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Cloning and expression analysis of ubiquitin carboxyl-terminal hydrolase isozyme L5 (*UCHL5*) gene in *Procambarus clarkii*

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ABSTRACT: This study cloned the full-length cDNA sequence of *Procambarus clarkii* ubiquitin carboxyl-terminal hydrolase isozyme L5 (*PcUCHL5*) gene using RT-PCR method. Then, the expression and subcellular localization of the *PcUCHL5* was investigated in tissues using qRT-PCR and *in situ* hybridization methods. The results indicated that *PcUCHL5* had a total length of 2392 bp and encoded 331 amino acids. The qRT-PCR results showed that *PcUCHL5* was significantly expressed in ovarian tissue ($p < 0.05$), with the highest expression level in stage III ovarian tissue during the ovarian development. Through *in situ* hybridization experiments, it was found that *PcUCHL5* mRNA had the strongest positive signal in primary oocytes with long-term yolk growth. The *PcUCHL5* gene might play an important role in the early ovarian development of crayfish, providing a theoretical basis for studying the regulatory mechanisms of *P. clarkii* egg cell growth and development.

KEYWORDS: *Procambarus clarkii*, *UCHL5*, ovary, clone expression, subcellular localization

INTRODUCTION

Procambarus clarkii is commonly known as crayfish or fresh water lobster. In 1929, Japan introduced it into the area near Nanjing, Jiangsu Province, China by ballast water [1].

P. clarkii has the advantages of short growth cycle, high disease resistance [2], rich nutrition, excellent taste [3], and being popular among consumers. Artificial breeding of *P. clarkii* has been widely carried out in all parts of China [4]. The breeding methods have been developed from releasing seedlings and monocultures, round trapping and round releasing to mixed culture of shrimp and fish [5], mixed culture of shrimp and crab, and rice and shrimp breeding [6], which has brought significant output value and profits to farmers. Although the breeding area has been expanded year by year, the mismatch between germplasm and market supply ability and the development demand of breeding industry has become the main factor restricting the development of *P. clarkii* industry due to the lack of seedling experience [7]. Therefore, it is of great significance to explore and analyze the reproductive development and regulatory mechanism of *P. clarkii* through molecular breeding technology to promote the development of breeding industry.

At present, some achievements have been made in the research of molecular breeding of *P. clarkii*. Using qRT-PCR method, Kang [8] reported that *RDH13* gene was specifically expressed in *P. clarkii* ovary and had a higher expression level in oocytes during vitellogenesis. Jiang et al [9] found that ribosomal protein *S24* (*RPS24*) gene played an important role in the early development of *P. clarkii* ovary and was significantly expressed in the ovary. Among all stages of ovary (from stage I to stage IV), the expression level of *PcRPS24* was the highest in stage I ovary. The *Dmc* gene was related to the occurrence and accumulation of yolk in the ovary of *P. clarkii* [10]. Sex differentiation is a hot research direction of molecular breeding. The expression of *PcSxl* in the ovary was significantly higher than that in the testes, suggesting that *PcSxl* could be related to sex differentiation of *P. clarkii* [11]. There was a significant difference in the expression of *PcDsx* gene between the tissues of juvenile male and female and the corresponding tissues of adult male and female *P. clarkii* [12]. Therefore, it was believed that *PcDsx* gene might be related to sex differentiation. Using RNA interference (RNAi) technology and other methods including qRT-PCR, Ge [13] found that the silencing of *IAG* gene would block the generation of spermatogonia and sperm, causing deformities of the first abdominal limb of *P. clarkii*. It was inferred that *IAG* gene might be related to sex differentiation and sex control function of *P. clarkii*.

In summary, molecular researches related to gonad development in *P. clarkii* have progressed rapidly. Studies showed that the ubiquitin carboxyl-terminal hydrolase isozyme (UCH) gene family was important for gonad development and disease treatment. Research results proved that UCH belongs to the deubiquitinating enzymes (DUBs) family [14], and DUBs

could remove ubiquitin from proteins to prevent the degradation of related functional proteins after ubiquitination [15]. In addition, deubiquitinase 3(DUB3) contributed to colorectal cancer metastasis and angiogenesis by regulating NF-B/HIF-1 pathway via EZH2 [16]. UCH could also remove ubiquitin from substrate proteins by releasing ubiquitin monomers [17]. Moreover, UCH gene family was closely related to oocyte development. i.e., *UCHL5* gene was specifically expressed in the ovary of *Scylla paramamosain*, with the highest expression in stage V ovary, followed by stage III ovary [18]. However, there has not been any study reported on functional analysis of *UCHL5* gene in *P. clarkii*. In this study, the full-length cDNA of *UCHL5* gene was cloned using RT-PCR method, and its expressions in different tissues including ovary at different developmental stages were analyzed by qRT-PCR method. At the same time, *in situ* hybridization was used to detect the location of *PcUCHL5* in the ovary to explore the mechanism of ovarian development at the molecular level, which could provide theoretical basis for the study of ovarian development of *P. clarkii*.

