

Reviewer Report

Title: Comparative genomics and transcriptomics of four Paragonimus species provide insights into lung fluke parasitism and pathogenesis

Version: Revision 1 **Date: 4/21/2020**

Reviewer name: James Wasmuth

Reviewer Comments to Author:

I thank the authors for the detailed response to my concerns.

Regarding the RNA-Seq data, I appreciate that these samples are precious. However, that does not compensate for the lack biological replicates. If insufficient material cannot be obtained than the experiment should be considered. I am unable to support the strategy to consider the pleural and two peritoneal samples as replicates for the "cavities", nor lung and liver to be considered replicates for "tissues". Figure S4 doesn't provide strong support for this division; the pearson correlation values are nearly identical for peritoneal B vs peritoneal A (0.93), as peritoneal A vs lung (0.92). Even if one were to ignore this, there remains the problem that "tissues" has only two replicates. Further, single samples are used to make claims as to expression, for example in line 302 and table 4. My resolute stance on the shortcomings of this analysis, is because the work presented will be cited by others with confidence and may lead to a snow-balling of over-interpretation that can have significant and negative impact on research into these important parasites. Due to the problems I list, I recommend that the expression section is removed from the analysis.

Regarding the measures of completeness, I thank the authors for providing more details. I agree with their use of the eukaryotic set of conserved genes in BUSCO. I disagree with the claim that "fragmented" genes "may or may not be considered complete." I challenge the authors to provide published examples of this. The paper on the Heterohabditis genome does also use the eukaryote set, but does not claim fragmented genes are complete. The authors, in their response, offer *S. mansoni*'s completeness of 73.8% as a comparison. In Wormbase-Parasite, all of the Schistosoma species have relative low scores on both CEGMA and BUSCO. It has been hypothesised that the blood flukes have lost a suite of genes previously thought to be highly conserved. Perhaps more impressive for the presented Paragonimus assemblies is the low proportion of duplicated complete genes. This suggests a low level of mis-assembly due to heterozygosity. I strongly encourage the authors to remove the fragmented genes from this "overall completeness" score in Table 1 and throughout the text.

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