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Genome-wide identification and transcriptome-based expression profiling of E2 gene family: Implication for potential roles in gonad development of *Crassostrea gigas*

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ABSTRACT

In this study, we investigated the role of E2 ubiquitin conjugating enzymes (E2) in the Pacific oyster *Crassostrea gigas*, with a focus on their involvement in gonad development. We identified 34 E2 genes clustered into nine subgroups and 24 subfamilies. The gene structure and intron-exon location were conserved within the same subfamily, but motif variation suggested functional diversity. Tissue transcriptome analyses revealed that most E2 genes were broadly expressed, with *UBE2CL* showing specific expression in the female gonad. Expression profiling of E2 genes during early embryo-larvae development stages suggested that five E2 genes were highly expressed in early embryo development, indicating their involvement in cell division processes. Furthermore, by profiling the expression of E2 genes in different gonadal developmental stages, we observed a gradual increase in expression for four genes during gametogenesis, with significantly higher expression in the female gonad at the maturation stage. Similarly, five E2 genes displayed elevated expression levels in the male gonad at the maturation stage, indicating their crucial roles in gonadal development and gametogenesis. Our study provides valuable insights into the potential functions of the E2 gene family in *C. gigas*, shedding light on the molecular mechanisms underlying gonad development in oysters.

1. Introduction

The ubiquitin-proteasome pathway (UPP) is a protein degradation pathway that is present ubiquitously in all tissues of the organism. This pathway plays a crucial role in post-translational modification of proteins, followed by the recognition and degradation of ubiquitinated substrate proteins by proteasomes (Wickliffe et al., 2011). In eukaryotes, this pathway is involved in almost all processes of cellular life activities, such as cell proliferation, cell differentiation, cell cycle, tumorigenesis, and DNA repair. The entire ubiquitin-proteasome pathway is composed of ubiquitin (Ub), ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), ubiquitin ligase enzyme (E3) and 26s proteasome pathway, which collectively mediate a multi-enzyme cascade reaction to promote the ubiquitination and subsequent degradation of target proteins (Hershko et al., 1983; Hou and Yang, 2013). The multi-enzyme cascade starts with the activation of Ub C-terminals by E1 in an ATP-dependent reaction, leading to the formation of a thioester-linked E1 ~ Ub conjugate (Stewart et al., 2016). The activated ubiquitin is then transferred from E1 to the active-site cysteine of E2 via a trans-thiolation reaction, resulting in the formation of an E2-ubiquitin intermediate. Subsequently, E3 ligates the target protein and binds to the E2-ubiquitin intermediate, leading to the transfer of Ub onto a lysine residue on the target protein. Additionally, the target proteins are modified by a ubiquitin or poly-ubiquitin chain through sequential ubiquitination cycles (Gao et al., 2017). Finally, the target protein with ubiquitin attached is degraded by the 26S proteasome (Nir et al., 2015).

E2 plays an important role in the ubiquitination process, acting as an intermediate link in the multi-enzyme cascade reaction of ubiquitin. E2 selectively binds to the ubiquitin ligase E3 to deliver ubiquitin to the target protein or directly delivers ubiquitin to the target protein. The ubiquitin-conjugating catalytic fold (UBC) domain, which is approximately 150 amino acids (aa) long and contains a lysine residue at its catalytic center to bind activated ubiquitin, is present in all E2 proteins. Numerous studies have demonstrated that the UBC domain mediates the

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Table 1
Information of E2 genes in *Crassostrea gigas*.

Gene name	Gene symbol	Chromosome location	Class	Number of amino acids	Molecular weight	Theoretical pI	Grand average of hydropathicity (GRAVY)	Subcellular localization
UBE2V1	LOC105346610	Chr6	I	144	16,296.52	7.72	−0.713	Nuclear
UBE2SL	LOC105348576	Chr6	II	395	45,704.55	6.43	−0.408	Nuclear
UBE2Q2L	LOC105327625	Chr3	II	375	43,233.76	5.26	−0.58	Nuclear
UBE2OL	LOC105342002	Chr10	II	1347	151,652.34	4.73	−0.639	Nuclear
BIRC6	LOC105338774	Chr10	IV	944	107,829.05	5.12	−0.528	Nuclear
UBE2D1	LOC105317402	Chr10	I	147	16,513.95	6.81	−0.295	Extracellular
UBE2IL	LOC117684193	Chr10	I	160	18,371.11	8.65	−0.614	Nuclear
UBE2A	LOC105340382	Chr7	III	173	19,829.73	4.41	−0.96	Nuclear
UBE2T	LOC105325483	Chr1	III	213	24,269	7.65	−0.672	Nuclear
UBE2TL	LOC117681002	Chr1	III	204	23,205.56	6.83	−0.711	Nuclear
UBE2E1	LOC105345258	Chr7	II	212	22,930.75	9.01	−0.551	Nuclear
UBE2M	LOC105343214	NW_022994901.1	I	182	20,945.1	7.61	−0.498	Nuclear
AKTIP	LOC105330744	Chr6	II	339	38,119.92	9.22	−0.43	Nuclear
UBE2Z	LOC105348658	Chr1	III	322	36,527.99	4.68	−0.612	Nuclear
UBE2JL	LOC105344154	Chr9	III	654	74,047.41	9.45	−0.622	Nuclear
UBE2G2	LOC105343566	Chr7	I	165	18,423.01	4.79	−0.311	Cytoplasmic
UBE2ML	LOC105340922	Chr7	I	194	21,853.29	4.33	−0.484	Cytoplasmic
UBE2J2	LOC105340557	Chr6	III	234	26,316.93	7.72	−0.357	Nuclear
UBE2R2	LOC105336710	Chr3	III	243	27,654.33	4.26	−0.895	Nuclear
UBE2CL	LOC105335915	Chr6	II	180	19,909.26	5.37	−0.593	Nuclear
UBE2D2B	LOC105335778	Chr10	I	152	17,523.09	4.93	−0.33	Cytoplasmic
UBE2N	LOC105335216	Chr8	I	153	17,294.97	6.13	−0.293	Cytoplasmic
UBE2L3	LOC105333930	Chr6	I	154	17,761.38	8.45	−0.727	Cytoplasmic
UBE2D	LOC105333673	Chr10	I	148	16,564.06	8.45	−0.469	Nuclear
UBE2Q2	LOC105332261	Chr6	II	335	38,178.36	4.34	−0.74	Nuclear
UBE2H	LOC105331747	Chr8	III	182	20,634.24	4.8	−0.521	Cytoplasmic
UBE2G1	LOC105330141	Chr6	I	168	19,200.83	5	−0.45	Cytoplasmic
UBE2KL	LOC105329848	Chr7	III	199	22,450.59	5.77	−0.36	Cytoplasmic
ZNF207	LOC105328208	Chr5	III	507	55,544.42	6.42	−0.632	Nuclear
UBE2U	LOC105327872	Chr10	I	331	38,139.61	5.49	−0.57	Cytoplasmic
UBE2S	LOC105321026	Chr10	III	208	23,081.66	8.79	−0.548	Nuclear
UBE2F	LOC105320746	Chr7	I	184	21,468.57	6.84	−0.614	Nuclear
UBE2W	LOC105319798	Chr10	I	151	17,098.62	7.67	−0.358	Nuclear
UBE2E3	LOC105318445	Chr7	II	180	19,859.66	9.3	−0.486	Nuclear

