Genome-Wide Identification and Expression Profiles of Myosin Genes in the Pacific White Shrimp, *Litopenaeus vannamei*

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As the main structural protein of muscle fiber, myosin is essential for multiple cellular processes or functions, especially for muscle composition and development. Although the shrimp possess a well-developed muscular system, the knowledge about the myosin family in shrimp is far from understood. In this study, we performed comprehensive analysis on the myosin genes in the genome of the Pacific white shrimp, *Litopenaeus vannamei*. A total of 29 myosin genes were identified, which were classified into 14 subfamilies. Among them, Myo2 subfamily was significantly expanded in the penaeid shrimp genome. Most of the Myo2 subfamily genes were primarily expressed in abdominal muscle, which suggested that Myo2 subfamily genes might be responsible for the well-developed muscular system of the penaeid shrimp. In situ hybridization detection showed that the slow-type muscle myosin gene was mainly localized in pleopod muscle and superficial ventral muscle of the shrimp. This study provides valuable insights into the evolutionary and functional characterization of myosin genes in shrimps, which provides clues for us to understand the well-developed muscular system of shrimp.

Keywords: myosin genes, muscle development, alternative splicing, penaeid shrimp, gene family

INTRODUCTION

Nearly all eukaryotic cells possess myosin proteins, which bind to filamentous actin and produce physical forces through ATP hydrolysis (Richards and Cavalier-Smith, 2005; Sebé-Pedrós et al., 2014). Myosin plays key roles in muscle composition and various cellular activities, including cytokinesis, organelle transport, cell polarization, intracellular transport and signal transduction (Hofmann et al., 2009; Bloemink and Geeves, 2011; Hartman et al., 2011; Pette and Staron, 2015). The Myo2 subfamily (Myosin 2), also known as myosin heavy chain (MYH or MHC), are the main component of the contractile muscle. They are considered to be conventional myosins, while the other subfamilies are considered to be unconventional myosins. The Myo2 subfamily genes can be classified into three groups, including fast-type, slow-type and non-muscle type. The Myo2 subfamily proteins can form large bipolar filaments through tail-directed homo-oligomerization, while the tails of the unconventional myosins typically direct binding to membrane and other proteins (Woolner and Bement, 2009).
Myosin consists of three domains, including a conserved motor (or head) domain with actin-binding activities, a short neck that serves as a binding site for myosin light chains, and a variable tail that generally mediates interaction with the motor “cargo” to determine the functional specificity of the motor (Berg et al., 2001; Richards and Cavalier-Smith, 2005). In general, it is very difficult to investigate the myosin gene family systemically due to their extensive alternative splicing events, large sizes and high copy numbers. Although crustaceans are a large, diverse group, limited myosin genes have been reported, except that 17 myosin genes in 13 crustaceans are a large, diverse group, limited myosin genes have been characterized in *Daphnia* (Odronitz and Kollmar, 2008). In addition, a few Myo2 sequences have been cloned from *Marsupenaeus japonicus*, *Penaeus monodon*, and *Litopenaeus vannamei*, respectively (Koyama et al., 2013). Hence, studies on the myosin gene family in crustaceans will enhance our understanding on their structure, function and evolution.

The Pacific white shrimp *L. vannamei* is one of the most economically important marine aquaculture species in the world (FAO, 2014). Based on the whole genome sequences (Zhang et al., 2019) and the transcriptome data, we performed a genome-wide analysis on the myosin gene family of *L. vannamei*. This study provides valuable resources for the shrimp myosin genes, which will increase our understanding on the crustacean muscular system.

**MATERIALS AND METHODS**

**Genome and Transcriptome Data**

The genomic data of *L. vannamei* (PRINA438564), *Lepeoptheirus salmonis* (GCA_001005205.1), *Parhyale hawaiiensis* (GCA_001587735.1), *Eulimnadia texana* (GCA_002872375.1), were obtained from NCBI GenBank and Ensemble database. Our previous research conducted RNA-seq on several libraries, (I) five larval stages, including embryo, nauplius, zoea, mysis and post-larvae (Wei et al., 2014); (II) eight molting stages, including the inter-molt (C), pre-molt (D0, D1, D2, D3, D4), and post-molt (P1 and P2) stages (Gao et al., 2015); (III) 16 adult tissues, including antennal gland, brain, hemocyt, epidermis, eyestalk, gill, hepatopancreas, heart, intestine, abdominal muscle, lymphoid organ, ovary, stomach, testis, thoracic ganglion, and abdominal ganglion (Zhang et al., 2018).

