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Chromosome-level genome assembly and annotation of *Pterygoplichthys pardalis*

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Suckermouth catfishes, with their evolved powerful features, have become notorious invasive species, causing significant damage to aquatic ecosystems. However, the lack of high-quality genomes severely restricts research on this group within the field. In this study, we *de novo* assembled the chromosome-level genome assembly of *Pterygoplichthys pardalis* using multiple platforms of sequencing data, including Illumina short reads, Nanopore long reads, and Hi-C sequencing reads, resulting in a 1.51 Gb genome assembly. Multiple evaluations, including read mapping ratio (98.52%), transcript mapping ratio (99.61%), conserved BUSCO gene set (98.8%), and N50 score (49.47 Mb), indicated the high continuity and accuracy of the genome assembly we generated. Genome annotation found that 0.97 Gb of genome sequences are repetitive sequences, accounting for 64.47% of the genome assembly. Further, 23,859 protein-coding genes were successfully predicted, 92.92% of which could be annotated in functional databases. This high-quality genome assembly of *P. pardalis* provides a valuable resource for understanding the genetic underpinnings of *P. pardalis*'s invasive success and offers critical data for future fisheries research and management.

Background & Summary

With their numerous evolved powerful features, suckermouth catfishes have emerged as one of the notorious invasive groups globally, with documented invasions across tropical and subtropical regions, including Southeast Asia, the southern United States, and Central America, exerting significant impacts on the ecosystem^{1–4}. Notably, *Pterygoplichthys pardalis*, native to the Amazon Basin, serves as a famous and typical representative of such an invasive group⁵. It has established invasive populations various countries, where it disrupts food webs, alters benthic habitats through burrowing, and damages fisheries infrastructure^{2,3,6,7}. More importantly, these invaders not only compete with native species over food resources, but they also aggressively prey on eggs and young fish, thereby leading to a decline in native fish populations and posing a significant threat to the integrity of the local ecological chain^{4,6}. Economic costs arise from levee erosion, reduced catch yields, and expensive eradication efforts^{2,3,6,7}, underscoring the urgency of understanding its biology to inform management strategies.

This omnivorous fish feeds on a wide variety of food sources, including algae, organic material, small invertebrates, and sediment particles^{1,8}, enabling exploitation of resource-poor environments. Amazingly, significant changes in their gastric system, which functions as an additional respiratory organ, enable them to thrive in environments with low levels of dissolved oxygen^{9,10}, which is the common feature of polluted or eutrophic habitats. Additionally, they possess the abilities to survive in cold temperatures and drought conditions by burrowing underground, even when the water level dips below the entrance of their burrows^{1,11}. Its rapid growth, high reproductive capacity, and lack of natural predators have facilitated its accidental introduction into non-native

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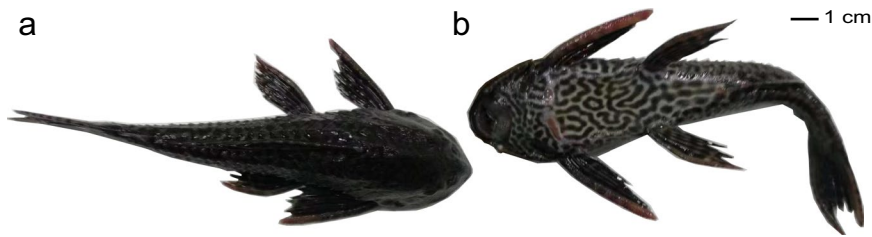


Fig. 1 A photo of the *P. pardalis* specimen used for the genome sequencing. **(a)** Dorsal view; **(b)** Ventral view.

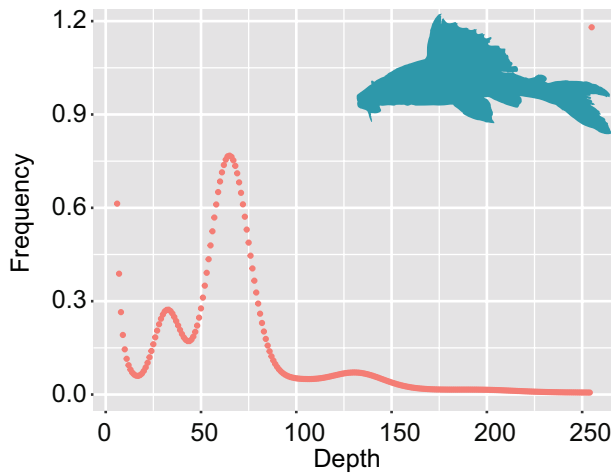


Fig. 2 Genomic information of *P. pardalis*. Survey of genomic characteristics. X-axis represents 17-mer depth, y-axis represents 17-mer frequency.

