

Károly Pálffy^{1*}, Tamás Felföldi², Anikó Mentés², Hajnalka Horváth¹, Károly Márialigeti²,
Emil Boros³, Lajos Vörös¹, Boglárka Somogyi¹

**Unique picoeukaryotic algal community under multiple environmental stress conditions
in a shallow, alkaline pan**

Original publication in: *Extremophiles* (2014) **18**: 111-119

¹Balaton Limnological Institute, Centre for Ecological Research, Hungarian Academy of Sciences,
Klebelsberg Kuno u. 3., H-8237 Tihany, Hungary

² Department of Microbiology, Institute of Biology, Eötvös Loránd University,
Pázmány Péter stny. 1/c, H-1117 Budapest, Hungary

³Kiskunság National Park Directorate,
Liszt Ferenc u. 19., H-6000 Kecskemét, Hungary

*corresponding author:

e-mail: palfy.karoly@okologia.mta.hu

telephone: +36-87-448244

fax: +36-87-448006

Abstract

Winter phytoplankton communities in the shallow alkaline pans of Hungary are frequently dominated by picoeukaryotes, sometimes in particularly high abundance. In winter 2012 the ice-covered alkaline Zab-szék pan was found to be extraordinarily rich in picoeukaryotic green algae ($42\text{--}82 \times 10^6$ cells ml⁻¹) despite the simultaneous presence of multiple stressors (low temperature and light intensity with high pH and salinity). The maximum photosynthetic rate of the picoeukaryote community was $1.4 \mu\text{g C } \mu\text{g chlorophyll } a^{-1} \text{ h}^{-1}$ at $125 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The assimilation rates compared with the available light intensity measured on the field show that the community was considerably light-limited. Estimated areal primary production was

180 mg C m⁻² d⁻¹. On the basis of the 18S rRNA gene analysis (cloning and DGGE), the community was phylogenetically heterogeneous with several previously undescribed chlorophyte lineages, which indicates the ability of picoeukaryotic communities to maintain high genetic diversity under extreme conditions.

Keywords: picoeukaryotes, alkaline pan, winter algal bloom, ice cover, primary production, genetic diversity

Introduction

Algae and cyanobacteria of the pico (<2 µm) size range, commonly referred to as photoautotrophic picoplankton (PPP), are frequently occurring members of the phytoplankton (Callieri and Stockner 2002), and are often major contributors to primary production in marine and freshwater environments (Stockner and Antia, 1986; Gaulke et al. 2010). Their discovery was closely related to the spreading application of epifluorescence microscopy and since that time our knowledge on these organisms has been steadily expanding. While *in situ* measurements and focused experimental efforts have provided a substantial amount of information on their ecology, their phylogenetic identification requires the use of molecular biological methods, since their small size mostly hinders microscopic identification on lower taxonomic levels (Callieri 2008). In several cases, the major picoplanktonic groups, picocyanobacteria and picoeukaryotes have been found to show characteristic temporal dynamics with their relative abundance highly depending on various physicochemical factors, such as light intensity, nutrient availability (Bec et al. 2011, Winder 2009) or temperature (Mózes et al. 2006).

There is an increasing amount of information on the ecology and phylogenetic community composition of autotrophic picoplankton found in the shallow lakes of Hungary (Vörös et al. 1991; Felföldi et al. 2009). Shallow alkaline pans in the central part of Hungary are particularly rich in PPP communities (Vörös et al. 2005). These turbid, hyposaline ponds with a pH of 9–10 and Na⁺ and HCO₃⁻ as dominant ions are extreme habitats for algae, in terms of light conditions in particular, because the high amount of suspended solids and humic substances characteristic for these waters always result in very low Secchi-disk transparency (1-2 cm) (Vörös et al. 2006; Felföldi et al. 2009). The various groups of autotrophic picoplankton show characteristic seasonal dynamics in these lakes: while

picocyanobacteria dominate in summer, winter communities exclusively consist of picoeukaryotes (Somogyi et al. 2009).

Weather conditions in winter 2012 allowed for the build-up of a 6-25 cm thick ice cover on these pans. In spite of the multiple environmental stress of constantly low temperature, low underwater light intensity and moderately high salinity and pH, the water of Zab-szék pan was bright green as a result of a highly abundant PPP community thriving under the ice. Since the occurrence of such phenomena is rarely documented in the temperate zone, the aim of the present study was to determine the abundance of this photoautotrophic picoplankton community, to explore its phylogenetic diversity, and to assess their efficiency as primary producers through measuring their photosynthesis along a light intensity gradient at ambient temperature.

Materials and methods

Site description, sampling and in situ measurements

Zab-szék is a shallow (5–40 cm deep), turbid alkaline pan with a surface area of 100 ha situated in the Kiskunság region in the central part of Hungary (geographical map is presented in Felföldi et al. 2009). In February 2012 the pan was completely covered with 6-25 cm thick ice. On 15 February water samples were taken at seven stations through holes cut in the ice (Fig. 1) with the simultaneous measurement of physical and chemical variables including temperature, pH, conductivity and dissolved oxygen (DO) concentration, all of which were determined with a WTW MultiLine portable field meter (P 8211). Light attenuation of the ice cover was measured with a LI-COR quantum sensor (2π).

