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The analysis of growth performance and expression of growth-related genes in natural gynogenic blunt snout bream muscle derived from the blunt snout bream (*Megalobrama amblycephala*, ♀) × Chinese perch (*Siniperca chuatsi*, ♂)

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ABSTRACT

Gynogenesis is an advanced breeding technique that can quickly establish pure lines, increase progenies genetic variation, cultivate strains with higher dominant traits. As a good model for cross order species hybridization, there are few reports on the study of natural gynogenetic blunt snout bream (GBSB), even on the differences in growth between gynogenesis progenies and their parent fish (blunt snout bream♀ and Chinese perch♂). In this research we compared the growth characteristics of the natural GBSB with its parents. It was found that GBSB had a faster growth rate and higher meat content than its female parent, and the diameter and density of muscle fibers were between those of paternal and maternal fish. We performed transcriptome sequencing of muscle tissues from GBSB and BSB, it identified 1353 significant DEGS in the muscle of the GBSB compared to the BSB, with 820 up- and 533 down-regulated genes. And the relative expression of *myosins* was notable rise in GBSB. In addition, the MRFs of gene structure, motifs, and phylogenetic relations were characterized in BSB and CP. It showed that MRFs and *mstn* central domain was highly conserved, but the number of conservative motifs in MRFs of CP is generally higher than that of BSB. Further, the relative expression of myosin complex, myogenic regulatory factors (MRFs), growth hormone/insulin-like growth factor (GH/IGF)-axis and most hypothalamic-pituitary-gonadal (HPG)-axis genes in GBSB were higher than that of the female fish and some were closer to that of the paternal fish. Through the distant hybridization between BSB and CP, a gynogenetic progeny with enhanced growth rate compared to the maternal fish was successfully generated. The research has contributed significant insights into the genes and molecular properties associated with muscle development, as well as their potential functional correlations in response to different functional requirements during the growth process of hybrid progeny formed by cross breeding.

1. Introduction

Being a vital economical freshwater fish in China, BSB boasts several advantages, including superior meat quality, rich nutrition, and rapid growth. It is extensively cultivated in numerous regions across the country (Chen et al., 2021). However, due to rapid domestication and overfishing, the germplasm resources of BSB are seriously threatened (Gao et al., 2012). Gynogenesis serves as a viable strategy to forestall the degeneration of species (Wang et al., 2019; Wu et al., 2022). During gynogenesis, the sperm normally enters the activated egg, its nucleus

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soon disappears and does not participate in the development of the egg. However, it has been shown that gynogenetic progenies contain fragments of paternal DNA(Gong et al., 2019; Liu, 2010; Mao et al., 2019). It was previously proposed that spermatozoa engage in limited genetic material exchange with the ovokaryon, a phenomenon known as the "heterospermic effect." At the DNA level, this may result in DNA mutations and recombination in the progeny. (Zhang et al., 2011). In terms of their phenotype, hybrids have the capacity to blend parental heterozygosity and exhibit heterosis (Mao et al., 2020). Therefore, it has the widespread adoption in numerous fish (Delomas et al., 2017; Wu et al., 2023). Fish gynogenesis can be categorized into two types: natural and artificial gynogenesis. Natural gynogenesis refers to the process where fish produce progeny through distant hybridization (Liu, 2022). If the parents are far related, it is difficult for the progeny produced by direct hybridization to survive, but the gynogenesis can be induced by the use of remote sperm in distant hybridization (Felip et al., 2001). However, the majority of contemporary fish hybridization experiments are carried out between subfamilies and families (Xiao et al., 2014). There are hardly any studies conducted on the exploration of gynogenesis between trans order species within fish. Previous studies have reported trans order hybridization of *Hypophthalmichthys molitrix* (♀) and *Pagrosomus major* (♂)(Zhang et al., 2014). However, what kind of heterosis is exhibited Zhang et al., 2011ish is worth further study.

In recent year, the advancements in transcriptome sequencing technology (RNA-seq) have shed light on the fish transcriptome. RNA-seq is now widely used to study the following questions in fish, such as mechanisms of muscle growth (Dos Santos et al., 2020), the expression of disease resistance genes (Wang et al., 2013), and MicroRNA expression associated with muscle quality traits (Paneru et al., 2017). All these shed new light on the molecular mechanisms underlying some biological processes in fish. However, the study of transcriptome-genome combination revealing rapid growth of trans order species has not been reported. The distant hybrid progeny may will show hybridization advantages, such as strong disease resistance and fast growth rate, which makes it have great commercial value and application prospect. Therefore, the growth advantages of GBSB are worth exploring in depth.

Muscle growth, characterized by a moderate to high degree of heritability. This process encompasses the simultaneous augment in muscle fiber number and size, with the equilibrium between these two aspects being determined by the developmental stage. (Robledo et al., 2017). It can be said that the growth of fish is the rapid proliferation and rapid hypertrophy of fish myofiber cells. The skeletal muscle of fish is mainly composed of muscle fibers, which are composed of myosin and filamin proteins(Wang et al., 2023). Myosin, a highly conserved and multifunctional protein, is broadly distributed across eukaryotes (Ojima, 2019). It is involved in phosphorylation and dephosphorylation, which can drive muscle contraction to produce force(Vandenboom, 2011). However, the expression of myosin light (*myl*) and heavy chain (*myh*) genes may change during muscle growth(Chen et al., 2023), Changes in *myhs* and *myls* expression levels may affect muscle fiber hypertrophy, muscle mass, and overall growth performance. In addition, the MRFs as a vital part in muscle development by adjusting the expansion and differentiation of muscle cells. (Buckingham and Rigby, 2014). The *mstn* significantly contributes to controlling muscle growth (Rescan, 2005). The *mstn* signaling specifically triggers Smad3 phosphorylation and enhances the interaction between Smad3 and *MyoD*, consequently suppressing the function of *MyoD* and halting muscle growth. (Zhu et al., 2022). Furthermore, the MRFs and *mstn* of gene structure, motifs, and phylogenetic relations were characterized. Myomaker, as a key factor in myoblast fusion, it promoter can bind to the *Myogenin* (*MyoG*) for myocyte fusion, muscle development and regeneration(Chen et al., 2020). The HPG and GH/IGF axis signal molecules play an important role in the regulation of fish growth(Dang et al., 2018; Liu et al., 2018). However, the regulation of HPG axis and GH/IGF axis on the formation of growth-dominant new fish from distant hybridization has not been widely reported, and it is worth further research.

To assess the influence of distant hybridization on the growth pace of progeny, we quantified the muscle morphology of GBSB, BSB, and CP. Furthermore, we conducted transcriptome sequencing on GBSB and BSB to identify growth-associated genes. Lastly, we analyzed and validated these genes. The research provides valuable perspectives on the growth advantage of GBSB in the aquaculture industry.

2. Materials and methods

2.1. Ethics statement

This research adhered to the Guide for the Care and Use of Laboratory Animals of Hunan Normal university. The fish samples were rendered unconscious using 100 mg/L MS-222 (Sigma) before dissection.

