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Mapping Growth-Related Quantitative Trait Locus (QTL) in Commercial Yellow River Carp (*Cyprinus carpio haematopterus*) during Overwintering

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Abstract: The common carp (*Cyprinus carpio*) is farmed globally; as a subspecies, the Yellow River carp (*C. c. haematopterus*) is widely farmed in northern China. Since the Yellow River carp grows slowly in winter, it would benefit breeders to implement a breeding program targeting growth in winter. In May 2017, we established a Yellow River carp family and measured the body length, height, thickness, and weight of individuals before (October 2017) and after winter (February 2018). Based on the genetic linkage map established in our laboratory, the quantitative trait locus (QTL) mapping of these four traits led to the detection of 29 growth-related QTLs, of which 9, 7, 3, and 10 QTLs were associated with body length, height, thickness, and body weight, respectively. One consensus QTL contained seven QTLs associated with four traits. Through the two major-effect QTLs (qBL-p-1 and qBL-p-2), we screened three candidate genes (*fnd3b*, *ghsr*, and *pld1*), which were associated with biological activities, such as fat formation, growth, and membrane transport. These results are helpful in exploring the growth regulation mechanism and providing a reference for genetic breeding with regard to the overwintering growth of Yellow River carp.

Keywords: *Cyprinus carpio haematopterus*; overwintering; growth; quantitative trait locus (QTL); candidate gene

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1. Introduction

The common carp (*Cyprinus carpio*) has a farming history of more than 8000 years in some parts of China [1]. Nowadays, the common carp is farmed globally with a production higher than 4.41 million tons in 2019 [2]. The Yellow River carp is a subspecies of the common carp that has a large yield in northern China. The rapid development of aquaculture has led researchers to focus their work on common carp genetics and breeding [3–6].

Among the methods used in breeding research, such as selective breeding and cross-breeding, quantitative trait locus (QTL) mapping is important, as it helps screen trait-related markers and assists breeding; studies on growth, feed conversion rate, disease resistance, sex, and muscle quality have been carried out using QTL [7]. Completion of the mapping of the carp genome resulted in the determination of the QTL location with increased accuracy [8,9]. Growth is one of the most important breeding traits. In research on growth-related QTL mapping, Laghari et al. [10] analyzed three traits of common carp F1 families

300 days post hatching, detecting four major-effect QTLs that explained more than 20% of the phenotypic variance. Peng et al. [11] mapped the body weight (BW), body length (BL), and carcass weight of Yellow River carp (*C. c. haematopterus*) 18 months after hatching (MAH) and obtained a total of 22 QTLs and several growth-related candidate genes. Feng et al. [12] identified 21 QTLs related to BW, total length, BL, body height (BH), or head length in Yangtze River common carp at 9 MAH using microsatellite and single nucleotide polymorphism (SNP) markers. By injecting passive integrated transponder (PIT) markers into common carp at 3 MAH and measuring their growth traits at 3 and 8 MAH, Su et al. [4] not only located 18 growth-related QTLs but also found a single shared QTL that partially controlled the BL, body depth, and body width. Wang et al. [13] detected a total of 14 major-effect QTLs and 17 candidate genes associated with BL, body thickness (BT), BH, or BW in three growth stages. However, there are few reports on cold-tolerant growth.

While studying the cold tolerance of common carp, Sun et al. [14] tagged 100 common carp before overwintering and identified living and dead individuals after overwintering. In that study, a genetic linkage map was constructed, and four putative markers associated with cold tolerance were identified, but only one marker could be mapped on a linkage group (LG). Liang et al. [15] detected a large number of cold-response genes in different tissues at different temperatures by transcriptomic analysis. Sun et al. [16] researched the effect of high and low temperatures on the common carp liver transcriptome. The results showed that the change in temperature had a great influence on the energy metabolism and metabolic process of cell membranes. Ge et al. [17] revealed that FoxO-related signal pathways play an important role in the formation and acclimation of the cold tolerance of Songpu mirror carp. Long et al. [18] analyzed the low-temperature-acclimation transcriptome of three common carp strains and found that low-temperature acclimation was consistent with the upregulation of genes related to cholesterol biosynthesis, and the content of total cholesterol in the brain increased significantly under cold stress.

Although many studies have been conducted on growth-related QTL mapping and cold tolerance, studies on growth-related QTL during the overwintering period have not yet been reported. However, during winter, the growth of Yellow River carp is almost stagnant. Prolonging the growth period of the Yellow River carp into the winter would play an important role in promoting the development of its farming. It is possible to screen QTL and candidate genes related to overwintering growth by using growth-related QTL mapping of overwintering. The QTL and candidate gene may also be related to cold tolerance. The aim of this study is to provide a reference for the growth and cold-tolerant breeding of Yellow River carp. To this end, we used high-density genetic maps of Yellow River carp [13] constructed by our group to locate growth-related QTL and to screen major candidate genes according to the BL, BH, BT, and BW of Yellow River carp during overwintering.