Laboratory measurements

Freshly collected samples were immediately transported into laboratory for additional measurements. Total suspended solids (TSS) content was determined gravimetrically after sample filtration on 0.4 μm pore size cellulose acetate filters. The concentration of chromophoric dissolved organic matter (CDOM) was determined as Pt colour. Briefly, water samples were filtered through GF-5 glass fibre filters, buffered with borate buffer, and subsequently absorption was measured against a blank at 440 and 750 nm (Kirk 1996) using a

Shimadzu UV 160A spectrophotometer. After correcting sample absorbance at 440 with that of the blank at 440 nm and the sample at 750 nm, Pt unit was calculated with the formula of Cuthbert and del Giorgio (1992). Chlorophyll *a* concentration was determined spectrophotometrically after hot methanol extraction using the absorption coefficients determined by Wellburn (1994).

The concentration of different nitrogen and phosphorus forms were also determined as follows. Ammonium (NH_4^+) concentration was measured with the indophenol-blue method (Mackereth et al. 1989), nitrate (NO_3^-) analysis was performed with the Cd-reduction method (Elliott and Porter 1971), while the amount of urea was determined as described by Newell et al. (1967). The amount of total phosphorus (TP) was measured according to Gales et al. (1966); briefly, all the phosphorus forms were transformed into PO_4^{3-} by dissolving the samples in $\text{K}_2\text{S}_2\text{O}_8$ for one hour at 121°C . After adding 5.4% ascorbic acid and a reagent solution (14% H_2SO_4 , 3% $(\text{NH}_4)_2\text{MoO}_4$ and 0.34% $\text{C}_4\text{H}_4\text{KO}_7\text{Sb}$) the absorbance of the mixture was measured at 890 nm using a SHIMADZU UV-VIS spectrophotometer. Soluble reactive phosphorus (SRP) was determined according to the method of Murphy and Riley (1962).

Microscopic analysis

Photoautotrophic picoplankton was examined from freshly collected samples immediately after transport to laboratory. 0.5–1 ml subsamples were filtered onto 0.4 μm pore sized black polycarbonate filters, which were subsequently embedded into 50% glycerol on a microscopy slide. The slides were examined with a Nikon Optiphot 2 epifluorescence microscope at 1,000x magnification using blue–violet (BV-2A) and green (G-2A) excitation light. 20 fields (~400 cells) were photographed with a Spot RT colour camera and PPP was counted on the images to avoid fluorescence fading. Picoeukaryotes fluoresce vivid red under blue–violet excitation and show no or only weak fluorescence when excited with green light. Picocyanobacteria can be distinguished from eukaryotes owing to the presence of phycobiliproteins, which exhibit greatly enhanced red fluorescence when using the green wave band. Phycoerythrin-rich picocyanobacteria fluoresce bright yellow, while phycocyanin-rich picocyanobacteria show weak red autofluorescence in blue–violet excitation light (MacIsaac and Stockner 1993). Photoautotrophic picoplankton abundance was converted

to biomass by measuring the dimensions of 200 cells with dia-illumination, calculating their biovolume and considering an average density of 1 g cm^{-3} .

Photosynthesis measurement

Photosynthesis of the photoautotrophic picoplankton community was measured using the ^{14}C -technique (Steemann Nielsen 1952). The sample collected at station 4 was diluted to $20 \mu\text{g l}^{-1}$ chlorophyll *a* with filtered water from the pan to reduce self-shading. 20 ml subsamples of the diluted sample were put into glass vials, preincubated for 1 hour, and after adding $\text{NaH}^{14}\text{CO}_3$ (0.084 MBq) incubated for 3 hours in triplicates in a water bath (1°C) under cool white fluorescent illumination of 6, 10, 26, 66, 222, 390, 706 and $1363 \mu\text{mol m}^{-2} \text{ s}^{-1}$. For dark carbon uptake three vials were incubated in darkness. Samples were subsequently filtered through $0.45 \mu\text{m}$ pore size cellulose-acetate membrane filters and put into HCl vapour to remove remaining inorganic ^{14}C . Next the filters were dissolved in 10 ml Bray scintillation mixture, after which radioactivity was measured with an LKB 1211-RACKBETA liquid scintillation counter. Photosynthesis-irradiance (P-I) curves were fitted using the model of Eilers and Peeters (1988) with the data analysis software OriginPro 7.5. Daily areal primary production was estimated using the P-I curves and the light intensity profile of the water column. Average ambient light intensity for each hour was calculated from the hourly sums of global radiation for the day of sampling provided by the Hungarian Meteorological Service, assuming that $1 \text{ W m}^{-2} = 4.6 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Wetzel and Likens 2000). Photosynthetically active radiation (PAR, 400-700 nm) was considered to be 47% of global radiation (Wetzel and Likens 2000). Underwater light intensity was calculated for each mm of the water column using the vertical light attenuation coefficient determined by the empirical formula of V.-Balogh et al. (2009) on the basis of the chlorophyll *a* concentration, total suspended solids and Pt colour. Light attenuation of the ice cover was also taken into account. Areal primary production can thus be estimated for each hour, assuming that (1) photoautotrophic picoplankton is able to yield net photosynthesis at light intensities as low as $2 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and (2) the P-I curve is constant throughout the day.

