A first genetic linage map construction and QTL mapping for growth traits in *Larimichthys polyactis*

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*Larimichthys polyactis* is a commercially important marine fish species in Eastern Asia, yet very few genetic resources exist. In particular, genetic linkage maps are critical tools for genetic breeding. In this study, we generated a high resolution linkage map from a family of 110 individuals and their parents by resequencing the individuals. 3,802 effective SNPs were mapped to 24 linkage groups (LGs). The map spanned 2,567.39 cm, with an average marker interval of 0.66 cm. We used the map to conduct QTL analysis for growth traits, and found 31 markers were significantly associated with growth-related traits. Specifically, three SNPs were identified for total length, nineteen SNPs for body length, and nine SNPs for body weight. The identified SNPs could explain 15.2–22.6% of the phenotypic variation. SNPs associated with growth traits were distributed on LG6 and LG11, and candidate genes included, *kif26b*, *bat1*, *gna1*, *gbgt1*, and *amfr*, which may regulate growth. The linkage map and mapped QTLs would be useful for improving the quality of *L. polyactis* via marker-assisted selection.

The small yellow croaker, *Larimichthys polyactis*, is a benthopelagic marine fish species that is endemic to the Bohai Sea, Yellow Sea and East China Sea, and is an ecologically and commercially important marine fish species. Artificial propagation of *L. polyactis* was achieved in 2015, and has contributed to the increased success of farming of this species in China. However, genetic studies of *L. polyactis* are limited to population structure and genetic diversity surveys, and transcriptome analysis under specific conditions, and there are few established microsatellite markers and mitochondrial genome sequences that are available.

Adult size is a critical economic trait for all aquaculture species, as larger fish are more valuable to consumers. Thus, the development of broodstock that can grow rapidly is of great interest. However, genomic resources for *L. polyactis* are not well developed, limiting our understanding of genes that contribute to growth in this species. Growth is a quantitative trait controlled by multiple genes, or quantitative trait loci (QTLs). Compared to traditional selective breeding programs that focus in selecting individuals with desirable traits, molecular based breeding programs accelerate the breeding process by using genetic markers that can be quantified faster than adult traits. Dense genetic maps are necessary for molecular breeding.

The development of next-generation sequencing (NGS) technologies have facilitated the sequencing and assembly of many fish genomes, such as *Cynoglossus semilaevis*, *Cyprinus carpio*, *Larimichthys crocea*, *Miticthys miyu*, *Paralichthys olivaceus*, *Lateolabrax maculatus*, *Epinephelus lanceolatus*, and *Carassius auratus*. Recently, the first reference genome of *L. polyactis* was sequenced and assembled (Xie et al., unpublished), and genome-scale SNP markers can now be identified through resequencing of the genome, which allows for the construction of high-density linkage maps. Genetic linkage maps are essential resources that can improve the quality of the genome and chromosomal assembly, and are essential for mapping QTLs. QTL mapping identifies genes that are related to phenotype variations within populations, and genes identified through QTL studies could be used to enhance breeding programs.

The objectives of this study were: (1) large-scale identification of SNPs and construction of a high-density SNP-based linkage map using genotyping by resequencing individuals of *L. polyactis*; (2) QTL mapping to detect...
SNPs associated with growth traits using the high-density map; and (3) identification of candidate genes that regulate growth related-traits of L. polyactis. This study establishes large-scale SNP markers and high-density linkage maps for L. polyactis, which can facilitate genome assembly, comparative genomics, and QTL mapping for economic traits in L. polyactis.

### Results

#### Values of the phenotypic traits. We used 110 L. polyactis progeny to generate the linkage map. The average values for total length, body length, and body weight were 16.62 ± 1.10 cm, 13.76 ± 0.87 cm, and 48.89 ± 10.33 g, respectively. These growth traits were strongly correlated with each other (r = 0.902–0.949, P < 0.01 for all) (Table 1). Body length and body weight had the highest correlation (r = 0.949). Of the progeny, 35 and 66 individuals were identified as males and females, respectively, with a sex ratio of 1:1.89. We were unable to identify the sex of the remaining nine individuals of the 110 progeny.

#### Construction of sequencing library and sequencing. A total of 112 sequencing libraries from two parents and 110 progenies were constructed and sequenced to generate 849.37 Gb of sequencing data, which resulted in 847.47 Gb of high-quality sequencing data with an average Q20 ratio of 97.09% and a GC content of 42.28%. The parents were sequenced at a higher level to enhance the chances of detecting more SNP markers. Finally, clean data covering 14.78 Gb with a GC% of 42.10 and 13.56 Gb with a GC% of 42.33 were obtained for the male and female parents, respectively. For each individual, the clean data ranged from 6.32 to 9.89 Gb, with an average of 7.45 Gb (Supplementary Table S1). From the paired end clean reads, 97,046,018 reads were obtained from the female parent and 105,785,790 reads were obtained from the male parent. Only reads that aligned to unique positions on the reference genome were retained for the subsequent SNP calling and genotyping (Supplementary Table S2).

