Genetics and Molecular Research

Molecular characterization and expression analysis of the β-actin gene from the ridgetail white prawn *Exopalaemon carinicauda*

J.P. Liang[1,2], Y. Wang[3], Q.Q. Ge[2], J.T. Li[2], P. Liu[2], J. Li[2] and G.X. Nie[1]

[1]Engineering Technology Research Center of Henan Province for Aquatic Animal Cultivation, College of Fisheries, Henan Normal University, Xinxiang, Henan, China
[2]Key Laboratory of Sustainable Development of Marine Fisheries, Ministry of Agriculture, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, Shandong, China
[3]Key Laboratory of South China Sea Fishery Resources Exploitation & Utilization, Key Laboratory of Aquatic Product Processing, Ministry of Agriculture, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, Guangdong, China

Corresponding author: J. Li
E-mail: lijian@ysfri.ac.cn

Genet. Mol. Res. 15 (2): gmr.15026872
Received July 20, 2015
Accepted January 4, 2016
Published April 25, 2016
DOI http://dx.doi.org/10.4238/gmr.15026872

ABSTRACT. Actin is a highly conserved protein that is found in all eukaryotic cells, and has been widely used as an internal control gene in gene expression studies. In this study, we cloned an actin gene (named *Ecβ-actin*) from *Exopalaemon carinicauda* and determined its expression levels. The full-length cDNA of *Ecβ-actin* was 1335 bp long, comprising a 1131-bp ORF encoding 376 amino acids, a 65-bp 5'-UTR, and a 139-bp 3'-UTR with a poly(A) tail. The A + T content was approximately 79% in the 3'-UTR of the *Ecβ-actin* mRNA. The 3'-UTR contained two repeats of the AUUUA motif. The putative protein Ecβ-actin showed high identity (97-99%) with other actins

from various species. Phylogenetic analysis revealed that Ecβ-actin belongs to Crustacea, although it formed a singleton sub-branch that was located a short distance from crabs and other shrimp species. *Ecβ-actin* expression was detected in the hepatopancreas, ovary, muscle, gill, stomach, and hemocytes, and was strongly expressed in the hemocytes and ovary of *E. carinicauda*. *Ecβ-actin* mRNA expression varied during ovarian development, with high levels observed at stages I and V. Therefore, caution should be taken when using the *Ecβ-actin* gene as an endogenous control gene.

Key words: *Exopalaemon carinicauda*; β-actin; Ovarian development; Internal control; Gene expression

INTRODUCTION

Actin, an important contractile protein in all eukaryotic cells, plays a key role in various cellular functions, including the maintenance of cytoskeletal structure, cell motility, cell division, intracellular movements, contractile processes, and transcription (Xiao et al., 2011). Actins, especially β-actin, are widely used as housekeeping genes because their expression is stable, making them suitable internal controls that can be used to normalize gene expression (Stürzenbaum and Kille, 2001). However, recent studies have shown that β-actin expression might change in response to biochemical stimuli during growth and differentiation, and in some disease states (Ruan and Lai, 2007). These findings challenge the reliability of β-actin when used as a reference gene.

β-actin genes have been studied in some fish species, such as *Epinephelus coioides* (Zhang et al., 2005), *Acipenser baerii* (Shi et al., 2010), and *Oreochromis aureus* (Hu et al., 2011). In addition, actin genes have been reported in crustaceans, including *Homarus gammarus* (Harrison and el Haj, 1994), *Gecarcinus lateralis* (Varadaraj et al., 1996), *Tigriopus japonicus* (Kim et al., 2003), *Macrobrachium rosenbergii* (Zhu et al., 2005b), *Litopenaeus vannamei* (Sun et al., 2007), *Sinopotamon yangtsekiense* (Gao et al., 2011), and *Scylla paramamosain* (Huang et al., 2012; Xu et al., 2013). The reliability of β-actin as an internal control was also discussed by these authors; however, few studies on the β-actin gene, and its suitability as a reference gene in *Exopalaemon carinicauda* have been reported.

The ridgetail white prawn *E. carinicauda* supports a large number of polyculture ponds in eastern China. However, the artificial-breeding technique for *E. carinicauda* is not advanced, which limits the further development of *E. carinicauda* cultures (Liang et al., 2014). Therefore, many studies have focused on gonadal development and immunity, including developmental and immune-related genes, which have been cloned and expressed using 18S as the reference gene (Li et al., 2012; Duan et al., 2013; Xu et al., 2014). Whether β-actin can be used as a reference gene in *E. carinicauda* is unknown as it has not yet been reported. The aim of the present study was to: 1) clone and characterize the β-actin gene (named *Ecβ-actin*) from *E. carinicauda*; 2) compare the β-actin amino acid sequence with β-actins known from other species; 3) analyze the pattern of *Ecβ-actin* mRNA expression in different tissues; 4) evaluate the *Ecβ-actin* expression profile in the ovary during ovarian development stages. These results are essential for determining whether *Ecβ-actin* can be used as an endogenous control gene in this species.

