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# Near complete T2T genome assembly of the banded goonch (*Bagarius rutilus*)

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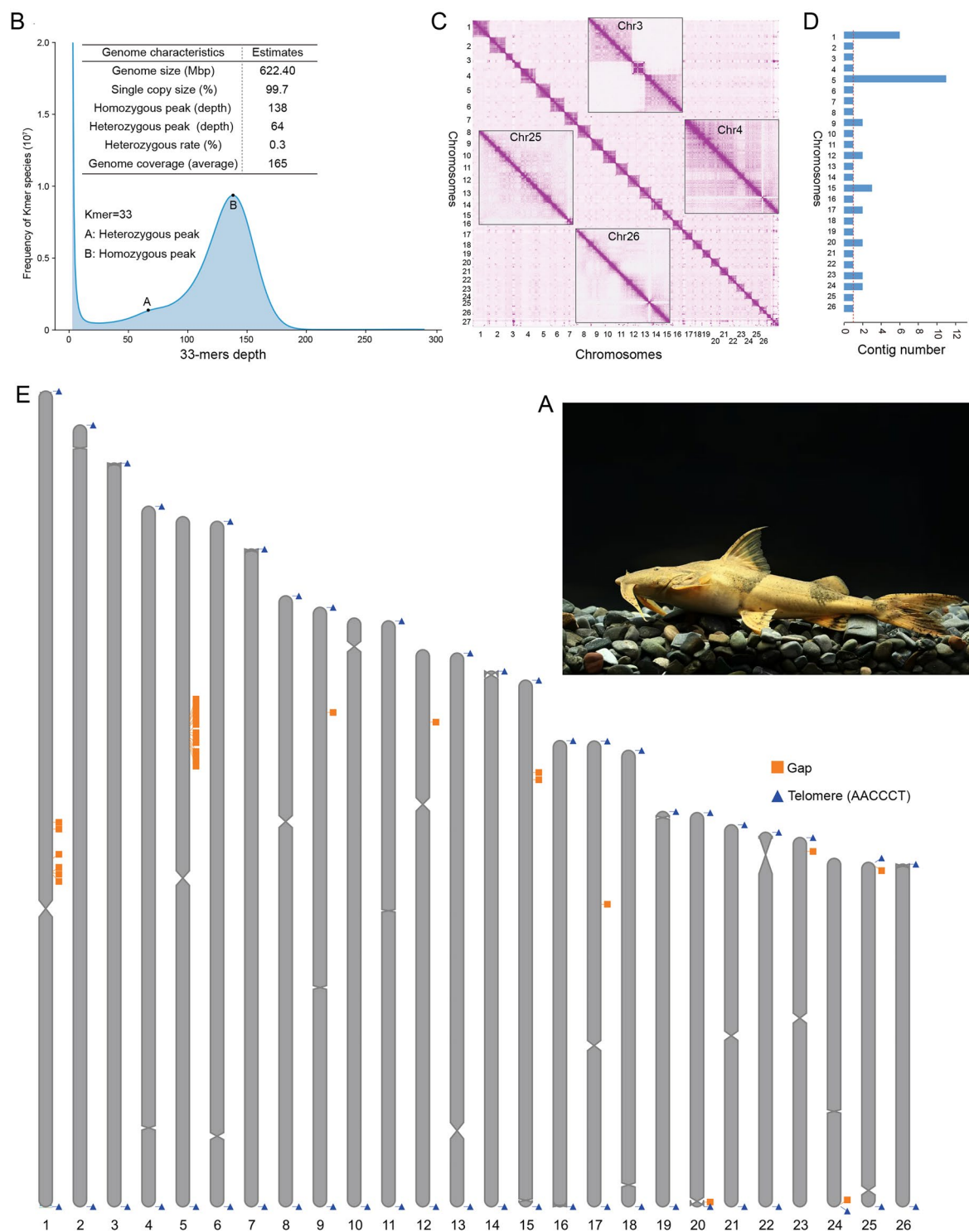
The order Siluriformes, a hyperdiverse teleost clade with over 3,000 species, exemplifies adaptive radiation through significant phenotypic innovations such as aerial respiration. However, the limited availability of high-quality genomes, particularly in underrepresented families such as Sisoridae, has hindered phylogenomic and mechanistic studies of their ecological diversification. Here, we present a nearly complete telomere-to-telomere (T2T) genome assembly of *Bagarius rutilus* (Banded Goonch) generated by a hybrid sequencing approach integrating PacBio HiFi, Oxford Nanopore ultra-long and Hi-C technologies. The 618.3 Mb genome (contig N50 = 21.06 Mb, scaffold N50 = 25.46 Mb) was resolved into 26 chromosomes, achieving a genome coverage of 98.17%. The quality of the assembly was validated by a BUSCO score of 97.5% (Actinopterygii\_odb10) and high conservation of synteny with other catfish species. Based on RNA sequencing, homology-based and *de novo* annotation, we identified 29,106 protein-coding genes. This chromosome-level genome represents one of the highest quality Siluriformes assemblies to date and provides a critical resource for reconstructing adaptive landscapes and exploring the genetic basis of phenotypic innovation in catfish.

