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PRIMARY RESEARCH PAPER

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

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Abstract A SSU rRNA gene clone library was constructed to establish the diversity of eukaryotic plankton in the African soda lake Nakuru during a phase of low salinity (9.7 ppt = hyposaline). Normally, the lake is mesosaline (up to 50 ppt) and its phytoplankton is dominated by few species of cyanobacteria, in particular *Arthrospira fusiformis*, which is the main food resource of Lesser Flamingos. Our study recovered a unique phytoplankton species composition characterized by a high diversity of monadoid and coccoid green algae. Out of 77 clones detected, 52 belonged to Chlorophyta. Many of the chlorophytes were transported from the catchment area into the lake through small seasonal rivers and an outflow of the Nakuru town sewage treatment plant. Other phylogenetic groups detected were Fungi, Cryptophyta, Jakobida, Alveolata, Stramenopiles, and Metazoa. Our findings reveal a hidden diversity, which would not have been detected by traditional observations.

Keywords Chlorophyta · Clone library · Lake Nakuru · Lesser Flamingo · Phytoflagellates · Soda lakes · SSU rRNA

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Introduction

The phytoplankton of saline-alkaline lakes in the Great African Rift Valley usually is dominated by cyanobacteria, mainly *Arthrospira fusiformis* (Voronichin) Komárek et Lund (often erroneously identified as "*Spirulina platensis*"), which forms the food base for hundreds of thousands of Lesser Flamingos (*Phoeniconaias minor* Geoffroy Saint Hilaire), the character birds of these unique biotopes. However, the quasi monospecific mass development of *Arthrospira* is unstable and is from time to time replaced by other cyanobacteria or eukaryotic algae (Vareschi, 1982; Melack, 1988; Ballot et al., 2004). Among the eukaryotic plankton, Vareschi (1982) frequently observed dominance by coccoid green algae with crescent-shaped cells. These selenastracean green algae are known to tolerate high salinity (Ferroni et al., 2007). Schagerl & Oduor (2008) have reported the presence of *Chlorella*, *Monoraphidium*, and *Scenedesmus* in the lake. In January 2010, *Arthrospira* in Nakuru was outcompeted by the green picoplankton *Picocystis salinarum* Lewin (Krienitz et al., 2012a).

During periods when the *Arthrospira* population was depressed, notable occurrences of benthic diatoms, cryptomonads, and euglenoids were recorded. While several benthic diatoms and euglenoids can be used as an alternative food source by Lesser Flamingos, most of the other algae are too small for ingestion by the birds (Tuite, 2000; Krienitz & Kotut, 2010).

The endorheic basin of Lake Nakuru is very shallow (maximum depth, 4.5 m) and experiences phases when the lake comes close to completely drying out alternating with periods when it gets flooded by inflows from rivers and surface run-off following heavy rainfall in the lake's catchment area as well as discharge from a sewage treatment plant. These dramatic fluctuations in water level result in wide changes in salinity and plankton composition. Variation in salt concentrations in environments like salt plains has been shown to promote the diversity of protists (Wilson et al., 2004; Buchheim et al., 2010).

We focussed on the hidden diversity of plankton communities that displace *Arthrospira* and other cyanobacteria, which are well adapted to the harsh and rapidly changing conditions of soda lakes. Our contribution adds new insights into plankton diversity, which certainly has significant effects on the structure and function of the food web. In this paper, we present results of a clone library of SSU rRNA genes (small subunit of the 18S rRNA gene) of eukaryotic plankton conducted during a phase of low salinity.