MATERIAL AND METHODS

Shrimp and culture conditions

Female *E. carinicauda* were obtained from a commercial farm in Qingdao, China. The average weight of the shrimp was 1.46 ± 0.51 g. Shrimp were cultivated in aerated seawater (salinity 30) for 7 days at 18°-22°C before the test.

Ovarian development was divided into five stages (Stages I-V) on the basis of the color and size of the ovary as observed through the external carapace (Li et al., 2014).

RNA preparation and first-strand cDNA synthesis

Total RNA was extracted from the hepatopancreas following homogenization in TRIzol Reagent (Invitrogen) following the protocol of the manufacturer. Then, RNA was analyzed on 1.5% agarose gels to determine its quantity by electrophoresis, and the purity and integrity was determined spectrophotometrically (A_{260}/A_{280}). First-strand cDNA was synthesized from 5 μg total RNA extracted from the hepatopancreas using M-MLV reverse transcriptase (Promega) according to the manufacturer instructions.

cDNA cloning for β-actin

A partial *Ecβ-actin* cDNA fragment was obtained using a pair of degenerate primers, F1 and R1 (Table 1), which were designed by the Primer Premier 5.0 program, according to the sequence alignment result of five cDNA sequences of β-actin from *M. rosenbergii* (GenBank accession No. AY626840), *Fenneropenaeus chinensis* (GenBank accession No. AY871269), *Eriocheir sinensis* (GenBank accession No. HM053699), and *L. vannamei* (GenBank Accession No. AY646096) using the DNAMAN software. The polymerase chain reaction (PCR) conditions were as follows: 94°C for 5 min, 30 cycles of 94°C for 30 s, 58.1°C for 30 s, 72°C for 1 min, 72°C for 10 min, and cooling to 4°C. Having isolated the partial *Ecβ-actin* sequence, the 5'- and 3'-ends of the mRNA were obtained by RACE using the gene-specific primers shown in Table 1. The PCR cycle was as follows: 94°C for 3 min, five cycles of 94°C for 30 s and 72°C for 3 min, five cycles of 94°C for 30 s, 70°C for 30 s, and 72°C for 3 min, 25 cycles of 94°C for 30 s, 68°C for 30 s and 72°C for 3 min, one cycle of 72°C for 10 min, then cooling to 4°C.

Table 1. Primers used for cDNA cloning and qPCR.

Primer name	Sequence (5'→3')
F1 (forward)	CACGGYATCATCACCAACTGGGA
R1 (reverse)	ATYTCCTTCTGCATGCGGTCAGC
3'-RACE	ACGGTCAGGTCATCACCATCGGTAACG
5'-RACE	CCATGTCGTCCCAGTTGGTGATGATAC
UPM	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT
Actin-F	CCGAGACATCAAGGAGAAGC
Actin-R	ATACCGCAAGATTCCATACCC
18S-F	GGGGAGGTAGTGACGAAAAAT
18S-R	TATACGCTAGTGGAGCTGGAA

The amplified PCR products were isolated from 1% agarose gel and the target band was purified using a PCR purification kit (Promega), ligated into a PMD_{18-T} vector (TaKaRa), and transformed in *Escherichia coli* TOP10' cells (Invitrogen). Recombinant bacteria were identified by blue/white screening and confirmed by PCR. The plasmids from several positive colonies were purified and sequenced.

Sequence and phylogenetic analysis

The amino acid sequence, molecular weight, and isoelectric point of the Ecβ-actin protein were deduced using DNASTAR. The deduced amino acid sequence of *Ecβ-actin* cDNA was compiled with other known β-actin sequences using the BLAST program in GenBank (http://www.blast.ncbi.nlm.nih.gov/Blast). Multiple-amino acid sequence alignments were carried out with DNAMAN. The phylogenetic tree was constructed with MEGA 4.0.

Expression profiles of *Ecβ-actin*

Total RNA was obtained from various tissues (hemocytes, gill, stomach, ovary, hepatopancreas, and muscle) of identical mature female *E. carinicauda.* RNA was extracted from the ovaries of three animals at ovarian stages I-V. Extracted total RNA was digested using RNase-free DNAse to remove any contamination. The RNA quantity was measured and the samples were made to the same concentration. First-strand cDNA was synthesized with 5 mg total RNA as described above.

The relative mRNA level of the *Ecβ-actin* gene was determined using an ABI 7500 real-time PCR system and SYBR^R Green Premix Ex Taq (TaKaRa). The PCR mixtures contained 5 μL SYBR^R Premix Ex Taq^{TM} II (2X) (TaKaRa), 0.4 μL each primer, 0.2 μL 50X ROX Reference Dye II, 1 μL cDNA, and 3 μL DEPC water. *Ecβ-actin* mRNA was amplified using the actin-F and actin-R primers, and 18S, was amplified using the 18S-F and 18S-R primers (GenBank accession No. HQ172894) to normalize the levels of expression between samples. The real-time quantitative PCR conditions were: 40 cycles of 95°C for 5 s, 60°C for 34 s, and 72°C for 34 s. Relative gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Gene expression levels were subjected to one-way analysis of variance (ANOVA) by SPSS 13.0, followed by the Duncan test to test for differences among the different samples. Significance was set at $P < 0.05$ (González-Rodríguez et al., 2012).

