Draft genomes of two Atlantic bay scallop subspecies *Argopecten irradians* irradians and *A. i. concentricus*

Xiao Liu¹, Chao Li², Min Chen³, Bo Liu², Xiaojun Yan¹, Junhao Ning³, Bin Ma⁴, Guilong Liu⁴, Zhaoshan Zhong³, Yanglei Jia¹, Qiong Shi⁶ & Chunde Wang²,³ ✉

The two subspecies of Atlantic bay scallop (*Argopecten irradians*), *A. i. irradians* and *A. i. concentricus*, are economically important aquacultural species in northern and southern China. Here, we performed the whole-genome sequencing, assembly, and gene annotation and produced draft genomes for both subspecies. In total, 253.17 and 272.97 gigabases (Gb) of raw reads were generated from Illumina Hiseq and PacBio platforms for *A. i. irradians* and *A. i. concentricus* respectively. Draft genomes of 835.7 Mb and 874.82 Mb were assembled for the two subspecies, accounting for 83.9% and 89.79% of the estimated sizes of their corresponding genomes, respectively. The contig N50 and scaffold N50 were 78.54 kb and 1.53 Mb for the *A. i. irradians* genome, and those for the *A. i. concentricus* genome were 63.73 kb and 1.25 Mb. Moreover, 26,777 and 25,979 protein-coding genes were predicted for *A. i. irradians* and *A. i. concentricus*, respectively. These valuable genome assemblies lay a solid foundation for future theoretical studies and provide guidance for practical scallop breeding.

**Background & Summary**

Two subspecies of the Atlantic bay scallop, the northern subspecies *Argopecten irradians* irradians (Lamarck, 1819) and the southern subspecies *A. i. concentricus* (Say, 1822), are widely cultured in China. The northern subspecies *A. i. irradians* is mainly cultured in northern waters, while the southern subspecies *A. i. concentricus* is generally cultured in southern waters¹. Both subspecies were introduced from the USA between the 1980s and 1990s. In general, these bay scallops grow fast but have short life spans (i.e. < 2.4 months)². These two subspecies are morphologically similar, although the ratio of shell width (W) to shell height (H) or shell length (L) of *A. i. concentricus* is remarkably higher than that of *A. i. irradians*—the average W/L ratio of adult *A. i. concentricus* and *A. i. irradians* are 0.59 and 0.45, respectively³. Although both subspecies are adapted to their natural habitats with wide temperature ranges, *A. i. irradians* is more tolerant to the cold northern waters but cannot survive in the southern warm waters, whereas *A. i. concentricus* is better adapted to the warm southern waters but stops growing at a temperature of 12°C or lower.

Successful diallel crossbreeding has been performed between the two subspecies, as well as between the Peruvian scallop (*Argopecten purpuratus*) and both of the two bay scallop subspecies⁴. In addition to high fertilization and hatching rates, the resulting F₁ hybrids exhibited excellent performance in production traits such as growth and survival, indicating a great potential in stock improvement via inter- or intra-specific hybridization between different subspecies or populations. To date, three high-performance strains, 'Bohai Red', 'QN-2' and 'QN Orange', with increases in average whole body weight of over 38% compared to unselected bay scallops, have been selected from the F₁ hybrids between the Peruvian scallop and *A. i. irradians*⁵.⁶. Recently, a new strain was

¹School of Fishery, Zhejiang Ocean University, Zhoushan, 316022, China. ²Marine Science and Engineering College, Qingdao Agricultural University, Qingdao, 266109, China. ³Yantai Institute of Coastal Zone Research and Center for Ocean Mega-Science, Chinese Academy of Sciences, Yantai, 264003, China. ⁴Yantai Spring-Sea Aquaseeds Co., Ltd., Yantai, 264006, China. ⁵Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, 266071, China. ⁶Shenzhen 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. ✉e-mail: chundewang2007@163.com
NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). DNA concentration was measured using a Qubit®
samples was checked by electrophoresis on 1% agarose gels. The purity of the DNA was also checked using a
been bred by self-fertilization in a scallop farm in Laizhou, Shandong Province, China. The quality of the DNA
were assembled into scaffolds using Platanus v1.2.414, and the gaps were filled using GapCloser15. Subsequently,
A routine assembly strategy was applied for the genome assemblies of both scallops. Briefly, all high-quality reads
for quality control. All downstream analyses were based on the high quality, clean reads identified here.
repeats, and low-quality sequences. In addition, the Q20, Q30, and GC content of the clean reads was determined
Trimmomatic. In this step, clean reads were obtained by removing reads containing adapter sequences, poly-N
1.2.1 (Pacific Biosciences, USA) for both subspecies. Raw reads in the FASTQ format were first processed using
PBJelly v14.1 was applied for additional gap filling with Pacbio reads16. Finally, all Illumina reads were employed
to correct the genome assemblies in Pilon v1.18 for two rounds17 (Table 1).