Materials and methods

Sampling

Lake Nakuru is a shallow, endorheic soda lake in the Gregory Rift Valley in Kenya at an altitude of 1,759 m above sea level, with a mean depth of 1.1 m, a maximum depth of 4.5 m, and a surface area of about 40 km^2 (Schagerl & Oduor, 2008). The lake is located in the Lake Nakuru National Park (http://www.kws.org/parks/parks_reserves/LNNP.html) and is world-famous for its dense population of Lesser Flamingos. Water inflow into the lake is mainly from rainfall (800–1,000 mm per year), surface run-off, and small seasonal rivers that include Makalia, Nderit, and Njoro (salinity <0.3 ppt). In addition, the lake also receives treated wastewater from the final sewage oxidation pond of the Nakuru town sewage treatment plant (salinity <1 ppt). Salinity measurements carried out over the period 2001–2011 yielded a mean of 24 ppt. A maximum salinity value of 51 ppt was recorded in January 2010 while a minimum salinity of 9 ppt was measured in November 2011. The sample for the present study was collected on November 1, 2011. Salinity, conductivity, and pH were measured directly in the field using a WTW Multiline P4 meter (Wissenschaftlich Technische Werkstätten Weilheim, Germany). The values recorded are as follows: salinity 9.7 ppt, conductivity 16.3 mS cm^{-1}, and pH 10.48. The site sampled is located near the foot of the Baboon Cliffs at the following coordinates: 00°21′88″S, 36°02′46″E. The field sample was taken from a few centimeters below the water surface.

Microscopy

For microscopy, 250 ml of the water was fixed with formaldehyde to achieve a final concentration of 1%. Phytoplankton numbers were 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 program OPTICOUNT (Opticount, 2008). The specific density of phytoplankton cells was assumed as 1 g cm^{-3}. Morphological examination of the phytoplankton taxa in the sample was carried out using a Nikon Eclipse E 600 light microscope with differential interference contrast.

Molecular analyses

For the molecular analyses, the fresh sample was filtered in the field through membrane filters with a pore size of 0.6 µm (Schleicher & Schuell GmbH, Dassel, Germany), air-dried, and transported to the laboratory in an ice-box. The filters were mechanically sliced into small pieces and rinsed with cell lysis buffer AP1 (Qiagen GmbH, Hilden, Germany). Cells were disrupted with the help of glass beads (Carl Roth GmbH, Karlsruhe, Germany; 0.7 mm) in the Tissuelyser II (Qiagen GmbH, Hilden, Germany). Genomic DNA was extracted in December 2011 using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) following the instructions given by the manufacturer.

Eukaryotic SSU rRNA genes were amplified by PCR (Polymerase Chain Reaction) with eukaryote-specific primers EukA and EukB (Medlin et al., 1988). Amplified rRNA gene products from several individual PCRs were pooled. The clone library was constructed according to Luo et al. (2009, 2011). The PCR was performed with an initial denaturation for 10 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min 30 s at 72°C, and finally an extension for 15 min at 72°C. 100 µl PCR products were cleaned using a QIAGEN purification kit, and then cloned with the Cloning kit (pGEM-T, Promega) following the manufacturer's directions. Libraries were screened for the whole 18S rRNA inserted by PCR with M13 primers.

Full-length sequencing was done with an ABI 3730 Sequencer with four conserved primers: two internal to the PCR products (570 F: 5′-CCA GCA GCC GCG GTA ATT C-3′; 905 F: 5′-GTC AGA GGT GAA ATT CTT GG-3′) and two targeted at the plasmid (M13F and M13R).

Poor-quality sequences and suspected chimeras were screened using BLAST with sequence segments separately, and then using the Chimera Check Program at Ribosomal Data Project II (Cole et al., 2003). The sequences that passed the Chimera screening were phylogenetically grouped and aligned using Clustal X v.1.83; alignments were manually checked using the "multicolor sequence alignment editor" of Hepperle (2003). Some ambiguously aligned positions were removed manually. The phylogenetic analysis was performed subsequently, and neighbor-joining (NEIGHBOR) with the Kimura two-parameter correction algorithm was calculated by Phylip 3.62 package. Support of branches of the tree was obtained by bootstrapping 1,000 datasets. The recent proposed revision of classification of eukaryotes was used in the designation of lineages of the phylogenetic tree (Adl et al., 2005).

SSU rRNA gene sequences reported in this study have been deposited in the GenBank database under the accession numbers JX296576–JX296639. The alignments are available from the authors on request.