## Background & Summary

The order Siluriformes (catfishes) is a quintessential model to understand the adaptive radiation in teleost fishes, comprising over 3,000 species (~25,000 protein coding genes) in more than 36 families that exhibit extraordinary phenotypic innovations<sup>1</sup>. These include the suprabranchial labyrinth organ in Clariidae for aerial respiration<sup>2</sup>, muscle-derived high-voltage bioelectric organs (>500 V) in Malapteruridae (electric catfish)<sup>3</sup>, keratinized feeding structures in Loricariidae (suckermouth armored catfish)<sup>4</sup>, and specialized hematophagous oral apparatus in parasitic lineages<sup>5</sup>. Such structural and functional innovations have enabled their colonization of diverse aquatic niches, ranging from freshwater to brackish ecosystems, and including benthic detritivory, filter feeding, blood parasitism, and even cave-dwelling adaptations<sup>3–6</sup>. Together, these features position Siluriformes as an unparalleled system for studying the mechanisms of adaptive radiation in teleosts.

Despite significant advances in catfish biology<sup>7–9</sup>, critical gaps in genomic resources remain, particularly within some families such as the Sisoridae, hindering comprehensive phylogenomic frameworks and mechanistic insights into their adaptive evolution. In this study we focused on a representative Sisoridae species *Bagarius rutilus* (banded goonch; Fig. 1A), which has restricted distribution ranges in Laos, Vietnam and China. In addition, this species is a second-class protected species in China and has important protection value. We present a near complete telomere-to-telomere (T2T) genome assembly of banded goonch using a multiple sequencing strategy with HiFi, ONT ultra-long and Hi-C technologies. The assembled genome spans approximately 618 megabase pairs (Mb) across 26 chromosomes (contig N50 = 21.06 Mb, scaffold N50 = 25.46 Mb) (Fig. 1B,C).

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**Fig. 1** Chromosome-level genome assembly of the banded goonch (*Bagarius rutilus*). (A) The picture of the banded goonch. (B) Summary of genome characteristics including estimated genome size and heterozygosity. (C) Hi-C interaction heatmap showing chromosomal compartmentalization and spatial proximity. Chromosomes 3, 4, 25 and 26 are magnified to highlight intrachromosomal interaction patterns. (D) Distribution of contig counts per chromosome, with regions to the left of the red line representing gap-free assemblies. (E) Ideogram of the banded goonch karyotype. Blue triangles at chromosome ends indicate putative telomeric regions, and orange rectangles indicate residual assembly gaps.

Seventeen chromosomes are completely free of gaps, six contain a single gap, and the remaining chromosomes contain more than one (Fig. 1D,E).

High continuity and accuracy were confirmed by the completeness of conserved core genes (BUSCO score: 97.5%) and syntenic alignment with related species (*Trichomycterus rosablanca*). This assembly represents one of the highest quality Siluriformes genomes, providing a critical genomic resource for resolving long-standing phylogenetic uncertainties<sup>10</sup> and elucidating the genetic basis of ecological specialization and protecting this species in the future.