interaction of E2 and E3 (Huang et al., 1999; Schulman et al., 2000; Zheng et al., 2000; Poyurovsky et al., 2007). While most E2 proteins contain only the UBC domain, some E2 proteins include N-terminal or C-terminal protein expansions beyond the UBC domain that mediate various reactions, resulting in different functions of E2 (Stewart et al., 2016). Additionally, some E2 proteins, such as *UBE2K*, have additional structural domains linked to the UBC domain that may interact with E3 or the substrate protein (Schelpe et al., 2016). E2 genes are highly conserved and present in all eukaryotes.

Many studies have indicated the importance of ubiquitination in gonadal development and the gametogenesis process. For example, in rice field eel (*Monopterus albus*), Ubiquitin C-terminal hydrolase-L1 (*Uch-L1*) plays a crucial role in both gametogenesis and gonadal transformation through the UPP pathway (Sun et al., 2008). In *Ascidian Ciona intestinalis*, *UBE2R* is highly expressed in germ cells at the late stage of spermatogenesis and is located at the head and tail of sperm, indicating its possible involvement in spermatogenesis or fertilization (Yokota et al., 2010). In mice (*Mus musculus*), *UBE2B* promotes H2A ubiquitylation and mediates AR's function in spermatogenesis (Mou et al., 2013). Additionally, *UBC9*, an essential E2 conjugating enzyme in the SUMO pathway, is involved in embryogenesis, gametogenesis, and sex modification in *Cynoglossus semilaevis* (Hu and Chen, 2013). *UBE2R* may also play a crucial role in crustaceans' oogenesis and spermatogenesis, thereby affecting gonadal development (Shen et al., 2009).

The Pacific oyster (*Crassostrea gigas*) is an important aquaculture species globally, possessing significant economic and ecological value. It is also an ideal model for studying the mechanisms of gonadal development in marine bivalves due to its unique reproductive characteristics, including a lack of sexual dimorphism, cyclical reproduction, and high fecundity. The gonadal development of Pacific oysters is closely linked to their meat quality and marketability. During the reproductive season, the drastic decrease in glycogen content caused by gonadal development leads to a decline in meat quality. Therefore, the regulatory mechanisms of gonadal development in Pacific oysters have garnered considerable attention from researchers. Despite this, our knowledge regarding the regulatory role of E2 genes in the gonadal development of Pacific oysters remains limited.

In this study, we conducted a genome-wide analysis of the E2 gene family in *C. gigas* to explore their phylogenetic relationships and gene structure. Furthermore, we investigated the gene expression of the E2 gene family to gain insights into their potential functions in *C. gigas*. This study represents the first investigation of the E2 family in *C. gigas*, and our findings shed light on the specific evolutionary taxonomic information of E2 in *C. gigas*. Our results provide insights into the potential roles of the E2 genes in *C. gigas* and contribute to the understanding of the regulatory mechanisms of gonadal development in *C. gigas*.

2. Materials and methods

2.1. Gene identification

The Pfam ID of the UBC domain was searched on NCBI. A hidden Markov model (HMM) file containing the UBC domain (PF00179) was downloaded from the Pfam database (El-Gebali et al., 2019; Mistry et al., 2020). HMMER v3.0 (http://hmmer.org/) was used to search for E2 conjugating enzymes (*UBE2*) genes in *C. gigas* (NCBI assembly GCF_902806645.1), with an e-value threshold of 1e-5. The protein sequences were extracted using TBtools (Chen et al., 2020). After manually removing redundant sequences, we submitted the candidate sequences to the SMART (Schultz et al., 2000), Pfam (Mistry et al., 2020), CDD databases (Schultz et al., 2000), as well as the NCBI Structure database (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) to verify the putative E2 proteins. The number of amino acids (aa), molecular weight (MW), isoelectric point (pI) and grand average of

