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Transcriptome analysis of the brain of the Chinese mitten crab, *Eriocheir sinensis*, for neuropeptide abundance profiles during ovarian development

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ABSTRACT

Neuropeptides, important messenger molecules, regulate various physiological processes, such as growth, development, and reproduction. In the present study, cDNA libraries from brains of *E. sinensis* were constructed and sequenced using the Illumina technique for transcript analysis and neuropeptides discovery. There were 233,887 transcripts assembled for 194,286 unigenes. According to the annotations of NCBI non-redundant protein (NR) database, 2487 (11.31%) unigenes were annotated successfully. In total, 1273 transcripts were assigned to the "signal transduction mechanisms" category using KOG analysis. The results of KEGG indicate signal transduction and translation pathways were the dominant and enriched signal pathways. Additionally, results indicated C2H2 was the main transcription factor (TF) family. Analysis of the assembled transcripts indicated there were 22 neuropeptide transcripts, such as allatostatin, crustacean female sex hormone, crustacean hyperglycemic hormone, diuretic hormone 31, and eclosion hormone. The detection of these neuropeptides provide for a basic understanding for future study of functions in development, reproduction, and sexual maturation in crustaceans.

1. Introduction

Neurosecretory cells produce various neuropeptides, which have important regulatory effects on biological functions (Jadhav et al., 2001). Normally, the neuropeptides synthesized and secreted by NSC have important functions in growth, oocyte development and ovarian maturation (Wang et al., 2016). Additionally, neuropeptides are important messenger molecules and function as neurotransmitters and modulators (Huybrechts et al., 2003). For example, gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide, regulating the reproductive function in the hypothalamo-pituitary-gonadal axis (Okubo and Nagahama, 2008; Nagasawa et al., 2017).

The crustacean eyestalk functions as an important neuroendocrine organ complex to regulate many physiological processes, including molting, growth, and reproduction. An eyestalk cDNA library has been constructed in many crustacean species and many neuropeptides have been identified, such as crustacean hyperglycemic hormone (CHH), molt-inhibiting hormone (MIH), and pigment dispersing hormone (PDH) (Yamano and Unuma, 2006; Ventura-López et al., 2017). The brain is also an important endocrine organ.

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The brain of crustaceans not only has a role of neuro-modulation, but also controls the release of hormones (Kulkarni et al., 1991). Many neuropeptides, such as allatostatin (AST), neuropeptide F (NPF), CHH, crustacean cardioactive peptide (CCAP), diuretic hormone 31 (DH31), and PDH have been found in the brain of various crustaceans, including the mud crab (*Scylla paramamosain*) (Bao et al., 2015), oriental river prawns (*Macrobrachium nipponense*) (Qiao et al., 2017), and mantis shrimp (*Neogonodactylus oerstedii*) (Donohue et al., 2017). In *S. paramamosain*, most of the predicted neuropeptides were in differential abundance in the cerebral ganglia at various vitellogenic stages, suggesting that these peptides are likely involved in vitellogenesis and ovarian maturation (Bao et al., 2015). In addition, by assessing the comparative transcriptomes of the eyestalk and cerebral ganglia from female *M. nipponense*, some biological functions related to reproduction were also predicted, such as photoreceptor activity and cuticle proteins (Qiao et al., 2017). Thus, brain neuropeptides should also be considered when studying reproduction and growth in crustaceans.

The Chinese mitten crab (*Eriocheir sinensis*), a euryhaline species native to East Asia, is one of the most important aquaculture crustaceans in China (Wang et al., 2006). Hence, controlling the crab's development and reproduction would be desirable. Growing evidence has indicated that the complex life history and breeding characteristics are closely associated with the neuropeptides (Morishita et al., 2010; Hiroshi et al., 2011). Thus, to control gonadal development in this species, individual maturity and reproductive behavior need to be understood as related to the brain neuropeptides of this crab.

In the present study, a brain transcriptome of *E.sinensis* was interrogated to identify and annotate the neuropeptide genes. To better predict the physiological functions, data were assessed using bioinformatics analysis. There was a focus on the neuropeptides and relevant receptors involved in gonadal maturity and reproduction. In addition, neuropeptide abundance profiles were analyzed so as to reveal its potential regulatory effects on ovarian development. The transcriptomic analysis provided basic data for further research on the regulatory mechanism in the reproductive neuroendocrine pathway.

