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DATA NOTE

Genome sequence of pacific abalone (Haliotis discus hannai): the first draft genome in family Haliotidae

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Abstract

Background: Abalones are large marine snails in the family Haliotidae and the genus Haliotis belonging to the class Gastropoda of the phylum Mollusca. The family Haliotidae contains only one genus, Haliotis, and this single genus is known to contain several species of abalone. With 18 additional subspecies, the most comprehensive treatment of Haliotidae considers 56 species valid [1]. Abalone is an economically important fishery and aquaculture animal that is considered a highly prized seafood delicacy. The total global supply of abalone has increased 5-fold since the 1970s and farm production increased explosively from 50 mt to 103464 mt in the past 40 years. Additionally, researchers have recently focused on

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abalone given their reported tumor suppression effect. However, despite the valuable features of this marine animal, no genomic information is available for the Haliotidae family and related research is still limited. To construct the *H. discus hannai* genome, a total of 580-G base pairs using Illumina and Pacbio platforms were generated with 322-fold coverage based on the 1.8-Gb estimated genome size of *H. discus hannai* using flow cytometry. The final genome assembly consisted of 1.86 Gb with 35 450 scaffolds (>2 kb). GC content level was 40.51%, and the N50 length of assembled scaffolds was 211 kb. We identified 29 449 genes using Evidence Modeler based on the gene information from ab initio prediction, protein homology with known genes, and transcriptome evidence of RNA-seq. Here we present the first Haliotidae genome, *H. discus hannai*, with sequencing data, assembly, and gene annotation information. This will be helpful for resolving the lack of genomic information in the Haliotidae family as well as providing more opportunities for understanding gastropod evolution.

Keywords: Abalone genome; Halotidae; Haliotis discus hannai

Introduction

Abalone is one of the most important marine gastropod mollusks that inhabits various coastal regions of the world. It is well known that abalone habitation impacts algal communications connected with the reef ecosystem, so they are often utilized for ecological research [2]. Among many abalone species, Haliotis discus hannai is a widely used ingredient in East Asian cuisine and is a valuable food resource due to its richness in protein and other nutrients (Fig. 1) [3, 4]. It is considered an important fishery industry animal. The total global supply of abalone has increased 5-fold since the 1970s. To prevent indiscreetly fishing abalones, legal landings from abalone fisheries have made fishery production decrease gradually from 19720 mt to 7486 mt but have made farm productions increase explosively from 50 mt to 103464 mt in the past 40 years [5]. Additionally, researchers have recently focused on H. discus hannai given its reported tumor suppression effect [6-8]. However, despite the valuable features of this marine animal, no genomic information is available. Therefore, the first draft genome in family Haliotidae has the potential to be utilized as a valuable resource for many researchers.

A single wild abalone (H. discus hannai) was collected from the brood stock at the Genetic and Breeding Research Center of the National Fisheries Research & Development Institute on Geoje Island, Korea for sampling. Hemolymph (10 ml) was withdrawn from the sole side foot muscle using a syringe. For genomic DNA extraction, hemocytes were harvested from fresh hemolymph by centrifugation at 3000 × rpm for 5 min at 4°C. Genomic DNA was extracted using a DNeasy Animal Mini Kit (Qiagen, Hilden, Germany). A total 39.38 μ g of DNA was quantified using the standard procedure of Quant-iT PicoGreen ds-DNA Assay Kit (Molecular Probes, Eugene, OR, USA) with Synergy HTX Multi-Mode Reader (Biotek, Winooski, VT, USA). Quality of DNA was also checked using ND-1000 spectrophotometer (Thermo Scientifc, Wilmington, DE, USA).

For whole genome shotgun sequencing and draft genome assembly, we used multiple sequencing platforms (Illumina Hiseq2000, Nextseq500 and Pacbio RS II) with seven different libraries. First, two paired-end libraries with insert sizes of 250 and 350 bp were constructed using Illumina TruSeq DNA Sample Prep. Kit (Illumina, San Diego, CA, USA). Mate pair libraries with insert sizes of about 3, 5, 8, and 10 k were constructed for



Figure 1. Example of a H. discus hannai, the pacific abalone.

