Chromosome-level genome assembly of largemouth bass (*Micropterus salmoides*) using PacBio and Hi-C technologies

Kuo He1,5, Liulan Zhao1,5, Zihao Yuan2,3,5, Adelino Canario4, Qiao Liu1, Siyi Chen1, Jiazhong Guo1, Wei Luo1, Haoxiao Yan1, Dongmei Zhang1, Lisen Li4 & Song Yang1✉

The largemouth bass (*Micropterus salmoides*) has become a cosmopolitan species due to its widespread introduction as game or domesticated fish. Here a high-quality chromosome-level reference genome of *M. salmoides* was produced by combining Illumina paired-end sequencing, PacBio single molecule sequencing technique (SMRT) and High-through chromosome conformation capture (Hi-C) technologies. Ultimately, the genome was assembled into 844.88 Mb with a contig N50 of 15.68 Mb and scaffold N50 length of 35.77 Mb. About 99.9% assembly genome sequences (844.00 Mb) could be anchored to 23 chromosomes, and 98.03% assembly genome sequences could be ordered and directed. The genome contained 38.19% repeat sequences and 2693 noncoding RNAs. A total of 26,370 protein-coding genes from 3415 gene families were predicted, of which 97.69% were functionally annotated. The high-quality genome assembly will be a fundamental resource to study and understand how *M. salmoides* adapt to novel and changing environments around the world, and also be expected to contribute to the genetic breeding and other research.

**Background & Summary**

The largemouth bass, *Micropterus salmoides* (Perciformes, Centrarchidae), is a native of North America introduced in other parts of the world, including the Iberian Peninsula, Italy, Mexico and China, either as a game or farmed fish1–3. It is now one of the top ten most common aquatic species in every continent, except Antarctica4,5, and has been listed among the top 100 invasive species6, with temperature and hydrologic changes as main predictors of its distribution1,7. Although its main habitat is freshwater lakes and rivers, it colonizes brackish waters, such as in the Gulf of Mexico and the Atlantic coasts of North America8. Largemouth bass has been introduced into China from the US in 19832, and it has become one of the main aquaculture species in China for its fast growth2,9.

The whole genome information is the basis for studying the nature of organisms, including advantages during biological invasions and adaptation to extreme environments such as hypoxia10–12, climate change13,14, temperature15,16 and salinity17,18. With the development of sequencing technology, genome research has been studied more deeply and accurately19. More and more fish genomes have been decoded, such as yellow perch (*Perca flavescens*)20, golden pompano (*Trachinotus ovatus*)21 and dark sleeper (*Odontobutis potamophila*)22, etc. Moreover, the Nile tilapia and Pacific bluefin tuna genome have been re-sequenced to improve the genome assembly and fill the previously missed gaps23,24. These genome studies have greatly elevated our understanding about genetics, environmental adaptive selection, and evolutionary history of the target species. These more detailed genomic data can also facilitate studies on nutritional requirements, disease control and prevention, and to improve traits of economic interest25–27.
In the present study, a novel high-quality chromosome-level genome assembly of largemouth bass was generated by single-molecule real-time sequencing combined with Illumina paired-end sequencing and Hi-C (Fig. 1). The final assembled genome size of *M. salmoides* was 844.88 Mb with an N50 contig length of 15.30 Mb and scaffold N50 length of 35.77 Mb. A total of 844.00 Mb assembled genome sequences were anchored on 23 chromosomes. The genome contained 38.19% repeat sequences and 2693 noncoding RNAs. A total of 26,370 protein-coding genes from 3415 gene families were predicted, of which 97.69% were functionally annotated.

**Methods**

**Ethics statement.** All experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in China. The sampled fish in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Animal Science and Technology of Sichuan Agricultural University, Sichuan, China, under permit No. DKY-YS13287.
Sequencing libraries. Tissues from a two-year-old adult female largemouth bass (body weight 1487 g, length 36 cm), obtained from an aquaculture farm of Chongzhou, Sichuan province, China, were used to construct genomic DNA sequencing libraries (muscle) and transcriptome sequencing libraries (liver, brain, muscle, heart, kidney, gill, and gonad). All the tissues were stored in liquid nitrogen until use.

