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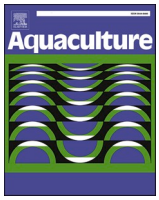

The formation and growth characteristics of an all-female hybrid crucian carp

Lujiao Duan, ChaoLiang Shen, Jingjing Lin, Xiaoxia Xiong, Huan Sun, Ziyi Huang, Xuanyi Zhang, Fanglei Liu, Jianming Yu, Qingfeng Liu *, Shaojun Liu *

State Key Laboratory of Developmental Biology of Freshwater Fish, Engineering Research Center of Polyploid Fish Reproduction and Breeding of the State Education Ministry, College of Life Sciences, Hunan Normal University, Changsha 410081, Hunan, People's Republic of China

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ABSTRACT

Germplasm innovation plays a critical role in fish genetic breeding. In this study, we obtained an all-female hybrid fish (AFWR) by integrating the technique of gynogenesis, distant hybridization and sex reversal. We conducted analyses of AFWR in terms of its morphological traits, chromosomal numbers, DNA content, gonadal development, muscle nutrition, and growth characteristics. The results showed that the morphological characteristics of AFWR were not significantly different from those of its original parents (WCC and RCC); AFWR were diploid with 100 chromosomes; the ovarian of AFWR was developing normally and produced mature egg during the breeding season; the muscle of AFWR had higher protein content and lower moisture compared to WCC and RCC, and the delicious amino acids content of AFWR was significantly higher than that of WCC. Furthermore, AFWR exhibited rapid growth and showed muscle hypertrophy and hyperplasia than WCC and RCC. Analyses of muscle transcriptomes showed that 8698 and 4619 differentially expressed genes (DEGs) were identified between AFWR and RCC, AFWR and WCC, respectively. Among the DEGs, growth-related genes were mainly enriched in insulin signaling pathway, TGF-beta signaling pathway, mTOR signaling pathway. Finally, twelve candidate genes for fast growth of AFWR were selected and identified by RT-qPCR. This research provided new scheme for the creation of new fish germplasm and will beneficial for the study of fish growth characteristics.

1. Introduction

High-quality germplasm is a driving force for fisheries development, breeding technology is the key to germplasm creation (Chen et al., 2023). Various fish breeding techniques have been utilized in the creation of fish germplasm, such as hybridization, gynogenesis, sex-controlled breeding (Liu et al., 2021b; Wang et al., 2019). Distant hybridization can result in phenotypic and genotypic changes in offspring by integrating the genomes of both parents. (Liu et al., 2022). Many hybrid fishes exhibiting heterosis have been widely utilized in aquaculture (Liu et al., 2018; Liu et al., 2020). Gynogenesis includes artificial gynogenesis and natural gynogenesis, which has been widely used in the improvement of economic and genetic characteristics of fishes (Arai, 2001a, 2001b). In recent years, gynogenetics has been applied in crucian carp (*Carassius auratus*) (Luo et al., 2011), common carp (*Cyprinus carpio*) (DELSHAD et al., 2005), grass carp (*Ctenopharyngodon idella*) (Wang et al., 2022), blunt snout bream (*Megalobrama amblycephala*) (Gong et al., 2019), mrigal carp (*Cirrhinus mrigala*) (Li et al., 2023), mandarin fish (*Siniperca chuatsi*) (Zhong et al., 2024), largemouth bass (*Micropterus salmoides*) (Wu et al., 2023), silver carp (*Hypophthalmichthys molitrix*) (Zou GuiWei et al., 2004), Lanzhou catfsh (*Silurus lanzhouensis*) (Li et al., 2022), etc. Feeding 17-α-methyltestosterone (MT) is an important way to achieve sex reversal in fish (Sun et al., 2006). In 1968, researchers used MT to achieve sex reversal in goldfish (Yamamoto and Kajishima, 1968). In XY sex-determined fish (such as crucian carp), all-female offspring can be obtained by artificial gynogenesis (Wen et al., 2020). However, due to the damage of eggs (cold or heat shocks) during the artificial gynogenesis, the mortality of offspring is high, which limits the practicality of artificial gynogenesis in large-scale fish breeding (Luo et al., 2011). Therefore, mating the female fish from the artificial gynogenesis with sex reversed male fish (genetically female fish) from the sex reversal is an effective method to create all-female fish on a large-scale.

Growth is an important economic trait of aquaculture. The faster

* Corresponding authors.
E-mail addresses: Liuqingfeng@hunnu.edu.cn (Q. Liu), lsj@hunnu.edu.cn (S. Liu).

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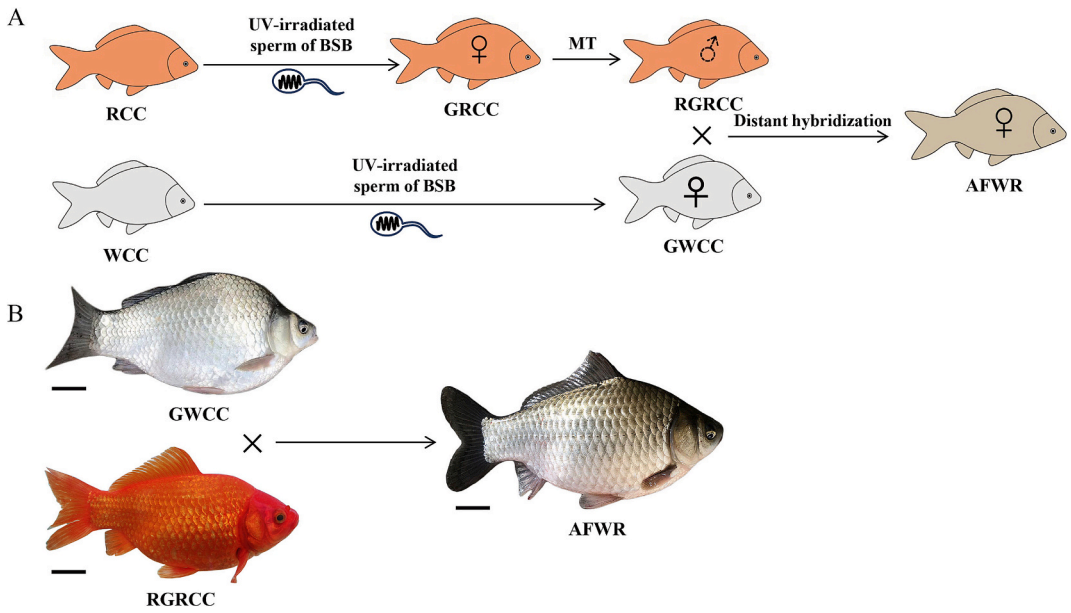

