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Abstract:	Background: The Peruvian scallop, Argopecten purpuratus, is mainly cultured in Southern Chile and had been introduced into China in last century. Unlike other Argopecten scallops, the Peruvian scallop normally has a long life span of up to 7-10 years. Therefore, researchers have been employing 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 (Gigabase) raw DNA reads were sequenced. The draft genome assembly of 724.78 Mb was generated (accounting for 81.87% of the estimated genome size 885.29 Mb), with a contig N50 size of 80.11 kb and scaffold N50 size of 1.02 Mb. Meanwhile, the repeat sequences were calculated to reach 33.74% of the whole genome, and a total of 26,256 protein-coding genes and 3,057 non-coding RNAs were predicted from the assembly. Conclusion: We generated a draft genome assembly of the Peruvian scallop, which will provide solid resource for further genetic breeding and evolutionary history analysis of this economically important scallop.	
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Draft genome of the Peruvian scallop *Argopecten purpuratus*

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Abstract

Background: The Peruvian scallop, *Argopecten purpuratus*, is mainly cultured in Southern Chile and had been introduced into China in last century. Unlike other *Argopecten* scallops, the Peruvian scallop normally has a long life span of up to 7-10 years. Therefore, researchers have been employing 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 (Gigabase) raw DNA reads were sequenced. The draft genome assembly of 724.78 Mb was generated (accounting for 81.87% of the estimated genome size 885.29 Mb), with a contig N50 size of 80.11 kb and scaffold N50 size of 1.02 Mb. Meanwhile, the repeat sequences were calculated to reach 33.74% of the whole genome, and a total of 26,256 protein-coding genes and 3,057 non-coding RNAs were predicted from the assembly. **Conclusion:** We generated a draft genome assembly of the Peruvian scallop, which will provide solid resource for further genetic breeding and evolutionary history analysis 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 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 stenotherm 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 2000's and had played an important role in stock improvement of *Argopecten* scallops via inter-specific hybridization [6, 7] with bay scallops.

Whole genome sequencing

Genomic DNA was extracted from muscle sample of a single *A. purpuratus* (Figure 1), which was obtained from a local scallop farm in Laizhou, Shandong Province, China. The traditional whole genome shotgun sequencing strategy was applied. 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), and sequenced on the Illumina HiSeq4000 platform with paired-end 150 bp. In addition, SMRTbell libraries were prepared using either 10-kb or 20-kb preparation protocols to optimize for the most high-quality and longest reads; subsequent sequencing was performed on PacBio Sequel instrument with Sequel^{TM} Sequencing Kit 1.2.1(Pacific Biosciences of California，USA). Finally, the 10X Genomics library was constructed and sequenced with paired-end 150 bp on the Hiseq platform. The Chromium™ Genome Solution（10X Genomics，USA）massively partitions and molecularly barcodes DNA using microfluidics, producing sequencing-ready libraries with >1,000,000 unique barcodes. In total, 463.19 gigabases (Gb) of raw reads were generated, including 75.72, 70.22, 19.21, 45.71, 28.34, 11.78, 18.01 and 194.20 Gb from the 250-bp, 450-bp, 2-kb, 5-kb, 10-kb, 20-kb libraries, Pacbio sequencing, and 10X Genomics library respectively. The raw reads were trimmed by removing adaptor sequences, ambiguous nucleotides and low-quality reads, and then these cleaned high-quality reads were used for subsequent genome assembling.

Estimation of the genome size and sequencing coverage

The 17-mer frequency distribution analysis [8] was performed on all the remaining clean reads to estimate the genome size of the Peruvian scallop using the following formula: genome size = k-mer number / peak depth. A total number of 6.22 × 10^{10} k-mers and the peak k-mer depth of 69 was employed to obtain the estimated genome size at 885.29 Mb (Table 1), and the estimated repeat sequencing ratio reaches 33.74%.

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

All the pair-end Illumina reads were first assembled into scaffolds using Platanus [9], and then were applied to fill the gaps by GapCloser [10]. Subsequently, the Pacbio data were used for additional gap filling by PBJelly v14.1 with default parameters [11], and then all the Illumina reads were employed for two rounds to correct the genome assembly by Pilon v1.18 [12]. After that, the 10X linked-reads were used to link scaffolds by fragScaff [13]. Finally, a draft genome of 724.78 Mb was assembled (accounting for 81.87% of the estimated genome size at 885.29 Mb), with a contig N50 size of 80.11 kb and a scaffold N50 size of 1.02 Mb (Table 1).