For short-read sequencing, genomic DNA was extracted from 500 mg of muscle using cetyl trimethylammonium bromide (CTAB) before chloroform purification. The genomic DNA was sonicated to a fragment size of 350 bp and the paired-end genomic library was prepared following the Illumina standard protocol, including terminal repair, polyA and adaptor addition, target fragment selection and PCR processes (Illumina, San Diego, CA, USA). The resulted library was quality checked using Agilent Bioanalyser 2100 and qPCR, and sequenced on an Illumina NovaSeq 6000 sequencing platform with paired-end 150 bp read layout.

For long-read sequencing, genomic DNA (~8 µg) was sheared into a large fragment by g-TUBE (Covaris), purified and recovered by AMPure PB magnetic beads, and used to construct single-molecule real-time bell (SMRTbell) sequencing libraries by the SMRTbell Template Prep Kit 2.0 (PacBio). The end-repaired fragments were size-selected using the Blue Pippin Size-Selection System (Sage Science, MA, USA), and damage-repaired using the SMRTbell Damage Repair Kit (PacBio). Then the products were combined polymerase using the PacBio DNA/Polymerase Kit before sequenced on the PacBio Sequel platform.

The full-length transcriptome was used to generate RNA data for gene prediction from a sample pool consisting of muscle, liver, gonad, kidney, gut, blood, and gills. Total RNA was extracted by TRIzol extraction reagent (Invitrogen, USA) according to the manufacturer's protocol. RNA purity was checked using the NanoPhotometer spectrophotometer (IMPLEN, CA, USA). The resulted library was quality checked using Agilent Bioanalyzer 2100 and qPCR, and sequenced on an Illumina NovaSeq 6000 sequencing platform with paired-end 150 bp read layout.

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| Library | Sequencing platform | Clean data (Gb) | Depth (×) | Contig N50 (Mb) | GC content (%) | Q20 (%) | Q30 (%) | Genome size (Mb) |
|---------|---------------------|-----------------|-----------|----------------|----------------|---------|---------|-----------------|
| Short reads Illumina NovaSeq 6000 | 58.51 | 66.94 | — | 40.88 | 96.63 | 93.36 | 874.14 |
| Long reads PacBio Sequel | 94.69 | 112.07 | 15.68 | 40.78 | — | — | 844.88 |
| Hi-C Illumina NovaSeq 6000 | 77.53 | 94.06 | 15.30 | 40.78 | 97.59 | 93.49 | 844.00 |

Table 1. *M. salmoides* genome sequencing statistics.

| Group | Cluster Num | Cluster Len | Order Num | Order Len |
|-------|-------------|-------------|-----------|-----------|
| Chr01 5 | 40,821,207 | 4 | 40,732,462 |
| Chr02 15 | 42,659,052 | 9 | 42,039,393 |
| Chr03 7 | 37,588,897 | 6 | 37,343,944 |
| Chr04 13 | 40,393,715 | 9 | 39,732,765 |
| Chr05 15 | 39,747,164 | 6 | 38,411,921 |
| Chr06 10 | 36,025,099 | 6 | 35,600,334 |
| Chr07 9 | 34,881,373 | 6 | 34,516,066 |
| Chr08 2 | 37,271,896 | 2 | 37,271,896 |
| Chr09 5 | 37,188,422 | 4 | 37,114,295 |
| Chr10 4 | 36,011,566 | 3 | 35,768,921 |
| Chr11 11 | 33,902,165 | 5 | 33,113,071 |
| Chr12 15 | 35,527,541 | 8 | 34,268,756 |
| Chr13 5 | 33,494,735 | 4 | 33,265,410 |
| Chr14 11 | 34,134,741 | 8 | 33,564,293 |
| Chr15 24 | 37,902,394 | 11 | 35,937,762 |
| Chr16 9 | 32,104,916 | 6 | 31,675,598 |
| Chr17 7 | 32,964,910 | 4 | 32,674,911 |
| Chr18 26 | 34,562,858 | 13 | 33,055,325 |
| Chr19 31 | 41,218,652 | 16 | 38,871,204 |
| Chr20 6 | 32,259,510 | 5 | 32,214,040 |
| Chr21 3 | 28,886,792 | 3 | 28,886,792 |
| Chr22 18 | 36,175,891 | 7 | 34,208,627 |
| Chr23 15 | 28,271,698 | 8 | 27,127,050 |
| Total (Ratio %) 266 (97.08) | 843995194 (99.9) | 153 (57.52) | 827394836 (98.03) |