Fig. 1. Breeding strategy of AFWR. (A) the AFWR was obtained by integrating the technique of gynogenesis, distant hybridization and sex reversal, (B) the appearance of GWCC, RGRCC and AFWR. RCC: red crucian carp, GRCC: gynogenetic red crucian carp, RGRCC: sex reversal GRCC, WCC: white crucian carp, GWCC: gynogenetic white crucian carp, AFWR: all-female hybrid fish, BSB: blunt snout bream, MT: 17-α-methyltestosterone. Bar = 5 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Countable traits and the ratios of measurable traits of AFWR, WCC and RCC[a].

Fish type	Countable traits						Measurable traits				
	LS	ULS	LLS	DF	ABF	ANF	WL / BL	BL / BH	BL / HL	HL / HH	CPL / CPH
AFWR	29–31	6–7	6–7	III + 19–20	9–10	III + 6–7	1.22 ± 0.01	2.18 ± 0.03	3.71 ± 0.15	1.20 ± 0.05	0.82 ± 0.01
WCC	32–34	6–8	5–7	III + 18–20	8–10	III + 6–7	1.24 ± 0.05	2.21 ± 0.13	3.71 ± 0.26	1.19 ± 0.04	0.85 ± 0.03
RCC	28–30	5–6	6–7	III + 18–20	8–9	III + 6–7	1.23 ± 0.03	2.20 ± 0.14	3.72 ± 0.20	1.18 ± 0.06	0.80 ± 0.02

Note: a Mean value ± SD. The abbreviations in the table respectively represent: LS, lateral scales. ULS, upper lateral scales. LLS, lower lateral scales. DF, dorsal fins. ABF, abdominal fins. ANF, anal fins. WL / BL, whole length / body length. BL / BH, body length / body height. BL / HL, body length / head length. HL / HH, head length / head height. CPL / CPH, caudal peduncle length / caudal peduncle height.

growth of fish means that it can shorten its farming time, save farming costs, and ultimately increase farming production and economic benefits. In fish, muscle weight is the primary determinant of body weight. Therefore, the characteristics of fast growth can be analyzed by detecting the development of muscle fibers and the protein and fat content of muscle (Liu et al., 2024). Many studies have indicated that fish growth is mainly affected by environment, genes, and the interaction between genes and environment (Lugert et al., 2016). Environment is the external factor and gene is the internal factor of growth trait regulation (Yin et al., 2020). Growth is a quantitative trait controlled by multiple genes, it is essential to identify major genes and utilize them in breeding programs for enhancing growth traits. High-throughput sequencing technology has shown strong advantages in the screening of growth-related candidate genes (Ren et al., 2016). Many key genes related to growth had been identified by high-throughput sequencing combined with gene editing techniques (Liu et al., 2019b; Liu et al., 2024; Moran et al., 2024).

In previous studies, we obtained the hybrid fish (WR) by crossing white crucian carp (*Carassius cuvieri*, WCC, ♀) with red crucian carp (*Carassius auratus* red var., RCC, ♂), WCC and RCC classified into different species in the genus of *Carassius*; and an improved fish (WR-II) was obtained by back-crossing of WR and WCC (Liu et al., 2019a). WR and WR-II have been widely used in China, because of their heterosis traits. Since female WR was needed in the process of creating WR-II, we want to obtain an all-female WR to improve production efficiency. In this study, we obtained the all-female WR (AFWR) by integrating the technique of gynogenesis, distant hybridization and sex reversal. Through the analysis of many characters of AFWR, it is confirmed that AFWR is a female diploid fish (2n = 100) with rapid growth and rich nutrition. Candidate genes for rapid growth in AFWR were identified through comparative analysis of the muscle transcriptome. This study showed a strategy for the creation of new fish germplasm and will beneficial for the study of fish growth characteristics.

2. Materials and methods

2.1. Generation of AFWR

WCC and RCC were collected from Hunan Normal University. The mature ovum of WCC and RCC were stimulated by blunt snout bream sperm inactivated through UV, the fertilized eggs were doubled with cold shock, and the GWCC (gynogenetic white crucian carp) and GRCC (gynogenetic red crucian carp) were obtained. The RGRCC (sex reversal GRCC) was obtained by feeding MT (100 μg/g, 60 days) to GRCC. The AFWR were obtained from the hybridization of GWCC and RGRCC.

