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Letter to the editor Open Access

Construction of a chromosome-level genome assembly for genome-wide identification of growth-related quantitative trait loci in *Sinocyclocheilus grahami* (Cypriniformes, Cyprinidae)

The Dianchi golden-line barbel, *Sinocyclocheilus grahami* (Regan, 1904), is one of the "Four Famous Fishes" of Yunnan Province, China. Given its economic value, this species has been artificially bred successfully since 2007, with a nationally selected breed ("*S. grahami*, Bayou No. 1") certified in 2018. For the future utilization of this species, its growth rate, disease resistance, and wild adaptability need to be improved, which could be achieved with the help of molecular marker-assisted selection (MAS). In the current study, we constructed the first chromosome-level genome of *S. grahami*, assembled 48 pseudo-chromosomes, and obtained a genome size of 1.49 Gb. We also performed QTL-seq analysis of *S. grahami* using the highest and lowest bulks (i.e., largest and smallest size) in both a sibling and random population. We screened two quantitative trait loci (QTLs) (Chr3, 14.9–39.1 Mb and Chr17, 4.1–27.4 Mb) as major growth-related locations. Several candidate genes (e.g., *map2k5*, *stat1*, *phf21a*, *sox6*, and *smad6*) were also identified, with functions related to growth, such as cell differentiation, neuronal development, skeletal muscle development, chondrogenesis, and immunity. These results built a solid foundation for in-depth MAS studies on the growth traits of *S. grahami*.

The Dianchi golden-line barbel (*Sinocyclocheilus grahami*, Cypriniformes, Cyprinidae) (Figure 1A) is a small-sized cyprinid with restricted distribution in the Dianchi basin and surrounding streams in Yunnan Province, China. Famed as a high-yield and nutrition-enriched fish, this species contains higher amount of protein, lipids, and essential amino acids than many common market fish, such as *Ctenopharyngodon idellus* (Valenciennes, 1844) (grass carp) and *Hypophthalmichthys nobilis* (Richardson, 1845) (bighead carp) (Zhao et al., 2013). However, the *S. grahami* population has declined sharply since the 1960s due to habitat damage, water pollution, and alien species invasion (Yang et al., 2007). As such, it was listed as an animal of Second-Class National Protection in 1989, and as an endangered fish in the "China Red Book of Endangered Animals" in 1998 (Le & Chen, 1998).

Since 2007, our team has successfully achieved the artificial breeding of *S. grahami* (Yang et al., 2007), which has not only helped in avoiding its wild extinction, but also opened a new era for its utilization. Moreover, after four generations of artificial selection, a new national breed ("*S. grahami*, Bayou No. 1") with accelerated growth and weakened inter-muscular bones was certified in 2018. This new breed highlights a successful paradigm of wildlife protection in China, i.e., from endangered species conservation to sustainable utilization. It is foreseeable that with "*S. grahami,* Bayou No. 1", focus will shift to the improvement of growth traits, which are among the most important economic characteristics in aquaculture (Yin et al., 2020).

Molecular marker-assisted selection (MAS) is an efficient method for trait improvement as it can effectively shorten selective breeding time and minimize possible negative risks to the environment (Yue, 2014). If high-precision chromosomal genomes are available, QTLs and candidate genes can be identified and applied for MAS. Commonly used methods for identifying QTLs and candidate genes in fish species studies include genome-wide association analysis (GWAS), QTL analysis, restriction site-associated DNA sequencing (RAD-seq), and transcriptome sequencing (RNA-seq) (Li et al.,

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2018; Liu et al., 2017; Salem et al., 2012; Yu et al., 2016). QTL-seq, which combines the advantages of both bulked segregant analysis (BSA) and whole-genome resequencing, is an innovative, rapid, and effective way in which to locate QTLs (Gu et al., 2018; Li et al., 2017; Takagi et al., 2013).

In the current study, we constructed a high-density linkage map of *S. grahami* using RAD-seq based on a full-sib family, including 169 offspring and their parents (F1 generation from domesticated Muyanghe population) cultivated in the Endangered Fish Conservation Center (EFCC) of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS). Based on the generated high-density genetic linkage map, we constructed a chromosome-level genome assembly with the assistance of our previous scaffold-level genome sequences (Yang et al., 2016). We also used the obtained chromosome-level genome as a reference and performed QTL-seq for growth based on sibling (~2 000 individuals) and random (~20 000 individuals) populations of *S. grahami*. All animal experiments were approved by the internal review board of KIZ, CAS (approval ID: SMKX-SQ-20170308-073). Detailed descriptions are provided in the Supplementary Materials and Methods.

