Chromosome-level genome assembly of golden pompano (Trachinotus ovatus) in the family Carangidae

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Golden pompano (Trachinotus ovatus), a marine fish in the Carangidae family, has a wide geographical distribution and adapts to severe environmental rigours. It is also an economically valuable aquaculture fish. To understand the genetic mechanism of adaptation to environmental rigours and improve the production in aquaculture, we assembled its genome. By combination of Illumina and Pacbio reads, the obtained genome sequence is 647.5 Mb with the contig N50 of 1.80 Mb and the scaffold N50 of 5.05 Mb. The assembly covers 98.9% of the estimated genome size (655 Mb). Based on Hi-C data, 99.4% of the assembled bases are anchored into 24 pseudo-chromosomes. The annotation includes 21,915 protein-coding genes, in which 95.7% of 2,586 BUSCO vertebrate conserved genes are complete. This genome is expected to contribute to the comparative analysis of the Carangidae family.

Background & Summary
The golden pompano, Trachinotus ovatus (Linnaeus 1758), belongs to Carangiformes and is widely distributed in tropical and subtropical oceans1. From a biogeographic perspective, this fish readily tolerates different environments. In addition, this fish has been one of the most important economic marine fish in China2. However, overfishing, diseases, and degeneration of genetic diversity have caused serious economic losses in T. ovatus production3. Many solutions, including selective breeding4, identification of trait-associated genes5, and dietary supplementation6, are adopted to overcome these problems and improve the production.

The golden pompano is a marine fish in the Carangidae family. One characteristic of this family is the indistinguishable sex chromosomes7. It is speculated that sex chromosomes in this family have not been largely differentiated, distinct from those with well-differentiated sex chromosomes8. Therefore, fish in this family could be used to analyse the initial evolution status of the sex-determination system. Another characteristic of this family is tolerance to high turbidity, rapid pH changes and low dissolved oxygen concentrations and crowding9. The Carangidae fish are potential candidates to study resistance to stress.

A high-quality genome assembly is necessary to understand the functional, ecological and evolutionary genomics of this species and other fish in the Carangidae family. In the present study, we presented a chromosome-level genome assembly of pompano using Illumina sequencing, Pacbio sequencing, and Hi-C technology (Fig. 1). We produced 105 Gb of cleaned Illumina reads of genomic DNA, 16.9 Gb Pacbio long reads, and 114.8 Gb cleaned data from a Hi-C library. The genome size was estimated to be 655 Mb (Fig. 2). A 647.5 Mb assembly of pompano was generated. The contig N50 length and scaffold N50 length were 1.80 Mb and 5.05 Mb, respectively. Based on 114.8 Gb Hi-C data, 99.4% of the assembly were anchored into 24 pseudo-chromosomes. The annotation includes 21,915 protein-coding genes.

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The coverage of the estimated genome size (98.9%), the proportion of bases anchored to the pseudo-chromosomes (99.4%), the RNA-seq alignment ratio (90.5%), the proportion of pompano protein-coding genes having homologues (97.5%), and the ratio of complete BUSCO vertebrate genes (95.7%) all indicate that this genome assembly is of high quality. The genome assembly and its annotated information would be useful for studies on environmental adaptations, resistance to disease and sex determination. This genome has already proven to be useful to mine functional genes underlying resistance to disease\textsuperscript{10,11}. It is the first chromosome-level genome in the Carangidae family and is expected to contribute to the study of the diversity, speciation, and evolution of this family.

**Methods**

**Ethics statement.** The sampled fish in this study was permitted by the Animal Care and Use Committee of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (No. SCSFRI96-253) and performed by the regulations and guidelines established with this committee.
A female pompano was collected in Xincun Bay, Hainan, China. Total genomic DNA was extracted using a DNA Extraction Kit (MAGEN Company, Guangdong, China) following the manufacturer’s protocols. The quality and quantity of total DNA were determined by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). We constructed two paired-end libraries (insert sizes of 500 and 700 bp) and three mate-pair libraries (insert sizes of 3, 5, and 14 kb) according to Illumina standard procedures (Illumina, San Diego, CA, USA). The libraries were sequenced on a HiSeq 2500 system with 250 bp PE mode or 100 bp PE mode (Table 1).