With this initial assembly, we applied the short insert library reads to map with the assembled genome using BWA software [14] to calculate the mapping ratio and assess the assembly integrity. In summary, 91.05% short reads were mapped onto the assembled genome with a coverage of 89.40%, indicating a good reliability of our genome assembly. CEGMA (Core Eukaryotic Genes Mapping Approach) 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, otherwise it is classified as partial. Through mapping to the 248 core eukaryotic genes, a total of 222 genes (89.52%) were identified. BUSCO (Benchmarking Universal Single-Copy Orthologs) 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 a good integrity of the genome assembly.

Repeat sequence analysis of the genome assembly

We searched transposable elements (TEs) in the assembled genome through *ab-initio* and homology based methods. For the former method, we applied RepeatModeler [17] (the parameter set as '--engine_db wublast') to build a specific repeat database. For the latter method, we employed known repeat library (Repbase) [18] to identify repeats with RepeatMasker [19] (the parameter set as '-a -nolow

-no_is -norna -parallel 3 -e wublast --pvalue 0.0001') and RepeatProteinMask (the parameter set as '-noLowSimple -pvalue 0.0001 -engine wublast') [19]. Tandem repeats finder (TRF) was used to find tandem repeats with the parameters setting as 'Match = 2, Mismatching penalty = 7, Delta = 7, PM = 80, PI = 10, Minscore = 50, MaxPeriod = 2,000' [20]. Finally, we summarized that the total repeat sequences are 294,496,811 bp, accounting for 40.63% of the assembled genome, and including 11.46% of tandem repeats, which is consistent with our above-mentioned estimation (Table 2).

Gene annotation

(1) 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 Pacific oyster (*Crassostrea gigas*), Mollusks (*Lottia gigantean*), Mosquito (*Anopheles gambiae*), Amphioxus (*Branchiostoma floridae*), Nematode (*Caenorhabditis elegans*), Ascidian (*Ciona intestinalis*), Fruit fly (*Drosophila melanogaster*), Leech (*Helobdella robusta*), Human (*Homo sapiens*), Octopuses (*Octopus bimaculoides*), Sea urchin (*Strongylocentrotus purpuratus*) were downloaded from Ensemble [21]. The protein sequences of homology species were aligned to the assembled genome with TBLASTn (e-value ≤ 10-5) [22] and predicted gene structures with GeneWise (the parameter set as '-genesf') [23]. The transcriptome data from muscle, sequenced by Illumina sequencing platform, were mapped onto our genome assembly with Tophat (the parameter set as '--max-intron-length 500000 -m 2 --library-type fr-unstranded') [24] and assembled to gene model with Cufflinks (the parameter set as '--multi-read-correct') [25] according to the pair-end relationships and the overlap between aligned reads. The *de novo* prediction of genes was carried out with four programs: Augustus (the parameter set as '-uniqueGeneId true --noInFrameStop=true --gff3 on –genemodel complete –strand both') [26], Genscan (the default parameter) [27], GlimmerHMM (the parameter set as ' -f -g') [28] and SNAP (the default parameter) [29]. All evidences of gene model were integrated using EvidenceModeler (EVM) [29]. Finally, we identified 26,256 protein-coding genes in the Peruvian scallop genome. A total of 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 evidence. The average transcript length, CDS length and intron length were calculated to be 10,534 bp, 1,418 bp and 1,505 bp respectively (Table 1).

(2) Gene functional annotation

Gene functions were predicted from the best BLASTP (e-value ≤ 10-5) hits in SwissProt databases [30]. Gene domain annotation was performed by searching the InterPro database [31]. All genes were aligned against Kyoto Encyclopedia of Genes and Genomes (KEGG) [32] to identify the best hits for pathways. Gene Ontology (GO) terms for genes were obtained from the corresponding InterPro entry [33]. Finally, among these annotated genes, 70.3% encoded proteins showed homology to proteins in the SwissProt database, 91.1% were identified in the non-redundant (Nr) database, 70.4% were identified in the KEGG database, 72.1% were identified in the InterPro, and a total of 92.1% could be mapped to the functional databases.

