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Picocystis salinarum (Chlorophyta) in saline lakes and hot springs of East Africa

Lothar Krienitz[1]*, Christina Bock[1,2], Kiplagat Kotut[3] and Wei Luo[4]

[1]*Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Alte Fischerhütte 2, D-16775 Stechlin-Neuglobsow, Germany*
[2]*University of Essen, Faculty of Biology, D-45141 Essen, Germany*
[3]*Plant and Microbial Sciences Department, Kenyatta University, PO Box 43844 GPO 00100 Nairobi, Kenya*
[4]*SOA Key Laboratory for Polar Science, Polar Research Institute of China, Jin Qiao Road 200136 Shanghai, P.R. China*

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The occurrence of *Picocystis salinarum* in saline inland waters of East Africa was investigated using a polyphasic approach of small-subunit (SSU) rDNA phylogeny and light microscope observations. Recent studies have found that *Picocystis* occasionally replaces the dominant cyanobacterium (*Arthrospira fusiformis*), which is the main food resource of Lesser Flamingos, in soda lakes of Bogoria and Nakuru. This article discusses the consequences of a high abundance (maximum cell numbers of > 3 billion cells l^{-1}) of *Picocystis* on food chains of African saline waters. During the study, we found a new morphotype of *Picocystis* characterized by larger cell sizes and absence of lobes in hot springs near Lake Magadi. SSU rRNA genes of *Picocystis* strains and uncultured field clones collected from Lake Nakuru were subjected to phylogenetic analyses together with other picoplankton from field and culture samples from saline, marine or freshwater. *Picocystis salinarum* from saline inland waters represents a link between marine and freshwater habitats from both an ecological and a phylogenetic point of view and is therefore of great interest.

Key Words: Hot springs, Lake Magadi, Lake Nakuru, *Picocystis*, Picoplankton, Prasinophytes, Saline lakes, SSU rRNA gene, Uncultured clones

INTRODUCTION

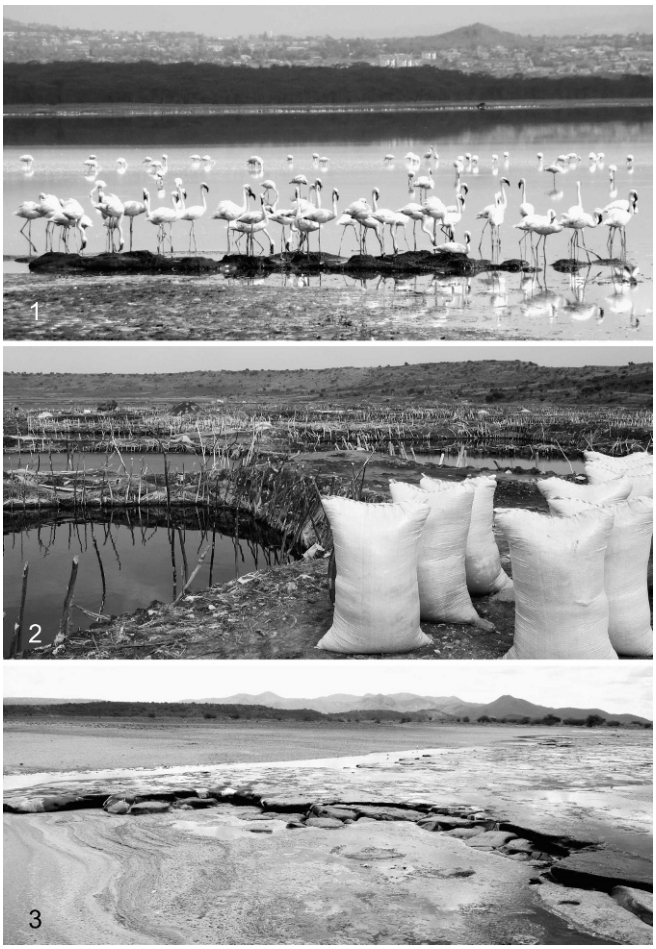
Picocystis salinarum Lewin (Lewin *et al.* 2000) is a unique chlorophyte with a separate phylogenetic lineage within the prasinophytes (Hepperle *et al.* 2001; Guillou *et al.* 2004). The locus classicus of *Picocystis* is a saline pond ($\sim$ 100‰ salt concentration) at the San Francisco Salt Works, California, USA. Later, it was found in Mono Lake ($\sim$ 85‰ salinity), USA (Hollibaugh *et al.* 2001), and in an alkaline lake in Inner Mongolia, P.R. China, by Ralph A. Lewin (Hollibaugh *et al.* 2001). Another strain of *Picocystis* (DGN-Z1) was isolated from a bloom of picoplankton in Dagenoer soda lake (188‰ salinity) in Inner Mongolia (Fanjing *et al.* 2009). *Picocystis* is characterized by an exclusive set of pigments with unusual carotenoid pattern (Lewin *et al.* 2000; Roesler *et al.* 2002). This tiny protist is difficult to recognize and distinguish in field samples and is probably often neglected or wrongly identified. Because of its key position as highly productive primary producer in food webs of saline habitats, it is necessary to focus more attention to its distribution, which is probably widespread. Recently, this alga was found to be very abundant in the soda lakes of the East African Rift Valley, lakes Bogoria and Nakuru (Krienitz & Kotut 2010). Based on morphological and molecular characteristics of field and culture samples, we compile our findings on *Picocystis* from several habitats of East Africa with elevated salinity. Our intention is to motivate phycologists and ecologists to deal with this inimitable organism and its involvement in food webs of extreme saline inland waters.

* Corresponding author (krie@igb-berlin.de).