The extracted DNA molecules were also used to construct two 20 kb libraries following the PacBio manufacturing protocols (Pacific Biosciences, CA, USA). The libraries were then sequenced with two cells on PacBio Sequel platform (Table 1).

The Hi-C technique has been applied into constructing chromosome-level assemblies. We prepared a Hi-C library for the chromosome assembly of pompano following the strategy of Rao et al.14. Briefly, the blood sample was fixed with fresh formaldehyde and then DNA-protein bonds were created. The restriction enzyme of MboI digested the DNA and the overlapping S' ends of the DNA fragments were repaired with a biotinylated residue.

Table 1. Data statistics of whole genome sequencing reads of pompano.

| Platform   | Insert size | Clean pairs | Total bases | Genome coverage (X) | SRA accession |
|------------|-------------|-------------|-------------|---------------------|---------------|
| Illumina   | 500 bp      | 44,554,312  | 19,894,674,143 | 30.3 | SRR8185380 |
| 700 bp     | 94,147,131  | 15,691,188,500 | 23.9 | SRR8185379 |
| 3K bp      | 24,639,173  | 5,597,129,699  | 8.5  | SRR8185378 |
| 5 Kbp      | 22,753,897  | 5,688,834,998  | 8.6  | SRR8185382 |
| 14 Kbp     | 149,292,822 | 28,171,641,480 | 42.9 | SRR8185385 |
| Hi-C (Illumina X ten) | 382,798,592 | 114,839,577,600 | 175.1 | SRR8168440 |
| Pacbio     | 2,278,176   | 16,879,861,540 | 25.7 | SRR7943174 |
| Total      | 272,622,581 | 206,762,907,960 | 315.3 |            |

Table 2. Estimation of genome size of pompano by k-mer analysis.

| K | Total number of k-mers | Number of erroneous k-mers | Peak in jellyfish counting | Estimated genome size (Mb) |
|---|------------------------|---------------------------|-----------------------------|---------------------------|
| 17 | 30,359,515,882 | 1,700,273,328 | 45 | 636.9 |
| 19 | 29,905,858,631 | 2,266,172,955 | 43 | 642.8 |
| 21 | 29,425,980,179 | 2,419,537,116 | 42 | 643.0 |
| 23 | 28,931,567,876 | 2,494,020,191 | 41 | 644.8 |
| 25 | 28,427,735,494 | 2,544,415,369 | 40 | 647.1 |
| 27 | 27,917,344,738 | 2,581,038,454 | 39 | 649.6 |
| 29 | 27,402,087,718 | 2,606,597,782 | 38 | 652.5 |
| 31 | 26,882,868,388 | 2,621,598,458 | 37 | 655.7 |

Table 3. Comparisons of other published Carangiformes assemblies.

Sampling and sequencing. A female pompano was collected in Xincun Bay, Hainan, China. Total genomic DNA was extracted using a DNA Extraction Kit (MAGEN Company, Guangdong, China) following the manufacturer’s protocols. The quality and quantity of total DNA were determined by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). We constructed two paired-end libraries (insert sizes of 500 and 700 bp) and three mate-pair libraries (insert sizes of 3, 5, and 14 kb) according to Illumina standard procedures (Illumina, San Diego, CA, USA). The libraries were sequenced on a HiSeq 2500 system with 250 bp PE mode or 100 bp PE mode (Table 1).

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The fragments close to each other in the nucleus during fixation were ligated. The Hi-C fragments were further sheared by sonication into smaller fragments of ~350 bp in size, which were then pulled-down with streptavidin beads. The Hi-C library for Illumina sequencing was prepared according to the manufacturer’s standard procedures. The library was sequenced on the Illumina HiSeq X Ten platform with 150 bp PE mode.