Table 2. The sequence distribution of each chromosome using Hi-C technology. Note: Chr01-23 represent 23 chromosomes; Cluster Num: the number of sequences located on a chromosome; Cluster Len: the length of sequence located on a chromosome; Order Num: the number of sequences of the direction can be determined; Order Len: the sequence length of the direction can be determined.
on the PacBio Sequel platform. Raw reads were processed into error corrected reads of insert (ROIs) using Iso-seq pipeline with minFullPass = 0 and minPredictedAccuracy = 0.90. Next, full-length, non-chemiric (FLNC) transcripts were determined by searching for the polyA tail signal and the 5′ and 3′ cDNA primers in ROIs. Full-length consensus sequences obtained from ICE (Iterative Clustering for Error Correction) were polished using Quiver. Finally, Full-length transcriptome sequencing yielded 20 Gb of clean data, including 26,369 high-quality consensus isoforms sequences with an average length of 2,895 bp.

Genome survey and assembly. The size, heterozygosity, and repetitive sequences in the *M. salmoides* genome were estimated by the analysis of k-mer frequency distribution of Illumina paired-end reads using the kmer_freq_stat script (Biomarker Technologies, Beijing, China), based on the formula $G = (N_{k\text{-mer}} - N_{\text{error}_{k\text{-mer}}})/D$ (where $G$: genome size; $N_{k\text{-mer}}$: the number of k-mers; $N_{\text{error}_{k\text{-mer}}}$: the number of depth 1 k-mers; $D$: the k-mer depth). After removing the k-mers with abnormal depth, a total of 49.16 M k-mers were obtained with a k-mers peak at a depth of 56 (Fig. 2). A total of 58.51 Gb high-quality filtered data was generated from the Illumina short read DNA library, with 66.94× genome coverage, a Q20 of 96.63% and a Q30 of 91.36% (Table 1). The genome size was estimated at 874.14 Mb, with 0.12% heterozygosity, 30.03% repetitive sequences, and 40.88% GC content (Table 1).

For long-read sequencing, reads longer than 500 bp generated by the PacBio Sequel platform were collected and a *de novo* genome was assembled initially using SMARTdenovo59 based on the data corrected by Canu v. 1.550. Subsequently, three rounds of refinement of the *de novo* genome were performed using Pilon31.
by Illumina short read sequencing data. Finally, the long-read SMRTbell library generated a total of 94.69 Gb (112.07 × genome coverage) with a reads N50 of 2880533 kb and an average read length of 24.75 kb. After error correction and assembly, an 844.88 Mb genome was assembled from 265 contigs with a N50 of 15.68 Mb (Table 1).

### Hi-C analysis and chromosome assembly

Hi-C libraries were prepared as previously reported\(^32,33\). Briefly, muscle tissue cells were fixed with formaldehyde to maintain the 3D structure of DNA in cells and the cells were digested using restriction endonuclease Hind III. Then, biotin-labeled bases were introduced using the DNA terminal repair mechanism. DNA (4 µg) was fragmented by a Covaris S220 focused-ultrasonicator (Gene Company Limited, Hong Kong) and 300–700 bp fragments were recovered. The DNA fragments containing interaction relationships were captured by streptavidin immunomagnetic beads for library construction. Library concentration and insert size were determined using the Qubit 3.0 and LabChip GX platforms (PerkinElmer), respectively. qPCR was used to estimate the effective concentration of the library. High quality Hi-C libraries were sequenced on the Illumina NovaSeq 6000 sequencing platform, and the sequencing data were used for chromosome-level assembly\(^34\). The software Burrows-Wheeler Aligner (BWA-MEM v. 0.7.10-r789) was used to align the sequencing pair-end clean reads with the sequence of the assembled genome to obtain the uniquely mapped read pairs\(^35\). The uniquely mapped read pairs were processed using HiC-Pro\(^36\). The genome contigs,
split into 50 kb segments, combined with uniquely matched Hi-C data, were clustered, ordered and directed onto the pseudochromosomes using LACHESIS with the following parameters: CLUSTER_MIN_RE_SITES = 30; CLUSTER_MAX_LINK_DENSITY = 2; CLUSTER_NONINFORMATIVE_RATIO = 2; ORDER_MIN_N_RES_IN_TRUN = 68; ORD-ER_MIN_N_RES_IN_SHREDS = 67. Finally, the chromosome assemblies were cut into 100 kb bins of equal lengths and the interaction signals generated by the valid mapped read pairs between each bin were visualized in a heat map.

