A chromosome-level genome assembly of the dark sleeper
Odontobutis potamophila

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Abstract

The dark sleeper, Odontobutis potamophila, is a commercially valuable fish that widely distributed in China and Southeast Asia countries. The phenomenon of sexual dimorphism in growth is conspicuous, which the males grow substantially larger and faster than the females. However, the high-quality genome resources for gaining insight into sex-determining mechanisms to develop sex-control breeding is still lacking. Here, a chromosomal-level genome assembly of O. potamophila was generated from a combination of Illumina reads, 10x Genomics sequencing, and Hi-C chromatin interaction sequencing. The assembled genome was 1,134.62 Mb with a contig N50 of 22.25 Mb and a scaffold N50 of 24.85 Mb, representing 94.4% completeness (BUSCO). Using Hi-C data, 96.49% of the total contig bases were anchored to the 22 chromosomes, with a contig N50 of 22.25 Mb and a scaffold N50 of 47.68 Mb. Approximately 54.18% of the genome were identified as repetitive elements, and 23,923 protein-coding genes were annotated in the genome. The assembled genome can be used as a valuable resource for molecular breeding and functional studies of O. potamophila in the future.

Keywords: Odontobutis potamophila; whole-genome sequence; sex-determination; gene annotation

Significance Statement

A great many of omics (transcriptome, proteomics and metabolomics) studies of Odontobutis potamophila have been reported in recent years. However, to solve the bottleneck problem in the breeding, the whole genome sequencing of O. potamophila is necessary. Here, a chromosomal-level genome assembly was generated, which would allow for the study of many biological questions.

Introduction

The dark sleeper Odontobutis potamophila is a commercially valuable fish that widely distributed in the river systems of China and Southeast Asia countries (Viet Nam, Japan and Korean) (Cheng et al. 2017; Hou et al. 2014; Zhang et al. 2015). The aquaculture of this species is potential value due to their high meat content, delicious taste and high profits (Wang et al. 2017; Li and Liu 2017). As an unique economic fish from China, artificial breeding of O. potamophila...
was explored in early 1990s, but a significant breakthrough was made for large scale seedling in 2009. However, the current culture model of *O. potamophila* was mainly mixed with other aquatic species, resulting in the production were not enough to meet the increasing consumption demand (Yan-Dong et al. 2015).

Recently, a great deal of studies involved in *O. potamophila* have been carried out on reproduction, farming and larvae rearing (Liu et al. 2008; Zhao et al. 2009). Phylogeny analysis via mitochondrial 12S rRNA sequence demonstrated that Chinese odontobutis mainly consisted of four species, comprising *O. potamophila*, *Odontobutis sinensis*, *Odontobutis haifengensis*, and *Odontobutis yaluensis*, respectively. Many scholars also conducted some research on the toxicology experiments of *O. potamophila*, and found that chlorpyrifos could cause serious damage to the gill and liver in the larval stage (Ding et al. 2012). In addition, Zhang et al. (2014) developed many polymorphic microsatellite markers for the purpose of kinship identification, linkage map construction, and genetic diversity analysis. Furthermore, many omics studies had been performed in recent years to analyze the regulatory mechanism of relevant economic traits (Wang et al. 2019).

Unfortunately, seedlings showed the phenomenon of germplasm decay during the course of production, including slow growth rate, weak disease resistance, increased disease incidence. On the other hand, *O. potamophila* showed a sexually dimorphic growth pattern, which the males grew substantially larger and at a quicker rate than the females (Cheng et al. 2017). Therefore, elucidation growth- or sex-regulatory mechanism, and breeding with rapid growth merit of *O. potamophila* is of great significance for the genetic management and scientific research. To solve these bottleneck problems in the breeding of *O. potamophila* and clarify the biological characteristics at molecular level, it was imperative to initiate the whole-genome sequencing. Here, we reported the whole genome sequence of *O. potamophila*, and the availability of reference genome will provide valuable resources for sex-control breeding and functional genomic research.