Following RAD-seq and quality control, a total of 286 Gb of raw reads were generated through an Illumina HiSeqX-Ten platform. In total, 25 646 single nucleotide polymorphism (SNP) markers were obtained across all samples in the full-sib family, with 18 874 SNPs detected in more than 70% of the progeny. Among them, 12 412 SNPs with normal Mendelian segregation patterns were retained to construct a linkage map, with classification into 48 linkage groups.

With the assistance of our previous scaffold-level *S. grahami* genome (Yang et al., 2016), we assigned the linkage groups into 48 pseudo-chromosomes. Our final chromosome-level assembly contained 12 412 high-quality SNPs, with a genome size of 1.49 Gb (see Supplementary Tables S1, S2), accounting for 84.99% of the scaffold-level assembly (1.75 Gb). Figure 1B shows the distributions of SNPs, genes, and GC content at 100 kb genomic intervals, as well as the one-to-one syntenic relationships of orthologous pairs. In total, 20 558 gene pairs (430 syntenic blocks) between *S. grahami* and *Danio rerio* (Hamilton, 1822) (zebrafish) were obtained after genome-wide alignments, thus supporting a 2:1 syntenic relationship between the two species for almost all chromosomes (Figure 1C).

Based on QTL-seq analysis (Takagi et al., 2013), two bulks from the sibling population (~2 000 individuals) were constructed, i.e., fastest-growth bulk consisting of 30 extremely large individuals and slowest-growth bulk consisting of 30 extremely small individuals. Two parallel bulks from the random population (~20 000 individuals) were also constructed, with 30 extreme-sized individuals contained in each bulk. In the four constructed bulks, the mean body lengths (and weights) of the fastest-and slowest-growth bulks were 47.83±2.71 mm (1.91±0.32 g) and 22.52±1.58 mm (0.2±0.05 g) in the sibling population, and 45.5±4.48 mm (1.88±0.79 g) and 20.83±2.11 mm (0.15±0.037 g) in the random population, respectively. In each population, body length and weight showed significant differences (*P*<0.01) between the two extreme bulks (Supplementary Figure S1).

After whole-genome resequencing and quality control of the four bulk-pooled samples from the two populations, a total of 1 584 million reads (150 bp in length) were retained for subsequent analyses. According to the SNP identification procedure in QTL-seq analysis, a total of 282 476 and 281 611 sliding windows were detected in the sibling and random populations, respectively, with more than 15 SNPs in each window. Correspondingly, the *F*st values of each window ranged from 0.001 to 0.138 and 0.001 to 0.188, and the lowest *F*st values for the top 1/1 000 windows (*F*st cutoff) were 0.085 and 0.098 in each population, respectively. After filtering those chromosomes that contained sliding windows (<4) with *F*st values higher than the cutoff, as well as those regions with only sporadic sliding window distributions, we retained 12 QTLs from the sibling population and 29 QTLs from the random population (Figure 1D, E).

Additionally, when we combined Fisher's Exact test and bulk frequency ratio (BFR) parameter (BFR≥4), 152 and 310 significant SNPs were identified in the sibling and random populations, respectively. The major SNPs (≥8) screened from the sibling population were distributed in Chr3, Chr17, Chr33, Chr34, and Chr46 (Figure 1F), while the major SNPs screened from the random population showed a relatively scattered distribution in Chr3, Chr10, Chr11, Chr13, Chr14, Chr15, Chr17, Chr18, Chr19, Chr24, Chr28, Chr30, Chr36, Chr37, Chr39, and Chr48 (Figure 1G).

To identify more reliable QTLs, we combined the significant SNPs identified from both the sliding windows and BFR parameter, and observed that growth-related QTLs converged in Chr3, Chr17, Chr33, Chr34, and Chr46 in the sibling population, and in Chr3, Chr11, Chr14, Chr15, Chr17, Chr18, Chr28, Chr36, Chr37, and Chr39 in the random population. Furthermore, when considering both sibling and random populations together, the overlapping QTL intervals between the two populations were in Chr3 (14.9–39.1 Mb) and Chr17 (4.1–27.4 Mb) (Figure 1H, I), suggesting that these QTLs represent the greatest possible growth-related regions in *S. grahami*.

Simultaneously, we identified several genes within the two major candidate QTL intervals that contained significant SNPs (BFR≥4), which were thus considered as candidate genes relevant to *S. grahami* growth. These genes included *mitogen-activated protein kinase kinase 5* (*map2k5*), which had two SNPs with BFR values of 8.4 and 5, respectively; *signal transducer and activator of transcription 1-alpha/beta* (*stat1*) and *PHD finger protein 21A* (*phf21a*), which contained SNPs with BFR values ≥5; and *SRY-box 6* (*sox6*), *SMAD family member 6* (*smad6*), *tetraspanin-3* (*tspan3*), and *unconventional myosin-Va* (*myo5a*), which all contained SNPs with BFR values ≥4 but <5 (see Supplementary Figure S2).

