Characterization of photosynthetic picoeukaryotes from the Southern Adriatic Pit

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BACKGROUND & AIMS

Photosynthetic picoeukaryotes (PPEs) with cell size less than 3 μm are widely distributed in the world oceans and play a critical role in oceanic primary production. Although PPEs are less numerous than their prokaryotic counterparts (*Prochlorococcus*, *Synechococcus*), due to their larger volume they can contribute greatly to global carbon cycling in the sea. Adriatic Sea, the northernmost part of the Mediterranean is a semi-enclosed oligotrophic basin where picophytoplankton is of an extreme importance for the functionality of the ecosystem. Until recently, our knowledge about phytoplankton community in Adriatic was limited to micro- and nanophytoplankton, while pico-fraction except of bacteria was neglected.

Aim of this study was to characterize cultured isolates of Adriatic PPEs using various types of methods: morphological (LM, TEM), chemical (lipid and pigment composition), physiological (growth rate) and phylogenetic characterization based on plastid 16S rRNA and small ribosomal unit 18S rRNA gene data.

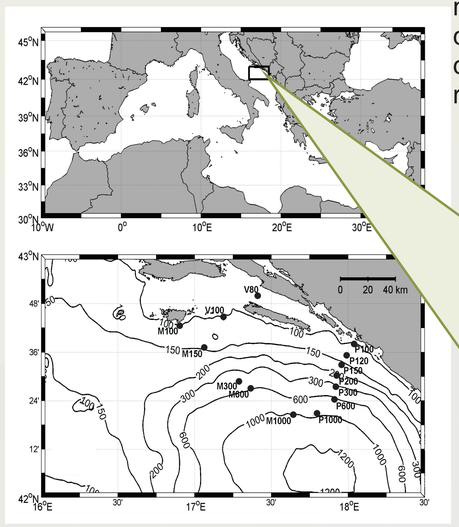


Figure 1. Map of the investigated area with stations.

During **BIOTA** cruise in February/March 2015, along two transects in Southern Adriatic Pit (Figure 1.), several clones of picoeukaryotes were isolated, cultivated and analysed using imaging, chemical and molecular methods. Photosynthetic picoeukaryotes were isolated from stations where flow cytometry analysis revealed their high abundances at greater depths (>280 m) well below photosynthesis active irradiance (PAR) values. After growth in mixed cultures, picoeukaryotic cells were filtered into a fresh medium through 3.0-μm-pore-size polycarbonate membrane filters and serial diluted by transferring in a repeated fashion a sub volume of a culture (1/10) to a fresh medium (9/10) to obtain statistically one cell per tube at the end of the series. Photosynthetic picoeukaryote plastid 16S rRNA gene and 18S rRNA gene were amplified with two set of primers - PLA491F/OXY1313R and SSU1/ITS1DR respectively.

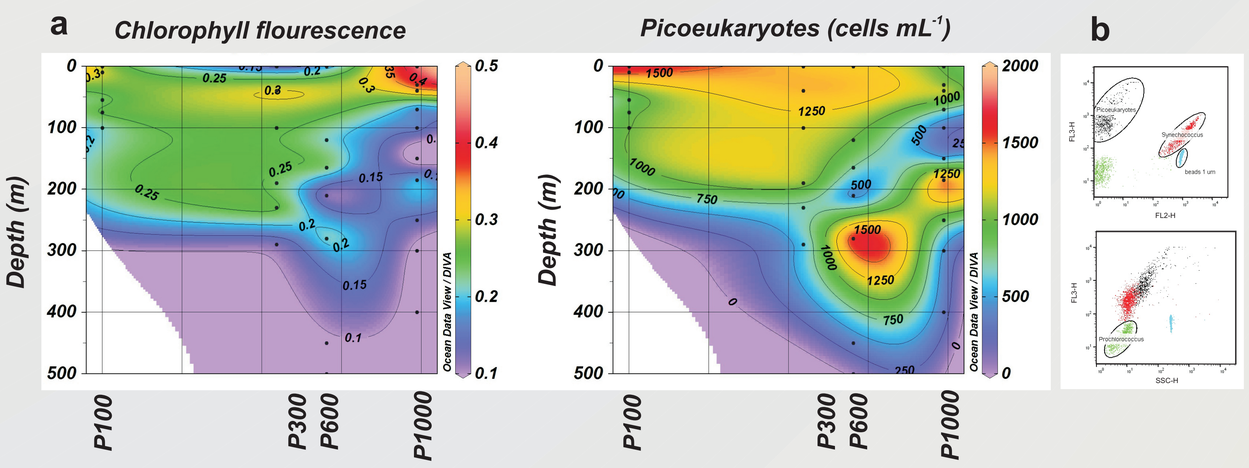


Figure 2. a - **P-transect** (P100 - P1000) ODV profiles of chlorophyll *a* fluorescence and abundances of total PPE community; **b** - Cytograms of deep samples from 280m, station P600 showing picophytoplankton populations (*Prochlorococcus*, *Synechococcus* and PPEs).