RESULTS

Molecular characterization of the *Ecβ-actin*

The partial *Ecβ-actin* cDNA fragment of 734 bp, and the full-length *Ecβ-actin* cDNA obtained by RACE are shown in Figure 1. The *Ecβ-actin* cDNA was 1335 bp long, including 65 bp of a 5'-untranslated region (5'-UTR), a 1131-bp open-reading frame (ORF) encoding 376 amino acids, and 139 bp of a 3'-UTR with a poly(A) tail. An ATG codon began at nucleotide 32 and a stop codon (TAA) began at nucleotide 1196. The codon usage of *Ecβ-actin* showed a 52% preference for C or G at the third position. A high A + T content of approximately 79% was observed at the 3'-UTR of *Ecβ-actin* cDNA. The 3'-UTR of the *Ecβ-actin* cDNA also

contained two repeats of the AUUUA sequence and AREs, which were located at nucleotide 1222-1226 and 1292-1296, respectively (Figure 2). The molecular mass of the Ecβ-actin protein was calculated as approximately 41,865 Da, and the estimated isoelectric point was 5.29. The full-length cDNA sequence of *Ecβ-actin* has been deposited at GenBank (accession No. JQ045354).

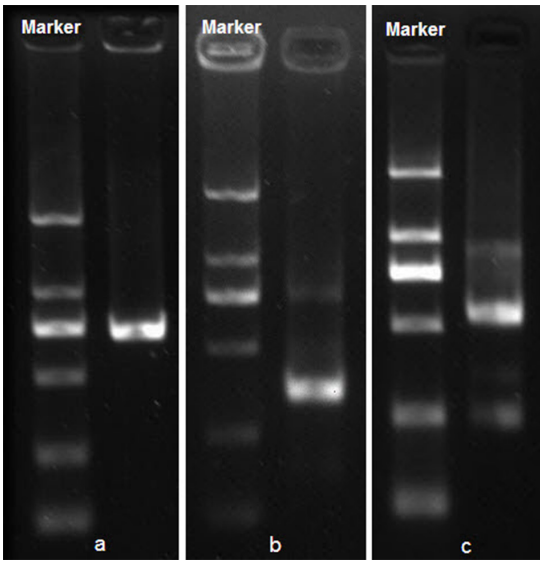

Figure 1. Electrophoretograms of *Ecβ-actin* fragments. **a.** Degenerate primer PCR. **b.** 5'-RACE PCR. **c.** 3'-RACE PCR.

```
   1 ACGCGGGGAG TGTACTTCAG CCTCAGGATA CGTCGTGACC TTTGTTAACG GCCTAAATCA CCAAA ATG TGT GAC GAA GAC GCA ACA GCC CTC
                                                                              M   C   D   E   D   A   T   A   L
  93 GTT GTT GAC AAT GGC TCC GGC ATG GTC AAG GCC GGT TTC GCC GGT GAT GAC GCC CCT CGC GCC GTC TTC CCT TCC ATC GTC
      V   V   D   N   G   S   G   M   V   K   A   G   F   A   G   D   D   A   P   R   A   V   F   P   S   I   V
 174 GGT CGC CCT CGT CAC CAG GGT GTG ATG GTC GGT ATG GGT CAG AAG GAC TCT TAT GTC GGC GAT GAG GCC CAG AGC AAG AGA
      G   R   P   R   H   Q   G   V   M   V   G   M   G   Q   K   D   S   Y   V   G   D   E   A   Q   S   K   R
 255 GGT ATC CTC ACC CTA AAG TAC CCA ATT GAG CAC GGT ATC GTA ACT AAC TGG GAT GAC ATG GAG AAG ATT TGG CAT CAT ACC
      G   I   L   T   L   K   Y   P   I   E   H   G   I   V   T   N   W   D   D   M   E   K   I   W   H   H   T
 336 TTC TAC AAT GAA CTT CGT GTT GCC CCC GAG GAG CAC CCG GTA CTC CTG ACA GAG GCT CCC TTG AAC CCT AAG GCT AAC AGG
      F   Y   N   E   L   R   V   A   P   E   E   H   P   V   L   L   T   E   A   P   L   N   P   K   A   N   R
 417 GAG AAG ATG ACC CAG ATT ATG TTT GAA ACT TTC AAC ACC CCA GCC ATG TAC GTC GCC ATC CAG GCT GTG CTG TCC CTG TAT
      E   K   M   T   Q   I   M   F   E   T   F   N   T   P   A   M   Y   V   A   I   Q   A   V   L   S   L   Y
 498 GCC TCT GGT CGT ACC ACT GGT ATC GTG CTG GAC TCT GGA GAT GGC GTC TCC CAC ACT GTG CCC ATT TAT GAG GGA TAT GCG
      A   S   G   R   T   T   G   I   V   L   D   S   G   D   G   V   S   H   T   V   P   I   Y   E   G   Y   A
