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 a 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).

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright ©2021 Editorial Office of Zoological Research, Kunming Institute of Zoology, Chinese Academy of Sciences
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 HiSeq X-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 Fst values of each window ranged from 0.001 to 0.138 and 0.001 to 0.188, and the lowest Fst values for the top 1/1 000 windows (Fst cutoff) were 0.085 and 0.098 in each population, respectively. After filtering those chromosomes that contained sliding windows (~4) with Fst 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 (28) 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, myo5e, and tspn3, 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 QTLs 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.

**ACKNOWLEDGMENTS**

We would like to thank all staff from the Endangered Fish Conservation Center (EFCC) of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS), for assisting us in the cultivation of fish materials.

Yang-Hui Yin1,2,3,4,†, Xin-Hui Zhang5,6,8,*, Xiao-Ai Wang1,2,3,†, Rui-Han Li5,6,8,*, Yuan-Wei Zhang1,2,3,†, Xin-Xin You5,6,*, Xin-Di Huang1,2,3,†, An-Lu Wu1,2,3,†, Mo Wang5,6,*, Xiao-Fu Pan1,2,3,*, Chao Bian5,6,*, Wan-Sheng Jiang5,6,*, Qiong Shi5,6,*, Jun-Xing Yang1,2,3,†

1 State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Innovative Academy of Seed Design, Chinese Academy of Sciences, Kunming, Yunnan 650224, China
2 Yunnan Key Laboratory of Plateau Fish Breeding, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650224, China
3 Yunnan Engineering Research Center for Plateau-Lake Health and Restoration, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650224, China
4 University of Chinese Academy of Sciences, Beijing 100049, China
5 Shenzhen Key Lab of Marine Genomics, Guangdong Provincial Key Lab of Molecular Breeding in Marine Economic Animals, BGI Academy of Marine Sciences, BGI Marine, BGI, Shenzhen, Guangdong 518083, China
6 BGI Education Center, University of Chinese Academy of Sciences, Shenzhen, Guangdong 518083, China
7 Key Laboratory for Conserving Wildlife with Small Populations in Yunnan, Faculty of Biodiversity Conservation, Southwest Forestry University, Kunming, Yunnan 650224, China
8 Hunan Engineering Laboratory for Chinese Giant Salamander’s Resource Protection and Comprehensive Utilization, and Key Laboratory of Hunan Forest and Chemical Industry Engineering, Jishou University, Zhangjiajie, Hunan 427000, China

*Authors contributed equally to this work
†Corresponding authors, E-mail: jiangwuchina@163.com; shiqiong@genomics.cn; yangjx@mail.kiz.ac.cn

**REFERENCES**

Barton NH, Keightley PD. 2002. Understanding quantitative genetic
variation. Nature Reviews Genetics, 3(1): 11–21.

Carter EJ, Cosgrove RA, Gonzalez I, Eisenmann JH, Lovett FA, Cobb LJ, et al. 2008. MEK5 and ERK5 are mediators of the pro-myogenic actions of IGF-2. Journal of Cell Science, 122: 3104–3112.

Chen TH, Chen CY, Wen HC, Chang CC, Wang HD, Chuu CP, et al. 2017. YAP promotes myogenic differentiation via the MEK5-ERK5 pathway. The FASEB Journal, 31(7): 2963–2972.

Dinev D, Jordan BW, Neufeld B, Lee JD, Lindemann D, Rapp UR, et al. 2001. Extracellular signal regulated kinase 5 (ERK5) is required for the differentiation of muscle cells. EMBO Reports, 2(9): 829–834.

Dinh ML, Dong Y, Hagiwara N. 2010. Evolutionary conservation of the role of Sox6 in terminal differentiation of skeletal muscle. Developmental Biology, 344(1): 533.

Estrada KD, Retting KN, Chin AM, Lyons KM. 2011. Smad6 is essential to limit BMP signaling during cartilage development. Journal of Bone and Mineral Research, 26(10): 2496–2510.

Gu XH, Jiang DL, Huang Y, Li BJ, Chen CH, Lin HR, et al. 2018. Identifying a major QTL associated with salinity tolerance in Nile tilapia using QTL-seq. Marine Biotechnology, 20(1): 98–107.

Jackson HE, Ono Y, Wang XG, Elworthy S, Ingham PW. 2015. The role of Sox6 in zebralight muscle fiber type specification. Skeletal Muscle, 5(1): 2.

Kim HG, Kim HT, Leach NT, Lan F, Ullmann R, Silahtaroglu A, et al. 2012. Translocations disrupting PHP21A in the Potokki-Shaffer syndrome region are associated with intellectual disability and craniofacial anomalies. The American Journal of Human Genetics, 91(1): 56–72.

Le PQ, Chen YJ. 1998. China Red Data Book of Endangered Animals: Fish. Beijing: Science Press. (in Chinese)

Lefebvre V. 2019. Roles and regulation of SOX transcription factors in skeletogenesis. Current Topics in Developmental Biology, 133: 171–193.

Li HL, Gu XH, Li BJ, Chen CH, Lin HR, Xia JH. 2017. Genome-wide QTL analysis identified significant associations between hypoxia tolerance and mutations in the GPR132 and ABCG4 Genes in Nile Tilapia. Marine Biotechnology, 19(5): 441–453.

