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ORIGINAL PAPER

Historical genetics on a sediment core from a Kenyan lake: intraspecific genotype turnover in a tropical rotifer is related to past environmental changes

Laura S. Epp · Kathleen R. Stoof · Martin H. Trauth · Ralph Tiedemann

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Abstract Using molecular genetic methods and an ancient DNA approach, we studied population and species succession of rotifers of the genus *Brachionus* in the Kenyan alkaline-saline crater lake Sonachi since the beginning of the 19th century as well as distribution of *Brachionus* haplotypes in recent and historic sediments of other lakes of the East African Rift System. The sediment core record of Lake Sonachi displays haplotypes of a distinct evolutionary lineage in all increments. Populations were dominated by a single mitochondrial haplotype for a period of 150 years, and two putatively intraspecific turnovers in dominance occurred. Both changes are concordant with major environmental perturbations documented by a profound visible change in sediment composition of the core. The first change was very abrupt and occurred after the deposition of volcanic ash at the beginning of the 19th century. The second change coincides with a major lake level lowstand during the 1940s. It was preceded by a period of successively declining lake level, in which two other haplotypes appeared in the lake. One of these putatively belongs to another species documented in historical and recent Kenyan lake sediments. The analysis of plankton population dynamics through historical time can reveal patterns of population persistence and turnover in relation to environmental changes.

Keywords Ancient DNA · Lake sediments · *Brachionus* · Rotifer · Population succession

L. S. Epp · K. R. Stoof · R. Tiedemann (✉)
Unit of Evolutionary Biology/Systematic Zoology, Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Strasse 24-25, Haus 26, 14476 Potsdam, Germany
e-mail: tiedeman@uni-potsdam.de

M. H. Trauth
Institute of Geosciences, University of Potsdam, Potsdam, Germany

Introduction

Genetic data obtained from historical sediments are a new source of information for former biotic assemblages in permafrost (Willerslev et al. 2003), cave deposits (Haile et al. 2007), glacial deposits (Willerslev et al. 2007), and lacustrine and marine cores (Coolen et al. 2004, 2006, 2007). Continuous genetic records not only provide the possibility to analyse changes in species assemblages, but also to trace the population structure and history of single species in the course of severe environmental change (Hadly et al. 2004). This exciting new area of research offers the possibility to analyse changes in biodiversity that can not be viewed with classical methods. In this study we use genetic data obtained from a sediment core and surface sediments of lakes of the eastern

branch of the East African Rift System to analyse population distribution and turnover of *Brachionus* rotifers in a tropical lake during the last 200 years.

Many plankton species, as the monogonont rotifers of the genus *Brachionus*, were traditionally regarded as displaying global "cosmopolitan" distributions owing to their high dispersal and colonization potential but recent molecular evidence has revealed a complex scenario for many species, indicating frequent geographic population structure and cryptic species (De Meester et al. 2002). Cryptic speciation has also been documented both for the halophilic *Brachionus plicatilis* (Ciros-Perez et al. 2001; Gomez et al. 2002; Suatoni et al. 2006) and the freshwater taxon *Brachionus calyciflorus* (Gilbert and Walsh 2005), and both taxa are now considered species complexes. A high degree of differentiation has also been found within putative biological species of *Brachionus*, both on a regional (Derry et al. 2003; Gomez et al. 2007) and on a global scale (Mills et al. 2007). De Meester et al. (2002) proposed that after initial colonization the first migrants are able to monopolize resources because of their rapid population growth, the presence of a resting egg bank and local adaptation (Monopolization Hypothesis), which explains the commonly found differentiation within plankton species in light of known high dispersal and colonization potential.

Molecular genetic analyses of zooplankton populations through historical time allow a retrospective view on the establishment of current diversity patterns. Thereby it becomes possible to determine timescales of population persistence, detect possible turnovers and track the effect of environmental changes. Most such studies conducted on zooplankton organisms have focused on the analysis of resting eggs of different *Daphnia* species. They have elucidated different historical processes such as in situ adaptation of lineages (Cousyn et al. 2001), long-time population persistence in a single lake (Mergeay et al. 2007) or past habitat invasions by novel lineages (Duffy et al. 2000; Mergeay et al. 2005, 2006).

Our study presents temporal molecular genetic data of tropical rotifers. The underlying tested hypothesis is that environmental changes may impact population stability, eventually translating into turnover in evolutionary lineages, as revealed by changes in genotype composition. It focuses on rotifers in Lake Sonachi, a Kenyan crater lake that has experienced dramatic changes in environmental conditions during the last 200 years. The paleolimnology of this lake was studied by Verschuren et al. (1999), who investigated the relationships between plankton community response (algae, chironomids, cladocerans) and lake depth or salinity. For these groups, community response to changing environmental conditions was found to be weak. Other zooplankton of the lake has not been studied intensely, but is considered to be scarce and sporadic in its occurrence (Verschuren et al. 1999). Rotifers have been reported twice from zooplankton samples, but they are not classical model organisms for paleolimnology. Because they, like many limnic organisms, build resting stages, they can be considered excellent target taxa for paleogenetic studies (Brendonck and De Meester 2003).

We investigated historical molecular genetic data for a continuous record from Lake Sonachi starting at the beginning of the 19th century, and from pithole samples from the shore of Lake Bogoria. To establish estimates of regional genetic diversity and differentiation among sites, we also obtained genetic data from a number of other lakes in the East African Rift Valley. We used complete environmental DNA isolations and designed specific amplification reactions targeting *Brachionus* rotifers to track dynamics in population persistence and turnover during historical time.

Description of sites studied

The study focused on Lake Sonachi, a small, shallow, alkaline-saline crater lake located at 1,884 m above sea level in the Rift Valley in Kenya. It is situated 3 km from the large freshwater Lake Naivasha, with which it is connected by subsurface flow. Both lakes share a history of lake level change (Verschuren et al. 1999). Since the beginning of the nineteenth century, lake levels have fluctuated frequently and reached maximum depths of 18 m during a highstand at the end of 19th century, and near desiccation in 2003.