DNA-based identification of phytoplankton community members

Extraction of total plankton genomic DNA was performed from a concentrated water sample collected at station 4 (centrifugation at 3000 g, 10 min) with the UltraClean™ Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) with slight modifications on the instructions given by the manufacturer as described in Borsodi et al. (2013). An approximately 1000 nt length fragment of the 18S rRNA gene was amplified by PCR using primers Euk328F and Chlo02R as described in Somogyi et al. (2013, accepted). Amplicons were examined by electrophoresis in 1% (w/v) agarose gel stained with GR Safe DNA Stain (Innovita, Gaithersburg, MD, USA). Prior to cloning and sequence analysis, PCR products were purified with the EZ-10 Spin Column PCR Product Purification Kit (Bio Basic, Markham, Canada). Clone library construction was performed with the pGEM-T Easy Vector System (Promega, Madison, WI, USA) according to the instructions given by the manufacturer. Sequence analysis was performed with clones having different restriction patterns generated with the endonucleases *Csp6I* and *Hin6I* (Fermentas, Vilnius, Lithuania). A Chlorophyta-specific denaturant gradient gel electrophoresis analysis (DGGE) with a gradient of denaturants from 40 to 70% was also carried out using a shorter fragment (approximately 400 nt length) amplified with primers Euk528F and Chlo02R as described by Keresztes et al. (2012). Sequencing reaction and capillary electrophoresis were performed by Biomi Ltd. (Gödöllő, Hungary). Trebouxiophycean clones were sequenced from both directions using primers M13F and M13R, while sequencing the DNA excised from dominant DGGE bands were performed with primer Chlo02R. Chromatograms were analyzed as described in Felföldi et al. (2009). Chimeric sequences were removed using mothur (Schloss et al. 2009). Sequence alignment of clones and sequences obtained from GenBank was performed with SINA (Pruesse et al. 2012), and phylogenetic analysis including the search for the best-fit model was conducted with the MEGA 5 software (Tamura et al. 2011). Chlorophyte clone sequences obtained in this study were submitted to GenBank under the accession numbers KC879102-KC879111.

Results and discussion

Physical and chemical characteristics

Basic physical and chemical data of the stations measured on the day of the sampling are presented in Tables 1 and 2. Average water depth of the pan was 29 cm, while ice cover thickness varied between 6 and 25 cm among the sampling stations (Table 1). Little variation was observed in pH with an average of 9.63, conductivity varied between a minimum of 8260 and a maximum of 9450 $\mu\text{S cm}^{-1}$, which is basically similar to the values measured during former studies on the lakes of this area (Felföldi et al. 2009; Somogyi et al. 2009). This high ion concentration indicated by the conductivity values allowed for water temperatures mostly below 0°C. DO concentration showed supersaturation (129–157 %), suggesting net phytoplankton photosynthesis under the ice cover.

TSS generally exceeded 100 mg l^{-1} , except at stations 2 and 5 with 56 and 94.5 mg l^{-1} , respectively (Table 2). CDOM concentration, expressed as Pt units, showed little variation with an average of 240 mg l^{-1} . Urea–N was the dominant N form, and SRP constituted a large part of the TP pool varying between 2234 and 3176 $\mu\text{g l}^{-1}$. The amount of biologically available P was one order of magnitude higher than that of the sum of the N forms, however, both N and P concentrations show that phytoplankton is not limited by nutrients, mainly due to the substantial external loads from aquatic birds (Boros et al. 2008).

Picoeukaryote abundance and primary production

Chlorophyll *a* concentration reached exceedingly high levels of 743–1039 $\mu\text{g l}^{-1}$ (Table 3), which, together with the TP concentration, implies strongly hypertrophic conditions. The phytoplankton was exclusively composed of photoautotrophic picoplankton (nano- and microplankton were not found). Chlorophyll *a* content varied between 0.7 and 1.0% of total biomass. In other shallow lakes of the region average relative chlorophyll *a* content of the phytoplankton varied between 0.25 and 0.78 % (Vörös and Padisák 1991). Since chlorophyll *a* content per unit biomass is inversely related to cell volume, we may assume that the higher values found in Zab-szék pan may be associated with the smaller average cell size in the community. On the other hand, extremely low light intensity can also cause increased cellular chlorophyll *a* content regardless of cell size. High chlorophyll contents per biovolume were also found in the deep hypolimnion of a high mountain lake during the winter cover period associated with small cells (Felip and Catalan 2000). The abundance of PPP in Zab-szék pan showed high values ranging from 42 to 82 $\times 10^6$ cells ml^{-1} and consisted of single-celled

picoeukaryotes. Similar values were found in winter 2006-2007 (Somogyi et al. 2009), which implies that this winter picoeukaryote bloom is a regularly occurring phenomenon in this pan.

Just as in the present study, winter photoautotrophic picoplankton assemblages have generally been found to be eukaryotic in other lakes, such as in the case of the large, shallow Lake Balaton. However, the maximum abundance of that PPP community was two orders of magnitude smaller than the values presented here (Vörös et al. 2009). Other saline lakes at various locations around the globe can similarly be favourable habitats for photoautotrophic picoplankton blooms (Fanjing et al. 2009; Wu et al. 2009; Krienitz et al. 2012), although some lakes have been found to be dominated also by other taxa above the pico size range (García et al. 1997; Harper et al. 2003; Schagerl and Oduor 2008). In several East African soda lakes picoeukaryote abundances can exceed 3 million cells ml⁻¹ (Krienitz et al. 2012), while Fanning et al. (2009) found a pico-sized green alga (*Picocystis* sp.) in a Mongolian soda lake in high abundance throughout the year, even under the ice, which interestingly resembles the situation presented here.

