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ORIGINAL PAPER

First record of picophytoplankton diversity in Central European hypersaline lakes

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Abstract Our survey has revealed that the phytoplankton in the anthropo-hypersaline lakes of the Transylvanian Basin (Romania) was often dominated by photoautotrophic picoplankton (PPP, cells with a diameter <2 μm). Therefore, the aim of this study was to identify PPP members both in the summer and the winter communities using molecular biological techniques, denaturing gradient gel electrophoresis (DGGE) and sequence analysis. The applied PCR–DGGE methods were highly specific to cyanobacteria and green algae. A total of 11 different plankton taxa were identified that were related to several distant taxonomic groups. PPP were represented by a simple community and consisted of two major genotypes, one from the green algal species *Picochlorum oklahomense* and the other related to marine *Synechococcus* isolates (Cyanobacteria). These marine PPP species were recorded for the first time in inland saline lakes from Europe. Besides picoplankton, several additional marine taxa (e.g. cryptophytes and haptophytes) were detected among the nanoplankton species. The presence of the identified marine and hypersaline species could be explained by wind, precipitation or waterfowl transfer; however, this latter could have smaller importance.

Keywords Hypersaline lakes · Transylvania · Photoautotrophic picoplankton · Molecular biodiversity · 16S/18S rRNA gene · *Picochlorum* · Marine *Synechococcus*

Abbreviations

SC	Specific conductance
DGGE	Denaturing gradient gel electrophoresis
PPP	Photoautotrophic picoplankton (or picophytoplankton)

Introduction

In the Transylvanian Basin, salt (NaCl) rich regions are relatively common. In these areas, salt mines were established in high numbers and most of them are situated in or near settlements. The majority of the salt lakes emerged in the last centuries by the collapse and flooding of abandoned salt mines. Currently, there are approximately 40 salt lakes in the Transylvanian Basin. The surface of these lakes is small, ranging between 380 and 12,100 m^2, but their depth ranges between 12 and 127 m (Bulgăreanu 1996; Alexe 2010). The inflow is mainly rain water. Most of these lakes

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are built around and are used as popular spas; therefore, they are exposed to high human impact. The bathing season covers almost half a year from May to September. Without human disturbance, these lakes tend to be meromictic. In the case of the Transylvanian hypersaline lakes, previous studies have mainly focused on their origin, physico-chemical parameters of the water and microbial characteristics of the mud (Muntean et al. 1996; Alinei et al. 2006). Their algal biota is poorly known. Ionescu et al. (1998) studied 23 anthroposaline lakes by traditional microscopy, and found 116 planktonic and benthic algal taxa: with approximately half of them identified as diatoms, 24 as cyanobacteria and 15 as green algal taxa. Only the diatom community of two mesosaline lakes near Turda was recently studied (Nagy et al. 2006; Nagy and Péterfi 2008).

Our preliminary survey with epifluorescence microscopy has demonstrated that the photoautotrophic plankton in these extreme habitats was often dominated by single-celled pico-sized (<2 μm) cyanobacteria or eukaryotic algae (Keresztes et al. 2011). This widespread functional group is usually referred to as photoautotrophic picoplankton or picophytoplankton (PPP) (Stockner 1991; Weisse 1993; Raven 1998; Callieri 2008). The detection and enumeration of PPP is relatively simple with epifluorescence microscopy (MacIsaac and Stockner 1993; Vörös et al. 1998), however, this technique is inadequate for the taxonomic identification of PPP members because of their minute cell size and the lack of distinct morphological features. Therefore, the application of molecular methods, such as denaturing gradient gel electrophoresis (DGGE), quantitative PCR, cloning, sequencing, fluorescent in situ and dot blot hybridization became widely used tools to identify taxa and characterize the diversity of PPP communities (e.g. Díez et al. 2001a, b; West et al. 2001; Marie et al. 2005; Becker et al. 2007; Sánchez-Baracaldo et al. 2008; Felföldi et al. 2009, 2011b; Somogyi et al. 2009, 2010; Zwirglmaier et al. 2008).

Our knowledge on PPP diversity in hypersaline environments is limited (salinity ≥50 ‰ , Hammer 1986), and these studies have focused mainly on solar salterns (Estrada et al. 2004; Řeháková et al. 2009; Wu et al. 2009) or saline ponds (Lewin et al. 2000) and soda lakes (Roesler et al. 2002; Krienitz et al. 2012). Additionally, recent studies have revealed that extreme or poorly studied environments could harbor previously unknown PPP taxa or genotypes (Lewin et al. 2000; Felföldi et al. 2011a; Somogyi et al. 2011). Currently, there is no data about the salt lakes in Europe. Therefore, the major aim of this study was to evaluate the unknown genetic diversity of phototrophic plankton assemblages in distinct salt lakes of the Transylvanian Basin having different salt concentration with special emphasis on the prokaryotic and eukaryotic PPP. To achieve this, Cyanobacteria- and Chlorophyta-specific primers were used to amplify the 16S rRNA and 18S rRNA genes, followed by DGGE and DNA sequence analysis.