2. Materials and Methods

2.1. Samples and Phenotypic Collection

In 2015, we established nine carp families. At 11 months after hatching (MAH), the growth was analyzed; one family with fast growth and one family with slow growth were screened. In May 2017, a female from a fast-growing and a male from a slow-growing Yellow River carp family established in 2015 were selected as parents to establish a family in the Aquaculture Base of Henan Normal University. In October 2017 (5 MAH), 300 carps were randomly selected to be anesthetized with MS-222 (tricaine methanesulfonate) and tagged with passive integrated transponder (PIT). The body length (BL), body height (BH), body thickness (BT), and body weight (BW) of the carps were measured. The carps were placed in a solution of 0.5% potassium permanganate for 15–20 min to remove potential pathogens and put into a cement pond to continue the culture. The average value, standard deviation of each trait, and Pearson correlation coefficient between traits were analyzed using SPSS 24.0 (IBM Corp, Armonk, NY, USA). The tagged carps were measured again in February 2018 (9 MAH), and approximately 0.3-g caudal fins were cut at the age of 17 MAH

for the following experiments (N = 209). The genomic DNA of the carp was extracted using the phenol-chloroform DNA extraction method and checked using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The range and average of water temperature during overwintering was 1–20 °C and 9.53 ± 5.80 °C. For apparent errors in the growth data of some individuals before and after overwintering, we only used the information of 201 carps for this experiment.

2.2. QTL Mapping

The DNA samples were sent to Shanghai Ouyi Biotechnology Co., Ltd. (Shanghai, China) for sequencing. The libraries of the parents and progeny were prepared following Qi et al. [19]. Read processing, SNP calling, and filtering were conducted following Qi et al. [19]. Chi-square tests were used to determine the markers' deviation from expected Mendelian segregation ratios. Population type was set as "backcross" (BC) for the maternal and paternal data sets and as "F2 intercross" for the HH data set [19]. SNPs that were heterozygous in both parents with progeny segregating as A:H:B (1:2:1) constituted the HH dataset. SNPs with genotypic score A or B in parent 1 and H in parent 2 and segregating as A:H or B:H (1:1) were combined, and B scores were converted to A scores to form the paternal (AH) dataset. Similarly, H:A and H:B markers were combined, and B scores were converted to A scores to yield the maternal (HA) dataset following Qi et al. [19].

The HA, AH, and HH genetic maps were constructed using a combination of MSTMap [20] and MAPMAKER (modified from Lander et al. [21] as described by Qi et al. [22]. The population type was set as "backcross" for the maternal and paternal datasets and as "F2 intercross" for the HH dataset. Co-segregating SNPs were added to the framework map to the same location as their representative marker using an in-house Python script. Linkage maps were drawn with MapChart [23]. Based on the genetic linkage maps of Yellow River carp established by our laboratory [13], the composite interval mapping model in WinQTLCart 2.5 [24] was used to map the QTL of net change in BL, BH, BT, and BW in Yellow River carp from 5 to 9 MAH. The population type for the QTL analysis was set as "SF2" for the HH maps and as "B2" for the maternal and paternal maps. A walk speed of 0.5 cM was used for QTL identification. The logarithm of the odds (LOD) threshold for significant QTL ($p \leq 0.05$) was determined by 1000 permutations. After the QTLs were located, all QTLs located within 1 Mb of each other on the physical map were identified as being consensus QTL.

QTL naming rules: q + the abbreviation of growth traits + map type (m: maternal map, p: paternal map, h: HH map) + QTL number. For example, qBL-m-1 represents the first QTL associated with body length in the maternal map; qBW-p-1 represents the first QTL associated with body weight in the paternal map; qBH-h-1 represents the first QTL associated with body height in the HH map.

2.3. Candidate Genes

Growth-related candidate genes were filtered from these QTLs with LOD > 10. Briefly, we screened the ±50 kb genome regions surrounding the significant SNPs based on the Yellow River carp reference genome [25] (BIGD Genome Warehouse, https://bigd.big.ac.cn/gwh/Assembly/497/show, accessed on 27 October 2021) and annotated the candidate genes by conducting BLAST analysis against the Swiss-Prot database.

3. Results

3.1. Growth Traits in Yellow River Carp Aged 5–9 MAH

Table S1 shows the measurements of four growth traits in Yellow River carp at the ages of 5 MAH and 9 MAH. Based on these data, we calculated the growth trait changes in Yellow River carp at 5 to 9 MAH. The net growth of BL, BH, BT, and BW of Yellow River carp at 5–9 MAH was 0.8 ± 0.7 cm, 0.0 ± 0.2 cm, 0.4 ± 0.4 cm, and 8.2 ± 5.9 g, respectively. There were great differences in growth among individuals; the highest and lowest increases in BW were 32.8 g and −0.8 g, respectively. The correlation of the four growth traits between

5 and 9 MAH, shown in Table 1, suggests that there was a moderately positive correlation between BW and BL (r = 0.361). There was a weak positive correlation between BL and BH (r = 0.187), while the correlation between other traits was not significant ($p > 0.05$). The results suggest that the increase in the BW of Yellow River carp at the age of 5–9 MAH was mainly associated with the increase in BL.