2.2. Gynogenesis and sampling

The experiment took place at the Fish Breed Base in Wugang, Hunan, China. Twenty of the sexually mature BSB and twenty of the sexually mature CP were randomly selected as parents between May and July 2021. The maternal fish received a single injection containing domperidone 1.0–2.0 μg/kg, LHRH-A2 10.0–12.0 μg/kg, and HCG 300-500 IU/kg. The paternal fish were given a single injection containing domperidone 2.0–5.0 μg/kg and LHRH-A2 2.0–5.0 μg/kg. After a span of 16 to 26 h, sperm were collected by gently pressing the abdomen of the CP and mature egg were obtained by squeezing the abdomen of the BSB. The sperms and eggs were mixed to obtain fertilized eggs, and the fertilized eggs were placed in a clean water incubation device with a water temperature of 22 °C ~ 25 °C for water incubation, and GBSB seedlings were obtained. At the same time, the sexually mature BSB and CP were selected to produce normal BSB and CP by self-cross respectively. The hatched fish fry is reared in ponds at a density of 400 fish per acre. BSB and GBSB were fed with high-protein diet, while CP was fed with fish fry of appropriate size to ensure adequate and suitable feed. The fish in each group were given adequate food, the weight of which accounted for 5% of the total weight of the fish to ensure adequate nutrition. Breeding conditions and Hatching conditions of GBSB is referred to (Wang et al., 2022; Wu et al., 2023).The muscle samples were taken from underside of the dorsal fin of five-month-old GBSB, BSB and CP. Sample was soaked in 4% paraformaldehyde for morphometric analysis and the fresh muscle from the dorsal of GBSB, BSB and CP was placed in liquid nitrogen for next step.

2.3. Histological analysis of muscle fibers

According to standard histological protocols and samples were dehydrated through a sequence of ethanol, the xylene permeates the samples, and embedded in paraffin. Sections of approximately 5 μm thickness were dyed using hematoxylin and eosin stain. and analyzed for fiber diameter under microscope (BX40F4, Olympus, Tokyo, Japan). Assuming muscle fibers are cylindrical, as per the method outlined in (Lee and McPherron, 2001). A diameter threshold of ≥10 μm was established for identifying fibers, as optical resolution below this limit rendered inadequate identification and accuracy in the analysis. The density of muscle fibers was determined by dividing the number of chosen muscle fibers by the occupied area.

2.4. RNA isolation and cDNA library development for transcriptome

RNA was extracted from GBSB and BSB muscle using Trizol (Ambion, USA) and based on the instruction of producer, the first strand of cDNA was generated in the M-MuLV system and the other one was synthesized under the DNA polymerase I system. Using AMPure XP beads to generate the cDNA library. Upon constructing the library, it underwent

preliminary quantification using the Qubit2.0 Fluorometer. The effective of library concentration was accurately measured through quantitative real-time PCR (qPCR). After ensuring the quality of library, pools of different libraries were formed based on their effective concentrations and target amount of offline data. Finally, Illumina sequencing was conducted to generate 150 bp paired end reads.

2.5. Assembly and annotation

Initial processing involved utilizing Perl scripts to manipulate the raw data and the clean reads were then employed for further analysis. Annotation files directly from the genome model, position data into GCA_018812025 *Megalobrama amblycephala* reference genome (https://www.ebi.ac.uk/ena/browser/view/GCA_018812025.1). The *HISAT2* (v2.0.5) software was utilized to build the index and the paired-end clean reads were aligned to the reference genome. By statistically comparing the distribution of reads on the reference sequence, etc. Gene functions were annotated to gain insights into the macro-level distribution of various gene functions. GO enrichment of DEGs was executed using the cluster Profiler (3.8.1) and $p<0.05$ were deemed significant difference. The KEGG offers resources for comprehending the high-level functions. We used cluster Profiler (version 3.8.1) to conduct a statistical analysis of the enrichment of DEGs in KEGG pathways. In addition, growth-related DEGs such as MRFs, myosin genes, GH/IGF and HPG axis

Table 1
Primer sequences used for qPCR.

species	Name	primer sequence (5′-3′)	primer sequence (5′-3′)
BSB and GBSB	mstna	CCACAGAACCGCAAGCCAT	CGATGTGCTGCCAGGAGTT
	mstnb	CCCATCTGGTGAACAAGGC	AGCAGCCACAGCGGTCTAC
	Myf5	TCTGAGGAGGACGAGCAC	CACCTTGGGAAGACGCTGG
	Myf6	GTCCGAGACAGGGTGCGA	TTGGATTGGGCACCGTCT
	MyoD	AACAACCAACGCTGACCGC	ATCTCGGGCTGGAGGCAT
	MyoG	TGTGTGGAGATGGACGGCT	CAGCCTCTGGTTGGGATTCAT
	myomaker	GTTGACTGCGGCTGTAAGGAT	CAAGAGCACCGAAGCAGAAC
	myomixer	GCAGATTAGCAGCCTCAGCC	ACAAGACGCAGCCCAGGAT
	GnRH2	AAGTGCCCAGTTTGCCAGC	GGCATCCAGCAGTATTGTCTTC
	GnRH3	CACTGGTCATACGGTTGGCTT	GCTGCTCCATTGGCGTGT
	FSHR	TCGTGGGAAATGTTGTTGTCTT	CTCCTGTCTGCCAGTCAATGC
	LHR	GTTGATGCTGGTGGCTCGT	AACGATGTCAGGAGCAAGAGG
	FSHb	GCTTCGTTGTTATGGTGATGCT	GCCACATTCCTCACTTTCCAT
	LHb	AGGGCTGTCCAAAATGTCTG	CAAGCGGACTGTCTCGTAGC
	myh6	TTAGGATGGACTTGGAGCGTAG	CTCGTCCTCAACCTTTCCATTC
	myh10	AGACCGCTCCCACAAACTACAG	CTCAAGAGTTCCCACATCATCC
	myh11a	GAGGAGGGTAAGAAGCGGC	GCTGTCCAAATCCATCAGTGTG
	myh11b	ACAGGTCAAGTCCAAGCAACG	TTTACGACGAGCAGCAGTGG
	myl1	CCAGGACCAGATGGAAGATTAC	AGCACCCATTACTGTTCCGTT
	myl4	CACTGTAATGGGTGCCGAACT	GCATCTTCCTGTCCTGCCATC
	myl6	GGATGTGATGCGTGCTCTG	GCAGCCATTAGCATCCTCAT
	myl7	GAAACCATCCTTGCTGCGT	CAGCCACATCTATTGGAGCG
	myl9a	ACGCCTTCACCTGCTTCG	CCGTGTTTGAGGATGCGAGT
	myl9b	GCACCAGGTCCCATCAACTT	CTCTCGCAGGTGGTCTTCG
	GH	TGTCGGTGGTGCTGGTTAGTT	GAGGCGGAAAGAGATGCG
	GHr	GGCGTCCAACAGACATCAG	GAGGTGTAGGCTCCATCGG
	IGF1	ATTGTGGACGAATGCTGCTT	CCTCGGCTTGAGTTCTTCTGAT
	IGF2a	CGAAACCTGTAAAGTCCGAGC	GAATCTGGCTTGCTCCTCATC
	IGF2b	GCTACAGTTTGTGTGCGAAGAC	CGTCCCTCTCTGACTTGGC
	mstna	AGCACAGCAAGCAGATGAGG	CTGAGGCTGAACAGGCAACA
	mstnb	TTGAGTGACCAAGAGACGCA	TGTTGTCATCTCCCAGCACG
	Myf5	CGACAGAGCGTGTGCTTCAT	TAGTTTTCCACCTGCTCCCG
	Myf6	GGATAATGTTCCGTCCGAGA	GCCACGGTCTTCCTCTTCAG
	MyoD	CTACAACGGGGACTCAGACG	CGCTCTTCAGACCGCCGT
	MyoG	ATGGGCTTGTGTGGGAGTCT	GGTTTGGGTTCATCAGGGTG
	myomaker	GTTTACGGCACGGCTCTCTC	CCTCACAGCCGTGGTCAAC
	myomixer	ATGCCAGCCGTTTTCCTCTT	GGCTTCTTTGGACTGCTGTG
	GnRH2	ATGTGCGTATCTCGGCTGGT	TGATGTGCCGAAAGAGTCCA
	GnRH3	ATGGAAGCGAGCAGCAGAGT	TCCCACACTTCTCTTTCCACC
	FSHR	ATCACCAGCAACACCCAATG	GGTCAAACAGAAAGCCGTGG
	LHR	TGAGGTGAATGGGAGCGACA	GGTGCGGCAAATGACATAGAG
	FSHb	CGGCTGTCATCTAACCAACG	GCGGTACACTCGCAGTTTCT
	LHb	ACCCTGTCATCAAGATACCGTT	GTAGGTTACGGTCGGGTCCA
CP	myh6	CAACCAAGTGAAAGCCGACA	GAAGCGACTCAAGCATCCGT
	myh10	TCATTATGCTGGGAGGGTGG	AAGTTGGGCTGGGTGTTGTG
	myh11a	GGACAGCAGTGCCCAACAAT	CCCCGTAGGCAAACTGTGAT
	myh11b	GCGACGGTGAACAAAGACGA	TCCTGTAGGCGTTGTCTGTGAT
	myl1	ACCATCATCAACAGCCCAAA	AGTTGACACAGCCGTTCTCG
	myl4	CGACCCACACAGCATCAAGA	CACCGCACTGAGAGAACGAG
	myl6	GCTCAAGGTCCTGGGCAATC	CTCGCAGTCCCTCCACAAAA
	myl7	ACATAAAACCCACCCCAGCA	CCTGTATCTGGGACTGCTCG
	myl9a	CCAAGGGAAAGACCACCAAG	GCGGGTGAACTCTGCGTAG
	myl9b	ACGAGTAGAGGGAGGAGCCG	ATCCCAAATGAATCCCAAGC
	GH	GCTGTCGGTGGTGTCTCTGG	TTTTGTTGAGTTGACGCTGCT
	GHr	CTCAGCCCTTCACAAAAACC	TGTGGTTGTCCTCAGTGGGTT
	IGF1	CGCTCTCTCCTTTCAGTGGC	CTTTGGAAGCAGCACTCGTC
	IGF2a	TACTGTGCCAAACCCGCTAA	TGCGGGCATCACGGGTA
	IGF2b	GCACTCATTTTGCCACACCT	CCGAGGCTATTTCCACAACG
	β-actin	ACCCACACCGTGCCCATCTA	CGGACAATTTCTCTTTCGGCTG