Materials and methods

Study sites and sample collection

Water samples were collected with Meyer bottles from ten saline lakes situated in five locations within the Transylvanian Basin in Romania (Fig. 1). In summer, the samples were taken from the mixed surface water, while in winter, the samples were collected in some cases from different depths.

Water temperature, specific conductance (SC) (with a HI 9033 multimeter, Hanna Instruments, Woonsocket, RI, USA) and pH (with a LDO-HQ20 multimeter, Hach, Loveland, CO, USA) were determined in the field. Salt concentration was estimated from SC using the following empirical equation based on data presented by Williams (1998):

$$C_{\mathrm{NaCl}} = 12.856 + 0.1609 \times \mathrm{SC} + 0.0046 \times \mathrm{SC}^2$$

where C_{NaCl} is the concentration of NaCl in g l^{-1} (range 21–311 g l^{-1}) and SC is the specific conductance of the water sample in mS cm^{-1} (range 30–225 mS cm^{-1}).

Unpreserved water samples were transferred to the laboratory in a thermo box under dark conditions for further analyses.

DNA extraction

Plankton samples for molecular analysis were filtered onto 0.2 μm pore size polycarbonate filters using gentle vacuum, and were kept at −20 °C until further processing. Filters were cut into small pieces, and the environmental genomic DNA was extracted using the UltraClean™ Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions, with the exception that the cell disruption step was carried out by shaking at 30 Hz for 4 min using Mixer Mill MM301 (Retsch, Haan, Germany).

PCR amplification

Both for the 16S rRNA and 18S rRNA gene, a two-step (nested) amplification protocol was applied, in the first reaction using general (bacteria- or eukarya-specific) and in the second reaction using Cyanobacteria- or Chlorophyta-specific primers. Amplification reactions were carried out with MyTaq™ Mix (Bioline Ltd., London, UK) according

Fig. 1 Geographical location of the sampling sites. Ocna Dej [Lake Cabdic (1)], Sic [Lake Băilor (2) and Lake Săpat (3)], Cojocna [Lake Băilor Cojocna (4) and Lake Durgău Cojocna (5)], Turda [Lake Tarzan (7), Lake Ocnei (8) and Lake Rotund (9)], Ocna Sibiului [Lake Ocnita-Avram Iancu (6) and Lake Fără Fund (10)]. *Squares* represent some main cities and *filled circles* mark sampling sites (with the numerical codes of lakes in parentheses)

Table 1 List of primers used

Primer name	Sequence (5′ → 3′)	Reference
27F	AGA GTT TGA TCM TGG CTC AG	Lane (1991)
1492R	TAC GGY TAC CTT GTT ACG ACT T	Lane (1991)
18S 1F	ACC TGG TTG ATC CTG CCA GT	Yamamoto et al. (2003)
18S 3R	CCT TCY GCA GGT TCA CCT AC	Yamamoto et al. (2003)
(GC-)CYA359F	(CGC CCG CCG CGC CCC GCG CCG GTC CCG CCG CCC CCG CCC G) GGG GAA TYT TCC GCA ATG GG	Nübel et al. (1997)
CYA781R	GAC TAC WGG GGT ATC TAA TCC CWT T	Nübel et al. (1997)
(GC-)EUK528F	(CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG) CCG CGG TAA TTC CAG CTC	Elwood et al. (1985)
CHLO02R	CTT CGA GCC CCC AAC TTT C	Simon et al. (2000)

to the manufacturer's instructions. In the first step, amplification of the 16S rRNA gene was carried out with 0.325 μM of 27F and 1492R primers (Table 1) with the following temperature profile: initial denaturation at 95 °C for 3 min, followed by 30 cycles of 15 s at 95 °C (denaturation), 15 s at 48 °C (annealing), 80 s at 72 °C (extension), and final extension at 72 °C for 30 min. For the 18S rRNA gene amplification, the first step was conducted with 0.325 μM of 18S 1F and 18S 3R primers (Table 1) using the following temperature profile: initial denaturation at 95 °C for 3 min, followed by 30 cycles of 15 s at 95 °C (denaturation), 15 s at 54 °C (annealing), 80 s at 72 °C (extension) and final extension at 72 °C for 30 min.

