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# Characterization of a novel ADP-ribosylation factor gene from *Macrobrachium nipponense* and its response to ammonia nitrogen stress

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ABSTRACT: ADP-ribosylation factors (Arfs) are guanosine triphosphate (GTP)-binding proteins that play essential roles in membrane trafficking, and they have been recently reported to be involved in innate immunity in crustaceans. However, little information is available on Arfs in the oriental river prawn *Macrobrachium nipponense* and their response to ammonia nitrogen stress. In this study, we identified a novel *M. nipponense* Arfn gene (*MnArfn*). The full-length cDNA of *MnArfn* was 1076 bp. It contained a 537 bp open reading frame (ORF) and encoded a 178 amino acid protein with a predicted molecular weight of 19.85 kDa. Sequence and phylogenetic analyses showed that MnArfn was an unidentified Arf, sharing 55–61% identity with other known Arfs. Quantitative real-time PCR (qPCR) indicated that all examined tissues (hepatopancreas, stomach, gill, heart, muscle, and eyestalk) expressed *MnArfn*. Hepatopancreas and gills, 2 organs involved in environmental stress management, had the highest expression. Under conditions of ammonia nitrogen stress, *MnArfn* expression in hepatopancreas and gills was significantly up-regulated at 6, 12, and 24 h. Western blotting experiments also revealed that MnArfn was distributed in all examined tissues, with the highest expression in hepatopancreas and gills, consistent with qPCR results. The findings from this study indicate that *MnArfn* may play an important role in the response of *M. nipponense* to ammonia nitrogen stress, which provides a new avenue to study the resistance mechanism(s) of crustaceans to ammonia nitrogen and to screen for individuals with resistance to unfavorable environments.

KEY WORDS: *Macrobrachium nipponense* · ADP ribosylation factor · Ammonia nitrogen stress

## 1. INTRODUCTION

The oriental river prawn *Macrobrachium nipponense* (Palaemonidae) (Cui et al. 2018) is an economically important aquacultural crustacean bred in freshwater and low-salinity estuarine regions in China (Cheng et al. 2017). However, with the expansion of culture practices and the deterioration of ecological environments (Duan et al. 2013, Price et al. 2015), environmental stresses have become the main restricting factor for the development of the prawn industry, and in some cases, large-scale economic losses have been reported (Moraes-Valenti & Valenti 2007, Lu et al. 2016). Ammonia nitrogen is

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the main toxic component in aquaculture water (Chen et al. 2016, Liang et al. 2016), and previous studies have reported that an elevated ammonia nitrogen level can impair the immune system (Chen & Kou 1992, Cheng & Chen 2002, Yue et al. 2010) and affect oxygen consumption (Chen & Lin 1992) in crustaceans. Moreover, the accumulation of excreted ammonia nitrogen can induce the expression of stress response-related and immune-related genes such as heat shock protein 90 (*HSP90*) (Li et al. 2012), ADP-ribosylation factor 1 (*Arf1*) (Duan et al. 2016), and a multiligand-binding protein, *gC1qR* (Sun et al. 2019). Therefore, a better understanding of the response of prawns to ammonia nitrogen stress is critical.

ADP-ribosylation factors (Arfs) are guanosine triphosphate (GTP)-binding proteins that play essential roles in membrane trafficking and cytoskeleton remodeling (Myers & Casanova 2008, Ding et al. 2015). Arfs are present in organisms including yeast, bacteria, vertebrates, and invertebrates (Ding et al. 2015). Based on sequence analyses, the 6 mammalian Arfs can be classified into 3 categories: class I comprises Arfs 1–3, class II comprises Arfs 4 and 5, and class III includes only Arf 6 (Zhang et al. 2010). Furthermore, different Arf proteins have different intracellular localizations and functions, depending on their membrane components and the diverse group of proteins they recruit (Donaldson & Honda 2005, D'Souza-Schorey & Chavrier 2006). Arfs exist in 2 conformations, namely GTP- and guanine diphosphate (GDP)-bound forms, and they are catalyzed by guanine exchange factors (GEFs) and GTPase activating proteins (GAPs). Recently, it has been reported that members of the small GTPase protein family, such as Rho, Ran (Liu et al. 2009), and Rab (Wu et al. 2008), are involved in the defense response in shrimp (He et al. 2004, Pan et al. 2005). Furthermore, shrimp Arfs have been found to participate in virus invasion and/or host defense (Wang et al. 2009).

Although several Arfs have been characterized from various organisms, the function of *M. nipponense MnArfn* in ammonia nitrogen stress is unclear. In this study, a novel Arf gene (*MnArfn*) from *M. nipponense* was identified and compared with known Afrs for the first time. The expression of *MnAfrn* gene in different tissues was also analyzed. We hope that our results will reveal the role of *MnAfrn* in ammonia nitrogen stress and provide a theoretical basis for future studies on the mechanism(s) of the *MnAfrn* gene.