Results

A total phytoplankton biomass of 34 mg l^{-1} was recorded in the lake. About 50% of the biomass was contributed by filamentous cyanobacteria, mainly the tiny Oscillatoriales from the family Pseudanabaenaceae, and few filaments of *Arthrospira* and *Anabaenopsis*. Besides cyanobacteria, phytoflagellates comprising cryptophytes, dinophytes, and chlorophytes were abundant. Microscopic identification of these flagellates was difficult. Most of them appeared to be closely related to *Chlamydomonas*. A few flagellates, each equipped with a lorica, were designated as members of *Pteromonas*. Coccoid chlorophytes belonging to the genera *Chlorella*, *Oocystis*, and *Scenedesmus* were also present in the sample in low quantities. The low biomass of *Arthrospira*, and dominance by small-celled phytoflagellates meant that the population of primary producers was not capable of meeting the dietary requirement of Lesser Flamingos, and this probably explained their absence from the lake during the sampling visit.

In total, 77 clones of the SSU rRNA genes comprising 17 phylotypes were completely sequenced (Table 1). The chimera detection software did not uncover any putative chimeras. The sample labeled as NKS (Nakuru Sample) had a rich diversity belonging to seven phylogenetic groups: Fungi, Cryptophyta, Jakobida, Alveolata, Stramenopiles, Chlorophyta, and Metazoa (Fig. 1). The Chlorophyta was found to be the most diverse group (Fig. 2). One Chytridiomycota related clone of the fungus clade was revealed. Two clones with a 99% similarity to *Guillardia theta* Hill et Wetherbee clustered with Cryptophyta. Six clones clustered with clade Jakobida, a group of heterotrophic nanoflagellates. Two clones belonging to Alveolata were the closest to Dinophyceae. One clone had a 92% similarity to *Stramenopile* sp. MESS13. Twelve clones represented the Metazoan clade (not incorporated into the phylogenetic tree) and were grouped with *Brachionus plicatilis* Müller with whom they had a 99% similarity. Chlorophyta took up 69% of all clones and were distributed into five different subclades. Our analysis revealed the presence of genotypes of coccoid chlorophytes such as *Chlorella*, *Oocystis*, and *Scenedesmus*, which were also recorded during microscopic observations. The monadoid (flagellated) members of different subgroups of Chlamydomonadaceae were very diverse in our clone library. Three clones established a sister clade to *Wislouchiella* and *Pteromonas* with 99% similarity. Another three clones were closely related to *Gungnir kasakii* (Nozaki) Nakada with 98% similarity. Three genotypes of *Chlamydomonas* were represented: *Ch. tetragama*

Table 1 Selected clones of eukaryotic plankton in Lake Nakuru and its similarity to published sequences