Table 1. Summary statistics of generated whole genome shotgun sequencing data.

Library name	Library type	Insert size	Platform	Read length	No. read	Total bp
250 bp	Paired-end	250	Nextseq500	150	876 529 480	131 440 418 087
350 bp	Paired-end	350	Hiseq2000	101	1 413 620 786	142 775 699 386
3 k	Mate-pair	3000	Nextseq500	150	580 064 464	85 689 154 056
5 k	Mate-pair	5000	Nextseq500	150	468 432 888	69 966 139 205
8 k	Mate-pair	8000	Nextseq500	150	335 132 792	50 109 845 012
10 k	Mate-pair	10 000	Nextseq500	150	569 376 096	85 080 237 236
20 k	P6-C4	20 000	Pacbio RS II	10 094		
(average)	1,573,020	15879626978				
Total						580 941 119 960



Figure 2. 19-mer distribution of using jellyfish with 350-bp paired-end whole genome sequencing data.

scaffolding process using Illumina Nextera mate-pair library construction protocol (Illumina). For high-quality genome assembly, long mate pair library with insert size over 40 kb is essential. We tried to construct a long mate pair library using 40 kb fosmid clone. However, efficiency of fosmid library construction was very low and we could not retain enough amount of clone. Therefore, Pacbio system was employed for final scaffolding process using long read. Pacbio long reads were generated using P6-C4 chemistry of Pacbio RS II system. Detailed information about the constructed library and generated sequencing data is provided in Table 1. Quality control process of generated raw data was conducted for downstream analysis. Quality of raw data was checked using FASTQC [9] and adapter sequences were removed via Trimmomatic [10], for paired-end libraries, and Nxtrim [11], for mate-pair libraries. K-mer frequency analysis of the abalone genome was conducted using a paired-end library with 350-bp insert size and the jellyfish [12] command-line program. The K-mer distribution of the paired-end library provides valuable information about the target genome. As a result, 19-mer distribution of H. discus hannai genome was generated (Fig. 2). Genome size estimation based on the 19-mer distribution was conducted through "Estimate genome size.pl" code (https://github.com/josephryan/estimate_genome_size.pl/wiki/ Estimate-genome-size.pl"). The estimated genome size of H. discus hannai using 19-mer distribution was about 1.65 Gb. Based on the 19-mer distribution of paired-end reads, there was a second peak located in the half x-axis of the main peak. This result indicates that the H. discus hannai genome had high heterozygous genetic character or probable DNA contamination from other organisms. Therefore, before genome assembly, raw reads from Hiseq2000 and Nextseq500 paired-end and mate pairs were preprocessed by bacterial sequences, duplicates, and ambiguous nucleotides. To remove the contaminant sequence, clean reads without adapter and low quality bases were mapped to bacterial and ocean metagenome databases downloaded from NCBI by applying the default setting run (-s 0.8 -l 0.5) of clc_mapper (https://www.qiagenbioinformatics.com/). After that, duplicates and ambiguous nucleotides were filtered out us-

Table 2. Summary statistics for the *H*. *discus hannai* draft genome (>2 kb).

Assembled genome	
Size (1n)	1.80 Gb
GC level	40.51%
No. scaffolds	35 450
N50 of scaffolds (bp)	211 346
N bases in scaffolds (%)	116 Mb (6.45%)
Longest (shortest) scaffolds (bp)	2 207 537 (2000)
Average scaffold length (bp)	50 870.65

 Table 3. Summary of identified repeat elements in the Haliotis discus hannai genome.

Repeat element	No. element	Length (%)
SINE	284485	96 155 199 (5.11%)
LINE	700 245	160 387 248 (8.53%)
LTR element	383770	55 149 794 (2.93%)
DNA element	58 022	14 563 432 (0.77%)
Small RNA	20 997	1 537 853 (0.08%)
Simple repeat	161246	32 547 245 (1.73%)
Low complexity	326 399	21 446 303 (1.14%)
Unclassifed	1 522 272	265 603 066 (14.1%)

ing clc_remove_duplicates (https://www.qiagenbioinformatics. com/). The resulting high-quality sequences were used in subsequent assembly. Error correction and initial contig assembly was conducted using clc_assembler within the CLC Assembly Cell (https://www.qiagenbioinformatics.com/products/clcassembly-cell/) software pipeline. Scaffolds were then built using the mate-pairs and Pacbio RS II reads sequentially by SSPACE [13] and PBJelly2 [14]. After scaffolding, we iteratively conducted gap filling process using Gapcloser [15] using -1 155 and -p 31 parameter option. Summary statistics for final assembly is provided in Table 2.

