DATA NOTE

Draft genome of the Peruvian scallop *Argopecten purpuratus*

Chao Li¹, Xiao Liu², Bo Liu¹, Bin Ma³, Fengqiao Liu¹, Guilong Liu¹, Qiong Shi⁴ and Chunde Wang*¹,*

¹Marine Science and Engineering College, Qingdao Agricultural University, Qingdao 266109, China, ²Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China, ³Qingdao Oceanwide BioTech Co., Ltd., Qingdao 266101, China and ⁴Shenzhen Key Lab of Marine Genomics, Guangdong Provincial Key Lab of Molecular Breeding in Marine Economic Animals, BGI Academy of Marine Sciences, BGI Marine, BGI, Shenzhen 518083, China

*Correspondence address. Chunde Wang, Marine Science and Engineering College, Qingdao Agricultural University, Qingdao 266109, China. Tel: +8613589227997; E-mail: chundewang2007@163.com http://orcid.org/0000-0002-6931-7394

Abstract

Background: The Peruvian scallop, *Argopecten purpuratus*, is mainly cultured in southern Chile and Peru was introduced into China in the last century. Unlike other *Argopecten* scallops, the Peruvian scallop normally has a long life span of up to 7 to 10 years. Therefore, researchers have been using it to develop hybrid vigor. Here, we performed whole genome sequencing, assembly, and gene annotation of the Peruvian scallop, with an important aim to develop genomic resources for genetic breeding in scallops.

Findings: A total of 463.19-Gb raw DNA reads were sequenced. A draft genome assembly of 724.78 Mb was generated (accounting for 81.87% of the estimated genome size of 885.29 Mb), with a contig N50 size of 80.11 kb and a scaffold N50 size of 1.02 Mb. Repeat sequences were calculated to reach 33.74% of the whole genome, and 26,256 protein-coding genes and 3,057 noncoding RNAs were predicted from the assembly.

Conclusions: We generated a high-quality draft genome assembly of the Peruvian scallop, which will provide a solid resource for further genetic breeding and for the analysis of the evolutionary history of this economically important scallop.

Keywords: *Argopecten purpuratus*; Peruvian scallop; genome assembly; annotation; gene prediction; phylogenetic analysis

Data Description

Introduction

The Peruvian scallop (*Argopecten purpuratus*), also known as the Chilean scallop, is a medium-sized bivalve with a wide distribution in Peru and Chile [1]. In Chile, the cultured scallops reach a commercial size of around 9 cm in shell height within 14–16 months [2]. It is a relatively stenothermic species as its natural habitat is largely under the influence of upwelling currents from Antarctica [3]. Unlike other *Argopecten* scallops, the Peruvian scallop normally has a long life span of up to 7–10 years [4, 5]. This scallop was introduced into China in the late 2000s and has played an important role in stock improvement of *Argopecten* scallops via interspecific hybridization with bay scallops [6, 7].

Whole genome sequencing

Genomic DNA was extracted from an adductor muscle sample of a single *A. purpuratus* (Fig. 1), which was obtained from a local scallop farm in Laizhou, Shandong Province, China. A whole genome shotgun sequencing strategy was then applied. Briefly, six libraries with different insert length (250 bp, 450 bp, 2 kb, 5 kb, 10 kb, and 20 kb) were constructed according to the standard protocol provided by Illumina (San Diego, CA, USA). In de-
The 17-mer frequency distribution analysis [8] was performed on the remaining clean reads to estimate the genome size of the Peruvian scallop using the following formula: genome size = k-mer number/peak depth. Based on a total number of 6.22 \(10^{10}\) k-mers and a peak k-mer depth of 69, the estimated genome size was calculated to be 885.29 Mb (Table 1) and the estimated repeat sequencing ratio was 33.74%.