**Sample collection.** A female *Bagarius rutilus* specimen was collected from Gasu Town, Xinping County, Yuxi City, Yunnan Province, China and ethically anesthetized with MS-222 (tricaine methanesulfonate) prior to dissection as previous study<sup>11</sup>. Skeletal muscle, heart, kidney and liver tissues were rapidly sampled, snap frozen in liquid nitrogen to preserve macromolecular integrity, and stored at  $-80^{\circ}\text{C}$  for subsequent genomic and transcriptomic analyses. Skeletal muscle was subjected to multi-platform sequencing, including short-read, high-fidelity long-read (PacBio Sequel II) and ultra-long nanopore technologies to ensure high quality genome assembly, while liver tissue was subjected to Hi-C sequencing for chromosome-scale scaffolding. In addition, RNA sequencing was performed on skeletal muscle, heart, kidney and liver tissues to construct a reference transcriptome and validate gene models.

**Genomic HiFi sequencing.** High molecular weight DNA was extracted from skeletal muscle using the MagAttract HMW DNA Kit (Qiagen) with optimized protocols: tissue homogenisation in ice-cold lysis buffer (2%  $\beta$ -mercaptoethanol) using a Potter-Elvehjem homogeniser (30 strokes at 200 rpm), extended Proteinase K digestion (18 h at  $56^{\circ}\text{C}$  with 15 rpm rotation) and MinElute column purification with pre-warmed EB buffer ( $65^{\circ}\text{C}$ ). For SMRTbell library construction, genomic DNA was mechanically sheared into 15–20 kb fragments using g-TUBE (Covaris;  $3 \times 6,000 \times \text{g}$  pulses), followed by dual enzyme damage repair (NEBNext FFPE: PreCR = 1:3,  $37^{\circ}\text{C}$  60 min) and BluePippin size selection (0.75% agarose, 15–25 kbp narrow window). Ligation efficiency was validated by SMRTbell specific qPCR (Kapa Biosystems). PacBio Sequel II sequencing in CCS mode (30 hours run time) included polymerase load optimisation (Binding Calculator v6.0) and magnetic bead enrichment (Sequel II Binding Kit 3.0) targeting 80% ZMW occupancy, resulting in 137 Gb HiFi reads (average 17.3 kbp) for downstream analyses.

**ONT Ultra-long sequencing.** Ultra-long DNA was extracted using an adapted protocol from the Nanobind CBB Big DNA Kit (Circulomics, #102-301-900), where tissue was embedded in low melting point agarose (1% in PBS) and subjected to proteinase K digestion (2 mg/mL,  $50^{\circ}\text{C}$ , 72 h). DNA was quantified using Qubit 4.0 (Invitrogen) and size profiled using the Femto Pulse System (Agilent). Library preparation was performed using the LSK-114 Ligation Sequencing Kit (Oxford Nanopore, #SQK-LSK114) with custom modifications, including DNA repair using Ultra II End-prep enzyme mix supplemented with 5% PEG 8000 (w/v), extended adapter ligation (30 min) with  $1.5 \times$  adapter concentration, and loading onto R10.4.1 flow cells pre-equilibrated with ELB supplemented with 0.05% Tween-20. Sequencing was performed on a PromethION P48 platform using MinKNOW v22.10.7 with adaptive sampling disabled, followed by basecalling using the Super Accurate (SUP) model in Dorado v0.3.0 and 49.57 Gb reads were generated.

**Short and Hi-C reads sequencing.** For short-read whole-genome sequencing, high-quality genomic DNA was sheared to an average fragment size of  $\sim 350$  bp using a Covaris S220 system. A paired-end library was constructed following the standard protocol of the MGIEasy FS DNA Library Prep Kit (MGI Tech) and subsequently sequenced on a DNBSEQ-T7 platform in paired-end 150 bp (PE150) mode.

Hi-C library preparation and sequencing fresh skeletal muscle tissue was cross-linked in 1% formaldehyde for 10 min at room temperature, followed by quenching with 0.125 M glycine. Cell nuclei were isolated and lysed, and chromatin was digested with the methylation-sensitive restriction enzyme DpnII at  $37^{\circ}\text{C}$  for 4 h to generate cohesive ends. Digested DNA fragments were biotinylated with biotin-14-dCTP and subjected to proximity ligation using T4 DNA ligase at  $16^{\circ}\text{C}$  for 4 hours. Ligated DNA was purified, sheared to 300–500 bp fragments using a Covaris S220 and enriched for biotinylated junctions by streptavidin bead capture. Libraries were constructed using the MGIEasy FS DNA Library Prep Kit, with fragment size distribution validated using the Agilent 2100 Bioanalyzer, and sequenced on a DNBSEQ-T7 platform (MGI Tech) in paired-end 150 bp mode.