Table 1. Pearson correlations between the growth traits of Yellow River carp at 5–9 months after hatching.

	Body Length	Body Height	Body Thickness	Body Weight
Body length	1	0.187 *	0.008	0.361 **
Body height		1	−0.005	0.107
Body thickness			1	0.068
Body weight				1

* and ** indicate that a correlation is significant at the 0.05 and 0.01 levels (2-tailed), respectively.

3.2. BL-Related QTL of Yellow River Carp at 5–9 MAH

We identified nine BL-associated QTLs of Yellow River carp at 5–9 MAH (Figure 1). As shown in Table 2, the LOD values range from 3.06 to 21.32, with a PVE (phenotypic variation explained) of 2.52% to 29.13% and additive effect of −3.49 to 0.59. Nine QTLs were located on three chromosomes (three QTLs on chromosome 4, five QTLs on chromosome 12, and one QTL on chromosome 13). There were two QTLs with LOD values greater than 10%: qBL-p-1 (LOD = 21.32, PVE = 29.13%) and qBL-p-2 (LOD = 18.47, PVE = 24.34%), located on chromosome 4. These QTLs were located at 20,559,100 bp and 12,719,902 bp on chromosome 4, respectively. Of the nine markers, only one marker (qBL-h-5) was located in an exonic region, while the others were located at intergenic regions.

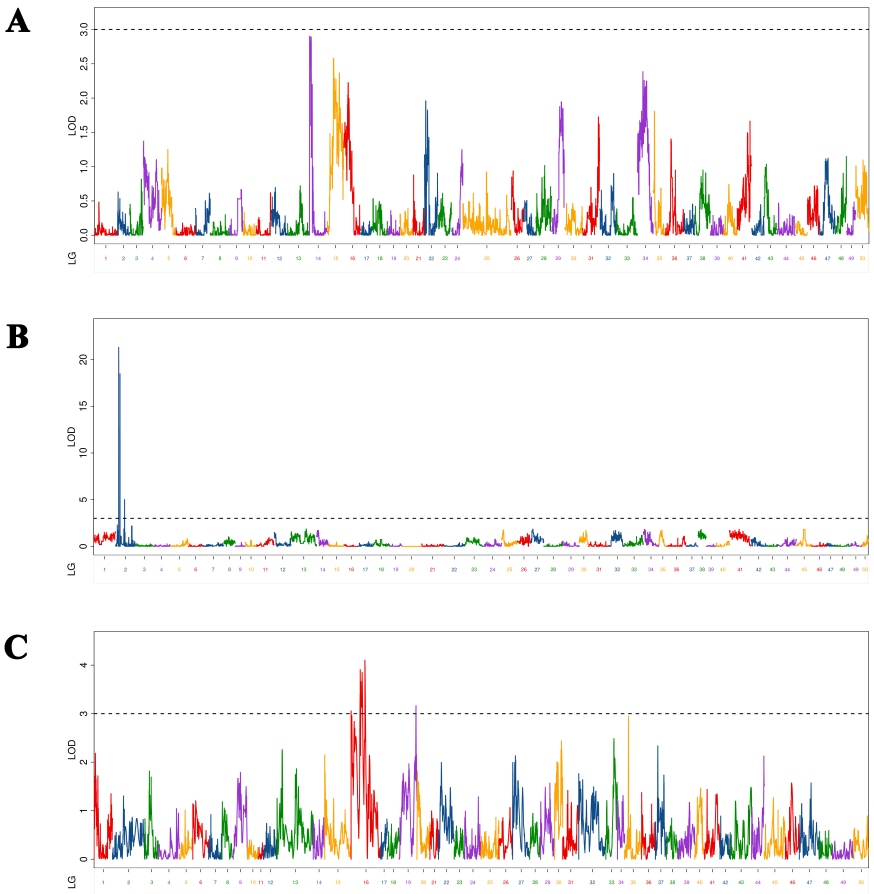

Figure 1. Distribution of body length-related QTLs on 50 linkage groups of Yellow River carp in the maternal (**A**), paternal (**B**), and HH maps (**C**). Different color indicates different linkage group.

Table 2. Information on body length-related QTLs in Yellow River carp at 5–9 months after hatching.

QTL Name	Linkage Group	Position (cM)	LOD	Additive Effect	PVE (%)	Marker Chromosome	Marker Position (bp)	Maker Region
qBL-p-1	LG2	30.6	21.32	−3.49	29.13	4	20,559,100	intergene
qBL-p-2	LG2	42.8	18.47	−3.19	24.34	4	12,719,902	intergene
qBL-p-3	LG2	86.2	5.02	0.59	7.06	4	23,459,588	intergene
qBL-h-1	LG16	0	3.06	−0.72	3.49	12	25,507,539	intergene
qBL-h-2	LG16	87.7	3.91	−0.93	5.71	12	25,496,788	intergene
qBL-h-3	LG16	94.0	3.66	−0.83	5.36	12	17,807,824	intergene
qBL-h-4	LG16	105.8	3.85	−0.86	5.34	12	1,083,098	intergene
qBL-h-5	LG16	132.7	4.10	−0.92	6.10	12	25,680,671	exon
qBL-h-6	LG19	161.9	3.17	−0.85	2.52	13	26,477,881	intergene

Marker indicates the nearest marker of QTL.