As seen on the photosynthesis–irradiance (P–I) curve in Fig. 2, the maximum photosynthetic rate of the picoeukaryote community was moderately low, reaching 1.4 µg C µg chlorophyll *a*⁻¹ h⁻¹ at 125 µmol m⁻² s⁻¹. The day of the sampling was overcast, which caused low light intensity values with a daily maximum of ~400 µmol m⁻² s⁻¹. The ice cover dramatically reduced ambient PAR by 96% on average, which meant a maximum light intensity of 18.2 µmol m⁻² s⁻¹ under the ice (Fig. 3), rapidly decreasing with depth. These light intensity values clearly show that the community was extremely light-limited. Winter primary production in this alkaline pan is primarily dependent on the amount of light rather than on nutrient availability, the same way as it was found in an ice-covered humic, boreal lake (Tulonen et al. 1994). On the basis of the P–I curve (Fig. 2) and assuming that the picoeukaryotes can potentially utilize PAR intensity as low as 2 µmol m⁻² s⁻¹, estimated areal primary production shows a maximum of 30 mg C m⁻² h⁻¹ and a daily sum of 180 mg C m⁻² d⁻¹ in Zab-szék pan, although this value can vary considerably as a function of ambient light intensity and also due to temporal changes in the P-I relationship. Similar winter values of 100–140 mg C m⁻² d⁻¹ were found in the mesotrophic eastern basin of Lake Balaton (Herodek and Tamás 1976; Herodek et al. 1982), in spite of a much smaller phytoplankton biomass, since lower light attenuation resulted in higher assimilation rates (1–4 µg C µg chl *a*⁻¹ h⁻¹) along a substantially deeper water column of 3.5 m. Even higher winter assimilation rates can be found in marine photoautotrophic picoplankton, such as those reported by Han and Furuya

(2000), however, both temperature and light attenuation were more moderate and favourable for photosynthesis. In comparison, the particularly low light conditions and temperature under the ice cover of the Zab-szék pan potentiated approximately $0.5 \mu\text{g C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$ at most for the picoeukaryotic community, which decreased to a negligible amount within the upper 10 cm. The ability of these picoeukaryotes to yield net primary production and reach such a high abundance under these extreme conditions within a few centimetres of water column makes this community particularly unique.

Community composition

In total, 85 clones were analyzed, which formed 37 clone groups based on their different restriction pattern. After removing putative chimeric sequences (6.3% of total number of clones), 23.8% of clones were affiliated with green algae. Most chlorophyte sequences (94.6%) were related to Trebouxiophycean isolates and formed three groups on the phylogenetic tree (Fig. 4). The main clone group (containing 42.1% of green algal clones, represented by clones AV-5, AV-12, AV-23 and AV-36) formed a cluster distant from all known strains, and showed the highest pairwise similarity values, 96.7-98.0%, to *Micractinium pusillum* SAG 48.93 or '*Nannochloris*' sp. AS 2-10. Within this cluster similarity ranged from 97.1 to 99.2%. Although there is no general pairwise similarity value of 18S rRNA gene that defines Trebouxiophycean genera, similarity values around 97-98% were accepted as genus level divergence among isolates in taxonomic revisions or recent species descriptions (Henley et al. 2004, Krienitz et al. 2004, Somogyi et al. 2011, 2013). Therefore this cluster may represent one or two previously unidentified genera.

Another group, represented with clones AV-17 and AV-60 showed 98.1-98.7% nucleotide similarity values with *Marvania* species. The third group of Zab-szék clones, was affiliated with the recently described *Chloroparva* and the revised *Pseudochloris* genera (Somogyi et al. 2011, 2013) with relatively low pairwise similarity values, 97.3-97.9%, and therefore represented additional potential new species or genera. Type strain of *Chloroparva pannonica* was isolated from a soda pan in the same region close to Zab-szék (Somogyi et al. 2011), an additional isolate from this genus was retrieved from Zab-szék itself (ACT 0602) and *Pseudochloris* isolates also originated from saline (marine) habitats (Henley et al. 2004; Somogyi et al. 2013).

The sequence of clone AV-16 showed 97.6% pairwise nucleotide sequence similarity to the small green flagellate, *Marsupiomonas pelliculata* PCC 441 (Pedinophyceae). Cells of this species are slightly larger than the picoplankton size, and have an approximate length of 3.0 μm , width of 2.3 μm and maximum thickness of 1.8 μm with a single 8-10 μm long flagellum (Jones et al. 1994). Members of this genus are inhabitants of marine and brackish environments, and are able to grow up to 3.8% salinity (Jones et al. 1994, Vaultot et al. 2008).

All other clones were distantly related to protists or invertebrates, most of these clones shared typical pairwise similarity values of 90-93% with cercozoan sequences (data not shown). Since there are known potential biases associated with the cloning of DNA fragments having different length (i.e. due to introns may present in the 18S rRNA gene of green algae; Palatinszky et al. 2011, Somogyi et al. 2011), and additionally the relatively low number of phytoplankton clones did not allow proper quantitative comparison of phototrophic taxa present in the sample, a DGGE analysis was also carried out. There were two major bands observable in the gel (data not shown). The shorter sequences from the excised bands (approximately 350 nt compared to the approximate 950 nt length of clone sequences) provided limited phylogenetic information compared to the sequence analysis of clones, but on the other hand, revealed the two major green algal genotype of the sample. One of these two dominant DGGE bands was identical with the sequence of clone AV-16, while the other major band was identical with clone AV-5 (data not shown).

The detected various chlorophyte genotypes indicated a genetically heterogenous community of phototrophic picoeukaryotes in Zab-szék pan. Our previous study focusing on picocyanobacteria in the soda pans of the same region also found relatively diverse communities (Felföldi et al. 2009). Similarly to our results, potentially new chlorophyte taxa were also described by Luo et al. (2013) in an African soda lake (Lake Nakuru), which suggests that the biodiversity of these unique water bodies is still unrevealed.

Conclusions

Our results on the picoeukaryote community in the alkaline pan Zab-szék in winter 2012 have shown that these organisms have the potential to actively photosynthesize and reach extraordinarily high abundance under multiple environmental stress conditions. Instead of the well-known picoeukaryote inhabitants of saline lakes and ponds, such as the members of the genera *Picochlorum* and *Picocystis* (Lewin et al. 2000; Henley et al. 2004; Fanjing et al.

