

## ***Interactive comment on “Protist communities in a marine oxygen minimum zone off Costa Rica by 454 pyrosequencing” by H. Jing et al.***

**Anonymous Referee #1**

Received and published: 3 September 2015

The manuscript by Jing et al focuses on the diversity of protist communities in the Costa Rica OMZ using pyrosequencing of 18S rRNA and rRNA genes. This work should be of interest to readers of biogeosciences because protists are important, yet understudied members of microbial ecosystems in OMZs. The authors present some interesting data, but the manuscript is very descriptive and the authors should do a better job of searching for the novel findings and re-focusing the manuscript on what the more interesting results mean. This could be achieved by a closer comparison to what has been previously reported for protists in other anoxic and low oxygen habitats. Furthermore, the authors should be a bit more specific about which specific groups they found and how their distributions compare to existing literature. For example, they authors state that they found ciliates but do not go into detail about which genera they found. Ciliates are highly diverse (estimated to contain ~4000 species) and so grouping at this

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level provides for only superficial analysis and discussion. Same applies for dinoflagellates and stramenopiles, for example did you find any MAST stramenopiles or MALV clades? If so which clades and how do they compare to what was found in other OMZs or anoxic waters? I recommend also for the manuscript to be carefully read and edited by an english native speaking colleague, because there are many grammatical and spelling mistakes. Specific comments are below.

Specific comments:

Title: The title as written is grammatically incorrect and should be re-written. Do you mean “Diversity of protist communities. . .” ?

Abstract: lines 14-16: As written these conclusions are very general and non-specific and do not focus on the novel aspects of the study (e.g., activity proxies with rRNA). Please re-write the conclusions to focus on the novel results and conclusions.

Page 13485, line 5; Page 13494, line 16, and Page 13500, line18: Correct authors name from “Oris” to “Orsi”.

Page 13485: line 23: Typo “picoeuaryotes”

Page 13486: line 14: Is this really the perfect place? Why do you say perfect? There are many OMZs with similar chemical stratification, and some (Arabian Sea) with even thicker OMZ core (1000 m).

Page 13487: lines 19-23: If you removed the RNA later then you through away a lot of the RNA that is lysed into solution from cells on the filters. This should be acknowledged, and some discussion of how this might have affected the authors results would be good.

Page 13487: lines 24-26: Please state manufacturer for DNase I and briefly the protocol followed.

Page 13488: line 1-5: Did you ever see amplification from DNA in these RNA controls?

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If so how did you proceed?

Page 13489: line 9: Please be more specific what is meant by cut off value of 60. What percent is this ?

13490: line 12: What do you mean by “remarkably higher” ? How much higher? You cite the table but would be good to state in the text for readers.

Page 13490: line 16: A lower protistan diversity in the anoxic water column was also found in the Cariaco Basin (Orsi et al 2011 ISME J).

Page 13490: line 18: What do these coverage values refer to? Is this percent of total sequences? Maybe it is better to report statistics for number of reads corresponding to each OTU?

Page 13490: lines 19-24: I am confused what this sentence is trying to communicate. Please clarify.

Page 13492: line 6: Did you find diatom rRNA in the OMZ core? If so that would be interesting to report. Edgcomb et al 2011 (ISME J) also found this in the Cariaco Basin and, while controversial, could indicate survival of diatoms in low oxygen waters without sunlight.

Page 13492: lines 15-18: Please also comment on whether you found any anaerobic ciliates, and if so which groups you found. For example, did you find any representatives of the Cariacotrichea (Orsi et al., 2011 IJSEM) ? This group has been proposed as an indicator species for low latitude OMZs (Orsi et al., 2012 ISME J) and thus it would be interesting to know if it is also found in the OMZ studied here, or whether it is more restricted to euxinic waters.

Page 13493: line 6: Define what you mean by “high numbers”.

Page 13493: line 8-10: Please specify which groups of ciliates you found (see above comment). This has important implications for the community structure and biogeog-

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raphy debates, so the discussion would benefit from this a lot.

Page 13493: lines 10-11: If you are referring to group I, II, III of Syndiniales please specify that.

Page 13494, line 9 and line 16: Actually the more correct reference for reduced protist diversity in low water column oxygen would be Orsi et al 2011 (ISME J), which validated the observed differences in richness with statistical estimates.

Page 13494: lines 17-20: To be more specific, I think is more likely due to the fact that the diversity of anaerobic protists is smaller compared to aerobic species (e.g. there are less species of anaerobes) . You could read the papers by Wilhelm Foissner as background. For this reason, if you look in a low oxygen environment, diversity will be lower simply because there are less species in existence.

Page 13495: lines 11-12: You should also read Orsi et al 2011 (ISME J) which found an abundance of hypoxic stramenopiles in the Cariaco Basin, further supporting this point. Edgcomb et al 2011 (ISME J) also found rRNA from stramenopiles in the anoxic waters, but these were from diatoms. The manuscript would benefit from a discussion of these studies as this is highly relevant.

Page 13495: lines 12-13: How or why do you think this happening?

Page 13495: lines 19-21: Not all Prorocentrales and Spirotrichea have a mutual symbiotic association with Rhizaria. Please specify which genera you actually found and whether these genera have been shown to live as symbionts.

Page 13495: line 28: You should also read Orsi et al 2013 (Frontiers in Extreme Microbiology), which found a prevalence of symbiotic relationships between ciliates and bacteria across a geographically broad range of oxygen depleted marine waters.

Page 13497: lines 24-29: This is an example of a redundant statement that appears multiple times throughout the manuscript. Please edit the text to remove redundancies.

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Page 13507: Figure 5: Can the authors comment on this apparent complete lack of a linear correlation between the rRNA and rDNA data?

Figure 6: What do the z scores represent in lay mans terms? The arrows point to two different heat blocks, so are the stacked histogram plots showing the average from both blocks? Can you break this up into separate stacked histograms for each block to look at differences between depths?

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