Basic reporting

The manuscript is well written, with an interesting topic and a logical structure. It is focused on demonstrating the baseline of the gut microbial diversity and composition variation between mucosa and digesta samples in three small species of fish. This will provide insights into fish residential gut microbiota identification. The introduction is appropriate to support the context, and the results are well discussed. If more information is provided as described below, I recommend that the manuscript can be published in Peer J.

Experimental design

The research question is well defined, relevant, and meaningful. The experiment is well designed, but more detailed information in methods is needed. Genomic data analysis seems solid, but in the reviewer's opinion, the taxonomic analysis can go deeper to a further level, such as the genus level.

Validity of the findings

All underlying data have been provided. The last section in the discussion can provide insights to further studies, but a clear conclusion can be helpful for summarising the highlights.

General comments

Introduction

Line 31. Please consider removing the word 'time' to keep consistent.

Line 64-66. Please support your statement with relevant references.

Materials and methods

Line 78-81. More detailed information in terms of fish size is needed, such as fish length, weight, etc.

Line 92. If emptied intestines are not washed with saline buffer, how do you make sure there is no digesta left?

Line 152. '>=' is not the correct format. Please change the symbol to '⩾'.

Line 153-155. It stated that phylogenetic trees were generated. Please indicate the result and include it in the supplementary section at least. If this is irrelevant, you should consider removing this sentence from the manuscript.

Line 185-200. The indication of nMDS and PERMANOVA analyses belongs to the β diversity analysis. These sentences are more suitable for the last section 'Analysis of diversity.

Line 199-200. How was the resemblance matrix of the dataset created? For example, the resemblance could be created by calculating the Euclidean distance or Bray-Curtis distance.

Line 202. The fish gut microbiome has been studied in many studies via 16S rRNA sequencing in recent years. Taxonomic composition at the family level may not be powerful or informative enough to explain the variation in the intestinal microbial community. One wonder why you only analysed the microbiota at the family level.

Result

Line 256-257. An additional nMDS figure showing the overall microbial similarity between the two sample types is recommended here.

Line 257-259. How do you identify the variance regarding sample type and location? And where is the detailed information?

Line 264. Where is the 'Table 3'? I assume it is the attached Table 1.

Line 261 -266. This part is a bit confusing. In the discussion (Line 302), you stated the location explained most of the variance. However, the result indicated that the explained variance was lower compared to digesta samples.

Line 267-269. "Most of the families" is not precise. Please demonstrate the result in number or percentage.

Discussion

Line 285-286. The word 'unique' is not appropriate to be used here. From my understanding, unique families indicate the families that are either detected in mucus or digesta only. so, there is no comparative degree.

Line 287 and line 366. Subtitles are unnecessary in the discussion section.

Line 319. Remove the word 'unique'.

Line 323. Please clarify the 'Salmonids' species. *Mycoplasmataceae* are not found to be dominating the gut of freshwater farmed Chinook salmon.

Line 330-331. Please reword this sentence. Detecting these families in digesta and mucus samples does not mean it can be detected in the surrounding environment. It can only be confirmed by analysing the microbiome from the rearing water.

Confidential notes to the editor

None.