2. Materials and methods

2.1. Animals and tissue collection

Healthy adult Chinese mitten crabs (150–200 g) were obtained from a local fisheries market and transported to the research laboratory. Three of the adult crabs were randomly selected and placed in an ice bath for 1 to 2 min until being anesthetized. Subsequently, brains were removed surgically, immediately frozen in liquid nitrogen, and then stored at −80 °C until required for library construction. In addition, early (EO) and late developing (LO) ovaries (Fang and Qiu, 2009), as well as different stages of ovarian development corresponding with early (EB) and late brain (LB) development were collected for RNA extraction.

2.2. RNA extraction

Total RNA was extracted using QIAzol Lysis Reagent (QIAGEN, China) according to the manufacturer's instructions. The extracted RNA was monitored on 1% agarose gels to detect its integrity. The RNA purity was assessed using the NanoPhotometer® spectrophotometer (IMPLEN, USA). The RNA concentration was measured using the Qubit® RNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, USA).

2.3. Library construction and transcriptome sequencing

The mRNA was purified from total RNA using poly T oligo-attached magnetic beads. First strand cDNA was synthesized using random hexamer primer and M-MLV Reverse Transcriptase (RNase H free). The second-strand cDNA was synthesized using buffer, dNTPs, RNase H and DNA polymerase I using the manufacturer's instructions. The short fragments were subsequently connected with sequencing adapters. After agarose gel electrophoresis, suitable fragments were selected as templates for PCR amplification. Lastly, PCR products were purified and library quality was assessed using the Agilent Bioanalyzer 2100 system. After cluster generation, all libraries were sequenced on an Illumina Hiseq platform at Novogene (Beijing, China). All of the analyses are based on the combined results of the three libraries.

2.4. De novo transcriptome assembly

The clean reads were obtained by removing reads containing adapter, reads containing ploy-N and low quality reads from raw data. Overlapping high-quality reads were assembled into contigs using Trinity (Version r20140413p1). The longest transcripts of each gene were defined as unigenes. Relative abundance of specific mRNAs in the transcriptome was estimated using RSEM (Version 1.2.15) with FPKM (expected number of fragments per kilobase of transcript sequences per million base pairs sequenced).

2.5. Functional annotation

To obtain comprehensive gene function information, unigene sequences were first aligned using Blastx with reference to the NCBI non-redundant protein database (NR), NCBI nucleotide sequences (NT), Gene Ontology (GO), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), and euKaryotic Ortholog Groups (KOG) protein databases (E-value, 10^{-5}), associating proteins with the greatest sequence similarity with the given unigenes along with thee protein functional annotations. Homology searches were conducted by query of the NR using the Blastx algorithm (E-value, 10^{-5}) (Altschul et al., 1997). For gene transcript annotation, the

Blastp method was used for amino acid sequence alignment, and then was used Blastx for nucleotide alignment. After NR and NT annotation, the Blast2GO program (Version 2.5) was used to obtain GO functional classification of all unigenes to understand the distribution of gene functions at the macro level. Using EC (Enzyme Commission number) terms, biochemical pathway information was collected by downloading relevant maps from the KEGG database (http://www.genome.jp/kegg/pathway.html). After obtaining the KEGG pathway annotations, unigenes were aligned to the KOG database to predict and classify potential functions based on known orthologous gene products. Every protein in KOG is assumed to evolve from an ancestor protein, and the whole database is built on protein coding genes from complete genomes as well as systematic evolutionary relationships of bacteria, algae and eukaryotic organisms (Tatusov et al., 2000). In addition, the prediction and classification of the transcription factor family (TF) were conducted using Itak and hmmscan, respectively.

2.6. Identification of SNP and SSR

There was comparison of results using samtools and picard-tools to arrange the chromosome coordinates and for removal of the repeated reads. The GATK2 (Version 3.2) software was used to perform SNP identifications. From the original results, SNPs with a mass value of less than 30 and a distance of less than 5 were filtered. The SSRs of the transcriptome were identified using MISA (http://pgrc.ipk-gatersleben.de/misa/misa.html). Perfect di-, tri-, tetra-, penta-, and hexa-nucleotide motifs were detected using MISA (Version 1.0).