## 2. MATERIALS AND METHODS

### 2.1. Ethics statement

This research was conducted in strict accordance with the International Guiding Principles for Biomedical Research Involving Animals 2012 (Council for International Organizations of Medical Sciences, www.cioms.ch), which specifies the care and permissible uses of experimental animals. The research did not involve endangered or protected species.

### 2.2. Animals

Healthy adult *Macrobrachium nipponense*, with an average weight of 3.71 ± 0.5 g (mean ± SD), were obtained from the Huangqian Reservoir in Taian, Shandong Province, China. Prawns were maintained in 800 l plastic tanks with aerated freshwater (temperature: 26 ± 0.5°C, pH: 7.8–8.2) and fed daily with a ration corresponding to 4% of the body weight for 1 wk before the experiments.

### 2.3. Ammonia nitrogen challenge and sampling

The ammonia nitrogen group and the control group each included 150 randomly selected prawns. According to the results of a 48 h median lethal concentration test, based on a pre-experiment containing 150 samples, an ammonia nitrogen dose of 98 mg $l^{-1}$ was used in this study. Nessler's reagent was used to measure the total ammonia nitrogen and $NH_4Cl$ levels, and then both components were adjusted to their desired concentrations. 150 healthy prawns were cultured under conditions of ammonia nitrogen stress for 96 h. Six individuals were selected from the treatment and control groups at 8 time points of 0, 3, 6, 12, 24, 48, 72, and 96 h (a total of 96 individuals were collected in this study) for hepatopancreas, gill, stomach, heart, muscle, eyestalk, and all samples were stored at −80°C for subsequent testing and analysis of each sample individually.

### 2.4. RNA preparation and cDNA synthesis

Total RNA from tissues collected at all time points was extracted according the manufacturer's instructions for TransZol UP (ET111-01, Trans Gen Biotech). RNA samples were evaluated by 1% agarose gel electrophoresis, and concentrations were determined

using a NanoPhotometer spectrophotometer (IMPLEN). First-strand cDNA was synthesized using a PrimeScript™ RT Reagent Kit (Takara) and the oligo dT primer from the PrimeScript™ kit. All cDNAs were stored at −20°C and used as templates in PCR.

### 2.5. Cloning the full-length *MnArfn* cDNA

A fragment of the *MnArfn* sequence was obtained from a hepatopancreas cDNA library available in our laboratory that was prepared as described by Yu et al. (2019). To obtain the full-length *MnArfn*, gene-specific primers (Arfn-5F1, Arfn-5F2, Arfn-3R1, Arfn-3R2) (Table 1) were designed for 5′- and 3′-RACE, and the 5′ and 3′ ends were obtained using a 3′-Full RACE Core Set ver. 2.0 kit and a 5′-Full RACE Kit (Takara), respectively. PCR conditions followed the manufacturer's instructions. PCR products were purified using a gel extraction kit (Sangon Biotech), cloned into the pMD18-T vector (Takara), and sequenced by Sangon Biotech.

### 2.6. Sequence analysis

BLAST (www.ncbi.nlm.nih.gov/BLAST/) was used to analyze and compare nucleotide and deduced amino acid sequences of MnArfn. The open reading frame (ORF) Finder program (www.ncbi.nlm.nih.gov/projects/gorf/orfig.cgi) was used to deduce the amino acid sequence. The molecular weight and isoelectric point of MnArfn were determined with the 'compute pI/Mw' tool (http://web.expasy.org/compute_pi/). Multiple sequence alignment was accomplished using the ClustalW Multiple Sequence Alignment program (www.ebi.ac.uk/clustalw/). A phylogenetic tree was constructed using MEGA 7.0 software, and the neighbor-joining method was used for phylogenetic analysis (www.megasoftware.net/mega7/).

### 2.7. Expression analysis with quantitative real-time PCR (qPCR)

qPCR was used to analyze *MnArfn* expression in the muscle, heart, hepatopancreas, gills, eyestalk, and stomach in a LightCycler®96 system (Roche Diagnostics). Primers (Arfn-F and Arfn-R) (Table 1) were designed based on the nucleotide sequence of *MnArfn*. *Macrobrachium rosenbergii* β-actin (GenBank KY038927.1) was used as an internal control. qPCR was carried out using the TB Green™ Advantage®qPCR Premix (Takara) according to the manufacturer's instructions. The qPCR cycling conditions were 95°C for 30 s, followed by 40 cycles at 95°C for 10 s and 56°C for 30 s. Each sample was assayed 6 times. The $2^{-\Delta\Delta ct}$ method was used to calculate the expression level of *MnArfn*.