3.3. BH-Related QTLs in Yellow River Carp at 5–9 MAH

In this study, seven BH-associated QTLs were identified in Yellow River carp at 5–9 MAH (Figure 2). As shown in Table 3, LOD values ranged from 3.12 to 5.89, with a PVE of 0.38% to 11.20% and additive effect of −0.33 to 0.26. Seven QTLs were located on seven chromosomes (chromosomes 2, 12, 13, 18, 24, 29, and 47, respectively). Among them, qBH-h-2 had the maximum LOD value (5.89), and PVE (11.20%) was mapped at 25,680,671 bp on chromosome 12 in the physical map. The QTL of qBH-h-1 with the minimum LOD value (3.12) and PVE (0.38%) was mapped at 13,961,785 bp on chromosome 18 in the physical map. Of these seven markers, two markers (qBH-h-2 and qBH-h-5) were located in exonic regions; qBH-h-6 was located in an intronic region; the other four markers were located in intergenic regions.

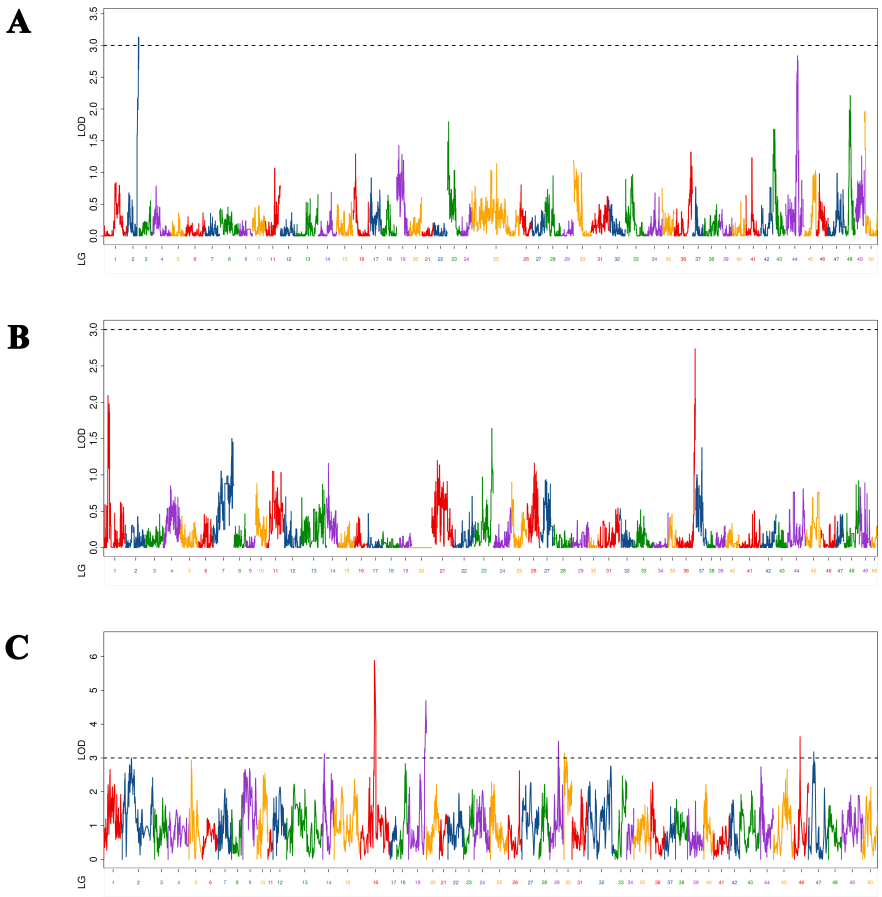

Figure 2. Distribution of body height-related QTLs on 50 linkage groups of Yellow River carp in the maternal (**A**), paternal (**B**), and HH maps (**C**). Different color indicates different linkage group.

Table 3. Information on body height-related QTLs in Yellow River carp at 5–9 months after hatching.

QTL Name	Linkage Group	Position (cM)	LOD	Additive Effect	PVE (%)	Marker Chromosome	Marker Position (bp)	Marker Region
qBH-m-1	LG2	91.7	3.13	0.05	5.62	2	27,571,216	intergene
qBH-h-1	LG14	15.2	3.12	0.12	0.38	18	13,961,785	intergene
qBH-h-2	LG16	132.7	5.89	−0.33	11.20	12	25,680,671	exon
qBH-h-3	LG19	166.2	4.70	−0.19	6.87	13	33,051,888	intergene
qBH-h-4	LG29	82.5	3.49	0.26	0.60	24	16,787,715	intergene
qBH-h-5	LG30	5.1	3.14	0.20	1.83	29	19,199,891	exon
qBH-h-6	LG46	53.9	3.63	−0.10	3.81	47	548,793	intron

Marker indicates the nearest marker of QTL.