MATERIAL AND METHODS

Sampling sites

Lakes Bogoria and Nakuru (Fig. 1), Kenya, are protected soda lakes in the Gregory Rift Valley, best known for their thousands and sometimes millions of Lesser Flamingos (Vareschi 1978; Harper *et al.* 2003; Krienitz & Kotut 2010). Lake Katwe (Fig. 2), Uganda, is a crater lake in close vicinity of the Queen Elizabeth National Park, situated between the large freshwater lakes Edward and George, and is used for salt extraction from its sediments (Pomeroy *et al.* 2003). Lake Magadi, Kenya, is a large salt pan divided into several lagoons (Schlüter 1993). Depending on the season and precipitation, the lake water can become a concentrated sodium carbonate brine. Most of the Magadi lagoons are dried out and covered by sodium sesquicarbonate (trona), which is used for industrial soda ash production by the Magadi Soda factory. Saline-alkaline hot springs (Coe 1966) discharge into alkaline lagoons from around the lake margins (Fig. 3). There being some limited surface inflows into the lake, the hot springs constitute the main water source for the lake's endorheic basin. In this study, we investigated a hot spring area in the southwestern lagoon with moderate temperatures of between 30°C and 40°C. The lakes and hot springs have been subjected to different phycological studies since 2001 and sampled at irregular intervals. This article focuses on occasions characterized by an abundance of *P. salinarum*. The sampling dates are given in Table 1. Salinity and pH were measured at each sampling date using a WTW Multiline P4 meter (Wissenschaftlich Technische Werkstätten Weilheim, Germany).



Figs 1–3. Study sites: habitats of *Picocystis salinarum*.
Fig. 1. Lake Nakuru. Lesser Flamingos nesting in the foreground with Nakuru town in the background.
Fig. 2. Lake Katwe. The lake area on the crater ground is divided into small ponds for salt production. In the foreground are sacks filled with salt.
Fig. 3. Hot springs in the southern basin of Lake Magadi. The lake is completely dried out, and the hot springs discharge water into the lake basin.

Microscopy and cultures

Phytoplankton samples were obtained from a few centimetres below the water surface. Samples for counting were immediately fixed with formaldehyde, while live subsamples for establishing unialgal cultures were left unpreserved. The phytoplankton was counted according to Utermöhl (1958) in sedimentation chambers (Hydro-Bios Apparatebau GmbH, Kiel, Germany) under an inverted microscope Eclipse TS 100 (Nikon Corporation, Tokyo, Japan). The phytoplankton biomass was calculated by geometric approximations using the computerized counting programme OPTICOUNT (Opticount 2008). The specific density of phytoplankton cells was taken as 1 g cm^{-3}.

Living original samples and rough cultures (containing 50% original sample water and 50% culture medium) were kept in 15-ml glass vials for later microscopical analyses and as inocula for isolating unialgal cultures. New strains were isolated in February 2010 by streaking the sample on 1.5% agar medium prepared with a Bourrelly medium

Table 1. *Picocystis salinarum* in East African inland waters.

Sampling site	Date	Salinity (‰)	pH	Cell number of *Picocystis* (l^{-1})	Biomass of *Picocystis* ($\mu g\ l^{-1}$)	Contribution of *Picocystis* to total phytoplankton biomass (%)	Associated main algal and cyanobacterial morphotypes
Lake Magadi South	09 Feb. 2002	29.7	9.40	3.59×10^6	7	< 1	Pennate diatoms
Lake Katwe	23 Jan. 2005	~ 300	9.72	4.15×10^6	9	< 1	*Arthrospira, Synechococcus*
Lake Bogoria	13 Feb. 2006	55.1	9.83	1.03×10^9	2200	4	*Arthrospira*
Lake Bogoria	01 Sep. 2006	52.0	9.99	3.41×10^9	7300	54	*Arthrospira*
Lake Nakuru	08 Jan. 2010	50.7	10.19	3.32×10^9	7100	68	Pennate diatoms
Lake Nakuru	17 Jan. 2010	61.6	10.20	3.46×10^9	7400	53	Pennate diatoms
Lake Magadi South	20 Jan. 2010	88.0	9.75	3.17×10^6	6	< 1	*Chroococcus*
Lake Magadi Check Point	20 Jan. 2010	14.0	10.13	2.90×10^6	4	< 1	*Anabaenopsis, Schroederia*
Hot Springs Magadi (35°C)	20 Jan. 2010	26.5	9.71	0.70×10^6	16	1.5	*Phormidium*

(Krienitz & Wirth 2006) modified by addition of Na_2CO_3 (0.3 g l^{-1}) and NaCl (15 g l^{-1}). Single colonies were picked by a glass capillary and transferred into the same medium without agar. The strains are maintained at the algal strain collection of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in suspensions at room temperature under a 14:10-hour light:dark regime. Two strains of *P. salinarum* (KR 2010/2 and KR 2010/4) were deposited in the Culture Collection of Algae of Charles University in Prague (CAUP M 301 and CAUP M 302).

The morphology of algae was examined using a Nikon Eclipse E600 light microscope with differential interference contrast. Microphotographs were taken with a Nikon Digital Camera DS-Fi1 and Nikon software NIS-Elements D (Nikon).

Molecular methods

A field sample from Lake Nakuru collected on 17 January 2010 was filtered using membrane filters with a pore size of 0.6 µm (Schleicher & Schuell GmbH, Dassel, Germany). The filters were mechanically sliced into small pieces and rinsed with cell lyses buffer AP1 (Qiagen GmbH, Hilden, Germany). Cells were disrupted with the help of glass beads (0.7 mm; Carl Roth GmbH, Karlsruhe, Germany) using the Tissuelyser II (Qiagen). Genomic DNA was extracted in February 2010 using the DNeasy Plant Mini Kit (Qiagen) following the instructions given by the manufacturer.

The clone libraries were constructed using two primer sets following the procedure described by Luo *et al.* (2009). Eukaryotic small-subunit (SSU) rRNA genes were amplified by polymerase chain reaction (PCR) with eukaryote-specific primers EukA and EukB (Medlin *et al.* 1988). Meanwhile, the second set of primers, EuK328f and CHLO02r (Zhu *et al.* 2005; Viprey *et al.* 2008) targeting the Chloroplastida, was used and yielded a fragment of about 950 base pairs. Amplified rRNA gene products from several individual PCRs were pooled and phylogenetic analyses subsequently carried out as described by Luo *et al.* (2006, 2009). A 15-µl aliquot of each PCR mixture containing amplified 18S rRNA was digested, respectively, with 10 U of the restriction endonuclease *Hae*III, in a total volume of 20 µl at 37°C for 4 hours. The restriction products were analyzed by agarose (2%, w/v) gel electrophoresis in TAE buffer containing 1 g ml^{-1} of ethidium bromide. Gels were photographed under UV light.