Eight tissues (blood, liver, muscle, brain, spleen, fin, ovary and stomach) were collected. Total RNA from each tissue was extracted and treated with DNase I (Thermo Fisher Scientific, Wilmington, DE, USA) to remove genomic DNA. The RNA integrity of each tissue was confirmed with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, USA). For each tissue, we constructed two RNA-sequencing libraries with an insert size of 300 bp and then sequenced them on the Illumina HiSeq platform with 150 bp PE mode.

**Read filtration and genome size estimation.** The genomic sequencing reads from five Illumina libraries were first cleaned to remove the adapters using Trimmomatic-0.35\(^{15}\). Then the quality trimming was performed using SolexaQA v3.7.1\(^{16}\) to filter the low-quality bases and short reads <25 bp. We produced 105 Gb of cleaned Illumina reads of genomic DNA. The RNA integrity of each tissue was confirmed with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, USA). For each tissue, we constructed two RNA-sequencing libraries with an insert size of 300 bp and then sequenced them on the Illumina HiSeq platform with 150 bp PE mode.

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Paclito sequencing generated ~16.9 Gb long reads (Table 1). The mean and N50 length were 7.4 and 12.2 kb, respectively. We corrected the Paclito long reads with reads from two Illumina paired-end libraries using proovread v2.14\(^{18}\). Additionally, the paired-end reads of the Hi-C library were trimmed by filtering adapters and removing reads of low quality with Trimmomatic-0.35\(^{15}\) and SolexaQA v3.7.1\(^{16}\), respectively. 382 million cleaned reads with the total bases of 114.8 Gb were generated from the Hi-C library.

### Table 4. Repeat content in pompano genome.

| Repeat elements       | Copies | Bases     | Percent (%) |
|-----------------------|--------|-----------|-------------|
| **Interspersed repeats** |        |           |             |
| SINE                  | 11,964 | 1,473,642 | 0.22        |
| Penelope              | 2,054  | 373,482   | 0.06        |
| LINE                  | 54,917 | 13,503,181| 2.08        |
| LTR                   | 15,038 | 2,965,180 | 0.46        |
| DNA transposon        | 161,301| 22,551,263| 3.48        |
| Unclassified          | 435,045| 69,429,000| 10.71       |
| **Subtotal**          | 680,319| 109,922,266| 16.96       |
| **Tandem repeats**    |        |           |             |
| Satellites            | 1,037  | 167,798   | 0.026       |
| Simple repeats        | 415,200| 18,131,460| 2.80        |
| Low complexity        | 50,191 | 2,814,637 | 0.43        |
| **Subtotal**          | 466,428| 21,113,895| 3.26        |
| Small RNA             | 2,167  | 188,301   | 0.029       |
| **Total**             | 1,148,914| 131,224,462| 20.25       |
Before genome assembly and gene annotation, we estimated the genome size by the k-mer analysis using 35.58 Gb filtered reads from the two paired-end Illumina libraries (500 bp and 700 bp libraries). The number of effective k-mers and the peak depth of a series of k values (17, 19, 21, 23, 25, 27, 29, and 31) were produced using Jeffyfish (v2.2) with the C-setting. The genome size was estimated following the formula \( \text{Genome Size} = \frac{\text{Total k-mers} - \text{Erroneous k-mers}}{\text{Peak}} \). The maximal genome size was calculated to be 655 Mb when a k-mer size was 31 (Table 2). The estimated genome size was within the range of previously reported sizes of other Carangidae fish (614.2 Mb~716.4 Mb, Table 3). Hence, the sequencing coverages of the cleaned Illumina reads, Pacbio reads, and Hi-C data were 114.5, 25.7, and 175.1-fold, respectively. The rate of genome heterozygosity estimated by GenomeScope (v1.0.0) was around 0.31% (Fig. 2). The low heterozygosity indicated this genome to be homozygous.