In total, 277.88 million read pairs (77.53 Gb clean data; 94.06 × coverage of the genome) were generated from the Hi-C library (Table 1), of which 77.26% were uniquely mapped on the assembled genome. Of the unique mapped read pairs, 60.67% were the valid interaction pairs (130.26 million), which were used for the next Hi-C assembly (Table S1). A total of 844.00 Mb (99.9%) assembled genome sequences were anchored on 23 chromosomes, and the order and direction of 827.39 Mb (98.03%) sequences could be determined. The detailed distribution of each chromosome sequence was shown in Table 2. The heat map of the Hi-C assembly interaction bins is consistent a genome assembly of excellent quality (Fig. 3). Finally, the genome size of _M. salmoides_ was assembled at 844.88 Mb, while contig N50 and scaffold N50 were 15.30 Mb and 35.77 Mb, respectively (Table 1).

**Repeats prediction.** The repetitive elements of the _M. salmoides_ genome were identified and annotated using RepeatModeler containing RECON and RepeatScout. The derived repetitive sequences were searched against curated libraries and the repetitive DNA element databases Repbase, REXdb and Dfam. The LTR retrotransposon retriever was applied to identify the output from LTRharvest and LTR_FINDER. The results were combined and deduplicated, and the repetitive elements were finalized by RepeatMasker. About 38.19% _M. salmoides_ genome was repetitive sequences, composed mainly of class II transposable elements (Table 3).

**Genes prediction and annotation.** The prediction of the genome gene structure was based on three different strategies: _ab initio_-based, homolog-based, and unigene-based. Genscan, Augustus v2.4, GlimmerHMM v3.0.4, GeneID v1.4 and SNAP (version 2006-07-28) were used to perform _ab initio_-based prediction. GeMoMa v1.3.1 was used for prediction based on homologous species. Hisat v2.0.4 and Stringtgie v1.2.3 were used for assembly based on reference transcripts, and TransDecoder v2.0.2 and GeneMarkS -t v5.1 were used for gene prediction. PASA v2.0.2 was used to predict unique genes based on unreferenced assembly of full-length transcriptome data. Finally, EVM v1.1.1 was used to integrate the prediction results obtained by the above three methods, and PASA v2.0.2 was used to modify the final gene models. A total of 26,370 protein-coding genes were predicted by integrating the prediction of _ab initio_, homology-based and RNA-seq strategies (Table S2), with average gene length of 14,483 bp, exon length of 2,601 bp, coding sequence of 1,724 bp and intron length of 11,882 bp (Table 4). Finally, 25,760 genes (97.69% of the total) were successfully annotated to GO, KEGG, KOG, TrEMBL, and NR database (Table S3).

Blastn searches using the Rfam database as input against the _M. salmoides_ genome was used to identify microRNA and rRNA and TRNAScan-SE was used to identify tRNA. Non-coding RNAs were predicted to be 2,639, including 633 microRNAs (miRNA) of 84 families, 230 rRNA genes of 4 families and 1,830 tRNA genes of 25 families (Table S4). Pseudogenes were predicted in the following way. The predicted protein sequences were used to search for homologous gene sequences (putative genes) through BLAT alignment. Then GeneWise was used to search for immature termination codons and code-shifting mutations in the gene sequences to obtain pseudogenes. In total, 986 pseudogenes were identified with a total length of 5,885,501 bp and an average length of 5,969 bp (Table S4).