The chromosome-level genome with 48 pseudo-chromosomes constructed here is consistent with previous karyotypic studies of *S. grahami* (Li et al., 1983; Xiao et al., 2002). In addition, the general 2:1 syntenic relationship between *S. grahami* and *D. rerio* demonstrated that the ancestor of *S. grahami* underwent another whole-genome duplication (WGD) after the teleost-specific genome duplication (TSGD), with the TSGD maintaining only 24–25 chromosomes in most extant fish, such as the model species

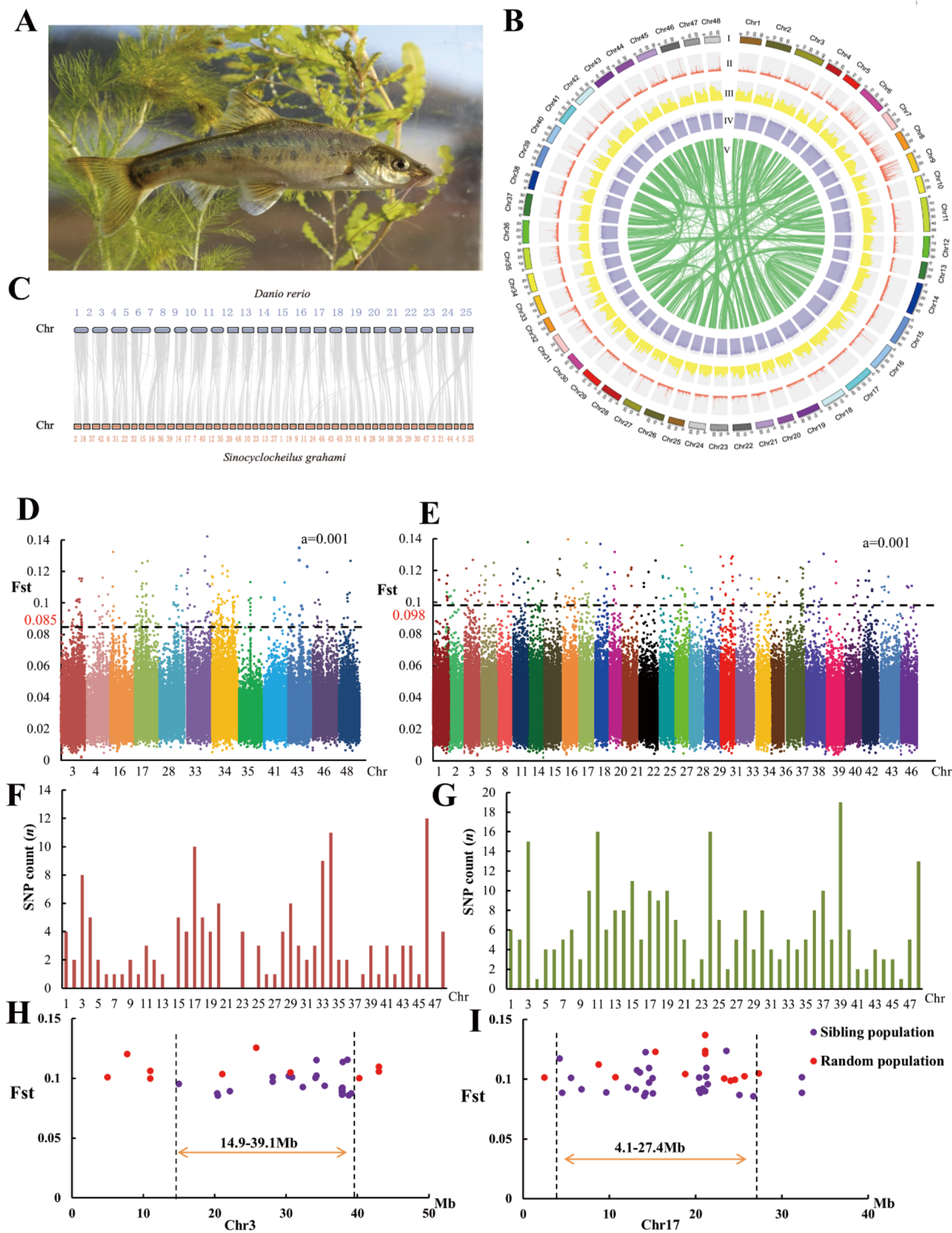

Figure 1 Chromosome-level genome and growth-related QTLs of *S. grahami*

A: Living specimen of *S. grahami*. B: Chromosome-level genome assembly showing length of each chromosome (I), density of SNP distribution in each 100 kb genomic interval (II), density of gene distribution in each 100 kb genomic interval (III), GC content in each 100 kb genomic interval (IV), and schematic of major inter-chromosomal relationships (V). C: General 2:1 syntenic relationship between *S. grahami* and *D. rerio*. D, E: Major significant QTL intervals identified by QTL-seq in sibling and random populations, respectively. X-axis represents different chromosomes (labeled with different colors); y-axis indicates *F*st values between two compared bulks. Each point represents *F*st value within a sliding window, with a=0.001 used as the threshold (calculated as *F*st=0.085 and 0.098 in sibling and random populations, respectively). F, G: Distribution of SNPs with high BFR (BFR≥4) in chromosome-level genome in sibling and random populations, respectively. X-axis represents different chromosomes; y-axis indicates number of SNPs identified in each chromosome. H, I: Overlapping QTL intervals between the two populations in Chr3 and Chr17, respectively. X-axis indicates localization in each chromosome; y-axis represents *F*st values between two groups. Double-sided arrow indicates the most overlapped QTL interval.

zebrafish (Xu et al., 2014).

In MAS practice, common QTLs between multiple populations/families are usually treated as valuable targets for containing relatively stable gene sites over long-term evolution (Barton & Keightley, 2002). Therefore, the two major candidate QTL intervals in Chr3 (14.9–39.1 Mb) and Chr17 (4.1–27.4 Mb) should be given priority in future MAS studies on *S. grahami*.