#### SNP discovery and genotyping. SNP calling of the two parents and F1 individuals was performed using GATK. A total of 777,570,223 markers were detected, of which 14,317,836 were retained once SNPs with more than 20% missing data in both genotype and individual were removed. After initial genotyping, the CP model was used in JoinMap5.0 to select for segregation types lm × ll (48,698 markers), nn × np (47,340 markers), and hk × hk (28,809 markers), which resulted in a total of 124,847 SNPs that were polymorphic in at least one parent and 92% of progeny (Table 2). Among the 24 LGs in the integrated map, LG16 was the largest group with a genetic distance of 212.87 cm and 249 effective SNPs, and LG17 was the shortest with 113 markers spanning 56.22 cm. The average effective SNP interval ranged from 0.50 to 0.99 cm, with an average distance of 0.66 cm (Table 3; Supplementary Table S3).

| Marker type | Mother genotype | Father genotype | Marker number | Percentage |
|-------------|-----------------|-----------------|--------------|------------|
| hh×hh | hh | hh | 28,809 | 23.07% |
| nn×np | nn | np | 47,340 | 37.92% |
| lm×ll | lm | ll | 48,698 | 39.01% |
| Total | | | 124,847 | 100.00% |

Table 2. Number of genotyping SNPs in each segregation types in F₁ individuals.

SNPs associated with growth traits using the high-density map; and (3) identification of candidate genes that regulate growth related-traits of L. polyactis. This study establishes large-scale SNP markers and high-density linkage maps for L. polyactis, which can facilitate genome assembly, comparative genomics, and QTL mapping for economic traits in L. polyactis.
QTL mapping of growth traits. The integrated linkage map with 3,802 effective SNPs in 24 LGs of *L. polyactis* was used to identify QTLs that associate with growth traits. The interval mapping model in the MapQTL package was used to analyze the total length, body length, body weight of 110 progeny. The estimated significant thresholds from permutation tests were 4.5 for total length, 4.8 for body length, and 3.9 for body weight.

The numbers of SNPs that were detected for each trait are shown in Table 4. We found three significant SNPs for total length with LOD scores of 4.74, 4.73 and 4.61, and these SNPs were located at 51.77, 52.23 and 52.69 of LG11. The proportion of phenotypic variation explained by these SNPs was 18.5%, 18.5% and 18.1%, respectively.

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**Figure 1.** The consensus linkage map of *L. polyactis*. The consensus map which contained 3,802 effective SNPs in 24 linkage groups was constructed through combing the male and female linkage maps.

**Table 3.** Characteristics of genetic maps and anchoring scaffolds of *L. polyactis*.

| Linkage | Maternal map | Paternal map | Integrated map |
|---------|--------------|--------------|----------------|
|         | No. of SNPs/ effective SNPs | Distance (cm) | No. of SNPs/ effective SNPs | Distance (cm) | No. of SNPs/ effective SNPs | Distance (cm) |
| LG1     | 2,519/58     | 61.52        | 2,861/70        | 131.9        | 5,317/213        | 144.85        | 0.68 |
| LG2     | 2,528/53     | 70.76        | 2,927/67        | 74.43        | 4,937/155        | 77.25        | 0.50 |
| LG3     | 2,452/59     | 77.56        | 2,638/51        | 56.99        | 4,783/135        | 81.85        | 0.61 |
| LG4     | 2,767/53     | 84.69        | 2,578/60        | 69.81        | 4,925/154        | 95.10        | 0.62 |
| LG5     | 2,582/66     | 99.05        | 2,267/62        | 62.84        | 4,543/175        | 129.36       | 0.74 |
| LG6     | 2,322/57     | 69.93        | 2,268/65        | 68.88        | 4,298/156        | 94.80        | 0.61 |
| LG7     | 2,329/69     | 83.70        | 2,332/77        | 88.25        | 4,299/152        | 75.87        | 0.50 |
| LG8     | 2,322/39     | 54.37        | 2,111/49        | 51.43        | 4,147/110        | 56.69        | 0.52 |
| LG9     | 2,495/67     | 96.83        | 1,958/62        | 82.04        | 4,182/172        | 105.68       | 0.61 |
| LG10    | 2,146/61     | 93.79        | 2,232/53        | 66.25        | 4,076/211        | 147.6        | 0.70 |
| LG11    | 2,104/51     | 91.46        | 2,161/62        | 67.02        | 3,980/161        | 85.14        | 0.53 |
| LG12    | 1,852/47     | 71.27        | 2,045/52        | 68.09        | 3,660/124        | 67.14        | 0.54 |
| LG13    | 2,000/51     | 70.15        | 1,932/68        | 82.12        | 3,630/156        | 98.99        | 0.63 |
| LG14    | 1,898/62     | 76.71        | 1,836/54        | 68.37        | 3,411/183        | 146.88       | 0.80 |
| LG15    | 1,959/55     | 159.06       | 1,679/40        | 71.03        | 3,430/129        | 127.83       | 0.99 |
| LG16    | 1,602/70     | 110.19       | 1,996/67        | 98.50        | 3,300/249        | 212.87       | 0.85 |
| LG17    | 2,347/51     | 56.14        | 1,931/63        | 56.88        | 3,438/113        | 56.21        | 0.50 |
| LG18    | 1,672/54     | 103.82       | 1,933/56        | 71.66        | 3,410/227        | 181.55       | 0.80 |
| LG19    | 1,835/46     | 86.89        | 1,729/59        | 68.25        | 3,331/128        | 99.70        | 0.78 |
| LG20    | 1,517/41     | 75.35        | 1,630/68        | 113.88       | 2,947/118        | 70.59        | 0.60 |
| LG21    | 1,610/40     | 57.33        | 1,656/45        | 54.16        | 3,044/117        | 64.57        | 0.55 |
| LG22    | 1,652/53     | 75.84        | 1,526/52        | 57.02        | 2,798/132        | 87.87        | 0.67 |
| LG23    | 1,419/59     | 62.50        | 1,520/67        | 88.51        | 2,689/213        | 152.07       | 0.71 |
| LG24    | 1,306/37     | 53.51        | 1,328/36        | 55.33        | 2,483/119        | 106.92       | 0.90 |
| Total   | 49,235/1,299 | 1942.42      | 49,074/1,405    | 1773.64      | 91,058/3,802     | 2,567.39     | -   |
| Average | 2051.46/54.13| 80.93        | 2,044.75/58.54  | 73.90        | 3,794.08/158.42  | 106.97       | 0.66 |

