Vol. 62: 177–190, 2011
doi: 10.3354/ame01465

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online January 19

Molecular characterisation of the small-eukaryote community in a tropical Great Lake (Lake Tanganyika, East Africa)

Anne-Laure Tarbe[1,*], Stephane Stenuite[1], Vanessa Balagué[2], Danny Sinyinza[3], Jean-Pierre Descy[1], Ramon Massana[2]

[1]Laboratory of Freshwater Ecology, URBO, Department of Biology, University of Namur, rue de Bruxelles 61, 5000 Namur, Belgium

[2]Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CSIC, Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

[3]Department of Fisheries (DOF), Ministry of Agriculture, Food and Fisheries, Mpulungu, Zambia

ABSTRACT: In aquatic environments, small eukaryotes (mainly algae and protozoa of 1 to 5 µm in size) are a key link in the carbon transfer to higher trophic levels, e.g. through primary production and grazing of picoplankton. However, the diversity of these microorganisms remains poorly investigated in freshwater habitats, and is still unknown in tropical aquatic systems. In this study, we investigated the small-eukaryote diversity in the oligotrophic Lake Tanganyika, one of the African Great Lakes, at different depths in the water column using denaturing gradient gel electrophoresis (DGGE) and gene clone libraries based on 18S rRNA genes. Each sample produced complex DGGE fingerprints clearly discriminating the epilimnion from the metalimnion. Analysis, using genetic libraries, confirmed the high level of small-eukaryote diversity in Lake Tanganyika. Organisms from 5 taxonomic groups (Stramenopiles, Alveolata, Cryptophyta, Kinetoplastea and Choanoflagellida) were dominant among the species detected. Some sequences were nearly identical to those recovered in temperate freshwaters in North America and Europe, suggesting a high dispersal ability in some small-eukaryote lineages. However, 49% of sequences were <95% similar to any sequence in GenBank. This may result from undersampling of freshwater systems, but also raises the possibility that perennially warm tropical waters harbour particular assemblages of planktonic small eukaryotes.

KEY WORDS: Small-eukaryote community · Tropical lake · 18S rRNA gene libraries

INTRODUCTION

Microbial eukaryotes are fundamental components of aquatic ecosystems, both as primary producers and as the main link between picoplanktonic carbon and the higher trophic levels through their grazing activities. They also play a crucial role in nutrient cycling (e.g. Arndt et al. 2000, Laybourn-Parry & Parry 2000). Because it is difficult to identify these microorganisms by their morphological features (e.g. Arndt et al. 2000), scientists used to classify them, in broad functional categories, as 'autotrophic' or 'heterotrophic'. The use of culture-independent molecular techniques based on small-subunit (SSU) rDNA allowed clear taxonomic classification and led to an increasing interest in characterising small-eukaryote diversity, distribution, and community assemblages in aquatic environments. Some 18S rRNA gene surveys reported assemblages that were putatively more complex than had been expected, with many potentially mixotrophic or parasitic small eukaryotes (e.g. Chen et al. 2008, Lefèvre et al. 2008). From the first published studies using those molecular methods (e.g. Díez et al. 2001a, Moon-van der Staay et al. 2001) to the present day, numerous

*Email: anne-laure.tarbe@fundp.ac.be.

undescribed lineages have been regularly uncovered. Thus, it appears that microbial eukaryote diversity remains underestimated. Actually, whereas a wide range of marine ecosystems have been investigated (e.g. Díez et al. 2001a, López-García et al. 2001, Edgcomb et al. 2002, Massana et al. 2004, Lovejoy et al. 2006), the small-eukaryote community in freshwater habitats remains poorly studied (e.g. Amaral Zettler et al. 2002, Lefranc et al. 2005, Richards et al. 2005, Lefèvre et al. 2007, Chen et al. 2008), despite the common divergence between freshwater and marine lineages within taxa (e.g. von der Heyden et al. 2004, Logares et al. 2007). In particular, small-eukaryote assemblages in tropical lakes have received no attention. These lakes differ from the temperate ones mainly by the so-called 'endless summer' (Kilham & Kilham 1990) that allows permanently high biological activity, with no winter period.

In many respects, Lake Tanganyika (LT), the oldest of the East African Great Lakes, is a good choice for addressing the small-eukaryote diversity in a tropical system. This oligotrophic lake presents relatively stable environmental conditions, and its assemblages of species, which evolved ~12 million years ago, have inspired many studies, addressing mostly fish and invertebrate biodiversity and endemicity (e.g. Salzburger et al. 2002, Marijnissen et al. 2006), and paleoclimatic reconstruction (e.g. Cohen et al. 2006, Stager et al. 2009). Like many oligotrophic environments (e.g. Li et al. 1992, Zubkov et al. 2000, Grob et al. 2007), LT also harbours a plankton community highly dominated by picoplankton — both autotrophic and heterotrophic (Descy et al. 2005, Stenuite et al. 2007, 2009). Flow cytometry counts allowed detection of picoeukaryote populations of around 2×10^3 cells ml^{-1} (Stenuite et al. 2009). In addition, analysis by microscopy showed that the main picoplankton grazers in LT were heterotrophic flagellates in the size class 2–5 µm, which contributed up to 76% of the total heterotrophic flagellate abundance that ranged between 0.30×10^3 and 1.83×10^3 cells ml^{-1} (Pirlot et al. 2005). Moreover, recent studies demonstrated that microbial grazing is of primary importance as a major factor controlling the productivity of LT (Pirlot et al. 2007). It is now clear that the small-eukaryote community plays a fundamental role in the pelagic food web of that tropical lake. An understanding of the ecological functioning of LT requires intensive characterisation of its small-eukaryote community. This question is all the more important for the lake since Stenuite et al. (2007) provided evidence of a decline in its primary production in recent decades, which could be linked to global warming (Verburg et al. 2003) and could eventually lead to substantial changes within the microbial communities. The ability to face global change could depend chiefly on the complexity of the food web in LT and on the existence within its communities of organisms capable of trophic shifts during environmental stress (e.g. McCann 2000).

We explored the diversity of small eukaryotes (<5 µm) in LT by analysing 18S rRNA gene sequences. Samples were collected from different regions and depth layers within the mixolimnion of the meromictic LT. A first screening of the overall small-eukaryote genetic diversity in the lake was carried out by the analysis and comparison of DGGE fingerprints. Two 18S rRNA gene clone libraries were then generated from well-discriminated depth layers (epilimnion and metalimnion) to unveil the small-eukaryote assemblages in seemingly different habitats that might harbour most of the diversity. Our survey raised fundamental ecological questions, such as the putative selection of particular small-eukaryote communities in tropical environments, and the relationship between the age and stability of a system and the complexity level of its microbial phylogenetic diversity.

MATERIALS AND METHODS

Study site. Located in East Africa, LT (Fig. 1) is a large oligotrophic and meromictic lake, with 2 deep basins (maximum depth 1470 m), that never mixes vertically beyond the oxycline (around 100–150 m depth). As in other tropical lakes, seasonality, in the form of a rainy and a dry season, is one of the main sources of variability in the ecology of the lake. The depth of the mixed layer varies with season and location. During the dry season (May–September), when southeast winds are blowing, a deep vertical mixing occurs in the oxic layer, and an upwelling takes place at the southern end (Coulter 1991, Plisnier et al. 1999). Then, vertical transport brings up nutrient-rich waters from below the thermocline (Coulter 1991). Conversely, during the rainy season (October–April), stratification of the mixolimnion occurs, resulting in low-nutrient conditions.

Physicochemical measurements and sample collection. Samples were collected at the southern part of the lake (off Mpulungu, Zambia; 08° 43.98' S, 31° 02.43' E), during 2 cruises in the rainy season of 2007 (on February 28 and March 9), when stratification occurred. During the first cruise, a sampling profile was performed in the pelagic zone by collecting water in a 5 l Niskin bottle, at 10 m intervals, in the upper 100 m of the water column, which represents most of the oxygenated layer. During the second cruise, sampling was repeated in the pelagic zone, in the epilimnion (pool of equivalent volumes of water from 0, 10, 20 and 30 m depth), and in the metalimnion (pool of

