Supplementary Materials

Supplementary Materials and Methods

Materials and RAD sequencing

In April 2016, we random selected one female and one male from the F1 generation (domesticated Muyanghe population) of *S. grahami* as a parental pair to construct a full-sib family through artificial reproduction. All individuals were cultivated in the Endangered Fish Conservation Center (EFCC) of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS), Kunming, Yunnan Province, China. After three months, the parents and 169 offspring were sampled to construct a high-density genetic linkage map.

Genomic DNA of 171 individuals was extracted for construction of restriction site-associated DNA (RAD) libraries in accordance with previously published protocols (Baird et al., 2008). In brief, each 30 μL enzyme reaction system, which included 1 μg of genomic DNA and 15 U of *EcoRI* (15 U/μL; Thermo Scientific, Waltham, MA, USA), was incubated at 65 °C for 10 min. Barcode adapters with sample-specific nucleotide codes were designed following the standard Illumina adapter design flow (Illumina, San Diego, CA, USA). A unique barcode adapter (10 μmol) for each DNA sample was added to each individual reaction system. Eleven pools were prepared and 300–500 bp fragments were collected. The corresponding 11 libraries were individually sequenced on a HiSeqX-ten platform (Illumina) using routine 150 bp paired-end sequencing.

Construction of genetic linkage map and chromosome-level genome assembly

Sequenced raw reads were filtered according to the following criteria: reads with >5% of unknown nucleotides and/or with excessive low-quality regions (<Q20) were removed using the SOAPnuke package (http://soap.genomics.org.cn/). Stacks (v0.99998) was employed for sequence mapping, stacks assembly, single nucleotide polymorphism (SNP) discovery, and genotyping according to previous research (Catchen et al., 2013). A minimum coverage of 10× for parental samples was applied to construct stacks and catalogue loci. Only those loci with <30% of missing data in the progenies were collected for further analysis (Wang et al., 2015). Linkage groups (LGs) were assigned for genetic map construction using JoinMap v4.1 (Van Ooijen, 2006), and the minimum logarithm of odds (LOD) score was set to more than 10 (Zhang et al., 2018, 2019). Scaffolds from our previously assembled genome (Yang et al., 2016) were integrated with this genetic map using Chromonomer v1.08 (Amores et al., 2014). Genome synteny analysis, in comparison with *Danio rerio* (zebrafish), was performed using MCScan, jcvi, and Circos software (Krzywinski et al., 2009; Tang et al., 2008, 2015).

Fish cultivation, bulk collection, sampling, and sequencing

In February 2018, a full-sib family population (generated from one female × one male, ~2 000 individuals) and a mixed family population (generated from multiple females × males, ~20 000 individuals) from farmed "*S. grahami,* Bayou No. 1" were constructed independently using artificial reproduction. The offspring from both

populations were cultivated for eight months in the EFCC. Rearing conditions for the full-sib and mixed-family populations were kept as consistent as possible, including similar stocking density, natural water temperature, and identical feeding frequency (twice every day) and fodder volume (~3% of fish weight). We then roughly separated the individuals of each population into large-, medium-, and small-sized groups (placed in different buckets) according to body size. In total, 500 individuals (200 extremely large individuals, 200 extremely small individuals, and 100 medium-sized individuals) from each population were transferred into two fish tanks (1 m×0.6 m×1 m) in the KIZ laboratory for further cultivation to observe growth. The individuals in the two fish tanks from the full-sib family and mixed-family were defined as the "sibling population" and "random population", respectively. Rearing conditions for the sibling and random populations were also kept as consistent as possible, including similar stocking density, natural water temperature, and identical feeding frequency (twice every day) and fodder volume (~3% of fish weight).

After two months of regular feeding, the fish were sampled, including measurement of body weight and length. Subsequently, we selected 30 extremely large individuals and 30 extremely small individuals as the two extremes of growth in each population. Finally, within the two examined populations, four bulks were obtained.