(3) Non-coding RNA annotation

The non-coding RNA genes, including miRNAs, rRNAs, snRNAs and tRNAs, were identified. The tRNAscan-SE software with eukaryote parameters [34] was employed to predict tRNA genes. The miRNA and snRNA genes in the assembled genome were extracted by INFERNAL software [35] against the Rfam database [36] with default parameters. Finally, 1,132 miRNAs, 1,664 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 sequenced species, including Human (*H. sapiens*), Amphioxus (*B. floridae*), Fruit fly (*D. melanogaster*), Red flour beetle (*T. castaneum*), Nematode (*Caenorhabditis elegans*), brachiopod (*Lingula anatine*), *Helobdella robusta Capitella teleta, Octopus bimaculoides, Lottia gigantean,* mollusk (*Aplysia californica*), Pacific Abalone (*Haliotis discus*), Pacific oyster (*C. gigas*), pearl oyster (*Pinctada fucata*), Yesso scallop (*Patinopecten yessoensis*), and cold seep mussel (*Bathymodiolus platifrons*), Brown mussel (*Modiolus philippinarum*) were analyzed. All data were downloaded from Ensemble [21] or NCBI [37]. For each protein-coding gene with alternative splicing isoforms, we only kept the longest protein sequence as the representative.

Gene family analysis was based on the homolog of gene sequences in related species, which was initially implemented by the alignment of an "all against all" BLASTP (with a cutoff of 1e-7), and subsequently the alignments with high-scoring segment pairs were conjoined for each gene pair by TreeFam [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 [39] with the optimized parameter of '-inflation 1.5'. All protein-coding genes from the examined 18 genomes were employed to assign gene families. In total, the protein-coding genes were classified into 45,268 families and 108 strict single-copy orthologs (Figure 2).

Phylogenetic analysis

Evolutionary analysis was performed using these single-copy protein-coding genes from the examined 18 species. Amino acid and nucleotide sequences of the ortholog genes were aligned by the multiple alignment software MUSCLE 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 (ML) deduced tree was inferred based on the matrix of nucleotide sequences using RAxML with default nucleotide substitution model-PROTGAMMAAUTO [41]. Clade support was assessed using bootstrapping algorithm in the RAxML package with 100 alignment replicates (Figure 3) [42]. The constructed phylogenetic tree (Figure 3) indicated that the Peruvian scallop and Yesso scallop are clustered closely first and then clustered with Oysters and Mussels, which is in consistent with their putative evolution relationships [43, 44].

The estimation of divergence time

The species divergence times were inferred with MCMCTree included in PAML v4.7a [45] with the parameter set as '--model 0 --rootage 1200 -clock 3', and evolutionary analysis was performed using single-copy protein-coding genes from the 18 examined species. Based on the phylogenetic tree (Figure 3), we estimated that the divergence between the Peruvian scallop and Yesso scallops happened at 113.6 Mya ago.

Conclusion

In the present study, we reported the first whole genome sequencing, assembly and annotation of Peruvian scallop (*A. purpuratus*), an economically important bivalve in 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 non-coding RNAs were predicted from the assembly. In the coming future, this generated genome assembly will provide solid support for deep biological studies. With availability of these genomic data, subsequent development of genetic markers for further genetic selection and molecular breeding of scallops could be realized. Our current genome data will also definitely facilitate the genetic evolutionary history analysis for the abundant scallops in the world.

Availability of Data

Supporting raw data have been deposited in NCBI with the project accession PRJNA418203.

Acknowledgements

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Conflicts of interest

The authors declare that they have no competing interests.

Author’s contributions

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

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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
The 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

Table 2. The prediction of repeats 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

Figure 1 Picture of a representative Peruvian scallop in China.