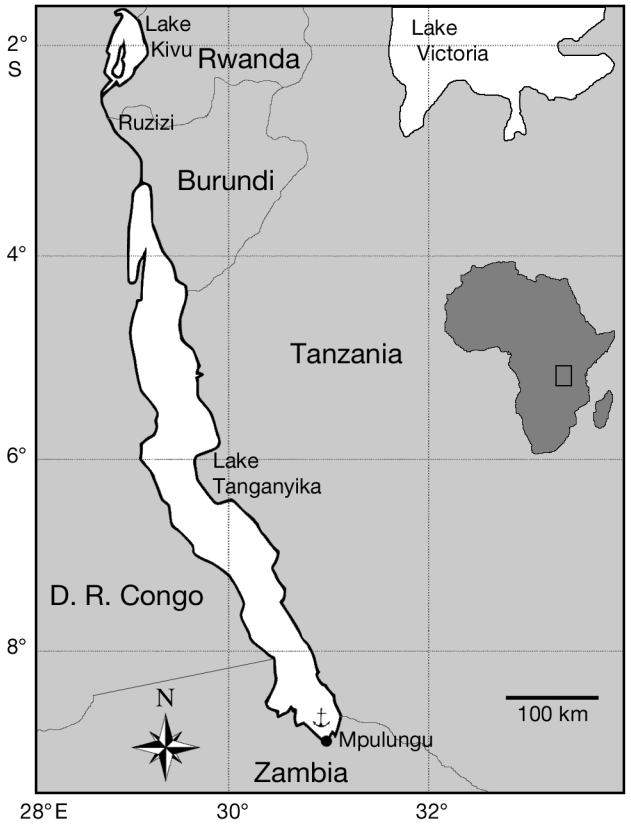

Fig. 1. Lake Tanganyika, with the location of the sampling station (Mpulungu, Zambia)

equivalent volumes of water from 40 and 50 m depth). A coastal sample (1 m below the surface, near the lake shore) was also collected. We performed limnological profiles (temperature, dissolved oxygen, pH and conductivity) with a multiparameter probe (Hydrolab DS4a), transparency and photosynthetically active radiation (PAR) downward attenuation measurements using Li-Cor LI1400 quantum sensors, and nutrient analysis using standard spectrophotometric techniques (American Public Health Association 1992). Chlorophyll *a* (chl *a*) concentrations were measured by high-performance liquid chromatography (HPLC) following the protocol of Descy et al. (2005). Samples for DNA extraction were pre-filtered through a net of mesh size 100 µm and collected in polycarbonate bottles. For the profile, 2 l of sample were successively filtered through polycarbonate filters (diameter 47 mm, Millipore) with pore sizes 10 µm, 5 µm and 0.6 µm. For pooled samples, 2 l of sample were first passed through a filter of pore size 10 µm and then collected on a polycarbonate filter (diameter 47 mm) of pore size 0.2 µm in order to get the size fraction between 10 µm and 0.2 µm. The size fraction between 5 µm and 0.2 µm was obtained by first filtering 2 l of sample through a filter of pore size 5 µm and then collecting on a polycarbonate filter of pore size 0.2 µm. Each filter was then placed in a cryovial containing 1.7 ml of lysis buffer (40 mM EDTA, 40 mM NaCl, 50 mM Tris-HCl pH 8.3, 0.75 M sucrose), frozen for a maximum of 2 wk at –20°C, and then stored at –80°C until the extraction of nucleic acid.

Nucleic acid extraction. Filters were first incubated with lysozyme (1 mg ml^{-1}, final conc.) at 37°C for 45 min and then digested with proteinase K (0.2 mg ml^{-1} final conc.) and sodium dodecyl sulphate (SDS, 1% final conc.) at 55°C for 1 h. Lysates were purified twice by extraction with an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1), and the residual phenol was removed by extraction with an equal volume of chloroform–isoamyl alcohol (24:1). Nucleic acid extracts were further purified, desalted and concentrated with Centricon-100 concentrator (Millipore) to a volume of 100 to 200 µl. The integrity of extracted DNA was checked by electrophoresis on a 0.8% agarose gel. The amount of total DNA was quantified spectrofluorometrically using Quant-it dsDNA assay kit (Invitrogen). Nucleic acid extracts were stored at –80°C until further analysis.

Denaturing gradient gel electrophoresis (DGGE). For DGGE, eukaryotic SSU rDNA-specific primers were used: Euk1F (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and Euk516r-GC (5′-ACC AGA CTT GCC CTC C-3′) with a 40 bp GC-clamp (Díez et al. 2001b); this protocol amplified a fragment of approximately 560 bp. Mixtures for PCR (50 µl) contained forward and reverse primers (each at 0.5 µM), each of the deoxynucleoside triphosphates at 200 µM, 0.15 µg ml^{-1} BSA, 1.25 U of *Taq* DNA polymerase (Promega), the PCR buffer supplied with the enzyme, and 10 ng of extracted DNA as template. The PCR protocol for DGGE started with a denaturation step at 94°C for 130 s, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s and extension at 72°C for 130 s. During the last cycle, the extension step lasted 12 min. An aliquot of each PCR product was electrophoresed in a 1% agarose gel stained with SybrSafe (Invitrogen) to check amplification, and quantified by comparison to a standard (Low DNA Mass Ladder, Invitrogen). DGGE was carried out with a DGGE-2000 system (CBS Scientific) as described by Muyzer et al. (1997). About 800 ng of each PCR product were loaded into individual lanes in a 6% polyacrylamide gel (acrylamide:bisacrylamide, 37.5:1), 0.75 mm thick, featuring a linear 45 to 65% gradient of DNA denaturing agent (100% was defined as 7 M urea and 40% deionized formamide). Electrophoresis was performed at 100 V and 60°C for 16 h in 1× TAE buffer (40 mM Tris base, 20 mM sodium acetate, 1 mM EDTA). Gels were stained for 45 min in 1× TAE buffer with SybrGold nucleic acid stain (Invitrogen), and visualized with UV

radiation using a Fluor-S MultiImager and the Multi-Analyst imaging software (Bio-Rad). The presence and intensity of DGGE bands was estimated using Diversity Database software (Bio-Rad), as previously described (Schauer et al. 2000). A distance matrix was then calculated using Euclidian distances, followed by a cluster construction with Statistica software, applying UPGMA (Unweighted Pair-group Average) amalgamation algorithm.

Cloning and sequencing of 18S rRNA genes. For clone libraries, PCRs were performed using eukaryote-specific primers Euk1F and EukR (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′), which amplified almost the entire SSU rRNA gene. PCR mixtures were prepared as explained in 'Materials and methods: DGGE' except that the total reaction volume was 100 µl and BSA was avoided. The PCR protocol consisted of an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 60 s, and extension at 72°C for 3 min. The last extension step was extended to 10 min. Two replicate PCR tubes were run for each sample to ensure an appropriate amount of product for cloning. An aliquot of each PCR product was electrophoresed in a 1% agarose gel and stained with SybrSafe as amplification control.

Amplicon replicates of the same sample were pooled and purified by electrophoresis on a 1.2% low-melt TAE agarose gel. Bands of interest were excised and melted at 65°C for subsequent ligation into the prepared vector (pCR 2.1) supplied with a TOPO-TA cloning kit (Invitrogen), according to the manufacturer's recommendations. After transformation of the cloning reaction into chemically competent One Shot® cells and incubation on Luria-Bertani (LB) agar plates containing 50 µg ml^{-1} of kanamycin, putative positive colonies were picked, grown in a multi-well plate containing LB medium, kanamycin and 7% glycerol, and stored at −80°C. The presence of the 18S rRNA gene insert was verified by PCR amplification of each colony, using the same primers and PCR procedure as described in 'Materials and methods: DGGE'. PCR products from positive clones were sent to Macrogen (Korea) for purification and sequencing with primer Euk528F (5′-GCG GTA ATT CCA GCT CCA A-3′), which resulted in sequences of about 800 bp. Sequences obtained were submitted to an NCBI BLAST analysis (Altschul et al. 1997) in order to compare them with the GenBank database for an approximate assessment of their phylogenetic affiliation. The closest match to each environmental partial 18S rRNA gene sequence was selected for tree construction. Suspected chimera artefacts were checked and effective chimeric sequences were discarded. Clones showing highly similar sequences (>99% similarity) were grouped with DOTUR software (Schloss & Handelsman 2005) and considered to belong to the same operational taxonomic unit (OTU). One representative of each OTU was sent back for sequencing with primers EukR, 336F (5′-CCG GAG AGG GAG CCT GA-3′), 516R (5′-ACC AGA CTT GCC CTC C-3′), and 1209F (5′-CAG GTC TGT GAT GCC C-3′) to obtain a complete 18S rRNA gene sequence. Nucleotide sequences obtained in the present study have been deposited in GenBank under accession numbers GU290066 to GU290116.

Phylogenetic analysis. Complete 18S rRNA gene sequences were aligned with representative sequences from different phylogenetic groups using MAFFT (Katoh et al. 2002) and highly variable regions were removed with GBlocks software (Castresana 2000). The resulting alignment was then submitted to JModelTest (Posada, 2008) for choosing the best model of nucleotide substitution. The GTR+i+γ model appeared to be the most appropriate model for our data set and was then used for maximum likelihood distance analysis (PhyML; Guindon & Gascuel 2003).

RESULTS AND DISCUSSION

Physicochemical parameters

The present study was conducted in the southern part of LT (Fig. 1) during the rainy season, when stratification of the oxygenated layer occurs (Fig. 2). Based on temperature, 3 different layers were clearly identified in the mixolimnion: epilimnion (from 0 to 30 m depth), metalimnion (30 to 60 m) and hypolimnion (~60 to 100 m). With a euphotic layer deeper than the epilimnion, the organisms in the upper layer benefited from a high exposure to light, and oxygen concentrations peaked between 15 and 25 m as a consequence of the high photosynthetic activity (Fig. 2). Inorganic nitrogen (N) and phosphorus (P) were higher in the metalimnion (Table 1), due to bacterial recycling of the sedimenting organic matter. The monimolimnion, not sampled here, corresponds to the whole water column <150 m which is permanently anoxic and harbours high concentrations of hydrogen sulphide (Edmond et al. 1993).