Before conducting gene prediction using the assembled sequence, repeat elements were identified using RepeatMasker [16] with Repbase [17]. RepeatModeler, which includes RECON [18], RepeatScout [19], and TRF [20], was used to create a custom database of H. discus hannai. After custom library construction, RepeatMasker with RMBlast was used for each genome with 'no_is' option, using repeat libraries from RepeatModeler and Repbase. Identified mobile elements are summarized in Table 3. Identified repeat elements were parsed for identifying more detailed information using a perl code named "One code to find them all" [21] and Fig. S1 shows the proportion of each mobile element. The genome size of H. discus hannai was 1.86 Gb, and this is the biggest genome among known gastropods. It is 5.31 and 2.02 times larger than genomes size of Lottia gigantea (0.35 Gb) and Aplysia californica (0.92 Gb) in the same Gastropoda class. In animals, the increase of genome size is commonly driven by transposable element, and this is a known genetic adaption mechanism to stressful environments [22]. Therefore, we conducted comparative analysis of repeat element against L. gignatea, a similar marine gastropod with large genome size difference from that of H. discus hannai, to identify the reason for this large difference. Fig. 3a shows the amount and proportion of identified repeat element from two marine gastropods.

The proportion of identified total repeat elements in H. discus hannai and L. gigantea is 30.76% and 22.25%, respectively. And the total amount of identified repeat elements in the H. discus hannai genome is almost six times larger than that of L. gigantea same as genome size. Such a linear relationship between genome size and the total proportion of repeat elements is consistent with a previous study [23]. The proportion, copy number, and divergence of each mobile element were identified and compared (Figs S2-6) for a deeper understanding of mobile elements in the two species. From the comparison, a notable finding has been observed on mobile elements: DNA transposable element, a Class II transposable element, exists in diverse forms in both species; however, retrotransposon element, a Class I transposable element, is much more abundant in H. discus hannai genome than in L. gigantea genome. Especially, the number of a non-LTR retrotransposon called LINE Element was exceptionally high. Fig. 3b illustrates the difference between the two species, using two signature mobile elements (H. discus hannai: LINE/I, DNA/TcMar-Tc1, L. gigantea: DNA/RC, DNA/Maverick) in each genome. DNA/RC and DNA/Maverick, two major mobile elements in L. gigantea genome, are observed in H. discus in somewhat similar distribution. On the other hand, the two signature mobile elements of H. discus hannai genome, LINE/I and DNA/TcMar-Tc1, are specifically abundant in H. discus hannai and seems to have expanded recently diverged compared to other elements. In sum, species specificity can be inferred from the distinctive patterns of repeat element expansion between the two species and the increased genome size of H. discus hannai may be associated with the non-LTR elements (especially LINE/I) contribution, in parallel to the human genome [23].

Genes were predicted through three different algorithms: ab initio, RNA-seq transcript based, and protein homology-based. For RNA-seq transcript based prediction, transcriptome data from six organ tissues (Table 4) were aligned to the assembled genome sequence using Tophat [24], and transcript structure was predicted through Cufflinks [25]. The homology-based method employs complete protein sequences from diverse taxonomical genomes, which is fit to our model. For H. discus hannai, the following eight species were utilized: L. gigantea, Crassostrea gigas, A. california, Strongylocentrtus purpuratus, Branchiostoma floridae, Danio rerio, Oncorhynchus mykiss, and Homo sapiens. Those protein sequences were aligned to the H. discus hannai genome using TBASTN (E-value \leq 1E-4) [26]. Next, the homologous genome sequences were aligned to the matched proteins using Exonerate [27] to predict the accurate spliced alignments. Table 5 summarizes the alignment results of known proteins in various species. For ab initio gene prediction, Augustus [28] was trained using RNA-seq data and known proteins by using the complete transcriptome as training matrix for HMM. Fgenesh [29] and Geneid [30] were also used. The parameters used and the number of predicted genes is provided in Table 6. Gene prediction data from each method was combined using EVM (Evidence Modeler) [31] to build a consensus gene set for the abalone genome. All gene models were converted to EVM compatible GFF3 format and merged to a consensus gene set. After consensus gene annotation was generated from EVM, manual curation was conducted for abandon genes from EVM to build a final consensus gene set of H. discus hannai. Manual curation was performed based on the genomic DNA mapping position of the RNA-seq sequence and the protein sequence of the related species. To determine the exon-intron edge of the gene, the genome mapping information of the transcriptome sequence was firstly reflected, and if not, the mapping information of the protein sequence of the related species was referred to secon-