**RNA sequencing.** Total RNA was extracted from tissue samples using TRIzol reagent (Invitrogen, 15596026) according to the manufacturer's protocol, followed by DNase I (NEB, #M0303) treatment to remove genomic DNA contamination. RNA quality and integrity were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies) to ensure an RNA Integrity Number (RIN)  $\geq 7.0$  for library construction.

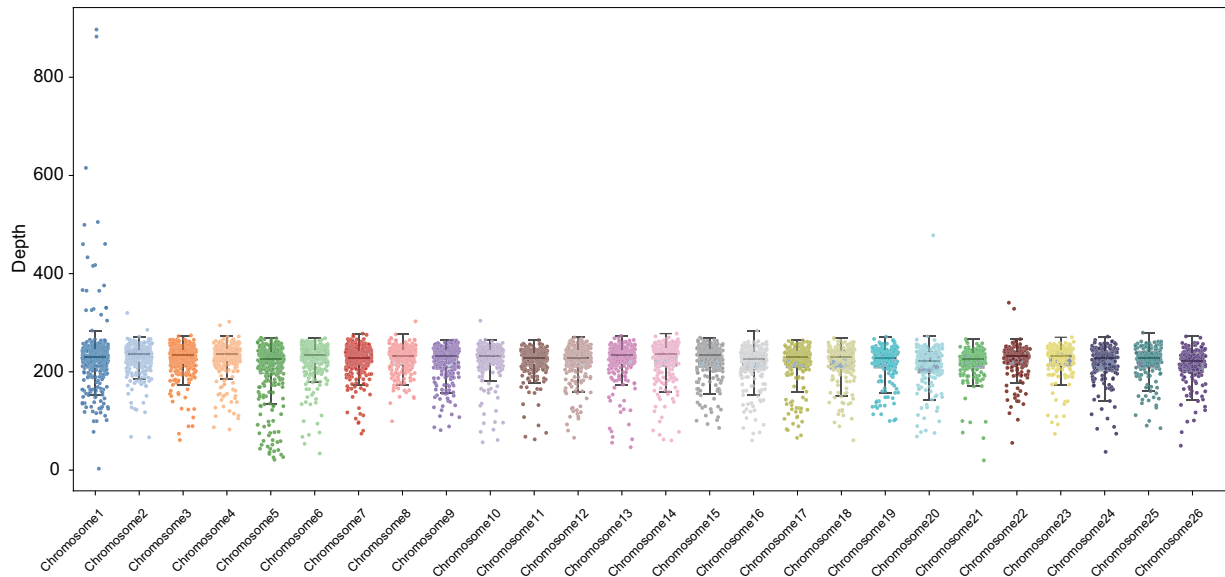
Poly(A) + mRNA was enriched using oligo (dT) magnetic beads and subsequently fragmented into  $\sim 250$  bp fragments. First-strand cDNA was synthesized using random hexamer primers and reverse transcriptase, followed by second-strand cDNA synthesis. The resulting cDNA was purified, end repaired, A-tailed and ligated to MGI-specific adaptors. After ligation, the cDNA was amplified by PCR using a limited number of cycles to minimize bias. The final libraries were assessed for size distribution using the Agilent 2100 Bioanalyzer and quantified using Qubit 4.0 (Invitrogen).

**Chromosome level genome assembly.** Initial genome characterization was performed by *K*-mer analysis using Jellyfish (v2.3.1; *k*-mer size = 33)<sup>12</sup> and GenomeScope (v2.0)<sup>13</sup>, which estimated a haploid genome size of 622 Mb with 0.3% heterozygosity (Fig. 1A–D). To ensure high quality input data for assembly, PacBio HiFi reads ( $\geq \text{Q20}$  accuracy, 15–20 kb) and Oxford Nanopore Technologies (ONT) ultra-long reads ( $N50 > 100$  kb) underwent rigorous pre-processing: HiFi reads were filtered with HiFiAdapterFilt<sup>14</sup> to remove adapter sequences, while ONT reads were quality trimmed with NanoFilt<sup>15</sup> (minimum Q score = 7, minimum length = 50 kb).

