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Genome-Wide Identification, Characterization and Expression Profiling of myosin Family Genes in Sebastes schlegelii

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Abstract: Myosins are important eukaryotic motor proteins that bind actin and utilize the energy of ATP hydrolysis to perform a broad range of functions such as muscle contraction, cell migration, cytokinesis, and intracellular trafficking. However, the characterization and function of myosin is poorly studied in teleost fish. In this study, we identified 60 myosin family genes in a marine teleost, black rockfish (Sebastes schlegelii), and further characterized their expression patterns. myosin showed diverse expression patterns in adult tissues, indicating they are involved in different types and compositions of muscle fibers. Among 12 subfamilies, S. schlegelii myo2 subfamily was significantly expanded, which was driven by tandem duplication events. The up-regulation of five representative genes of myo2 in the skeletal muscle during fast-growth stages of juvenile and adult S. schlegelii revealed their active role in skeletal muscle fiber synthesis. Moreover, the expression regulation of myosin during the process of myoblast differentiation in vitro suggested that they contribute to skeletal muscle growth by involvement of both myoblast proliferation and differentiation. Taken together, our work characterized myosin genes systemically and demonstrated their diverse functions in a marine teleost species. This lays foundation for the further studies of muscle growth regulation and molecular mechanisms of indeterminate skeletal muscle growth of large teleost fishes.

Keywords: myosin gene family; myo2; skeletal muscle growth; myoblast differentiation; Sebastes schlegelii

1. Introduction

Myosins are a large family of cytoskeletal motor proteins that bind filamentous actin and utilize the energy of ATP hydrolysis to play an important part in divergent biological process, such as muscle contraction, cell motility and contractility, cytokinesis, and intracellular trafficking [1-3]. Myosin was firstly described in rabbits [4]. Subsequently, cardiac muscle and smooth muscle myosin was reported in studies conducted by Bailey and Cohen et al. [5,6]. Myosins are typically composed of three domains, a conserved head located in the N-terminal that binds to actin filaments, a short neck as a binding site for myosin light chain, and a tail located in the C-terminal generally binding to the motor “cargo” to determine the functions of the motor [7,8]. Eukaryotes contain up to 35 myosin subfamilies based on an analysis of 2269 Myosin motor domains from 328 organisms [9]. Generally, the myo2 subfamily, as known as myosin 2 (myosin heavy chain, MYH or MHC), is considered to be the conventional myosin, which is the main component of skeletal muscle whereas the other myosin subfamilies are considered to be unconventional myosin [10].
Myosin family genes have been widely identified to be functional in multiple biological processes [2]. For conventional myosin, MYH2 and MYH7 have been shown to play an important role in the formation of muscle fibers and are stably expressed in different types of muscle fibers [11]. MYH11 is proven to be a useful marker to define myoid cells in mouse testis [12]. For unconventional myosin, myosin 3 plays a key role in regulating stereocilia lengths required for normal hearing [8]. Myosin 5 is essential in intracellular transport of organelles, mRNA and other cargo [13]. Among the many functions of myosin genes, their role in the organization of muscle fibers is best characterized, as described in mammal, shrimp, and silkworm [14–16]. A recent study reported that knocking down of myosin heavy chain caused the inhibition of sarcomeric organization of thin filaments in larval musculature in oysters, suggesting the vital role of MYH in muscle development of aquatic economic species [17].

In the aquaculture industry, skeletal muscle growth is closely related to the growth trait of cultured fish, which will directly impact the economic benefit. Over the past decade, multiple strategies have been applied to improve the fish production, including genetic engineering breeding and interspecific hybridization [18]. Illustrating the molecular mechanism of skeletal muscle growth will contribute to making strategies to promote the growth of cultured fish. One early study was carried out to demonstrate the MYH classification, evolution, and expression in fish [19]. More recent studies focused on some specific members of myosin and characterized their expression and function in fish [20–23]. Despite the great process of characterization of myosin genes in teleost fish, the genomewide phylogenetic identification and characterization of complete myosin family genes in teleost is still poorly conducted.

Black rockfish (*Sebastes schlegelii*), distributing in China, Japan, and Korea, is an important marine teleost species. The postnatal growth of *S. schlegelii* exhibit an indeterminate growth pattern, involving both the recruitment and hypertrophy of muscle fibers [24]. The availability of chromosome-level genome sequence [25] provides us the opportunity to analyze myosin genes in genome-wide scale. In this study, we identified and characterized 60 myosin genes in *S. schlegelii* and further characterized their expression patterns. Our work provides valuable information for further detailed functional analysis of myosin in the muscle growth of large teleost fish.

## 2. Materials and Methods

### 2.1. Ethics Statement

This study was approved by the College of Marine Life Sciences, Ocean University of China Institutional Animal Care and Use Committee on 10 October 2018 (Project Identification Code: 20181010).

