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Molecular Characteristics of *Scylla paramamosain* Ubiquitin-Conjugating Enzyme E2 and Expression Analysis During Oogenesis

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Ubiquitination is an essential post-translational modification of proteins and involved in many physiological processes, including sperm differentiation, oocyte maturation, and embryonic development. Among them, the ubiquitin-conjugating enzyme E2 plays a crucial role in gametogenesis. However, the participation of E2 in the regulation of the gonad development of crustaceans is still very limited, especially for oogenesis. In this research, we isolated and identified full-length complementary DNA of ubiquitin-conjugating enzyme E2 from mud crab *(Scylla paramamosain) Sp-Ubc2* detected distribution in different tissues by real-time quantitative PCR, and described expression patterns during oogenesis by *in situ* hybridization. The results demonstrated that the deduced proteins with a ubiquitin-conjugating (UBC) catalytic domain was clustered with other arthropods. In addition, the *Sp-Ubc2* was widely distributed in different tissues of female individuals and kept the highest expression level in the ovary. Moreover, the expression level of *Sp-Ubc2* changed significantly during oogenesis and reached peak in secondary and tertiary vitellogenesis stages. At the same time, the *Sp-Ubc2* signal in the ovary gradually concentrated in nuclei. These results indicated that *Sp-Ubc2* might play important roles in the cell cycle of oogenesis. This study would provide insights into the regulatory mechanism of gonad development in *S. paramamosain*, and it also enriched the theoretical basis of ubiquitin family genes involved in crustacean gonad development.

Keywords: ***Scylla paramamosain*, *Sp-Ubc2*, oogenesis, tissue expression, ubiquitination**

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INTRODUCTION

Ubiquitin is a highly conserved protein composed of 76 amino acid residues, which is widely present in eukaryotes (Swatek and Komander, 2016). The ubiquitin can be covalently attached to a target protein to influence protein stability and biological function, which is called ubiquitination (Choo and Zhang, 2009). As an important type of post-translational modification of proteins, ubiquitination is mainly involved in the intracellular translocation of proteins, chromosomal organization, DNA repair, cell cycle control, and apoptosis (Dye and Schulman, 2007; Gao et al., 2017). At present, there are at least three enzymes of ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2, and ubiquitin ligase E3 that are necessary for ubiquitination (Neutzner and Neutzner, 2012). In the

TABLE 1 | Primers used in the study.

Primer	Sequence	Purpose
5'-RACE	GGGTGGAGTGGAAGGGTA	5'- and 3'-RACE
3'-RACE	CTGTCCTCTATGTTCTAC	
Sp-Ubc2-F1	TAACTGCGTCGAACTCAGAGT	Head to toe PCR
Sp-Ubc2-R1	CATCTGTTACATGACAACAGTG	
Sp-Ubc2-F2	CGCCCTACAAAGAACCCAGA	RT-qPCR
Sp-Ubc2-R2	GGGTGGAAGAGAGGTGGTTC	
18S-F	ATGATAGGGATTGGGGTTTGC	
18S-R	AAGAGTGCCAGTCCGAAGG	
Sp-Ubc2-F3	ACCCTTCCACTCCACCCA	ISH
Sp-Ubc2-R3	AGTAAATAAAAGTCACAG	

process of ubiquitination, E1, E2, and E3 cooperate to transfer ubiquitin molecules to target proteins, in which E1 activates the ubiquitin in an ATP-dependent manner and delivers to E2. Then, E3 transfers the E2-bound activated ubiquitin to the target protein (Hershko et al., 1983).

For ubiquitin enzymes, E2 contains a ubiquitin-conjugating (UBC) domain, spanning 140–200 amino acids in length, which mediates the interaction between E2 and E3 (Gao et al., 2017). In ubiquitination, E2 plays a key role in regulating the fate and function of target proteins (Li et al., 2020). E2 genes exist as a multigene family that has been identified in model organisms, including *Saccharomyces cerevisiae* (Seufert et al., 1990), *Caenorhabditis elegans* (Jones et al., 2002), *Homo sapiens* (Jiang and Beaudet, 2004), *Arabidopsis thaliana* (Kraft et al., 2005), and

