. 2016. Transcriptome analysis and gene function annotation of Bambusa emeiensis shoots based on high-throughput sequencing technology. Chinese Journal of Biotechnology 32(11):1610–1623 DOI 10.13345/j.cjb.160095.

Chen H, Xiao GQ, Chai XL, Lin XG, Fang J, Teng SS. 2017. Transcriptome analysis of sex-related genes in the blood clam Tegillarca granosa. PLOS ONE 12(9):e0184584 DOI 10.1371/journal.pone.0184584.

De-Santis C, Jerry DR. 2007. Candidate growth genes in fish—where should we be looking? Aquaculture 272(1–4):22–38 DOI 10.1016/j.aquaculture.2007.08.036.

DeMeester SL, Buchman T, Cobb JP. 2001. The heat shock paradox: does NF-κB determine cell fate? Faseb Journal 15(1):270–274 DOI 10.1096/fj.00-0170hyp.

Devlin RH, Nagahama Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic physiological and environmental influences. Aquaculture 208(3–4):191–364 DOI 10.1016/S0044-8486(02)00057-1.

Dewey CN, Li B. 2011. RSEM: accurate transcript quantification from RNA-seq data with or without a reference genome. BMC Bioinformatics 12(1):323 DOI 10.1186/1471-2105-12-323.

Di AS, Rossi P, Geremia R, Sette C. 2002. The MAPK pathway triggers activation of Nek2 during chromosome condensation in mouse spermatocytes. Development 129(7):1715–1727 DOI 10.1242/dev.129.7.1715.

Dong XJ. 2018. Phylogenetic analysis of NBS gene family in five Triticeae species based on transcriptome. MA thesis, Shandong Agricultural University.

Dong ZD, Li XY, Liao J, Zhang N, Guo YS, Wang ZD. 2020. Comparative transcriptome analysis of male and female liver of Oryzias curvinotus. Oceanologia et Limnologia Sinica 51(5):1203–1213 DOI 10.11693/hyhz20191200274.

Du XX, Wang B, Liu XM, Liu XB, He Y, Zhang QQ, Wang XB. 2017. Comparative transcriptome analysis of ovary and testis reveals potential sex-related genes and pathways in spotted knifejaw Oplegnathus punctatus. Gene 637:203–210 DOI 10.1016/j.gene.2017.09.055.

Dumont JN, Brummett AR. 1985. Egg envelope in vertebrates. Developmental Biology 1:235–288 DOI 10.10107/978-1-4615-6814-8_5.

Dutney L, Elizur A, Lee P. 2017. Analysis of sexually dimorphic growth in captive reared cobia (Rachycentron canadum) and the occurrence of intersex individuals. Aquaculture 468(1):348–355 DOI 10.1016/j.aquaculture.2016.09.044.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Amit I. 2013. Trinity: reconstructing a full-length transcriptome without a genome from RNA-seq data. Nature Biotechnology 29:644 DOI 10.1038/nbt.1883.

Hart SN, Therneau TM, Zhang YJ, Poland GA, Kocher JP. 2013. Calculating sample size estimates for RNA sequencing data. Journal of Computational Biology 20(12):970–978 DOI 10.1089/cmb.2012.0283.

Haunerland NH, Spener F. 2004. Fatty acid-binding proteins—insights from genetic manipulations. Progress in Lipid Research 43(4):328–349 DOI 10.1016/j.plipres.2004.05.001.

Her GM, Chiang CC, Wu JL. 2004. Zebrafish intestinal fatty acid binding protein (I-FABP) gene promoter drives gut-specific expression in stable transgenic fish. Genesis 38(1):26–31 DOI 10.1002/gene.10248.

Hou G, Wang JR, Chen ZZ, Zhou JL, Huang WS, Zhang H. 2020. Molecular and morphological identification and seasonal distribution of eggs of four Decapterus fish species in the northern South China Sea: a key to conservation of spawning ground. Frontiers in Marine Science 7:590564 DOI 10.3389/fmars.2020.590564.
Institute of Zoology, Chinese Academy of Sciences, Institute of Oceanology, Chinese Academy of Sciences & Shanghai Ocean University. 1962. *Annals of fishes Insouth China Sea*. Beijing: Science Press, 387–389.

Jung KW, Kim SY, Okagaki LH, Nielsen K, Bahn YS. 2011a. Ste50 adaptor protein governs sexual differentiation of *Cryptococcus neoformans* via the pheromone-response MAPK signaling pathway. *Fungal Genetics and Biology* 48(2):154–165 DOI 10.1016/j.fgb.2010.10.006.