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**QTL mapping of growth traits.** The integrated linkage map with 3,802 effective SNPs in 24 LGs of *L. polyactis* was used to identify QTLs that associate with growth traits. The interval mapping model in the MapQTL package was used to analyze the total length, body length, body weight of 110 progeny. The estimated significant thresholds from permutation tests were 4.5 for total length, 4.8 for body length, and 3.9 for body weight.

The numbers of SNPs that were detected for each trait are shown in Table 4. We found three significant SNPs for total length with LOD scores of 4.74, 4.73 and 4.61, and these SNPs were located at 51.77, 52.23 and 52.69 of LG11. The proportion of phenotypic variation explained by these SNPs was 18.5%, 18.5% and 18.1%, respectively.
use of the F1 generation as the mapping group, the number of non-redundant markers that mapped onto the (0.89)45. Compared with other fish genetic maps, our genetic map is relatively high in quality. The high quality group, and more than 200 individuals should be genotyped.

We constructed the first genetic map of L. polyactis P. olivaceus inter-locus distance of 0.66 cm. The value of the average locus distance was larger than 199825, various linkage maps have been developed for aquaculture species using microsatellites, as well as SNPs. Hypophthalmichthys nobilis QTL mapping28, population genetic analysis29, and genome-wide association studies (GW AS) 30. In the past, organism have been more suitable for genetic studies26, especially in high-density genetic linkage mapping27, Compared to microsatellites with long flanking DNA sequences, SNP markers that are most abundant in an expensive. Today, sequencing an individual's genotype is simple, quick, and reproducible31, and a number of high density genetic linkage map. Since the publication of the first genetic linkage map of tilapia in 199835, various linkage maps have been developed for aquaculture species using microsatellites, as well as SNPs. To further identify potential causative genes underlying growth traits, we screened the reference genome and identified protein-coding genes by mapping the corresponding tags of SNPs in QTL regions to the scaffold assembly followed by retrieval of the corresponding gene from the gene annotation file. Thirteen genes were thus identified, including kinesin-like protein KIF26B (kif26b), E3 ubiquitin-protein ligase AMFR-like (amfr), Nucleolar RNA helicase 2 (ddx21), elongation factor for RNA polymerase II (ell), polypeptide N-acetylglucosaminyltransferase 2 (gaint2), globoside alpha-1,3-N-acetylgalactosaminyltransferase 1 (gbgt1), glucosamine 6-phosphate N-acetyltransferase (gna1), Homeodomain-interacting protein kinase 3 (hipk), solute carrier family 6 member 2 (slc6a2), B(0, +)-type amino acid transporter 1 (bat1), serine/threonine/tyrosine interacting protein (styx), ArfGAP with GTPase domain, ankyrin repeat and PH domain 2 (styx) (Supplementary Table S5). Of these, the most significant SNP located in the QTLs on LG11 that was associated with total length, body length and body weight revealed kif26b as a potential candidate gene that may regulate growth in L. polyactis. The candidate genes of amfr, ddx21, gbgt1, gna1, bat1, styx detected on LG11 were also shared for body length and body weight. These candidate genes may contribute to the genetic control of growth traits. The exact function of these genes in L. polyactis needs further study.

Discussion

High density genetic linkage map. Since the publication of the first genetic linkage map of tilapia in 199835, various linkage maps have been developed for aquaculture species using microsatellites, as well as SNPs. Compared to microsatellites with long flanking DNA sequences, SNP markers that are most abundant in an organism have been more suitable for genetic studies26, especially in high-density genetic linkage mapping27, QTL mapping28, population genetic analysis29, and genome-wide association studies (GWAS)30. In the past, developing SNP markers and studying their patterns in large populations were technologically challenging and expensive. Today, sequencing an individual's genotype is simple, quick, and reproducible31, and a number of high-density linkage maps have been constructed for aquaculture species, such as P. olivaceus32, Lates calcarifer33,44, C. carpio35, C. auratus36,37, Megalobrama amblycephala38, and Colossoma macropomum39. Here, we constructed the first genetic map of L. polyactis using 94,169 SNPs, and the integrated map consists of 24 LGs, which corresponded to 3,802 effective loci. The total length of the integrated map was 3,794.08 cm, with an average inter-locus distance of 0.66 cm. The value of the average locus distance was larger than P. olivaceus (0.47)35, L. crocea (0.54)45, C. auratus (0.44)49, Seriola aureovittata (0.30)49, but smaller than_dicentrarchus labrax (0.72)45, Hypophthalmichthys nobilis (0.75)45, C. carpio (0.75)33, Scophthalmus maximus (0.72)44, and Sillurus meridionalis (0.89)45. Compared with other fish genetic maps, our genetic map is relatively high in quality. The high quality linkage map that we generated has enabled us to determine the number and position of the QTLs for growth traits, and will allow for fine-mapping of QTLs in molecular breeding of L. polyactis. We also constructed the paternal and maternal linkage maps of the L. polyactis, and these data are valuable resources for genome selection (GS) and GWAS in L. polyactis. Due to the limited the number of individuals that were sequenced, and the use of the F1 generation as the mapping group, the number of non-redundant markers that mapped onto the map was relatively small. Therefore, in the subsequent research, the F2 generation should be used as the mapping group, and more than 200 individuals should be genotyped.