Samples were also retrieved from six other lakes situated in the eastern branch of the East African Rift System in Kenya (Fig. 1; Table 1). The lakes of the Kenyan Rift Valley are in close proximity, but display strikingly different hydrological and environmental features and range in alkalinity from pH 11

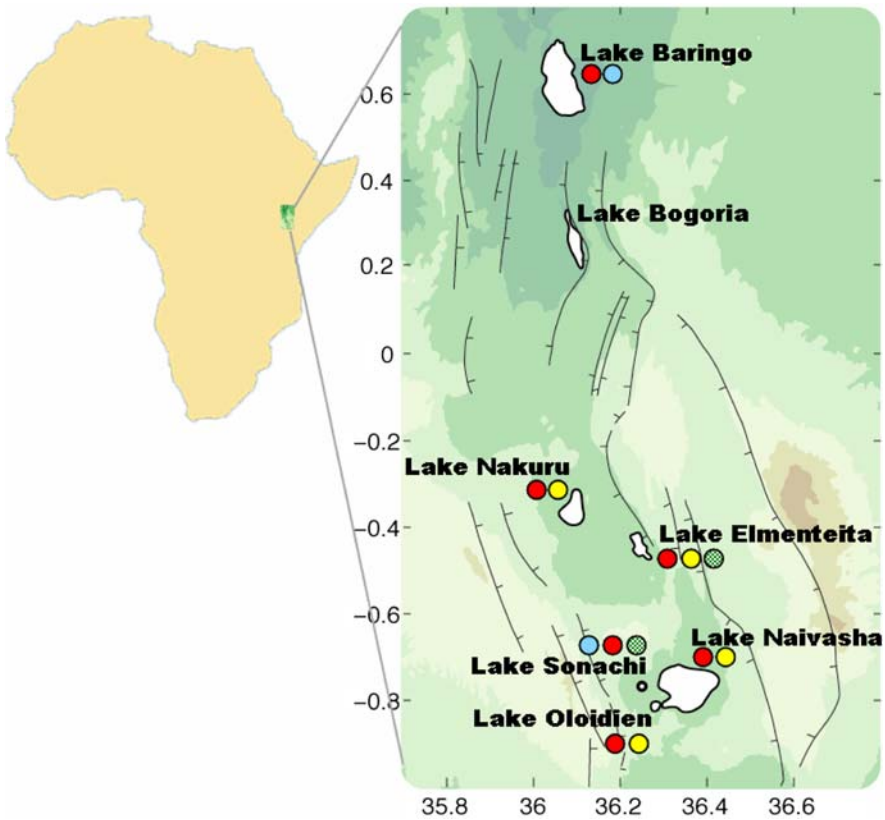

Fig. 1 Sampling sites in Kenyan Rift Valley Lakes. *Brachionus* haplotypes of the short COI-153 bp amplicon found in surface samples are depicted as *colored circles*. Color code is as follows: *blue* haplotype A, *red* haplotype B, *yellow* haplotype C, *dotted green* haplotype D

(Lake Elmenteita) to pH 8 (Lake Naivasha) and in depth from less than 1 m to 15 m. The lakes have experienced drastic changes in water level and environmental conditions both on geological time-scales (Trauth et al. 2005) and during the last hundreds of years (Verschuren 2001).

Materials and methods

Sampling

Samples used in this study comprise a sediment core from Lake Sonachi (core GCL2), surface sediment samples from different lakes in the Kenyan Rift Valley and pithole samples from a site adjacent to Lake Bogoria (Fig. 1; Table 1). In all cases, great care was taken to avoid contamination during sampling.

The sediment core from Lake Sonachi was taken with a KC kajak sediment core sampler (KC-Denmark A/S), using a transparent coring tube, and extracted in the field. The core was sampled in increments of 1.2 cm (samples GCL2-1 and GCL2-2) or 2.1 cm (all remaining samples, Table 3). These larger sampling increments were used because the sediment was very soft and we could thereby ensure proper separation of the samples used in molecular genetic analyses. The samples were taken from the center of each new slice, using fresh instruments (disposable plastic knives) and gloves for each sample. They were stored in 15-ml sterile sample tubes in Queens Tissue Buffer (Seutin et al. 1991, 20% DMSO, 0.25 M EDTA, saturated with NaCl, pH 8.0). Samples were mixed with the buffer by shaking and stored in the dark. After returning to the laboratory, the samples were kept in the dark at 10°C.

Surface sediment samples constitute integrated samples taken from within the lakes and from the shoreline. The samples from within the lakes were taken with an Ekman–Birge grabber from a boat. A subsample of the large Ekman–Birge sample was taken with a clean spatula, after the grabber was hauled on the boat. The shoreline samples were taken

Table 1 Sampling localities

Locality	Sample type	Geographical coordinates	Altitude (m)	pH[a]	Conductivity[a]
Lake Sonachi	Core, surface	0°47′S 36°16′E	1,894	10.4	23 mS/cm
Lake Oloidien	Surface	0°50′S 36°17′E	1,890	9.86	5.6 mS/cm
Lake Naivasha	Surface	0°47′S 36°21′E	1,890	8.5	320 μS/cm
Lake Elmenteita	Surface	0°27′S 36°15′E	1,786	10.5	37 mS/cm
Lake Nakuru	Surface	0°22′S 36°05′E	1,770	10.5	23 mS/cm
Lake Bogoria	Pithole	0°15′N 36°06′E	990	10.5	77 mS/cm
Lake Baringo	Surface	0°36′N 36°04′E	965	9	900 μS/cm

[a] Measured in August 2005

with a tulip planter, with subsequent subsampling with a spatula. The samples were treated and stored as described above.

Pithole samples were taken at Lake Bogoria, approximately 15 m from the shore of the lake in August 2005. The sides of the hole were cleaned with a spatula, and samples were obtained at 20 and 39 cm depth. The samples were stored as described above.

Dating

The bottom of the Lake Sonachi core GCL2 consists of a volcanic ash layer, confirmed by microscopic inspection. This layer, although previously interpreted as a dessication horizon, was dated by Verschuren (1999) to the year 1812 using ^{210}Pb, on cores dated to 1988 at the top. This date coincides with the documented latest volcanic eruption in the area at the beginning of the 19th century (Clarke et al. 1990), only 15 km from the Lake Sonachi, at Olkaria (Ololbutot lava flow). A similar, stiff layer has been described in sediment cores from Lake Naivasha (Verschuren 2001).

As an exact correlation with cores published by Verschuren (1999) based on organic carbon measurements appeared ambiguous we assumed a linear depth-age correlation, for the purpose of discussing the approximate timeframe of events. The top of the core was set to 2007, which was the time of the sampling. It should be noted that adopting sedimentation rates proposed by Verschuren (1999) gives an identical age for an important lithological change estimated to have occurred in the mid 1940s.

Age approximation for the Lake Bogoria pithole samples was carried out by applying the sedimentation rates established by Tiercelin et al. (1987) for a sediment core retrieved in close vicinity to the site sampled here. The sample Bog 5 (20 cm depth) thus dates to 200–300 years and the sample Bog 3 (39 cm depth) to approximately 500 years.