generation to diverse habitats and to understand their wide variation in life span as well as the development of male sterile
In a previous study, we have sequenced and assembled the genome of the Peruvian scallop12. The genomic
data of Argopecten scallops and their hybrids will allow us to investigate the evolutionary relationships among
Argopecten scallop species and subspecies, to study the molecular mechanisms underlying scallop adaptations
to diverse habitats and to understand their wide variation in life span as well as the development of male sterile
gonads in their F1 hybrids.
In this study, we sequenced and assembled the genomes of the two bay scallop subspecies, A. i. irradians and
A. i. concentricus. Together with the genomic data of the Peruvian scallop, the results of our present study and
subsequent genome-wide association studies will eventually facilitate the breeding progress in these Argopecten
 scallops.

Methods

Sample collection and genomic DNA extraction. Genomic DNA was extracted from the adductor
muscle of a single specimen from a pure line of A. i. irradians (Fig. 1a) and A. i. concentricus (Fig. 1b), which have
been bred by self-fertilization in a scallop farm in Laizhou, Shandong Province, China. The quality of the DNA
samples was checked by electrophoresis on 1% agarose gels. The purity of the DNA was also checked using a
NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). DNA concentration was measured using a Qubit®
DNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). A total of 1.5 μg DNA per sample was
used for subsequent sample preparations.

DNA library preparation and whole genome sequencing. Sequencing libraries were generated using the Truseq Nano DNA HT Sample Preparation Kit (Illumina, USA) following the manufacturer’s instructions. Index codes were used to cross-index the sequences and samples, that is, the DNA samples were fragmented by sonication and then end-polished, A-tailed, and ligated with the full-length adapter for Illumina sequencing followed by PCR amplification. The resulting PCR products were purified (AMPure XP system) and the sequence libraries were analyzed for size distribution by Agilent2100 Bioanalyzer and quantified using real-time PCR.

These libraries were sequenced on an Illumina HiSeq4000 platform using a 150-bp paired-end sequencing
protocol. Additional sequencing was performed on a PacBio Sequel instrument with a SequelTM Sequencing Kit
1.2.1 (Pacific Biosciences, USA) for both subspecies. Raw reads in the FASTQ format were first processed using
Trimmomatic. In this step, clean reads were obtained by removing reads containing adapter sequences, poly-N
repeats, and low-quality sequences. In addition, the Q20, Q30, and GC content of the clean reads was determined
to quality control. All downstream analyses were based on the high quality, clean reads identified here.

Genome assembly. To estimate the genome size of both subspecies, a routine 17-mer frequency distribution
analysis13 was performed according to the following formula: genome size = k-mer number/peak depth (Table 1).
A routine assembly strategy was applied for the genome assemblies of both scallops. Briefly, all high-quality reads
were assembled into scaffolds using Platanus v1.2.414, and the gaps were filled using GapCloser15. Subsequently,
PBJelly v14.1 was applied for additional gap filling with Pacbio reads16. Finally, all Illumina reads were employed
to correct the genome assemblies in Pilon v1.18 for two rounds17 (Table 1).