genes from transcriptome data.

2.6. Quantification and analysis of DEGs

The FPKM (Fragments Per Kilobase of exon model per Million mapped fragments) for each gene was computed using Feature Counts (1.5.0-p3) on account of the length of gene. Differential expression analysis of genes between two groups was conducted using *DESeq2* (1.20.0). Genes with a Fold Change ≥2 and Padj ≤0.05 were deemed significantly differentially expressed by the DESeq test. Furthermore, the Benjamini-Hochberg method was applied to filter genes exhibiting significantly varied expression levels in two groups.

2.7. MRFs and mstn of BSB and CP sequence and correlation analysis

The genome data were obtained from the CP reference genome (https://www.ebi.ac.uk/ena/browser/view/GCA_011952085.1). The MEME suit software was employed to detect conserved motifs within the identified BSB and CP MRFs, as well as mstn proteins (Bailey et al., 2015). A phylogenetic tree for MRFs and *mstn* was generated using the maximum likelihood method, with iqtree (2.2.2.7) employed for conducting ML phylogenetic tree analysis. (Lanfear et al., 2020). These contents mentioned above were visualized using the TBtools.

2.8. Quantitative analysis of related genes

Six muscle samples were selected in each group, RNA was obtainted from muscle using Trizol (Sangon), followed by the synthesis of first-strand cDNA with the help of PrimeScript RT Kit (Monad). Genes for growth and development as follows. The qPCR was performed for myosin-related genes, MRFs, HPG axis, and GH/IGF axis. We used *Primer Premier 5* to design primers and amplified the mRNA sequences of these genes (Table 1), which were synthesized by Sangon Biotech Co., LTD. *β-actin* as the reference gene. The qPCR assay was executed with Six sample duplicates and the technical replicate repeated three times for each sample. The qPCR steps are predenaturation at 95 °C for 30 s, denaturation at 95 °C for 10 s, anneal at 60°Cfor 30 s, generally 40 cycles, dissolution curve using the instrument default program. The relative mRNA expression was computed using $2^{-\Delta\Delta Ct}$ (Bustin et al., 2009).

2.9. Statistical analysis

The SPSS software (20.0, USA) was used to process the related data. The related data were represented by the mean ± standard deviation (SD), and we used analysis of variance (ANOVA) to determine whether there are significant differences between different samples. **P*-value <0.05 was statistically significant and ***P*-value <0.01.

3. Results

3.1. The body weight, visceral mass weight and comparison of countable characters

To reveal growth differences and appearance difference between GBSB and parents, the body weight and visceral mass weight of BSB, GBSB and CP at five-month-old and one-year-old were compared and their morphological characteristics were measured. As shown in Table 2, the average body weight of GBSB was higher than BSB ($p < 0.05$), while the weight of visceral mass was no significant difference from that of maternal fish. It indicated that GBSB is heavier than BSB when the visceral mass is the same weight, so the meat content is higher, and the growth of muscle is faster. As shown in Table 3, the countable characters of GBSB and BSB are basically the same, basically no difference.

Table 2
Weight of BSB, GBSB and CP and weight of visceral mass (Unit: gram).

	Starting weight	5-month-old	Visceral mass weight	One-year-old	Visceral mass weight
BSB	0.005	36.95 ± 5.17a	4.93 ± 0.40a	276.60 ± 22.78a	48.45 ± 14.49a
GBSB	0.005	55.10 ± 4.76b	3.80 ± 1.11a	382.50 ± 17.08b	50.51 ± 18.26a
CP	0.005	651.02 ± 38.23c	68.22 ± 5.21b	1053.21 ± 81.33c	142 ± 21.08b

The data is presented as the mean ± SD. Values with distinct superscripts (a, b, c) indicate significant differences ($p < 0.05$, $n = 3$).

Table 3
Comparison of countable characters of GBSB and BSB (unit: slice or strip).

	Lateral line scale	scale above lateral line	scale below lateral line	Dorsal fin strip	Pelvic fin strip	Anal fin strip
BSB	55–60	13–15	7–8	III + 8–9	9–10	III + 24–25
GBSB	52–54	11–13	7–8	III + 8–9	8–9	III + 26–28

Capitalized Roman numerals represent the number of hard spines, and Arabic numerals represent the number of fins.

3.2. The muscle fiber morphology, diameter, and density

The growth of muscles is related to the size and quantity of muscle fibers. In order to reveal the differences of muscle fibers between GBSB and parental fish, the muscle size and fibers number were compared among the three groups. The results of HE staining were shown in Fig. 1 and the muscle fiber diameter and density were listed in Table 4. The muscle fiber diameter of GBSB was slightly higher than BSB and lower than CP ($p < 0.05$). But the muscle fiber density of GBSB was slightly lower than BSB and higher than CP. Obviously, the growth of muscle fibers in the progeny of gynogenesis was better than BSB.