Sequencing platform	Total bases (bp)	Average length(bp)	Coverage (X)
Short-insert library	146,148,898,800	150	96.94
Nanopore library	218,068,467,938	23,709	144.65
Hi-C library	149,241,882,900	150	98.99
RNA library	9,703,545,250	150	—

Table 1. Statistics of the sequencing data generated in this study.

habitats, where it rapidly establishes invasive populations^{1,12–16}. These traits, coupled with a lack of natural predators in non-native ranges, enable *P. pardalis* to monopolize niches, displace native species, and degrade ecosystems^{12,13}. Eventually, once these invaders establish a population, eradicating them becomes challenging. Despite scientists, including ichthyologists, ecologists, and evolutionary biologists, have been studying for decades^{1,7,14,17}, genetic mechanisms underlying *P. pardalis*’s adaptability remain poorly understood. Only a single mitochondrial genome (NCBI Accession: NC_058365)¹⁸ and a very fragmented nuclear draft genome (contig N50: 4.15 kb)¹⁹ are insufficient for resolving these complex traits. More importantly, this situation significantly limits insights into molecular drivers of invasiveness and constrains comparative analyses with native and invasive relatives.

To address these challenges, we present a chromosome-level genome assembly (1.51 Gb) of *P. pardalis* by integrating Illumina short reads, Nanopore long reads, and Hi-C data. By combining multiple annotation strategies, we ultimately determined that 0.97 Gb of the genome are repetitive sequences, which account for 64.47% of the total genome, and we successfully predicted 23,859 protein-coding genes in the *P. pardalis* genome. These findings not only provide a high-quality genome resource for *P. pardalis*, but also facilitate large-scale comparative genomic studies and enable prevention- and control-oriented applications.

Methods

Data acquisition. The catfish samples used in this study were purchased from an ornamental fish wholesale market in Xi’an, China (Fig. 1). The remaining samples of this specimen (Catfish_01) have been cryopreserved at –80 °C in the Biodiversity Repository of the Institute of Basic and Translational Medicine at Xi’an Medical University. All animal specimens were collected legally in accordance with the policy of the Animal Care and Use Ethics of the institution. Genomic DNA was extracted from the muscle tissue of one suckermouth catfish (*P. pardalis*) using the Blood & Cell Culture DNA Mini Kit (Qiagen, Hilden, Germany). To obtain a high-quality