 579 CTT CCT CAC GCC ATC CTC CGT CTG GAC TTG GCA GGA CGT GAC TTA ACA GAC TAC CTC ATG AAG ATC CTT ACC GAA CGT GGC
      L   P   H   A   I   L   R   L   D   L   A   G   R   D   L   T   D   Y   L   M   K   I   L   T   E   R   G
 660 TAC ACA TTC ACT ACC ACT GCT GAG CGA GAA ATC GTC CGA GAC ATC AAG GAG AAG CTC TGC TAT GTT GCC CTG GAC TTC GAG
      Y   T   F   T   T   T   A   E   R   E   I   V   R   D   I   K   E   K   L   C   Y   V   A   L   D   F   E
 741 CAG GAA ATG ACC ACT GCC GCC TCC TCC TCC CTT GAG AAG TCC TAC GAG CTT CCC GAC GGT CAG GTC ATC ACT ATT GGT
      Q   E   M   T   T   A   A   S   S   S   S   L   E   K   S   Y   E   L   P   D   G   Q   V   I   T   I   G
 822 AAC GAG AGG TTT AGG TGC CCC GAA GCT CTC TTC CAA CCA TCC TTC CTG GGT ATG GAA TCT TGC GGT ATC CAC GAG ACT ACT
      N   E   R   F   R   C   P   E   A   L   F   Q   P   S   F   L   G   M   E   S   C   G   I   H   E   T   T
 903 TAC AAC TCA ATC ATG AAA TGC GAC GTA GAC ATC CGT AAG GAT TTG TAT GCC AAC ACT GTC CTG TCT GGA GGC ACC ACA ATG
      Y   N   S   I   M   K   C   D   V   D   I   R   K   D   L   Y   A   N   T   V   L   S   G   G   T   T   M
 984 TAC CCA GGA ATT GCT GAC AGA ATG CAG AAG GAA ATC ACT GCC CTG GCA CCT TCC ACC ATG AAG ATT AAG ATC ATC GCA CCT
      Y   P   G   I   A   D   R   M   Q   K   E   I   T   A   L   A   P   S   T   M   K   I   K   I   I   A   P
1065 CCC GAG CGC AAA TAC TCC GTC TGG ATC GGC GGA TCT ATC TTG GCC TCT CTA TCC ACA TTC CAG CAG ATG TGG ATT AGC AAA
      P   E   R   K   Y   S   V   W   I   G   G   S   I   L   A   S   L   S   T   F   Q   Q   M   W   I   S   K
1146 CAA GAA TAT GAT GAG TCT GGA CCA TCC ATT GTA CAC AGG AAA TGC TTC TAA GTCAATATCT TTATTTTTGC TTAAAATTTAGATTAAG
      Q   E   Y   D   E   S   G   P   S   I   V   H   R   K   C   F   *
1234 TGTGTAGATTACA CGCAGATTTT CTATATAAAT TCTACACTTA ATTATAAGGA TGCTAATTTAATTAAATTAATTTTGCAAAAAAAAAAAAAAAAAAAAAAA
```

Figure 2. Nucleotide and deduced amino acid sequences of β-actin from *Exopalaemon carinicauda*. The start and stop codons are indicated by open boxes. A consensus polyadenylation signal is underlined. The sequences complementary to AU-rich elements of the mRNA are shaded.

Homology analysis of *Ecβ-actin*

Bioinformatic analyses revealed that Ecβ-actin shares high identity with the β-actins of invertebrates and vertebrates (Table 2). Compared to other known β-actins, they are highly conserved (Figure 3).

Table 2. Sequence identity of the *Exopalaemon carinicauda* β-actin protein with other known β-actin proteins.

Species	Identity (%)	GenBank accession No.
Macrobrachium rosenbergii	99	AAU04441.1
Litopenaeus vannamei	99	AAG16253.1
Fenneropenaeus chinensis	99	ABB05541.1
Eriocheir sinensis	98	ADH43622.1
Aedes albopictus	98	ABG46341.1
Rachycentron canadum	98	ABX71624.1
Oncorhynchus mykiss	97	NP_001117707.1
Bos taurus	97	AAI42414.1
Capra hircus	97	AFN25402.1