Fish from the four bulks were first euthanized using MS-222. Muscle tissues from each individual were collected and kept under sterile conditions. Subsequently,

genomic DNA of each individual was extracted using an Animal Genomic DNA Kit (Tsingke Biological Technology, Beijing, China) in accordance with the manufacturer's instructions. DNA concentration and quality of each sample were measured using a Nanodrop and Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA). We collected the final bulk samples using a pooled strategy: i.e., extracted an equal amount of DNA from each of the 30 individual samples in the same bulk to build a pooled sample for that bulk. In total, four pooled samples corresponding to the four bulks were obtained, and these samples were then labelled in a paired-end 150 bp library and sequenced using the Illumina Hiseq X-Ten platform. The sequencing depth of each sample was generally more than 30× the genome coverage.

QTL-seq analysis of growth-related QTLs

After next-generation sequencing, the short reads were filtered using SOAPnuke (http://soap.genomics.org.cn/). We then removed polymerase chain reaction (PCR) duplications using Picard (http://broadinstitute.github.io/picard/). The unique mapped reads were then used to perform QTL analysis and SNP identification. QTL-seq analysis was conducted according to Gu et al. (2018) with slight modification. The clean whole-genome sequencing (WGS) data were individually mapped to the *S. grahami* reference genomes using bowtie2 (Langmead & Salzberg, 2012) with default parameters. SAMtools and PoPoolation2 (Kofler et al., 2011) were employed to calculate genome-wide Fst values between two groups with different extreme traits

using the sliding window approach, with the following parameters: min-qual 30, --min-count 3, --min-coverage 15, --max-coverage 2 000, --min-covered-fraction 0, --window-size 10 000, --step-size 5 000, and --pool-size 30. Those Fst values identified in a window with less than 15 SNPs were further filtered. An empirical value of a=0.001 was used as the genome-wide significant threshold (Gu et al., 2018; Li et al., 2017). Genome-wide significant QTL regions were defined as regions with at least the four top Fst values. Additionally, sliding windows with significant Fst values and sporadic distributions in the genome were excluded from further analysis.

Significant SNP identification

Similar to QTL-seq analysis, SAMtools and PoPoolation2 were employed for identification of SNPs (Yao et al., 2017). Those SNP loci with more than two allele variants were discarded. Initial SNPs were screened to improve quality by setting the following two factors: 1) minimum reads in each group ≥15; and 2) total minor allele reads count >3. Significant SNPs were identified by Fisher's Exact test, and the threshold was set with a false discovery rate (FDR) *P*-value of ≤0.05. In addition, the BFR parameter was calculated for each allele. A BFR value of 4 was considered the cutoff for identification of SNPs linked to growth-related traits (Ramirez-Gonzalez et al., 2015; Wang et al., 2013; Yao et al., 2017).