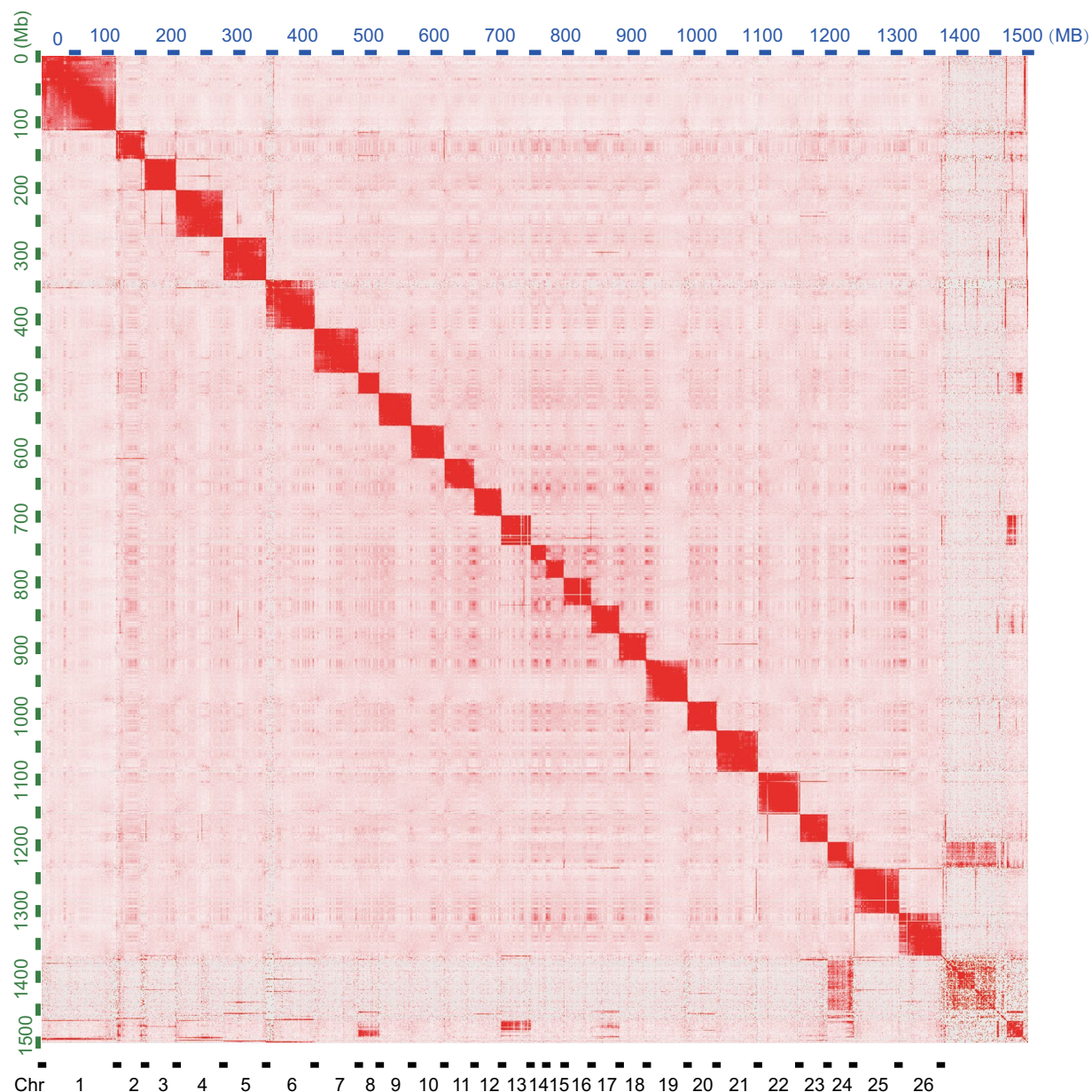


Fig. 3 Heatmap of chromosomal interactions. Blocks represent contact between corresponding locations.

chromosome-level genome assembly, data from multiple sequencing platforms were acquired: 1) A short-insert paired-end library was prepared and sequenced on the Illumina NovaSeq. 6000 platform; 2) A Nanopore library was prepared and sequenced across 26 flow-cells using the Nanopore PromethION 48 (Oxford Nanopore, Oxford, UK); 3) A Hi-C library was constructed and sequenced using the Illumina NovaSeq. 6000 platform; 4) To support genome annotation, total RNAs was extracted from muscle using a TRIzol Kit (Life Technologies) and subsequently used for library construction and sequencing on the Illumina NovaSeq. 6000 platform. All library construction and genome/transcriptome sequencing processes were conducted in biotechnology companies according to their standard workflows. In total, we got 146.15 Gb of Illumina paired-end short-read data (Table 1), 218.07 Gb of Nanopore long-read sequencing data (Table 1), and 149.24 Gb of high-throughput chromosome conformation capture (Hi-C) sequencing data (Table 1).

Quality control of sequencing data. To facilitate high-quality genome assembly, we performed strict quality control processes. For Illumina reads, adaptor sequences and polymerase chain reaction (PCR) duplicates were removed from all paired-end reads with Perl scripts²⁰. Additionally, any Illumina reads containing more than 5% unknown bases or exceeding 30 low-quality bases, along with their paired-end reads, were discarded²¹. For Nanopore reads, only reads with a mean quality score >7 were retained and used for subsequent analysis²¹.

