Hydrobiologia (2013) 703:79–93
DOI 10.1007/s10750-012-1346-z

PRIMARY RESEARCH PAPER

Mass developments of the cyanobacteria *Anabaenopsis* and *Cyanospira* (Nostocales) in the soda lakes of Kenya: ecological and systematic implications

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Received: 3 August 2012 / Revised: 24 September 2012 / Accepted: 1 October 2012 / Published online: 19 October 2012

Abstract The food web of the saline–alkaline lakes of East Africa is characterised by a unique interaction between the Lesser Flamingos as consumer birds and the cyanobacterium *Arthrospira fusiformis* as the primary producer. However, this interaction is disturbed frequently by alterations of the phytoplankton community. During the period 2001–2012, species of the cyanobacteria *Anabaenopsis* and *Cyanospira* were observed in four soda lakes of the African Rift Valley and compared to the entire phytoplankton biomass and composition. Their morphology was highly variable. Each species preferred a distinct range of salinity: *C. capsulata* 30–40 ppt, *C. rippkae* 25–35 ppt, *A. arnoldii* and *A. abijatae* 10–30 ppt, and *A. elenkinii* 0–15 ppt. Occasional dominance of *Anabaenopsis* and *Cyanospira* in the lakes investigated shows that members of these genera are serious competitors of *A. fusiformis*, the main food for Lesser Flamingos. Furthermore, mass developments of *C. capsulata* adversely affected food uptake by the flamingos at Lake Bogoria because they formed mucilaginous colonies that clogged the food filter system. From field samples of the three lakes, uncultured *Anabaenopsis* and *Cyanospira* spp. clones were obtained and subjected to phylogenetic analyses. The 16S rRNA gene sequencing data put into doubt the differentiation of *Anabaenopsis* and *Cyanospira* into separate genera as recently suggested.

Keywords *Anabaenopsis* · *Cyanospira* · Lesser Flamingo · Plankton succession · Soda lakes · Bogoria · Elmenteita · Nakuru · Oloidien

Introduction

The soda lakes Bogoria, Elmenteita, Nakuru and Oloidien in the Kenyan part of the Great African Rift Valley support a dense population of the Lesser Flamingo (*Phoeniconaias minor* Geoffroy Saint Hilaire). More than one million individuals of this spectacular bird have been observed frequently at these unique ecosystems (Brown, 1959; Harper et al., 2003; Childress et al., 2008). The preferred food resource for the flamingos is the quasi-monospecific mass development of the cyanobacterium *Arthrospira fusiformis* (Voronichin) Komárek et Lund (referred to

Handling editor: Luigi Naselli-Flores

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in the past as '*Spirulina platensis*') (Vareschi, 1978; Vareschi & Jacobs, 1985). However, because of ecosystem degradation and other unknown reasons, this archetypal interaction between the primary producer and consumer bird has been disturbed considerably within the last few decades. The dense populations of *Arthrospira* have been shown to be unstable as they crash periodically (Tuite, 1981; Melack, 1988; Schagerl & Oduor, 2008). Following episodes of *Arthrospira* crashes, benthic diatoms act as the alternative food resource for the Lesser Flamingo. However, because benthic diatoms have lower productivity and nutritional value (Tuite, 2000), they cannot sustain the dense flamingo population that sometimes establishes in these lakes. *Arthrospira* is often outcompeted by other cyanobacteria or eukaryotic algae which cannot be ingested because they do not meet the size requirements of highly specialized bills of the Lesser Flamingos. Such food organisms can be too small to be trapped by the filter system, such as the chlorophyte *Picocystis* (Krienitz et al., 2012). Alternatively, they can be too large to be ingested by Lesser Flamingos, as in the case of mucilaginous colony-forming cyanobacteria taxa belonging to the relationship of *Anabaenopsis* (Krienitz & Kotut, 2010). During the last decade, *Arthrospira* populations in lakes Nakuru and Elmenteita were associated with different species of the genus *Anabaenopsis* or *Cyanospira* (Ballot et al., 2004, 2008; Oduor & Schagerl, 2007a; Kotut & Krienitz, 2011). This phenomenon had earlier been observed by Rich (1932) in samples collected in 1929. Lake Oloidien, a former bay of Lake Naivasha, the salinity of which has witnessed a steady increase, regularly hosted *Anabaenopsis* during its freshwater phase. However, *Arthrospira* has recently achieved dominance and this has resulted in the lake attracting hundreds of thousands of Lesser Flamingos during the last few years.