Genome assessment. Following the initial assembly, the integrity of both genome assemblies was assessed by mapping the reads from short-insert libraries onto the assembled genomes using Burrows-Wheeler Aligner
Two methods were employed to identify transposable elements (TEs) in the assembled genomes. When using the \textit{ab-initio} method, RepeatModeler was used to build a species-specific repeat database (parameters set as ‘–engine\_db wublast’\textsuperscript{22}). When using a homology-based method, RepeatMasker\textsuperscript{23} was employed to identify repeats with known repeat libraries (Repbase)\textsuperscript{24} using the following parameters: ‘-a -nolow -no_is -norna -parallel 3 -e wublast–pvalue 0.0001’ , along with RepeatProteinMask (the parameter set as ‘-noLowSimple -pvalue 0.0001 -engine wublast’\textsuperscript{23}), and the repbase data were collected from a comprehensive database of undifferentiated species (RepBase Metadata Database RELEASE 20170127). In addition, tandem repeats were identified using Tandem Repeats Finder (TRF) with the parameters setting as ‘Match = 2, Mismatching penalty = 7, Delta = 7, PM = 80, PI = 10, Minscore = 50, MaxPeriod = 2,000’\textsuperscript{25} (Table 2).

**Repeat annotation.** Two methods were employed to identify transposable elements (TEs) in the assembled genomes. When using the \textit{ab-initio} method, RepeatModeler was used to build a species-specific repeat database (parameters set as ‘–engine\_db wublast’\textsuperscript{22}). When using a homology-based method, RepeatMasker\textsuperscript{23} was employed to identify repeats with known repeat libraries (Repbase)\textsuperscript{24} using the following parameters: ‘-a -nolow -no_is -norna -parallel 3 -e wublast–pvalue 0.0001’ , along with RepeatProteinMask (the parameter set as ‘-noLowSimple -pvalue 0.0001 -engine wublast’\textsuperscript{23}), and the repbase data were collected from a comprehensive database of undifferentiated species (RepBase Metadata Database RELEASE 20170127). In addition, tandem repeats were identified using Tandem Repeats Finder (TRF) with the parameters setting as ‘Match = 2, Mismatching penalty = 7, Delta = 7, PM = 80, PI = 10, Minscore = 50, MaxPeriod = 2,000’\textsuperscript{25} (Table 2).

**Gene annotation.** \textit{de novo prediction.} Protein-coding genes in the assembled genomes were annotated using \textit{de novo} prediction by homology with transcriptome data-based evidence. Four programs were employed for the \textit{de novo} prediction of genes, including Augustus v3.2.1 (with the following parameters: ‘-uniqueGeneId true –noInFrameStop = true –gff3 on –genemodel complete –strand both’)\textsuperscript{26}, Genscan (using default parameters)\textsuperscript{27}, GlimmerHMM (with the following parameters: ‘-f -g’)\textsuperscript{28}, and SNAP (using default parameters)\textsuperscript{29}.  

**Homology-based annotation.** Protein sequences from mosquito (\textit{Anopheles gambiae}), Amphioxus (\textit{Branchiostoma floridae}), nematode (\textit{Caenorhabditis elegans}), Ascidian (\textit{Ciona intestinalis}), Pacific oyster (\textit{C. gigas}, also known as \textit{Magallana gigas}), fruit fly (\textit{Drosophila melanogaster}), leech (\textit{Helobdella robusta}), human (\textit{Homo sapiens}), owl limpet (\textit{Lottia gigantea}), octopus (\textit{Octopus bimaculoides}), and sea urchin (\textit{Stronglylocentrotus}

| Type                  | Repeat Size (bp)   | % of genome     |
|-----------------------|-------------------|-----------------|
|                       | A. i. irradians    | A. i. concentricus | A. i. irradians | A. i. concentricus |
| TRF                   | 126,153,959       | 135,900,220     | 15.10          | 15.53              |
| RepeatMasker          | 309,417,572       | 326,918,089     | 37.02          | 37.37              |
| RepeatProteinMask     | 31,422,581        | 30,821,540      | 3.76           | 3.52               |
| Total                 | 389,681,429       | 412,788,948     | 46.63          | 47.19              |

Table 2. Prediction of repeat elements in the two genome assemblies of bay scallop.
purpuratus) were used for homology-based searches against the two genome assemblies using TBLASTn (e-value ≤ 10−5)30. The final gene structures were predicted using GeneWise (with the following parameters: ‘-genes’)31.