### 2.8. Statistical analysis

Statistical analysis was performed using SPSS Ver 21.0 (IBM) and GraphPad Prism 8.0 (GraphPad Software) software. We used 1-way ANOVA and

Table 1. Primers used in this study. *Eco*R-I and *Xho*-I restriction enzyme sites for Arfn-pro-F and Arfn-pro-R, respectively, are underlined

| Primers | Sequence (5′–3′) | Purpose |
|---|---|---|
| Arfn-5F1 (outer primer) | TCC GTC CAC TTC TAC CAC | 5′ RACE |
| Arfn-5F2 (inner primer) | CAC GGG CGA ACG ATA AAC | |
| Arfn-3R1 (outer primer) | ACG CCT GAA ACG CCC TTG GT | 3′ RACE |
| Arfn-3R2 (inner primer) | AAG GAT TGG TCT TGA | |
| Arfn-F | CAT CCT GAG CAG CAT TCT | qPCR |
| Arfn-R | CCT TGT CTG TGC TGT CTA CG | |
| β-actinF[a] | TAT GCA CTT CCT CAT GCC AT | |
| β-actinR | AGG AGG CGG CAG TGG TCA T | |
| Arfn-pro-F | TAT CGG ATC C GAA TTC ATG GGT GCC ATC CTG AGC | protein analysis |
| Arfn-pro-R | GGT GGT GGT G CTC GA GTC ACT CTG TGA CCG CCT TGG | |

[a]GenBank accession number KY038927.1

Tukey's multiple range tests to compare the expression levels of *MnArfn* in the hepatopancreas and gills to evaluate the differences between the control and treatment groups. All data used for plotting met the ANOVA assumptions (normality and homogeneity of variances).

### 2.9. Recombinant MnArfn protein expression and antiserum preparation

Based on the full-length *MnArfn* cDNA, primers (Arfn-pro-F and Arfn-pro-R) were designed to amplify the mature peptide. *Eco*R-I and *Xho*-I restriction enzyme sites, underlined in Table 1, were selected for Arfn-pro-F and Arfn-pro-R, respectively. The fragment was cloned into the pMD18-T vector (Takara) and sequenced. The fragment was then digested and inserted into the pET-30a (+) vector. The recombinant plasmid (pET30a (+)-MnArfn) was transformed into *E. coli* BL21 (DE3) cells (Trans Gen Biotech). After induction of expression with IPTG at 25°C, bacterial cells were harvested, re-suspended in phosphate-buffered saline, and sonicated. The His-tag Protein Purification Kit (Beyotime Biotechnology) was used, according to the manufacturer's instructions, to purify the recombinant MnArfn. The polyclonal antibody was prepared using the purified protein by Affinity Biosciences (Changzhou, China).

### 2.10. Western blot analysis

Proteins were isolated from the muscle, heart, hepatopancreas, gills, eyestalk, and stomach. Protein concentrations were determined with the Enhanced BCA Protein Assay Kit (Beyotime Biotechnology), according to the manufacturer's instructions. Samples (each containing 20 µg of protein) were separated on 12% SDS-PAGE gels, and proteins were transferred onto nitrocellulose membranes. Membranes were blocked with QuickBlock™ Blocking Buffer (Beyotime Biotechnology) and subsequently incubated with the polyclonal antibody against MnArfn. Membranes were washed 3 times with Tris-buffered saline containing 0.5% Tween-20 (TBST). Anti-rabbit horseradish peroxidase-conjugated IgG (1/1000) (Beyotime Biotechnology) was used as the secondary antibody. Membranes were washed 3 times with TBST. The MnArfn protein was visualized using the Affinity® ECL Kit (picogram) and quantified using a chemiluminescence imaging system (GDL-DOC EQ, Bio-Rad).

## 3. RESULTS

### 3.1. Sequence analysis

The complete nucleotide sequence of *MnArfn* from *Macrobrachium nipponense* was determined by reverse-transcription PCR (RT-PCR) and RACE methods. The full-length cDNA of *MnArfn* was 1076 bp. It contained a 316 bp 5′ untranslated region (UTR), a 537 bp ORF, and a 217 bp 3′ UTR with a poly(A) tail. The ORF encoded a 178 amino acid protein, while no signal peptide was found. The deduced molecular weight was 19.85 kDa, and the theoretical isoelectric point was 4.96. Furthermore, MnArfn had a conserved N-terminal myristoylation site of $G_2$, P-loop ($^{24}$GLDGVGKT$^{31}$), switch region 1 ($^{40}$GKVVQTIPTIGF$^{51}$), interswitch region ($^{52}$NVETVEYKNISFTVW$^{66}$), and switch region 2 ($^{67}$DLPSQCKMRPLWRHY$^{81}$). The cDNA sequence of the *MnArfn* gene was submitted to GenBank with accession number MN747148.1 (Fig. 1).