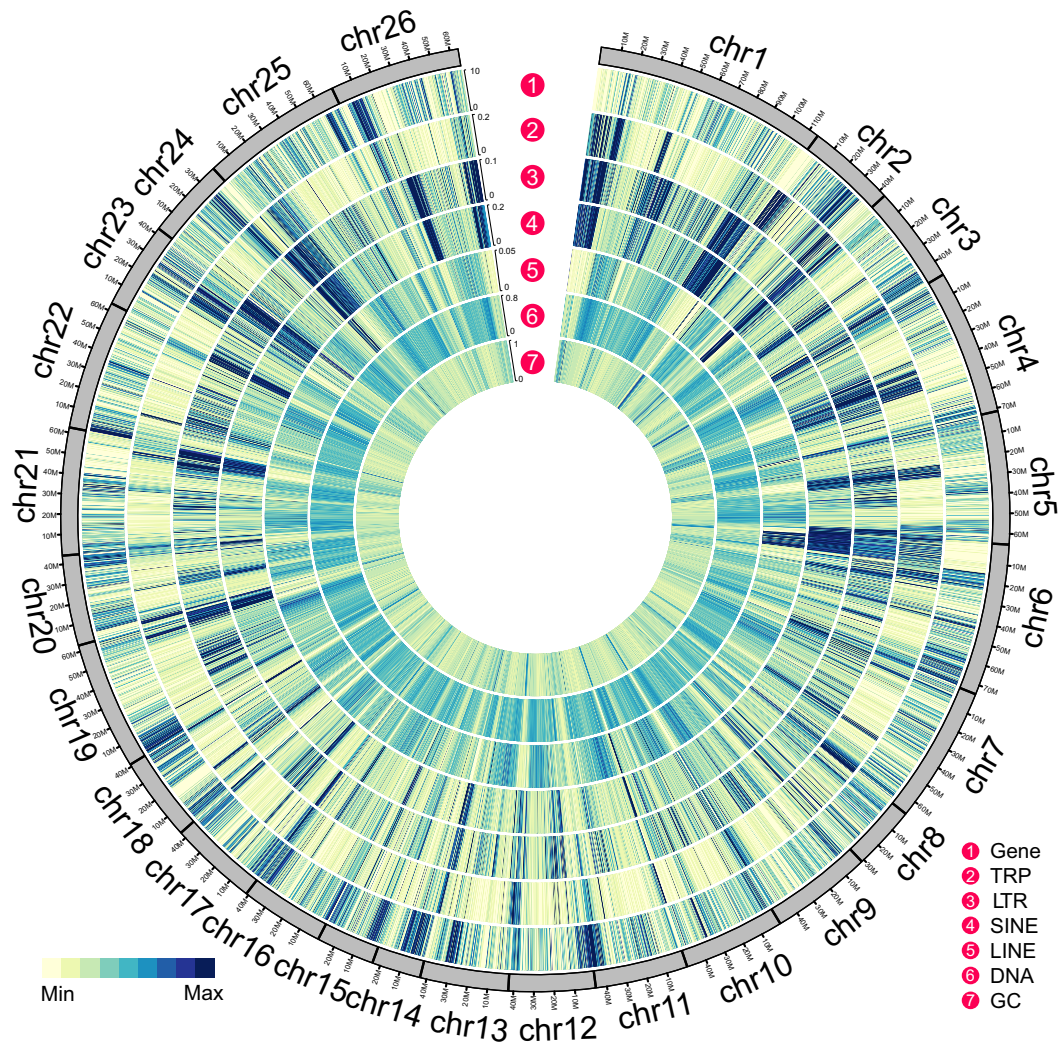


Fig. 4 Distributions of genomic elements in *P. pardalis* genome. Outer to inner ring are distributions of protein-coding genes, tandem repeats (TRP), long terminal repeats (LTR), short interspersed nuclear elements (SINE), long interspersed nuclear elements (LINE), DNA elements, and GC content, respectively.

Chromosome ID	Length (bp)	Chromosome ID	Length (bp)
Chr1	114,651,524	Chr14	23,724,721
Chr2	43,195,137	Chr15	28,276,377
Chr3	47,361,500	Chr16	40,897,388
Chr4	71,254,436	Chr17	43,031,752
Chr5	64,993,512	Chr18	40,891,462
Chr6	74,398,770	Chr19	62,825,890
Chr7	67,112,199	Chr20	44,018,593
Chr8	31,065,881	Chr21	62,176,789
Chr9	49,448,268	Chr22	63,455,860
Chr10	49,469,991	Chr23	42,514,100
Chr11	45,172,604	Chr24	39,030,154
Chr12	42,234,017	Chr25	69,423,165
Chr13	42,988,544	Chr26	64,198,399
Total length of long scaffolds (bp)		1,367,811,033	
Total genome size (bp)		1,507,578,907	
Ratio of long scaffolds in whole genome		90.73%	

Table 2. Statistics of chromosomal level assembly of *P. pardalis*.

