MOLECULAR MICROBIAL EUKARYOTE DIVERSITY IN FIVE RIFT VALLEY LAKES AND DESCRIPTION OF THREE CILIATES SPECIES FROM LAKE BOGORIA, KENYA

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DEDICATIONS

In loving memory of my late father, Henry Martin Ong'ondo (1944 – 1983)

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List of Abbreviations and Acronyms

BLAST	–	Basic Local Alignment Search Tool
DNA	–	Deoxyribonucleic acid
HNF	–	Heterotrophic nanoflagellate
MEGA	–	Molecular Evolutionary Genetics Analysis
NCBI	–	National Centre for Biotechnology Information
NGS	–	Next generation sequencing
OTU	–	Operational taxonomic unit
PCR	–	Polymerase chain reaction
rRNA	–	Ribosomal Ribonucleic Acid
SSUrRNA	–	Small subunit ribosomal ribonucleic acid
UNEP	–	United Nations Environmental Programme
V9	–	Hypervariable region 9
WTW	–	Wissenschaftlich-Technische Werkstätten

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CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background

Current information of freshwater ecosystems and biodiversity is incomplete and lacks a global coverage (UNEP, 1999). This is specifically true for the vast majority of tropical and subtropical habitats. In Kenya, the (Gregory) Rift Valley contains several lakes ranging in size from the 250 km long Lake Turkana at the north, to small (< 1 km) volcanic crater lakes, which are all endorheic in depressions on the valley floor or inside volcanic craters (Talling and Talling, 1965). Most of the lakes, as a consequence of the endorheic nature, are saline to a greater or lesser degree. The importance of the lakes both from an environmental and socio-economic perspective cannot be understated with many of the lakes being a source of income from tourism and/or are critical habitats formigratory birds such as flamingo (Schagerl and Oduor, 2008). The ecological implications of climate change, development activities, and increasing high demand for water and other resources on these aquatic ecosystems by the ever increasing human population are still poorly understood.

A number of the Rift Valley lakes (e.g., Nakuru and Bogoria) are designated Ramsar sites and are nationally protected under the Kenya Wildlife Conservation and Management act (Part VI) 2013, with the prime objective of ensuring optimum returns in termsof cultural, aesthetic and scientific gains. These unique ecosystems remain largely understudied especially in terms of the processes controlling distribution and biodiversity of microbial eukaryotic species, community structure and food web dynamics (e.g., Vareschi, 1982; Duckworth *et al*., 1996; Talling, 2001; Williams, 2002). The focus of past studies was traditionally on functional categories (e.g., trophic chain levels) rather than on biodiversity and species composition. Further, the focus was on large metazoan, specifically vertebrate species, whereas the dominant microbial compounds remain understudied. Though several protist species have been isolated and described and taxonomic inventories of freshwater protists have been constructed, there is an increasing interest in integrating the existing taxonomic data (Weisse, 2002; Finlay, 2004).The knowledge of the biodiversity of microbial eukaryotes in the lakes will be important in conservation and management of these ecosystems.

The application of a morphological species concept in microbial taxonomy and recurrent observation of the same protist morphotypes in freshwater systems from different localities promoted the view of a cosmopolitan protist population and led to the concept that the global protist species richness could be relatively low (e.g., Finlay and Clarke, 1999; Finlay, 2002).The most sensible view is that protist distribution is not fundamentally different to that of other organismsand overall fits the 'moderate endemicity model'(Foissner, 1999, 2007, 2009; Martiny *et al*., 2006; Vyverman *et al*., 2007).The introduction of molecular tools into microbial eukaryotes research gave remarkable outcome with studies using these techniques demonstrating the molecular diversity of protists was much higher than that of plants and metazoa (e.g., Moreira and López-García, 2002; Berney *et al*., 2004; Cavalier-Smith, 2004 ; Guillou *et al*., 2004 ; Countway *et al*., 2005 ; Slapeta *et al*., 2005), and much greater than suggested by their morphological diversity as observed by light microscopy; even taking into account statistical extrapolations of morphospecies diversity from large, globally distributed data sets such as that for ciliates by Chao *et al*. (2006). It also demonstrated that there might be micro-organisms with restricted distribution (Papke *et al*., 2003).

The molecular phylogeny and diversity of microbial eukaryotes in the Rift Valley lakes is seldom studied. Molecular tools offer a cheaper alternative for studying microbial eukaryote diversity in such environment as opposed to traditional morphology based taxonomic methods. This study is an effort to advance on the understanding of the diversity of eukaryotic protists in the Rift Valley lakes in Kenya by using a combination of high-throughput next-generation sequencing (NGS) techniques and classical morphological methods. It investigated the microbial eukaryotic diversity, molecular phylogeny and taxonomy of ciliates in five lakes. It employed modern and comprehensive molecular approaches to unveil fundamental information for the understanding of the taxonomy and community structure of microbial eukaryotes in the lakes. Furthermore, the study significantly contributes to the understanding ofthese ecosystems and the dynamics of other microbial components in the lakes.

1.2 Ecology of the rift valley lakes

The lacustrine environments in the Rift Valley are strongly influenced by tectonic, volcanic and climate-driven processes (Tiercelin and Lezzar, 2002). Differences in basin geometries and drainage patterns have resulted in highly contrasting lake characters and sedimentary environments, ranging from shallow and alkaline – saline (e.g., Bogoria, Nakuru, Elmenteita) to freshwater lakes (e.g., Naivasha and Baringo) (Bergner *et al*., 2009). The high alkalinity is attributed to lava which was discharged during the volcanic period accompanying the

formation of the Rift Valley (Millbrink, 1977). Evaporation and reduction in volume; and changes in climatic conditions has resulted in concentration of soda in the lakes (Oduor *et al*., 2003). The ecology and geochemistry of African saline lakes are reviewed by Eugster and Hardie (1978), Beadle (1981), Melack (1981), and Livingstone and Melack (1984). While much of thepresent knowledge, is generally derived from studies on their chemistry (e.g. Talling and Talling, 1965; Wood and Talling, 1988), biodiversity (Harper *et al*., 2003), primary production (e.g. Melack and Kilham, 1974) and food-webs (Vareschi and Jacobs, 1984; Yasindi *et al*., 2002).

The biodiversity and community structure of most Rift Valley lakes are primarily controlled by salinity and the degree of environmental stability (Wood and Talling, 1988; Kebede*et al*., 1994). Some of the lakes (e.g., Lake Nakuru) are among the most productive aquatic environments with productivity rates exceeding 10 g C m^{-2} d^{-1} (Schagerl and Oduor, 2008), presumably due to high ambient temperatures, high light intensities and unlimited access to CO_2 in these carbonate-rich waters (Melack and Kilham, 1974). Despite the high production of each trophic level (Vareschi, 1987), the alkaline – saline lakes have a limited species complement, specifically in macro-organisms (Grant *et al*., 1990; Duckworth *et al*., 1996). The inhabiting specialized biota tolerates high water temperature, high salinity, and low oxygen concentrations (Hecky and Kilham, 1973; Melack, 1981; Talling, 1992). The extreme environment prevailing in some of the lakes tends to inhibit the growth of diverse plankton community but is conducive for exploitation by well-adapted organisms (Oduor and Schagerl, 2007a, b).The inhabiting specialized biota tolerates high water temperature, high salinity, and fluctuations in oxygen concentrations (Hecky and Kilham, 1973).

The alkaline – saline lakes that have been frequently investigated include lakes Bogoria and Nakuru (e.g., Kilham, 1981, Vareschi and Jacobs, 1984; Finlay *et al*., 1987; Yasindi *et al*., 2002; Harper *et al*., 2003, Oyoo-Okoth *et al*., 2011; Ongondo *et al*., 2013). Bacterial concentrations in these lakes are extremely high with numbers ranging between 1.0 x 10^7 ml^{-1} and 2.0 x 10^9 ml^{-1} (Kilham, 1981; Yasindi *et al*., 2002). The phytoplankton productivity is mainly contributed by the enormous biomass of the cyanobacterium *Arthrospira fusiformis* (Vorochinin)which dominates the species-poor phytoplankton community most of the time (Ballot *et al*., 2004). A large consumer population which includes birds, zooplankton, and fish is supported by the phytoplankton biomass directly and indirectly (Vareschi and Jacobs, 1984). The bird population is dominated by the lesser flamingo *Phoeniconaias minor*Geoffroy, the greater flamingo *Phoenicopterus ruber ruseus* Pallas and the pelican *Pelecanus onocrotalus* L. (Vareschi, 1978). The zooplankton community is composed of the

rotifers *Brachionus dimidiatus* Bryce, *B. plicatilis* Muller and *Hexartha jenkinae*, the copepod *Lovenulaafricana* Daday and a few insect species (Vareschi and Vareschi, 1984; Yasindi *et al*., 2002; Harper *et al.*, 2003). The introduced tilapine fish (*Oreochromis alcalicus grahami* Boulenger) is present in L. Nakuru but not in L. Bogoria.

Grazing exerted by microbial eukaryotes, usually dominated by phagotrophic protists, is considered the most relevant phytoplankton mortality factor in most aquatic systems (Calbet and Landry, 2004). In lakes Bogoria and Nakuru, protists (including ciliates such as *Frontonia* sp. and *Condylostoma* sp.) are among the important taxa that graze on the primary producers (Yasindi *et al*., 2002; Burian *et al*., 2013; Ong'ondo *et al*., 2013); with reported cases of high mean ciliate abundance, biomass and production in L. Nakuru overlapping with low abundance of the cyanophyte *A. fusiformis* (Yasindi *et al*., 2002). Other ciliate genera recorded in the plankton include: *Acineria, Cyclidium,Dileptus, Euplotes,Halteria, Holophrya, Lagynophrya,Monodinium, Rimaleptus, Sphaerophyra, Spathidium, Trachelius, Vorticella* and some unidentified taxa (Yasindi *et al*., 2007; Ong'ondo *et al*., 2013).

1.3 Thesis outline

The broad objective of this study was to determine the molecular eukaryotic protist diversity, community structure and taxonomy of ciliates in five Rift Valley lakesnamely Lake Baringo, Lake Turkana, Lake Oloidien, Lake Elmenteita and Lake Bogoria. The high-througput 454 pyrosequencing technique was used as a tool to reveal the protistan molecular diversity and distribution patterns from the marker gene 18SSU rRNA, and to infer phylogenetic relationships between the taxa. The materials and methods are described in Chapter 2. The diversity and community composition of eukaryotic protists and the phylogenetic affiliation of ciliates are analysed in Chapter 3. Chapter 4 focuses on the molecular microbial diversity and ciliate community assessment from morphology in Lake Bogoria. Chapter 5 describes biodiversity and composition of Chlorophytes in the lakes. Chapter 6 provides a taxonomic description of three new ciliate species from Lake Bogoria and Chapter 7 the overall conclusions.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Study sites

The summary of the study lakes are given below and in figure 1.

2.1.1 Lake Baringo

Lake Baringo (0° 36´ N, 36° 04´ E) lies approximately60 km north of the equator at an altitude of 975 m above sea level. The surface area is approximately130 km^2 with wide fluctuations asa consequence of climatic changes (Kallqvist, 1987; Oduor *et al*., 2003).The lake is characterized by high turbidity and highly variable lake levels resulting from prolonged periods of drought interspersed with heavy rainfall (Anderson, 2002). Lake Baringo is a 'Ramsar' conservation site, owing to factors that include its provision of critical habitat and refuge for more than 500 bird species, including migrants of global conservation significance, and for its invaluable habitat for fish, including the endemic *Oreochromis niloticus baringoensis* Trewavas (Ramsar, 2002). Several studies on the physico-chemistry, phytoplankton, primary production, floraand fauna have been carried out (e.g., Beadle, 1932; Vacelet *et al*., 1991; Patterson and Wilson, 1995).The phytoplankton community is at times mainly dominated by the cyanobacterium *Microcystis aeruginosa* and due to the high turbidity the phytoplankton biomass is low, ranging between 1.5 and 8.2 mg l^{-1} (Ballot *et al*., 2003). High mean total phosphorus concentration (1.0 mg l^{-1}) and mean total nitrogen concentration (2.8 mg l^{-1}) typical for hypertrophic lakes were recorded in the lake (Ballot *et al*., 2003).The fish community comprises seven species, of which *O. n. baringoensis*, *Clarias gariepinus*and *Protopterus aethiopicus*are the main species that are commercially exploited (Odada *et al*.,2006; Britton *et al*., 2008).

2.1.2 Lake Turkana

Lake Turkana (2° 27´ - 4° 40´ N, 36° 07´ E) is located in a closed basin within the East African Rift Valley in the arid north-west region of Kenya. It has been one of the least studied of the African Great Lakes owing to its remote location (Cohen, 1986). Bathymetric survey of the lake between 1972 and 1975 reported the lake was 257 km long; had variable width from 13 – 44 km (average31 km); mean depth of 31 m ($Z_m \approx 114$ m); and a surface area of approximately 6,750 km^2 (Ferguson and Harbott, 1982). The water is slightly saline and lake levels are mainly sustained by the inflows of the Omo River (about 90%), with the other notable inflow coming from Kerio and Turkwel Rivers (Ferguson and Harbott, 1982).

The lake waters are well mixed and well oxygenated in the upper layers, due to the prevailing high winds; and the water exhibits high organic and inorganicturbidity on both a seasonal and continuousbasis (Cohen, 1986). The mean conductivity of the lake is 3,500 – 3,800 μScm^{-1} and alkalinity varies between 17 – 21 meq l^{-1} (Cohen, 1986; Kolding, 1993). More recent studies confirmed conductivity levels inthe main lake have remained fairly constant over the last 30 years (e.g., Avery & Eng, 2012). Cyanobacteria, mainly *Microcystis aeruginosa* dominate the phytoplankton in open waters that are characterised bylow species diversity (Kolding, 1993). Benthic invertebrates include: the soft-bottom associated corixid (*Micronecta* sp.) and theostracod (*Hemicypris kliei*); rocky-bottom assemblages' dominatedby various gastropods and insects; and muddy-bottom assemblages dominated by the ostracods *Hemicypris intermedia* and *Sclerocypris cf. Clavularis*, several gastropodand chironomid species (Cohen, 1986). Fourty eight (48)fish specieshave been recorded in the lake, ten of which wereendemic to the lake(Hopson, 1982). The food web is detritus based, indicated by a high abundance of planktonic ciliates; detritivorous zooplankton dominated by *Trophodiaptomus banforanus* and prawns (*Macrobranchium niloticum* and *Caridinia nilotica*); and a large community of mud-shifting/benthic feeding fish (E.g., *Labeo horie*, *Barbus bynni*, *Citharinus cithara* and *Disticodus niloticus*) (Kolding, 1993).

2.1.3 Lake Oloidien

Lake Oloidien (0° 45´ S, 36° 20´ E) is a former bay of Lake Naivasha located at the south west corner of the bigger lake, at an altitude of 1890 m above sea level (Kalff, 1983). It is a hydrologically closed basin with a surface area of approximately 5.5 km^2 (Kalff, 1983). The water level is maintained only by rainfall, evaporation, and sub-surface inflow from L. Naivasha through a permeable sill (Verschuren *et al.*, 2000). The lake has shifted from freshwater conditions towards an alkaline – saline lake with the conductivity values changing considerably. In the period 1979/1980 when the two lakes were connected, the conductivity was 660 µS cm^{-1}, which was only twice higher than in L. Naivasha but the conductivity values are now between 3,890 and 5,270 µS cm^{-1}(Kalff and Watson, 1986;Ballot *et al.*, 2004). The total alkalinity ranges from 39 to 65 meq l^{-1}(1,970 – 3,250 mg $CaCO_3$ l^{-1}); Secchi-depth around 0.22 m; pH range from 9.3 to 9.9; Total phosphorus concentrations from 0.4 – 1.0 mg l^{-1}; and total nitrogen concentrations from 0.9–6.3 mg l^{-1}(Ballot *et al.*, 2009). Phytoplankton and zooplankton community exhibit substantial fluctuations with the phytoplankton community consisting mainly of cyanobacteria typical for alkaline – saline lakes and dominated by *Chroococcus minutus*, unidentifiable thin filamentous Oscillatoriales, *Arthrospira fusiformis*and *Anabaenopsis elenkinii* (Ballot *et al.*, 2009).

2.1.4 Lake Elmenteita

Lake Elmenteita (0°27'S, 36°15'E) lies at 1776 m above sea level. The surface area is about 20 $km^2$and mean depth range from 0.65 – 1.1 m (Melack, 1988). The lake lies in an area with a semi-arid climate, with amean annual rainfall of about 700 mm and the high rates of evaporation from the lake's surface result in a precipitation-evaporation deficit of approximately minus 600 mm per year (Hastenrath and Kutzbach, 1983). The deficit is balanced by runoff from a number of small streams, the Kariandusi River, and the Mbaruk/Meroronyi River (Cohen and Nielsen, 1986). Very high evaporation rates during the drier seasons leads to a reduction in the total surfacearea, an increasein alkalinity (1200 $mgCaCO_3l^{-1}$); pH above 9; and high concentrationof solutes, mainly carbonates, chlorides and sulphates (Mwaura,1999). Ecological and limnological information; and climate statistics pertinent on the lake are summarized in Melack (1976, 1981 and 1988), Tuite (1981), Vareschi (1982), Melack *et al*. (1982) and Kalff(1983).

2.1.5 Lake Bogoria

Lake Bogoria (00° 15´ N, 36° 07´ E) is situated at an altitude of 963 m above sea level and covers an asymmetric half graben within the axial depression of the Gregory Rift Valley (Schlüter, 1997). It is about 17 km long and 3.5 km wide, with a maximum depth of approximately 10 m (Hickley *et al.,* 2003). Previous studies covered various aspects on the ecology and limnology of the lake such as microbial, phytoplankton and zooplankton communities (Harper *et al.*, 2003; Burian *et al.*, 2013); ciliated protists (Yasindi *et al.*, 2007; Ong'ondo *et al.*, 2013); bathymetry (Hickley *et al*., 2003); major cations, anions and certain trace elements (Jirsa *et al.*, 2013).

2.2 Sample collection

Water samples were collected from one point atacentral pelagic zone of each lake. The samples were obtained from pre-determined depths (i.e., surface, 0.5 m, 1 m, 2 m and 3 m) using a 2-litremodified Schindler type sampler (Figure 2.2 f), collected in a triplicate of sterile plastic bottles and taken to the shore where filtration was done. The water was filtered through 0.1 µm polycarbonate filters, the filter placed in sterile plastic petri-dishes and air-dried before storing in a cooler box for transportation to the laboratory at Egerton University where the filters were stored as – 20 ° C until further processing.

Samples for ciliate analyses were collected at 0 – 5 m depths in the pelagic zone between September and December 2011 and in October 2012. A detailed description of the sampling procedures and sample processing is provided in Ong'ondo *et al.,* (2013). Beside preserved

samples, additional raw water was collected in clean 250-ml plastic bottles for the observation of the living ciliates.

Additionally, environmental parameters were determined on site and water samples for further determination of chemical and biotic parameters collected. The classification of the lakes employed in this study is from Hammer *et al.*, (1983) that is based on the total dissolved solutes in the water i.e., freshwater (< 3g l^{-1}); Hyposaline (3 – 20 g l^{-1}); Mesosaline (20 – 50 g l^{-1}) and hypersaline (> 50g l^{-1}). The results presented in this work (text, figures and tables) are subsequently arranged in an increasing order of salinity based on this classification.