Figure 3. Repeat element information of H. discus hannai compared to L. gigantean. (a) Total amount and ratio of identified repeat element classified into eight classes (DNA, LINE, SINE, LTR, Low complexity, Satellite, Simple repeat, and Unknown) from each genome. (b) Distribution of gene copy number of the two highly possessed repeat elements in each genome based on the divergence. Heat maps indicate the total amount of repeat element divided into 20 levels based on the divergence.

darily to confirm the gene model. Finally, genes that were not translated into protein sequences in the final gene model were removed. A total of 29449 genes was predicted in the *H. discus hannai* genome and summary statistics for the consensus gene set is provided in Table 7. To evaluate the quality of the *H. discus hannai* draft genome, we conducted paired-end read remapping and BUSCO (Benchmarking Universal Single-Copy Orthologs) analysis. 94.89% of paired-end reads with a 350-bp insert size were successfully mapped to the assembled genome and assembled genome contains 609 complete and 130 fragmented genes in BUSCO analysis. The detailed information of BUSCO analysis is summarized in Table 8.

In summary, here we report the first annotated Haliotidae genome of *H. discus hannai* based on various genetic evidence. We expect that the *H. discus hannai genome* presented here, which is the first genome to be sequenced in the family Haliotidae,

Library name	Library type	Platform	Read length	No. read	Total bp
Blood	Paired-end	Hiseq2000	101	53 525 950	5 406 120 950
Digestive duct	Paired-end	Hiseq2000	101	56 485 666	5 705 052 266
Gill	Paired-end	Hiseq2000	101	66 415 882	6708 004 082
Hepatopancreas	Paired-end	Hiseq2000	101	58467 176	5 905 184 776
Mantle	Paired-end	Hiseq2000	101	65741776	6 639 919 376
Ovary	Paired-end	Hiseq2000	101	60997 100	6 160 707 100
Total		*			36 524 988 550

Table 4. Summary statistics of generated transcriptome data for six organ tissues using Illumina platform.

Table 5. Summary statistics of protein alignment using tBlastn for protein based evidence gene structure.

Species	Туре	Element	Total count	Count/ gene	Total length, bp	Mean length, Bp	Genome coverage, %
Homo sapiens	Protein	Transcript	18 792		109 068 639	5803.99	5.80
-	(69 002)	Exon	77 320	4.11	12667395	163.83	0.67
Danio rerio	Protein	Transcript	11605		68796463	5928.17	3.66
	(42 474)	Exon	47 300	4.08	7 978 167	168.67	0.42
Oncorhynchus mykiss	Protein	Transcript	15 901		55 043 032	3461.61	2.93
	(53 876)	Exon	46 040	2.90	7 567 059	164.36	0.40
Lottia gigantea	Protein	Transcript	29 345		177 851 531	6060.71	9.47
	(23 851)	Exon	118 165	4.03	20 583 999	174.20	1.10
Crassostrea gigas	Protein	Transcript	32 978		231 175 282	7009.98	12.30
	(28 0 27)	Exon	140 784	4.27	23649828	167.99	1.26
Aplysia californica	Protein	Transcript	10 570		67 396 621	6376.22	3.59
	(29 096)	Exon	45 737	4.33	7 797 503	170.49	0.42
Strongylocentrotus purpuratus	Protein	Transcript	9116		46 270 640	5075.76	2.46
	(38 7 30)	Exon	34 572	3.79	5 627 082	162.76	0.30
Branchiostoma floridae	Protein	Transcript	27 438		125 307 206	4566.92	6.67
-	(58 493)	Exon	92426	3.37	15 483 164	167.52	0.82

Table 6. Summary statistics for ab initio gene prediction results using various programs and parameters.