Hybrid assembly, scaffolding, and chromosome anchoring. The error-corrected long reads were assembled using Canu v1.7 with the default parameters of correctedErrorRate as 0.039. The contigs were further connected into longer contigs with the error-corrected long reads using Opera-LG. The contigs were further scaffolded using mate-pair libraries, and the gaps in the scaffolds were closed with reads from the paired-end libraries using Platanus v1.2.4. The gaps in the assemblies were further filled with the raw long reads using LR_Gapcloser v1.0. The final genome sequences were polished by pilon v1.22 using cleaned Illumina short reads to correct errors in base level. A ~647.5 Mb genome assembly of pompano with 373 scaffolds was constructed. The assembly covered 98.9% of estimated genome regions. The contig N50 length and scaffold N50 length were 1.80 Mb and 5.05 Mb, respectively. A total of 137 scaffolds, longer than 1.26 Mb, covered over 90% of the assembly (Table 2).

To anchor scaffolds into pseudo-chromosomes, HiCUP v0.6.1 was firstly used to map and process the reads from the Hi-C library. Two reads of pairs were mapped to the polished scaffolds using Bowtie 2 with the default parameters. If both two reads from one pair were uniquely mapped to the assembly, this pair was retained for the downstream filtration. HiCUP removed invalid pairs which were generated from contiguous sequences, circularization, dangling ends, internal fragments, re-ligation, PCR duplication, and fragments of wrong size. Based on the refined alignments, we clustered 321 scaffolds into pseudo-chromosomes using Lachesis v1.0. It is reported that pompano genome consists of 24 chromosomes by linkage group analysis and karyotyping. Therefore, the pseudo-chromosome number was set as 24. Finally, Lachesis ordered and oriented 259 scaffolds into 24 pseudo-chromosomes, corresponding to 69.4% and 99.4% of the assembly by sequence number and base count, respectively. The average pseudo-chromosome length was 26.84 Mb. The unanchored 114 scaffolds were much short with an average length of 33.3 kb, covering only 0.6% of the assembly. To validate the correction of the
Hi-C scaffolding to pseudo-chromosome level, we constructed an interaction matrix with cleaned reads from the Hi-C library using HiC-Pro (default parameters and LIGATION_SITE = GATC). The genome was divided into bins of equal size of 100 Kb, and the number of contacts was determined between each pair of reported bins. A contact map plotted with HiCPlotter confirmed the genome structure and quality (Fig. 3). Compared with other Carangidae fish, it is the first chromosome-level assembly in this family (Table 3).

![Fig. 5](alignment_frequency_distribution.png)

**Fig. 5** Alignment frequency distribution of Pacbio long reads and Illumina short reads.

![Fig. 6](insert_size_distribution.png)

**Fig. 6** Distribution of insert sizes of sequencing reads in five libraries.
De novo repeat prediction and classification. Before predicting protein-coding genes, we masked the repetitive regions of the assembly using a combination of ab initio and homology-based approaches. RepeatModeler v1.0.11 (http://www.repeatmasker.org/RepeatModeler/) was used to construct a pompano-specific repeat library consisting of 1,134 consensus repeats. By using RepeatMasker v4.0.7 (http://repeatmasker.org/cgi-bin/WEBRepeatMasker), the repeat regions of this assembly were masked first with the Repbase teleost repeat library34 and then with the pompano-specific library. The results from the stepwise method identified 131.22 Mb of repeat sequences, included 109.9 Mb of interspersed repeats and 21.1 Mb of tandem repeats. Among classified interspersed repeats, DNA transposons were more abundant than retrotransposons. The repeats accounted for 20.25% of the assembly (Table 4), close to that of published diploid fish genomes35–37.

Gene prediction and functional annotation. Based on the repeat-masked assembly, we predicted gene models by integrating ab initio predictions, homologue prediction, and RNA-seq models. First, Fgenesh38 was used to construct de novo gene models. Second, we aligned fish proteins from the Ensembl database 39 to the assembly using BLAT40. All fish proteins annotated in Ensembl database were downloaded to construct an Ensembl fish protein set. The proteins having alignments with over 70% coverage were re-aligned to the assembly using GeneWise41 for accurately spliced alignments. Third, a total of 32 Gb of clean RNA-seq reads from eight tissues trimmed by Trimmomatic-0.3516 and SolexaQA v3.7.116 were used to construct RNA-seq based gene models. RNA-seq reads were mapped to the genome using HISAT242, and the alignments were input to Cufflinks43 to predict transcripts. All three sets of gene models were merged to form a comprehensive consensus gene set using Cuffmerge43. For each model, the longest transcript was selected as the representative transcript. The coding region and protein sequence of the representative transcript were predicted using Transdecoder (https://transdecoder.github.io/). A consensus pompano gene set consisted of 21,915 protein-coding genes. The protein-coding gene number and structures were comparable with that of published Carangiformes genomes (Table 3).