| Item                      | Count     |
|---------------------------|-----------|
| Gene Number               | 26,370    |
| Gene Length (bp)          | 381,932,021 |
| Average Gene Length (bp)  | 14,483.58 |
| Exon Length (bp)          | 68,599,926 |
| Average Exon Length (bp)  | 2,601.44  |
| Exon Number               | 260,466   |
| Average Exon Number       | 9.88      |
| CDS Length (bp)           | 45,485,238 |
| Average CDS Length (bp)   | 1,724.89  |
| CDS Number                | 253,748   |
| Average CDS Number        | 9.62      |
| Intron Length (bp)        | 313,332,095 |
| Average Intron Length (bp)| 11,882.14 |
| Intron Number             | 234,096   |
| Average Intron Number     | 8.88      |

**Table 4.** The basic information statistics of assembled genome.
Data Records
The sequencing data (Full-length transcriptome, Hi-C, Illumina and PacBio) have been deposited in SRA (Sequence Read Archive) database as SRR12886575, SRR12886576, SRR12886577, and SRR12886578. The assembly genome data was deposited in GenBank. The assembly genome data, gene CDS and Exon data and functional annotations were also stored in Figshare.

Technical Validation
The assembly was evaluated using three criteria: the mapping of Illumina reads, core gene integrity, and BUSCO assessment. The Benchmarking Universal Single Copy Orthologs were searched in CEGMA v2.5.8 and BUSCO v 3.0.69 to evaluate the conserved core genes in the genome. The Illumina reads fully (99.54%) mapped to the assembled genome, including 97.78% of paired-end reads. A total of 445 out of 458 conserved eukaryotic core genes from the CEGMA database were found in the assembled genome (Table S5). Finally, 97.49% of the complete BUSCOs were included in the assembled genome (Table S5). In summary, this is a high-quality de novo assembly reference genome.

Code availability
All commands and pipelines used in data processing were executed according to the manual and protocols of the corresponding bioinformatics software.