### Table 1: Summary of the Peruvian scallop genome assembly and annotation

| Genome assembly | Parameter       |
|-----------------|-----------------|
| Contig N50 size (kb) | 80.11           |
| Scaffold N50 size (Mb) | 1.02           |
| Estimated genome size (Mb) | 885.29         |
| Assembled genome size (Mb) | 724.78         |
| Genome coverage (%) | 303.83          |
| Longest scaffold (bp) | 11,125,544      |
| Genome annotation | Parameter       |
| Protein-coding gene number | 26,256          |
| Average transcript length (kb) | 10.53          |
| Average CDS length (bp) | 1,418.29        |
| Average intron length (bp) | 1,505.92       |
| Average exon length (bp) | 201.09          |
| Average exons per gene | 7.05            |

### De novo genome assembly and quality assessment of A. purpuratus genome

All the pair-end Illumina reads were first assembled into scaffolds using Platanus_v1.2.4 (Platanus, RRID:SCR_015531) [9], and the gaps were then filled by GapCloser_v1.12-r6 (GapCloser, RRID:SCR_015026) [10]. Subsequently, the PacBio data were used for additional gap filling by PBJelly_v14.1 (PBJelly, RRID:SCR_012091) with default parameters [11], and then all of the Illumina reads were used to correct the genome assembly by Pilon_v1.18 (Pilon, RRID:SCR_014731) for two rounds [12]. After that, the 10X linked-reads were used to link scaffolds by fragScaff_140 324.1 [13]. First, in order to solve the issue of heterozygosity, in our assembly process we chose 19-kmer to draw k-mer distribution histogram and classified all the kmers into homozygous kmer and heterozygous kmer according to the coverage depth. Second, we utilized 45-kmer to construct the de Bruijn figure and combine the bubbles for heterozygous sites, according to the sequences with longer length and deeper coverage depth. Then, the pair-end information was used to determine the connection between the heterozygous parts and filter the contigs lacking support. Finally, the heterozygous contigs and homozygous contigs were distinguished based on contig coverage depth. After assembly, the reads from short insert length libraries were mapped onto the assembled genome. Only one peak was observed in the sequencing depth distribution analysis with the average sequencing depth of 148.2×, which is consistent with the sequencing depth, indicating high quality of the assembled scallop genome. Finally, a draft genome of 724.78 Mb was assembled (accounting for 81.87% of the estimated genome size of 885.29 Mb), with a contig N50 size of 80.11 kb and scaffold N50 size of 1.02 Mb (Table 1).

With this initial assembly, we mapped the short insert library reads onto the assembled genome using BWA_0.6.2 (BWA, RRID:SCR_010910) software [14] to calculate the mapping ratio.
and assess the assembly integrity. In summary, 91.05% of the short reads were mapped onto the assembled genome with a coverage of 89.40%, indicating high reliability of genome assembly. CEGMA, RRID:SCR_015055 defines a set of conserved protein families that occur in a wide range of eukaryotes and presents a mapping procedure to accurately identify their exon-intron structures in a novel genomic sequence [15]. A protein is classified as complete if the alignment of the predicted protein to the HMM profile represents at least 70% of the original KOG domain, or otherwise classified as partial. Through mapping to the 248 core eukaryotic genes, 222 genes (89.52%) were identified. BUSCO_v3 (Benchmarking Universal Single-Copy Orthologs; RRID:SCR_015008) provides quantitative measures for the assessment of genome assembly completeness, based on evolutionarily informed expectations of gene content from near-universal single-copy orthologs [16]. We confirmed that 89% of the 843 single-copy genes were identified, indicating good integrity of the genome assembly.