**Isolation and Annotation of Myosin Genes**

The full protein sequences of the *L. vannamei* genome were searched against the myosin head (motor domain) (PF00063) to find all the candidate myosin genes by using HMMPER3.0 (Potter et al., 2018). All possible myosin transcripts were collected from the transcriptome data. Then, the redundant sequences were removed using CAP3 program (Huang and Madan, 1999). The candidate sequences were submitted to SMART (Letunic et al., 2014) and InterPro (Finn et al., 2016) databases to determine the integrity of the motor domain. More myosin sequences used in this study were collected from CyMoBase1 and (Hammesfahr et al., 2010).

**Gene Structure and Alternative Splicing**

The characteristics of myosin genes, including the location, gene length, open reading frame (ORF), exon number and the number of deduced amino acids were analyzed in detail. To illustrate the structure of myosin genes, all alternative spliced exons were characterized by mapping all variant transcripts to the shrimp genome.

**Phylogenetic Analysis**

The amino acid sequences of the conserved myosin head domain were aligned by MUSCLE program (version 3.8.31) (Edgar, 2004) with default parameters. A neighbor-joining (NJ) phylogenetic tree with 1000 bootstrap replicates was constructed by MEGA7.0 program (Kumar et al., 2016), and visualized using iTOL (Letunic and Bork, 2007). To investigate the evolution of the myosin gene family of shrimp, a class occurrence tree of 29 arthropods was generated by using the method described by Odronitz et al. (2009).

**Transcription Regulatory Element Identification**

To investigate whether the expanded muscle-type Myo2 genes are associated with muscle composition or development, transcription regulatory elements of muscle-type Myo2 genes were predicted. In detail, the promoter regions, located at the 2 kb upstream of the transcriptional start site, were first extracted from the shrimp genome. The transcription regulatory elements were then predicted by Signal Scan2 with TRANSFAC database and the PATCH algorithm integrated in the GeneXplain platform. In order to decrease the false positive rate, we intersected the predicted results of Signal Scan and GeneXplain.

**Expression Patterns at Early Development Stages and Different Tissues of Adults**

In previous studies, we conducted Digital Gene Expression Profiling (DGE) to sequence 20 larval stages and 16 adult tissues of *L. vannamei*, and the RPKM (reads per kilobases per million reads) values of all transcripts were calculated (Zhang et al., 2018). The RPKM values of myosin genes were extracted and normalized with log2 conversion. Heat maps were created using TBtools software (Chen et al., 2018).

**In situ Hybridization**

In this study, *LvMYH5* was characterized to be a slow-type muscle Myo2 gene. To further distinguish fiber types and muscle distribution of the abdominal muscle of shrimp, *in situ* hybridization was performed according to the protocol...
A total of 29 myosin genes were identified in *L. vannamei*, including 15 muscle-type Myo2 genes, one non-muscle type Myo2 gene and 13 unconventional myosin genes (Table 1). Among them, five muscle-type myosin genes had been developed in our laboratory (Zhang et al., 2018). Briefly, primers LvMYH5-pF with T7 promoter sequence and LvMYH5-R (Supplementary Table 1) were designed to amplify the cDNA fragments as the template of *LvMYH5* to synthesize sense probe. Primers LvMYH5-pR with T7 promoter sequence and LvMYH5-F were designed as the template to synthesize antisense probe. PCR products were purified using MiniBEST DNA Fragment Purification Kit (Takara, Japan). The Digoxigenin (DIG)-labeled sense and antisense RNA probes were transcribed by TranscriptAid T7 High Yield Transcription Kit (Thermo Fisher Scientific, United States) and synthesized using DIG RNA Labeling Mixture (Roche, Germany), respectively, and stored at −80°C.