Analysis of DGGE fingerprints

We first performed a DGGE gel to compare the small-eukaryote assemblages from the different depths in a vertical profile (Fig. 3A). Each sample produced a complex fingerprint composed of a large number of bands. Some bands were unique to epilimnetic or metalimnetic samples. The cluster analysis clearly

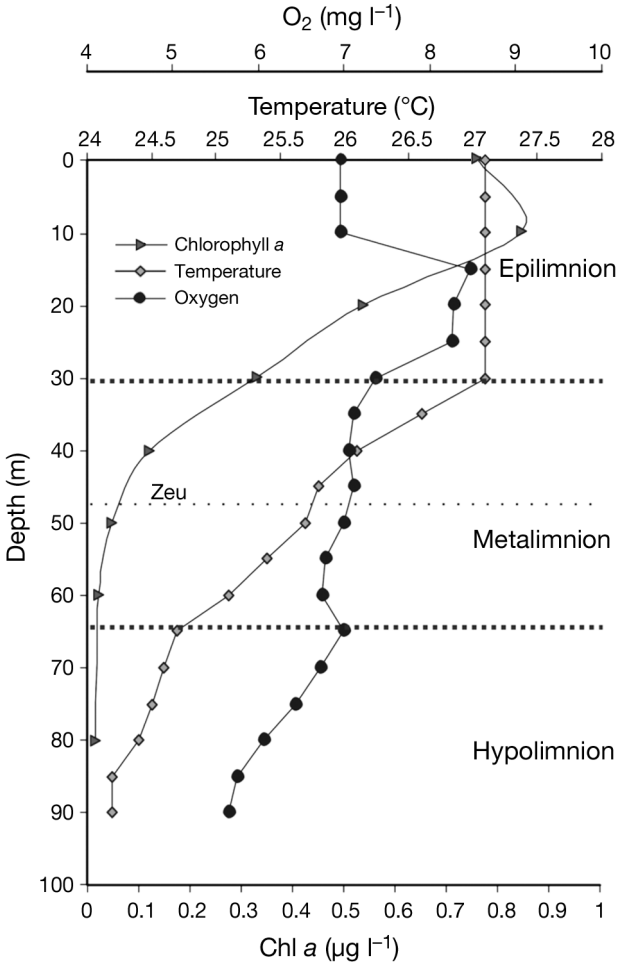

Fig. 2. Vertical profiles of temperature, chlorophyll *a* and dissolved oxygen in the pelagic sampling area (February 28, 2007). The depth of the euphotic layer (Zeu) is shown. See Table 1 for the physicochemical features of pooled water samples

Table 1. Physicochemical features of the pooled water samples used to generate clone libraries of eukaryotic 18S rRNA genes (TRK07E and TRK07M)

Feature	TKR07E–Epilimnion	TKR07M–Metalimnion
Depth (m)	0 to 30	40 & 50
Soluble reactive P (µM)	0.69	24.68
Dissolved inorganic N (µM)	0.98	2.88
Dissolved Si (µM)	36.88	35.15

discriminated between samples from the epilimnetic layer and deeper samples, presumably as a result of different light and resource conditions and their influence on planktonic communities. The diversity within the size class 0.2–5 µm was compared to a broader size fraction (0.2–10 µm) with a second DGGE, for 3 pooled water samples (epilimnion, metalimnion and coastal samples) (Fig. 3B). As expected (Pirlot et al. 2005), most of the diversity was retrieved in the 0.2–5 µm fraction, except for some unique bands displayed in the 0.2–10 µm fraction. Once sequenced, most of these bands corresponded to sequences affiliated to either ciliates or copepods (data not shown). Regarding the sampled region, obvious differences appeared between epilimnion and metalimnion, as previously observed, whereas coastal and epilimnion samples were more similar, revealing analogous assemblages in the upper waters of LT in both coastal and pelagic areas. Since many studies have produced evidence of the unexpected diversity of small eukaryotes (especially flagellates) within the 0.2–5 µm size fraction in various environments (e.g. Lefranc et al. 2005, López-García et al. 2001, Massana et al. 2002, Massana et al. 2004, Richards et al. 2005), and because of differences in the small-eukaryote composition between epilimnion and metalimnion, we constructed genetic clone libraries from the 0.2–5 µm fraction of both layers.

Small-eukaryote diversity in Lake Tanganyika

A total of 91and 100 18S rRNA gene clones were obtained from the epilimnion (TKR07E) and the metalimnion (TKR07M), respectively, partially sequenced (~800 bp) and submitted to a BLAST search. After the removal of putative chimeric sequences and metazoan sequences, 88 and 93 18S rRNA gene clones remained and were considered further in this analysis. To correct for putative sequencing errors and intragenomic or intraspecific variability, partial sequences exceeding 99% of similarity were grouped into the same OTU using the DOTUR software. This resulted in 51 OTUs. Rarefaction curves, calculated from the total number of effective clones, did not reach saturation, suggesting an undersampling of small-eukaryote diversity in these samples (data not shown). The metalimnion displayed about 30% more OTUs than the epilimnion (34 vs. 23 OTUs, respectively) for a similar number of clones analysed. This observation should be considered with caution, as the metalimnion is known as a slow sedimentation layer which could harbour free or detritic DNA from dead sinking organisms. The 2 sampled zones shared only 12% of OTUs, which confirmed the very different assemblages between the 2 water layers as seen before in the DGGE gels, together with the severe undersampling of our libraries. Analysis of more clones may have revealed further diversity, especially within the less abundant taxa, and an increased overlapping between the 2 layers sampled. Nevertheless, the very different light regime, oxygen and nutrient concentrations, and chlorophyll *a* biomass between

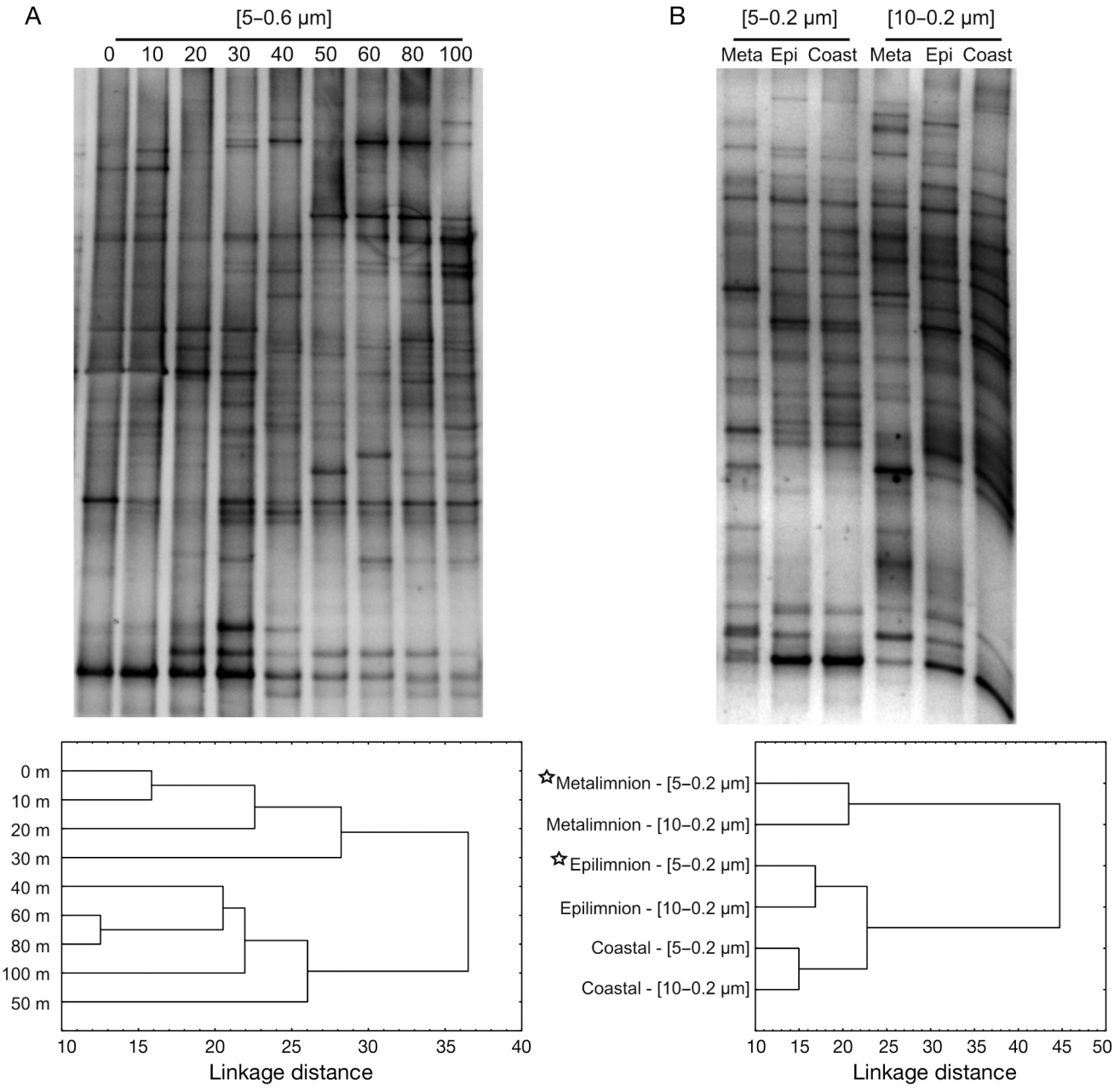

Fig. 3. Denaturing gradient gel electrophoresis (DGGE) gels and cluster analysis of small-eukaryote communities based on DGGE bands. Similarities are expressed by Euclidian distances. The clustering was performed using UPGMA amalgamation algorithm. (A) DGGE of a vertical profile; (B) DGGE on the different pooled samples. ☆: samples analysed using genetic libraries

the 2 water layers could well explain their peculiar OTUs. In terms of small-eukaryote abundance, counts by microscopy performed during the same sampling campaign had already shown differences in the contribution of pigmented cells within the 2 habitats, with 14.0% of the total small-eukaryote abundance in the epilimnion (total = 1.85×10^3 cells ml^{-1}) vs. 9.5% in the metalimnion (total = 1.22×10^3 cells ml^{-1}) (Tarbe 2010). A clone representative of each OTU was completely sequenced and phylogenetic trees were constructed for the main groups (Table 2). Sequences not included in the phylogenetic analysis are presented in Table 3. Some of these sequences could not even be affiliated to a given supergroup, corroborating observations made during the last decade in marine environments (see Massana & Pedrós-Alió 2008), where new diversity was detected at almost all phylogenetic scales, from species to supergroups (e.g. Picobiliphytes; Not et al. 2007). Five taxonomic groups represented 82.4% of the detected diversity in the 2 pooled samples: Stra-