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   1 GGGCAGTGTCGCATTCTATTCCTTCTGAGAGAGTAACTGCGTCGAACTCAGAGTGATTGA  60
  61 CTCGCCACTatgtctggaattgccattgctcgtctggctgaagagagaaaggcatggagg 120
   1           M  S  G  I  A  I  A  R  L  A  E  E  R  K  A  W  R   17
 121 aaagatcatccttttgggttcattgctcgccctacaaagaacccagatggcaccctgaac 180
  18 K  D  H  P  F  G  F  I  A  R  P  T  K  N  P  D  G  T  L  N   37
 181 ctcatgaactgggagtgtgccatccctggcaagaagggaacaccgtgggagagtggcctg 240
  38 L  M  N  W  E  C  A  I  P  G  K  K  G  T  P  W  E  S  G  L   57
 241 tacagattgcgcatgatcttcaaagatgattacccttccactccacccaagtgcaaattt 300
  58 Y  R  L  R  M  I  F  K  D  D  Y  P  S  T  P  P  K  C  K  F   77
 301 gaaccacctctcttccaccccaatgtttacccatcaggaacagtgtgtctgtcactgctg 360
  78 E  P  P  L  F  H  P  N  V  Y  P  S  G  T  V  C  L  S  L  L   97
 361 gatgaagagaaggactggaggcccgccattacaatcaagcagattcttttgggaattcag 420
  98 D  E  E  K  D  W  R  P  A  I  T  I  K  Q  I  L  L  G  I  Q  117
 421 gaccttctcaacgatcccaacatcaaggaccctgcacaggctgaggcgtacaccatctac 480
 118 D  L  L  N  D  P  N  I  K  D  P  A  Q  A  E  A  Y  T  I  Y  137
 481 tgccaaaaccggttggaatatgaaaagagagtacgggcacaggccaaggcaatgtctgcc 540
 138 C  Q  N  R  L  E  Y  E  K  R  V  R  A  Q  A  K  A  M  S  A  157
 541 cctttgagtaaATCCTGGACGTAAAGTTGAAACGACGCTGACTTTGTGCTCCCCGATTT 600
 158 P  F  E  *                                                  160
 601 ACATTTGCTTATTAGGTATAACTAAGTAGGTAGATCTTGGGGCAACTGGATGTTCTGTCA 660
 661 AGAAACTGGACATGTTTCACTGTTGTCATGTAACAGATGAGCTTACATGGTCAGACAGTG 720
 721 TTTGGATACATGAGGATTTTAATTTTTATTCATTCTATACATTTCTTTCCTGTGTATGAA 780
 781 TCTGCCTGTGTATTGGAAAACTTCTCAGCTTCCTGATTTCCAGAATCTTTCAAACGTTAT 840
 841 CCCTGACAGGTCAGAGTGAGAAGTGATTTACTGTGACTTTTATTTACTTTTTTATGTACC 900
 901 TGTCCTCTATGTTCTACTTTTGAGTCATTAGTGGATATTAATAAAGCTATTTTTATCAAA 960
 961 AAAAAAAAAAA 971
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FIGURE 1 | The full length of *Sp-Ubc2* cDNA and deduced amino acid sequence. The underlined sequences showed the E2 catalytic domain; 8 motifs in the domain were labeled with yellow shading; the polyadenylation signal (AATAA), active cysteine, and amino acids interacted with E3 were marked red, box, and bold, respectively.

Drosophila melanogaster (Michelle et al., 2009; Nagy et al., 2012). Currently, E2 genes in more and more economic species are cloned and researched. In plants, E2 responds to environmental stress and influence development. For example, E2 is involved in abiotic stresses and hormone treatments in rice (Zhiguo et al., 2015) and maize (Jue et al., 2015), while E2 changes the fruit-ripening process and response to cold and heat stress in tomato (Wang et al., 2014) and grape (Gao et al., 2017). In animals, the levels of E2 are increased when the fish or crustaceans are exposed to pesticide and an oxidative state (Horst et al., 2007; Sanz et al., 2012). However, limited information can describe the specific function and mechanism of E2 in these species.

Additionally, the ubiquitin enzymes, especially for E2, were found to possibly regulate the development process in *Drosophila* (Ohlmeyer and Schüpbach, 2003; Chen et al., 2009; Lu et al., 2013). The ubiquitination, a highly selective proteolysis pathway, is also the basis of cell cycle regulation. In addition, the eukaryotic cell cycle depends on precise and rigorous regulation of various factors, the core of which is the periodic synthesis and destruction of cyclins (Johnson and Walker, 1999). Cyclin plays a crucial role in mitosis and meiosis and is an important regulator of egg or sperm development (Jeong et al., 2011). Therefore, cyclin ubiquitination can mediate germ cell proliferation, differentiation, and apoptosis. In a previous study, the *Drosophila* E2-encoding gene–mediated degradation of Cyclin A was essential for the maintenance of germline stem cells (Chen et al., 2009). Subsequently, the conserved E2 polyubiquitin gene was proven that was essential for male meiotic cell cycle progression and germ cell differentiation in *Drosophila* (Lu et al., 2013). Like *Drosophila*, E2 was identified and characterized in the testes of the giant tiger shrimp (*Penaeus monodon*) as other sex-related genes and suggested that this gene involved gonadal development (Leelatanawit et al., 2008). Subsequently, the UBE2r showed significantly different expression levels in developing the testis and ovary of penaeid shrimp (*Marsupenaeus japonicus*), suggesting that UBE2r had an important role in oogenesis and spermatogenesis (Shen et al., 2009). Therefore, E2 is also related to the development and regulation of aquatic organisms.