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References
1. Bae, M.-J., Murphy, C. A. & García-Berthou, E. Temperature and hydrologic alteration predict the spread of invasive largemouth Bass (Micropterus salmoides). *Sci. Total Environ.* 659, 58–66, https://doi.org/10.1016/j.scitotenv.2018.05.001 (2018).
2. Bai, J., Dijar, L.-C., Quan, Y. & Liang, S. Taxonomic status and genetic diversity of cultured largemouth bass *Micropterus salmoides* in China. *Aquaculture* 278, 27–30, https://doi.org/10.1016/j.aquaculture.2008.03.016 (2008).
3. García-Berthou, E. et al. Introduction pathways and establishment rates of invasive aquatic species in Europe. *Can. J. Fish. Aquat. Sci.* 62, 453–463, https://doi.org/10.1139/f05-017 (2005).
4. García-Berthou, E. Ontogenetic diet shifts and interrupted piscivory in introduced largemouth bass (*Micropterus salmoides*) gill and liver: Aggravation of oxidative stress, inhibition of immunity and promotion of cell apoptosis. *Fish and Shellfish Immunology* 98, 923–936, https://doi.org/10.1016/j.fsi.2019.11.056 (2020).
5. Lowe, S., Browne, M., Boudjelas, S. & Pooper, M. D. 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. *The Invasive Species Specialist Group*, 12 pp, https://www.researchgate.net/publication/273442552_100_of_the_World's_Worst_Invasive_Alien_Species_A_Selection_From_the_Global_Invasive_Species_Database (2000).
6. Letizia, C. M. et al. The role of alien fish (the centrarchid *Micropterus salmoides*) in lake food webs highlighted by stable isotope analysis. *Freshwat. Biol.* 63, 1130–1142, https://doi.org/10.1111/fwb.13122 (2018).
7. Glover, D. C., Devries, D. R. & Wright, R. A. Effects of temperature, salinity and body size on routine metabolism of coastal largemouth bass *Micropterus salmoides*. *J. Fish Biol.* 81, 1463–1478, https://doi.org/10.1111/j.1095-8649.2012.03385.x (2012).
8. Zhou, Y.-L., Ge, L.-E., Wang, W.-G., Li, M.-J. & Lin, S.-M. High dietary lipid level alters the growth, hepatic metabolism enzyme, and anti-oxidative capacity in juvenile largemouth bass *Micropterus salmoides*. *Fish Physiol. Biochem.* 46, 125–134, https://doi.org/10.1007/s10695-019-00705-7 (2020).
9. Sun, J. L. et al. Interactive effect of thermal and hypoxia on largemouth bass (*Micropterus salmoides*) gill and liver: Aggravation of oxidative stress, inhibition of immunity and promotion of cell apoptosis. *Fish and Shellfish Immunology* 98, 923–936, https://doi.org/10.1016/j.fsi.2019.11.056 (2020).
10. Gou, X. et al. Whole-genome sequencing of six dog breeds from continuous altitudes reveals adaptation to high-altitude hypoxia. *Genome Res.* 24, 1308–1315, https://doi.org/10.1101/gr.171876.113 (2014).
11. Huerta-Sánchez, E. et al. Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. *Nature* 512, 194–197, https://doi.org/10.1038/nature13408 (2014).
12. Sun, J. L. et al. Acute hypoxia changes the mode of glucose and lipid utilization in the liver of the largemouth bass (*Micropterus salmoides*). *Sci. Total Environ.* 713, 135157, https://doi.org/10.1016/j.scitotenv.2019.135157 (2020).
13. Hoffmann, A. A. & Sgro, C. M. Climate change and evolutionary adaptation. *Nature* 470, 479–485, https://doi.org/10.1038/nature09670 (2011).
14. Dam, H. G. Evolutionary adaptation of marine zooplankton to global change. *Annual review of marine science* 5, 349–370, https://doi.org/10.1146/annurev-marine-122111-172229 (2013).
15. Cruz, A. L. B. et al. Similar temperature dependencies of glycolytic enzymes: an evolutionary adaptation to temperature dynamics? *BMC Syst. Biol.* 6, 151, https://doi.org/10.1186/1752-0509-6-151 (2012).
16. Chen, Z. & Nàrum, S. R. Whole genome resequencing reveals genomic regions associated with thermal adaptation in redband trout. *Mol. Ecol.* 30, 162–174, https://doi.org/10.1111/mec.15717 (2021).
17. Sun, C. et al. Chromosome-level genome assembly for the largemouth bass *Micropterus salmoides* provides insights into adaptation to fresh and brackish water. *Molecular Ecology Resources* 21, 301–315, https://doi.org/10.1111/1755-0998.13256 (2021).
18. Xiong, Y. et al. Comparisons of Salinity Adaptation in Terms of Growth, Body Composition, and Energy Budget in Juveniles of Rainbow and Steelhead Trouts (*Oncorhynchus mykiss*). *J. Oceanic Univ. China* 18, https://doi.org/10.18201/ji10802-199-3779-4 (2019).
19. Gao, Y. et al. Single-molecule Real-time (SMRT) Isoform Sequencing (Iso-Seq) in Plants: The Status of the Bioinformatics Tools to Unravel the Transcriptome Complexity. *Current Bioinformatics* 14, 566–573, https://doi.org/10.2174/157489614666190204151746 (2019).
20. Peron, R. et al. Characterization of a Y-specific duplication/insertion of the anti-Mullerian hormone type II receptor gene based on a chromosome-scale genome assembly of yellow perch, *Perca flavescens*. *Molecular Ecology Resources* 20, 531–543, https://doi.org/10.1111/1755-0998.13133 (2020).
21. Zhang, D. C. et al. Chromosome-level genome assembly of golden pompano (*Trachinotus ovatus*) in the family Carangidae. *Scientific Data* 6, 216, https://doi.org/10.1038/s41597-019-0238-8 (2019).
22. Jia, Y. et al. A Chromosome-Level Genome Assembly of the Dark Sleeper Odontobutis potamophila. *Genome Biology and Evolution* 13, eava271, https://doi.org/10.1093/gbe/eva271 (2021).
23. Conte, M. A., Gammerdinger, W. J., Bartie, K. L., Pennman, D. J. & Kocher, T. D. A high quality assembly of the Nile Tilapia (*Oreochromis niloticus*) genome reveals the structure of two sex determination regions. * BMC Genomics* 18, 341, https://doi.org/10.1186/s12864-017-3723-5 (2017).
24. Suda, A. et al. Improvement of the Pacific bluefin tuna (*Thunnus orientalis*) reference genome and development of male-specific DNA markers. *Scientific Reports* 9, 14450, https://doi.org/10.1038/s41598-019-50978-4 (2019).
25. Shi, C. M., Zhao, H., Zhai, X. L., Chen, Y. J. & Lin, S. M. Linseed oil can decrease liver fat deposition and improve antioxidant ability of juvenile largemouth bass, Micropterus salmoides. *Fish Physiol. Biochem.* 45, 1513–1521, https://doi.org/10.1007/s10695-019-00636-3 (2019).