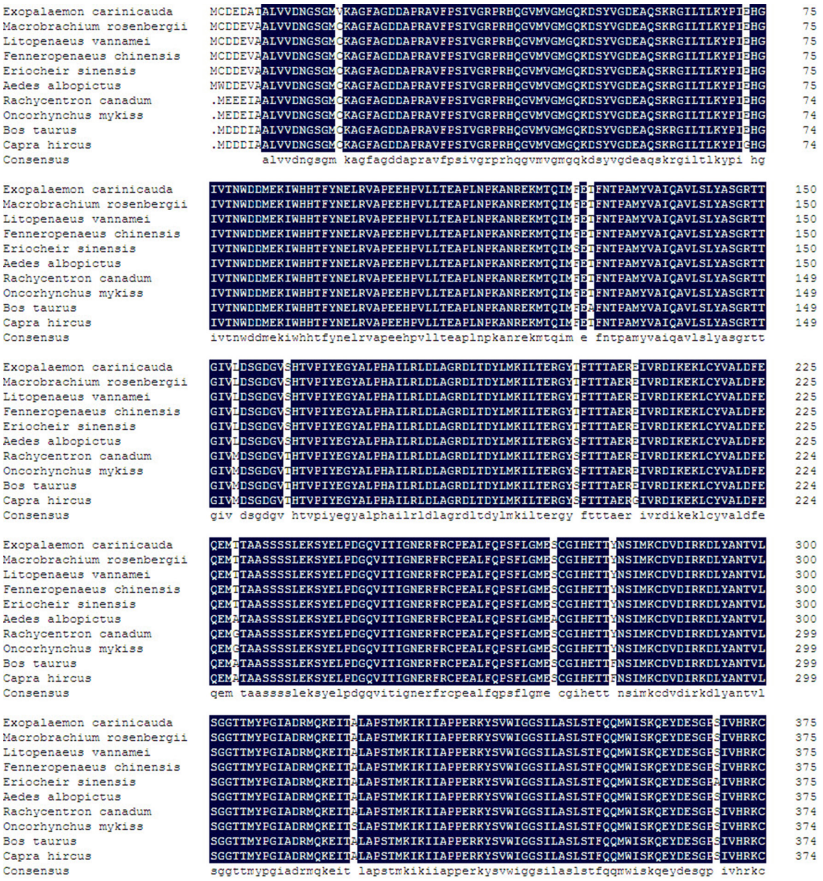

Figure 3. Multiple alignment of the deduced amino acid sequence of β-actin from *Exopalaemon carinicauda.*

Phylogenetic analysis of *Ecβ-actin*

A molecular phylogenetic tree was constructed using MEGA 4.0 (Figure 4) based on the amino acid sequences of β-actin. β-actin proteins from 24 species were divided into two distinct branches in an unrooted tree containing invertebrates and vertebrates. Along the invertebrate branch, shrimps, crabs, and insects formed distinct groups. Along the second branch of vertebrates, all fish and terrestrial animals were clustered together and formed two small branches, respectively. The phylogenetic analysis was consistent with traditional taxonomy. Ecβ-actin should belong to Crustacea, but it was a singleton sub-branch, a short distance away from crabs and other shrimp species.

Figure 4. Phylogenetic tree of β-actin amino acid sequences constructed by the neighbor-joining method. *Scylla paramamosain* (ADG03642.1), *Polyrhachis vicina* (ACX37099.1), *Gallus gallus* (CAA25004.1), *Acanthopagrus schlegelii* (AAR84618.1), *Carassius auratus* (BAA92339.2), *Struthio camelus* (AIE38011.1), *Paramisgurnus dabryanus* (AFA41501.1), *Drosophila melanogaster* (AAA28314.1), *Anas platyrhynchos* (ABR87937.1), *Platichthys flesus* (AAF63665.1), *Cirrhinus molitorella* (AAY25518.1), *Sus scrofa* (AAS55927.1), *Phascolarctos cinereus* (AAY53911.1), *Ornithodoros moubata* (BAE46505.1), *Aedes albopictus* (ABG46341.1), *Bos taurus* (AAI42414.1), *Capra hircus* (AFN25402.1), *Eriocheir sinensis* (ADH43622.1), *Fenneropenaeus chinensis* (ABB05541.1), *Litopenaeus vannamei* (AAG16253.1), *Macrobrachium rosenbergii* (AAU04441.1), *Oncorhynchus mykiss* (NP_001117707.1), *Rachycentron canadum* (ABX71624.1), *Exopalaemon carinicauda* (AEY68535.1).

Tissue expression of the *Ecβ-actin* transcript

Levels of *Ecβ-actin* expression in different tissues are shown in Figure 5. mRNA transcripts of *Ecβ-actin* were widely detected in all tested tissues including the hemocytes, gill, stomach, ovary, hepatopancreas, and muscle. The highest expression was observed in the hemocytes, in which levels 2-90 times higher than those in other tissues were observed ($P < 0.05$). *Ecβ-actin* was expressed at moderately high levels in the ovary, and the lowest expression was found in the hepatopancreas, muscle, gill, and stomach.