### 3.2. Sequence alignment and phylogenetic analysis

The deduced MnArfn amino acid sequence shared identities with Arfs from invertebrates and vertebrates, including *Penaeus japonicus* (60.67%/Arfn), *Litpenaeus vannamei* (60.92%/Arfn), *Stylophora pistillata* (59.78%/Arf4), *Sparus aurata* (57.87%/Arf4), *Cimex lectularius* (56.98%/Arf2), *Bigelowiella natans* (55.62%/Arfn), *Rhinatrema bivittatum* (60.34%/Arf5), and *Anarrhichthys ocellatus* (59.66%/Arf4). Multiple sequence alignments of MnArfn with other known Arfs indicated that MnArfn was highly conserved and contained an N-terminal myristoylation site of $G_2$. However, the protein sequences of the P-loop ($^{24}$GLDGVGKT$^{31}$), switch region 1 ($^{40}$GKVVQ TIPTIGF$^{51}$), and switch region 2 ($^{67}$DLPSQCKMR-PLWRHY$^{81}$) of MnArfn differed from those of the other known Arf proteins (Fig. 2). In addition, Fig. 2 shows regions or sequences directly related to protein function, including the GXXXXGKT region, DVGG sequence, and NKQD sequence.

Phylogenetic analysis showed that MnAfrn clustered with Arf1 of *P. japonicus*, suggesting their evolutionary homology. MnAfrn did not cluster with the Japanese shrimp Afr4, indicating that MnAfrn may be a new Afr1 (Fig. 3).


Fig. 1. Nucleotide and amino acid sequences of MnArfn. Important sites are marked as follows; double red underline: codon (ATG) and termination codon (TAG); black arrowhead: N-terminal myristoylation site of $G_2$; black underline: P-loop ($^{24}$GLDGVGKT$^{31}$); red underline: switch region 1 ($^{40}$GKVVQTIPTIGF$^{51}$); yellow underline: interswitch region ($^{52}$NVETVEYKNSFTVW$^{66}$); green underline: switch region 2 ($^{67}$DLPSQCKMRPLWRHY$^{81}$)

### 3.3. Tissue distribution of MnArfn

qPCR and Western blotting were performed to examine the tissue distribution and expression of MnArfn. The results of qPCR showed that *MnArfn* was expressed in all examined tissues, including the muscle, heart, gills, hepatopancreas, eyestalk, and stomach. The highest expression was detected in the hepatopancreas, followed by the gills, whereas the lowest expression was detected in the eyestalk (Fig. 4A). Western blotting results also showed that MnArfn was expressed in all examined tissues. Here as well, the highest level was observed in the hepatopancreas, whereas the lowest level was in the eyestalk (Fig. 4B).

### 3.4. mRNA expression of *MnArfn* in hepatopancreas and gills after ammonia nitrogen stress

The temporal expression of *MnArfn* in hepatopancreas and gills of *M. nipponense* under ammonia nitrogen stress was examined by qPCR. Compared with the control group, the *MnArfn* level in the