### 2.2. Identification of Myosin Family Genes in *S. schlegelii*

To identify myosin family genes in *S. schlegelii*, the genome and transcriptome database, which are available at CNSA (CNGB Nucleotide Sequence Archive) under the accession ID CNP0000222 were searched against the Myosin amino acid sequences from some representative species, including *H. sapiens, M. musculus, O. latipes, O. niloticus, L. oculatus*, and *G. aculeatus*. TBLASTN algorithm search was performed and sequences with the e-value below e\(^{-5}\) were collected. Then, the putative myosin sequences were used as query to blast 89 transcriptome assembly of *S. schlegelii*. Only the sequences got hits in at least one transcriptome was regarded as the candidate myosin genes. Candidate myosin family members were submitted to SMART [26] and NCBI-CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) database to predict motor domain. The intracellular location of the proteins was predicted by WoLF PSORT (https://www.genscript.com/psort/wolf_psort.html, accessed on 7 January 2021). The chromosome position, coding strand of myosin genes was identified based on genome annotation file. Tandem duplication analysis was conducted according to two criteria: (a) the similarity of aligned sequences was > 70%; (b) two genes were located in the same chromosomal region within 100 kb [27,28].
2.3. Molecular Characters of S. schlegelii Myosin Genes

The physiochemical properties of identified myosin genes, including the molecular weight (Mw) and theoretical isoelectric point (pl), were calculated by the ExPasy site (https://web.expasy.org/protparam/, accessed on 7 January 2021) [29,30]. The exon-intron structure of myosin genes was determined by comparison of genome and transcriptome sequences following the GT-AG rule [31]. The schematic diagram of gene structure was displayed using GSDD 2.0 program (http://gsds.cbi.pku.edu.cn/, accessed on 30 January 2021). Protein motif analysis was carried out with Motif Elicitation (MEME) program (http://meme-suite.org/tools/meme, accessed on 30 January 2021).

2.4. Phylogenetic and Synteny Analysis

Multiple sequences alignments of Myosin proteins were conducted by Clustal W [32]. The Neighbor-Joining method was utilized to perform phylogenetic analysis by MEGA 7.0 [33] and visualized using iTOL website (http://itol.embl.de, accessed on 20 January 2021). The conserved synten of myosins and their adjacent genes among spotted gar, teleost fish species, and mammals was analyzed based on the genomics website (http://www.genomicus.biologie.ens.fr/genomicus-82.01/cgi-bin/phyloview, accessed on 13 May 2021) and the schematic diagram was drawn by hand.

2.5. Expression Profiling of Myosin Genes at Early Developmental Stages, Different Tissues, and During in Vitro Myoblast Differentiation Process

In our previous studies, we built a large number of transcriptome database of S. schlegelii, including different tissues and early developmental stages [25]. In this study, a total of 89 transcriptomes, including 63 tissue libraries and 26 early developmental stages were utilized to characterize the expression profiles of S. schlegelii myosin genes (Table S1 and Table S2). We also successfully established a continuous skeletal muscle cell line from juvenile rockfish muscle in one of related study. The cell line consisted of a high ratio of myoblasts and could differentiate into myotubes upon differentiation medium treatment. The transcriptomes of muscle cells at different time points after differentiation medium induction were generated and processed (BioProject ID: PRJNA661185) (This data will be published in other study). TPM (Transcripts Per Million reads) value of myosin genes were extracted (Table S3). Heat maps were created by TBtools [34].

2.6. qRT-PCR Validation of Myosin Expression

Total RNA from all collected samples were isolated using TRIzol reagent (Invitrogen, California, USA) according to the manufacturer’s protocol. After removing genomic DNA with DNase I (Takara, Dalian, China), cDNA synthesis was performed with Reverse Transcriptase M-MLV Kit (Takara, Dalian, China). The quantity and quality of cDNA were tested by spectrophotometry and agarose gel electrophoresis (Coolaber, Beijing, China), respectively. Specific primers for real-time PCR were designed using Integrated DNA Technologies (http://sg.idtdna.com/pages/home, accessed on 2 February 2021). qRT-PCR was performed on LightCycler480 (Roche, San Francisco, USA) using the NovoStart®SYBR qPCR SuperMix Plus (Novoprotein, Shanghai, China) in a condition with 95 °C for 5 min, 45 cycles (95 °C for 15 s) and 60 °C for 45 s. The EIF5A1 gene in S. schlegelii was used as a reference gene to normalize the expression of target genes. The relative expression levels of target genes were calculated based on 2^(-△△Ct) comparative Ct method. Statistical analysis was conducted using one-way analysis of variance followed by the least significant difference test using SPSS2.0 (IBM, Armonk, NY, USA), and differences with P < 0.05 were treated as significant. Each experiment was performed with triplicates.