DNA isolation

DNA isolation was carried out using the PowerSoil™ DNA Isolation Kit (MoBio Laboratories, California). The PowerSoil™-bead solution was transferred from the bead solution tube to a sterile Eppendorf tube. The samples and buffer were mixed well just prior to isolation and 500 μl of each sample/buffer-mixture were transferred to the bead solution tube. The sample was centrifuged at 12,000 rpm for 10 min and the supernatant was removed (remaining sediment weight 150–200 mg). The PowerSoil™-bead solution and the buffer C1 included in the kit were added and the sample vortexed briefly. Subsequent release of the DNA from the cells and/or sediment matrix was carried out by first incubating the sample with Proteinase K (55°C, 1 h), then heating it to 70°C for 10 min and finally bead-beating with the Mini-BeadBeater (Glen Mills, NJ) for 50 s at 3,600 rpm. Before continuing with the isolation procedure, 5 μl of RNA-solution (1 μl/μg) were added to facilitate precipitation of nucleic acids. The remaining protocol was carried out according to the manufacturer's instruction.

All laboratory procedures were carried out adhering to strict precautions necessary to ensure authenticity of results as proposed by Gilbert et al. (2005) for work with ancient DNA. The isolation of core samples and pithole samples was carried out in an ancient DNA lab physically separated from any other molecular biology laboratory and employing rigorous controls against contamination. Isolation was carried

out for 3 or 5 samples at a time, with the addition of one negative control, for which the isolation was carried out using only the chemicals. Samples were isolated under a Hepa-filtered UV-hood, with UV-sterilisation effective for at least 1 h before and after samples had been unpacked and processed at the workspace. Gloves were changed between touching samples and between all subsequent laboratory steps. Surface sediment samples were isolated in a dedicated DNA-isolation lab after thorough cleaning of surfaces with DNA-ExitusPlus (AppliChem), with no samples except the ones isolated present at the time of isolation. The sediment samples of different lakes were isolated on different days each.

Primer design

After retrieving *Brachionus* sequences for the mitochondrial cytochrome oxidase I gene (COI) with universal primers (Folmer et al. 1994) from a subset of samples (GC37, GC27 and OB13), two sets of nested primers specific for *Brachionus* sequences were designed using the program OLIGO® (Rychlik and Rychlik 1989). These primers amplify a short amplicon of 153 base pairs (bp) excluding primers (COI-153 bp) and a longer amplicon (encompassing the short amplicon) of 433 bp excluding primers (COI-433 bp, Table 2). The chosen primers were checked for specificity by conducting a BLAST search in NCBI. Both forward and reverse primers were identical only to respective sequences of *Brachionus* "*plicatilis*" entries.

Primers used for sequencing were identical to the internal nested primers, but for the shorter 153 bp amplicon sequencing primers with a 40 bp tail (Binladen et al. 2007) were employed.

PCR, cloning and sequencing

Short amplicons were retrieved from all sediment core samples. Two independent PCR reactions of each core sample were sequenced directly. A subset of sediment core PCR products was cloned. These were samples that displayed ambiguities in the sequences directly retrieved from the PCR product and samples from adjacent strata in the core. PCR products from the topmost sample comprising the water–sediment boundary were also cloned.

The short amplicons were also retrieved from surface sediment samples from all the lakes and from the pithole samples from Lake Bogoria. Long amplicons were retrieved from a subset of surface sediments from Lake Nakuru, Lake Oloidien, and Lake Sonachi. A subset of surface sediment sample PCR products and the pithole sample PCR products were cloned. In total, 252 clones originating from 20 separate cloning experiments were sequenced for core and surface samples. All positive PCR products were also sequenced directly. Sequences were deposited in GenBank (Acc. Nos. GQ169692-GQ169703).

All PCR reactions were performed in a total volume of 37.5 µl, containing 1 mM Tris–HCl, pH 9.0, 5 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.13 µM of both forward and reverse primers, and 0.75 U *Taq* polymerase (Qbiogene). Amplifications were performed in a Biometra T-Gradient or T3000 (general PCR lab), or a Biometra T-Personal (ancient DNA lab) thermocycler according to the following reaction profile: the PCR tubes were transferred from ice to the cycler heated to 94°C, followed by 1 cycle at 94°C for 2 min, 35 cycles at 94°C for 30 s, at the primer-specific annealing

Table 2 Primers designed for this study

Primer name	Primer sequence	Used in combination with	T_A (°C)	Amplicon name
B_COI_1	TTT GGT ATT TGA GCG GGC	B_COI_457R	58	COI-153 bp
B_COI_3[a]	TGG TAT TTG AGC GGG CCT	B_COI_454R[a]	50[a]	
B_COI_275F	TCC TGT CTT CAG CTA TTG ATG C	B_COI_457R	54	COI-433 bp
B_COI_278F[a]	TGT CTT CAG CTA TTG ATG CAG G	B_COI_454R[a]	54	
B_COI_457R	GAT CAA GCG AAA CTC TTT TTG T			
B_COI_454R[a]	TCA AGC GAA ACT CTT TTT GTA GT			

[a] Primers and conditions used in nested PCR reactions

temperature (T_A in Table 2) for 45 s, 72°C for 2 min, and a final extension at 72°C for 15 min.

The first PCR of the core samples and pithole samples was carried out in a separate cycler in the ancient DNA facility (this lab and this cycler are never used for recent samples), with PCR set-up and reaction performed under separate UV-sterilised workstations. A negative control was included in each PCR. About 3 or 5 µl of DNA isolate were used. The PCR-products were then immediately transferred to the post-PCR area of the general lab. Set-up of nested PCRs, using 1 or 3 µl of the initial PCR product, was always conducted under a UV-sterilised hood. PCR tubes containing the first reaction were only opened immediately before transfer of the PCR product to the nested PCR tube and closed immediately afterwards. Therefore, PCR products of the first reaction were not checked on an agarose gel before the set-up of the nested PCR, in order to minimize handling/opening of PCR tubes. Gloves were changed after opening and closing each PCR tube to prevent cross-contamination. A negative control was included in the nested PCR as well. After completion of the nested PCR reaction, the PCR products were checked on an agarose gel together with the initial PCR.

Set-up of surface sediment PCRs was performed in a dedicated and thoroughly cleaned pre-PCR lab. Samples of different lakes were never touched with the same gloves to exclude cross-contamination between samples. About 3 µl of DNA isolate of the surface sediments were used.

The PCR products to be cloned were subjected to a reconditioning PCR (Thompson et al. 2002), using 5 µl of PCR product and the internal nested primers. Cloning was performed using the Topo TA cloning kit (Invitrogen). Depending on the success of the cloning experiment, as many positive clones as available, up to a maximum of 20, were sequenced.

