Chromosome genome assembly and annotation of the yellowbelly pufferfish with PacBio and Hi-C sequencing data

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Pufferfish are ideal models for vertebrate chromosome evolution studies. The yellowbelly pufferfish, \textit{Takifugu flavidus}, is an important marine fish species in the aquaculture industry and ecology of East Asia. The chromosome assembly of the species could facilitate the study of chromosome evolution and functional gene mapping. To this end, 44, 27 and 50 Gb reads were generated for genome assembly using Illumina, PacBio and Hi-C sequencing technologies, respectively. More than 13 Gb full-length transcripts were sequenced on the PacBio platform. A 366 Mb genome was obtained with the contig of 4.4 Mb and scaffold N50 length of 15.7 Mb. 266 contigs were reliably assembled into 22 chromosomes, representing 95.9\% of the total genome. A total of 29,416 protein-coding genes were predicted and 28,071 genes were functionally annotated. More than 97.7\% of the BUSCO genes were successfully detected in the genome. The genome resource in this work will be used for the conservation and population genetics of the yellowbelly pufferfish, as well as in vertebrate chromosome evolution studies.

Background & Summary
The yellowbelly pufferfish (FishBase ID: 24266), \textit{Takifugu flavidus}, is an economically and ecologically important fish species in coastal regions of East Asia, including the East China Sea, Yellow Sea and Bohai Bay\textsuperscript{1,2}. The yellowbelly pufferfish is also a temperate bottom fish that exhibits only short-distance seasonal migration\textsuperscript{3}. The yellowbelly pufferfish is caught and cultivated as a delicious fish species with high market value\textsuperscript{2,4,5}. However, due to environmental deterioration and overfishing, the wild populations of the species have declined in the last decade\textsuperscript{6,7}. Additionally, a low survival rate in artificial breeding has greatly limited the development of the marine aquaculture of the yellowbelly pufferfish\textsuperscript{8,9}. Previous studies of the yellowbelly pufferfish have mainly focused on behavioural\textsuperscript{1}, morphological and growth characteristics, temperature and salinity effects on embryos and larval development\textsuperscript{9}, and molecular marker development\textsuperscript{10}. A genome of \textit{Takifugu flavidus} was published in 2014\textsuperscript{11}; however, this genome was a fragmented draft with contig and scaffold N50 lengths of 2.7 kb and 305.7 kb, respectively. A high-quality reference genome of the yellowbelly pufferfish could facilitate and prompt conservation genetics research and investigation of the molecular mechanisms of important economic traits of the species.

The genomes of pufferfish have also played an important role in studies of vertebrate genome evolution due to the compactness of genus \textit{Takifugu} genomes\textsuperscript{12-15}. Previous studies have shown that the number of repetitive

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elements in pufferfish is significantly reduced\textsuperscript{14,15}. The genome of *Takifugu rubripes*, the other pufferfish in genus *Takifugu*, exhibits conserved linkages with humans, implying the preservation of chromosomal segments from the common vertebrate ancestor\textsuperscript{13–15}. Another pufferfish genome, that of *Tetraodon nigroviridis*, was the second pufferfish genome reported\textsuperscript{14}. Although the yellowbelly pufferfish genome has been published, the genome was constructed using short reads from SOLiD next-generation sequencing, and the sequences have not been assembled at the chromosomal level\textsuperscript{11}. The elucidation of genomic evolution among pufferfish species, and comparison with other vertebrates such as humans, will require further chromosomal genome assemblies for pufferfish species.

In this work, we applied a combined strategy involving Illumina, PacBio and Hi-C technologies to generate sequencing data for chromosomal genome construction for the yellowbelly pufferfish (Fig. 1). More than 98% of BUSCO genes were detected, and the contig and scaffold N50 lengths reached 4.4 and 15.7 Mb, respectively, demonstrating the outstanding completeness and sequence continuity of the genome. A total of 29,416 protein-coding genes were predicted in the assembled genome, and more than 95% of those genes were successfully functionally annotated. We believe that the chromosomal genome assembly constructed in this work will not only be valuable for ecology, conservation and aquaculture studies of the yellowbelly pufferfish but will also be of general interest in the evolutionary investigation of teleosts and vertebrates.