3.3. Sequencing and annotation of unigenes and DEGs enrichment analysis

In this study, the raw reads were deposited in the National Center of Biotechnology Information (NCBI) Sequence Read Archive (SRA) with access number PRJNA893089. To further reveal the skeletal muscle growth differences between juvenile GBSB and BSB, we analyzed the transcriptome data of both. In the GO analysis, 17,132 unigenes with GO annotations were assigned to 45 functional phrases. The primary terms included binding, catalytic activity, and cellular process (Fig. 2). We mapped 18,739 unigenes to reference KEGG pathways to determine growth-related biological pathways. These comprised 139 main pathways. We identified 1353 significant DEGs in the muscle of the GBSB versus BSB groups, consisting of 820 upregulated and 533 downregulated genes (Fig. 3). In the 30 significant terms composed of GO enriched differential genes, it emerged that myosin complexes play a vital part in cell composition (Fig. 4A). The mapping between GO ID and GO term is shown in Supplementary Table 1. Then DEGs were enriched by KEGG, and 30 pathways with the most significant differences were selected for analysis, among which cardiac muscle contraction was significantly different (Fig. 4B). The mapping between KEGG ID and Description is displayed in supplementary Table 2. Moreover, the expression of myosin II in the Tight junction pathway was significantly increased in GBSB (Fig. 5). Based on the transcriptome data, the expression of growth-related genes displayed in Fig. 6. As a result, we found that except for *mstnb*, *myh10*, and *myl4*, the expression of growth-related genes in GBSB noticeably amplified as compared to BSB.

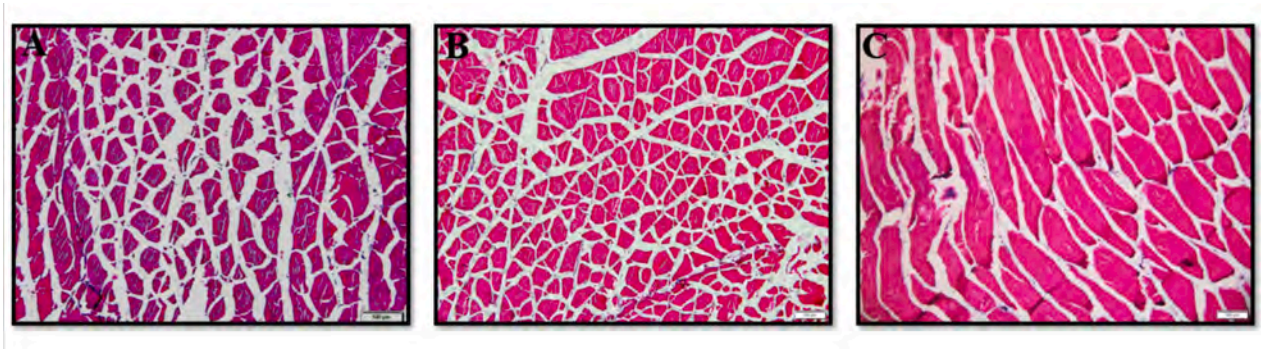

Fig. 1. The photomicrographs of muscle fiber tissue in BSB, GBSB and CP, respectively. Note: A: BSB; B: GBSB; C: CP.

Table 4
Muscle fiber diameter and density in GBSB、BSB and CP for one year.

	Fiber Diameter (μm)	Fiber Density (N/mm^2)
BSB	33.56 ± 0.23c	162.39 ± 2.61a
GBSB	38.00 ± 0.08b	138.19 ± 2.26b
CP	59.61 ± 0.58a	101.00 ± 2.57c

The data is presented as the mean ± SD. Values with distinct superscripts (a, b, c) indicate significant differences ($p < 0.05$, n = 3).

3.4. Phylogenetic tree, motifs, and gene structure of MRFs and mstn

In order to further search for the reasons for GBSB's growth advantages, we compared the MRFs, *mstn* and *myomaker* conserved motifs and gene structure of CP and BSB (Fig. 7). The evolutionary analysis of BSB and CP revealed that MRFs and *mstn* of the same kinds were inclined to group together in one embranchment, suggesting a close phylogenetic connection between them. According to the result of the MEME analysis, conserved patterns were detected in the MRFs and *mstn* sequences of both BSB and CP, most motifs were in the central domain of the gene, indicating that the MRFs and *mstn* central domain was highly conserved. In addition, the number of conservative motifs in *mstn* of BSB is higher than that of CP, but the number of conservative motifs in MRFs of CP is generally higher than that of BSB, especially *myomaker*. The above analysis suggested that the faster growth of the CP than the BSB may be related to the structure of these genes.

3.5. qPCR validation

In order to verify transcriptome data and determine the relative expression of growth genes, we measured the GH/IGF axis, HPG axis, MRFs, and myosin-related genes mRNA level. As is shown in Fig. 8, the expression of MRFs in GBSB was observably higher than that in BSB, but it was lower to that in CP. And the expression of *mstnb* in BSB was higher than that in GBSB and CP. Except for myosin light chain 1 (*myl1*), almost all myosin light chains (*myls*) and heavy chains (*myhs*) were expressed higher in GBSB than in BSB. Even some genes mRNA level was close to CP, such as *myl6/9b*. For the GH/IGF-axis, almost expression of all genes in GBSB was higher than that in BSB, even closer to that in CP (except GH). For the HPG axis, the expression of *GnRH2*, *FSHb* and *LHb* was higher in GBSB compared with BSB. The qPCR outcomes for these genes align largely with the transcriptome data, indicating that GBSB had higher expression levels of GH/IGF-axis, HPG-axis, MRFs, and myosin-related genes and lower expression levels of *mstn* compared with maternal fish.

