

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.

Clare Bird et al.

clare.bird@ed.ac.uk

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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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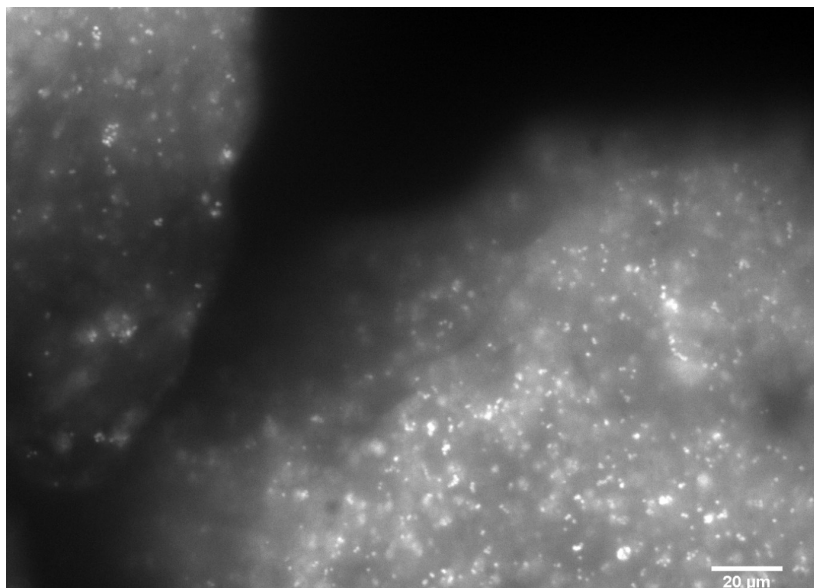


Fig. 1. A *G. bulloides* cell under a TRITC filter set. The pigment phycoerythrin, characteristic of *Synechococcus*, autofluoresces demonstrating the presence of these cyanobacterial cells.

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