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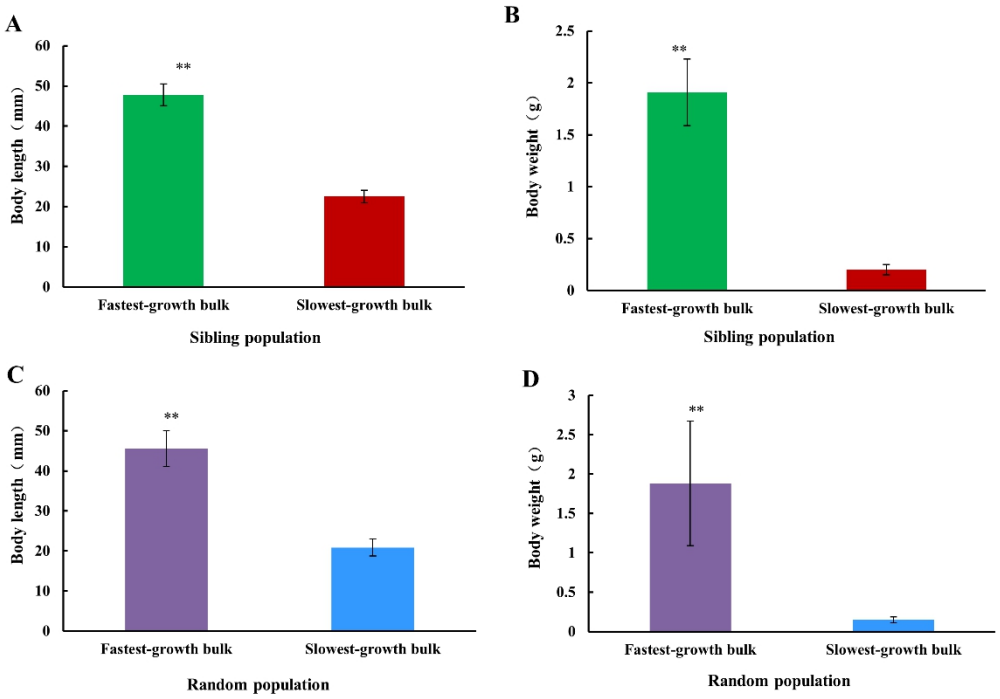
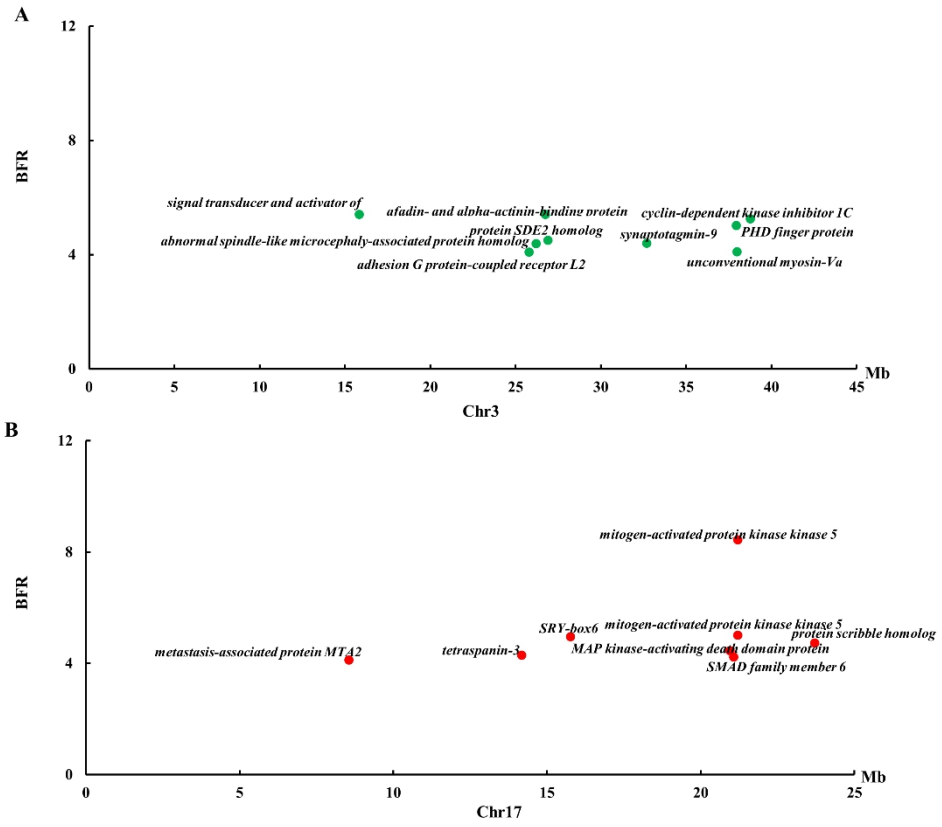
Supplementary Figures



Supplementary Figure S1 Phenotypic variations in examined fish. Body length (**A**) and body weight (**B**) variations in sibling population. Body length (**C**) and body weight (**D**) variations in random population. All variations were statistically significant (**, P<0.01).



Supplementary Figure S2 Growth-related candidate genes. Genes with significant SNPs (BFR≥4) in intervals (14.9–39.1 Mb) of Chr3 (**A**) and genes with significant SNPs (BFR≥4) in intervals (4.1–27.4 Mb) of Chr17 (**B**).

Supplementary Tables

Supplementary Table S1 Summary of assembled chromosome-level genome of *S. graham*

