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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.

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1. 16S rRNA gene metabarcoding and fluorescence microscopy can reveal the presence of bacteria in the cell (possibly digesting foods or endobionts), but cannot suggest ecological interaction between host and bacteria. How could you say the bacteria as endobionts?

Future work will elucidate the nature of the relationship between Synechococcus and G. bulloides and until a benefit is demonstrated to either party we have refrained from using the term symbiont. We feel that the term endobiont is wholly appropriate in this

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instance. The definition of an endobiont is of an organism that lives either below a surface (such as a sea bed) or inside another organism. It does not imply (beneficial) ecological interactions (although interactions must occur at a molecular level). We have used the term endobiont as our evidence suggests that Synechococcus are alive inside the G. bulloides cell. Synechococcus cell counts in TEM images demonstrate large numbers of Synechococcus cells inside G. bulloides, and in addition, that 5% of these cells are going through cellular division, i.e. they are reproducing.

2. The 16S rRNA gene metabarcodings were coming from amplicon sequences. Amplicon sequences are biased by primer, thus ratio of amplicon sequences did not mean the ratio of the bacteria community inside the cell.

The primer set used in this study is that designed and used by the Earth Microbiome Project (Gilbert et al., 2010). The biases in this primer set are well known and have recently been corrected for (Apprill et al., 2015; Walters et al., 2016; Parada et al., 2016). The primer set has been tested with mock communities (Parada et al., 2016) and compares well with FISH results (Apprill et al. 2015) giving a good representation of the bacterial assemblages targeted. The bias in this primer set does not include an over amplification of Synechococcus. Therefore we believe that the proportions of Synechococcus demonstrated by this method are accurate and taken with the TEM cell counts, do reflect the true proportions of Synechococcus within the G. bulloides cell.

3. Also, the TEM image of possible Synechococcus is difficult to observe thylakoid membrane. It is unclear for me to distinguish them as Synechococcus.

The thylakoid membranes can be observed circling the periphery of the cell, but we acknowledge that the clarity of these is not perfect. However, the carboxysomes, only found in cyanobacteria, are very clear, and the cell division in a single plain is also obvious. Both are characteristic of Synechococcus. TRITC excitation of G. bulloides cells under fluorescence microscopy (see new uploaded image), also demonstrates

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the presence of phycoerythrin-containing bacteria throughout the cell. Phycoerythrin is a pigment characteristic of Synechococcus and therefore all the evidence points to these cells being Synechococcus.

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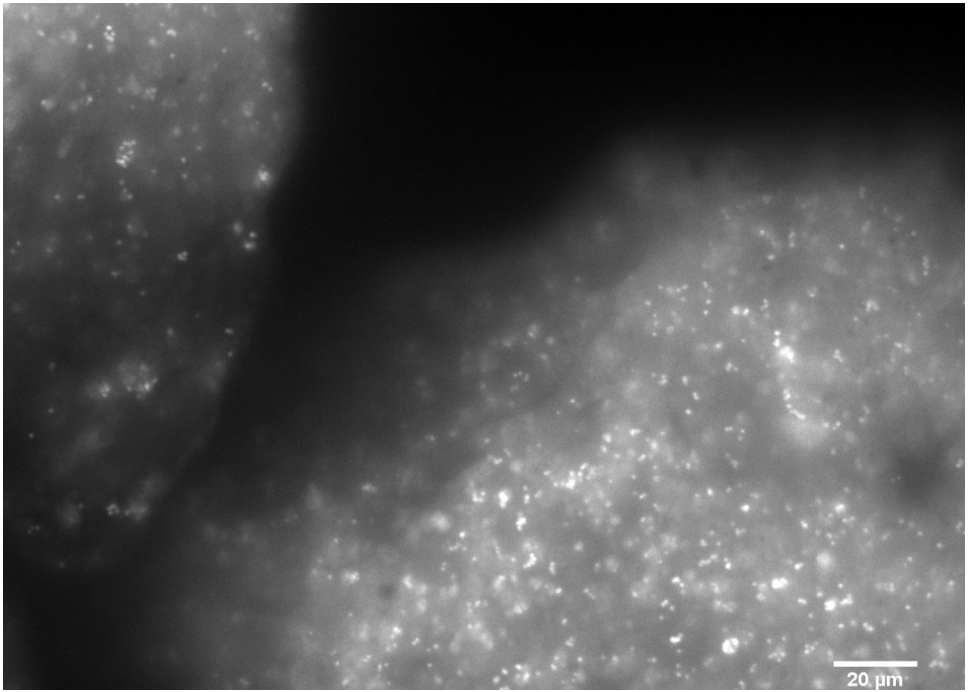

Fig. 1. A G. bulloides cell under a TRITC filter set. The pigment phycoerythrin, characteristic of Synechococcus, autofluoresces demonstrating the presence of these cyanobacterial cells.

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