Li N, Zhou T, Geng X, Jin YL, Wang XG, Liu SK, et al. 2018. Identification of novel genes significantly affecting growth in catfish through GWAS analysis. Molecular Genetics and Genomics, 293(3): 587–599.

Li SS, Wang RF, Liu GZ, Wang XG, Li CY. 1993. A karyotypic study of eight species of freshwater teleost fish. Hereditas (Beijing), 5(4): 25–28. (in Chinese)

Liu HY, Fu BD, Pang MX, Feng X, Yu XM, Tong JO. 2017. A high-density genetic linkage map and QTL fine mapping for body weight in crucian carp (Carassius auratus) using 2b-RAD sequencing. G3: Genes, Genomes, Genetics, 7(8): 2473–2478.

Lv WH, Zhong XJ, Xu KN, Cao DC, Yan YQ, Sun XW. 2016. QTL variations for growth-related traits in eight distinct families of common carp (Cyprinus carpio). BMC Genetics, 17(1): 65.

Mauro A, Ciccarelli C, De Cesaris P, Scooglio A, Bouché M, Molinaro M, et al. 2002. PKCα-mediated ERK, JNK and p38 activation regulates the myogenic program in human rhabdomyosarcoma cells. Journal of Cell Science, 115(18): 3587–3599.

Najjar I, Fagard R. 2010. STAT1 and pathogens, not a friendly relationship. Biochimie, 92(8): 425–444.

Nishimoto S, Kusakabe M, Nishida E. 2005. Requirement of the MEK5-ERK5 pathway for neural differentiation in Xenopus embryonic development. EMBO Reports, 6(11): 1064–1069.

Rancura KGO, Monteiro MR, Carvalho RF, Martins C, Wasko AP, Casaletti L, et al. 2008. Brain distribution of myosin Va in rainbow trout Oncorhynchus mykiss. Acta Zoologica, 89(1): 29–36.

Salem M, Vallejo RL, Leeds TD, Pati Y, Liu SX, Sabbagh A, et al. 2012. RNA-Seq identifies SNP markers for growth traits in rainbow trout. PLoS One, 7(5): e36264.

Song H, Yan YL, Titus T, He XJ, Postlethwaite JH. 2011. The role of stat1b in zebralight hematopoiesis. Mechanisms of Development, 128(7–10): 442–456.

Takagi H, Abe A, Yoshida K, Kosugi S, Natsume S, Mitsuoka C, et al. 2013. QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. The Plant Journal, 74(1): 174–183.

Wang RJ, Sun LY, Bao LS, Zhang JR, Jiang YL, Yao J, et al. 2013. Bulk segregant RNA-seq reveals expression and positional candidate genes and allele-specific expression for disease resistance against enteric septicaemia of catfish. BMC Genomics, 14(1): 929.

Xiao H, Zhang RD, Feng JG, Ou YM, Li WX. 2002. Nuclear DNA content and ploidy of seventeen species of fishes in Sinocyclocheilus. Zoological Research, 23(3): 195–199. (in Chinese)

Xu P, Zhang XF, Wang XM, Li JT, Liu GM, Kuang YY, et al. 2014. Genome sequence and genetic diversity of the common carp, Cyprinus carpio. Nature Genetics, 46(11): 1212–1219.

Yang JX, Chen XN, Bai J, Fang DM, Qiu Y, Jiang WS, et al. 2016. The Sinocyclocheilus cavelfish genome provides insights into cave adaptation. BMC Biology, 14(1): 1.

Yang JX, Pan FX, Li ZY. 2007. Preliminary report on the successful breeding of the endangered fish Sinocyclocheilus grahami endemic to Dianchi Lake. Zoological Research, 28(3): 329–331. (in Chinese)

Yao J, Wang RJ, Liu ZJ. 2017. Genetic analysis using RNA-Seq: bulk segregant RNA-Seq. In: Liu ZJ. Bioinformatics in Aquaculture: Principles and Methods. Hoboken: John Wiley & Sons Ltd, 169–178.

Yeh HY, Klesius PH. 2012. Channel catfish, Ictalurus punctatus (Rafinesque), tetraspanin membrane protein family: identification, characterization and phylogenetic analysis of tetraspanin 3 and tetraspanin 7 (CD231) transcripts. Fish Physiology and Biochemistry, 38(6): 1553–1563.

Yin YH, Jiang WS, Pan XF, Yang JX. 2020. Recent progress in growth trait of aquaculture fish. Journal of Fishery Sciences of China, 27(4): 463–484. (in Chinese)

Yu H, You XX, Li J, Liu HK, Meng ZN, Xiao L, et al. 2016. Genome-wide mapping of growth-related quantitative trait loci in orange-spotted grouper (Epinephelus coioides) using double digest restriction-site associated DNA sequencing (ddRADseq). International Journal of Molecular Sciences, 17(4): 501.

Yue GH. 2014. Recent advances of genome mapping and marker-assisted selection in aquaculture. Fish and Fisheries, 15(3): 376–396.

Zhao YP, Pan XF, Yang JX, Chen XY, Li ZY, Wang XA. 2013. Analysis of the nutritional components in muscle of Sinocyclocheilus grahami and S. tingen. Zoological Research, 34(6): 636–639. (in Chinese)