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Biogeosciences Discussions

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

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Dear Biogeosciences Editor

Thanks or considering our manuscript, please find below specific responses to the referees comments:

Referee no 1:

P. 17: basionym is misspelled as basyonim. Corrected

P. 17. Please see Article 33.4 and related ICBN rules; the proposed combination (Biecheleria cincta) is invalid as proposed.

We edited the proposed combination

P. 18. Regarding Dinobryon faculiferum, we found the same thing but never got around to publishing it. We lost the culture. Also, we obtained gene sequences for the marine Dinobryon that did fit with the freshwater spp (also unpublished). I think it is a new genus. We never observed this to form colonies, and I didn't see where the authors commented on its colony or single-cell status.

Light microscopy photographs of strains RCC2290 and RCC2292 clearly show that these strains belong to Dinobryon faculiferum because of the size and the lorica shape typical of these species (Throndsen 1997). It is not a colonial species as indicated on page 14 line 382 ("In RCC2292, RCC2293 and RCC2294 cells are solitary") .

p. 23. I consider the remarks about nitrate concentration to be incomplete. First, the nitrate measurements are for "standing crop" of nitrate, not its flux. That is, if microbes are rapidly shuffling nitrate molecules, then a small amount of free nitrate in the water doesn't measure the nitrate activity of the flux. A large "standing crop" of nitrate in the water provides no information about the use of the nitrate. Perhaps most importantly, all algae will utilize ammonia and the flux for ammonia can be very high – almost none measurable in the water but because of the high flux, it is sufficient for growth. In summary, total nitrogen in the system is an indicator of potential system production because all cells have nitrogen and they have nitrogen in roughly the same amounts (of course, higher in organisms with biliproteins, lower in cells with lots of fat, etc.). One should keep in mind the great story that unveiled during the discovery and culture of Prochlorococcus. It was found in greatest cell numbers when nitrate values were high, and therefore it was concluded that it needed lots of nitrate. However, it requires ammonia (rarely nitrite), it cannot use nitrate, and the high nitrate measurements were completely misleading.

During the MALINA cruise both nitrate and ammonium were detected at very low concentrations and other papers on this special issue claim that nitrogen was limiting (e.g. Ortega et al.). We thus believe that surface waters in the Beaufort Sea were limited in inorganic nitrogen during the MALINA cruise. A new sentence has been added (l.

BGD

9, C3778–C3790, 2012

483-485) to refer to the Ortega paper.

Table 1. This information could be placed in a supplemental table; it has little impact on the meat of the manuscript

Table 1 shows the number of strains obtained at different stations using a range of isolation techniques. We believe this information should be in the main text.

Table 1. Salinity is a unitless measure; the units cancel in the ratio; psu is not recognized.

We removed the columns containing salinity and temperature because these data appear in our companion paper (Balzano et al., 2012)

Fig. 1 could be a supplemental image in my opinion; the latitude – longitude coordinates are more valuable, I think. Also, within minutes, the water mass has moved, something new has replace it, ...

We removed Figure 1 as the same information is reported in Table 1.

Fig. 2. The colour images are beautiful; the graytones of the background could be balanced a bit, but excellent images.

We prefer to keep images as close to the original photographs as possible.

Does the paper address relevant scientific questions within the scope of BG? I am not sure what question was asked?

The paper investigates the diversity of flagellates within the Arctic

Does the paper present novel concepts, ideas, tools, or data? There isn't anything novel, but the work is solid.

We find a number of novel genera and species, although this occur very often in this kind of studies

Are substantial conclusions reached? They found one Micromonas in the samples, but

BGD

9, C3778–C3790, 2012

I am not sure if this is substantial.

We found a high genetic homogeneity within the dominant Arctic photosynthetic picoplankter. This ecotype occurs throughout the Arctic under different conditions of light, temperature and nutrients. We started this study thinking that genetic differentiaton could occur between surface and deep strains, at least at the ITS level. However the genetic homogeneity found here suggests physiological plasticity for Arctic Micromonas that can adapt to both surface high light and deep low light conditions.