MATERIALS AND METHODS

Test materials

Twelve healthy *P. clarkii* adults consisting of nine (9) females and three (3) males, body length of (8.24 ± 0.20) cm and body weight of (22.29 ± 1.33) g, were selected. After dissection, three groups of nine tissue samples: muscles, hepatopancreas, brain, gills, blood, heart, intestine, ovary, and testis were individually taken from three crayfish samples and placed in EP tubes. Each tube was placed in a cryovial and quickly frozen in liquid nitrogen to prevent RNA degradation. After taking out from liquid nitrogen, the sample were quickly store in a −80 °C freezer for future use. According to a previous report [19], the development process of *P. clarkii* was divided into five (5) stages (I, II, III, IV, and V) according to shape and color of the ovary. Dissect another 120 adult female crayfish. Select three pieces of ovarian tissues of *P. clarkii* at developmental stages from I to V and put them into one tube. Take three groups and preserve them in the same way. Reverse transcription reagents (PrimeScript RT reagent Kit with gDNA Eraser), high-fidelity enzymes (PrimeSTAR Max DNA Polymerase) and real-time fluorescence quantitative reagents (SYBR® Premix Ex Taq™) used in the experiments were purchased from Baori Doctor Biological Technology (Beijing) Co., Ltd. TRIzol reagent was purchased from Wuhan Savier Biological Technology Co., Ltd. DNA product purification kit was purchased from Tiangen Biochemical Technology (Beijing) Co., Ltd.

Primer design and synthesis

According to the *UCHL5* gene sequence of the *Procrazii crazii* genome sequencing result (NC_059599.1) published in GenBank, specific primers were designed by Primer Premier X software (Table S1), and all primers were commissioned to be synthesized by Sangon Bio-Engineering (Shanghai) Co., Ltd.

Total RNA extraction and cDNA synthesis

Trizol method was used to extract total RNA from muscle, liver and ovary at different developmental stages. NanoDrop 2000 was used to measure the concentration and purity of total RNA, and 1.5% agarose gel electrophoresis was used to examine the integrity of RNA. RNA was reverse transcribed into cDNA using a reverse transcription kit and reaction program. The reaction procedure comprised two steps as follows: first step, 2 min at 42 °C; second step, 15 min at 37 °C followed by 5 s at 85 °C. After the reaction, the products were kept at −20 °C for later use.

PcUCHL5 gene cloning

The *PcUCHL5* gene was cloned by RT-PCR method. The reaction system was 20 μl, the mixed cDNA of the nine tissues was used as template (2 μl); and the primers *PcUCHL5* F/*PcUCHL5* R 1 μl each, high fidelity enzyme 10 μl, and sterile water 6 μl were added. The PCR amplification program was as follows: predenaturation at 98 °C for 1 min; 35 cycles of 98 °C for 10 s, 58 °C for 30 s, and 72 °C for 40 s. The cells were extended at 72 °C for 5 min and stored at 4 °C. 1.5% agarose gels were prepared, and PCR amplification products were examined by electrophoresis. The PCR products were purified using purification kit. The purified PCR products were digested and transformed into top 10 competent cells for overnight culture, and single colonies were selected and cultured in liquid medium. Positive clones were verified by PCR, and the bacterial solution was sent to Sangon Bioengineering (Shanghai) Co., Ltd. for sequencing.

Bioinformatics analysis of *PcUCHL5* gene

The nucleotide and amino acid sequences of *PcUCHL5* gene were analyzed using the Sequence Manipulation Suite (SMS) (http://www.bio-soft.net/sms/). ProtParam (https://web.expasy.org/protparam) was used for molecular weight and theoretical isoelectric point analysis. NCBI Conserved Domains (https://www.ncbi.nlm.nih.gov/cdd/) tools, online analytical *PcUCHL5* domain structure search by NCBI Blast *PcUCHL5* homology of amino acid sequence, GeneDoc software was used for amino acid multiple sequence alignment analysis. Amino acid sequences of 15 species including *Cherax quadricarinatus*, *Eriocheir sinensis*, zebrafish (*Danio rerio*), and mouse (*Mus musculus*) were selected. The phylogenetic tree was constructed by Neighbor-Joining method in MEGA 11 software.

Expression of *PcUCHL5* gene in different tissues and ovarian tissues of *P. clarkii* at different developmental stages

The qRT-PCR reaction system was 20 µl, and each tissue sample was set up in triplicates. The reaction program was divided into two steps: (1) predenaturation at 95 °C for 30 s; (2) denaturation at 95 °C for 5 s and renaturation at 60 °C for 34 s; and set up 40 cycles. The melting curve program was as follows: denaturation at 95 °C for 15 s, renaturation at 60 °C for 1 min, and denaturation at 95 °C for 15 s. SPSS 27.0 and Excel software were used for statistical analysis of quantitative data. One-way ANOVA and least significant difference (LSD) were used to compare the differences between different data groups. $p < 0.05$ was considered as significant difference. $p < 0.01$ was considered highly significant, and finally GraphPad Prism 9 software was used for mapping.

Subcellular localization

In order to determine the expression and localization of *PcUCHL5* gene in oogonia, primary oocytes and secondary oocytes of each stage, three ovarian tissues of stages II, III, and IV were dissected and immediately placed into 2 ml EP tube containing 1 ml *in situ* hybridization fixative (Wuhan Cyvier Biotechnology Co., Ltd.). Based on the CDS sequence of the *PcUCHL5* gene, the probe sequence was designed using Primer Premier X software (Table S1), The first primer is a sense probe, and the last two primers are antisense probes. The SweAMI FISH probe was commissioned to be synthesized by Wuhan Saiwell Biotechnology Co., Ltd. Ovarian tissue samples were performed tissue fixation, dehydration, sectioning, digestion, prehybridization, hybridization, DAB staining, dehydration, and sealing treatment. Firstly, a sense probe was used to hybridize with a nucleic acid sequence that was completely complementary to the target sequence; thereby, generating a detectable signal. Then, complementary binding between the antisense RNA probe and the target RNA was utilized to ensure that no detection signal was generated under ideal conditions; thus, verifying the reliability of the experimental results. Observations were done through a microscope. Images were collected and analyzed.

