Reviewer Report

Title: The Gene-Rich Genome of the Scallop Pecten maximus

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Reviewer Comments to Author:

The authors presented a high-quality scallop genome of Pecten maximus. Using PacBio long reads followed by scaffolding with 10x Chromium and Hi-C, they generated the genome assembly of the chromosomal level. After gene annotation, the authors analyzed the Hox gene cluster and neurotoxins. The sequencing method is state-of-the-art, and the manuscript is well presented. I have comments mostly on their genome assembly and gene annotation methods as follows.

Major comments:

- 1. There are 67,741 gene models (even after filtering) found in the P. maximus genome. This number is very high among animals. I noticed that the authors performed gene prediction based on a non-masked genome. Would it introduce prediction errors? To my knowledge, people usually predict genes using a masked genome. That is to avoid the misprediction of genes from repetitive elements. From my experience, using the gene prediction program, Augustus, with UTR setting is not very good for non-model species. I am concerning that gene annotation with UTR prediction might be troublesome. It is particularly the case when the authors got 215,598 putative genes.
- 2. Following the first comment, how could the authors make sure that they have a haplotype genome assembly using the long-read approach? Is there a step that the authors can assure that two highly variable allele scaffolds can be collapsed into one? This possible redundancy is a particular concern when the species has high heterozygosity. Is it possible that 67,741 gene models predicted in the P. maximus genome is due to having a redundant diploid genome?
- 3. Assembly Assessment: What is the primary reason that P. maximus is much larger than Crassostrea gigas and Lottia gigantea? If that is not due to the repeats, what about the intergenic region or intron size among these species?
- 4. For those scaffolds with blast similarity to Proteobacteria, do all the genes on those scaffolds have blast hits to Proteobacteria genes? Panel C in Figure 2 is difficult to see, especially for the color code. Maybe consider to zoom-in a bit and adjust data visualization (e.g., circle size). I definitely can see that some circles have high GC (>0.4) and coverage (>100). Are those possible contamination (their colors are not easily visible)?
- 5. Could the authors explain why P. maximus has 518 species-specific orthogroups? This number seems to be unreasonably high compared to those in other molluscs. Similar concern for the unassigned genes (158,024 genes in P. maximus compared to 2,000-7,000 in other species).
- 6. Did the authors perform any test to assess whether P. maximus has the whole-genome duplication (WGD)? Only one example of the Hox gene cluster is not convincing to exclude the possibility of WGD. Minor comments:
- 1. There are some small typos and format issues. But without line numbers labeled, it is difficult to

point them out. The authors should add line numbers for the revised version.

- 2. Repeat elements -> "Repetitive elements" for consistency.
- 3. c.f. -> "cf."

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