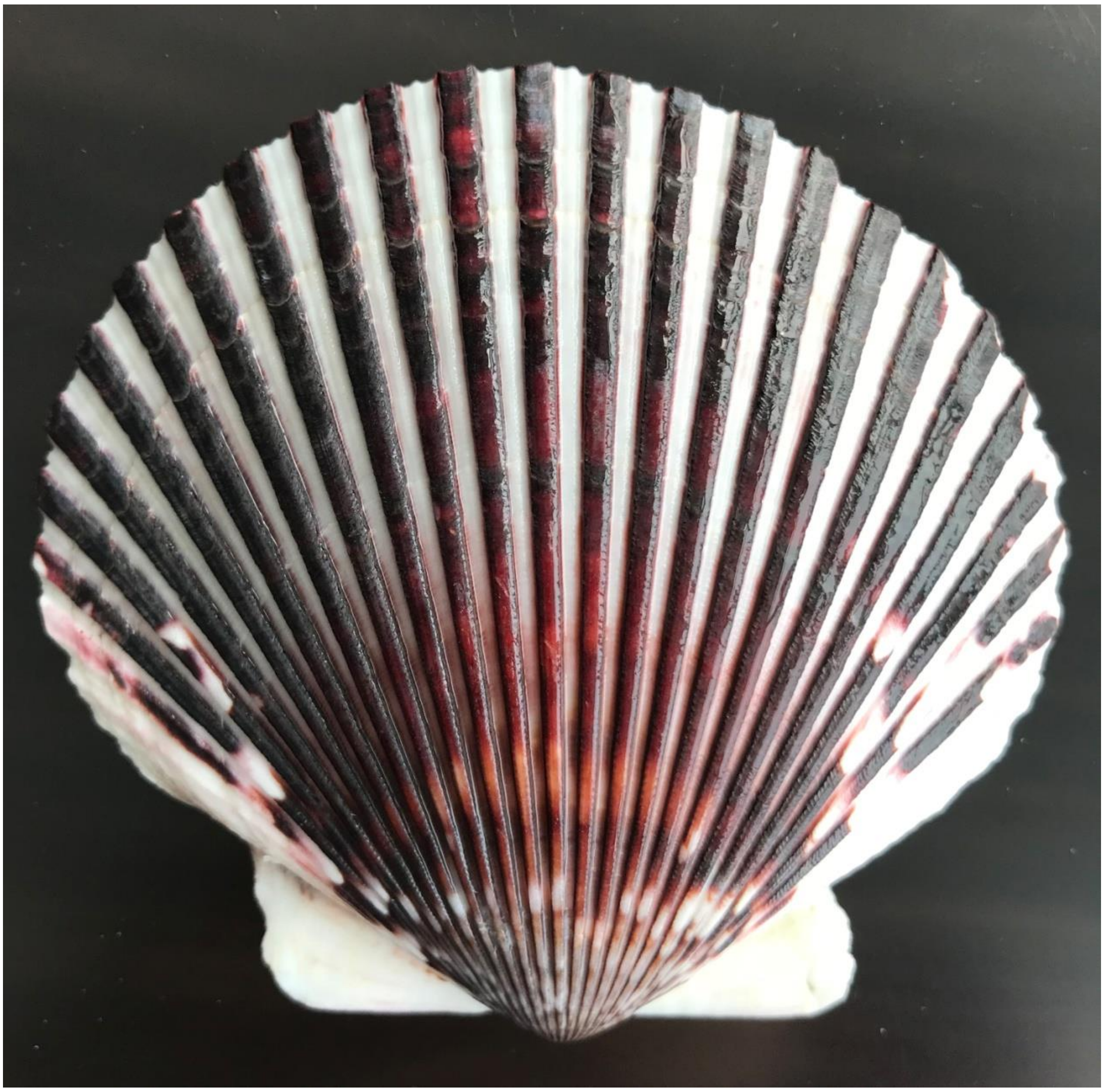


Figure 2. Distribution of genes in different species. Abbreviations: Aca, *Aplysia californica;* Apu, *Argopecten purpuratus;* Bfl, *Branchiostoma floridae;* Bpl, *Bathymodiolus platifrons;* Cel, *Caenorhabditis elegans;* Cgi, *Crassostrea gigas; Cte, Capitella teleta;* Dme, *Drosophila melanogaster;* Has, *Homo sapiens;* Hdi, *Haliotis discus;* Hro, *Helobdella robusta;* Lan, *Lingula anatine;* Lgi, *Lottia gigantean;* Mph, *Modiolus philippinarum;* Obi, *Octopus bimaculoides;* Pfu, *Pinctada fucata;* Tca, *Tribolium castaneum*.