Transverse sections with 5–7 µm thickness were prepared from polyformaldehyde fixed abdominal muscle of juvenile shrimp (about 2 cm in body length) embedded in paraffin (Sigma, Germany), and hybridization was performed following the general protocol of DIG RNA labeling kit (SP6/T7) (Roche, Germany). The final concentration of both anti-sense and sense RNA probes were 1.5 ng/µl. Subsequently, alkaline phosphatase-conjugated anti-DIG antibody and NBT/BCIP (DIG Nucleic Acid Detection Kit, Roche) were used to detect RNA probes and observed under Nikon Eclipse 80i microscope (Nikon, Japan).

### RESULTS

#### Identification of Myosin Genes

A total of 29 myosin genes were identified in *L. vannamei*, including 15 muscle-type Myo2 genes, one non-muscle type Myo2 gene and 13 unconventional myosin genes (Table 1). Among them, five muscle-type myosin genes had been

| Myosin name | Genome position | Position (bp) | Exon | No. of aa | Unigenes |
|-------------|-----------------|---------------|------|----------|----------|
| LvMyo1A     | LVANscaffold_1022 | 139066:159076  | 23   | 1081     | Unigene0007554 |
| LvMyo1B     | LVANscaffold_2218 | 313258:311201  | 20   | 1032     | Unigene0049576 |
| LvMyo1C     | Unavailable      | Unavailable    | Unavailable | 1177    | cl78174_g1 |
| LvMyo1D     | LVANscaffold_2215 | 143463:1447901 | 21   | 1177     | cl83379_g1 |
| LvMYH1      | LVANscaffold_3160 | 506207:527443  | 24   | 1972     | c81769_g2 |
| LvMYH2      | LVANscaffold_2946 | 891867:984764  | 24   | 1972     | c81769_g2 |
| LvMYH3      | LVANscaffold_2986 | 675866:686178  | >18  | 1911     | CL120.Contig45_All |
| LvMYH4      | LVANscaffold_904 | 105970:114423  | 11   | 1124     | AB759099.1 |
| LvMYH5      | LVANscaffold_1838 | 9836:15995     | >8   | 1198     | AB759100.1 |
| LvMYH6      | LVANscaffold_2713 | 277521:272168  | 22   | 1909     | AB759104.1 |
| LvMYH7      | LVANscaffold_2713 | 44648:62854    | 22   | 1909     | AB758444.1 |
| LvMYH8      | LVANscaffold_2713 | 169683:186166  | 21   | 1909     | Unigene7586_All |
| LvMYH9      | LVANscaffold_904 | 190184:192834  | >21  | 1911     | CL120.Contig24_All |
| LvMYH10     | LVANscaffold_904 | 26105:36210    | >14  | 1915     | CL120.Contig4_All |
| LvMYH11     | LVANscaffold_904 | 184800:190271  | 19   | 1913     | CL120.Contig83_All |
| LvMYH12     | LVANscaffold_904 | 236788:225555  | >20  | 1911     | CL120.Contig50_All |
| LvMYH13     | LVANscaffold_660 | 671473:678211  | >14  | 1911     | CL120.Contig15_All |
| LvMYH14     | LVANscaffold_1774 | 1247154:1253260| >18  | 1929     | CL120.Contig53_All |
| LvMYH15     | LVANscaffold_903 | 446522:459841  | >18  | 1909     | CL120.Contig13_All |
| LvMYH16     | LVANscaffold_1774 | 1258456:126373| >8   | 1321     | CL120.Contig78_All |
| LvMyo5      | LVANscaffold_441 | 69978:76891    | 37   | 1853     | c81514_g1 |
|             |                  |               |      |          | Unigene0049576 |
| LvMyo6      | LVANscaffold_1654 | 616383:638879  | 29   | 1257     | c83722_g3 |
| LvMyo7A     | LVANscaffold_1737 | 4719:14556    | 40   | 2158     | c8358_g1 |
| LvMyo9      | LVANscaffold_2760 | 317572:400490 | 33   | 2057     | c83307_g1 |
| LvMyo10     | LVANscaffold_1465 | 684100:748649 | 33   | 2057     | c8358_g1 |
| LvMyo15     | LVANscaffold_2015 | 255392:276113 | 31   | 2037     | c75923_g3 |
| LvMyo18     | LVANscaffold_1503 | 325742:153935 | 31   | 2037     | c75923_g3 |
| LvMyo20     | LVANscaffold_609 | 944458:956768  | >18  | 1010     | Unigene0091838 |
| LvMyo21     | LVANscaffold_2019 | 461705:493440 | 26   | 1269     | c83156_g1 |
| LvMyo22     | LVANscaffold_1625 | 630095:647523  | >35  | 1963     | c83429_g1 |
reported previously, including \textit{LvMHC1}, \textit{LvMHC2}, \textit{LvMHC4}, \textit{LvMHC5}, and \textit{LvMHC6} (Koyama et al., 2012, 2013). They were designated as \textit{LvMYH1}, \textit{LvMYH7}, \textit{LvMYH4}, \textit{LvMYH5}, and \textit{LvMYH6}, respectively, in this study to avoid confusion with major histocompatibility complex genes. The gene structure of these 29 myosin genes showed high complexity with the sizes ranging from 6.159 to 171.807 kb that corresponded to encode proteins of 1,010-2,158 aa (Table 1). Phylogenetic analysis indicated that these myosin genes were clustered into 14 distinct groups, namely 14 subfamilies, \textit{Myo1}, \textit{muscle-type Myo2}, \textit{non-muscle type Myo2}, \textit{Myo3}, \textit{Myo5}, \textit{Myo6}, \textit{Myo7}, \textit{Myo9}, \textit{Myo15}, \textit{Myo18}, \textit{Myo19}, \textit{Myo20}, \textit{Myo21}, and \textit{Myo22}. \textit{Myo1} can be further divided into four subfamilies, \textit{Myo1A}, \textit{Myo1B}, \textit{Myo1C}, and \textit{Myo1D}. These myosin genes within
Fifteen muscle-type Myo2 genes exhibited conspicuous lineage-specific expansion in the shrimp genome. Among them, eight Myo2 genes were tandemly distributed in two clusters (Figure 2), *LvMYH9, LvMYH10, LvMYH4, LvMYH11*, and *LvMYH12* were located on the Scaffold 904, *LvMYH7, LvMYH8*, and *LvMYH6* were located on the Scaffold 2713. Besides, at least 19 fragmental muscle-type Myo2-like sequences or pseudogenes were located on the Scaffold 903, Scaffold 2713 and Scaffold 3755 (Figure 2). The others scattered in the shrimp genome (Table 1).