2.7. Histological Examination

For histological examination, the samples of muscle from juvenile at different days post parturition (20 dpp, 35 dpp, 50 dpp, 75 dpp, and 90 dpp) and adult S. schlegelii at two different ages (1.5-year-old and 2.5-year-old) were collected and fixed in 4% paraformal-
dehyde (PFA) overnight at room temperature. Then, the samples were transferred to methanol (30%, 50%, 70%, 80%, 90%, 2 h, respectively), and finally stored in 100% methanol. After dehydrating and embedding in paraffin, tissue blocks were sectioned at 6 μm, and stained with hematoxylin and eosin (Solarbio, Beijing, China).

3. Results

3.1. Identification of Myosin Genes in S. schlegelii

The Myosin amino acid sequences from some representative species, including *H. sapiens*, *M. musculus*, *O. latipes*, *O. niloticus*, *L. oculatus*, and *G. aculeatus* were used to search against *S. schlegelii* genome and transcriptome database. In total, 60 *myosin* genes in *S. schlegelii* were identified, including 27 conventional *myosin* genes and 33 unconventional *myosin* genes. *S. schlegelii* myosin genes varied largely in length and physicochemical properties. The length of coding sequences of *myosin* genes ranged from 2862 to 11,313 bp, with corresponding protein length ranged from 954 to 3771 amino acids. Based on primary protein sequences, the molecular weights and isoelectric points of Myosins were predicted. The molecular weight of Myosin proteins ranged from 109.70 to 435.08 kDa with the isoelectric point ranging from 5.42 to 9.43. The number of transcripts produced by each gene was also counted. We found that 29 *myosins* had more than one transcript. *myo7b* and *myo9ab* produced the highest number (seven) of transcripts. The detailed information of *myosin* genes was summarized in Table 1.

| Gene   | Chromosome | CDS (bp) | Amino Acid (aa) | Mw (Kda) | PI | Location | Number of Transcript |
|--------|------------|----------|-----------------|----------|----|----------|---------------------|
| Myo1b  | Chr 20     | 3513     | 1171            | 135.52   | 9.43 | C, N, M, Cs | 4                   |
| Myo1c  | Chr 4      | 2928     | 976             | 112.43   | 9.23 | C, N, M, Cs, Es | 4                   |
| Myo1cl | Chr 12     | 3204     | 1068            | 122.32   | 8.98 | C, N, Cs, Es | 1                   |
| Myo1d  | Chr 18     | 3528     | 1176            | 134.19   | 9.3  | C, N, M | 3                   |
| Myo1e  | Chr 2      | 3297     | 1099            | 124.93   | 9.21 | Es, M | 1                   |
| Myo1f  | Chr 6      | 3219     | 1073            | 122.63   | 9.23 | C, N, M, Cs | 3                   |
| Myo1g  | Chr 11     | 3033     | 1011            | 115.55   | 7.96 | C, N, M, Cs | 1                   |
| Myo1h  | Chr 8      | 3090     | 1030            | 118.74   | 8.6  | C, N, M | 3                   |
| Myo1h1 | Chr 17     | 3102     | 1034            | 119.84   | 9.13 | C, N, M | 1                   |
| Myo3a  | Chr 21     | 5112     | 1704            | 194.21   | 6.52 | C, N, M | 2                   |
| Myo3b  | Chr 20     | 4017     | 1339            | 152.55   | 8.45 | C, N, M, Cs | 2                   |
| Myo5a  | Chr 2      | 5577     | 1859            | 214.27   | 8.84 | C, N, M | 3                   |
| Myo5b  | Chr 6      | 4662     | 1554            | 176.03   | 9.12 | C, N, M | 1                   |
| Myo5c  | Chr 9      | 10089    | 3353            | 388.34   | 8.43 | C, N, M | 1                   |
| Myo6   | Chr 14     | 2862     | 954             | 109.7    | 8.59 | C, N, M, Cs | 1                   |
| Myo6l  | Chr 22     | 3273     | 1091            | 125.21   | 8.47 | C, N, M | 2                   |
| Myo7a  | Chr 11     | 6330     | 2110            | 243.81   | 8.92 | C, N, M | 3                   |
| Myo7b  | Chr 4      | 5940     | 1980            | 228.49   | 8.88 | C, N, M | 1                   |
| Myo7b  | Chr 6      | 6696     | 2232            | 256.07   | 8.88 | C, N, M | 2                   |
| Myo7bb | Chr 7      | 6252     | 2084            | 237.81   | 8.16 | C, N, M | 2                   |
| Myo9a  | Chr 2      | 7689     | 2563            | 291.95   | 8.45 | C, N, M | 4                   |
| Myo9ab | Chr 9      | 7224     | 2408            | 274.34   | 8.58 | C, N, M | 7                   |
| Myo9a  | Chr 6      | 7464     | 2488            | 282.91   | 7.63 | C, N, M | 3                   |
| Myo9b  | Chr 7      | 5862     | 1954            | 223.1    | 8.6  | C, N, Cs | 5                   |
| Myo10  | Chr 20     | 5763     | 1921            | 220.93   | 5.57 | C, N, M | 1                   |
| Myo10l | Chr 14     | 6030     | 2010            | 227.9    | 6.8  | C, N, M | 2                   |
| Myo10l1| Chr 7      | 5745     | 1915            | 218.78   | 6.13 | C, N, M | 2                   |
| Myo15a | Chr 5      | 9417     | 3139            | 352.11   | 9.02 | C, N, M | 1                   |
| Myo15ab| Chr 13     | 10,929   | 3643            | 406.73   | 8.35 | C, N, M | 1                   |
| Myo16  | Chr 20     | 5730     | 1910            | 210.75   | 8.41 | C, N, M | 2                   |
| Myo18a | Chr 4      | 8160     | 2720            | 304.89   | 6.78 | C, N, M | 6                   |
| Myo18ab| Chr 12     | 6903     | 2301            | 259.71   | 6.82 | C, N, M | 5                   |
3.2. The Evolutionary Relationship of Myosin Genes