26. Camus, A., Griffin, M., Armwood, A. & Soto, E. A Spontaneous Outbreak of Systemic Edwardsiella piscicida Infection in Largemouth Bass Micropterus salmoides (Lacepède, 1802) in California, USA. *J. Fish Dis.* 42, 759–763, https://doi.org/10.1111/jfd.12961 (2019).

27. Zhu, Q., Wang, Y. & Feng, J. Rapid diagnosis of largemouth bass ranavirus in fish samples using the loop-mediated amplification method. *Mov. Cell Probes* 52, 101569, https://doi.org/10.1016/j.mcp.2020.101569 (2020).

28. Krolach, J. et al. Real-time DNA sequencing from single polymerase molecules. *Methods Enzymol.* 472, 431–455, https://doi.org/10.1016/bs.mie.2017.09.011 (2020).

29. Liu, H., Wu, S., Li, A. & Ruan, J. SMARTdenovo: a de novo assembler using long noisy reads. *GigaByte* 2021, 1–9, https://doi.org/10.4647/gigaabyte.15 (2021).

30. Koren, S. et al. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res.* 27, 722–736, https://doi.org/10.1101/gr.215087.116 (2017).

31. Walker, B. J. et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9, e121633, https://doi.org/10.1371/journal.pone.0121633 (2014).

32. Bao, S. P. et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159, 1665–1680, https://doi.org/10.1016/j.cell.2014.11.021 (2014).

33. Gong, G. et al. Chromosomal-level assembly of yellow catfish genome using third-generation DNA sequencing and Hi-C analysis. *Gigascience* 7, https://doi.org/10.1093/gigascience/giy120 (2018).

34. Burton, J. N. et al. Chromosome-scale scaffolding of de novo genome assemblies based on transcriptom interactions. *Nat. Biotechnol.* 31, 1119–1125, https://doi.org/10.1038/nt.2013.277 (2013).

35. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760, https://doi.org/10.1093/bioinformatics/btp324 (2009).

36. Servant, N. et al. HIS-C-Pro: an optimized and flexible pipeline for Hi-C data processing. *Genome Biology* 16, 259, https://doi.org/10.1186/s13059-015-0881-x (2015).

37. Bao, Z. & Eddy, S. R. Automated de novo identification of repeat sequence families in sequenced genomes. *Genome Res.* 12, 1269–1276, https://doi.org/10.1101/gr.88502.0 (2002).

38. Price, A. L., Jones, N. C. & Pevzner, P. A. De novo identification of repeat families in large genomes. *Bioinformatics* 21, i351–i358, https://doi.org/10.1093/bioinformatics/bti018 (2005).

39. Bao, W., Kojima, K. K. & Kohany, O. RepBase Update, a database of repetitive elements in eukaryotic genomes. *Nucleic Acids Res.* 40, D122–D139, https://doi.org/10.1093/nar/gkd131 (2012).

40. Alioto, T., Blanco, E., Parra, G. I. & Guigo, R. Using geneid to Identify Genes. *Bioinformatics* 20, ii215–ii225, https://doi.org/10.1093/bioinformatics/btm194 (2004).

41. Bao, Z. & Eddy, S. R. Automated de novo identification of repeat sequence families in sequenced genomes. *Genome Res.* 12, 1269–1276, https://doi.org/10.1101/gr.88502.0 (2002).

42. Ou, S. & Jiang, N. LTR_retriever: A Highly Accurate and Sensitive Program for Identification of Long Terminal Repeat Retrosopons. *Bioinformatics* 25, 2021–2030, https://doi.org/10.1093/bioinformatics/btq220 (2009).