Table 2. Phylogenetic affiliations of the representative clones of each operational taxonomic unit (OTU) used for tree construction

Taxon	OTU	Closest uncultured relative (% similarity)	Closest cultured relative (% similarity)
Chrysophyceae	TKR07M.80	AY919815–LG46-06 (96.4)	*Paraphysomonas butcheri* (97.3)
	TKR07M.65	AY919815–LG46-06 (96.2)	*Paraphysomonas butcheri* (97.0)
	TKR07M.100	AY919815–LG46-06 (98.9)	*Mallomonas annulata* (95.4)
	TKR07E.13	AY651077–JBAF33 (99.6)	*Uroglena* sp. (96.0)
	TKR07E.62	AY919824–LG73-06 (99.4)	*Ochromonas* sp. (98.8)
	TKR07E.55	AY082987–RT5iin35 (94.8)	*Poterioochromonas* sp. (97.3)
	TKR07M.117	AY919824–LG73-06 (99.8)	*Ochromonas* sp. (98.7)
	TKR07M.17	AY082987–RT5iin35 (95.3)	*Poterioochromonas* sp. (96.8)
	TKR07M.13	AY919811–LG43-07 (98.7)	*Chrysamoeba mikrokonta* (92.1)
	TKR07M.92	EF024391–Elev_18S_1047 (93.5)	*Mallomonas akrokomos* (94.5)
	TKR07M.14	AB275091–CYSGM-8 (96.3)	*Oikomonas* sp. (94.9)
	TKR07M.39	AB275091–CYSGM-8 (97.0)	*Oikomonas* sp. (95.9)
	TKR07M.70	EF023425–Amb_18S_766 (92.9)	*Ochromonas* sp. (92.7)
Bicosoecida	TKR07M.62	AF363207–ME1-24 (87.4)	*Pseudobodo tremulans* (87.9)
	TKR07M.93	EU162647–PSH9SP2005 (90.5)	*Bicosoeca petiolata* (96.4)
	TKR07E.25	EU162646–PSA11SP2005 (91.8)	*Adriamonas peritocrescens* (94.2)
	TKR07M.28	EU162647–PSH9SP2005 (90.5)	*Bicosoeca petiolata* (98.7)
	TKR07M.83	EU162647–PSH9SP2005 (90.9)	*Bicosoeca petiolata* (98.1)
Cryptophyta	TKR07E.51	DQ244012–PFF1AU2004 (95.9)	*Cryptomonas marssonii* (89.2)
	TKR07E.87	DQ244012–PFF1AU2004 (98.1)	*Goniomonas* sp. (88.3)
	TKR07E.37	DQ244012–PFF1AU2004 (98.5)	*Goniomonas* sp. (88.6)
	TKR07E.42	AY919764–LG23-04 (95.6)	*Cryptomonas marssonii* (95.7)
	TKR07M.116	EF527142–SIF_1D6 (97.7)	*Cryptophyta* sp. (98.5)
Dinophyceae	TKR07M.54	AM050344–*Pfiesteria*-like dinoflagellate (94.9)	*Pfiesteria*-like sp. (94.8)
	TKR07M.139	AM050344–*Pfiesteria*-like dinoflagellate (95.3)	*Pfiesteria*-like sp. (95.3)
	TKR07E.28	DQ103869–M3_18H10 (93.2)	*Gymnodinium beii* (95.2)
	TKR07E.24	EF527111–SIF_1B11 (89.5)	*Dinophyceae* sp. (89.6)
Ciliophora	TKR07M.125	DQ244028–PAA2AU2004 (93.7)	*Strobilidium caudatum* (93.0)
	TKR07E.90	DQ244028–PAA2AU2004 (95.7)	*Strobilidium caudatum* (94.7)
	TKR07E.48	AY919679–LG01-11 (98.5)	*Strombidium* sp. (97.3)
	TKR07E.8	DQ244028–PAA2AU2004 (93.2)	*Varistrombidium* sp. *Kielum* (92.8)
	TKR07M.137	DQ244022–PAB6AU2004 (99.4)	*Colpoda* sp. (99.7)
Cercozoa	TKR07M.24	AY642696–P1.23 (90.8)	*Cercomonas plasmodialis* (89.6)
	TKR07E.83	EF024169–Elev_18S_508 (91.9)	*Dimorpha*-like sp. (89.9)
Amoebozoa	TKR07M.132	EF526824 – NIF_3H1 (84.7)	*Echinamoeba exundans* (99.0)
Kinetoplastea	TKR07M.1	AY753949–L12.4 (90.5)	*Ichthyobodo necator* (84.7)
	TKR07E.70	AY753949–L12.4 (90.5)	*Ichthyobodo necator* (84.5)
	TKR07M.27	AY753949–L12.4 (89.9)	*Ichthyobodo* sp. (85.4)
	TKR07M.36	AY753948–L4.9 (93.6)	*Ichthyobodo necator* (88.9)
	TKR07M.129	AY753971–L5.5 (88.3)	*Bodo saltans* (88.8)
Chytridiomycota	TKR07M.19	DQ244013–PFD11AU2004 (94.2)	*Rhizophydium* sp. (94.3)
Choanoflagellida	TKR07M.45	EF024012–Amb_18S_1490 (91.1)	*Monosiga ovata* (91.1)
	TKR07E.73	EF024012–Amb_18S_1490 (89.8)	*Desmarella moniliformis* (89.0)
	TKR07E.14	EF024015–Amb_18S_1493 (90.0)	*Salpingoeca amphoridium* (90.0)
	TKR07E.2	AB275066–DSGM-66 (92.6)	*Salpingoeca pyxidium* (90.8)

Table 3. Best GenBank matches to Lake Tanganyika 18S rRNA gene sequences not included in the phylogenetic analysis

Taxon	OTU representative	No. clones in libraries TKR07E	TKR07M	Closest relative (% similarity)
Cryptophyta	TKR07E.71	2		AJ421146–*Teleaulax amphioxeia* nucleomorph (96.6)
Unclassified fungi	TKR07M.87		1	DQ536471–*Endogone lactiflua* (78.3)
Unclassified eukaryotes	TKR07M.79		1	EF024491–Elev_18S_790 (83.4)
	TKR07E.41	2		EF024299–Elev_18S_700 (79.9)
	TKR07M.122		1	FJ157338–kor_250804_27 (79.8)
	TKR07M.106		1	EF172851–SSRPB60 (79.8)

menopiles (35.3%), Choanoflagellida (7.8%), Cryptophyta (11.8%), Alveolata (17.6%) and Kinetoplastea (9.8%). Chlorophyceae, a genetic group composed of phototrophic forms, were absent in LT libraries, suggesting that the Chlorophyceae signal recovered in this lake from pigment studies (Descy et al. 2005) was mainly from larger cells or colonies (Cocquyt & Vyverman 2005, Descy et al. 2010). In addition, contrary to some similar studies conducted in temperate lakes (Lefèvre et al. 2007, 2008, Lepère et al. 2008), fungi were very scarce in LT, with only 2 sequences detected. Even though ciliates are generally larger than 5 µm (Pirlot et al. 2005), and are therefore not included in the small-eukaryote definition, they were kept in the analysis as a comparison to similar studies. The BLAST search of the representative sequence of each OTU revealed a loose distribution of LT sequences with their close relatives in GenBank (Fig. 4). Thus, 25.5% of OTUs had a sequence similarity with GenBank entries of between 98 and 100%, whereas 21.6% of OTUs were between 88 and 92% (49% with <95% similarity). Interestingly, 5 OTUs were below 84% similarity and could not even be assigned to a given eukaryotic supergroup. Regarding the specific phylogenetic groups, very new sequences, most of them with less than 90% similarity, often affiliated to Kinetoplastea and Choanoflagellida, known as undersampled groups. Other retrieved groups, such as Cryptophyta, Chrysophyceae and Bicosoecida, consisted of both highly similar and more distant taxa. Considering the origin of the best matches in GenBank from LT sequences (Figs. 5 & 6), LT small-eukaryote assemblages appeared as a good example of the well-known barrier between microbial communities in freshwater and marine environments (e.g. Logares et al. 2007, von der Heyden et al. 2004). On the other hand, even if environmental clones from temperate freshwater systems appeared first, the average similarity was still rather low, which is indicative of an obvious undersampling of freshwater systems. All freshwater high matches, derived from either the oligotrophic Lake George (USA) or the oligomesotrophic Lake Pavin (France), supported the concept of an environmental selection of taxa based on the trophic status (e.g. Dolan 2005, Lefranc et al. 2005, Lepère et al. 2006). Finally, the closest cultured match from LT sequences was rather distant, except for some Bicosoecida and Dinoflagellata sequences, indicating that the small-eukaryote diversity of LT is far from being represented in culture collections.