2.2. Morphological traits, body weight and muscle fiber analyses

Under the same conditions, 300 fish from each species (AFWR, WCC and RCC) were cultured separately in a 150 m^2 pond for 1 year. 30 fish were randomly selected from each group to measure morphological traits and body weight. The measurable traits and the countable traits

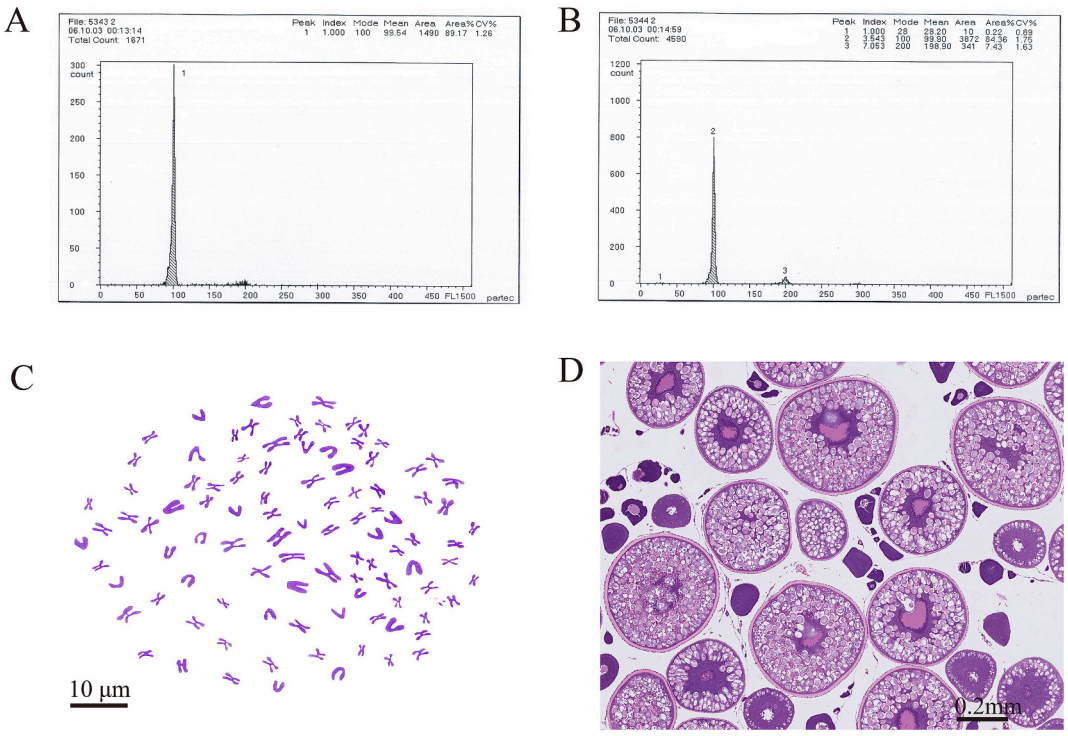

Fig. 2. Ploidy and fertility of AFWR. (A) the DNA content of RCC (99.54), (B) the DNA content of AFWR (99.90), (C) the metaphase chromosome phase of AFWR, bar = 10 μm, (D) the histology of ovaries of AFWR (stages II or III), Bar = 0.2 mm.

Table 2
Muscle proximate composition of AFWR, WCC and RCC.

Fish type	Moisture (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Ash (g/100 g)	Carbohydrates (g/100 g)	Energy value (KJ/100 g)
AFWR	72.30^{bc}	3.90	17.70^{b}	1.80^{c}	79.30	1669.97
WCC	75.90^{a}	3.65	15.90^{ac}	1.60^{c}	78.85	1724.59
RCC	76.70^{a}	3.70	16.70^{b}	2.70^{ab}	76.90	1707.22

Notes: 1. The data in the table are mean values. 2. $^{a, b}$ and ^{c} represent significant differences from AFWR, WCC and RCC, respectively ($P < 0.05$).

Table 3
Muscle amino acid content of AFWR, WCC and RCC. (g/100 g).

Amino acid	AFWR	WCC	RCC
Asp	1.74	1.49	1.73
His	0.52	0.53	0.71
Glu	2.48	2.15	2.42
Val	0.83	0.65	0.78
Ser	0.74	0.66	0.73
Gly	0.85	0.83	0.87
Met	0.39	0.42	0.50
Thr	0.68	0.65	0.76
Arg	0.93	0.89	1.01
Tyr	0.50	0.49	0.57
Ala	1.06	0.86	1.02
Phe	0.73	0.63	0.72
Ile	0.77	0.61	0.70
Leu	1.32	1.12	1.32
Lys	1.64	1.15	1.68
Σ DAA	6.13^{b}	5.33^{ac}	6.04^{b}
Σ EAA	6.36^{b}	5.23^{ac}	6.46^{b}
Σ TAA	15.18^{b}	13.13^{ac}	15.52^{b}

Notes: 1. Data is average. 2. Σ DAA: Total delicious amino acids; 3. Σ EAA: Total essential amino acids; 4. Σ TAA: Total amino acids. 5. $^{a, b}$ and ^{c} represent significant differences from AFWR, WCC and RCC, respectively ($P < 0.05$).

can refer to previous study (Liu et al., 2019a). Furthermore, 5 fish of each species were selected for paraffin section of muscle. The method of muscle fiber analysis can be referred to the previous report (Zhong et al., 2016).

2.3. Ploidy level and gonadal structure analysis

The ploidy level of AFWR was identified by the chromosome number and the DNA content. DNA content was detected by the flow cytometer (Partec) (10 samples of each fish). The number of chromosomes in AFWR was confirmed using a chromosome count test of kidney tissue (10 fish). The gonadal development of AFWR was detected by histological sectioning, and 10-month old AFWR was used for sampling. The methods of chromosome counting and gonad sectioning can be referenced from previous studies (Liu et al., 2019a).

2.4. Muscle nutrient

The protein, fat, moisture, ash, and amino acids of AFWR, WCC and RCC were detected. Protein content was detected by Kjeldahl method, fat content was detected by Soxhlet extraction method, moisture content was detected by vacuum freeze-drying, amino acids content was detected by high-performance liquid chromatograph (Agilent).

2.5. Muscle transcriptome analysis

9 samples (3 AFWR, 3 WCC and 3 RCC) were used for transcriptome sequencing. The RNA was utilized for sequencing library construction and sequencing. Following the extraction of clean reads, comparative analysis was performed using the RCC genome as the reference. DEGs with padj<0.01 and |log2foldchange| > 1.5 were identified using the DESeq2R software package in the AFWR vs. WCC, and AFWR vs. RCC comparisons. The growth-related genes were obtained by GO and KEGG analysis.