**Materials and Methods**

**Sample collection and sequencing**
The dark sleeper *O. potamophila* was obtained from the Balidian breeding base of Zhejiang Institute of Freshwater Fisheries in 2019 (Huzhou, China). The muscle tissues were dissected from a single female individual for DNA extraction using the phenol/chloroform extraction method. Library preparation and sequencing were performed by an external service (Novogene Co., Ltd., Beijing, China). High quality genomic DNA were randomly sheared (insert size 350 bp) through Covarisisg-TUBE, and paired-end (PE) libraries were constructed for sequencing on the PromethION platform at Novogene (Beijing, China). To aid genome annotation, eight tissues from the same individual, including gill, heart, brain, muscle, intestine, skin, and ovary, were collected for RNA extraction and Transcriptome sequencing. Subsequently, sequencing libraries were prepared using NEBNext®UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer’s recommendations. Finally, the library preparations were sequenced on an Illumina platform and 125 bp/150 bp paired-end reads were generated.

**Genome estimation and assembly**

For a general judgment of genome size, we used *K*-mer analysis to estimate genome size from the mathematical perspective. As a result, the genome size of *O. potamophila* was estimated to approximately 1156.17 Mb by the *K*-mer frequency distribution. The Illumina sequence reads was then assembled using Soapdenovo software as described below: I) Fragments randomly sheared into different insert sizes; II) Represent read sequence overlap using de Bruijin graph; III) Remove erroneous connections on the graph; IV) Break at repeat boundaries and out contigs; V) Scaffold construction; VI) Gap closure (Li et al. 2010). Finally, the resulting assembly contigs were connected to linked-reads from 10X Genomics-derived sequencing data to yield a draft *O. potamophila* genome assembly (Adey et al. 2014).

To further improve the accuracy of the assembly, Hi-C libraries were constructed to generate a chromosome-level assembly of the genome. Hi-C clean data was mapped to the draft assembled sequence from 10X Genomics using BWA software (Servant et al. 2015), and the low quality reads were removed by SAMTOOLS. Last, the valid Hi-C reads pairs were applied for clustering, ordering, and orienting to finish assembly at a chromosome-level. Further, the completeness of the *O. potamophila* genome was evaluated by Core Eukaryotic Genes Mapping Approach (CEGMA) (Parra et al. 2007) and Benchmarking Universal Single-Copy Orthologs (BUSCO)
Repeat analysis and non-coding gene annotation

The repeat sequences in the genome mainly consisted of tandem repeat and interspersed repeat (Ge et al. 2019). Here, repetitive sequences annotation were performed by homology searches against known repeat databases and de novo prediction. Homology searches repetitive elements in the *O. potamophila* genome depended on the RepBase database (http://www.girinst.org/repbase/) with Repeatmasker and repeatproteinmask software (Bao et al. 2015). For the second method, De novo repetitive element database was firstly constructed by LTR_FINDER (Xu and Wang 2007), RepeatScout (Price et al. 2005), RepeatModeler (Smit and Hubley 2010) with default parameters, then employing the RepeatMasker (Tempel 2012) to annotate repeat elements with the database.

Non-coding RNAs, including miRNA, snRNA, tRNA and rRNA, also have important biological functions (Hombach and Kretz 2016). For example, MicroRNAs (miRNAs) are small endogenous RNAs that regulate gene-expression posttranscriptionally in many different cellular pathways and systems (Lu and Rothenberg 2018). Transfer RNAs (tRNAs) and Ribosomal RNAs (rRNAs) are thought to directly involve in protein synthesis (Jarroux et al. 2017). Small nuclear RNA (snRNA) is proven to participate in pre-mRNA splicing (Shi 2017). These non-coding RNAs were identified and annotated across the *O. potamophila* genome. The tRNAs were predicted using the program tRNAscan-SE (Lowe and Chan 2016). High conserved rRNAs were annotated using BLASTN (Camacho et al. 2009), and other ncRNAs were identified by searching against the Rfam database with default parameters using the infernal software (Daub et al. 2015).