3.4. BT-Related QTLs in Yellow River Carp at 5–9 MAH

We identified three BT-associated QTLs in Yellow River carp at 5–9 MAH (Figure 3). As shown in Table 4, the LOD values range from 3.26 to 3.78, with a PVE of 1.88% to 7.26% and an additive effect of −0.19 to 0.18. Three QTLs were located on three chromosomes (chromosomes 4, 12, and 31). Among the QTLs, qBT-h-2 had the maximum LOD value (3.79), and PVE (7.26%) was mapped at 25,680,671 bp on chromosome 12 in the physical map. Meanwhile, qBT-h-1, qBT-h-2, and qBT-h-1 were located in intronic, exonic, and intergenic regions, respectively.

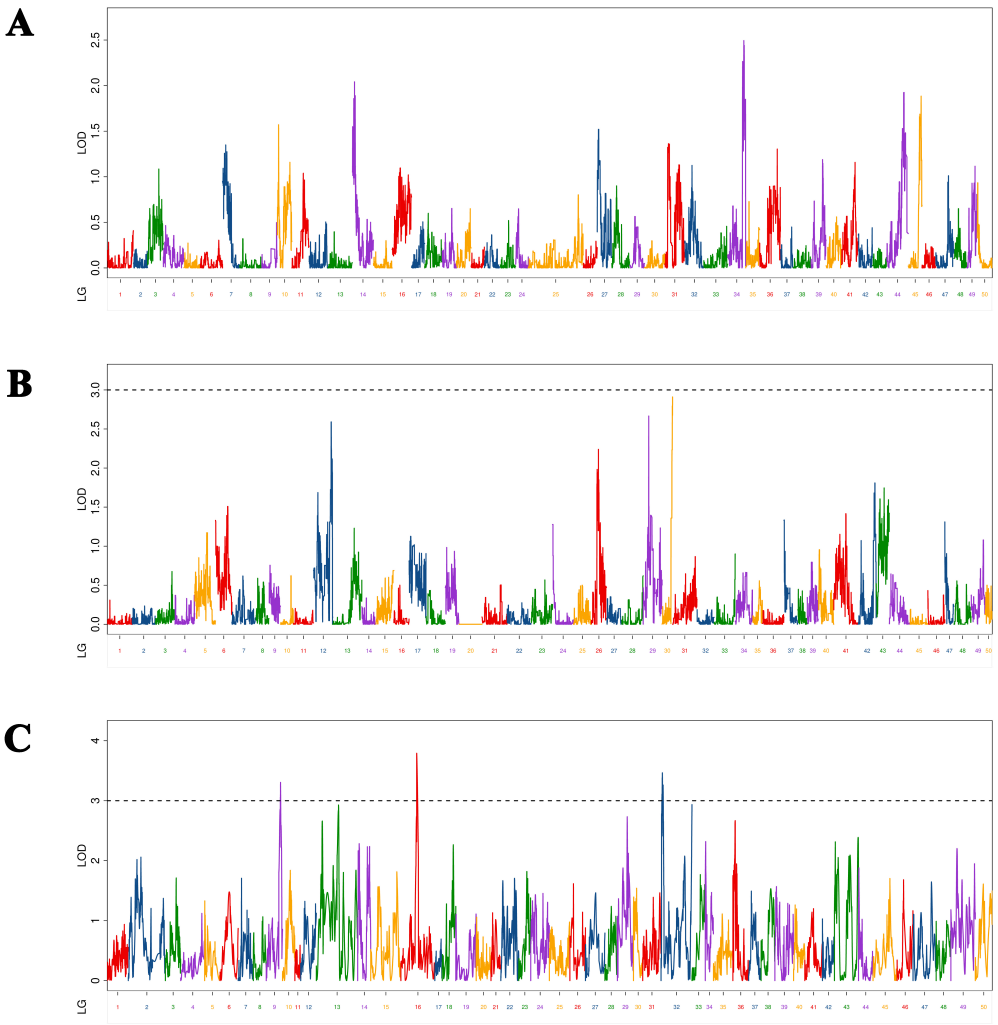

Figure 3. Distribution of body thickness-related QTLs in 50 linkage groups of Yellow River carp in the maternal (**A**), paternal (**B**), and HH maps (**C**). Different color indicates different linkage group.

Table 4. Information on body thickness-related QTLs in Yellow River carp at 5–9 months after hatching.

QTL Name	Linkage Group	Position (cM)	LOD	Additive Effect	PVE (%)	Marker Chromosome	Marker Position (bp)	Marker Region
qBT-h-1	LG9	119.90	3.31	0.18	3.13	4	17,045,253	intron
qBT-h-2	LG16	132.70	3.79	−0.19	7.26	12	25,680,671	exon
qBT-h-3	LG32	17.20	3.26	0.15	1.88	31	24,691,636	intergene

Marker indicates the nearest marker of QTL.