In this paper, we report on phytoplankton composition changes in the four saline lakes over a period of 12 years with emphasis on abundance of *Anabaenopsis* and *Cyanospira*. It has become evident that members of these genera play a key role in the succession of primary producers in these habitats. Their extremely variable morphology has made species delineation very difficult. We therefore used 16S rRNA gene clones obtained from uncultured monospecific field samples to analyse the molecular-phylogenetic properties of the taxa. The species and genus conception of *Anabaenopsis* and the closely related *Cyanospira* is discussed and areas for further studies on these ecologically important cyanobacteria are identified.

Materials and methods

Site description and sampling

The lakes under investigation (Table 1) are situated in the eastern part of the Great African Rift Valley in the territory of Kenya. Lakes Bogoria, Elmenteita and Nakuru are meso- to hypersaline, alkaline soda lakes, whereas Lake Oloidien, a former bay of the freshwater Lake Naivasha, has in the course of the study period changed from fresh to hyposaline conditions. Lakes Bogoria and Oloidien are deeper than Elmenteita and Nakuru, which nearly dried out several times during the last decade. Owing to the wide fluctuation in the volume and level of water in lakes Elmenteita and Nakuru, they were characterised by greater amplitudes of variation in their physico-chemical properties. The four lakes were studied 19 times at irregular intervals in the period from 2001–2012. Salinity and pH were measured at each sampling date using a WTW Multiline P4 meter (Wissenschaftlich Technische Werkstätten Weilheim, Germany). Phytoplankton samples were collected from a few centimetres below the water surface at a distance of about 10 m from the shoreline and immediately fixed with formaldehyde (final concentration 1%). The geographic coordinates of the main sampling points are given in Table 1.

Microscopy

Phytoplankton were counted according to Utermöhl (1958) and photographically documented in sedimentation chambers (Hydro-Bios Apparatebau GmbH, Kiel, Germany) under the inverted microscope Eclipse TS 100 (Nikon Corporation, Tokyo, Japan). The phytoplankton biomass was calculated by geometric approximations using the computerized counting program Opticount (Sequentix, 2006). The specific density of the phytoplankton cells was taken as 1 g cm^{-3}.

Table 1 Short characteristics of the lakes studied

	Lake Bogoria	Lake Elmentaita	Lake Nakuru	Lake Oloidien
Main sampling point	00°13′83″N 36°05′55″E	00°27′34″S 36°15′33″E	00°21′88″S 36°03′46″E	00°49′00″S 36°15′52″E
Surface area (km^2)	~34	~20	~40	~6
Maximum depth (m)	10	3.1	4.5	6
pH	9.9–10.6	9.7–10.5	10.1–10.5	9.3–10.4
References to read more details	Harper et al. (2003); Ballot et al. (2004)	Melack (1988); Schagerl & Oduor (2008); Ballot et al. (2004)	Vareschi (1978); Schagerl & Oduor (2008); Ballot et al. (2004)	Kalff & Watson (1986); Verschuren et al. (2000); Ballot et al. (2009)

Estimation of flamingo numbers

A visual estimate of the number of flamingos was made at each site and sorted into three categories for lakes Bogoria, Elmenteita and Nakuru (>500,000, >100,000 and <100,000 individuals) and four categories for Lake Oloidien (>100,000, >20,000, >10,000 and <1,000 individuals). These rough estimates were carried out at strategic points at each lake (for more details, see Krienitz & Kotut, 2010).