A hybrid assembly strategy integrating HiFi, ONT ultra-long and Hi-C reads was used to achieve chromosome-scale continuity. Primary contigs were assembled from HiFi and ONT ultra-long reads using Hifiasm (v0.24.0-r702)<sup>16</sup>, using

| Term             | Contig assembly | Hi-C assembly |
|------------------|-----------------|---------------|
| Genome size (bp) | 618,684,347     | 618,684,347   |
| N50              | 21,064,696      | 25,467,879    |
| N90              | 14,454,354      | 16,050,002    |
| Chromosomes      | —               | 26            |
| Contigs          | 153             | 119           |
| Max length (bp)  | 33,978,905      | 35,568,020    |
| Min length (bp)  | 6,637           | 6,637         |

**Table 1.** Statistics of the genome assembly.



**Fig. 2** Distribution of HiFi read sequencing depth across each chromosome. Each point represents the mean sequencing depth calculated in a 100 kb window.

HiFi accuracy for base-level resolution and ONT reads to resolve long repetitive regions. Chromosome scaffolding was then performed using Hi-C data: reads were aligned to the draft assembly using BWA (v0.7.12)<sup>17</sup>, and chromatin interaction patterns were used to iteratively correct scaffold orientation and linkage using yahs (v1.2a.1)<sup>18</sup>. This step anchored 98% of the assembly into chromosome-scale pseudomolecules. Finally, we used quarTeT<sup>19</sup> (v1.2.5) to fill gaps based on the HiFi and ONT reads, and the telomeres were also identified by quarTeT (Fig. 1D).

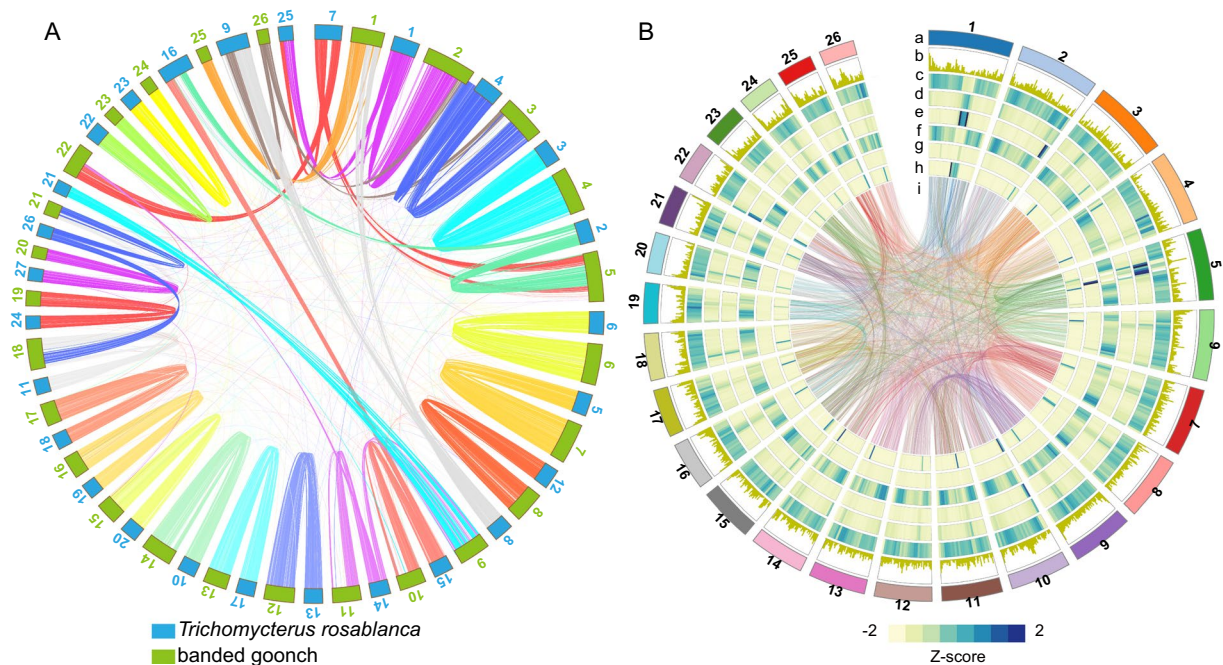
The completeness and accuracy of the assembly was validated using BUSCO (v5.5.0; actinopterygii\_odb10)<sup>20</sup>, which identified 97.5% of conserved actinopterygii genes, confirming a comprehensive representation of gene space. Spatial consistency and chromosomal integrity were further verified by generating a Hi-C contact matrix with Juicer (v1.6)<sup>21</sup> and visualising interaction patterns in Juicebox (v1.11.08)<sup>22</sup> (Fig. 1B). Finally, the assembled genome size was approximately 618 Mb across 26 chromosomes. Of these, 17 were completely gap-free, six contained only a single gap, and three had more than one gaps (Fig. 1B,C and Table 1).

