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Interactive comment on "16S rRNA gene metabarcoding reveals a potential metabolic role for intracellular bacteria in a major marine planktonic calcifier (Foraminifera)" *by* Clare Bird et al.

Anonymous Referee #1

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The manuscript by Bird et al. describes the first evidence for intracellular bacteria in planktonic foraminifera, marine protists. The authors use different approaches, microscopy, gene sequencing and metabarcoding, to investigate the presence and identity of bacteria within a planktonic foraminifer from two locations in the California Current. The main bacterial genus found is Synechococcus, which shows higher abundances inside the foraminiferal cell compared to the water column and seems to be actively dividing within the host. The authors thus consider it an endobiont within one genotype of the foraminifer Globigerina bulloides and they discuss potential metabolic roles of this bacterial genus in the association. The manuscript in general is well written

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and presents the methods in great detail. The number of figures and the content of the supplements are suitable for the understanding of the MS. Being the first description of intracellular bacteria within a planktonic foraminiferal cell, I consider the manuscript of great importance for the understanding of the ecology of planktonic foraminifera, which further affects their application as proxies in paleontological studies. However, there are some issues regarding the interpretation of the data as well as some small technical issues, as I point out below, that need to be addressed by the authors.

General comments: Title: The title is too general and promises something that cannot be shown yet by the data. Metabarcoding only reveals the presence of the bacteria not their metabolic role. The title further seems to refer to all planktonic foraminifera in general, while the study only analyzes one genotype of one morphospecies.

Methodology: In part 4.1.4 of the discussion you mention the possibility of a primer bias against a certain group of bacteria introduced by the PCR based approach for the detection and identification of bacteria. I wonder if this may not be a more general problem in the study, making certain groups of bacteria appear more abundant than they actually are, since they are amplified more easily with the chosen primers than other groups. This issue needs to be discussed in the MS.

Discussion: Showing a metabolic role for intracellular bacteria in a eukaryote host sure is a difficult task. So for now this part should remain rather speculative and not appear for example in the title, as mentioned above. Yet, I agree that referring to the bacteria as endobionts is legitimate, as this purely describes their presence within another organism. As mentioned in part 4.1.2, Synechococcus are present in deep-living benthic foraminifera as well as diatoms. In both cases photosynthesis would not play any role in the association between the bacteria and the hosts. I wonder if Synechococcus just uses the hosts as some kind of protection, more or less “infesting” them. In this regard, I am not sure how the authors conclude that G. bulloides actively and species-specifically takes up the bacteria from the water column. I think there are no data yet to show how the bacteria really end up in the foraminiferal cell.

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Detailed comments: Abstract: Line 2: Maybe edit to: “This marine protist is commonly used in micropaleontological investigations. . .” Lines 4-5: The reasoning why the authors chose G. bulloides to search for bacteria symbiosis is not completely clear. What does “atypical geochemical shell signature” and “divergent ecology” mean?

Introduction: Page 4 Lines 18-20: This sentence needs clarification: “. . .by more than any other extant, surface-dwelling species. . .Such large deviations. . .” Written like this, the statement needs quantification on how large the deviations actually are. Line 31: Is there only this one genotype in the sampling area? If yes then paleontological analysis on that morphospecies from that area should not contain any noise due to genotypes as mentioned in the paragraph before. Page 5 Line 1: Globigerina bulloides: In general the genus name should be written out only once at the beginning of each chapter and then afterwards abbreviated. Line 3-4: “We demonstrate. . .” I don’t think it is really demonstrated here that the bacteria are actively taken up by the foraminifera and I also don’t think it can be said yet if the association is really SPECIES-specific.

Material and Methods: Page 5 Line 18: I think it would be helpful to put the sampling point for the bacteria analysis in the water column in Figure 1. Line 22: How do the dates chosen for sampling relate to the oceanography and the changes in foraminifera abundances? Line 26: “species level”: I assume this refers to morphospecies as the genotypes (which seem to be the actual species) cannot be differentiated morphologically as you mentioned before. Page 6: Line 2: I wonder how it is possible to make sure that all external contaminants are removed. By putting the shell in RNALater I assume that also contaminant DNA gets preserved. How is it possible to separate the foraminifera cell from the contaminants? Line 9: Maybe mention here which genes were amplified. Page 7: Line 7: I am not sure I understand which samples were pooled for the sequencing. The different individuals? How are they told apart again later on?

Results: Page 9: Line 29: In Table S1 the N. dutertrei individual is called DUT59. Page 10: Line 4: I think it could be helpful to show the unstained G. bulloides in the supplementary files to have a comparison between stained and unstained images. Of

course, even better would be a comparison to a stained species without (or with less) bacteria (e.g. N. dutertrei) to see the difference. Page 12: Line 3: I wonder how reliable the comparison between the bacteria in the foraminifera and the water column really is as the water column data were not taken together with the foraminifera sampling. I think it is necessary to further comment on these bacteria data to show how stable they are over time and how reliable it is to assume they were still valid at the time of sampling.

Discussion: Page 15: Line 30: "...with the majority of OTUs (>97%)..."

References: In general species names must be in italics.

Figure 1: The zoomed-out map is very small. I suggest making it larger and enhancing the contrast of the colors to make it more useful.

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