Jung H, Lyons RE, Dinh H, Hurwood DA, McWilliam S, Mather PB. 2011b. Transcriptomics of a giant fresh-water prawn (*Macrobrachium rosenbergii*): de novo assembly, annotation and marker discovery. *PLOS ONE* 6(12):e27938 DOI 10.1371/journal.pone.0027938.

Kanamori A, Naruse K, Mitani H, Shima A, Horii H. 2003. Genomic organization of ZP domain containing egg envelope genes in medaka (*Oryzias latipes*). *Gene* 305(1):35–45 DOI 10.1016/s0378-1119(02)01211-8.

Kobayashi Y, Nagahama Y, Nakamura M. 2013. Diversity and plasticity of sex determination and differentiation in fishes. *Sexual Development* 7(1–3):115–125 DOI 10.1159/000342009.

Kong XL, Li M, Chen ZZ, Gong YY, Zhang J, Zhang P. 2019. Development and evaluation of di-/tri-nucleotide-repeated microsatellites by RAD-seq in *Decapterus macrosoma*. *South China Fisheries Science* 15(3):97–103 DOI 10.12131/20180256.

Langmead B. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* 10(3):R25 DOI 10.1186/gb-2009-10-3-r25.

Li YH, Wang HP, Yao H, O’Bryant P, Waly EA. 2017. *De novo* transcriptome sequencing and analysis of male, pseudo-male and female yellow perch, *Perca flavescens*. *PLOS ONE* 12(2):e0171187 DOI 10.1371/journal.pone.0171187.

Litscher ES, Wassarman PM. 2007. Egg extracellular coat proteins: from fish to mammals. *Histology & Histopathology* 22(3):337–347 DOI 10.14670/HH-22.337.

Litscher ES, Wassarman PM. 2018. The fish egg’s zona pellucida. *Current Topics in Developmental Biology* 130:275–305 DOI 10.1016/bs.ctdb.2018.01.002.

Liu XJ, Wang H, Gong ZY. 2006. Tandem-repeated zebrafish *zp3* genes possess oocyte-specific promoters and are insensitive to estrogen induction. *Biology of Reproduction* 74:1016–1025 DOI 10.1095/biolreprod.105.049403.

Liu HY, Zhang N, Yan HW, Liu Q, Xing K, Chen L, Sui YZ, Lin Y, Li CJ, Guo LY. 2016. Vitellogenin (Vg) gene in mantis shrimp *Oratosquilla oratoria*. *Journal of Dalian Ocean University* 31(2):131–139 DOI 10.16535/j.cnki.dlhxyb.2016.02.003.

Lou FR, Yang TY, Han ZQ, Gao TX. 2018. Transcriptome analysis for identification of candidate genes related to sex determination and growth in *Charybdis japonica*. *Gene* 677:10–16 DOI 10.1016/j.gene.2018.07.044.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15(12):550 DOI 10.1186/s13059-014-0550-8.

Ma Q, Liu SF, Zhuang ZM, Lin L, Sun ZZ, Liu CL, Su YQ, Tang QS. 2011. Genomic structure, polymorphism and expression analysis of growth hormone-releasing hormone and pituitary adenylate cyclase activating polypeptide genes in the half-smooth tongue sole (*Cynoglossus semilaevis*). *Genetics and Molecular Research* 10(4):3828–3846 DOI 10.4238/2011.

Ma DY, Ma AJ, Huang ZH, Wang GN, Wang T, Xia DD, Ma BH. 2016. Transcriptome analysis for identification of genes related to gonad differentiation, growth, immune response and marker discovery in the turbot (*Scophthalmus maximus*). *PLOS ONE* 11(2):e0149414 DOI 10.1371/journal.pone.0149414.
Mac LI, Nimpf J, Schneider WJ. 1994. Avian riboflavin binding protein binds to lipoprotein receptors in association with vitellogenin. *Journal of Biological Chemistry* **269**(39):24127 DOI 10.1016/S0021-9258(19)51057-2.

Mittapalli O, Bai X, Mamidala P, Rajarapu SP, Bonello P, Herms DA. 2010. Tissue-specific transcriptomics of the exotic invasive insect pest emerald ash borer (*Agrilus planipennis*). *PLOS ONE* **5**(10):e13708 DOI 10.1371/journal.pone.0013708.

Ottolenghi C, Pelosi E, Tran J, Colombino M, Douglass E, Nedorezov T, Antonio C, Antonino F, Schlessinger D. 2007. Loss of *wnt4* and *foxl2* leads to female-to-male sex reversal extending to germ cells. *Human Molecular Genetics* **16**(23):2795–2804 DOI 10.1093/hmg/ddm235.