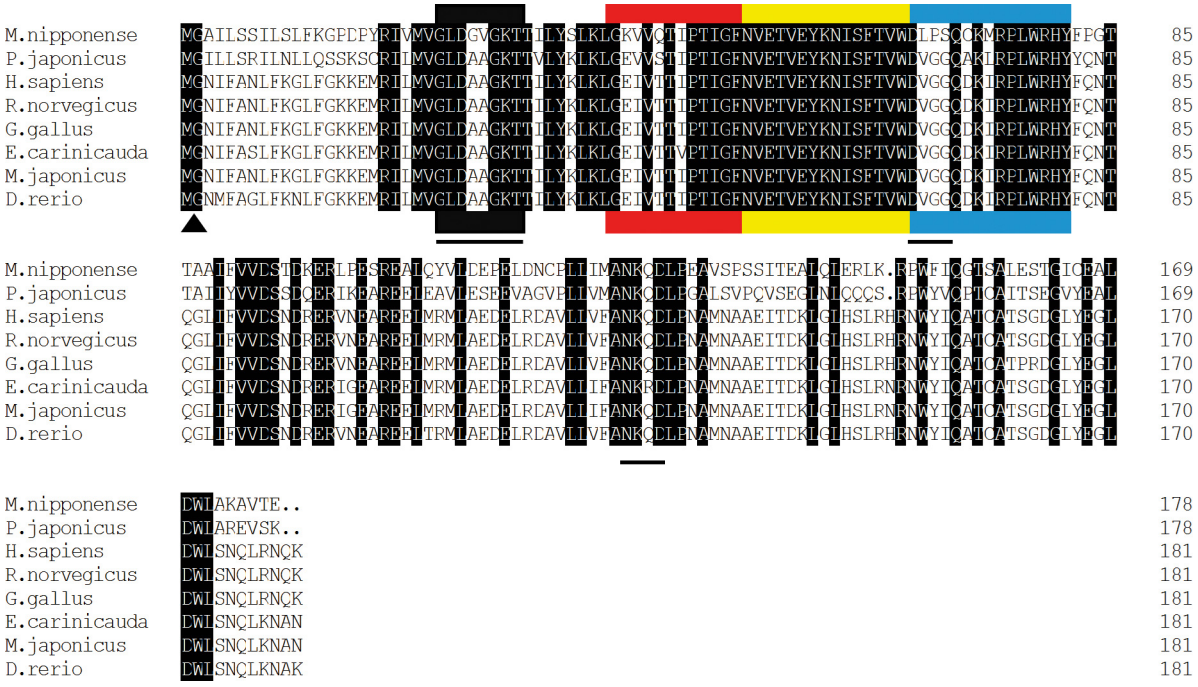


Fig. 2. Partial results of multiple alignment of MnArfn with other proteins of the Arf family: *Penaeus japonicus* (GenBank accession number ADE42874.1), *Homo sapiens* (NP_001649.1), *Rattus norvegicus* (NP_071963.1), *Gallus gallus* (NP_00100 6352.1), *Exopalaemon carinicauda* (AQM40226.1), *Marsupenaeus japonicus* (ADE42873.1), *Danio rerio* (AAH66632.1). The P-loop (24GLDGVGKT31), switch region 1 (40GKVVQTIPTIGF51), interswitch region (52NVETVEYKNISFTVW66), and switch region 2 (67DLPSQCKMRPLWRHY81) are shown as a black, red, yellow, and blue regions, respectively. GXXXXGKT region, DVGG sequence, and NKQD sequence are marked with black underlines

hepatopancreas increased in response to ammonia nitrogen stress, with the highest level measured at 6 h ($p < 0.01$), indicating that ammonia nitrogen stress can induce *MnArfn* expression. The *MnArfn* level decreased at 12 h and reached the lowest level at 72 h (Fig. 5A). The *MnArfn* level was significantly increased in the gills at 6 h ($p < 0.01$). Subsequently, it decreased at 12 h and reached the lowest level at 72 h (Fig. 5B).

### 3.5. Recombinant expression and purification

Recombinant MnArfn expression was induced by IPTG, and it was overexpressed as a soluble protein in *E. coli* BL21 (DE3) cells at 25°C. The deduced molecular weight of recombinant MnArfn was 19.85 kDa, consistent with the SDS-PAGE results (Fig. 6).

## 4. DISCUSSION

With the rapid development of intensive aquaculture models, the content of ammonia nitrogen in aquaculture environments has become the second major factor restricting the development of aquaculture besides dissolved oxygen (Ebeling et al. 2006). The production of ammonia nitrogen comes not only from the process of protein metabolism in aquatic animals, but also from the decomposition of feed by microorganisms in the culture environment (Chang et al. 2015). Previous studies have demonstrated that suboptimal environmental conditions can weaken the immune function of crustaceans and increase their susceptibility to certain diseases and infections (Li & Chen 2008). Ammonia nitrogen can induce oxidative stress by increasing the generation of reactive oxygen species (Chang et al. 2015). Furthermore, ammonia nitrogen stress can decrease peroxinectin and prophenoloxidase gene levels by 50 and 60%, respectively, in *Penaeus stylirostris* (Le Moullac & Haffner 2000). High concentrations of ammonia nitrogen can suppress immune function in shrimp, thereby damaging their organs (Cheng & Chen 2002). We previously reported that high concentrations of ammonia nitrogen impair organs and the immune system of prawns (Sun et al. 2020). To sum up, a high concentration of ammonia nitrogen in the aquaculture environment directly impairs the health