3.5. BW-Related QTLs in Yellow River Carp at 5–9 MAH

We identified 10 BW-associated QTLs in Yellow River carp at 5–9 MAH (Figure 4). As shown in Table 5, the LOD values range from 3.00 to 5.58, with a PVE of 2.07% to 8.85% and an additive effect of −52.80 to 52.62. These QTLs were located on three chromosomes (one QTL on chromosomes 13 and 33 and two QTLs on chromosomes 12, 29, 34, and 39). Among them, qBW-h-1 with the maximum LOD value (5.58) was mapped at 25,680,671 bp on chromosome 12 in the physical map. Of the ten markers, qBW-h-3 was located in exonic regions; there were four markers (qBW-p-1, qBW-h-1, qBW-h-2 and qBW-h-5) located in intronic regions; the other five markers were located in intergenic regions.

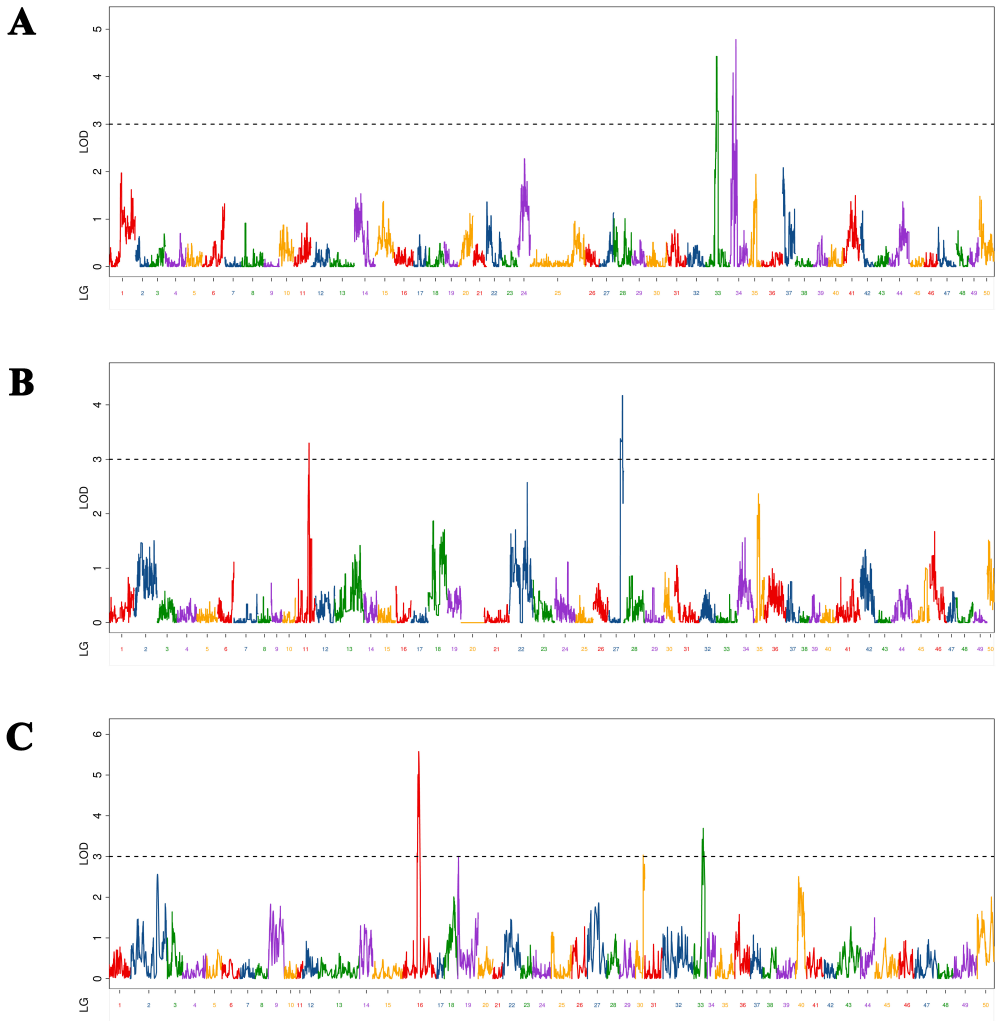

Figure 4. Distribution of body weight-related QTLs on 50 linkage groups of Yellow River carp in the maternal (**A**), paternal (**B**), and HH maps (**C**). Different color indicates different linkage group.

Table 5. Information on body weight-related QTLs in Yellow River carp at 5–9 months after hatching.

