



Peer Community In Genomics

A comprehensive look at *Xenopus* gut microbiota: effects of feed, developmental stages and parental transmission

Wirulda Pootakham based on peer reviews by **Vanessa Marcelino** and 1 anonymous reviewer

Thibault Scalvenzi, Isabelle Clavereau, Mickael Bourge, Nicolas Pollet (2021) Gut microbial ecology of *Xenopus* tadpoles across life stages. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Genomics.
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It is well established that the gut microbiota play an important role in the overall health of their hosts (Jandhyala et al. 2015). To date, there are still a limited number of studies on the complex microbial communities inhabiting vertebrate digestive systems, especially the ones that also explored the functional diversity of the microbial community (Bletz et al. 2016).

This preprint by Scalvenzi et al. (2021) reports a comprehensive study on the phylogenetic and metabolic profiles of the *Xenopus* gut microbiota. The author describes significant changes in the gut microbiome communities at different developmental stages and demonstrates different microbial community composition across organs. In addition, the study also investigates the impact of diet on the *Xenopus* tadpole gut microbiome communities as well as how the bacterial communities are transmitted from parents to the next generation.

This is one of the first studies that addresses the interactions between gut bacteria and tadpoles during the development. The authors observe the dynamics of gut microbiome communities during tadpole growth and metamorphosis. They also explore host-gut microbial community metabolic interactions and demonstrate the capacity of the microbiome to complement the metabolic pathways of the *Xenopus* genome. Although this study is limited by the use of *Xenopus* tadpoles in a laboratory, which are probably different from those in nature, I believe it still provides important and valuable information for the research community working on vertebrate's microbiota and their interaction with the host.

References:

Bletz et al. (2016). Amphibian gut microbiota shifts differentially in community structure but converges on habitat-specific predicted functions. Nature Communications, 7(1), 1-12. doi: <https://doi.org/10.1038/ncomms13699>

Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Reddy, D. N. (2015). Role of the normal gut microbiota. World journal of gastroenterology: WJG, 21(29), 8787. doi: <https://dx.doi.org/10.3748%2Fwjg.v21.i29.8787>

Scalvenzi, T., Clavereau, I., Bourge, M. & Pollet, N. (2021) Gut microbial ecology of Xenopus tadpoles across life stages. bioRxiv, 2020.05.25.110734, ver. 4 peer-reviewed and recommended by Peer community in Geonmics. <https://doi.org/10.1101/2020.05.25.110734>

Reviews

Evaluation round #2

DOI or URL of the preprint: <https://doi.org/10.1101/2020.05.25.110734>

Version of the preprint: version 2

Authors' reply, 04 March 2021

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Decision by **Wirulda Pootakham**, posted 27 February 2021

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

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Reviewer #1: 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?

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

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

Comments from Reviewer #2: 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.

Reviewed by anonymous reviewer 1, 11 February 2021

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.

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?

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

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

Reviewed by Vanessa Marcelino, 28 January 2021

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.

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.

Evaluation round #1

DOI or URL of the preprint: <https://www.biorxiv.org/content/10.1101/2020.05.25.110734v1>

Authors' reply, 08 January 2021

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Decision by Wirulda Pootakham, posted 09 August 2020

Please revise the manuscript

This study provides an important resource to the field. The authors did a thorough study on the vertebrate microbiota; however, there are issues raised by reviewers that need to be addressed before I can recommend this preprint. I would like to ask the authors to carefully go through each reviewer's comments and try to address them.

Reviewed by anonymous reviewer 1, 15 July 2020

Scalvenzi and colleagues provide a comprehensive analysis of the *Xenopus*' microbiota along its distinct developmental stages. Important questions such as the variation of the bacterial community composition along these stages, along the gut, but also the impact of diet on *Xenopus*' microbiota, and the source of transmission of the community were investigated. An interesting parallel is made between mammals and *Xenopus* microbiota, with some major lineages and functions being conserved. I believe that this thorough piece of work could prove important for the communities working on vertebrate's microbiota, on *Xenopus* evo-devo, or host-microbiota associations, and establish a reference for future investigations.

The article reads generally well, and conclusions drawn seem well-supported by the data.

Just a small point on my reviewer's experience: I have to admit that it was quite hard to find figures (some were duplicated in the main text PDF file – provoking some confusion at 1st sight), sup figures, and sup tables. Figures and tables are not all numbered in the combined PDF files, and there seems to be some issues with sup figures numbering (see below). Putting the legend next to each figure - and a title by each table would have been a great help to ease up the reading. Please also note for next submission that all sup files could have been submitted independently to bioRxiv.

Here follow specific points of discussion:

1) I was a bit surprised that archaea were not mentioned at any point of the study. They are increasingly recognized as being part of mammals microbiota (humans, apes, cows...)- yet in relatively little abundance (see for instance Koskinen 2017, mBio). As a parallel is drawn between mammal/vertebrate microbiota and that of *Xenopus*, I was wondering whether they had popped out at some point in the metagenomic/metatranscriptomic analyses. Unfortunately, it has been shown that universal primers often used for 16S amplification miss a huge portion of archaeal diversity and that others should be used (see Raymann 2017, mSphere), but metagenomic/metatranscriptomic study could have revealed them.