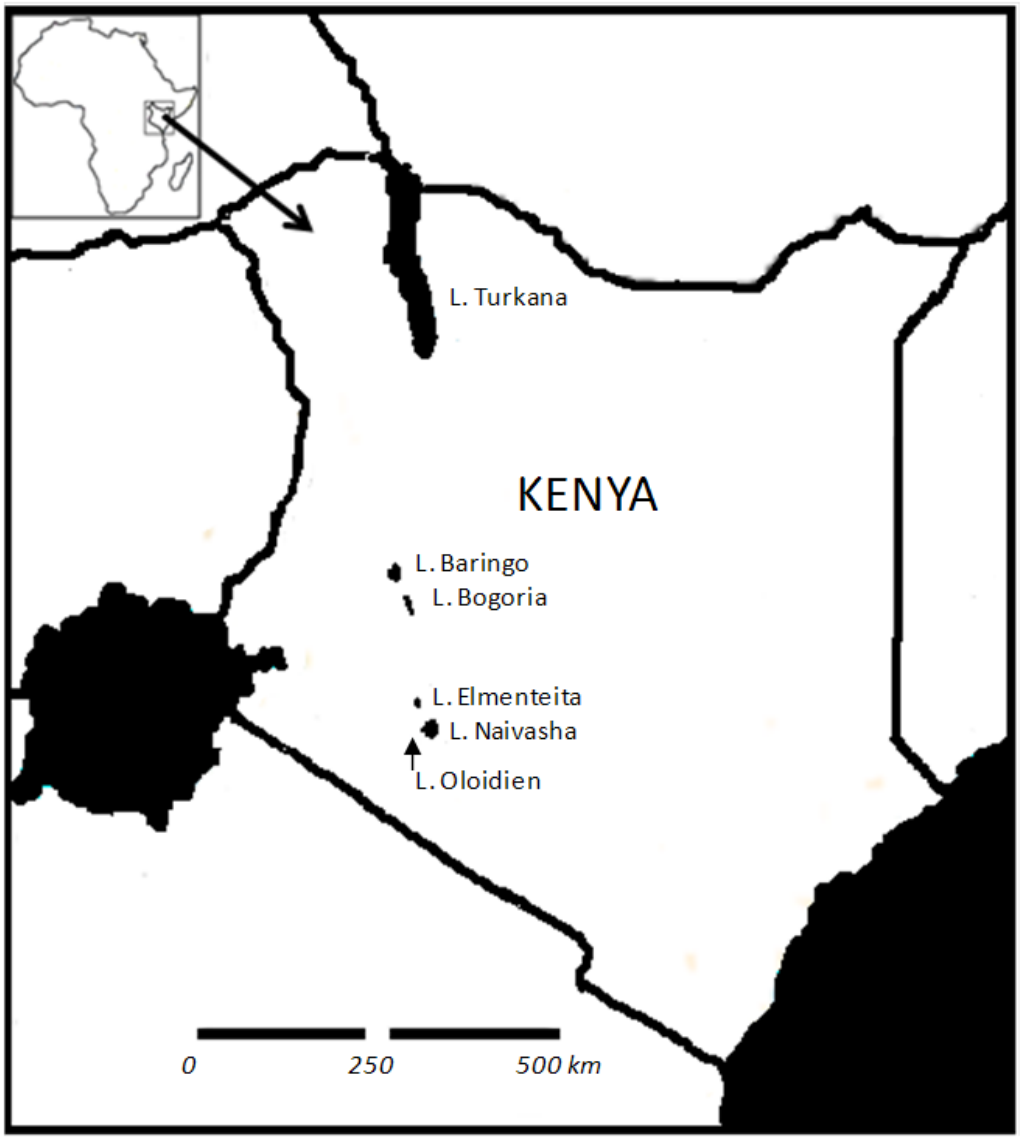

Figure 2.1: Map of Africa and Kenya showing the study sites

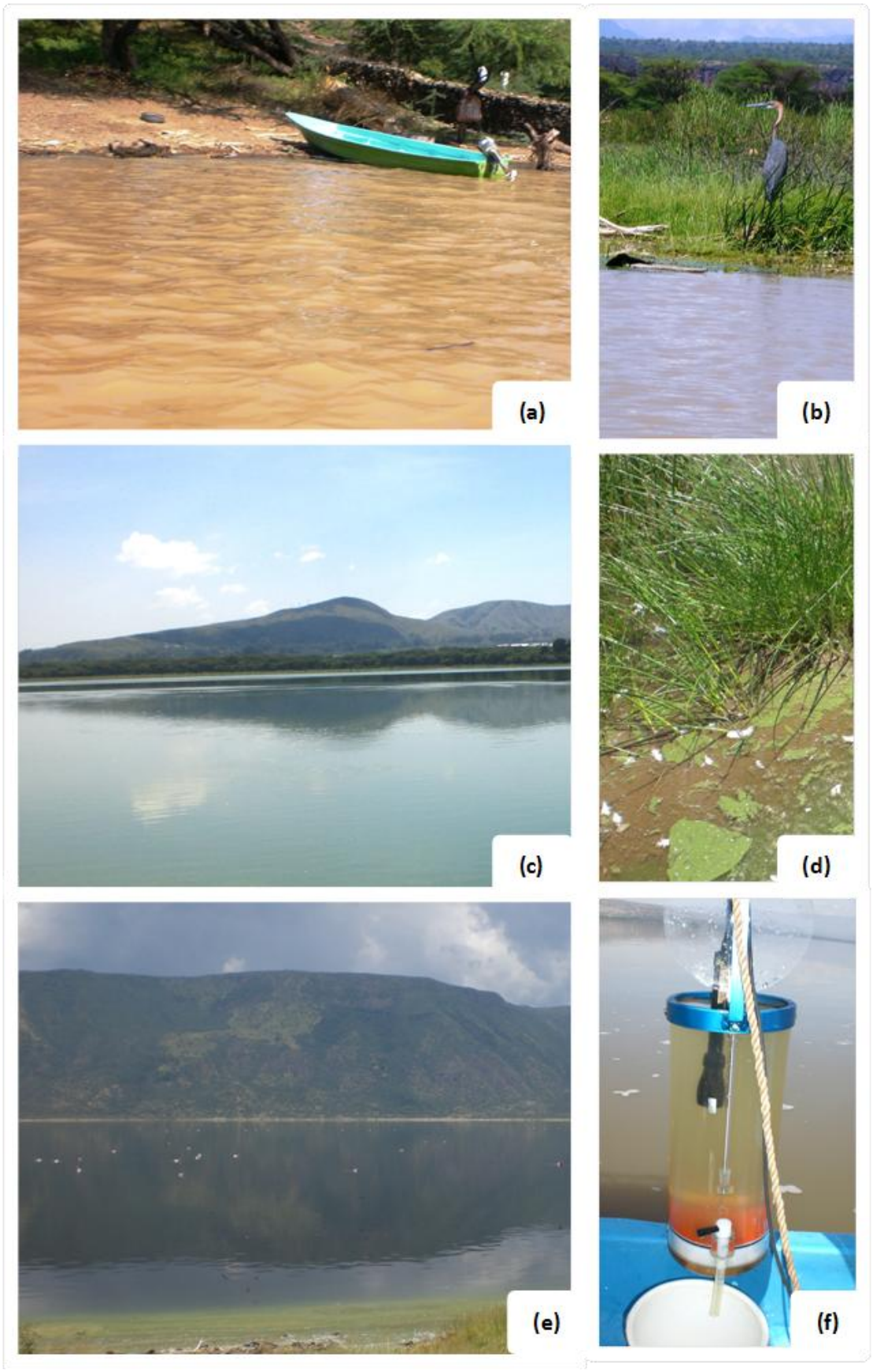

Figure 2.2: Photographs of some of the studysites. Lake Baringo **(a, b)**; Lake Oloidien **(c, d)**; Lake Bogoria **(e)**; and the modified Schindler type sampler used for collecting water samples from the lakes **(f)**.

2.3 Microscopy

Light microscope observations of ciliates samples were conducted with light microscopes equipped with differential interference contrast (DIC) optics.

2.3.1 Live observations

The living ciliates were observed in detail to record the main characteristics at magnifications ranging from 100x – 1,000x using an inverted microscope equipped with differential interference contrast (DIC; Nikon Eclipse Ti-S, Japan). For the examination of their swimming behaviour, the ciliates were observed in a glass depression slide under a stereo microscope (Wild Leitz) at 12.5x – 50x magnifications. Drawings of live cells were based onfree-hand sketches.

2.3.2 Fixation and staining

The ciliates fixed in 5 % Bouin's fluid were impregnated with protargol according to Foissner*et al*. (1999) and Montagnes and Lynn (1993) to reveal their infraciliature and other important morphological structures for their taxonomic identification.Drawings of protargol - impregnated specimens were made with a camera lucida.As protargol stain unfortunately is not commercially available any more, I additionally prepared and used protargol stain following the protocol of Pan*et al*., (2013a). Silver carbonate was prepared according to the protocol by Foissner *et al*., (1999).

2.3.3 Counts and measurements

Counts and measurements were carried out at magnifications of 400x – 1,000x with additional help of image analysis systems (camera Nikon DS-Fi1, software NIS-elements D 3.2; LUCIA D, Laboratory Imaging, Czech Republic, www.lim.cz).

2.4 DNA extraction, amplification and sequencing

At the General Botany laboratory in Duisburg-Essen University, DNA from the filters was extracted by suspending in 10 ml of phosphate lysis buffer containing 4% cetyltrimethyl-ammonium bromide (CTAB); tris(hydroxymethyl)aminomethane (Tris), 0.1 mM; Ethylenediaminetetraacetic acid (EDTA), 0.05 mM; Sodium chloride (NaCl), 0.1 mM; and Buffer, pH 8.0. After the lysis, proteins were removed using chloroform-isopropanol mixture and the DNA precipitated with isopropanol.The DNA yields from three filters per depth at each lake were pooled before performing PCR and the quality was checked by 1% agar gel electrophoresis. The DNA yield was quantified using UV–Vis spectrophotometer (Nanodrop ND-1000, Nanodrop Technologies, USA) before storing at -20 °C until further analysis.

DNA amplification were performed through standard PCR cycles in a MasterMix® thermocycler as follows: an initial denaturing step at 95 °C for 3 minutes, followed by 35 cycles of 30 sec each at 98 °C, 45sec annealing at 60 °C, and 45 sec final extension at 72 °C. The PCR mixture included 1 – 5 µl DNA template (x 10 diluted), 10 µl Buffer (x 10), 1 µl dNTP, 1 µl forward primer, 1 µl reverse primer, 25 nMol Taq polymerase and double distilled water to make it to 50 µl. Broad spectrum eukaryotic primers were used for sequencing the V9 hypervariable region. The primers were: forward (5'– GTACACACCGCCCGTC–3') and reverse (5'– GTAGGTGAACCTGCAGAAGGATCA – 3'). The forward primers used were tagged, enabling the identification of each sample. PCR products from eight reactions perprimer pair were pooled, cleaned and eluted in 30 µl elution buffers according to the protocol (MinElute PCR purification kit, Qiagen). The PCR products were then finally pooled and sent for pyrosequencing (Roche 454 GS FLX) at Eurofin Biotechnology Laboratory (Germany).

2.5 High-throughput data processing and analysis

The 454 sequencing data were corrected for pyrosequencing errors using established pipelines (Medinger *et al*., 2010; Nolte *et al*., 2010) with the short and noisy reads discarded completely and low-quality base calls trimmed from longer sequences. The primers, sequencing adaptors and barcode tags were then removed from raw sequencing reads, with the relevant metadata (sample site or primer name) inserted into FASTA headers files. Finally, the 454 reads were aligned to reference sequences from the National Centre for Biotechnology Information (NCBI) database and assigned to operational taxonomic unit (OTU). The OTUs clusters were defined by the best match for each read using the Basic Local Alignment Search Tool (BLAST) of the nucleotide database. Diversity was defined as the number of OTUs (OTU richness) in each lake.

2.6 Phylogenetic placement of the 454 reads

In a first step, a dataset of 159 SSU rRNA gene reference sequences with 1854 bases, representing the major clades of Ciliophora, was initially aligned using ClustalW (Thompson *et al*. 1994) and manually adjusted visually. The reference sequences were chosen according to the nearest NCBI blast results of the 454 reads for Ciliophora. A similar approach was employed for phylogenetic placement of Chlorophyta where a dataset of 160 SSU rRNA gene reference sequences with 1854 bases was used. RAxML 7.2.7 with the General Time Reversible model of nucleotide substitution (Stamatakis et al. 2005) was used to create the phylogenetic reference trees. The 454 pyrosequencing reads ("query" sequences) were manually aligned and merged with the reference alignment. Query sequences were then

placed on a phylogenetic tree by using pplacer v1.1 (Matsen *et al*. 2010). Lineages which contained only reference sequences were deleted from the alignments and subsequent phylogenetic trees containing 94 reference sequences for Ciliophora and 94 reference sequences for Chlorophyta were calculated with the same settings as described above.

2.7 Stastical analysis

The microbial eukaryote sequences were normalized and rarefied, and the protist community taxonomic richness computed on the basis of the rarefied total number of protist amplicons from the five study sites. Hellinger transformation, Ward cluster analysis, Principal Component Analysis (PCA) and other multivariate analyses of the physico-chemical parameters of the sites were conducted to account for the variability of the observed values. The statistical analyses were performed in R using the package 'vegan' (R-project: www.r-project.org) and the graphs drawn in Sigma plot.

CHAPTER 3

3.0 MOLECULAR DIVERSITY OF EUKARYOTIC PROTISTS

3.1 Introduction

The application of molecular techniques such as real-time PCR and analyses of molecular sequence data have become increasingly important in investigating biodiversity at the community level (Stoeck *et al*., 2006). The most commonly used markers have been parts of the genes coding for ribosomal ribonucleic acid (rRNA), in particular 18SSUrDNA (Pawlowski *et al*., 2012). RNA molecules are ubiquitous in all cellular life forms and highly expressed, making them useful in the application of molecular methods to study the biodiversity of organisms (Head *et al*., 1998). Specific primers have been used to amplify fragments of rRNA operons and other genes in order to detect an organism or group of organisms in phylogenetic studies (e.g., Holben *et al*., 2002). Ecophylogenetic research offers numerous applications, such as designating conservation priorities and estimating the effects of biodiversity on ecosystem functioning (Provete, 2013).

Understanding protist diversity highlights the role of these organisms in major biogeochemical cycles and illustrates how diversity affects the stability and resilience of biological communities (Caron, 2005). Studies show that environmental factors such as physical, chemical and biological local parameters have significant effects in shaping microbial composition at a regional scale(e.g., Martiny *et al*. 2006). For example, variations in salt concentrations in environments such as salt plains have been shown to promote protist diversity (Wilson *et al.*, 2004; Buchheim *et al.*, 2010). The alkaline – saline Rift Valley lakes offer remarkable environments for the biodiversity study of planktonic protists within a wide range of physico – chemical conditions. The Rift Valley lakes can beregarded as discrete patches subjected to different natural selection regimes with many of the lakes exhibitingstrong differences in abiotic environmental factors within short geographic distances; thus providing ideal study sites for protist community analyses (Luo *et al*., 2013).

I investigated the protisttaxonomic richness and genetic diversity in five Rift Valley lakes in Kenya. The objective was to determine the effect of the physico – chemical environment of the respective lake onto species community structuring and genetic diversity. Therefore, water samples from the lakes ranging in salinity from freshwater to mesosaline were obtained between September and December 2011. The molecular eukaryote diversity in the samples was determined by pyrosequencing of the V9 region of the 18s SSU rRNA. I hypothesized

that the protists in the lakes, as illustrated by a detailed study of ciliates, were restricted in their genetic diversity and distribution by the environmental gradients found in the five alkaline – saline lakes.

3.2 Results

3.2.1 Environmental parameters

The main environmental parameters determined are summarized in Table 3.1. Based on the concentration of dissolved solutes (i.e., salinity), lakes Baringo and Turkana were classified as freshwater, lakes Elmenteita and Oloidien were hyposaline, and Lake Bogoria was mesosaline. Conductivity in the lakes increased with salinity and the lowest values were recorded in Lake Baringo, and the highest in Lake Bogoria (Table 3.1). Other notable differences among the lakes were observed in their oxygen concentrations. Further variables determined thatcould influence protist dynamics, were the chlorophyll *a* (Chl *a*) concentration (indicative of phytoplankton biomass) and the Secchi depth (Table 3.1). Chlorophyll *a* was lowest in Lake Turkana and highest in the more eutrophic lakes (Baringo, Oloidien and Bogoria).

Table 3.1: Meanphysico-chemical variables and Chlorophyll *a* concentration measured between September and December 2011 in the respective lakes.

Lake	**Baringo**	**Turkana**	**Oloidien**	**Elmenteita**	**Bogoria**
Variables/Classification	Freshwater	Freshwater	Hyposaline	Hyposaline	Mesosaline
Depth (m) - Sampling point	6.5	10.0	3.8	1.6	6.0
pH	8.3	8.5	9.9	9.9	10.1
Temperature (°C)	25.9	28.2	24.5	23.6	30.9
Oxygen (mg l^{-1})	6.4	7.0	18.4	8.3	14.0
Conductivity (µS cm^{-1})	564	3,290	7,064	8,640	52,140
Salinity (‰)	0.3	1.7	3.9	4.8	34.1
Secchi depth (cm)	27.5	44.5	14.0	20.0	54.5
Chlorophyll *a* (µg l^{-1})	248.6	18.0	583.4	87.4	249.0

3.2.1 Overall protist diversity

The 18SSU rDNA gene from the samples produced 96,555 reads (sequences) meeting the quality control criteria, of which 33.8 % were metazoan and other non-target sequences, such as Viridiplantae (Streptophyta) and Fungi (Basidiomycota). The remaining (eukaryotic protist) sequences were dominated at a higher level of taxonomic assignment, approximately

corresponding to kingdoms and phyla by Alveolata (27.2 %). Other abundant sequences included RNA sequences from protist groups that have not been isolated i.e., Stramenopiles (14.2 %), environmental sequences (ES; 13.2 %), Fungi (11.2 %), Chlorophyta (11.1 %), and Cryptophyta (9.1 %). Groups that occurred in abundances of less than 5 % included Excavata (mainly Euglenozoa), Rhizaria and Choanoflagellida. Others (6.2 %) included: Amoebozoa, Apusozoa, Centroheliozoa, Ichthyosporea, Katablepharidophyta, Haptophyceae and Nucleariidae (Figure 3.2).

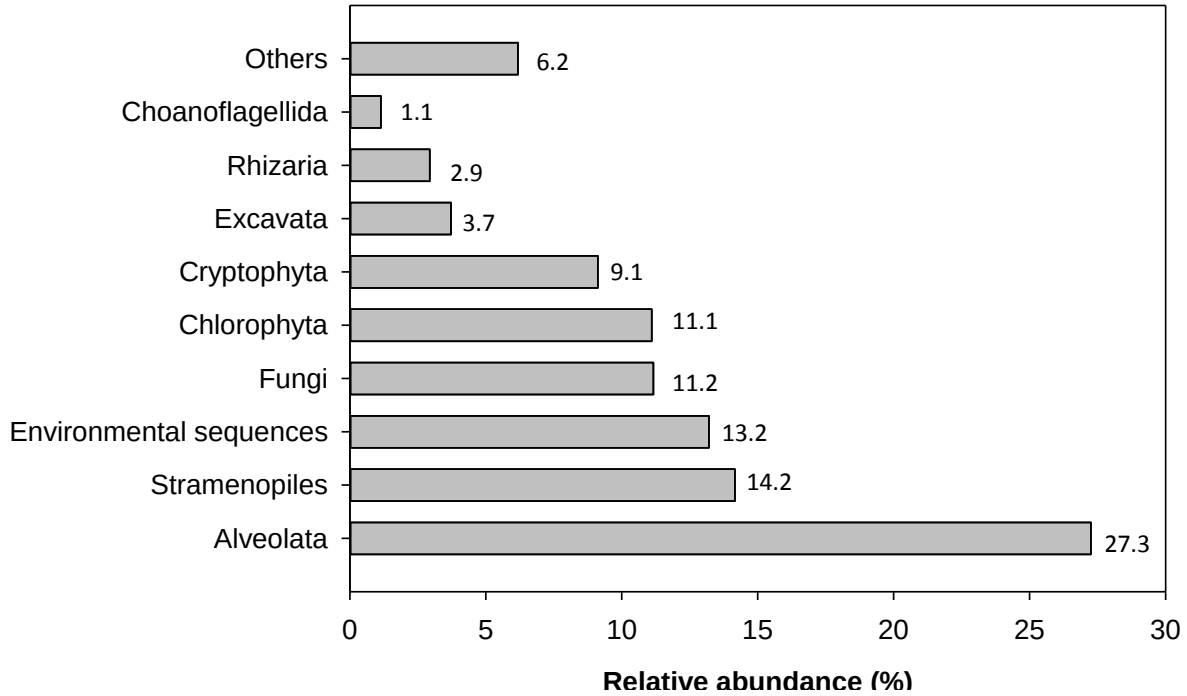

Figure 3.2: Overall relative rRNA gene amplicon abundance of protists in the lakes.

3.2.3 Protist diversity in the lakes

Protist diversity was expressed as the relative proportion of sequences of the major taxonomic groups (Figure 3.3). The groups included Alveolates (Ciliophora, Dinophyceae, Apicomplexa and unspecified alveolate sequences), accounting for 22 % to 39 % of all sequences detected, Cryptophytes (5 – 23 %), ES (3 – 18 %), Stramenopiles (9 – 16 %),Fungi (5 – 15 %), Chlorophytes (1 – 15 %), Excavates (0 – 7 %), Rhizaria (0 – 4 %), Choanoflagellida **(**1 – 2 %) and other groups such as Amoebozoa, Apusozoa, Centroheliozoa, Ichthyosporea, Ketabrepharidophyta Haptophyceae and Nucleariidae(3 – 15 %) (Figure 3.3).

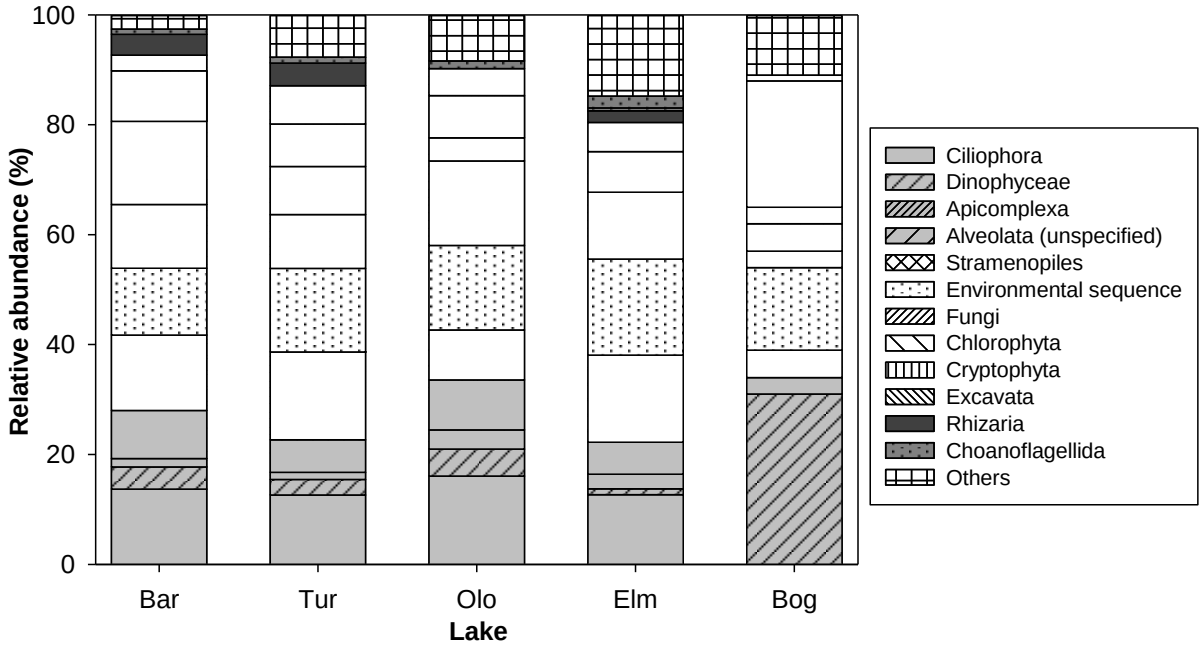

Figure 3.3: The relative abundance of amplicons from the major protist groups in the lakes. (Lake codes: Bar - Baringo; Tur – Turkana; Olo – Oloidien; Elm – Elmenteita; and Bog – Bogoria).