RESULTS

Sequence characteristics of *PcUCHL5* cDNA

The full-length *PcUCHL5* cDNA sequence was cloned by RT-PCR (Fig. 1), and the electrophoretic band was visible above the DL2000 DNA marker. Bioinformatics analysis showed that the *PcUCHL5* gene was 2392 bp in length, including 68 bp in the 5′ untranslated region, 1328 bp in the 3′ untranslated region, and 996 bp in the open reading frame (ORF), encoding 331 amino acids. The predicted molecular weight of the protein was 38.1 kDa. The theoretical isoelectric point was 5.41. L258–L303 is the unique C-terminal domain of *UCHL5* gene (Fig. 2).

Fig. 1 PCR amplification of *PcUCHL*: DL2000 DNA marker (left lane); *18SrRNA* (middle lane); gene of *PCUCHL5* (right lane).

Amino acid homology comparison and phylogenetic tree construction of *PcUCHL5*

Multiple sequence alignment of *UCHL5* C-terminal domain proteins showed that the *PcUCHL5* protein had high similarity with *UCHL5* proteins from other species, and the highest similarity was *Homarus americanus* (86.33%). The similarities with *Cherax quadricarinatus*, *Eriocheir sinensis*, and *Scylla paramamosain* were 85.52%, 79.62%, and 79.09%, respectively. The similarity with vertebrates was low, i.e., 46.81% with *Homo sapiens* and 45.83% with zebrafish (*Danio rerio*), *Mus musculus*, and *Xenopus laevis* (Fig. 3). These results indicated that the *UCHL5* homolog protein is conserved during evolution and that *P. clarkii* has a high homology with decapod shrimp crabs.

The *UCHL5* gene protein sequences of shrimps and crabs, fish and amphibians in different taxonomic positions were analyzed. The results showed that the phylogenetic tree could be divided into two major branches, with *P. clarkii* clustered into one branch with other invertebrates such as *Homarus americanus* and *Eriocheir sinensis*. *Homo sapiens*, *Mus musculus*, and other vertebrates were in the same group. Additionally, the results showed that *P. clarkii* was closely related to decapod shrimps and crabs and had low homology with amphibians, mammals, fish, and other animals (Fig. 4).

Analysis of *PcUCHL5* mRNA tissue expression

The expression levels of *PcUCHL5* gene in different tissues were investigated by qRT-PCR method, and the results showed that *PcUCHL5* gene was expressed in all tissue samples. The expression level of *PcUCHL5* gene in the ovary was the highest and also significantly higher than that in other tissues ($p < 0.05$), followed by the expression level in the testes. The expressions of *PcUCHL5* gene in the intestine and the testes were similar, with the lowest found in the gills (Fig. 5).

