

**Full length cloning and phylogenetic analyses of TCTP and ferritin genes from
Fenneropenaeus indicus (H. Milne Edwards)**

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Abstract

Elucidation of bacterial afflictions in commercially important aquaculture reared shrimps through molecular analyses is pivotal for prevention and/or control of disease outbreaks. In this study we examined the phylogenetic relatedness and compared the possible immune related functional roles of both translationally controlled tumor protein (TCTP) and ferritin genes with the previous studies. Both of them were substantially upregulated in the Indian white prawn *Fenneropenaeus indicus* postlarvae due to bath challenging with a virulent strain, *Vibrio harveyi* D3. Full-length cloning of these genes by RACE-PCR yielded 727 bp long TCTP and 1212 bp long ferritin gene sequences. Their open reading frame was 507 bp and 510 bp respectively. The TCTP ORF coded for 168 amino acids with three substitutions at positions 37, 141, 155, and ferritin ORF coded for 170 amino acids with no species specific substitutions. Phylogenetic analysis suggested the closest relatedness of both TCTP and ferritin from *F. indicus* to *F. chinensis*. In addition to reporting the full length sequences of these immune relevant genes, this study is useful to recognize their conserved natures which perhaps make them important defense related proteins in the innate immune system of *F. indicus*.

Keywords: *Fenneropenaeus indicus*; *Vibrio harveyi*; TCTP; Ferritin; Gene expression;

RACE-PCR

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Introduction

The Indian white prawn, *Fenneropenaeus indicus* (H. Milne Edwards) contributes substantially to marine shrimp aquaculture in India. Among reared shrimps, microbial infections are usually associated with high incidence of mortality rates and, larval deformities (Vandenberghe, Thompson, Gomez-Gill & Swings 2003). The highly virulent and infectious luminous vibriosis caused by *Vibrio harveyi* in farmed shrimps represents a serious threat in hatcheries causing mass mortalities, and in rearing and production of marine shrimp aquaculture facilities (Lavilla Pitog, Baticados, Cruz-Lacierda & De La Pena 1990; Saulnier, Haffner, Goarant, Levy & Ansquer 2000).

Lacking immunologic memory, the invertebrate immune system is dependent on specific innate molecules for its defense against external pathogens (Lee & Söderhäll 2000). From a previous study (Nayak, Ajay, Ramaiah, Meena & Sreepada 2011) it was observed that the two most important immune related genes, viz., translationally controlled tumor protein (TCTP) and ferritin genes were substantially upregulated in *F. indicus* postlarvae as a result of bath challenge with *V. harveyi* D3. As the expression levels of TCTP are reported to be significantly upregulated in response to a large number of extracellular stimuli there have been a large number of studies on this aspect (see Bommer & Thiele 2004). This protein has been credited to perform a large array of functions and different activities. It has a significant role in important cellular processes, such as cell growth, cell cycle progression, and malignant cell transformation (Gachet, Tournier, Lee, Lazaris-Karatzas, Poulton & Bommer 1999; Liu, Peng, Cheng, Yuan, Yang-Yen 2005; Yang, Yang, Xiong, Yan, Wang, Nishino, Mirkovic, Nguyen, Wang & Yang 2005). Known as also p23 or fortillin, TCTP is a highly conserved and widely expressed protein throughout the animal and plant kingdoms (Mulenga & Azad 2005; Venugopal 2005). It is reported to protect cells under toxic conditions and from apoptosis (Graidist, Fujise, Wanna, Sritunyalucksana & Phongdara 2006; Hsu, Chern, Cai, Liu & Choi 2007). Previously it was known to be an tumor-related protein as observed in mouse erythroleukemic cells Yenofsky, Cereghini, Krowczyńska & Brawerman 1983; Chitpatima, Makrides, Bandyopadhyay & Brawerman 1988). Furthermore, it sometimes acts as an extracellular cytokine having a lot of significance in the field of medical pathology (Chitpatima *et al.* 1988).

Ferritin has been reported from a wide range of organisms starting from prokaryotes to eukaryotes (Harrison & Arosio 1996). Ferritins are basically iron storage proteins found throughout the animal Kingdom and belong to a large family of ferritin-like diiron-carboxylate proteins (Theil 1987). Ferritin

is related to innate immune responses as its synthesis is regulated by cytokines responsible for inflammatory responses at transcriptional and translational levels (Konjin, Hershko & Izak 1979; Rogers, Bridges, Durmowicz, Glass, Auron & Munro 1990) Being an iron storage protein, ferritin is very crucial in the metabolism as well as in the detoxification of iron. Its expression has been reported to be associated with the role of maintaining iron homeostasis inside the cell (Marcelis 1980). Ferritin acts effectively against bacterial pathogens by failing the pathogens chances of binding to iron in the host tissue (Ong, Wang, Zhu, Ho & Ding 2005). Earlier studies of this transcript in shrimps (Pan, He, Yang, Liu & Xu 2005; Zhang, Li, Wang, Zhang, Zhou & Xiang 2006) had revealed upregulation following challenge with white spot syndrome virus (WSSV).