3.2.4 Taxonomic richness

After rarefaction, the clustering based on a 97 % sequence identity criterion, produced between 13 – 148 OTUs. The taxonomic richness decreased significantly (t-test, $P < 0.001$) along a salinity gradient and was highest in the freshwater lakes Baringo and Turkana, and lowest in the mesosaline Lake Bogoria (Figure 3.4). The taxonomic richness of the protists varied with depth in each lake but did not present a clearly defined pattern (Figure 3.4).

Lake Baringo: Highest taxonomic richness (148 OTUs) was recorded at the surface and 91 OTUs in the combined depth sample (2 + 3 m). The taxonomic richness decreased to about 80 OTUs at 0.5 and 46 OTUs at 1 m depths, respectively (Figure 3.4).

Lake Turkana: The highest taxonomic richness was recorded at 1 m depth (94 OTUs). There were 55 OTUs detected in the combined depth sample (2 + 3 m) and 29 OTUs at 0.5 m depths, respectively. The number of amplicons found in the surface sample was very few (around 20 OTUs) (Figure 3.4).

Lake Oloidien: Taxonomic richness was 31 OTUs at 0.5 m and 33 OTUs in the combined depth sample (2 +3 m), respectively. The number of amplicons detected in the surface sample and in 1 m were not included in the graph as they were few (Figure 3.4).

Lake Elmenteita: 51 OTUs were recorded at 1 m and 18 OTUs at 0.5 m depths, respectively. In the surface samples, only a few OTUs were found (Figure 3.4).

Lake Bogoria: 13 – 16 OTUs were detected in the four depths sampled with the highest value (16 OTUs) recorded at 0.5 m.

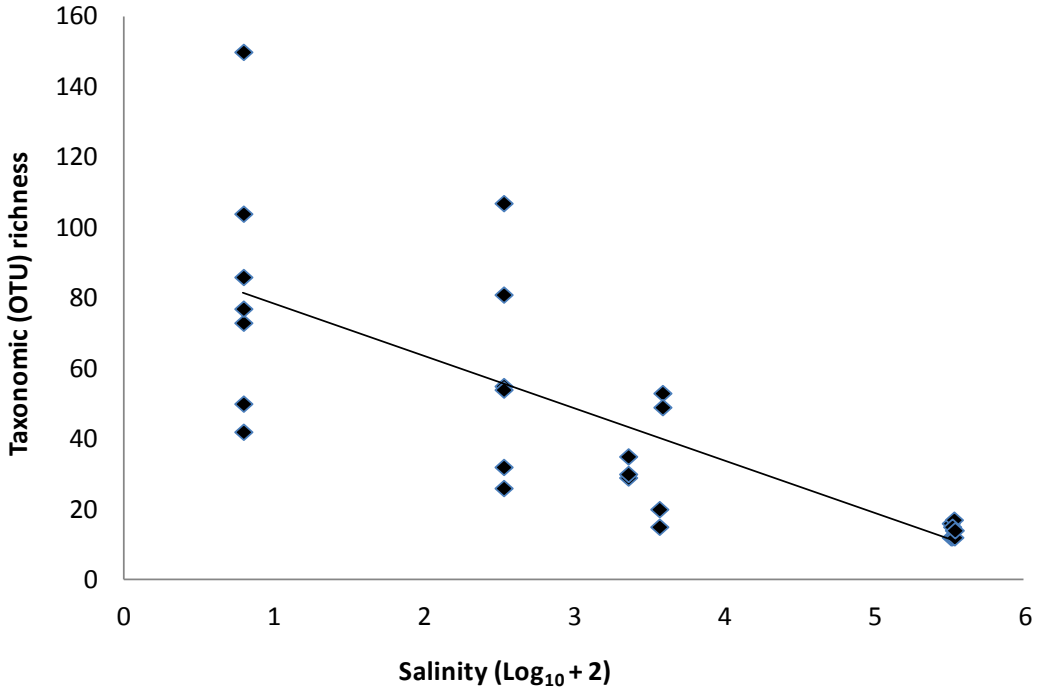

Figure 3.4. Rarefied taxonomic (OTU) richness of the protist groups (OTUs; black diamond-shapes, y-axis) and a salinity gradient (line; x-axis; units = g l^{-1}) at different depths in the sampled lakes.

3.2.4 Spatial distribution

In the principal correspondent analysis (PCA) where the major taxonomic protist groups were compared to the environmental variables, results show that lakes that had a similar community structures appeared close together in the ordination space while lakes located further from each other had different community structures (Figure 3.5). According to their dominant protists, the lakes were distinctly divided into five clusters that corresponded to freshwater (Lakes Baringo and Turkana), hyposaline (Lakes Oloidien and Elmenteita) and the mesosaline Lake Bogoria (Figure 3.5). The 1st axis and the sum of all axes in the PCA explained a significant portion of the variance in the data.The environmental variables influencing the distribution of the protist communities were salinity (r^2 = 0.982), pH (r^2 = 0.925), temperature (r^2 = 0.925), light attenuation (Secchi depth; r^2 = 0.693) and oxygen concentration (r^2 = 0.616). (Figure 3.5).

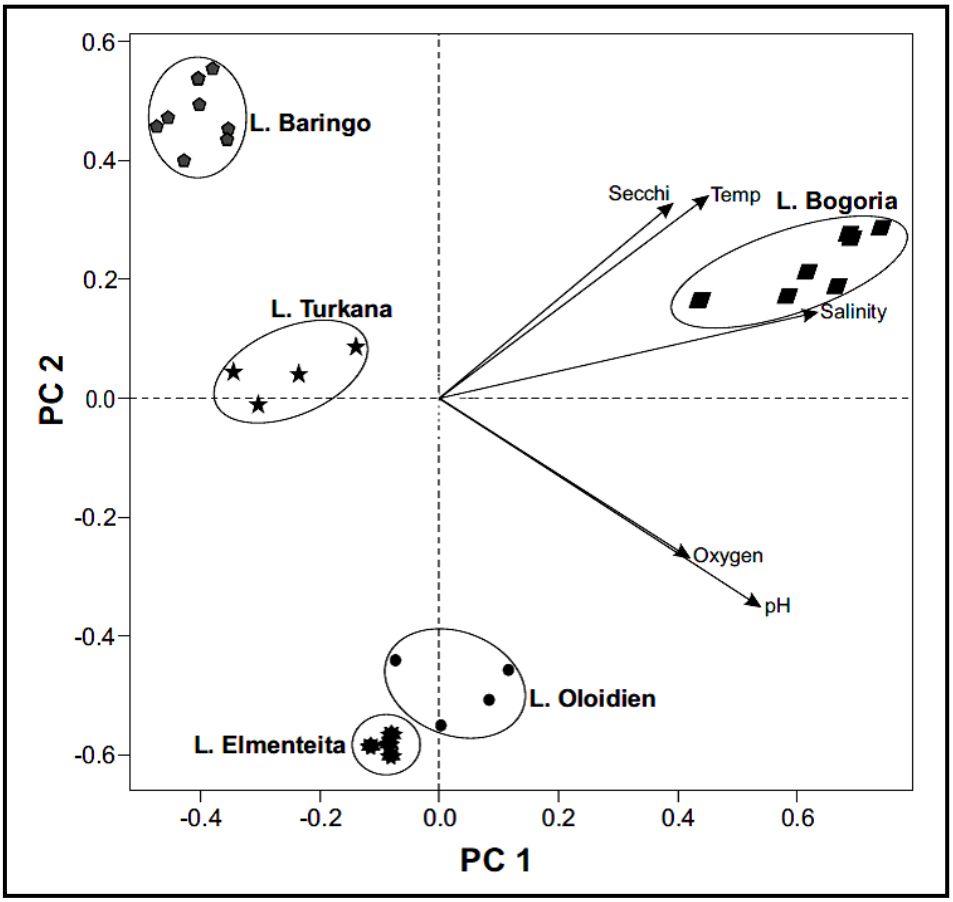

Figure 3.5: Biplot of samples based on protist taxon (OTU) composition and main environmental variables affecting their distribution.

3.2.5 Phylogenetic affiliation of the ciliates

Phylogenetic relationships of ciliates from the lakes as inferred by a SSU rRNA gene tree from a combined dataset are shown in Figure 3.6. The phylogenetic relations inferred from the sequences provided consistent support for all the ciliate classes. The major ciliates classes (Lynn, 2008) were recorded except for the class Armophorea. The final alignment of the sequences from the lakes comprised 76 phylotypes including Oligohymenophorea (25 phylotypes), Spirotrichea (24), Litostomatea (12), Prostomatea (4), Phyllopharyngea (3), Colpodea (3), Plagiopylea (2), Nassophorea (1), Karyorelictea (1) and Heterotrichea (1).

Oligohymenophoreans and spirotrichs were found in all the five lakes, Prostomatea, Plagiopylea, Phyllopharyngea and Colpodea were detected in the freshwater environment, litostomateans in the freshwater and hyposaline lakes, Nassophorea, Karyorelictea and Heterotrichea in the hyposaline lakes (Figure 3.6).

Spirotrichea: Most spirotrich phylotypes were recorded from the freshwater lakes Baringo and Turkana (20 and 16, respectively) and only 4 – 6 phylotypes in the hyposaline and mesosaline lakes. Four Halteriidae phylotypes closely related to *Halteria* sp. and *Meseres*

corlissi were present in all the lakes except for the mesosaline Lake Bogoria. A stichotrichid ciliate closely affiliated to *Stichotricha* sp. was found in all the lakes except Lake Turkana. One oxytrichid phylotype identical to *Crytohymena citrina* was detected in four lakes except Lake Bogoria. The other three oxytrichid phylotypes had a high sequence similarity to *Gastrostyla* sp., *Paraurosomoida* sp. and *Oxytrichia* sp. However, one Oxytrichidae phylotype (ES ID013) was placed at the base of the clade and appeared to be distantly related to the other sequences. The families Strombidiidae, Strobilidiidae and Tintinnidae were recorded exclusively from the freshwater lakes. Phylotypes from these families had a high sequence similarity to *Novistrombidium* sp., *Rimostrombidium* sp. and *Tintinidium fluviatile*. One Euplotidae phylotype with high sequence similarity to *Moneuplotes* sp. and *Euplotes rariseta* was present in all the lakes except Lake Elmenteita.

Oligohymenophorea: Scuticociliates with high sequence similarity to *Cyclidium glaucoma* and *Ancistrum crassum* were the most abundant phylotypes. Altogether, nine out of 12 phylotypes occurred in the freshwater Lake Baringo. However, except for three phylotypes that formed a clade with *C. glaucoma*, the others were aligned in a clade that nested among the families Cyclidiidae, Ancistridae and Philasteridae (Figure 3.6). Other oligohymenophoreans included four Vorticellidae, three Trichodinidae, a Philasteridae and a Tetrahymenidae that were recorded mainly from the freshwater environment. One Cinetochilidae phylotype was recorded in the hyposaline and mesosaline lakes.

Litostomatea: Phylotypes from this class were only recorded in the freshwater and mesosaline environments with eight recorded from Lake Baringo. Enchelyidae, Spathidiidae and Tracheliidae with a high sequence similarity to *Spathidium* sp., *Arcuospathidium* sp., and *Dileptus* sp. occurred in the freshwater lakes. Litonotidae, Lacrymariidae and one Isotrichidae phylotype were recorded from the hyposaline lakes.

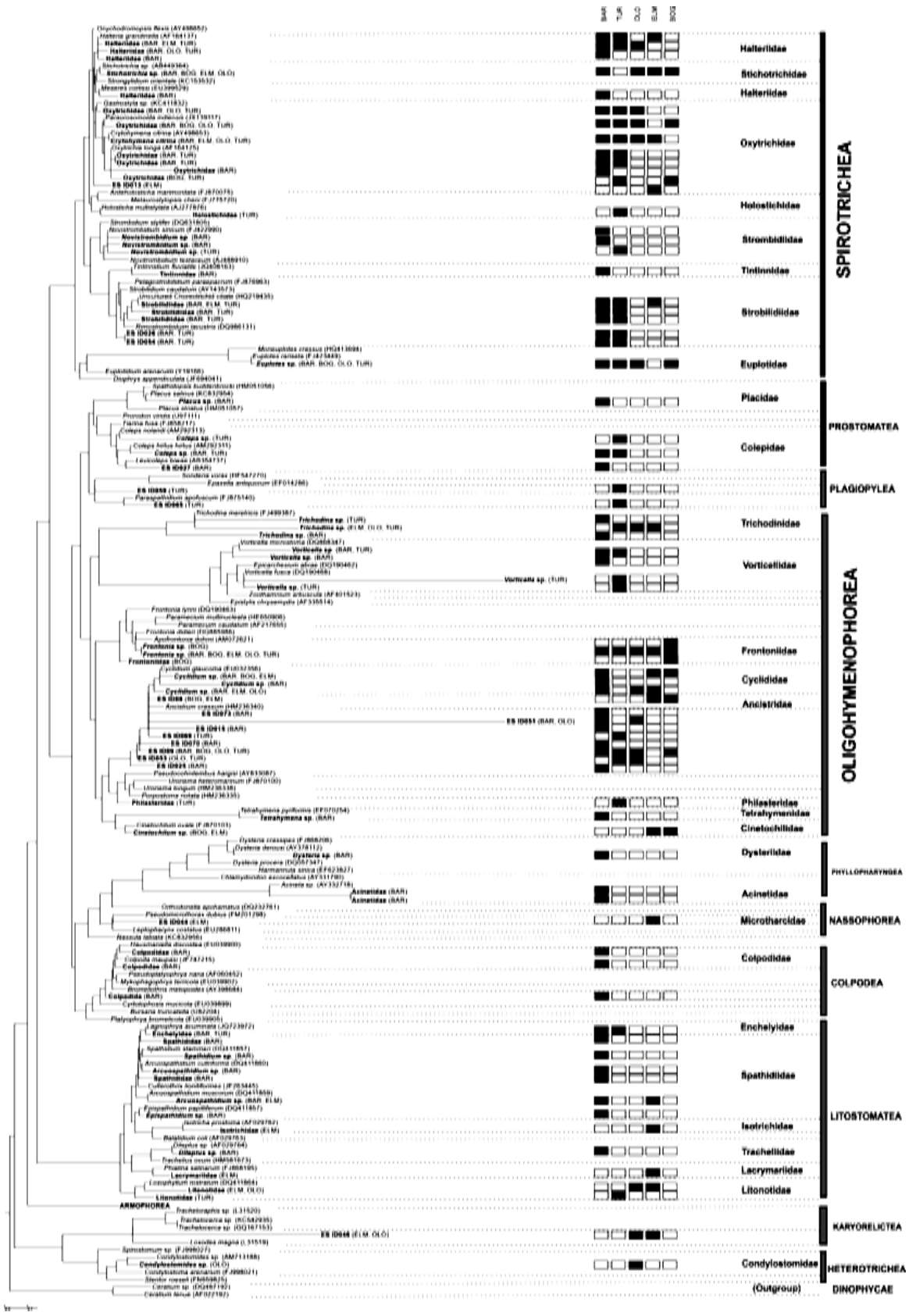

Figure 3.6. Phylogenetic tree for ciliate 18S rRNA gene sequences (n = 74) obtained from the lakes studied. The class and family are indicated on the right hand side. The shaded boxes indicate the presence of the phylotype in the studied lake. Bar = 0.10 fixed point mutation per nucleotide position. (Lake codes: Bar - Baringo; Tur – Turkana; Olo – Oloidien; Elm – Elmenteita; and Bog – Bogoria).

3.3 Discussion

A central goal of ecology is understanding the spatio-temporal patterns of biodiversity, elucidating the mechanisms controlling biodiversity and determining environmental factors maintaining biodiversity (Green and Bohannan, 2006; Lepère*et al.*, 2013). The alkaline - saline lakes investigated in this study cover a range of environmental conditions that affect microbial eukaryote biodiversity.

3.3.1 Molecular protist diversity and taxonomic richness

Protist diversity in the lakes was dominated by five major taxonomic groups with Alveolates being the most abundant and widely distributed (Figure 3.6). Similar results have been observed in environmental studies in freshwaters (e.g., Richards *et al.*, 2005, Lefèvre *et al*., 2007). On the other hand, a molecular study of plankton using the cloning methods in the alkaline – salineLake Nakuru (at low salinity of 9 g l^{-1}) identified 77 clones of the SSU rRNA genes comprising 17 phylotypes belonging to seven phylogenetic groups, namely Alveolata, Chlorophyta, Cryptophyta, Fungi, Jakobida, Stramenopiles, and Metazoa (Luo *et al.*, 2013). The study of Luo et al., (2013) based on clones unlike the present study that used 454 pyrosequencing, even though carried out in different lakes, reported similar protist groups. Some researchers have questioned results based on environmental PCR cloning and pyro-sequencing because of possible biases such as preferential amplification of certain taxa and the over-representation of taxa with high target gene copy number (e.g., Suzuki and Giovannoni, 1996; Potvin and Lovejoy, 2009; Heywood *et al.*, 2011). A comparison of multiple clone libraries constructed using artificial and natural protist assemblages, demonstrated primer-specific biases in the 18SSU rRNA gene clone library composition (Potvin and Lovejoy, 2009).The number of 18SSU rDNA gene copies in protists can vary by at least four orders of magnitude, with some correlation to genome size (Prokopowich*et al*., 2003) and cell size (Zhu *et al*., 2005).

The low taxon richness detected in some of the samples (e.g., Oloidien at 0 m depth) are possibly due to errors during the DNA extraction process and PCR amplification of DNA. There remains the possibility that some specificgroups did not amplify with the primer sets used due to sequence mismatches or inhibitory DNA secondary or tertiary structures (Potvin and Lovejoy, 2009). Also, the sampling effort in each lake (from only one location) and the results obtained from the pyrosequencing represent only a rough estimation of the genetic diversity as species diversity and populationdensities can vary (spatially and temporally) by several orders of magnitude (Bik *et al.*, 2012).

Although molecular methods theoretically opened the possibility of studying microbialdiversity independently of morphological identificationand cultivation (Šlapeta *et al.*, 2005), the field is not yet mature (Stoeck *et al.*, 2013). Additionally, the accuracy of BLAST-derived taxonomy depends on the database coverage for a given taxonomic group with divergent lineages, a feature that is common in poorly characterized environmental samples (Bik *et al.*, 2012). The limited eukaryote reference databases and inconsistent taxonomic levels currently hinder the development of robust computational pipelines for marker gene data (Christen, 2008).Ultimately, a much larger collection of full-length eukaryotic reference sequences (or whole genomes) will be necessary for identifying erroneous reads, and for providing a strong link between sequence data and morphology (Bik *et al.*, 2012). For example, environmental data sets have yielded novel molecular taxonomic insights into the magnitude and composition of the eukaryotic biosphere in a range of habitats (e.g., Nolte *et al.*, 2010; Edgcomb*et al.*, 2011).Therefore, despite the above challenges, pyrosequencing and other next generation approach are a step forward (e.g., in the detection of rare species, or diversity patterns without detailed taxonomic information), though the diversity may underestimates its true value due to incomplete coverage (Hill *et al*., 2003).

3.3.2 Spatial distribution of the protist community

According to the cosmopolitan view of the microbial world, spatial patterns of microbial diversity are driven by environmental heterogeneity (Green and Bohannan, 2006). Thus, one might expect to find similar microbial communities in similar habitats and differentiated microbial communities along an environmental gradient. Multiple studies have shown that environmental heterogeneityis the primary factor underlying microbial distance–decay relationships (e.g., Martiny *et al*., 2006). This is possibly the case in the Rift Valley lakes studied considering the difference in number of protist species recorded in Lake Baringo in comparison to Lake Bogoria (Figure 3.4). Even though the distance between the two lakes is barely 20 km, the two represent a freshwater habitat and a mesosaline habitat. When coupled with environmental data, the distance–decay relationship offers a means to assess the relativeimportance of environmental heterogeneity and dispersalhistory in controlling the spatial scaling of biodiversity (Legendre *et al*., 2005). Although it is accepted widely that macro-organismcommunity composition decays with increasing distancebetween samples (Tuomisto*et al*., 2006) little is known about microbialcommunity turnover rates (Green and Bohannan, 2006).

The main environmental parameters affecting the protist communities in the investigated lakes were salinity, pH, temperature, light attenuation and oxygen concentration (Figure 3.5).

Salinity was one of the most important factors explaining protist diversity both in terms of species richness and community composition. This finding fits to the literature data as salinity is considered one of the most important environmental factors structuring microbial assemblages (Lozupone and Knight, 2008; Casamayor and Barberán, 2010). Some species common inthe lakes (e.g., the ciliate *Cyclidium*) occur from freshwater lakes to almost saturation and the lake biotas subjected to increasing stress respond by local extinctions (Yasindi *et al*., 2007). The biota of such lakes are extremely limited in taxon numbers but population densities may be such that total biomass is as high as in high-diversity freshwater systems (Finlay *et al*., 1987; Yasindi *et al*., 2002).

Salt-water biota tend to have specific lower and upper salinity thresholds but a reasonable number of halophilic species appear only at minimum conductivities of 2,000 - 3,000 µS cm^{-1} (Hammer, 1986). Several salinity stress adaptation strategies such as synthesis of stress proteins(Smurov*et al*., 2013) have been developed in microorganisms inhabiting high salinity environments, mostly in prokaryotes (Oren, 2002a, b).The first signs of reaction to change are shifts in species abundance within communities followed by a rapid selection for opportunists and generalists, capable of feeding on a wide variety of food types, and resistant to high salinity and low dissolved oxygen e.g., *Cyclidium* spp (Finlay *et al*., 2006). An effective strategy to escape extinction is to produce resting stages (cysts) (e.g., Montresor *et. al*., 2006) and the switching from active to latent or pseudo-latent life may last for years (Chambouvet*et al*., 2011).