**Methods**

**Sample collection.** A female yellowbelly pufferfish (Fig. 2), reared in the fish breeding centre of Fujian Normal University in Fuzhou City of Fujian Province was used for genome sequencing and assembly. Fresh white muscle, eye, skin, gonad, gut, liver, kidney, blood, gall bladder and air bladder tissues were collected and quickly frozen in liquid nitrogen for one hour. White muscle tissues were used for DNA sequencing for genome assembly, while all tissues were used for transcriptome sequencing.

**DNA and RNA sequencing.** Genomic DNA from white muscle tissue was extracted using the standard phenol/chloroform extraction method for DNA sequencing library construction. The integrity of the genomic DNA molecules was checked using agarose gel electrophoresis. Both the Illumina HiSeq X Ten platform and the PacBio SEQUEL platform were applied for genomic sequencing to generate short and long genomic reads, respectively. For the Illumina X Ten platform (San Diego, CA, USA), a paired-end library was constructed with an insert size of 250 base pairs (bp) according to the protocol provided by the manufacturer. As a result, 44 Gb (~120X coverage of the estimated genome size, Table 1) of accurate short reads were generated, which were further cleaned using the HTQC utility\textsuperscript{16}. Adapter sequences and reads with more than 10% N bases or low-quality bases (≤5) were filtered from the sequencing data. After filtering, 41.8 Gb (~110X, Table 1) of cleaned data were retained for the following analysis. To obtain sufficient sequencing data for genome assembly, we constructed two 20 kb DNA libraries using the extracted DNA and the standard Pacific Biosciences (PacBio, Menlo Park, CA) protocol, and fragments were selected using the Blue Pippin Size-Selection System (Sage Science, MA, USA). The library was sequenced using the PacBio SEQUEL platform. After removing adaptors, we obtained 27.2 Gb subreads (~73X,
Table 1) for genome assembly. The genomic sequencing data used for subsequent genome assembly are summarized in Table 1.

We also performed RNA sequencing to generate transcriptome data for gene model prediction. To include as many tissue-specific transcripts as possible, multiple tissues were collected, as indicated in the Sample Collection section. TRIzol reagent (Invitrogen, USA) was used to separately extract RNA from all of the collected tissues, including white muscle, ocular, skin, gonad, intestine, liver, kidney, blood, gall bladder and air bladder tissues. RNA quality was checked with a NanoDrop ND-1000 spectrophotometer (Labtech, Ringmer, UK) and a 2100 Bioanalyzer (Agilent Technologies, CA, USA). Then, RNA molecules were equally mixed for transcriptome sequencing on the PacBio SEQUEL platform. First, cDNA was prepared using the SMARTer PCR cDNA Synthesis Kit (Clontech) from 1 µg of purified RNA. The Iso-Seq libraries were constructed from the BluePippin (Sage Science, MA, USA) size-selected cDNA with a size range of 0.6–3 kb according to the PacBio SEQUEL library construction protocol. Two SMRT flow cells were used for long-read transcriptome sequencing, and the resulting data used for gene prediction are summarized in Table 1.

**De novo assembly of the yellowbelly pufferfish genome.** For the Next Generation Sequencing (NGS) short reads, the Kmer-based method was used to perform genome survey analysis to estimate the genome size, heterozygosity and repeat content of the yellowbelly pufferfish genome. We counted the number of each 17-mer with Jellyfish, and the frequency distribution is plotted in Fig. 3. The yellowbelly pufferfish genome size was then estimated from the frequency distribution to be 377 Mb.

The Falcon package was used to assemble the yellowbelly pufferfish genome with PacBio long reads, using the parameters length_cutoff = 10 kb and pr_length_cutoff = 8 kb. The procedures of long-read self-correction, corrected read alignment, sequence graph construction, and contig assembly were performed in Falcon. To correct random sequencing errors in the Falcon output, two steps of genome sequence polishing were applied: we first used arrow to polish the genome using long sequencing data, and two rounds of polishing using NGS short reads were then applied with Pilon. Finally, we obtained a final contig assembly of 366 Mb with a contig N50 length of 4.4 Mb (Table 2).