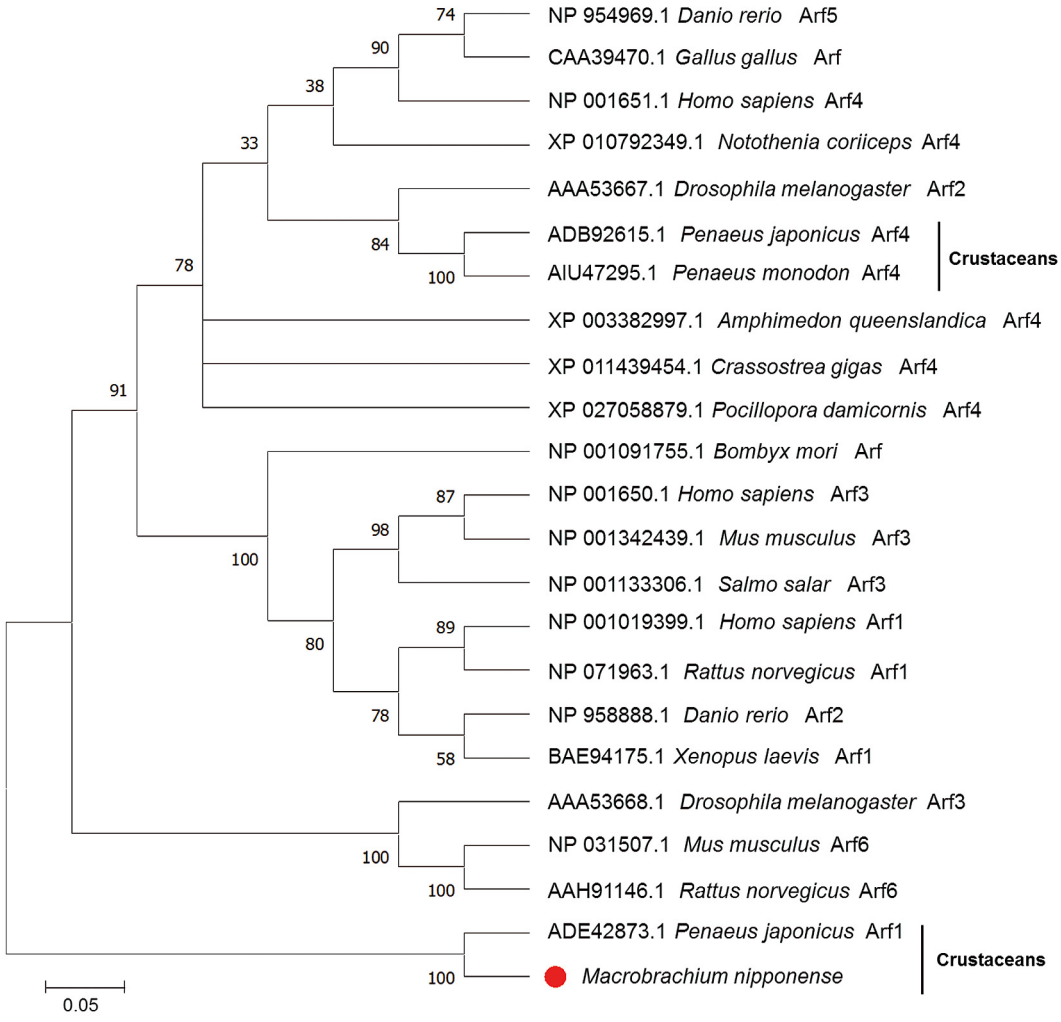


Fig. 3. Phylogenetic analysis of the Arf family from different species, along with their GenBank accession numbers

of aquatic animals, especially *Macrobrachium nipponense*, and further affects the healthy development of the production and aquaculture industry.

The hepatopancreas of prawns is comparable to the fat of insects and the liver of mammals, which serves as a sensitive indicator of the environment and the general health of organisms (Sousa & Petriella 2000, Gross et al. 2001). Gills, as the respiratory organ of prawns, are in direct contact with freshwater; thus, they provide the first-line defense and play a crucial role in the response to biotic and abiotic factors (Cui et al. 2017). Therefore, genes that are either up- or down-regulated in response to ammonia nitrogen stress should be studied to obtain a better understanding of how hepatopancreas and gills respond to environmental toxicants.

Studying the interacting mechanisms between aquatic organisms and environments, such as aquatic environment changes and bacterial and viral infection, is very important for aquatic production. Arfs reported in *Penaeus monodon* may be closely related to ammonia nitrogen stress and the immune process of pathogenic infection (Duan et al. 2016). Similar reports suggested that Arfs may be associated with viral infections (Zhang et al. 2010). Arfs are members of the Ras gene superfamily and are highly conserved in biological evolution, especially the N-terminal myristoylation site of G2 and the interswitch region. The P-loop is also expressed as the GXXXXGKT region (human Arf1 [hArf1], 24th–31st amino acids) associated with GTP dissociation. In this study, although the amino acid sequence of MnArfn is not identical to that of hArf1, the amino acid at the key position has not changed, and the third amino acid in this region is not glycine which plays a key role in the dissociation of GTP in normal Ras genes;