Term	Size (bp)	Number
N90	23,724,721	26
N80	40,891,462	22
N70	42,988,544	27
N60	45,172,604	14
N50	49,469,991	11
Max length (bp)	114,651,524	—
Total size (bp)	1,507,578,907	—
Total number (>100bp)	414	—
Total number (>10kb)	414	—

Table 3. Statistics of assembly information of *P. pardalis*.

Library	Eukaryota	Metazoa	Actinopterygii
Complete BUSCOs (C)	252	939	3367
Complete and single-copy BUSCOs (S)	250	920	3328
Complete and duplicated BUSCOs (D)	2	19	39
Fragmented BUSCOs (F)	1	4	34
Missing BUSCOs (M)	2	11	239
Total BUSCO groups searched	255	954	3640
Summarize	98.80%	98.40%	92.50%

Table 4. Completeness assessment of *P. pardalis* genome by BUSCO.

Type	Total Number	Mapped Number	Mapped ratio(%)
Genomic short reads	803,190,085 (reads)	791,332,947 (reads)	98.52%
Transcripts	103,116	102,716	99.61%
Nanopore	16,359,196 (reads)	16,341,304 (reads)	99.89%

Table 5. Statistics of the mapping ratio of the reads and transcripts to the *P. pardalis* genome.

Term	Size (bp)	Number
N90	383	61,534
N80	706	39,794
N70	1,161	27,349
N60	1,693	19,365
N50	2,267	13,634
Max length (bp)	82,524	—
Total size (bp)	112,286,016	—
Total number (>100bp)	103,116	—
Total number (>10kb)	268	—

Table 6. Statistics of transcript assembly by Bridger software.

Genome size estimation. A *k*-mer based strategy was employed to estimate the genome size of *P. pardalis*. Using all the cleaned short-insert Illumina reads, a 17-mer was selected for this analysis (<https://github.com/fanagislab/kmerfreq>). The genome size can be calculated using the formula: $G = Knum/Kdepth$. *G* represents the estimated genome size, *Knum* denotes the total count of 17-mers, and *Kdepth* represents the peak depth of the 17-mers²². The genome of *P. pardalis* was estimated to be approximately 1.48 Gb, with a considerable level of heterozygosity (Fig. 2).

Genome assembly. The genome assembly was performed with the following steps: 1) Long reads from the Nanopore platform were used for the contig-level assembly using NextDenovo (v2.2; <https://github.com/Nextomics/NextDenovo>). Key parameters were carefully set to ensure optimal assembly, including a read cutoff of 1k, a seed cutoff of 59754, and a blocksize of 5 g. 2) Cleaned short reads generated from the Illumina short-insert library were mapped onto the assembled contigs using BWA (v0.7.17)²³. To further enhance the accuracy of the assembly at the single-base level, we performed two iterations of correction using Pilon (v1.22)²⁴. 3) We mapped the Hi-C sequencing reads to the corrected contigs, and subsequently utilized Juicer (v1.5.7)²⁵ and 3D *de novo*

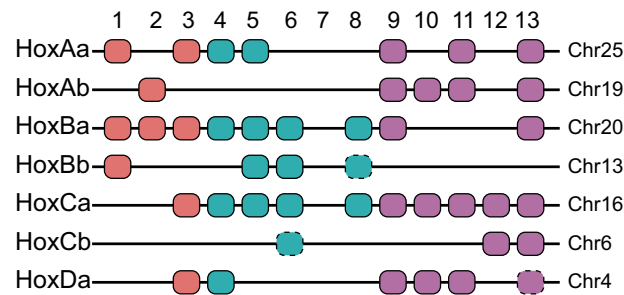


Fig. 5 Hox gene clusters in *P. pardalis* genome. Solid line represents functionally annotated gene in the database, dotted line represents that only the gene fragment could be found.

Type	Repeat size (bp)	Percent of genome (%)
Trf	110,789,900	7.348862
Repeatmasker	358,195,998	23.759685
Proteinmasker	135,338,199	8.977188
De novo	911,029,930	60.430000
Total	971,995,156	64.473916

Table 7. Statistics of the repetitive sequences annotated by each method of the *P. pardalis* genome.