To evaluate the quality of the assembled genome, the completeness and accuracy were assessed via BUSCO analysis and short-read mapping. The completeness of the assembled yellowbelly pufferfish genome was assessed by using BUSCO v3.0 with the vertebrata_odb9 database. We found that 95.7% and 2.7% of 2,586 BUSCO genes were completely and partially BUSCO genes were detected in the genome. We also aligned NGS short reads to the genome and found that more than 98.5% of the reads were reliably aligned, showing a high mapping ratio for the short-read sequencing data.

**Chromosome construction using interaction information from Hi-C data.** In this work, we applied the Hi-C technique for chromosome construction for the yellowbelly pufferfish. Although the Hi-C technique was first introduced to quantify genome-wide interactions, the method exhibits suitability for chromosome assembly and has been successfully applied in many genomic projects. In our study, we used 0.2 ml of blood from the same individual used for genome sequencing for Hi-C library construction and sequencing using the
same method as in a previous study\textsuperscript{25,26}. From the Hi-C library sequencing, approximately 50.7 Gb of data were generated (Table 1). The sequencing reads were mapped to the polished yellowbelly pufferfish genome with Bowtie 1.2.2\textsuperscript{27}. We independently aligned the two read ends to the genome and only selected the read pairs for which both ends were uniquely aligned to the genome. The hiclib Python library\textsuperscript{28} and a previously reported method\textsuperscript{24} were applied to filter the Hi-C reads, and the interaction frequency was quantified and normalized among contigs. Lachesis\textsuperscript{29} with default parameters was then applied to cluster contigs with the agglomerative hierarchical clustering method using the interaction matrix between sequences. Among the 169 million read pairs generated from Hi-C sequencing, 59 million read pairs (34.9\%) provided valid interaction information for chromosome assembly. As a result, the contigs from the yellowbelly pufferfish were successfully clustered into 22 groups, which were further ordered and oriented into chromosomes. Finally, 271 contigs were reliably anchored on chromosomes, accounting for 95.9\% of the total genome. The contig and scaffold N50 values reached 4.4 and 15.7 Mb (Tables 2 and 3), respectively, providing the first chromosomal genome assembly for the yellowbelly pufferfish.

### Gene model prediction and functional annotations.

Repeat elements were annotated in the yellowbelly pufferfish before gene model annotation. We applied Tandem Repeat Finder (TRF)\textsuperscript{30}, LTR\_FINDER\textsuperscript{31}, PILER\textsuperscript{32} and RepeatScout\textsuperscript{33} for the \textit{ab initio} prediction of repeat elements in the genome. Thereafter, RepeatMasker and RepeatProteinMask (http://www.repeatmasker.org) were used to search the genome sequences for known repeat elements, with the genome sequences used as queries against the Repbase database\textsuperscript{34}. The repetitive element annotations are listed in Table 4.

Gene annotation was performed on the repetitive-element-masked genome. A combined strategy of homology-based, \textit{ab initio} and transcriptome-based gene prediction methods was used. Protein sequences of \textit{Astyanax mexicanus}, \textit{Danio rerio}, \textit{Gadus morhua}, \textit{Ictalurus punctatus}, \textit{Oryzias latipes}, \textit{Takifugu rubripes}, \textit{Tetraodon nigroviridis} and \textit{Oreochromis niloticus} were downloaded from Ensembl\textsuperscript{35}. Proteins from the closely related fish species were mapped to the yellowbelly pufferfish genome using TBLASTN\textsuperscript{36}. The alignments were joined with Solar, and GeneWise\textsuperscript{37} was used to predict the exact gene structure of the corresponding genomic regions. Augustus\textsuperscript{38} was also used for the \textit{ab initio} prediction of genes in the repeat-masked genome. Finally, the full-length transcriptome sequences generated from PacBio sequencing were aligned to the genome using the
TopHat package\textsuperscript{39}, and gene structure was predicted using Cufflinks\textsuperscript{40}. All gene models were merged, and redundancy was removed by MAKER\textsuperscript{41}, leading to a total of 29,416 protein-coding genes.