2009; Wu et al. 2009; Krienitz et al. 2012), *Chloroparva pannonica* has been found to be the only characteristic species of these alkaline pans until now. On the other hand, the present study has found a genetically diverse community with a relatively high number of previously unidentified green algal taxa.

The formation of such a winter community may be caused by the combination of various factors, e.g. high inorganic nutrient content (Boros et al. 2008) and the putative lack of efficient grazers at low temperature (simultaneous absence of bottom-up and top-down control). One could also raise the question: What enables these picoeukaryotes to gain absolute dominance in these hypertrophic water bodies? This may be the outcome of adaptation to this peculiar environment, however, this assumption cannot be confirmed on the basis of a single sampling and without more sophisticated laboratory experiments. Testing this hypothesis would require a comprehensive ecophysiological study on the isolated strains representing the community, with a focus on the effect of light, temperature and salinity on the enzyme content and/or activity of the cells. A comparison with phylogenetically related taxa from other habitats would also be desirable.

Acknowledgements

The authors are gratefully indebted to Balázs Németh, Zsolt Gyula Keresztes, Tamás Sági and Éva Koltai for technical assistance. This work was financially supported by the NKTH-OTKA CNK 80140 and the EnvEurope Life Environment LIFE08ENV/IT/000339 projects. B.S. and T.F. were supported by the Bolyai János Research Grant (Hungarian Academy of Sciences).

References

- Bec B, Collos Y, Souchu P, Vaquer A, Lautier J, Fiandrino A, Benau L, Orsoni V, Laugier T (2011) Distribution of picophytoplankton and nanophytoplankton along an anthropogenic eutrophication gradient in French Mediterranean coastal lagoons. *Aquat Microb Ecol* 63:29–45
- Boros E, Nagy T, Pigniczki C, Kotymán L, V-Balogh K, Vörös L (2008) The effect of aquatic birds on the nutrient load and water quality of soda pans in Hungary. *Acta Zool Hung* 54:207–224

- Borsodi AK, Felföldi T, Máthé I, Bognár V, Knáb M, Krett G, Jurecska L, Tóth EM, Márialigeti K (2013) Phylogenetic diversity of bacterial and archaeal communities inhabiting the saline Lake Red located in Sovata, Romania. *Extremophiles* 17:87–98
- Callieri C, Stockner G (2002) Freshwater autotrophic picoplankton: a review. *J Limnol* 61:1–14
- Callieri C (2008) Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. *Freshwat Rev* 1:1–28
- Cuthbert ID, del Giorgio P (1992) Toward a standard method of measuring color in freshwater. *Limnol Oceanogr* 37:1319–1326
- Eilers PHC, Peeters JCH (1988) A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecol Model* 42:199–215
- Elliott RJ, Porter AG (1971) A rapid cadmium reduction method for the determination of nitrate in bacon and curing brines. *The Analyst* 96:522–527
- Fanjing K, Qinxian J, Jia E, Mianping Z (2009) Characterization of a eukaryotic picoplankton alga, strain DGN-Z1, isolated from a soda lake in inner Mongolia, China. *Nat Resour Env Iss* 15:185–189
- Felföldi T, Somogyi B, Márialigeti K, Vörös L (2009) Characterization of photoautotrophic picoplankton assemblages in turbid, alkaline lakes of the Carpathian Basin (Central Europe). *J Limnol* 68:385–395
- Felip M, Catalan J (2000) The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: decoupling in their spatial and temporal maxima. *J Plankton Res* 22:91–105
- Gales ME JR., Julian EC, Kroner RC (1966) Method for quantitative determination of total phosphorus in water. *J Amer Water Works Assoc* 58:1363–1368
- García CM, García-Ruiz R, Rendón M, Niell FX, Lucena J (1997) Hydrological cycle and interannual variability of the aquatic community in a temporary saline lake (Fuente de Piedra, Southern Spain). *Hydrobiologia* 345:131–141
- Gaulke AK, Wetz MS, Paerl HW (2010) Picophytoplankton: A major contributor to planktonic biomass and primary production in a eutrophic, river-dominated estuary. *Estuar Coast Shelf S* 90:45–54
- Han M-S, Furuya K (2000) Size and species-specific primary production and community structure of phytoplankton in Tokyo Bay. *J Plankton Res* 22:1221–1235