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                                                                                                                AGGCAGGG 8
GAGCAGCAGCAGCCTAGCCGCCGCCGCCGCCAGTGTTGACAAGACAAGAGCAGCATCATCATGGTGGTGTCTGACGCTGGCAACTGGTGCCTCATCGAGAGTGACCCCGGGGTCTTCACC 128
                                                                  M  V  V  S  D  A  G  N  W  C  L  I  E  S  D  P  G  V  F  T  20
GACCTCATACACAAGTTTGGGGTTAAAGGTGTTCAAGTGGAAGAAATCTGGAGCTTAGATGATGATTCCTTCATAAATCTTAAGCCTGTGCATGGTCTCATTTTCTTGTTCAAATGGCAG 248
 D  L  I  H  K  F  G  V  K  G  V  Q  V  E  E  I  W  S  L  D  D  D  S  F  I  N  L  K  P  V  H  G  L  I  F  L  F  K  W  Q  60
CAAGAGGAACAGCCGTCTGGTACAGTGGTGCAGGATAATCGGCTGGATAAGATATTCTTCGCAAAACAGATGATAAACAATGCTTGTGCCACCCAAGCAATCCTATCGATATTGCTTAAC 368
 Q  E  E  Q  P  S  G  T  V  V  Q  D  N  R  L  D  K  I  F  F  A  K  Q  M  I  N  N  A  C  A  T  Q  A  I  L  S  I  L  L  N  100
ACAAAACACGTGGACCTACAGCTTGGATCTACTCTTTCTGAGTTTAAGGAGTTTACACAGACATTTGATGCTCACATGAAGGGGTTAGCACTCTCCAATTCAGATACCATTCGCAACGTC 488
 T  K  H  V  D  L  Q  L  G  S  T  L  S  E  F  K  E  F  T  Q  T  F  D  A  H  M  K  G  L  A  L  S  N  S  D  T  I  R  N  V  140
CACAACTCATTTGCCAGGCAGACTCTCTTCGAGTTTGACAAGCAACAACCTTCAGAAGATGACGATGTGTTCCACTTTGTGGGTTATATTCCCATCGAAGGCCGTCTGTATGAACTGGAT 608
 H  N  S  F  A  R  Q  T  L  F  E  F  D  K  Q  Q  P  S  E  D  D  D  V  F  H  F  V  G  Y  I  P  I  E  G  R  L  Y  E  L  D  180
GGCCTCAAGGATGGTCCAATAGATTTGGGACCCATATCTCCTGGTACAGATTGGCTCACAGTCGTACAGCCAGTTATTCAGCGAAGAATTCAAAAATACAGTGAAGGTGAAATTCACTTT 728
 G  L  K  D  G  P  I  D  L  G  P  I  S  P  G  T  D  W  L  T  V  V  Q  P  V  I  Q  R  R  I  Q  K  Y  S  E  G  E  I  H  F  220
AACCTTATGGCTCTTGTCAGCGATCGTAAAATGGTTATTGAGAGGAATATTACACAGTTGCAGAGAGAAATTGAGGAGAGTGAAATGGACACATCCATTCAAGAGGAGGAATTGGCACGT 848
 N  L  M  A  L  V  S  D  R  K  M  V  I  E  R  N  I  T  Q  L  Q  R  E  I  E  E  S  E  M  D  T  S  I  Q  E  E  E  L  A  R  260
CTACGAGCAACATTGGAGTCTGAGGAAAACAAGCGAGCTCGTTGGCAAGTTGAGAATATTCGCCGTAAACACAATTACCTTCCACTCATTGTCAACATGATGAAGATTCTGGCAGAGGAA 968
 L  R  A  T  L  E  S  E  E  N  K  R  A  R  W  Q  V  E  N  I  R  R  K  H  N  Y  L  P  L  I  V  N  M  M  K  I  L  A  E  E  300
GGAAAGTTATTGCCAATCTATCAGAATGCTCGGGAAAAGGCACGAGCACGCCATGAGAAATCTAAAGAAAAGTCCAAAGAAAAGAGCAGTGCTTGACAGGGAACAACACAAACTATAAAA 1088
 G  K  L  L  P  I  Y  Q  N  A  R  E  K  A  R  A  R  H  E  K  S  K  E  K  S  K  E  K  S  S  A  *                          331
GTTACAAACTGGAAACAAACTTTGGTGCAGTTGGTAATTATCAAGAAGGAAGTATCGGTGTTGAGTACCAATCTATACAATCCGTCGAGTGTTGAGCGTTGGAAACTGGAATGGTCACGT 1208
TTGTGCTGAAAAAAAAACTTTTTTGCAAAGGTATCTTACTAAAATTTATATCAAATGCTTATAGATTTTAATCTTTAAGGATTGTTAGTTATTTGCATTAAAAAGCAGTGTTAGATGTTA 1328
AAATCTAAAAGATGTTTGGAGTTAATTGTATGTTTTTGACATCTGTAATTGGCAGCTTAAATTGTTATGGAATTTTGAGCAATTTAGGTGGGTTTTATTCGCTTCCTTTTTAAACATACG 1448
AATGTATGATATTTTACAAATATATTTTGATCTTGCTGCATACAATGTGTACCATTTCCTCAATAAAGTCTTATGATGTGGCAAGTGTACCAAATTTGTGTTATTTATTATTACATTTGT 1568
GCATAGCAATATTGTATTATTCTATCCAGAAAAATTGTCTGTGGTGTTTTTGCATTTAATGGCACATACGAGACTAATGAAAGAATCTCGACTGTTGTCTAGGTATACAGCACAGTATTT 1688
TAATGTTATTCAGCCCCAATTAAGTATTTCCCACGTTAATTTTTCTGTTTGTATCTGATTCCTTGTTTACTTCAGAACTTCTCTCCAAAGAAGAAGTCTTTAGATTTTGCTCATTTTACT 1808
TGATTTTTCATGAAATCACTGACCATCTGTTACCCTACCAGTTTGGGATCTTAAAAGTTAACATTGTGTGAACAGGGATTTTTAATAATTTCTGCAATCTTACCATACATTGTATCAAAC 1928
CAAAATTCAGCATGTCTTTCAATTAATTTATAAGGAAATTACAAATAAAACAATAATATTGTCTGTAAAACCAAGTGATTATTTTGTAGAATCTATTTCAATGCCAAATCATCTGCTCCC 2048
GGAGTCATCATATCTTGAAGAAAATCCACAATACATTTATTAGGTCTTGCTTTAATTCATCATTATTGGCTTCACAAGAAACCTCTTAACAGTGTTCTTTTACATTTCCTGGTGTGGGAT 2168
AATTGTAACTGCAGCATTGTATACATCAAAGAGATGTAGCCACATTCCTCTGTTTATTTGAATATGCATGAGATGCAATGCAGAACTTGTACTTTAGCTACATGGTCAGACTGACAACAC 2288
CTCTTGCTCCAGGACACCAGCGGCTTGTGTCGAGGCCCAGTGCTGTGGTGCAGCTCCTTAATGTTATCCATATTGATATTTAATAAATAAACTGCAAAATTCTC               2392
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Fig. 2 Full length cDNA sequence and amino acid sequence of *UCHL5* gene of *P. clarkii*. The gray shaded area represents the conservative C-terminal domain.

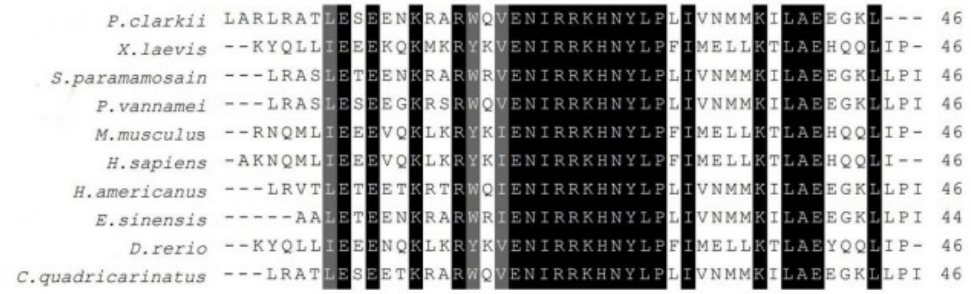

Fig. 3 Multiple amino acid sequence alignment of C-terminal domain of *P. clarkii* and other species. Black: Highly similar area; Gray: Relatively highly similar area.