The mud crab (*Scylla paramamosain*) is one of economically important marine crabs with commercial and nutritional

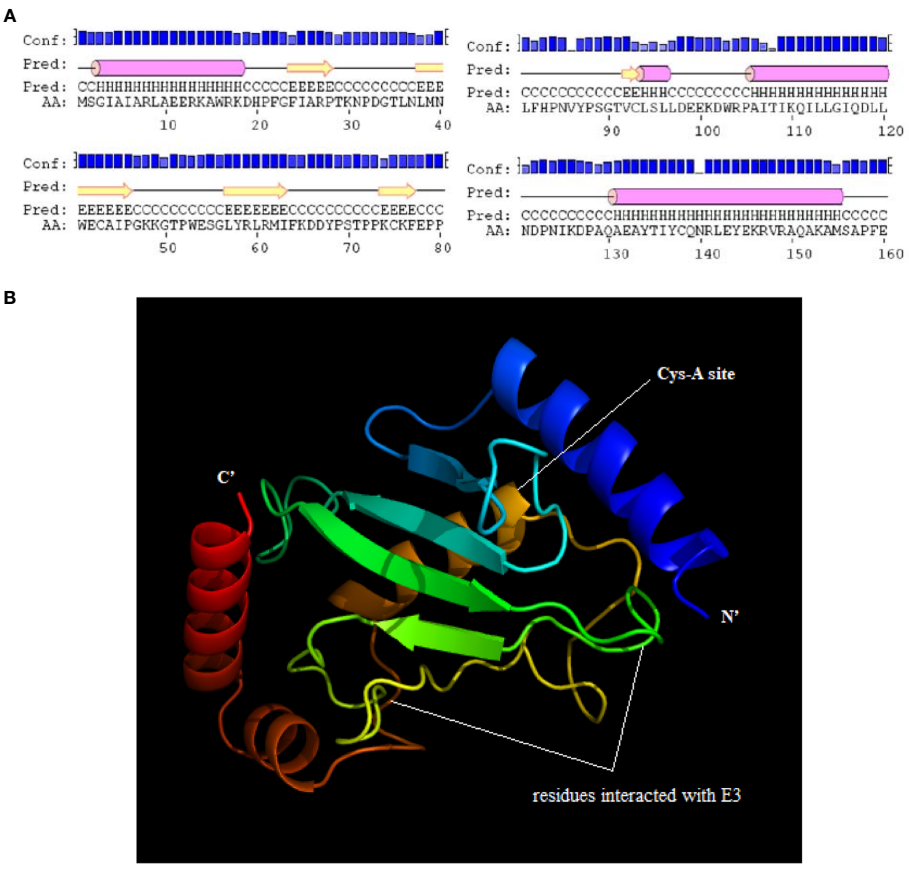

FIGURE 2 | The predicted two-dimensional (2D) and three-dimensional (3D) structure of *Sp*-UBC 2. **(A)** the Pred showed the predicted 2D structure. **(B)** The predicted 3D structure of *Sp*-UBC 2. Conf: credibility; AA: amino acid sequence; cylinder:α helix; arrow: extended strand; line: random coil.