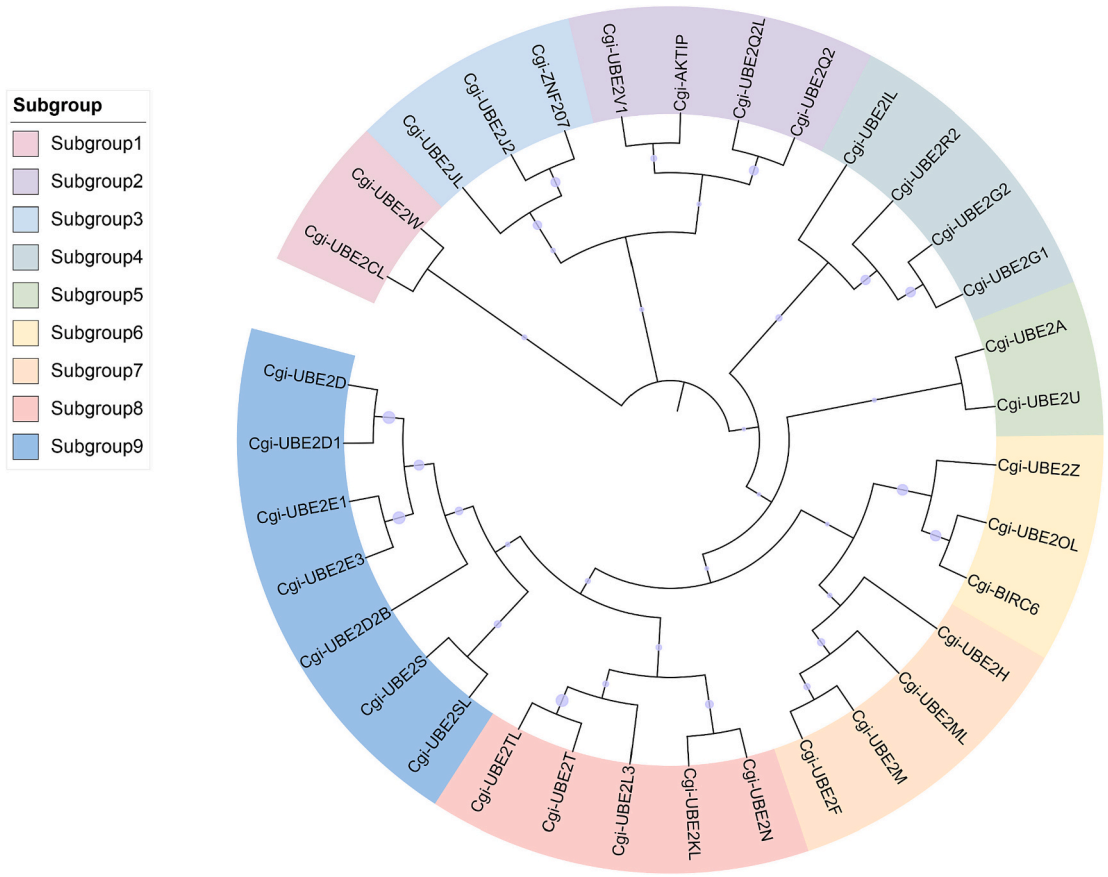

Fig. 1. Phylogenetic relationships of E2 genes in *Crassostrea gigas*. Different colors represent different subgroups of E2 genes.

hydropathicity (GRAVY) of E2 family proteins were predicted by ExPASy ProtParam (https://web.expasy.org/protparam/).

2.2. Phylogenetic analysis and classification of the E2 gene family

To investigate the evolutionary relationship among the E2 gene family members, we conducted a phylogenetic analysis using the amino acid sequences of E2 from several representative species, including *Homo sapiens*, *Mizuhopecten yessoensis* and *Danio rerio*. The information of E2 genes is shown in Supplemental Table S1. The MUSCLE algorithm implemented in MEGA 11 (Kumar et al., 2018) was used for sequence alignment, and redundant sequences were manually removed. Subfamilies and subgroups were identified by constructing phylogenetic trees using the Neighbor-joining (NJ) method with a bootstrap of 1000 replicates in MEGA 11. The phylogenetic trees were visualized using iTOL v6.0 (Letunic and Bork, 2007).

2.3. Gene structure and protein sequence analysis

The conserved motif in the E2 family proteins were searched using MEME (http://meme-suite.org/tools/meme). The parameter was set as follows: the Motif Site distribution was set any number of sites per sequence; the Maximum Number of Motifs was set 10; the Minimum Motif Width was set 6; the Maximum Motif Width was set 50; and other parameters was set default. And the resulting motif was visualized by TBtools (Chen et al., 2020). CDS and UTR regions were analyzed using TBtools.

2.4. Expression patterns of E2 genes across various tissues and developmental stages in C. gigas

Transcriptome data from the National Center for Biotechnology Information (NCBI) with the accession numbers SRP014559 (PRJNA146329) and SRP112367 (PRJNA394546) were downloaded and analyzed to investigate the expression patterns of the E2 gene family in *C. gigas* across different developmental stages and tissues. The developmental stages included different larval stages and gonadal developmental stages. The larval developmental stages comprised of egg stage (E), two cells stage (TC), four cells stage (FC), early morula stage (EM), morula stage (M), blastula stage (B), free swimming stage (FS), early gastrula stage (EG), trochophore stage (T), early D-shaped larva stage (ED), D-shaped larva stage (D), early umbo larva stage (EU), umbo larva stage (U), later umbo larva stage (LU), competent pediveliger for metamorphosis stage (P), spat stage (S) and juvenile stage (J). The gonadal developmental stages included undifferentiated stage (S0), early development stage (S1) and maturation stage (S3). Tissues analyzed comprised of adductor muscle (Amu), digestive gland (Dgl), female gonad (Fgo), male gonad (Mgo), gill (Gil), hemolymph (Hem), labial palp (Lpa) and outer edge of mantle (Man). The expressional heatmaps of E2 genes were generated using omicshare tools (https://www.omicshare.com/tools/home/report/reportheatmap.html). The FPKM values of E2 genes are shown in Supplemental Tables S2–S4. We used tau (τ) to identify the tissue specificity of E2 genes of *C. gigas*, as described previously (Kryuchkova-Mostacci and Robinson-Rechavi, 2017). The value of tau (τ) was calculated using TBtools (Chen et al., 2020).

