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## ***Interactive comment on “Diversity of cultured photosynthetic flagellates in the North East Pacific and Arctic Oceans in summer” by S. Balzano et al.***

### **Anonymous Referee #2**

Received and published: 24 July 2012

Referee comments on manuscript “Diversity of cultured photosynthetic flagellates in the North East Pacific and Arctic Oceans in summer” (bg-2012-198) by Balzano et al.

July 24th, 2012

### General comments

The paper “Diversity of cultured photosynthetic flagellates in the North East Pacific and Arctic oceans in summer” by Balzano et al. reports an attempt to describe the genetic diversity of autotrophic flagellated phytoplankton in high-latitude cold waters, by isolating and culturing a large number of strains, and characterizing them genetically as well as morphologically. In this respect, the authors substantially add to the existing knowledge on microbial genetic diversity in marginal environments. They also significantly

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contribute to the limited knowledge on flagellated species diversity in these environments, hitherto largely described through microscopic analyses on preserved samples (with low taxonomic resolution for several taxa), and, more recently, through metagenomic analyses of environmental samples, the taxonomic resolution of which depend not only on the potential bias of applied gene regions and primers, but also on the lack of baseline sequence data for uncultured taxa in particular. This paper thus combines traditional and more novel tools to produce new data on species diversity, information that will be useful for understanding the structure and function of high-latitude marine ecosystems as well as for predicting the impact of future change in these regions.

The overall presentation is well structured with a clear description of methods and results. The results section tends to include some comments that could fit better in the discussion. The following discussion, however, is relevant and many interesting aspects are covered. An aspect which could add to the quality of the discussion is the aspect of seasonality. This is mentioned in the introduction, but largely overlooked in the discussion. Seasonality is extreme in polar regions, which will bear consequences for the species diversity sampled. On a more general basis, the paper lacks a section stating the main conclusions of this work, which could be added after the discussion and also included in the abstract. In the present form of the paper, the conclusions appear scattered in the text. I have several minor and technical concerns which can easily be addressed, all specified below.

The authors state that the other species groups found in large numbers during the MALINA cruises (e.g. diatoms) will be presented in a separate paper. To some extent one could wish that the species diversity results would have been compiled and presented altogether, in order to give a better overview of the autotrophic community structure in the area sampled, but I guess this was considered a data compilation too extensive for one paper. As it is now, partly the same results will eventually be published in three papers. I look forward to seeing the results also on the other parts of the (culturable) phytoplankton community in press!

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## Specific comments

p. 6220, lines 2-4: A few words on the objective (stated in the introduction) could be added here. This would make the abstract even more to the point.

p. 6222, lines 13-14: (Tables 1 and 2, Fig. 1) The authors largely refer to published data from the Canadian Arctic, and this study is based on material sampled in the NE Pacific, the Bering Strait, the Chukchi and Beaufort Seas. Why then, are the two stations (ARC11, ARC12), which seem to have been sampled from within the Chukchi Sea, referred to as “Arctic Ocean” stations? This is very general and perhaps even somewhat misleading regarding the relatively small geographical region of the Arctic Ocean sampled. Or are the stations defined based on hydrographical conditions?

p. 6223, lines 22-25: How were cultures or surface samples maintained between sampling and isolation? The authors describe the light conditions in terms of intensity ( $\mu\text{E m}^{-2}\text{s}^{-1}$ ), but do not mention the photoperiod used during the incubation. Where the cultures kept at similar conditions during the whole period of 1-6 months between sampling and isolation/analysis?

p. 6225, lines 8, 14, 15: Are all primers used listed as 5'-3'? This could be stated in the text.

p. 6225, lines 28- p. 6226, line 1: Is there a reason behind mixing taxonomic levels here? Why not mention all the classes defined later (e.g. Table 2) within Chlorophyta etc. – or were they all grouped together at this stage of the analysis?

p. 6226, lines 7-8: This information (first sentence) could be given already in section 2.3 where all molecular analyses are described.

p. 6228, lines 4-15: Most of this information is already given in the methods section, i.e., not necessary to repeat here. As commented on before, it would be more specific to name the (Arctic Ocean) regions sampled.

p. 6228, line 24: There is hardly any doubt concerning the meaning of “size” in this

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sentence, but it would be even clearer to use “diameter” (if this is what is reported).

p. 6229, lines 4-5: This is an interesting divergence and the sequences from tropical and temperate *Micromonas* could preferably be named in the text (as in line 8 with strain ID), and more specifically marked in Fig. 3, with the suffix tropical/temperate as done with e.g. the *Nephroselmis* sequences.

p. 6229, lines 9-19: Was there any morphological analysis performed on this *B. prasinus* strain before it was lost? There seem to be several morphological features characteristic of *B. prasinus* which could further confirm the genetic identification (e.g. Eikrem & Thronsen (1990). The ultrastructure of *Bathycoccus* gen. nov. and *B. prasinus* sp. nov., a non-motile picoplanktonic alga (Chlorophyta, Prasinophyceae) from the Mediterranean and Atlantic. *Phycologia* 29: 344-350).