To understand the evolutionary relationship and classification of myosin, the phylogenetic analysis was conducted by Neighbor-Joining method. As shown in Figure 1A, 60 S. schlegelii myosins were classified into 12 subfamily clusters. Myo1 and Myo2 were the first two largest subfamilies in S. schlegelii, including 7 and 27 members, respectively. Compared to mammals and other teleost species, Myo2 subfamily in S. schlegelii were significantly expanded ($p = 0.000003$), which could be further divided into eight subfamilies. Among Myo2 subfamily in S. schlegelii, almost half of the genes were clustered with MYH2 genes from other species, suggesting the expansion of MYH2 contributed the expansion of S. schlegelii conventional myosin genes. The member and number of Myo2 genes in some representative teleost fish were concluded in Figure 1B.

3.3. Functional Domains and Gene Structure of Myosin Genes

The putative functional domains were identified using SMART and Pfam databases. As shown in Figure 2, all S. schlegelii myosins had a motor domain (myosin head), which located in the N-terminus. In addition to myosin head, all Myo2 genes had a Myosin-N domain, except Ss_10001285. Furthermore, the domain types and numbers of myosin genes were similar within the same subfamily members but divergent among different subfamilies.

Table 1. Cont.

| Gene          | Chromosome | CDS (bp) | Amino Acid (aa) | Mw (Kd) | PI  | Location         | Number of Transcript |
|---------------|------------|----------|-----------------|---------|-----|------------------|---------------------|
| Myo19         | Chr 4      | 2913     | 971             | 110.4   | 8.27| C, N, M, Es      | 1                   |
| Ss MYH6       | Chr 22     | 5802     | 1934            | 222.63  | 5.62| C, N, Cs, Es     | 1                   |
| Ss MYH7ba     | Chr 10     | 5712     | 1904            | 219.7   | 6.19| C, N, M, Cs      | 1                   |
| MYH7bb        | Chr 1      | 5850     | 1950            | 224.95  | 5.95| C, N, Cs         | 1                   |
| Ss MYH9a      | Chr 5      | 5904     | 1968            | 228.58  | 5.26| C, N, M, Cs      | 1                   |
| Ss MYH9b      | Chr 13     | 5928     | 1976            | 229.45  | 5.5 | C, N, M, Cs      | 1                   |
| Ss MYH10      | Chr 5      | 5985     | 1995            | 231.74  | 5.42| C, N, M, Cs      | 2                   |
| Ss MYH10l     | Chr 13     | 5793     | 1931            | 223.77  | 5.46| C, N, M, Cs      | 2                   |
| Ss MYH11a     | Chr 5      | 5916     | 1972            | 227.02  | 5.43| C, N, M, Cs      | 2                   |
| Ss MYH11b     | Chr 13     | 5850     | 1950            | 224.29  | 5.48| C, N, M, Cs      | 1                   |
| Ss MYH14      | Chr 19     | 5763     | 1921            | 220.93  | 5.57| C, N, M          | 4                   |
| Ss MYH16      | Chr 13     | 5760     | 1920            | 220.35  | 5.86| C, N, M, Cs      | 1                   |
| Ss 10008025   | Chr 7      | 11,313   | 3771            | 435.08  | 5.92| C, N, Cs, Es     | 1                   |
| Ss 10008026   | Chr 7      | 5877     | 1959            | 225.07  | 5.78| C, N, Cs, Es     | 1                   |
| Ss 10008027   | Chr 7      | 5823     | 1941            | 223.39  | 5.8 | C, N, Cs, Es     | 1                   |
| Ss 10001285   | Chr 9      | 4020     | 1340            | 154.38  | 5.57| C, N, M, Cs      | 1                   |
| Ss 10001286   | Chr 9      | 5817     | 1939            | 221.86  | 5.8 | C, N, Cs, Es     | 1                   |
| Ss 10001287   | Chr 9      | 5664     | 1888            | 216.24  | 5.49| C, N, Cs, Es     | 2                   |
| Ss 10002778   | Chr 1      | 5751     | 1917            | 219.67  | 5.58| C, N, M, Cs      | 1                   |
| Ss 10002779   | Chr 1      | 5937     | 1979            | 227.43  | 5.86| C, N, M, Cs      | 4                   |
| Ss 10002780   | Chr 1      | 5811     | 1937            | 222.69  | 5.75| C, N, M, Cs      | 1                   |
| Ss 10013951   | Chr 13     | 5787     | 1929            | 222.94  | 5.78| C, N, M, Cs      | 1                   |
| Ss 10013952   | Chr 13     | 5736     | 1912            | 220.14  | 5.66| C, N, M, Cs      | 1                   |
| Ss 10015508   | Chr 9      | 5571     | 1857            | 212.53  | 5.66| C, N, M, Cs      | 5                   |
| Ss10015614    | Chr 9      | 5886     | 1962            | 224.78  | 5.58| C, N, M, Cs      | 1                   |
| Ss 10015615   | Chr 9      | 5829     | 1943            | 222.54  | 5.5 | C, N, M, Cs      | 1                   |
| Ss 10021428   | Chr 13     | 5778     | 1926            | 220.78  | 5.76| C, N, M, Cs      | 3                   |
| Ss 10021429   | Chr 13     | 5841     | 1947            | 223.01  | 5.67| C, N, M, Cs      | 1                   |
Figure 1. The phylogenetic analysis and classification of myosin genes. (A) Phylogenetic tree of myosin genes was constructed with Neighbor-Joining method by MEGA7 (bootstrap = 1000). Twelve subfamilies were decorated by different colors. (B) Characterization of Myo2 subfamily in teleost fishes. The phylogenetic tree was conducted by MEGA 7.0 using CO1 sequences of these species. The dotted border represented gene loss in the species.
Figure 2. A schematic diagram of *S. schlegelii* myosin gene domains. The maximum likelihood method was used to construct the phylogenetic tree of *myosin* genes (left panel). The rectangle with different colors represented different domains (right panel). The scale bar indicated 500 amino acid residues.