Molecular studies

For molecular studies, monospecific samples of *Anabaenopsis* or *Cyanospira* spp. determined by light microscopy were collected from the pelagic habitat of Lake Bogoria (19.01.2011), Lake Elmenteita (12.07.2008) and Lake Nakuru (13.07.2008). The collected field samples were scrubbed from dried filters with a sterile scalpel and genomic DNA was extracted using Dynabead DNA Direct System I (Invitrogen/Dynal Biotech, Oslo, Norway) following the steps outlined in the manufacturer's manual. The polymerase chain reaction (PCR) was performed to obtain a partial sequence of 16S rRNA gene in a Peltier Thermal Cycler PTC 200 (MJ Research Inc., San Francisco, USA). The volume and concentrations of the PCR cocktail were used as described by Dadheech et al. (2012a). The primers pA and B23S (cyanobacterial specific) were used for amplification of the 16S rRNA gene (Edwards et al., 1989; Gkelis et al., 2005). The PCR protocol with an annealing temperature of 50°C was used as described in Ballot et al. (2008). The amplified products were cleaned using the QIAquick PCR purification column according to the manufacturer's protocol and examined on 1% agarose gel. Cleaned PCR products were cloned using the Zero Blunt® Topo® PCR cloning kit (Invitrogen, Germany) according to the manufacturer's instructions. Selected positive clones were cycle sequenced with different primers pA, pC, pE, pDr, pFr (Edwards et al., 1989) and WAW1486R (Wilmotte et al., 1993) to retrieve a partial sequence (>1,200 bp) of 16S rRNA gene. The uncultured *Anabaenopsis* or *Cyanospira* spp. clones were designated as LNK (Lake Nakuru), LBK (Lake Bogoria) and LEK (Lake Elmenteita). Both strands were sequenced on ABI 3100 Avant Genetic Analyzer using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Applera, Deutschland, GmbH, Darmstadt, Germany) as described in the manufacturer's manual.

The sequences of 16S rRNA gene belonging to *Anabaenopsis* and *Cyanospira* taxa were retrieved from the nucleotide NCBI database and aligned with sequences obtained in this study using the software CLUSTALX ver. 2.0 (Larkin et al., 2007). Sequences reported in this study have been deposited in the GenBank database under accession numbers (JX462677–JX462679). Alignment was checked visually using the Manual Sequence Alignment Editor, Align v05/2008 (Hepperle, 2008). The 16S rRNA gene sequence similarity (identity matrix) was calculated from all positions of the alignment including gaps using the program Align. For the phylogenetic analysis, 16S rRNA gene fragment of 1276 nucleotides was used. The phylogenetic tree was constructed by the maximum likelihood (ML) method using the program MEGA 5 (Tamura et al., 2011) with default settings, applying a HKY model of nucleotide substitution, which gave the best fit to this dataset. Confidence

values for the edges of the ML tree were computed by bootstrapping of 1,000 replications. *Gloeobacter violaceus* PCC 7421 (AF132790) was chosen as the outgroup.

The genetic potential of the field samples investigated to produce a variety of cyanotoxins was assessed using different primer sets. Primers and PCR protocol for each genetic locus HEPF/HEPR and DQmcyF/DQmcyR for microcystin/nodularin (Jungblut & Neilan, 2006; Al-Tebrineh et al., 2011), FAA/RAA for *mcy*B (Neilan et al., 1999), AnaC-genF/AnacC-genR for anacystin (Rantala-Ylinen et al., 2011) and sxtA-F/sxtA-R for saxitoxin (Al-Tebrineh et al., 2010) were employed for amplification.

Results

The phytoplankton biomass and composition as well as the occurrence of *Anabaenopsis* and *Cyanospira* exhibited very different patterns in all the four soda lakes studied (Figs. 1, 2, 3 and 4). In total, three species of *Anabaenopsis* and two species of *Cyanospira* were observed (Table 2). Each lake had a special dominant species of *Anabaenopsis* or *Cyanospira*, and there was no species common to all the lakes. The morphology of these species was highly variable. Their main diacritic characteristics are the shape and size of the vegetative cells, the presence and shape of heterocytes and akinetes as well as the general organisation of the colonies, including the production of mucilage (Table 2).