| Traits | Linkage | Position | Number of significant SNPs | LOD threshold value | LOD value of detected SNPs | Exp%a |
|--------|---------|----------|---------------------------|---------------------|---------------------------|-------|
| Total length | LG11 | 51.77–52.69 | 3 | 4.5 | 4.61–4.74 | 18.10–18.50 |
| Body length | LG11 | 42.61 | 1 | 4.8 | 5.17 | 19.50 |
| Body weight | LG6 | 71.93 | 1 | 3.9 | 4.30 | 16.9 |
| | LG11 | 51.32–52.69 | 4 | 3.9 | 3.96–4.32 | 16.40–17.10 |
| | LG11 | 54.98–56.35 | 4 | 3.9 | 3.91–4.03 | 15.20–15.70 |

Table 4. Detected QTLs for growth-related traits of L. polyactis. a percentage of explained phenotypic variation.
Figure 2. Total length (a), body length (b) and body weight (c) QTL mapping and association analysis in *L. polyactis* among all linkage groups. The x- and y-axes correspond respectively to relative position on the linkage groups and the LOD value. The red horizontal line represents a linkage group-wise logarithm of odds (LOD) significance threshold of 4.8 for total length, 4.5 for body length, and 3.9 for body weight.
Candidate growth-related genes. To improve aquaculture of *L. polyactis*, it is important to identify markers linked to economically important traits to breed superior parental stock. QTL analyses enable us to find the markers associated with the genetic variation for growth-related traits and the candidate genes involving physiological processes for the traits, which can be applied to breeding programs. We found strong correlations ($r \geq 0.902$) among the three growth-related traits that were measured, and this finding was similar to previous work on other aquaculture species\(^{26-40}\). QTL analyses of growth-related traits have been conducted in some aquaculture species, such as *Oncorhynchus mykiss*\(^{41}\), *Salmo salar*\(^{42}\), *P. olivaceus*\(^{43}\), *C. semilaevis*\(^{44}\), *tilapia*\(^{45}\), and *L. calcarifer*\(^{46}\). As growth traits are quantitative traits controlled by multiple genes, growth-related QTLs are mapped to several LGs in most cases. For example, O’Malley et al.\(^{47}\) identified QTLs for body weight in rainbow trout on 10 different LGs, Gutierrez et al.\(^{48}\) and Reid et al.\(^{49}\) identified QTLs for body weight in two LGs and six LGs of Atlantic salmon, respectively. In the present study, multiple QTLs were found on two LGs for growth-related traits, and three SNPs on LG11 were shared for total length, body length and body weight, suggesting that this QTL affects all three growth-related traits in *L. polyactis*. Eight SNPs that were associated with body weight and body length had the same distribution pattern on LG11. One SNP detected for body weight was located on LG6, and this SNP was unique to body weight. In all, QTLs related to different growth traits were generally located on the same LGs, though a few were found on different LGs.

QTL mapping enables us not only to detect genetic markers associated with important traits, but also to identify candidate genes that regulate traits of interest\(^{50}\). For example, Wang et al.\(^{51}\) identified *cathepsin D*, *KCTD15*, and *csm22* as genes that affect body weight, body length, and total length of the Asian seabass using QTL mapping\(^{52}\). Wringe et al.\(^{53}\) found several major candidate growth genes (e.g., *GH2* and *Pax7*) in *Oncorhynchus mykiss*. Tao and Boulding\(^{54}\) found *GH* was significantly associated with growth rate of *Salvelinus alpinus*. Sun et al.\(^{55}\) identified two SNPs in *MSTN* that were significantly associated with body weight and Fulton’s factor in the common carp. Liu et al.\(^{56}\) identified a SNP in the UTR of *MSTN* that was strongly associated with total length, body length, and body weight of *Aristichthys nobilis*. In the present study, candidate genes identified from the growth-related QTLs may play critical roles in the genetic regulation of development, cell-proliferation, energy metabolism, and immunity. For example, *kif26b* was identified from the significant QTL intervals on LG11 as a candidate gene that was consistently associated with all three growth traits. As a kinesin family gene, *kif26b* plays an important role in the compact adhesion between mesenchymal cells\(^{61}\). It was also reported that overexpressing *kif26b* promoted cell proliferation and migration, while silencing of *kif26b* had the opposite effect\(^{62,63}\). Therefore, we hypothesized that *kif26b* may regulate growth traits through promoting cell proliferation. Another candidate gene, *bat1*, associated with body length and body weight, belongs to the family of amino acid transporters associated with type II membrane glycoproteins\(^{64}\), and plays an important role in the protein digestion and absorption pathway. The candidate gene, *gna1*, also associated with body length and body weight, is a key enzyme in the pathway for biosynthesis of UDP-N-acetylglucosamine, an important donor substrate for N-linked glycosylation\(^{65}\), which is important in amino sugar and nucleotide sugar metabolism. The gene *gfb1*, which encodes the glycosyltransferase 1, regulates glycosphingolipid biosynthesis\(^{66}\), which is important in globoside biosynthesis and metabolism. Finally, *amfr* is a ubiquitin protein ligase implicated in protein processing in the endoplasmic reticulum\(^{67}\). According to previous reports, we speculated that these genes may be involved in the growth regulation of *L. polyactis*, which requires further careful study to be verified. In addition, there are likely to be other undetected genes that regulate growth-related traits that need to be studied further using a genetic map with a higher density of SNPs.