Clone number	Closest match	Sequence similarity (number of bases, %)	Taxonomic group	References
Fungus				
NKS 146	Uncultured Chytridiomycota clone PFG9SP2005	1678/1804 (93%)	Chytridiomycota	Lefèvre et al. (2008)
Cryptophyta				
NKS 9, 111, 170	*Guillardia theta* CCMP2712	1758/1775 (99%)	Geminigeraceae	Douglas et al. (1991)
Jakobida				
NKS 82 (96, 97, 105, 177, 188)	*Andalucia godoyi* isolate AND28	970/1079 (90%)	Jakobida	Lara et al. 2007
Alveolata				
NKS 106, 180	*Cryptoperidiniopsis broadyi* sp. Folly C5	1636/1810 (90%)	Dinophyceae	Steidinger et al. (2006)
Stramenopile				
NKS 100	*Stramenopile* sp. MESS13	1678/1828 (92%)	Stramenopiles	Park & Simpson (2010)
Chlorophyta				
Trebouxiophyceae				
NKS 3 (18, 138, 144, 163)	*Chlorella sorokiniana* Prag A14	1787/1801 (99%)	Trebouxiophyceae	Huss et al. (1999)
Oocystaceae				
NKS 72	*Oocystis marssonii* Krienitz 96/10	1737/1782 (97%)	Oocystaceae	Hepperle et al. (2000)
Scenedesmaceae				
NKS 5 (8, 16, 102, 168, 178)	*Scenedesmus armatus* var. *subalternans* CCAP276/4A	1771/1781 (99%)	Scenedesmaceae	Unpublished
Chlamydomonadaceae				
NKS 98 (115, 119)	*Wislouchiella planctonica* UTEX LB 1030	1769/1785 (99%)	Phacotaceae	Hepperle et al. (1998)
NKS 112 (116, 139)	*Gungnir kasakii* NIES-1360	1709/1749 (98%)	Haematococcaceae	Nakada et al. (2008)
NKS 157 (75, 87, 114, 174)	*Chlamydomonas tetragama* NIES 446	1735/1779 (98%)	Chlamydomonadaceae	unpublished
NKS 176	*Chlamydomonas raudensis* CCAP 11/131	1714/1748 (98%)	Chlamydomonadaceae	Pocock et al. (2004)
NKS 172	*Chlamydomonas reinhardtii* strain SAG 53.72	1510/1622 (93%)	Chlamydomonadaceae	Fulnečková et al. (2012)
NKS 175	*Oogamochlamys ettlii* UTEX 2218	1712/1777 (96%)	Chlamydomonadaceae	Pröschold et al. (2001)
NKS 4 (7, 13, 14, 15, 24, 53, 55, 60, 61, 63, 65, 85, 86, 103, 107, 123, 149, 151, 153, 158, 159, 166, 187, 190, 192)	Uncultured Chlorophyta clone AY2009B6	1711/1754 (98%)	Uncultured Chlorophyta	Monchy et al. (2011)
Metazoan				
NKS 23 (84, 95, 140, 147, 150, 152, 155, 156, 173, 179, 189)	*Brachionus plicatilis*	1801/1810 (99%)	Brachionidae	Aguinaldo et al. (1997)