3.3.3 Phylogenic affiliation of the ciliates

My results indicate that spirotrichs, oligohymenophoreans and Litostomateans were well represented in the five target lakes (Figure 3.6). The results are comparable to those of a morphological study of 17 tropical lakes where Spirotrichea (mainly *Strobilidium* sp., *Strombidium* sp. and *Halteria* sp.) and Oligohymenophorea (*Cyclidium* sp., *Cristigera* sp., and *Pleuronema* sp.) dominated the ciliate communities together with other classes such as Phyllopharyngea, Heterotrichea and Prostomatea being relatively abundant (Yasindi *et al.*, 2007). Furthermore, studies of temperate lakesindicated that Spirotrichea (Halteriidae, Choreotrichidae, and Strombidiidae) and Prostomatea (Prostomatida) typically dominated(e.g., Beaver and Crisman, 1989; Müller *et al.*, 1991; Sonntag *et al.*, 2006). For example, *Halteria grandinella* is a cosmopolitan found in a wide range of habitats (see compilation in Foissner *et al.*, 1999) and *Cyclidium glaucoma* is known to tolerate a wide range of salinities (Finlay *et al*., 2006).

The ciliate amplicons were not identical to reference sequences at species level in the phylogenetic tree except for a few (e.g., *Cyrtohymena citrina*). This may be as a result of insufficient taxon sampling (Philippe *et al.*, 2004); an artefact especially important in protistan phylogeny because a significant number of known eukaryotic taxa presently lack representative rRNA gene sequences in databases (Epstein and López-García, 2008). For example, several protistan taxa only reached stable phylogenetic position once a significant number of sequences of other members of that group were included (Berney *et al.*, 2004). Consequently, a strong emphasis on morphological and environmental data collection, guide trees and reference sequence databases, and open-access repositories for high-throughput data sets are urgently needed to make substantial progress with high-throughput eukaryotic studies (Bik *et al.*, 2012).

The molecular data presented in this study indicates that many of the morphotypes in the alkaline – saline lakes (e.g., *Cyclidium* spp.) may contain many distinct lineages and further work is needed to determine whether each of them represents a different evolutionary peak adapted to particular habitat features (Guggiari and Peck, 2008). Other studies that involved molecular investigations using environmental sampling of 18SSU rDNA haplotypes also indicated a high number of ciliate phylotypes (e.g., Stoeck *et al.*, 2006; Doherty *et al.*, 2007).In contrast to such cryptic species, there are also cases in ciliates in which there is only limited genetic variation, at least as measured by 18SSU rDNA divergence, in the light of considerable morphological variation (Greslin *et al.*, 1989;Ardell *et al.*, 2003).Some ciliate species display heritable scrambling that can cause instant, or at least rapid, speciation as extensive gene scrambling disrupt pairing of homologous chromosomes during meiosis (Dunthorn *et al*., 2008).

My result strongly supports gene sequence comparisons and their increasingly predominant application to reconstruct phylogenetic relationships. In theory, tree-insertion methods, e.g., pplacer (Matsen *et al.*, 2010) circumvent many of the issues that confound BLAST assignments, by providing an additional line of evidence to supplement BLAST hits (particularly where reference sequences are unclassified or misnamed), helping to identify divergent lineages (long branch taxa with no close reference sequences) and aiding the development of phylogenetic species concepts to delineate OTUs as putative species (Bik *et al.*, 2012).

CHAPTER 4

4.0 MOLECULAR MICROBIAL DIVERSITY AND CILIATE MORPHOSPECIES IN LAKE BOGORIA

4.1 Introduction

The microbial biodiversity and community structure of the Rift Valley lakes in Eastern Africa are primarily controlled by salinity and the degree of environmental stability (Talling, 2001). The lakes have exceptionally high ion concentrations and experience huge fluctuations in water volume that alter the chemical equilibrium and influence the bioavailability of elements for the aquatic biota (Jirsa *et al.*, 2013). The lakes are also characterized by high production within each trophic level (Vareschi and Jacobs, 1985) and the endemic specialized biota tolerates high water temperature, high salinity, and fluctuations in oxygen concentrations (Melack and Kilham, 1974).

Several well-known extreme alkaline environments such as Mono Lake in California, have been extensively examined for their bacterial and archaeal diversity but remain underexplored with respect to microbial eukaryotic diversity (Humayoun *et al*., 2003). However, knowledge of the morphological, functional and ecological diversity of microbial eukaryotes is essential for a number of practical reasons (Baldauf, 2008). Protists -microbial eukaryotic organisms assembled for convenience and mostly unicellular in structure - are important components in the pelagic food webs, and play numerous other roles (e.g., environmental quality indicators), but are often neglected or overlooked in biodiversity studies(Corliss, 2002). Heterotrophic protists, typically < 100 µm in length including flagellates, ciliates and amoeba, serve as both major decomposers and mediators of nutrient recycling in aquatic ecosystems (Fenchel, 2008; Desvilettes and Bec, 2009). The feeding mode, food size consumed and protist behaviours show large variations, indicating that the protists are a highly heterogeneous group (Boenigk and Arndt, 2002).

Prokaryotic microbes and macro-organisms have already been in the focus of diversity studies in Rift Valley alkaline – saline lakes in the past (e.g., Duckworth *etal.*, 1996; Harper *et al*., 2003). However, only a few studies have focused on the protistan diversity in these lakes (e.g., Yasindi *et al*., 2002; Ong'ondo *et al.*, 2013). In this study, I investigated the protistan diversity in Lake Bogoria using molecular methods (454 pyro-sequencing) and determined the ciliated protist taxonomic composition and abundance using classical microscopy methods. The first hypothesis considered was that the diversity of protists was

restricted by the extreme environment of this lake and the second hypothesis was that the ciliate molecular diversity was much higher than that obtained from traditional methods based on morphological criteria ('morphospecies concept').

4.2 Results

4.2.1 Protist taxonomic richness and abundance

The 18SSU rRNA gene produced approximately 18,600 protist and fungal amplicons that were assigned to 46 OTUs representing 9 major groups that included: Alveolata, Cryptophyta, Stramenopiles, Chlorophyta, Fungi, Ichtyosporea, Jakobida, Choanoflagellida, Katablepharidophyta and two uncultured protist sequences. The most abundant amplicons were Alveolata (61.9%) and Cryptophyta (37.5 %) with two taxa (the ciliate *Frontonia* sp. and the cryptophyte *Guillardia theta*) dominating and accounting for 87.9 % of the amplicons (Table 4.1). The Shannon diversity and evenness indices were on average 1.3 and 0.2 respectively.

Table 4.1: Summary of protist groups, sequences abundance, relative abundance, and similarity of 18S rRNAs sequences to known protist taxa

				Similarity of sequence to identified species (%)			
Group	**Sequences**	**Abundance (%)**	**OTUs**	**> 97**	**95 - 97**	**90 - 95**	**< 90**
Alveolata	11520	61.9	13	7	3	3	0
Cryptophyta	6983	37.5	12	1	7	4	0
Stramenopiles	59	0.3	9	3	0	5	1
Others	39	0.2	12	3	2	4	3
TOTAL	**18601**	**100.0**	**46**	**14**	**12**	**16**	**4**

4.2.2 Protistan and fungal phylogenetic affiliations

The degree of novelty in the Lake Bogoria amplicons was explored by looking at 18SSU rRNA gene identity value >97% in databases (BLASTN search, October 2013). The results are summarized in Table 4.1. The most similar identified gene sequences were:

Cryptophyta: Apart from *G. theta* (6 OTUs), the other six cryptophyte OTUs were related to: *Hanusia phi* (95 %), *Goniomonas* sp. (93 %), *Chroomonas* sp. (97 %) and *Cryptomonas* sp (92 %).

Stramenopiles had a total of 9 OTUs related to an uncultured stramenopile clone NKS100 (97 %); *Nannochloropsis* sp. (93 %), the marine groups sequences MESS13, MESS14 and ME5 (94 %), an uncultured bicosoecid clones (90 – 94 %), an uncultured ochromonad clone (100 %) and an uncultured *Synura* clone (91 %).

Fungi: There were three OTUs with one being 100 % similar to an uncultured fungus while the second OTU was similar to both *Paraphaeosphaeria* sp. and *Paraconiothyrium* sp. (99 %). The third OTU was unrelated to known eukaryote sequence (< 90 %).

Dinophyceae: Two OTUs were recovered, with the first similar toan uncultured *Peridinium* clone (99 %) and the second linked to*Woloszynskia leopoliensis* (92 %)

Chlorophyta: There were two OTUs with high similarity to *Dunaliella salina* (100 %) and *Chlamydomonas globosa* (100 %).

Ichthyosporea: Two OTUs unrelated to known eukaryotes sequence(91 % and 87 %).

Others groups included: one OTU of unclear affliation linked to an uncultured Katablepharidaceae clone (90 %); one OTU similar to*Jakobida libera* (99 %):oneOTU with unclear affiliation linked to Ichthyosporea (90 %); and lastly, a fungal OTU (97 %).

4.2.3 Ciliate molecular abundance, morphospecies and taxonomic composition

The ciliate species assigned as molecular OTUs was higher than those identified by classical morphospecies methods (11 vs. 6, respectively). There was intraspecific variation in the amplicons that resulted in more than one molecular OTU being assigned to *Cyclidium* spp. (3 OTUs) and *Frontonia* spp. (2 OTUs). Other 'species' identified on the basis of the amplicons included: *Paramecium* sp., *Paraurostyla* sp., *Moneuplotes* sp., *Stichotrichia* sp. *Cinetochilum* sp. and an uncultured ciliate clone. On the other hand, at least two ciliate morphospecies, the dileptid *Rimaleptus* sp. and an unidentified stichotrich ciliate identified by microscopy were not captured by molecular sequencing (Table 4.2).

Frontonia sp. had high amplicons abundance (accounting for 89.85 %) but was present at only 16.8 % in abundance determined by microscopy. On the contrary, *Cyclidium* spp. had a higher morphospecies abundance (68.52 %) but only represented by 0.96 % of the amplicons. *Rimaleptus* sp., was observed under the microscope but the 454 sequences did not have amplicons belonging to this taxon. The ciliates categorised as unidentified in the microscopy methods could not be identified from morphology and may be representives of taxa revealed by the molecular methods i.e., *Paraurostyla* sp., *stichotrichia* sp. and the uncultured ciliate clone.

The Shannon diversity and evenness indices returned slightly different values for the two methods. The diversity and evenness were on average 0.7 and 0.3, respectively for the molecular methods and 0.9 and 0.5, respectively for microscopy methods; reflecting the dominance of a few taxa, notably *Cyclidium* spp and *Frontonia* spp.

Table 4.2: Comparison of data from molecular (amplicon abundance and similarity to NCBI sequences) and abundance values determined by microscopy. A dash (-) denotes no values were determined; N/A – Not applicable.

Taxa	Number of amplicons	Amplicon Similarity to NCBI sequence (%)	Morpho - species abundance (cells/litre)	Amplicon abundance (%)	Relative morpho - species abundance (%)
Cinetochilum sp.	1	94.63	7600	0.01	13.44
Cyclidium glaucoma 1	15	97.84	38750	0.13	68.52
Cyclidium glaucoma 2	92	97.1	-	0.80	-
Cyclidium sp.	3	95.68	-	0.03	-
Frontonia sp.1	10185	98.11	9500	88.45	16.80
Frontonia sp. 2	161	97.96	-	1.40	-
Moneuplotes sp.	308	98.57	300	2.67	0.53
Paramecium sp.	2	94.00	-	0.02	-
Paraurostyla sp.	223	96.88	-	1.94	-
Stichotrichia sp.	524	96.64	-	4.55	-
Uncultured ciliate	1	100	-	0.01	-
Rimaleptus sp.	0	N/A	100	0	0.18
Unidentified ciliate	0	N/A	300	N/A	0.53

4.3 Discussion

4.3.1 Protist and fungal taxonomic richness and sequence abundance

The high abundance of Alveolata and Cryptophyta amplicons suggest that estimating eukaryotic protist and fungal taxonomic richness based on sequence abundance are likely biased. Medinger *et al*., (2010) also reported a general overestimation of alveolates by NGS with the overestimation varying substantially among the species analysed, thus suggesting the NGS of rDNA was not suited for estimation of absolute species abundance. Other studies on environmental PCR (e.g., Potvin and Lovejoy, 2009) also recorded similar biased representation of various phylogenetic groupsraising the possibilities that PCR-based methods may under – represent some microbial eukaryote groups, especially those with fewer

ribosomal gene copies relative to protists with high copy numbers (Amaral-Zettler, 2013). Additionally, resting stages (e.g. cysts) contributed to NGS-based abundance though that was not taken into acccount in morphological observations (Medinger *et al.*, 2010). Single-cell NGS methods confirmed possibilities that traditional, environmental PCR-based methods may have under represented group of protists and showed that variation in abundance of species with different rDNA repeat counts couldseverely affect the abundance estimates of all species in the sequence library(Medinger *et al.*, 2010).

Shannon diversity and evenness indices were low, reflecting the dominance of only a few protists groups in the lake. At a local scale, species diversity is often expressed as indices that weigh both the richness and equitability of a sample (Hill *et al*., 2003). Alpha diversity, usually expressed as diversity indices (e.g., Shannon diversity index) based on number of OTUs (OTU richness) is a metric often employed in biodiversity studies to distinguish communities (Amaral-Zettler, 2013). Apart from the Shannon diversity index, species richness of a community can also be estimated using non-parametric methods e.g., Chao or ACE estimator (Chao, 1984; Chao & Lee, 1992). However, the non-parametric methods may under-estimate the true microbial diversity when data is limiting such as in high throughput sequences. Overall, such diversity indices should be treated with caution as the number of rRNA copies can vary dramatically even within species (Averbeck and Eickbush, 2005) confounding the ability to correlate the number of reads generated in a marker gene survey with the number of individuals in a sample (Bik *et al*., 2012). Early proof-of-concept control experiments in nematodes revealed a strong consistency in rRNA patterns but highlighted the difficulty of correlating OTUs with biological species and defining absolute abundances as the rRNA read number per individual was highly variable, even within a single specimen (Porazinska *et al.*, 2009). It may be possible to amend this problem by using either group-specific PCR primers or by restricting the analysis to species with similar rDNA repeat numbers(Medinger *et al.*, 2010). Alternatively, the comparison of environmental communities should only be in terms of relative taxon abundance i.e., normalizing sequence reads per OTU (Christen, 2008).

4.3.2 Protist and fungal taxon phylogenetic affiliations

The levels of similarity of some protist and fungal amplicons to sequences in availabe databases were low. This may be attributed to the level of resolution attainable using the eukaryotic V9 hypervariable region as it does not readily differentiate between species, highlighting the importance of accurately cataloguing sequence data from described protist species in databases as suggested, for example, in the protistan barcoding initiative

(Pawlowski *et al*., 2012). Nevertheless, the molecular methods are useful in uncovering 'novel' diversity in microbial eukaryotes, for example, Stramenopiles in Lake Bogoria had high OTU richness but a low similarity to identified sequences, a result similar to other studies that suggested the largest unknown diversity of this group was likely present in freshwater systems (e.g., Shalchian-Tabrizi *et al*., 2008). Other studies from saline environments also found taxa considered to contain extremely novel sequences(e.g., Triadó-Margarit and Casamayor, 2013; Amaral-Zettler, 2013).Additionally, many protist and fungal amplicons distant from known species or genera were detected in this study; similar observations have been found even in freshwater systems that have been traditionally studied for long periods (Berney *et al*., 2004).It is possible that a number of such phylotypes correspond to described species or groups for which the SSU rRNA sequence is not yet available (Baldauf, 2003). However, for some protist groups such as ciliate, SSU rRNA sequences for representatives of all classes and virtually all subclasses are available in databases (Lynn and Small, 2002).

4.3.3 Ciliate molecular abundance, morphospecies and taxonomic composition

The ciliate morphospecies taxon abundances did not reflect the true taxon-assigned amplicon abundances. Though the morpho-species concept is considered the standard for ciliates (Foissner *et al*., 2002), the amplicons revealed a diversity of the ciliates that could not be observed by microscopy (morphological methods).This is congruent with the observation that many well studied morphotypes, including those with sufficient biogeographical sampling, are known to represent a variety of morphologically indistinguishable species (Foissner, 2006).The occurrence of more than one amplicon for *Cyclidium* phylotypes may be due to disparate rates of morphological and molecular evolution of the ciliate genomes (Dunthorn *et al*., 2008). In other studies using molecular methods (e.g., Guggiari and Peck, 2008), it was observed that clones isolated from a morphospecies were molecularly distinct and such ecotypes might represent distinct species or possibly incipient species (Weisse *et al*., 2007).

Frontonia sp. had about 98 % amplicon similarity with *F. Didieri*18SSU rRNA sequences from NCBI database, but a closer observation using classical microscopy methods indicated there were considerable morphological differences between the two species and the species from Lake Bogoria may be an undescribed species (Ong'ondo *et al.*, 2013; see also Chapter 6). In general, the V9 region of the 18SSU rRNA gene as used in this study is not necessarily able to differentiate between ciliate taxa at species level and is more appropriate for indicator analysis (Amaral-Zettler, 2013).The species identification in protists based on the morphospecies concept (Adl *et al*., 2007), has the advantage of using morphological

characters in the identification of ciliates, but it requires a wealth of experience and high quality microscopes to be able to differentiate the species. Adl *et al*., (2007) suggested the emphasis for species delineation be placed on the combination of phylogenetic analysis of sequence data plus physiological adaptations to a multidimensional niche space i.e., an ecologically relevant parameter (Whittaker, 1972). Furthermore, in obtaining a standard set of information in species delineation, there should be a combination of standardized requirements for microscopy (e.g., use of digital still-images of live specimens or digital video showing patterns of motility, and scanning or transmission electron micrographs), DNA sequence information, habitat and feeding preferences, and where possible, a description of life cycle stages (Adl *et al*., 2007).

There is widespread acceptance that identification of protist species using light microscopy alone is no longer sufficient or adequate (e.g., Adl and Gupta, 2006). On the other hand, incongruities between morphotype and phylotype abundances in the protist community have been found in other studies (e.g., Medinger *et al*., 2010; Stoeck *et al*., 2013). Therefore, the combined use of classical morphological methods and molecular technique provided better results that are not achieved by one or the other method. It shows that even in a single lake, more one species that are morphologically similar but differ in the genotype may occur, and thus enabling niche separation.

CHAPTER 5

DIVERSITY AND COMPOSITION OF CHLOROPHYTES

5.1 Introduction

The diversity and community composition of chlorophytes in the alkaline – saline Rift Valley lakes in Kenya remains poorly understood. Previous studies of phytoplankton (e.g., Schagerl and Oduor, 2008; Krienitz *et al*., 2012) were based on light microscopy and at present, there is limited knowledge on the molecular phylogenetic diversity and taxonomic affiliation of algal groups in the lakes. The phytoplankton is usually dominated by cyanobacteria that from time to time are replaced by eukaryotic algae (Vareschi, 1982; Ballot *et al*., 2004; Schagerl and Oduor, 2008). The eukaryotic algae, observed in the lakes include: coccoid green algae (Vareschi, 1982); *Chlorella, Monoraphidium* and*Scenedesmus* (Schagerl and Oduor, 2008); *Picocystis salinarum*, and cryptomonads (Krienitz *et al*., 2012; Luo *et al*., 2013). Furthermore, studies have indicated that the phytoplankton diversity in lakes significantly affected the structure and function of the food web; and the biodiversity of the eukaryotic phytoplankton was higher at low salinity compared to hypersaline conditions (e.g., Luo *et al*., 2013).

Green algae (Chlorophyta) are common inhabitants of marine, freshwater and terrestrial environments (Leliaert *et al*., 2012). Chlorophytes exhibit a remarkable morphological diversity and have played a crucial role in the global ecosystem for hundreds of millions of years (Falkowski *et al*., 2004; Leliaert *et al*., 2011). Traditionally, four classes are recognized within the group: the freshwater or terrestrial Trebouxiophyceaeand Chlorophyceae, the coastal Ulvophyceae, and the unicellular, predominantly marine planktonic Prasinophyceae (Leliaert *et al*., 2012). The introduction of phylogenetic methods into the systematic of algae and the alignment of eco-function groups with true species identities using the bar-coding concept has led to a fundamental revision of the concept of higher taxonomic lineages and provided a better understanding of interactions between the chlorophytes and their environment (Bock *et al*., 2011; Leliaert *et al*., 2012). In addition, the incorporation of phylogenetic information into ecology enables ecological questions to be addressed in an evolutionary context, leading to a deeper understanding of processes that give rise to patterns of biodiversity (Donoghue, 2002).

Phototrophic phytoplanktons exhibit a remarkable capability for adaptation and acclimation that allows them to inhabit niches representing spatial-temporally varying biological extremes

of light, salinity, pH, and water potential (Krumbein*et al*.,2004). The observation that many morphospecies of heterotrophic nanoflagellates occurred in both freshwater and marine habitats encouraged the view that salinity could be a relatively unimportant evolutionary barrier (Patterson and Lee, 2000). The alkaline – saline Rift Valley lakes offer a remarkable environment to test this hypothesis. I analysed the phylogeny and taxonomic affiliation of Chlorophyta from five Rift Valley lakes of different salinity. I hypothesized that the diversity of green algae in the lakes followed the pattern of spatially variation in physico – chemical environmental parameters which was reflected by differences in algal phylotypes in the lakes.

5.2 Results

5.2.1 Chlorophyta distribution

The distribution of chlorophytes in the lakes followed the pattern of spatially variation in the physico – chemical environment with most of the phylotypes recovered from the freshwater lakes Baringo and Turkana (Figure 5.1).

Lake Baringo: There were 63 chlorophyte phylotypes with chlorophyceae being the most abundant (50). Trebouxiophyceae had nine phylotypes and there was one phylotype each from Pedinomonadaceae, Chlorodendrophyceae, Nephroselmidophyceae and Mamiellophyceae.

