To: The Committee for PhD studies in Limnology
Faculty of Science, University of South Bohemia
Czech Republic
Sept. 30th, 2023

This PhD thesis "Ecology of Gemmatimonadota" by Ms. Mujakic extensively addressed the ecological importance and genomic potential of Gemmatimonadota bacteria in various environments. The research topic is highly timely given that at the moment there is so little information regarding this metabolically versatile and numerically abundant bacterial phylum in almost all types of natural environments. I believe the work presented in this thesis represents a significant improvement in our understanding of these environmental Gemmatimoadota. In general, the thesis is well-written and organized. A large part of the work has been published and properly documented with regard to Ms. Mujakic's contribution to each work.

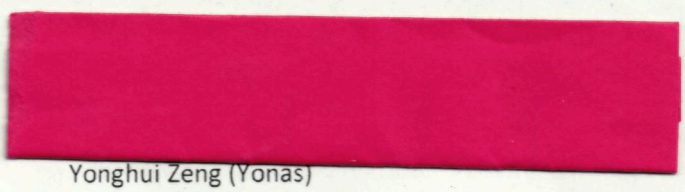
I am very impressed by Ms. Mujakic's achievements during her PhD study. She was involved in nine research articles in total with two as the first author and one more to be published, all in mainstream international scientific journals in the field of environmental microbiology. She also presented her work at many international conferences and attended overseas research stays. These experiences in publishing and collaborations demonstrate her qualify as both an individual PhD student and a researcher working in a team. I have no doubt she can move on to the next stage of defending her PhD thesis. Before that, I wish my concerns could be properly addressed.

1. Thesis title: there is a large part of the thesis addressing the genomics of Gemmatimonadota, and actually this thesis does not employ that much conventional methodology in ecology to address the pattern, dynamics, and physiology of Gemmatimonadota. Maybe the title should be changed to "Ecology and genomics of Gemmatimonadota" to better reflect the content.
2. Introduction, section 1.3. The references here are a bit outdated and does not reflect current knowledge, especially on recent advancement in high-throughput cultivation and culturomics.
3. Figure 1, add a bit more information on what the Type 1 and type 2 reaction centers are.
4. Figure 2 and associated content, currently not only short reads are used for metagenomics. More and more studies involve both short and long reads technologies to carry out metagenomics in order to higher quality and more complete MAGs.
5. Page 9, section 1.6. It should be emphasized that the phototrophic Eremiobacteriota is still lacking culture representatives and even the genome representing the phototrophic capacity is not fully resolved, in other words, not a complete chromosome. By these standards, I would argue it should be considered a pending phototrophic phylum.
6. Page 10, please elaborate a bit more on why you think AAP "have lost any carbon fixation capacity". It may apply to certain Proteobacteria but definitely not all AAPs.
7. Page 12, it is worth highlighting the detailed locations where these two phototrophic Gemma were isolated. Here you mentioned HGT many times and indeed HGT may play a significant role in the evolution of phototrophic Gemma. I recommend adding a section on what is HGT and how HGT contributes to the evolution of AAPs.
8. Figure 4, *R. gelatinosus* is NOT a gammaproteobacteium.
9. Aims and objectives, page 15. I think this part can be better organized to present a better flow of the design and execution of this thesis. I kind of get lost with my mind bumping back and forth when reading ...overarching aim...main ideas...also wanted to...finally....the

objective.... I also have a feeling it is not fully addressed regarding why it is important to study this particular group of bacteria. Such importance should be highlighted here or form an individual section in the previous part.

10. Page 21, section 3.1. A short summary of how different groups of phototrophic Gemma should be included here. How many of these MAGs are at the high-quality level (>50% complete, <10% contamination)? This is more important information for generating an overview of this part of thesis. Please also add some explanations why small-sized free-living Gemma was mostly observed in the hypolimnion. In general, a summary should go beyond a summary of only methods and results. The same also applied to other summaries in this thesis.
11. Page 38. This paper consists of the most important part of this thesis. I think these supplementary figures deserve space to be shown in this thesis so readers can easily find the most important information without looking up the original deposits.
12. Page 45, section 3.2. Please detail the yearly range of the information you collected. This field is moving fast. This can give a good overview of how your review can contribute to the topic.
13. Page 76. For the pufM ASV that were classified as Gemmatimonadota, please add a phylogenetic tree to support this, which seems missing in this thesis. Since PGC of phototrophic Gemma largely originated from Proteobacteria, their pufM genes are often intricated and hard to distinguish.
14. Page 83, section 3.4. Please highlight how many MAGs are potentially phototrophic Gemma. A missing topic in this section is the Gemmatimonadota bacteria that contain both pufM and rhodopsin genes. Please refer to the paper DOI: 10.1128/mBio.02641-20 (Table 1).
15. Page 129, Section 4. I think this section is not intended to repeat the question asked previously. Instead, please focus more on conclusions and prospects as the title suggests. The observation of Gemma associated with other organisms is a highlight of this work. Please detail which organisms it is associated, i.e. Microcystis. Previous data all come from omics surveys. This is the very first direction observation.
    In general, I recommend expanding a bit more on this section, especially on future prospects, e.g. how long reads can help address MAGs issues, how culturomics can contribute to the finding of more Gemma species, how functional gene-based CARD-FISH can generate novel insights in the ecophysiology of Gemma. It is also a nice place to share any failures in attempting to culture more Gemmas in the lab, if any.

I believe my concerns are manageable. Based on the high scientific achievements and merits demonstrated in this PhD study, I recommend Ms. Mujakic receiving her doctoral degree after a successful defending of her thesis.



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