2.7. Real-time quantitative PCR (q-PCR) of neuropeptide genes predicted from transcriptome analysis

Based on published literature and the transcriptomic dataset, there was a prediction of neuropeptides existing in the brain of *E. sinensis*. Nine neuropeptides genes were randomly selected and the abundance in the EB, LB, EO, and LO were analyzed by q-PCR. The q-PCR was conducted using a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad) in a 20 μL reaction mixture containing 1 μL cDNA template, 10 μL 2 × TB Green Premix Ex Taq II (TaKaRa, Japan), 0.25 μL of each primer (10 μM) and 8.5 μL nuclease-free water. A reaction without cDNA was used as the negative control. The housekeeping gene *β-actin* was amplified as an internal reference. The mRNA relative abundances were calculated using the $2^{-\triangle\triangle CT}$ method. The statistical significance was measured with one-way ANOVA using SPSS (version 20.0) and the differences were considered to be significant if $P < 0.05$.

3. Results and discussion

3.1. Transcriptome sequencing output, assembly and function annotation

In total, RNA sequencing produced 22,233,587 raw reads from the three brain libraries on average. After removing adaptors and low quality reads, there were 21,564,558 clean reads. Through sequence assembly, 233,887 transcripts and 194,286 unigenes were obtained. As shown in Fig. 1, a size distribution analysis of the total number of transcript contigs indicated there were 101,941 transcripts (43.59%) of less than 301 bp, 63,607 transcripts (27.2%) ranging from 301 to 500 bp, 35,964 transcripts (15.38%) ranging from 501 to 1000 bp, 18,581 transcripts (7.94%) ranging from 1001 to 2000 bp and 13,794 transcripts (5.9%) that were greater than 2000 bp in length. Similarly, a size distribution analysis of the unigenes indicated 92,962 unigenes (21.07%) of less than 301 bp, 55,071 unigenes (12.48%) ranging from 301 to 500 bp, 274,442 unigenes (62.19%) ranging from 501 to 1000 bp, 11,811 unigenes (2.68%) ranging from 1001 to 2000 bp and 7000 unigenes (1.59%) that were greater than 2000 bp in length.

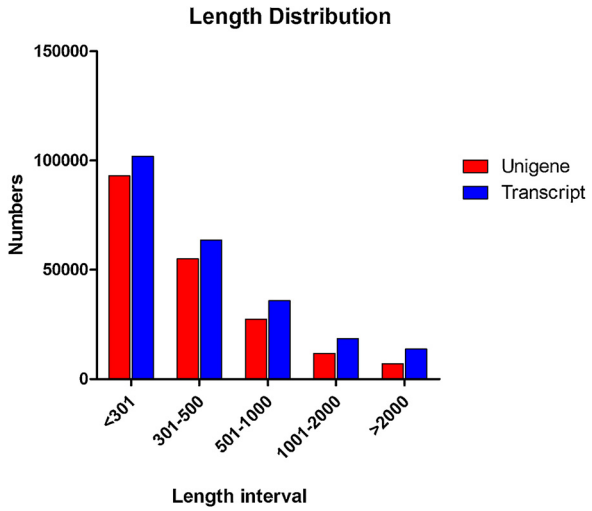

Fig. 1. Length distribution of transcripts and unigenes in *Eriocheir sinensis* brain.