A suppression subtractive hybridization (SSH) experiment between healthy and *Vibrio harveyi* challenged *F. indicus* postlarvae had revealed high representation of both these transcripts in the subtracted library (Nayak *et al.* 2011). Earlier qPCR analysis of these proteins had also shown upregulation in both *Penaeus monodon* and *F. indicus* postlarvae challenged with *V. harveyi* (Nayak, Singh, Ramaiah & Sreepada 2010; Nayak *et al.* 2011). In view of the general lack of molecular information on immune related genes from this commercially valuable shrimp species, full length cloning (RACE) of both TCTP and ferritin gene and sequencing were carried out to realize their possible functional role(s) and their phylogenetic relatedness/diversity.

Materials and methods

Immune challenge of postlarvae

Ten day old post-larvae (PL) of *F. indicus* obtained from a commercial hatchery that maintained the PL in cisterns (50 cm x 50 cm x 20 cm [height]) were allowed for 2-3 hrs in a glass tank filled with 10-15 litres of 0.45 µm passed seawater to reduce bacterial load. They were then transported in air-packed polythene bags filled with 20L filtered seawater. After their reaching the laboratory too, they were held in a 20 L aquarium tank filled with filtered seawater under continuous aeration. They were fed twice daily with artificial feed and maintained throughout the 4 day experimental period under aeration at 25°C and 28 psu salinity.

The immune challenge experiment was identical to the one we followed for *P. monodon* (Nayak *et al.* 2010). The *F. indicus* PL were challenged with the same strain of *V. harveyi* D3 (GenBank accession No. HM486479). For the immune challenge replicates of two glass containers of 2-liter capacity were

filled with filtered autoclaved seawater. In each container 50 PL14 were acclimatized for a day under aeration. Briefly, 24 h old cultures in sea water nutrient broth were used for the experimental challenge.

Total RNA isolation

Total RNA was isolated from the hepatopancreas of freshly dissected *F. indicus* using Trizol reagent (Sigma USA) following the instructions of the manufacturer. The concentration and purity of RNA samples were determined spectrophotometrically by measuring the absorbance at 260nm. An aliquot of RNA sample was also run on 1% formaldehyde agarose gel for checking structural integrity.

Full length cloning using RACE-PCR

To clone the full length cDNA of TCTP and ferritin gene 3' RACE and 5' RACE was carried out using a Gene racer Kit (Invitrogen, USA) following the instructions of the manufacturer. cDNA synthesis was carried out using reagents provided with the Gene racer kit (Invitrogen, USA). Separate reactions for amplifications of 5' and 3' end fragments were carried out using gene specific primers and Gene racer primers (provided by the manufacturer). The gene specific primers used to amplify the ferritin and TCTP cDNA were designed from the corresponding EST fragments obtained from a previously constructed *F. indicus* subtracted cDNA library. Primers were designed using primer express software (ABI, USA). Full length TCTP gene was amplified by using the gene specific primer TPF 5' ATATGCGTCTGCAGCAAACCGGCTT3' and TPR 5' AAGACTGGCCGCTCTTCTCCATG3' along with the Gene racer primers provided by the manufacturer. Similarly for amplifying the ferritin gene FERF 5' ACGTTGCTCTGCCGGGTTTCG 3' and FERR 5' CAGCCGGACCAGCACGTTTCA 3' were used. The 3' RACE and 5' RACE PCR products obtained were later cloned separately into TOPO vector (Invitrogen, USA). The ligation products were transformed into *E. coli* Top10 cells by electroporation. From each cloned library, 10- 12 positive clones were picked out randomly and checked for inserts by carrying out PCR with gene specific primers. Plasmid isolation from the clones was done using a Pure Link plasmid DNA purification kit (Invitrogen, USA). The 5' and 3' RACE products were later sequenced bi-directionally using M13 forward and reverse primers.

Sequence analysis

All raw sequences were manually screened for vector sequences using Vecscreen tool available at NCBI. After removal of vector sequences the sequences were analyzed using blastx and blastn algorithms. The complete nucleotide sequence of the gene was obtained after aligning the 5' and 3' end

cDNA sequences. The open reading frame for the obtained nucleotide sequence was determined by using the ORF Finder tool available at NCBI. The full length sequences were submitted at Genbank. Multiple sequence alignment of the deduced protein sequence of TCTP and ferritin along with other homologous sequences from representative species was carried out using Clustal X software following the profile method (Thompson, Gibson, Plewniak, Jeanmougin & Higgins 1997). Sequence editing was carried out using the Bioedit software. Protein sequences used for the phylogenetic analysis of TCTP and ferritin were retrieved from the GenBank database (Table 2a and 2b). Separate phylogenetic trees for the both the proteins were constructed using Neighbour joining method using Mega-4 by default settings of the program. To get the most probable tree, the sequences were boot-strapped 1000 times. Branches with <50% bootstrap support were collapsed in the final tree.

Results

Bath challenge of F. indicus with Vibrio harveyi

Besides, lethargy and loss of appetite, close to 45% mortality was observed in the *F. indicus* post larvae that were bath challenged by adding 2.5- 3.2 ($\times 10^6$) ml⁻¹ *V. harveyi* D3 cells (Table 1). No mortality was observed in the control set.