### Table 2: The prediction of repeat elements in the Peruvian scallop genome

| Type          | Repeat size (bp) | % of genome |
|---------------|------------------|-------------|
| TRF           | 83,037,380       | 11.46       |
| RepeatMasker  | 237,471,691      | 32.76       |
| RepeatProteinMask | 21,719,425   | 3.00        |
| Total         | 294,496,811      | 40.63       |

**Gene annotation**

**Annotation of protein coding genes**

The annotation strategy for protein-coding genes integrated de novo prediction with homology and transcriptome data-based evidence. Homology sequences from African malaria mosquito (Anopheles gambiae), ascidian (Ciona intestinalis), Florida lancelet (Branchiostoma floridae), fruit fly (Drosophila melanogaster), human (Homo sapiens), leech (Helobdella robusta), nematode (Caenorhabditis elegans), octopus (Octopus bimaculoides), owl limpet (Lottia gigantea), Pacific oyster (Crassostrea gigas), and sea urchin (Strongylocentrotus purpuratus) were downloaded from Ensembl [21]. The protein sequences of homology species were aligned to the assembled genome with TBlastn (Basic Local Alignment Search Tool; e-value ≤10\(^{-5}\)) [22], and gene structures were predicted with GeneWise, RRID:SCR_015054 (the parameter set as “-genef”) [23]. The transcriptome data were generated from adductor muscle, hepatopancreas, and mantle on Illema platifrons; Cel, Caenorhabditis elegans; Cgi, Crassostrea gigas; Cte, Capitella teleta, Dme, Drosophila melanogaster; Hsa, Homo sapiens; Hdi, Halocynthia roretzi; Hro, Helodella robusta; Lan, Lottia anatina; Lgi, Lottia gigantea; Mph, Modiolus philippinarum; Obi, Octopus bimaculoides; Pfu, Pinctada fucata; Pye, Patinopecten yessoensis; Tca, Tribolium castaneum.

![Figure 2: Distribution of genes in different species. Abbreviations: Aca, Aplysia californica; Agu, Argopecten purpuratus; Bfl, Branchiostoma floridae; Bpl, Bathymodiolus platifrons; Cel, Caenorhabditis elegans; Cgi, Crassostrea gigas; Cte, Capitella teleta, Dme, Drosophila melanogaster; Hsa, Homo sapiens; Hdi, Halocynthia roretzi; Hro, Helodella robusta; Lan, Lottia anatina; Lgi, Lottia gigantea; Mph, Modiolus philippinarum; Obi, Octopus bimaculoides; Pfu, Pinctada fucata; Pye, Patinopecten yessoensis; Tca, Tribolium castaneum.](https://example.com/figure2.png)
gene model were integrated using EvidenceModeler 2012-06-25 (EVM) [29]. Finally, we identified 26,256 protein-coding genes in the Peruvian scallop genome. In detail, 26,513 genes were predicted through the de novo method, 19,394 genes were annotated by RNA transcripts or raw RNA reads, and 15,608 genes were supported by homolog evidences. The average transcript length, CDS length, and intron length were 10,534 bp, 1,418 bp, and 1,505 bp, respectively (Table 1).

**Gene functional annotation**

Gene functions were predicted from the best BLASTP (e-value \( \leq 10^{-5} \)) hits in SwissProt databases [30]. Gene domain annotation was performed by searching the InterPro (InterPro, RRID:SCR_006695) database [31]. All genes were aligned against Kyoto Encyclopedia of Genes and Genomes (KEGG, RRID:SCR_012773) [32] to identify the best hits for pathways. Gene ontology terms for genes were obtained from the corresponding InterPro entry [33]. Finally, among these annotated genes, 70.3% of encoded proteins showed homology to proteins in the SwissProt database, 91.1% were identified in the nonredundant database, 70.4% were identified in the KEGG database, 72.1% were identified in the InterPro database, and 92.1% could be mapped onto the functional databases.

**Noncoding RNA annotation**

The noncoding RNA genes, including miRNAs, rRNAs, snRNAs, and tRNAs, were identified. The tRNAscan-SE,2.0 (tRNAscan-SE, RRID:SCR_010835) software with eukaryote parameters [34] was used to predict tRNA genes. The miRNA and snRNA genes in the assembled genome were extracted by INFERNAL,1.1.2 software [35] against the Rfam (Rfam, RRID:SCR_007891) database [36] with default parameters. Finally, 1132 miRNAs, 1664 tRNAs, 41 rRNAs, and 220 snRNAs were discovered from the Peruvian scallop genome.