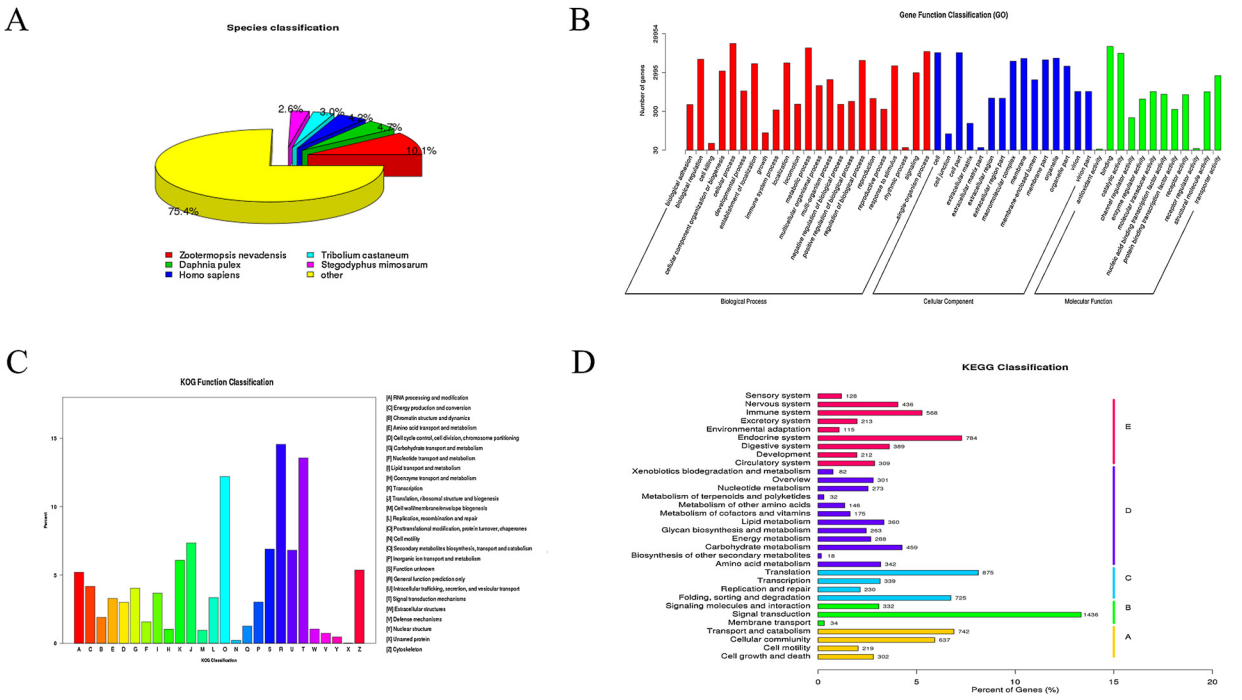

Fig. 2. Functional annotations of *Eriocheir sinensis* brain transcriptome; A) Species distribution of transcripts against the nr database in NCBI; B) Functional Gene Ontology (GO) classification of the unigenes; C) Histogram presentation of clusters of orthologous groups (KOG) Classification; D) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of unigenes (A, Cellular Processes; B, Environmental Information Processing; C, Genetic Information Processing; D, Metabolism; E, Organismal System).

By comparing with NR database, the gene functional information and the sequence similarity can be obtained between the Chinese mitten crab and matched species. A total of 21,989 unigenes (11.31% of total unigenes) from *E. sinensis* were annotated in NR based on matches with sequences with known function in many species. Annotated unigenes had a great amount of similarity (10.1%) with the dampwood termite (*Zootermopsis nevadensis*), as well as a lesser similarity with some other species: 2.6% with the spider (*Stegodyphus mimosarum*), 3.0% with beetles (*Tribolium castaneum*), 4.2% with humans (*Homo sapiens*), and 4.7% with water fleas (*Daphnia pulex*) (Fig. 2A).

The GO could provide a structured and controlled vocabulary for describing gene products in three categories: biological processes, cellular components and molecular functions. As shown in Fig. 2B, 29,954 unigenes (15.41% of total unigenes) were annotated in GO, comprised of 81,461 transcripts categorized into the biological process category, 30,652 transcripts to the cellular component category and 55,398 transcripts to the molecular function category. The assigned unigenes were divided into 58 functional terms, of which, “cellular process” and “metabolic process” were the most abundant in the biological category. In the cellular component category, “cell” and “cell part” comprised the largest proportion. In the molecular function category, many unique sequences were likely to possess “binding” and “catalytic activity”.

Functional annotation using the KOG database classified 9385 transcripts into 26 categories. The greatest representation of biological processes included “General function prediction only” (1366), “Signal transduction mechanisms” (1273), and “Posttranslational modification, protein turnover, chaperones” (1145). The 648 unigenes were assigned to the “function unknown” category (Fig. 2C). These genes may be foundational for future research directions of neuropeptides.