Full length cloning of TCTP gene and sequence analysis

The 302 bp length EST fragment of TCTP gene identified from *F. indicus* was used as template to design primers for full length amplification of this gene. On blastx analysis, the identified *F. indicus* complete gene (Accession No gb|JG772274|) was identical to a previous TCTP sequence (gb|ACR58988.1|). The identified fragment had 98% similarity to *Litopenaeus vannamei* fragment (Accession No. gb|ABY55541.1|) and *Fenneropenaeus merguensis* (Accession No.gb|AAV84282.1|), also it was found to have 97 % homology to *Penaeus monodon* TCTP fragment (Accession No. gb|AAO61938.1|).

The complete TCTP nucleotide sequence obtained after RACE PCR was 727 bp in length. Upon analysis of the complete RACE PCR product, it was found that the ORF of this gene consisted of 507 bp coding for 168 amino acids (Fig 1). The calculated iso-electric point (pI) was 4.3 and molecular weight was 19.23kDa. Highly conserved nature of TCTP was evident from the aminoacid sequence alignment of TCTP sequences from different arthropod representatives (Fig 2.). When compared within the penaeid shrimp species particular amino acid substitutions were observed at positions 37, 141 and 155 in

the sequence alignment (Fig 2). At position 37 a valine to isoleucine substitution was found and at position 141, aspartic acid to valine substitution was observed. A third substitution was observed at position 155 where aspartic acid was substituted by histidine (Fig 2).

Phylogenetic analyses of the TCTP gene

The phylogenetic tree constructed in this study clearly shows that the crustacean members distributed in four different groups (Fig 3). *F. indicus* TCTP along with other penaeid family representatives clustered to a separate clade and the protein sequences, taken for the tree construction, clustered with 100% bootstrap value. The homology of different arthropod TCTP sequences as compared to the identified *F. indicus* TCTP sequence in terms of percent identity are presented in Table 2a. The phylogenetic tree constructed clearly shows the high homology between TCTP sequences from *F. indicus* and *F. chinensis* which have clustered in the same node (Fig. 3). From the phylogenetic tree it can also be clearly seen that *Aedes albopictus* and *Tigriopus japonicus* did not fall into any of the four groups. A high similarity shared by TCTP from *F. indicus* with other penaeid species suggests its conserved nature within the penaeid family.

Full length cloning of ferritin gene

After 3' and 5' ends RACE PCR, the full-length cDNA fragment of *F. indicus* ferritin gene was found to be 1212 bp (Accession No gb|JN651914|). The full length nucleotide sequence and the deduced amino acid sequence are shown in (Fig 4). Upon Blastx analysis the obtained ferritin fragment had 98% identity at the amino acid level with *F. chinensis* (ABB05537.1) and *L. vannamei* (AAX55641.1). The identified fragment also shared 96% similarity with previously deposited *P. monodon* (gb|ABP68819.1) sequence in the Genbank. Upon analysis of the complete nucleotide sequence of ferritin it was found that that the open reading frame (ORF) of this gene was 510 bp in length. The 5' untranslated region (5'-UTR) was found to be of 130 bp and 3' untranslated region (3'-UTR) was 568 bp. The ORF encoded 170 amino acids with a predicted molecular weight (MW) about 19385.81 Da and a theoretical isoelectric point (PI) of 5.03. The deduced amino acids sequence also shared high similarity with those of *Pacifastacus leniusculus* (CAA62186), *Macrobrachium rosenbergii* (ABY75225.1), *Scylla paramamosain* (ADM26622.1) and *Procambarus clarkii* (AEB54659.1). Amino acid sequence alignment of the identified *F. indicus* ferritin gene with other representative species however showed no significant amino acid substitutions (Fig 5). An iron responsive element sequence (IRE) which is

specific features of the ferritin protein was found to be located at positions 5 to 30 bases towards the 5'UTR region (Fig 4).

Phylogenetic analyses of the ferritin gene

The phylogenetic tree constructed in this study clearly shows that the *F. indicus* ferritin along with other shrimp species representatives clustered to a separate clade (Fig 6). The homology of different representative ferritin sequences deposited in Genbank as compared to the identified *F. indicus* ferritin sequence in terms of percent identity are presented in (Table 2b). Upon analysis of the phylogenetic tree it was seen that there was high homology between ferritin sequences from *F. indicus* and *F. chinensis* which have clustered in the same node (Fig 6). Ferritin from mammalian representatives comprising of *Danio rerio*, *Mus musculus* and *Homo sapiens* formed a separate cluster. In this phylogenetic tree the *Drosophila melanogaster* ferritin sequence was found to be an outlier.

Discussion

The bath challenge of *F. indicus* post-larvae with *V. harveyi* D3 resulted in lethargic behavior and mortalities similar to those observed in *P. monodon* (Nayak *et al.* 2010). In this study, the full length genes of TCTP and ferritin from *F. indicus* were cloned individually using RACE PCR technique. Since the full length cDNAs are important in characterizing and correct annotation of genomic sequences, this technique has great significance in elucidating the functional profiles of any given protein molecule (Satoh, Doi, Nagata, Kishimoto, Suzuki, Otomo, Kawai, Nakamura, Hirozane-Kishikawa & Kanagawa 2007).