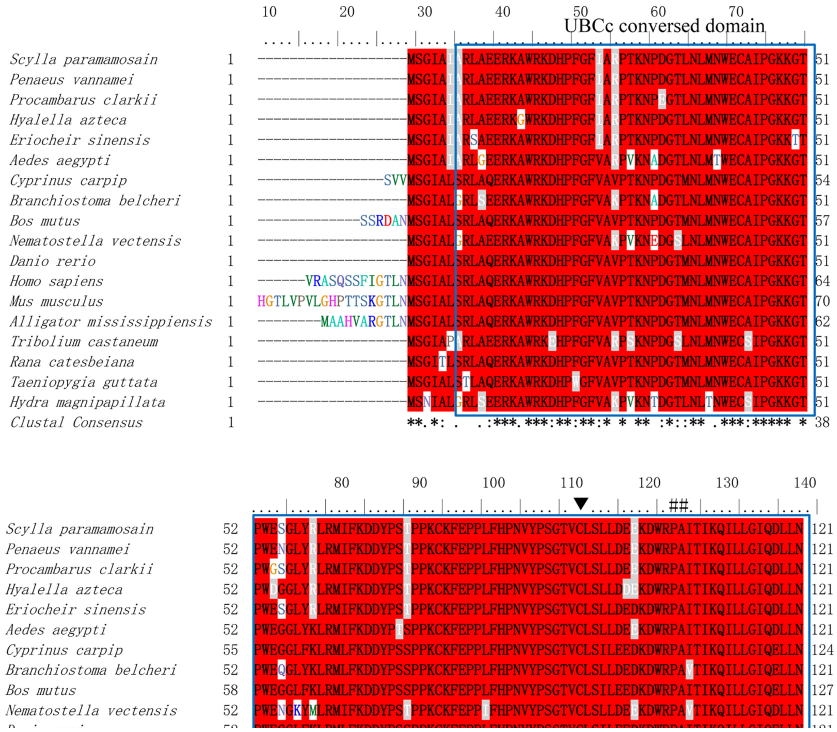

FIGURE 3 | The homology analysis of *Sp*-UBC 2 in *S. paramamosain* and other organisms. The sequence used in homology analysis was as follows: *Scylla paramamosain*: ACJ03792; *Penaeus vannamei*: AFU93453; *Procambarus clarkii*: AFV69238; *Hyalella azteca*: XP_018014422; *Eriocheir sinensis*: ADW82097; *Ceratosolen solmsi marchali*: XP_011500210; *Aedes aegypti*: XP_001654033; *Cyprinus carpip*: KTG33102; *Cyprinus carpip*: KTG33102; *Bos mutus*: ELR62878; *Nematostella vectensis*: XP_001640489; *Danio rerio*: NP_571908; *Homo sapiens*: BAD92225; *Mus musculus*: EDL22414; *Alligator mississippiensis*: XP_019355948. blue box: the conversed UBCc domain; ▼: the conversed Cys site; ##: the residue of E2 interaction with E3.

values (Wan et al., 2019). *S. paramamosain* has the advantages of a large body size and rapid growth rate, which has been one of artificial cultivation crabs in China and South Asia (Chen and Wang, 2019; Wan et al., 2021). In recent years, the demand for *S. paramamosain* has been increasing, while the amount of wild-caught resources has been decreasing, which significantly restricts the healthy and sustainable development of *S. paramamosain* aquaculture. Therefore, the breeding techniques related to improving spawning and the breeding survival rate and decreasing the cost have become the current main tasks in the aquaculture production of the mud crab. Additionally, a better understanding of the molecular regulatory mechanisms involved in gonad development, sex determination, and gametogenesis would be helpful for crab aquaculture. In a previous study, we identified and described the expression profiles of ubiquitin C-terminal hydrolases *Sp-uchl3* and *Sp-uchl5* during the gonad of *S. paramamosain*; it indicated that these genes participated in the development process (Han et al., 2018). In this study, we aimed to identify the E2 of *S. paramamosain* (*Sp-Ubc2*). Then, we detected the distribution of *Sp-Ubc2* in different tissues of female individuals and the expression level of *Sp-Ubc2* at different development stages of ovary by real-time quantitative PCR (RT-qPCR). At the same time, we described the specific expression patterns of *Sp-Ubc2* during oogenesis by *in situ* hybridization (ISH). This results of this project would lay a foundation for further research about the molecular mechanism of the gonad development of crab, especially for oogenesis.

MATERIALS AND METHODS

Animals and Tissue Collection

S. paramamosain was purchased from a local jimei market (Xiamen, China), and a total of 25 were used for this study. According to the external morphology, color, gonadosomatic index (GSI), and histological feature of previous studies, the ovary at different development stages was classified into five periods: O1 (proliferation, GSI=0.57 ± 0.47), O2 (pre-vitellogenesis, GSI=2.19 ± 0.21), O3 (primary vitellogenesis, GSI=3.68 ± 0.20), O4 (secondary vitellogenesis, GSI=7.81 ± 0.94), and O5 (tertiary vitellogenesis, GSI=10.49 ± 0.49) (Jia et al., 2013; Wang et al., 2021). At least three crabs were used for the experiments at each developmental stage. The various tissues including muscle, testis, ovary, brain, eyestalk, gill, heart, hepatopancreas, intestines, and stomach were dissected and stored in liquid nitrogen for future use. In addition, some ovaries at different developmental stages were preserved in

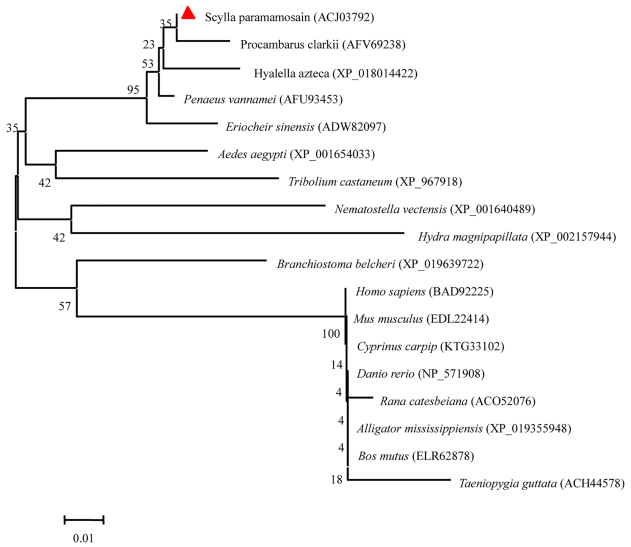

FIGURE 4 | The phylogenetic analysis of *Sp*-UBC 2 in *S. paramamosain* and other organisms. The GenBank codes of species were described in brackets. The *Sp*-UBC 2 was marked by a red triangle. The numbers adjacent to nodes indicated the bootstrap percentage value for 1,000 replicates.

liquid nitrogen for RNA extraction, and the others were fixed in 4% paraformaldehyde solution for ISH.