Five unialgal strains of *P. salinarum* (KR 2003/27 and KR 2003/26 from Lake Magadi, KR 2010/4 from Lake Magadi hot springs and KR 2010/1 and KR 2010/2 from Lake Nakuru) were sequenced in the course of this study. After disruption of the cells with the Tissuelyser and extraction of genomic DNA using the DNeasy Plant Mini Kit, the PCR of the SSU of rRNA gene, purification and gene sequencing were carried out as outlined by Bock *et al.* (2011). Subsequent phylogenetic analyses included sequences from five new strains of *Picocystis* described above, eight uncultured clones from Lake Nakuru, three *Picocystis* sequences from the United States and China as well as 33 sequences of the Chlorophyta, mostly of picoplankton size, and two charophycean algae (as out-group) obtained from the GenBank (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov). The designation of strains and clones, their origin and accession numbers of their sequences are provided in Table 2.

The SSU alignment was constructed manually using the alignment editor Align (Hepperle 2002). Introns were excluded. In-group sequences were chosen according to previously published analyses of Hollibaugh *et al.* (2001), Guillou *et al.* (2004) and Marin & Melkonian (2010). The Charophyceae *Chara foetida* and *Nitella capillaris* were selected as out-groups following the analyses of Guillou *et al.* (2004). The alignment used for phylogenetic analyses consisted of 1593 characters and 53 sequences. Each alignment was analyzed by distance [neighbour joining (NJ)] and maximum parsimony (MP) using PAUP* (portable version 4.0b10; Swofford 2002). The MP analyses were performed with heuristic search options based on simple taxon addition, tree-bisection-reconnection branch swapping algorithm and Multrees options enabled. The maximum likelihood (ML) analyses were calculated using Treefinder (Jobb 2008) with the model and parameters proposed by Treefinder under AICc criteria (J3:G:5). To test the confidence of the trees topologies, we carried out bootstrap analyses for NJ, MP and ML (1000 replicates each). Bayesian analyses were performed using MrBayes version 3.1. with covarion settings (Huelsenbeck & Ronquist 2001). Two runs with four chains of Markov chain Monte Carlo iterations were performed for 2,000,000 generations with tree sampling every 100 generations. The stationary distribution was assumed when the average standard deviations of split frequencies between two runs was lower than 0.01. The first 25% of the calculated trees were discarded as burn-in. A 50% majority-rule consensus tree was calculated for posterior probabilities.

RESULTS

Picocystis established subdominant populations in lakes Magadi (February 2002) and Katwe (January 2005), both of which were dominated by pennate diatoms and *Arthrospira*/*Synechococcus*, respectively. With cell numbers of about 3–4 million l^{-1} and a biomass of 7–9 µg l^{-1}, the contribution of *Picocystis* to the total phytoplankton biomass was below 1% (Table 1). Field samples from these lakes contained only spherical cells of *Picocystis*. Cultures of *Picocystis* obtained from these samples had the typical lobate cell morphology. In February 2006, a population of *Picocystis* was for the first time recorded in Lake Bogoria. Rough cultures of these field samples were characterized mostly by lobate cells (Fig. 4). Cell numbers of *Picocystis* in February 2006 were more than 1 billion l^{-1} but contributed only 4% to the total phytoplankton biomass in the sample from Bogoria, which was dominated by *Arthrospira*. In September 2006, the *Arthrospira* population decreased considerably, while the *Picocystis* population went up to 3.4 billion cells l^{-1}, which accounted for about 54% of total phytoplankton biomass.

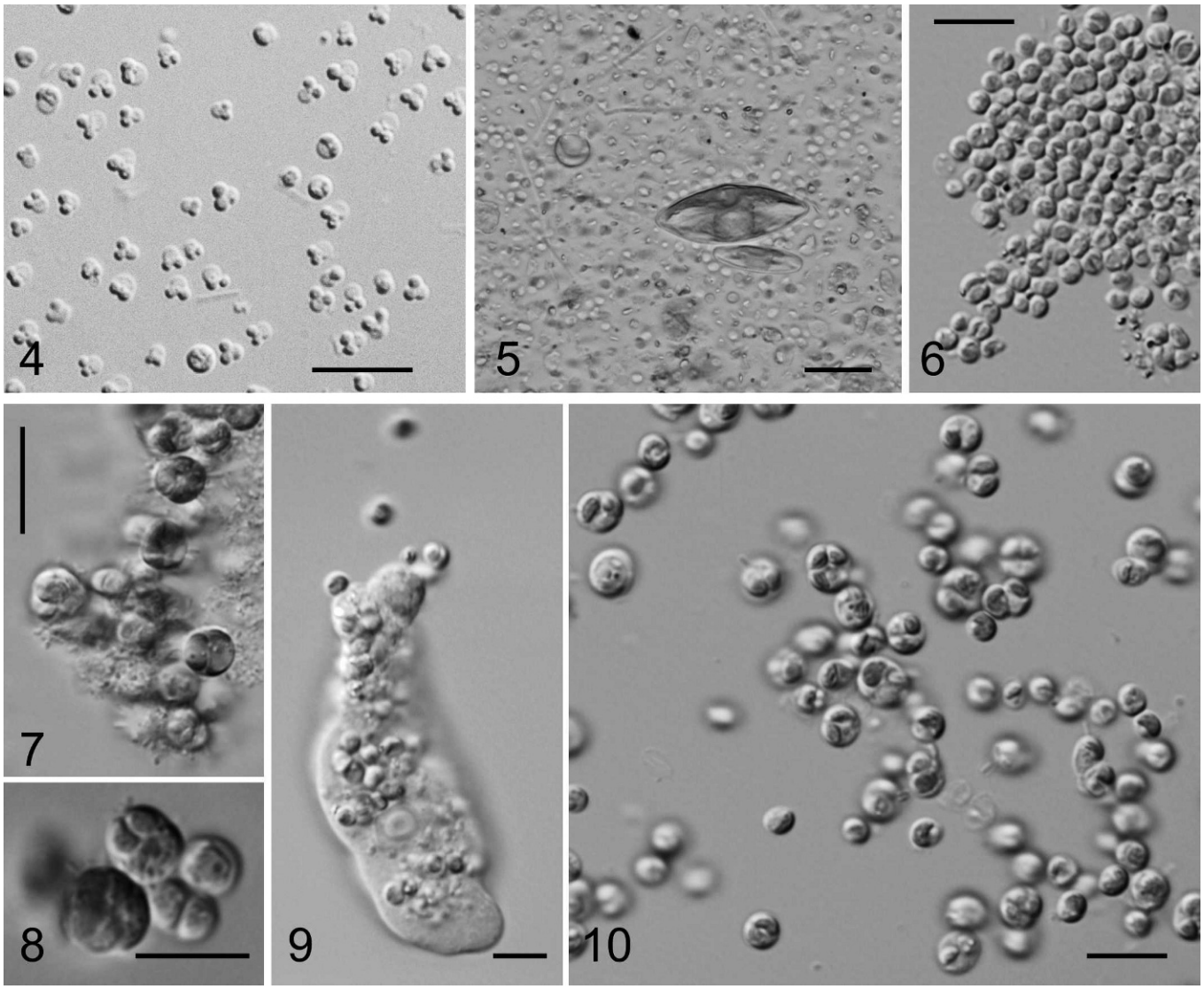
In January 2010, a very unique situation was observed at Lake Nakuru after the lake nearly dried out (in August/September 2009). Following a partial refill by heavy rains