**HiFi reads depth calculation.** HiFi reads were aligned to the reference genome using Minimap2 (v2.25)<sup>23</sup> with the -ax map-hifi option. The resulting BAM file was then used to calculate the sequencing depth at each base position using the bedtools (v2.25.0)<sup>24</sup> genomecov utility. To quantify the distribution of reads along each chromosome, we computed the average read depth across consecutive, non-overlapping 100 kb windows using the bedtools make windows and map utilities. This process yielded the mean coverage for each 100 kb genomic bin, enabling the visualisation of read distribution patterns at the chromosomal level (Fig. 2).

**Annotation of genome assembly.** Repetitive elements in the banded goonch genome assembly were annotated through a combination of homology-based and *de novo* approaches. Homology detection was performed using RepeatProteinMask<sup>25</sup> for protein-level alignment of transposable elements (TEs) and RepeatMasker<sup>26</sup> for DNA-level identification. Tandem repeats were predicted with TRF<sup>27</sup> using parameters optimized for sensitivity (trf 2 7 7 80 10 50 2000 -d -h). To capture species-specific repeats, a custom repeat library was constructed using RepeatModeler (v1.73)<sup>25</sup>, which generated consensus sequences through iterative classification. Non-overlapping annotations from homology-based, tandem repeat, and *de novo* predictions were merged into a unified dataset using bedtools (v2.25.0)<sup>24</sup>, resolving redundant overlaps. Collectively, repetitive elements comprised 26.70% of the genome assembly (Table 2).

| Class   | Count   | Masked (bp) | Percentages |
|---------|---------|-------------|-------------|
| DNA     | 537     | 93,143      | 0.02%       |
| LINE    | 86,198  | 30,945,334  | 5.01%       |
| LTR     | 7,193   | 5,873,997   | 0.95%       |
| SINE    | 121,887 | 20,867,110  | 3.38%       |
| TIR     | 246,033 | 57,008,848  | 9.23%       |
| Other   | 143,984 | 34,762,877  | 5.62%       |
| unknown | 63,584  | 15,363,299  | 2.49%       |
| Total   | 669,416 | 164,914,608 | 26.70%      |

**Table 2.** Statistics of repeat elements in genome assembly.



**Fig. 3** Comparative synteny and genomic architecture of the banded goonch (*Bagarius rutilus*). **(A)** Circos plot illustrates collinear relationships between the banded goonch (blue tracks) and the *Trichomycterus rosablanca* (green tracks). Connecting ribbons represent conserved homologous regions. **(B)** Circular visualization of genomic features across the banded goonch genome, analyzed in 500-kb non-overlapping windows. The color scale of the heatmap represents the Z-score normalized values for each data track. a: chromosomes, b: gene frequency across the genome, c: density of DNA transposable elements, d: density of long interspersed nuclear elements (LINEs), e: density of long terminal repeats (LTRs), f: density of short interspersed nuclear elements (SINEs), g: density of other transposable elements, h: density of unknown genomic elements, i: the collinearity relationships within genome.