Post sequencing analyses and phylogenetic inference

Sequences retrieved both from direct sequencing of PCR products and from cloning were aligned using the Clustal W module with default settings as implemented in BioEdit (Hall 1999), and haplotypes were defined. To exclude polymerase errors being considered true variation, only such haplotypes that were found in at least two clones from a single reaction or in independent PCR reactions were considered. If sequences derived directly from PCR products displayed single ambiguities in the chromatograms and these coincided with variation discovered in cloning experiments, the PCR products were inferred to contain both haplotypes (see e.g., NK25 from Lake Nakuru, Table 3). Nucleotide statistics and amino acid translation were performed using Mega 4.1 (Tamura et al. 2007).

Alignments were constructed from the inferred haplotypes of both amplicons and *Brachionus* sequences deposited in GenBank (*Brachionus* "*plicatilis*" as used by Suatoni et al. 2006 and all other *Brachionus* sequences named to the species level). The alignments were collapsed to contain only unique sequences using the program Collapse 1.2 (available from http://darwin.uvigo.es). Phylogenetic inference was carried out with MrBayes (Huelsenbeck and Ronquist 2001, GTR+I+G model of nucleotide substitution) and with Maximum Likelihood as implemented in RaxML (Stamatakis et al. 2008) for alignments of both amplicons, using *Keratella quadrata* (AF499084) as an outgroup. Prior to running the Bayesian analysis, the most appropriate model of nucleotide substitution (GTR+I+G) was identified using a hierarchical likelihood ratio test in Modeltest 3.7 (Posada and Crandall 1998). Two independent runs with one cold and three heated chains were run for 1,000,000 generations. We sampled every 500 generations and discarded the first 500 trees as burnin to create a consensus tree from the remaining trees.

A 95% probability parsimony network was constructed in TCS (Clement et al. 2000) with the long COI-433 bp Kenyan *Brachionus* sequences.

Results

Haplotype definition, diversity, and phylogeny estimation

Four different haplotypes A–D were identified from the sequences of the short COI-153 bp amplicon in our samples (Table 3). Haplotypes A–C were very similar, with A and C differing from B by only one mutation, respectively (uncorrected pairwise *p*-distance 0.7%), and all substitutions in third codon

Table 3 Samples and *Brachionus* haplotypes

Locality	Samples	Sampling	COI-153 bp				COI-433 bp							
			A	B	C	D	I-1	I-2	I-3	I-4	I-5	I-6	I-7	I-8
Lake Sonachi	GCL2-1	Water—0 cm		x_{15}										
Sediment core	GCL2-2	1–1.2 cm		x										
	GCL2-3	1.2–3.3 cm		x										
	GCL2-4	3.3–5.4 cm		x										
	GCL2-5	5.4–7.5 cm		x_{20}										
	GCL2-6	7.5–9.6 cm	x_3	x_{17}										
	GCL2-7	9.6–11.7 cm	x_9	x_7		x_1								
	GCL2-8	11.7–13.8 cm	x_{12}	x_4		x_3								
	GCL2-9	13.8–15.9 cm	x_{19}											
	GCL2-10	15.9–18 cm	x											
	GCL2-11	18–20.1 cm	x											
	GCL2-12	20.1–22.2 cm	x											
	GCL2-13	22.2–24.3 cm	x											
	GCL2-14	24.3–26.4 cm	x_{19}											
	GCL2-15	26.4–28.5 cm	x_{12}	x_6										
	GCL2-16	28.5–30.6 cm		x_{19}										
Surface	GC17	Shoreline		x										
	GC18	Shoreline				x	x							
	GC27	Shoreline		x_2			x							
	GC29	Boat		x										
	GC37	Boat	x_1					x						
	GC41	Boat		x					x					
	GC49	Boat	x											
	GC45	Boat	x	x				x_1	x_6					
Lake Oloidien	OB09	Shoreline		x	x									
Surface	OB13	Shoreline		x										
	OB14	Shoreline					x							
Lake Naivasha	NSA26	Boat		x										
Surface	NSA38	Boat		x										
	NSA44	Boat		x										
	NSA20	Boat		x_{11}	x_5									
Lake Elmenteita	ELM18	Wading		x								x		
Surface	ELM19	Wading		x		x						x	x	
	ELM17	Wading		x_4		x_9						x		
Lake Nakuru	NK3	Wading			x									
Surface	NK4	Wading			x_2									
	NK25	Wading		x	x							x	x	
	NK26	Wading		x	x							x	x	
	NK33	Wading		x_8	x									
	NK34	Wading		x	x							x	x	
	NK35	Wading		x	x				x_5	x_2	x_2	x_1	x_2	x_2
Lake Bogoria	BOG 5	20 cm depth			x_3	x_1								
Pithole	BOG 3	39 cm depth		x_3										

Table 3 continued

Locality	Samples	Sampling	COI-153 bp				COI-433 bp							
			A	B	C	D	I-1	I-2	I-3	I-4	I-5	I-6	I-7	I-8
Lake Baringo	BAR 5	Boat		x										
Surface	BAR 10	Boat		x										
	BAR 18	Boat		x										
	BAR 22	Boat		x										
	BAR 29	Boat	x_4	x_{12}										

x detected by sequencing subscript gives number of clone sequences, where occurence was verified by cloning of PCR product

position. Haplotype D, however, differed strongly from the three former, with 30 variable sites out of 154 and a *p*-distance of 23.72%. It displayed three amino acid differences in a translated amino acid alignment.

Sequences of the long COI-433 bp amplicon displayed higher diversity, with a total of 8 haplotypes defined (Table 3). These longer sequences displayed 9 variable sites out of 369 aligned positions, and pairwise *p*-distances range from 0.3 to 1.9%. An amino acid translation in an alignment with the translation deposited in GenBank (AY785229) revealed two amino acid substitutions between haplotypes 1 and 2 and one amino acid substitution between haplotype 1 and the other haplotypes found. In the sequence stretch overlapping with the short sequences, all 8 COI-433 bp haplotypes matched to COI-153 bp haplotypes A–C, while none showed identity to the divergent COI-153 bp haplotype D.