Are the scientific methods and assumptions valid and clearly outlined? I think they are valid and clearly outlined. Are the results sufficient to support the interpretations and conclusions? As I mentioned above, the nitrate interpretations are dubious at best. But when one conducts a floristic study, it is almost always impossible to expand the study in any meaningful way with regard to biochemistry, ecology, molecular biology, etc. It is a floristic study, full stop.

We replied about nitrates above.

Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)? If one obtains the cultures, then yes. If one goes to the sample sites and recollections, then perhaps yes, perhaps no. Do the authors give proper credit to related work and clearly indicate their own new/original contribution? I think so. Does the title clearly reflect the contents of the paper? Yes Does the abstract provide a concise and complete summary? Yes Is the overall presentation well structured and clear? Yes, some material could be placed as supplemental materials.

We reduced some text and placed some material in the Supplemental Material

Is the language fluent and precise? Yes, it is very well written. Are mathematical formulae, symbols, abbreviations, and units correctly defined and used? Yes, as far as I could determine. Should any parts of the paper (text, formulae, figures, tables)

BGD

9, C3778–C3790, 2012

be clarified, reduced, combined, or eliminated? There is some repetition that could be removed.

Done

Are the number and quality of references appropriate? I think so. Is the amount and quality of supplementary material appropriate? OK Referee no 2

We reduced both the results and discussion sections. We also wrote a short conclusion for this work as suggested. Diatoms have been investigated in more details (28S rRNA gene sequencing, electron microscopy) compared to other phytoplankters and adding the results from these identifications here would make the paper very long and hard to follow. The diatom data will be soon submitted in a separate paper. Please find below responses to 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.

Done

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?

We replaced Arctic Ocean with Chukchi Sea where appropriate

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 (μE m-2s-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

BGD

9, C3778–C3790, 2012

sampling and isolation/analysis?

We included the photoperiod used as well as the culturing conditions

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

Corrected

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?

All Chlorophyta classes were grouped together at this stage of the analysis. We harmonized taxonomic levels in the revised version using the division level : "(Chlorophyta, Cryptophyta, Alveolata, Heterokontophyta, Haptophyta)" – see line 159 of revised paper.

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

We give this information here because the full 18S rRNA gene was sequenced only for a subset of strains based on the preliminary phylogenetic analyses described before.

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. We reduced this information

p. 6228, line 24: There is hardly any doubt concerning the meaning of "size" in this sentence, but it would be even clearer to use "diameter" (if this is what is reported).

Corrected

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

BGD

9, C3778–C3790, 2012

strain ID), and more specifically marked in Fig. 3, with the suffix tropical/temperate as done with e.g. the Nephroselmis sequences.

In Fig. 2 (previously Fig. 3), we indicated the geographic origin for Micromonas strains.

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

No, unfortunately the strain was lost before we could perform any morphological analysis. The genus Bathycoccus has a very low variability at both 18S and ITS level.

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?

We are currently doing EM analyses for these strains and the results will be reported in the future.

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

Done

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?

Done

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).

Done

p. 6232, lines 1-2: As for the Mamiellophyceae strains; was there no time for EM on

BGD

9, C3778–C3790, 2012

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.

We agree that electron microscopy (EM) would have added significant information to our paper. However as EM is highly time consuming and we chose not to use it for all strains in the present study aimed at presenting an overview of the diversity of the arctic flagellates. We hope to perform such detailed studies in the future for a selected number of strains.

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

Done. We also added a few words about salinity adaptation.

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.

Corrected

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.

Done

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

Six. This is now included in the text

BGD

9, C3778–C3790, 2012

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?

We agree that the first sentence of Section 4.1 reports one of the most interesting results of the present study. However as suggested by referee no 3 we started the discussion with a short paragraph reporting the success of the techniques used here, the limitations (we did not do EM for all our strains) and the suggestions for future studies.

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?

Yes, corrected

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.

Yes mixotrophy among nano and microplankton might explained the higher diversity found for these groups especially in nutrient-depleted waters. The papers referred often suggest that mixotrophy allows the survival of phytoplankton in oligotrophic waters. To the best of our knowledge mixotrophy has never been reported for Micromonas, which is the dominant picoplankter from our study. We do not know whether the strains isolated here can grow through mixotrophy or if such strategy just allows their survival.