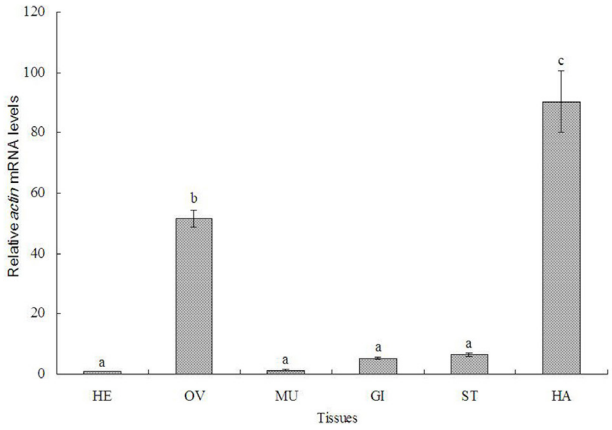

Figure 5. *Ecβ-actin* transcripts in hepatopancreas (HE), ovary (OV), muscle (MU), gill (GI), stomach (ST), and hemocytes (HA).

Expression of the *Ecβ-actin* transcript during ovarian maturation

Ecβ-actin cDNA expression in the ovary differed as the ovary developed. *Ecβ-actin* mRNA was expressed at high levels in the ovary at stages I and V, and at low levels at stages II, III, and IV (Figure 6).

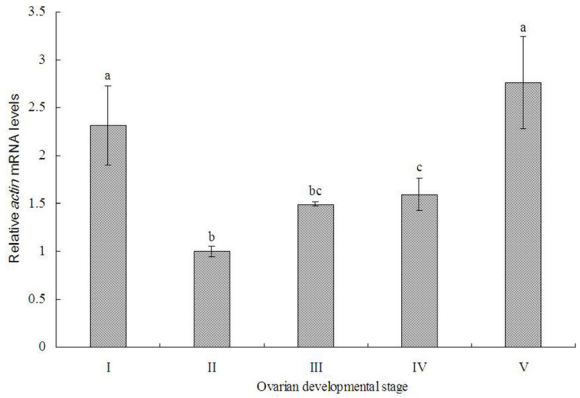

Figure 6. *Ecβ-actin* transcripts in the ovary of *Exopalaemon carinicauda* during maturation.

DISCUSSION

β-actin genes have previously been reported in other crustaceans. In the present study, *Ecβ-actin* was cloned, for the first time, from the ridgetail white prawn, *E. carinicauda*. Full-length *Ecβ-actin* cDNA was 1335 bp long, comprising a 1131-bp ORF that encoded 376 amino acids. The ORF of *Ecβ-actin* had a high G + C content (approximately 52%), reflecting a preference towards codons with G or C at the third position, while the 3'-UTR had an A + T content of approximately 79%. A similar preference has been found in *G. lateralis* (Varadaraj et al., 1996), *M. rosenbergii* (Zhu et al., 2005b), *L. vannamei* (Sun et al., 2007), and *S. paramamosain* (Huang et al., 2012). However, the G + C content of actin mRNA is low in Artemia and *H. americanus*, and is often replaced by either A or T (Macias and Sastre, 1990; Harrison and el Haj, 1994).

AUUUA sequences within the 3'-UTR of cDNA named AU-rich elements (AREs) might affect the stability of some mRNAs in the 3'-UTR (Brawerman, 1993). Two copies of the AUUUA sequence are present in the 3'-UTR of *Ecβ-actin* cDNA (Figure 1). AREs have been recognized as potent destabilizing elements in a wide variety of short-lived mRNAs, like those of proto-oncogenes and cytokines (Guhaniyogi and Brewer, 2001; Dearth and DeWille, 2003). The A + U-rich sequences in the 3'-UTR of *Ecβ-actin* mRNA are typical of AREs. The AUUUA sequences have been observed in the 3'-UTR of actin mRNA from other animals such as *G. lateralis* and *Sinonovacula constricta* (Varadaraj et al., 1996; Feng et al., 2011). The role of AREs in the 3'-UTR of *Ecβ-actin* mRNA needs further study.

Sequence analysis indicated that Ecβ-actin protein shares high identity (97-99%) with other vertebrate and invertebrate β-actins. Phylogenetic analysis revealed that β-actins can be divided into four distinct branches: terrestrial vertebrates, aquatic vertebrates, insects, and crustaceans. Ecβ-actin should belong to Crustacea, but it formed a singleton sub-branch, which was a short distance away from crabs and other shrimp species. The relationships between different animals as shown in the phylogenetic tree are in good agreement with the traditional classification scheme based on morphology.

In the present study, *Ecβ-actin* expression was observed in many tissues, including the hepatopancreas, ovary, muscle, gill, stomach, and hemocytes, with a higher level detected in the ovary and hemocytes compared with other tissues. Previous studies have demonstrated that β-actin is mainly expressed in the muscle tissue of other crustaceans, such as *H. americanus* (Harrison and el Haj, 1994), *M. rosenbergii* (Zhu et al., 2005b), and *S. paramamosain* (Xu et al., 2013). However, expression of β-actin is barely detectable in the skeletal muscle of *E. coioides* (Zhang et al., 2005). Therefore, *Ecβ-actin* might not be suitable as an endogenous control gene for use to normalize gene expression between different tissues.

