

Interactive comment on “Marine bacteria in deep Arctic and Antarctic ice cores: a proxy for evolution in oceans over 300 million generations” by P. B. Price and R. C. Bay

Anonymous Referee #1

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General comment:

It is generally accepted that the major sources of microbial cells transported by atmospheric circulation and deposited with snow on glacial surfaces are terrestrial dust, marine surface aerosols and volcanic ashes. The manuscript of Price and Bay is focused on bacteria of marine origin and more specifically on the autotrophic photosynthetic picocyanobacteria *Prochlorococcus* and *Synechococcus*, which are dominant in tropical and temperate oceans and are also found in polar environments. Furthermore, the authors raise a very important question whether glacial microorganisms undergo any evolution. The far-reaching idea is that because microbial cells are trapped and pre-

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served chronologically at different depths for very long times maintaining extremely low metabolic activity without division (e.i. have extremely low mutation rate), comparative studies of their genomes vs. depth can provide a new tool for studying microbial evolution prior deposition, in this case in the ocean. Before pursuing this intriguing possibility, the authors present in this manuscript an impressive in its scale detailed analysis of cyanobacteria in glacial ice from 8 sites in Greenland and Antarctica. They used a range of fluorescence-based methods and instruments to detect these microorganisms including their previously developed Berkeley fluorescence spectrophotometer (BFS), epifluorescence microscopy (EFM), flow cytometry (FCM), as well as differential interference contrast (DIC). The precise tuning of each method during application and the use of reference pure cultures of well studied marine isolates of *Prochlorococcus* and *Synechococcus* for step-by-step parameter optimization should be especially mentioned as a methodological achievement contributing to the successful detection of previously hard to find cyanobacteria in glacial ice. The results from measurements of autofluorescence of tryptophan (Trp) and chlorophyll (Chl) as proxies of total microbial content and presence of cyanobacteria are well and logically presented in the text and in two tables and nine figures. The discussion uses these new data and existing literature to present intriguing interpretations and a new view on the annual fluorescence co-modulation of Chl and Trp in glacial ice from both poles and how its intensity may be dependent on climate and seasonal distribution of phototrophs in ocean waters. This paper provides an example of convincing experimental evidence in support of a novel hypothesis built on previous key theoretical ideas of Dr. Price such as the concept of specific microbial habitats within the ice and the cold origin of life on Mars and Earth.

Specific Comments:

The introduction presents a concise but general description of glacial ice as a microbial habitat. It would be helpful to the reader if the specific goals of this particular study were included at the end of the introduction.

Material and methods

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- What were the media in which the provided reference cultures of *Prochlorococcus* and *Synechococcus* were grown and were the media tested for background autofluorescence?

- A systematic list of all sites from which the analyzed samples originated is needed in the Material and methods sections. According to the text of the abstract 2 Arctic and 6 Antarctic sites were studied. A more detailed list of 7 sites is presented on p. 6542 in relation to Fig. 1, whereas 8 sites are listed on p. 6548 for the analyzes on Fig. 9.

Results and discussion

When discussing the prospect of studying the evolutionary changes of picocyanobacterial cells before they were frozen deep in glacial ice, where they do not further evolve for a number of reasons (summarized in the text), the authors rightfully emphasize on prior evolution in marine waters. However, a number of studies have shown that these photosynthetic microorganisms are dominant in another specific glacial environment, not mentioned in the text, the cryoconite holes on glacial surface, where cyanobacteria are active drivers of microbial photosynthesis and primary production, also capable of growth, especially during the summer. This is relevant to the seasonal fluctuations of Chl fluorescence observed in glacial depth.

Suggested technical corrections:

- Add subtitles: Results on p.6542 before sub-section 3. Chl autofluorescence emitted from unmelted glacial ice and Discussion at the top of p.6551 to be consistent with sections Introduction and Materials and methods.

- Omit the numbering of sub-sections (1-7).

- Change subtitle on p. 6543 to Differential Interference Contrast (DIC) and Epifluorescence Microscopy (EFM) in melted glacial ice and cultures.

- Add phycoerythrin before (PE) on line 11, p.6546. This abbreviation was previously introduced only on the abstract.

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- References are needed on the same p. 6546, lines 19-22.

- Add a description of the wiggly red line in the legend of Fig.2, similar to the one in the text.

- The axes on Fig 8 should be labeled in the same way as those on Fig. 6, 7.

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