Table 2. Strains and uncultured clones from field samples used in this study for phylogenetic analyses.

Species/clone	Strain/clone designation	Origin	Accession number	Reference
Prasinophyceae				
Picocystis salinarum	L7	Mono Lake, USA	AF153313	Hollibaugh *et al.* (2001)
Picocystis salinarum	SSFB	San Francisco Salt Works, USA	AF125167	Hollibaugh *et al.* (2001)
Picocystis salinarum	IM214	Saline lake, Inner Mongolia, China	AF153314	Hollibaugh *et al.* (2001)
Picocystis salinarum	KR 2003/26	Lake Magadi, Kenya	DQ267704	This study
Picocystis salinarum	KR 2003/27	Lake Magadi, Kenya	DQ267705	This study
Picocystis salinarum	KR 2010/1	Lake Nakuru, Kenya	HM990667	This study
Picocystis salinarum	KR 2010/2 (CAUP M 301)	Lake Nakuru, Kenya	HM990668	This study
Picocystis salinarum	KR 2010/4 (CAUP M 302)	Hot Springs Magadi, Kenya	HM990669	This study
Picocystis salinarum, uncultured clone	Nakuru_10	Lake Nakuru, Kenya	HM990659	This study
Picocystis salinarum, uncultured clone	Nakuru_14	Lake Nakuru, Kenya	HM990660	This study
Picocystis salinarum, uncultured clone	Nakuru_20	Lake Nakuru, Kenya	HM990661	This study
Picocystis salinarum, uncultured clone	Nakuru_21	Lake Nakuru, Kenya	HM990662	This study
Picocystis salinarum, uncultured clone	Nakuru_22	Lake Nakuru, Kenya	HM990663	This study
Picocystis salinarum, uncultured clone	Nakuru_32	Lake Nakuru, Kenya	HM990664	This study
Picocystis salinarum, uncultured clone	Nakuru_33	Lake Nakuru, Kenya	HM990665	This study
Picocystis salinarum, uncultured clone	Nakuru_82	Lake Nakuru, Kenya	HM990666	This study
Unidentified coccoid green alga	RCC 287	Equatorial Pacific	AY425302	Guillou *et al.* (2004)
Unidentified coccoid green alga	CCMP 1205	Marine, collection site unknown (Trident cruise)	U40921	Potter *et al.* (1997)
Environmental sequence	RA001219.46	English Channel, Roscoff, Astan	AY425303	Guillou *et al.* (2004)
Environmental sequence	OLI11059	Equatorial Pacific	AJ402345	Guillou *et al.* (2004)
Environmental sequence	OLI11305	Equatorial Pacific	AJ402358	Guillou *et al.* (2004)
Environmental sequence	OLI11345	Equatorial Pacific	AJ402359	Guillou *et al.* (2004)
Bathycoccus prasinos	ALMO2	Alboran Sea	AY425314	Guillou *et al.* (2004)
Bathycoccus prasinos	BLA77	Mediterranean Sea	AY425315	Guillou *et al.* (2004)
Ostreococcus tauri	BCC17000	Mediterranean Sea	GQ426344	Grimsley *et al.* (2010)
Ostreococcus tauri	CB6	Mediterranean Sea	GQ426346	Grimsley *et al.* (2010)
Ostreococcus sp.	RCC 344	North Atlantic	AY425307	Guillou *et al.* (2004)
Ostreococcus sp.	RCC 393	Thyrrenian Sea	AY425311	Guillou *et al.* (2004)
Coccoid green alga	CCMP 1407	North Atlantic	U40919	Potter *et al.* (1997)
Prasinococcus sp.	CCMP 1202	North Atlantic	AF203401	Fawley *et al.* (2000)
Pycnococcus provasolii	RCC 244	Mediterranean Sea	AY425305	Guillou *et al.* (2004)
Pycnococcus provasolii	CCMP 1199	North Atlantic	AF122889	Fawley *et al.* (2000)
Chlorophyceae				
Bracteacoccus minor	UTEX 66		U63097	Lewis (unpublished)
Monoraphidium dybowskii	SAG 202-7e	Spring in place of pilgrimage, Lourdes, France	Y16939	Krienitz *et al.* (2001)
Mychonastes homosphaera	CCAP 211/8E	Lake Erken, Sweden	X73996	Huss *et al.* (1999)
Mychonastes rotundus	CCAP 211/99	Small dam 'Rhinopool', Nakuru National Park, Kenya	GQ477048	Krienitz *et al.* (2011)
Trebouxiophyceae				
'*Chlorella minutissima*'	SAG 1.80	Mangrove swamp, Bermuda	AB006046	Hanagata (unpublished)
Chlorella vulgaris	SAG 211-11b	Pond near Delft, Netherlands	X13688	Krienitz *et al.* (2004)
Chloroparva pannonica	ACT 0608	Böddi-szék soda pan, Hungaria	FJ013257	Somogyi *et al.* (2011)
Choricystis minor	SAG 251-1	Grandprês, Canada	X89012	Krienitz *et al.* (1996)
Choricystis minor	SAG 17.98	Basin in greenhouse, Göttingen, Germany	AY762605	Karsten *et al.* (2005)
Dictyosphaerium ehrenbergianum	CCAP 222/1A	Pond near Cambridge, UK	GQ176854	Krienitz *et al.* (2010)
Marinichlorella kaistiae	KAS 007	South Sea, Korea	AB176665	Aslam *et al.* (2007)
Marvania geminata	SAG 12.88	Freshwater, Slovakia	AF124336	Bhattacharya & Foth (unpublished)
Marvania coccoides	CCAP 251/1b	Freshwater, Cambridge, UK	AB080301	Yamamoto *et al.* (2003)
Nannochloris bacillaris	Ogawa *et al.* (1995)	Lake Nakatuna, Japan	AB080300	Yamamoto *et al.* (2003)
Nannochloris eukaryotum	UTEX 2502	Marine aquarium, Croatia	AB080304	Yamamoto *et al.* (2003)
Parachlorella beijerinckii	SAG 2046	Nonnenbach brook, Germany	AY323841	Krienitz *et al.* (2004)
Picochlorum atomum	CCAP 251/7	Marine, fish tank, Cyprus	AB080303	Yamamoto *et al.* (2003)