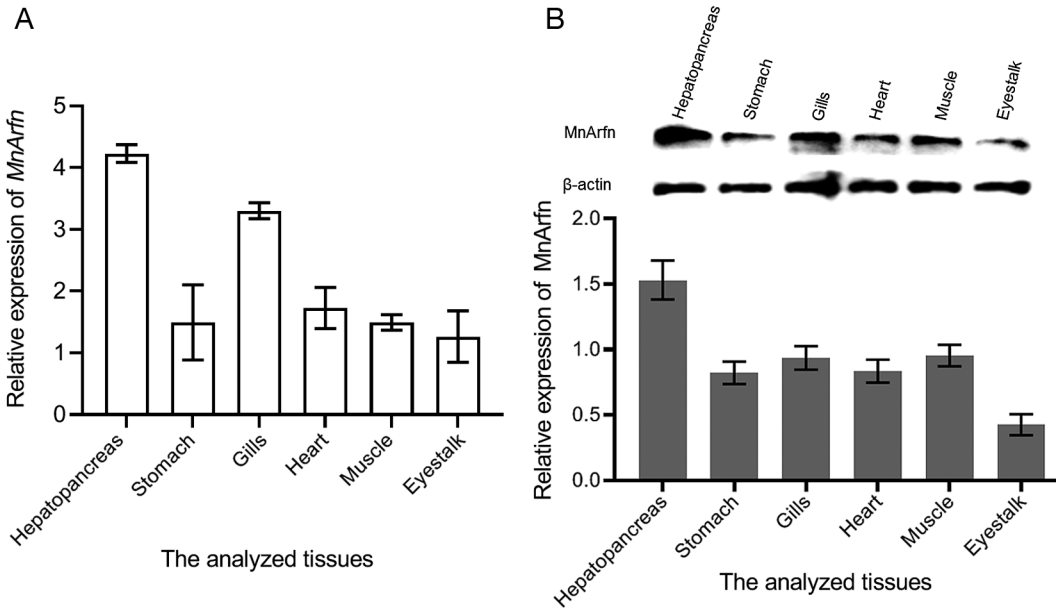


Fig. 4. Expression of *MnArfn* in different tissues of *Macrobrachium nipponense* and analyzed by (A) qPCR and (B) Western blotting. β-actin was used as the control; data are shown as means ± SE (N = 3)

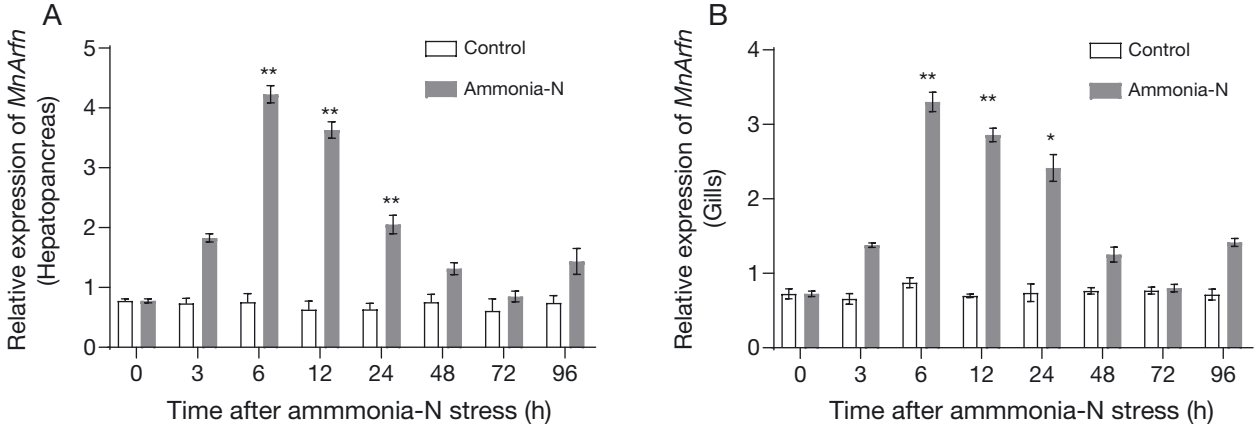


Fig. 5. Expression of *MnArfn* in (A) hepatopancreas and (B) gills of *Macrobrachium nipponense* at different times after ammonia nitrogen stress. Data are shown as means ± SE (N = 6). Asterisks represent significant differences (*$p < 0.05$, **$p < 0.01$)

therefore, we speculated that it does not affect the biological activity of this region (Botstein et al. 1988). The DVGG sequence (hArf1, 67–70th amino acids) in switch region 2 is thought to be involved in the regulation of GTP binding to $Mg^{2+}$, but the MnArfn sequence is not completely identical, suggesting that it may cause differential binding activities between GTP and $Mg^{2+}$. The NKQD sequence (hArf1, 126–129th amino acid) is thought to be involved in binding with the guanine ring, and MnArfn is consistent with all species to which it was compared.