BGD

9, C3778–C3790, 2012

We moved all the discussion related to mixotrophy in Section 4.5.

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) 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.

The Barents Sea has been suggested by previous studies to act as a physical barrier for microbial dispersion, this might explain the geographical isolation of Arctic marine microbes. However to the best of our knowledge it is not known why Arctic marine microbes are less likely to be globally dispersed than Arctic microbes from air or freshwater. We are not aware of any studies clarifying this aspect.

We made all technical corrections suggested except the one related to Figure 1. This figure has been moved to Supplementary Figures as suggested by referee 1. Latitude and longitude of the stations are already indicated in Table 1.

Referee no 3

We agree that coupling microscopy and molecular techniques will improve the taxonomic references for the increasing genetic diversity found in seawater by linking genotypes with morphotypes. However as electron microscopy is highly time consuming, and different taxa may require different preparation techniques we could not analyse by EM all the genotypes found here. Below the responses to specific comments:

l.5: change phytoplankton enrichment to algal medium enrichment

Done

l.10. Heterokontophyta. Change to Ochrophyta, which is synonymous, but preferred by Algaebase (www.algaebase.org).

BGD

9, C3778–C3790, 2012

The term Heterokontophyta is more used than Ochrophyta in the literature. Many recently published papers in journals dealing with phytoplankton taxonomy (e.g. Phycologia and Journal of Phycology) use the term Heterokontophyta.

p. 6223, l. 25. Change "hand pipetting" to "single cell capillary or pipette (as appropriate) isolation" if this was the method used

Done

p. 6224, l. 10. If "Hand isolation" is the same as hand pipetting, use the same term and give if possible a reference to this method.

Corrected, we also included a reference for single cell pipette isolation.

6225, l. 28. Give the names at the same taxonomic level, e.g. at the division level.

Done

p. 6228: Undescribed Mamiellaceae: It would indeed be interesting with examinations by transmission electron microscopy (TEM) of these strains to clarify their identity. Whole mounts are probably enough to show both the flagellar hairs and the scale morphology. p. 6232: Prymnesiophyceae: Again I would encourage using TEM (whole mounts) to clarify the identity of the Haptolina strains. p. 6236: Again I would encourage using TEM (whole mounts) or SEM to clarify the identity of the Pedinellales strains.

We could not characterise all the strains by electron microscopy because this technique is time consuming and the preparation of samples for EM may vary according to the taxon. We plan to perform such studies in the future on selected strains.

p. 6235: I suggest to add Chrysophyceae before Dinobryon and Dictyochophyce before Pedinellales to have an equal taxonomic level for all taxa within Ochrophyta.

Done

BGD

9, C3778–C3790, 2012

p. 6237: Further identification and description of the unidentified pelagophytes (SEM and thin sectioning/TEM) is encouraged in a following manuscript. It could be mentioned what is needed in order to identify these species further.

We added a short paragraph at the end of the discussion mentioning what is needed to further identify the MALINA strains.

Tables: Use the latest taxonomy and confer with Algaebase.

We verified that the latest taxonomy is used, except the usage of Ochrophyta instead of Heterokontophyta for which we do not agree.

Fig. 2. The pictures are beautiful, but it would be easier to find out which species are shown if they are numbered a, b, c, etc.

Corrected

Fig. 3. The font should be larger to be able to read this figure.

We made the font larger

Fig. 6. Ceratium spp. were used as out group, but is an ingroup of dinoflagellates. Argue why this is possible (or use another out group). Make sure that the names follow the latest taxonomy (confer with Algaebase). Use italic for all species names.

Ceratium is indeed a dinoflagellate but it is phylogenetically far from the Suessiales as well as the other genera used in this phylogenetic tree. See Siano et al. 2010.

Supplementary material: Table 1. The text from "Culture were enriched by either.... is unclear and needs to be rewritten.

Rewritten References Balzano, S., D. Marie, et al. (2012). "Composition of the summer photosynthetic pico and nanoplankton communities in the Beaufort Sea assessed by T-RFLP and sequences of the 18S rRNA gene from flow cytometry sorted samples." Isme Journal 6: 1480-1498.

BGD

9, C3778–C3790, 2012

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Interactive comment on Biogeosciences Discuss., 9, 6219, 2012.