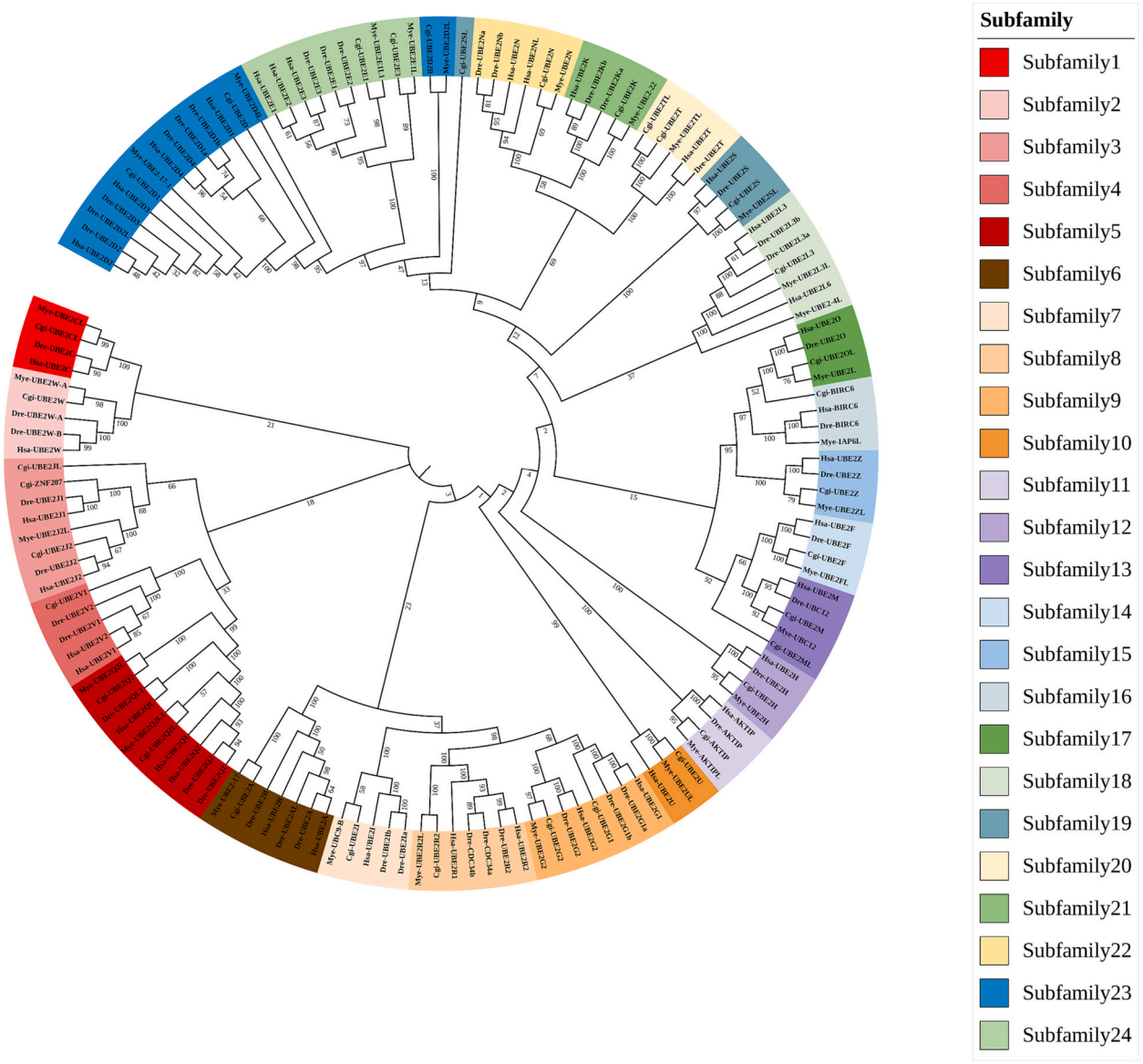

Fig. 2. Phylogenetic tree of E2 genes in *C. gigas* (Cgi) and representative species, including *H. sapiens* (Has), *M. yessoensis* (Mye) and *D. rerio* (Dre). The phylogenetic tree was constructed using the Neighbor Joining method (NJ) with 1000 bootstrap replicates. Different colors represent different subfamilies of E2 genes.

2.5. Quantitative real-time PCR

In order to investigate the E2 genes involved in the gonadal development of *C. gigas*, four genes potentially associated with gonadal development and gametogenesis were randomly selected based on transcriptomic data for the quantitative real-time PCR (qRT-PCR). The *C. gigas* individuals used in this study were obtained from Rongcheng, Shandong Province, China in January, March, and June 2022. Since these four genes may be involved in female gonadal development based on the transcriptome data, total RNA was extracted from the female gonad tissues of *C. gigas* using RNA-easy Isolation Reagent (Vazyme, China). The RNA was then reversed transcribed into cDNAs using an Evo M-MLV RT Mix kit with gDNA Clean (Accurate Bio, China). The reverse-transcribed reaction system included 2.0 μL gDNA clean mix, 1 μg RNA, and 4.0 μL 5× EVO M-mix, and a suitable volume of RNase-free water was added to the cDNA synthesis reaction system (20 μL). Four primers were designed using Primer5 software (Premier Biosoft International, USA) (Table S5) for qRT-PCR test. And these four primers were designed to span the intron region. The qPCR test was performed on a LightCycler 480 real-time PCR instrument (Roche, USA) using the SYBR Green Premix *Pro Taq* HS qPCR Kit PCR Kit (Accurate Bio, China). Ef1-α (Huan et al., 2016) was used as an internal control. All primers are listed in Supplementary Table S5. The relative expression levels of E2 genes were calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The data were analyzed using SPSS 21.0, and one-way ANOVA was used for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Identification of E2 genes in C. gigas

Totally 34 E2 genes were identified in *C. gigas*, and their respective information is shown in Table 1. The lengths of the E2 coding proteins in *C. gigas* ranged from 144 (*UBE2V1*) to 1347 (*UBE2OL*) aa, with MW ranging from 16296.52 (*UBE2V1*) to 151652.34 (*UBE2OL*) Da. The theoretical pI ranged from 4.26 (*UBE2R2*) to 9.45 (*UBE2JL*). Twenty-four E2 proteins were found to be localized in the nucleus, nine in the nucleus cytoplasmic, and one (*UBE2D2B*) in the extracellular compartment.