Qi XX, Li WJ, Shi ZY, Shang C, Zhou ZR, Shang P. 2015. Study on the expression of EF-hand gene in *Hyriopsis cumingii*. *Acta Agriculturae Zhejiangensis* **27**(12):2078–2085 DOI 10.3969/j.issn.1004-1524.2015.12.06.

Rennie MD, Purchase CF, Lester N, Collins NC, Shuter BJ, Abrams PA. 2008. Lazy males? Bioenergetic differences in energy acquisition and metabolism help to explain sexual size dimorphism in percids. *Journal of Animal Ecology* **77**(5):916–926 DOI 10.1111/j.1365-2656.2008.01412.x.

Ribas L, Robledo D, Gómez-tato A, Viñas A, Martínez P, Pfiferrer F. 2016. Comprehensive transcriptomic analysis of the process of gonadal sex differentiation in the turbot (*Scophthalmus maximus*). *Molecular & Cellular Endocrinology* **422**:132–149 DOI 10.1016/j.mce.2015.11.006.

Robledo D, Ronza P, Harrison PW, Losada AP, Bermudez R, Pardo BG, Redondo MJ, Sitjà-Bobadilla A, Quiroga MI, Martínez P. 2014. RNA-seq analysis reveals significant transcriptome changes in turbot (*Scophthalmus maximus*) suffering severe enteromyxosis. *BMC Genomics* **15**(1):1149 DOI 10.1186/1471-2164-15-1149.

Sebastian B, Thomas T, Thomas M, Uwe S, Martin M. 2017. MISA-web: a web server for microsatellite prediction. *Bioinformatics (Oxford, England)* **33**:2583–2585 DOI 10.1093/bioinformatics/btx198.

Shan BB, Liu Y, Yang CP, Li Y, Wang LM, Sun DR. 2021a. Comparative transcriptome analysis of female and male fine-patterned puffer: identification of candidate genes associated with growth and sex differentiation. *Fishes* **6**(4):79 DOI 10.3390/fishes6040079.

Shan BB, Liu Y, Yang CP, Zhao Y, Sun DR. 2021b. Comparative transcriptomic analysis for identification of candidate sex-related genes and pathways in Crimson seabream (*Parargyrops edita*). *Scientific Reports* **11**(1):1077 DOI 10.1038/s41598-020-80282-5.

Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**(19):3210–3212 DOI 10.1093/bioinformatics/btv351.

Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran TJ, Sinclair AH. 2009. The avian z-linked gene *DMRT1* is required for male sex determination in the chicken. *Nature* **461**(7261):267–271 DOI 10.1038/nature08298.

Specker JL, Sullivan CV. 1994. Vitellogenesis in fishes: status and perspectives. In: *Perspectives in Endocrinology*. Ottawa: National Research Council, 304–315.

Sun SX, Han ZQ. 2019. A study of crustacean phylogeny based on genomic and transcriptome data. *Journal of Zhejiang Ocean University (Natural Science)* **38**(6):469–474 DOI 10.3969/j.issn.1008-830X.2019.06.001.

Sánchez CC, Weber GM, Gao G, Cleveland BM, Yao J, Rexroad CE. 2011. Generation of a reference transcriptome for evaluating rainbow trout responses to various stressors. *BMC Genomics* **12**:626 DOI 10.1186/1471-2164-12-626.
Taborsky G. 1980. Iron binding by phosvitin and its conformational consequence. *Journal of Biological Chemistry* 255(7):2976–2984 DOI 10.1016/S0021-9258(19)85837-4.

Tian CX, Li ZY, Dong ZD, Huang Y, Du T, Chen H, Jiang DN, Deng SP, Zhang YL, Wanida S. 2019. Transcriptome analysis of male and female mature gonads of silver sillago (*Sillago sihama*). *Genes* 10(2):129 DOI 10.3390/genes10020129.

Toguyeni A, Fauconneau B, Boujard T, Fostier A, Kuhn ER, Mol KA, Baroiller JF. 1997. Feeding behaviour and food utilization in tilapia, *Oreochromis niloticus*: effect of sex ratio and relationship with the endocrine status. *Physiology & Behavior* 62(2):273–279 DOI 10.1016/s0031-9384(97)00114-5.

Torstensen B, Nanton D, Olsvik P, Sundvold H, Stubhaug I. 2009. Gene expression of fatty acid-binding proteins, fatty acid transport proteins (cd36 and FATP) and β-oxidation-related genes in Atlantic salmon (*Salmo salar L.*) fed fish oil or vegetable oil. *Aquaculture Nutrition* 15(4):440–451 DOI 10.1111/j.1365-2095.2008.00609.x.