Protein-coding gene prediction and annotation

Gene models were established using a combination of *ab initio*, homology-based and RNA-Seq assisted prediction. For gene predication based on *ab initio*, we employed Augustus (v3.2.3), Geneid (v1.4), Genescan (v1.0), GlimmerHMM (v3.04) and SNAP (2013-11-29) to predict protein-coding genes in *O. potamophila* genome (Stanke et al. 2004; Majoros et al. 2004; Korf 2004). Regarding homology-based prediction, proteins sequences of *Gadus morhua*, *Ctenapharyngodon idellus*, *Cyprinus carpio*, and *Larimichthys crocea*, were downloaded from Ensembl/NCBI/others. Subsequently, potential gene structures were aligned to the homologous
genome for all alignments with GenWise software (v2.4.1) (Birney and Durbin 2000). For transcriptome-based prediction, RNA-seq data from different tissues were generated with Trinity (v2.1.1) for the genome, and exons region and splice positions were aligned to genome fasta using Hisat (v2.0.4)/TopHat (v2.0.11) with default parameters (Kim et al. 2013; Trapnell et al. 2012). Functional annotation of the predicted genes were performed using public databases of SwissProt, InterPro, NR from NCBI and Kyoto Encyclopedia of Genes and Genomes (KEGG). The motifs and domains were annotated using InterProScan by searching against protein databases, such as ProDom, PRINTS, Pfam, SMART, PANTER and PROSITE.

**Comparative genome analysis**

Gene families were analyzed using OthoMCL for identification species-specific and shared genes between *O. potamophila* and other ten fish species (Feng et al. 2006), including *Larimichthys crocea*, *Danio rerio*, *Gasterosteus aculeatus*, *Oreochromis niloticus*, *Takifugu rubripes*, *Cyprinus carpio*, *Cynoglossus semilaevis*, *Ctenopharyngodon idellus*, *Oncorhynchus mykiss*, and *Oryzias latipes*. To examine *O. potamophila* evolution, single-copy genes from the above analysis were selected for multi-alignment using MUSCLE (Robert 2004) to build super alignment matrix, and then a phylogenetic tree was constructed by RAxML software with ML TREE method. Subsequently, divergence time was estimated using PAML software (Yang et al. 2007).

**Results and Discussion**

**Genome Assembly and Statistics**

Here, we performed the whole-genome sequencing of *O. potamophila* with Oxford Nanopore technology on PromethION platforms. To estimate the genome size and heterozygosity of *O. potamophila*, 17-mers were counted as 42,778,163,910 from clean reads, and the size of genome was approximately 1,156.17 Mb with 0.29% heterozygosity by survey analysis (Table S1, S2, Fig. 1). The detailed genome sequencing information were summarized, and a total of 181.27G (coverage of 160.41X) clean data were produced after quality filtration from a single genomic DNA library (Table 1). Accordingly, a final 1,134.62 Mb draft genome assembly was obtained, covering 98.14% of the estimated genome sizes. The N50s of contigs and scaffolds of the *O. potamophila* genome were 22.25 Mb and 24.85 Mb, respectively. The GC content of the
assembly genome was estimated to be 43.26% (Table S3).

Table 1 Statistics of the genome sequencing data

| Pair-end libraries | Insert size | Total data (G) | Read length (bp) | Sequence coverage (X) |
|--------------------|-------------|----------------|------------------|-----------------------|
| Nanopore           | -           | 130.43         | -                | 115.42                |
| Illumina reads     | 350         | 50.84          | 150              | 44.99                 |
| 10X                | 600         | 121.55         | -                | 107.57                |
| Hi-C               | -           | 113.07         | -                | 100.06                |
| Total              | -           | 415.89         | -                | 368.04                |

To improve the genome sequencing read-level accuracy, we used a combination of linked-reads and proximity ligation in this study. Using Hi-C data, 96.49% (1,058,372,153) of the total contig bases (1,096,900,524) were anchored to the 22 chromosomes, with a contig N50 of 22.25 Mb and a scaffold N50 of 47.68 Mb (Tables S1, Fig S1). Furthermore, the completeness of the *O. potamophila* genome was evaluated by Core Eukaryotic Genes Mapping Approach (CEGMA) and Benchmarking Universal Single-Copy Orthologs (BUSCO), respectively. Using CEGMA method, 95.56% of the 248 core genes were identified in the genome (Table S4), and BUSCO results revealed that 94.4% complete and 1.2% partial of the 2586 vertebrate BUSCO genes were captured (Table S5). Taken together, our results indicated that the genome assembly was complete and of high quality.