To further investigate the gene function, all transcripts were submitted to KEGG analysis, and 230 metabolic pathways were predicted. The number of transcripts in different pathways ranged from 1 to 344 (Table S1 in Supplementary data). The top 32 pathways are shown in Fig. 2D, and two major pathways (Signal transduction and Translation) contained more than 2311 transcripts. In a cell, various signal transducers recognize and interact with each other, transmit signals and form signal transduction pathways. For example, the signal transductions were initiated by c-fos in the pituitary, which include cAMP pathway, MAPK pathway, Ca˜(2+)/calmodulin-dependent kinases pathway and nuclear factor of the activated T-cell (NFAT) pathway (Chen et al., 2015). These predicted pathways are likely to be useful in future investigations focusing on the function in the reproductive system of *E. sinensis*.

Altogether, 1386 genes were predicted to be transcription factors (Table S2 in Supplementary data). The results indicated that more than half of the genes (771) were predicted to be transcription factors in the C2H2 family. Other transcription factors that were predicted include bHLH, C3H, HB, HMG, Orphans, PHD, and TRAF. The C2H2 protein is known as the having classical zinc finger structure and, together with other factors, participates in controlling transcription of target genes (Iuchi, 2001).

Table 1
The counts of putative single nucleotide polymorphisms (SNP) in the transcriptomes of *E. sinensis* brain.

Library	Total SNP	Non coding SNP	Coding SNP	Synonymous	Non-synonymous
Crab brain1	315,580(100%)	245,235(77.71%)	70,345(22.29%)	57,016(18.07%)	13,329(4.22%)
Crab brain2	350,191(100%)	270,384(77.21%)	79,807(22.79%)	65,261(18.64%)	14,546(4.15%)
Crab brain3	322,658(100%)	251,654(77.99%)	71,004(22.01%)	57,561(17.84%)	13,443(4.17%)

The three brain samples were biological repetitions. The differences between synonymous and non-synonymous SNPs are that the change of the coding sequence caused by a synonymous SNP, which would not affect the amino acid sequence of the translated protein. Whereas a non-synonymous SNP changes the amino acid sequence of translated protein sequence.

3.2. Identification of SNP and SSR

The SNP refers to a genetic marker formed by the mutation of a single nucleotide in the genome. This type of marker is abundant in quantity and rich in polymorphisms. A total of 329,477 SNPs were detected in the present study, comprised of 255,758 noncoding and 73,719 coding SNPs (Table 1). A total of 63,879 SSRs were obtained after conducting the SSR detection on all unigenes. The distribution of different motifs contained di- (62.13%), tri- (34.6%), tetra- (3.21%), penta- (0.03%), and hexa-nucleotides (0.02%) (Fig. S1 in Supplementary data). Di-nnucleotide motifs were the most prevalent, including AC/GT, AG/CT, AT/AT, and CG/CG. Of these, AC/GT and AG/CT were the predominant types, with proportions of 55.95% and 38.96%, respectively (Table S3 in Supplementary data).

3.3. Identification of neuropeptide precursors

The search for neuropeptide precursors was based on candidate genes that have been validated. According to the sequence analysis in the present study and that from previous studies, 22 neuropeptides existed in the *E. sinensis* transcriptome (Table 2), 16 of which were also detected in *S. paramamosain.* Although some important neuropeptide precursors were not detected, including GnRH, Inotocin, Insulin, Proctolin, RFamide, SIFamide, and FMRFamide, the receptor for these compounds was detected (Table 2). In the GnRH signaling pathway in vertebrates, GnRH could be coupled to its receptor, GnRHR, then activate downstream pathways after stimulating a series of biochemical cascades (Krsmanovic et al., 2003; Roelle et al., 2003; Shah et al., 2003). The insulin receptors are essential for the development and steroidogenic function of adult Leydig cells (Neirijnck et al., 2018). It is more difficult to confirm the absence than the presence of a gene, as the gene sequence may not be conserved or the sequenced transcripts may be incomplete, thus the target gene was not predicted. From results of the present, there were some important neuropeptides detected that were previously identified in other crustaceans, such as CHH, CCAP, PDH, and RPCH.