Table 2. Continued

Species/clone	Strain/clone designation	Origin	Accession number	Reference
Picochlorum maculatum	CCAP 251/3	Marine	AB080302	Yamamoto *et al.* (2003)
Picochlorum oklahomensis	UTEX 2795	Saline pool, Salt Plains National Wildlife Refuge Oklahoma, USA	AY422073	Henley *et al.* (2004)
Charophyceae (as out-group)				
Chara foetida		Botanical Garden Cologne, Germany	X70704	Steinkötter *et al.* (1994)
Nitella capillaris		Botanical Garden Cologne, Germany	AJ250111	Marin & Melkonian (1998)



Figs 4–10. *Picocystis salinarum* in field samples and cultures. Scale bars = 5 μm.
Fig. 4. Dense rough culture of *Picocystis* from Lake Bogoria 3 weeks after transfer of field material into culture medium. Most cells exhibit the characteristic lobate morphology.
Fig. 5. Field sample from Lake Nakuru containing spherical cells of *Picocystis*, pennate diatoms and many grains of silt.
Fig. 6. *Picocystis salinarum*, strain KR 2010/1, isolated from a sample collected in Lake Nakuru.
Figs 7, 8. Lumpy aggregates of mother cells of *Picocystis* in field samples from Magadi hot springs.
Fig. 9. *Picocystis* ingested by an amoeba, observed in a field sample from Magadi hot springs.
Fig. 10. *Picocystis salinarum*, strain KR 2010/4, isolated from a sample collected in Magadi hot springs.

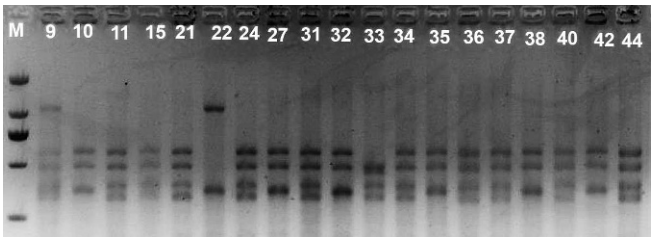


Fig. 11. RFLP diagram of 19 positive clones from field samples collected on 17 January 2010 from Lake Nakuru (Kenya) with restriction endonuclease *Hae*III and PCR primers EukA and EukB. Lane number represented the clone number. Lane M was DNA marker DL2000 (TaKaRa, Dalian, China). Though five band patterns were shown among these clones, the clone sequences with different band patterns were identical to *Picocystis salinarum.*

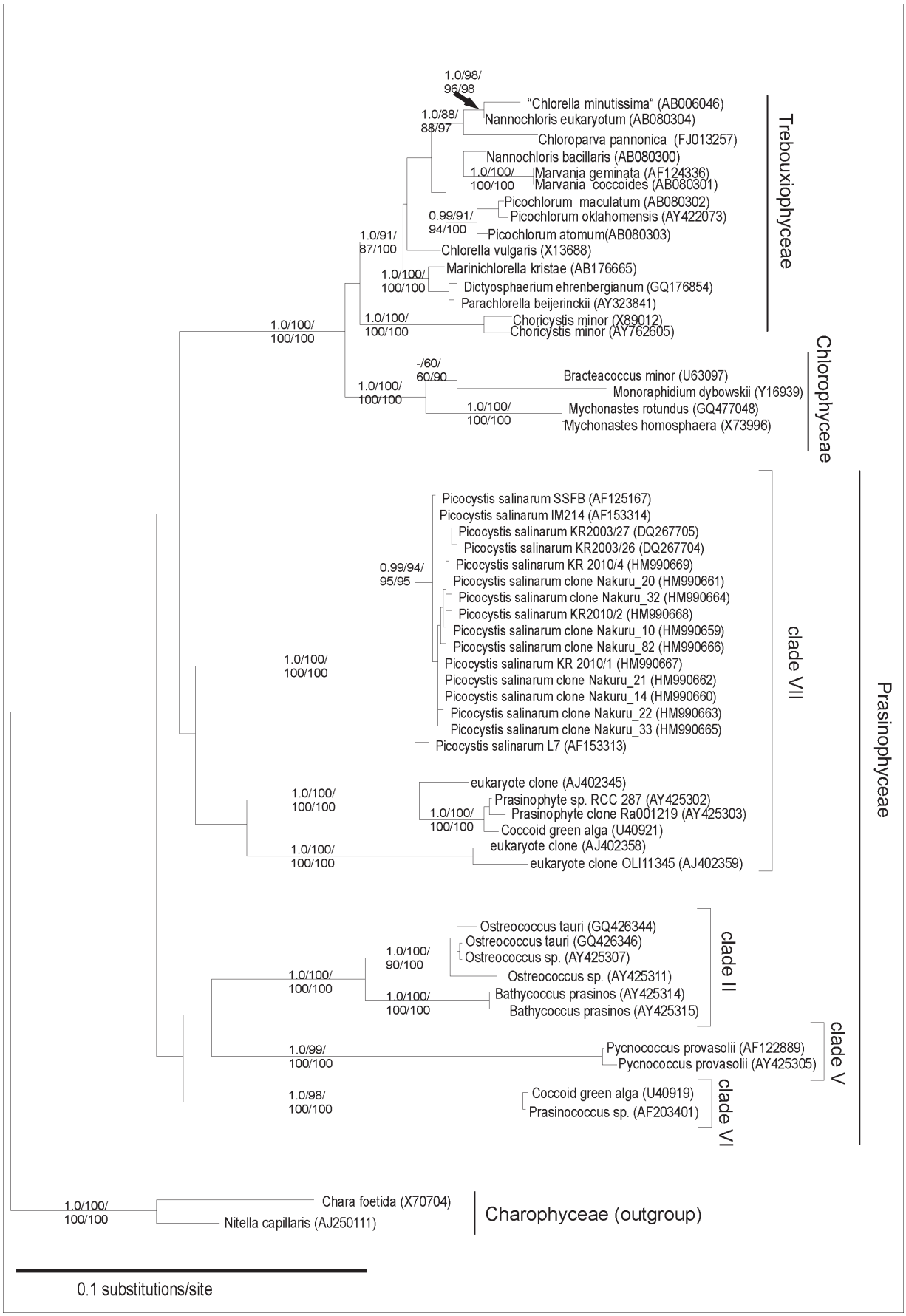
(starting in December 2009), the *Arthrospira* population collapsed completely and was replaced by *Picocystis*, pennate diatoms (from the sediment), several flagellates (cryptophytes) and *Synechococcus* morphotypes. The number of *Picocystis* ranged from 3.3 to 3.5 billion cells l^{-1} with a biomass of more than 7000 µg l^{-1} (53–68% of the total phytoplankton biomass). The cells present were oval to spherical, not lobate and difficult to identify because of the high inorganic turbidity of the water (Fig. 5). Even in cultures, no lobate cells were observed (Fig. 6). At the same time, *Picocystis* was detected in the two lagoons of Lake Magadi with cell densities similar to that of year 2002 (3–4 million l^{-1}). However, a different association of dominant phytoplankton was observed. Green picoplankton was recorded in a hot spring draining into a dry lagoon at the southern part of the lake. The hot spring had a temperature of 35°C. Other hot springs in the same area with higher temperatures were not inhabited by such picoplankton. Molecular analysis of a picoplankton strain isolated from the hot springs confirmed that the isolate was *Picocystis*. *Picocystis*-like cells in Magadi hot spring occurred in aggregations (Figs 7, 8) and served as food for amoebae (Fig. 9). Cells from field samples and cultures (Fig. 10) of *Picocystis* from the hot spring were considerably larger than those growing in lakes and never produced lobate cells. Whereas the vegetative cells of *Picocystis* from lake plankton had a diameter of 1.5–2 µm and mother cells did not exceed 2.5 µm, the vegetative cells from hot spring samples were 2.5–3.5 µm with mother cells (producing two or four autospores) having a maximum diameter of 5 µm.