Moreover, according to the hypothesis that SNPs with greater BFR values locate at loci with more dominant relationships to target traits (Wang et al., 2013; Yao et al., 2017), we identified several candidate genes related to growth within these two QTL intervals. For example, the top candidate gene *map2k5* is highly conserved among various species. It is an upstream kinase of *extracellular signal regulated kinase 5* (*erk5*) with abundant expression in skeletal muscle (Dinev et al., 2001), and the *map2k5-erk5* pathway is critical in muscle cell differentiation (Dinev et al., 2001), myogenesis (Chen et al., 2017; Mauro et al., 2002), neural differentiation (Nishimoto et al., 2005), and growth factor mediation (Carter et al., 2009). Therefore, it is reasonable to infer that *map2k5* mutations may have affected the *map2k5-erk5* pathway and induced differences in growth between the fastest- and slowest-growth bulks. Of course, other candidate genes, such as *stat1*, *phf21a*, *smad6*, *sox6*, *myo5a*, and *tspan3*, may also have played important roles in the differences in growth between bulks as they are involved in functions related to neuronal development, endochondral bone formation, chondrogenesis, skeletal muscle development, myeloid development, cell differentiation, and immunity (Dinh et al., 2010; Estrada et al., 2011; Jackson et al., 2015; Kim et al., 2012; Lefebvre, 2019; Najjar & Fagard, 2010; Rancura et al., 2008; Song et al., 2011; Yeh & Klesius, 2012).

Nevertheless, we also identified several QTL differences between the two populations. In theory, these QLTs should not be excluded as candidate QTLs, but further research is required. Lv et al. (2016) identified 165 QTLs for growth-related traits in eight distinct families of *Cyprinus carpio* (Linnaeus, 1758) (common carp), and found only 36 QTLs were common in more than two families, with all others found to be distinct among families. Thus, both previous study and our present research suggest that differences between populations/families would, to a great extent, represent population/family-specific QTLs as growth is a complex trait affected by many genes. From this point of view, to improve MAS efficiency in complex traits such as growth, more populations/families with both simple and complex genetic backgrounds should be investigated.

DATA AVAILABILITY

All relevant sequences were deposited in the National Center for Biotechnology Information (NCBI) under accession No. PRJNA702560 and China National GeneBank (CNGB) under accession No. CNA0019203.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

J.X.Y., W.S.J., and Q.S. designed the study. Y.H.Y., X.A.W., Y.W.Z., A.L.W., X.D.H., M.W., and X.F.P. collected the samples and performed the laboratory work. Y.H.Y., X.H.Z., R.H.L., X.X.S., X.X.Y., and C.B. performed data analysis. Y.H.Y., X.H.Z., X.A.W., and R.H.L. drafted the paper. W.S.J., J.X.Y., and Q.S. revised the paper. All authors read and approved the final version of the manuscript.

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