- Harper DM, Childress RB, Harper MM, Boar RR, Hickley PH, Mills SC, Otieno N, Drane T, Vareschi E, Nasirwa O, Mwatha WE, Darlington JPEC, Escuté-Gasulla X (2003) Aquatic biodiversity and saline lakes: Lake Bogoria National Reserve, Kenya. *Hydrobiologia* 500:259–276
- Henley WJ, Hironaka JL, Guillou L, Buchheim MA, Buchheim JA, Fawley MW, Fawley KP (2004) Phylogenetic analysis of the 'Nannochloris-like' algae and diagnoses of *Picochlorum oklahomensis* gen. et sp. nov (Trebouxiophyceae, Chlorophyta). *Phycologia* 43:641–652
- Herodek S, Tamás G (1976) A fitoplankton tömege, termelése és a Balaton eutrofizálódása. *Hidrológiai Közlöny* 56/5:219–228
- Herodek S, Vörös L, Tóth F (1982) The mass and production of phytoplankton and the eutrophication of Lake Balaton. *Hidrológiai Közlöny* 62/5:220–229 (in Hungarian with English summary)
- Jones HLJ, Leadbeater BSC, Green JC (1994) An ultrastructural study of *Marsupiomonas pelliculata* gen. et sp. nov., a new member of the Pedinophyceae. *Eur J Phycol* 29:171–181
- Keresztes ZG, Felföldi T, Somogyi B, Székely G, Dragoş N, Márialigeti K, Bartha C, Vörös L (2012) First record of picophytoplankton diversity in Central European hypersaline lakes. *Extremophiles* 16:759–769
- Kirk JTO (1996) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge
- Krienitz L, Hegewald EH, Hepperle D, Huss VAR, Rohr T, Wolf M (2004) Phylogenetic relationship of *Chlorella* and *Parachlorella* gen. nov. (Chlorophyta, Trebouxiophyceae). *Phycologia* 43:529–542
- Krienitz L, Bock C, Kotut K, Luo W (2012) *Picocystis salinarum* (Chlorophyta) in saline lakes and hot springs of East Africa. *Phycologia* 51:22–32
- Lewin RA, Krienitz L, Goericke R, Takeda H, Hepperle D (2000) *Picocystis salinarum* gen. et sp. nov. (Chlorophyta) – a new picoplanktonic green alga. *Phycologia* 39:560–565
- Luo W, Kotut K, Krienitz L (2013) Hidden diversity of eukaryotic plankton in the soda lake Nakuru, Kenya, during a phase of low salinity revealed by a SSU rRNA gene clone library. *Hydrobiologia* 702:95–103

- MacIsaac EA, Stockner JG (1993) Enumeration of phototrophic picoplankton by autofluorescence microscopy. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, pp 187–197
- Mackereth FJH, Heron J, Talling JF (1989) Water analysis: some revised methods for limnologists. Freshwater Biological Association Scientific Publications 36 (Ambleside)
- Mózes A, Présing M, Vörös L (2006) Seasonal dynamics of picocyanobacteria and picoeukaryotes in a large shallow lake (Lake Balaton, Hungary). *Int Rev Hydrobiol* 91:38–50
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
- Newell BS, Morgan B, Candy J (1967) The determination of urea in seawater. *J Mar Res* 25:201–202
- Palatinszky M, Nikolausz M, Sváb D, Márialigeti K (2011) Preferential ligation during TA-cloning of multitemplate PCR products - A factor causing bias in microbial community structure analysis. *J Microbiol Meth* 85:131–136
- Pruesse E, Peplies J, Glöckner FO (2012) SINA: accurate high throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28:1823–1829.
- Schagerl M, Oduor SO (2008) Phytoplankton community relationship to environmental variables in three Kenyan Rift Valley saline-alkaline lakes. *Mar Freshwater Res* 47:189–197
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75:7537–7541.
- Somogyi B, Felföldi T, Vanyovszki J, Ágyi Á, Márialigeti K, Vörös L (2009) Winter bloom of picoeukaryotes in Hungarian shallow turbid soda pans and the role of light and temperature. *Aquat Ecol* 43:735–744
- Somogyi B, Felföldi T, Dinka M, Vörös L (2010) Periodic autotrophic picoplankton predominance in a large, shallow alkaline lake (Lake Fertő/Neusiedlersee). *Ann Limnol – Int J Lim* 46:9–19
- Somogyi B, Felföldi T, Solymosi K, Makk J, Homonnay ZG, Horváth G, Turcsi E, Böddi B, Márialigeti K, Vörös L (2011) *Chloroparva pannonica* gen. et sp. nov.

- (Trebouxiophyceae, Chlorophyta) - a new picoplanktonic green alga from a turbid, shallow soda pan. *Phycologia* 50:1–10
- Somogyi B, Felföldi T, Solymosi K, Flieger K, Márialigeti K, Böddi B, Vörös L (2013) One step closer to eliminating nomenclatural problems of minute coccoid green algae: *Pseudochloris wilhelmii* gen. et sp. nov. (Trebouxiophyceae, Chlorophyta) *Eur J Phycol* (accepted)
- Steemann Nielsen E (1952) The use of radioactive carbon (C-14) for measuring organic production in the sea. *Journal – Conseil Permanent International Pour L'Exploration De La Mer* 18:117–140
- Stockner JG, Antia NJ (1986) Algal picoplankton from marine and freshwater: a multidisciplinary perspective. *Can J Fish Aquat. Sci* 43:2472–2503
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739
- Tulonen T, Kankaala P, Ojala A, Arvola L (1994) Factors controlling production of phytoplankton and bacteria under ice in a humic, boreal lake. *J Plankton Res* 16:1411–1432
- V.-Balogh K, Németh B, Vörös L (2009) Specific attenuation coefficients of optically active substances and their contribution to the underwater ultraviolet and visible light climate in shallow lakes and ponds. *Hydrobiologia* 632:91–105
- Vaulot D, Eikrem W, Viprey M, Moreau H (2008) The diversity of small eukaryotic phytoplankton ($\leq 3 \mu\text{m}$) in marine ecosystems. *FEMS Microbiol Rev* 32:795–820
- Vörös L, Gulyás P, Németh J (1991) Occurrence, dynamics and production of picoplankton in Hungarian shallow lakes. *Int Rev Hydrobiol* 76:617–629
- Vörös L, Padisák J (1991) Phytoplankton biomass and chlorophyll-*a* in some shallow lakes in central Europe. *Hydrobiologia* 215:111–119
- Vörös L, V.-Balogh K, Boros E (2005) Picoplankton predominance in soda lakes. *Hidrológiai Közlöny* 85/6:166–168 (in Hungarian with English summary)
- Vörös L, Boros E, Schmidt A, V.-Balogh K, Németh B, Somogyi B, Mózes A (2006) Physical and chemical environment of phytoplankton in soda pans having white coloured water. *Hidrológiai Közlöny* 86/6:139–141 (in Hungarian with English summary)
- Vörös L, Mózes A, Somogyi B (2009) A five-year study of autotrophic winter picoplankton in Lake Balaton, Hungary. *Aquat Ecol* 43: 727–734