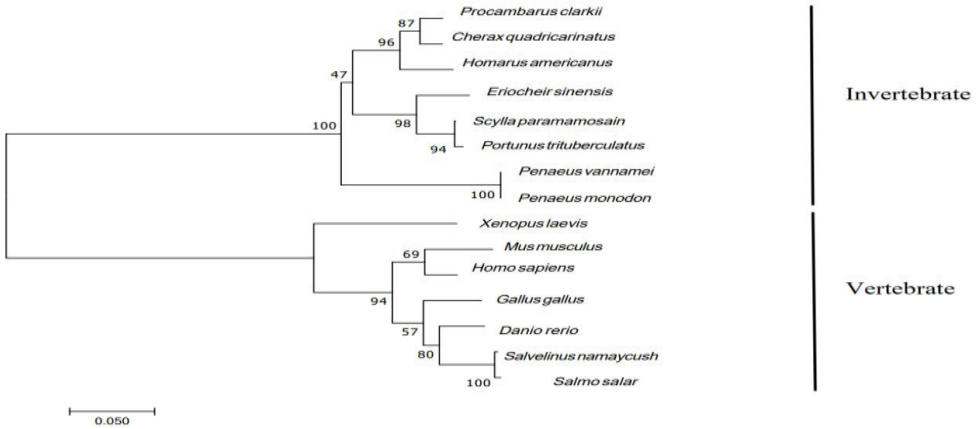

Fig. 4 Phylogenetic tree of *UCHL5* protein sequence based on neighbor-joining (NJ) method.

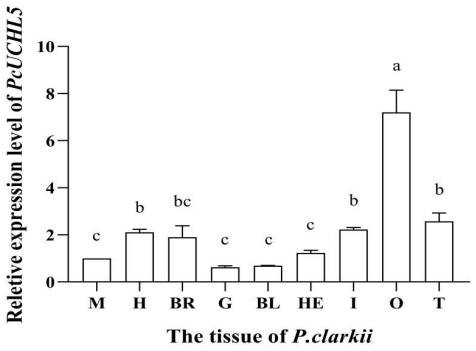

Fig. 5 Expression characteristics of *PcUCHL5* gene in different tissues. M, Muscles; H, Hepatopancreas; BR, Brain; G, Gills; BL, Blood; HE, Heart; I, Intestine; O, Ovary; T, Testis. Different letters indicate significant differences $p < 0.05$).

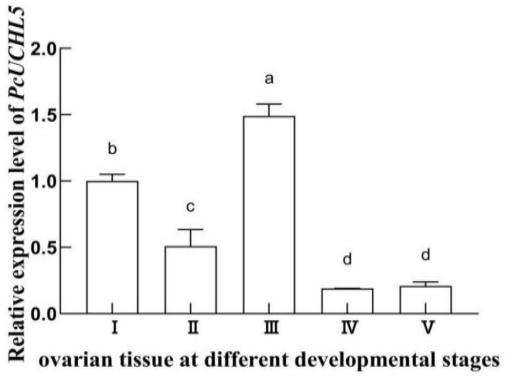

Fig. 6 Expression patterns of *PcUCHL5* gene in different stages of ovarian development. Different letters indicate significant differences $p < 0.05$).

Expression pattern of *PcUCHL5* during ovarian development

In order to study the expression level of *PcUCHL5* gene in *P. clarkii* ovarian tissues at different developmental stages, qRT-PCR was used, with *18SrRNA* as the reference gene. The results showed that the expression of *PcUCHL5* gene in stage III ovary was the highest, and it was significantly higher than those in ovarian tissues of the other developmental stages ($p < 0.05$). The expression level was significantly higher in early ovarian development than in later ovarian development (Fig. 6). It was speculated that the *PcUCHL5* gene promoted the production of vitellogenic substances in the ovary.

Subcellular localization of *PcUCHL5* in ovarian tissue

The results of *in situ* hybridization were shown in Fig. 7, where Fig. 7(1) and Fig. 7(2) representing images of the same site and a positive result under the sense probe; and Fig. 7(3) and Fig. 7(4) representing images of the same site and a negative result under the antisense probe. The results indicated that using the antisense RNA probe for negative control experiments did not produce any positive signals, and the experimental results were reliable. The results of *in situ* hybridization experiments showed that *PcUCHL5* mRNA was expressed in follicular cells, oocytes, cytoplasm, and nucleus around oocytes at various stages of early development. Positive signals were strong in early oocytes; primary oocytes in primary, micro growth, and large growth stages; and in mature primary oocytes. The weak positive signal in secondary oocytes (Fig. 7) indicated that *PcUCHL5* gene could play an important role in early egg development.