Repeat analysis and Genome annotation

The identification of repetitive elements showed that a total of 587,699,328 bp repeat sequence were identified in the *O. potamophila* genome, which accounted for 53.58% of the genome. Among them, 0.49% of the genome was identified as tandem repeat, and long terminal repeat retrotransposons (48.75%) were the most abundant TEs in *O. potamophila*, followed by long interspersed elements (LINEs, 15.49%) (Table S6). The gene model prediction method was applied to the protein-coding gene annotation in the *O. potamophila* genome. For genome annotation, approximately 24,748 protein-coding genes were identified, and a total of 23,923 genes (96.7%) were annotated by at least one public database (Table S7). Furthermore, four types of non-coding RNAs were predicted across the *O. potamophila* genome, comprising 1,876 miRNAs, 1,569 rRNA, 4,139 tRNAs and 654 snRNAs (Table S8).
Comparative genome analysis

To investigate the phylogenetic position of *O. potamophila* with other published fish species, OrthoMCL was used for orthologue group identification. Clustering analysis revealed that 7,974 gene families and 1,182 single-copy genes were shared by *O. potamophila* and other fish species. Moreover, a total of 1,024, 460, 259, and 267 gene families were found specific to *O. potamophila*, *L. crocea*, *G. aculeatus*, and *C. semilaevis*, respectively (Fig. 1). Using these single-copy orthologues, we constructed a phylogenetic tree by RAxML software with ML TREE method. Phylogenetic analysis showed that Cyprinidae family (*D. rerio*, *C. idellus* and *C. carpio*) clustered one branch, and *O. potamophila* was closely related to *C. semilaevis*, and the estimated divergence time was approximately 125 million years ago (Fig. 1).

**Figure 1** Assemblies and evolution of *Odontobutis potamophila* genome. a) an adult dark sleeper. b) 41-mer frequency distribution in the genomes. The X-axis is the Kmer depth, and Y-axis represents the frequency of the Kmer for a given depth. c) Venn diagram of shared and unique orthologous gene family for four selected vertebrate genomes. Each number represents the number of orthologous gene families shared by the indicated genomes. d) Phylogenetic relationship of *O. potamophila* and 10 other fish species genome using 1,182 single copy orthologous genes. The divergence time is given in millions of years in blue color. The relative rates of molecular evolution are expressed as the branch lengths. Estimates of divergence times
(millions of years) calculated from the rate of sequence similarity are indicated at each node.

Conclusions

In the present study, we represented the chromosome-level genome sequencing, assembly, and annotation of *O. potamophila* using multiple sequencing platforms. The draft genome assembly was 1,134.62 Mb with a contig N50 of 22.25 Mb and a scaffold N50 of 24.85 Mb. The genome was functionally annotated to generate 24,748 protein-coding genes. The availability of the high-quality reference genome resource will be valuable for functional studies, especially elucidating on sex-determining mechanisms.

Availability of supporting data

Sequence data is available in the Sequence Read Archive (SRA) on NCBI (BioProject: PRJNA649018).

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Y.J. and Z.G. conceived and designed the study. Y.J. and J.Z. prepared the collected the samples. S.L., M.C., and S.C. performed the data analysis. Y.J. and J.Z. edited the figure and table. Y.J., J.Z., and F.L. wrote the manuscript. All authors discussed the results and commented on the manuscript and Z.G. revised the manuscript.

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Literature Cited

Adey A, et al. 2014. In vitro, long-range sequence information for de novo genome assembly via transposase contiguity. Genome Res. 24(12):2041-2049.

Bao W, Kojima KK, Kohany O. 2015. Repbase Update, a database of repetitive elements in eukaryotic genomes. Mob DNA 6:11.
Birney E, Durbin R. 2000. Using GeneWise in the Drosophila annotation experiment. Genome Res. 10(4):547–548.

Camacho C, et al. 2009. BLAST+: Architecture and applications. BMC Bioinformatics 10(1):421.

Cheng Z, et al. 2017. Integrated analysis of mRNA-seq and miRNA-seq reveals the potential roles of sex-biased miRNA-mRNA pairs in gonad tissue of dark sleeper (Odontobutis potamophila). BMC Genomics 18(1):613.

Daub J, et al. 2015. Rfam: annotating families of non-coding RNA sequences. Methods Mol Biol. 1269:349–363.

Ding Z, et al. 2012. Acute toxicity of chlorpyrifos (CPF) to Odontobutis potamophila juveniles. Journal of Fishery ences of China 19:528-535.

Feng C, et al. 2006. OrthoMCL-DB: querying a comprehensive multi-species collection of ortholog groups. Nucleic Acids Res. 34(Database issue):D363-368.