As a first indicator of small-eukaryote functional diversity in LT, we extrapolated as far as possible the trophic roles of the retrieved small eukaryotes, assuming that these phylotypes might display trophic behaviours similar to their closest cultured match (Fig. 7; Lefèvre et al. 2008).

Firstly, the ratio between colourless OTUs and pigmented OTUs in this oligotrophic lake (around 2.3, data not shown) would be between what was found in oligomesotrophic and eutrophic freshwater systems according to Lefranc et al. (2005). In LT, pigmented cells would consist of Chrysophyceae (9 OTUs), Cryptophyta (3 OTUs) and Dinoflagellata (2 OTUs), a different assemblage from those observed by these authors in both oligotrophic and oligomesotrophic systems. These authors recovered Chlorophyta representatives,

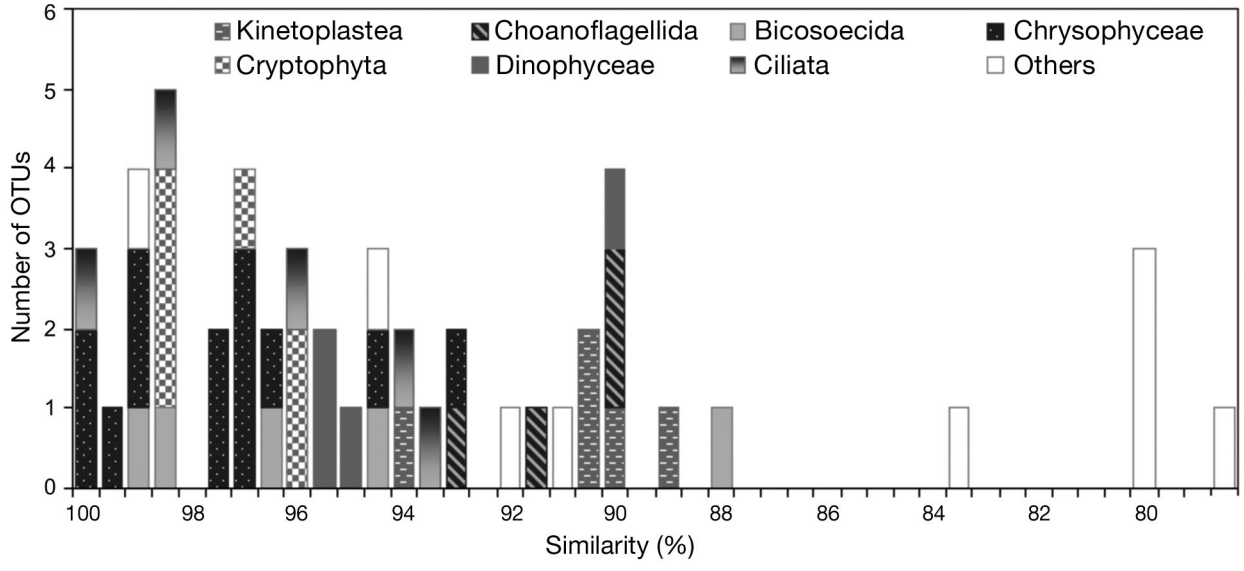

Fig. 4. Overview of the similarity of 1 representative sequence per operational taxonomical unit (OTU) from this study against GenBank sequences, binned by 0.5% similarity intervals (*x*-axis). The *y*-axis shows the number of OTU sequences in each bin

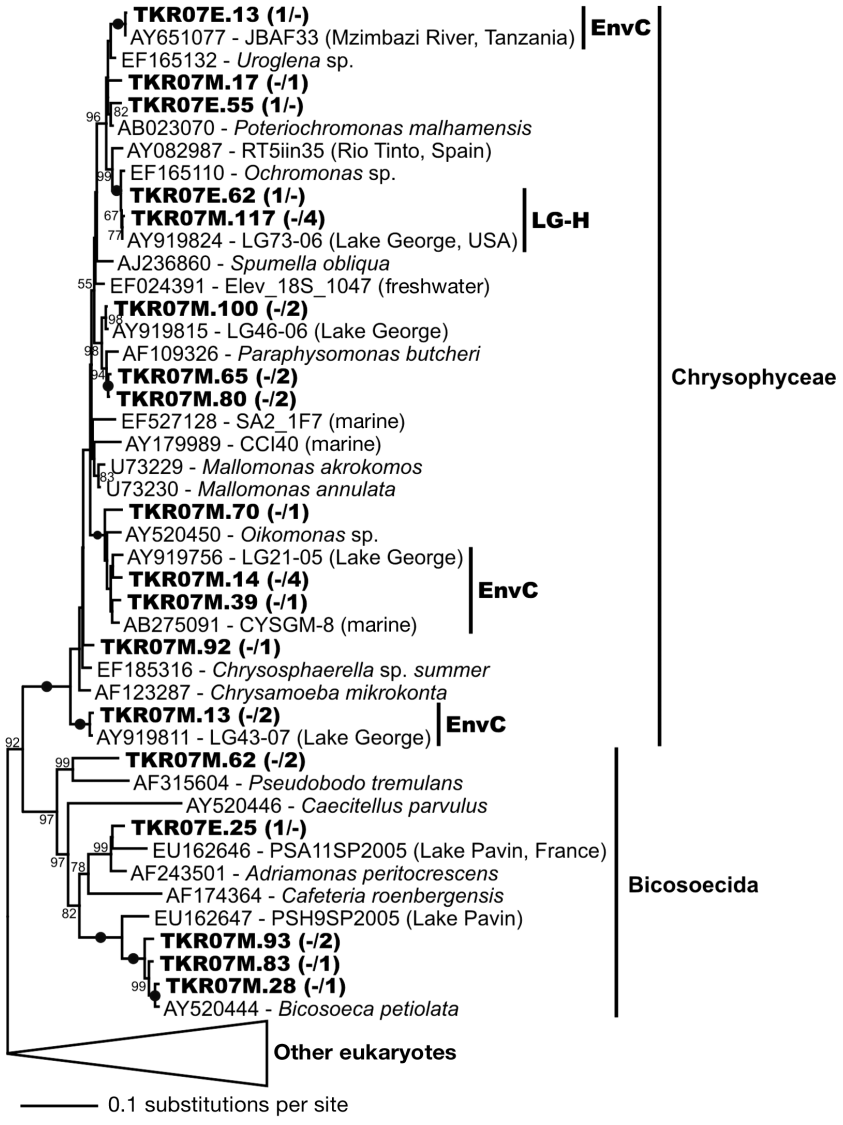

Fig. 5. Diversity of small eukaryotes of the Stramenopiles sampled from Lake Tanganyika (LT). The 18S rRNA gene evolutionary distance tree under maximum likelihood criteria was inferred from an alignment of 114 taxa. The tree was constructed by using a GTR+i+γ DNA substitution model determined by JModelTest (proportion of invariable sites, i = 0.195; variable-site gamma distribution shape parameter, γ = 0.500), and based on 1505 positions. Bootstrap percentages from 100 replicates are given at nodes when support exceeds 50%. A black dot on a branch indicates 100% support value. Environmental representative sequences from LT are in **bold**. The occurrence of each LT operational taxonomic unit (OTU) is marked with figures in brackets, representing the number of corresponding clones recovered in epilimnion/metalimnion. The best BLAST environmental freshwater, environmental marine and culture matches to LT OTUs were included in the analysis. EnvC: new environmental clades containing LT OTUs; LG-H: environmental clade previously described by Richards et al. (2005)

whereas no pigmented Chrysophyceae were detected in the oligotrophic Lake Godivelle, and they detected Haptophyceae phylotypes, but no Dinoflagellata in the oligomesotrophic Lake Pavin. Beyond the undersampling of freshwater systems that still keep us from asserting solid conclusions, such relatively diverging results allow us to consider tropical systems as different from temperate ones.

Secondly, potential parasitism and saprotrophy would represent some 41% of the LT retrieved clones that grouped with known taxa (13% of the affiliated OTUs), sustaining observations made in French freshwater lakes (e.g. Lefèvre et al. 2008, Mangot et al. 2009). These would correspond mainly to the phytotypes affiliated to Prokinetoplastida. Bacterivorous organisms included, colourless organisms would thus reach 76% of the retrieved and affiliated clones in LT (74% when ciliates were discarded). This is quite in agreement with observations by epifluorescence microscopy in which pigmented flagellates represented less than 15% of total small-eukaryote abundance (Tarbe 2010). Obviously, these observations must be considered with extreme caution, keeping in mind that affiliation of LT phylotypes to known small-eukaryote species was often quite low.