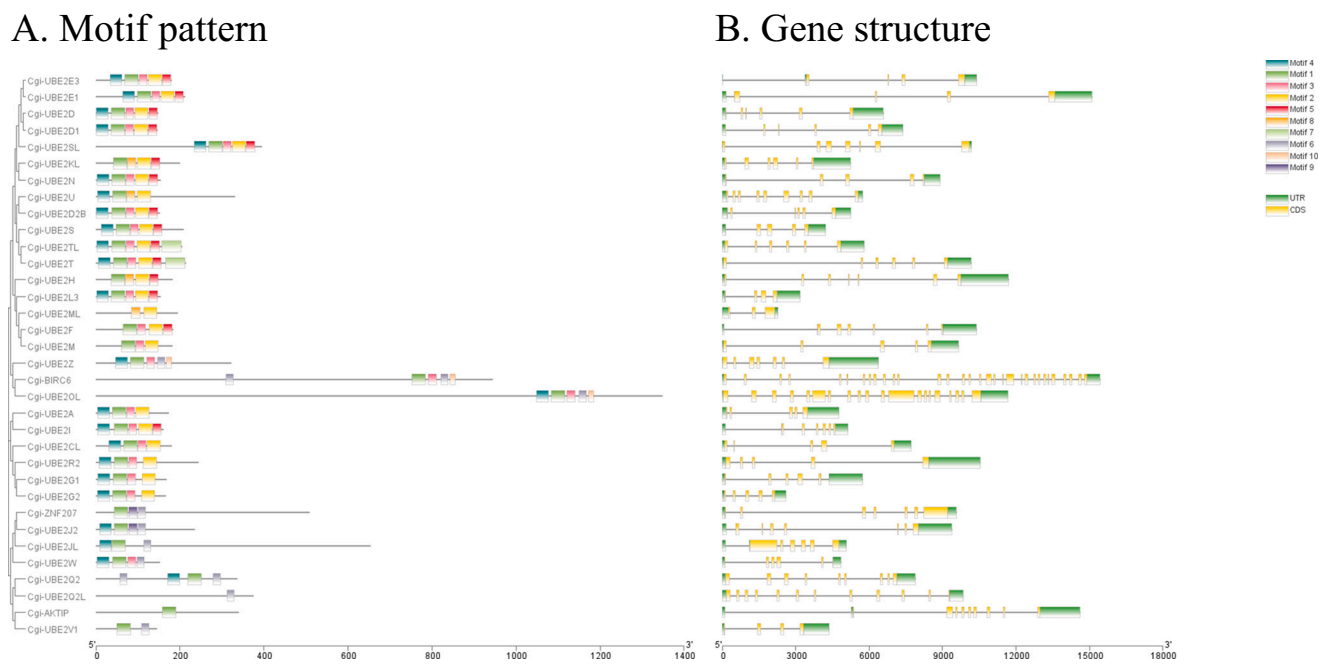

Fig. 3. Conserved motifs and gene structures of E2 genes based on phylogenetic relationships. (A) Motifs are displayed as colored boxes. (B) Exons and introns of E2 genes. Exons are represented by yellow boxes, introns are shown as black lines, and untranslated regions (UTR) are denoted by green boxes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Phylogenetic analysis and classification of the E2 gene family

The human E2 gene family is classified into four classes based on the presence or absence of additional extensions to the conserved domain (van Wijk and Timmers, 2010). Similarly, we classified the E2 gene family in *C. gigas* into four classes, namely class I (13), class II (10), class III (7) and class IV (1) (as shown in Table 1). Interestingly, two E2 genes (*UBE2CL* and *UBE2K*) have domains other than UBC, with *UBE2CL* containing a FOX domain at the N-terminal and *UBE2K* having a UBA domain at the C-terminal.

Based on our phylogenetic tree analysis, the E2 gene family of *C. gigas* was classified into nine subgroups, each containing distinct members (Fig. 1). To further investigate the evolutionary relationships of the E2 family in *C. gigas*, protein sequences from four different species (*C. gigas*, *H. sapiens*, *M. yessoensis* and *D. rerio*) were used to construct a phylogenetic tree. The resulting tree according to human nomenclature (van Wijk and Timmers, 2010), classified the E2 gene family into 24 subfamilies, with *UBE2D*, *UBE2E*, *UBE2S*, *UBE2T*, *UBE2M*, *UBE2G*, *UBE2Q* and *UBE2J* subfamilies each having two members, while the other subfamilies have one gene member (Fig. 2).

3.3. Gene structure and protein sequence analysis

In this study, motifs were investigated to reveal the structural and functional diversity of ubiquitin-conjugating enzymes. Ten motifs were identified (Fig. 3A), with lengths varying from 14 to 47 aa. Among them, motif 4 was identified as the C-terminal domains of E2 proteins. Motif 1, 2, 3, 4 and 5 were highly conserved and present in most E2 proteins. The phylogenetic tree with motifs indicated that closely related genes had the same motifs, suggesting similar functions and structures. Some genes had unique motifs, such as motif 9, which was found in both *cgi-ZNF207* and *cgi-UBE2J2*, indicating that they may have same functions.

The structural analysis of E2 genes in *C. gigas* revealed that the genes were encoded by a varying number of exons, ranging from four to 32 (Fig. 3B). The intron-exon location and number were found to be similar within the same subfamily of E2 genes. The unique intron size of each E2 gene may be the insertion of transposable elements into the intronic regions (Sela et al., 2010). The distribution of E2 genes on *C. gigas* chromosomes was investigated, and 33 E2 genes were assigned to eight chromosomes, with one gene assigned to an unplaced scaffold (Table 1). Chromosome 10 contained the largest number of E2 genes (nine genes), while chromosome 1, 3, 8, and 9 contained one to three E2 genes. Chromosome 6 contained eight E2 genes, and seven E2 genes were located on chromosome 7.

3.4. Expression profiling of E2 genes in different larval developmental stages in C. gigas

The E2 genes in *C. gigas* exhibited diverse expression patterns during different larval development (stages E to J). Notably, the expression levels of *UBE2CL*, *UBE2E1*, *UBE2D1*, *UBE2SL* and *UBE2S* gradually deceased during development (Fig. 4). The expression of these five genes was higher between egg stage (E) and early gastrula stage (EG) than in other stages, indicating their involvement in the cell division process.

3.5. Expression profiling of E2 genes in different tissues in C. gigas

To determine the spatial expression pattern of E2 genes, transcriptional profiles of eight tissues were characterized in *C. gigas* (Fig. 5). The results showed significant differences in expression levels of E2 genes across different tissues. Notably, heatmap of transcriptome data revealed that high expression levels of E2 genes were found in female gonad and hemolymph. To measure the tissue specificity of E2 gene expression, the Tau (τ) value was calculated for every gene in the transcriptome data from six different tissues (Table S6). The values of τ range from 0 to 1, where 0 indicates a ubiquitously expressed genes and 1 indicates highly tissue-specifically expressed genes (Kryuchkova-Mostacci and Robinson-Rechavi, 2017). Tissue-specific genes are those with an average $\tau > 0.8$ between individuals of the same species. According to our analysis of the E2 genes, *UBE2CL* was tissue-specific in female gonad, whose value of $\tau = 0.81$. Four genes (*UBE2T*, *UBE2TL*, *AKTIP* and *UBE2E1*) of E2 were tissue-enriched ($0.6 < \tau < 0.8$) genes in *C. gigas*, which were found that these genes clustered together in female gonad based on transcriptional profiles, plausibly representing tissue-