In Lake Bogoria, a *Cyanospira*-like cyanobacterium showed episodic appearances in 2009 and 2011 (Fig. 1). During the period from 2001–2006, *A. fusiformis* dominated the phytoplankton exclusively. During this phase, salinity showed stable levels of between 43 and 50 ppt. However, upon attaining a salinity maximum of 55 ppt in July 2008, the *Arthrospira* population was replaced by picoplanktonic green alga *Picocystis salinarum*. Subsequently, rainfall in the catchment area of Lake Bogoria diluted the lake water resulting in a salinity decline to 39 ppt in 2009. The impact of the salinity decline was the replacement of *Picocystis* by *Cyanospira* and a small population of *Arthrospira*. A second peak of *Cyanospira* was observed in January 2011 following the drop in salinity to 33 ppt. The dense blooms of *Cyanospira* were seen aggregating close to shoreline of the lake (Fig. 5a). The *Cyanospira* population in Lake Bogoria differed from other members of the *Anabaenopsis*/*Cyanospira* group in the other lakes by their colony, size and possession of dense and wide mucilaginous envelopes. In general, the colonies had a diameter of several millimetres and were visible to the naked eye (Fig. 5b). Negative staining with Indian ink revealed the presence of a dense slimy cover (Fig. 5c). Within the colonies, long chains of akinetes were present (Fig. 5d). Molecular investigations confirmed its species identity as *Cyanospira capsulata* (see phylogenetic tree). The Lesser Flamingos were not able to ingest these large mucilaginous colonies and the majority of the birds left the lake, resulting in low bird numbers.

In Lake Elmenteita, three species of *Anabaenopsis* were found regularly from 2001 to 2009 (Fig. 2). These species were recovered even when the lake had nearly dried out, resulting in a salinity increase to saturation levels of about 300 ppt in 2009. The contribution of *Anabaenopsis* to the phytoplankton biomass of the lake reached a maximum value of 88% of the total biomass in 2002. During this period, the dominant species of *Anabaenopsis* was *A. arnoldii* (Fig. 6a). After the rains and refilling of the lake in 2009, the salinity decreased to levels below 10 ppt and no *Anabaenopsis* was observed in the lake at this time of low salinity levels. In the period after January 2010, the phytoplankton biomass level remained below 30 mg l^{-1} and no flamingos visited the lake during this period. Overall, owing to the low biomass of *Arthrospira*, the number of flamingos at Lake Elmenteita was considerably lower than at the other three lakes (Fig. 2).

In Lake Nakuru, during the period between 2001 and 2006, stable populations of *Arthrospira* and *Anabaenopsis* accompanied by high to medium numbers of flamingos were observed (Fig. 3). In this lake, three different species of *Anabaenopsis* and one species of *Cyanospira* were observed with *A. abijatae* being the dominant taxon (Fig. 6b). *A. arnoldii* was frequently present in the plankton, whilst *A. elenkinii* and *C. rippkae* (Fig. 6c) only made periodic appearances with low individual counts during the last two years. The highest contribution of *Anabaenopsis* and *Cyanospira* to the total phytoplankton biomass was about 40% in 2001, 2003 and 2006. In August 2006, very unstable conditions, characterised by wide fluctuation in water level and a salinity level that oscillated