All sequences revealed highest similarity with rotifers of the genus *Brachionus* in a BLAST search compared to all sequences published in Genbank (http://blast.ncbi.nlm.nih.gov). Haplotypes A–C of the COI-153 bp amplicon and all 8 haplotypes of the COI-433 bp amplicon had highest similarity with a sequence of *Brachionus dimidiatus* (Acc. No. EU 046249), whereas haplotype D of the COI-153 bp amplicon was most similar to sequences ascribed to *Brachionus* "*plicatilis*". Phylogeny estimation conducted with an alignment of the Kenyan haplotypes together with the sequences belonging to the species complex *Brachionus* "*plicatilis*" (Suatoni et al. 2006) and all other available haplotypes belonging to the genus *Brachionus* using *Keratella* as an outgroup resulted in a phylogeny mostly concordant with that of Suatoni et al. (2006) for both amplicons (Fig. 4). All our Kenyan sequences are included in a monophyletic clade with the published *Brachionus* "*plicatilis*" sequences. The haplotypes A–C of the COI-153 bp amplicon (corresponding to haplotypes I-1–I-8 for COI-433 bp) form a highly supported monophyletic clade with the sequence of *B. dimidiatus* in all analyses, referred to as Kenya I. Haplotype D, referred to as Kenya II, is well separated from this, showing closest phylogenetic affinities with clade 4 of *Brachionus plicatilis sensu* Suatoni et al. (2006). Hence, the Kenyan haplotypes belong to two distinct evolutionary lineages (Kenya I and II). Given the

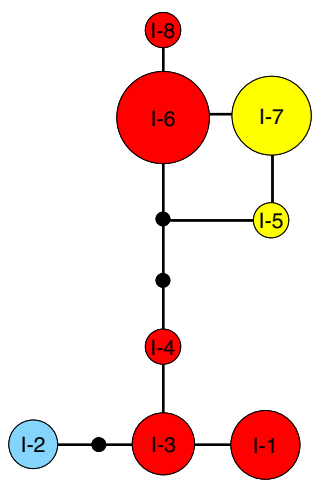

Fig. 2 Haplotype network constructed with the long COI-433 bp haplotypes. Color coding is equivalent to the respective short COI-153 bp haplotype sequences (A *blue*, B *red*, C *yellow*). The three short haplotypes A, B, and C display marked differences, especially between A and B. Circles are sized according to the frequency with which the haplotypes occurred in the data set

close ties of the Kenya I-sequences to *B. dimidiatus* and adopting the rationale of Suatoni et al. (2006) for the delineation of species within *Brachionus*, the two lineages found in this study very likely reflect two different species.

A Maximum Parsimony Network using TCS (Clement et al. 2000) was constructed for the eight haplotypes of the COI-433 bp amplicon (Fig. 2). The relationship between these haplotypes and haplotypes A, B, C defined for the short COI-153 bp amplicon is as follows: COI-153 bp haplotype A is represented by a single longer sequence (COI-433 bp haplotype I-2), separated from sequences belonging to COI-153 bp haplotype B by two to seven mutational steps. COI-153 bp haplotype B is comprised of 5 different COI-433 bp haplotypes (I-1, I-3, I-4, I-6, I-8) situated centrally in the network and containing the most common COI-433 bp haplotype I-6. Sequences defined as COI-153 bp haplotype C (COI-433 bp haplotypes I-5, I-7) are close to the B types, but definite evolutionary relationships cannot be determined, as indicated by a loop in the network (Fig. 2).

Geographical distribution of haplotypes

The geographical distribution of the short sequence haplotypes in surface sediments of the different lakes is depicted in Fig. 1 and listed in Table 3. Our approach does not allow for a quantitative assessment of abundance, as single samples are composed of an unknown number of individuals. More than one haplotype was recorded from each lake. Haplotype A was found only in Lake Sonachi and Lake Baringo, while haplotype C was not found in any Lake Sonachi or Lake Baringo sample. Haplotype D was found only in Lake Elmenteita and in Lake Sonachi. The samples from the Bogoria pithole contained haplotypes B (at 39 cm) and haplotypes C and D (at 20 cm). The Lake Sonachi core GCL2 contained haplotypes A, B, and D.

Vertical and chronological succession of haplotypes in Lake Sonachi

The bottom of the core GCL2 is formed by $a > 1$ cm thick gray fine-grained layer, containing almost exclusively fresh volcanic ash and minor constituents of organic matter. Although previously interpreted as a desiccation horizon (Verschuren et al. 1999), we consider this layer as a volcanic ash from a recent eruption in the Naivasha Basin. The best candidate for this eruption is the last eruption of the Olkaria Volcanic Complex that generated the Ololbutot lava flow about 15 km SSE of Lake Sonachi. This eruption was indirectly ^{14}C dated by using carbonised tree branches from beneath a pumice flow to 180 ± 50 years before present (Clarke et al. 1990). This date corresponds to the age established by Verschuren for the top of this layer (1812 AD $\pm$ 29 years). The volcanic glass does not show any evidence for weathering as it typically occurs in alkaline Kenyan rift basin lakes (Trauth et al. 2003). Rhyolithic and trachytic ashes deposited in high-pH environments are expected to show yellowish and greenish colorations due to the occurrence of authigenic weathering products such as smectites and zeolithes (Trauth et al. 2003). Since the closed-basin Lake Sonachi would have experienced higher alkalinities and salinities during an episode of a lower lake level we conclude that the lake level was high during eruption and deposition of the volcanic ash at around 180 ± 50 years ago. This interpretation is in good agreement with the lake level record of Lake Naivasha (Verschuren 2001, Fig. 3), which is hydrologically connected with Lake Sonachi via groundwater flow (Verschuren 1999). The lake level of Lake Naivasha was decreasing rapidly during this time but had not yet reached its final lowstand (Verschuren 2001).

Direct sequencing of two independent PCR products and/or cloning experiments of each sample from the different strata revealed a single dominant haplotype (either A or B) for most samples (Table 3; Fig. 3), with two shifts in dominance occurring throughout the core. The first shift happened between the lowermost sample GCL2-16 and GCL2-14 (from B to A), with both types present in GCL2-15. The second shift occured between GCL2-9 and GCL2-5 (from A to B). The interjacent samples GCL2-8, GCL2-7, and GCL2-6 contained both haplotypes, and in addition, GCL2-8 and GCL2-7 contained the divergent haplotype D. The lower shift (GCL2-16 to GCL2-14) coincides with the period after the deposition of volcanic ash. The upper shift (GCL2-9 to GCL2-5) coincides with a period of rapidly declining lake level resulting in a historic lowstand in 1946 (Verschuren 1999).