Then we searched for homologues of pompano proteins by aligning them against the Swiss-Prot database, TrEMBL database44 and Ensembl fish protein set with Blastp (e value of $10^{-5}$). Homologue searches found that 21,365 of pompano genes had homologues in at least one database (Table 5). The KEGG biological pathways and Gene Ontology terms of each gene were annotated using the KEGG Automatic Annotation Server 45 and Blast2GO46, respectively. Among the identified protein-coding genes, 20,594 genes were annotated to have at least one Gene Ontology (GO) term, and 7,956 genes were mapped to KEGG pathways. Finally, 21,365 genes (97.5%) were assigned to at least one of five databases (Table 5).

Quality assessment of genome assembly and gene annotation. The quality of the assembly was evaluated using multiple indicators. (1) To estimate the quality value (QV) of the assembly, the cleaned reads from two paired-end libraries were mapped to the assembly with BWA48 and then the pipeup file produced by SAMtools48 were input to Referee49 to calculate a quality score for every position. Referee provided a higher scoring base to an erroneous position and this reference base was considered to be an error. We estimated that this genome had one error per 1000 base pairs with a quality value of 30. (2) We validated the assembly by comparing the cleaned read spectrum from two paired-end libraries with the copy number in the assembly using KAT toolkit40. The k-mer showed the homozygous distribution without a heterozygous peak (Fig. 4), consistent with the low heterozygosity observed by GenomeScope (Fig. 2). The main content occurred once, suggesting that the

| Tissue       | Cleaned pairs | Total bases | Alignment ratio | SRA accession |
|--------------|--------------|------------|-----------------|--------------|
| Blood        | 10,639,911   | 2,631,736,943 | 90.67%          | SRR8656488   |
| Liver        | 16,235,470   | 4,029,392,277 | 89.20%          | SRR8656489   |
| Muscle       | 14,800,607   | 3,677,971,940 | 94.05%          | SRR8656490   |
| Brain        | 14,983,402   | 3,714,276,260 | 82.65%          | SRR8656491   |
| Spleen       | 8,778,246    | 2,178,602,070 | 93.22%          | SRR8656484   |
| Fin          | 25,750,965   | 6,390,342,718 | 93.52%          | SRR8656485   |
| Ovary        | 19,151,732   | 4,749,798,341 | 91.98%          | SRR8656486   |
| Stomach      | 18,574,229   | 4,604,137,153 | 87.94%          | SRR8656487   |
| Total        | 128,914,562  | 31,976,257,702 | 90.49%          |              |

Table 6. Mapping ratio of RNA-seq reads from eight tissues.

| BUSCO benchmark                      | Number | Percentage (%) |
|--------------------------------------|--------|----------------|
| Complete BUSCOs                      | 2,473  | 95.7%          |
| Complete and single-copy BUSCOs      | 2,438  | 94.3%          |
| Complete and duplicated BUSCOs       | 35     | 1.4%           |
| Fragmented BUSCOs                    | 45     | 1.7%           |
| Missing BUSCOs                       | 68     | 2.6%           |
| Total BUSCO vertebrate genes         | 2,586  | 100%           |

