

Interactive comment on “Picoplankton diversity in the South-East Pacific Ocean from cultures” by F. Le Gall et al.

Anonymous Referee #3

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This paper presents a huge culturing effort for marine picoeukaryotes. These small protists are ecologically relevant in marine systems, but they are only partly represented in culture collections. Molecular environmental analyses of this assemblage have shown the discrepancy for some groups (alveolates, stramenopiles, picobiliphytes) between in situ populations and culture collections, whereas other groups, like prasinophytes, appear to be well represented by cultures. So, culturing efforts such as the one presented here are required for a better characterization of marine picoeukaryotes. Another remarkable aspect of this study is that cultures were initiated with seawater collected during an oceanographic cruise, including coastal upwelled waters and open sea ultra-oligotrophic waters. After screening 1900 precultures, the authors isolated 212 strains, which were all characterized morphologically and about half of them by sequencing

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the 18S rDNA. Most of these strains were very small (2-3 μm), so true picoeukaryotes. This represents an interesting contribution of biological material, and strains have been deposited in culture collections and are available for further studies. It will help to mention in the introduction if similar culturing efforts have been done before (when, where, and how many strains). However, as mentioned by the authors, the study failed in the two most interesting and novel aspects, the isolation of picoeukaryotes only known from molecular surveys and an extensive strain isolation from the ultraoligotrophic waters. Sadly, the two more promising strains by their novelty were lost, probably due to the difficulty to keep them into culture. So, this study also points to the necessity to apply novel culturing strategies. Only a few 18S rDNA sequences are shown in Fig. 4, implying that those represent the whole collection of strains isolated. However, it would be interesting to know which is the variability seen (at the 18S rDNA sequenced) by the repeated cultures. Was it always 100%? For instance, only 2 rDNA sequences are shown for the 38 *Pelagomonas* strains. Are the other 36 identical to one or the other?

Specific comments Page 2701, line 5-7. Picoplankton was first proposed in 1978 to include essentially bacteria. A few years later it was seen that also included protists. Rephrase, since it seems that picoplankton was defined in base of the recently discovered small protists. Page 2703, line 1-3. ?Five phylogenetic divisions? but six groups are listed. Page 2704, line2. A better reference for the rice-based media is A.J. Cowling 1991. Free-living heterotrophic flagellates: methods of isolation and maintenance, including sources of strains in culture. In: D.J. Patterson and J. Larsen, eds., *The Biology of Free-Living Heterotrophic Flagellates*, Clarendon Press, Oxford, pp. 477-492 Page 2704, line 18-19. Change ?to target the rarer cells? to ?in order that rarer cells form visible populations in the cytogram? Page 2705, line 11. Transporting photosynthetic cultures growing at 20°C in an ice box (dark and around 0°C) is probably not ideal. There is a better way to do it? Were many cultures lost during the transport? Page 2707-2708. I don?t see why cultures during the initial growth phase are more stable. Is this something general? Page 2710, lines 23-26. Rephrase: there are 16 Phaeocystis strains, 3 from upwelling regions and 5 for the east of the gyre. What about

the remaining 8? Page 2711, line 6-8. I don?t understand the reasoning, because I believe that the same sample could include 2 (and many more) different taxonomical different Pelagomonas Table 1. Change ?min? and ?max? by ?surface? and ?DCM?, respectively

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