2) lines 121-124: “we analyzed the distribution of bacterial populations by relative size (forward scatter) and nucleic acid fluorescence, we found that the samples from young tadpoles differed markedly from those of mature tadpoles in their cytometric profiles (Figure 1A).” I'm not much familiar with flow cytometry techniques... but could these results translate into distributions by cell size on e.g. barplots? To me, this seems easier to compare and grasp differences between distributions than by visualizing the type of graph on Fig 1A. I don't find evident the differences in communities in terms of cell size between the earlier tadpole stage and the others, except that the number of cells is much lower for the former. Could for instance “population A” of the earlier stage correspond to “population C” at the NF56 stage? Are the differences between distributions significant? What statistical test was performed?

3) A related question: maybe I missed those, but on which grounds were defined the “clusters”/ “bacterial populations” based of flow cytometry results presented in Fig 1A? I don't see any details provided either in Methods or in Sup Methods.

4) Fig. 1A: what are the cells at around 10^2 forward scattered, and 10^1 PI? Do they correspond to dead cells? Otherwise, couldn't they form another population than the “A” one?

5) Line 122: please clarify for non FACS specialists, that you are talking about cell sizes.

6) I think the results on Fig S3C could call for a small comment on inter-individual variations at the same developmental stage, given than samples are then pooled for analyses as shown on e.g. Fig. 2A.

7) If I may, I have some suggestions for Figure 2BCD: maybe could different colours distinguish between developmental stages? Also, for homogeneity-sake, could the same terms always designate the same developmental stages? “Premet” instead of “tadpole” for instance, on all panels? Maybe the legend could display the developmental stages' in the “right order” to help non-specialists follow?

8) Line 206 - ... : it is not alpha diversity that is displayed on Fig 2B, but a measure of phylogenetic diversity (Faith' PD index). It could be appropriate to precise which metrics exactly were used when mentioning the results, and upon 1st appearance, slip a few words about the exact meaning of each metrics.

9) Line 211: which Sup Table? Aren't they all numbered? Line 238, 255, 263,...: same comment

10) Please explain what are exactly the metrics used for “community structure” and “community membership” on Fig. 3? Why are the distance types used different between Fig 2C and Fig 3B?

11) Line 256: “Feces and skin microbiomes were clearly separated from the other samples, but not between them.” Maybe would be worth mentioning that these are also the ones that vary the most among a same organ?

12) Line 329, 331, 412: “SES MPD”, “SES MNTD”, “RPK”, please explain acronym upon 1st appearance.

13) Fig. S8B: What is exactly the metrics used? what is measured? “w”.

14) How much gDNA and RNA material was used for prepping the metagenomic/metatranscriptomic sequencing libraries?

15) Programs used to assemble and map the reads of the metagenomic analyses could be briefly mentioned along Results. Line 554: the general method to assess the source of microbial communities is only mentioned in Discussion – this could have been introduced earlier. In a general manner, methods used could be briefly listed along main text so that the reader understands where the results come from.

16) Please check sup figures numbering: Figure S9 and S10 rather S7 and S8. Please also check that all sup figures are cited in main text – I am not sure this was the case.

17) The assembly statistics are not given in a very extensive manner, but given the median size of assembled contigs (~800bp), it seems quite sub-optimal. I was wondering what was the proportion of assembled contigs predicted as being part of a CDS? This could give an idea of the proportion of assembled reads that has been analysed.

18) Line 405: it is unclear to me what scaffolds were analysed: all assembled ones? or the 25 longest only? Please clarify (results for 10 are shown on fig S9-S10).

19) It is said line 403-404 that each of the 10 (or 25? unclear, see above) longest scaffolds “seems to be derived from a different genome”: what exactly goes in this direction? I don’t think this can be assessed from results shown on Fig S9 and S10 (GC% and reads coverage along scaffolds). Could it be possible to assign taxonomy to the longest scaffolds? It is said on line 681 that the BGI provided taxonomic affiliation for metagenomic data, is that correct?

20) Fig. S10: reads mapping is quite uneven for the scaffold at the bottom, with a shift towards lower coverages toward the right of the contig, making this scaffold look suspicious. Could the authors comment on that?

21) Line 672: “July 6th to 9th” from which year?

22) Line 676: the name is “Silva”.

23) Line 691: please check again sup fig numbers.

Reviewed by **Vanessa Marcelino**, 04 July 2020

The manuscript from Scalvenzi and colleagues reports a comprehensive study on the microbiome of *Xenopus*, an important model organism. The authors report significant changes in microbiome composition during developmental stages. The study also addressed the impacts of diet and parental transmission of microbes. The majority of the study was based on amplicon sequencing, with metagenomics and metatranscriptomics data from a subset of samples constituting a nice addition to the study. Overall, this thorough study provides an important resource to the field. There are however several issues that need to be addressed before I can recommend this paper for acceptance.