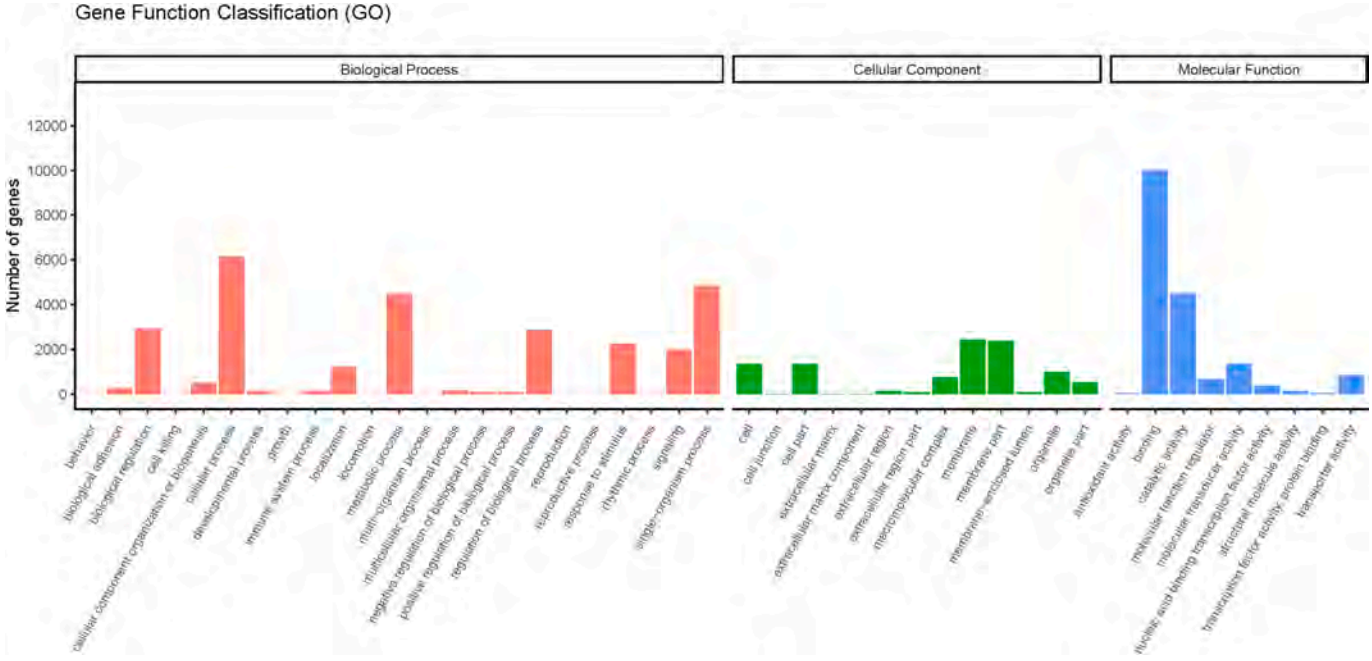

Fig. 2. Gene ontology (GO) classification of differentially expressed unigenes between GBSB and BSB. Unigenes were annotated in three categories: biological process, molecular function, and cellular component.

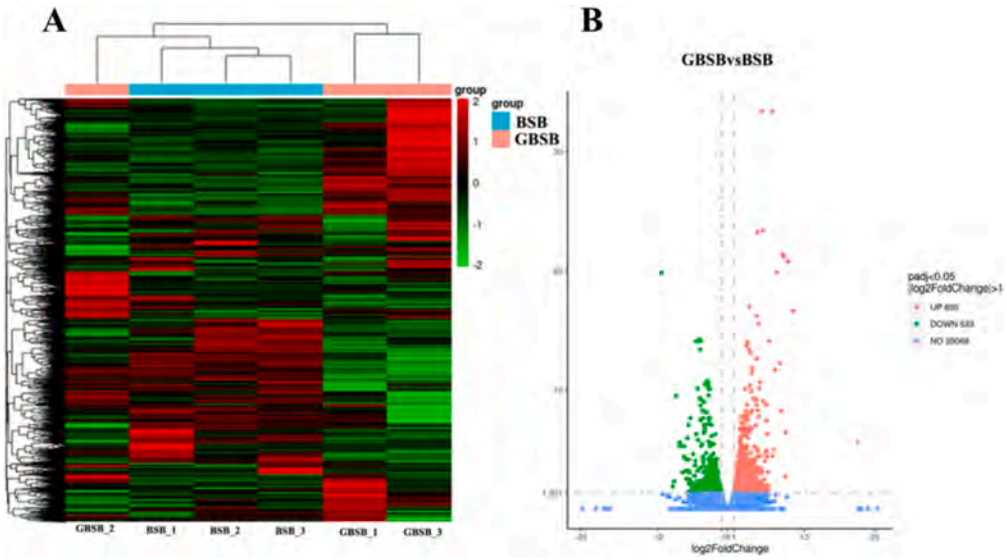

Fig. 3. (A) Cluster analysis of DEGs in muscle transcriptomes of GBSB and BSB groups. The red and green reveal the up-and down-regulated DEGs; (B) Gene expression profiles in the muscle. The red and green represent the up-and down-regulated DEGs, blue means no differential genes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

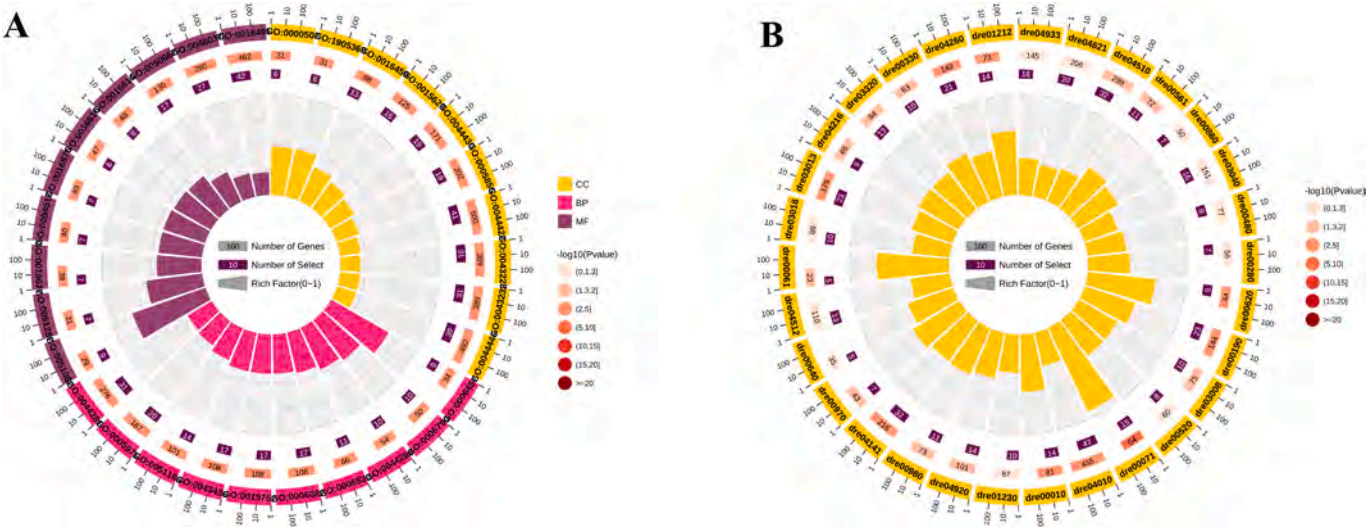

Fig. 4. The GO enrichment and KEGG pathways of DEGs. (A) The GO enrichment of DEGs focuses on 30 significant terms in GBSB relative to BS, biological process (BP), cell component (CC) and molecular function (MF) were 10 terms each. Each GO ID on the circle represents a GO term. (B) The KEGG pathways of DEGs focuses on 30 significant terms in GBSB relative to BSB. Each KEGG ID on the circle represents a KEGG pathway.

4. Discussion

In order to reveal the differences in growth between natural gynogenic progenies and maternal fish. We recorded the growth of BSB and GBSB and determined their number of traits and measured GBSB, BSB and CP of muscle fiber characteristics. The evolutionary relationships, conserved motifs, and gene structure of MRFs and *mstna/b* in GBSB and CP were analyzed. In addition, we also took the muscle tissue of GBSB and BSB for transcriptome sequencing and conducted quantitative detection of genes related to growth. The data indicated that the GBSB grow rapidly compared with BSB. The muscle fiber feature of GBSB were between BSB and CP. Analysis of MRFs and *mstna/b* mRNA levels revealed large differences in these genes between GBSB and CP. Transcriptome data showed that GBSB and BSB differ in the expression of some muscle growth genes that control growth. Meanwhile, conserved motifs and gene structure analysis of MRFs, *mstn* and *myomaker* revealed differences between the CP and BSB. We speculate that the expression in MRFs, GH/IGF-axis, myosin genes and HPG-axis of GBSB, which in turn caused GBSB to grow faster than BSB, and it possible that the heterospermic effect of the male parent may be involved.