Phylogenetic tree topologies

Stramenopiles

Among the 18 OTUs that grouped with Stramenopiles (Fig. 5), 5 affiliated to the Bicosoecida. According to morphological observations, this heterotrophic biflagellate group is considered to be abundant in freshwater habitats (Arndt et al. 2000), and this is confirmed by recent 18S rRNA gene studies (e.g. Richards et al. 2005, Lefèvre et al. 2008). Most of the LT Bicosoecida sequences affiliated to *Bicosoeca petiolata*, a loricate flagellate seemingly globally dispersed in freshwater environments. The other 13 Stramenopiles OTUs were all related to Chrysophyceae and generally closely related to sequences recovered in Lake George, suggesting a widespread distribution of these taxa in oligotrophic freshwater environments. For instance, TKR07E.62 and TKR07M.117 were almost identical to LG73-06, the representative of a novel Chrysophyceae clade (LG-H) unveiled by Richards et

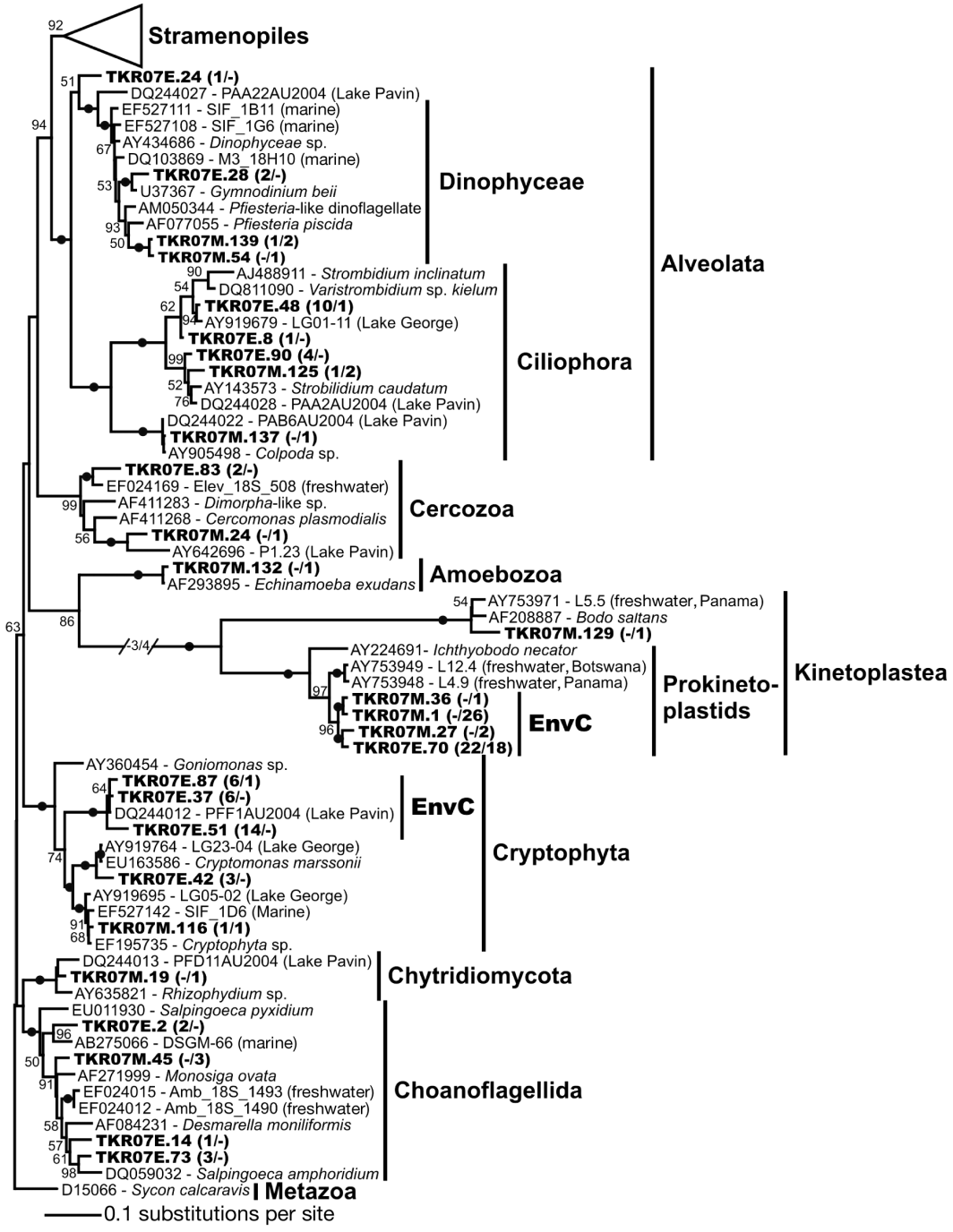

Fig. 6. Diversity of small eukaryotes, other than Stramenopiles, sampled from Lake Tanganyika. Proportional length reduction of Kinetoplastea branch is labelled. See legend to Fig. 5. for details

al. (2005). However, in our study, all these sequences appeared to be closely related to *Ochromonas* sp., which rules out the hypothesis of a really new clade. Other LT OTUs formed new clusters with Lake George sequences that were previously alone in GenBank. Thus, TKR07M.100 grouped with LG46-06 in a new phylogenetic cluster; TKR07M.14 and TKR07M.39 clustered with LG21-05 and a marine clone in another new cluster. According to the BLAST search (Table 2), the majority of the LT Chrysophyceae OTUs were related to heterotrophic taxa (*Oikomonas* sp., *Paraphysomonas butcheri*) or mixotrophic taxa (*Ochromonas* sp., *Proteriochromonas* sp.). The phylogenetic tree confirms the affiliation of these OTUs to either

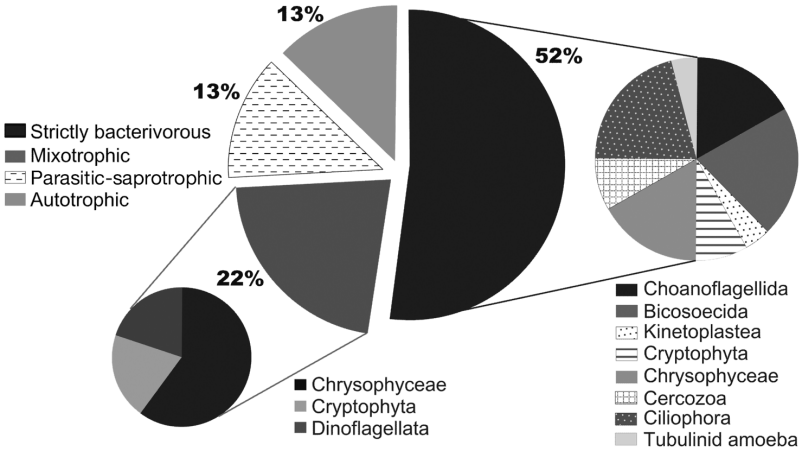

Fig. 7. Relative frequencies of OTUs (n = 46, unclassified eukaryotes and cryptophyte nucleomorphs not included) retrieved from the 0.2–5 µm fraction of the epilimnion and the metalimnion in the south basin (Mpulungu) of Lake Tanganyika during the rainy season of 2007, and putative affiliation to broad functional groups

heterotrophic or mixotrophic strains. Even if the reported affiliations of LT sequences to known species were generally quite low, these observations seem reliable, considering that trophic features of Chrysophyceae are often similar within the same genus. Only 3 OTUs (TKR07M.13, TKR07M.92 and TKRM.100) were closer to autotrophic taxa (*Chrysamoeba mikrokonta* and *Mallomonas* spp.) according to the BLAST search. For these latter OTUs, affiliation with autotrophic taxa is not so clear in the tree. With regard to their spatial distribution, most Stramenopiles OTUs were recovered in the metalimnion. This is not surprising, as the metalimnion features a high bacterial abundance (Stenuite et al. 2009) and a particular light regime, which can favour mixotrophy as an alternative to phototrophy.

Other eukaryote groups

Four LT OTUs grouped with Choanoflagellida (Fig. 6), the heterotrophic flagellates closest to metazoans. Three of the LT OTUs were better related to taxa reported as freshwater species (Carr et al. 2008), while TKR07E.2 grouped with a marine environmental clone. Although they are reported to be ubiquitously distributed in aquatic environments, no Choanoflagellida were encountered in the eutrophic Lake Aydat, while Choanoflagellida OTUs were retrieved in oligotrophic (Lake Godivelle) or oligomesotrophic (Lake Pavin) systems of the same area (Lefranc et al. 2005), suggesting a putative limitation of their dispersal in nutrient-rich environments.

Six LT OTUs clustered with Cryptophyta. One of them, associated to a nucleomorph, was not included in the tree (Table 3). From the remaining ones, 2 were related to autotrophic taxa (*Cryptophyta* sp., *Cryptomonas marssonii*), while the other 3 were closer, but distantly related, to *Goniomonas* sp., reported as a heterotrophic bacterivorous flagellate genus. These latter OTUs (TKR07E.51, TKR07E.87 and TKR07E.37) seemed to form a new clade with 1 Lake Pavin clone. The low proportion of cryptophyte clones in LT is in agreement with pigment analysis (Descy et al. 2005) which revealed a weak contribution of pigmented cryptophytes to autotrophic plankton biomass in this lake. The large majority of Cryptophyta OTUs were retrieved from the epilimnion.