Transcriptome-based annotation. Transcriptome data from different tissues including kidney, hepatopancreas, and haemolymph were mapped onto each genome assembly using Tophat (with the following parameters: ‘--max-intron-length 500000 -m 2–library-type fr-unstranded’)15, and used for gene modeling using Cufflinks (with the following parameters: ‘--multi-read-correct’)15 according to the pair-end relationships and the overlaps between aligned reads.

Gene set integration. Following de novo prediction, homology-based annotation, and transcriptome-based prediction, we integrated the gene models using EvidenceModeler (EVM)30 to generate a comprehensive and non-redundant gene set (Table 1).

Functional assignment. Gene function annotation was performed by aligning the predicted protein sequences against various protein databases—including the SwissProt34 and NCBI non-redundant (Nr) databases—using BLASTP (e-value ≤ 10−5). Gene domain annotation was performed by searching the InterPro database25. All genes were aligned against the Kyoto Encyclopedia of Genes and Genomes (KEGG)36 database to identify gene pathways. Gene Ontology (GO) terms of the genes were obtained from the corresponding InterPro entry27.

Ortholog and gene family expansion analysis. The protein-coding genes from both scallop genome assemblies and seventeen other sequenced species including Pacific oyster, owl limpet, Amphioxus, nematode, fruit fly, leech, human, octopus, red flour beetle (Tribolium castaneum), polychaete (Capitella teleta), brachiopod (Lingula anatina), sea slug (Aplysia californica), abalone (Haliotis discus), pearl oyster (Pinctada fucata), Yesso scallop (P. yessoensis), deep-sea vent mussel (B. platifrons) and shallow-water mussel (M. philippinarum) were analyzed. All data were downloaded from the Ensembl18 or NCBI39 databases. Gene family analysis was performed based on the homologs of the protein-coding genes in the related species, which was initially implemented by the alignment of an “all against all” BLASTP. Subsequently, alignments with high-scoring segment pairs (HSPs) were conjoined for each gene pair by Solar40 to process the mapping results. To identify homologous gene-pairs, more than 30% coverage of the aligned regions in both homologous genes was required. Finally, homologous genes were clustered into gene families by OrthoMCL41. A p-value cut-off of 1e-5 was chosen for putative orthologs or paralogs, which were converted into a graph for the nodes of representative protein sequences. The resulting graph is represented by a symmetric similarity matrix to which an MCL algorithm was applied (with the following parameters: “-inflation 1.5”) to regulate cluster tightness (Fig. 2a,b).

Genome evolution analysis. Phylogenetic analysis. To trace the evolutionary position of A. i. irradians and A. i. concentricus, a dataset containing 107 single-copy protein-coding genes retrieved from the 19 species mentioned above was used for phylogenetic tree construction and divergence time estimation. Protein sequences for these single-copy genes were aligned by MUSCLE22 one by one, and then were concatenated to the final data-set. ProtTest29 was used to select the best-fit model for this dataset. Then, the phylogenetic tree was reconstructed using the RAxML method (version 7.2.3)44 with LG + G + I + F model with the proportion of invariable sites 0.07 and Gamma shape parameter 0.83. The clade containing H. sapiens and B. floridana was set as outgroup. Clade support was assessed using the bootstrapping algorithm in the RAxML with 1000 alignment replicates.

Estimation of divergence time. Species divergence time was inferred based on the same dataset containing 107 single-copy protein-coding genes from the 19 species using the MCMCTree function included in PAML v4.7a46 with the following parameters: “-model 0-rootage 1200 -clock 3”. For their divergence time estimation, reference divergence times obtained from TimeTree database46 were used as time scales to calibrate the divergence time of A. i. irradians and A. i. concentricus. These include the divergence times between T. castaneum and D. melanogaster (307–414 Mya), between P. yessoensis and C. gigas (>330 Mya), between C. teleta and D. melanogaster (531–581 Mya), between C. teleta and L. gigantean (351–381 Mya), between C. gigas and L. gigantean (500–550 Mya), between H. robusta and C. teleta (450–602 Mya), between P. fucata and C. gigas (>330 Mya), and between B. platifrons and M. philippinarum (39–132 Mya).