To gain insights into structures of *myosin* genes, we conducted the exon-intron structure analysis based on genome and transcriptome data following the GT-AG rule (Figure 3, left panel). The genomic structure was divergent among different *myosin* subfamilies. The motif analysis of *S. schlegelii* *myosin* genes was further performed (Figure 3, right panel). Motif1, motif4, and motif7, locating in myosin head domain, were present in all *myosin* genes. The number and category of motifs were divergent in different subfamilies, but was relatively conserved within the same subfamily. For instance, most *Myo2* subfamily genes shared the same motifs but differed from the motifs conserved in *Myo1* subfamily genes (Figure 3, right panel).
3.4. Chromosome Distribution and Synteny Analysis of Myosin Genes

The positions of myosin genes on the corresponding chromosomes of *S. schlegelii* were indicated in Figure 4. All myosin genes could be mapped onto 21 chromosomes of *S. schlegelii*. Chromosome 13 contained the highest number of myosin genes (9), whereas no myosin genes was located on chromosome 15, chromosome 23 and chromosome 24 (Figure 4). To understand the consequences of teleost genome duplication (TGD) on myosins evolution, we conducted synteny analysis of myosins and their adjacent genes among spotted gar (*Lepisosteus oculatus*), whose lineage diverged before TGD, four teleost fish species (*Gasterosteus aculeatus*, *Oryzias latipes*, *Orechromis niloticus* and *S. schlegelii*) and mammals (*Homo sapiens* and *Mus musculus*). We found 14 myosins (*myo1hl*, *myo1cl*, *myo6l*, *myo7ab*, *myo7bb*, *myo9ab*, *myo9bb*, *myo10l*, *myo15ab*, *MYH7bb*, *MYH9b*, *MYH10l*, and *MYH11*) were generated by TGD whereas the expanded MYH2 genes specifically emerged in tandem duplication form in *S. schlegelii* (Figure S1). Fifteen myosin genes, including three MYH7 genes and 12 MYH2 genes were clustered into six tandem duplication event regions on chromosome 1 (one cluster), chromosome 7 (one cluster), chromosome 9 (two clusters), and chromosome 13 (two clusters) (Figure S1 and Table 2).
Figure 4. Chromosome location and synteny analysis of *myosin* genes. The boxes with different colors represented different chromosomes of *S. schlegeli*. The red lines represented the syntenic relationships of *myosin* genes in *S. schlegeli*.