From the 9 OTUs affiliated to Alveolata, 3 grouped with Dinophyceae and 5 with Ciliophora, whereas the precise affiliation of TKR07E.24 was unclear. LT Dinophyceae OTUs were more closely related to cultured strains: TKR07E.28, recovered only in the epilimnion library, affiliated to the autotrophic *Gymnodinium beii*, while TKR07M.54 and TKR07M.139 clustered with the genus *Pfiesteria*, in which individuals can display various morphologies and trophic modes within the same species. These latter OTUs were thus considered as mixotrophic in Fig. 7. Within the Ciliata, the taxa affiliated to LT OTUs (*Strobilidium* sp., *Strombidium* sp. and *Colpoda* sp.) corroborated the microscope-based observations (data not shown). Some of the LT ciliates were highly related (>99% similarity) to environmental clones from Lake Pavin (France) or Lake George (USA), showing that these lineages supported both temperate and tropical conditions and were thus widely distributed.

Four of the 5 Kinetoplastea OTUs in LT (TKR07M.1, TKR07E.70, TKR07M.27 and TKR07M.36), with less than 91% similarity with GenBank entries, seemed to form a novel clade within the Prokinetoplastids. They were closer, but still very distantly related, to the genus *Ichthyobodo*, known as a fish parasite. The last OTU grouped with Neobodonids. As evidence of the undersampling of that group, all LT Kinetoplastea OTUs were poorly related to sequences listed in GenBank, even when these corresponded to environmental clones sampled in systems geographically close to LT (L12.4, Botswana). This is not surprising given the putatively high evolution rate of Kinetoplastea (e.g. von der Heyden & Cavalier-Smith 2005). The very low affiliation percentage of Kinetoplastea OTUs, combined with the high evolution rate in this group, could be indicative of some small-eukaryote endemicity in LT.

Small-eukaryote assemblages in perennially warm waters

While temperate lakes are governed mainly by physical constraints, forcing their communities to adapt to changing and sometimes hostile environments, the 'endless summer' environmental conditions, peculiar to tropical great lakes, are less limiting for communities, and allow a biological control of nutrient conditions and elemental cycles (Kilham & Kilham 1990). Freshwater systems exerting different ecological pressure on their biota should thus harbour particular microbial communities. That issue could be confirmed only by sampling tropical systems.

Our study produced a first molecular characterisation of the small-eukaryote assemblage in a tropical great lake, whose functioning greatly relies on the microbial food web (Pirlot et al. 2007). Through the construction of 18S rRNA gene clone libraries, we attempted to address fundamental ecological questions, such as (1) the role of the environment in the composition of the community, and (2) the relationship between the age of an ecosystem and the functional diversity of its microbial assemblages. Such issues are of great importance to reach reliable conclusions on the genetic and functional diversity of microbial communities and their potential role in their changing environment.

Five taxonomic groups dominated the retrieved diversity in the oligotrophic LT: Stramenopiles (35% of the detected OTUs), Alveolata (18%), Cryptophyta (12%), Kinetoplastea (10%) and Choanoflagellida (8%). As in other freshwater environments (e.g. Lefranc et al. 2005), no LT sequences grouped with novel Alveolata or Stramenopiles lineages. The *Telonemia* phylum, recovered in Lake Pavin (Lefèvre et al. 2008), was absent in LT (K. Schalchian-Tabrizi pers. comm.). Most of the LT sequences were poorly related to taxa identified in temperate freshwater systems, including lakes with a similar trophic level, and generally grouped in specific clades, which can be indicative of the severe undersampling of tropical systems. This was very clear for Kinetoplastea and Choanoflagellida, known to be particularly undersampled. However, low similarity also occurred for some sequences in better-sampled groups, such as Chrysophyceae, Bicosoecida and Dinophyceae. In our survey, all of these latter groups contained sequences whose closest relative was below 93% similarity (96% for Cryptophyta). From a biogeographical point of view, such a divergence between the communities of LT and temperate lakes could be indicative of some selection of taxa by tropical conditions. A sampling of other tropical systems should corroborate this hypothesis. Conversely, some LT OTUs shared 99% or even more similarity with Lake George or Lake Pavin sequences (Table 2), supporting the high rate of microbial dispersal and the relative ubiquity of some lineages. Apart from physical conditions, trophic status might be another important factor for microbial communities (Lefranc et al. 2005).When we compared the gross assemblage found in LT with small-eukaryote (0.2–5 µm) communities from temperate freshwater systems, similarities and differences appeared at various levels. For example, in the dimictic oligotrophic Lake George (USA; Richards et al. 2005), 3 phyla highly dominated the detected diversity: Stramenopiles, Cryptophyta and Alveolates. In the oligotrophic Lake Godivelle (France; Lefranc et al. 2005), Alveolata, Stramenopiles and Cryptophyta also dominated, together with Fungi. The small-eukaryote diversity in the shallow Lake Taihu, China, consisted mainly of Stramenopiles, Alveolata, Cercozoa and Cryptophyta in the oligotrophic sampling sites; whereas Chlorophyta were represented more in the nutrient-rich areas (Chen et al. 2008). Finally, in the oligomesotrophic Lake Pavin (France; Lefranc et al. 2005, Lefèvre et al. 2007, 2008), Alveolata, Cryptophyta, Stramenopiles, Fungi and Cercozoa were the main taxonomic groups recovered. Overall, Chrysophyceae were always well represented in the freshwater libraries, confirming their valuable role in freshwater environments (Arndt et al. 2000). Worth mentioning is the difficulty in drawing conclusions on microbial biogeography, as data on other tropical lakes are missing and there have been only few studies on small-eukaryote diversity in temperate lakes covering a whole year cycle.

Surprisingly, in LT the Kinetoplastea (39% clones) represented an important fraction of the diversity. Most of the Kinetoplastea phylotypes were distantly affiliated to a fish parasite. If their parasitism were to be established, and their abundance within the community confirmed, this would bring new insight into the functioning of the microbial food web. Parasitism has already been described as a potentially important niche in temperate lakes through the abundance of Fungi (e.g. Lefèvre et al. 2008, Lepère et al. 2008). In LT, Kinetoplastea, if parasitic, could play a functional role similar to that of Fungi. Additional surveys on different tropical systems are needed to test whether Kinetoplastea are substantial constituents of the small-eukaryote communities in these environments. Choanoflagellida also contributed a substantial part (5% clones) of the small-eukaryote diversity. The absence of the Choanoflagellida in most of the other freshwater surveys was related mainly to their larger size (e.g. Richards et al. 2005), which could have excluded them from the analysis. In LT, analysis by microscopy showed that Choanoflagellida were small enough to pass through filters of pore size 5 µm. Con-

versely, some of the life stages of LT small eukaryotes could have been removed by size fractionation. This could be the case for fungi which develop hyphae and sporangia. Small-eukaryote diversity in LT could thus be even greater than we found in our study.

According to Fig. 7, alternative trophic modes could represent a large contribution to the microbial food web in LT. Yet, even if oligotrophic, this lake is one of the world's most productive freshwater systems (Coulter 1991). It is expected that this lake, 12 million years old, evolved high trophic efficiency by developing a complex and diverse food web, with high microbial taxonomic and functional diversity. The fundamental role of the microbial food web in sustaining LT trophic efficiency has already been suggested in recent studies (Sarvala et al. 2003, Pirlot et al. 2007). Mixotrophy and parasitism could be additional strategies to exploit all sources of available carbon in this very old, oligotrophic environment, and their potential importance in that system suggests a high level of complexity within the microbial food web, optimizing the exploitation of resources.

Of course, these figures must be considered with caution, as extracellular DNA, or DNA from dead organisms, can be included in the library. Further analysis of diversity based on rRNA would be more indicative of the functional diversity of LT (e.g. Not et al. 2009). Nevertheless, the SSU rDNA approach brings us significant information on the diversity that can be found in a system.

Considering the high small-eukaryote diversity detected in LT compared with that in temperate regions, it seems obvious that tropical systems should be more systematically focused upon in future studies in order to reach an estimate of global small-eukaryote diversity.

Acknowledgements. The authors are indebted to Dr. Harris Phiri, from the Department of Fisheries (DOF), Mpulungu, Zambia. Thanks are also due to all the technical staff of the DOF for their precious help in the field. This study was supported by the FNRS (National Fund for Scientific Research) through the PhD scholarship to A.L.T. and to project GEMMA (CTM2007-63753-C02-01/MAR, MEC) to R.M.