The CHH, as a regulator of multiple physiological effects, is involved in reproduction, molting, and osmoregulation. In support of the function of CHH in reproduction, CHH stimulates oocyte growth in *Homarus americanus in vitro.* (Tensen et al., 1989). In the shore crab (*Carcinus maenas*), an increase in cGMP concentration after the incubation with CHH was observed in the gill, midgut, and hindgut glands, which was correlated with a relatively greater glucose concentration (Lacombe et al., 1999). Results from the present study revealed that the CHH precursor amino acid sequences (154 aa) possessed 72% identity with the *E. sinensis* CHH (Accession number ABB46291.1). The reason for this could be attributed to a difference in hypotype. The CHH peptides sequences contained a highly conserved common sequence: RSAEGFGRMERLLSQLR.

The CCAP is a C-terminal amidated nonapeptide hormone that has been widely found in crustaceans and insects (Chen et al., 2016). The CCAP peptide was first isolated from pericardial organs of *C. maenas*. Furthermore, the results suggested that CCAP is a neurohormone that is stored in relatively large amounts in the pericardial organ, and, upon release into the hemolymph, functions to regulate heart tissues (Stangier et al., 1987). Results of previous studies indicate that CCAP has an important role in the spermatheca (Da and Lange, 2006) and oviduct (Donini and Lange, 2002) functions. The predicted CCAP precursor amino acid sequences (164 aa) were 72% identical to the *C. maenas* CCAP (Accession number ABB46291.1). The CCAP precursor amino acid sequences had the conserved peptide sequences of KRPFCNAFTGCGKKRS, suggesting structural conservation among decapod crustaceans.

The PDH is a family of octadecapeptides that has been isolated from several crustacean species (Rao and Riehm, 1993). The PDH molecule functions on retinular-shielding pigments and retinular photoreceptor cells, modulating the entrance of light, and changing their electrical properties. The predicted PDH proteins had a high amino acid sequence similarity (83%) with the *Callinectes sapidus* PDH (Accession number Q23755.1). The PDH peptide shared the conservation of NSELINSILGLPKVMNDA.

The RPCH is a peptide hormone that was first discovered in crustaceans, and is involved in the distribution of pigments and light-dark adaption. In decapod crustaceans, RPCH is synthesized by the X-organ and sinus gland, both of which are located in the eyestalks (Mangerich et al., 1986). The RPCH was first identified within eyestalks of the pink shrimp (*Pandalus borealis*). Subsequently, RPCH sequences were identified in several other crustacean species, including crabs and crayfish (Yang et al., 1999). Results of the present study revealed that the RPCH precursor nucleotide sequences possessed 75% similarity with the *S. paramamosain* RPCH (Accession number AGW45011.1).

Table 2
Putative neuropeptide derived from the brain transcriptomes of *E. sinensis*.