Material and methods
Mapping family and DNA extraction. A F1 full-sib family of *L. polyactis* was generated for genetic mapping at the breeding station of Marine Fishery Institute of Zhejiang Province (Xishan Island, Zhoushan, China) in April 2017. Larva was cultured as described in Liu et al.\(^{2}\). The female and male parents were from Xiangshan Harbour wild stocks. Muscle samples of the parents were collected and stored in 100% ethanol, and kept in a −20 °C freezer. Seven months after hatching, 110 full-sibling offspring were randomly sampled for linkage analysis, and total length (cm), body length (cm), and body weight (g) were measured. The sex of each individual was identified based on anatomical observation of gonads. Muscle tissue from each individual was collected and preserved in 100% ethanol for DNA extraction.

Genomic DNA was extracted using the TIANGEN Marine animal DNA extraction kit (TIANGEN, Beijing, China). The concentration of the extracted genomic DNA was quantified using a NanoDrop 2000 (Thermo, USA) and the DNA integrity of each individual sample was evaluated by 1% agarose gel electrophoresis. DNA samples were stored at −20 °C for further experiments.

Sequencing library preparation and next generation sequencing. SNP identification and genotyping were performed by resequencing of the genome of *L. polyactis* individuals. Sequencing libraries were constructed following Nunes et al.\(^{59}\) with slight modifications. Genomic DNA of each individual was fragmented into about 350 bp fragments by a Covaris crusher. Sequencing libraries were generated using a TruSeq Library Construction Kit following the manufacturer’s instructions. Digested DNA fragments were examined by electrophoresis and were then ligated with barcode adaptors. Ligation products were pooled to select for 350–600 bp fragments using Pippin Prep (Sage Science, USA) after clean up with a QIAquick PCR Purification Kit (Qiagen, Germany). Libraries were enriched by PCR using Phusion High-Fidelity DNA Polymerase (New England Biolabs, USA). Finally, libraries were cleaned using the QIAquick PCR Purification Kit (Qiagen, Germany) and quantified using the KAPA Library Quantification Kit (Kapa Biosystems, USA) for paired-end sequencing on an Illumina HiSeq™ PE150 platform (Illumina, USA), which produced single-end raw reads of 150 bp.
Sequence data analysis and genotyping of SNPs. Barcodes were used to sort the raw reads from each individual. To ensure that reads were reliable, raw data (raw reads) in FASTQ format were processed through a series of quality control (QC) procedures with in-house C scripts. QC standards were as follows: (1) reads with $\geq 10\%$ unidentified nucleotides (Ns) were removed, (2) reads containing the HaeIII or EcoRI sequences were removed, and (3) reads with $>50\%$ of bases having a Phred quality $<5$ were removed. The clean reads from each individual were aligned against the reference genome (Xie et al., unpublished) using the Burrows-Wheeler Aligner (BWA) software (settings: mem -t 4 -k 32 -M -R). If multiple read pairs had identical external coordinates, the pair with the highest mapping quality was retained. SNP calling was performed for parents and progeny using the Broad Institute's open-source GATK software (-type UnifiedGenotyper). Unreliable SNPs were eliminated by a filtering process. A Perl script was used to filter the SNPs that had more than two genotypes. Any SNP with more than 20% missing data in both genotype and individual were removed from further analysis.

Linkage map construction. Linkage analysis was conducted by taking a pseudo-test cross strategy. SNPs were divided into three categories according to their segregation patterns: AB × AA or AB × BB (1:1 segregation only in female parent), AA × AB or BB × AB (1:1 segregation only in male parent), and AB × AB (1:2:1 segregation in both parents). Markers showing significant segregation distortion ($P < 0.001$) were removed. The remaining SNPs were used to construct the genetic map with the package Lep-MAP (https://sourceforge.net/projects/lep-map3/)\(^\text{69}\). Genetic maps were constructed following methods described in Shao et al.\(^\text{32}\). The genotype data were filtered manually to remove obvious Mendelian errors from the offspring. The group LOD value ranged from 4 to 20, depending on the linkage group. Maps were generated by regression mapping using Lep-MAP and the Kosambi mapping function in Lep-MAP was used to convert the recombination frequencies into map distances in centiMorgans (cm). The order of markers was obtained using the order markers module using 5' anchored PCR. An integrated map was constructed using male and female maps that were originally generated using only one input file. To speed up the computation, constant rates for genotype errors and recombination were used. Finally, marker positions were established using the order markers module.