Type	Repbse TEs		TE protiens		De novo		Combined TEs	
	Length (bp)	% in genome	Length (bp)	% in genome	Length (bp)	% in genome	Length (bp)	% in genome
DNA	264,951,677	17.57	32,487,312	2.15	455,290,359	30.20	499,766,710	33.15
LINE	61,003,156	4.05	73,956,432	4.91	118,936,260	7.89	144,008,691	9.55
SINE	3,835,338	0.25	0	0.00	26,813,655	1.78	29,267,760	1.94
LTR	33,661,995	2.23	28,705,578	1.90	61,223,416	4.06	78,002,083	5.17
Other	3,955,503	0.26	228,157	0.02	65,567,572	4.35	67,325,227	4.47
UnKnown	1,273,026	0.08	0	0.00	217,454,064	14.42	218,339,022	14.48
Total	358,195,998	23.76	135,338,199	8.98	911,029,930	60.43	940,547,025	62.39

Table 8. Statistical of the predicted transposable element in the *P. pardalis* genome.

Term	Number	Percentage(%)
InterPro	19,394	81.28589
GO	14,887	62.39574
KEGG	16,633	69.71374
Swissprot	20,504	85.93822
TrEMBL	21,968	92.07427
Cog	7,425	31.12033
NR	22,143	92.80775
Annotated	22,169	92.91672
Unanotated	1,690	7.083281
Total	23,859	

Table 9. Statistics of functional annotation for protein coding genes.

assembly (v180922)²⁶ to perform chromosome-level genome assembly. Eventually, we successfully assembled the 1.51 Gb chromosome-level reference genome, with a total of 26 chromosomes and a scaffold N50 length of 49.47 Mb (Figs. 3, 4, Tables 2, 3). Notably, the assembled genome size closely aligned with the estimated size based on *k-mer* analysis (1.48 Gb) (Fig. 2), indicating the high-integrity of the genome assembly we acquired. To further evaluate the quality of the genome assembly, multiple strategies were employed, including the BUSCO (v5.2.2, Vertebrata_odb10)²⁷ score (98.8%) (Table 4), the mapping ratio of short-insert reads (98.52%) (Table 5),

Library	metazoa
Complete BUSCOs (C)	837
Complete and single-copy BUSCOs (S)	807
Complete and duplicated BUSCOs (D)	30
Fragmented BUSCOs (F)	47
Missing BUSCOs (M)	70
Total BUSCO groups searched	954
Summarize	87.70%

Table 10. Completeness assessment of *P. pardalis* gene by BUSCO.