- Winder M (2009) Photosynthetic picoplankton dynamics in Lake Tahoe: temporal and spatial niche partitioning among prokaryotic and eukaryotic cells. *J Plankton Res* 31:1307–1320
- Wellburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiol* 144:307–313
- Wetzel RG, Likens GE (2000) *Limnological Analyses*. Springer, New York
- Wu QL, Chatzinotas A, Wang J, Boenigk J (2009) Genetic diversity of eukaryotic plankton assemblages in Eastern Tibetan lakes differing by their salinity and Altitude. *Microb Ecol* 58:569–581

Table 1 Depth, ice cover, pH, conductivity and dissolved oxygen concentration and saturation measured at the sampling stations in the alkaline Zab-szék pan in winter 2012

Station	Coordinates	Water depth (cm)	Ice thickness (cm)	Temperature (°C)	pH	Conductivity ($\mu\text{S cm}^{-1}$)	O ₂ (mg l ⁻¹)	O ₂ %
1	N46° 49.59' E19° 10.30'	n.a.	11	+1.0	9.73	8260	20.2	145
2	N46° 49.61' E19° 10.29'	n.a.	15	-1.0	9.64	8810	20.0	135
3	N46° 49.64' E19° 10.25'	26	12	-0.3	9.58	8800	21.0	145
4	N46° 49.66' E19° 10.23'	30	6	-0.5	9.58	8900	19.1	129
5	N46° 49.70' E19° 10.16'	24	12	-0.6	9.61	8780	n.a.	n.a.
6	N46° 49.72' E19° 10.15'	30	25	-1.1	9.57	9450	23.1	157
7	N46° 49.73' E19° 10.15'	37	14	-1.4	9.73	n.a.	n.a.	n.a.

n.a. – not analyzed

Table 2 Concentration of total suspended solids (TSS), Pt colour and different nitrogen and phosphorus forms in the alkaline Zab-szék pan in winter 2012

Station	TSS (mg l ⁻¹)	Pt colour (mg l ⁻¹)	NH ₄ ⁺ -N ($\mu\text{g l}^{-1}$)	NO ₃ ⁻ -N ($\mu\text{g l}^{-1}$)	Urea-N ($\mu\text{g l}^{-1}$)	SRP ($\mu\text{g l}^{-1}$)	TP ($\mu\text{g l}^{-1}$)
1	150.8	242.3	34.5	175.8	192.4	3176	3250
2	56.0	240.6	16.9	36.7	175.9	2443	2815
3	107.0	244.8	20.3	33.6	139.9	2436	3147
4	131.0	245.6	23.2	21.8	219.2	2368	3276
5	94.5	244.8	35.0	110.9	179.8	2233	3084
6	103.5	255.7	20.0	95.2	163.9	2431	3309
7	110.5	n.a.	13.4	63.3	314.8	n.a.	n.a.

n.a. – not analyzed

Table 3 Chlorophyll *a* concentration, photoautotrophic picoplankton (PPP) abundance, biomass and chlorophyll *a* content in the alkaline Zab-szék pan in winter 2012

Station	Chlorophyll <i>a</i> ($\mu\text{g l}^{-1}$)	PPP abundance ($10^6 \text{ cell ml}^{-1}$)	PPP biomass (mg l^{-1})	$\mu\text{g chlorophyll } a$ $\text{mg}^{-1} \text{ PPP biomass}$
1	992	82.37	145	6.84
2	743	42.26	74	9.98
3	977	58.68	103	9.46
4	1039	62.66	110	9.42
5	777	47.36	83	9.32
6	821	54.07	95	8.63
7	979	56.93	100	9.77



Fig. 1 Green-coloured water of the ice-covered alkaline Zab-szék pan as a sign of phytoplankton bloom in winter 2012

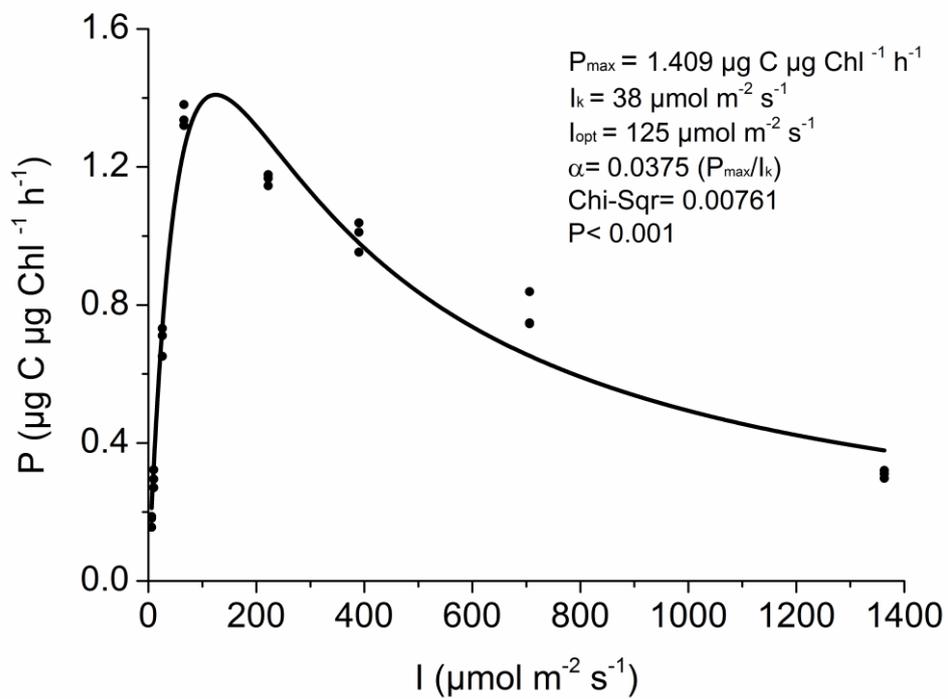


Fig. 2 Chlorophyll *a* specific photosynthesis-irradiance curve of the picoeukaryotic community sampled from Zab-szék pan in winter 2012