p. 6230, lines 5-6: For the potentially undescribed *Mamiellophyceae* strains; was there no time for EM on these strains, or will this be reported elsewhere?

p. 6230, line 15: A reference to Fig. 3 could be in place here.

p. 6231, lines 8-12: Again, a reference to Fig. 3 could be in place here. And perhaps a few words on the *Carteria* I – a clade sensu Suda et al., 2005?

p. 6231, lines 13-15: If cells are pear-shaped, cell size could be reported as length and width (as done previously in the text).

p. 6232, lines 1-2: As for the *Mamiellophyceae* strains; was there no time for EM on the *Pyramimonas* strains, or will this be reported elsewhere? I find it a bit surprising that the authors do not go in to depth with the morphological part, even though this would be highly valuable in cases where gene sequencing cannot resolve the phylogeny to species level. This would also add more new knowledge concerning the diversity of the cold-water flagellates investigated here. I do know, however, that this is time-consuming work.

p. 6232, lines 3-12: This paragraph could perhaps fit better in the Discussion section?

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Anyway, in the context of adaptation to different salinities, it is also interesting that the MALINA genotypes cluster with an uncultured chlorophyte from the Baltic Sea (Fig. 3).

p. 6232, lines 1-3: If cells are pear-shaped, cell size could be reported as length and width (as done previously in the text). I also believe “wider” is more appropriate than “larger” on line 3.

p. 6232, line 14: It would be nice if the authors could sum up the number of corresponding genotypes (18S) found already in the beginning of this paragraph, as done with several other groups in the text.

p. 6235, lines 19-20: Also here; how many genotypes?

p. 6238-6239, section 4.1 and preceding paragraph: The first sentence in section 4.1 appears to be the one of the most important results. Perhaps this could start the discussion on autotrophic microbial diversity revealed by different techniques?

p. 6238, lines 15-16: This sentence is somewhat unclear. Do the authors mean that the Rhodomonas genotype could be associated with a species observed by LM in the cell counts of the MALINA cruise?

p. 6241-6242, section 4.4: These are interesting thoughts, but some sort of conclusive statement is lacking. I.e., are the authors suggesting that mixotrophy or heterotrophy enables higher species diversity among nano- and microplankton in nutrient-deplete waters? Do the papers referred to confirm this relationship? What about mixotrophy or heterotrophy among picoplankton, could such strategies affect species diversity or rather allow for survival during unfavourable conditions? (see e.g. Iversen & Seuthe (2010) Seasonal microbial processes in a high-latitude fjord (Kongsfjorden, Svalbard): I. Heterotrophic bacteria, picoplankton and nanoflagellates. Polar Biology.) This part of the discussion is linked to section 4.3 and could be further elaborated.

p. 6242-6243, section 4.5: The discussion on endemic lineages is very interesting and I appreciate the fact that the authors consider the yet unknown part of microbial (genetic)

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diversity in different oceanic regions. Concerning the debate on the biogeography of Arctic microbes; is there any explanation to why marine eukaryotes “are less likely to be globally dispersed”? If this is true, is it related to dispersal barriers, adaptive divergence? Such perspectives could add an interesting dimension to the discussion and references on this matter are needed.

#### Technical corrections

p. 6230, line 7: Replace chlorophyta with Chlorophyta.

p. 6231, line 8: Replace “large” with “wide”; more in line with rest of the text.

p. 6231, line 10: Replace *C. obstusa* with *C. obtusa* (as in Fig. 3).

p. 6231, line 23: Replace *Trichocystys* with *Trichocystis*. This subgenus is also wrongly referred to as *Trichocystys* in Fig. 3, please correct this typing error.

p. 6235, line 1: Should this be Siano et al., 2009? Otherwise, a reference is missing from the list.

p. 6235, line 15: Replace Basyonim with Basionym.

p. 6235, line 24: Replace large with wide. Large is perhaps more descriptive in French when it comes to the width of an object. . .

p. 6237, lines 8 and 14: Reference to suppl. Fig. should be corrected to S1.

p. 6250, Table 1: The CTD in the second column is presumably given in metres (m), which could be added.

p. 6252, Fig. 1: Latitudinal and longitudinal lines would be good in this figure!

p. 6254, line 12: Haptophyta could perhaps be replaced with Prymnesiophyceae, which is used in the text (p. 6232) and in Fig. 3. However, in the discussion (e.g. p. 6241, line 13), Haptophyta is used again. It would be clearer to be more consistent throughout the paper.

p. 6255, Fig. 3: Please note the “Haptolina” (*H. hirta*, *H. fragaria*) typing errors in the Prymnesiophyceae section, and the “Cryptomonas” (*C. acuta*) in the Cryptophyceae section. Please double-check all species names in the text, in the tables and figures; I have not checked them all. . .

p. 6258, Fig. 5d: Perhaps the EAV referred to in the text could be shown more clearly with the help of an arrow in the figure?

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