

Reviewer Report

Title: Chromosome-level assembly of the mustache toad genome using third-generation DNA sequencing and Hi-C analysis

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Reviewer Comments to Author:

Here, Yongxin Li and colleagues reported the chromosome-level genome with the full annotation of the mustache toad, *Vibrissaphora ailaonica*, using conventional paired-end short read, sufficient amount of PacBio long reads and chromosome conformation capture (Hi-C) data. Although there are several amphibian genomes reported previously, many of them do not have chromosome-level genomes, so I think this is definitely a valuable resource to the community, especially to study the synteny of amphibian genome. So I would like to recommend accepting this manuscript for publication after resolving some issues as mentioned below:

- On page 5, more details for RNA-Seq library prep construction method should be provided (poly-A capturing or ribosome-depletion? Which library kit do they use?). Also, even the authors mentioned that 9 tissues were dissected from the biospecimen they sequenced the genome (page 4, line 85), it is not clear whether all those tissues were used in this 'mixed RNA-Seq' experiment. Please provide more details for this experiment.
- On page 5, the authors mentioned that they used four Hi-C libraries. Are they constructed from the same samples (blood), with the same parameter (four technical replicates)? Or using different samples? If they used a different parameter to construct these four libraries, it should be specified.
- Authors claimed that they deposited the data on PRJNA523649, but it looks like they uploaded one single file for each data set. Because they used different libraries, at least for paired-end seq (Table S1) and HiC-seq (Table S4), it would be better to provide those raw data separately.

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