Table 2. The tandem duplication events of *myosin* genes identified in *S. schlegeli*.

| Cluster Number | Gene ID     | Chromosome | Start Site | End Site  |
|----------------|-------------|------------|------------|-----------|
| 1              | Ss_10021428 | 13         | 9553704    | 9568469   |
|                | Ss_10021429 | 13         | 9589690    | 9601364   |
| 2              | Ss_10015614 | 9          | 11373314   | 11383630  |
|                | Ss_10015615 | 9          | 11388379   | 11398341  |
| 3              | Ss_10013951 | 13         | 17872608   | 17905057  |
|                | Ss_10013952 | 13         | 17911150   | 17926242  |
| 4              | Ss_10008025 | 7          | 29692553   | 29729078  |
|                | Ss_10008026 | 7          | 29738637   | 29750296  |
|                | Ss_10008027 | 7          | 29758471   | 29783643  |
| 5              | Ss_10002778 | 1          | 34544361   | 34556333  |
|                | Ss_10002779 | 1          | 34564613   | 34580184  |
|                | Ss_10002780 | 1          | 34589861   | 34601724  |
| 6              | Ss_10001285 | 9          | 11434203   | 11445087  |
|                | Ss_10001286 | 9          | 11448018   | 11458669  |
|                | Ss_10001287 | 9          | 11466155   | 11477014  |

3.5. Expression Patterns of Myosin Genes in Adult Tissues and Early Developmental Stages

The expression levels of *myosin* genes in ten adult tissues (Heart, Liver, Spleen, Kidney, Brain, Gill, Muscle, Intestine, Testis, and Ovary) and six early developmental stages (1-cell, 32-cell, blastula, gastrula, somite, and pre-hatching) were evaluated by TPM (Transcripts per million) values from 89 transcriptome database (Figure 5A). In adult tissues, the expression patterns of *myosin* genes were divergent. For example, seven *myo2* genes (Ss_10001285, Ss_10001286, Ss_10001287, Ss_10015614, Ss_10015615, Ss_10015508, and Ss_10002780) were highly expressed in muscles, whereas the rest of 20 *myo2* genes were highly expressed in heart, brain, intestine, and gonads, respectively. In addition to conventional *myosin* genes, some unconventional *myosin* genes also showed tissue-specific expression patterns.
All *S. schlegelii* myosin genes could be detected with the dynamic expression patterns during the early developmental stages (Figure 5B). Most myosin genes showed the highest expression level at the pre-hatching stage, including all MYH2 genes and most other Myo2 subfamily genes. In addition, some Myo2 genes showed distinct expression patterns. For instance, the expression of MYH16 showed the highest level at gastrula, whereas the expression levels of MYH7, MYH14, and MYH6 began to increase at somite stage, and Ss_10015508 expressed incredibly high at blastula stage.

### 3.6. Myo2 Genes Participate in the Muscle Growth in Juvenile and Adult *S. schlegelii*

We firstly performed the histological observation of muscle from juvenile *S. schlegelii* at different days post parturition (20 dpp, 35 dpp, 50 dpp, 75 dpp, and 90 dpp). As shown in Figure 6A, the sarcomere gradually widened and muscle fibers gradually became longer and thicker with the growth of *S. schegelli*. 

**Figure 5.** Expression profiles of myosin genes in adult tissues (A) and early developmental stages (B). The color scale represented the TPM (Transcripts-per-million) value. The red and blue color represented the relatively higher and lower TPM (Transcripts-per-million) value, respectively.
To further explore the roles of myosin genes in the growth process of \textit{S. schlegelii}, we selected several muscle-highly-expressed and significantly expanded \textit{Myo2} genes (\textit{Ss\_10001286}, \textit{Ss\_10021429}, \textit{Ss\_10015615}, \textit{10002780} of \textit{MYH2} and \textit{Ss\_10008027} of \textit{MYH7}), and measured their expression levels in the muscle of juvenile fish. \textit{Ss\_10001286}, \textit{Ss\_10002780} and \textit{Ss\_10021429} were significantly up-regulated from 75 dph to 90 dpp, while the expression levels of \textit{Ss\_10015615} and \textit{Ss\_10008027} increased since 35 dpp (Figure 6B).

In adult \textit{S. schlegelii}, the diameter and cross-sectional area of muscle fibers increased rapidly from 1.5 to 2.5 years old (Figure 6C). Except for \textit{Ss\_10002780}, the other four selected genes showed an extremely significant increase of expression in 2.5-year-old fish (Figure 6D).