Lake Turkana: A total of 19 phylotypes were recorded with Chlorophyceae and Trebouxiophyceae each having seven phylotypes. Pedinomonadaceae had three while Mamiellophyceae and Pyramimonadaceae were represented by one phylotype each (Figure 5.1).

Lake Oloidien: There were only four phylotypes with two Chlorophyceae phylotypes and one each for the Trebouxiophyceae and Pyramimonadaceae.

Lake Elmenteita: There were nine phylotypes with five Trebouxiophyceae, three chlorophyceae and one Pyramimonadaceae.

Lake Bogoria: Two Chlorophyceae phylotypes (*Dunaliella* sp. and *Chlamydomonas* sp.) were recorded.

5.2.2 Diversity and phylogenetic affiliation

74 amplicons of the 18SSU rRNA gene sequences of Chlorophyta were obtained from the lakes. Most of the amplicons (58) were affiliated with Chlorophyceae (mainly Volvocales and Sphaeropleales). Trebouxiophyceae (11) and Prasinophyceae (five) were the other major

chlorophyte groups (Figure 5.1). Many of the amplicons (50) had similarities ranging from 85 – 97 % to known chlorophyte 18SSU rRNA sequences from the NCBI database.

5.2.2.1 Chlorophyceae

The phylotypes recovered mainly belonged to two major clades of Chlorophyceae i.e., Volvocales and Sphaeropleales (Figure 5.2a, b). The Volvocales, consisting mainly of unicellular biflagellate motile green algae, were the most abundant phylotypes in the lakes. The phylotypes were placed within clades belonging to the genera *Chlorogonium, Dunaliella, Chlamydomonas, Wislouchiela* and *Phocatus*. The Sphaeropleales belonged to coccoid green algae from the genera *Pseudopediastrum*, *Scenedesmus*, *Monoraphidium* and *Mychonastes.* Pediomonaphyceae was represented by the genus *Pediomonas*while several phylotypes aligned in the basal position to the major clades within Chlorophyceae and were assigned at a higher taxonomic level than the genera in the clade (Figure 5.2a, b).

5.2.2.2 Trebouxiophyceae

The phylotypes were affiliated to the genera *Heynigia*, *Botryococcus*, *Chloricystis*, *Apatococcus* and several lineages of the *Oocytis* clade. There were also a number of amplicons that were assigned to higher taxa levels (i.e., Trebouxiophyceae) as they were placed at the base of clades in which they were aligned (Figure 5.2c).

5.2.2.3 Prasinophyceae

There were five phylotypes affiliated to Chlorodendrophyceae, Nephroselmidophyceae, Pyramimonadaceae and Mamiellophyceae (Figure 5.2c).

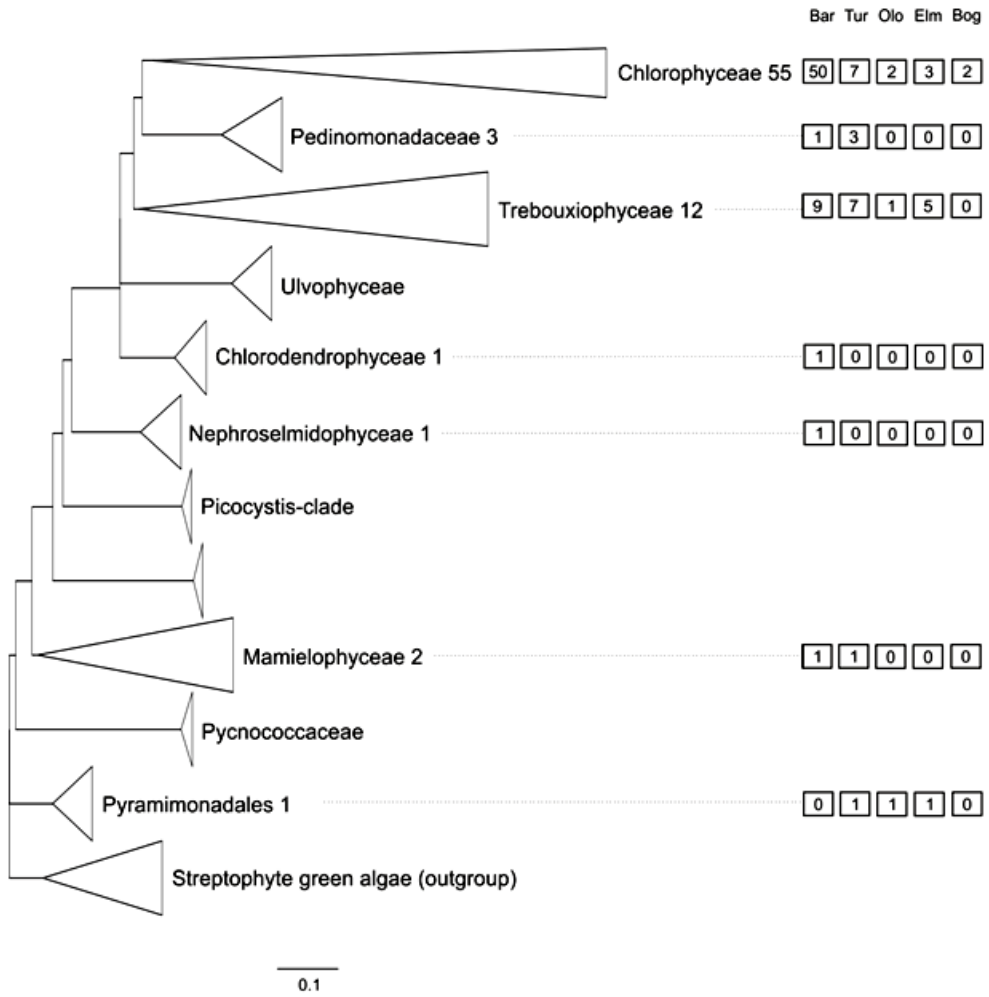

Figure5.1: Collapsed phylogenetic tree for the 18S rRNA gene sequences (n = 74) obtained from the lakes studied. The number of phylotypes assigned to each phylogenetic clade and the abundance of the high-rank taxonomic groups are indicated on the right hand side. Bar = 0.10 fixed point mutation per nucleotide position.(Lake codes: Bar - Baringo; Tur – Turkana; Olo – Oloidien; Elm – Elmenteita; and Bog – Bogoria).

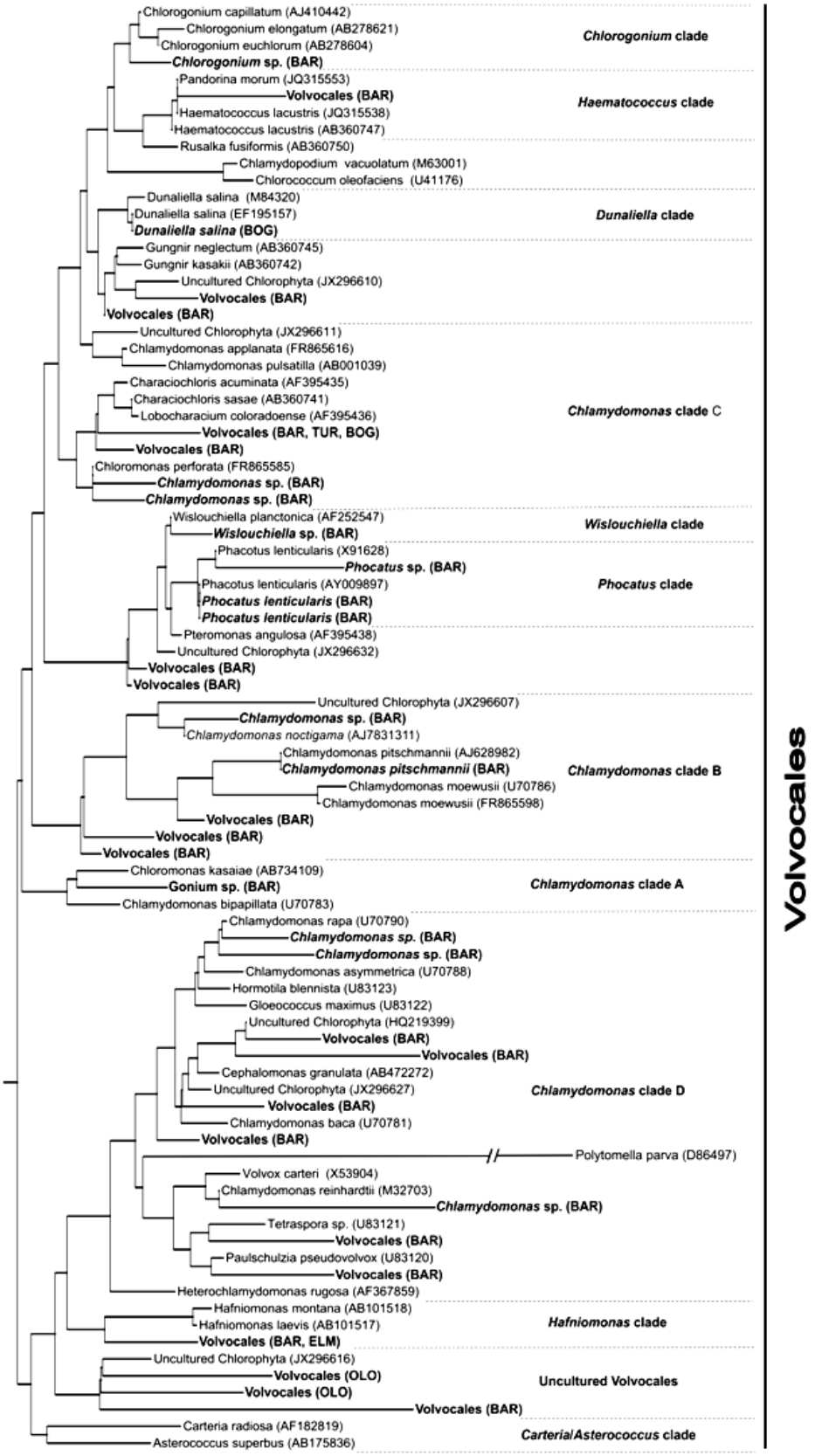

Figure5.2a: phylogenetic tree for the 18S rRNA gene sequences for the Volvocales obtained from the lakes. The sequences from this study are in bold and the lake codes from where the amplicon originated are in brackets after the taxa names. Bar = 0.04 fixed point mutation per nucleotide position. (Lake codes: Bar - Baringo; Tur – Turkana; Olo – Oloidien; Elm – Elmenteita; and Bog – Bogoria).

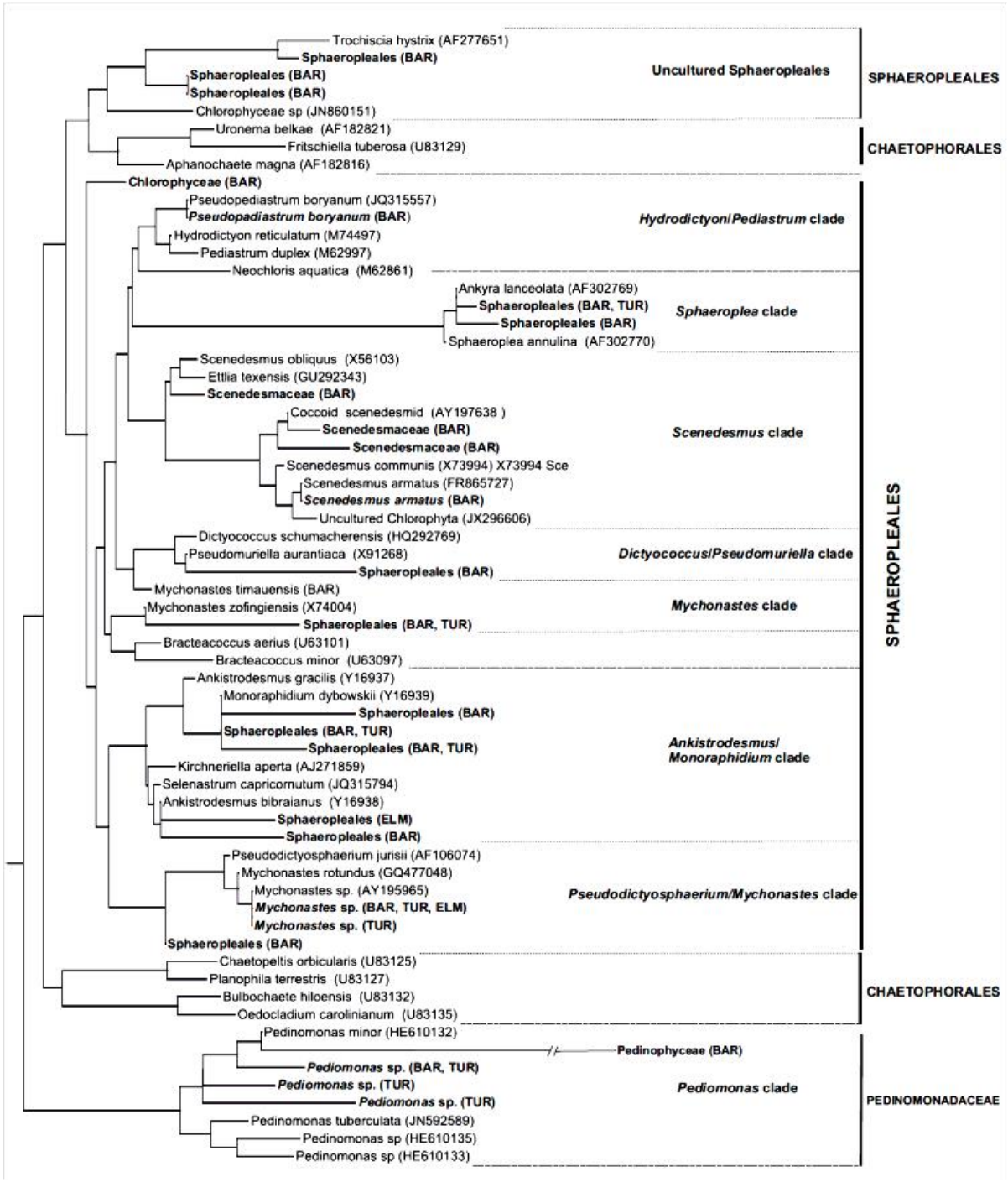

Figure5.2b: Phylogenetic tree for the 18SSU rRNA gene sequences for the Sphaeopleales and Pedinomonadaceae obtained from the lakes. The sequences from this study are in bold and the lake codes from where the amplicon originated are in brackets after the taxa names. Bar = 0.04 fixed point mutation per nucleotide position. (Lake codes: Bar - Baringo; Tur – Turkana; Olo – Oloidien; Elm – Elmenteita; and Bog – Bogoria).

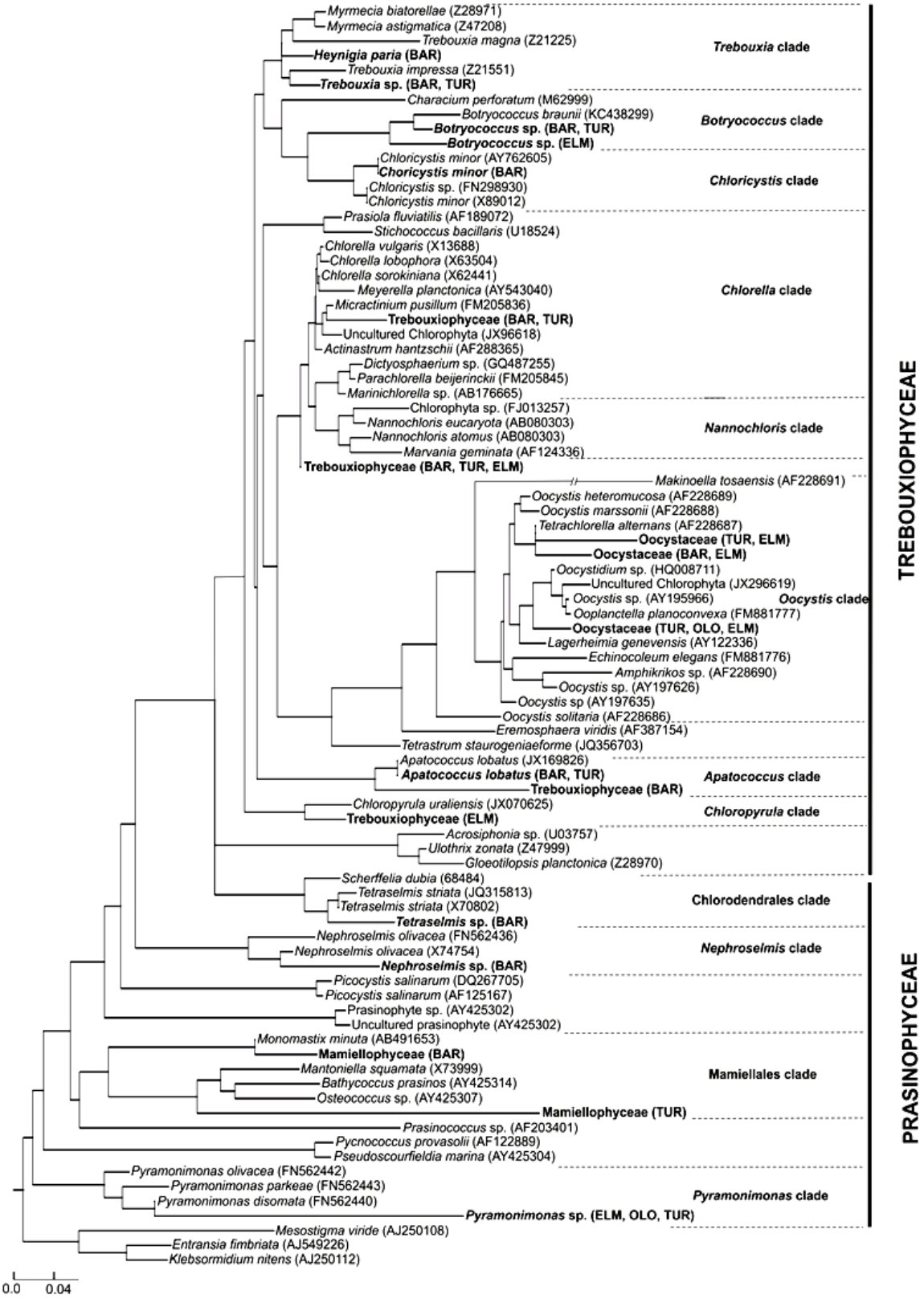

Figure5.2c: Phylogenetic tree for the 18S rRNA gene sequences for the Trebouxiophyceae and Prasinophyceae obtained from the lakes. The sequences from this study are in bold and the lake codes from where the amplicon originated are in brackets after the taxa names. Bar = 0.04 fixed point mutation per nucleotide position. (Lake codes: Bar - Baringo; Tur – Turkana; Olo – Oloidien; Elm – Elmenteita; and Bog – Bogoria).

5.3 Discussion

The majority of green algae thrive in freshwater or terrestrial habitats, but some microscopic forms (prasinophytes) are abundant in marine phytoplankton (Friedl and Rybalka, 2012). Phylogeneticstudies indicate a clear evolutionary separation between marine and freshwater lineages, suggesting that the two types of environments are generally inhabited by distantly related groups of microbes (e.g., Hahn, 2006; Logares, 2009). However, my results should be interpretedconsidering the moderate number of samples analysed per lake, the inherent under-sampling, and low similarities in some cases between the amplicons and sequences from the NCBI database.

5.2.1 Chlorophyta distribution

The distribution of chlorophytes in the Rift valley lakes appear to be strongly influenced by salinity as most of the phylotypes were recorded in the freshwater lakes. It indicates a high diversity in the freshwater lakes compared to the alkaline – saline lakes in agreement with general ecological principles where more extreme environments, such as the alkaline – saline Lake Bogoria, are expected to have a relatively simple ecosystem structure as the number and metabolic diversity of the micro-organisms adapted to life at high salt concentration is limited (Oren, 2002). In Lake Bogoria, the poor taxonomic representation of Chlorophyta could also be as a result of competitive exclusion by Cyanobacteria (e.g., *Arthrospira fusiformes*) that periodically occur in high biomass in the lake (Schagerl and Oduor, 2008). Furthermore, my results are contrary to the view that salinity could be a relatively unimportant evolutionary barrier (Larsen and Patterson, 1990) even though the distribution of a majority of chlorophytes shows they thrive in freshwater or terrestrial habitats (Friedl and Rybalka, 2012). Most of the Chlorophytes phylotypes were recovered from the freshwater lakes (Baringo and Turkana), but one of the challenges of determining the effects of environmental factors (such as salinity) in a natural community is the fact that taxa present respond to other factors, including availability of nutrients (Greenwald & Hurlbert, 1993).

5.2.2 Diversity and phylogenetic affiliation

5.2.2.1Chlorophyceae

Most of the amplicons in the lakes were affiliated with Chlorophyceae (mainly Volvocales and Sphaeropleales) which showed a high diversity by forming a number of clades (Figure 5.2a, b). This result are similar to other analyses using 18SSU rDNA that found strains of *Chlamydomonas* and *Chloromonas*formed seven different clades and confirmed the polyphylyof the two genera (e.g., Buchheim*et al.*, 1996; Pröschold *et al.*, 2001).

Chloromonas, is traditionally distinguished from its close relative, *Chlamydomonas*, by lacking pyrenoids in its chloroplasts (Ettl, 1970, 1976). Others studies found a highdegree of divergence among well-defined clades in the genus *Chlamydomonas* (Pröschold *et al*., 2001). In this study, there were many sequences that were classified as uncultured Chlorophyceae and were aligned basal to well defined clades of Volvocales.