Nested PCRs were performed similar to the protocols described above, but with Cyanobacteria- or Chlorophyta-specific primer combinations. The second PCR for Cyanobacteria was conducted using CYA359F (with GC clamp) and CYA781R primers (Table 1) with the following temperature profile: initial denaturation at 95 °C for 1 min, followed by 30 cycles of 15 s at 95 °C (denaturation), 15 s at 60 °C (annealing), 20 s at 72 °C (extension), and final extension at 72 °C for 30 min. For the amplification of green algae, the EUK528F (with GC clamp) and CHLO02R primers (Table 1) were used with the following temperature profile: initial denaturation at 95 °C for 1 min, followed by 30 cycles of 15 s at 95 °C (denaturation), 15 s

at 55 °C (annealing), 20 s at 72 °C (extension), and final extension at 72 °C for 30 min.

The PCR products were examined by 1 % agarose gel electrophoresis stained with GR Safe DNA Stain (Innovita Inc, Gaithersburg, MD, USA).

DGGE and DNA sequence analysis

DGGE analysis (with a denaturing gradient from 30 to 50 %) and sequencing the reamplified DNA of representative bands were performed as described by Felföldi et al. (2009). Errors of automatic base calling on chromatograms were manually corrected, which was followed by the removal of the primer sequences using the MEGA 5.05 software (Tamura et al. 2011). Obtained sequences were compared to the GenBank nucleotide database using the Blast search program (Altschul et al. 1997), and were submitted to GenBank under the following accession numbers: JQ654468–JQ654484 (in the case of identical sequences originating from the same lake, only one selected representative sequence was submitted).

Phylogenetic analysis was conducted using the MEGA 5.05 software.

Results and discussion

In most sampling sites, lake water was mixed in summer due to the intensive bathing, while in winter, lakes were highly stratified (Table 2). Near the water surface, the specific conductance was usually lower (36–101 mS cm^{-1}) in winter than in summer (32–195 mS cm^{-1}). The estimated salt concentration (NaCl) of the samples ranged between 22 and 212 g l^{-1} (Table 2). Accordingly, most of the lakes can be considered hypersaline, the salt concentration of their water (excluding the diluted surface layers) exceeded that of the world oceans ($\sim$35 g l^{-1}). Due to the surface freshwater input (rainwater), their salinity increased from the surface to the deepest layers, as the lakes have underwater salty beds (Table 2).

A total of 27 samples from ten hypersaline lakes were investigated with DGGE analysis using the 16S rRNA and 18S rRNA genes. Reamplification and sequencing of representative 16S rRNA bands resulted in 15 sequences of unambiguous quality related to cyanobacteria and eukaryotic plastids (Figs. 2, 3). While 11 unambiguous 18S rRNA gene sequences were obtained from excised bands, and all of them were affiliated with green algae (Figs. 4, 5). Overall, both PCR–DGGE protocols were highly specific for the phototrophic community, cyanobacteria and eukaryotic algae.

According to the 16S rRNA analysis, sequence C17 from summer surface water of Lake 1 was grouped with marine *Synechococcus* isolates (Cyanobacteria) (100 % pairwise sequence similarity with *Synechococcus* sp. RS9918 from clade VIII sensu Fuller et al. 2003, Fig. 3; Table 3). Members of the picocyanobacterial genus *Synechococcus* are commonly distributed, many strains have been isolated and studied (sometimes under incorrect names) and their whole taxonomy awaits comprehensive revision (Komárek 2010). Picocyanobacterial sequences retrieved from continental habitats are separated from the obligate marine picophytoplankton clade (comprising *Prochlorococcus* and marine *Synechococcus*) (Crosbie et al. 2003). Previous reports on saline or hypersaline environments, such as the Great Salt Lake (UT, USA), the hypersaline Mono Lake (CA, USA), East Tibetan saline lakes (China) or hyposaline soda pans (Hungary), have indicated that the members of their picocyanobacterial communities were distantly related to the above-mentioned marine clade (Budinoff and Hollibaugh 2007; Xing et al. 2009; Wu et al. 2010; Felföldi et al. 2011a). *Synechococcus* phylotypes of the marine picophytoplankton clade are well-known inhabitants of seas and oceans (e.g. Fuller et al. 2003; Zwirglmaier et al. 2008), although the saline environment and the high dispersive potential of these microorganisms could explain their presence in the investigated continental lakes.

Another 16S rRNA gene sequence group retrieved that was related to pico-sized algae contained sequence C2 originating from Lake 6 in summer and sequence C8 from Lake 4 in winter (Fig. 3; Table 3). These sequences were closely related (99.4 % pairwise similarity) to *Picochlorum* sp. (Chlorophyta) plastids. The presence of this genus was also verified by means of the 18S rRNA analysis: sequences E19 and E20 (from Lake 1 in winter) as well as sequence E12 (from Lake 6 in summer) showed 100 % nucleotide similarity to *Picochlorum oklahomense* UTEX 2795 (Figs. 4, 5; Table 3), which was isolated from an ephemeral saline pool (Henley et al. 2004). Members of the picoeukaryotic genus *Picochlorum* were found in various marine and saline environments (Henley et al. 2004), and were also detected in the heliothermal Lake Ursu that is located in Sovata, Romania (István Mathé, Tamás Felföldi and Károly Márialigeti, unpublished results). The presence of this genus in the hypersaline lakes of the Transylvanian Basin corresponds well with the broad halotolerance of *P. oklahomense* (Henley et al. 2002).

