

## Reviewer Report

**Title: Genome of the ramshorn snail *Biomphalaria straminea* - an obligate intermediate host of schistosomiasis**

**Version: Original Submission**    **Date: 9/5/2021**

**Reviewer name: Coen Adema**

### Reviewer Comments to Author:

This characterization of the genome of *Biomphalaria straminea*, invasive from South America to Hong Kong and main land China, provides a valuable contribution to interpret biology of freshwater snail species of the genus *Biomphalaria* that vector the infectious disease human schistosomiasis. Caused by trematode parasites of the genus *Schistosoma*, the impact of schistosomiasis on global human health is second only to malaria. The data complement the previously reported *Biomphalaria glabrata* genome and is highly likely to drive extensive comparative genome analyses to inform disease vector biology and biology of molluscs in general.

The study performs logical analyses of the genome involving repetitive content, Hox gene complement and organization, synteny with available molluscan assemblies, gene expansions, presence of genes of metabolic/hormonal pathways. The latter includes an experimental work component.

Although several sequencing approaches are applied, the work did not yield a chromosome level assembly. The authors should discuss why not, and identify likely challenges to chromosome-level assembly for this snail species (repetitive content/genome size?). The reviewer does not perceive this as weakness, rather an indication of complexity of gastropod (molluscan?) genomes.

Also, comparison with other molluscan genomes is valuable but the presentation of the results is less effective because it does not clarify the taxonomic relations of the molluscs included in the study. It is recommended to provide a figure to clarify the taxonomy of Cephalopoda, Bivalvia and Gastropoda in this study. Within Gastropoda, also identify prosobranchs, heterobranchs, eupulmonates and Hygrophila.

Consistent use of deuterostomes, exdysozoan plus lophotrochozoan protostomes and prebilateria would improve clarity.

Finally, the description of the relevant parasitology is incorrect in places, see specific comments for corrections.

Overall, the methods appropriate to the aims of the study, additional details are needed to describe the experimental work with live snails.

The conclusions are adequately supported by the data, although the use of a single stimulus for gene expression of ecdysteroid pathway genes does not seem to equate systemic analysis, also considering the lack of DE.

Quality of language is generally acceptable, specific comments follow below for editing of language in the manuscript.

No specific methods section is provided but this reviewer is not concerned.

specific comments

P1 Title, intermediate vector is incorrect, change to (obligate) intermediate host or vector

P2 Abstract

L6 B. stramina IS native to South America

L7 Reservoir is a parasitological term used for alternative host species, other than the main hosts for a parasite. It is not appropriate for the snail host. Suggest replace "reservoir" by "vector host"

L9 suggest edit hinders to limits

L15-16, indicate range of "other molluscs", does it include the classes gastropoda, bivalvia and cephalopoda?

L18, again specify certain mollusc lineages

L21 suggest edit to biology related to snail vectors of schistosomiasis

P3

L2 240 million are actually infected, these would require treatment, not preventive treatment

L9-10 these eggs do NOT circulate to the liver and leave the body. Eggs are deposited in blood venules and cross the intestinal wall to leave the body with faeces. Eggs that fail to do so are called reflux eggs and these circulate to the liver where they cause pathology. Please correct.

L10 delete contaminated

L12 delete further

L14-15 edit infected by to potential vectors for

L17-18, For native range also cite "DeJong RJ, et al. Evolutionary relationships and biogeography of Biomphalaria (Gastropoda: Planorbidae) with implications regarding its role as host of the human bloodfluke, Schistosoma mansoni. Mol Biol Evol. 2001 Dec;18(12):2225-39. doi: 10.1093/oxfordjournals.molbev.a003769. PMID: 11719572."

P4

L4--- Indicate whether parasite was introduced by infected snails, humans or other reservoir hosts.

P5

L3 Unclear with methods(P19,L13-18) indicate how many snails were used in total, only 1 or more because of using 1 adult snail for each of the sequencing methods. Also indicate source of snails; field isolate (date, latitude longitude of collection), wild type or lab isolate (inbred, outbred, kept for how many generations?).