RESULTS

Flow cytometry analysis revealed total PPE community abundance varying among stations, but most interesting station was P600 where they dominated samples from greater depths (>280 m) well below photosynthesis active irradiance (PAR) values (**Figure 2**). PPEs abundance reached up to 4 x 10^6 cells L^{-1} in environmental samples. In laboratory growth experiment their abundance reached up to 3 x 10^7 cells mL^{-1} in the exponentional phase.

Morphological analysis of four isolated clones did not reveal any difference in LM or TEM. Both, LM and TEM distinghuished picoeukaryote features that are present in other Trebouxiophyceae (**Figure 3, 4**). Detected pigments in all cultivated clones were chlorophyll *a*, *b*, lutein, β-carotene, violaxanthin and neoxanthin.

Phylogenetic analysis of 16S rRNA partial sequences revealed 99-100% similarity with genera *Nannochloris*, *Picochlorum* and some uncultured eukaryote clones while 18S rRNA partial sequences showed bigger similarity towards genus *Picochlorum* (**Figure 5, 6**).

Lipid composition demonstrated clear domination of glycolipid sulfoquinovosyldiacylglycerol, on average 34% among all lipid classes (**Figure 7**).

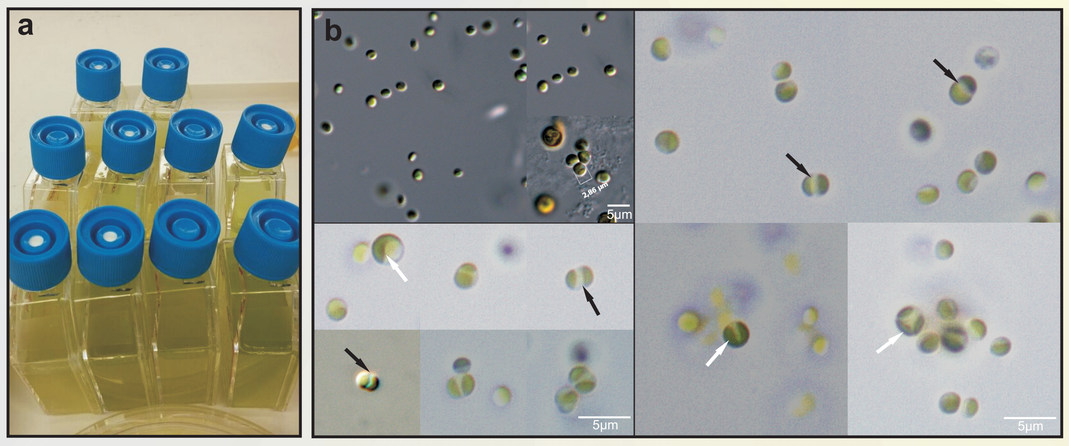


Figure 3. a - Batch cultures of various picoeukaryotes from BIOTA cruise; **b** - light micrographs of studied strains of *Picochlorum* sp.; white arrows indicating one large lobbed chloroplast, black arrows indicating cells in division (autosporulation) and mother cell wall.

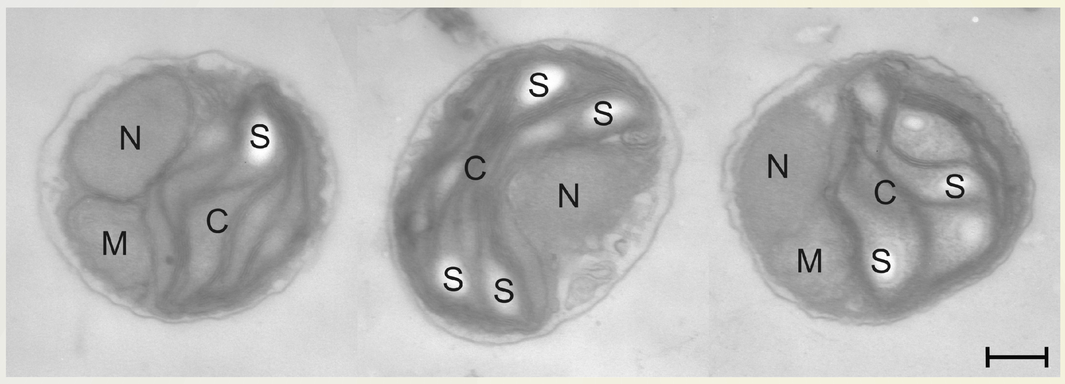


Figure 4. Transmission electronic micrographs of *Picochlorum* sp. N – nucleus, C – chloroplast, M – mitochondrion, S – starch inclusions. Scale bar 300 nm.