RNA Isolation and cDNA Synthesis

The total RNA of different tissues was isolated as described in the previous study (Han et al., 2018). The residual DNA contamination was eliminated using RNase-free DNase I at 37°C for 30 min. Additionally, the first-strand cDNA was reverse-transcribed using the oligo-dT-adaptor primer and Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Promega, Madison, WI, USA), following the instruction.

Cloning of *Sp-Ubc2*

Based on our Expressed Sequence Tags (EST) database from our laboratory, the partial fragment of *Sp-Ubc2* cDNA sequences was obtained. According to the above fragment, the rapid-amplification of cDNA ends (RACE) specific primers were designed. Following the manual, 5'- and 3'-RACE were used to isolate the full-length cDNA sequence using the SMARTer RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, CA, USA). Then, the open reading frame (ORF) was validated by head-to-toe PCR. The PCR products were separated by electrophoresis on 1% agarose gel, then purified and subcloned into pMD™19-T Vector (Takara, Beijing, China). Finally, the bacteria solution was sent for sequencing at Sangon (Shanghai, China). All primers used in this study are listed in **Table 1**.

Bioinformatics Analysis

The bioinformatics of sequence was done as described in the previous study (Han et al., 2018), including the analysis of signal peptide, isoelectric point, molecular weight, cellular localization, phosphorylation sites, multiple alignments, three-dimensional structure, and phylogenic tree.

Real-Time Quantitative PCR and *In Situ* Hybridization

The expression of *Sp-Ubc2* in different tissues of female individuals and different ovary development stages was quantified by RT-qPCR as described in the previous study (Han et al., 2018). The results were analyzed by the comparative Ct method. In this method, the expression of *Sp-Ubc2* was normalized against 18S expression, generating a ΔCt. Relative expression was calculated according to the equation, $2^{-\Delta\Delta Ct}$.

The *Sp-Ubc2* mRNA distribution was detected in different ovary development stages by ISH. The ovary samples were dehydrated through a graded series of ethanol, embedded in paraffin wax, and cut into 7 μm for H&E staining or *in situ* hybridization. In addition, the protocols of ISH followed the methods described previously (Zhang et al., 2003), with a few modifications as detailed below. Briefly, approximately 600-bp *Sp-Ubc2* fragment was inserted into the pGEM-T Easy vector (Promega, Madison, WI, USA). Sense and antisense RNA probes were synthesized by *in vitro* transcription from a vector under the drive of the T7 or SP6 promoter with the Digoxigenin (DIG) RNA Labeling Kit (Roche, Mannheim, Germany). The sections were dewaxed, rehydrated, and digested, then performed with the probes at 65°C for 14 h. Stained samples were observed and photographed through a Nikon Eclipse E600 microscope, using a Nikon DC50NN imaging system.

Statistical Analysis

The software SPSS 26 was used for statistical analyses. The data of RT-qPCR expression were subjected to one-way ANOVA, followed by Tukey's test. All data are presented as mean ± standard error (SE) of the different tissues or development stages. Differences were considered significant at $P < 0.05$.

RESULTS

Molecular Characterization of *Sp-Ubc2* cDNA

A part fragment of *Sp-Ubc2* was obtained from the EST library constructed by our laboratory, and the remaining unknown regions were isolated through 3'- and 5'-RACE. The full-length 971-bp *Sp-Ubc2* cDNA (Genbank : FJ265877) was produced by