The NCBI non-redundant protein (nr) database and the SwissProt database with an E-value threshold of 1e-5 were used for the functional annotation of the protein-coding genes using BLASTX and the BLASTN utility\textsuperscript{42}. Functional ontology and pathway information from the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases was assigned to the genes using Blast2GO\textsuperscript{43}. Ultimately, 28,017 genes (95.24\% of the total) of the yellowbelly pufferfish were functionally annotated (Table 5).

| Chr  | Chr length (bp) | Contig number | Gene number |
|------|----------------|---------------|-------------|
| Chr1 | 28,838,366     | 15            | 2,171       |
| Chr2 | 19,632,357     | 11            | 1,243       |
| Chr3 | 19,136,632     | 13            | 1,361       |
| Chr4 | 18,781,179     | 28            | 1,639       |
| Chr5 | 18,395,123     | 16            | 1,444       |
| Chr6 | 16,875,900     | 15            | 1,440       |
| Chr7 | 16,703,359     | 13            | 1,189       |
| Chr8 | 16,202,710     | 8             | 1,268       |
| Chr9 | 15,776,270     | 8             | 1,063       |
| Chr10| 15,676,631     | 7             | 1,215       |
| Chr11| 15,654,207     | 13            | 1,091       |
| Chr12| 15,631,021     | 10            | 1,269       |
| Chr13| 15,542,920     | 11            | 1,272       |
| Chr14| 15,503,328     | 17            | 1,341       |
| Chr15| 15,463,098     | 11            | 1,395       |
| Chr16| 14,247,604     | 12            | 1,103       |
| Chr17| 13,381,174     | 14            | 986         |
| Chr18| 13,174,367     | 19            | 1,324       |
| Chr19| 12,605,058     | 6             | 993         |
| Chr20| 12,303,402     | 10            | 991         |
| Chr21| 11,708,235     | 5             | 868         |
| Chr22| 9,947,389      | 9             | 747         |

Table 3. Summary of the assembled chromosomes of the yellowbelly pufferfish.

| No. of TEs   | Length (bp) | % of total TEs | % of genome |
|--------------|-------------|----------------|-------------|
| 300,773      | 60,927,544  | 100            | 16.63       |
| Class I: Retroelement | 77,720       | 29,919,159     | 49.11       | 8.17         |
| LTR retrotransposon  | 20,782     | 10,394,098     | 17.06       | 2.84         |
| Ty1/Copia    | 865         | 227,841        | 0.37        | 0.06         |
| Ty3/Gypsy   | 7,440       | 3,670,541      | 6.02        | 1.00         |
| Other        | 12,477      | 6,495,716      | 10.66       | 1.77         |
| Non-LTR retrotransposon | 51,417       | 18,060,948     | 29.64       | 4.93         |
| LINE         | 37,274      | 16,042,878     | 26.33       | 4.38         |
| SINE         | 14,143      | 2,018,070      | 3.31        | 0.55         |
| Unclassified retroelement | 5,521      | 1,464,113      | 2.40        | 0.40         |
| Class II: DNA transposon | 39,742    | 11,514,017     | 18.90       | 3.14         |
| TIR          |             |                |             |              |
| CMC[DTC]     | 3,477       | 365,955        | 0.60        | 0.10         |
| hAT          | 8,606       | 3,318,856      | 5.45        | 0.91         |
| Mutator      | 406         | 110,998        | 0.18        | 0.03         |
| Tc1/Mariner  | 9,293       | 3,015,854      | 4.95        | 0.82         |
| PIF/Harbinger| 2,035       | 672,257        | 1.10        | 0.18         |
| Other        | 6,632       | 1,014,243      | 1.66        | 0.28         |
| Helitron     | 74          | 12,208         | 0.02        | 0.00         |
| Tandem repeats | 194,185    | 19,801,588     | 32.50       | 5.41         |
| Unknown      | 1,412       | 870,602        | 1.43        | 0.24         |

Table 4. Repetitive element annotations in the yellowbelly pufferfish.
Data records

The sequencing dataset and genome assembly were deposited in public repositories. The previous genome assembly can be accessed under accession number AOOT01000000 in NCBI44. The genomic Illumina sequencing data, genomic PacBio sequencing data, transcriptomic PacBio sequencing data, and the genomic Hi-C sequencing data were deposited as SRP162225 in NCBI45. The final chromosome assembly and genome annotation were submitted to NCBI Assembly with accession number RHFK00000000 46. The functional annotation files are also available at figshare47.