Expression of the *Ecβ-actin* gene was analyzed across ovarian developmental stages and was found to be significantly higher at stages I and V than at other stages of ovarian development. In shrimp and crab, the oogonia pass through different maturation stages before becoming a mature oocyte. Mitosis mainly occurs in immature oocytes, while the mature oocytes are found in the metaphase of the first meiosis. This phenomenon indicates that the first meiosis occurs at maturation stage, and the second meiosis is completed after spawning (Yano, 1988; Minagawa and Sano, 1997; Zhu et al., 2005a). There is evidence that female *E. carinicauda* have the potential to carry more than one batch of eggs within a reproductive period. A large number of mitotic oogonia were also observed in stage I and V ovaries from *E. carinicauda*, while their daughter cells increased in size upon entering the first meiosis at the

maturation stage in ripe ovaries (Li et al., 2014). These results imply that the Ecβ-actin might be involved in oogonia mitosis, and is associated with SWI/SNF-like chromatin-remodeling complexes and histone modifiers (Chen and Shen, 2007). *Ecβ-actin* might not be suitable for use as an endogenous control gene during ovarian development of *E. carinicauda*.

In conclusion, we successfully cloned the full-length cDNA of β-actin from *E. carinicauda*. *Ecβ-actin* was found to be differentially expressed in the gill, stomach, hepatopancreas, ovary, muscle, and hemocytes. *Ecβ-actin* expression differed between ovarian developmental stages, and expression levels were higher at stages I and V than at other stages. *Ecβ-actin* gene might not be suitable for use as a reference gene in RT-PCR analyses of gene expression.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the Modern Agro-Industry Technology Research System (#CARS-47), the Special Fund for Agro-Scientific Research in the Public Interest (#201103034), the National Natural Science Foundation of China (#31502185), and the PhD Scientific Research Initiation Foundation of Henan Normal University (#QD13054).

REFERENCES

Brawerman G (1993). mRNA degradation in eukaryotic cells: an overview. Control of Messenger RNA Stability. Academic Press, San Diego, CA, 149-159.

Chen M and Shen X (2007). Nuclear actin and actin-related proteins in chromatin dynamics. *Curr. Opin. Cell Biol.* 19: 326-330. http://dx.doi.org/10.1016/j.ceb.2007.04.009

Dearth LR and DeWille J (2003). An AU-rich element in the 3' untranslated region of the C/EBP δ mRNA is important for protein binding during G_0 growth arrest. *Biochem. Biophys. Res. Commun.* 304: 344-350. http://dx.doi.org/10.1016/S0006-291X(03)00597-7

Duan Y, Liu P, Li J, Li J, et al. (2013). Immune gene discovery by expressed sequence tag (EST) analysis of hemocytes in the ridgetail white prawn *Exopalaemon carinicauda*. *Fish Shellfish Immunol.* 34: 173-182. http://dx.doi.org/10.1016/j.fsi.2012.10.026

Feng BB, Zhong YM, Niu DH, Chen H, et al. (2011). Molecular characteristics and expression analysis of β-actin 1 gene from *Sinonovacula constricta*. *J. Fish. China* 35: 650-659.

Gao AB, Wang L and Yuan H (2011). Amplification of cDNA and primary molecular phylogeny based on β-actin gene sequences of a freshwater crab, *Sinopotamon yangtsekiense*. *J. Shanghai Ocean Univ.* 20: 173-178.

González-Rodríguez G, Colubi A and Gil MÁ (2012). Fuzzy data treated as functional data: A one-way ANOVA test approach. *Comput. Stat. Data Anal.* 56: 943-955. http://dx.doi.org/10.1016/j.csda.2010.06.013

Guhaniyogi J and Brewer G (2001). Regulation of mRNA stability in mammalian cells. *Gene* 265: 11-23. http://dx.doi.org/10.1016/S0378-1119(01)00350-X

Harrison P and el Haj AJ (1994). Actin mRNA levels and myofibrillar growth in leg muscles of the European lobster (*Homarus gammarus*) in response to passive stretch. *Mol. Mar. Biol. Biotechnol.* 3: 35-41.

Hu HY, Jia YY and Tang YK (2011). Cloning and gene construction analysis of β-actin in *Oreochromis aureus*. *J. Shanghai Ocean Univ.* 20: 185-190.

Huang JR, Huang HY, Ye HH and Li SJ (2012). Cloning and Analysis of β-actin Gene from *Scylla paramamosain*. *J. Xiamen Uni*. (*Nat. Sci*.) 51: 274-279.