The Sphaeropleales were the other group of green algae in the lakes. Sphaeropleales including the genus *Scenedesmus* species (coccoid green algal) are common in fresh and brackish waters, particularly in nutrient-rich conditions (Trainor and Egan, 1990). They are an ecologically important group of green algae because they may constitute a major part of the green algal biomass in phytoplankton (Lürling, 2003). *Scenedesmus* is morphologically well defined by its elliptical to spindle-shaped cells that are grouped together, usually 2, 4 or 8 of them in one to three rows, though unicells may also be common (Hegewald, 1997; Kessler *et al*., 1997). The genus *Scenedesmus* has a large number of described taxa (with up to 1300, enumerated in Hegewald and Silva, 1988), but its actual diversity has been controversial (Komárek and Fott, 1983). The proliferation on a numerous species, varieties and forms of *Scenedesmus* may in large part be a reflection of morphological plasticity within the genus and the reliability of narrow circumscriptions of taxa (Trainor and Egan, 1990). The 18SSU rRNA sequence analyses suggest that *Scenedesmus* species may be split in at least two lineages that correspond to the subgenus *Desmodesmus* and another lineage that unites the subgenera *Scenedesmus* and *Acutodesmus* (Kessler *et al*., 1997).

There were four Pedinophyceae phylotypes recovered and they formed a clade at the base of the Sphaeropleales. This is consistent with other studies that reported Pedinophyceae had no clearaffiliation with other groups of chlorophytes (e.g., Melkonian, 1990; Moestrup, 1991). Only three genera (*Pedinomonas*, *Resultor*, and *Marsupiomonas*), of these small, naked flagellateslacking scales on their unique flagellum have been described (Moestrup, 1991). The presence oftwo basal bodies, each associated with two microtubularroots, suggests that they are not primarily uniflagellates (Melkonian, 1990). Although the persistent telophase spindleduring mitosis and the eye-spot located opposite the flagellainsertion have been considered to be ancient featuresshared with some prasinophyceans, an affinity with the Ulvophyceaehas been proposed based on the configuration ofthe flagellar apparatus and the structure of the stellatepattern of the flagellar transition region (Melkonian, 1990; Moestrup, 1991).

5.2.2.2 Trebouxiophyceae

Only a dozen amplicons of this group were recorded in the studied lakes probably because membersof Trebouxiophyceae are mostly found in drier habitats (e.g., soil), or areaerophytic algae that dominate aeroterrestrial biofilms of many regions (Gustavs*et al*., 2011; Friedl and Rybalka, 2012). The 18SSU rDNA phylogenies resolved four well supported Trebouxiophyceae clades in the lakesthat comprised of multiple genera, i.e., Trebouxiales, *Choricystis*/*Botryococcus*-clade, Chlorellales (*Oocystis* clade), and *Apatococcus* clade. The Trebouxiophyceae exhibit a highdegree of morphological heterogeneity (Lewis and McCourt, 2004), and are characterized by an ellipticalcell shape, small cell size, and unequal autospores (Darienko*et al*., 2010). Ellipsoidal '*Chlorella*-like' species show high phenotypic plasticity which has led to differentspecies designations in the literature (Darienko*et al*., 2010). The genera *Chlorella* and *Nannochloris*, small (less than 3 µm) roughly spherical planktonic green algae, reveal so few morphological characters that it is difficult or impossible to separate and identify them using morphological criteria alone (Krienitz *et al*., 1999; Finlay, 2004). Only when morphological and ultrastructural features are used in combination with biochemical (e.g., production or absence of secondary carotenoids), physiological and molecular (e.g., complete SSU rRNA gene sequences) characters, can the taxa be separated unambiguously (Huss *et al*., 1999).

Interestingly, the *Trebouxia* clade had one phylotype (*Heynigia paria*) which was newly described in an earlier study in Lake Baringo (Bock *et al*., 2011). A second phylotype affiliated to *Trebouxia* was found in both freshwater lakes (Baringo and Turkana) suggesting that members of Trebouxiales are planktonic in these habitats or they may have been deposited in the lakes from aerial-borne dust particles. Two other phylotypes (*Botryococcus* and *Chloricystis*) have been reported in the phytoplankton (Fawley *et al*., 2005), while the larger *Oocystis* clade that had three phylotypes occur in the freshwater and hyposaline lakes. *Apatococcus* sp., reported in Lake Baringo and Turkana are abundant in aeroterrestrial biofilms, especiallyin urban areas (Rindi and Guiry, 2004), where they cause extensive discolorations on walls and roofs (Darienko *et al*., 2010). Similar results have been reported in other studies that found many ellipsoidal '*Chlorella*-like' species are widely distributed in all kinds of natural and artificial terrestrialhabitats, freshwater and marine environments, and as lichen symbionts in temperate climates (e.g., Rindi *et al*., 2010; Gray *et al*., 2007). Additionally, several new genera which belong to the *Chlorella* clade have been described from freshwater phytoplankton, e.g., *Meyerella* (Fawley *et al*., 2005), *Hegewaldia* (Pröschold *et al*., 2010), *Heynigia*, and *Hindakia* (Bock *et al*., 2011).

5.2.2.3 Prasinophyceae

The phylogenetic position and arrangement of prasinophytes clades in this study was similar to other studies that placed them at the base of the Chlorophyta with a paraphyletic assemblage (e.g., Guillou *et al*., 2004). Prasinophytes phylotypes affiliated to Chlorodendrophyceae, Nephroselmidophyceae, Pyramimonadaceae and Mamiellophyceae were recorded in the Rift Valley lakes, though they are predominantly found in the marine environment (Yamaguchi *et al*., 2011). Previous studies on lake planktons that relied on microscopy may have missed these groups as numerous prasinophytes are very small and belong to the picoplanktonic fraction, i.e. organisms with a diameter of less than 3 μm (Stockner, 1988). My results concur with other studies (e.g., Marin and Melkonian, 2010) that reported Prasinophyte environmental sequences originating from freshwater habitats. Molecular work based on the nuclear SSU rDNA revealed the presence of a large number of sequences from prasinophytes in oceanic waters (e.g., Romari & Vaulot, 2004; Rodríguez et al., 2005), and the use of such methods in the study of phytoplanktons in lakes may reveal a higher biodiversity of prasinophytes. Unfortunately, not much is currently known about the morphology and ecophysiology of the environmental Prasinophyte sequences, thus hindering further discussion about the significance of these organisms in the freshwater environment (Marin and Melkonian, 2010). Consequently, isolation, sequencing, and describing representatives of the picoplanktonic prasinophytes is necessary in order to increase our knowledge of this key algal class (Marin and Melkonian, 2010).

CHAPTER 6

6.0 DESCRIPTION OF THREE NEW CILIATE SPECIES, *Frontonia alkalina* sp. candidatus (Ciliophora, Peniculia), *Euplotes endoroisi* sp. candidatus (Ciliophora, Hypotrichia) and *Condylostoma bogoriense* sp. candidatus (Ciliphora, Heterotrichia)

6.1 Introduction

Ciliates are unique among microbial groups in that their diverse morphology, abundance and relatively large sizes have enabled the creation of a comprehensive morphology-based taxonomy (Dunthorn and Katz, 2008). Studies of ciliate taxonomy, diversity and ecology have traditionally utilized microscopy-based methods of identification (e.g., Petz *et al*., 1995; Foissner *et al*., 1999). The recurrent observation of the same morphotypes from different localities in freshwater and marine habitats led to the assumption that the global ciliate species richness could be relatively low (Finlay and Clarke, 1999). Furthermore, molecular studies have shown that species that are at the 18SSU rRNA level almost identical and morphologically indistinguishable may carry unique genetic diversity and phenotypic traits that enable specialized exploitation of resources in their natural habitat (Nanney *et al.*, 1998; Finlay, 2002; Dunthorn *et al*., 2008; Pfandl *et al.*, 2009).

Ciliates are of considerable importance in aquatic ecosystems, as they are the major consumers of algae and bacteria and constitute a nutritional resource for other protists, invertebrates, and even fish larvae (e.g., Finlay and Esteban, 1998; Posch *et al.*, 1999; Sherr and Sherr, 2002; Zingel *et al.*, 2012). Ecological studies indicate that ciliates occur in high abundance in the pelagic of tropical African lakes (e.g., Hecky and Kling, 1981; Finlay *et al.*, 1987; Yasindi *et al.*, 2002; Yasindi *et al.*, 2007; Ong'ondo *et al.*, 2013). Specifically, the ciliate assemblage in Lake Bogoria, an endorheic alkaline-saline lake in the Gregorian Rift Valley in Kenya is characterized by the presence of relatively few ciliate morphospecies found throughout the year (Yasindi *et al.*, 2007; Ong'ondo *et al.*, 2013). Earlier studies of ciliates in the lake mainly focused on the ecology and detailed species identification was lacking under the assumption that most of the species were already described (e.g., Yasindi *et al*., 2007). Here, I describe three new species belonging to the species-rich genera *Frontonia, Euplotes* and*Condylostoma* that are common in habitats ranging from marine to freshwater.

In detail, the genus *Frontonia* Ehrenberg 1833 comprises more than 40 species mainly described from morphology (e.g., Kahl 1930 – 1935; Bullington, 1939; Dragesco, 1960;

Roque, 1961; Gil& Perez, 1964) while more recent (re-)descriptions and reviews also included genetic sequence data (e.g., Long *et al*., 2005; Fokin *et al*., 2006; Gao *et al*., 2008; Fan *et al*., 2011). However, many species in this genusstill lack detailed morphological studies and consequently they are poorly defined (Fan *et al*., 2011). The genus *Frontonia* belongs to the subclass Peniculia in the class Oligohymenophorea (Lynn, 2008). *Frontonia* can be frequently found in marine, brackish and freshwater habitats (Gao *et al*., 2008) and are widely distributed also in tropical African lakes (Dragesco and Dragesco-Kernéis, 1991; Yasindi *et al*., 2007), in subtropical and temperate water bodies (e.g., Beaver and Crisman, 1989) and in brackish and marine environments (e.g., Fan *et al*., 2011; Pan *et al*., 2013b). Some of the most important characters to distinguish *Frontonia* species are their cell size and shape, number of somatic ciliary rows, the number and location of the excretory pores and the presence/absence of collecting canals of the contractile vacuole or the shape of the postoral suture (Foissner *et al*., 2002).

Euplotes Ehrenberg 1830 is a highly diverse and cosmopolitan genus.Almost 150 species and sub-species have been established or assigned to *Euplotes* based mainly on morphological and morphogenetic characters (e.g., Curds, 1975; Song and Wilbert, 2002; Alekperov *et al*., 2005; Jiang *et al*., 2010a, b). However, only around 70 of these are acknowledged as legitimate species (Borror and Hill, 1995; Petz *et al*., 1995; Foissner *et al*., 2002). *Euplotes* can be found in nearly any marine, estuarine, freshwater, or edaphic habitat (Schwarz *et al*., 2007). The genus *Euplotes* belongs to the subclass Euplotida in the class Spirotrichea (Lynn, 2008). One major difficulty encountered by taxonomists considering this genus is that newly described species are either inadequately described or appear to be environmental variants (Pierson, 1943). Important taxonomic characteristics cited by Tuffrau (1960), Carter (1972) and Borror (1972) included the number of frontoventral cirri (FVC), the pattern of dorsal interkinetal system, the number of dorso-lateral kineties, and the form of the macronucleus. Among the recognized species, the FVC are considered to be remarkably constant in number and position (Washburn and Borror, 1972).

The genus *Condylostoma* Bory 1826 belongs to the family Condylostomatidae (Kahl in Doflein & Reichenow, 1929). The comparison of *Condylostoma* congeners is compounded by overlapping taxonomic features that include extremely variable morphological aspects (e.g., Size and body shape (especially the appearance of buccal field and the posterior end of cell), habitat, number of ciliary rows (= somatic kineties) and the ratio of buccal field:body length body shape, size, number of kinety rows) and few characters for use in circumscribing

species (Song *et al*., 2003). Additionally, some described species have insufficient taxonomic details and numerous misinterpretations and/or are inadequately described (Foissner et al. 2002). For example, the structure of apical membrane (frontal cirri) should bea good indication of the species distinction but is one characteristic that has been used in varying terms (Song and Wilbert, 1997).

I based the descriptions of the ciliates on the morphospecies concept using classical silver impregnation methods. For the *Frontonia* described here, the molecular phylogeny inferred from the 18 SSU rRNA is already available (Ong'ondo *et al.*, 2013).

6.2 Results

6.2.1 *Frontonia alkalina* sp. cand. (Fig. 6.1 a – i, Fig. 2 a – e; Table 6.1 and Table 6.2)

Diagnosis
Size about 165 × 97 µm *in vivo*; ellipsoidal in ventral view and ovate with acuminate anterior and rounded posterior endin lateral view, dorsoventrally flattened.On average 120 ciliary rows; 3 – 5 vestibular kineties, 4 – 6 postoral kineties; peniculi 1 - 3 each with four rows of basak bodies. Two dorso-lateral sub-equatorial contractile vacuoles without collecting canals, each with 2 – 4 excretory pores. Postoral suture extends from posterior end of buccal cavity and ends sub-terminally at about 1/8 of dorsal side as an inclined stripe.

Type location
Lake Bogoria (holotype and five paratypes) and Lake Nakuru (paratypes).

Type material
One holo- and fiveparatype slides with protargol – impregnated specimens have been deposited at the Biologiezentrum Linz (LI), Austria. Relevant specimens are marked by black ink circles on the cover glass.

Etymology
The species name '*alkalina*' derived from the alkaline nature (pH ~10) of the water in the lake from which the ciliate was isolated.

Description
Size *in vivo* 123 – 215 x 70 – 130 µm with mean of 165 x 97 µm and 101 – 143 x 55 – 95 µm with mean of 116 x 70 in protargol-impregnated specimens (Lake Bogoria population; referred to as 'Bogoria' in the following); and 100 – 193 x 51 – 125 µm with mean of 139 x 80 µm in protargol-impregnated specimens from Lake Nakuru (referred to as 'Nakuru' in the following; Table 1). The mean length: width ratio of both the ventral and lateral view of the

ciliate is 1.3 – 2.1:1 *in vivo* and 1.3 – 2.5:1 in protargol-impregnated specimens for both populations. Shape ellipsoidal in ventral view and ovate in lateral view with an acuminate anterior and broadly rounded posterior, dorsoventrally flattened (Fig. 6.1a, b). Location of the nuclear apparatus variable (43 – 83µm distance from the anterior end in Bogoria; and 31 – 85 µm distance from the anterior end in Nakuru), in the mid- to posterior region in most cells (Fig. 6.1 c, d). Macronucleus ovoid to ellipsoid *in vivo*, about 35 – 54 x 22 – 37 µm, on average 46 x 31 µm (Bogoria, Fig. 1c); in protargol-impregnated specimens prolate spheroid to curved elongate, 18 – 31x 10 – 17 µm (Bogoria) and 17 – 46 x 17 – 27 µm (Nakuru). Micronuclei pyriform, 1 – 2 located at anterior or lateral indentations of the macronucleus, 4 – 6 x 2 – 4 µm (Bogoria) and 4 – 7 x 4 – 5 µm (Nakuru) in protargol-impregnated specimens (Fig. 6.1c). Two contractile vacuoles without collecting canals, with 2 – 4 excretory pores each, located in sub-equatorial dorso-lateral right hand side of cell (Fig. 6.1d). Extrusomes form a conspicuous fringe, located in between two kinetids of a ciliary row, fusiform and about 5 µm in length, about 15 µm when ejected.

Cytoplasm colourless, at times with large yellowish to greenish food vacuoles of about 10 – 25 µm in diameter containing ingested cyanobacteria (*Arthrospira fusiformis*) and coccoid cyanophytes (Fig. 6.1a). Large food vacuoles sometimes contain other ciliates or remnants of rotifer trophi (Fig. 6.1f). Numerous crystalline inorganic particles are scattered in the cytoplasm (Fig. 6.1c).

Ciliates move by gliding (e.g., on the bottom of a petri-dish) or swim fast forward by rotating about the longitudinal axis.

Somatic cilia about 10 µm long *in vivo* (Bogoria). 4 – 6 postoral ciliary rows, five on average. 110 – 130 somatic ciliary rows forming a suture in the ventral midline of the ciliate (Fig. 6.2d, e) including about 25 ciliary rows on the dorsal side that originate from the postoral suture and end before the preoral suture by running diagonally abut in 2 – 3 longitudinal ciliary rows (Fig. 6.2c).Preoral suture extends across the cell apex from the buccal cavity and terminates sub-apically about 1/6 of cell length on dorsal side. About 7 – 10 gradually shortened ventral ciliary rows on the left side of the oral apparatus (Fig. 6.1i). Postoral suture extends from the posterior end of the buccal cavity to about 1/8 of dorsal side of cell ending sub-terminallyas an inclined stripe (Fig. 6.2c).

Oral apparatus located in anterior third of the ciliate occupying about 20 % of cell length (Fig. 6.1a, d). Buccal cavity arch-shaped with three slightly curved peniculi (Fig. 6.1i), on average 23, 21 and 18 µm in length. Each of the three peniculi has four rows of basal bodies.

Oral kineties consist of three vestibular kineties, paroral membrane with two rows of basal bodies at right margin of vestibular ciliary stripe and about 5 – 6 postoral kineties (Fig. 6.1i).

Occurence and ecology

Frontonia alkalina was found in the pelagic of the lake in average numbers of 7,000 ind. l^{-1} at the depths sampled (0 – 5 m). Also recorded in the same samples was *Acineria incurvata*, *Cyclidium glaucoma*, *Cinetochilum ovale*, *Euplotes* sp. and *Rimaleptus* sp.

Frontonia spp. have been also frequently recorded from other alkaline – saline lakes in Kenya, such as Lake Nakuru (Finlay *et al.*, 1987; Yasindi *et al.*, 2002; Ong'ondo *et al.*, 2013), Lake Sonachi (Yasindi *et al.*, 2007), Lake Elmenteita and Lake Simbi (GO pers. obs.). *Frontonia alkalina* most probably forms cysts and is likely dispersed by birds (e.g., flamingos) that migrate between various alkaline – saline lakes in the East Africa Rift Valley. Molecular analysis of the 18SSU rRNA gene indicated identical populations of *F. alkalina* in Lake Bogoria and Lake Nakuru (Ong'ondo *et al.*, 2013). Intraspecific size differences between individuals of asingle population have been reported as a common phenomenon in *Frontonia* species (e.g., Fan *et al.*, 2011). The cell size difference of the two populations may be caused by differentenvironmental conditions, especially salinity, which is roughly twice as high in Lake Bogoria as in Lake Nakuru (Ong'ondo *et al.*, 2013).

F. alkalina was successfully maintained in raw culture at room temperatures (20 °C) and initially the ciliate fed on naturally available food, i.e., the cyanobacterium *A. fusiformis*. When the cyanobacterium was exhausted, the ciliates fed on diatoms that subsequently developed in the raw cultures. Due to evaporation, the salinity increased up to 100 ‰ and consequently the abundance of *F. alkalina* decreased drastically. After dilution of the culture by addition of distilled water, the *F. alkalina* population recovered again. However, the cells were sensitive to sudden changes in osmotic pressure and direct dilution in petri-dishes led to the cells bursting. In the alkaline - saline lakes, the salinity may fluctuate between 5 – 90 ‰ and conductivity between 9 – 160 mS cm^{-1} at 25°C as a result of considerable variations in lake water levels and volume as has been recorded from Lake Nakuru (Vareschi, 1982).

Comparison with most related congeners

Frontonia alkalina resembles *Frontonia microstoma*, *Frontonia anatolica*, *Frontonia didieri*, and *Frontonia fusca* in terms of shape, size, post-oral ciliary rows, number of somatic ciliary rows, number and position of contractile vacuoles, excretory pores and collecting canals and the postoral suture (Table 6.2).

Frontonia alkalina is ellipsoid to ovate in shape (vs. Slender, 'club-shaped' and dilated in *F. microstoma*) and is smaller, i.e., 123 – 215 µm vs. 200 – 300µm *in-vivo*. *F. alkalina* has 4 – 6 postoral ciliary rows and about 7 – 9 shortened somatic ciliary rows bordering the left side of the buccal cavity (vs. 4 postoral ciliary rows and no shortened ciliary rows in *F. microstoma*). *F. alkaline* has two large contractile vacuoles with 2 – 4 excretory pores vs. two contractile vacuoles with 1 – 2 excretory pores per vacuole in *F. microstoma* (Roque, 1960).

Frontonia alkalina differs in size from *F. anatolica* (123 – 215 x 70 – 130 µm vs. 101– 134 x 47–67 µm), the number of somatic kineties (110 – 130 vs. 78 – 105), the contractile vacuoles without collecting canals vs. about 6 – 7 conspicuous collecting canals; 2 – 4 vs. 1 excretory pore per contractile vacuole in *F. anatolica* (Yildiz and Senler, 2013).

Frontonia alkalina is similar to *F. fusca* in havingsome mediandorsal ciliary rows that abut to the anterior suture bymerging into each other. *F. alkalina* differs from *F. fusca* in size *in vivo*(123 – 215 x 70 – 130 µm vs. 90 – 170 × 45– 75 µm), number of somatic kineties (110 – 130 vs. 75–92); lack of a pigment spot on right side of anterior dorsal side that is present in *F. fusca*. Contractile vacuoles without collecting canals vs. 6 – 9 collecting canals in *F. fusca* (Fokin, 2008).

Frontonia alkalina differs from *F. didieri* by size *in vivo* (120 – 215 x 70 – 130 µm vs. 100 – 150 x 45 – 80 µm), number of somatic kineties (110 – 130 vs. 61 – 71); number of contractile vacuoles (2 vs. 1) without collecting canals vs. about eight conspicuous collecting canals in *F. didieri* (Long *et al.*, 2008).

Table 6.1 Morphometric data on *Frontonia alkalina* based on living (L) and protargol-impregnated (P)(Foissner's method) specimens. Measurements in micrometre (µm). CV = coefficient of variation in %; M = median; Max = maximum; Mean = arithmetic mean; Min = minimum; n = number of cells investigated; SD = standard deviation; SE = standard error of arithmetic mean; Me = method; Pop = Population from Bogoria (B) and Nakuru (N).