Sequences C7 and C13 from the winter sample of Lake 5 showed moderate similarity (95.6 %) to *Rhizochromulina* sp. CCMP 1253 (Heterokontophyta) and to an undescribed pico-sized flagellate, dictyochophyte sp. RCC332 (Heterokontophyta) (Fig. 3; Table 3). Plastid 16S rRNA gene sequences of these strains were completely identical within the investigated region, even though they differ significantly in their morphology and their 18S rRNA sequences

Table 2 List of investigated lakes and selected environmental variables

Location	Lake (code)	GPS coordinates	Sampling date	Sample code	Sampling depth (m)	T (°C)	SC (mS cm^{-1})	Estimated salt concentration (g l^{-1})	pH
Ocna Dej	L. Cabdic (1)	N47°07.712′ E23°51.900′	22-07-2010	1S0	0.1	28.2	80	55	8.9
			07-02-2011	1W0	0.1	−1.2	56	36	7.5
				1W1	1	5.6	84	59	7.9
Sic	L. Băilor (2)	N46°55.913′ E23°54.073′	22-07-2010	2S0	0.1	29.9	67	44	8.8
			07-02-2011	2W0	0	1.4	48	31	8.6
				2W2	2	2.5	80	55	8.5
	L. Săpat (3)	N46°54.287′ E23°54.165′	22-07-2010	3S0	0.1	38.5	195	219	8.7
Cojocna	L. Băilor Cojocna (4)	N46°44.907′ E23°50.441′	22-07-2010	4S0	0.1	30.5	133	116	8.3
			07-02-2011	4W0	0.1	−0.5	89	64	8.6
	L. Durgău Cojocna (5)	N46°44.836′ E23°50.442′	22-07-2010	5S0	0.1	30.5	129	110	8.0
			07-02-2011	5W0	0.1	−1.7	69	46	8.9
				5W1.5	1.5	8.5	132	114	7.4
Turda	L. Tarzan (7)	N46°34.472′ E23°48.549′	18-08-2010	7S0	0.1	28.8	32	22	ND
			08-02-2011	7W0	0.1	−0.6	36	25	9.2
				7W2.5	2.5	8.2	52	34	7.3
	L. Ocnei (8)	N46°35.158′ E23°47.282′	29-08-2010	8S0	0.1	ND	68	45	8.9
			08-02-2011	8W0	0.1	2.0	82	57	8.9
				8W3	3	9.0	122	101	8.5
				8W4	4	17.0	213	256	7.0
	L. Rotund (9)	N46°35.099′ E23°47.21′	29-08-2010	9S0	0.1	ND	66	44	8.4
			08-02-2011	9W0	0.1	−0.3	27	20	8.1
Ocna Sibiului	L. Ocnita-Avram Iancu (6)	N45°52.444′ E24°03.983′	22-07-2010	6S0	0.1	31.0	145	133	8.6
			09-02-2011	6W0	0.1	−0.2	74	50	9.5
				6W1.5	1.5	10.3	154	147	8.8
	L. Fără Fund (10)	N45°52.578′ E24°04.064′	22-07-2010	10S0	0.1	30.8	178	187	8.4
			09-02-2011	10W0	0.1	−0.4	101	76	9.4
				10W1.5	1.5	6.6	183	196	9.1

T temperature, *SC* specific conductance, *ND* no data

In the sample codes, the first character represents the code of the lake, the second character marks the sampling period (*S* summer, *W* winter) and the third represents the sampling depth (in meters)

(online available, unpublished data from Roscoff Culture Collection; Vaulot et al. 2004). Therefore, sequences C7 and C13 may represent an unknown dictyochophyte genotype (a potential picoflagellate), but their identification was not possible based on partial 16S rRNA gene sequences.

Other genotypes detected in this study were closely or distantly related to larger eukaryotic algae. Plastid 16S rRNA gene sequences were affiliated with the members of the genera *Guillardia* (Cryptophyta), *Isochrysis* (Haptophyta), *Amphora* and *Navicula* (Heterokontophyta; Table 3). *Guillardia theta* was found with 99.1 % pairwise similarity from Lake 1 and *Isochrysis* sp. were identified with 100 % pairwise similarity from Lakes 8 and 9 in winter (Figs. 2, 3; Table 3). According to our best knowledge, members of both genera are exclusively known from marine habitats. Retrieved diatom sequences were indistinguishably closely related, based on the investigated region of the plastid 16S rRNA gene, to a clade formed by members of the genera *Amphora*, *Cymbella* and *Navicula*.