Neuropeptide/ Neuropeptide receptor	Peptide precursor	Transcript ID	Length (nt)	FPKM	E value	Similarity	Hit species
Neuropeptide							
AST	AST	c156083_g3	468	30.63	1E-30	83%	*Panulirus interruptus*
	AST	c156083_g2	254	63.51	7E-31	86%	*Panulirus interruptus*
	AST	c156083_g1	2,686	68.98	3E-79	61%	*Panulirus interruptus*
AST-A	AST-A1	c130288_g1	915	20.2	2E-15	71%	*Scylla paramamosain*
AST-B	AST-B	c136549_g1	1,388	158.99	4E-142	78%	*Scylla paramamosain*
AST-C	AST-C	c125819_g1	840	95.44	2E-46	74%	*Scylla paramamosain*
CFSH	CFSH	c212742_g1	448	2.56	2E-89	74%	*Callinectes sapidus*
CHH	CHH	c144982_g1	1,872	1.08	3E-69	86%	*Eriocheir sinensis*
CCAP	CCAP	c147598_g1	1,581	16.8	7E-69	72%	*Carcinus maenas*
DH 31	DH 31	c151059_g11	1,780	239.92	3E-66	81%	*Scylla paramamosain*
EH	EH	c149692_g5	1,576	41.23	7E-33	96%	*Scylla paramamosain*
FLRFamide	FLRFamide	c146434_g1	1,705	93.16	4E-60	70%	*Scylla paramamosain*
GPα2	GPα2	c140215_g1	1,309	22.44	8E-48	74%	*Cryptotermes secundus*
GPβ5	GPβ5	c137772_g1	1,496	28.09	1E-57	79%	*Penaeus monodon*
insulin-like peptide	insulin-like peptide 1	c96588_g1	335	29.82	8E-18	56%	*Cherax quadricarinatus*
	insulin-like peptide 2	c143007_g1	1,086	24.01	2E-12	80%	*Saqmariasus verreauxi*
Myosuppressin	Myosuppressin	c138316_g1	545	51.15	9E-48	91%	*Scylla paramamosain*
NEC	NEC 1	c73757_g2	278	0	2E-19	65%	*Zootermopsis nevadensis*
Neuroparsin	Neuroparsin 1	c125807_g1	758	470.13	7E-33	77%	*Scylla paramamosain*
	Neuroparsin	c124434_g1	1,303	3.06	3E-17	47%	*Metapenaeus ensis*
	Neuroparsin	c135397_g1	455	3.69	2E-17	73%	*Portunus trituberculatus*
NPF	neuropeptide F2	c123677_g1	939	16.41	2E-38	88%	*Scylla paramamosain*
Orcokinin	Orcokinin 1	c82406_g1	841	251.15	5E-50	73%	*Scylla paramamosain*
PDH	PDH 1	c108254_g1	480	26.34	1E-27	83%	*Callinectes sapidus*
RPCH	RPCH	c155975_g2	406	99.76	4E-12	75%	*Scylla paramamosain*
Sulfakinin	Sulfakinin	c145415_g1	811	26.21	2E-14	92%	*Scylla paramamosain*
Tachykinin	Tachykinin	c135694_g1	640	144.15	4E-50	61%	*Scylla paramamosain*
Receptor							
GnRHR	GnRHR	c132147_g1	1,899	0.78	3E-149	64%	*hyalella azteca*
InotocinR	InotocinR	c142545_g1	2,780	1.86	1E-103	47%	*Limulus Polyphemus*
InsulinR	Insulin-like R	c155343_g1	5,272	5.11	0	38%	*Macrobrachium rosenbergii*
ProctolinR	Proctolin-1 R like	c155373_g3	3,998	6.71	1E-88	49%	*Centruroides sculpturatus*
RFamideR	RFamideR-like	c15025_g1	340	1.04	8E-24	57%	*Agrilus planipennis*
SIFamideR	SIFamideR-like	c136559_g1	2,030	1.11	5E-145	56%	*Zootermopsis nevadensis*
FMRFamideR	FMRFamideR-lile	c155080_g1	2,575	5.34	2E-81	53%	*Limulus Polyphemus*

AST, Allatostatin; CFSH, Crustacean female sex hormone; CHH, crustacean hyperglycemic hormone; CCAP, crustacean cardioactive peptide; DH31, diuretic hormone 31; EH, eclosion hormone; GP, glycoprotein hormone; NEC, Neuroendocrine convertase; NPF, neuropeptide F; PDH, pigment dispersing hormone; RPCH, red pigment concentrating hormone; GnRHR, gonadotropin-releasing hormone receptor.

3.4. Differential abundance of neuropeptides imply a regulatory role during ovarian maturation

The effects of neuropeptides on maturation and spawning in decapod crustaceans are relevant. In many studies, results indicate that the ovarian development is regulated, not only by neuropeptides generated from eyestalks, but also by those from the brain as well (Bao et al., 2015; Qiao et al., 2017). To explore the potential relationship between neuropeptides and ovarian development in *E. sinensis*, the relative of abundances of nine neuropeptide mRNA transcripts in the brain and ovary were examined during ovarian development.

As shown in Fig. 3, q-PCR results revealed that six neuropeptides [PDH FLRFamide, glycoprotein hormone β5 (GP β5), AST-B, CHH, and DH 31] were present in the ovary and brain. Obviously, the relative abundance mRNA transcripts for these six neuropeptides were greater in the brain than in the ovary. It was deduced that the ovarian maturation may require large amounts of neuropeptides, and neuropeptides in the brain may have a compensation effect. Three neuropeptide mRNA transcripts [CCAP, RPCH and myosuppressin] were present in the brain but not yet in the ovary, which may be attributed to these genes being expressed only in specific developmental stages. Interestingly, the trend of transcript abundance for neuropeptides was consistent between the brain and ovary. Namely, it was maintained at relatively greater abundances in early developmental stage followed by a significant decrease during the mature developmental stage (except RPDH). For example, the relative abundance of CHH in EB was eight times that in LB. Results of the present study were similar with those of previous studies. In *S. paramamosain*, the relative abundance of β-PDH-1 mRNA transcripts was consistent between the eyestalk and ovary, and was maintained in relatively greater abundances from the previtellogenic to the vitellogenic stage, and then there was lesser abundances during the mature stage (Huang et al., 2014). The mRNA transcript abundance profile of β-PDH-1 in the eyestalk and ovary indicated that β-PDH-1 may have an important function in ovarian maturation (Huang et al., 2014). A similar result was reported in the swimming crab (*Portunus trituberculatus*) (Xie et al., 2014). During ovarian development, the relative abundance of Pt-CHH1 mRNA transcript in eyestalks was less during the previtellogenic stage and subsequent developmental stages, and there were the least abundances of transcript at the mature stage, suggesting that Pt-