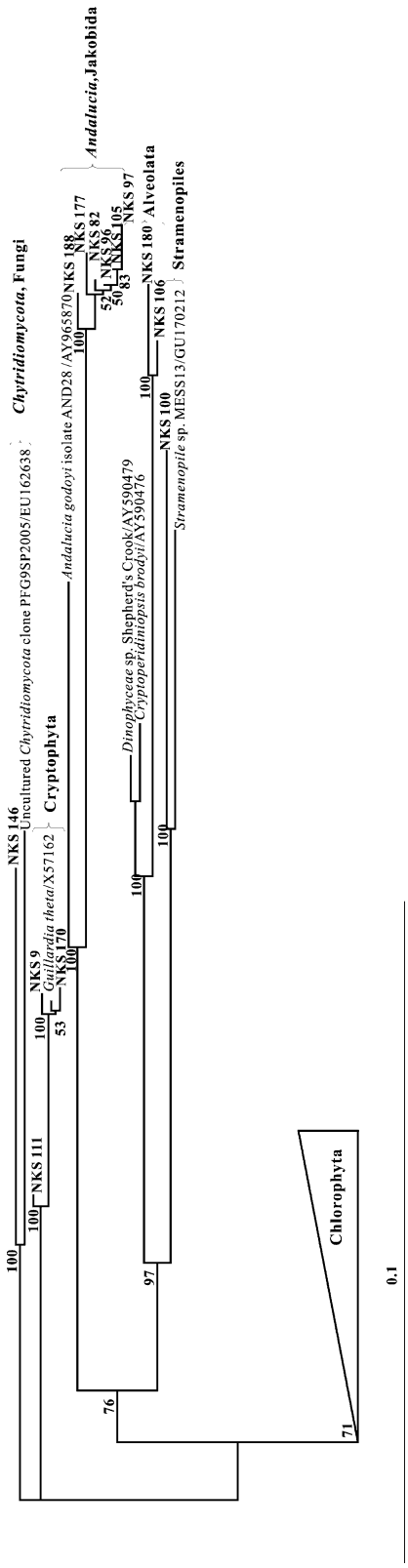

Fig. 1 Phylogeny tree of SSU rRNA gene sequences from eukaryotic plankton of Lake Nakuru (Kenya) by Neighbor-joining analysis. Labels in *bold* indicate clones found in this study. Main lineages were Fungi, Cryptophyta, Jakobida, Alveolata, Stramenopiles, Chlorophyta, and Metazoa (not involved into the tree analysis). Bootstrap support values (>50%) of neighbor-joining analysis (1,000 replicates) are marked in the tree. The *scale bars* represent nucleotide substitutions per site; the actual value depends on the branch lengths in the tree

(Bohlin) Wille, *Ch. raudensis* Ettl, and *Ch. reinhardtii* Dangeard. One clone was very close to *Oogamochlamys ettlii* Pröschold, Marin, Schlösser et Melkonian with a 96% similarity. Twenty-six clones clustered with an uncultured Chlorophyta clone AY2009B6 with a 98% similarity.

Discussion

Molecular approaches presently provide new insights into biodiversity and its ecological function. This is of special importance to small organisms, which would otherwise not be detected and/or correctly identified. Such protists are not easily recognizable, hence can only be provisionally identified by microscopy. To our knowledge, the only genetic study dealing with eukaryotic phytoplankton of Lake Nakuru was the SSU rRNA gene analysis of field clones and cultured strains of the prasinophycean green alga *Picocystis salinarum* (Krienitz et al., 2012a). The mass development of *Picocystis* in the lake was recorded in January 2010 when salinity reached a maximum value of 51 ppt (hypersaline conditions; Hammer et al., 1983). *Picocystis* was accompanied by only few sediment diatoms and tiny cyanobacterial filaments (Krienitz et al., 2012a).

The present study revealed the phytoplankton properties at the opposite end of the salinity range in the lake (a low salinity of about 10 ppt). In this hyposaline environment, the biodiversity of eukaryotic phytoplankton was higher compared to hypersaline conditions. However, the situation under both scenarios (hypersaline and hyposaline conditions) was very unfavorable to the Lesser Flamingos, this was because food was not available; hence the birds had to leave the lake.

The consequence of considerable changes in the physicochemical properties of the water body was the invasion of the phytoplankton community by "new" taxa. Where do these taxa come from? The plankton community is often recruited from taxa which are hidden