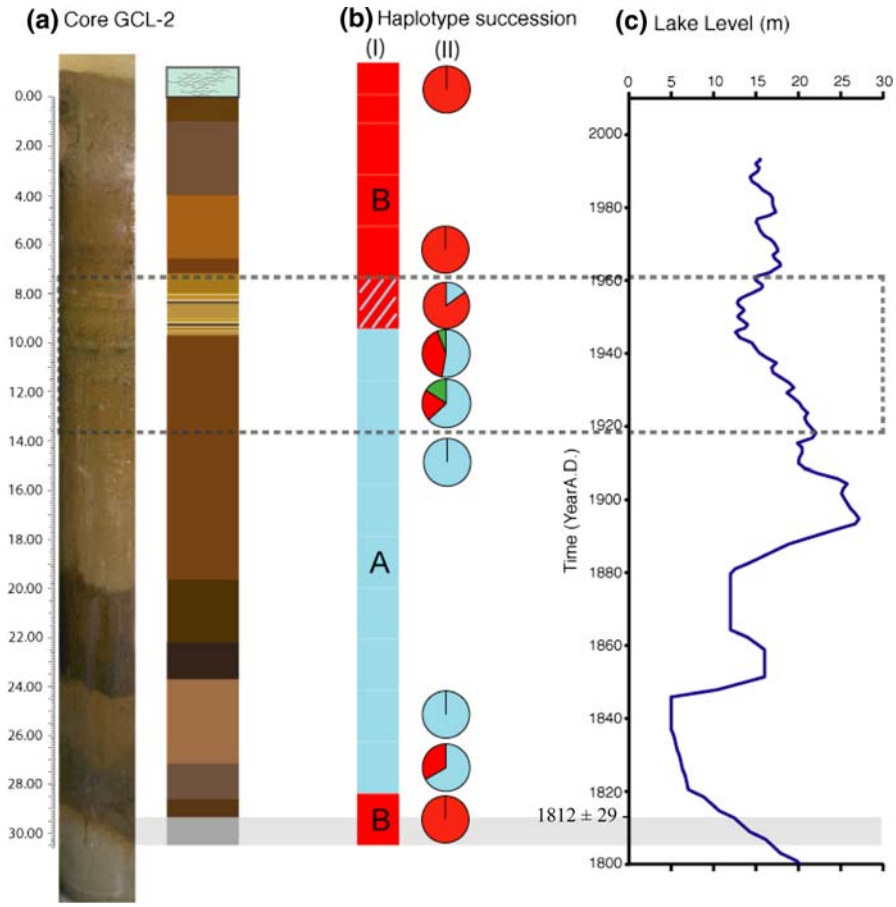

Fig. 3 Lake Sonachi sediment core GCL2 with haplotype succession and corresponding lake level changes. **a** Sediment core and sketch revealing distinct changes in sediment composition. **b** Haplotype succession of *Brachionus* rotifers. I Haplotypes visible when PCR products were sequenced directly. II Proportions of clone sequences. Color coding: *red*: B, *blue*: A, *green*: D. The haplotypes A and B putatively belong to the same species, whereas D is a distinct evolutionary lineage. **c** Lake level changes of Lake Naivasha, which can be extrapolated to Lake Sonachi (Verschuren 1999). *Grey horizon* indicates tephra layer of the Ololbutot eruption (Clarke et al. 1990). The *dotted line* indicates the time frame, in which the second haplotype displacement occurred

In summary, there were long times of persistence of single types, interrupted by shifts. The oldest sample (GCL2-16) revealed haplotype B (in 19 clones sequenced). The sample just above, GCL2-15 showed a transition and displayed both haplotypes B (6 clones) and A (12 clones). The following samples GCL2-14–GCL2-9, representing approximately the years from the 1830s to 1920, displayed only haplotypes A (also confirmed in cloning of the samples closest to the shifts, i.e., GCL2-14 (19 clones) and GCL2-9 (19 clones)). Samples GCL2-8 and GCL2-7 (approximately 1920–1945 AD) contained the three haplotypes A, B, and D, with the relative frequency of A diminishing compared to B, and D more frequent in the lower sample (Table 3; Fig. 3). In the successive sample, GCL2-6, just above the lowstand of the mid 1940s, haplotype B was dominant (17 clones), but haplotype A was still present (3 clones). Sample GCL2-5 displayed only haplotype B (20 clones), as did the top-most sample GCL2-1 (15 clones), which constituted the present sediment–water interface.

Discussion

Disentangling the relative role of dispersal, ecological factors, and historical constraints in shaping present biodiversity is a fascinating endeavor that is becoming more and more feasible using molecular genetic data. Traditional notions on plankton organisms involve frequent global "cosmopolitan" distributions with no or little differences between geographically widespread populations, rapid colonization of new habitats and fluctuating population dynamics. Studies relying on molecular genetic data have revealed substantial genetic differentiation between populations of different geographic localities in a number of plankton species (Brendonck et al. 2000; Vanoverbeke and De Meester 1997),

even though experimental evidence does point to high dispersal potential (Green and Figuerola 2005). Possible mechanisms leading to this situation were described by De Meester et al. (2002), who suggested that initial colonization by one certain lineage paired with the typical clonal reproduction can lead to rapid adaptation and inhibit colonization by following divergent lineages (Monopolization Hypothesis).

Elucidating the temporal dynamics leading to the patterns observed in recent populations is now becoming possible using molecular genetic approaches, especially for organisms that build resting stages deposited in sediments (Brendonck and De Meester 2003). Such studies can reveal how long distinct populations persist in an ecosystem (Mergeay et al. 2007) and on what timescales populations can change (Duffy et al. 2000; Mergeay et al. 2006). The molecular genetic analyses conducted to date have most often focused on cladoceran ephippia (Cousyn et al. 2001; Mergeay et al. 2005, 2006, 2007).

The present study presents a continuous genetic record of rotifers inferred to belong to the genus *Brachionus* in a single Kenyan lake (Lake Sonachi) since the beginning of the nineteenth century and links genetic changes to environmental changes in the lake. The zooplankton community of this lake is reported to be poor in species and apparently erratic in its occurrences, but has only been sporadically sampled (Verschuren et al. 1999). Species so far recorded by direct observation are the rotifers *B. dimidiatus* in 1929 (de Beauchamp 1932) and *B. quadridentatus* in 1993 (Green 1993), and the calanoid copepod *Paradiaptomus africanus* in the 1930s (Beadle 1932; Lowndes 1936). The reduction of rotifer fauna to only one species in this lake reflects a pattern revealed by Green (1993), who showed that lakes with salinities above 2,000 μS/cm had a maximum of three dominant rotifer species present. Most commonly the dominant rotifer in highly alkaline-saline East African waters is *B. dimidiatus* (Green and Mengestou 1991; Nogrady 1983). Overall, the rotifer fauna of East Africa, even though more intensively studied than that of other regions of Africa, is still not completely documented, as revealed by the recent discovery of new and endemic species in Kenya, among others a new species of *Brachionus* (Segers et al. 1994).