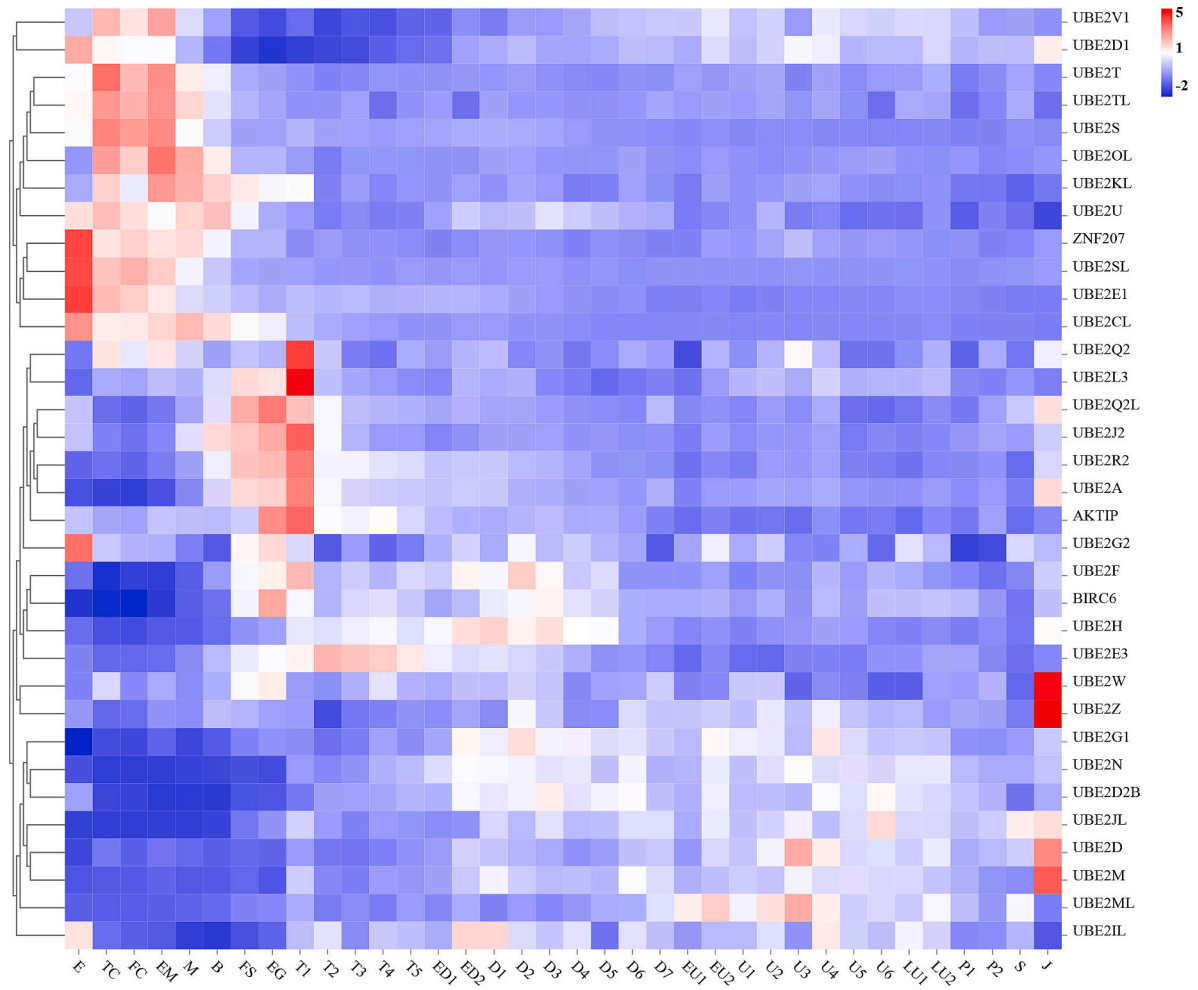

Fig. 4. Heatmap illustrating the expression profiles of E2 gene during different embryo-larval developmental stages. Abbreviations: egg stage (E), two cells stage (TC), four cells stage (FC), early morula stage (EM), morula stage (M), blastula stage (B), free swimming stage (FS), early gastrula stage (EG), trochophore stage (T1-T5), early D-shaped larva stage (ED1-ED2), D-shaped larva stage (D1-D7), early umbo larva stage (EU1-EU2), umbo larva stage (U1-U6), later umbo larva stage (LU1-LU2), competent pediveliger for metamorphosis 1 stage (P1-P2), spat stage (S) and juvenile stage (J).

specific functions. Twenty-nine genes ($\tau < 0.6$) of E2 were broadly expressed. Consequently, E2 genes may have tissue-specific expression patterns in adult tissues of *C. gigas*.

3.6. Expression profiling of E2 genes in different gonadal developmental stages

The transcriptional profiles of three gonadal developmental stages in *C. gigas* (Fig. 6 and Table S3) revealed that *UBE2S*, *UBE2T*, *UBE2E* and *UBE2CL* were significantly up-regulated during female gonad development, with their expression levels gradually increasing from undifferentiated stage (S0) to the maturation stage (S3). Conversely, *UBE2ML* was significantly down-regulated throughout the process of gonadal developmental. In male gonads, five E2 genes (*UBE2A*, *UBE2R2*, *UBE2D*, *UBE2G1* and *UBE2U*) showed a gradual increase in expression from S0 to S3, with significantly higher expression levels at S3 compared to S0.

3.7. Validation of E2 genes by qRT-PCR

A total of four E2 genes (*UBE2S*, *UBE2T*, *UBE2CL* and *UBE2E1*), which might be related to gonadal development in the expression profiling, were selected for qRT-PCR to determine their expression levels. For qRT-PCR analysis, the expression levels of four E2 genes (*UBE2S*, *UBE2T*, *UBE2CL* and *UBE2E1*) were consistent with sequencing data (Fig. 7).

4. Discussion

In this study, we conducted a comprehensive analysis of the E2 gene family in *C. gigas* and identified 34 E2 genes, which are conserved with a high number of introns (Irimia et al., 2007). The number of E2 genes in *C. gigas* is less than in *H. sapiens* (38) and *D. rerio* (45), but more than in *M. yessoensis* (28) and *D. melanogaster* (27) (Jones et al., 2001). Our findings suggest that the protein family expansion during evolution, where lower eukaryotes have lower numbers of E2 enzymes than higher ones (van Wijk and Timmers, 2010), may not hold true for all cases. The phylogenetic analysis indicated that E2 genes are highly conserved among different species, and subfamilies of different species are divided into the same branch. These findings suggest that E2 genes in *C. gigas* may have multiple functions.