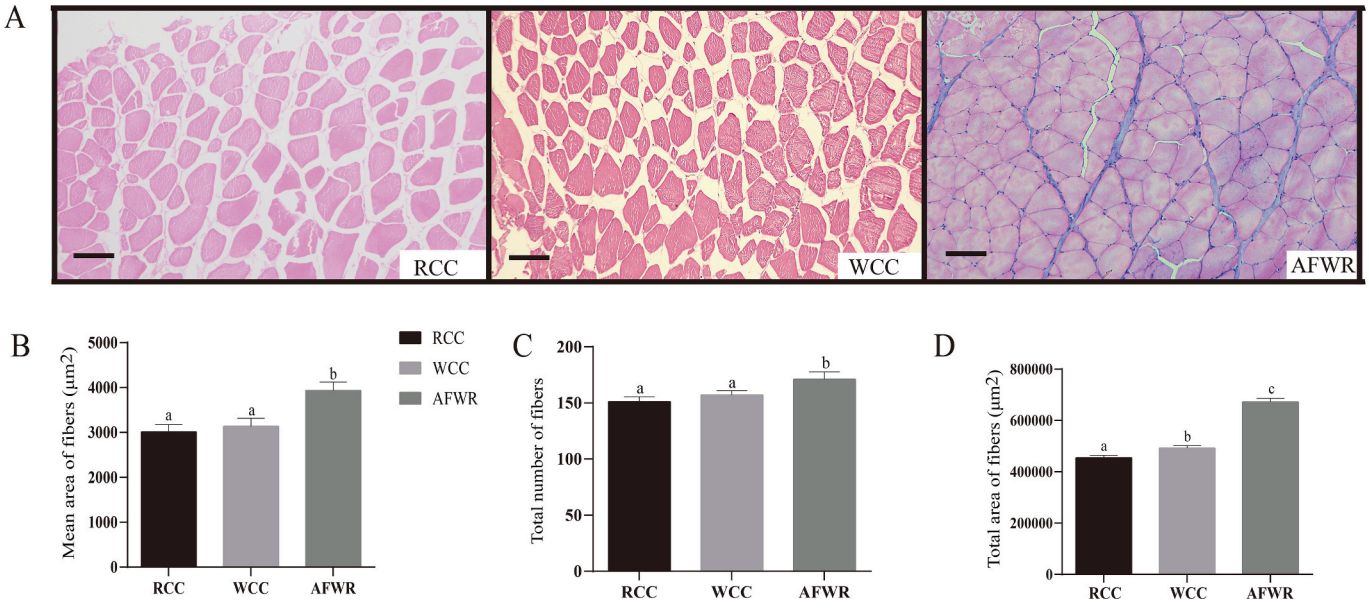

Fig. 3. Muscle fiber analysis of AFWR. (A) Paraffin sectioning of RCC, WCC, and AFWR, Bar = 100 μm, (B) the mean area of fibers,(C) the total number of fibers, (D) the total area of fibers. a, b, and c represent significant differences ($P < 0.05$).