The sequences retrieved in this study all fell within the clade of the *Brachionus* "*plicatilis*" species complex when subjected to phylogenetic analyses. Specifically, our most abundant and diverse Kenyan clade I shows close affinities to a published sequence of *B. dimidiatus* (Fig. 4). Therefore we consider it extremely likely that we have detected this species, which has also been described from the lake (de Beauchamp 1932). Unfortunately, we were so far not able to confirm the presence of rotifer resting eggs in the core by direct microscopical observation. Future work will certainly enable linking sequences to morphologically defined species by plankton collection and systematic retrieval of genetic data for morphologically distinguishable species.

Using genetic data from historical sediments as a record of past biodiversity changes requires the DNA in the sediment to be stratified. In the analysed core we found a very discrete stratigraphy of haplotypes, and the different haplotypes from the different strata in the Lake Bogoria pithole point to a stratigraphic layering there as well. This pattern implies that the problem of DNA leaching reported for DNA studies from other non-frozen sediments (Haile et al. 2007) is not apparent in our study. We assume that the mixture of haplotypes seen in the "surface" samples might constitute an artifact, as these samples might integrate over several years, rather than documenting the population present at a certain point in time. The mixing of haplotypes can be explained by sampling bulk samples taken from a mixture of depths up to about 8 cm, or from reworking of surface sediments, especially at the shoreline, or from both processes. Our investigation clearly demonstrates that genetic data in the sediment record studied is stratigraphically structured and can be used to analyse the temporal population dynamics in plankton species. We assume that the DNA detected via PCR amplification originates from resting eggs deposited in clear succession with little turbation occurring.

The data from the sediment core reveal interesting patterns that have not been described to date for a zooplankton species. First of all, dominance of mitochondrial haplotype A was persistent for nearly 150 years in Lake Sonachi. Evidently, this does not rule out the possibility that other haplotypes of the same species were present in lower numbers and were not detected by our approach. It is also highly unlikely that the species was present at all times, as

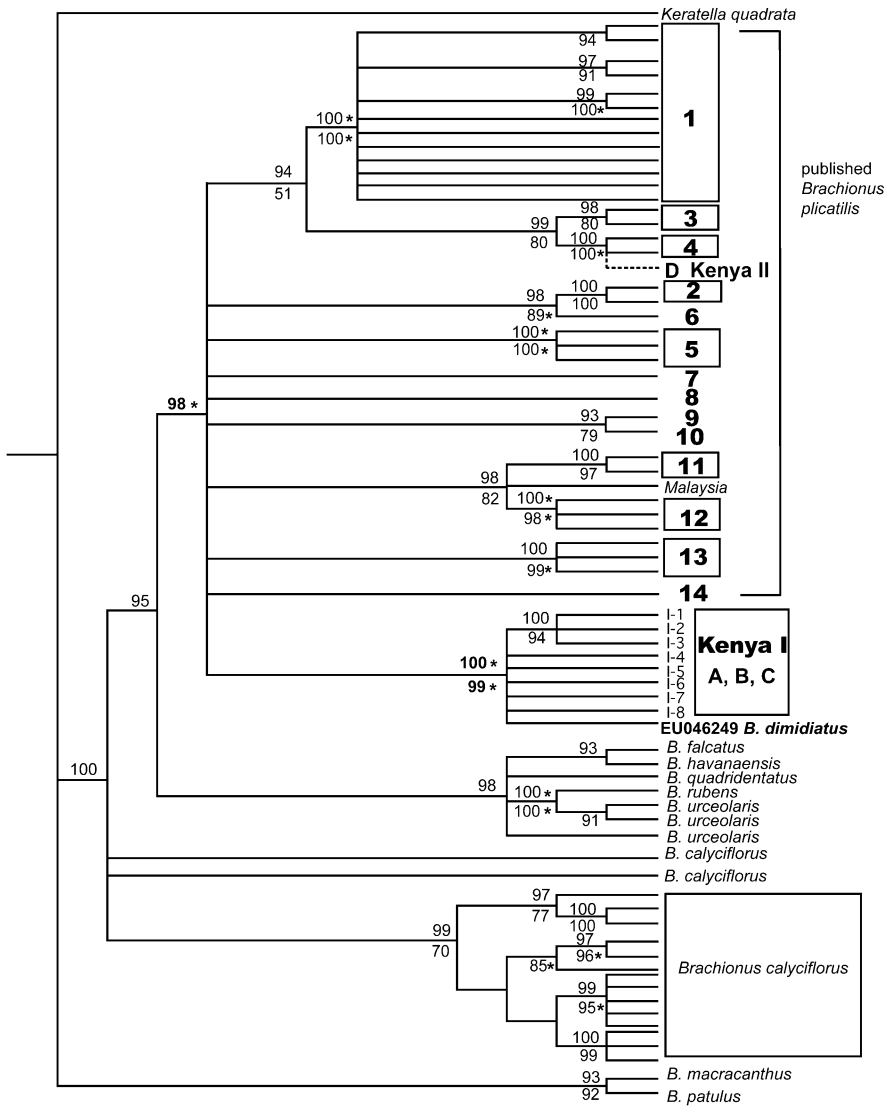

Fig. 4 Combined tree of the Bayesian- and Maximum Likelihood-phylogenetic inferences of the COI-433 bp sequences using *Keratella quadrata* as an outgroup. Support values for each of the clades are given for both the Bayesian inference (*above the branch*) and Maximum Likelihood (*below the branch*). Highly supported clades for the COI-153 bp alignment are indicated by an *. Numbered clades of *Brachionus plicatilis* and the sequence named Malaysia correspond to clades obtained in an analysis of Suatoni et al. (2006)

high levels of seasonal and annual variation have been documented in recent East African rotifer populations (Nogrady 1983) and all records imply that zooplankton occurrence in Lake Sonachi is not continuous (Green 1993; Verschuren et al. 1999). We document a long-term trend in dominance of a certain haplotype in a lake with unstable short-term environmental conditions (Verschuren 1999; Verschuren et al. 1999) and presumably high population fluctuation of the analysed species as implied by its putatively sporadic occurrence (Verschuren et al. 1999). As the neighboring lakes are all in close proximity, we can also assume a high potential for dispersal from neighboring populations (Havel and Shurin 2004). Nonetheless, re-occurrence of the species during this period of haplotype stability is more likely to be accomplished by repopulation from resting stages present in the topmost sediment layers.

Secondly, the two major changes in haplotype dominance are both correlated with major changes in sediment composition of the core and correspond to distinct environmental changes and events. The first change, from haplotype B to A, occurred after the deposition of volcanic ash in the lake and during a period of decreasing lake level. It was seemingly abrupt, as only the sample just above the tephra displayed a mixture of both haplotypes. The second displacement was less abrupt, with more than one

sample containing multiple haplotypes. This change corresponds to a period of decreasing lake level and subsequent lake level lowstand approximately in the mid-1940s.