Chr ID	Length of Chr (Mb)	No. of anchored genes	No. of anchored scaffolds
Chr1	31.5	760	49
Chr2	37.7	909	49
Chr3	44.3	1149	85
Chr4	23.5	517	25
Chr5	26.1	631	23
Chr6	40.5	1064	69
Chr7	28.1	746	42
Chr8	28.6	599	43
Chr9	29.7	644	49
Chr10	26.3	599	33
Chr11	52.2	1171	102
Chr12	31.9	673	59
Chr13	25.9	632	33
Chr14	48.6	1050	79
Chr15	43.3	1099	63
Chr16	40.1	1093	59
Chr17	43.1	1004	61
Chr18	35.4	826	62
Chr19	33.8	778	53
Chr20	23.2	665	38
Chr21	26	678	45
Chr22	27.1	653	42
Chr23	25.9	607	40
Chr24	24.3	587	41
Chr25	23.8	639	40
Chr26	29.6	742	46
Chr27	27.1	646	39
Chr28	30.4	611	54
Chr29	29.1	702	42
Chr30	25.4	698	26
Chr31	29.8	845	66
Chr32	22.2	558	41
Chr33	29.5	690	34
Chr34	28.1	704	39
Chr35	32.3	686	47
Chr36	34.9	823	47
Chr37	33.4	955	45
Chr38	28.8	703	47
Chr39	32.9	844	32
Chr40	28.2	763	43
Chr41	30.3	751	32
Chr42	31.8	994	42
Chr43	30.3	818	28
Chr44	27.9	775	44
Chr45	30.5	846	37
Chr46	28.1	694	30
Chr47	23.3	648	31

Chr48	23.1	655	34
Linked	1,490	36,924	2,210
Unlinked total	263.8	5,185	29,894
Linked percent	84.99%	87.68%	6.8%

Supplementary Table S2 Inter-chromosomal relationships among various chromosomes

Pairs		Pairs	
Chr1	Chr19	Chr20	Chr48
Chr2	Chr18	Chr21	Chr44
Chr3	Chr11	Chr22	Chr32
Chr4	Chr5	Chr24	Chr46
Chr6	Chr31	Chr25	Chr3
Chr7	Chr40	Chr26	Chr29
Chr8	Chr28	Chr30	Chr47
Chr9	Chr11	Chr33	Chr41
Chr10	Chr23	Chr36	Chr39
Chr12	Chr35	Chr37	Chr42
Chr13	Chr27	Chr38	Chr34
Chr14	Chr17	Chr43	Chr45
Chr15	Chr16		

Note: Chromosomes (Chr) marked in red (Chr3 and Chr11) indicate existence of multiple inter-chromosomal relationships.

Supplementary Table S3 Sliding windows and Fst values for Chr3 and Chr17

Sibling population							Random population						
Chr. ID	Peak position of the window	Scaffold ID	Peak position of the window	SNP number in the window	Average minimum coverage	Fst values	Chr. ID	Peak position of the window	Scaffold ID	Peak position of the window	SNP number in the window	Average minimum coverage	Fst values
Chr3	14928780	NW_015505571.1	230000	19	23.1	0.09540	Chr3	4953259	NW_015505722.1	250000	23	18.3	0.10114
Chr3	20329698	NW_015505348.1	1290000	23	21.3	0.08744	Chr3	7717161	NW_015505378.1	520000	46	19.5	0.12041
Chr3	20409698	NW_015505348.1	1370000	23	20.7	0.08576	Chr3	10952504	NW_015505210.1	3105000	45	19.1	0.10007
Chr3	22089096	NW_015505693.1	555000	64	25.6	0.08938	Chr3	10957504	NW_015505210.1	3100000	32	20.2	0.10638
Chr3	28082878	NW_015505511.1	220000	52	23	0.09729	Chr3	21039491	NW_015505403.1	835000	40	19.2	0.10366
Chr3	28087878	NW_015505511.1	215000	74	24.2	0.10134	Chr3	25796891	NW_015505736.1	605000	36	19	0.12574
Chr3	30369504	NW_015506250.1	220000	22	21.5	0.10212	Chr3	30595764	NW_015506287.1	85000	25	18.8	0.10509
Chr3	30805764	NW_015506287.1	295000	22	21.8	0.10118	Chr3	40222322	NW_015506578.1	185000	37	19.4	0.10029
Chr3	32315842	NW_015505292.1	615000	29	23	0.09276	Chr3	42988202	NW_015506014.1	405000	23	19.6	0.10992
Chr3	34070941	NW_015505229.1	1140000	28	23.9	0.10059	Chr3	42993202	NW_015506014.1	400000	22	19.4	0.10589
Chr3	34215941	NW_015505229.1	1285000	43	22.9	0.11545							