Technical Validation

The quality of the DNA and RNA molecules and libraries used for genomic sequencing and transcriptome sequencing was validated before sequencing. The extracted DNA spectrophotometer ratios (SP) were 260/280 ≥ 1.6 for both Illumina and PacBio sequencing. DNA samples >2µg and 20µg were used for Illumina and PacBio sequencing, respectively. The concentration and quality of the total RNA were evaluated using a NanoVue Plus spectrophotometer (GE Healthcare, NJ, USA). RNA samples with a total RNA amount ≥10µg, RNA integrity number ≥8, and rRNA ratio ≥1.5 were used to construct the sequencing library.

To validate our genome assembly, we compared the new genome to the previous genome. The new genome contained significantly fewer ambiguous bases (0.02 Mb) than the previous genome (67.9 Mb), but the size (366 Mb) of the new genome was approximately 20 Mb larger than that of the previous genome (346 Mb). Considering the estimated genome size of 377 Mb determined from the Kmer-based method, our new genome exhibited high completeness compared to the previous genome. The contig and scaffold N50 values of the newly assembled genome were almost 4,000 and 50 times higher than those of the previous genome, indicating a remarkable improvement in the sequence continuity of our assembly. We attributed the completeness and the continuity of the new genome to the application of PacBio long reads in the genome assembly. To further validate the improved continuity, we aligned genome fragments to our new genome with the NUCmer utility and found that more than 76% of the contigs were reliably mapped to the new genome with alignment ratios greater than 95%. Figure 4 provides two examples of alignments of scaffold sequences from the previous genome assembly to our new genome assembly. (a) Alignments on contig5 in the new genome. (b) Alignment on contig74 in the new genome. The X axis represents the scaffolds from the previous genome, and the Y axis represents the contig sequences assembled in this work. The straight and reverse alignments of the scaffold sequences are shown in blue and red, respectively.

Usage Notes

The contig sequences were assembled into chromosomes using interaction information from Hi-C sequencing data; therefore we used 100bp to represent the unknown gap sizes among contigs in the chromosome sequences.

Table 5. The statistics of functional annotation of protein-coding genes. Note that “at least one database” here refers to genes with at least one hit in multiple databases.

| Database   | Number | Percent (%) |
|------------|--------|-------------|
| Nr         | 27,859 | 94.7        |
| GO         | 16,533 | 56.2        |
| KEGG       | 27,700 | 94.2        |
| SwissProt  | 23,881 | 81.2        |
| At least one database | 28,017 | 95.2        |
| Total      | 29,416 |             |

Fig. 4 Two examples of the alignment of scaffolds from the previous genome assembly to our new yellowbelly pufferfish genome assembly. (a) Alignments on contig5 in the new genome. (b) Alignment on contig74 in the new genome. The X axis represents the scaffolds from the previous genome, and the Y axis represents the contig sequences assembled in this work. The straight and reverse alignments of the scaffold sequences are shown in blue and red, respectively.

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Usage Notes

The contig sequences were assembled into chromosomes using interaction information from Hi-C sequencing data; therefore we used 100bp to represent the unknown gap sizes among contigs in the chromosome sequences.
Code availability
No specific code or script was used in this work. All commands used in the processing were executed according to the manual and protocols of the corresponding bioinformatics software.