Most of the research on the structure and function of Arfs has been conducted by investigating its mutants, which also provides a novel approach to study the functional changes caused by the sequence changes in switch region 2 that differ from other Arfs. In this study, the full-length cDNA of *MnArfn* was identified from *M. nipponense*, and this is the first report on the involvement of the *MnArfn* gene in the ammonia nitrogen stress response of *M. nipponense*. Sequence and phylogenetic analyses indicated that MnArfn belongs to the Arf family and showed 55–62% identity with Arfs of other species. The phylogenetic tree, which included Arfs of crustaceans, mammals, fish, birds, and other animals, showed that *MnArfn* had high homology with the Arf1 of *Penaeus*

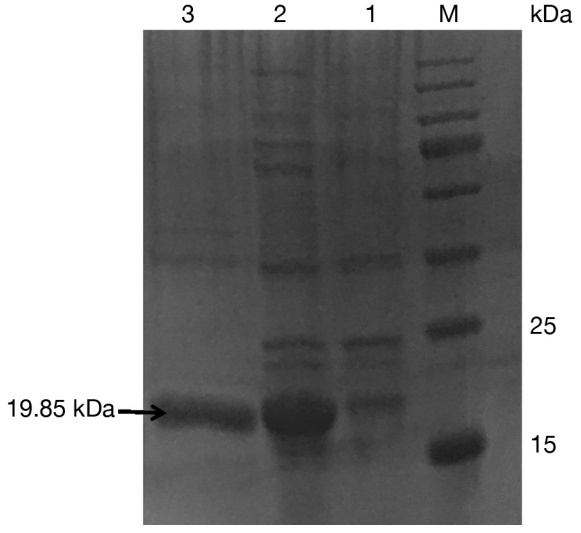


Fig. 6. SDS-PAGE analysis of recombinant MnArfn. Lane M: Protein marker; lane 1: expression without IPTG induction; lane 2: induced MnArfn-pET30a; lane 3: purified recombinant MnArfn protein

*japonicas* (Fig. 3). These findings suggest that *MnArfn* is a new member of the Arf family in *M. nipponense*. We also hypothesize that *MnArfn* plays an important role in the ammonia stress response.

To examine the function of *MnArfn* in *M. nipponense*, its expression in tissues was examined by qPCR. *MnArfn* was expressed in all examined tissues, especially in hepatopancreas and gills, consistent with the tissue distribution of Arfs in *Macrobrachium rosenbergii* (Arf1/2) (Ding et al. 2015), *Exopalaemon carinicauda* (Arf1) (Duan et al. 2016), and *Penaeus monodon* (Arf4) (Shekhar & Gomathi 2017), indicating that *MnArfn* has many functions. In this study, we observed a significant increase of *MnArfn* expression in hepatopancreas and gills under conditions of ammonia nitrogen stress. As important immune organs, hepatopancreas and gills function in the first-line defense against environmental stressors and pathogens (Vallet-Gely et al. 2008, Huang & Ren 2015). Previous studies have reported that immune-related genes, such as *a2M* (Ho et al. 2009), *NM23* (Duan et al. 2015), *HSP90* (Zhao et al. 2011), and *CTL* (Xiu et al. 2015), are highly expressed in hepatopancreas and gills of prawns and shrimps. The high expression of *MnArfn* in hepatopancreas and gills may indicate that it plays an important role in the ammonia stress response. Under conditions of ammonia nitrogen stress, the *MnArfn* level was significantly up-regulated, reaching its highest and lowest levels at 6 and 72 h, respectively. This immediate increase in the *MnArfn* level after exposure to ammonia nitrogen stress indicates that the protein functions in the early response by suppressing stress. Western blotting results also indicated that MnArfn was expressed in all examined tissues, again with the highest levels in the hepatopancreas, followed by the gills, consistent with the qPCR results. Taken collectively, *MnArfn* may protect prawns from ammonia nitrogen stress and plays an essential role in regulating ammonia stress of *M. nipponense*.

## 5. CONCLUSION

This is the first report of the identification and cloning of the *Macrobrachium nipponense* Arfn gene *MnArfn*. The results of this study provide a foundation for further investigations of the stress tolerance mechanism of the *MnArfn* gene. *MnArfn* was highly expressed in hepatopancreas and gills. Under ammonia nitrogen stress, the expression of *MnArfn* in metabolism-related tissues showed that the expression of *MnArfn* could be induced and might play a role in the stress regulation of prawns. These results provide a new avenue and theoretical basis for follow-up studies on the mechanism(s) of crustacean resistance to external adverse environments and provide a new perspective to screen for stress-resistant individuals and cultivating more resistent varieties.

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