The amino acid sequence alignment of the TCTP protein suggested three significant substitutions which are specific for *F. indicus* (positions 37, 141 and 155; Fig 2). These results accorded with previous observation of (Rajesh, Kiruthiga, Rashika, Priya & Narayanan 2010). A valine to isoleucine substitution at position 37, aspartic acid to valine substitution at position 141 and aspartic acid to histidine substitution at position 155 were unique to the TCTP gene in *F. indicus vis a vis* other penaeid shrimps. Substitution of histidine might be very significant as histidines are known to impart anti-inflammatory and antioxidative properties to proteins (Seo, Kwak & Lee 2010; Chen, Muramoto, Yamauchi, Fujimoto & Nokihara 1998). In this regard observations by (Mohankumar & Ramasamy 2006; Mathew, Ashok Kumar, Anandan, Viswanathan & Devadasan 2007) are useful to infer that when shrimps undergo severe oxidative stress under viral and/or microbial challenges, such substitutions might prove effective in dealing with the pathogen. Furthermore, the antimicrobial activity

of histidine containing peptides against pathogenic bacteria is also well known (Mason, Gasnier, Kichler, Pre´vost, Aunis, Metz-Boutigue & Bechinger 2006). These substitutions might impart TCTP a definite role in defense against bacterial pathogens. Thus expression of TCTP in *F. indicus* postlarvae might protect them from bacterial challenges and usher in their survival due to or, during microbe-induced stress.

Recent studies using SSH, have recognized the protective role of TCTP as an important defense molecule in *V. harveyi* challenged *P. monodon* (Bangrak, Graidist, Chotigeat & Phongdara 2004; Nayak *et al.* 2010). Recently elevated levels of TCTP expression has been reported in WSSV challenged *F. indicus* (Rajesh *et al.* 2010). Though, TCTP expression is sometimes reported to decrease during the terminal stage of the infection as in a case of WSSV challenge (Graidist *et al.* 2006), its manifold expression in challenged *F. indicus* postlarvae might be of greater relevance to the affected haemocytes during the processes of apoptosis. Along with antioxidant and innate immune responses, the increased expression of TCTP signifies its importance as an integral part of host response against bacterial challenges in cell death/apoptosis mechanisms.

From the complete nucleotide sequence of the *F. indicus* TCTP, it is clear that the structural conservation and uniqueness of this molecule might play significant roles in many biochemical and signaling pathways. Although few but significant amino acid substitutions were observed in this study, these substitutions might play crucial roles in the defense of microbial challenged shrimps and thereby have relevance with the protective functions associated with this molecule. While direct evidences on its anti-apoptotic properties are essential, results from this study do imply its prominent role in innate defense mechanisms of shrimps. This study is useful to recognize the importance of up-regulation of anti-apoptotic mechanisms in shrimp as a result of bacterial pathogens. From this present study, it is clear that TCTP plays a critical role in the survival of the *F. indicus* against *V. harveyi* infection/challenge.

While extensive studies have been carried out on ferritins in vertebrates, very little is known about their sequence, distribution and expression in crustaceans and fishes. Many studies earlier have shown that ferritin synthesis of ferritins is directly affected by inflammatory conditions including those caused by bacterial infections (Orino & Watanabe 2008). The 1212 bp RACE PCR product obtained in this study had very high similarity to *F. chinensis* ferritin sequence (ABB05537.1). Detailed structure of ferritin gene shows the presence of iron-responsive element (IRE) region which is an important factor in

assigning functional importance as an iron binding protein in the invertebrates. This region of ferritin gene codes for a very unique iron regulatory protein (IRPs) that can help in translational regulation of ferritin gene by binding to iron responsive elements (IREs) located in the 5' untranslated regions (5'-UTR) of the gene (Durand, Carvalho, Goudard, Pieri, Fower & Cotrat 1999). The IRE was found to be located at positions 5 to 30 bases towards the 5'UTR region. The IRE regions are very conserved and found in almost all major vertebrates and invertebrates. Higher levels of ferritin have been already shown in our previous study in *F. indicus* (Nayak *et al.* 2011). From this it can be hypothesized that higher expression levels of ferritin gene signifies its defense mechanism against the invading bacteria as has been shown earlier by Marcelis (1980). In essence binding of free iron is considered bacteriostatic and bacterial cell growth is inhibited if no iron is available for synthesis of essential iron-containing compounds.

The importance of ferritin is due to its multifaceted roles in diverse number of biological processes like detoxification (Munro & Linder 1978), inflammation (Roeser, Jacobs & Worwood 1980) development regulation (Levenson & Fitch 2000) and neuronal differentiation (VanLandingham & Levenson 2003). Its immunogenic properties can also be contributed to its unique sequence structure which permits it to act as a iron sequestering machinery (Ong, Wang, Zhu, Ho & Ding 2005). Their multimeric structure consists of an assembly of 24 individual subunit proteins and each of the subunit has a definite functional role (Harrison & Arosio 1996). The core formed by this protein is able to bind upto 4500 iron atoms (Harrison & Arosio 1996). Rise in the levels of ferritin expression has also been reported as a signal or marker for the commencement of inflammatory processes and neoplasms (Moroz, Chetrit, Kahn & Modan 1997; Ozen, Uygur, Sahin, Tekgul, Ergen & Remzi 1995). Accordingly, ferritin is also well known to play a unique role in the innate immunity processes against viral infection (Zhang *et al.* 2006). Although the perfect function of this molecule as an antibacterial agent in shrimps is still to be proved, this study has brought out its relevance in the *F. indicus* as a major defense protein against bacterial agents like *Vibrio harveyi*.