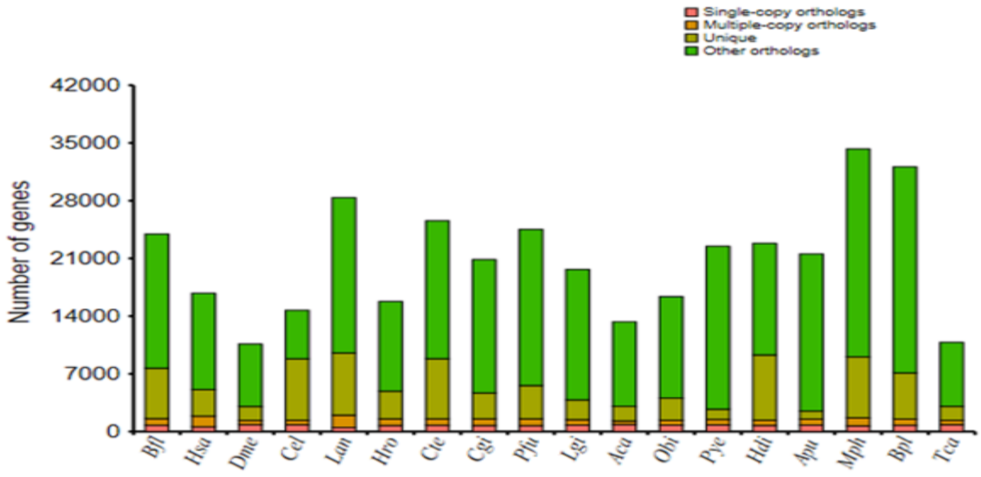
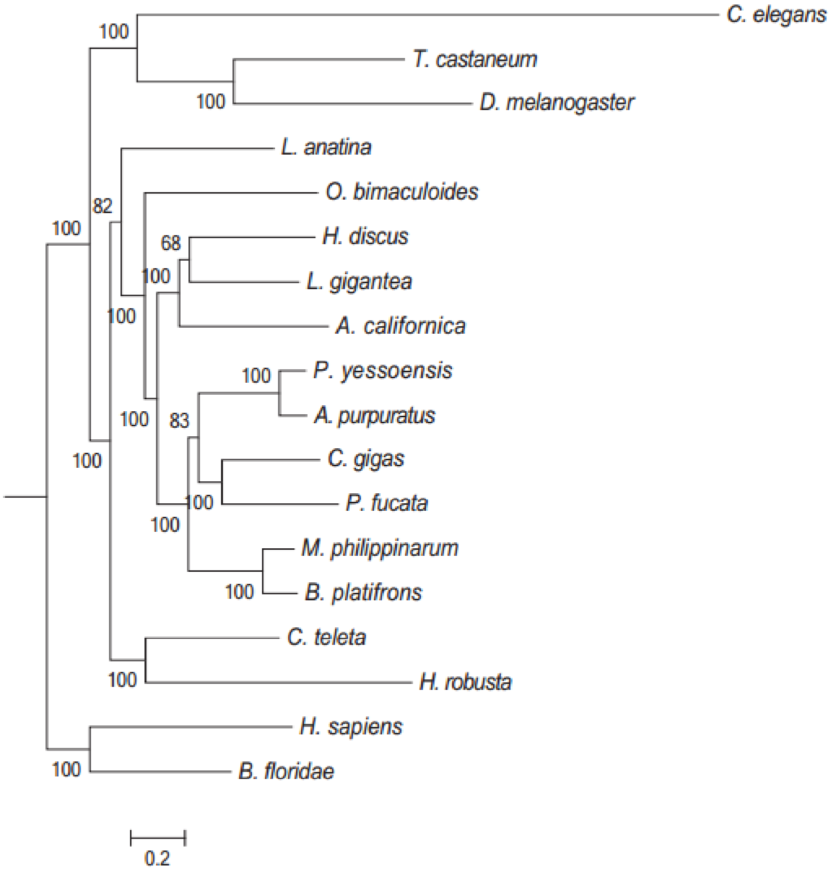


Figure 3. Bootstrap support of phylogenetic tree. A ML tree was constructed by RAxML based on 108 single-copy protein-coding genes of the related species. The total number of bootstrap was 100.


C. elegans
T. castaneum
D. melanogaster
L. anatina
O. bimaculoides
H. discus
L. gigantea
A. californica
P. yessoensis
A. purpuratus
C. gigas
P. fucata
M. philippinarum
B. platifrons
C. teleta
H. robusta
H. sapiens
B. floridae
100
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82
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68
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83
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