The potential effect of confounders and sample size is unclear There are multiple co-variates and it is important to be upfront about those. For example: the authors sequenced 16S amplicons from both RNA and DNA, why? Are the statistical results still significant when this is taken into account? The genetic material was preserved in ethanol for some samples, while fresh DNA/RNA was used for others. I would like to see evidence that this differential treatment does not affect the conclusions of the paper. Given that the Methods section is at the end of the manuscript, it is important to mention number of replicates for each experiment while describing the results.

The metagenomics and metatranscriptomics aspects were performed with only 5 samples, all from the same developmental stage, and therefore it does not contribute to “analyse the succession of microbial communities and their activities across different body habitats” as the abstract (and other parts of the manuscript) suggest. This small sample size is only mentioned in the methods section, at the end of the manuscript. The manuscript would benefit from being upfront about this limitation.

Gender bias: Line 63 reads “... these results were challenged by Eugène Wollmann and his wife, who finally observed...” Referring to a female scientist as just someone’s wife is sexist. Cite both first names or none. Also – I think the reference is Wollman and Wollman, 1915 (rather than Wollman 1913, but please check). Likewise, in line 503, the word “man” can be replaced by the more neutral word “humans”.

The manuscript needs **English proofreading**.

I also have questions regarding the interpretation of some of the results:

X. tropicalis microbiome transmission: The authors conclude that the skin of the parents is one of the main drivers of microbial transmission, but they did not sample an important source of microbes – the surrounding water. Considering that the adult frog skin microbiome is likely to be influenced by the surrounding water, it is possible that their conclusion about the parental transmission is circumstantial, and that the surrounding environment is the single most important contributor.

Xenopus gut microbial diversity during development and metamorphosis: L.148 – 149: How were these OTUs defined (which % similarity)? L 149- 150: The rarefaction curve is far from reaching a plateau. In fact, the overall sequencing depth is low. The authors should be upfront about this limitation.

Xenopus tropicalis gut microbiota genes catalog The conclusion that the *Xenopus* gut microbiome is similar to the human microbiome is most likely an artefact. The majority of known bacterial genes are from human-derived bacteria, therefore the results observed here are not surprising. I invite the authors to compare the available gut metagenomes of any other vertebrate (especially the ones raised in captivity). The conclusion that the *Xenopus* gut microbiome is similar to the human gut microbiome is only relevant if the authors can show that this is not the case for other vertebrates.

L 402-403: I don’t see how you can make the conclusion that the scaffolds come from different genomes (you mean organisms?) based on coverage.

Metabolic profile of the Xenopus gut microbiota: Were vertebrate and human-contaminant sequences removed from the analyses? This is essential to ensure that the observed metabolites were derived from the microbiome.

It is not clear to me how the metabolic map connecting the host and the microbiome pathways was performed, and the interpretation of these results is even less clear. The paper states that “Our data highlighted the important capacity of the microbiota to complement the metabolic pathways of the *Xenopus* genome” (L. 573 – 574). I think this an over statement. The data does not indicate if the metabolites produced by the microbiota can be used by the host (or vice versa). Supplementary figure S11 (the metabolic map) is really impressive, but not mentioned anywhere in the main text.

The discussion around bile acids is interesting, it seems highly speculative but the authors acknowledge that this is just one possible interpretation of the results.

Evolution This study does not provide insights in the ‘evolution of microbiomes’ as suggested by the title, and it also does not help to understand the “evolutionary associations between amphibians and their microbiota.” as stated in the conclusion. There is no evidence for an evolutionary association between host and microbes here. Perhaps succession is a better word.

Specific comments:

Ethics approval: Please mention the issuing institution of this ethics approval.

The R scripts (L. 678) are a plus, really nicely done. Please add the link in the “availability of data and material”.

Line 681 - 682: “We used the taxonomic assignment results provided by the BGI for the metagenomic analysis.”. Please provide more details, this is not reproducible.

The bias in microbiome composition that is caused by keeping animals in captivity is only superficially

addressed in the last paragraph of the discussion, without any qualitative or quantitative assessment. How does the microbiome composition observed here compares with the one observed in wild anurans? I suggest performing this comparison and adding this information in the results section.

Supplementary Materials:

Supplementary tables are hard to find and what I think is Sup table 1 seems to be incomplete (it does not show number of sequences obtained per sample as mentioned in L. 668).

Did you perform rRNA depletion during library preparation?

DNA extraction from animals fixed in ethanol - what was this used for? Metagenomics?

Metagenome assembly: the text states "We used the Genotoul hardware and software services (<http://bioinfo.genotoul.fr>) to perform the assembly and used other high memory usage software." Please specify which other software were used. Sentences like this make the whole manuscript sound like a coarse draft.

Figure S3 is also really good and make the whole study much clearer. I would try to reduce the text in the results section (which is rather long) and replace it with this figure.