Data Records
The whole genome sequences of A. i. irradians and A. i. concentricus were deposited in public repositories. The raw sequencing and transcriptomic data were deposited in NCBI Sequence Read Archive, under the SRA study accession SRP1745267. This whole-genome project including the assembly fasta, annotation and protein sequences was uploaded to Dryad (https://doi.org/10.5061/dryad.hdr7sqvdr)48. All genome annotation and phylogenetic tree files were uploaded to Figshare (https://doi.org/10.6084/m9.figshare.c.4356239)49. The genome assemblies are also available at the NCBI Assembly website50,51.

Technical Validation
To produce high-quality draft genome assemblies, we applied whole-genome sequencing, assembly, and annotation of the two bay scallop subspecies. The whole genome shotgun sequencing strategy was used for both bay scallop subspecies. We constructed six sequencing libraries including two short-insert libraries (250 bp and 450 bp) and four long-insert libraries (2, 5, 10, and 20 kb) for A. i. irradians and A. i. concentricus, respectively. For A. i.
irradians, a total of 253.17 gigabases (Gb) of raw reads were generated while a total of 272.97 Gb of raw reads were generated for _A. i. concentricus_. For _A. i. irradians_, a total of $3.86 \times 10^{10}$ k-mers with a peak k-mer depth of 38 were employed to obtain an estimated genome size of 996.07 Mb (Table 1). In _A. i. concentricus_, a total of $4.97 \times 10^{10}$ k-mers and a peak k-mer depth of 50 were employed to obtain the estimated genome size of 974.3 Mb. Finally, draft genomes of 835.7 Mb and 874.82 Mb were assembled for _A. i. irradians_ and _A. i. concentricus_, respectively (Table 1), which accounted for 83.9% and 89.79% of their corresponding estimated genome size (Table 1). For the genome assembly of _A. i. irradians_, the contig N50 was 78.54 kb and the scaffold N50 was 1.53 Mb; meanwhile, the contig and scaffold N50s of the _A. i. concentricus_ genome assembly were 63.73 kb and 1.25 Mb, respectively (Table 1). 99.46% of all short reads could be mapped onto the assembled genome of _A. i. irradians_ with a coverage of 90.46%. Similarly, in _A. i. concentricus_, 99.4% of all short reads could be mapped onto the assembled genome.
with a coverage of 86.41%. These mapping results suggest good reliability for both genome assemblies, which are close to the assembly of the Peruvian scallop genome in our previous study but better than those of other related bivalve species12.

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. Our evaluation results demonstrated that both genome assemblies covered 231 (93.15%) of the 248 Core Eukaryotic Gene sequences, indicating a high level of completeness within the two genome assemblies. A related assessment identified 91% of the 843 single-copy genes in both genome assemblies. These data indicate the high integrity of both genome assemblies. A total of 389,681,429 and 412,788,948 bp of repeat sequences were predicted in the A. i. irradians and the A. i. concentricus genomes, respectively. These repeat sequences accounted for 46.43% and 47.17% of the corresponding genome assemblies (Table 2). A total of 26,777 protein-coding genes were predicted in A. i. irradians with an average transcript length of 11.86 kb. The public functional databases Swissprot, interpro and NR were used for gene prediction and annotation. Similarly, a total of 25,979 protein-coding genes were predicted in A. i. concentricus with an average transcript length of 12.17 kb (Table 1). In total, 24,943 (93.2%) and 24,428 (94%) predicted proteins could be functionally annotated in A. i. irradians and A. i. concentricus, respectively, using public databases. In total, the protein-coding genes were classified into 48,052 gene families and 107 strict single-copy orthologs (Fig. 2a). Compared to other examined species, 1,949 and 1,769 gene families were exclusively presented in A. i. irradians and A. i. concentricus, respectively (Fig. 2b).

The evolutionary position and divergence time of A. i. irradians and A. i. concentricus were elucidated in this study. The results of the phylogenetic tree showed that outgroup clade containing H. sapiens and B. floridana located in the basal position of the whole tree with high confidence (bootstrap value = 100%). Meanwhile, we found that A. i. irradians and A. i. concentricus clustered together with the 100% bootstrap value (Fig. 3a), and then merged as a sister group to P. yessoensis, as it did in the divergence time tree (Fig. 3b). It shows that this clade has a close relationship with the other two clades containing C. gigas, P. fucata and M. philippinarum, B. platifrons. Besides, we estimated the divergence times of A. i. irradians and A. i. concentricus using single-copy protein-coding genes from the 19 examined species (Fig. 3b). The result showed that the divergence time between the Northern subspecies (A. i. irradians) and the Southern subspecies (A. i. concentricus) happened at ~26.4 Mya ago, and the analysis suggested that the ancestor of A. i. irradians, A. i. concentricus and P. yessoensis originated ~85.9 Mya.