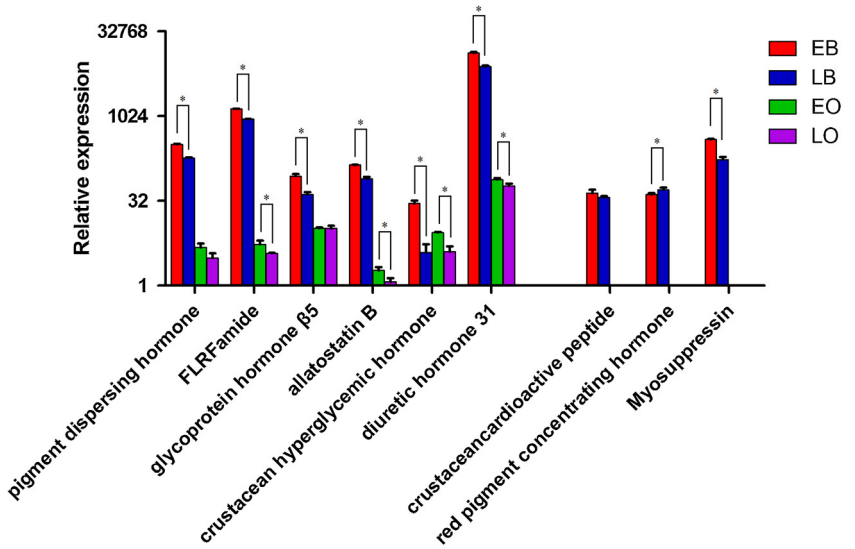

Fig. 3. Abundance patterns from mRNA transcripts of nine neuropeptides in the brain and ovary were confirmed using q-PCR; *β-actin* was used as an internal reference; Results of q-PCR are shown as the mean + SD of three biological replicates; EB: brain at the early development stage of ovary; LB: brain at the late development stage of ovary; EO: early ovary; LO: late ovary; Asterisks indicate a difference ($P < 0.05$).

CHH1 may be involved in inhibition of ovarian development (Xie et al., 2014). In *S. paramamosain*, the relative mRNA transcript abundances of 21 neuropeptides at different vitellogenic stages were quantified. Most of these also had differential mRNA transcript abundances in the cerebral ganglia at various vitellogenic stages, suggesting that there was likely involvement in vitellogenesis and ovarian maturation. Of the 21 neuropeptides, thirteen had the least mRNA transcript abundances in the late vitellogenic stages (Bao et al., 2015). In addition, results of the present study indicated that the relative abundances of mRNA transcripts of some neuropeptides (FLRF amide, AST-B, CHH, and DH 31) were consistent with findings in previous studies in *S. paramamosain*, indicating that the neuropeptides may be involved in the ovarian maturation.

4. Conclusions

In the present study, a comprehensive analysis was performed of the *E. sinensis* brain transcriptome and there was identification of neuropeptides or receptors. Totally, there were 22 neuropeptides identified, of which, some have been identified to be associated with gonadal development, such as CHH, CCAP, and PDH. In addition, many neuropeptides had a differential mRNA transcript abundance in the brain and ovary during developmental maturation, suggesting the neuropeptides may be involved in ovarian maturation. Taken together, the results of this study can be used as a reference about neuropeptide distribution for other Decapoda species, and provide foundational information for future molecular and functional investigations of neuropeptides.

Conflict of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anireprosci.2018.12.010.

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