

## Evolution and functional analysis of the *Pif97* gene of the Pacific oyster *Crassostrea gigas*

Xiaotong WANG<sup>1#</sup>, Xiaorui SONG<sup>1#</sup>, Tong WANG<sup>1</sup>, Qihui ZHU<sup>1</sup>, Guoying MIAO<sup>1</sup>, Yuanxin CHEN<sup>2</sup>, Xiaodong FANG<sup>2</sup>, Huayong QUE<sup>1</sup>, Li LI<sup>1\*</sup>, Guofan ZHANG<sup>1</sup>

<sup>1</sup> Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

<sup>2</sup> BGI-Shenzhen, Shenzhen 518083, China

**Abstract** Mollusc shell matrix proteins (SMPs) are important functional components embedded in the shell and play a role in shell formation. A SMP (*Pif177*) was identified previously from the nacreous layer of the Japanese pearl oyster *Pinctada fucata*, and its cleavage products (named pfPif97 and pfPif80 proteins) were found to bind to the chitin framework and induce aragonite crystal formation and orient the c axis. In this study, a homologue of *pfPif177* was cloned from the mantle of the Pacific oyster *Crassostrea gigas*, containing the homologue of *pfPif97* only and not *pfPif80*. This finding hints at the large divergence in gene structure between the two species. This homologue (*cgPif97*) shares characteristics with *pfPif97*, and suggests that the biological functions of these two proteins may be similar. The expression pattern of *cgPif97* in different tissues and development stages indicates that it may play an important role in shell formation of the adult oyster. The morphology of the inner shell surface was affected by injected siRNA of *cgPif97* and the calcite laths of the shell became thinner and narrower when the siRNA dose increased, suggesting that the *cgPif97* gene plays an important role in calcite shell formation in *C. gigas*. In conclusion, we found evidence that the *Pif177* gene evolved very fast but still retains a similar function among species [Current Zoology 59 (1): 109–115, 2013].

**Keywords** Mollusca, *Crassostrea gigas*, Pif97 gene, Biominerization, Shell formation, Calcite

Molluscan shell matrix proteins (SMPs) are important functional components embedded in the shell and contribute to shell formation. A number of SMPs have been identified in several species, with partial functional verification (Heinemann, et al., 2006, Marie et al., 2007, Marin et al., 2007, Moradian-Oldak et al., 1992, Shen et al., 1997, Suzuki et al., 2004, Weiss et al., 2000). However, the functional similarities and differences among SMP homologues in different species remain unclear. It has been reported that only a small part of the shell genes identified in Tropical abalone *Haliotis asinina* have identifiable homologues in the full genome of the patellogastropod *Lottia scutum*, suggesting that SMPs evolved quickly and there are significant molecular differences in the ways in which gastropods synthesize their shells (Jackson DJ, et al., 2006). For Class Bivalvia, it is not known if a similar mechanism exists.

The novel SMP *Pif177* was identified from the nacreous layer of the Japanese pearl oyster *Pinctada fucata*. The *Pif177* gene encodes a precursor protein that can be post-translationally cleaved into Pif97 and Pif80, named pfPif97 and pfPif80 respectively. The pfPif97

protein binds to the chitin framework through the chitin-binding type-2 domain and is involved in the formation of the lamellar sheet. The pfPif80 protein induces aragonite crystal formation and regulates the orientation of the c axis (Suzuki et al., 2009). Though the Japanese pearl oyster and Pacific oyster *Crassostrea gigas* belong to different Orders in the Subclass Pteriomorpha, it has recently been shown that they are close species (Kocot et al., 2011, Smith et al., 2011, Wang et al., 2011a). The crystal structure of calcium carbonate in their shells is very different, Japanese pearl oysters are mainly composed of aragonite and Pacific oysters calcite. Aragonite and calcite are two structural isomers of calcium carbonate, suggesting that the gene(s) controlling the shell formation process may be very different. The comparison of *Pif177* genes from Japanese pearl oysters and Pacific oysters may be useful to understand the evolution of shell formation in bivalves.

In this study, a homologue of *pfPif177* was cloned from the mantle of the Pacific oyster and named *cgPif177*. Sequence characteristics and expression patterns in different tissues and developmental stages were

Received Feb. 14, 2012; accepted Feb. 29, 2012.

\* Corresponding author. E-mail: lili@qdio.ac.cn. <sup>#</sup> These authors contributed equally to this work.

© 2013 Current Zoology

evaluated for this homologue. Last, its biological function in shell formation was analyzed using RNAi methods.

## 1 Materials and Methods

### 1.1 Gene cloning

Total RNA was isolated from the mantle of one adult wild oyster (Weihai, China). The homologue of *pfPif177* was cloned by rapid amplification of cDNA ends. Related primers are listed in Table S1.

### 1.2 Sequence analysis

For the purposes of this study, the *Pif177* gene was viewed as two segments (named *pfPif97* and *pfPif80*), meaning that gene *pfPif97* refers to that segment that leads to the protein *pfPif97*.