Previous studies have shown that heterologous sperm may influence to heterotic progeny in the process of distant hybridization (Mao et al., 2020). Our study in GBSB and BSB discovered that the growth pace of GBSB surpassed BSB, with GBSB exhibiting superior muscle mass and fiber diameter compared to BSB. In general, the higher muscle fiber diameter resulted in the lower density(Tang et al., 2021). Previous studies have shown that muscle fiber diameter increases with body weight during growth phase Atlantic salmon and the growth of fish body is directly proportional to the cross section of muscle fiber and inversely proportional to the density of muscle fiber(Johnston et al., 2000). Although the environment may affect muscle growth(Johnston et al., 2003), the growth environment of GBSB, BSB and CP is basically the same. Thus, it may indicate that the GBSB has obvious growth advantage over the maternal fish. This phenomenon is also reflected in the

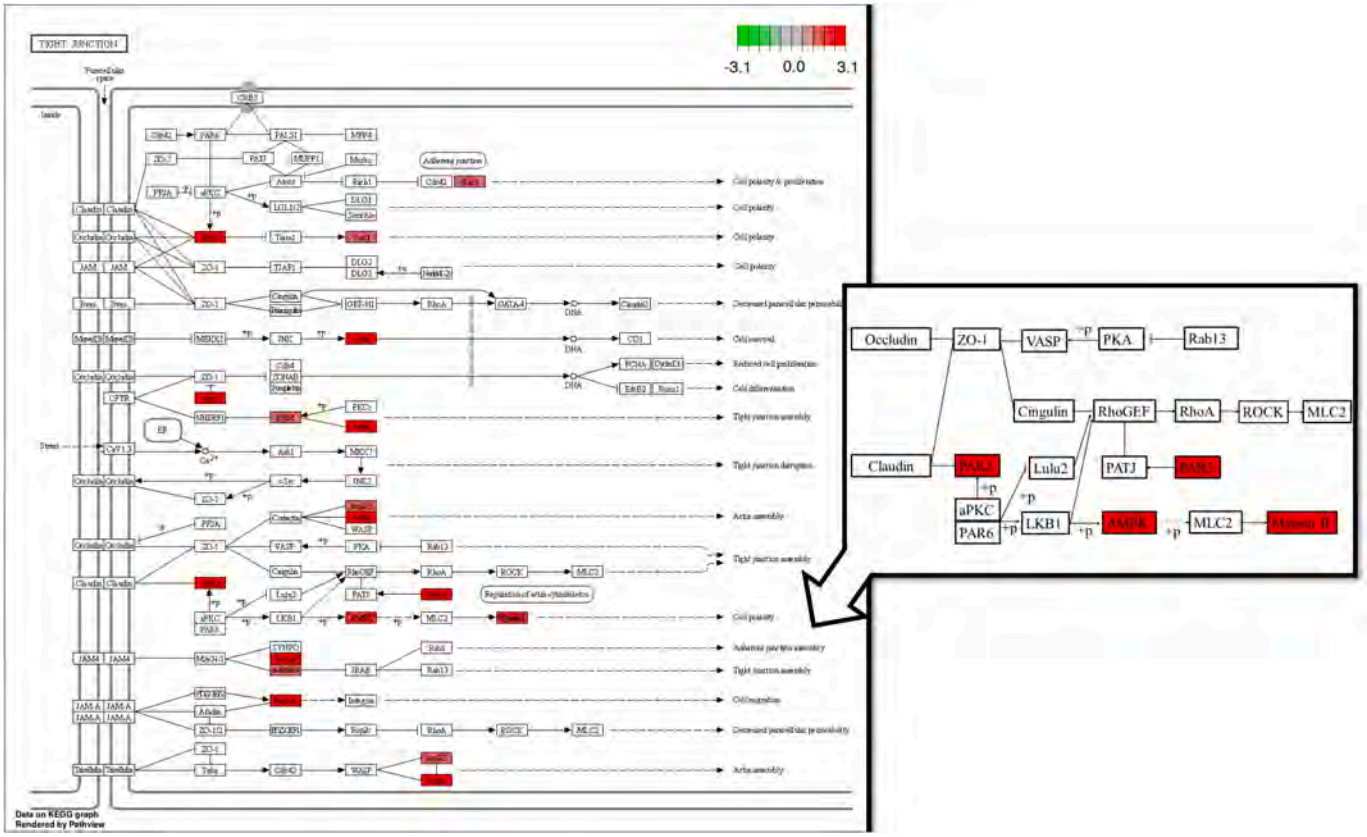

Fig. 5. The Tight junction signaling pathway, red represents the up-regulation of GBSB relative to BSB related genes. The image on the right is a local magnification of the left pathway. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

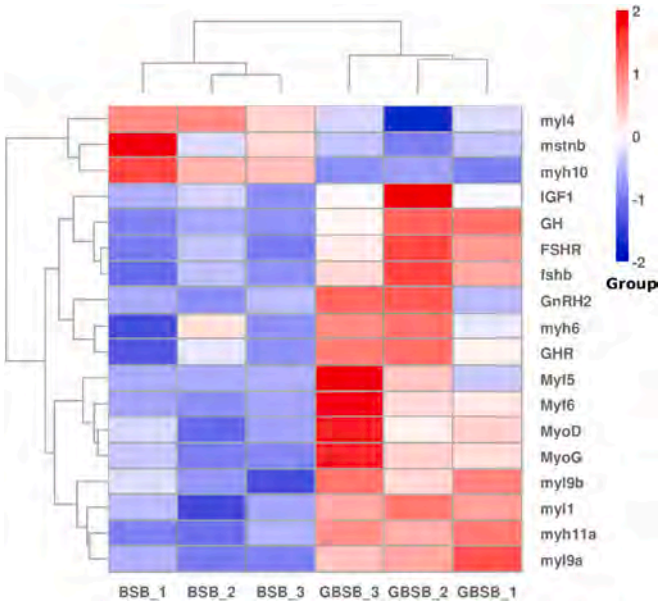

Fig. 6. Cluster analysis of Growth-related DEGs of GBSB vs. BSB groups. The red and blue reveal the up-and down-regulated DEGs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

allotetraploid gibel carp(Li et al., 2016). We speculate that the biological impact of CP-induced heterologous sperm allows the growth rate of natural GBSB to outpace that of BSB.

Studies have shown that the expression of fish myosin heavy chain gene is related to the potential physiological function, the expression of specific *myhs* isoforms determines the contractile properties of muscle fibers and may impact fish performance, including rainbow trout and torafugu(Alami-Durante et al., 2010; Asaduzzaman et al., 2013). In our study, we noted that most GBSB myosin light chain (*myl*) and heavy chain (*myh*) gene expression were generally higher than BSB. The result is consistent with what we found in *European Sea Bass Larvae* (Alami-Durante et al., 2011), which may be due to distant hybridization disrupting the original gene expression (Ostberg et al., 2015). *Myhs* plays a pivotal role in the structure and function of muscle development and early growth in CP, serving as the primary genetic contributor(Chen et al., 2023). While, *Myls* primarily participates in the control of energy metabolism, with significant expression in muscle tissues (Wu et al., 2021). In summary, the *myls* and *myhs* are integral components of the myosin protein complex, essential for muscle contraction and growth in fish. Their expression levels and interactions play key roles in regulating muscle function, muscle growth, and overall growth performance in fish. Thus, we speculate that the faster growth of juvenile GBSB may be related to adjust *myls* and *myhs*.