Based on the Chlorophyta-specific 18S rRNA gene analysis, we found sequences related to the genera *Chlamydomonas, Dunaliella, Ankyra* and *Hormotila/Chlorococcum* (Fig. 5; Table 3). Some of these taxa also harbor species that inhabit marine or saline aquatic habitats. Members of the large polyphyletic genus *Chlamydomonas* are known both form freshwater and marine habitats (Harris 2009; Leliaert et al. 2012), while *Dunaliella* species are well-known inhabitants of saline aquatic environments (Estrada et al. 2004; Řeháková et al. 2009; Wu et al. 2009).

Regarding the 18S rRNA gene-based DGGE (Fig. 4), the band representing the genus *Chlamydomonas* (E3) was observable in most of the samples investigated, while *Ankyra*- and *Hormotila*-related genotypes were restricted only to a few samples (bands E21, E22, E24, E25 and E28). Genotypes related to *Picochlorum* (bands E12, E19 and E20) and *Dunaliella* (band E5) were located in the middle part of the gel and showed highly similar melting properties with multiple band patterns. Similarly in the case of the 16S rRNA gene-based DGGE (Fig. 2), four sequences

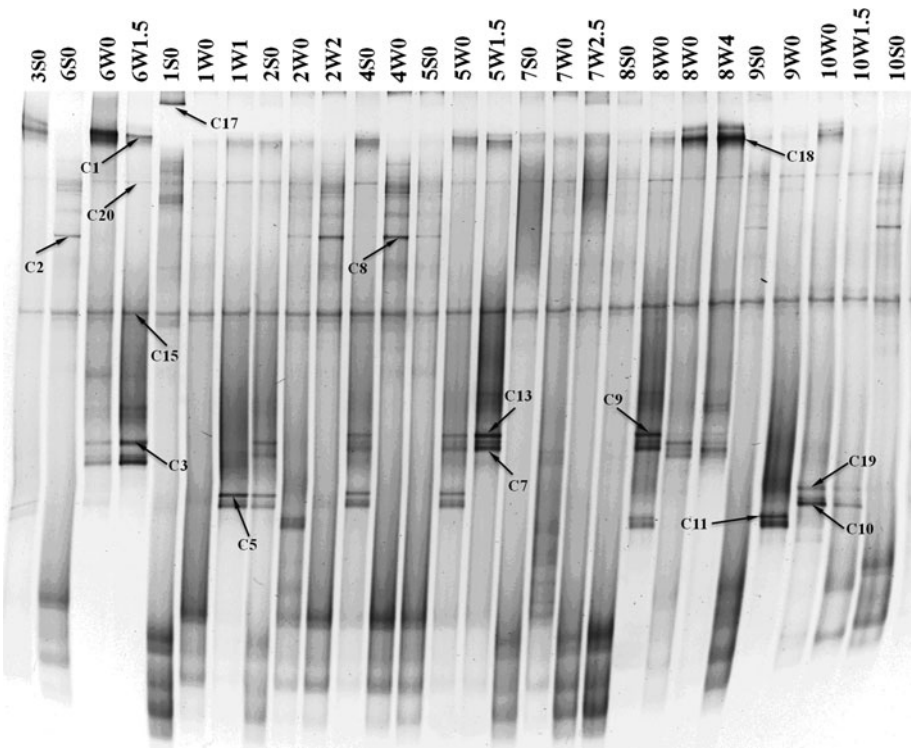

Fig. 2 DGGE profile of saline lake samples based on the 16S rRNA gene. *Arrows* indicate the excised, reamplified and sequenced bands. For sample codes, see Table 2