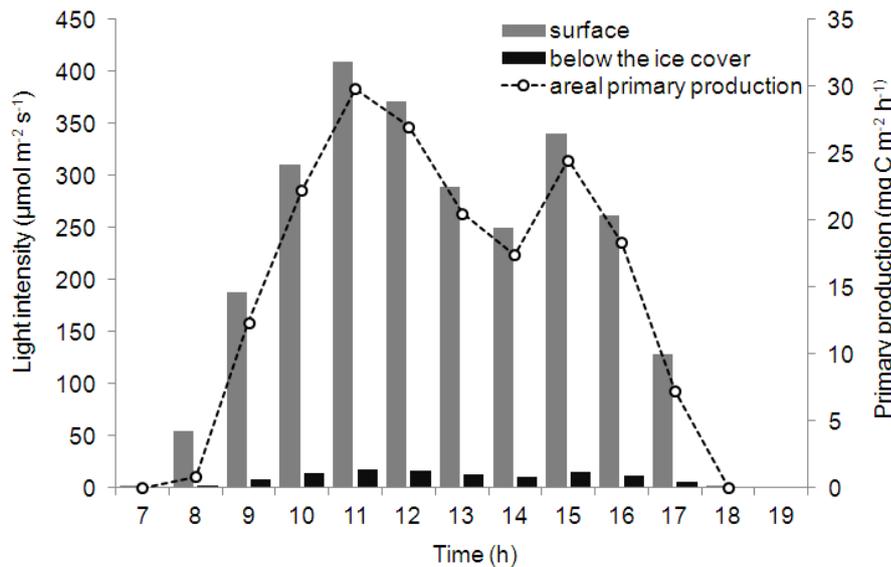


Fig. 3 Daily variation in the intensity of photosynthetically active radiation on the surface and directly below the ice cover and the estimated areal primary production of photoautotrophic picoplankton in the alkaline Zab-szék pan in winter 2012

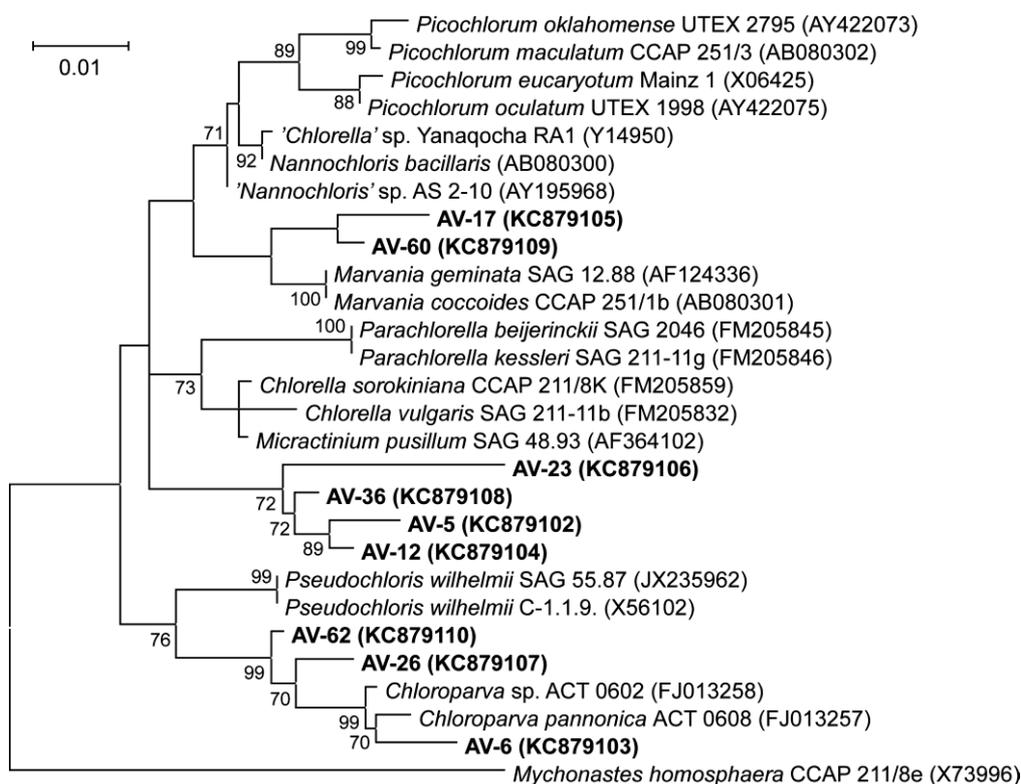


Fig. 4 Maximum likelihood phylogenetic tree of Trebouxiophyceyan clones retrieved from Zab-szék sample and related strains. Analysis was based on 932 nucleotide positions with the Tamura-Nei substitution model (gamma distributed rates among sites). Bootstrap values lower than 70% are not shown. Sequences determined in this study appear in bold letters