From the field sample from Lake Nakuru, we established a clone library targeting eukaryotes containing 77 positive *Picocystis* clones from a total of 143 selected clones. We sequenced eight positive clones that might represent different genotypes under RFLP. However, all the sequenced clones, such as clone Nakuru_22 (Fig. 11), represented only *P. salinarum*. Using another primer set designed to amplify Chloroplastida, we subjected 86 positive clones from a total of 97 selected clones to RFLP. Nine positive clones with different RFLP pattern were sequenced but had 99% similarity to *P. salinarum*; thus, the sequences were not used in the phylogenetic analysis. The clone libraries based on both primer sets suggested that the dominant *Picocystis* genotype was *P. salinarum*.

Together with the five sequences of unialgal cultures of *Picocystis* from Kenya, eight uncultured clones from Lake Nakuru established a clade accompanied by two close sister clades containing the three classical *P. salinarum* strains SSFB, IM 214 and L7 (Fig. 12). This *Picocystis* lineage together with four clones and two unidentified strains from the Atlantic or Pacific Ocean, belonged to the prasinophycean clade VII according to Guillou *et al.* (2004). However, the sister relationship of *Picocystis* and the unidentified prasinophyceans was not supported in our analyses. Next to clade VII, members of four genera of picoplanktonic prasinophytes from marine habitats formed three other clades, which also were designated according to Guillou *et al.* (2004): *Bathycoccus* and *Ostreococcus* in clade II, *Pycnococcus* in clade V and *Prasinococcus* in clade VI (= class Mamiellophyceae). Parallel to the prasinophytes was a large clade comprising several lineages of Chlorophyceae and Trebouxiophyceae, all of which included members of picoplankton: *Mychonastes*, *Monoraphidium*, *Choricystis*, *Picochlorum*, *Chloroparva*, *Marvania*, '*Nannochloris*' and '*Chlorella*' from contrasting habitats.