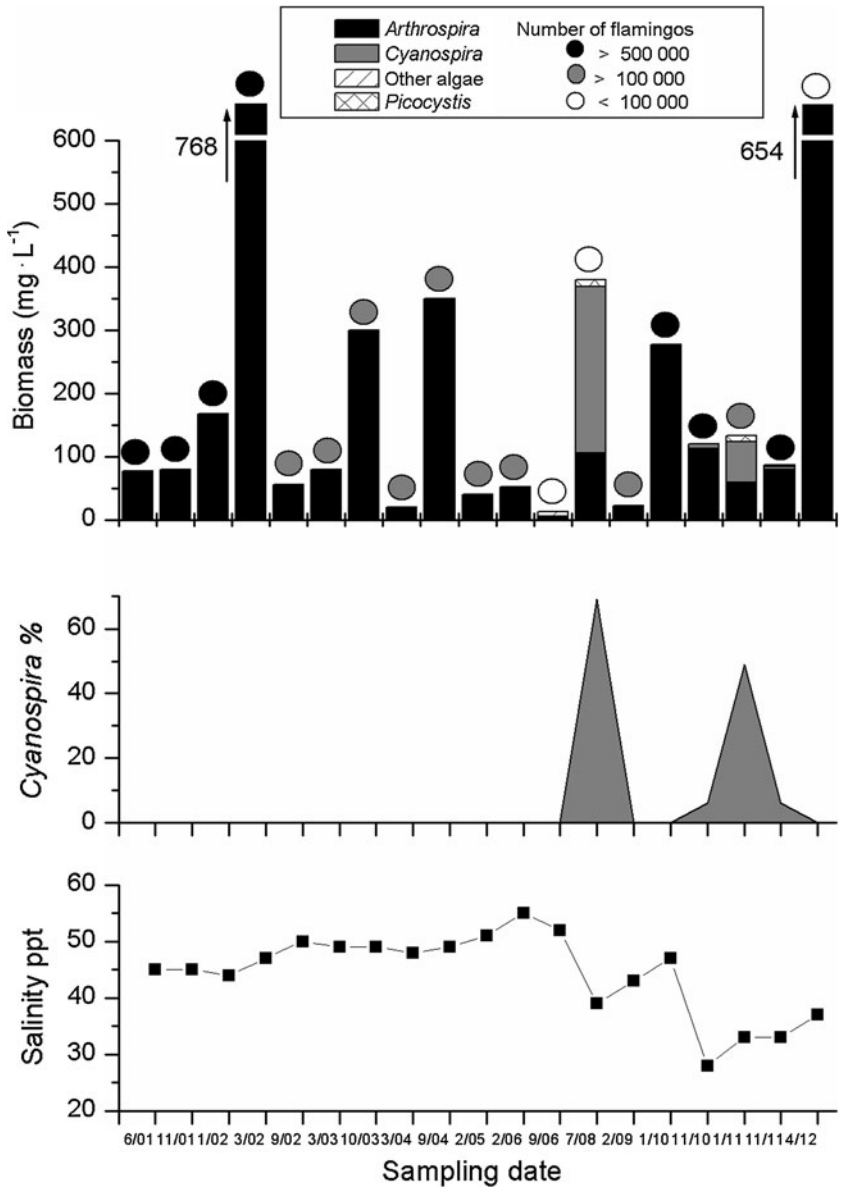

Fig. 1 Phytoplankton succession, contribution of the dominant taxa to phytoplankton biomass, number of flamingos, percentage contribution of *Cyanospira* to total phytoplankton biomass, and salinity levels in Lake Bogoria during the period 2001–2012

between 36 and 43 ppt and later dropped to a value of about 20 ppt, were witnessed in the lake. In January 2010, salinity rose to a maximum value of 51 ppt. At this peak salinity level, the cyanobacteria were outcompeted by the picoplanktonic green alga *P. salinarum*. In 2011, the rainfall received was above average leading to a dramatic increase in water level. The consequence of this was that salinity declined to levels of around 10 ppt. Since 2006, the conditions at the lake have not been supportive to the life of Lesser Flamingos with their numbers being generally low during this period. No flamingo was observed at the lake in November 2011 and April 2012 (Fig. 3).

In Lake Oloidien, a dramatic change in phytoplankton composition and flamingo numbers was observed during the study period (Fig. 4). In 2001, coccoid green algae dominated the algal community, accompanied by coccoid cyanobacteria such as *Chroococcus*. In the period from 2002–2005, the abundance levels of chroococcalean cyanobacteria