Functional domain (IPR) prediction was performed at <http://prosite.expasy.org/> and signal peptide at <http://genome.cbs.dtu.dk/services/SignalP-2.0/>.

### 1.3 Expression patterns

The transcriptome data for eight tissues from one individual and for 38 developmental stages (unpublished data; the name of the tissues and developmental stages are presented in Table S2) were acquired using the RNA-seq method. The expression patterns of *cgPif97* in the different tissues and developmental stages were determined based on its RPKM value (reads per kilobase of gene model per million mapped reads) in this transcriptome data.

### 1.4 RNAi experiment and scanning electron microscope (SEM) observation

In order to investigate the function of *cgPif97* in the process of shell formation a RNAi experiment was carried out.

Twenty-four *C. gigas* individuals from one population with shell height (the distance from middle of the hinge line to the opposing valve margin) of 6–7 cm were chosen as experimental animals. The control group contained six animals and each of the three experimental groups had six individuals. For this experiment, siRNA was designed based on the sequence of *cgPif97*: its sense chain was GCGAGAGAUUUGGGUAUUATT and its antisense chain was UAAUACCCAAAUCU-CUCGCTT.

In order to open the oyster shell without hurting the animal, all oysters were anesthetized in 8% MgSO<sub>4</sub> seawater solution (Wang X, et al., 2011b). After 12 h, the shells of the oysters were opened. The adductor muscle tissues of the control group were injected with 50 μl PBS (Suzuki M, et al., 2009). Individuals in the experimental groups were injected with one of the fol-

lowing treatments: 4 μg siRNA + 50 μl PBS; 16 μg siRNA+ 50 μl PBS; or 26 μg siRNA + 50 μl PBS.

After 6 d all oysters were killed and their mantle tissues and shells sampled. Total RNAs were extracted from the mantle tissues and prepared for real-time quantitative PCR experiments. The quantities of *cgPif97* mRNA from 4 groups were identified using the TaKaRa DRR039A Real Time PCR Kit and the Applied Biosystems 7500 Fast Real-Time PCR System with the Comparative Ct Method (ΔΔCT Method), and normalized with *beta-actin* mRNA to compensate for variations in input RNA amounts. Each cDNA sample was amplified in 3 duplicates. The primers used in the real-time quantitative PCR experiment are listed in Table S1.

The inner surface of the shell from each oyster was observed under a SEM (SM-5610-LV, Joel Ltd.). The morphology of the inner shell surface was photographed and the images of different individuals were compared.

## 2 Results

### 2.1 Gene cloning

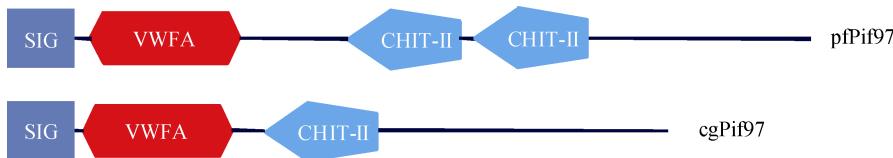
After assembly of the fragments of 5' RACE, 3' RACE and middle segment, a 1667 nt cDNA sequence was acquired with 41 nt 5' UTR and 93 nt 3' UTR. The length of predicted cds was 1533 nt and that of the deduced amino acid sequence 510 AA (Fig. S1). The Genbank accession number is JQ619625.

### 2.2 Sequence characteristics

The deduced protein contained 510 AA, shorter than the *pfPif97* protein containing 525 amino acids (AA). The blast results indicate that only the homologue of *Pif97* of *P. fucata* was found in the cloned gene and named *cgPif97*. Fig. 1 shows a diagram of their functional domains. The *pfPif97* protein contained one VWFA domain and two CHIT\_BIND\_II domains, whereas the *cgPif97* protein contained one VWFA domain and one CHIT\_BIND\_II domain. Both proteins contained one signal peptide. Although one of the CHIT\_BIND\_II domains was lost in the *cgPif97* protein the categories of functional domains present in the two proteins were the same.