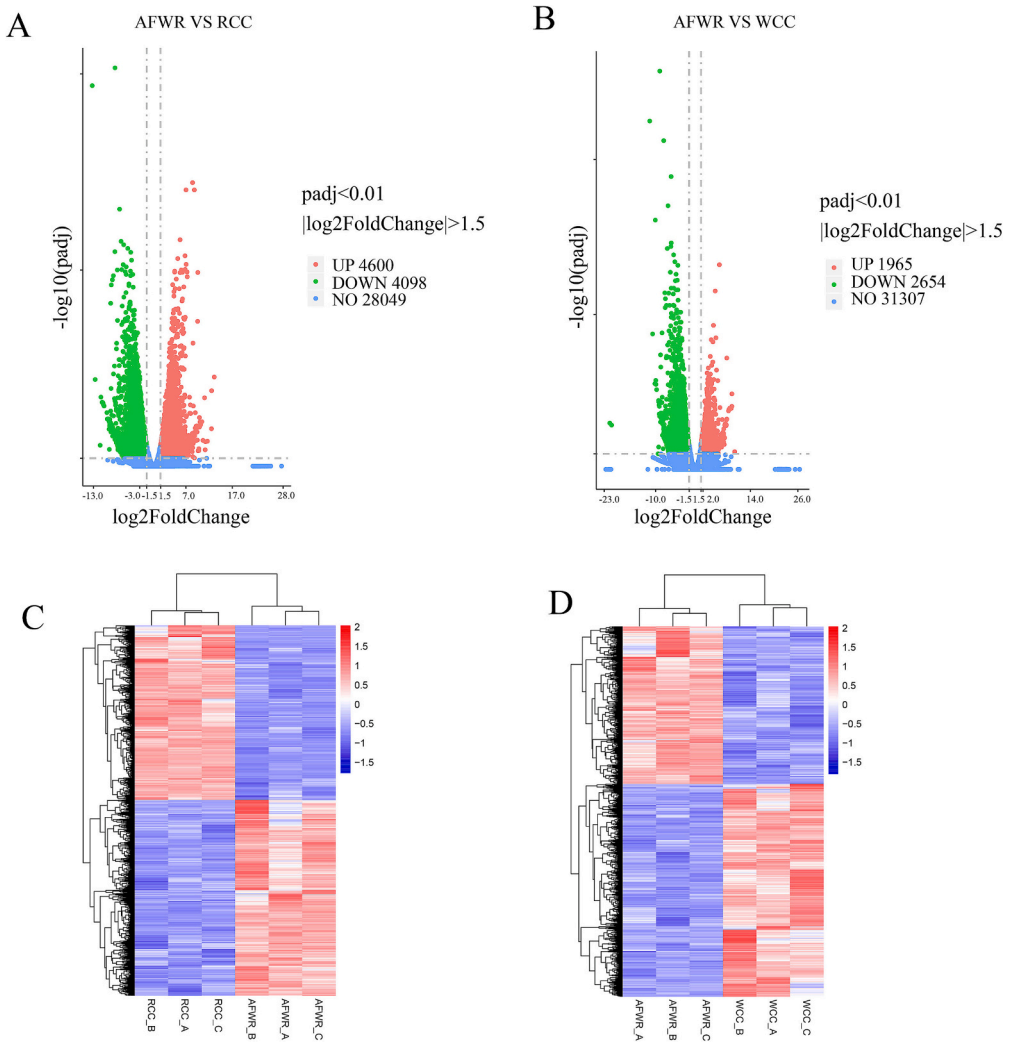

Fig. 4. Identification of DEGs and clustering analysis of DEGs. (A) DEGs in the AFWR vs. RCC, (B) DEGs in the AFWR vs. WCC, (C) detailed hierarchical clustering of DEGs in AFWR vs. RCC, (D) detailed hierarchical clustering of DEGs in AFWR vs. WCC.

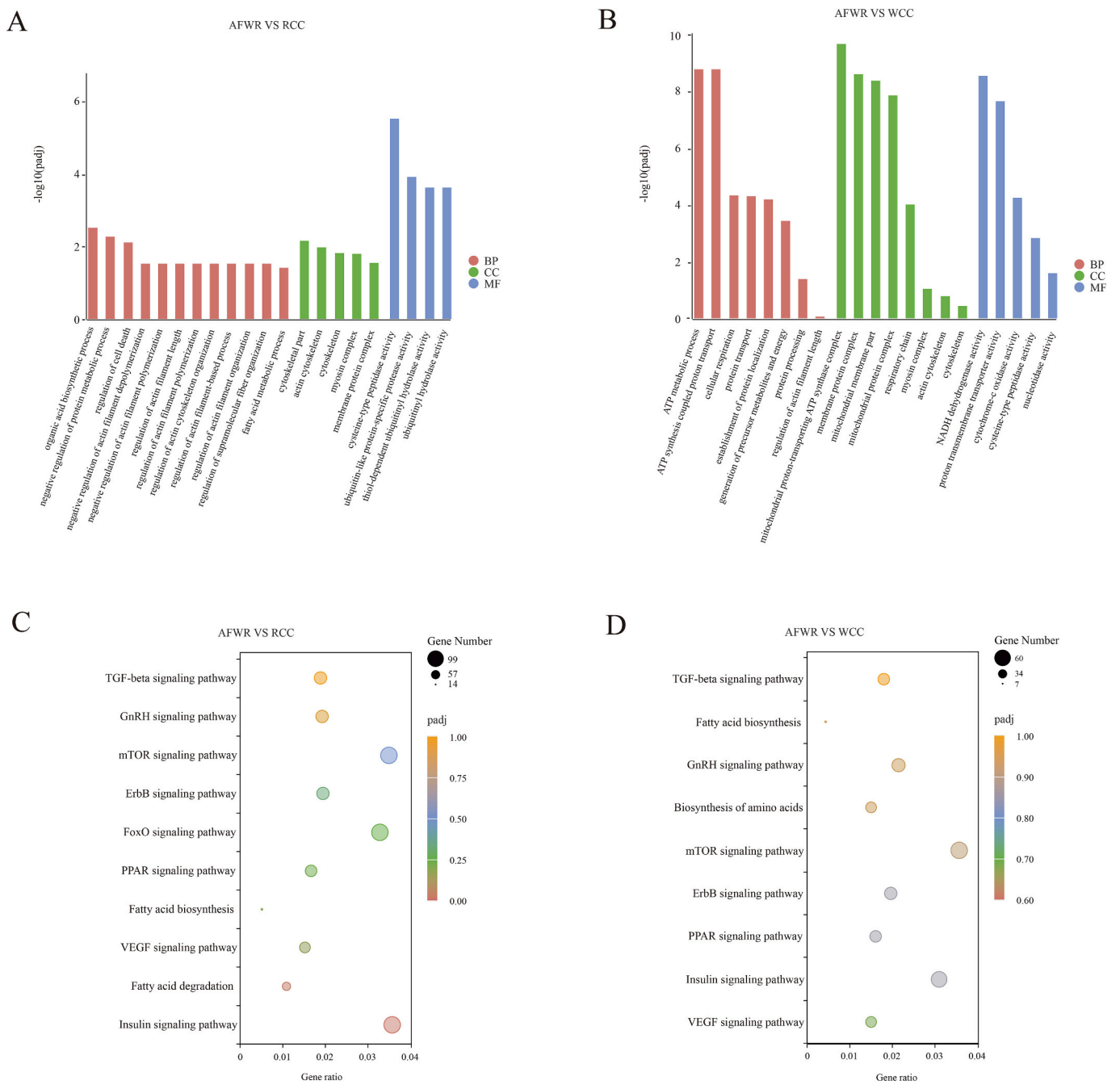

Fig. 5. GO and KEGG analysis of DEGs. (A) GO clustering of DEGs in AFWR vs. RCC, (B) GO clustering of DEGs in AFWR vs. WCC, (C) KEGG clustering of DEGs in AFWR vs. RCC, (D) KEGG clustering of DEGs in AFWR vs. WCC.

2.6. Real-time quantitative PCR

Total RNA and cDNA were obtained from muscle by the commercial kit (Omega). The protocol was consistent with previous reports (Liu et al., 2019b), with three biological replicates analyzed for each sample, the primers are listed in Table S1. In hybrid fishes, the expression levels of genes were represented by the total expression of the homeolog pair.

3. Results

3.1. Creation of AFWR and its morphological traits

AFWR was obtained by integrating the techniques of gynogenesis, distant hybridization and sex reversal (Fig. 1A). The GWCC was obtained by gynogenesis, the RGRCC was obtained by gynogenesis and sex reversal, and the AFWR was obtained by the hybridization of GWCC and RGRCC (Fig. 1B). There was no significant difference ($p > 0.05$) between AFWR and its original parents for all countable traits and all measurable traits (Table 1). Some of the measurable traits of AFWR were intermediate between original parents, such as lateral scales, upper lateral scales, and abdominal fins (Table 1).