**Myosin Functional Domain**

Generally, homologous myosin genes of different species possess similar domain architecture (Richards and Cavalier-Smith, 2005). All myosin genes identified in this study contained a large ATPase motor domain which hydrolyzes ATP, except for *LvMYH4*, which encodes a headless myosin. In addition, all the myosin genes contained at least one isoleucine-glutamine (IQ) motif except for *LvMyo6* (Figure 3). The myosin genes could be classified into different groups with diverse functions based on the remaining domains and variable number of IQ motifs. *LvMyo5* contained six IQ domains and a unique C-terminal DIL domain. *LvMyo19, LvMyo20,* and *LvMyo21* had truncated tail regions. Notably, all muscle-type Myo2 proteins contained an N-terminal SH3-like fold, a large ATPase motor domain,
an IQ motif, and a large C-terminal myosin tail. However, the N-terminal SH3-like domain was absent in unconventional myosin genes of *L. vannamei*. Compared with *DamMyo18* and *DapMyo18*, the upstream PDZ domain was absent in *PhawMyo18* and *LvMyo18*.

**Phylogenetic Analysis**

As expected, the crustacea formed a distinct clade from other arthropods, and *L. vannamei* was most closely related to *P. hawaiensis* belonging to Malacostraca clades (Figure 4). In addition, arthropods contained 14 classes (groups) of myosin.
genes including muscle-type Myo2, non-muscle Myo2 (MYH2), Myo5, Myo6, Myo7A, Myo15, Myo18, Myo20, and Myo21 with a few exceptions. For example, non-muscle type Myo2, Myo6, and Myo15 were absent in Ixodes scapularis, and Myo18 and Myo21 were not found in Tetanychus urticae and Strigamia maritima, respectively. Most of these myosin genes were present in crustaceans, so we inferred that the last common crustacean ancestor had already contained these classes of myosin genes.