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References
1. Shi, Y. H. et al. Growth, development and behavior ecology of tawny puffer (Takifugu fluviatus) larvae and juveniles. *Journal of Fisheries of China* 34, 1509–1517 (2010).
2. Zhong, J. X. et al. Studies on artificial propagation and larva-rearing of Fugu flavidus. *Marine Sciences* 33, 1–7 (2009).
3. Zhang, G., Shi, Y., Zha, Y., Liu, J. & Zang, W. Effects of salinity on embryos and larvae of tawny puffer Takifugu fluviatus. *Aquaculture* 302, 71–75, https://doi.org/10.1016/j.aquaculture.2010.02.005 (2010).
4. Liao, X. X. et al. Analysis of Lipid & Fatty Acid Composition in Different Tissues of Adult Female and Male Takifugu fluviatus. *Food Science* 35, 133–137 (2014).
5. Tao, N. P., Wang, L. Y., Gong, X. & Liu, Y. Comparison of nutritional composition of farmed pufferfish muscles among Fugu obscurus, Fugu fluviatus and Fugu rubripes. *Journal of Food Composition & Analysis* 28, 40–45 (2012).
6. Stump, E., Ralph, G. M., Comerov-Raynal, M. T., Matsuuria, K. & Carpenter, K. Global conservation status of marine pufferfishes (Tetraodontiformes: Tetraodontidae). *Global Ecology & Conservation* 14, e00388 (2018).
7. Liu, Y., Qin, Z., Liu, H., Chao, L. & Tong, A. The complete mitochondrial genome sequence of Takifugu fluviatus (Tetraodontiformes: Tetraodontidae). *Mitochondrial DNA A DNA Mapp Seq Anal* 27, 613–614 (2014).
8. Shi, Y. H., Zhang, G. Y., Zha, Y. Z., Liu, J. Z. & Zang, W. L. Effects of temperature on fertilized eggs and larvae of tawny puffer Takifugu fluviatus. *Aquaculture Research* 41, 1741–1747 (2010).
9. Shi, Y. Z., Zhang, G., Liu, J. & Zang, W. Effects of temperature and salinity on oxygen consumption of tawny puffer Takifugu fluviatus juvenile. *Aquaculture* 42, 301–307, https://doi.org/10.1111/j.1365-2109.2010.02638.x (2011).
10. Ma, H., Chen, S., Liao, X., Xu, T. & Ge, J. Isolation and characterization of polymorphic microsatellite loci from a dinucleotide-enriched genomic library of obscure puffer (Takifugu obscurus) and cross-species amplification. *Conservation Genetics* 10, 955–957, https://doi.org/10.1007/s10592-008-9560-2 (2009).
11. Gao, Y. et al. Draft Sequencing and Analysis of the Genome of Pufferfish Takifugu fluviatus. *DNA Research* 21, 627–637, https://doi.org/10.1093/dnares/dsu025 (2014).
12. Gao, Y. et al. Draft Sequencing and Analysis of the Genome of Pufferfish Takifugu fluviatus. *DNA Research* 21, 627–637 (2014).
13. Volf, I. N., Brasch, I. & Froshauer, A. Fish Genomes, Comparative Genomics and Vertebrate Evolution. *Current Genomics* 7, 43–57 (2006).
14. Jallon, O. et al. Genome duplication in the teleost fish Tetraodon nigroviridis reveals the early vertebrate proto-karyotype. *Nature* 431, 946 (2004).
15. Aparicio, S. et al. Whole-Genome Shotgun Assembly and Analysis of the Genome of Fugu rubripes. *Science* 297, 1301–1310 (2002).
16. Yang, X. et al. HTQC: a fast quality control toolkit for Illumina sequencing data. *Bmc Bioinformatics* 14, 1–4 (2013).
17. Liu, R. et al. Estimation of genomic characteristics by analyzing k-mer frequency in de novo genome projects. *Quantitative Biology* 35, 62–67 (2013).
18. Marçais, G. & Kingsford, C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics* 27, 764 (2011).
19. Chiu, C. S. et al. Phased Diploid Genome Assembly with Single Molecule Real-Time Sequencing. *Nature Methods* 13, 1050–1054 (2016).
20. Chiu, C. S. et al. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nature Methods* 10, 563 (2013).
21. Walker, B. J. et al. Pilon: An Integrated Tool for Comprehensive Mutational Variant Detection and Genome Assembly Improvement. *Plos One* 9, e112963 (2014).
22. Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V. & Zdobnov, E. M. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31, 3210 (2015).
23. Belaghzal, H., Dekker, J. & Gibcus, J. H. Hi-C 2.0: An optimized hi-c procedure for high-resolution genome-wide mapping of chromosome conformation. *Methods* 123, 56–65 (2017).