LITERATURE CITED

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Amaral Zettler LA, Gomez F, Zettler E, Keenan BG, Amils R, Sogin ML (2002) Microbiology: eukaryotic diversity in Spain's River of Fire. Nature 417:137

American Public Health Association (APHA) (1992) Standard methods for the examination of water and wastewater. A.P.H.A., New York, NY

Arndt H, Dietrich D, Auer B, Cleven EJ, Gräfenhan T, Weitere M, Mylnikov AP (2000) Functional diversity of heterotrophic flagellates in aquatic ecosystems. In: Leadbeater BSC, Green JC (eds) The flagellates: unity, diversity and evolution. Taylor & Francis, London, p 240–268

Carr M, Leadbeater BSC, Hassan R, Nelson M, Baldauf SL (2008) Molecular phylogeny of choanoflagellates, the sister group to Metazoa. Proc Natl Acad Sci USA 105: 16641–16646

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17:540–552

Chen M, Chen F, Yu Y, Ji J, Kong F (2008) Genetic diversity of eukaryotic microorganisms in Lake Taihu, a large shallow subtropical lake in China. Microb Ecol 56:572–583

Cocquyt C, Vyverman W (2005) Phytoplankton in Lake Tanganyika: a comparison of community composition and biomass off Kigoma with previous studies 27 years ago. J Gt Lakes Res 31:535–546

Cohen AS, Lezzar KE, Cole J, Dettman D and others (2006) Late Holocene linkages between decade–century scale climate variability and productivity at Lake Tanganyika, Africa. J Paleolimnol 36:189–209

Coulter GW (1991) Lake Tanganyika and its life. Oxford University Press, London

Descy JP, Hardy MA, Stenuite S, Pirlot S and others (2005) Phytoplankton pigments and community composition in Lake Tanganyika. Freshw Biol 50:668–684

Descy JP, Tarbe AL, Stenuite S, Pirlot S and others (2010) Drivers of phytoplankton diversity in Lake Tanganyika. Hydrobiologia 653:29–44

Díez B, Pedrós-Alió C, Massana R (2001a) Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. Appl Environ Microbiol 67:2932–2941

Díez B, Pedrós-Alió C, Marsh TL, Massana R (2001b) Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Appl Environ Microbiol 67:2942–2951

Dolan JR (2005) Biogeography of aquatic microbes. Aquat Microb Ecol 41:39–48

Edgcomb VP, Kysela DT, Teske A, Gomez AV, Sogin ML (2002) Benthic eukaryotic diversity in the Guaymas Basin hydrothermal vent environment. Proc Natl Acad Sci USA 99:7658–7662

Edmond JM, Stallard RF, Craig H, Craig V, Weiss RF, Coulter GW (1993) Nutrient chemistry of the water column of Lake Tanganyika. Limnol Oceanogr 38:725–738

Grob C, Ulloa O, Claustre H, Huot Y, Alarcón G, Marie D (2007) Contribution of picoplankton to the total particulate organic carbon concentration in the eastern South Pacific. Biogeosciences 4:837–852

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Katoh K, Misawa K, Kuma K, Myata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30: 3059–3066

Kilham P, Kilham SS (1990) Endless summer: internal loading processes dominate nutrient cycling in tropical lakes. Freshw Biol 23:379–389

Laybourn-Parry JEM, Parry JD (2000) Flagellates and the microbial loop. In: Leadbeater BSC, Green JC (eds) The flagellates: unity, diversity and evolution. Taylor & Francis, London, p 216–239

Lefèvre E, Bardot C, Noël C, Carrias JF, Viscogliosi E, Amblard C, Sime-Ngando T (2007) Unveiling fungal

zooflagellates as members of freshwater picoeukaryotes: evidence from a molecular diversity study in a deep meromictic lake. Environ Microbiol 9:61–71

Lefèvre E, Roussel B, Amblard C, Sime-Ngando T (2008) The molecular diversity of freshwater picoeukaryotes reveals high occurrence of putative parasitoids in the plankton. PLoS ONE 3:e2324

Lefranc M, Thénot A, Lepère C, Debroas D (2005) Genetic diversity of small eukaryotes in lakes differing by their trophic status. Appl Environ Microbiol 71:5935–5942

Lepère C, Boucher D, Jardillier L, Domaizon I, Debroas D (2006) Succession and regulation factors of small-eukaryote community composition in a lacustrine ecosystem (Lake Pavin). Appl Environ Microbiol 72:2971–2981

Lepère C, Domaizon I, Debroas D (2008) Unexpected importance of potential parasites in the composition of the freshwater small-eukaryote community. Appl Environ Microbiol 74:2940–2949

Li WKW, Dickie PM, Irwin BD, Wood AM (1992) Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. Deep-Sea Res I 39: 501–519

Logares R, Shalchian-Tabrizi K, Boltovskoy A, Regenfors K (2007) Extensive dinoflagellate phylogenies indicate infrequent marine–freshwater transitions. Mol Phyl Evol 45: 887–903

López-García P, Rodriguez-Valera F, Pedrós-Alió C, Moreira D (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409:603–607

Lovejoy C, Massana R, Pedrós-Alió C (2006) Diversity and distribution of marine microbial eukaryotes in the Arctic Ocean and adjacent seas. Appl Environ Microbiol 72: 3085–3095

Mangot JF, Lepère C, Bouvier C, Debroas D, Domaizon I (2009) Community structure and dynamics of small eukaryotes targeted by new oligonucleotide probes: new insight into the lacustrine microbial food web. Appl Environ Microbiol 75:6373–6381

Marijnissen SAE, Michel E, Daniels SR, Erpenbeck D, Menken SBJ, Schram FR (2006) Molecular evidence for recent divergence of Lake Tanganyika endemic crabs (Decapoda: Platythelphusidae). Mol Phylogenet Evol 40: 628–634

Massana R, Pedrós-Alió C (2008) Unveiling new microbial eukaryotes in the surface ocean. Curr Opin Microbiol 11: 213–218

Massana R, Guillou L, Díez B, Pedrós-Alió C (2002) Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean. Appl Environ Microbiol 68: 4554–4558

Massana R, Balagué V, Guillou L, Pedrós-Alió C (2004) Picoeukaryotic diversity in an oligotrophic coastal site studied by molecular and culturing approaches. FEMS Microbiol Ecol 50:231–243

McCann KS (2000) The diversity–stability debate. Nature 405:228–233

Moon-van der Staay SY, De Wachter R, Vaulot D (2001) Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. Nature 409:607–610

Muyzer G, Brinkhoff T, Nübel U, Santegoeds C, Schäfer H, Wawer C (1997) Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. In: Akkermans ADL, van Elsas JD, de Bruijn FJ (eds) Molecular microbial ecology manual. Kluwer Academic, Dordrecht, p 1–27

Not F, Valentin K, Romari K, Lovejoy C and others (2007) Picobiliphytes: a marine picoplanktonic algal group with unknown affinities to other eukaryotes. Science 315:253–255

Not F, del Campo J, Balagué V, de Vargas C, Massana R (2009) New insights into the diversity of marine picoeukaryotes. PLoS ONE 4:e7143

Pirlot S, Vanderheyden J, Descy JP, Servais P (2005) Abundance and biomass of heterotrophic micro-organisms in Lake Tanganyika. Freshw Biol 50:1219–1232

Pirlot S, Unrein F, Descy JP, Servais P (2007) Fate of heterotrophic bacteria in Lake Tanganyika (East Africa). FEMS Microbiol Ecol 62:354–364

Plisnier PD, Chitamwebwa D, Mwape L, Tshibangu K, Langenberg V, Coenen E (1999) Limnological annual cycle inferred from physical-chemical fluctuations at three stations of Lake Tanganyika. Hydrobiologia 407:45–58

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Richards TA, Vepritskiy AA, Gouliamova DE, Nierzwicki-Bauer SA (2005) The molecular diversity of freshwater picoeukaryotes from an oligotrophic lake reveals diverse, distinctive and globally dispersed lineages. Environ Microbiol 7:1413–1425

Salzburger W, Meyer A, Baric S, Verheyen E, Sturmbauer C (2002) Phylogeny of the Lake Tanganyika cichlids species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. Syst Biol 51:113–135

Sarvala J, Badende S, Chitamwebwa D, Juvonen P and others (2003) Size-fractionated $\delta^{15}N$ and $\delta^{13}C$ isotope ratios elucidate the role of the microbial food web in the pelagial of Lake Tanganyika. Aquat Ecosyst Health Manage 6: 241–250

Schauer M, Massana R, Pedrós-Alió C (2000) Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular fingerprinting. FEMS Microbiol Ecol 33:51–59

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71:1501–1506

Stager JC, Cocquyt C, Bonnefille R, Weyhenmeyere C, Bowerman N (2009) A late Holocene paleoclimatic history of Lake Tanganyika, East Africa. Quat Res 72:47–56

Stenuite S, Pirlot S, Hardy MA, Sarmento H, Tarbe AL, Leporcq B, Descy JP (2007) Phytoplankton production and growth rate in Lake Tanganyika: evidence of a decline in primary productivity in recent decades. Freshw Biol 52: 2226–2239

Stenuite S, Tarbe AL, Sarmento H, Unrein F and others (2009) Photosynthetic picoplankton in Lake Tanganyika: biomass distribution patterns with depth, season and basin. J Plankton Res 31:1531–1544

Tarbe AL (2010) Les protistes: acteurs-clés du réseau trophique pélagique au Lac Tanganyika. PhD thesis, University of Namur

Verburg P, Hecky RE, Kling H (2003) Ecological consequences of a century of warming in Lake Tanganyika. Science 301:505–507

von der Heyden S, Cavalier-Smith T (2005) Culturing and environmental DNA sequencing uncover hidden kinetoplastid biodiversity and a major marine clade within ancestrally freshwater *Neobodo designis*. Int J Syst Evol Microbiol 55:2605–2621

von der Heyden S, Chao E, Cavalier-Smith T (2004) Genetic diversity of goniomonads: an ancient divergence between marine and freshwater species. Eur J Phycol 39:343–350

Zubkov M, Sleigh M, Burkill P, Leakey R (2000) Picoplankton community structure on the Atlantic Meridional Transect: a comparison between seasons. Prog Oceanogr 45: 369–386

Editorial responsibility: Daniel Vaulot, Roscoff, France

Submitted: January 20, 2010; Accepted: October 3, 2010
Proofs received from author(s): December 23, 2010