

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

Anonymous Referee #2

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General evaluation

This paper addresses the composition of microbial eukaryotes on a vertical profile in the Coasta Rica dome where there is a pronounced OMZ. The authors used 18S rRNA metabarcoding starting both from DNA and RNA. Unfortunately the 18S region they used is not the most resolutive (V4 or V9 would be more resolutive). The manuscript is failry descriptive but still provides interesting data. I do not suggest publication at this stage. The paper should be revised in depth and then resubmitted. The major change needed include a better taxonomic treatment. In particular one of the major eukaryotic group (Syndiniales) is never mentionned. I doubt very much it was not present and abundant. Synurophyceae is typically a freshwater group but found there. It could well be present but this will have to be demonstrated. These problems are probably

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an artefact from the data analysis. All OTUS should assigned to the genus level as much as possible. Also the authors seem not to master fully eukaryotic phylogeny as evidenced by the numerous typos in the taxa as well as the incoherence on some of the level used. Some of the taxonomic assertion are plainly wrong such as the fact that all dinoflagelaltes are photosynthetic (see below). The introduction needs some rewriting as its failry vague. I would focus much more on what is known about protists in low oxygen regions rather than be very general. In short, the authors should read much more what is known in the taxonomy and oceanographic litterature about the taxa they mention and then rewrite the paper.

Specific remarks

p. 13484 l. 25. I do not hink that the Bachy paper is he best reference to support this sentence. Instead he shows that diversity can be OVERestimated by molecular méthods. "Until last decade" does not sound right to me

p.13485 l. 5. Cite de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahe, F., Logares, R., Lara, E. et al. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science. 348:1261605.

l. 15. mixotrophic protists are not parasitic (I do not know of any parasitic protist that would be also photosynthetic and feeding on bacteria)

l. 21 "anoxic waters has caused significant changes"... does not sound right... "can cause" ?

p. 13487 l.9. Please provide a supplementary figure with the vertical profiles of all physical and oxygen parameters at the sammpled station.

p. 13488. l. 7. The region chosen for the metabarcoding is not the best choice for eukaryotes because it is much less variable than other regions such as the V4 and V9 used in almost all other studies.

p. 13489. l. 1 which 18S data sets ? Be more precise.

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- l. 8. Why assignation was done vs. Silva bacteria while we are dealing with eukaryotes ?
- l. 13. Which R package ?
- p. 13490, a lot of error in spellings. "Haptophyta", "Viridiplantae", "Dinophyceae". Please check all taxonomic names very carefully everywhere ! Also only genus and species have to be in italics all other rank names are in normal font.
- l. 3. "Phylum" (singular) and not "phylae"
- l. 25. Among the Dinophyceae you never mention the Syndiniales (what you call latter parasitic group I, II, III and which appears at the OTU level but not in the global assignation). This is often the most abundant group in eukaryotic metabarcodes sets. Please clarify this point.
- p. 13492. l. 5 It is interesting that Eustigmatophyceae have been found because they are rarely appear in clone libraries or metabarcoding
- l. 13. Please give a more precise assignation of the dominant OTUs possibly down to the genus level.
- l. 16. Correct "Eumas..."
- p. 13493 l. 7. Not all dinoflagelaltes are photosynthetic even within orders that are mostly photosynthetic. Your treatment of the dinos must be much more thorough because at present it is very vague.
- l. 11 The parasitic groups belong to order Syndiniales... see Guillou, L., Viprey, M., Chambouvet, A., Welsh, R.M., Kirkham, A.R., Massana, R., Scanlan, D.J. et al. 2008. Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). *Environ. Microbiol.* 10:3349–65.
- l. 19. Provide references about mixotrophy. Which Procoentrum and prasinophytes are mixotrophic ???

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- p. 13494. l.12-15. Leakage may also affect rRNA. This really depends on the life time of the rRNA molecule vs the DNA but in contrast to mRNA, rRNA can have a long life time and therefore be found in the leaked material through the filter.
- l. 26. Dinoflagellates are the dominant group in barcodes studies see: de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahe, F., Logares, R., Lara, E. et al. 2015. Eukaryotic plankton diversity in the sunlit ocean. *Science.* 348:1261605
- p. 13495. l. 1. Not all Gymnodiniales are mixotrophic by far...
- l. 4. resting cysts would be expected to be found in the sediments not the water column.
- l. 10. Synurophyceae are a freshwater group. Their presence in the OMZ should be demonstrated and discussed with reference to other work on this group;
- l. 21. Apicomplexa are a parasitic group usually not found in marine waters. Please detail exactly which Apicomplexa are found in this profile and discuss with respect to the literature.
- p. 13496. l. 16. What is the PX-Clade of Stramenopile ? Where found before ? Discuss...
- p. 13497 l. 15. As said before many dinos are not photosynthetic so it is necessary to be much more detailed on what dinos exactly were found, go back to the literature and estimate what is their trophic behavior.
- l. 20-30. Please shorten.
- Fig. 6. It is impossible to read the labels... Split into 2 subfigures with larger size.

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