### 3.7. Myosin Genes Involved in Myoblast Differentiation

To further investigate the roles of \textit{myosin} genes in the muscle development, we concluded the expression patterns of \textit{myosin} genes during the process of myoblasts differentiation. As shown in Figure 7, \textit{myosin} genes were differently regulated during this process. \textit{Myo1e}, \textit{Myo3a}, \textit{Myo5b}, and \textit{Ss\_10013951} exhibited the earliest response to differentiation treatment, showing the highest expression level at 24 h. Later on, \textit{Myo15a}, \textit{Myo1f}, \textit{Myo1c}, and \textit{Myo6a} were up-regulated at 48 h, and then decreased. Most \textit{Myo2} genes showed the highest expression level at 72 h after differentiation induction. However, a large number of
myosin genes were significantly down-regulated during differentiation process. In addition, the expression levels of Myo7aa and Myo1hl did not change, suggesting that they are not involved in myoblasts differentiation.

4. Discussion

Skeletal muscle development and growth contribute to the size and weight of cultured animals. With the development of aquaculture industry, many strategies have been applied to promoting the muscle growth, and the genetic studies on muscle growth got more and more attention [35–37]. Myosin are a large and conserved family of cytoskeletal motor proteins that bind actin and participate in a broad range of biological processes [1,38]. However, little is known about myosin genes in teleost. In this study, we isolated and characterized 60 myosin family genes in S. schlegelii based on genome and transcriptome datasets, and initially elucidated the involvement of myosin genes in myoblasts differentiation and muscle growth of S. schlegelii.

4.1. Diverse Functions of Myosin Genes in S. schlegelii

The gene expression patterns provide insight to the function and biological activity of target genes. Our results showed myosin genes were ubiquitously expressed in different tissues with diverse expression levels but with subfamily-specific pattern. Previous
studies concluded that muscle function was determined by its structure and fiber type composition [39], so we speculated the divergent expression patterns of S. schlegelii myosin genes might be related to different types and compositions of muscle fibers in different tissues. Interestingly, we found the tandem duplicated MYH2 genes of S. schlegelii were extremely highly expressed in skeletal muscle, suggesting their potential roles in skeletal muscle development and growth which directly determine the size of fish body. In addition, other representative MYHs showed conserved expression patterns between fish and mammals. For example, MYH6 tend to be expressed in atrial muscle and MYH7 is abundant in ventricular muscle in mammals, the mutation of MYH6 or MYH7 will lead to the lesions of heart [40–43]. In S. schlegelii, we also identified the highest expression level of MYH7b and MYH6 in the heart, suggesting their potential conserved function between teleost and mammals. In addition, MYH11b was detected to be abundant in S. schlegelii intestines, which contain a lot of smooth muscle. This is consistent with the previous report that MYH11 functions in smooth muscle in human [44]. For unconventional myosin, quite a few number of genes were detected the highest levels in brain and gonad of S. schlegelii, suggesting their potential roles in neuron and reproductive systems, as described in previous studies [45]. Taken together, the myosin subfamily genes may conduct conserved functions between human and teleost. Most myosin genes started to be highly expressed at pre-hatching stage, suggesting the muscle fibers synthesis was significantly active during this period.

4.2. Expansion of Myo2 Subfamily and Their Involvement in Skeletal Muscle Growth in S. schlegelii

Teleost had undergone the TGD during the evolution, resulted in two or more copies of genes existing in teleost genome [46]. Generally, gene expansion results in strengthened phenotype and drives the evolutionary process of adaption [47]. In S. schlegelii, we identified and characterized 60 myosin genes, containing 27 conventional myosin genes, which belong to Myo2 subfamily. Compared to other teleost species, S. schlegelii Myo2 subfamily showed significant expansion (Figure 1), which was driven by the tandem duplication events (Figure 4). Previous studies have shown massive expansion of gene family in teleost might be related to the vital roles of family members in certain biological processes. For example, the expansion of NLR family suggest that NLRs have a more substantial role in the innate immunity in haddock [48]. The expansion of tdrd family may be related to their important function in germline in Japanese flounder [49]. Members of Myo2 subfamily have been identified to play an important role in muscle development in teleost but in different regulatory ways. For example, in torafugu, MYH_{M86-1} and MYH_{86-2} were reported to have markedly different expression patterns in muscle [20]. Three embryonic MYHs were located in the same or different cranial muscles of common carp larvae [50]. In S. schlegelii, some of MYH2 genes generated by tandem duplication showed distinct expression patterns. As the marker of mature skeletal muscle differentiation, most MYH2 genes expressed highly in skeletal muscle whereas several MYH2 genes such as Ss_10002778 and Ss_10002779 expressed highly in testis, Ss_10021428 highly in gill, Ss_10013951 and Ss_10013952 highly in brain, suggesting the expanded MYH2 genes might gain the neo-function in smooth muscle and gland cells instead of the traditional role in skeletal muscle. This result provides the evidence that gene duplication is the important source for the emergence of evolutionary novelties and neofunctionalization of gene families. Taken together, we speculate that the expansion of Myo2 subfamily genes contribute to development and growth of both skeletal muscle and some other important organs in S. schlegelii. The specific roles for each member need further observation.