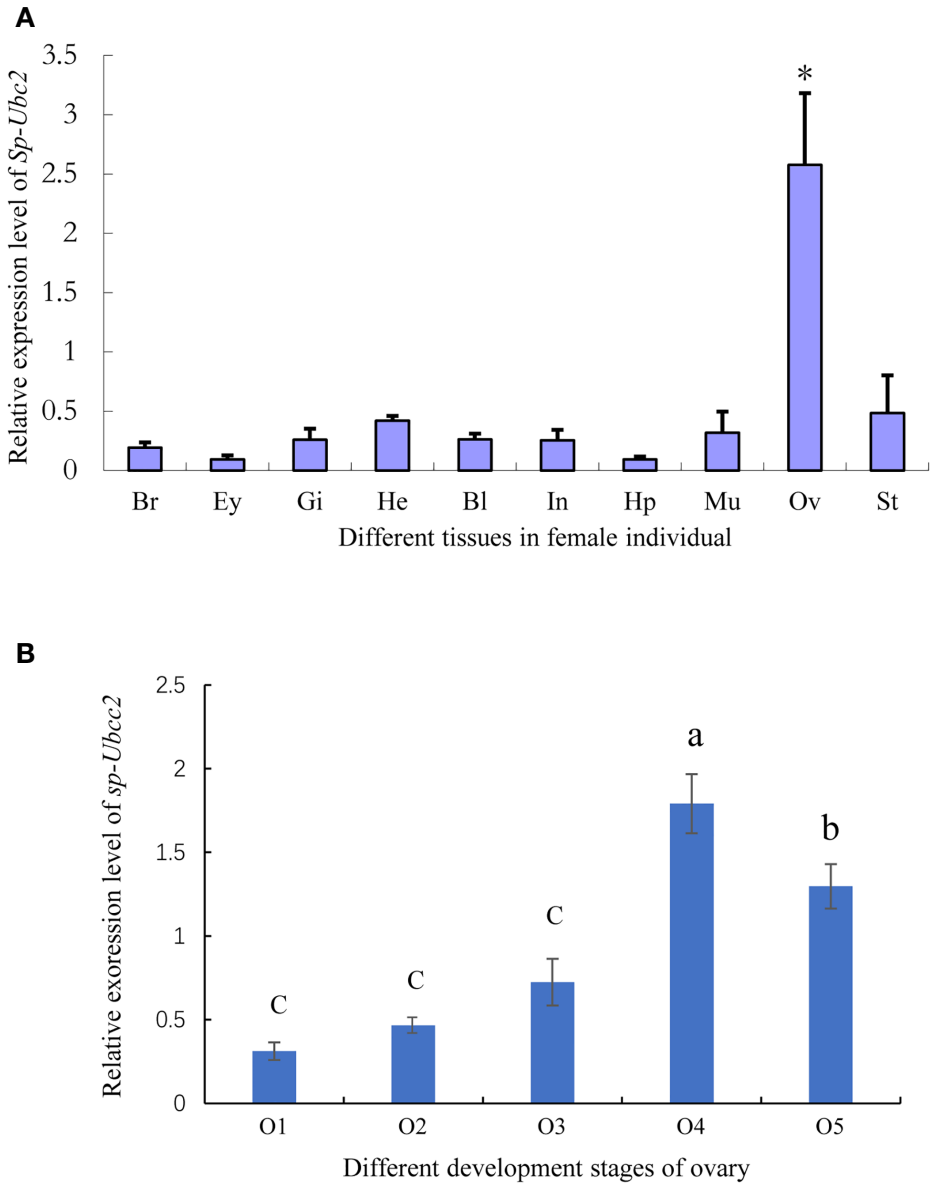

FIGURE 5 | The expression level of *Sp-Ubc2* mRNA in female tissues. **(A)** The distribution of *Sp-Ubc2* in different female tissues. **(B)** The expression pattern of *Sp-Ubc2* during oogenesis. Br, brain; Ey, eye; Gi, gill; He, heart; Ha, hemolymph; Bl, blood; In: intestine; Hp, hepatopancreas; Mu, muscle; Ov, ovary; St, stomach; O1, O2, O3, O4, and O5 represented different developmental ovaries; * in **(A)** indicated the significant difference ($p < 0.05$); different letters in **(B)** indicated statistically significant differences ($P < 0.05$).

overlapping the sequences of all fragments, which consisted of a 5'UTR of 69 bp, an ORF of 483 bp, and a 3'UTR of 419-bp (**Figure 1**). Then, the ORF was validated by head-to-toe PCR, which encodes 160 amino acids. Additionally, the molecular mass and theoretical isoelectric point (pI) of deduced protein sequence were 18.3kDa and 8.64, respectively. Sequence analysis by ProScan revealed that Sp-Ubc2 protein has 8 motifs, including 1 cAMP- and cGMP-dependent protein kinase phosphorylation site (K^{48}-T^{51}), 1 protein kinase C site (T^{108}-K^{110}), 2 casein kinase II phosphorylation sites(T^{51}-E^{54}, S^{95}-D^{98}), 1 N-myristoylation phosphorylation site(G^{3}-R^{8}), 1 N-myristoylation sites (P^{46}-K^{49}), and 2 ubiquitin conjugation enzyme active sites (F^{82}-L^{96}, F^{82}-L^{97}) (**Figure 1**).

Structure and Phylogenetic Analysis of *Sp*-UBC 2 Protein

The result predicted by the software of PSORT II indicated that *Sp*-UBC 2 protein may be mainly located in the nucleus (60.9%). The two-dimensional (2D) structure of *Sp*-UBC 2 showed 4-α helix, 5 extended strands, and several random coils (**Figure 2A**). In addition, the three-dimensional (3D) structure of *Sp*-UBC

