

Review of Moal et al: Intriguing diversity among diazotrophic picoplankton

This manuscript presents data on diazotrophic picoplankton distributions in the Mediterranean Sea. The topic is a relevant one and some interesting data is included, particularly regarding *Bradyrhizobium*. The latter should be the main thrust of the paper. Unfortunately the work relating to the recovered plastid sequences is flawed and should be removed entirely. Thus, it is not surprising to me that these plastid sequences are being obtained as a result of non-specific amplification following using a nested PCR protocol. The authors indeed state that in silico studies show the Nitro821 to still be specific for unicellular cyanobacterial diazotrophs, adding weight to the idea that the plastid sequences are a result of mis-priming. The only way to know the obtained 16S rRNA sequences have an identical sequence in the Nitro821 primer region is to find a sequence identical to theirs in Genbank but obtained with a different primer pair that is further toward the end of the 16S rRNA gene than the Nitro821 primer.

The manuscript also contains many typographical errors which need to be addressed (see below)

As it stands the manuscript thus needs major revision.

Specific comments:

Title: Change to 'Intriguing diversity among diazotrophic picoplankton along a Mediterranean transect: a dominance of rhizobia'. (At the very least remove all mention of plastids)

Throughout the manuscript italicise gene names (*nifH* etc). Also, use *Bradyrhizobium* throughout which is the genus name.

Abstract

Line 2: delete been

Line 17: delete the sentence starting 'Surprisingly, the....'

Lines 19-22: re-write these sentences: *Bradyrhizobium* sequences dominated *nifH* clone libraries from picoplanktonic size fractions. A few sequences of γ -proteobacteria were also detected in the central Mediterranean Sea.

Line 25: add comma after particles

Line 26: and using photosynthetic activity....

Line 26-27: Delete sentence beginning: 'Among UCYN further work...'

Introduction:

p8781

Line 5: compared to Redfield (not Redfield's one)

Line 11: punctual peaks

Line 13: replace 'massive' with a more appropriate description (or delete massive)

Line 15: re-write as follows: However, the importance of diazotrophy has been argued to be inconsistent with the known phosphate-starved conditions found in the Mediterranean Sea.....

Line 17: controlled by phosphate...

Line 25: larger ones (not large one)

p8782

Line 11 replace semi-colon with comma (after Foster et al., 2007)

Line 13-14: In addition to being free-living, UCYN have also been suggested to produce mucilage,.....

Line 17: role in global....

Line 20: delete comma after Archaea

Line 21: be specific which subunit of nitrogenase the *nifH* gene encodes

p8783

Line 3: the help of the quantitative....

Line 3-4: technique using the *nifH* gene,

Line 9: especially in the phylum Cyanobacteria

Line 10: ...which quantifies *nifH* gene copies per millilitre....

Lines 14-15: '....to investigate for the hypothetical picoplanktonic UCYN' needs re-phrasing since it is poor English

Line 19-20: ...as low concentration targets may be diluted too much during the different extraction steps.

Line 21-24: DNA, a nested approach using the *nifH* gene was introduced....

(Line 21 it is unclear what partially unsuccessful means here explain why)

Line 25: Planktonic diazotrophic activity has long been attributed....

Line 26:expensive process via photosynthesis (i.e. delete owing to)

Line 27-28:acquire an autonomous carbon

p8784

Line 1: function has also recently been proven to be missing within the UCYN-A group (Zehr et al., 2008).....

Line 3: expression patterns...

Line 6: the entire diazotrophic community....

Lines 8-10: delete these lines and replace with: To begin to address this we used here a combination of approaches: i) to assess the distribution.....

Line 20: diazotroph distributions and

p8786

Line 9: were counterstained with...

Line 17: delete distribution (so sentence reads '....Nitro821-hybridized cells on filters ...')

Line 18: '....on triplicate counts...'

Line 20: This homogeneity allowed us to

p8787

Line 1: '....emission wavelengths of...

Line 11: '....using DNA extraction (Mazard et al., 2004).'

Line 13: '.....was discarded using a vacuum.....

Line 18: '.....following the DNA extraction.....