QTL mapping for growth traits. Pearson's correlations of the three growth-related traits were performed for all progeny. The QTLs of growth traits were identified using MapQTL 5.0\(^\text{70}\) using Interval Mapping (IM). Automatic cofactor selection (backward elimination, $P < 0.05$) was used to select significantly associated markers as cofactors. The LOD significance threshold levels were determined by Permutation Tests with 1,000 permutations at significance level of $P < 0.05$. QTLs with LOD scores exceeding the genome-wide LOD threshold at $P < 0.05$ were considered to be significant. The location of each QTL was determined based on its LOD peak location and the surrounding region. MapQTL 5.0 was used to identify QTL reference values, including phenotypic variation and positive or negative additive effect for growth traits. The candidate genes in the QTL intervals were regarded as candidate genes of particular interest.

Ethics statement. This study was approved by the Animal Care and Use committee of Centre for Applied Aquatic Genomics at Zhejiang Academy of Agricultural Sciences. The methods were carried out in accordance with the approved guidelines.

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References
1. FishBase. https://www.fishbase.org (2014).
2. Liu, F. et al. Interspecific hybridization and genetic characterization of Larimichthys polyactis (♀) and L. crocea (♂). Aquacult. Int. 27, 663–674 (2019).
3. Li, Y., Han, Z., Song, N. & Gao, T. New evidence to genetic analysis of small yellow croaker (Larimichthys polyactis) with continuous distribution in China. Biochem. Syst. Ecol. 50, 331–338 (2013).
4. Chen, B. Y. et al. Broodstock cultivation and spawning induction techniques in small yellow croaker Pseudosciaena polyactis. Fish. Sci. 35, 250–254 (2016) (In Chinese with English Abstract).
5. Zhang, C., Ye, Z., Wan, R., Ma, Q. & Li, Z. Investigating the population structure of small yellow croaker (Larimichthys polyactis) using internal and external features of otoliths. Fish. Res. 153, 41–47 (2014).
6. Zhang, Y. et al. Mitochondrial DNA variation and population genetic structure in the small yellow croaker at the coast of Yellow Sea and East China Sea. Biochem. Syst. Ecol. 71, 236–243 (2017).
7. Liu, B. J., Zhang, B. D., Gao, T. X. & Liu, J. X. Signatures of temporal genetic differentiation of the small yellow croaker (Larimichthys polyactis) revealed by microsatellite markers. Fish. Res. 194, 50–54 (2017).
8. Liu, L. W. et al. In-depth transcriptome analysis of Larimichthys polyactis, de novo assembly, functional annotation. Mar Genom. 33, 27–29 (2017).
9. Chen, W. & Cheng, Q. Development of thirty-five novel polymorphic microsatellite markers in Pseudosciaena polyactis (Perciformes:Sciaenidae) and cross-species amplification in closely related species Pseudosciaena crocea. Biochem. Syst. Ecol. 47, 111–115 (2013).
10. Liu, B. J., Gao, T. & Liu, J. X. Development of 17 novel polymorphic microsatellites in the small yellow croaker Larimichthys polyactis. Conserv. Genet. Resour. 6, 397–399 (2013).
11. Ma, C. Y., Ma, H. Y. & Ma, L. R. Development and testing of 13 polymorphic microsatellite markers in Larimichthys polyactis (Sciaenidae) using 5' anchored PCR. Genet. Mol. Res. 10, 1455 (2011).
12. Cheng, Y., Wang, R., Sun, Y. & Xu, T. The complete mitochondrial genome of the small yellow croaker and partitioned Bayesian analysis of Sciaenidae fish phylogeny. *Genet. Mol. Biol.* 35, 191–199 (2012).

13. Kim, J. K. et al. Genetic structure of *Larimichthys polyactis* (Pisces: Sciaenidae) in the Yellow and East China Seas inferred from microsatellite and mitochondrial DNA analyses. *Anim. Cells Syst.* 16, 313–320 (2012).

14. Tong, J. G. & Sun, X. W. Genetic and genomic analyses for economically important traits and their applications in molecular breeding of cultured fish. *Sci. China Life Sci.* 58, 178–186 (2015).

15. Yue, G. H. Recent advances of genome mapping and marker-assisted selection in aquaculture. *Fish Fish.* 15, 376–396 (2014).

16. Chen, S. L. et al. Whole-genome sequence of a flatfish provides insights into ZW sex chromosome evolution and adaptation to a benthic lifestyle. *Nat. Genet.* 46, 253–260 (2014).

17. Xu, P. et al. Genome sequence and genetic diversity of the common carp. *Cyprinus carpio*. *Nat. Genet.* 46, 1212–1219 (2014).

18. Ao, J. Q. et al. Genome sequencing of the perciform fish *Larimichthys crocea* provides insights into molecular and genetic mechanisms of stress adaptation. *PloS Genet.* 11, e1005118 (2015).

19. Xu, T. et al. The genome of the miyii croaker reveals well-developed innate immune and sensory systems. *Sci. Rep.* 6, 21902 (2016).

20. Shao, C. et al. The genome and transcriptome of Japanese flounder provide insights into flatfish asymmetry. *Nat. Genet.* 49, 119–124 (2017).

21. Shao, C. et al. Chromosome-level genome assembly of the spotted sea bass, *Lateolabrax maculatus*. *GigaScience* 7, giy114 (2018).