Results from this study demonstrate the structural uniqueness of TCTP and ferritin genes and brings forth their important functions in *F. indicus* innate immunity. Both these two proteins share exclusive sequence structure conservation among their related species implying a strong evolutionary relationship of these genes within different shrimp-species. Although few but significant amino acid substitutions were observed in *F. indicus* TCTP gene, they might be vital in the defense of larval stages of *F. indicus*. From the total absence of shrimp-species specific substitution in the ferritin gene, it is ascribable that its

protective roles are highly similar to the ones reported from other shrimps. Although direct evidences on anti-apoptotic properties of *F. indicus* TCTP or that of ferritin in multifaceted protective functions would expand our understanding, results from this study in general imply their prominent role in innate defense mechanisms of shrimps.

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1	TAC TGG GCC GAC ACG AGA CAA GCA TCT CCA CCA GTT GAG AGT ATT	46
47	AGC GAC GAT CAT CTA GCC GCC <u>ATG</u> AAG GTC TTC AAG GAT ATG CTC	91
	M K V F K D M L	
92	ACA GGT GAT GAG ATG TTC ACC GAC ACC TAT AAG TAT GAG GAG GTG	136
	T G D E M F T D T Y K Y E E V	
137	GAT GAT GCC TTC TAC ATG GTC ATT GGA AAA AAT ATT ACT ATT ACT	181
	D D A F Y M V I G K N I T I T	
182	GAG GAT AAC ATT GAG CTA GAG GGA GCC AAT CCA TCA GCT GAA GAG	226
	E D N I E L E G A N P S A E E	
227	GCT GAT GAA GGC ACT GAC ACT ACT AGT CAG TCT GGC GTA GAT GTA	271
	A D E G T D T T S Q S G V D V	
272	GTT ATA TAT ATG CGT CTG CAG GAA ACC GGC TTC CAA GTA AAG AAG	316
	V I Y M R L Q E T G F Q V K K	
317	GAT TAT CTT GCA TAC ATG AAA GAA TAC CTA AAG AAT GTA AAG GCA	361
	D Y L A Y M K E Y L K N V K A	
362	AAG TTG GAA GGC ACA CCT GAA GCT TCA AAG TTA ACA TCT ATC CAG	406
	K L E G T P E A S K L T S I Q	
407	AAG CCT CTT ACA GAC CTT TTG AAG AAG TTC AAG GAC TTG CAA TTC	451
	K P L T D L L K K F K D L Q F	
452	TTC ACT GGA GAA TCA ATG GTA CCC GAT GGC ATG GTT GTT CTC ATG	496
	F T G E S M V P D G M V V L M	
497	GAT TAC AAA GAC ATT CAT GGA GAA GAG CGG CCA GTC TTA TAC TTC	541
	D Y K D I H G E E R P V L Y F	
542	CCA AAA TAC GGT CTA ACA GAG GAG AAA CTA <u>TAA</u> ACG TTA TTT ATT	586
	P K Y G L T E E K L *	
587	TCT GAG TTA TAA TGC AGC CCT CGT CAT CTG GAC TCC CAG GGT CAT	631
632	GAA AGC CGA TTG TTC ATG GTC CTT TTT AAT TTA ATT TTA ATA TAC	676
677	TCA GAT AAA TTT AGT CAT CCT GCC CAA AAA AAA AAA AAA AAA AAA	721
722	AAA AAA 727	

Fig 1. Complete nucleotide and deduced amino acid sequence of *Fenneropenaeus indicus* TCTP gene (Accession No gb|JG772274|) obtained in this study using RACE-PCR. Letters in bold and underlined indicate the start and stop codons, arrows indicate the annealing sites of gene specific primers used in the RACE-PCR