QTL Name	Linkage Group	Position (cM)	LOD	Additive Effect	PVE (%)	Marker Chromosome	Marker Position (bp)	Marker Region
qBW-m-1	LG33	72.20	4.43	1.67	7.91	33	12,876,549	intergene
qBW-m-2	LG34	23.10	4.08	−4.32	7.29	34	3,466,694	intergene
qBW-m-3	LG34	42.40	4.78	4.47	8.85	34	5,507,998	intergene
qBW-p-1	LG11	112.30	3.30	−1.51	6.30	12	24,883,274	intron
qBW-p-2	LG27	117.50	4.17	−1.57	6.91	29	1,341,522	intergene
qBW-h-1	LG16	132.70	5.58	−52.80	5.00	12	25,680,671	intron
qBW-h-2	LG19	5.60	3.00	52.62	2.07	13	36,453,503	intron
qBW-h-3	LG30	64.80	3.03	41.59	4.90	29	14,096,508	exon
qBW-h-4	LG33	70.00	3.42	42.25	3.20	39	4,996,409	intergene
qBW-h-5	LG33	77.90	3.69	46.25	4.17	39	18,795,619	intron

Marker indicates the nearest marker of QTL.

3.6. Consensus QTL at 5–9 MAH

We screened one consensus QTL (C-QTL) containing seven QTLs (qBL-h-1, qBL-h-2, qBL-h-5, qBH-h-2, qBT-h-2, qBW-p-1, and qBW-h-1). Among these, the nearest marker position of qBL-h-5, qBH-h-2, qBT-h-2, and qBW-h-1 was the same at 25,680,671 bp on chromosome 12. The marker positions of qBL-h-1, qBL-h-2, and qBW-p-1 were 25,507,539 bp, 25,495,788 bp, and 24,883,274 bp, respectively. As shown in Table 6, C-QTL may control four growth traits (BL, BH, BT, and BW) at the same time, thereby being pleiotropic.

Table 6. Information on the consensus QTL in Yellow River carp at 5–9 months after hatching.

Consensus QTL	QTL Name	Growth Trait	LOD	PVE (%)	Marker Name	Marker Chromosome	Marker Position (bp)
C-QTL	qBL-h-1	Body length	3.06	3.49	Tag_65111r	12	25,507,539
	qBL-h-2	Body length	3.91	5.71	Tag_65107	12	25,495,788
	qBL-h-5	Body length	4.10	6.10	Tag_65171	12	2,568,0671
	qBH-h-2	Body height	5.89	11.20	Tag_65171	12	25,680,671
	qBT-h-2	Body thickness	3.79	7.26	Tag_65171	12	25,680,671
	qBW-p-1	Body weighe	3.30	6.30	Tag_64958	12	24,883,274
	qBW-h-1	Body weighe	5.58	5.00	Tag_65171	12	25,680,671

3.7. Candidate Genes

According to the standard of candidate gene screening, three candidate genes (Table 7) related to growth were screened at 5–9 MAH. These three candidate genes on chromosome 4 are closely related to qBL-p-2. Among them, Fibronectin type III domain-containing protein 3B (Fnd3b), also termed factor for adipocyte differentiation 104 (Fad104), may be a positive regulator of adipogenesis [26]. Growth hormone secretagogue receptor type 1 (Ghsr) is a receptor for ghrelin; coupled to G-alpha-11 proteins, it can stimulate growth hormone secretion. The gene also binds other growth hormone-releasing peptides (Ghrp) (e.g., Met-enkephalin and Ghrp-6) as well as non-peptide, low-molecular-weight secretagogues (e.g., L-692,429, MK-0677, and adenosine) [27,28]. Phospholipase D1 (Pld1) functions as phospholipase selective for phosphatidylcholine, which is implicated as a critical step in numerous cellular pathways, including signal transduction, membrane trafficking, and the regulation of mitosis [29,30].

Table 7. Candidate genes related to major effect QTLs in Yellow River carp at 5–9 months after hatching.

QTL	Chromosome	Gene Start	Gene End	Gene ID	Gene Name	Annotation
qBL-p-2	4	12,653,254	12,741,288	HHLG4g0456	*fnd3b*	Fibronectin type III domain-containing protein 3B
		12,749,810	12,751,776	HHLG4g0457	*ghsr*	Growth hormone secretagogue receptor type 1
		12,767,533	12,788,064	HHLG4g0458	*pld1*	Phospholipase D1

4. Discussion

Our study on the growth-related QTLs of Yellow River carp during overwintering showed that the family grew slowly during the overwintering period. There were significant differences in the added value of BW among individuals, and the increase in BW resulted mainly from the increase in BL. At present, there are some reports about the

growth-related QTL of fish at a certain growth stage. For example, Sauvage et al. [31] tagged brook charr (*Salvelinus fontinalis*) with PIT at the age of 5 months, collected the growth data of four different growth periods (May 2009, July 2009, August 2009, and November 2009), and mapped the growth rate-related QTLs in different growth periods. Zhang et al. [32] measured the growth rate of common carp at three time points (3, 4, and 5 MAH) and identified 15 genomic regions associated with growth rate. Wang et al. [13] carried out a QTL mapping study on four growth traits of Yellow River carp at the age of 13, 17, and 13–17 months. However, there is no research on the QTL location related to the growth of fish during overwintering. This may be due to the slow growth of carp during overwintering; some scholars think that the research is of little significance. Although carp grow slowly during overwintering, there are great differences among individuals. Thus, we can screen the major-effect QTL during overwintering by detecting the QTL related to growth traits. These QTL may also be related to the cold tolerance of carp.