Line 21: give a reference for the general 27F/1515R primers

p8788

Line 1: '.....in 50 µl volumes. The first PCR used fixed cells on filters as

Line 10: '.....according to the manufacturer's instructions.'

p8789

Line 5: How is the number of 12 *Crocospaera* cells per PCR reaction obtained?

Line 9: '.....primarily quantified using the TSA-FISH technique.

Line 17: '.....using the TSA-FISH technique (Fig. 3).

Line 26: '.....concentrated around the dinoflagellate nucleus.....

p8790

Line 1: delete community

Line 4: DCM depth

Line 11-12: 16S rDNA clone libraries

Lines 13-19: Delete the sentences beginning 'One sequence from station A....' to the end of the paragraph since this is non-specific PCR amplification.

p8791

Line 12: '.....plankton net haul data....

Line 27: delete semi colon after (Goeble et al., 2008) and replace with a comma

p8792

Line 1: 'This latter morphological'

Line 4: 'phylogenetic analysis belonged to group A...

Line 7 and following lines: DELETE ALL OF SECTION 4.2 since it is incorrect

p8794

Line 23: replace dominating with dominant

Line 24: delete 'belonging to a group of'.

Line 24: *Bradyrhizobium*

Line 25-26: In addition to *Bradyrhizobium*,.....

p8795

Line 9: freshwater (one word)

Line 12: rhizobia

Lines 18-19: '...to contribute to two-thirds of...'

Line 21-22: I think remarkable is a little overstating the finding – interesting would be better

Line 23: whose closest relative is the freshwater strain....

Line 28-29: Despite being diazotrophic, phototrophic free-living bacteria.....

p8796

Line 1: (Madigan, 1995; Riemann et al., 2010). We hypothesise though that similar to their closest relatives, the...

Line 3: '....acquire an independent source....'

Line 6: '....proposes hypotheses for the...'

Line 11: '....cyanobacterial| development temperature,.....'

Line 15: '....are considered to be at an ecological advantage compared to....'

Line 18: '....than deeper and nitrate-enriched ones.....'

Line 22-27: Delete these sentences since this is standard biochemistry

p8797

Line 2: inorganic phosphate, whose concentrations were....

Line 5: '...which is considered as the...'

Line 6: replace 'marine ocean' with 'marine waters'

Line 8: '...been shown to grow in deprived....'

Line 11: 'Moreover, the phosphate enrichment....

Line 13: The requirements of these different cell types towards phosphate are not reflected

Lines 15-17: re-write as follows: '....UCYN-B possess a broad spectrum of genes encoding i) a high affinity transport system to acquire inorganic phosphate and ii) for the scavenging of phosphomonoesters, the.....

Lines 18-20: re-write as follows: 'In addition, we provide in this study (Table 3) an analysis of the UCYN-A genome with respect to P which, despite its reduced size, has a similar P genetic toolbox to that of other picoplanktonic.....

Line 22: '....only marine cyanobacterium that has the potential.....

P8798

Lines 1-2: re-write as follows: 'While iron and phosphate seem to control the development of *Trichodesmium* and *Richelia*, temperature has recently been suspected to limit UCYN....

Line 7: delete 'the one'

Line 9: seem slightly relaxed

Line 10-11: We suspect carbon

Line 13: '....making it probably dependent on an unknown....'

Line 20-24: delete from 'Although it has not....' to the end of the paragraph

Line 27: '...show a significant degree...

Line 29: 'limiting factors' not 'the limiting factors'

p8799

Line 3-4: (i) similar to their terrestrial....

Line 4: symbioses

Line 5-6: (ii) *Bradyrhizobium*, the most widely distributed diazotroph in this....

Line 7: delete the phrase 'which by many ways resemble plastids with their genome size and metabolic pathways'

Lines 9-13: delete from 'This close relationship' to the end of the paragraph

Line 14: re-write as follows: 'To answer these hypotheses, further work will be

Line 16: environments.

Lines 16-17: delete the sentence Among UCYN,....

Table 2: Delete all the plastid 16S sequences

Fig 2 Legend :*C. watsonii*....time on different types of DNA template collected.....

Fig. 5 Just show the cyanobacterial sequences and delete the rest of this phylogenetic tree