Characteristics	**Pop**	**Me**	**Mean**	**M**	**SD**	**CV**	**min**	**max**	**n**
Body, length	B	L	165.0	161.6	22.0	13.3	123.0	215.0	36
	B	P	116.0	116.1	10.9	9.5	101.0	143.0	54
	N	P	139.0	148.8	21.0	15.1	100.0	193.0	62
Body, width in ventral view	B	L	97.0	95.6	15.4	15.9	70.0	130.0	36
	B	P	70.0	67.4	10.2	14.7	55.0	95.0	54
	N	P	80.0	92.7	17.2	21.5	51.0	125.0	62
Body, width in lateral view	B	P	62.2	63.3	8.1	9.8	50.0	83.0	21
	N	P	66.6	63.2	12.6	12.2	51.0	103.0	22
Body length:width, ratio in ventral view	B	P	1.7	1.7	0.2	12.6	1.3	2.1	54
	N	P	1.8	1.7	0.2	14.0	1.3	2.5	61
Body length:width, ratio in lateral view	B	L	1.7	1.7	0.2	12.7	1.3	2.1	36
	B	P	1.7	1.7	0.2	10.8	1.4	2.1	21
	N	P	1.6	1.6	0.2	9.0	1.3	2.0	22
Anterior end to macronucleus, distance	B	P	60.3	59.9	10.1	12.2	43.0	83.0	22
	N	P	57.4	58.4	15.7	18.5	31.0	85.0	22
Anterior end to buccal cavity, distance	B	P	17.3	17.0	4.6	16.8	9.0	27.0	22
	N	P	17.9	16.3	5.1	19.5	10.0	26.0	22
Buccal cavity, length	B	L	29.0	28.7	3.4	12.0	21.0	33.0	17
Buccal cavity: Body length, ratio	B	L	0.2	0.2	0.0	19.6	0.1	0.3	17
Macronucleus, length	B	L	46.0	48.0	5.7	12.3	35.0	54.0	13
	B	P	23.6	23.3	3.4	14.6	18.0	31.0	31
	N	P	30.0	29.3	7.5	24.9	17.0	46.0	28
Macronucleus, width	B	L	31.0	31.4	4.2	13.5	22.0	37.0	13
	B	P	13.0	13.6	1.8	13.7	10.0	17.0	31
	N	P	22.0	22.0	3.5	15.7	17.0	27.0	8
Micronucleus, length	B	P	4.6	4.6	0.7	14.6	4.0	6.0	21
	N	P	5.3	4.9	1.1	19.8	4.0	7.0	13
Micronucleus, width	B	P	3.0	3.2	0.5	16.0	2.0	4.0	21
	N	P	4.0	3.7	0.6	15.4	4.0	5.0	4
Peniculus 1, length (chord of organelle)	B	P	23.0	23.0	1.2	5.4	21.0	25.0	26
Peniculus 2, length (chord of organelle)	B	P	21.0	20.3	1.4	6.8	19.0	23.0	25
Peniculus 3, length (chord of organelle)	B	P	18.0	18.1	1.2	6.8	17.0	21.0	25
Somatic ciliary rows, number	B	P	120	120	6.6	5.5	110	130	25

Postoral kineties, number	B	P	5.0	5.0	0.5	10.0	4.0	6.0	25
Vestibular kineties, number	B	P	3.2	3.0	0.5	15.0	3.0	5.0	25
Peniculus 1, number of ciliary rows	B	P	4.0	4.0	0.0	0.0	4.0	4.0	25
Peniculus 2, number of ciliary rows	B	P	4.0	4.0	0.0	0.0	4.0	4.0	25
Peniculus 3, number of ciliary rows	B	P	4.0	4.0	0.0	0.0	4.0	4.0	25

Table 6.2: Comparison of main characteristicsto the closest congeners of *Frontonia alkalina*.

Characteristics	*Frontonia alkalina*	*F. microstoma*	*F. anatolica*	*F. fusca*	*F.didieri*
Body, length (*in vivo*)	123 – 215	200 – 300	101 – 134	90 – 170	100 – 150
Body, width (*in vivo*)	70 – 130	-	47 – 67	45 – 75	45 – 80
Shape	Ovate to ellipsoid	Acuminate anterior and ovoid posterior	Elliptical	elongate obovoidal	Elliptical
Buccal cavity, length	21 – 33	-	16 – 21	22 – 27	-
Buccal cavity, mean length in % of mean body length	10 - 30	-	15 – 20	20	14
Postoral kineties, number	4 – 6	4	3 - 5	4	3 – 5
Somatic kineties, number	110 – 130	110 – 120	78 – 105	75 – 92	61 – 71
Excretory pore, number	4 – 8	2 – 4	1	1 – 3	1 – 4
P1, P2 & P3, number of kinety rows	4 + 4 + 4	4 -5 + 4 - 5 , 3 - 4	4 + 4 + 2	4 + 4 + 3	4 + 4 + 3
Contractile vacuole, number	2	2	2	2 – 3	1
Collecting canals, number	0	0	6 - 7	6 – 9	8
Habitat	Alkaline-saline lake	brackish	Alkaline lake	Brackish-marine puddle	Marine
Source		Roque, 1961	Yildiz & Senler, 2013	Fokin, 2008	Long *et al*., 2008

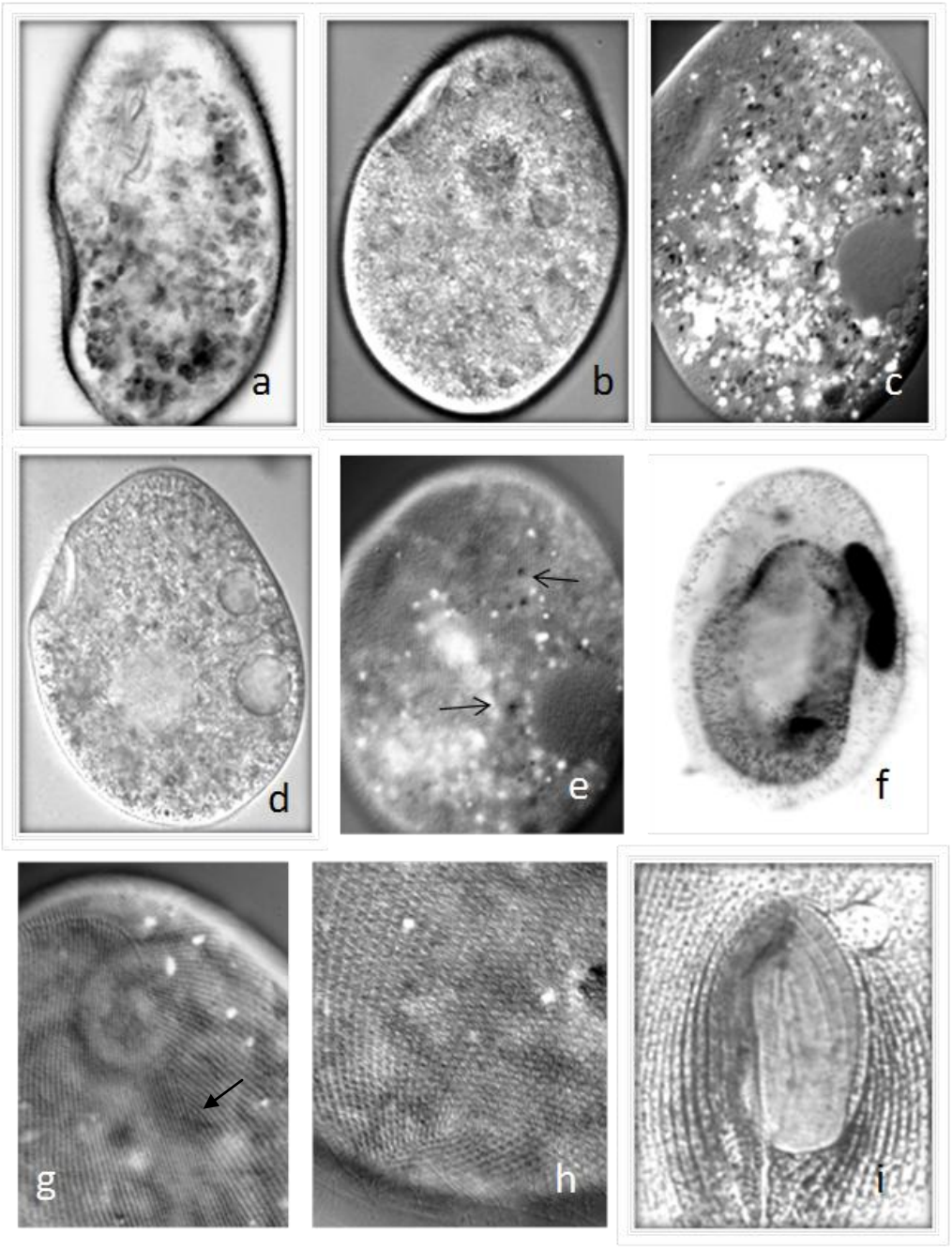

Figure 6.1: (a) Ventral view of *F. alkalina* cell isolated from natural population with many coccoid cyanophytes are visible in the cytoplasm; **(b)** Lateral view of the ciliate showing position of the buccal cavity, the general shape with an acuminate anterior and rounded posterior; **(c)** Lateral view of the ciliate showing the macronucleus and two micronuclei, dark ellipsoid body; and numerous inorganic particles - bright spots; **(d)** Lateral view of the ciliate showing contractile vacuoles; **(e)** Dorsal view of the ciliate with arrows showing excretory pores; **(f)** Ciliate cell with a smaller individual inside, a case of cannibalism?; **(g)**Anterior dorsal region showing the preoral suture and with arrow showing position of ciliary rows that terminate before the preoral suture by fusion of each other;**(h)** Posterior view of the dorsal side showing extent of postoral suture; **(i)**Buccal cavity of the ciliate. Living cells, DIC **(a – e, g & h)**; Protargol-impregnated **(f)**; Cell impregnated with silver carbonate **(i).**

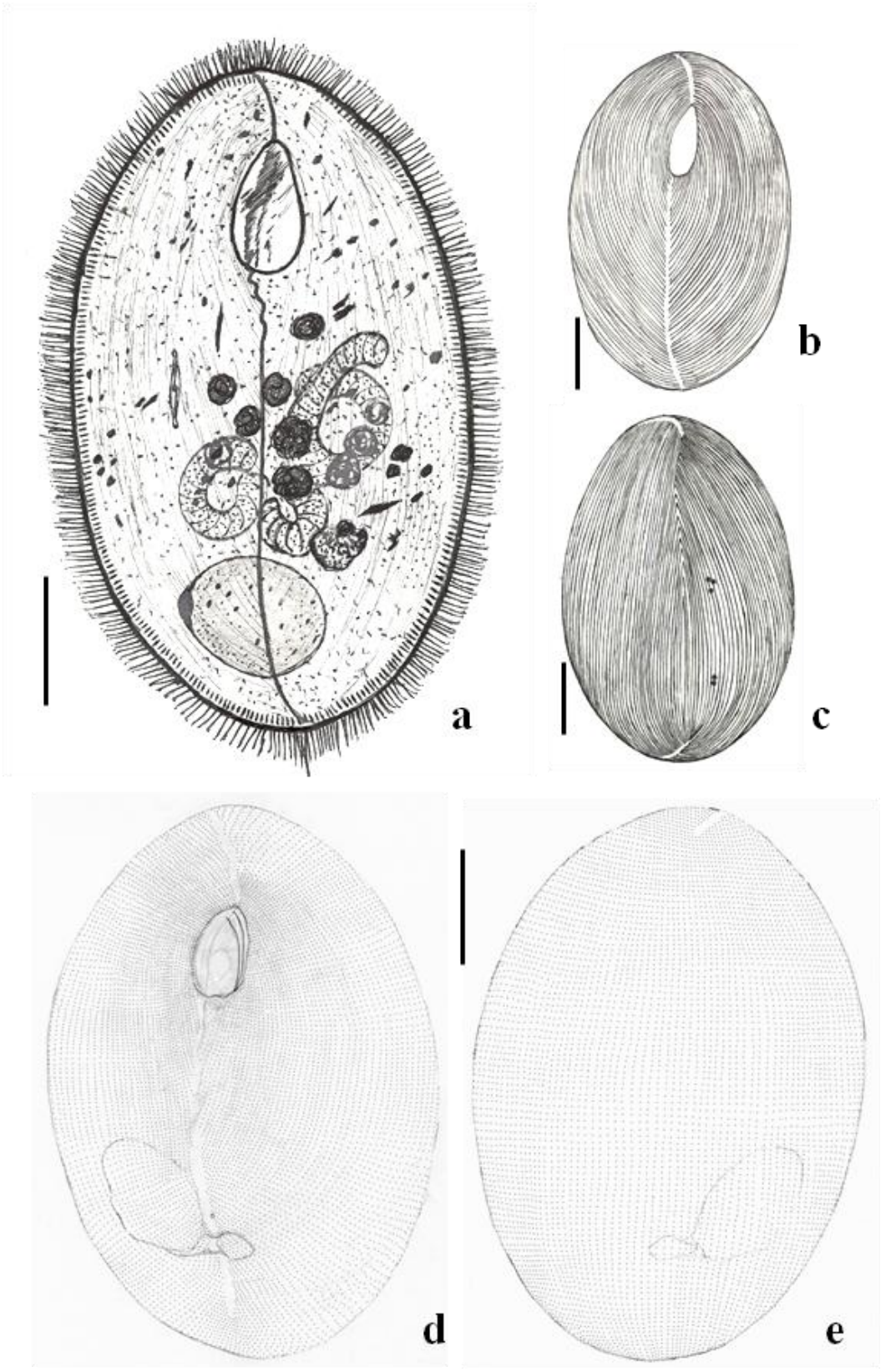

Figure 6.2: (a) Details of the morphology of *Frontonia alkalina* from life; **(b)** Ventral view of cell showing buccal cavity and ciliary rows. **(c)**Dorsal view showing extent of the pre-oral, post-oral sutures and position of excretory pores black dots; **(d)** Arrangement of the basal kinety on ventral; and **(e)** dorsal sides. Drawings from ciliates impregnated in protargol; Scale bars = 30μm

6.2.2 *Euplotes endoroisi* sp. cand. (Fig. 6.3 a – g; Table 6.3 and 6.4)

Diagnosis

Size about 58 x 39 µm *in vivo*. Cell outline ovoid with anterior end narrowly rounded with a distinct notch at the right side. Macronucleus curved- to inverted C-shape. 7 – 8 frontoventral cirri, 4 transverse, 2 marginal cirri and 1 caudal cirrus. Buccal field about two thirds of cell length with about 29 adoral membranelles. 8 –10 dorsolateral ciliary rows with about 14 dikinetids in mid–dorsal row. 5 – 6 conspicuous dorsal ridges.

Type location

Lake Bogoria

Type material

One holo- and five paratype slide with protargol – impregnated specimens have been deposited at the Biologie Zentrum Linz (LI), Austria.

Etymology/Dedication

The species is dedicated to the Endorois, a small indigenous minority community believed to be the original inhabitants of the area around Lake Bogoria.

Description

Size 52 – 63 x 33 – 44 µm *in vivo*, on average 58 x 39 µm, 26 – 43 x 17 – 30 µm, on average 32 x 22 µm in protargol – impregnated specimens (Table 3). Cell outline ovoid with both left and right margins convex (Fig. 3d). Anterior end narrowly rounded with a distinct notch on the collar at right side where the adoral zone of membranelles begins, whereas the posterior end is rounded. Cell flattened dorsoventrally with ventral side slightly convex and dorsal side strongly arched and bearing 5 – 6 prominent ridges (Fig. 3e, f). Three conspicuous ridges on ventral side extend posteriorly to the transverse cirri (Fig. 3a, c). Buccal field occupies 60 – 75% of the total cell length. Cytoplasm colourless often with a few food vacuoles and a large contractile vacuole located at the posterior ventralside. Macronucleus curved to slightly inverted C-shape. Paroral membrane small, typically composed of many irregularly arranged kinetosomes, located below the buccal lip (Fig. 3d). Adoral zone composed of 28 – 32 membranelles. 7 – 8 frontoventral cirri, 4 transverse cirri, 2 marginal cirri located on left side of the cell posterior to buccal field and one single caudal cirrus (Fig. a, d, g). 8 – 10 dorsal ciliary rows extending almost the entire length of cell except for the left most which includes about 5 dikinetids. Mid-dorsal ciliary row with about 14 dikinetids (Fig. 3b). Movement moderately fast by crawling or slightly jerking, with the two marginal cirri used for forward propulsion.

Euplotes endoroisi exhibits changes in the number and arrangement of the fronto – ventral and transverse cirri at 1/VI and 2/VI following the system of numbering by Wallengren (1900). These groups of cirri are not observed in the early streak phase of morphogenesis (Fig. 3g). Based on the similarity in the position of cirri in this species and *E. moebiusi*, but differences in their numbers and arrangement, this species may be a result of allopatric speciation of *E. moebiusi* that occur in neighbouring Lake Nakuru (Ong'ondo *et al.*, 2013) or a result of genetic drift, mutation or hybridization.

Occurence and Ecology

Euplotes endoroisi was found in densities of about 400 ind. l^{-1} in the uppermost 3 m of Lake Bogoria but not in deeper anoxic depths (above the sediment layer). Other ciliates, namely *Acineria incurvata, Condylostoma* sp., *Cyclidium glaucoma, Cinetochilum* sp., *Frontonia alkalina*, and *Rimaleptus* sp. were also present in the same samples.*Euplotes moebiusi* has also been reported from this lake before (Ong'ondo *et al*., 2013) but was absent in these samples.

Comparison with related species

Euplotes endoroisi should be compared with four other morphotypes possessing seven to nine frontoventral cirri and a variable number of marginal and/or caudal cirri, namely *Euplotes raikovi, Euplotes parkei, Euplotes orientalis* and *Euplotes affinis.* All of these small marine species have five transverse cirri while *E. endoroisi* invariably has four (Table 2).

Euplotes raikovi differs from *E. endoroisi* in size (40 – 56 x 41 – 49 vs. 22 x 32 µm), number of adoral membranelles (22 – 29 vs. 28 – 32),frontoventral cirri (7 vs. 7 – 8), transverse cirri (5 vs. 4), caudal cirri (2 vs. 1), dorsal kineties (6 – 7 vs. 8 – 10),and dikinetids in the mid-dorsal row (10 – 13 vs.12 – 16)(Agamaliev, 1965; Washburn and Borror, 1972; Jiang *et al.*, 2010b).

Euplotes parkei differs from *E.endoroisi* in size (about 40 x 30 µm vs. 52 – 63 x 33 – 44 µm), number of adoral membranelles (18 vs. 28 – 32),frontoventral cirri (8 vs. 7 – 8), transverse cirri (5 vs. 4), caudal cirri (2 vs. 1), dorsal kineties (8 vs. 8 – 10),and dikinetids in the mid-dorsal row (11 vs.12 – 16)(Curds, 1974).

Euplotes orientalis differs from *E. endoroisi* in size (35– 45 x 23 – 31 µm vs. 52 – 63 x 33 – 44 µm), number of adoral membranelles (18 – 25 vs. 28 – 32), frontoventral cirri (8 vs. 7 – 8), transverse cirri (5 vs. 4), dorsal kineties (6 – 7 vs. 8 – 10),and dikinetids in the mid-dorsal row (7 vs. 12 – 16)(Jiang *et al.*, 2010b).

Table 6.3: Morphometric data on *Euplotes endoroisi*. Data based on life specimen (L) and protargol impregnated specimen (P). Length and width measurements in μm. CV = coefficient of variation in %; Max = maximum; Mean = arithmetic mean; Min = minimum; n = number of cells measured; SD = standard deviation.

Characteristic		Mean	Median	SD	CV	Min	Max	n
Body, length	L	58.0	58.2	3.5	6.0	52.0	63.0	21
	P	32.0	30.6	4.1	12.8	26.0	43.0	38
Body, width	L	39.0	38.1	3.4	8.7	33.0	44.0	21
	P	22.0	21.3	3.4	15.5	17.0	30.0	38
Body length:width ratio	L	1.5	1.5	0.1	8.4	1.3	1.8	21
	P	1.7	1.7	0.2	13.4	1.3	2.2	42
Adoral zone, length	P	21.8	21.2	2.3	10.4	19.0	28.0	32
Adoral zone:Body length ratio (%)	P	69.0	69.0	4.3	6.3	60.0	75.0	32
Adoral membranelle, number	P	29.0	28	1.3	4,6	28	32	37
Fronto-ventral cirri, number	P	7.3	7	0.5	6.4	7	8	40
Transverse cirri, number	P	4	4	0	0	4	4	40
Marginal cirri, number	P	2	2	0	0	2	2	40
Caudal cirri, number	P	1	1	0	0	1	1	40
Dorso-ventral kinety rows, number	P	8.9	9	0.7	7.5	8	10	33
Dikinetids in mid-dorsal kinety, number	P	14.4	14	0.9	6.5	12	16	36
Leftmost kinety on ventral side, number	P	4.6	5	0.7	14.1	4	6	33

Table 6.4: Comparison of *Euplotes endoroisi* with some related congeners. AZM, adoral zone of membranelles; DK, dorsal kineties; FVC, frontoventral cirri; RC, reduced cirri.