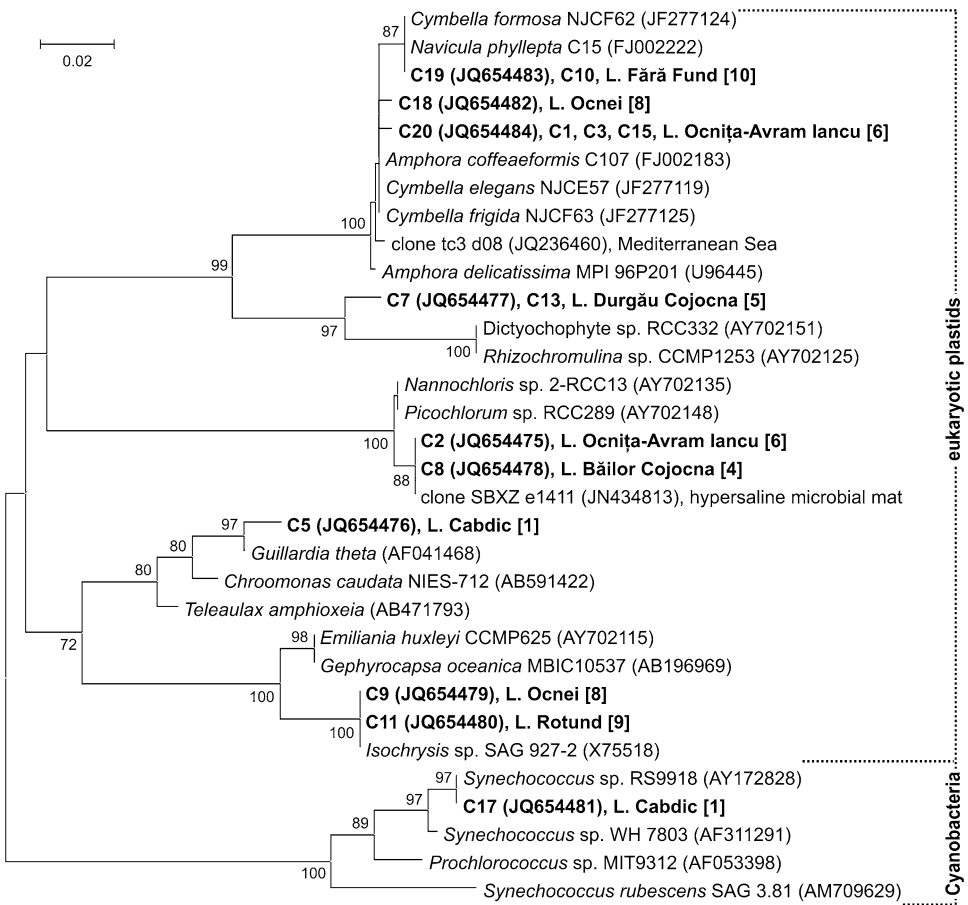

Fig. 3 Neighbour joining phylogenetic tree of sequences recovered from 16S rRNA gene-based DGGE. Evolutionary distances were calculated using the Maximum Composite Likelihood method. The tree is based on 297 unambiguously aligned nucleotide positions. Bootstrap values lower than 70 were removed from the branches. Sequences determined in this study appear in *bold letters*. Lake codes are given in *square brackets*

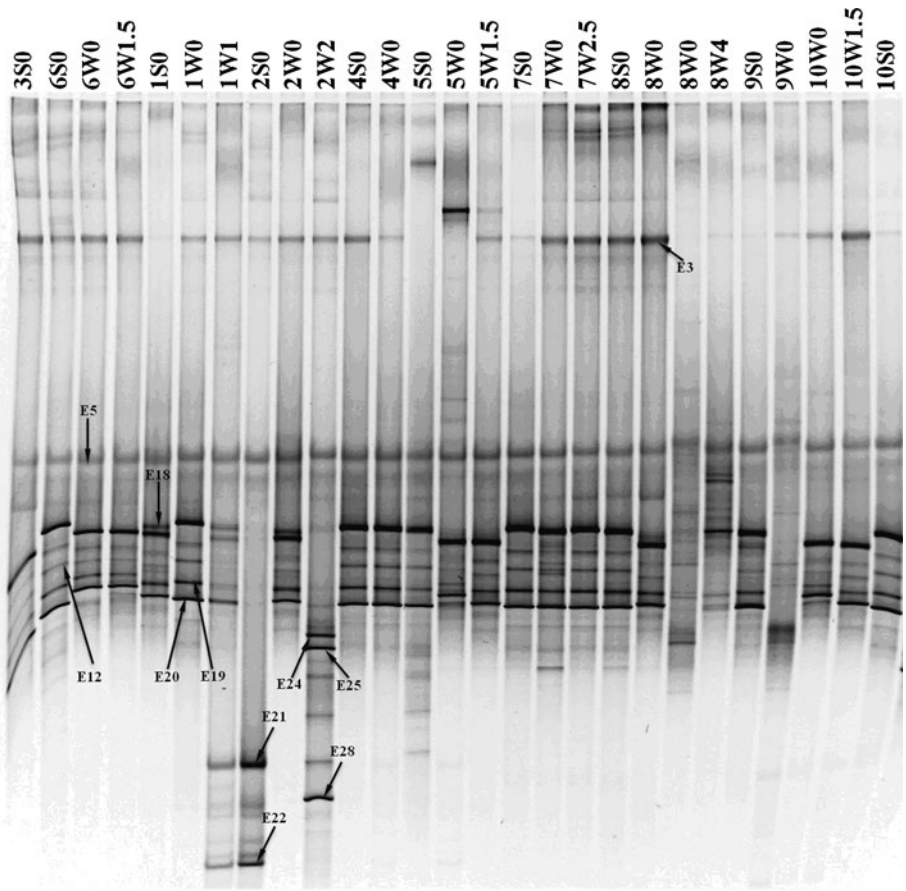

Fig. 4 DGGE profile of saline lake samples based on the 18S rRNA gene. *Arrows* indicate the excised, reamplified and sequenced bands. For sample codes, see Table 2