DISCUSSION

Bioinformatics analysis of *PcUCHL5* gene

In this study, the expression pattern of *UCHL5* gene was systematically revealed in the tissues of *P. clarkii*, and the nucleotide and the amino acid sequences of the gene were analyzed. The full-length cDNA of *UCHL5* was 2392 bp, encoding 331 amino acids with a predicted molecular weight of 38.1 kDa and a theoretical isoelectric point of 5.41. Multi-protein sequence alignment showed that the *UCHL5* protein sequence of *PcUCHL5* had a high homology with the *UCHL5* protein sequence of *C. quadricarinatus*, *H. americanus*, and *E. sinensis*. Phylogenetic analysis showed that *PcUCHL5* had a high homology with the *UCHL5* protein sequence of shrimps and crabs. It had the C-terminal domain of *UCHL5* gene family. The C-terminal domain proteins of *P. clarkii* shared about 80% homology with shrimp-crabs. The homology with vertebrates was low, about 45%. These results suggested that the gene might have a similar function in shrimps and crabs. In similar studies, the *UCHL5* protein sequence of the *E. carinicauda* showed the highest homology (83%) with the protein sequence of *Litopenaeus vannamei*, and it gathered with shrimps and crabs to form a branch such as *L. vannamei* and *Portunus trituberculatus*, with a conserved C-terminal domain [20]. Studie on other ubiquitin genes related to *P. clarkii* ovary development were also reported, Shi et al [21] studied the effect of ubiquitin-conjugative enzyme *E2* gene on *P. clarkii* ovary development and found that this gene had a high homology with the arthropod *UB-E2* gene. Phylogenetic analysis showed that it was clustered with *L. vannamei* and other shrimp species. These results indicated that the *UCHL5* gene of *P. clarkii* had the closest sequence homology with shrimp crabs and

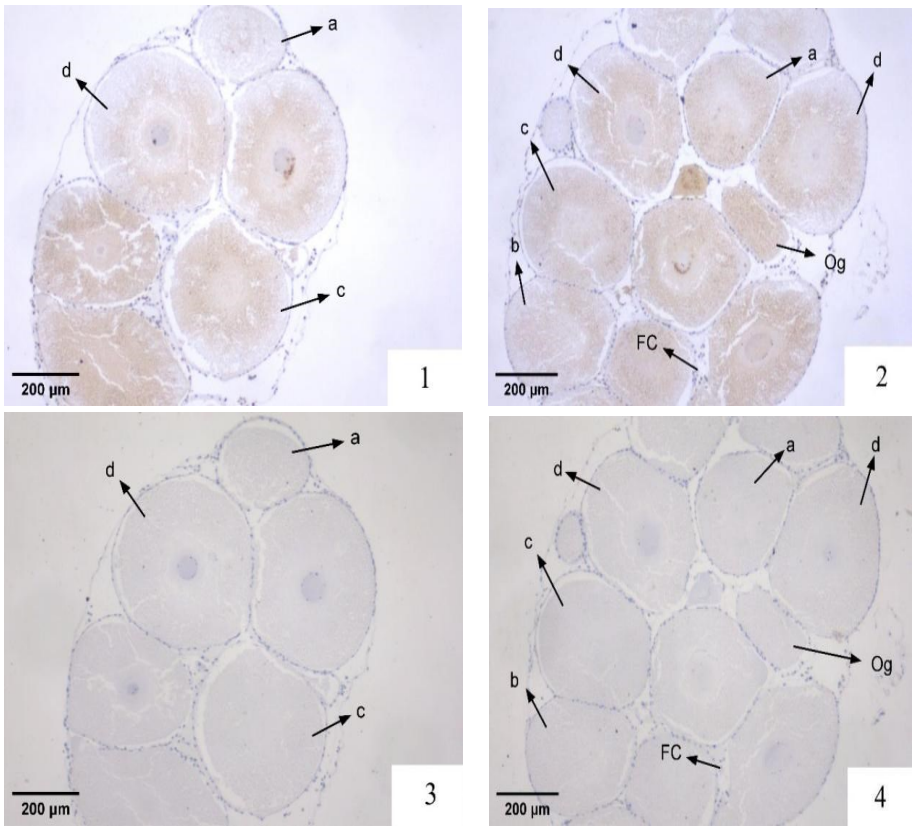

Fig. 7 *In situ* hybridization analysis of *PcUCHL5* mRNA. FC, Follicular cells; Og, Oogonia; a, Long term primary oocytes in small offspring; b, Large growth stage primary oocytes; c, Mature primary oocytes; d, Secondary oocytes. Yellow is a positive signal.

similar sequence characteristics with other ubiquitin hydrolase genes of *P. clarkii*.

Tissue expression analysis of *PcUCHL5* gene

qRT-PCR results showed that *PcUCHL5* gene was expressed at the highest level in ovarian tissues and significantly higher than in other tissues. *UCHL5* gene could remove ubiquitin from the substrate protein by regulating the deubiquitination of immune-related proteins, which played an important role in the body's immune response [22]. In this study, the expression of *UCHL5* gene was high in the intestinal tissue of *P. clarkii*, which is one of its main immune organs, suggesting that *UCHL5* also has a potential function in the related immune response of the body. UCH family plays an important role in the development of oocytes. Researchers found that *UCHL1* pointed mutant protein on the maturation of mouse oocytes, and it could be related to the formation of polar bodies during oocyte division [23]. *UCHL1* and *UCHL3* genes were highly expressed in oocytes at the second meiotic metaphase of rhesus monkey (*Macaca mulatta*) ovarian tissue [24] and were associated with the functions of oocyte cortex and meiotic spindle. The expression of *UCHL5* gene in the ovary was the highest and significantly higher than those in other tissues ($p < 0.05$). Interestingly, qRT-PCR analysis showed that the expression level of *PcUCHL5* gene was the highest in the ovary and was significantly higher than those in other tissues ($p < 0.05$). In different stages of ovarian development, the expression of *PcUCHL5* gene was the highest in stage III, and it began to decline to the lowest level in stages IV and V, suggesting that *PcUCHL5* gene was associated with the vitellogenin production process of early oocytes.