Alternative Splicing
Among the 29 myosin genes, four genes underwent alternative splicing (Figure 5 and Supplementary Table 2). The unspliced transcript of LvMYH2 encoded 1,972 aa protein, and the corresponding spliced transcripts encoded 1,982 and 1,970 aa when the exon 20 was retained and exon 4 was mutually excluded, respectively. LvMyo5 possessed at least three splicing events, the unspliced transcript encoded 1,853 aa and the corresponding spliced transcript encoded 1,833, 1,804 and 1,846 aa with the exon 7, 24 and 28 were skipped, respectively. In the case of LvMyo6, an exon skipping event occurred in exon 22, and the corresponding spliced transcript encoded 1,238 aa, whereas the unspliced transcript encoded 1,257 aa. As for LvMyo18, the unspliced transcript generated 2,037 aa in length. Exon 8 of LvMyo18-A1 transcript was retained, and the corresponding spliced transcript encoded 2,061 aa. Alternatively, a novel 660 bp intron between exon 28 and exon 29 of LvMyo18-A2 transcript was retained. Furthermore, an alternative acceptor site was found in exon 29 of LvMyo18-A3 transcript, and the corresponding spliced transcript encoded 2,066 aa.

Transcription Regulatory Elements of the Muscle-Type Myo2 Promoters
A large number of muscle development-related transcription factors binding to the upstream promoter sequences of 10 LvMYHs were identified, including TATA-binding protein (TBP), GATA-1, activator protein 1 (AP1), specificity protein 1 (Sp1), serum response factor (SRF), myogenic differentiation 1 (MyoD) and myogenin (Figure 6). LvMYH1, LvMYH2, LvMYH3, LvMYH6, LvMYH8, LvMYH9, LvMYH11, LvMYH12, LvMYH14, and LvMYH15 possessed 11, 5, 8, 13, 12, 11, 14, 18, 14, and 7 binding sites of muscle-related transcription factors, respectively. Among them, LvMYH12 contained the highest number of binding sites of five muscle-related transcription factors. In addition, all analyzed LvMYH genes possessed at least one binding site of MyoD, except for LvMYH2 and LvMYH15. The LvMYH3, LvMYH9, LvMYH11, and LvMYH14, might be activated by myogenin (Figure 6).

Temporal and Spatial Expression Patterns of Myosin Genes
We analyzed the temporal and spatial expression patterns of myosin genes based on the transcriptome of tissues or development stages (Figure 7). The results revealed that seven Myo2 subfamily genes, LvMYH3, LvMYH6, LvMYH8,
LvMYH9, LvMYH10, LvMYH13, and LvMYH15, were specifically highly expressed in the abdominal muscle. LvMYH12 was exclusively expressed in stomach, and LvMYH16 was particularly abundant in heart and epidermis. The expression levels of LvMYH11 and LvMYH14 were very low in all tissues. Similarly, the unconventional myosin genes also showed tissue-specific expression patterns; for instance, LvMyo5 was specifically expressed in hemocyte, and LvMyo21 exhibited high expression level in eyestalk.

All myosin genes were detectable with dynamic expression patterns during the whole developmental stage. Most of muscle-type Myo2 genes were highly expressed between the larvae in membrane (Lim) stage and the post-larva stage (P1) (Figure 8). For instance, LvMYH2, LvMYH3, and LvMYH10 were expressed from Lim stage to nauplius 6 (N6) stage, and LvMYH6, LvMYH8, LvMYH9, and LvMYH15 were highly expressed from zoea 1 (Z1) to P1 stage. However, unconventional myosin genes exhibited a distinct pattern, LvMyo1A, LvMyo1B, LvMyo1D, LvMyo9, LvMyo15, and LvMyo19 were mainly expressed from zygote to gastrula stage, and LvMyo7A and LvMyo20 were expressed from limb bud embryo 1 (Lbe1) stage to N6 stage.