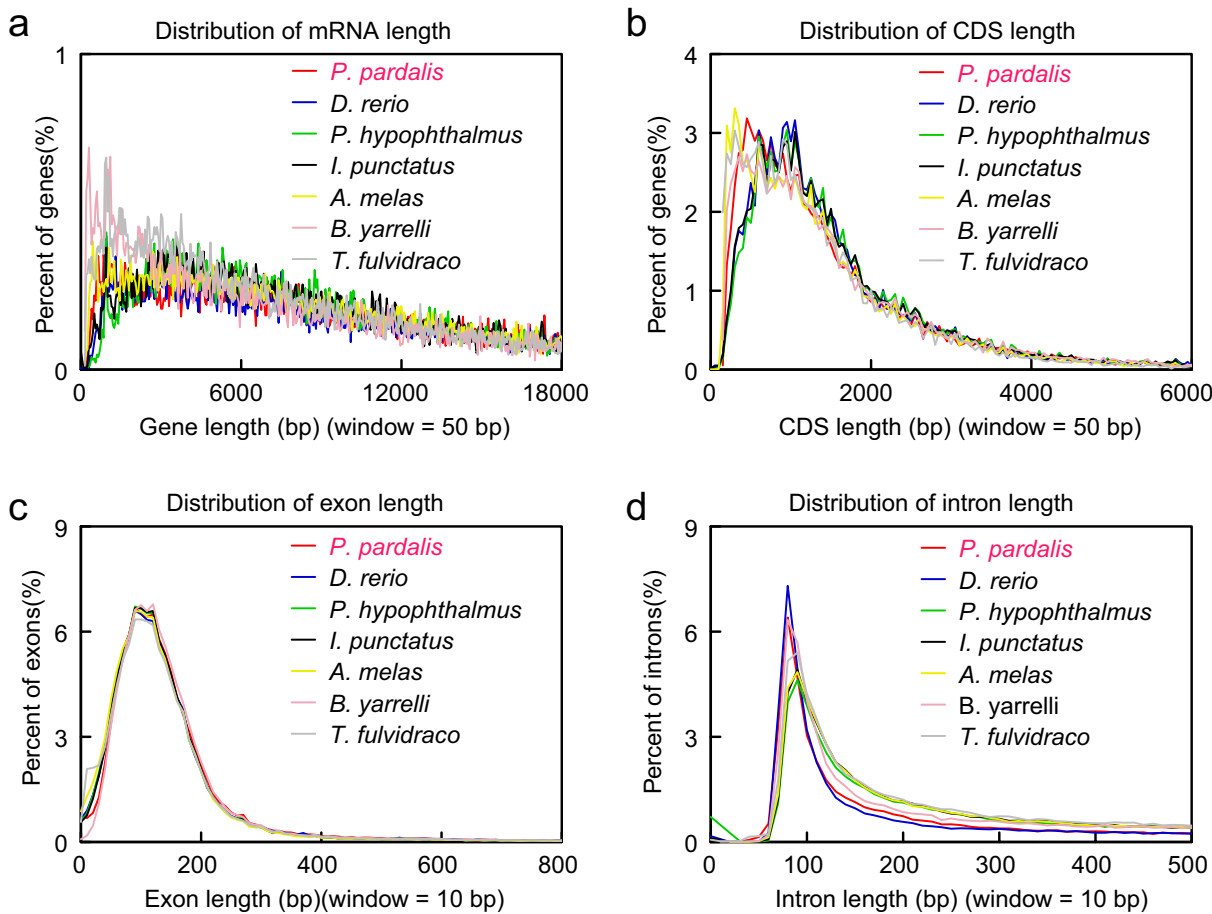


Fig. 6 Quality comparison of protein-coding genes between *P. pardalis* and other species. Quality of gene annotation based on (a) gene length, (b) CDS length, (c) exon length, and (d) intron length, respectively.

transcripts (99.61%) (Tables 5, 6), Nanopore (99.89%) (Table 5), QV value (31.59), as well as the Hox clusters (Fig. 5). Among them, the Nanopore reads were remapped with minimap2 (v2.26-r1175), and the QV scores were assessed by Merqury (v3.0.1; <https://github.com/marbl/merqury>). All these results indicate that the *P. pardalis* genome assembly exhibits both high integrity and accuracy.

Genome annotation. Tandem repetitive sequences within the genome were identified using Tandem Repeat Finder (v4.07)²⁸. Non-interspersed repeats in the genome were annotated using RepeatMasker (v4.1.0)²⁹. Transposable elements (TEs) in the genome were annotated at both the DNA and protein levels. A *de novo* repeat library at the DNA level was constructed using RepeatModeler (v1.0.4; GitHub - Dfam-consortium/RepeatModeler: De-Novo Repeat Discovery Tool) enabling the identification of potential novel repetitive sequences. The genome assembly was searched against Repbase (v23.06) using RepeatMasker (v4.1.0)²⁹ to detect homologous repetitive sequences, providing a more comprehensive picture of the repetitive sequence content. RM-BLASTX within RepeatProteinMask (v4.1.0) was employed to query the TE protein database at the protein level. We found that 0.97 Gb of the genome length consisted of repetitive sequences, which accounts for 64.47% of the genome assembly of *P. pardalis* (Table 7). Among them, DNA elements (499.77 Mb; 33.15%) constitute the

largest proportion of transposable elements (TEs; Table 8), which were followed by the long interspersed nuclear elements (LINEs; 144.01 Mb; 9.55%), long terminal repeats (LTRs; 78.00 Mb; 5.17%), and short interspersed nuclear elements (SINEs; 29.27 Mb; 1.94%), in the *P. pardalis* genome (Table 8).