Kim IC, Kim YJ, Song SJ, Lee JS, et al. (2003). The intertidal harpacticoid copepod *Tigriopus japonicus* (Crustacea: Copepoda) beta-actin gene: cloning, sequence and intraspecies variation. *DNA Seq.* 14: 279-284. http://dx.doi.org/10.1080/1085566031000141135

Li J, Han J, Chen P, Chang Z, et al. (2012). Cloning of a heat shock protein 90 (HSP90) gene and expression analysis in the ridgetail white prawn *Exopalaemon carinicauda*. *Fish Shellfish Immunol.* 32: 1191-1197. http://dx.doi.org/10.1016/j.fsi.2012.03.008

Li ZG, Zhang CS, Li FH and Xiang JH (2014). Histological study on the gonadal development of *Exopalaemon carinicauda* (Holthuis, 1950). *J. Fish. China* 38: 362-370.

Liang JP, Li J, Li JT, Liu P, et al. (2014). Accumulation and elimination of enrofloxacin and its metabolite ciprofloxacin in the ridgetail white prawn *Exopalaemon carinicauda* following medicated feed and bath administration. *J. Vet. Pharmacol. Ther.* 37: 508-514. http://dx.doi.org/10.1111/jvp.12115

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct\ method}$. *Methods* 25: 402-408. http://dx.doi.org/10.1006/meth.2001.1262

Macias MT and Sastre L (1990). Molecular cloning and expression of four actin isoforms during Artemia development. *Nucleic Acids Res.* 18: 5219-5225. http://dx.doi.org/10.1093/nar/18.17.5219

Minagawa M and Sano M (1997). Oogenesis and ovarian development cycle of the spiny lobster *Panulirus japonicus* (Decapoda: Palinuridae). *Mar. Freshw. Res.* 48: 875-888. http://dx.doi.org/10.1071/MF97170

Ruan W and Lai M (2007). Actin, a reliable marker of internal control? *Clin. Chim. Acta* 385: 1-5. http://dx.doi.org/10.1016/j.cca.2007.07.003

Shi ZY, Cheng QQ and Song JK (2010). Cloning and sequence analysis of *Siberian sturgeon* full-length β-actin gene cDNA and application of genes as an internal reference. *J. Fish. Sci. China* 17: 1173-1182.

Stürzenbaum SR and Kille P (2001). Control genes in quantitative molecular biological techniques: the variability of invariance. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 130: 281-289. http://dx.doi.org/10.1016/S1096-4959(01)00440-7

Sun PS, Soderlund M, Venzon NC, Jr., Ye D, et al. (2007). Isolation and characterization of two actins of the Pacific white shrimp, *Litopenaeus vannamei*. *Mar. Biol.* 151: 2145-2151. http://dx.doi.org/10.1007/s00227-007-0646-8

Varadaraj K, Kumari SS and Skinner DM (1996). Actin-encoding cDNAs and gene expression during the intermolt cycle of the Bermuda land crab *Gecarcinus lateralis*. *Gene* 171: 177-184. http://dx.doi.org/10.1016/0378-1119(96)00018-2

Xiao X, Li M, Wang K, Qin Q, et al. (2011). Characterization of large yellow croaker (*Pseudosciaena crocea*) β-actin promoter supports β-actin gene as an internal control for gene expression modulation and its potential application in transgenic studies in fish. *Fish Shellfish Immunol.* 30: 1072-1079. http://dx.doi.org/10.1016/j.fsi.2011.02.008

Xu WF, Liu P, Li JT, Li J, et al. (2014). Cloning and expression of gene vasa during ovarian development cycle in *Exopalaemon carinicauda*. *Oceanol. Limnol. Sin.* 45: 574-582.

Xu Z, Ma HY, Ma CY, Feng NN, et al. (2013). Cloning and tissue expression of β-actin in the mud crab (*Scylla paramamosain*) and its utility as an endogenous control. *Biotechnol. Bull.* 8: 105-112.

Yano I (1988). Oocyte development in the kuruma prawn *Penaeus japonicus*. *Mar. Biol.* 99: 547-553. http://dx.doi.org/10.1007/BF00392562

Zhang WM, Zhang Y, Zhang LH, Wang SG, et al. (2005). Nucleotide sequence and mRNA expression analysis of β-actin gene in the orange-spotted grouper *Epinephelus coioides*. *Fish Physiol. Biochem.* 31: 373-383. http://dx.doi.org/10.1007/s10695-005-4743-7

Zhu DF, Wang CL and Yu HW (2005a). Oogenesis, oocyte activation and early cleavage of *Portunus trituberculatus* (miers). *Oceanol. Limnol. Sin.* 36: 423-429.

Zhu XJ, Dai ZM, Liu J and Yang WJ (2005b). Actin gene in prawn, *Macrobrachium rosenbergii*: characteristics and differential tissue expression during embryonic development. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 140: 599-605. http://dx.doi.org/10.1016/j.cbpc.2004.12.010