**In situ Hybridization**

Shrimp abdominal muscle could be divided into four functional muscle groups, the superficial ventral muscle, the lateral muscle, the dorsal muscle and the main ventral muscle. The morphological organization of the skeletal muscle was displayed by the hematoxylin-eosin (H&E) staining (Figure 9A). In situ hybridization detection showed that LvMYH5, characterized as a marker gene of slow-type skeletal muscle Myo2, was mainly expressed in pleopod muscle and superficial ventral muscle (Figure 9C). They were attached between yokes of thin cuticle lying transversely across the posterior portion of each abdominal segment beneath the ventral nerve cord, and presumably function to hold the articular cuticle between the abdominal segments in place. However, no significant signal was observed in flexor and extensor muscle (Figure 9B).
FIGURE 8 | The expression profiles of myosin genes during development of *L. vannamei*. Log2-transformed expression values were used to create the heat map. The red or blue colors represent the higher or lower relative abundance of each myosin gene, respectively. On the right are myosin genes; the bottom lists different larval stages of *L. vannamei*, from left to right: zygote, 2 cells (C2), 4 cells (C4), 32 cells (C32), blastula (blas), gastrula (gast), limb bud embryo 1 (Lbe1), limb bud embryo 2 (Lbe2), larva in membrane 1 (Lim1), larva in membrane 2 (Lim2), Nauplius 1 (N1), Nauplius 2 (N2), Nauplius 3 (N3), Nauplius 6 (N6), Zoea 1 (Z1), Zoea 2 (Z2), Zoea 3 (Z3), Mysis 1 (M1), Mysis 2 (M2), Mysis 3 (M3), and Post-larva 1 (P1).

DISCUSSION

The Evolutionary Relationship of Myosin Genes in Arthropods

The evolutionary relationship of myosin family is limited in crustaceans due to the lack of Crustacea genome and difficulties for myosin gene identification. With the decoding of more and more Crustacea genomes, we are now in a position to validate the evolution of myosin genes proposed previously in arthropods and gain new insights in crustaceans. In accordance with previous study (Ovronitz et al., 2009), all myosin subfamilies are shared in arthropods, suggesting that the common ancestor of arthropod has already owned all myosin classes (Ovronitz and Kollmar, 2007). Meanwhile, the evolution of arthropods is likely accompanied by taxon- or species-specific losses of certain myosin classes. For example, Maxillopoda and Arachnida appeared to have lost *Myo3A* and *Myo19* classes, and Drosophilidae have lost *Myo1D*, *Myo3A*, *Myo9*, and *Myo19* classes. As Crustacea, Maxillopoda may have lost *Myo1A* and *Myo1B* after separating from Branchiopoda and Malacostraca. The potential lack of the *Myo3* class in *L. vannamei* and *L. salmonis* may be specific characteristic of species. In agreement with the results of several comparative genome analyses (Hill, 2005; Colbourne et al., 2011; Chipman et al., 2014; Kao et al., 2016), the Crustacea clade is more closely related to the Hexapoda clade, but the Myriapoda clade is closer relative to the Arachnida.

Gene Expansion of Muscle-Type Myo2 Subfamilies

Gene family expansion generally results for a strengthened phenotype. In the *L. vannamei* genome, the muscle-type *Myo2* subfamilies showed significant expansion. Eight of 15 muscle-type *Myo2* genes are tandemly duplicated. In addition, numerous fragmental muscle-type *Myo2*-like sequences or pseudogenes
near the LvMYH cluster were also detected. Furthermore, most of the expanded muscle-type Myo2 genes possess binding sites of various myogenesis related transcription factors, such as Sp1 (Biesiada et al., 1999), AP-1 (Andreucci et al., 2002), myogenin (Hasty et al., 1993) and MyoD (Tapscott et al., 1988). It was reported that LvMYH1 and LvMYH7 were located in all fibers of abdominal muscles containing extensor and flexor muscles by in situ hybridization (Koyama et al., 2012, 2013). These results indicate that these expanded muscle-type Myo2 genes might be associated with muscle composition or development. In accordance with our previous study (Zhang et al., 2018), this study reveals the pleopod muscle and superficial ventral muscle of L. vannamei contain slow-type oxidative muscle fibers.

