

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.

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The authors wish to thank T. Grebert for taking the time to read this manuscript and for his useful and constructive comments, which the authors have enjoyed discussing here.

1. The title is misleading as it is not supported by any result presented in this study, and should be changed.

The authors accept this in full. Future work will determine the metabolic role of the cyanobacteria identified within the foraminiferal cell in this study. Therefore the authors

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acknowledge that the title should be amended to “16S rRNA gene metabarcoding reveals the presence of intracellular cyanobacteria in a major marine planktonic calcifier (Foraminifera)”

2. Synechococcus clade I also contains green-light specialists (e.g. strains ROS8604 or SYN20, see Pittera et al. 2014, ISME J, doi 10.1038/ismej.2013.228). Similarly, only two strains of clade IV have been characterized for pigmentation. The claim that all clade I and IV Synechococcus are chromatic acclimators (discussion 4.1.5 and 4.2) is thus false for clade I and should be moderated for clade IV.

Thank you for this valid point. The text in discussion sections 4.1.5 and 4.2 will be modified accordingly to more accurately state that some strains within clade I and those so far characterized for pigments in clade IV are chromatic adapters. The rest of the discussion regarding the potential benefit of chromatic adaption to the host, remains valid.

3. In paragraph 4.1.1, you claim that PCR amplification of 16S RNA and rbcL "provides additional evidence that Synechococcus DNA [is] not grossly degraded by nucleases". However, you do amplify 16S marker gene for metabarcoding of bacteria that you claim are digested by the host. You thus use the same result to draw diametrically opposed conclusions.

This is an excellent point that does need clarification. DNA degradation in “dietary samples” limits the size of DNA fragments that can be successfully amplified. For investigation of prey items target fragments should be limited to ~100-250 bp. Whilst longer fragments can be utilised, they will limit the success of amplification, so that sequences will not always be obtained (see Pompanon et al., 2012 doi: 10.1111/j.1365-294X.2011.05403.x).

In our study using 16S rRNA gene metabarcoding, we have amplified a fragment of 253 bp which will therefore give us information not only on intact bacteria but also on those bacteria that have been phagocytosed for food. Since the diet of foraminifera is

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not wholly known this information is also of value. TEM imaging has then enabled us to further discriminate between food and endobiont.

We also PCR amplified partial *Synechococcus* 16S rRNA genes of 422 bp from *G. bulloides* total DNA for cloning and Sanger sequencing. This is a longer fragment than ideal for identifying prey (i.e. >250 bp) suggesting that the *Synechococcus* DNA is more intact than might be expected if it were the DNA of prey bacteria. Therefore the authors consider this to be supporting evidence (not stand alone evidence) that *Synechococcus* are living cells, endobionts, and not prey bacteria. The term “not grossly degraded by nucleases” is used in the manuscript to avoid over exaggeration of the significance of this data.

The authors consider that a further sentence in section 4.1.1 regarding the sizes of target DNA in helping to discriminate between prey and endobiont bacteria will be a helpful addition to the discussion.

4. In paragraph 4.1.3, you observe 100% identity between 16S RNA and *rbcL* markers between “endobionts” and clade IV *Synechococcus* CC9902. However, strains CC9902 and BL107 share 100% and 98.9% nucleotide identity for these markers, yet share an average nucleotide identity of 91.3% for the core proteins (see Dufresne et al 2008, *Genome Biology*, doi 10.1186/gb-2008-9-5-r90), lower than the threshold value commonly used for bacterial species definition (94%). The conclusion “This strongly supports a strategy of horizontal rather than vertical transmission” should thus be moderated, as two strains exhibiting high degrees of similarity for these markers can be quite divergent for the rest of their genome: these markers thus provide evidence but are not sufficient to totally exclude genetic drift between internal and free-living *Synechococcus*.

Unlike in the example given above, both the 16S rRNA gene and the *rbcL* gene amplified and cloned from total *G. bulloides* DNA gave 100% identity to *Synechococcus* CC9902. However, we accept this point and will moderate our conclusion in section

4.1.3 accordingly with the caveat that there is a high degree of diversity among strains seemingly closely related through analysis of 16S and rbcL phylogenies.

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