L5 is the complete mitogenome included as one of the scaffolds

P6

L3, here and in figure, explain "spike in activity", is this a large number of sequences with only few lethal mutations, how is this shown in figure 1C?

P7

L1 "genes coopted to novel structures", explain, are these genes coding for proteins with novel structures or proteins with novel functions?

L5 "another oyster" provide Genus and species

L6, indicate that Mollusca belong to lophotrochozoa, a main lineage of protostomes along with ecdysozoa and indicate that the vertebrates belong to deuterostomia.

L10, and elsewhere (e.g. P9, L3-6) , define clustered and linked, what is the difference?

L12 identify L. gigantea as gastropod

L13 indicate level of ancestor (gastropod, molluscan or lophotrochozoan...)

P10

L19 indicate classes for limpet and oyster (Gastropoda and Bivalvia, respectively)P11

L14 define widely found, perhaps as broadly distributed?

P14

L13 suggest include "or assemblies of additional Biomphalaria species"

L14 what are potential impacts?

L16 "good markers", to what extent?

L18-21. This interesting comparative genome analyses may deserve a figure?

L22-23 define "mollusc genomes", perhaps "as available from GenBank for gastropod, bivalve and cephalopod molluscs.

P15

L1-2 does the number of pseudochromosomes resemble the number of chromosomes shown by karyotyping *B. glabrata* (see e.g Adema et al., 2017)

L2 edit genus to genera, and perhaps "of other classes" (see previous comment)

L3-4. cite studies by Campbell and DeJong, mentioned above.

L8 identify *Achatina* as eupulmonata gastropod, also this species of stylommatophora (landsnail) was proposed to have undergone whole genome duplication, does this complicate the synteny comparison?

L11 indicate that this would be an ancestor of the Hygrophila and Eupulmonata

L12-14, for accuracy, text actually lists molluscs with greater phylogenetic distance (prosobranch gastropods and Bivalvia), revise accordingly.

L16-17 provide citation for divergence and ancestor?

P16

L7-10. Suggest to remove fig 3C, the text adequately conveys the lack of altered expression. Indicate what other organisms do respond to the stimulus employed, suggesting differences in biology?

L19 define high memory scores

P17

L7, Is this pattern similar or both *Biomphalaria* species?

L11 suggest edit "in certain" to "among"

L16 "its adaptation" to what?

L17 (also P18, L3-4) Are these HSPs (types and numbers) in accordance with the *B. glabrata* genome, Adema et al 2017?

P18

L15 suggest edit "can change" to "changed"

P19,

L3 suggest edit "holding" to "having"

L4 "dynamics" is not clear, revise to other word?

L6 edit to ..... *glabrata* as well as among other molluscan taxa. Or similar

P23

L1-2 How were the initial sequences used for the searches selected?

L14-17, add identifiers for the species used as deuterostomes, and ecdysozoan or lophotrochozoan protostomes, also indicate oyster is bivalvia, limpet is gastropod.

P25

L9 define adult by shell diameter size, or confirmed by reproductive maturity. How was culture initiated? was this from a multiple individual collected from the field or from a single snail by selfing? For how long was the culture maintained at time of experiments?

P26

provide primers used for the gene targets. Was target specificity tested? Myoglobin may have sequence similarity with the multi domain sequences of Biomphalaria hemoglobin.

Figure 4

identify in figure branches of heterobranchs, prosobranchs, gastropods, bivalves and cephalopods. (similar to fig 5b)

Again can the number of (pseudo)chromosome clusters be equated with chromosome numbers recorded for Biomphalaria sp?

Fig 7B indicate branches for lophotrochozoan protostomes, ecdysozoan protostomes and deuterostomes

## **Methods**

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

## **Conclusions**

Are the conclusions adequately supported by the data shown? Choose an item.

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