The ubiquitin-proteasome pathway and ubiquitin-related genes are known to play crucial roles in various aspects of reproductive development and gametogenesis. Ubiquitin-related genes are expressed widely in animal gametogenesis, which plays an essential role in homologous recombination (Vyas et al., 2013), sex chromosome inactivation (An et al., 2010), oocyte meiotic resumption (Oh et al., 2010), the first polar body extrusion (Pomerantz and Dekel, 2013) and sperm-egg fusion during meiosis (Shin et al., 2013). In this study, we found that *UBE2CL* is specifically expressed in the female gonads of *C. gigas* and is significantly upregulated during female gonad development. This suggests that *UBE2CL* may have important functions in oogenesis. Additionally, we

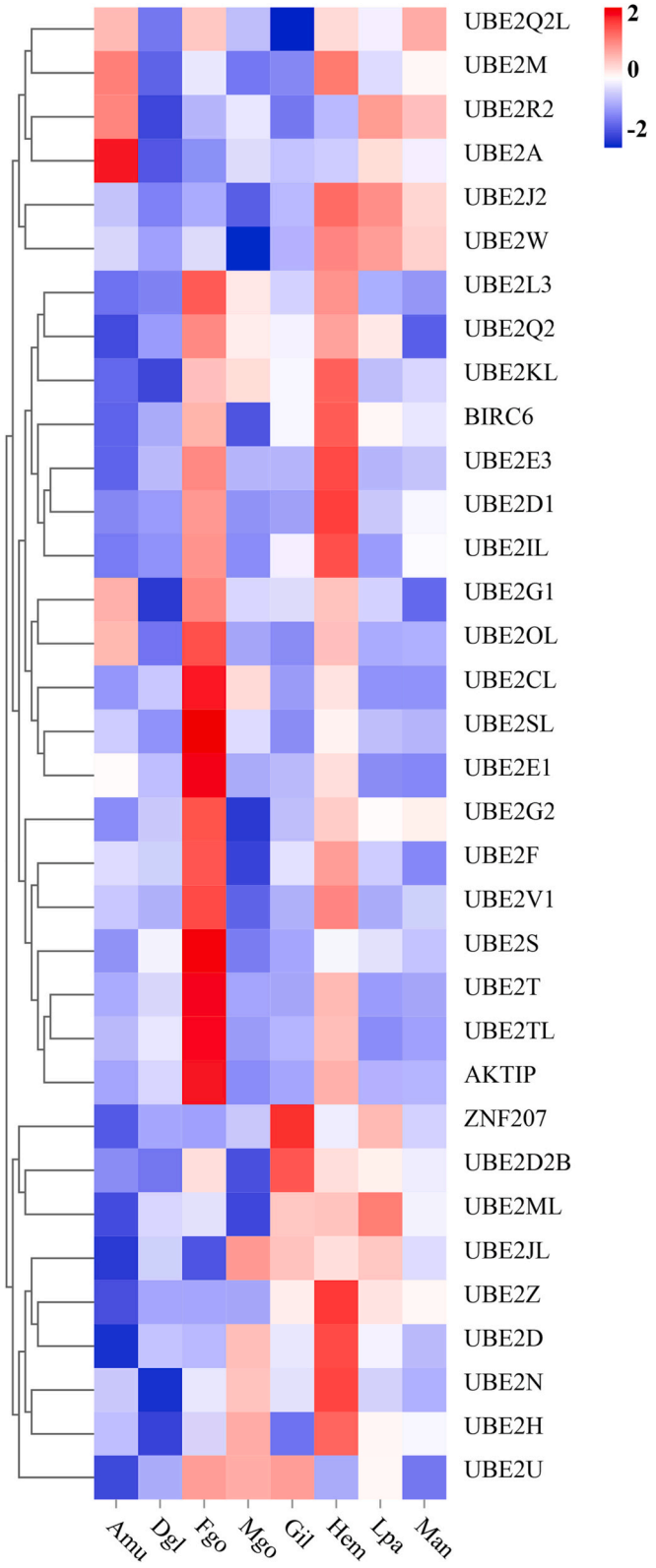

Fig. 5. Heatmap displaying the expression profiles of E2 gene in different tissues. Abbreviations: adductor muscle (Amu), digestive gland (Dgl), female gonad (Fgo), male gonad (Mgo), gill (Gil), hemolymph (Hem), labial palp (Lap) and outer edge of mantle (Man).

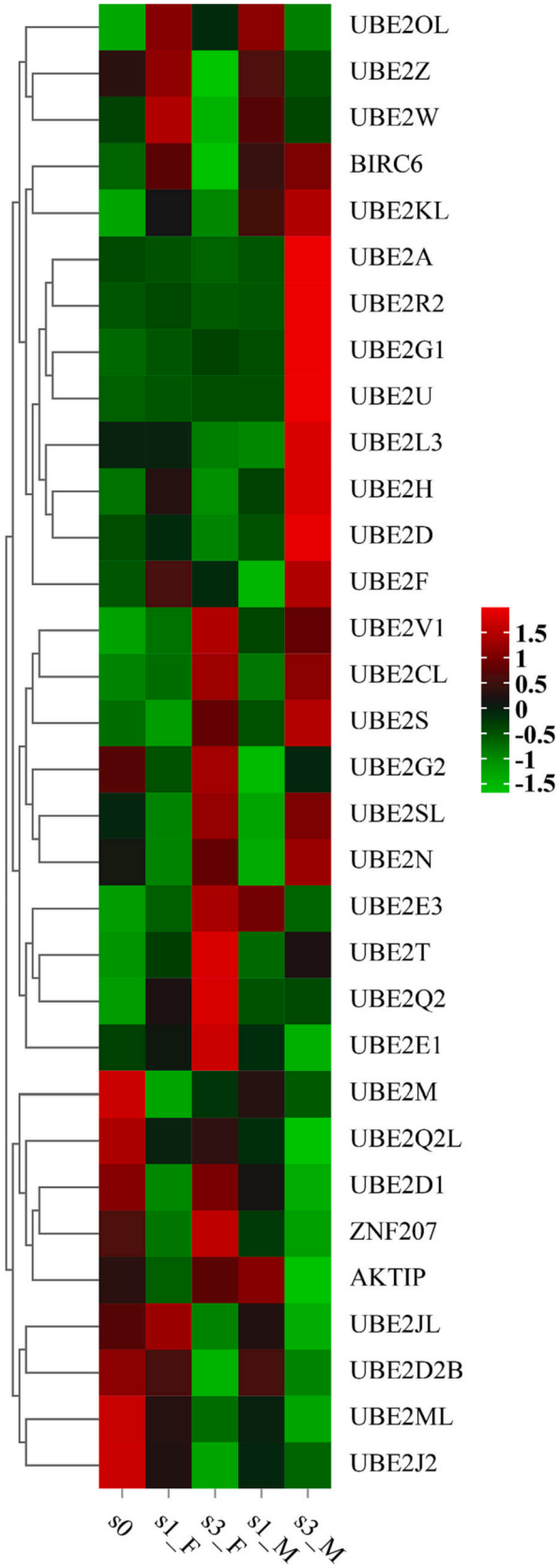

Fig. 6. Heatmap depicting the expression profiles of E2 gene at different gonadal developmental stages. Abbreviations: undifferentiated stage (S0), female early development stage (S1_F), female maturation stage (S3_F), male early development stage (S1_M), male maturation stage (S3_M).