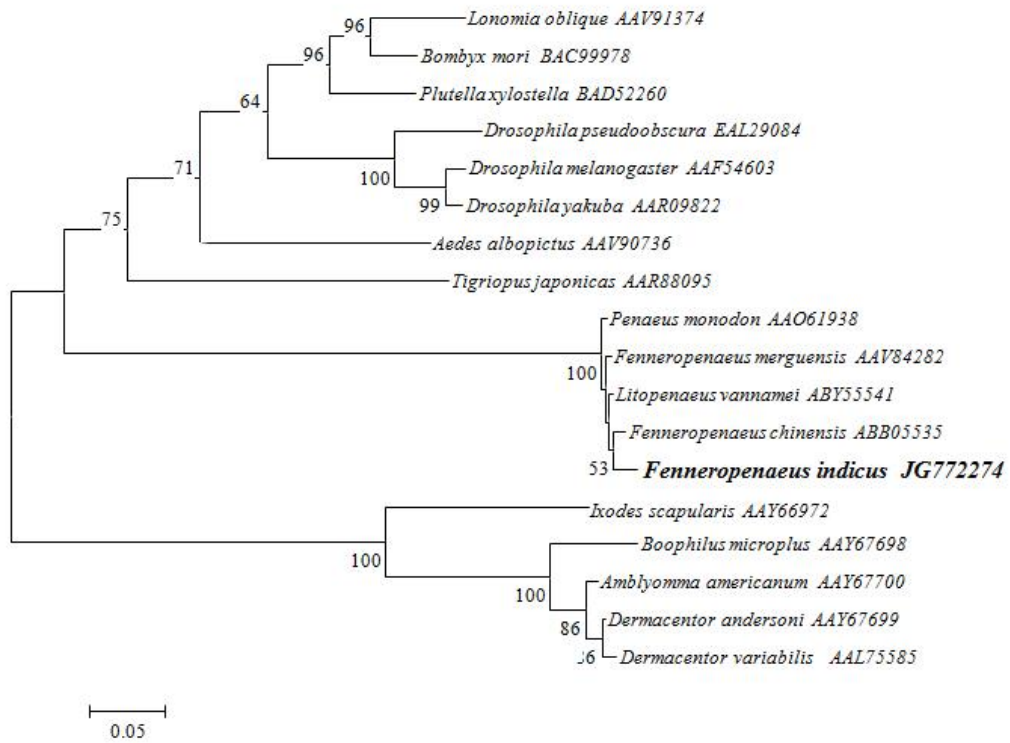


Fig 3. Phylogenetic tree of translationally controlled tumor protein (TCTP) gene in arthropods. The tree was constructed using neighbor joining method using Mega-4. The percentages of boot strap support are shown on the branches. These values were calculated from 1000 bootstrap re-samplings.


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1   GTGCTCCGGGTCACCAGTGTGTGGACGAGTACCTTTAAGCGAACCTCTGGAAACCTCTCCTTTTGGGA
70  CCTCTTCCGTCAACTTTATTACCTCACGTACCATTACCGCTCATCCAAGAGACGATCAAG ATG GCT
    M   A
138 TCT CAG GTT CGT CAG AAC TAC CAC GAA GAC TGC GAA GCT TCT ATC AAC AAA CAG
    S   Q   V   R   Q   N   Y   H   E   D   C   E   A   S   I   N   K   Q
192 ATC AAC ATG GAA CTG TAC GCT TCT TAC GTT TAC CTG TCT ATG GCT CAC TAC TTC
    I   N   M   E   L   Y   A   S   Y   V   Y   L   S   M   A   H   Y   F
246 GAA CGT GAC GAC GTT GCT CTG CCG GGT TTC → GCT AAA TTC TTC AAA GAC TCT TCT
    E   R   D   D   V   A   L   P   G   F   A   K   F   F   K   D   S   S
300 GAC GAA GAA CGT GAA CAC GCT CAG ATC TTC ATG AAA TAC CAG AAC AAA CGT GGT
    D   E   R   E   H   A   Q   I   F   M   K   Y   Q   N   K   R   G
354 GGT CGT ATC GTT CTG CAG CAG ATC GCT GCT CCG TCT ATG CAG GAA TGG GGT ACC
    G   R   I   V   L   Q   Q   I   A   A   P   S   M   Q   E   W   G   T
408 GGT CTG GAC GCT CTG CAG GCT GCT CTG GAC CTG GAA AAA CAG GTT AAC CAG TCT
    G   L   D   A   L   Q   A   A   L   D   L   E   K   Q   V   N   Q   S
462 CTG CTG GAA CTG CAC GGT ACC GCT TCT GGT AAC AAC GAC CCG CAC CTG ACC AAA
    L   L   E   L   H   G   T   A   S   G   N   N   D   P   H   L   T   K
516 CTG CTG GAA GAC GAA TAC CTG GAA GAA CAG GTT GAC TCT ATC AAA AAA ATC GGT
    L   L   E   D   E   Y   L   E   E   Q   V   D   S   I   K   K   I   G
570 GAC ATG ATC ACC CGT CTG AAA CGT GCT GGT CCG GCT GGT CTG GGT GAA TAC ATG
    D   M   I   T   R   L   K   R   A   G   P   A   G   L   G   E   Y   M
624 TTC GAC AAA GAA CTG AAA TAA ATCGATCGATGAAAATCAAGCCCTATTCTTGTGTAATTTTAC
    F   D   K   E   L   K   *
688 GGGAAATGTCATCAGTAACAAAAAGGAAAAAAGTCAAACCGTATATTACCACTGCTTTTTTACACCTGAAGC
759 ATCACAAAGGTTACATTGTATTTTGGATAGTATTGGACATTGCACACTAATCAGTAACATCAGTCTGTACA
830 AATGGTGCTTTATTTCGTGTAAGACACTCCCATCCAAATTCTGCAATTAGGTTGAACTTGGTAAACGGATTA
901 CTAATCTGGTAAAYAACCCGAAGCAATTAGAATTGGGAATTGAGATATAATAGTTAAAAGTTGATTTGTGG
972 AACACCCTTCATGTTGATTAATGTAATTCATATAGATTGAGAATTAGTGCAAACAGTGTTAGATCCTTTGA
1043 CAACAGATATAGGTATGTTACTATTCTTATCCAAGGTAATTTATTGGTTGTAATCTTGCATTTAATATGC
1114 TTGGTTAGTAMCAACATTGCTTGCGGTGTTGGCTTCTGGAAACTGGATGTTTCATCACTTCATTTTATCAT
1185 TACATTAAGGGTCGCTAAAAA

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Fig 4. Complete nucleotide and deduced amino acid sequence obtained from *Fenneropenaeus indicus* ferritin cDNA (gb|JN651914|). The start codon (ATG) and the stop codon (TAA) are indicated by underlined letters. The annealing sites of gene specific primers used in the RACE-PCR are shown by arrows. The IRE site is shown in rectangular box.