Program	Matrix	Element	Total count	Count/ gene	Total length, bp	Mean length, bp	Genome coverage, %
Augustus	Custom parameter (RNAseq)	Gene	88 825	3.92	367 066 732	4132.47	19.54
		CDS	348 528		76388076	219.17	4.07
	Custom parameter (H.discus hannai IsoSeq)	Gene	90 396	4.11	395 511 710	4375.32	21.05
		CDS	371487		78 508 401	211.34	4.18
	Custom parameter (H.discus discus IsoSeq)	Gene	84 322	3.97	346 455 180	4108.72	18.44
		CDS	335 103		72527841	216.43	3.86
	Custom parameter (BUSCO)	Gene	111 058	4.24	626 749 935	5643.45	33.36
		CDS	470 839		84333972	179.11	4.49
	Custom parameter (CEGAM)	Gene	76 504	4.95	393 121 657	5138.58	20.92
		CDS	378 485		63 424 677	167.58	3.38
	Custom parameter (Protein)	Gene	22420	3.43	184 289 721	8219.88	9.81
		CDS	76 848		20291739	264.05	1.08
Fgenesh	Custom parameter	Gene	184 051	3.46	1366 924 540	7426.88	72.75
		CDS	636 568		98055591	154.04	5.22
Geneid	Ciona intestinalis	Gene	789 540	1.41	436 990 370	553.47	23.26
		CDS	1 112 959		140 976 492	126.67	7.50

will provide useful genomic information for many researchers. H. discus hannai is a cold-water abalone breed that has difficulties dealing with the change in their inhabitable latitude, which is due to global warming and the resulting increase in the rate of sudden perishing. Genomic information of abalone is essential information that can be used for genetic breeding to improve productivity and genetic engineering for the heat resistance breed. It can also provide valuable information for future genomic studies, because only limited genome information about marine animals and mollusks is currently available. Table 7. Summary statistics for the consensus gene set of Haliotis discus hannai genome.

Element	No. elements	Exon/transcript	Avg. length	Total length	Genome coverage
Gene	29449	-	2705	79661536	4.2%
Exon	74745	2.54	280	20985 298	1.1%
Intron	45 296	1.54	1295	58 676 238	3.1%

Table 8. Summary statistics of Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis for H. *discus hannai* genome based on Metazoans DB.

Categories	#Genes	Percentage
Complete single-copy BUSCOs	609	72.2
Complete duplicate BUSCOs	48	5.7
Fragmented BUSCOs	130	15.4
Missing BUSCOs	104	12.3

Evolutionary signatures recorded in the abalone genome can be identified through future comparative genomic studies and we expect our result will provide more insight into Haliotidae and marine mollusk evolution.

Availability of supporting data

Raw data is available in project accession PRJNA317403 in the NCBI database. Further supporting data can be found in the *GigaScience* GigaDB [32].

List of abbreviations

EVM - Evidence Modeler BUSCO - Benchmarking Universal Single-Copy Orthologs

Competing interests

All authors report no competing interests.

Author contributions

Sampling - Bo-Hye Nam, Young-Ok Kim, Dong-Gyun Kim Sequencing - Bo-Hye Nam, Hee Jeong Kong, Woo-Jin Kim, Jeong-Ha Kang, Ji-Young Moon, Choul Ji Park, Duk Kyung Kim

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Funding and experimental design – Jung Youn Park, Cheul Min An, Seoae Cho, Heebal Kim

Additional files

Figure S1. Tree map for sum of repeat element for H. discus hannai.

Figure S2. Comparison of SINE element distribution of H. discus hannai and L. gigantea.

Figure S3. Comparison of LINE element distribution of *H. discus* hannai and *L. gigantea*.

Figure S4. Comparison of LTR element distribution of *H. discus* hannai and *L. gigantea*.

Figure S5. Comparison of DNA transposon element distribution of H. discus hannai and L. gigantea.

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