Muscle growth depends on the regulation of key genes such as MRFs and *mstn* (Zanou and Gailly, 2013). We noted that the gene structure and conserved motifs of MRFs and *mstn* of the BSB and CP are quite different. The number of conserved motifs in most MRFs of CP is higher than that of BSB. In particular, the *myomaker* of BSB have a limited quantity of conserved motifs and are extremely susceptible to influences. In addition, the conserved motifs in the *mstn* of BSB are evenly distributed at one end, while the conserved motifs in the *mstn* of CP are scattered at both ends and we found that the UTR and CDs length in the *mstn* of BSB were higher than those of CP. In general, we speculate that the difference in the number and location of parental motif and gene structure is very likely to affect the gene expression of the progeny, because motif is significant for protein function (Barik, 2022). However, there are few

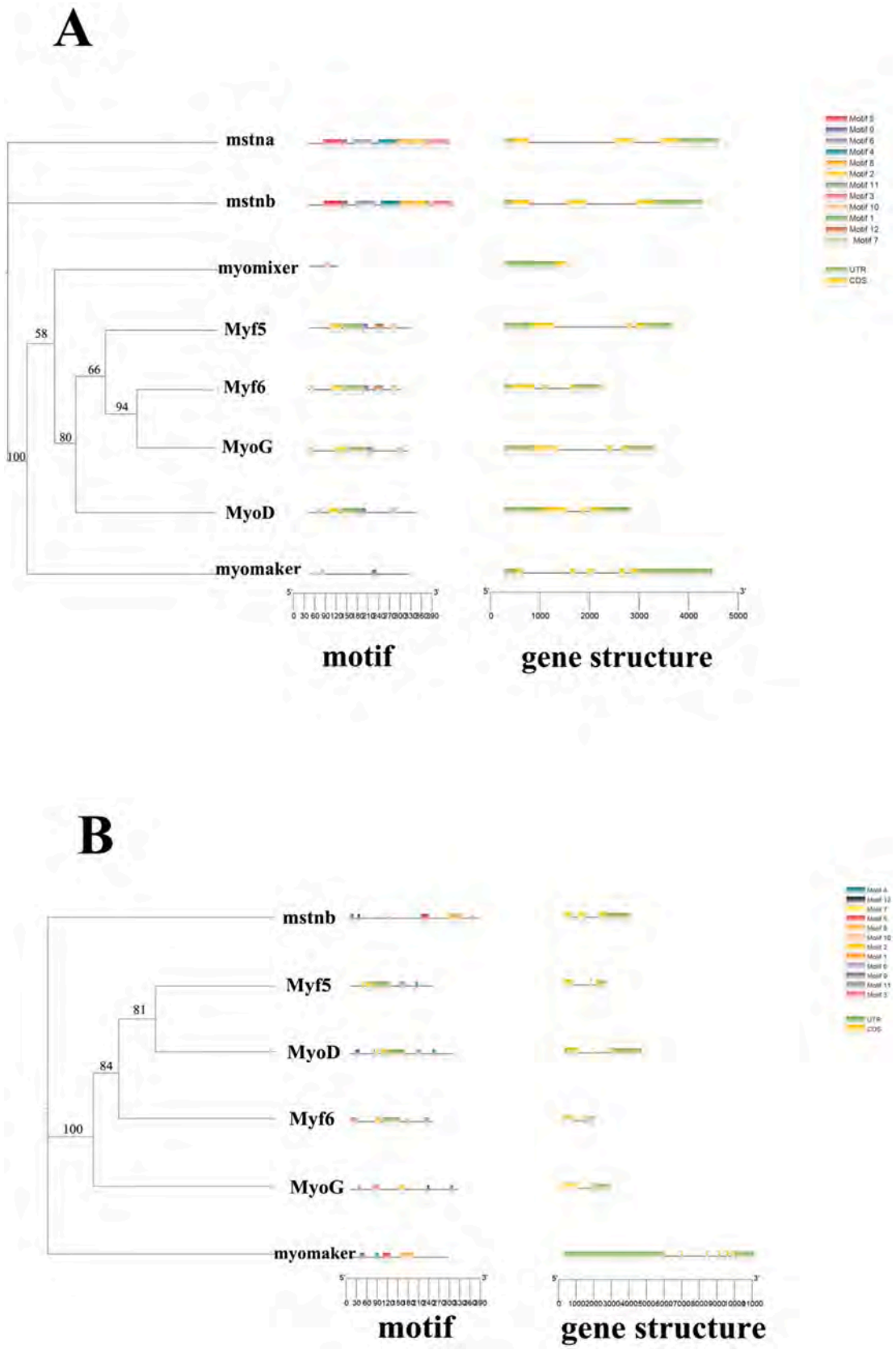

Fig. 7. Phylogenetic relationship, motifs, and gene structure analysis of MRFs and *mstn* in BSB(A) and CP(B). Colored boxes indicate different motifs and gene structure.