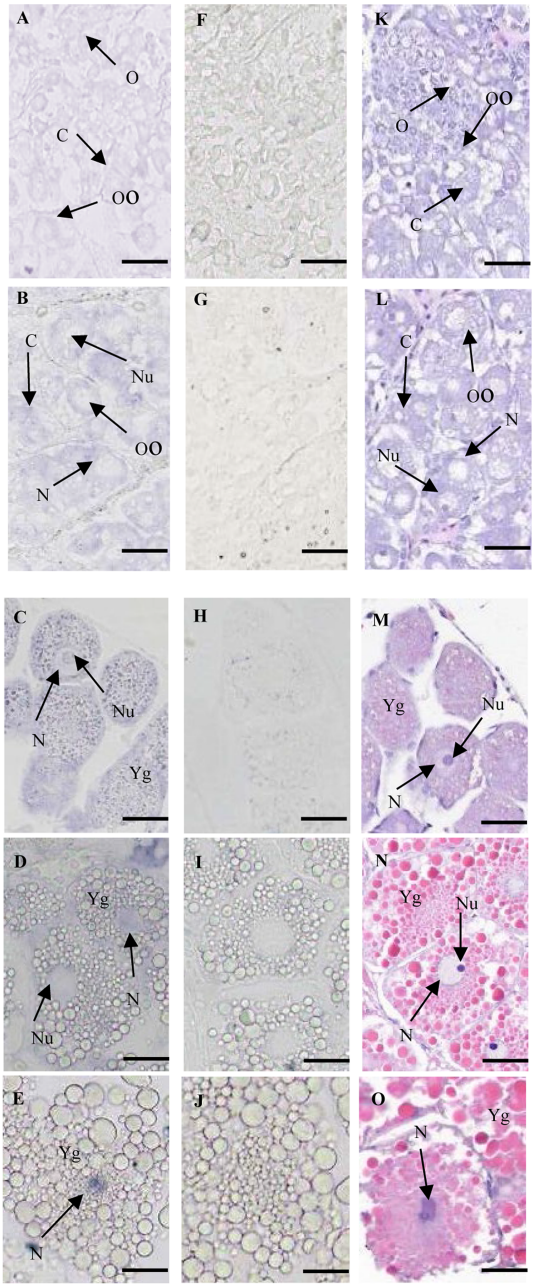

FIGURE 6 | The expression pattern of *Sp-Ubc2* mRNA during oogenesis. **(A–E)** The paraffin section of ovary was hybridized with the *Sp-Ubc2* antisense probe. **(F–J)** The paraffin section of ovary was hybridized with the *Sp-Ubc2* sense probe. **(F–J)** The paraffin section and **(H, E)** of ovary. **(A, F,** and **K)**: proliferation stage; **(B, G,** and **L)**: pre-vitellogenesis; **(C, H, M)**, primary vitellogenesis, **(D, I, N)**, secondary vitellogenesis, **(E, J, O)**, tertiary vitellogenesis; O, oogonia, Oo, oocytes, Cy, cytoplasm, N, nuclei, Nu, nucleolus, Yg, vitellogenin. scale bar, 100 μm.

2 was at the SWISS-MODEL server using an automatically matched template (protein data bank code : 2pe6A); the structure of α-helix and β-sheet was clearly observed in this picture (**Figure 2B**). Moreover, a Cys-site was also found in the domain (**Figure 2B**).

The homology analysis was conducted using *Sp*-UBC 2 with UBC 2 from other organisms, which indicated that the catalytic domain and active Cys-A site were evolutionarily conserved across mammals, arthropods, fishes, birds, echinozoa, and fungi (**Figure 3**). The phylogenetic analysis of UBC 2 protein demonstrated that the above species were clustered into three clusters: arthropod, cnidarian, and vertebrate. The *Sp*-UBC 2 was clustered in the first cluster with other arthropods, such as *Procambarus clarkii*, *Hyalella Azteca*, and *Penaeus vannamei* (**Figure 4**).

The Distribution of *Sp-Ubc2* in Female Different Tissues

RT-qPCR analysis showed that the *Sp-Ubc2* distributed various tissues of female individuals, including the heart, brain, muscle, and ovary (**Figure 5A**). However, the expression level of *Sp-Ubc2* in the ovary was the highest, compared with other tissues (**Figure 5A**). There was a significant difference between the ovary and other tissues, which revealed that the high expression of *Sp-Ubc2* might play crucial roles in ovary development in *S. paramamosain*.

The Expression Pattern of *Sp-Ubc2* During Oogenesis

According to the RT-qPCR, the ovary was found to have the highest level of *Sp-Ubc2*. For the specific expression level of *Sp-Ubc2* at various development stages, further RT-qPCR detection demonstrated that during the ovary oogenesis, the expression level was highest in the O4 stage, followed by the O5 stage and lower and stable between O1 and O3 (**Figure 5B**).

Moreover, the localization of *Sp-Ubc2* was investigated in the ovary by ISH during oogenesis. In accordance with histology, the stages of stained cells were identified (**Figures 6K–O**). As control, the tissue section hybridized with the sense probe was not stained (**Figures 6F–J**). From proliferation to pre-vitellogenesis, a positive signal could be observed in the nuclei and cytoplasm of oogonia, so was the result in oocytes (**Figures 6A, B**). In primary vitellogenesis, *Sp-Ubc2* mRNA was almost distributed evenly in whole oocytes (**Figure 6C**). Meanwhile, from secondary vitellogenesis to tertiary vitellogenesis, a positive signal was mainly observed in the nuclei (**Figures 6D, E**).