**Global gene family classification**

Protein-coding genes from the Peruvian scallop and other sequence species, including Brachiopod (Lingula anatina), brown mussel (Modiolus philippinarum), California sea hare (Aplysia californica), cold seep mussel (Bathymodiolus platifrons), Florida lancelet (B. floridae), fruit fly (D. melanogaster), human (H. sapiens), leech (H. robusta, Capitella teleta), nematode (C. elegans), octopus (O. bimaculoides), owl limpet (H. robusta), Pacific abalone (Haliothis discus), Pacific oyster (C. gigas), pearl oyster (Pinctada fucata), red flour beetle (Tribolium castaneum), and Yesso scallop (Patinopecten yessoensis) were analyzed. All data were downloaded from Ensembl [21] or National Center for Biotechnology Information (NCBI) [37]. For each protein-coding gene with alternative splicing isoforms, only the longest protein sequence was kept as the representative.

Gene family analysis based on the homolog of gene sequences in related species was initially implemented by the alignment of an “all against all” BLASTP (with a cutoff of 1e-7) and subsequently followed by alignments with high-scoring segment pairs conjoined for each gene pair by TreeFam 3.0 [38]. To identify homologous gene pairs, we required more than 30% coverage of the aligned regions in both homologous genes. Finally, homologous genes were clustered into gene families by OrthoMCL-5 (OrthoMCL DB: Ortholog Groups of Protein Sequences, RRID:SCR_007839) [39] with the optimized parameter of “-inflation 1.5.” All protein-coding genes from the 18 examined genomes were used to assign gene families. In total, the protein-coding genes were classified into 45,268 families and 108 strict single-copy orthologs (Fig 2).

**Phylogenetic analysis**

Evolutionary analysis was performed using these single-copy protein-coding genes from the 18 examined species. Amino acid and nucleotide sequences of the ortholog genes were aligned using the multiple alignment software MUSCLE (MUSCLE, RRID:SCR_011812) with default parameters [40]. A total number of 108 single-copy ortholog alignments were concatenated into a super alignment matrix of 242,085 nucleotides. A maximum likelihood method deduced tree was inferred based on the matrix of nucleotide sequences using RAxML v8.0.19 (RAxML, RRID:SCR_006086) with default nucleotide substitution model PROTGAMMAAUTO [41]. Clade support was assessed using bootstrapping algorithm in the RAxML package with 100 alignment replicates (Fig 3) [42]. The constructed phylogenetic tree (Fig 3) indicated that the Peruvian scallop and Yesso scallop were clustered closely first and then clustered with oysters and mussels, which is in consistent with their putative evolution relationships [43-46].

**The estimation of divergence time**

The species divergence times were inferred with MCMCTree included in PAML v4.7a (PAML, RRID:SCR_014932) [47] with the parameter set as “burn-in = 1000, sample-number = 1000 000, sample-frequency = 2,” and evolutionary analysis was performed using single-copy protein-coding genes from the 18 examined species. Based on the phylogenetic tree (Fig 3), the molecular clock was calibrated based on the fossil records according to previous studies [48-50]. Finally, we estimated that...
the divergence between the Peruvian scallop and Yesso scallop happened at 113.6 million years ago.

Conclusions

In the present study, we report the first whole genome sequencing, assembly, and annotation of the Peruvian scallop (A. purpuratus), an economically important bivalve in Chile, Peru, and China. The assembled draft genome of 724.78 Mb accounts for 81.87% of the estimated genome size (885.29 Mb). A total of 26,256 protein-coding genes and 3,057 noncoding RNAs were predicted from the genome assembly. This genome assembly will provide solid support for in-depth biological studies. With the availability of these genomic data, subsequent development of genetic markers for further genetic selection and molecular breeding of scallops could be realized. The current genome data will also facilitate genetic analyses of the evolutionary history of the abundant scallops in the world.