**Code availability**

In the study, we did not use any custom specific code. The command line for each step is executed as indicated for each step of all bioinformatics procedures.

Received: 15 April 2019; Accepted: 3 March 2020; Published online: 23 March 2020

**References**

1. Zhang, F., He, Y. & Yang, H. Introduction engineering of bay scallop and its comprehensive effects. *Engineering Science*. 2, 30–35 (2000).
2. Blake, N. J. & Shumway, S. E. Chapter 17 Bay scallop and calico scallop fisheries, culture and enhancement in eastern North America. *Developments in Aquaculture & Fisheries Science*. 35, 945–964 (2006).
3. Liu, X. et al. Morphology and genetic differentiation in offsprings of four transplanted bay scallop populations. *Oceanologia et Limnologia Sinica*. 37, 61–68 (2006).
4. Zhang, H., Liu, X., Zhang, G. & Wang, C. Growth and survival of reciprocal crosses between two bay scallops, Argopecten irradians Say and A. i. irradians lamarmor. *Aquaculture*. 272, 588–593 (2007).
5. Wang, C. et al. Introduction of the Peruvian scallop and its hybridization with the bay scallop in China. *Aquaculture*. 310, 380–387 (2011).
6. Wang, C. et al. Selection of a new scallop strain, the Bohai Red, from the hybrid between the bay scallop and Peruvian scallop. *Aquaculture*. 479, 250–255 (2017).
7. Xu, D. et al. Selection of a new scallop strain, QN-2, from the backcross of Peruvian scallop × bay scallop F1 hybrids with bay scallops. *Aquaculture Research*. 50(12), 3692–3699 (2019).
8. Zhang, G. et al. The oyster genome reveals stress adaptation and complexity of shell formation. *Nature*. 490, 49–54 (2012).
9. Wang, S. et al. Scallop genome provides insights into evolution of bilaterian karyotype and development. *Nat. Ecol. Evol*. 1, 120 (2017).
10. Sun, J. et al. Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. *Nat. Ecol. Evol*. 1, 121 (2017).
11. Takeuchi, T. et al. Draft genome of the pearl oyster *Pinctada fucata*: a platform for understanding bivalve biology. *DNA Res*. 19, 117–130 (2012).
12. Li, C. et al. Draft genome of the Peruvian scallop *Argopecten purpuratus*. *Gigascience*. 7, https://doi.org/10.1093/gigascience/giy031 (2018).
13. Marçais, G. & Kingsford, C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics*. 27, 764–770 (2011).
14. Kajitani, R. et al. Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. *Genome Research*. 24, 1384–1395 (2014).
15. Luo, R. et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience*. 1, 18 (2012).
16. English, A. C. et al. Mind the gap: upgrading genomes with Pacific Biosciences RS long-read sequencing technology. *Plos ONE*. 7, e47768 (2012).
17. Walker, B. I. et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *Plos One*. 9, e112963 (2014).
18. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 25, 1754–1760 (2009).
19. Li, H. et al. The sequence alignment/map format and SAMtools. *Bioinformatics*. 25, 2078–2079 (2009).
20. Parra, G., Bradnam, K. & Korf, I. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. *Bioinformatics*. 23, 1061–1067 (2007).
Acknowledgements
This work was supported by the National Natural Science Foundation of China (No. 31572618 and 31972791 granted to C. Wang and No. 41676152 granted to X. Liu), and the Earmarked Fund for Agriculture Seed Improvement Project of Shandong Province (No. 2017LZGC009 granted to C. Wang and X. Liu) and Earmarked Fund for Shandong Modern Agro-Industry Technology Research System (No. SDAIT-14 granted to C. Wang). The original brood stocks of A. i. concentricus were provided by Zhigang Liu.

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

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to C.W.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