At present, the research on the cold tolerance of fish is mainly focused on the transcriptomic analysis of the common carp [18,20], Nile tilapia (*Oreochromis niloticus*) [33], yellow drum (*Nibea albiflora*) [34], *Onychostoma macrolepis* [35], etc. However, there are few studies on cold-tolerance-related QTL. At present, there is only one report on the location of cold-tolerance-related QTL in common carp [14]. In this study, the dead and surviving fish were classified into two groups after overwintering. Four RAPD markers that may be associated with cold tolerance were screened, but only one was located in the linkage group. In this study, 300 fish were initially marked, but only 198 fish were used because the samples were collected at the age of 17 MAH for QTL mapping. There were still 207 fish at the age of 17 MAH, but the growth data of nine fish were obviously wrong during the overwintering period, so these growth data were excluded. In future research, we should develop devices that can automatically and accurately obtain the phenotypic traits of fish and so improve the accuracy of the results [36].

In this study, as different maps (maternal, paternal, and HH) are constructed with different markers, the QTL mapping results of the same trait on these maps are very different. Twenty-nine QTLs related to overwintering growth were located and distributed on 12 chromosomes. The characteristics of a large number and dispersion of QTL were similar to those mentioned by other studies on common carp growth-related QTL [10–12,37]. The largest number of growth-related QTLs in common carp was 165, distributed in 30 linkage groups [38], indicating that common carp growth-related traits were determined by multiple genes. The results of the present study show that these growth-related, complex traits are controlled by a large number of genes. It is generally assumed that the higher the LOD value, the higher the confidence, and the higher the PVE, the greater the role of QTL. In this study, there were only two BL-related QTLs that had LOD > 10 and PVE > 20%, which were values similar to those obtained by a study on the Yangtze River common carp growth-related QTL [12]. In that study, the authors found that one BW-related QTL had LOD > 10 and PVE > 20%. However, although several QTLs have been found in studies of Yellow River carp [11] and common carp [10] with PVE >20%, none had LOD >10%. Therefore, assumptions regarding whether there was a QTL that had a significant effect on different growth traits of common carp at different growth stages were quite different. However, these QTLs with significant effects are the ones we should focus on, as they can be used in molecular-assisted breeding, similar to the two QTLs that had significant effects and were screened in this study. The molecular markers and genes will help increase overwintering growth and prolong the growth period. In this study, C-QTL contained seven QTLs associated with four growth traits and had obvious pleiotropy. In previous studies, certain QTLs appeared pleiotropic in different periods [13] or different families [38]. The consensus QTLs may be related to housekeeping genes if their PVE is very low. In this study, the PVE of C-QTL was low, which may have little effect on overwintering growth. Although many markers were located in genes, the major QTLs were located in intergenic regions.

Among the three overwintering growth-related genes screened in this study, *fnd3b* is a protooncogene involved in the occurrence and development of cancer [39,40]. The protein Fnd3b expressed in this gene is a positive regulator of adipogenesis, related to adipocyte differentiation, and is an RNA-binding protein [41,42]. Other studies have shown that Fnd3b affects human craniofacial development [43] and is related to the domestication of chickens [44]. Ghsr, the receptor of growth hormone secretory hormone (Ghrelin) and growth hormone-releasing peptide, is a G protein coupling protein [45]. The Ghsr/Ghrelin system is an important pathway for the regulation of growth hormone secretion [46,47]. Ghsr has been found to play an important role in energy balance and metabolism as well as digestion and GH secretion in fish [48]. However, in the study of fish QTL, no QTL related to Ghsr was found. Pld1 is an enzyme widely distributed in bacteria, fungi, animals, and plants [49]. It plays a key role in signal transduction, membrane transport, and cytoskeletal regulation [50–52]. Lipid catabolism plays an important role in enhancing the cold tolerance of fish [53]. Zheng et al. [54] studied the QTL related to muscle fat content and abdominal fat in common carp and located 18 genome-wide QTLs with suggestive significance. This result was different from the genes found in this study, which might be due to the different traits examined in the two studies. These three genes could be used as important candidates to study the growth and metabolism of Yellow River carp during overwintering.

In conclusion, this is the first study on the growth-related QTL mapping in overwintering of Yellow River carp. In this study, 29 QTLs, including two major-effect QLTs and one C-QTL related to overwintering growth traits, were detected. Additionally, we screened three genes related to overwintering growth (*fnd3b*, *ghsr*, and *pld1*), which were related to fat formation, growth, membrane transport, etc. The results of this study provide information for the breeding of Yellow River carp to improve growth and cold tolerance during overwintering.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fishes7040166/s1, Table S1: Body length, height, thickness, and weight of the family at the age of 5, 9, and 5–9 months after hatching (MAH).

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