Availability of supporting data

Supporting data are available in the GigaScience database [52]. Raw data have been deposited in NCBI with the project accession PRJNA418203. BioSample accessions: SAMN08022140 (genome); SAMN08731415 (transcriptome; muscle); SAMN08731411 (transcriptome; mantle); and SAMN08731410 (transcriptome, hepatopancreas).

Abbreviations

BLAST: Basic Local Alignment Search Tool; KEGG: Kyoto Encyclopedia of Genes and Genomes; NCBI: National Center for Biotechnology Information.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (31572618 grant to C.W. and 41676152 grant to X.L.) and the earmarked fund for Shandong Modern Agro-Industry Technology Research System (SDAIT-14) grant to C.W.

Author contributions

C.W., X.L., and C.L. designed the project. B.M., F.L., and G.L. collected the samples and prepared the quality control. C.L., C.W., and X.L. were involved in the data analyses. C.W., X.L., C.L., and Q.S. wrote the manuscript. All authors read and approved the final manuscript.

References

1. Dall WH. The mollusca and branchiopoda. Report of dredging operation, Albatros' 1891. Bulletin Mollusca Comparative Zoology 1909;37:147–294.
2. Gonzalez ML, Lopez DA, Perez MC, et al. Growth of the scallop, Argopecten purpuratus (Lamarck, 1819), in southern Chile. Aquaculture 1999;175(3-4):307–16.
3. Genética D, Morfológica Y, Dos E, et al. Genetic and morphological differentiation between two pectinid populations of Argopecten purpuratus from the northern Chilean coast. Estudios Oceanologicos 2001;1:51–60.
4. Disalvo LH, Alarcon E, Martinez E, et al. Progress in mass culture of Chlamys (Argopecten) purpurata Lamarck (1819) with notes on its natural history. Revista Chilena de Historia Natural 1984;57:35–45.
5. Estabrooks SL. The possible role of telomerases in the short life span of the bay scallop, Argopecten irradians irridans Lamarck. J Shellfish Res 2007;26(2):307–13.
6. Wang C, Liu B, Li J, et al. Inter-specific hybridization between Argopecten purpuratus and Argopecten irradians. Marine Sci 2009;33(10):84–75.
7. Wang C, Liu B, Li J, et al. Introduction of the Peruvian scallop and its hybridization with the bay scallop in China. Aquaculture 2011;310(3-4):380–7.
8. Marçais G, Kingsford C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics 2011;27(6):764–70.
9. Kajitani R, Toshimoto K, Noguchi H, et al. Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res 2014;24(8):1384–95.
10. Luo R, Liu B, Xie Y, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. GigaSci 2012;1(1):18.
11. English AC, Richards S, Han Y, et al. Mind the gap: upgrading genomes with PacBio Biosciences RS long-read sequencing technology. PLoS One 2012;7(11):e47768.
12. Walker BJ, Abeel T, Shea T, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 2014;9(11):e112963.
13. Adey A, Kitzman JO, Burton JN, et al. In vitro, long-range sequence information for de novo genome assembly via transposable contiguity. Genome Res 2014;24(12):2041–9.
14. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25(14):1754–60.
15. Parra G, Bradnam K, Korf I. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics 2007;23(9):1061–7.
16. Simão FA, Waterhouse RM, Ioannidis P, et al. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 2015;31(19):3210–2.
17. Grundmann N, Demester L, Makalowski W. T.Eclass—a tool for automated classification of unknown eukaryotic transposable elements. Bioinformatics 2009;25(10):1329–30.
18. Jurka J, Kipitonov VV, Pavlichev A, et al. RepBase Update, a database of eukaryotic repetitive elements. Cytogenet Genome Res 2005;110(1-4):462–7.
19. Tarailograovac M, Chen N. Using RepeatMasker to identify repetitive elements in genomic sequences. Current Protocols in Bioinformatics 2009; Chapter 4 Unit 4:Unit 4.10.
20. Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 1999;27(2):573–80.
21. Kersey PJ, Allen JE, Allot A, et al. Ensembl Genomes 2018: an integratedomics infrastructure for non-vertebrate species. Nucleic Acids Res 2017;46:D802–D808.
22. Kent WJ. BLAT—The BLAST-Like Alignment Tool. Genome Res 2002;12(4):656–64.
23. Birney E, Clamp M, Durbin R. GeneWise and Genomewise. Genome Res 2004;14(5):988–95.
24. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 2009;25(9):1105–11.
25. Trapnell C, Williams BA, Pertea G. Transcript assembly and
quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation, 2010; 28(5):511–515.