3.2. Ploidy and fertility of AFWR

The DNA contents of RCC (used as control) and AFWR were shown in Fig. 2A and Fig. 2B. The ratio of DNA content of AFWR to RCC is 1.00 (99.90/99.54 = 1.00), indicating that AFWR is a diploid fish. We detected 100 metaphase cell division phase of AFWR and confirmed that

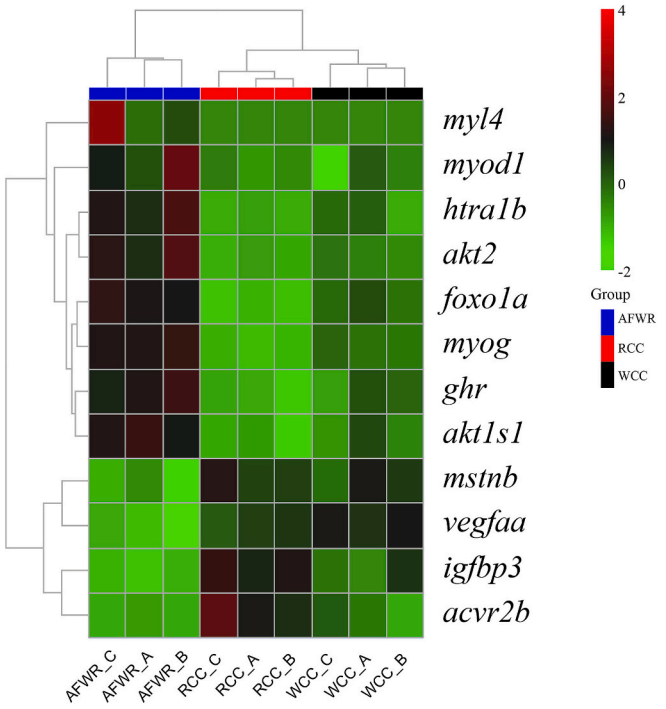

Fig. 6. Clustering of growth-related DEGs. The red indicates up-regulated, the green indicates down-regulated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

AFWR has 100 chromosomes (Fig. 2C, Table S2) (2n = 100). At 10-month old, the ovaries of AFWR develop normally and were mainly composed of oocytes at stages II or III (Fig. 2D). During breeding season, AFWR can produce mature eggs.

3.3. Muscle nutrient of AFWR

The results of muscle nutrient for AFWR, WCC and RCC were presented in Tables 2 and 3. The moisture of AFWR (72.30 g/100 g) was significant lower than those of WCC (75.90 g/100 g) and RCC (76.70 g/100 g) ($p < 0.05$), the protein of AFWR (17.70 g/100 g) and RCC (16.70 g/100 g) were significant higher than that of WCC (15.90 g/100 g) ($p < 0.05$), the ash of AFWR (1.80 g/100 g) and WCC (1.60 g/100 g) were significant lower than that of RCC (2.70 g/100 g) ($p < 0.05$), the total delicious amino acids, total essential amino acids, and total amino acids of AFWR (6.13 g/100 g, 6.36 g/100 g, 15.18 g/100 g) and RCC (6.04 g/100 g, 6.46 g/100 g, 15.52 g/100 g) were significant higher than those of WCC (5.33 g/100 g, 5.23 g/100 g, 13.13 g/100 g) ($p < 0.05$).

3.4. Body weight and muscle fiber analysis of AFWR

The mean body weights of AFWR, WCC and RCC were 350.3 ± 15.1 g, 293.2 ± 10.7 g, and 269.7 ± 10.6 g, respectively. The mean body weight of AFWR was significant higher than those of WCC (16.30 % faster than WCC) and RCC (23.00 % faster than RCC) ($p < 0.05$). Muscle fiber section of AFWR, WCC and RCC was shown in Fig. 3A. The mean fiber area of AFWR (3932.3 ± 190.8 μm^2) was significant higher than those of WCC (3135.0 ± 178.6 μm^2) and RCC (3010.8 ± 166.2 μm^2) (Fig. 3B); the total fiber number of AFWR (171.0 ± 6.7) was significant higher than those of WCC (157.0 ± 3.9) and RCC (151.0 ± 4.6) (Fig. 3C); the total fiber area of AFWR (672,426.0 ± 13,623.0 μm^2) was significant higher than those of WCC (492,179.0 ± 10,153.0 μm^2) and RCC (454,631.0 ± 9685.0 μm^2) (Fig. 3D). In short, AFWR exhibited rapid growth and showed muscle hypertrophy and hyperplasia than WCC and RCC.

3.5. Muscle transcriptome analysis of AFWR

Based on the characteristics of hypertrophy and hyperplasia of AFWR, muscle transcriptome analysis of AFWR, WCC and RCC was performed. We obtained 4.01 × 10^8 (60.23 GB) clean reads (Table S3), the repeatability of the same fish is presented in Fig. S1. Through transcriptome comparative analysis, we obtained 8698 differentially expressed genes (DEGs; upregulated: 4600, downregulated: 4098) between AFWR and RCC (Fig. 4A and C), 4619 DEGs (upregulated: 1965, downregulated: 2654) between AFWR and WCC (Fig. 4B and D). Growth-related genes were obtained by GO and KEGG analysis of DEGs, such as genes annotated with the terms myosin complex (GO:0016459), actin cytoskeleton (GO:0015629), regulation of actin filament length (GO:0030832), TGF-beta signaling pathway (ko04350), insulin signaling pathway (ko04910), mTOR signaling pathway (ko04150), VEGF signaling pathway (ko04370) (Fig. 5). The twelve candidate genes (myosin light chain 4 (*myl4*), myogenic differentiation antigen 1 (*myod1*), high-temperature requirement A serine peptidase 1B (*htra1b*), protein kinase 2 (*akt2*), forkhead box O1B (*foxo1b*), myogenin (*myog*), growth hormone receptor (*ghr*), AKT1 substrate 1 (*akt1s1*), myostatin b (*mstnb*), vascular endothelial growth factor Aa (*vegfaa*), insulin-like growth factor-binding protein 3 (*igfbp3*), activin a receptor type 2b (*acvr2b*)) for fast growth of AFWR were selected (Fig. 6) and identified by RT-qPCR (Fig. 7). The results of RT-qPCR verified the accuracy of the transcriptome.