The muscle of shrimp accounts for more than half of the total body weight (Chen and Chen, 2001), but the mechanism of its well-developed muscular system is unknown. Our previous study has also indicated large expansion of actin gene family in L. vannamei (Zhang et al., 2018), which encodes structural proteins of muscle fiber together with muscle-type myosin heavy chains. Taken together, we speculate that the well-developed muscular system of L. vannamei might be associated with the massive expansion of muscle-type actin genes and muscle-type Myo2 genes.

### Alternative Splicing of Shrimp Myosin Genes

In most arthropods, MYH1 with multiple complex alternative splicing patterns was observed to be involved in muscle composition (Bernstein et al., 1986; Rozek and Davidson, 1986; Nyitray et al., 1994; Odronzit and Kollmar, 2008; Kollmar and Hatje, 2014). For example, the D. duplex MYH1 (Mhc1) contains nine sets of mutually exclusive spliced exons (MXEs), and Drosophila melanogaster MYH1 contains five clusters of MXEs and 480 combinations of alternative splicing patterns are possibly existed (Odronzit and Kollmar, 2008). Consistent with the report that crustacean L. salmonis contains 17 Myo2 genes without MXEs (Kollmar and Hatje, 2014), no MXE has been found in the 15 muscle-type Myo2 genes in this study. This implies that the last common ancestor of crustacean might have developed a MXE-less muscle-type Myo2 gene and followed by extensive gene duplications. Multiple, but not alternatively spliced myosin heavy chain genes are therefore a common characteristic of crustacean (Kollmar and Hatje, 2014). This might be caused by the loss of introns in the ancient muscle-type myosin heavy chain gene during arthropods evolution (Odronzit and Kollmar, 2008). However, unconventional myosin genes exhibited higher diversity due to the exon skipping in L. vannamei. It indicated that alternative splicing could potentially yield unconventional myosin variants with diverse functions.

### Special Expression Patterns of Shrimp Myosin Genes

Most of muscle-type Myo2 genes of L. vannamei were primarily expressed during larva in membrane and post-larvae stages. Combined with our previous study on actin gene family (Zhang et al., 2018), the whole early development stage of shrimp can be grouped into three periods. In the beginning of early development period (zygote to gastrula), most muscle-type Myo2 and actin genes are not expressed, then begin to be expressed in the second period (limb embryo and larvae in membrane stage),
and their expression levels reach to the peak and remain at high levels in the third period (zoea to post-larvae stage). According to the report by Hertzler and Freas (2009), major ventral pleonal muscle and dorsal pleonal muscle of *L. vannamei* formed at the zoea stage, while pleopod muscle and major pleonal muscle formed in Mysis stage. Therefore, the high expression of muscle-type Myo2 and actin genes in these stages is compatible with the formation and development of pleonal and pleopod muscle.

The genes regulating muscle development, such as transcription factors, start to be expressed and to activate the co-expression of actin and myosin gene clusters at the nauplius stage. It promotes growth of muscle fibers in the larvae stage. Furthermore, compared with multiple alternative splicing of a single gene, the gene clusters have equivalent effects on improving the expression of transcripts, and strengthening the function of proteins (Talavera et al., 2007). Therefore, it is reasonable to conclude that the gene expansion of muscle-type Myo2 and actin genes may contribute to abundant fast-type muscle fibers and the explosive force of the shrimp abdominal muscles.

**CONCLUSION**

In summary, we identified 29 myosin genes in the *L. vannamei* genome, and classified them into 14 subfamilies. Their genome localization, gene structure, protein domains, trans-acting elements in promoter regions and molecular evolution were comprehensively analyzed. Their temporal and spatial expression profiles provide insights into their important functions in muscle composition and development. Moreover, the expanded muscle-type Myo2 subfamily may explain the well-developed muscular system of *L. vannamei*. Collectively, this study will provide important clues for future research on the function of myosin genes in shrimp.

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ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Welfare ethics of experimental animals and safety inspection system of animal experiments, laboratory animal management and ethics Committee of IOCAS. The protocol was approved by the laboratory animal management and ethics Committee of IOCAS.

**AUTHOR CONTRIBUTIONS**

JX, FL, and XnZ conceived and designed the study. XiZ and CL collected the data. XIZ conducted the bioinformatics analyses, performed all experiments and wrote the manuscript. JY, XnZ, and FL revised the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.00610/full#supplementary-material
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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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