DISCUSSION

At the beginning of our studies on *Picocystis*, identification of this tiny sphere was a big challenge, and we had to sharpen our visual tools to be able to do so. Based on the experience gained during our collaboration with the late Ralph A. Lewin (Scripps Institution of Oceanography in La Jolla, CA, USA), who established the strains from San Francisco Salt Works, Mono Lake and Inner Mongolia, we learned that the best criterion to discriminate *Picocystis* from other picoplankton was the trilobate cell morphology in older cultures. Two lateral lobes contained the chloroplast, and the third lobe contained the nucleus (Lewin *et al.* 2000). Later, we found out that these lobate cells can also occur in field samples, making their identification in the natural environment easier (Krienitz & Kotut 2010). Nevertheless, a simple spherical morphology was mostly observed in our samples. Field samples from Magadi hot springs and a strain isolated from the same sample (KR 2010/4) had cells that were considerably larger and never produced lobes, and the mother cells often produced four autospores. We recommend that further studies be carried out to confirm whether this morphotype represents a new species. Generally, the basis for distinguishing between species in the genus *Picocystis* is presently the subject of discussion among picophytoplanktologists. Roesler *et al.* (2002) referred to a personal discussion with T. Hollibaugh, who suspected that a new strain (ML) of *Picocystis* from Mono Lake could be a separate species. We tried to analyse the ITS2 region of rRNA gene of our African strains. Compensatory base changes in the conserved regions of this gene are good markers for species delineation (Coleman 2007; Müller *et al.* 2007) and have already been successfully applied to other picoplankton species, such as *Mychonastes* (Krienitz *et al.* 2011). However, our efforts to obtain usable ITS2 sequences from the African material have been unsuccessful.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96/98
"Chlorella minutissima" (AB006046)
1.0/88/
88/97
Nannochloris eukaryotum (AB080304)
Chloroparva pannonica (FJ013257)
Nannochloris bacillaris (AB080300)
1.0/100/
100/100
Marvania geminata (AF124336)
Marvania coccoides (AB080301)
Picochlorum maculatum (AB080302)
0.99/91/
94/100
Picochlorum oklahomensis (AY422073)
Picochlorum atomum(AB080303)
1.0/91/
87/100
Chlorella vulgaris (X13688)
Marinichlorella kristae (AB176665)
1.0/100/
100/100
Dictyosphaerium ehrenbergianum (GQ176854)
Parachlorella beijerinckii (AY323841)
1.0/100/
100/100
Choricystis minor (X89012)
Choricystis minor (AY762605)
Trebouxiophyceae
1.0/100/
100/100
-/60/
60/90
Bracteacoccus minor (U63097)
Monoraphidium dybowskii (Y16939)
1.0/100/
100/100
1.0/100/
100/100
Mychonastes rotundus (GQ477048)
Mychonastes homosphaera (X73996)
Chlorophyceae
Picocystis salinarum SSFB (AF125167)
Picocystis salinarum IM214 (AF153314)
Picocystis salinarum KR2003/27 (DQ267705)
Picocystis salinarum KR2003/26 (DQ267704)
0.99/94/
95/95
Picocystis salinarum KR 2010/4 (HM990669)
Picocystis salinarum clone Nakuru_20 (HM990661)
Picocystis salinarum clone Nakuru_32 (HM990664)
Picocystis salinarum KR2010/2 (HM990668)
Picocystis salinarum clone Nakuru_10 (HM990659)
Picocystis salinarum clone Nakuru_82 (HM990666)
1.0/100/
100/100
Picocystis salinarum KR 2010/1 (HM990667)
Picocystis salinarum clone Nakuru_21 (HM990662)
Picocystis salinarum clone Nakuru_14 (HM990660)
Picocystis salinarum clone Nakuru_22 (HM990663)
Picocystis salinarum clone Nakuru_33 (HM990665)
Picocystis salinarum L7 (AF153313)
clade VII
eukaryote clone (AJ402345)
1.0/100/
100/100
Prasinophyte sp. RCC 287 (AY425302)
1.0/100/
100/100
Prasinophyte clone Ra001219 (AY425303)
Coccoid green alga (U40921)
1.0/100/
100/100
eukaryote clone (AJ402358)
eukaryote clone OLI11345 (AJ402359)
Ostreococcus tauri (GQ426344)
Ostreococcus tauri (GQ426346)
1.0/100/
90/100
Ostreococcus sp. (AY425307)
1.0/100/
100/100
Ostreococcus sp. (AY425311)
1.0/100/
100/100
Bathycoccus prasinos (AY425314)
Bathycoccus prasinos (AY425315)
clade II
Pycnococcus provasolii (AF122889)
1.0/99/
100/100
Pycnococcus provasolii (AY425305)
clade V
1.0/98/
100/100
Coccoid green alga (U40919)
Prasinococcus sp. (AF203401)
clade VI
Prasinophyceae
1.0/100/
100/100
Chara foetida (X70704)
Nitella capillaris (AJ250111)
Charophyceae (outgroup)
0.1 substitutions/site

Because of its fast growth and high primary production, picophytoplankton can play a key role in food webs of freshwater, marine and saline habitats (Stockner & Antia 1986; Raven 1999). The best-studied saline alkaline lake hosting *Picocystis* is Mono Lake east of Sierra Nevada, situated in the temperate zone, where it switches between monomictic and meromictic stages and is characterised by a high productivity (Hollibaugh *et al.* 2001; Melack 2002; Roesler *et al.* 2002). *Picocystis* in Mono Lake can grow under a wide range of environmental conditions and can survive drastic changes in growth conditions, such as those associated with low light and oxygen availability. It contributes about 50% of the biomass of primary producers and is one of the main food resources for the brine shrimp *Artemia monica* Verrill (Roesler *et al.* 2002). In our cultures, we observed that cell numbers of *Picocystis* can increase 2–3-fold per day (not shown). These growth rates are comparable to the upper growth limit of the picoplankton of the marine embayments of Hawaii, where a doubling rate 0.97 to 3.62 per day has been reported (Bienfang *et al.* 1984). The main primary producer in the African soda lakes is the cyanobacterium *Arthrospira fusiformis* (Voronichin) Komárek & Lund, also known to be a fast-growing species. Vonshak (1997) recorded doubling times of 11–20 hours in cultures under saline conditions at 35°C. Further studies should be carried out to establish the nature of interaction (e.g. competition) between these key producers, which differ considerably in size and fall in different phytoplankton functional groups. Based on the functional groups of Reynolds *et al.* (2002) and Padisák *et al.* (2009), the microplanktonic *Arthrospira* belongs to the functional group S2, while the picoplanktonic *Picocystis* belongs to group K. The main primary consumers of African soda lakes, the Lesser Flamingos, which occur in flocks of several hundreds of thousands at Bogoria and Nakuru, require microplanktonic food algae for their survival. This is because the filter apparatus in their bills cannot retain the tiny picoplankton (Jenkin 1957; Vareschi 1978; Krienitz & Kotut 2010). Hence, the mass development of *Arthrospira* represents the main food source for the Lesser Flamingos. The replacement of *Arthrospira* by *Picocystis* adversely affects the Lesser Flamingos, as it drastically reduces the food supply to the consumer birds. In the face of diminishing food supply, the starving birds have to migrate to other lakes in the region that can provide a sufficient biomass of *Arthrospira* or benthic diatoms (Tuite 2000). However, the dense population of *Picocystis* could be an attractive food source for zooplankton, especially the rotifers. Vareschi & Vareschi (1984) described the crustacean *Lovenula africana* Daday and rotifers such as *Brachionus dimidiatus* Bryce and *B. plicatilis* Müller as main representatives of zooplankton in Lake Nakuru. Hence, the rotifers could benefit from an increase in the biomass of the picoplankton.