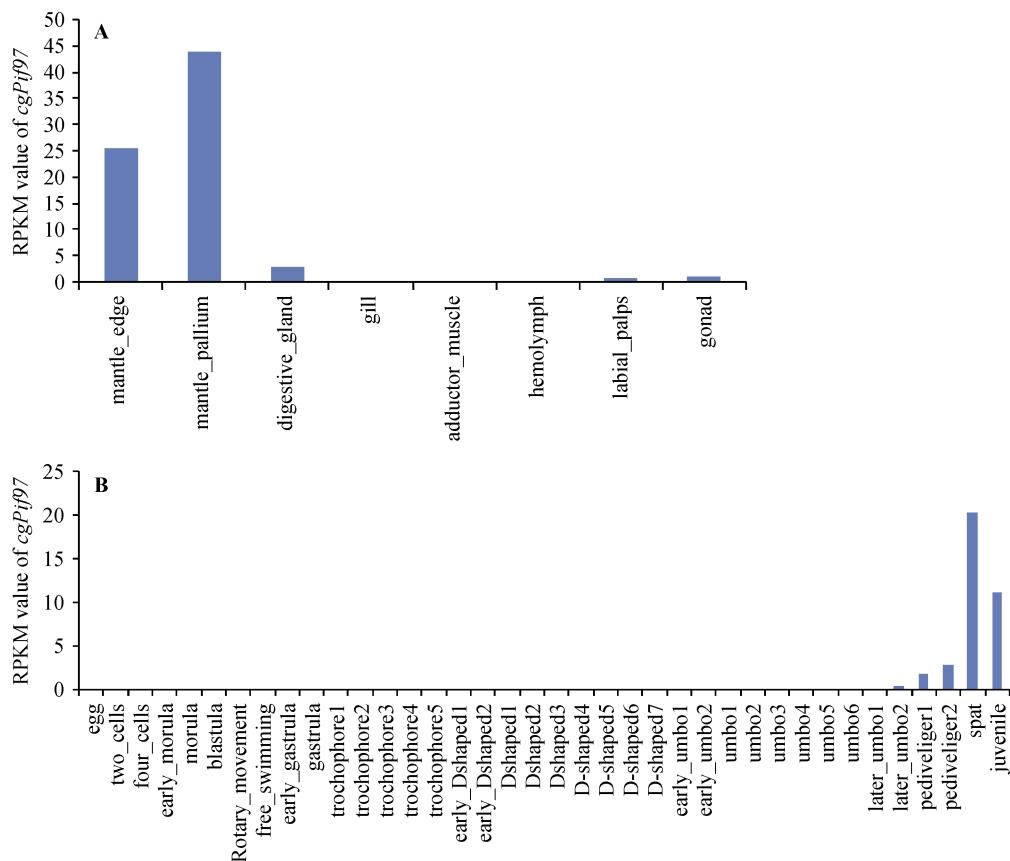
### 2.3 Expression patterns

The expression patterns of *cgPif97* were examined in eight different tissues and at 38 different development stages. The expression level of the *cgPif97* gene in the mantle was far higher than in other tissues, and it was also higher in the mantle pallium than in the mantle edge (Fig. 2A). In addition, expression of the *cgPif97* gene began at the late umbo2 stage and increased to higher levels at the spat and juvenile stages (i.e., ex-



**Fig. 1 The functional domains of Pif97 of *P. fucata* and *C. gigas***

Signal peptide was predicted at <http://genome.cbs.dtu.dk/services/SignalP-2.0/> and InterProScan domain (IPR) at <http://prosite.expasy.org/>. SIG, signal peptide; VWFA, Von Willebrand factor type A domain; CHIT-II, chitin-binding type-2 domain. Panel A, Pif97 of *P. fucata*: SIG, 1-22 AA; VWFA, 29-202 amino acids (AA); CHIT\_BIND\_II, 255-319 AA and 322-372 AA; Panel B, Pif97 of *C. gigas*: SIG, 1-19 AA; VWFA, 11-179 AA; CHIT\_BIND\_II, 185-241 AA.



**Fig. 2 Expression patterns of cgPif97 in tissues and different developmental stages**

A. The X-axis shows eight different tissues. B. The X-axis shows 38 different developmental stages.

pression reached a high level only after settlement and metamorphosis) (Fig. 2B).

#### 2.4 RNAi experiment and SEM observation

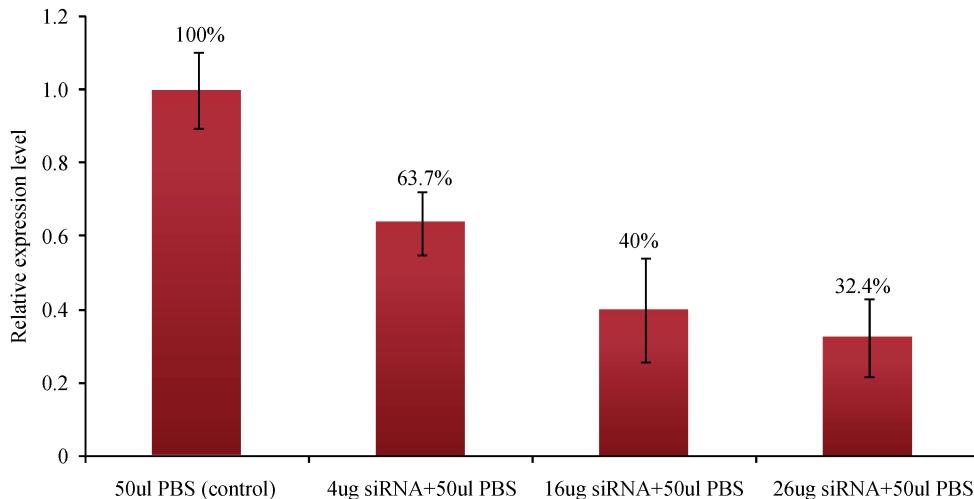
Increasing the injection dosage of siRNA resulted in a decrease in mRNA expression of cgPif97 in the mantle ( $P < 0.001$ ) (Fig. 3). The expression level of cgPif97 in the control group was defined as 100%, and those in the three experimental groups were 63.7%, 40%, and 32.4% respectively. This meant that cgPif97 mRNA expression was repressed by 36.3%, 60%, and 67.6% after exposure to 4  $\mu$ g, 16  $\mu$ g, and 26  $\mu$ g of siRNA, respectively.