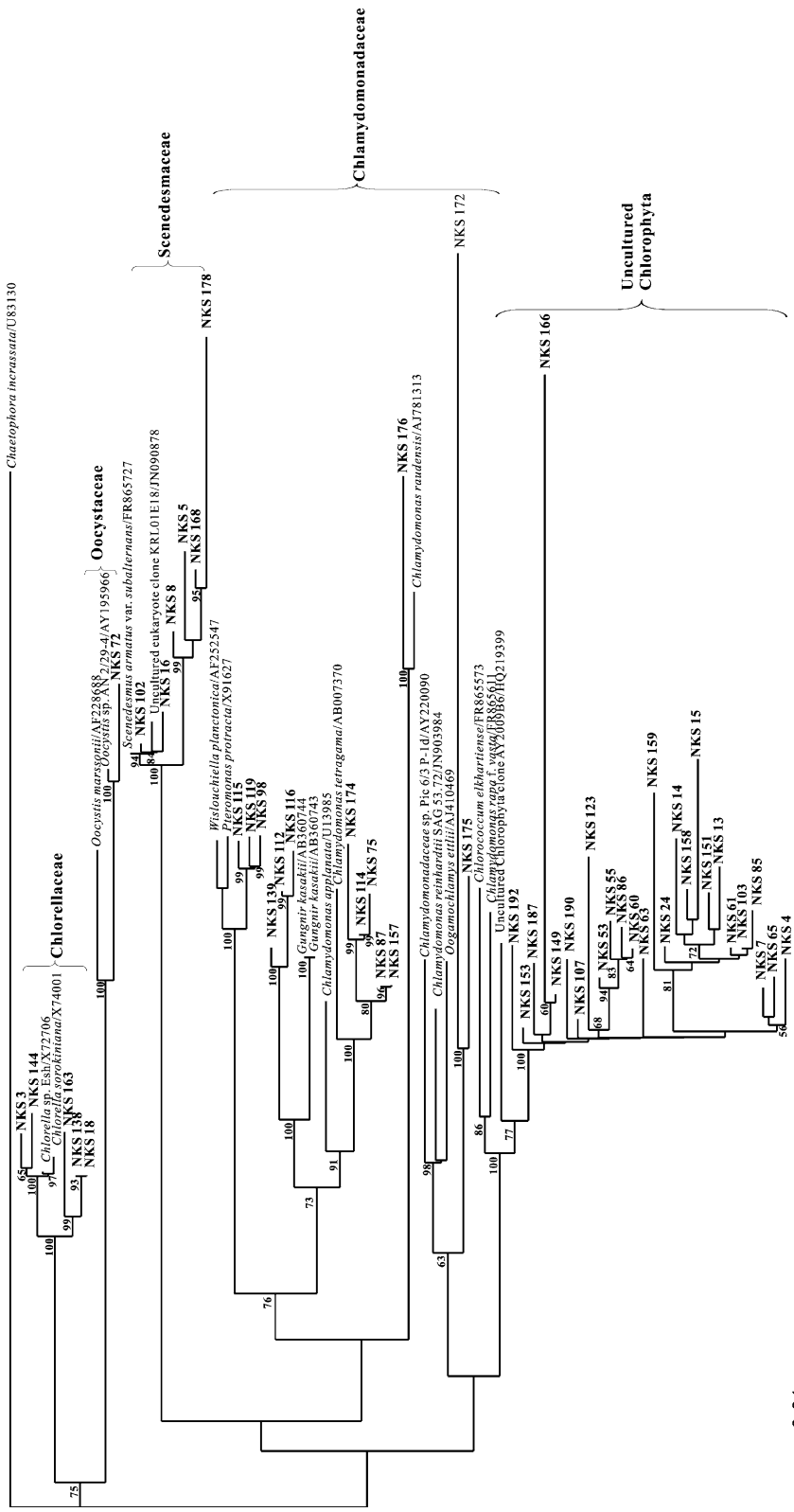

Fig. 2 Phylogenetic tree of the predominant main phylum Chlorophyta with *Chaetophora incrassata* as outgroup. 1,861 Positions were considered from an alignment of total sequences. Bootstrap support values (>50%) of neighbor-joining analysis (1,000 replicates) are marked in the tree. The *scale bars* represent nucleotide substitutions per site; the actual value depends on the branch lengths in the tree

in the "ecological memory" of the lake. Some of these taxa were probably present in the phytoplankton in the past (but were not recorded) and are preserved in the sediments or other niches. In response to changes in environmental conditions, these taxa can establish new populations during plankton successions and can also influence the present or future responses of the community. This phenomenon has been observed in different lakes in Europe (Padisák, 1992; Padisák et al., 2010). The second source is the introduction from outside the lake by erosion from the catchment area or transportation from other regions by different vectors such as water, air, animals, especially water birds, or man (Padisák, 2009). For those taxa transported from freshwater or soil habitats to the soda lake, it is a great advantage to adapt to the saline conditions. On the other hand, organisms transported by water birds or wind from the sea can easily adapt to the saline conditions of saline inland lakes. However, under hypersaline conditions they are also confronted by a much higher salinity than in the sea where it is about 35 ppt (Sommer 1994).

Evidence from published observations on the distribution of the different groups recorded in our study confirm that the taxa closely related to our clones occur in different habitats including freshwater, saline, or marine environments as described below.

Chytridiomycota

One clone of the aquatic fungi group was detected in Lake Nakuru. These parasitic chytrids are very common in freshwaters and have considerable effects on phytoplankton (Kagami et al., 2007). However, they can tolerate saline conditions (Voronin, 2008).