Chr3	34220941	NW_015505229.1	1290000	71	22.8	0.10240							
Chr3	35384929	NW_015506197.1	235000	101	22.6	0.09394							
Chr3	37792486	NW_015506553.1	230000	38	22.7	0.09171							
Chr3	37797486	NW_015506553.1	225000	30	23.6	0.08915							
Chr3	37817486	NW_015506553.1	205000	22	24.1	0.08636							
Chr3	37857486	NW_015506553.1	165000	18	21.1	0.09220							
Chr3	37862486	NW_015506553.1	160000	20	21.7	0.08782							
Chr3	37867486	NW_015506553.1	155000	24	22.7	0.08988							
Chr3	37927486	NW_015506553.1	95000	37	24.6	0.11378							
Chr3	38587585	NW_015505995.1	565000	43	23	0.11553							
Chr3	38772814	NW_015507056.1	80000	130	26.1	0.08600							
Chr3	39097913	NW_015506129.1	245000	20	23.4	0.08748							
Chr17	4193602	NW_015505604.1	455000	24	22.8	0.11741	Chr17	2442171	NW_015505951.1	1230000	23	19	0.10145
Chr17	4493602	NW_015505604.1	155000	24	23.4	0.08871	Chr17	8711637	NW_015505419.1	1145000	22	20.3	0.11238
Chr17	5544797	NW_015506040.1	410000	39	22.7	0.10127	Chr17	10674542	NW_015505897.1	40000	40	19.1	0.10187
Chr17	6745427	NW_015505902.1	40000	22	24.2	0.09180	Chr17	15322323	NW_015505255.1	920000	18	20.2	0.12295

Chr17	9600415	NW_015506101.1	355000	25	23.2	0.08895	Chr17	18757422	NW_015505203.1	2515000	25	18.8	0.10456
Chr17	12113958	NW_015505697.1	445000	16	23.4	0.09318	Chr17	21066719	NW_015506055.1	320000	31	19.4	0.12391
Chr17	13019057	NW_015505794.1	460000	65	22.8	0.09152	Chr17	21071719	NW_015506055.1	315000	41	19.8	0.13718
Chr17	13179057	NW_015505794.1	620000	29	22.6	0.10775	Chr17	21076719	NW_015506055.1	310000	35	19.7	0.12153
Chr17	13492323	NW_015505255.1	2750000	27	24.1	0.10590	Chr17	23277903	NW_015505315.1	1705000	20	19.3	0.10065
Chr17	14022323	NW_015505255.1	2220000	22	23	0.08626	Chr17	24042903	NW_015505315.1	940000	29	20.6	0.09892
Chr17	14177323	NW_015505255.1	2065000	109	22.4	0.12267	Chr17	24547903	NW_015505315.1	435000	43	19.3	0.09956
Chr17	14182323	NW_015505255.1	2060000	194	26.9	0.08854	Chr17	25628002	NW_015505740.1	645000	17	19.7	0.10250
Chr17	14562323	NW_015505255.1	1680000	52	23.7	0.09720	Chr17	27305986	NW_015505398.1	765000	28	19.6	0.10502
Chr17	14567323	NW_015505255.1	1675000	44	24.1	0.10956							
Chr17	14962323	NW_015505255.1	1280000	34	24.6	0.10104							
Chr17	15007323	NW_015505255.1	1235000	18	22.5	0.08828							
Chr17	20391719	NW_015506055.1	995000	84	23.2	0.09112							
Chr17	20396719	NW_015506055.1	990000	48	24	0.10146							
Chr17	20536719	NW_015506055.1	850000	53	22.9	0.08871							
Chr17	20806719	NW_015506055.1	580000	33	22.7	0.09231							

Chr17	21076719	NW_015506055.1	310000	40	24.7	0.09012
Chr17	21211719	NW_015506055.1	175000	64	22.3	0.10239
Chr17	21216719	NW_015506055.1	170000	61	22.4	0.10939
Chr17	21381719	NW_015506055.1	5000	26	23.4	0.09598
Chr17	23542903	NW_015505315.1	1440000	16	20.6	0.12386
Chr17	25063002	NW_015505740.1	80000	20	23.1	0.087
Chr17	26665986	NW_015505398.1	125000	36	24.3	0.08585
Chr17	32284487	NW_015505469.1	1230000	22	22.2	0.08879
Chr17	32289487	NW_015505469.1	1235000	22	23.4	0.10174