Reviewer Report

Title: **Draft genome assembly of the invasive cane toad, Rhinella marina**

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Reviewer name: **Taejoon Kwon**

Reviewer Comments to Author:

Here Edward et al., reported the draft genome of cane toad Rhinella marina. Although there are several genomes recently published, still amphibian is highly understudied in genomics, so I think this data would be valuable resource to expand our knowledge in many perspectives, including the evolutionary history of this species' invasion in new environment. Authors analyzed their data thoughtfully, and released all data in public repository, so I think this manuscript is satisfactory for GigaScience as a Data Note. However, there are some points required to be clarify before publication (see below), so I think authors should revise the following points before publication:(1) Although BUCSO analysis can be used for genome completeness, it is based on protein coding genes, so I think 'Assessment of genome completeness' would be better to be merged with 'Genome annotation and gene prediction' section. (2) In previous publication with R. marina transcriptome (Richardson, et al., GigaScience, 2018; doi: 10.1093/gigascience/gix114; Ref #18 on current manuscript), it was reported that 1.7% of BUCSO genes were fragmented, and 7.4% of them were missing on their 62,202 CDS transcripts. These numbers look better than genome-based result described in this manuscript (7.5% of fragmented, and 9.5% of missing). Authors may need to discuss the difference among these two annotations.(3) The analysis of 'unknown function' genes with published de novo transcriptome (p.9 line 229-) seems to have a circularity. Authors used all RNA-seq data already on their annotation, which are also used for de novo transcriptome construction (p.9 line 206). So instead of analyzing their matched length, I recommend to analyze their expression level from RNA-seq data. If 'unknown function' genes were mis-annotated genes as authors thought, it should have lower level of evidence for expression, compared to 'known function' genes. Some minor points:(1) '3 s.f' (significant figure) notation on table headers make the reader confused. It is obvious to recognize by looking at numbers on table, so it would be better to remove it. (2) In Table 4, qPCR value is also the average of two experiments (p.8, line 190-191), so it would be fair to present min/max values for that.

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