Microscopic inspection of the tephra layer in the sample GCL2-16 revealed that it contains organic material, but not much. We therefore assume that the tephra contains dispersed organic material deposited just before the eruption of the ash and that this is still present in the samples GCL2-16 and GCL2-15. Thus, the haplotype B might represent the population present before the ash deposition. Possibly, the population did not outlast the tephra deposition, the genetic detection of B is due to remaining resting eggs from the former population, and the lake was repopulated by *Brachionus* with the haplotype A within the next 10–20 years. Alternatively, the population with haplotype B, although outlasting the tephra deposition, was severely reduced, such that displacement by a population with haplotype A was easily possible. Finally, the tephra deposition might have had such an impact that no relict populations are detectable above or within the layer. The haplotype B would then indicate an initial, but unstable population, that could not become established during this period of declining lake level, and a very rapid displacement of B by A occurred.

In any scenario we assume that the tephra deposition significantly reduced the rotifer population that might have been present before. Impacts of tephra deposition on plankton communities have been previously documented in a number of instances (Barker et al. 2000; Telford et al. 2004). Immediate changes in abiotic factors resulting from volcanic ash include the solution of novel salts, a short-term reduction of light in the lake and changes in nutrient resources availability, namely reduction of phosphorus and increase of silica (Telford et al. 2004). The reduction of phosphorus is due to the tephra acting as a physical barrier for phosphorus regeneration from organic material in the sediment. The decline of the *Brachionus* population could be due to an analogous process, i.e., the tephra acting as a physical barrier inhibiting the hatching of rotifer resting eggs. It is also possible that the viability of the resting eggs was reduced by the depositional process. Garcia-Roger et al. (2006) have ascribed differences in density, viability, and deterioration of resting egg banks to differences in environmental conditions.

The second haplotype change from A to B coincides with a major lowstand in the 1940s. The change was preceded by a period from approximately 1920–1945, in which the three haplotypes A, B and D were recovered from the samples. During this period, haplotype A remained dominant. The sample just above the lowstand (GCL2-6, Table 3) contained only haplotypes B and A, with an arising dominance of B. Samples younger than this lowstand display only haplotype B. The period, in which the three haplotypes became apparent, coincides with the start of the progressive decline in water level (Fig. 3), but the exact mechanism of the displacement cannot be elucidated by the present data. Current resolution is not sufficient to exactly determine the date of the first appearance of a new haplotype. Likewise, as any sample integrates over about one decade, we are unable to truly distinguish between coexistence of haplotypes in the water column and short term (e.g., seasonal or annual) succession of haplotypes. We also have no data on possible adaptive differences of the populations involved. The haplotypes A and B were inferred to belong to the same evolutionary lineage (Kenya I; inferred to constitute *B. dimidiatus*) and were genetically very similar, but haplotype D is clearly separated and putatively constitutes another species (as tentatively indicated by our phylogenetic analysis: Kenya II in Fig. 4). While adaptive differences might exist between these two lineages/species, the geographical distribution of haplotypes in surface sediments does not imply adaptive differences of the haplotypes A and B in relation to salinity or pH. Lake Baringo and Lake Sonachi both display these haplotypes, but exhibit very different abiotic environmental conditions (Fig. 1; Table 1).

This means that the change in itself might not be adaptive, but all evidence points to the change being induced by environmental change. This also holds true for the displacement related to the tephra deposition. While we cannot principally rule out the possibility of a correlation between haplotype and adaption of the respective lineage to different microenvironments, we see extinction and re-colonization as a plausible scenario to explain intraspecific haplotype turnover related to environmental change: Both ash deposition at the beginning of the 19th century and historical long-lasting lowstands could have caused the local rotifer fauna to become diminished or even extinct, such that re-colonization

eventually brought in a different evolutionary lineage (i.e., carrying a different haplotype). Our data also indicate that in the absence of such drastic events, the same haplotypes, and hence lineages, persisted for a long time and might even have re-appeared after shorter unfavorable periods. This scenario would be in accordance with the Monopolization Hypothesis (De Meester et al. 2002), where immigration into a vital local population is prevented.

For the displacement in the 1940s a situation can be imagined in which an initial decline in population due to either less favorable conditions in the water column or to lower hatching success enabled new populations (haplotype B) or even other species (haplotype D) to colonize the habitat from other lakes. The appearance of new populations and/or species can result in a situation of inter- and intraspecific competition and further population decline. Different species of *Brachionus* have been shown to display lower growth rates when jointly cultured with other species (Fernandez-Araiza et al. 2005).

For the cladoceran *Daphnia barbata*, Mergeay et al. (2007) established a high degree of genetic continuity between populations in the same lake that were fully separated in time, thereby suggesting that re-colonization occurred from the resting egg bank even after periods of prolonged absence of at least 50 years. In the case of our two rotifer populations carrying haplotype B at the bottom of the core and after the 1940s, we cannot determine whether the new population is derived from the older one. Data from the longer COI-433 bp sequences shows that there is variation within the haplotype B, and it is well possible that the two B haplotypes separated in time are not identical. Moreover, viability of rotifer resting eggs has been shown to decrease dramatically from the top of a sediment core to 10 cm in depth (Garcia-Roger et al. 2006). The abrupt displacement of B by A after the ash deposition makes it seem improbable that surviving resting stages of the former B population could recolonize the lake after over 100 years and hence provides evidence for a re-colonization by imigration/dispersal. Sequences assigned to haplotype B were found in surface sediments from all lakes, and we therefore cannot determine the source of colonization at the moment. Future studies employing more variable markers, such as microsatellite analysis of individual resting eggs, can reveal a more detailed picture of geographical genetic differentiation and source populations in colonization events.

Our results imply that the population structure of *Brachionus* in lakes of the East African Rift System is determined by historical colonization events following dramatic environmental changes. While the exact mechanisms of displacement and sources of colonization remain to be analysed further, this paleogenetic study has uncovered two events that induced population turnover in a rotifer species within the last 200 years: tephra deposition after a volcanic eruption and an exceptional lake level lowstand. Such events are typical for the East African Rift Valley and likely are of prime importance in shaping biodiversity patterns in the area. The analyses of historical dynamics within single species can uncover biotic responses to environmental pressure at a fine scale and reveal associated cues and mechanisms. Such analyses are possible using sediment cores as records of continuous stratified genetic data for plankton organisms such as rotifers, which have proven a new and interesting model for palaeolimnology.

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