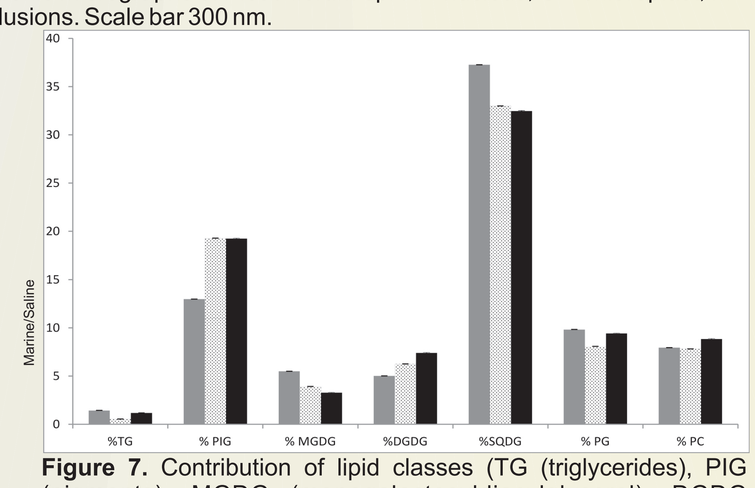
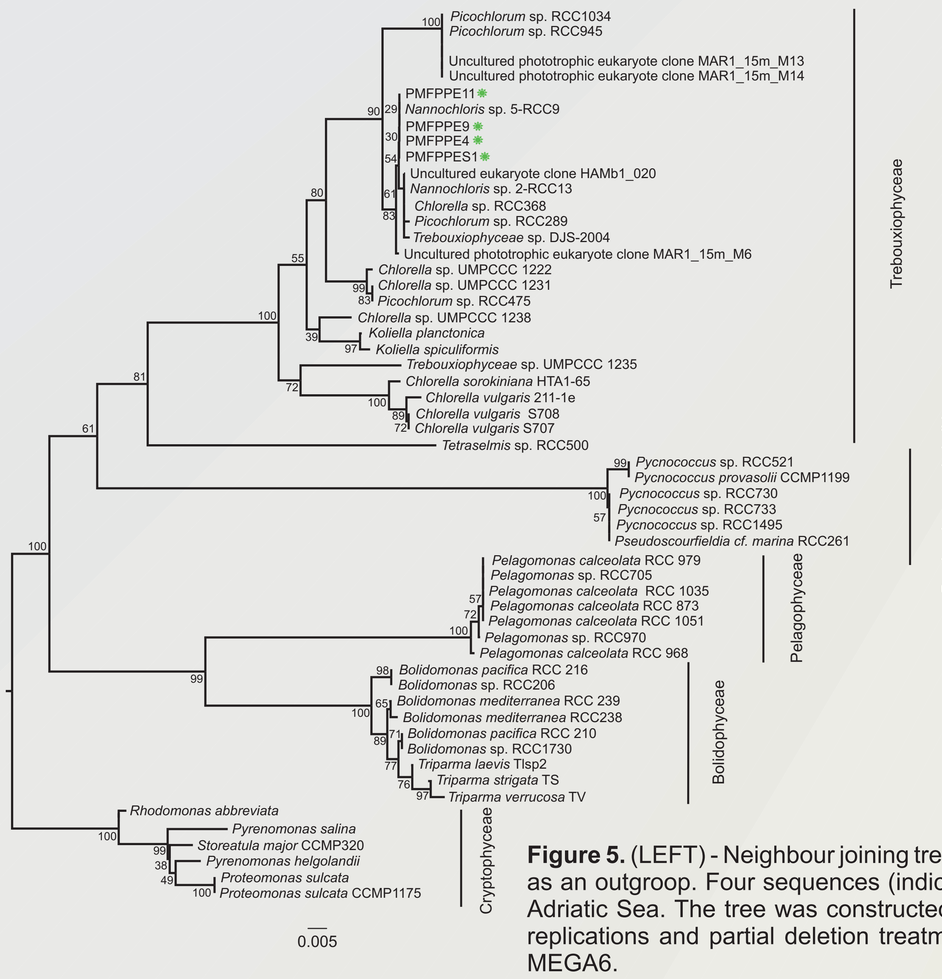