4. Discussion

Breeding technology is crucial in the development of fish germplasm (Wang et al., 2024). Hybridization, gynogenesis, androgenesis, sex-controlled breeding, selective breeding, gene editing and other technologies have been widely used in aquaculture (Chen et al., 2023). In previous studies, we obtained the WR and WR-II by the distant hybridization, they have the characteristics of fast growth, strong disease resistance, high hatching rate and high fertilization rate, and they have been widely used in fisheries (Liu et al., 2019a). Especially in recent years, the market demand for WR-II is steadily increasing. In the production process of WR-II, the number of eggs in 2–3 WR can correspond to the number of sperm in one WCC. Therefore, enhancing the yield of WR-II hinges on increasing the population of female WR. So, we want to create an all-female WR to improve production efficiency. To obtain all-female WR, the female WCC and sex reversed male RCC (genetically female RCC) were needed. Utilizing a combination of artificial gynogenesis and sex reversal techniques is a crucial method for producing all-female fishes (Boney et al., 1984; Nagy et al., 1981; Wu et al., 1990). WCC and RCC belong to XY sex-determined fishes (Luo et al., 2011), so we obtained the RGRCC by the combination of artificial gynogenesis and sex reversal technique, and GWCC was obtained by gynogenesis. Then the AFWR was obtained by the hybridization of GWCC and RGRCC. The AFWR was diploid with 100 chromosomes, it exhibited typical gonadal development and produced fully mature eggs. In short, based on the characteristics of gynogenesis, distant hybridization and sex reversal, the AFWR was successfully created.

Hybridization usually result in heterosis, for example, triploid fish with fast growth and diploid hybrids with strong hypoxic tolerance were produced by hybridization (Liu et al., 2021a; Ren et al., 2019). More and more researches have indicated that there are some advantageous traits in gynogenetic fish, which may be related to the heterosperm effect (Wu et al., 2023). For example, gynogenetic mandarin fish with rapid growth (Wu et al., 2023), gynogenetic grass carp with rapid growth and strong disease resistance (Mao et al., 2020), gynogenetic mrigal carp with low-temperature resistant (Li et al., 2024; Li et al., 2023). In previous study, the WR exhibit rapid growth than WCC (10.00 % faster than WCC) and RCC (20.23 % faster than RCC), and showed hypertrophy than WCC and RCC (Liu et al., 2024). However, in this study, the AFWR grows 16.30 % faster than WCC and 23.00 % faster than RCC, and AFWR showed