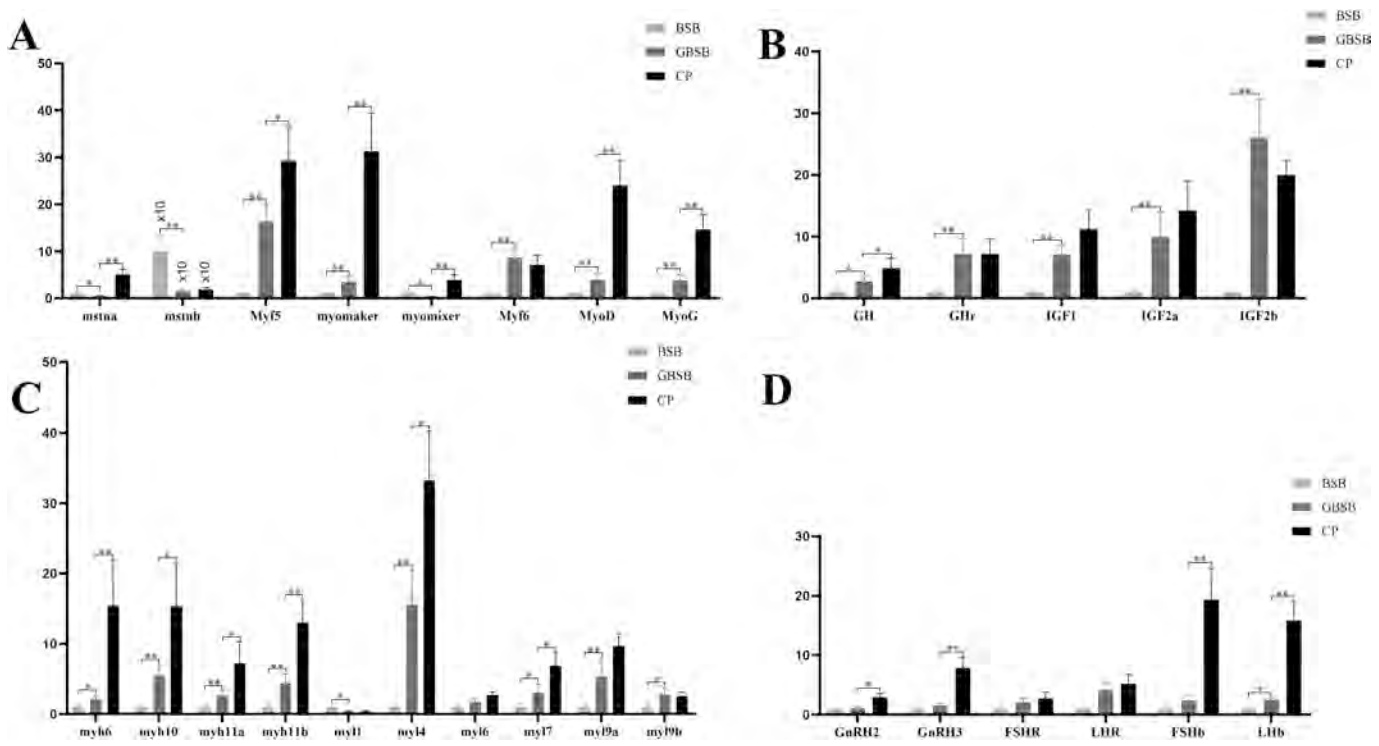

Fig. 8. (A) Differential expression of MRFs in BSB, GBSB and CP; (B) Differential expression of GH/IGF-axis genes in BSB, GBSB and CP; (C) The expression of myosin light chain and heavy chain in BSB, GBSB and CP; (D) Differential expression of HPG-axis genes in BSB, GBSB and CP. The values are expressed as the mean ± standard deviation ($n = 6$) of the normalized transcript levels of each gene. Statistical difference between time points is indicated by different letter notations ($p < 0.05$). **p*-value <0.05, ***p*-value <0.01. The numbers on the graph represent the amplification of the relative expression of the gene directly below.

reports on the above results, the relevant mechanism needs to be further explored. In addition, we investigated the expression of muscle growth genes in both progeny and parents. The mRNA levels of *Myf5*, *MyoD*, *MyoG*, and *myomaker* in GBSB fell between those of BSB and CP. *Michelato* et al. noted that increased *MyoD* and *MyoG* mRNA levels led to enhanced muscle development in Nile tilapia juveniles *Oreochromis niloticus*.(Michelato et al., 2017). Meanwhile, the high expression pattern of *MyoG* enhances fish growth in hybrid striped bass(Childress et al., 2016). Obviously, the high expression of *MyoD* and *MyoG* in the muscle of progenies of gynogenesis has a similar promoting effect on muscle growth. In addition, previous research has demonstrated that *mstn* increased expression during rainbow trout development, peaking at 140 g (Garikipati et al., 2006; Johansen and Overturf, 2005). Lower expression of *mstnb* in GBSB than BSB indicated that GBSB muscle grow faster. There is a difference in the weight of GBSB and BSB in the same period of our selection, we speculate it may be one of the reasons for the difference in *mstn* expression between the two. All in all, our data may suggest that the low expression of GBSB relative to BSB of *mstnb* is one of the reasons for accelerating its rapid muscle development.

The development of fish skeletal muscle is adjusted by the GH/IGFs-axis. Such as, the IGF2 can mediate up-regulation of *MyoD* expression (Vélez et al., 2017). Previous reports showed that the high expression of GH/IGF-axis genes in hybrid catfish and hybrid striped bass(Won et al., 2016; Zhao et al., 2019). Similarly, we detected the expression of GH/IGFs-axis in GBSB were significantly higher than BSB. Furthermore, previous research has shown that the HPG axis plays a vital part in teleost development and reproduction(Blanco, 2020) and other studies have suggested the HPG axis and the GH/IGF axis have an interaction (Tenuta et al., 2021). In this research, the expression of some HPG axis genes in GBSB were higher than BSB and much lower than CP. The occurrence of this situation could be because the expression of HPG axis genes in GBSB also influences hybrid muscle growth. However, the exact reason behind this needs to be further explored. In summary, the high expression of GH/IGF and HPG-axis gene is related to the higher muscle growth rate of progenies in natural gynogenesis compared to the maternal parent, but the regulatory mechanism needs further research.

In conclusion, the growth rate of GBSB obtained by distant hybridization was better than that of BSB. This might be attributed to the varying expression of genes related to muscle development between GBSB and BSB (Fig. 9). These outcomes of the transcriptome and qPCR will augment our comprehension of the molecular mechanisms underlying differential growth in distant hybrid fish. In addition, GBSB breaks the trans order species reproductive boundary and integrates many superior traits from parents. It has important biological significance in biological evolution and fish breeding. In the future, our research will focus on growth-related genes and trans order species hybridization. Obviously, trans order species hybridization can help us understand the relationships between different fish species and the mechanisms of genetic exchange and variation and can be used to introduce new genetic variants and genome combinations, which can improve the productivity and resilience of economically important fish species. We speculate that by hybridizing different species, we can create progeny with desirable traits, such as faster growth rates, improved disease resistance, and better meat quality. Nevertheless, how does the heterospermic effect affect growth related gene expression of the gynogenetic progeny still need to be further explored.

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CRediT authorship contribution statement

Ping Wu: Supervision, Methodology, Funding acquisition. **Wangwang Ji:** Writing – original draft, Validation, Investigation. **Ya Zeng:** Formal analysis. **Jie Tang:** Data curation. **Chang Wu:** Methodology. **Qinbo Qin:** Methodology. **Ting Yi:** Writing – review & editing. **Yi Zhou:** Visualization. **Rurong Zhao:** Visualization. **Min Tao:** Visualization. **Chun Zhang:** Investigation. **Chenchen Tang:** Investigation. **Kaikun Luo:** Resources, Investigation. **Yuequn Wang:** Writing – review &

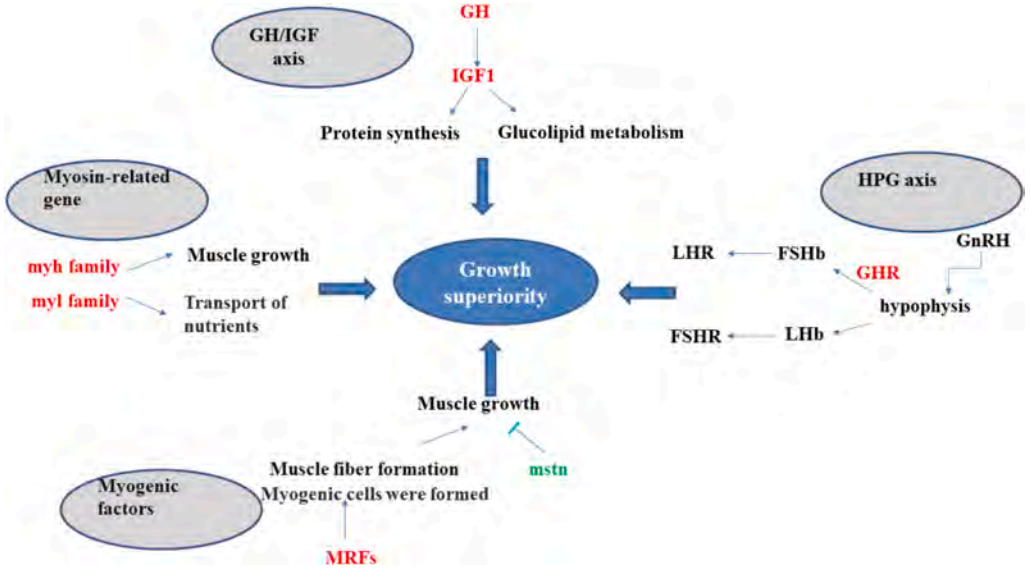

Fig. 9. The predicted map of DEGs regulated growth superiority. Red and green represent the up- and down-regulation of DEGs, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

editing. **Shaojun Liu:** Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

All authors confirm that they do not possess any known financial conflicts of interest or personal connections that might have seemed to sway the research presented in the manuscript.

Data availability

The data that has been used is confidential.

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