The morphology of the inner shell surface also was

affected by siRNA. SEM images revealed that the calcite laths of the oyster shell became thinner and narrower as the siRNA dose increased (Fig. 4).

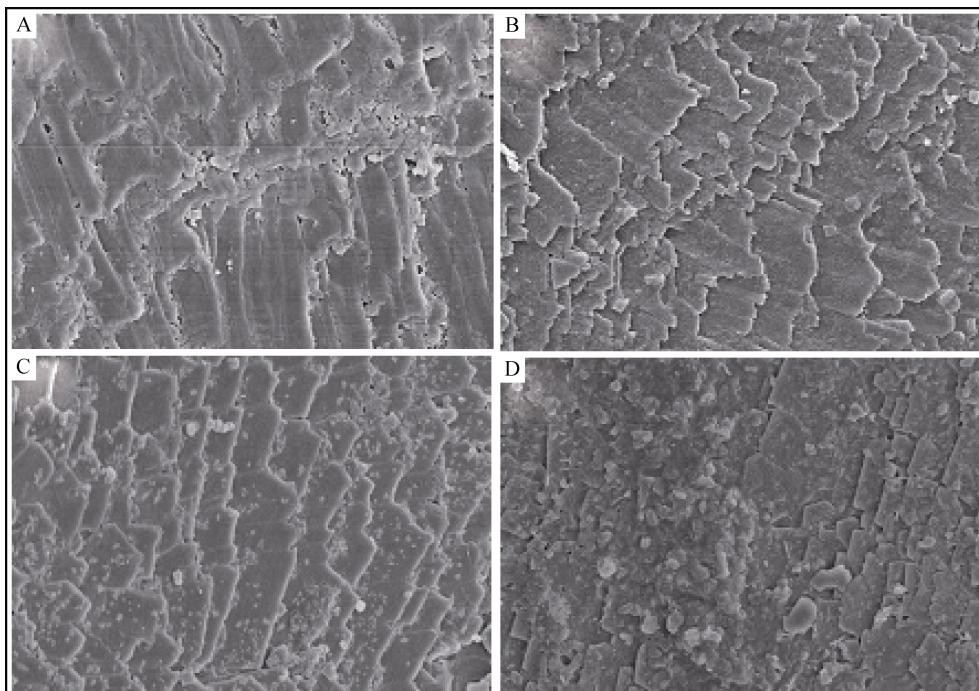
### 3 Discussion

The cgPif97 and pfPif97 proteins both have one VWFA domain and one CHIT\_BIND\_II domain, although the cgPif97 protein contains a second CHIT\_BIND\_II domain. This similarity in domains suggests that the biological function of cgPif97 may be similar to that of pfPif97. However, the homologue of the pfPif80 gene was not found in the cloned gene based on amino acid sequence, and the cloned full length



**Fig. 3 Expression level of *cgPif97* was inhibited by siRNA in a dose-dependent manner**

Significant differences can be seen in each treatment group compared with the control ( $P < 0.001$ ).



**Fig. 4 SEM of the shell inner surface of *C. gigas* under different treatments**

A. 50  $\mu$ l PBS as the control. B. 4  $\mu$ g siRNA + 50  $\mu$ l PBS. C. 16  $\mu$ g siRNA + 50  $\mu$ l PBS. D. 26  $\mu$ g siRNA + 50  $\mu$ l PBS. Viewed at a magnification of 5000 times.

cDNA was not enough long and could not possibly contain the *cgPif80* gene. Further, we searched the whole oyster genome and gene set (unpublished data) based on amino acid sequences, and were unable to locate the *cgPif80* gene (data not shown). Because of the functional importance of *pifPif80*, we believe the *cgPif80* protein may evolve very fast and exist in a different sequence in the Pacific oyster with similar biomineratization function and not lost completely. In fact, positive selection analysis for *pifPif97* and *cgPif97* was consid-

ered but then abandoned because of the huge sequence divergence between these two genes (Fig. S2). The *Pif80* and *Pif97* genes evolved very fast between the Japanese pearl oyster and Pacific oyster, supporting the hypothesis that shell matrix proteins evolve rapidly (Jackson et al., 2006). The faster evolution of *Pif80* seemed reasonable because of its function that directs aragonite crystal formation and c axis orientation (Suzuki et al., 2009) and the huge difference in shell crystal structure between Japanese pearl oysters (mainly

ragonite) and Pacific oysters (mainly calcite).