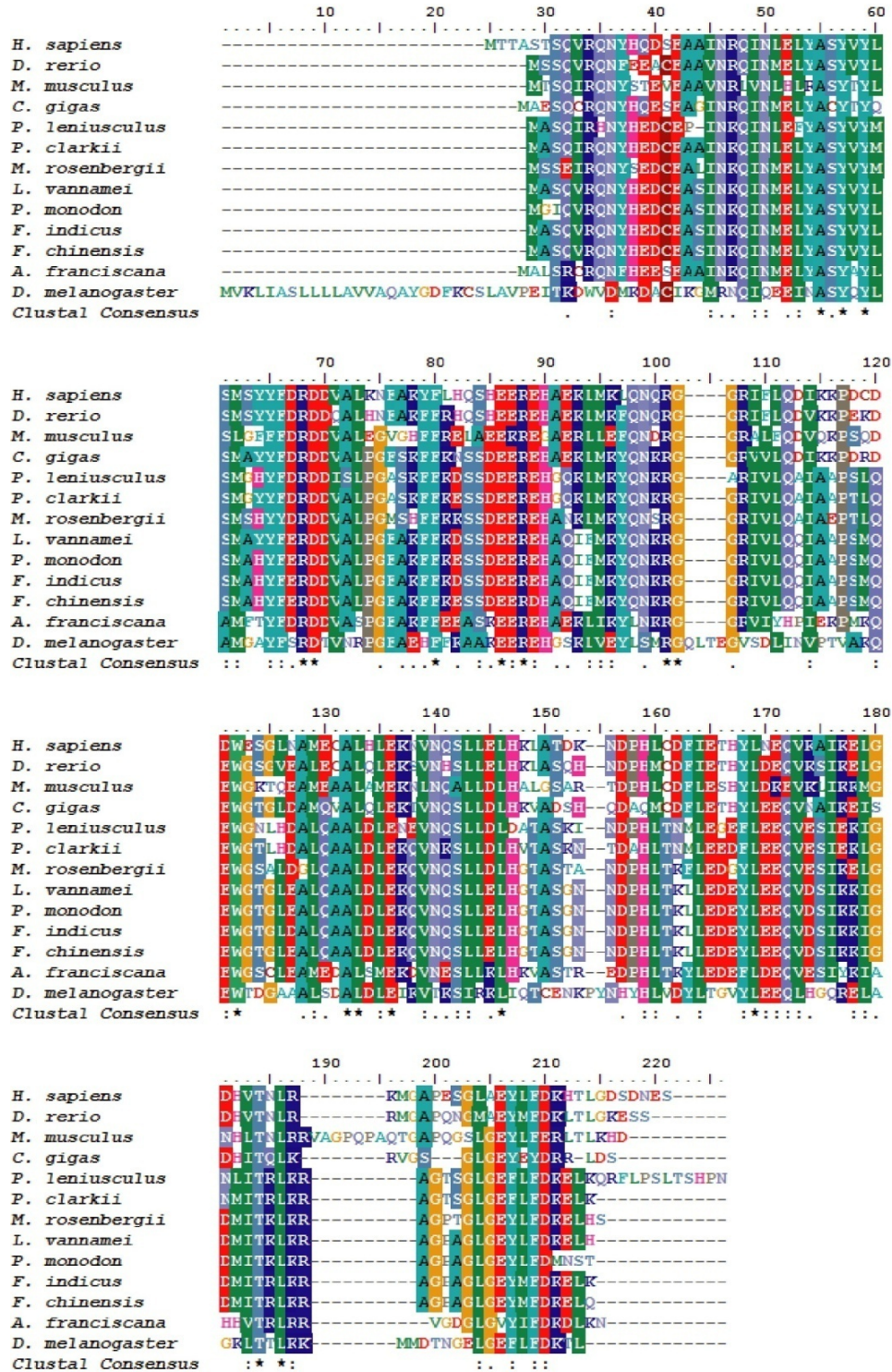


Fig 5. Multiple sequence alignment of *Fenneropenaeus indicus* ferritin amino acid sequence obtained in this study (Accession No gb|JN651914|) with other representative ferritin sequences. Identities are marked in asterisk below and similarities are shaded in colored codes.