Cryptophyta

These small-celled auto- and mixotrophic flagellates were frequently observed in Lake Nakuru (Vareschi 1982; Schagerl & Oduor, 2008) and provisionally placed in the genera *Cryptomonas* or *Rhodomonas*. The closest match to clone NKS 170 is *Guillardia theta*, a cryptophyte from coastal waters of North America (Hill & Wetherbee, 1990).

Jakobida

This is a recently discovered group of heterotrophic nanoflagellates. Members of the genus *Andalucia* have a cell length of 3–5 μm and were found in marine sediments or soils (Simpson & Patterson, 2001; Lara et al., 2006).

Alveolata

The closest match was next to Dinophyceae. The heterotrophic dinoflagellate *Cryptoperidiniopsis broadyii* Steidinger, Landsberg, P. L. Mason, Vogelbein, Tester et Litaker is known from estuaries of North America (Steidinger et al., 2006).

Stramenopiles

This group was weakly recovered and is represented by one clone in the clone library related to the halotolerant heterotrophic flagellates of Placididea (Park & Simpson, 2010).

Chlorophyta

The green algal clones contributed the majority of clones in the clone library of Lake Nakuru. However, we have not been able to find taxonomic relatives for 26 clones of uncultured Chlorophyta. This is possibly because these clones represent new taxa, or that they exist in classical morphological descriptions whose SSU rRNA genes have not been analyzed. The remaining 26 clones of Chlorophyta belonged to three different families: Chlorellaceae, Scenedesmaceae, and Chlamydomonadaceae.

The coccoid members exhibiting a close relationship with *Chlorella*, *Oocystis*, and *Scenedesmus* are common in freshwater and soil environments and are known to survive also in extreme conditions such as a high salinity and temperature (Kessler, 1982). Furthermore they can easily be introduced from other sites. One of the main sources of coccoid green algae for Lake Nakuru is the final sewage oxidation pond of Nakuru town sewage treatment plant, which is occasionally colonized by dense populations of *Chlorella* and *Scenedesmus* relatives (Kotut et al., 2010). This pond was found to accommodate the highest biodiversity of Chlorellaceae in Kenyan waters ever studied (Krienitz et al., 2012b).

The monadoid (flagellated) members of different subgroups of Chlamydomonadaceae were diverse in our clone library. Three clones established a sister clade to *Wislouchiella* and *Pteromonas*; phytoflagellates

equipped with a lorica (Phacotaceae). They are related to *Pteromonas protracta* Lemmermann. *Pteromonas* was often found in numbers exceeding one million cells ml^{-1} in an artificial dam in the Lake Nakuru National Park built for watering wildlife (Krienitz unpubl. observation). This dam is located at a distance of about 2 km from the eastern shore of Lake Nakuru and could be the source of *Pteromonas*. Closely associated to the loricated green flagellates is the clade of naked *Dunaliella* relatives which included three clones with a close match to *Gungnir kasakii*. Members of the genus *Gungnir* are rare and have been studied exclusively in freshwater (Nakada et al., 2008). The other lineages of Chlamydomonadaceae contained clones related to subgroups of this family with a typical chlamys. Most of the chlamydomonads originate from freshwaters and soils (Pröschold et al., 2001). *Ch. raudensis* is psychrophilic and found in the Antarctica (Pocock et al., 2004) and a few members of Chlamydomonadaceae are known to be halotolerant (Leliaert et al., 2012). Combined morphological, ecological, and molecular investigations of the green flagellates will be essential to give a clear picture of their systematics and role in saline-alkaline ecosystems.

Studies involving samples taken regularly along a salinity gradient will enhance our understanding of how the shift in this unique community of microphytes in Lake Nakuru reflects the ecological situation. Our survey may help to build the bridge between classical morphological and ecological methods with molecular phylogenetic applications in ongoing studies on the famous soda lakes of East Africa.

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