In general, SMPs are thought to be secreted from the mantle, thus the corresponding genes are thought to be highly expressed in the mantle (Jackson et al., 2010). The particularly high expression of the *cgPif97* gene in the mantle (Fig. 2A) agrees with this premise. High expression of *cgPif97* in the mantle combined with high expression during late developmental stages (after settlement and metamorphosis) suggests that *cgPif97* may play an important role in shell formation in the adult oyster. The mantle of molluscs can be divided into two parts, the (1) inner mantle (mantle pallium) adhering to shell inner surface and (2) outer mantle (mantle edge) adhering to the shell edge. In previous studies the mantle edge was proven for shell elongation and the mantle pallium for shell thickening in the Japanese pearl oyster (Sudo et al., 1997). Here, higher expression of the *cgPif97* gene in the mantle pallium than mantle edge (Fig. 2B) means that the gene is possibly more important for shell thickening than for shell elongation.

With increased dosage of siRNA the mRNA expression of *cgPif97* in the mantle decreased (Fig. 3). This suggests that siRNA repressed the expression of the *cgPif97* gene and that the effect was dose dependent. When *cgPif97* expression was repressed by siRNA, the morphology of the oyster inner shell surface changed (Fig. 4), suggesting that the *cgPif97* gene plays an important role in oyster shell formation. Because the main crystal structure of calcium carbonate in *C. gigas* shells is calcite and the scanned area of the inner shell surface was also composed of calcite, it is likely that the *cgPif97* gene plays an important role in calcite shell formation in this oyster species. Interestingly, the calcite laths of the oyster shell became thinner and narrower when siRNA was added in the first two treatment groups, yet irregular mineralization was not detected (Fig. 4B,C). This result suggests that the *cgPif97* gene did not affect calcite crystallization; instead, it may function to bind the chitin framework and participate in the formation of the lamellar sheet, not crystal formation as is the case for *pjPif97*. The irregular mineralization observed in the third treatment group (Fig. 4D) may be due to the large amount of siRNA used, which possibly prevented the formation of the basic organic frame.

In conclusion, though the Pif97 gene evolved quickly, it still plays an important role in calcite shell formation in *C. gigas* and this is similar to the known function in aragonite shell formation of *P. fucata*.

**Acknowledgments** This research was supported by the Na-

tional Basic Research Program of China (973 Program) (2010CB126401), the National Natural Science Foundation of China (40730845), the earmarked fund for Modern Agro-industry Technology Research System, Taishan Scholar Program of Shandon and Taishan Scholars Climb Program of Shandong.

## References

- Heinemann F, Treccani L, Fritz M, 2006. Abalone nacre insoluble matrix induces growth of flat and oriented aragonite crystals. *Biochem. Biophys. Res. Commun.* 344: 45–49.
- Jackson DJ, McDougall C, Green K, Simpson F, Worheide G et al., 2006. A rapidly evolving secretome builds and patterns a sea shell. *BMC Biol.* 4: 40.
- Jackson DJ, McDougall C, Woodcroft B, Moase P, Rose RA et al., 2010. Parallel evolution of nacre building gene sets in molluscs. *Molecular Biology and Evolution* 27: 591–608.
- Kocot KM, Cannon JT, Todt C, Citarella MR, Kohn AB et al., 2011. Phylogenomics reveals deep molluscan relationships. *Nature* 477: 452–456.
- Mann S, 1988. Molecular recognition in biomineralization. *Nature* 332: 6.
- Marie B, Luquet G, Pais De Barros JP, Guichard N, Morel S et al., 2007. The shell matrix of the freshwater mussel *Unio pictorum* (Paleoheterodonta, Unionida): Involvement of acidic polysaccharides from glycoproteins in nacre mineralization. *FEBS J.* 274: 2933–2945.
- Marin F, Luquet G, Marie B, Medakovic D, 2007. Molluscan shell proteins: Primary structure, origin, and evolution. *Current topics in Developmental Biology* 80: 209–276.
- Moradian-Oldak J, Frolov F, Addadi L, Weiner S, 1992. Interactions between acidic matrix macromolecules and calcium phosphate ester crystals: relevance to carbonate apatite formation in biomineralization. *Proc Biol. Sci.* 247: 47–55.
- Shen X, Belcher AM, Hansma PK, Stucky GD, Morse DE, 1997. Molecular cloning and characterization of lustrin A, a matrix protein from shell and pearl nacre of *Haliotis rufescens*. *J. Biol. Chem.* 272: 32472–32481.
- Smith SA, Wilson NG, Goetz FE, Feehery C, Andrade SC et al., 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. *Nature* 480: 364–367.
- Sudo S, Fujikawa T, Nagakura T, Ohkubo T, Sakaguchi K et al., 1997. Structures of mollusc shell framework proteins. *Nature* 387: 563–564.
- Suzuki M, Murayama E, Inoue H, Ozaki N, Tohse H et al., 2004. Characterization of Prismalin-14, a novel matrix protein from the prismatic layer of the Japanese pearl oyster *Pinctada fucata*. *Biochem. J.* 382: 205–213.
- Suzuki M, Saruwatari K, Kogure T, Yamamoto Y, Nishimura T et al., 2009a. An acidic matrix protein, Pif, is a key macromolecule for nacre formation. *Science* 325: 1388–1390.
- Wang X, Li L, Xu F, Zhang G, 2011a. Tropomyosin is a nice marker gene for phylogenetic analysis of molluscs. *Molecular Biology Reports*: 1–5.
- Wang X, Li L, Xu F, Zhang G, 2011b. Genomic DNA extraction