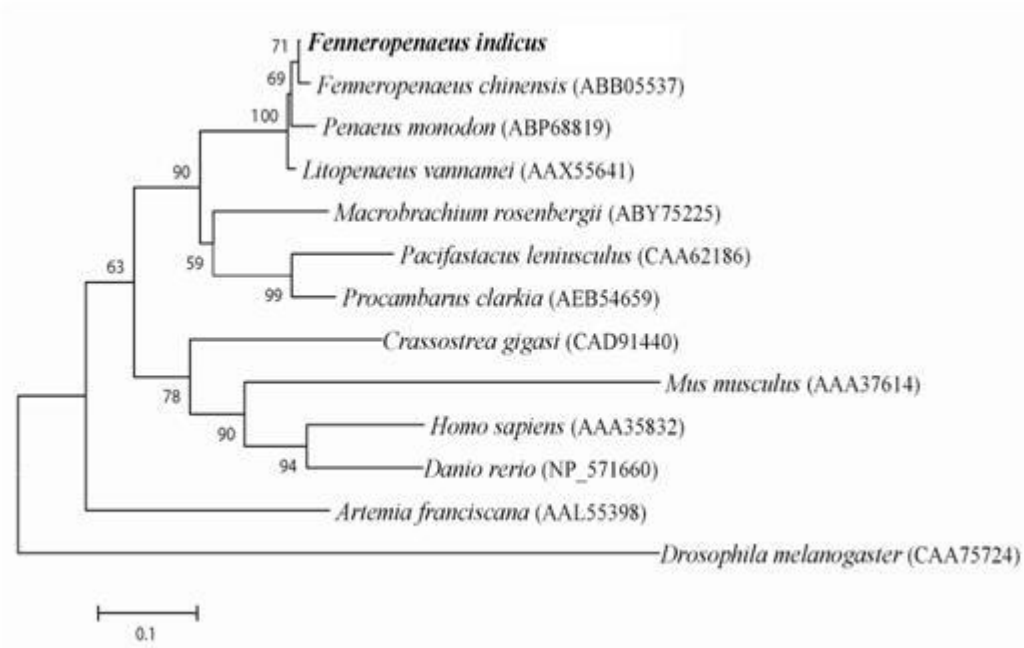


Fig 6. Phylogenetic tree of ferritin gene. Phylogenetic tree was constructed from the deduced amino-acid sequences of ferritin along with other several representative organisms. The tree was constructed using Neighbour joining method using Mega-4. The percentages of bootstrap support are shown on the branches. These values were calculated from 1000 bootstrap re-samplings.

Table 1. Observed mortality of *Fenneropenaeus indicus* post-larvae (PL14) bath challenged with *Vibrio harveyi* D3 isolate.

Experimental group	Total animals (no.)	Mortality (number died [~%]) at time (h)				
		0	24	48	72	96
Bath challenged	150*	0	23 [15]	45 [30]	60 [40]	68 [45]
Control	150*	0	0	0	0	0

*Experiment done with 50 PL per trial; mortalities in bath challenged sets are cumulative averages from three independent trials

Table 2a. Accession numbers of arthropod translationally controlled tumor protein (TCTP) sequences retrieved from GenBank and used for the present study

Accession No.	Arthropod representative	No. of aminoacids	% ID
ABY55541	<i>Litopenaeus vannamei</i>	168	98.2
ABB05535	<i>Fenneropenaeus chinensis</i>	168	97.6
AAO61938	<i>Penaeus monodon</i>	168	97.6
AAV84282	<i>Fenneropenaeus merguensis</i>	168	97.6
AAR88095	<i>Tigriopus japonicus</i>	172	52.9
AAV90736	<i>Aedes albopictus</i>	171	50.2
AAV91374	<i>Lonomia oblique</i>	172	48.2
BAC99978	<i>Bombyx mori</i>	172	50
BAD52260	<i>Plutella xylostella</i>	172	51
AAF54603	<i>Drosophila melanogaster</i>	172	55.8
AAR09822	<i>Drosophila yakuba</i>	172	55.8
EAL29084	<i>Drosophila pseudoobscura</i>	172	54
AAV66972	<i>Ixodes scapularis</i>	173	44
AAV67698	<i>Boophilus microplus</i>	173	41.9
AAV67699	<i>Dermacentor andersoni</i>	173	43.1
AAV67700	<i>Amblyomma americanum</i>	173	43.1
AAL75585	<i>Dermacentor variabilis</i>	173	43

Table 2b. Accession numbers of ferritin amino acid sequences retrieved from GenBank and used for the present study

Accession No.	Representative organism	No. of amino acids	% ID
AAA35832	<i>Homo sapiens</i>	183	55
NP571660	<i>Danio reiro</i>	177	57
AAA37614	<i>Mus musculus</i>	183	44
CAD91440	<i>Crassostrea gigas</i>	171	60
CAA62186	<i>Pacifastacus leniusculus</i>	181	68
AEB54659	<i>Procambarus clarkii</i>	170	77
ABY75225	<i>Macrobrachium rosenbergii</i>	171	78
ABB05537	<i>Fenneropenaeus chinensis</i>	170	98
AAX55641	<i>Litopenaeus vannamei</i>	170	98
ABP68819	<i>Penaeus monodon</i>	170	95
AAL55398	<i>Artemia franciscana</i>	171	60
CAA75724	<i>Drosophila melanogaster</i>	205	31