DISCUSSION

The present study successfully isolated and identified the *Sp-Ubc2* homologue of *S. paramamosain*. The deduced *Sp*-UBC 2 proteins had the UBC catalytic domain containing 8 motifs, as observed in penaeid shrimp (Shen et al., 2009) and grape (Gao et al., 2017). In addition, phylogenetic analysis revealed that the *Sp*-UBC 2 was clustered with other arthropods. The 2D and 3D structure of *Sp*-UBC 2 further predicted that the 4-α helix, β-sheet, and Cys-site presented in the UBC catalytic domain, which might be involved in various processes such as cell cycle progression, organelle biogenesis, and transcriptional regulation (DiAntonio et al., 2001).

Regarding the tissue distribution of *Sp-Ubc2*, RT-qPCR analysis showed that the *Sp-Ubc2* mRNA is widely distributed in various tissues of female individuals. This suggested that *Sp-Ubc2* participated in many physiological processes such as chromosomal organization, DNA repair, and cell cycle control, similar to *Homo sapiens* (Jiang and Beaudet, 2004) and *D. melanogaster* (Michelle et al., 2009; Nagy et al., 2012). Surprisingly, the *Sp-Ubc2* demonstrated the highest expression level in the ovary, which was significantly different from other tissues. Moreover, the expression level of *Sp-Ubc2* changed prominently as the developing ovary. In secondary and tertiary vitellogenesis stages, the expression level of *Sp-Ubc2* reached its peak. The results were consistent with the previous research in penaeid shrimp, which all indicated that the expression of *Sp-Ubc2* plays a more important role in the development process of the ovary (Shen et al., 2009). For the specific expression pattern of *Sp-Ubc2* during oogenesis, the *Sp-Ubc2* mRNA was observed in the nuclei and cytoplasm of oogonia and oocytes at proliferation, pre-vitellogenesis, and primary vitellogenesis stages, while the positive signal was gradually concentrated in nuclei from secondary vitellogenesis to tertiary vitellogenesis. According to some reports, ubiquitination contributed to several regulatory mechanisms of gametogenesis. In ascidians, the ubiquitin system participated in the elimination of defective sperm in epididymis and paternal mitochondria in fertilized eggs (Sakai et al., 2004). During early *Drosophila* oogenesis, the ubiquitin ligase complex was essential for cell cycle control that ensured proper transport of centrosomes into the oocyte for maintaining the development fate (Braun et al., 2021). Therefore, this change of *Sp-Ubc2* in the distribution position of ovary further suggested that the crucial function of *Sp-Ubc2* was related to the cell cycle.

In addition, a number of other small proteins can be covalently attached to target proteins in a manner similar to ubiquitination, such as SUMO (small ubiquitin-like modifier) (Dohmen, 2004). In recent years, SUMOylation has emerged in almost all aspects of cellular physiology (Liu et al., 2017). Like ubiquitination, there are three corresponding enzymes (E1, E2, and E3) involved in SUMOylation. Among them, the Ubc9 is only an E2-conjugating enzyme currently found, which could mediate SUMOylation through directly binding SUMO to target proteins (Sakaguchi et al., 2007). It has been proven that the Ubc9 is associated with various processes during spermatogenesis and oogenesis. In Chinese mitten crab (*Eriocheir sinensis*), the *EsUbc9* expression in the ovary were low in the early stage, reached the highest level at stage III, and then gradually decreased in stage IV, which suggested that SUMOylation might play an important role during oogenesis (Wang et al., 2012). Ubiquitination and SUMOylation are all important ways of protein post-translational modification, which are widely involved in regulating protein function and various aspects of cell life activities. Polyubiquitination degrades proteins, while SUMOylation mainly regulates protein interaction and localization (Bossis and Melchior, 2006). Under certain circumstances, SUMOylation and ubiquitination can both synergistically regulate protein function and antagonize each other (Choi et al., 2008; Kang et al., 2008). Therefore, more SUMOylation during the oogenesis of *S. paramamosain*.

In conclusion, we firstly reported of *Sp-Ubc2* in *S. paramamosain*. We obtained the full-length cDNA of *Sp-Ubc2* with UBC domain. Then, we detected that the *Sp-Ubc2* was widely distributed in different tissues of female individuals and kept the highest expression level in the ovary. Furthermore, we found that during oogenesis, the expression level of *Sp-Ubc2* was higher in O4 and O5 stages than other stages, and the *Sp-Ubc2* signal was gradually concentrated in nuclei, which indicated that *Sp-Ubc2* might play important roles in the cell cycle. This study would provide insights into the regulatory mechanism of gonad development in *S. paramamosain*, and it also enriched the theoretical basis of ubiquitin family genes involved in crustacean gonad development.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

All of the study design and animal experiments were conducted in accordance with guidelines of Jimei University's Animal Care and Use Committee (2011-59).

AUTHOR CONTRIBUTIONS

YW conceived and designed the study. KH and YD contributed to the experimental work and original data preparation. LZ contributed to the manuscript writing and revision. ZZ and CR contributed with the experimental materials. All authors read and approved the final manuscript.

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