In the hot springs of Lake Magadi, *Picocystis* seems to be a food resource for amoebae and possibly the endemic fish *Oreochromis grahami* Boulenger, which feeds on cyanobacteria and algae (Coe 1966; Seegers & Tichy 1999). Normally, the fish cannot filter such tiny green algae; however, the *Picocystis* in the springs formed clumps that reached an edible size. This was apparently not the case in Lake Nakuru, where *Picocystis* exhibits a solitary life form and is therefore too small to be captured by the filtration system of the fish. The only fish species in Lake Nakuru, *O. grahami* was introduced into the lake in about 1960 and feeds mainly on *Arthrospira* (Vareschi 1979).

The water samples from our study lakes containing *Picocystis* showed a wide range of salinity, with the lowest value of 14‰ recorded in one of the lagoons of Lake Magadi and the highest value of ~ 300‰ recorded in Lake Katwe. This range covers all the reported categories of saline lakes. According to Hammer *et al.* (1983), three categories of saline lakes can be recognized: hyposaline (3–20‰), mesosaline (20–50‰) and hypersaline (> 50‰). Ocean water with an average salinity of 35‰ (Sommer 1994) falls within the mesohaline category. The highest abundance of *Picocystis*, with cell numbers of between 1.0 and 3.5 billion cells l^{-1}, occurred in lakes Bogoria and Nakuru, with salinities of between 50.7 and 61.6‰. Roesler *et al.* (2002) demonstrated experimentally that *Picocystis* from Mono Lake was able to grow in a salinity range of 0–260‰, with a peak growth at a salinity of 40‰. *Picocystis* is able to produce osmolytes as an adaptive way to reduce the stress associated with fluctuations in ionic strength. Ciulla *et al.* (1997) have documented the existence of such substances in aerobic bacteria living in Mono Lake.

To date, the presence of *Picocystis* has been confirmed in inland waters but not in marine habitats. However, the closest relatives of *Picocystis* come from the sea. In the phylogenetic tree, several field samples and two strains from the Atlantic and Pacific regions analysed by Guillou *et al.* (2004) established a sister clade to the *Picocystis* lineage. Guillou *et al.* (2004) combined both lineages into clade VII as a new clade of prasinophytes, as suggested by Marin & Melkonian (2010). However, so far, the sister relationship between different clades of prasinophytes is not well supported (Guillou *et al.* 2004). Other intensively studied marine coccoid prasinopytes of picoplankton size, such as *Bathycoccus prasinos* Eikrem & Throndsen, *Ostreococcus tauri* Courties & Chrétiennot-Dinet, *Prasinococcus capsulatus* Miyashita & Chihara and *Pycnococcus provasolii* Guillard (Courties *et al.* 1998; Fawley *et al.* 2000; Guillou *et al.* 2004; Grimsley *et al.* 2010), established neighbour clades in the tree. This high diversity of picoplanktonic chlorophytes in the sea (Viprey *et al.* 2008) confirms the famous thesis of Potter *et al.* (1997) that convergent evolution masks extensive biodiversity among marine coccoid picoplankton.

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Fig. 12. Phylogenetic tree comparing SSU rRNA genes of *Picocystis* strains, field clones and other picoplankton from field and culture samples from saline, marine or freshwater habitats. Phylogenetic tree inferred from maximum likelihood (ML) analysis with bootstrap support (BP) and posterior probabilities (PP) indicated at the nodes. Left number from Bayesian analyses, followed by ML, MP and NJ. Hyphen indicates BP values lower than 50% and PP values lower than 0.95. Branch length represents substitutions per site.

It is important to note that a high diversity of coccoid picoplankton lineages occurs not only in the Prasinophyceae but also in the Trebouxiophyceae. '*Chlorella minutissima* Fott & Nováková, SAG 1.80' isolated from a mangrove swamp (Bermuda) is related to '*Nannochloris eukaryotum* Wilhelm, Eisenbeis, Wild & Zahn, UTEX 2502' from a marine aquarium (Croatia). The taxonomy of both strains remains unresolved and requires further investigations (Somogyi *et al.* 2011). In the genus *Picochlorum*, two picoplankton species from the sea are well known [*P. atomus* (Butcher) Henley, Hironaka, Guillou, M. Buchheim, J. Buchhheim, M. Fawley & K. Fawley and *P. maculatum* (Butcher) Henley, Hironaka, Guillou, M. Buchheim, J. Buchhheim, M. Fawley & K. Fawley)], while one species is known from the salt pans of Oklahoma, USA (*P. oklahomenis* Hironaka; Henley *et al.* 2004). *Chloroparva pannonica* Somogyi, Felföldi & Vörös is a characteristic picoplanktonic chlorophyte from Hungarian salt pans (Somogyi *et al.* 2010). *Marinichlorella kaistiae* Z. Aslam, W. Shin, M.K. Kim, W.-T. Im & S.T. Lee, which is accommodated in the *Parachlorella* clade of Chlorellaceae (Bock *et al.* 2011), is a marine nanoplankter (Aslam *et al.* 2007). Other clades of the Trebouxiophyceae contain picoplankton from freshwaters: the autosporine *Choricystis minor* (Skuja) Fott (Krienitz *et al.* 1996, 1999; Hepperle & Krienitz 2001), *Nannochloris bacillaris* Naumann (Yamamoto *et al.* 2003) and the budding *Marvania* (Yamamoto *et al.* 2003).

Within Chlorophyceae, until now, only two lineages containing picoplankters were found. *Mychonastes* species were described from brackish and fresh waters (Simpson & Van Valkenburg 1978; Krienitz *et al.* 1999, 2011). The tiny, rod-shaped *Monoraphidium dybowskii* (Woloszynska) Hindák & Komárková-Legnerová has the potential to produce mass developments in small stagnant inland waters, especially in village ponds and tanks (Krienitz *et al.* 2001).

More than five decades after Rodhe (1955) first drew the attention of limnologists to the existence of tiny 'μ-algae', three decades after the 'sturm und drang' phase of research on marine and freshwater picoplankton in the late 1970s and early 1980s (reviewed by Stockner & Antia 1986) and two decades after Stockner's (1991) 'view from the summit', we can safely observe that a tremendous volume of information on the ecology and phylogeny of the picoplankton has been collected, which considerably exceeds the expectations of the pioneers. However, many questions remain unanswered and provide new challenges to picoplanktologists. *Picocystis salinarum* from saline inland waters represents a link between marine and freshwater habitats from both an ecological and a phylogenetic point of view and is therefore of great interest.

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