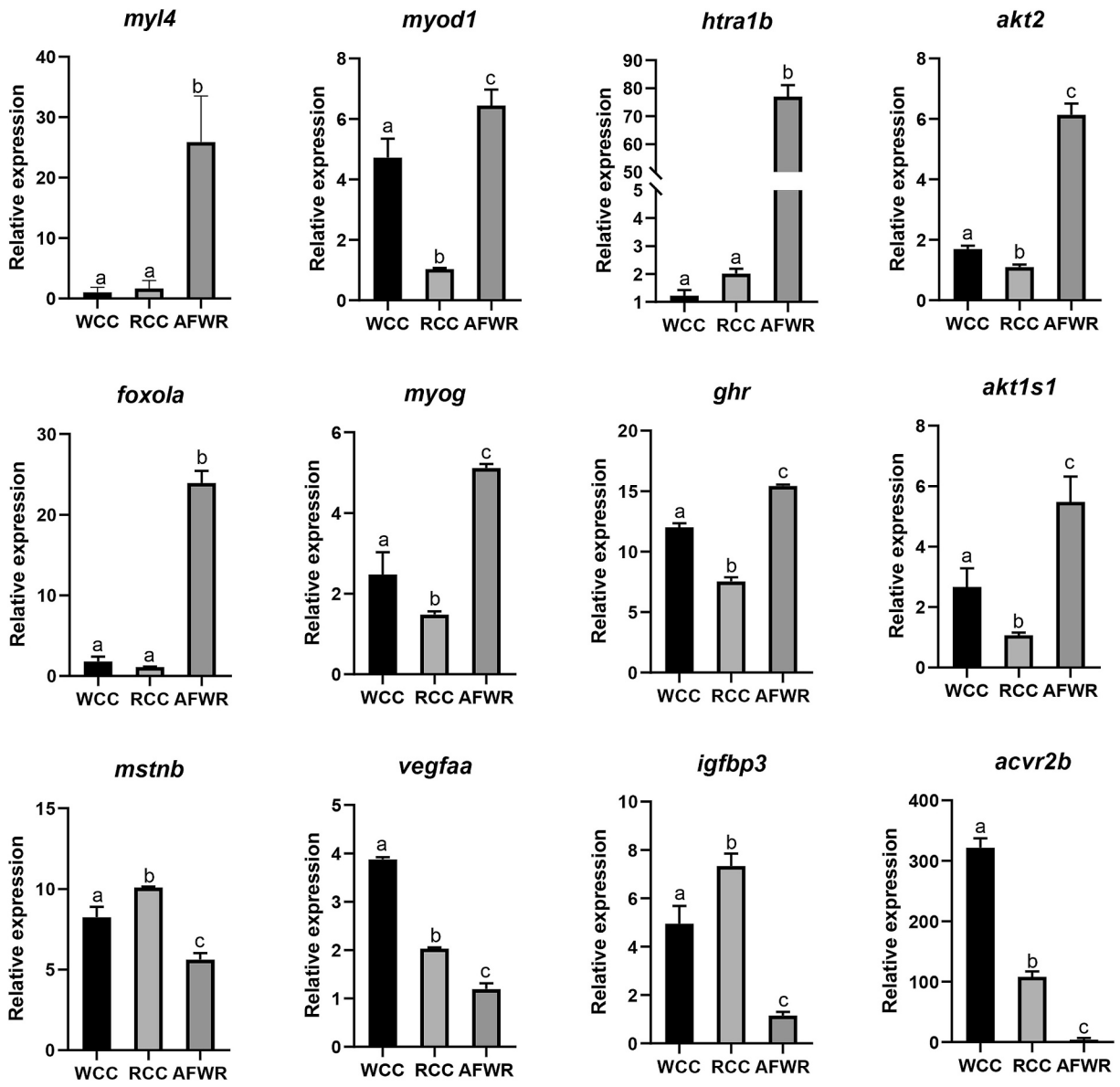

Fig. 7. The relative mRNA levels of growth-related genes were detected by RT-qPCR. a, b, and c represent significant differences ($P < 0.05$).

muscle hypertrophy and hyperplasia than WCC and RCC. Additionally, the muscle of AFWR displayed elevated protein and delicious amino acids content in comparison to its parents. Based on these results and the creation process of AFWR, we think that the advantage of AFWR may be caused by heterosis and heterosperm effect.

Growth is an important economic trait in genetic breeding of fish, it is a quantitative trait controlled by multiettple genes (Lugert et al., 2016). Finding the major genes of growth can provide important theoretical guidance for the genetic breeding of fish. The growth advantage of AFWR provides a good material for screening candidate genes for fast growth. Through transcriptome comparative analysis, 8698 DEGs were found between AFWR and RCC, 4619 DEGs were found between AFWR and WCC. Among these DEGs, growth-related genes were mainly enriched in insulin signaling pathway, TGF-beta signaling pathway, mTOR signaling pathway, VEGF signaling pathway. And twelve candidate genes for fast growth of AFWR were selected and identified by RT-qPCR. Of the 12 candidate genes, four (*mstnb*, *vegfaa*, *igfbp3*, and *acvr2b*) were up-regulated in AFWR and eight (*myl4*, *myod1*, *htra1b*, *akt2*, *foxo1a*, *myog*, *ghr*, and *akt1s1*) were down-regulated in AFWR. *Mstnb*, *acvr2b*, *myod1*, *myog*, and *myl4* regulate muscle growth and development (Joyce, 2023; McPherron and Lee, 2002; Zanou and Gailly, 2013). *Mstnb* and *acvr2b* are negative regulators of muscle growth. In mice, cattle and fishes, *mstnb* deficiency results in muscle hypertrophy and hyperplasia, which promotes rapid growth (Berry et al., 2002; Chisada et al., 2011; Khalil et al., 2017; McPherron and Lee, 2002); the absence of *acvr2* in zebrafish can also cause hypertrophy and hyperplasia of muscle tissue (Che et al., 2022). *Myod1* and *myog* are target genes of *mstnb* and *acvr2*, and they are positive regulators of muscle growth (Chisada et al., 2011). In Nile tilapia and hybrid striped bass, the high expression of *myod* and *myog* can enhance fish growth (Childress et al., 2016; Michelato et al., 2017). *Myl* (myosin liLilght chain) is highly expressed in muscle tissue and is involved in regulating energy metabolism (Wu et al., 2021). In this study, AFWR exhibited showed muscle hypertrophy and hyperplasia than WCC and RCC. Therefore, we speculate that the characteristics of muscle hypertrophy and hyperplasia in AFWR may be caused by these genes. *Akt2* and *akt1s1* positively regulate protein synthesis and muscle development (Schiaffino et al., 2013). So, the high protein in AFWR muscle tissue may be related to the high expression of *akt2* and *akt1s1*. Furthermore, the expression patterns of *ghr* (up-regulation) and *igfbp3* (down-regulation) in AFWR, which have a regulatory effect on growth

hormone (Triantaphyllopoulos et al., 2020), also promote the growth of AFWR. In general, some key genes in AFWR that regulate muscle growth, protein synthesis and growth hormone were activated or inhibited to promote its growth.

In summary, we obtained an all-female hybrid fish (AFWR) by integrating the technique of gynogenesis, distant hybridization and sex reversal. We found that AFWR is a diploid fish with 100 chromosomes, it is all-female and can produce mature eggs. The muscular nutritional value of AFWR is better than that of its parents. Furthermore, AFWR exhibited rapid growth and showed muscle hypertrophy and hyperplasia than WCC and RCC. Twelve candidate genes for fast growth of AFWR were selected and identified by RT-qPCR. The AFWR has important application value in fish genetic breeding, it provided a new way for the creation of new fish germplasm.

CRediT authorship contribution statement

Lujiao Duan: Writing – original draft, Data curation. **ChaoLiang Shen:** Data curation. **Jingjing Lin:** Data curation. **Xiaoxia Xiong:** Data curation. **Huan Sun:** Investigation. **Ziyi Huang:** Writing – review & editing. **Xuanyi Zhang:** Resources, Methodology. **Fanglei Liu:** Software. **Jianming Yu:** Resources, Formal analysis. **Qingfeng Liu:** Writing – review & editing, Funding acquisition, Conceptualization, Data curation. **Shaojun Liu:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplemental Information includes Fig. S1, and Table S1-S3, and can be found in this article online. Supplementary data to this article can be found online at [https://doi.org/10.1016/j.aquaculture.2024.742085].

Data availability

The clean reads of transcriptomes have been uploaded to the NCBI (Accession Nos. SRR29662862, SRR29662861, SRR29662860, SRR25395549, SRR25395550, SRR25395553, SRR29761170, SRR29761169, and SRR29761168).

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