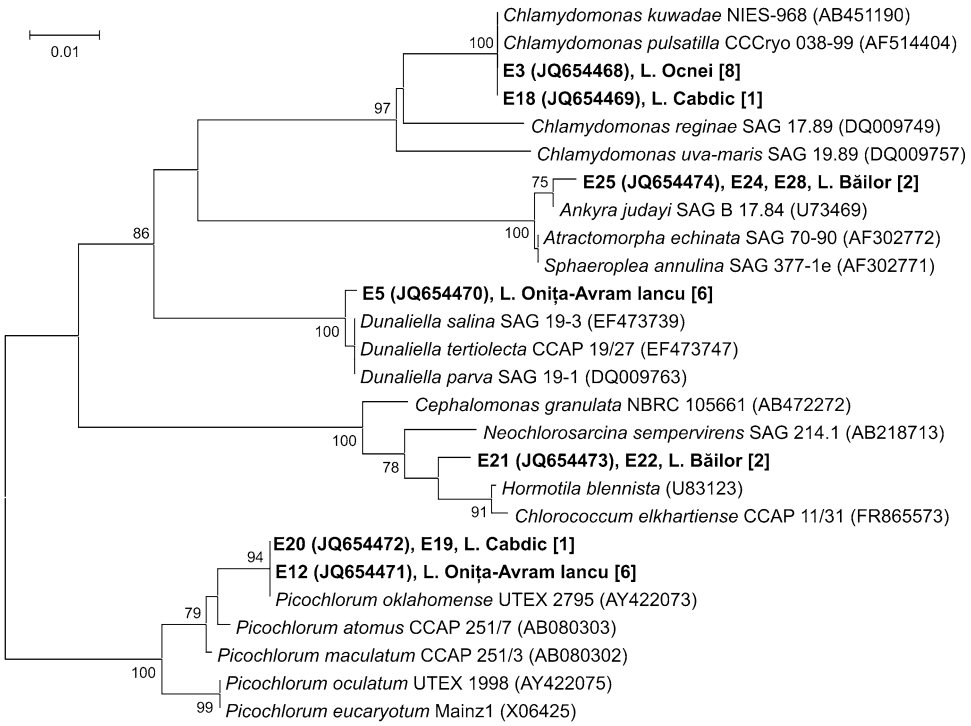

Fig. 5 Neighbour joining phylogenetic tree of sequences recovered from 18S rRNA gene-based DGGE. The tree is based on 331 unambiguously aligned nucleotide positions. For other details, see the legend of Fig. 3

Table 3 Phylogenetic affiliation of sequences obtained from the studied Transylvanian saline lakes and the origin of most related isolates

Sequence (Lake code)	Closest relatives (Accession number)	Similarity (%)	Division	Habitat	Reference
C17 (1)	*Synechococcus sp.* RS9918 (AY172828)*	100	Cyanobacteria	Marine	Fuller et al. (2003)
C5 (1)	*Guillardia theta* (AF041468)	99.1	Cryptophyta	Marine	Douglas and Durnford (1989)
C9 (8), C11 (9)	*Isochrysis* sp. SAG 927-2 (X75518)	100	Haptophyta	Marine	Huss et al., unpublished
C18 (8), C1, C3, C15, C20 (6)	*Amphora coffeaeformis* C107 (FJ002183)	99.7	Heterokontophyta	Marine/ Brackish	Rampen et al. (2009)
C10, C19 (10)	*Navicula phyllepta* C15 (FJ002222)	100	Heterokontophyta	Marine	Rampen et al. (2009)
C7, C13 (5)	*Rhizochromulina sp.* CCMP1253 (AY702125)	95.6	Heterokontophyta	Marine	Fuller et al. (2006)
	Dictyochophyte sp. RCC332 (AY702151)*	95.6	Heterokontophyta	Marine	Fuller et al. (2006)
E12 (6), E19, E20 (1)	*Picochlorum oklahomense* UTEX 2795 (AY422073)*	99.7	Chlorophyta	Hypersaline	Henley et al. (2004)
C2 (6), C8 (4)	*Picochlorum sp.* RCC289 (AY702148)*	99.4	Chlorophyta	Marine	Fuller et al. (2006)
	Nannochloris sp. 2-RCC13 (AY702135)*	99.4	Chlorophyta	Marine	Fuller et al. (2006)
E3 (8), E18 (1)	*Chlamydomonas pulsatilla* CCCryo 038-99 (AF514404)	100	Chlorophyta	Marine/ Snow	Leya et al., unpublished
	Chlamydomonas kuwadae NIES-968 (AB451190)	100	Chlorophyta	Freshwater	Nakada and Nozaki (2009)
E5 (6)	*Dunaliella salina* SAG 19-3 (EF473739)	99.7	Chlorophyta	Hypersaline	Di Giuseppe and Dini, unpublished
	Dunaliella tertiolecta CCAP 19/27 (EF473747)	99.7	Chlorophyta	Unknown	Di Giuseppe and Dini, unpublished
	Dunaliella salina SAG 19-1 (DQ009763)	99.7	Chlorophyta	Saline lake	Buchheim et al., unpublished
E24, E25, E28 (2)	*Ankyra judayi* SAG B17.84 (U73469)	99.7	Chlorophyta	Freshwater	Buchheim et al. (2001)
E21, E22 (2)	*Hormotila blennista* (U83123)	98.8	Chlorophyta	Freshwater	Booton et al. (1998)