Subcellular localization of *PcUCHL5* gene

Subcellular localization is an effective way to confirm the expression location and expression signal strength of target genes in tissue cells [25]. In order to further understand the subcellular localization of *PcUCHL5* gene in the ovary of *P. clarkii*, *in situ* hybridization analysis was done, and the results showed that the positive signal of *PcUCHL5* mRNA was strong in the growing primary oocytes, and the hybridization signal was evenly distributed in the cytoplasm and nucleolus of the cells. The hybridization signal was weak in mature primary and secondary oocytes, but much

stronger in the nucleolus than in the cytoplasm. The expression of *PcUCHL5* mRNA in follicular cells was low. Previous studies showed that the *PcC1q* gene of *P. clarkii* promoted the production of vitellogenin and was specifically expressed in follicular cells around oocytes [26]. In addition, it was found that *PcRDH11*, a gene related to ovarian development, was specifically expressed in the follicular cells around the early oocytes, but it was transferred to the cytoplasm in the late vitellogenesis [27]. These results indicated that there were differences in the specific expression positions of genes related to ovarian development. The ubiquitin-binding enzyme *E2r* gene was also found closely related to the ovarian development of *P. clarkii* [28]. In the *in situ* hybridization experiment, it was found that *Pc-UBE2r* gene was evenly distributed in the cytoplasm of immature oocytes in the ovarian tissue, and then gradually migrated to the oocyte nucleus and around the follicular cells, which was similar to the results of this study. Therefore, it was speculated that the regulatory mechanism of ovarian development by ubiquitin genes was similar. Vitellogenesis is a necessary condition for the maturation of crustaceans ovary [29]. For *P. clarkii*, a large amount of yolk began to deposit in the cytoplasm in stage III ovarian development [30]. The *P. clarkii* stage III ovarian tissue was mainly composed of primary oocytes in the growth stage, and *PcUCHL5* was specifically expressed in stage III ovarian tissue, indicating that it could be closely related to vitellogenesis.

CONCLUSION

In summary, the sequence characteristics and the tissue expression pattern of UCHL5 gene were cloned and analyzed. *PcUCHL5* gene was specifically expressed in the stage III ovary of the *P. clarkii*, and the *in situ* hybridization showed that the positive signal of *PcUCHL5* gene was the strongest in vitellogenic oocytes. It was, hence, speculated that *PcUCHL5* gene might play a regulatory role in vitellogenesis. The results provided a theoretical basis for related molecular breeding researches in the future.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.026.

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REFERENCES

1. Yue GH, Li JL, Bai ZY, Wang CM, Feng F (2010) Genetic diversity and population structure of the invasive alien red swamp crayfish. *Biol Invasions* **12**, 2697–2706.
2. Gong SY, Lv JL, Sun RJ, Li LP, He XG (2008) The study on reproductive biology of *Procambarus clarkii*. *Freshwater Fish* **38**, 23–25.
3. Tian J, Xu QQ, Tian L, Hu W, Yang CG, Gao WH (2017) The muscle composition analysis and flesh quality of *Procambarus crarkia* in the Dongting lake. *Acta Hydrobiol Sin* **41**, 870–877.
4. Li CJ, Tu ZC, Wen PW, Wang H (2022) Present situation and future development trend of crayfish processing in China. *STFI* **43**, 463–470.
5. Pan YZ, Tu GP (2003) Preliminary study on the cultivation technology of *Procambarus clarkii*. *Freshwater Fish* **33**, 35–36.
6. Xu B, Wei KJ, Ma BS, Xu J, Zhu XY, Liu W (2021) Comparison of growth performance, body composition and serum biochemical indexes of *Procambarus clarkii* larvae in four farming models. *Freshwater Fish* **51**, 84–90.
7. Yuan C, Lu ZL, Huang BS, Wang DP, Li WH (2021) *Procambarus clarkii* breeding technology and prospects in Guangxi. *FIS* **36**, 267–274.
8. Kang PF (2017) Transcriptome analysis of ovary and functional analysis of *RDH13* gene in red swamp crayfish *Procambarus clarkii*. Central China Normal University, China.
9. Jiang HX, Li QZ, Zhang R, Wang L, Zhang M, Yu M, Qiao ZG, Li XJ (2021) Cloning and expression analyses of ribosomal protein *S24* (*RPS24*) gene in *Procambarus clarkii*. *Freshwater Fish* **51**, 97–105.
10. Wang L, Wang H, Xue C, Zhu CK, Wu N, Chang GL, Li JL (2021) Molecular cloning and expression analysis of *Dmc* gene related to gonadal development in *Procambarus clarkii*. *Aquaculture Res* **52**, 1321–1326.
11. Fei JM, Shi LL, Li YH (2021) Cloning and expression analyses of sex-lethal gene in *Procambarus clarkii*. *J Huazhong Agric Univ* **40**, 120–128.
12. Shi LL, Chen JH, Shi RX, Fei JM, Zhang L, Li YH (2021) Molecular cloning and expression analysis of *PcDsx* in *Procambarus clarkii*. *J Huazhong Agric Univ* **40**, 129–136.
13. Ge HL (2022) Functional study on RNA interference on *IAG* gene of *Procambarus clarkii* by dsRNA and siRNA. Wuhan, Huazhong Agricultural University, China.
14. Cao Y, Yan X, Bai X, Tang F, Si PH, Bai C, Tuoheti K, Guo LF, et al (2022) *UCHL5* promotes proliferation and migration of bladder cancer cells by activating c-Myc via AKT/mTOR signaling. *Cancers* **14**, 5538.
15. Jia XB, Li Q (2020) Research advances on deubiquitinating enzymes involved in the development of hepatocellular carcinoma. *Chin J Clin Oncol* **47**, 260–264.
16. De ZY, Yang H, Shao KB, Wei JL, Jun MH, Jiang J (2022) DUB3 contributes to colorectal cancer cell migration andangiogenesis via NF-KB/HIF-1α. *ScienceAsia* **48**, 359–366.
17. Lin Y, Pei P, Wang S (2019) Progress in histone ubiquitination and deubiquitination with chromatin homeostasis and gene expression regulation. *Prog Modern Biomed* **19**, 1578–1582.
18. Han KH, Dai YB, Zhang ZP, Zou ZH, Wang YL (2018) Molecular characterization and expression profiles of Sp-uchl3 and Sp-uchl5 during gonad development of *Scylla paramamosain*. *Molecules* **23**, 213.