Table 7. BUSCO evaluation of the pompano genes compared with the vertebrate gene set.
homozygous regions were not expanded. Furthermore, the absent k-mers (black) at the frequency of average sampling depth was low (Fig. 4), suggesting a high level of assembly completeness. The assembly correctly represented kmer spectrum from the cleaned Illumina reads. (3) We aligned Pacbio long reads to the repeat-masked assembly using Minimap2 and retained those alignments having read coverages over 90%. Almost 98.9% of long reads were uniquely aligned, suggesting that few homozygous contents were duplicated (Fig. 5). The cleaned Illumina reads were aligned to the repeat-masked assembly using BWA. With the coverage threshold of 90%, over 96.3% of reads were uniquely aligned, also supporting few duplicated homozygous contents (Fig. 5). These two distributions were consistent with the main unique content in the KAT analysis. (4) The insert size distributions of paired-end/mate-pair libraries by aligning reads to the genome using BWA were consistent with the estimated insert sizes (Fig. 6). (5) The clean RNA-seq reads from multiple tissues had an average alignment ratio of 90.5% to the assembly using HISAT2 (Table 6). All the indicators suggested a high-quality genomic resource for the further analysis. The indistinguishable sex chromosome is one characteristic of this family. This chromosome-level assembly would provide a reference to identify sex chromosome and study the evolution of sex chromosome.

The completeness of pompano genes was evaluated by using BUSCO software. The pompano genes were compared with the 2,586 BUSCO vertebrate conserved gene set. Comparing pompano genes with the vertebrate gene set revealed that 95.7% of the vertebrate genes were identified as complete. The ‘complete and single-copy BUSCOs’ genes accounted for 94.3% of the total genes, and the ‘complete and duplicated BUSCOs’ genes represented 1.4% (Table 7).
Comparison of pompano genome with other Carangiformes genomes. We then compared the pompano genome with other four Carangiformes genomes, including three Carangidae genomes (*Seriola quinquergadiata*, *Seriola dumerili*, and *Seriola rivoliana*) and one Echeneidae genome (*Echeneis naucrates*) using Mashmap252 (mapping segment length = 500 Kbp, and perc_ identity = 75%). The genomic sequences of three Carangidae fish showed synteny to pompano genome (Fig. 7a–c). We found that the 24 pseudo-chromosomes of *Echeneis naucrates* had clear one-to-one relationship to pompano pseudo-chromosomes (Fig. 7d), suggesting that these two genomes did not experience chromosome fission and fusion events. These results revealed that the pompano genome will contribute to the study of the genome evolution of the Carangidae family and the Carangiformes order.

Data Records

All sequencing data, genome assembly, predicted gene models and functional annotation were deposited in public repositories. The Illumina genomic sequencing reads, Pacbio long reads, Hi-C data, and RNA-seq reads of eight tissues were deposited in Sequence Read Archive at NCBI SRP13669774. The chromosome-level assembly was available in the GenBank at NCBI UWUD01000000085. The assembled contigs, scaffolds, gene structure, homologs, and functional annotations were stored in Figshare86.

Technical Validation

Three metrics, including peak length, total amount, and concentration were used to estimate the degradation level and quality of DNA samples. To construct Illumina libraries, the peak length of the isolated DNA was ≥20 kbp and total DNA ≥5 µg with minimum 50 ng/µL. For PacBio libraries, the peak length was ≥40 kbp and total DNA ≥7 µg with minimum 70 ng/µL. To construct the RNA-seq library of each tissue, the RNA integrity was ≥7.0 and total RNA >10 µg with rRNA ratio ≥1.5.

Code availability

Canu in the genome assembly and BLAT alignment in the gene prediction were utilized with specific parameters, described in Methods. The other bioinformatics tools were run with the default parameters. There were no any custom specific codes.

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Author contributions
Zhang D.C., Li J.T. and Jiang S.G. conceived the project. Li J.T., Zhang D.C., Guo H.Y. and Zhu K.C., Xiao J., Li S.Q. and Zhang Y. participated in genome assembly and data analysis. Zhang N., Liu B.S. and Guo L. extracted the genomic DNA and performed genome sequencing. Li J.T., Zhang D.C., Jiang S.G., Guo H.Y. and Zhu K.C. prepared the manuscript.

Competing interests
The authors declare no competing interests.
Additional information

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