43. Ou, S. & Jiang, N. LTR_FINDER_parallel: parallelization of LTR_FINDER enabling rapid identification of long terminal repeat retrotransposons. *Mob. DNA* 10, 48, https://doi.org/10.1093/mobdbx/txz041 (2019).

44. Ou, S. & Jiang, N. LTR_FINDER: parallelization of LTR_FINDER enabling rapid identification of long terminal repeat retrotransposons. *Mob. DNA* 9, 101569, https://doi.org/10.1093/mobdbx/txz041 (2019).

45. Tarailo-Graovac, M. & Chen, N. Using RepeatMasker to Identify Repetitive Elements in Genomic Sequences. *Curr. Protoc. Bioinformatics* 25, https://doi.org/10.1002/047129593X.ta15.01 (2009).

46. Driscoll, D. A. & Hardy, C. M. Dispersal and phylogeography of the agamid lizard Amphibolurus nobbi in fragmented and continuous habitat. *Mov. Ecol.* 14, 1613–1629, https://doi.org/10.1111/j.1365-294X.2005.02309.x (2005).

47. Stanke, M. & Waack, S. Gene prediction with a hidden Markov model and a new intron submodel. *Bioinformatics* 19, i215–i225, https://doi.org/10.1093/bioinformatics/bti1080 (2003).

48. Majchrows, W. H., Pertea, M. & Salzberg, S. L. TigrScan and GlimmerHMM: two open source ab initio eukaryotic gene-finders. *Bioinformatics* 20, 2878–2879, https://doi.org/10.1093/bioinformatics/btf315 (2004).

49. Aho-Toigo, T., Blanco, E., Parra, G. I. & Guigo, R. Using geneid to Identify Genes. *Curr. Protoc. Bioinformatics* 64, e56, https://doi.org/10.1002/cpbi.56 (2018).

50. Shapiro, M. D. et al. Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* 428, 717–723, https://doi.org/10.1038/nature02415 (2004).

51. Keilwagen, J. et al. Using intron position conservation for homology-based gene prediction. *Nucleic Acids Res.* 44, e89, https://doi.org/10.1093/nar/gkw992 (2016).

52. Keilwagen, J., Hartung, F., Paulini, M., Twardziok, S. O. & Grau, J. Combining RNA-seq data and homology-based gene prediction for plants, animals and fungi. *BMC Bioinformatics* 19, 189, https://doi.org/10.1186/s12859-018-2203-5 (2018).

53. Kim, D., Langmead, B. & Salzberg, S. L. HISAT: a fast spliced aligner with low memory requirements. *Fish Physiol. Biochem.* 45, 1513–1521, https://doi.org/10.1007/s10695-019-00636-3 (2019).

54. Pertea, M. et al. Rfam: annotating non-coding RNAs in complete genomes. *Nucleic Acids Res.* 33, D70–D82, https://doi.org/10.1093/nr/nri215 (2005).

55. Heese, R., Chu, J. S.-C., Wang, K., Pei, J. & Chen, N. GenBlastA: enabling BLAST to identify homologous gene sequences. *Genome Res.* 19, 143–149, https://doi.org/10.1101/gr.082081.108 (2009).

56. NCBI Sequence Read Archive https://identifiers.org/ncbi/insdc.sra:SRR12886575 (2021).

57. NCBI Sequence Read Archive https://identifiers.org/ncbi/insdc.sra:SRR12886576 (2021).

58. NCBI Sequence Read Archive https://identifiers.org/ncbi/insdc.sra:SRR12886577 (2021).

59. NCBI Sequence Read Archive https://identifiers.org/ncbi/insdc.sra:SRR12886578 (2021).
Author contributions
Song Yang managed the grants, supervised the laboratory work, and led the design of this study. Kuo He, Liulan Zhao and Zihao Yuan performed bioinformatics and also drafted the manuscript. Adelino Canario, Qiao Liu, Siyi Chen, Jiazhong Guo, Wei Luo, and Lisen Li revised the manuscript. Dongmei Zhang, Haoxiao Yan participated in the tissue sampling. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to S.Y.

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