22. Zhou, Q. et al. A chromosome-level genome assembly of the giant gourami (*Epinephelus lanceolatus*) provides insights into its innate immunity and rapid growth. *Mol. Ecol. Resour.* 19, 1322–1332 (2019).

23. Chen, Z. L. et al. De novo assembly of the goldfish (*Carassius auratus*) genome and the evolution of genes after whole-genome duplication. *Sci. Adv.* 5, 547 (2019).

24. Carlson, B. M., Omusko, S. W. & Gross, J. B. A high-density linkage map for Astyanax mexicanus using genotyping-by-sequencing technology. *G3*-*Genes Genom. Genet.* 5, 241–251 (2015).

25. Kocher, T. D., Lee, W. J., Sobolewski, H., Pennman, D. & McAndrew, B. A genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*). *Genetics* 148, 1225–1232 (1998).

26. Vignal, A., Milan, D., SanCristobal, M. & Eggen, A. A review on SNP and other types of molecular markers and their use in animal genetics. *Genet. Sel. Evol.* 34, 275 (2002).

27. Ren, P. et al. Genetic mapping and quantitative trait loci analysis of growth-related traits in the small abalone *Haliotis discolor* using restriction-site-associated DNA sequencing. *Aquaculture* 454, 163–170 (2016).

28. Yu, H. et al. Gains in QTL detection using an ultra-high density SNP map based on population sequencing relative to traditional RFLP/SSR markers. *PloS ONE* 6, e17595 (2011).

29. Yañé, J. M. et al. Genomewide single nucleotide polymorphism discovery in Atlantic salmon (*Salmo salar*): validation in wild and farmed American and European populations. *Mol. Ecol. Resour.* 16, 1002–1011 (2016).

30. Correa, K. et al. Genome-wide association study for resistance to Caligus rogercresseyi in Atlantic salmon (*Salmo salar* L.) using a 50K SNP genotyping array. *Aquaculture* 472, 61–65 (2017).

31. Elshire, R. J. et al. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS ONE* 6, e19379 (2011).

32. Shao, C. et al. Genome-wide SNP identification for the construction of a high-resolution genetic map of Japanese flounder (*Paralichthys olivaceus*): applications to QTL mapping of *Vibrio anguillarum* disease resistance and comparative genomic analysis. *DNA Res.* 22, 161–170 (2015).

33. Wang, L. et al. Construction of a high-density linkage map and fine mapping of QTL for growth in Asian seabass. *Sci. Rep.* 5, 16358 (2015).

34. Liu, P., Wang, L., Wong, S. M. & Yue, G. H. Fine mapping QTL for resistance to VNN disease using a high-density linkage map in Asian seabass. *Sci. Rep.* 6, 32122 (2016).

35. Peng, W. et al. An ultra-high density linkage map and QTL mapping for sex and growth-related traits of common carp (*Cyprinus carpio*). *Sci. Rep.* 6, 26693 (2016).

36. Liu, H. et al. A high-density genetic linkage map and QTL fine mapping for body weight in crucian carp (*Carassius auratus*) using 2b-RAD sequencing. *G3*-*Genes Genom. Genet.* 7, 2473–2487 (2017).

37. Kuang, Y. Y. et al. The genetic map of goldfish (*Carassius auratus*) provided insights to the divergent genome evolutions in the Cyprinidae family. *Sci. Rep.* 6, 34849 (2016).

38. Wang, S. M. et al. Construction of a high-density linkage map and fine mapping of QTLs for growth and gonad related traits in blunt snout bream. *Sci. Rep.* 7, 46509 (2017).

39. Numes, J. R. et al. Large-scale SNP discovery and construction of a high-density genetic map of *Colosoma macropomum* through genotyping-by-sequencing. *Sci. Rep.* 7, 46112 (2017).

40. Ao, J. et al. Construction of the high-density genetic linkage map and chromosome map of large yellow croaker (*Larimichthys crocea*). *Int. J. Mol. Sci.* 16, 26237–26248 (2015).

41. Nguyen, N. H., Rastas, P. M. A., Premachandra, H. K. A. & Knibb, W. First high-density linkage map and single nucleotide polymorphisms significantly associated with traits of economic importance in Yellowtail Kingfish *Seriola lalandi*. *Front. Genet.* 9, 127 (2018).

42. Palaiokostas, C. et al. A new SNP-based vision of the genetics of sex determination in European sea bass (*Dicentrarchus labrax*). *Genet. Sel. Evol.* 47, 68 (2015).

43. Fu, B., Liu, H., Yu, X. & Tong, J. A high-density genetic map and growth related QTL mapping in bighead carp (*Hypophthalmichthys nobilis*). *Sci. Rep.* 6, 28679 (2016).

44. Wang, W. et al. High-density genetic linkage mapping in turbot (*Scophthalmus maximus L.*) based on SNP markers and major sex- and growth-related regions detection. *PloS ONE* 10, e0120410 (2015).

45. Xie, M. et al. Restriction site-associated DNA sequencing for SNP discovery and high-density genetic map construction in southern catfish (*Silurus meridionalis*). *R Soc. Open Sci.* 5, 172058 (2018).