Picoplanktonic taxa were marked with asterisk

retrieved from different bands in sample lane 6W1.5 were identical. These phenomena could be explained with the formation of both homo- and heteroduplex molecules (Ferris and Ward 1997), and that hindered a detailed computational pattern analysis. Accordingly, no clear seasonal pattern separation was observable in the investigated samples (e.g. compare the samples 4S0, 4W0 and 5S0 or 7S0, 7W0, 7W2.5 and 8S0 in Fig. 4).

Cyanobacteria [e.g. *Phormidium, Arthrospira* (*Spirulina*) and *Aphanothece*], diatoms (e.g. *Amphora, Navicula* and *Nitzschia*) and unicellular green algae (e.g. *Dunaliella* and *Asteromonas*) are common in hypersaline environments (salt lakes, hypersaline lagoons and solar salterns; Seckbach 2007). For example, in hypersaline, alkaline lakes of East Africa, cyanobacteria *Arthrospira platensis, Arthrospira fusiformis* and *Anabenopsis abijatae* are the most abundant phytoplankters (Harper et al. 2003; Schagerl and Oduor 2008; Kotut and Krienitz 2011). Based on a comprehensive study of the diatom flora of North American salt lakes, the most halotolerant taxa are *Navicula subinflatoides*, *Amphora coffeiformis*, *Synedra fasciculate*, *Nitzschia communis* and *Nitzschia frustulum v. perpusilla* (Blinn 1993). In the Great Salt Lake (UT, USA), *Aphanothece halophytica* and *Nodularia spumigena* are the dominant cyanobacteria (Roney et al. 2009), but microscopic green algae such as *Dunaliella salina* and *Dunaliella viridis* are also common members of the phytoplankton (Post 1977). The green algal genus *Dunaliella* is also widespread in other hypersaline lakes [e.g. Honda Lake (Spain), Xiaochaidan Lake (China) and Dead Sea (Israel)] (Oren et al. 1995; López-Gonzáles et al. 1998; Wu et al. 2009) and solar salterns (Elevi Bardavid et al. 2008; Oren 2010). Within the pico-sized green algae, the members of the genera *Picochlorum* and *Picocystis* are the most well-known inhabitants of hypersaline lakes and ponds (Lewin et al. 2000; Henley et al. 2004; Wu et al. 2009; Krienitz et al. 2012). The presence of some taxa detected in the Transylvanian lakes by our analysis (e.g. *Picochlorum* and

Dunaliella) agrees well with former studies on hypersaline environments; but additionally, we have retrieved that marine taxa (cryptophytes, haptophytes and picocyanobacteria) are also characteristic inhabitants of these aquatic ecosystems.

Conclusions

In summary, PCR–DGGE was highly specific to cyanobacteria and green algae, and no heterotrophs were retrieved in our analysis (that could be a significant bias in similar analyses). We found eleven algal taxa in the Transylvanian saline lakes and these taxa were related mainly to marine or hypersaline species. PPP was represented by the marine picophytoplankton clade of *Synechococcus* (Cyanobacteria) and the marine/hypersaline genus *Picochlorum* (Chlorophyta). This is the first record on both groups in Central Europe. Additionally, a putative dictyochophyte picoflagellate may also be present. Within larger algae, the occurrence of marine cryptophytes and haptophytes was also verified. The presence of the identified marine and hypersaline species could be explained by wind, precipitation and waterfowl transfer. However, this latter could have smaller importance because of the urban environment and small surface of the studied lakes.

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