Prediction and functional annotation of protein-coding genes. Protein-coding genes were predicted based on three distinct strategies. For *de novo*-based prediction, the transcripts of *P. pardalis* muscle tissue were assembled based on RNA-seq data using Bridger (r2014-12-01)³⁰. Subsequently, the assembled transcripts were filtered and underwent primary prediction using the PASA pipeline (v2.1.0)³¹ and AUGUSTUS (v2.5.5)³². Protein sequences, including *Bagarius yarrelli* (GCA_005784505.1), *Ameiurus melas* (GCA_012411365.1), *Ictalurus punctatus* (GCF_001660625.1), *Pangasianodon hypophthalmus* (GCF_009078355.1), *Tachysurus fulvidraco* (GCF_003724035.1), *Hemibagrus wyckioides* (GCA_019097595.1), *Silurus meridionalis* (GCF_014805685.1), *Clarias magur* (GCA_013621035.1), *Danio rerio* (GCF_000002035.6), *Pelteobagrus fulvidraco* (<http://gigadb.org/dataset/100506>), and *Glyptosternon maculatum* (<https://doi.org/10.1093/gigascience/giy104>), were downloaded for homology-based prediction. To further refine the coding gene prediction, we selected the longest transcript for each gene and removed those with premature termination sites. Using the Basic Local Alignment Search Tool (BLAST) (v2.2.26; <https://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/2.2.26/>) with an *e-value* threshold of 1e-5, we then performed homology-based annotation using GeneWise (v2.4.1)³³. For transcript-based prediction, RNA-seq reads were mapped to the assembled genome using BLAT (v34)³⁴ and spliced alignments were subsequently linked using the PASA pipeline (v2.1.0)³¹. Finally, the predicted coding genes obtained from the three strategies were integrated using EvidenceModeler (r2012-06-25)³⁵. We successfully predicted 23,859 protein-coding genes in the *P. pardalis* genome (Table 9), with the BUSCO score of 87.7% (metazoa_odb10, Table 10). To validate the quality of these predicted protein-coding genes, we conducted a comparative analysis of length distributions across many gene structures, including mRNA (Fig. 6a), coding sequences (CDS) (Fig. 6b), exons (Fig. 6c), and introns (Fig. 6d), between *P. pardalis* and other species. Our results indicated that the predicted protein-coding genes in *P. pardalis* exhibited comparable quality to those previously reported in other species (Fig. 6).

For functional annotation, all the predicted protein-coding genes were aligned to multiple databases, including InterPro (<https://www.ebi.ac.uk/interpro/>), Gene Ontology (GO) (<https://geneontology.org/>), Kyoto Encyclopedia of Genes and Genomes (KEGG) (<https://www.kegg.jp/>), UniProt/SwissProt (<https://www.uniprot.org/>), UniProt/TrEMBL (<https://www.uniprot.org/>), and the Non-Redundant Protein Sequence Database (NR) (<https://ftp.ncbi.nlm.nih.gov/blast/db>). We found the majority of the predicted genes (22,169; 92.92%) had homologous genes in various public databases (Table 9).

Data Records

All the raw sequencing data, including Nanopore and Illumina reads, have been uploaded to the NCBI database (National Center for Biotechnology Information, <https://www.ncbi.nlm.nih.gov>) under the BioProject accession number PRJNA1165483³⁶. The genome assembly and annotation files were uploaded to the Dryad Digital Repository (<https://doi.org/10.5061/dryad.bk3j9kdgh>)³⁷ and Genbank dataset (GCA_050231285.1)³⁸.

Technical Validation

The final assembly (1.51 Gb) of *P. pardalis* is slightly larger than the estimated genome size (1.48 Gb), which may be caused by the genome heterozygosity (Fig. 2). Three distinct strategies were employed to predict protein-coding genes. Using Hi-C technology, we successfully assembled 26 chromosomes of *P. pardalis* (Fig. 3), which is consistent with the result of a karyotype experiment in a previous study³⁹. Genome annotation further revealed that the length and proportion of repetitive sequences in *P. pardalis* (0.97 Gb and 64.47%) are obviously higher than those of other catfish species (*I. punctatus*: 0.27 Gb and 34.92%, *P. hypophthalmus*: 0.27 Gb and 36.90%, *H. wyckioides*: 0.32 Gb and 40.12%, *S. meridionalis*: 0.30 Gb and 40.12%, *G. maculatum*: 0.25 Gb and 32.76%, and *P. fulvidraco*: 0.28 Gb and 38.47%) (Fig. 4), indicating that the expansion of repetitive regions is the main reason for the large genome of *P. pardalis*.

Code availability

No specific code or script was used in this study. All data processing commands were executed according to the official manuals and standard protocols of the respective software. The Methods section includes the software versions, URLs, and parameters.

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Author contributions

L.X. and Y.Z. supervised the project. W.X. collected the samples. W.X. and Y.L. performed the sequencing of PacBio, Illumina, and Hi-C data. W.X., H.X., Y.L. and H.J. performed the genome assemble and annotation analysis. W.X. prepared all graphical illustrations. W.X. wrote the manuscript. L.X., H.X., Y.W., Y.Y., X.L. and W.F. revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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