46. Gjerde, B. & Gjedrem, T. Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. *Aquaculture* 36, 97–110 (1984).

47. Luo, W. et al. Genetic parameter estimates for growth-related traits of blunt snout bream (*Megalobrama amblycephala*) using microsatellite-based pedigree. *Aquac. Res.* 45, 1881–1888 (2014).

48. Jiao, W. et al. High-resolution linkage and quantitative trait locus mapping aided by genome survey sequencing: building up an integrative genomic framework for a bivalve mollusc. *DNA Res.* 21, 83–101 (2013).

49. Weinge, B. F. et al. Growth-related quantitative trait loci in domestic and wild rainbow trout (*Oncorhynchus mykiss*). *BMC Genet.* 11, 63 (2010).

50. Gutierrez, A. P. et al. Genetic mapping of quantitative trait loci (QTL) for body-weight in Atlantic salmon (*Salmo salar*) using a 6.5K SNP array. *Aquaculture* 358–359, 61–70 (2012).

51. Song, W. et al. Construction of high-density genetic linkage maps and mapping of growth-related quantitative trait loci in the Japanese Flounder (*Paralichthys olivaceus*). *PloS ONE* 7, e50404 (2012).

52. Song, W. et al. Construction of a high-density microsatellite genetic linkage map and mapping of sexual and growth-related traits in half-smooth tongue sole (*Cynoglossus semilaevis*). *PloS ONE* 7, e52097 (2012).
53. Liu, F. et al. A genome scan revealed significant associations of growth traits with a major QTL and GHR2 in tilapia. Sci. Rep. 4, 7256 (2014).
54. O’Malley, K. G., Sakamoto, T., Danzmann, R. G. & Ferguson, M. M. Quantitative trait loci for spawning date and body weight in rainbow trout: testing for conserved effects across ancestrally duplicated chromosomes. J Hered 94, 273–284 (2003).
55. Reid, D. P., Szanto, A., Glebe, B., Danzmann, R. G. & Ferguson, M. M. QTL for body weight and condition factor in Atlantic salmon (Salmo salar): comparative analysis with rainbow trout (Oncorhynchus mykiss) and Arctic char (Salvelinus alpinus). Heredity 94, 166–172 (2005).
56. Mackay, T. F., Stone, E. A. & Ayroles, J. J. The genetics of quantitative traits: challenges and prospects. Nat. Rev. Genet. 10, 565–577 (2009).
57. Wang, C. M. et al. A high-resolution linkage map for comparative genome analysis and QTL fine mapping in Asian seabass. Lates calcarifer. BMC Genom. 12, 174 (2011).
58. Tao, W. J. & Boulding, E. G. Associations between single nucleotide polymorphisms in candidate genes and growth rate in Arctic char (Salvelinus alpinus L.). Heredity 91, 60–69 (2003).
59. Sun, Y. H., Yu, X. & Tong, J. Polymorphisms in myostatin gene and associations with growth traits in common carp. Int J Mol Sci. 13, 14956–14961 (2012).
60. Liu, L., Yu, X. & Tong, J. Molecular characterization of myostatin (MSTN) gene and association analysis with growth traits in the bighead carp (Aristichthys nobilis), Mol. Biol. Rep. 39, 9211–9221 (2012).
61. Uchiyama, Y. et al. Kil26b, a kinocin family gene, regulates adhesion of the embryonic kidney mesenchyme. Proc. Natl. Acad. Sci. U. S. A. 107, 9240–9245 (2010).
62. Teng, Y., Guo, B., Mu, X. & Liu, S. KIF26B promotes cell proliferation and migration through the FGF2/ERK signaling pathway in breast cancer. Biomed. Pharmacother. 108, 766–773 (2018).
63. Yang, X., Zhang, L. & Xie, L. Upregulation of KIF26B, cell migration and proliferation of Human ovarian cancer cell lines in vitro, and patient outcomes from Human bioinformatic analysis. Med. Sci. Monit. 24, 3863–3872 (2018).
64. Chairougdua, A. et al. Identification of an amino acid transporter associated with the cystinuria-related type ii membrane glycoprotein. J. Biol. Chem. 274(41), 28845–28848 (1999).
65. Wang, J., Liu, X., Liang, Y. H., Li, L. F. & Su, X. D. Acceptor substrate binding revealed by crystal structure of human glucosamine-6-phosphate N-acetyltransferase 1. FEBS Lett. 582(20), 2973–2978 (2008).
66. Jacob, F. et al. Expression of GBGT1 is epigenetically regulated by DNA methylation in ovarian cancer cells. BMC Mol. Biol. 15, 24 (2014).
67. Fang, S. Y. et al. The tumor autocrine motility factor receptor, gp78, is a ubiquitin protein ligase implicated in degradation from the endoplasmic reticulum. Proc. Natl. Acad. Sci. 98, 14422–14427 (2001).
68. Li, H. et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).
69. Rastas, P. Lep-MAP3: robust linkage mapping even for low-coverage whole genome sequencing data. Bioinformatics 33, 3726–3732 (2017).
70. Van Ooijen, J. W. MapQTL Version 5: software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Plant Research International, Wageningen, The Netherlands (2009).

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Author contributions
F. L. conducted preparation of the sequence data, bioinformatics analysis and manuscript preparation. W. Z., Q. X. and H. C. were involved in one or more processes of DNA extraction or bioinformatics analysis. Wantu Xu were involved in fish culture. F. L. and B. L. conceived, designed, and guided the research. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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