Protein-coding genes were annotated using an integrative approach that combined *ab initio* prediction, homology-based evidence, and transcriptomic support, as in previous studies<sup>28–30</sup>. RNA-seq data from skeletal muscle, heart, kidney and liver were assembled using rnaSPAdes (v3.13.1)<sup>31</sup>, and putative coding sequences were identified from the assembled transcripts using TransDecoder (v5.5.0)<sup>32</sup>. For homology-based prediction, protein sequences from seven close species (*Ictalurus punctatus*<sup>33</sup> [GCA\_001660625.3], *Ictalurus furcatus*<sup>34</sup> [GCA\_023375685.2], *Hemibagrus wyckioidei*<sup>35</sup> [GCA\_019097595.1], *Silurus meridionalis*<sup>36</sup> [GCA\_014805685.1], *Clarias dussumieri*<sup>37</sup> [GCA\_041682065.1]. Finally, EVIDENCEModeler (v1.1.1)<sup>38</sup> integrated predictions from all three methods, prioritizing transcriptome-derived evidence (weight = 10), followed by homology (weight = 5) and *ab initio* predictions (weight = 1), yielding a consensus set of 29,106 protein-coding genes with high confidence.

**Syntenic alignment with *Trichomycterus rosablanca*.** The genome of *Trichomycterus rosaceus*<sup>39</sup> (assembly accession: GCA\_030014385.1) was retrieved from the National Center for Biotechnology Information (NCBI) database for the comparative syntenic analysis. The protein-coding genes within this genome were then annotated using the aforementioned genome annotation pipeline. This annotated genome was then used to perform a whole-genome synteny comparison with our target species using JCVI<sup>40</sup>. To refine the results, the identified syntenic blocks were filtered based on two criteria: (1) blocks containing fewer than five genes were discarded and (2) only syntenic gene pairs with sequence identities greater than 75% were retained for subsequent analysis (Fig. 3A,B).

## Data Records

HiFi, ONT ultra-long, Hi-C and RNA-seq raw reads were deposited in the China National GeneBank Database (CNGBdb) under the accession numbers CNP0007058<sup>41–49</sup>. The genome assembly and annotation were also can be found in figshare database ([https://figshare.com/articles/dataset/Genome\\_genome\\_annotation\\_information\\_and\\_protein\\_sequence\\_of\\_Bagarius\\_rutilus/28660733](https://figshare.com/articles/dataset/Genome_genome_annotation_information_and_protein_sequence_of_Bagarius_rutilus/28660733))<sup>50,51</sup> and GenBank (JBRFMU000000000.1)<sup>52</sup>.

## Technical Validation

The integrity of the genomic DNA was verified by agarose gel electrophoresis and its concentration quantified using a Qubit 4.0 Fluorometer with the 1 × dsDNA High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific). The final genome assembly demonstrated a high degree of completeness, as evidenced by a BUSCO score of 97.5%, and exhibited strong syntenic collinearity with the genome of the related catfish species *Trichomycterus rosaceus*. To further assess the accuracy and contiguity of the assembly, the raw sequencing reads were mapped back, yielding high alignment rates and coverage depths: 99.71% for HiFi reads (at 223 × mean coverage), 99.85% for ONT ultra-long reads (at 41 × mean coverage), and 100% for Hi-C reads, confirming the high quality of the final genome assembly.

## Data availability

All raw sequencing data including HiFi, ONT ultra-long, Hi-C and RNA-seq raw reads and genome assembly were deposited in the China National GeneBank Database (CNGBdb) under the accession numbers CNP0007058<sup>41–49</sup>, which includes Illumina short reads (CNX1195290)<sup>41</sup>, Hi-C data (CNX1195291)<sup>42</sup>, ONT ultra-long reads (CNX1195292)<sup>43</sup>, PacBio HiFi reads (CNX1195293)<sup>44</sup>, RNA-seq data (CNX1195294–CNX1195297)<sup>45–48</sup> and genome assembly (CNA0423406)<sup>49</sup>. The genome assembly and annotation information were also can be found in figshare database<sup>50,51</sup> and GenBank (JBRFMU000000000.1)<sup>52</sup>.

## Code availability

No specific script was used in this work. The corresponding bioinformatics software and the specific versions of software have been described in Methods.

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## Author contributions

Y.L., F.Z., J.L., M.D., J.L. and W.C. conceived and designed the project. C.L., H.Y. and Y.L. collected the samples. Y.L. and F.Z. performed the DNA and RNA extraction, library preparation, and genome sequencing. W.C. performed the bioinformatics analysis and visualized the results. Y.L., F.Z., J.L., M.D., J.L. and W.C. wrote the manuscript. Y.L. and W.C. revised and edited the manuscript. All authors have read and approved the final version of manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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