We selected five skeletal muscle-highly-expressed MYH genes and analyzed their expression levels in the fast-growth stages of juvenile and adult S. schlegelii. Our current results showed the expression levels of MYHs were significantly up-regulated from 50 dpp or 75 dpp, corresponding to the timepoints of significant increase of number and size of muscle fibers. In juvenile torafugu, MYHs was confirmed to be involved in mosaic hyperplasia and stratified hyperplasia, which contributed to the juvenile skeletal muscle
growth [20], the similar conclusion was also obtained in some other species, such as shrimp, carp, and Atlantic cod [15,51,52]. In adult S. schlegelii, the selected genes showed significantly higher expression in 2.5-year-old than in 1.5-year-old fish, suggesting more active synthesis of muscle fibers in 2.5-year-old. This is in accordance with our recent finding that 2.5-year-old fish grows faster than 1.5-year-old fish (the details of growth pattern will be reported separately). To sum up, Myo2 genes are involved in skeletal muscle fibers development and contribute to the skeletal muscle growth of juvenile and adult S. schlegelii.

4.3. Myosin Genes Participate in Myoblast Differentiation of S. schlegelii

Myosin genes of S. schlegelii were identified to be involved in muscle fiber growth in our studies; however, the mechanisms by which step myosin is involved in muscle development is unclear. As described in previous studies, myoblast proliferation and differentiation promotes the formation of muscle fibers, and then contributes to the growth of skeletal muscles in cultured animals [53]. The culture and differentiation induction of S. schlegelii myoblast in vitro, and the availability of transcriptome data at different timepoints during myoblast differentiation give us a chance to analyze the involvement of myosin genes during myoblast differentiation. As our results showed, myosin genes displayed quite different response in the process of myoblast differentiation. Most Myo2 subfamily genes were upregulated in this process, especially the MYH2 genes cluster, which was identified significantly expanded and highly expressed in the skeletal muscle in S. schlegelii. As the late differentiation marker [54], it is easy to understand the up-regulation of MYH2 with the differentiation process of myoblast cells in S. schlegelii, which has also been widely demonstrated in other species [55–57].

However, quite a few of myosin genes, containing both conventional and unconventional myosin, were down-regulated during cell differentiation. For muscle fiber development and growth, except for the contribution of increased protein synthesis and increased cell size due to cell differentiation, the formation of new myoblast by cell proliferation also plays a vital role [58]. It has been widely identified that myosin could be involved in cell proliferation. For example, knockdown of myosin 6 inhibits proliferation of hepatocellular carcinoma cells and oral squamous cell carcinoma cells [59,60]. myosin 16, an unconventional member, is proved to have a role in regulation of cell cycle and cell proliferation [61]. In Drosophila, on-muscle myosin 2 is required for cell proliferation during wing morphogenesis [62]. In our study, the down-regulation of myosin during cell differentiation suggest their potential roles in cell proliferation in S. schlegelii. Taken together, S. schlegelii myosin family genes participate in skeletal muscle growth by involvement both in myoblast proliferation and differentiation.

5. Conclusions

We identified and characterized 60 myosin family genes in S. schlegelii and demonstrated the expansion of myo2 subfamily in this species. The expression profiling of myosin provides the evidence that they are involved in different types and compositions of muscle fibers in different tissues. Moreover, the expanded myo2 genes actively participate in the skeletal muscle growth both in juvenile and adult S. schlegelii. During myoblast differentiation, myosin responded differently, revealing their divergent functions in myoblast proliferation and differentiation. Taken together, our work characterized myosin genes systemically and demonstrated their diverse functions in a marine teleost species. This lays foundation for the further studies of muscle growth regulation and molecular mechanisms of indeterminate skeletal muscle growth of large teleost fishes.
Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/genes12060808/s1, Figure S1. Synteny analysis of myosins and adjacent genes among spotted gar (Lepisosteus oculatus), medaka (oryzias latipes), threespine stickleback (Gasterosteus aculeatus), human (Homo sapiens) and mouse (Mus musculus). Orthologs of each gene were shown in the same color. Gene orientation is indicated by the direction of the arrows. Table S1: The TPM values of Sebastes Schlegelii myosins in different tissues. Table S2: The TPM values of Sebastes Schlegelii myosins during different developmental stages. Table S3: The TPM values of Sebastes Schlegelii myosins during myoblast differentiation in vitro.

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Data Availability Statement: The datasets of whole genome sequencing of S. schlegelii and RNA-seq of tissues and developmental stages were available in CNSA (CNGB Nucleotide sequence archive) with the accession ID CNP0000222. The RNA-seq dataset of S. schlegelii myoblasts differentiation was submitted to NCBI SRA (BioProject ID: PRJNA661185).

Conflicts of Interest: The authors declare that there is no conflict of interest.

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