from *in vivo* sampled tissue of Pacific oyster *Crassostrea gigas*.

The Israeli Journal of Aquaculture-Bamidgeh 63: 1–3.

Weiss IM, Kaufmann S, Mann K, Fritz M, 2000. Purification and

characterization of perlucin and perlustrin, two new proteins from the shell of the mollusc *Haliotis laevigata*. Biochem. Biophys Res. Commun. 267: 17–21.

**Table S1** The primers for RACE of *cgPif97* cDNA

Sequence (5'→3')	Size (bp)	Applications
GGCCACGCGTCGACTAGTACG <sub>10</sub>	29	5' RACE
CCACAACACCAGCCATAGGAACC	23	5' RACE
GTATGAGAGCAGGGACAGAGAAGG	23	5' RACE
GGCCACGCGTCGACTAGTACT <sub>16</sub>	36	3' RACE
GTTACGGGTGGGTCCAAGGTCA	23	3' RACE
CTCGTGTACCGAACATAAAAG	21	3' RACE
AGATTTCACAAACCCCTCGTAC	21	To amplify partial cDNA of <i>cgPif97</i>
AGGGGTCTGGGTTATTGTAC	20	To amplify partial cDNA of <i>cgPif97</i>
CCGACGGTCAGGTACATCAC	19	Used to amplify the internal reference gene in real-time PCR
CTCATCGTACTCCCTGCTTG	19	Used to amplify the internal reference gene in real-time PCR
TTTCCAGAACATAGTTGACGGC	20	Used to amplify <i>cgPif97</i> gene in real-time PCR
AGGAACCCCCATCTCGTTG	19	Used to amplify <i>cgPif97</i> gene in real-time PCR

**Table S2** The list of the tissues and developmental stages used in the study of mRNA expression

Tissues	Developmental stages
mantle edge	egg
mantle pallium	two cells
digest gland	four cells
gill	early morula stage
adductor muscle	morula stage
hemolymph	blastula stage
labial palps	Rotary movement
gonad	free swimming, early gastrula stage, gastrula stage, trophophore 1–5, early D-shaped larva 1,2, D-shaped larva 1–7, early umbo larva 1,2, umbo larva 1–6, later umbo larva 1,2, pediveliger 1,2, spat, juvenile

AATGATTTAAGTTAACAGGACTGCCACATTGGCTAGGAA**ATG**AAGGCTTCCATCTTGTATTCTATAATCTGTGA  
 TTCATTCTCGGACAACGCGCTGACCCCTTCCGAAAGGTTGCAAAGCGCAGATGACGCTGTTTGTAAATCGATGGATC  
 AGACAGTATCTCTGAGTCAGATTCCAAACCCCTCGTACGTCATTCCAGAATAGTTGACGGCTTCATATTGGCTCTGGAG  
 AAACAGGATGGGCATCATCGTGTACAGTAAGGTTGTTGCTCTCTGTCGGCTCTCATACGATCCGGTGTATCTAAAAGAT  
 CAAGCCAGCATCATGCCCTCATCCTAGAGAGGGTACCAACACCCACCTGGTATAGAGGAGATGATAGATATGTTAAAAAGA  
 CAAACGAGATGGGGTCTCATGGCTGGTGTGACTGATGGAATATCTAAAGAAAAGGAAAAACCCCTACTACAGTCAA  
 GATTAGCGAGAGATTGGGTTAAACATGTTCTCGGCTGGCGTGTACACGGAGGAAGGAAACTAAGGAAATCTGCT  
 TCGAATCTGTACGGTATCAAAGTGGACTCTTGTGACGAAGTGTGAAATTCTACAAACCTTGTGCAATTGTTGCTC  
 AAACAAATGTATGATGCCCTGGTGTATGCCCTACCCAAACGCGTATGCAAAACTGTAGACTCTACTGGAAGTGTGAGGGTG  
 AGGAATCAAAGCTCACCTGTTGCCCAGGGTTCTCTCGGCACCACTCCAGCTCTGTATACCTGATCCAAATGCGTG  
 GAACCCCTGCGGAGACGGGGTCCCATGCAATAAAAGACCATCCGTATACCAACCTACAATTATGAAGAAACTATTGAAGG  
 TTACCGGTTGGTCCAAAGGTCACTGCCACCTGGGACGGCTATGACGGGTACCTGGGTGTACAAATAACCCAGACCCCTC  
 CCCCTCCCTAGAAGAGTTGAGTTAGTTAGTCACATTCCATCGACATTGACTGTGCGACACATCGGTAACGGCTTCTA  
 ATAAAAAAATCATGGGTCACAGTACAAGAACAGGGTAGCCCTTTCGAAGGTAAGCCAACACTCGTGTACCGAACATACA  
 AAGGTACCTCGGATCCAACCTCTGGTCAAATGAGATAAAGGAGTTCTAGCTTGAACCCAGGGCTTCAGTAACG  
 GAGATTGCTACACCCCTGACCCCTCAACTGATCAAAAGATTCAACTACAAGAGTTGAGAACTCATATAGGCAA  
 CGCACGGGTTACTATTCAACCAATTCAAACCATGGAATGAAGTGACGTTAGCCATGATGGGAACACTCTAGCTGGATC  
 CGTGAATGGTATTCAAGACGAGAGAGGGTCAATGGACCAATTCAAGAACGGCGTATGGAATTTCATCGGGTCTGTGATG  
 GGTACAAGCATTTCCAGGGATATGGACTATGTCACAATTATAATGCCCAATATGAACGTTACAACAAGT**TAA**TGA  
 ATTGGGAAGATTGTGCTGTGATATTCAAAACCTCTACTAACAAATGATAAAGTCTTATCAGGAAAAAA  
 AAAAAAA