Figure 7. Contribution of lipid classes (TG (triglycerides), PIG (pigments), MGDG (monogalactosyldiacylglycerol), DGDG (digalactosyldiacylglycerol), SQDG (sulfoquinovosyldiacylglycerol, PG (phosphatidylglycerol), PC (phosphatidylcholin)) to total lipids given in percentages. Data are given for samples PMFPPE9 (grey), PMFPPE11 (dotted) and PMFPPE4 (black).



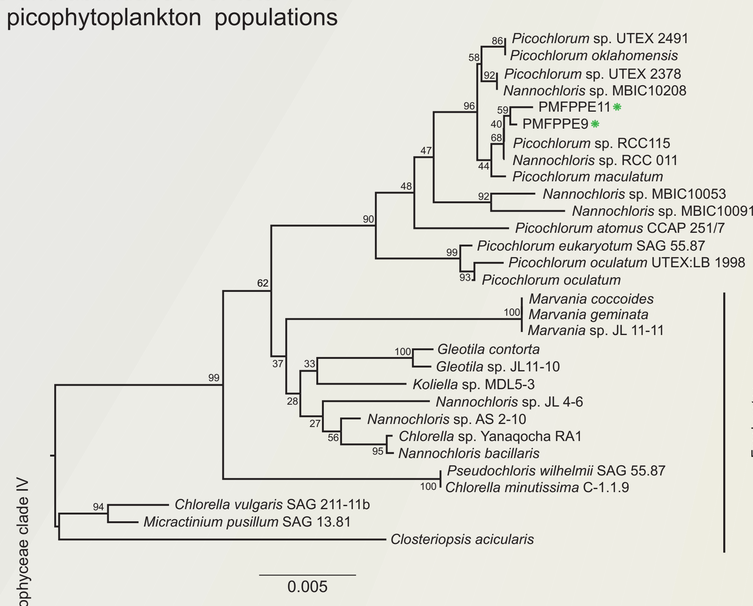


Figure 6. (RIGHT) - Neighbour joining tree of 18S rRNA partial sequences from *Nannochloris*-like taxa (indicated with green star) rooted with *Chlorella vulgaris*, *Micractinium pusillum* and *Closteriopsis acicularis* as the outgroup (-Ln likelihood – 2959.666). Two sequences (indicated with green star) were retrieved from cultivated strains from Adriatic Sea. Tree was constructed using Jukes-Cantor (JC + G) model with 1,000 bootstrap replications and partial deletion as treatment for missing data. Evolutionary analyses were conducted in MEGA6.

Figure 5. (LEFT) - Neighbour joining tree of plastid 16S rRNA gene sequences rooted with Cryptophyceae as an outgroop. Four sequences (indicated with green star) were retrieved from cultivated strains from Adriatic Sea. The tree was constructed using Jukes-Cantor (JC + G) model with total 1,000 bootstrap replications and partial deletion treatment for missing data. Evolutionary analyses were conducted in MEGA6.

DISCUSSION & CONCLUSION

Combining various methods in investigating Adriatic PPE strains allowed us to characterize our cultivated picoeukaryote as ***Picochlorum* sp.**. Morphology results distinguished our strains from sister genus *Nannochloris* with type of cell division (autosporulation in opposite od binary fission, respectively) while phylogeny analysis differently positioned Adriatic isolates based on two molecular markers (16S DNA and 18S DNA).

Based on **16S rRNA** gene sequence phylogeny four Adriatic strains are more likely to be *Nannochloris* that can be explained with marker preface to environmental samples. On the other hand, phylogeny based on **18S rDNA** gene , as shown in Fig. 6, Adriatic strains PMFPPE9 and PMFPPE11 are more likely to be *Picochlorum* sp.

Finally, lipid analysis of monocultures revealed very high lipid content with majority of **SQDG** which contains sulfur and sugar instead of phosphate. Since PPE is isolated from oligotrophic environment and cultivated in F2 medium with high phosphorous content we presume that high sulfolipid contentrepresents **evolutionary adaptation** of this picoeukaryote by synthesing high content of SQDG instead of phospholipids no matter on the environmental condition where they grow.

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