Ge H, et al. 2019. De novo assembly of a chromosome-level reference genome of red-spotted grouper (Epinephelus akaara) using nanopore sequencing and Hi-C. Mol Ecol Resour. 19(6):1461-1469.

Hombach S, Kretz M. 2016. Non-coding RNAs: Classification, Biology and Functioning. Adv Exp Med Biol. 937:3-17.

Hou X, et al. 2014. Genetic diversity of Odontobutis potamophila from different geographic populations inferred from mtDNA control region. Mitochondrial DNA 25(5):400-406.

Jarroux J, Morillon A, Pinskaya M. 2017. History, Discovery, and Classification of IncRNAs. Adv Exp Med Biol. 1008:1-46.

Kim D, et al. 2013. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 14(4):R36.

Korf I. 2004. Gene finding in novel genomes. BMC Bioinformatics 5(1):59.

Liu M, Hu X, Han Q, Luo Y. 2008. Variation of the proteinic enzymes activities during the embryonic and larval development of Odontobutis potamophila. Freshw Fish 38:39-41.

Li Q, Liu Z. 2016. New complete mitochondrial genome of the Odontobutis potamophila (Perciformes, Odontobutidae): genome description and phylogenetic performance. Mitochondrial DNA A DNA Mapp Seq Anal. 27:163-164.
Li R, et al. 2010. *De novo* assembly of human genomes with massively parallel short read sequencing. Genome Res. 20(2):265-272.

Lowe TM, Chan PP. tRNAscan-SE On-line: integrating search and context for analysis of transferRNA genes. Nucleic Acids Res 2016;44(W1):W54–7.

Lu TX, Rothenberg ME. 2018. MicroRNA. J Allergy Clin Immunol. 141(4):1202-1207.

Majoros WH, Pertea M, Salzberg SL. 2004. TigrScan and GlimmerHMM: two open source ab initio eukaryotic gene-finders. Bioinformatics 20(16):2878–2879.

Parra G, Bradnam K, Korf I. 2007. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics 23:1061–1067.

Price AL, Jones NC, Pevzner PA. 2005. *De novo* identification of repeat families in large genomes. Bioinformatics 21:i351-i358.

Robert CE. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792-1797.

Servant N, et al. 2015. HiC-Pro: an optimized and flexible pipeline for Hi-C data processing. Genome Biol. 16:259.

Shi Y. 2017. The Spliceosome: A Protein-Directed Metalloribozyme. J Mol Biol. 429(17):2640-2653.

Smit AFA, Hubley R. RepeatModeler Open-1.0 (Seattle: The Institute for Systems Biology) 2010.

Simao FA, et al. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210.

Stanke M, et al. 2004. AUGUSTUS: a web server for gene finding in eukaryotes. Nucleic Acids Res. 32:309–312.

Tempel, S. 2012. Using and Understanding RepeatMasker. Methods Mol Biol. 859:29-51.

Trapnell C, et al. 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 7(3):562-578.

Xu Z, Wang H. 2007. LTR_FINDER: an efficient tool for the prediction of full-length LTR retrotransposons. Nucleic Acids Res. 35:W265-W268.

Wang PP, et al. 2017. Characterization and development of 56 EST-SSR markers derived from
the transcriptome of *Odontobutis potamophila*. Genet Mol Res. 16:10.4238.

Wang T, et al. 2019. Integrated analysis of proteomics and metabolomics reveals the potential sex determination mechanism in *Odontobutis potamophila*. J Proteomics 208:103482.

Yan-Dong D, et al. 2015. Analysis of morphological variations among four different geographic populations of *Odontobutis potamophila*. Mar Fisheries 37:24-30.

Yang Z. 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. Mol Biol Evol. 24:1586-1591.

Zhang HW, et al. 2015. Development and validation of single nucleotide polymorphism markers in *Odontobutis potamophila* from transcriptomic sequencing. Genet Mol Res. 14:2080-2085.

Zhang L, et al. 2014. Development and characterization of 42 novel polymorphic microsatellite markers for *Odontobutis potamophila* from EST sequences. Conserv Genet Resour. 6:469-472.

Zhao X, et al. 2009. Effects of starvation on ovarian development in female *Odontobutis potamophila* during over-wintering period. J. Fish. China 33:70-77.