

We are sincerely thankful to you for taking the time to review this manuscript, you have provided us with a fair, constructive and generous review. Your comments and remarks have led to an improved manuscript. We know that reviewer's work is crucial to the success of a scientific publication, and we have a profound respect for your work and that of all our colleagues involved, including readers.

Round #2

by Wirulda Pootakham, 2021-02-27 09:24

Manuscript: <https://doi.org/10.1101/2020.05.25.110734> version version 2

Minor revision required

Dear Authors,

Thank you for revising the manuscript. Both reviewers are happy with the revision. I've attached their comments below. Once these comments are addressed, I would be happy to recommend this preprint.

Thank you very much.

Regards, WP

Reviews

Reviewed by anonymous reviewer, 2021-02-11 09:50

I would like to thank the authors who made a great deal of effort to address the reviewers' points, including performing re-analyses of the reads to obtain newly improved assemblies that seem much better and robust for functional analyses. I'd also like to acknowledge the efforts made by the authors to design highly informative figures, and to provide the code for their statistical analyses. The entire manuscript greatly improved and provides more clarity on the methods used, the results obtained and their limitations. The paper reads well and is well-focused even though many aspects were explored in this study. I'm sure this study will stand as a reference on *Xenopus* and amphibians' microbiota. I have no further comments and recommend this paper for publication.

Answer : We expressed our thanks in the acknowledgements as an honest sign of respect for your work as a reviewer.

Here are only a few suggestions of modifications:

L. 692, 701: Since you have all the details, maybe could you provide info in Mat&Meth about the amount of DNA and RNA material obtained before sequencing/amplification?

Answer : Thank you for this question. We added this information in the materials and methods.

Line 695: We obtained 10-15 μg of total RNA and $\sim 1 \mu\text{g}$ of DNA per filtered tadpole gut.

Fig S7_C => please provide in legend the meaning for the abbreviations "TGA" and "SL", or mention they are the strains' names?

Answer : Thank you for this remark. We added the information as follows :

Abbreviations SL and TGA refer to *X. tropicalis* strain's names: SL for Sierra Leone strain and TGA for a laboratory population of *Adiopodoume* strain (Ivory Coast) outbred to *Uyere* strain (Nigeria).

There were still some typos, the manuscript should be scrutinized for these.

Answer : We edited the text to remove typographic and other errors that were left.

Reviewed by Vanessa Marcelino, 2021-01-28 05:23

The manuscript has markedly improved. My previous concerns with confounder effects has been clarified, the current conclusions and discussion are coherent and the paper reads much better.

It is unfortunate to receive a passive-aggressive answer to my previous comment on the manuscript's sentence "used other high memory usage software". Submitting a paper with incomplete reference to the methods, and then mocking the reviewer's comments about it, shows lack of respect for reviewers' time – who are trying to help improve this manuscript.

Answer : Communication in a non-native language is difficult (Berenbaum, M.R. PNAS 2020 ; 117 :4-6 <https://www.pnas.org/cgi/doi/10.1073/pnas.1920932117>). We expressed our thanks in the acknowledgements as an honest sign of respect for your work as a reviewer. We hope very much that you have been pleased by your impact, rather than by what seems to be a misappraisal of one of your sentence.

One minor comment: L403: "Metagenomic and metatranscriptomic sequencing gave similar taxonomic profiles for bacteria in concordance with the 16S rRNA gene metabarcoding approach, while giving a finer taxonomic resolution (Figure 7A)." The figure however does not show a comparison with the 16S rDNA data, it also does not show a better taxonomic resolution (reported at phylum level). If a formal comparison

between methods has been done, please indicate where to find the results. Otherwise I suggest deleting "in concordance with the ..." Please also indicate (e.g. in the legend) whether the taxonomic profiles in Figure 7A were based on reads or MAGs.

Answer : Thank you for raising this point. We followed your suggestion and deleted this part of the sentence since we do not dare performing a formal comparison between results derived from 16s rRNA targeted sequencing and shotgun sequencing. The results of comparing 16s rRNA targeted sequencing using different primer pairs shows that it is already a complex endeavour (Abellan-Schenyder et al., mSphere Feb 2021, 6 (1) e01202-20; DOI: 10.1128/mSphere.01202-20). We spoke about a finer taxonomic resolution because we assembled full-length 16S rDNA gene.

The sentence now reads (line 405):

Metagenomic and metatranscriptomic sequencing gave similar taxonomic profiles for bacteria according to reads matching 16s rRNA gene sequences (Figure 7A).