**Fig. S1** The sequence structure of *cgPif97*

The upstream highlighted was 5' UTR, the downstream highlighted 3' UTR. The bold "ATG" was initiation codon and the bold "TAA" termination codon.

pif97-cds	Pinctada fucata .txt	AATGATTTTAACAGGACTGCCACATTGGCTAGGAA	0
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus		40
pif97-cds	Pinctada fucata .txt	.ATCGTCAAGTCATATTCCAGTTCTGCTTGTGTCACC	39
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	ATCGTCAAGTCATATTCCAGTTCTGCTTGTGTCACC atg a gt c t tt t t t t	80
pif97-cds	Pinctada_fucata_.txt	CGCGTTTTGTGTCGGTCAATACTGTGTAAGTCAGAAGA	79
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	CGCGTTTTGTGTCGGTCAATACTGTGTAAGTCAGAAGA g tc ttt gg ctga t ga	120
pif97-cds	Pinctada fucata .txt	CAGGA.....GAGTGAGGGCAACGGGA	104
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	CAGGA.....GAGTGAGGGCAACGGGA AGGTGTCGCAAGACGGCAGATGAGCTGTTTGTGTAAGGA ag ga gt gt t t ga	160
pif97-cds	Pinctada fucata .txt	CGGCTTGTGTCAGTACCAAGATTTGCTAACATCTG	144
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	CGGCTTGTGTCAGTACCAAGATTTGCTAACATCTG g tc ga t tc tga agattt aa ct	200
pif97-cds	Pinctada_fucata_.txt	AAACAGGCCATTCTGCTCATCGACGTGGCTTCAGCATCG	184
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	AAACAGGCCATTCTGCTCATCGACGTGGCTTCAGCATCG CGTAGCTOCATTCAGAGATACTGAGCGGTTCACTATGAG ccat t a at gt ggc t at g	240
pif97-cds	Pinctada fucata .txt	ATGACAATDARTCGACTGGATGGTACATAGGATCTC	224
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	ATGACAATDARTCGACTGGATGGTACATAGGATCTC GCTCTGGACAAACCGGTTGGATCATGCTGTTCAATAA aaa c g t gg at tca tac g	280
pif97-cds	Pinctada_fucata_.txt	CGAAGTTCTGGACTCAATACOCTTCAAGGCGAATGCGA	264
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	CGAAGTTCTGGACTCAATACOCTTCAAGGCGAATGCGA AGGTTGTCGCTCTGCTCGCTCTCATACGATCGCTCG g gtt c ctc tcc t a cgtat g	320
pif97-cds	Pinctada fucata .txt	GACCTTGCTGGACTATTGCAATCATGAAGAGACGGCTGT	304
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	GACCTTGCTGGACTATTGCAATCATGAAGAGACGGCTGT TATCTAAAAGATCAAGCCPCCATCATGCCTCTTCGCTG a ct g catg a cc a g	360
pif97-cds	Pinctada_fucata_.txt	CCCCATCCAACATTTTAAAGGATCGCGAGGCTAAAG	344
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	CCCCATCCAACATTTTAAAGGATCGCGAGGCTAAAG AGGGTCCAAACGCCACCTTGGATATAGAGGAGCATAPAGA ccaa c gg at g ga a a	400
pif97-cds	Pinctada fucata .txt	ATGTTTTCTTAAACAGGAAATACATGTCGCAATTAT	384
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	ATGTTTTCTTAAACAGGAAATACATGTCGCAATTAT TATTTTAA.....AAAGAGAAGGAGCATGGTGTGATGAT atgttt aa ag a a atg g c tat	437
pif97-cds	Pinctada_fucata_.txt	ACTATGAAATTAGGGGGGATAITGCGGATAGAGAAGTGA	424
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	ACTATGAAATTAGGGGGGATAITGCGGATAGAGAAGTGA GCTGGTGTTCCTCACTGATGGAATACTAGAGAAGG ct t t g gat t ta agaa g	477