Characteristics	*E.endoroisi*	*E. raikovi*	*E. parkei*	*E. orientalis*	*E. affinis*
Cell, length	33 - 44	40 - 56	41	35 - 45	38.4
Cell, width	17 - 30	41 - 49	30	23 - 31	25.8
Macronucleus, Shape	Inverted C - or curved bar	C - shaped		inverted U-shaped	
Adoral membranelles, number	28 - 32	22 - 29	18	18 - 25	18 - 20
AZM: Body length, %	~70	65	~67	65	~67
FVC, number	7 - 8	7	8	8	9
TC, number	4	5	5	5	5
MC + CC, number	2 + 1	1 + 2	4	2 + 2	3
Dorso-lateral kinety, rows	8 – 10	6 - 7	8	6 - 7	
Dikinetids in mid-dorsal kinety, number	12 -16	10 - 13	11	7	
Dorsal ridges , number	5 - 6	5	6	5 - 6	5
Ventral ridges, number	3	3		3	
Habitat	Freshwater	Marine	Marine	Marine	Freshwater
Source		Jiang *et al*., 2010b	Curds, 1974	Jiang *et al.*, 2010b	Curds, 1974

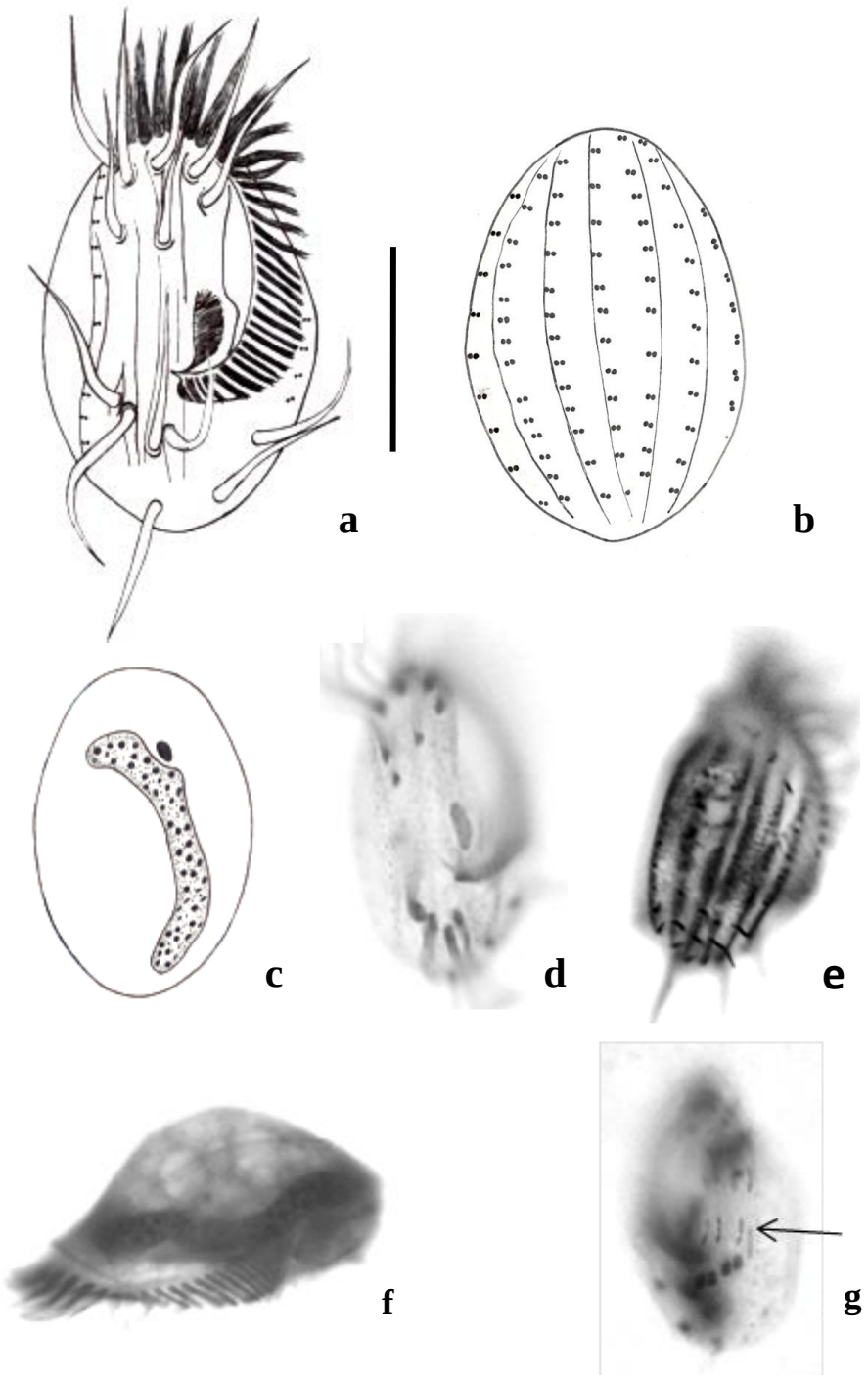

Figure 6.3: Detailed morphology of *Euplotes endoroisi* showing **(a)** the ventral; and **(b)** dorsal; **(c)** Sketch of the ventral view of cell showing shape and position of nuclei; **(d)** Specimen with 8 fronto-ventral cirri; **(e)** Dorsal view showing five prominent ridges; **(f)** Lateral view showing strongly arched dorsal of cell; **(g)** Early streak phase of morphogenesis with arrow showing developing transverse cirri. Protargol-impregnated impregnated specimen**;** Scale bar = 25µm.

6.2.3 *Condylostoma bogoriense* spec. cand. (Fig. 6.4 a – f; Table 6.5; and Table 6.6)

Diagnosis

Size about 285 × 95 µm in protargol impregnated specimen. Body elongate sac-shaped with buccal cavity about 40% of body length. Macronucleus moniliform with 15 – 26 nodules, on average 18 nodules. 54 – 69 somatic ciliary rows, and 115 – 155 adoral membranelles. Frontal membranelles single row of 6 – 8 cirri.

Type location

Alkaline – saline Lake Bogoria (00° 15'N, 36° 06'E).

Type material

One holo- and five paratype slide with protargol – impregnated specimens, respectively, have been deposited at the Biologiezentrum Linz, Austria.

Etymology

The species is named after the locality in which it was discovered.

Description

Body size is 224 – 400 by 68 – 123 µm with a mean of 283 x 95 µm after protargol impregnation. Cell elongate sac-shaped with posterior end gradually tapered and curved (Fig. 6.4a, b). Macronucleus aligned with right margin of the body, with distinct nodules connected by fine threads and composed of 15 – 26 with many small nucleoli (Fig. 6.4a, d). Each macronucleus nodule about 12 x 7 µm in protagol-impregnated specimen. Small (≤ 2 µm) and numerous micronuclei attached to/in close proximity to macronucleus nodules.

Somatic cilia arranged longitudinally in 54 – 69 ciliary rows with dikinetids paired basal bodies (Fig. 6.4f; Table 6.5). Ciliary rows usually commence near the buccal field and include several shortened rows on both ventral and dorsal sides i.e., not originating or ending at the anterior or posterior ends (Fig. 6.4e).

Oral apparatus conspicuous, occupy about 40% of body length, with proximal portion extending spirally into the cytopharynx. Paroral membrane located on the inner right side of buccal cavity, conspicuously long and smoothly undulated (Fig. 6.4c). Frontal cirri with six to eight basal elements arranged in a row at right apex of oral apparatus (Fig. 6.4c). Adoral zone originates from anterior right side of cell, conspicuously cover left side of buccal field and consisting of 115 – 155 membranelles (Table 6.5).

Occurrence and ecology

The feeding mode is generally omnivorous with little preference for prey species. In a grazing experiment, *Condylostoma bogoriense*, exhibited a more uniform ingestion rate, with a wide rangeof particles ingested, but a higher grazing rate on *A. fusiformis* (Burian *et al*., 2013).

Comparison with related species

Condylostoma bogoriense can be distinguished from *Condylostoma spatiosum* by having a smaller size (< 300 µm vs. > 400 µm, mean length); buccal field about 2/5 of body length (vs. 1/3); One frontal cirrus vs. two positioned in parallel; about 63 somatic kineties (vs. 58), with many kineties on both ventral and dorsal side strongly shortened or fragmented vs. 2 – 5 on ventral side in *C. spatiosum*. Consistently smaller macronucleus nodules (11.5 x 7 µm vs. 36 x 19µm) composedof 15 – 26 fragments (vs.13–22); and different habitat (saline lake vs. marine)(Shao *et al*., 2006).

Compared with *Condylostoma magnum*, *C. bogoriense* is smaller (< 300 µm vs. > 400 µm, mean length); buccal field about 2/5 of body length (vs. 1/3 – 1/4); One row of frontal cirrus with 6 – 8 brushes of cilia vs. two frontal cirriarranged in a row; about 115 – 155 membranelles in AZM vs. 150 – 200; about 63 somatic kineties (vs. 47 – 56), macronucleus nodules composedof 15 – 26 fragments vs.12 – 19 in *C. magnum*; and different habitat (saline lake vs. marine) (Song and Wilbert, 1997).

C. bogoriense can be distinguished from *Condylostoma curva* Burkovsky 1970 by having 115 – 155 membranelles in AZM vs. 68 – 108; about 63 somatic kineties with shortened kineties on both ventral and dorsal sides vs. 22 – 32, with all kineties on dorsalside extend complete length of cell; macronucleus nodules composedof 15 – 26 vs. 5 – 13 in *C. curva*; and different habitat (saline lake vs. marine)(Song *et al*., 2003).

Compared with *Condylostoma minutum*, *C. bogoriense* has 115 – 155 adoral membranelles vs. 67 – 103; about 63 somatic kineties (vs. 26 – 33), macronucleus nodules composedof 15 – 26 vs. 9 – 18 in *C. minutum*, different habitat (saline lake vs. marine) (Chen *et al*., 2007).

C. bogoriense differs from *C. granulosum* Bullington 1940 in number of macronucleus nodules 15 – 26 vs. 8 – 9 in *C. granulosum*; the number of somatic kineties (54 –69 vs. about 30); and different habitat (saline lake vs. marine).

C. bogoriense has about 63 somatic kineties (vs. 26 – 34); one row of frontal cirri with 6 – 8 brushes vs. 4 – 5 in *C.arenarium sensu* Kahl 1932 and the buccal cavity cover about 40% of body length vs. 20%.

Table 6.5: Morphometric data of *Condylostoma bogoriense*. Data based on protargol impregnated specimens. Length and width measurements in μm. CV = coefficient of variation in %; Max = maximum; Mean = arithmetic mean; Min = minimum; n = number of cells measured; SD = standard deviation

Characteristic	Mean	Median	SD	CV	Min	Max	n
Body, length	283.0	271.5	41.1	14.5	224.0	400.0	32
Body, width	95.0	91.6	16.5	17.4	68.0	123.0	32
Body length : width ratio	3.1:1	3:1	0.5	17.8	2.3	4.2	32
Buccal field, length	108.0	103.4	14.6	13.6	86.7	154.3	31
Buccal field: Body length (%)	38.0	38.6	4.3	11.5	28.6	44.2	30
Macronuclear nodule, number	19.0	18.5	3.0	15.9	15	26	32
Macronuclear nodule, length	11.0	11.9	2.9	25.4	4.4	16	32
Macronuclear nodule, width	7.0	7.5	1.8	26.2	2.2	9.2	32
Adoral membranelle, number	132	130	10.0	7.6	115	155	27
Frontal Cirri, number	1	1	0.0	0.0	1	1	25
Somatic kineties, number	63.0	63.0	4.8	7.6	54	69	29

Table 6.6: Comparison of *Condylostoma bogoriense* to the closest congeners.Measurements *in-vivo* except for *Condylostoma bogoriense*.

Characteristic	*C. bogoriense*	*C. spatiosum*	*C. magnum*	*C. curva*	*C. granulosum*	*C. minutum*
Body, length,	224 – 400	328 – 636	450 – 800	150 – 350	590 – 2140	200 – 400
Body, width	68 – 123	148 – 248		97 – 133		80
Buccal field, length	87 – 154	96 – 200		61		25 - 33%
Buccal field : body length, ratio	~2/5	~1/3 – 1/4	~1/3 – 1/4	~1/3 – 2/5	~1/3	
Macronuclear nodules, number	15 – 26	13 – 22	12 – 19	5 – 13	6 – 1 3	9 – 18
Adoral membranelle, number	115 – 155	113 – 153	~150 – 200	68 –108	123 – 210	67 – 103
Frontal Cirri, rows	1	2	2	4 – 8	1 – 3	1
Somatic Kineties, number	54 – 69	51 – 63	47 – 56	22 – 32	28 – 65	26 – 33
Habitat	Freshwater	Marine	Marine	Marine	Marine	Marine
Data source	original	Shao *et al.* 2006	Song and Wilbert, 1997	Song *et al.* 2003	Petz *et al.* 1995	Chen *et al.* 2007

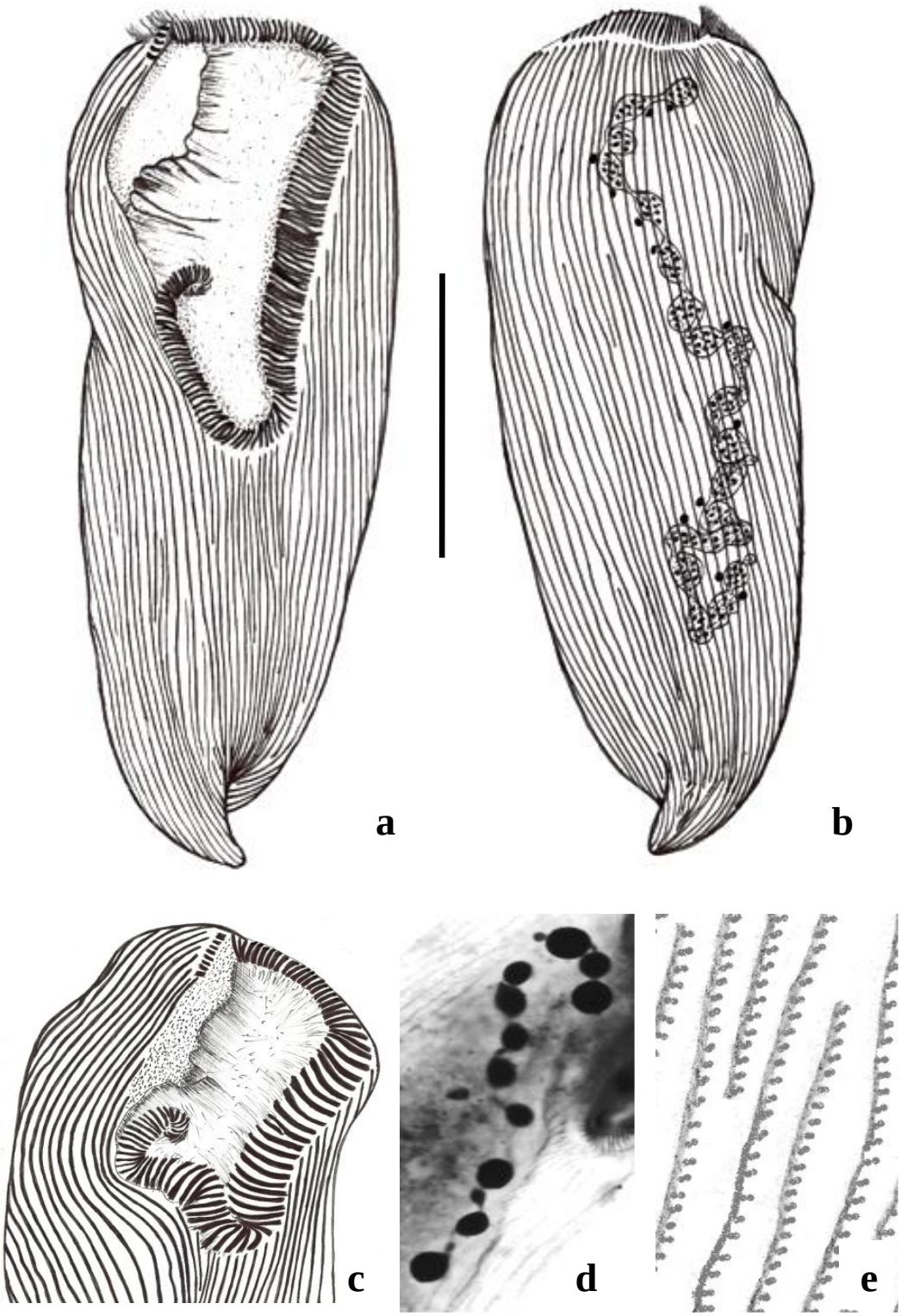

Figure 6. 4: Details morphology of *Condylostoma bogiriense* showing **(a)** ventral; and **(b)** dorsal views. **(c)** Anterior ventral view showing buccal cavity and frontal membrane; **(d)** Macronuclear nodules; **(e)** Ciliary rows showing dikinetids. Protargol-impregnated impregnated specimen:Scale bar = 100 µm.

SUMMARY

The molecular diversity, phylogeny and taxonomic composition of protist communities from five Rift Valley lakes in Kenya were investigated using classical microscopy and 454 pyrosequencing techniques. Overall, I obtained 96,555 amplicons (average length 250 bp) of which 33.8 % were metazoan and other non-target organisms. The remaining eukaryotic protist amplicons were assigned to operational taxonomic units (OTUs) defined by the best match for each amplicon using the Basic Local Alignment Search Tool (BLAST) of nucleotides from the National Center for Biotechnology Information (NCBI) database. Protistan taxonomic richness was high, and the 18SSU rRNA gene amplicons were assigned to nine high-rank taxa grouped into 746 representative OTUs at 97% similarity to the reference sequences. The most abundant sequences were affiliated to Alveolata (27.2 % of total sequences, mostly Ciliophora). Other abundant sequences were: Stramenopiles (14.2 %), Fungi (11.2 %), Chlorophyta (11.1 %), Cryptophyta (9 %) and 13.2 % unclassified environmental sequences (ES). Following rarefaction to 1000 reads per lake, the amplicons yielded between 13 – 148 OTUs per sample. The protistan diversity exhibited specific spatial distribution patterns in the lakes as a function of the environmental variables, notably salinity. In-depth analyses focussing on ciliates and chlorophytes revealed a diversity characterized by a high number of amplicons in the freshwater lakes. Additionally, I provide here a taxonomic descriptions of three ciliate species (*Frontonia alkalina* spec. candidatus, *Euplotes endoroisi* spec. candidatus and *Condylostoma bogoriense* spec. candidatus), from Lake Bogoria, based on morphological characters. This work gives a broad overview of the molecular diversity of protistan eukaryotic assemblages from the five lakes spanning a range from freshwater to mesosaline (0.3 – 34.1 ‰ salinity). It unveils the alkaline – saline Rift Valley lakes as an important reservoir of eukaryotic protists diversity and shows that our understanding of the community structure and molecular diversity of microbial eukaryotes in these tropical lakes is still limited.

ZUSAMMENFASSUNG

Die molekulare Diversität, Phylogenie und taxonomische Zusammensetzung von Protisten-Gesellschaften aus fünf Rift Valley Seen in Kenia wurden mit klassischer Mikroskopie und der 454 Pyrosequenzierungs-Technik untersucht. Insgesamt erhielt ich 96.555 Amplikons (Durchschnittslänge 250 bp), von denen 33,8% Metazoen und andere Nicht-Zielorganismen waren. Die restlichen eukaryotischen Amplikons wurden taxonomischen Einheiten (operational taxonomic units – OTUs) zugeordnet, die durch den besten Treffer für jedes Amplikon des Basic Local Alignment Search Toolfür Nukleotide (BLAST) in der National Center for Biotechnology Information Datenbank (NCBI) definiert wurden. Die taxonomische Vielfalt der Protisten war hoch und die 18s-rRNA-Amplikons wurden neun hochrangigen taxonomischen Gruppen zugeordnet. Die 746 eingruppierten OTUs hatten eine Übereinstimmung mit den Referenzsequenzen von 97%. Die meisten gefundenen Sequenzen wurden den Alveolata zugeordnet (27,2% der Gesamtsequenzen, zumeist Ciliophora). Weitere taxonomische Gruppen, denen viele Sequenzen zugeordnet wurden, waren: Stramenopiles (14,2%), Fungi (11,2%), Chlorophyta (11,1%) und Cryptophyta (9%). Auch nicht weiter klassifizierte Sequenzen (unclassified environmetal Sequentes – ES) machten mit 13,2% einen größen Teil der Gesamtsequenzen aus. Nach der Analyse über rarefaction ergab sich bei einem Level von je 1000 Einzelsequenzen eine Zuordnung zu 13 bis 148 OTUs pro Probe. Die Diverstität der Protisten zeigte spezifische räumliche Verteilungsmuster in den Seen, die mit vorliegenden Umweltvariablen, insbesondere dem Salzgehalt, korrelierten. Vertiefende Analysen mit dem Fokus auf Ciliaten und Chlorophyta zeigten eine Vielfalt, die durch eine hohe Anzahl von Amplikons in den Süßwasserseen gekennzeichnet war. Des Weiteren führe ich eine taxonomische Beschreibungen der drei Ciliatenspezies (*Frontonia alkalina* sp. cand., *Euplotes endoroisi* sp. cand. und *Condylostoma bogoriense* sp. cand.) aus dem Lake Bogoria basierend auf morphologischen Merkmalen durch. Die vorliegende Arbeit gibt einen breiten Überblick über die molekulare Vielfalt der Protisten-Gesellschaften der fünf untersuchten Seen, die den Bereich von Süßwasser bis mesosalin umfassen (0,3 - 34,1 ‰ Salzgehalt). Sie zeigt die Rift Valley Seen als wichtiges Reservoir eukaryotisch-protistischer Vielfalt und macht deutlich, dass unser Verständnis der Community-Struktur und molekularen Diversität mikrobieller Eukaryoten in diesen tropischen Seen noch begrenzt ist.

CONCLUSION

This study contributed to our understanding of the community structure and molecular diversity of microbial eukaryotes in tropical lakes. The application of next generation sequencing (NGS) techniques such as 454 sequencing based on the 18s RNA demonstrated that the identification of protists using the molecular methods provide more taxonomic depth compared to the classical morphospecies concept with the molecular phylotypes indicating a higher diversity and intra-specific variation of the microbial eukaryotes. Additionally, the taxonomy of ciliates in Lakes Bogoria illustrated that there was possibilities of description of more ciliate species from the alkaline-saline Rift Valley lakes even though these species had close relations to marine congeners. Overall, the combined use of classical morphological methods and molecular techniques provided better results that could not have been achieved by one or the other method only.

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