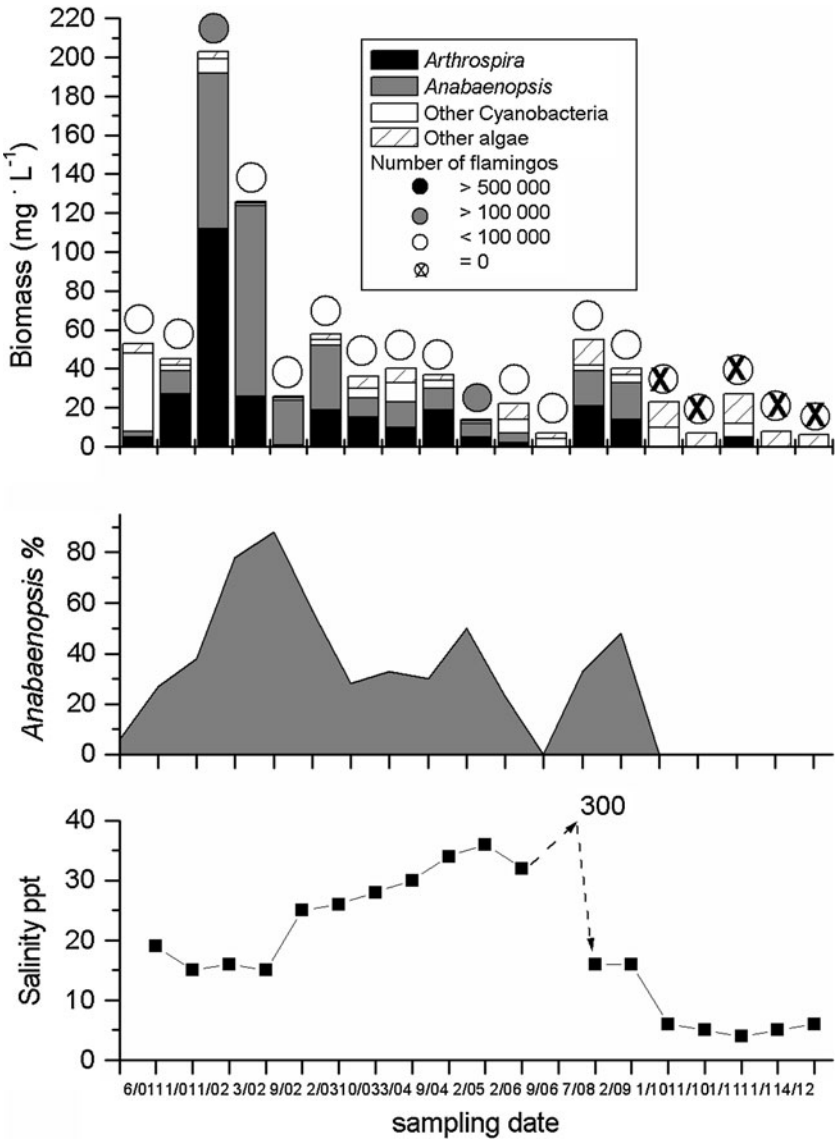

Fig. 2 Phytoplankton succession, contribution of the dominant taxa to phytoplankton biomass, number of flamingos, percentage contribution of *Anabaenopsis* to total phytoplankton biomass, and salinity levels in Lake Elmenteita during the period 2001–2012

fluctuated within a narrow range with slight progressive increase over time. However, with time, *Arthrospira* started being noticeable in the plankton, albeit in low concentration. Between 15 and 30% of the phytoplankton biomass during this period was due to *Anabaenopsis elenkinii*. Within this period, the salinity progressively increased from levels of below 2 ppt to around 3 ppt. From 2006 onwards, after salinity rose to above 5 ppt, *Arthrospira* assumed dominance of the plankton with a biomass of more than 100 mg l^{-1}. In the first years of *Arthrospira* assuming dominance, populations of *Anabaenopsis* remained at a subdominant level. However, towards the end of the study period, *Anabaenopsis* completely disappeared from the plankton. The mass development of *Arthrospira* appeared to have attracted the Lesser Flamingos into the lake resulting in a buildup in flamingo numbers from tens of thousands to more than 100,000 individuals.

The phylogenetic tree (Fig. 7), which included sequences of *Anabaenopsis* and *Cyanospira* that are presently available in GenBank, revealed the presence of two subclades (A, B). Subclade-A contained three species, *C. rippkae*, *A. abijatae* and *C. capsulata*,