Tranpell C, Williams BA, Pettea G, Mortazavi A, Kwan G, Baren MJ, Salzberg SL, Wold BJ, Pachter L. 2010. Transcript assembly and quantification by RNA-seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology* 28(5):511–515 DOI 10.1038/nbt.1621.

Unuma T, Kurokawa T, Yamamoto T, Akiyama T, Suzuki T. 1998. A protein identical to the yolk protein is stored in the testis in male red sea urchin, *Pseudocentrotus depressus*. *The Biological Bulletin* 194(1):92–97 DOI 10.2307/1542517.

Valle D. 1993. Vitellogenesis in insects and other groups: a review. *Memórias do Instituto Oswaldo Cruz* 88(1):1–26 DOI 10.1590/S0074-02761993000100005.

Wallace RA. 1985. *Vitellogenesis and oocyte growth in nonmammalian vertebrates*. New York: Springer, 127–177.

Wang LK, Feng ZX, Wang X, Wang XW, Zhang XG. 2010. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* 26(1):136–138 DOI 10.1093/bioinformatics/btp612.

Wang YJ, Lou FR, Shui BN. 2019. Transcriptome data *de novo* assembly and gene annotation analysis of *Nibea albi flora* through high throughput sequencing. *Journal of Zhejiang Ocean University (Natural Science)* 38(6):475–480, 494 DOI 10.3969/j.issn.1008-830X.2019.06.002.

Wang N, Wang RK, Wang RQ, Chen SL. 2018. Transcriptomics analysis revealing candidate networks and genes for the body size sexual dimorphism of Chinese tongue sole (*Cynoglossus semilaevis*). *Functional & Integrative Genomics* 18:327–339 DOI 10.1007/s10142-018-0595-y.

Wassarman PM. 1988. Zona pellucida glycoproteins. *Annual Review of Biochemistry* 57(1):415–442 DOI 10.1146/annurev.bi.57.070188.002215.

Wei J, Ramanathan P, Martin IC, Moran C, Taylor RM, Williamson P. 2013. Identification of gene sets and pathways associated with lactation performance in mice. *Physiological Genomics* 45(5):171–181 DOI 10.1152/physiogenomics.00139.201.

Wei Q, Zhang YY, Schouteden C, Zheng YX, Zhang Q, Dong JH, Wonesch V, Ling K, Dammermann A, Hu J. 2016. The hydrolethalus syndrome protein HYLS-1 regulates formation of the ciliary gate. *Nature Communications* 7:12437 DOI 10.1038/ncomms12437.

Wu D. 2018. Molecular cloning of three vitellogenin homologs in *Scatophagus argus* and their expressions after estrogen exposure. MA thesis, Shanghai Ocean University.
Xie C, Mao XZ, Huang JJ, Ding Y, Wu JM, Dong S, Kong L, Gao G, Li CY, Wei LP. 2011. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Research* **39**(W316–W322) DOI 10.1093/nar/gkr483.

Yang LD, Wang Y, Zhang ZL, He SP. 2015. Comprehensive transcriptome analysis reveals accelerated genic evolution in a Tibet Fish, *Gymnodiptychus pachycheilus*. *Genome Biology and Evolution* **7**(1):251–261 DOI 10.1093/gbe/evu279.

Yang L, Zhang XF, Tan YG, Zhang P. 2009. Analysis on the catch composition of light purse seine in the northern South China Sea. *South China Fisheries Science* **5**(6):65–70 DOI 10.3969/j.issn.1673-2227.2009.06.012.

Zhang L, Li Y, Lin LS, Yao Z, Yan L, Zhang P. 2016a. Fishery resources acoustic assessment of major economic species in south-central of the South China Sea. *Marine Fisheries* **38**(6):577–587.

Zhang Y, Lou FR, Han ZQ. 2019. Comparative transcriptome study between Qingdao and Zhoushan populations of *Oratosquilla oratoria*. *Journal of Zhejiang Ocean University (Natural Science)* **38**(3):195–204 DOI 10.1371/journal.pone.0173238.

Zhao XY, Sun W, Zhang JP, Lei T, Guo WS. 2020. A chemical kinetics model of working cycle for an axonemal dynein. *Acta Scientarium Naturalarium Universitatis Neimongol* **51**(5):509–515 DOI 10.13484/j.nmgdxxbz.20200509.

Zhu YF. 2008. Roles of calcium binding protein in neural system diseases. *Chinese Journal of Biochemistry and Molecular Biology* **24**(5):413–418 DOI 10.3969/j.issn.1007-7626.2008.05.005.