pif97-cds	Pinctada fucata .txt	AATGTCGTTGGATCAAGGATAITAGCGGAAATGTTGAGA	464
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	AATGTCGTTGGATCAAGGATAITAGCGGAAATGTTGAGA AAAATAACGCTACTAAGGAAAGTTGCGAAAGTATTGTC aa a t t a c gc ag ga g	517
pif97-cds	Pinctada_fucata_.txt	TATTAACTTGCGCTTGGATTCGGGCCAATGGTGAAG	504
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	TATTAACTTGCGCTTGGATTCGGGCCAATGGTGAAG TATTAACTTGCGCTTGGATTCGGGCCAATGGTGAAG tattaa t t c t gg t gg a ga	557
pif97-cds	Pinctada fucata .txt	AGAGACACATAGAGACCATGGATATGATAGAGACCAAG	544
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	AGAGACACATAGAGACCATGGATATGATAGAGACCAAG GAAGACGAACTAGAGACCATGGATATGATAGAGACCAAG aga ga ta g at gc t at ga ca g	597
pif97-cds	Pinctada_fucata_.txt	CATACTTTATGGATGACGAAAGATGTTAACATCAGAAAAT	584
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	CATACTTTATGGATGACGAAAGATGTTAACATCAGAAAAT c c	598
pif97-cds	Pinctada_fucata .txt	GAAAGAAATCCCTGATTATCTATGCAAAAT	624
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	GAAAGAAATCCCTGATTATCTATGCAAAAT .....TATCAAAGCT .....TATCAAAGTG tatcaaag	668
pif97-cds	Pinctada fucata .txt	AGAAAGCCAAAGTACAGTGGGAAAAAGTCAAACTG	664
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	AGAAAGCCAAAGTACAGTGGGAAAAAGTCAAACTG GAGTCCTTGTGAGAGCTCTGAAATTTTCAAAACTTG ag a g a a tc aac tg	648
pif97-cds	Pinctada_fucata_.txt	CGAAAGAAGTGTGATATGGACCGGGGTTAAAGTGGAG	704
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	CGAAAGAAGTGTGATATGGACCGGGGTTAAAGTGGAG TCCACTTGTGATGTCACAAACAGGTAACTGAAAAATGTA g ag att t a gat gca cc gg	685
pif97-cds	Pinctada fucata .txt	ATTCGTTGTC.....ACTGAAACGTCAGCGACATTCGATA	742
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	ATTCGTTGTC.....ACTGAAACGTCAGCGACATTCGATA TCTGAACTGCTAACAAACAGGTAACTGAAAAATGTA tc tgc ac aaac a cga aaa a a	725
pif97-cds	Pinctada fucata .txt	AGGUAAGGAAGTACAGAAGTCAAGGATATGTCGTTGTC	782
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	AGGUAAGGAAGTACAGAAGTCAAGGATATGTCGTTGTC CTCTACTGAACTGTCAGGAGTGTGCGAACTCAAACTGCGCT a gaaat ag g gat gta	765
pif97-cds	Pinctada_fucata_.txt	TGAATGGTATGTCAGTACAGGATATGGACAGTCCTGCCACA	822
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	TGAATGGTATGTCAGTACAGGATATGGACAGTCCTGCCACA CTTGTCCCAAGGTTTCTTCTGCCAGTCCTGCCACA t t gg c tcc	805
pif97-cds	Pinctada fucata .txt	AGGTGTCAGGAATTGGTAAATGTCAGCAATGTCGTTGTCAT	862
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	AGGTGTCAGGAATTGGTAAATGTCAGCAATGTCGTTGTCAT CTGAACTGCTCCCAATGTCAGGAAACGCGGAGAC gt t t aatg g aa tg ga	845
pif97-cds	Pinctada_fucata_.txt	CCCTTGCGATTCAGTCAATGTCAGGCCATTTGGCTAGTA	902
Pif97-JQ619625_Crassostrea_gigas.txt	Consensus	CCCTTGCGATTCAGTCAATGTCAGGCCATTTGGCTAGTA GAGGGTCCCGATTCAGTCAATGTCAGGCCATTTGGCTAGTA a a C aa G C G t t C A A C	885