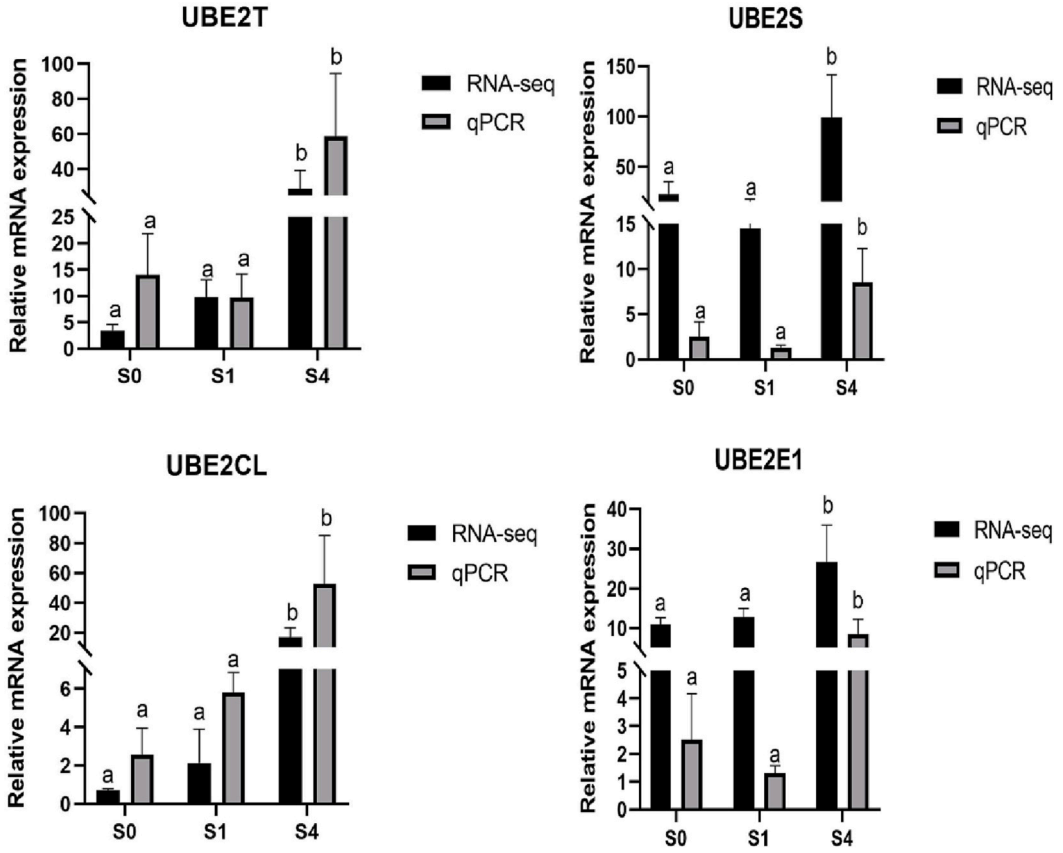

Fig. 7. Validation of RNA-Seq results using qRT-PCR. Transcript expression levels of selected genes were normalized to the reference gene Ef1-α.

observed that *UBE2T*, *UBE2S* and *UBE2E1* significantly increase during the transition from the early development stage to maturation stage in females, indicating their potential role in female gonadal development. Several studies have shown that *UBE2C* and *UBE2S* promote chromosome segregation in oocytes and regulate APC/C activity during meiosis of mouse oocytes. These E2 genes are relevant to spindle formation, and if overexpressed, can accelerate the completion of the first meiotic cytokinesis (Sako et al., 2014; Ben-Eliezer et al., 2015). *UBE2S* and *UBE2C* also play a role in the escape from MII arrest in porcine oocytes by ensuring certain protein ubiquitination (Fujioka et al., 2018). *UBE2E1* has been shown to regulate H2A ubiquitination (Wheaton et al., 2017), which is associated with cell cycle progression, and abnormal ubiquitination of H2A results in decreased cell growth rate (Hu et al., 2014). *UBE2T* has been shown to promote the G2/M transition by regulating cyclin B1 and cyclin-dependent kinase 1 in human (Liu et al., 2019). Therefore, these genes are likely involved in the development of the ovary and oocyte maturation of *C. gigas*.

In addition, we found that five E2 genes (*UBE2A*, *UBE2R2*, *UBE2D*, *UBE2G1* and *UBE2U*) were significantly highly expressed in the male gonad at maturation stage of *C. gigas*. *UBE2A* has been reported to be essential for the ubiquitination of histone H2B (Zhang and Yu, 2012), which is correlated with transcriptional elongation of active genes and plays a role in DNA double-strand break repair (Nakamura et al., 2011). *UBE2D* promotes DNA repair in human, allowing the cell cycle to proceed normally and facilitating proper cell division (Schmidt et al., 2015). Therefore, it is possible that the E2 genes abundant in the male gonad are related to reproduction function.

This study also identified high expression levels of *UBE2CL*, *UBE2E1*, *UBE2D1*, *UBE2SL* and *UBE2S* during early embryo development stages, which suggests their involvement in the process of early embryonic development. For example, *UBE2D1* and *UBE2E1* have been reported to assist Ring1B·BMI1 in catalyzing ubiquitination of H2A, which is first detected at the syncytial blastocyst stage in Diptera, and is closely associated with the initiation of zygotic transcription (Ruder et al., 1987). Therefore, these E2 genes may play a role in the function of early embryonic development.

5. Conclusions

The study provides valuable insights into the E2 gene family in *C. gigas*, and their potential roles in gametogenesis and larval development. The identification and characterization of these genes could pave the way for further research on their functions and potential applications in aquaculture and related fields. Overall, this study adds to our understanding of the genomic and developmental mechanisms that underlie the complex life cycle of oysters and contributes to the broader field of evolutionary genomics.

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CRediT authorship contribution statement

Xiaoyu Zou: Investigation, Formal analysis, Data curation, Writing.
Hong Yu: Supervision, Resources, Funding acquisition, Review & Editing.
Qi Li: Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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