19. Song GT, He JX, Wu BL, Chen J, Huang L, Wang X, Wu S (2018) Study on development of female reproductive system and histological structure of *Procambarus clarkii*. *Acta Agric Jiangxi* **30**, 68–75.
20. Gao W, Dai Q, Zhang P, Song CY, Zhu SS, Lai XF, Gao H, Yan BL (2022) Molecular cloning of the ubiquitin carboxyl-terminal hydrolase isozyme L5 and its functional analysis during ovarian development in *Exopalaemon carinicauda*. *Prog Fish Sci* **43**, 163–171.
21. Shi BT, Qian ZJ, Wang H, Lu W, Niu DH, Li JL (2018) Cloning and expression analysis of ubiquitin-conjugating enzyme *E2* gene of *Procambarus clarkii*. *J Shanghai Ocean Univ* **27**, 814–824.
22. Ge JF (2018) Study on the function and mechanism of ubiquitin carboxyl-terminal hydrolase isozyme LS in the development of gliomas. Zhejiang University, China.
23. Xu YJ, Zhang Y, Shi Y, Ying HR, Wang WW, Hu TT, Song D, Song D, et al (2021) C90S and I93M mutations of ubiquitin C-terminal hydrolase L1 do not affect mouse oocyte maturation. *Academic J Nav Med Univ* **42**, 1217–1223.
24. Mtango NR, Sutovsky M, Vandevoort CA, Latham KE, Sutovsky P (2012) Essential role of ubiquitin C-terminal hydrolases UCHL1 and UCHL3 in mammalian oocyte maturation. *J Cell Physiol* **227**, 2022–2029.
25. Zhou N, Wang YK, Hong KH, Wei J, Yu LY, Zhu XP (2022) Molecular cloning, expression profile and subcellular localization of nanos1 gene from *Macrobrachium rosenbergii*. *South China Fish Sci* **18**, 52–59.
26. Tan HY, Shao GM, Kang PF, Wang YF (2017) Full-length normalization subtractive hybridization analysis provides new insights into sexual precocity and ovarian development of red swamp crayfish *Procambarus clarkii*. *Aquaculture* **468**, 417–427.
27. Kang PF, Mao B, Fan C, Wang YF (2019) Transcriptomic information from the ovaries of red swamp crayfish (*Procambarus clarkii*) provides new insights into development of ovaries and embryos. *Aquaculture* **505**, 333–343.
28. Qian ZJ, Zhu TL, Jiang HC, Shi BT, Li JL (2016) Molecular cloning and expression analysis of ubiquitin-conjugating enzyme *E2r* in *Procambarus clarkii*. *J Shanghai Ocean Univ* **25**, 641–651.
29. Kluebsoongnoen J, Panyim S, Udomkit A (2020) Regulation of vitellogenin gene expression under the negative modulator, gonad-inhibiting hormone in *Penaeus monodon*. *Comp Biochem Physiol A Mol Integr Physiol* **243**, 110682.
30. Shui Y, Guan ZB, Zhao CY, Xu ZH, Zhou X (2013) Comparative proteomic studies on the ovarian development stages III and IV of *Procambarus clarkii*. *JAS* **41**, 179–183.

Appendix A. Supplementary data

Table S1 Details of UCHL5 gene primers.

Primer	Sequence (5′–3′)	Application
UCHL5-F	CCGCCAGTGTTGACAAGACAA	RT-PCR
UCHL5-R	GAGAATTTTGCAGTTTATTTA	
UCHL5-QF	GTGCCACCCAAGCAATCCTA	qPCR
UCHL5-QR	TGTGGACGTTGCGAATGGTA	
18SrRNA-F	TGCATCACGTCTCTGACCGC	RT-PCR
18SrRNA-R	TCGCAGTAGTTCGTCTTGCG	
18SrRNA-QF	CTGCGACGCTAGAGGTGAAA	qPCR
18SrRNA-QR	GGATCGCTAGTTGGCATCGT	
UCHL5-Pro	TGTTTTCCTCAGACTCCAATGTTGCTC GGTGAGCCCAAGGTAGTCAGGATAGG TTGTAGGTTTTCTCGTAGCACTTCTCGT	Probe of CISH