26. Stanke M, Waack S. Gene prediction with a hidden Markov model and a new intron submodel. Bioinformatics 2003;19(suppl 2):215–25.

27. Salamov AA, Solovyev VV. Ab initio gene finding in Drosophila genomic DNA. Genome Res 2000;10(4):516–22.

28. Majaros W, Pertea M, Salzberg SL. TigrScan and GlimmerHMM: two open source ab initio eukaryotic gene-finders. Bioinformatics 2004;20(16):2878–9.

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

30. Bairoch A, Apweiler R. The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000. Nucleic Acids Res 2000;28(1):45–48.

31. Mulder N, Apweiler R. InterPro and InterProScan: tools for protein sequence classification and comparison. Methods Mol Biol 2007;396:59.

32. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 2000;27(1):29–34.

33. Sherlock G. Gene Ontology: tool for the unification of biology. Canadian Institute of Food Science & Technology Journal 2009;22(4):415.

34. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 1997;25(5):955–64.

35. Nawrocki EP, Kolbe DL, Eddy SR. Infernal 1.0: inference of RNA alignments. Bioinformatics 2009;25(10):1335–7.

36. Griffiths-Jones S, Moxon S, Marshall M, et al. Rfam: annotating non-coding RNAs in complete genomes. Nucleic Acids Res 2004;33(Database issue):D121–4.

37. Sayers EW, , Barrett T, Benson DA, et al. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 2010, 38(Database issue):D1–D13.

38. Ruan J, Li H, Chen Z, et al. TreeFam: 2008 update. Nucleic Acids Res 2008;36(Database issue):D735–D40.

39. Li L, Stoeckert CJ, Roos DS. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res 2003;13(9):2178–89.

40. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004;32(5):1792–7.

41. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 2014;30(9):1312–3.

42. Sun J, Zhang Y, Xu T, et al. Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. Nat. ecol. evol. 2017;1(5):121.

43. Shi W, Zhang J, Jiao W et al. Scallop genome provides insights into evolution of bilaterian karyotype and development. Nat Ecol Evol 2017;1(5):120.

44. Benton M, Donoghue P, Asher R. Calibrating and constraining molecular clocks. In: H SB Kumar S (eds), The Timetree of Life. Oxford University Press, 2009, pp. 35–86.

45. Mergl M, Massa D, Plauchut B. Devonian and carboniferous brachiopods and bivalves of the Djado sub-basin (North Niger, SW Libya). Journal of the Czech Geological Society 2001;46(3):169–88.

46. Erwin DH, Laflamme M, Tweedt SM, et al. The Cambrian conundrum: early divergence and later ecological success in the early history of animals. Science 2011;334(6059):1091–7.

47. Li C, Liu X, Liu B, et al. Supporting data for “draft genome of the Peruvian scallop Argopecten purpuratus.” GigaScience Database 2018. http://dx.doi.org/10.5524/100419