24. Nicolas, S. et al. HiC-Pro: an optimized and flexible pipeline for Hi-C data processing. *Genome Biology* 16, 259 (2015).
25. Xu, S. et al. A draft genome assembly of the Chinese sillago (Sillago sinica), the first reference genome for Sillaginidae fishes. *GigaScience* 7, gyi108–gyi108, https://doi.org/10.1093/gigascience/gyi108 (2018).
26. Gong, G. et al. Chromosomal-level assembly of yellow catfish genome using third-generation DNA sequencing and Hi-C analysis. *GigaScience* 7, gyi120–gyi120, https://doi.org/10.1093/gigascience/gyi120 (2018).
27. Langmead, B. Aligning short sequencing reads with Bowtie. (John Wiley & Sons, Inc., 2010).
28. Imakaev, M. et al. Iterative correction of Hi-C data reveals hallmarks of chromosome organization. *Nature Methods* 9, 999 (2012).
29. Burton, J. N. et al. Chromosome-scale scaffolding of de novo genome assemblies based on chromatin interactions. *Nature Biotechnology* 31, 1119–1125 (2013).
30. Benson, G. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Research* 27, 573 (1999).
31. Zhao, X. & Hao, W. LTR-FINDER: an efficient tool for the prediction of full-length LTR retrotransposons. *Nucleic Acids Research* 35, W265–W268 (2007).
32. Edgar, R. C. & Myers, E. W. PILER: identification and classification of genomic repeats. *Bioinformatics* 21(Suppl 1), i152 (2005).
33. Price, A. L., Jones, N. C. & Pevzner, P. A. De novo identification of repeat families in large genomes. *Bioinformatics* 21(Suppl 1), i351 (2005).
34. Jurka, J. et al. Repbase Update, a database of eukaryotic repetitive elements. *Cytogenetic & Genome Research* 110, 462–467 (2005).
35. Flick, P. et al. Ensembl 2014. *Nucleic Acids Research* 42, D749–D755 (2014).
36. Gertz, E. M. et al. Comparison-based statistics and translated nucleotide searches: Improving the TBLASTN module of BLAST. *Bmc Biology* 4, 41 (2006).
37. Birney, E., Clamp, M. & Durbin, R. GeneWise and Genomewise. *Genome Research* 14, 988 (2004).
38. Stanke, M. et al. AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Research* 34, 435–439 (2006).
39. Trapnell, C., Pachter, L. & Salzberg, S. L. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25, 1105–1111 (2009).
40. Ghosh, S. & Chen, C. K. K. Analysis of RNA-Seq Data Using TopHat and Cufflinks. *Methods in Molecular Biology* 1374, 339 (2016).
41. Cantarel, B. L. et al. MAKER: an easy-to-use annotation pipeline designed for emerging model organism genomes. *Genome Research* 18, 188–196 (2008).
42. Lobo, I. Basic Local Alignment Search Tool (BLAST). *Journal of Molecular Biology* 215, 403–410 (2008).
43. Conesa, A. et al. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21, 3674 (2005).
44. Gao, Y. Takifugu flavidus, whole genome shotgun sequencing project. GenBank, https://identifiers.org/ncbi/insdc:AOOT01000000 (2013).
45. NCBI Sequence Read Archive, https://identifiers.org/ncbi/insdc.sra:SRP162225 (2018).
46. Xiao, S. Takifugu flavidus isolate HTHZ2018, whole genome shotgun sequencing project. GenBank, https://identifiers.org/ncbi/insdc:RHFK00000000 (2018).
47. Xiao, S. Function annotation of Takifugu flavidus genome genes. figshare. https://doi.org/10.6084/m9.figshare.9944087.v1 (2019).

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Author contributions
Z.H., X.H. and Y.C. conceived and designed the study; G.L., Z.L., J.Z., Y.Z. and Y.G. collected the samples; Y.Z., D.C. and T.X. performed DNA sequencing and Hi-C experiments; Y.X. and J.C. performed RNA sequencing; Y.Z., D.C. and S.X. estimated the genome size, assembled the genome, and assessed the assembly quality; S.X. and Y.Z. performed the genome annotation and functional genomic analysis. S.X., Z.H. and Y.Z. wrote the manuscript. All authors read, edited, and approved the final manuscript for submission.

Competing interests
The authors declare no competing interests.

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