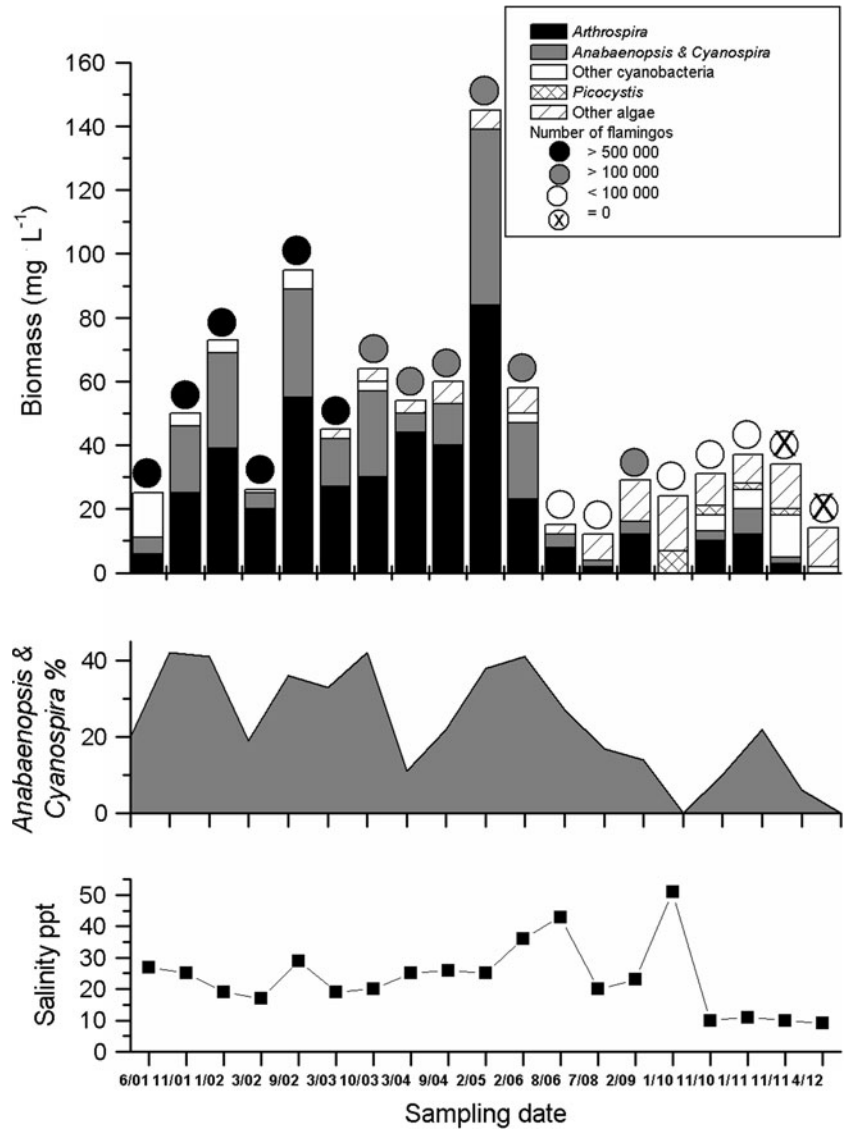

Fig. 3 Phytoplankton succession, contribution of the dominant taxa to phytoplankton biomass, number of flamingos, percentage contribution of *Anabaenopsis* and *Cyanospira* to total phytoplankton biomass, and salinity levels in Lake Nakuru during the period 2001–2012

whilst subclade-B had a total of four species, *A. circularis*, *A. elenkinii*, *A. nadsonii* and another species whose identity could not be determined. The clone sequences of our field samples corresponded to *A. abijatae* (Lake Nakuru), *C. capsulata* (Lake Bogoria) and *A. elenkinii* (Lake Elmenteita). Both subclades were strongly associated (>80% bootstrap support) in the phylogenetic tree. The subclade-A and subclade-B were also supported by high bootstrap values of 99 and 85%, respectively. Most lineages in the subclades exhibited high bootstrap values. A comparison of the 16S rRNA gene sequences of different species revealed a very high level of similarity, which was higher than 98% in all cases. Specific primers used for amplification of genetic loci responsible for production of cyanotoxins (microcystin, anatoxin and saxitoxin) did not reveal the presence of these loci. We therefore concluded that the three field samples studied did not contain cyanotoxins.

Discussion

The phytoplankton changes in the four soda lakes investigated during the period from 2001 to 2012 were

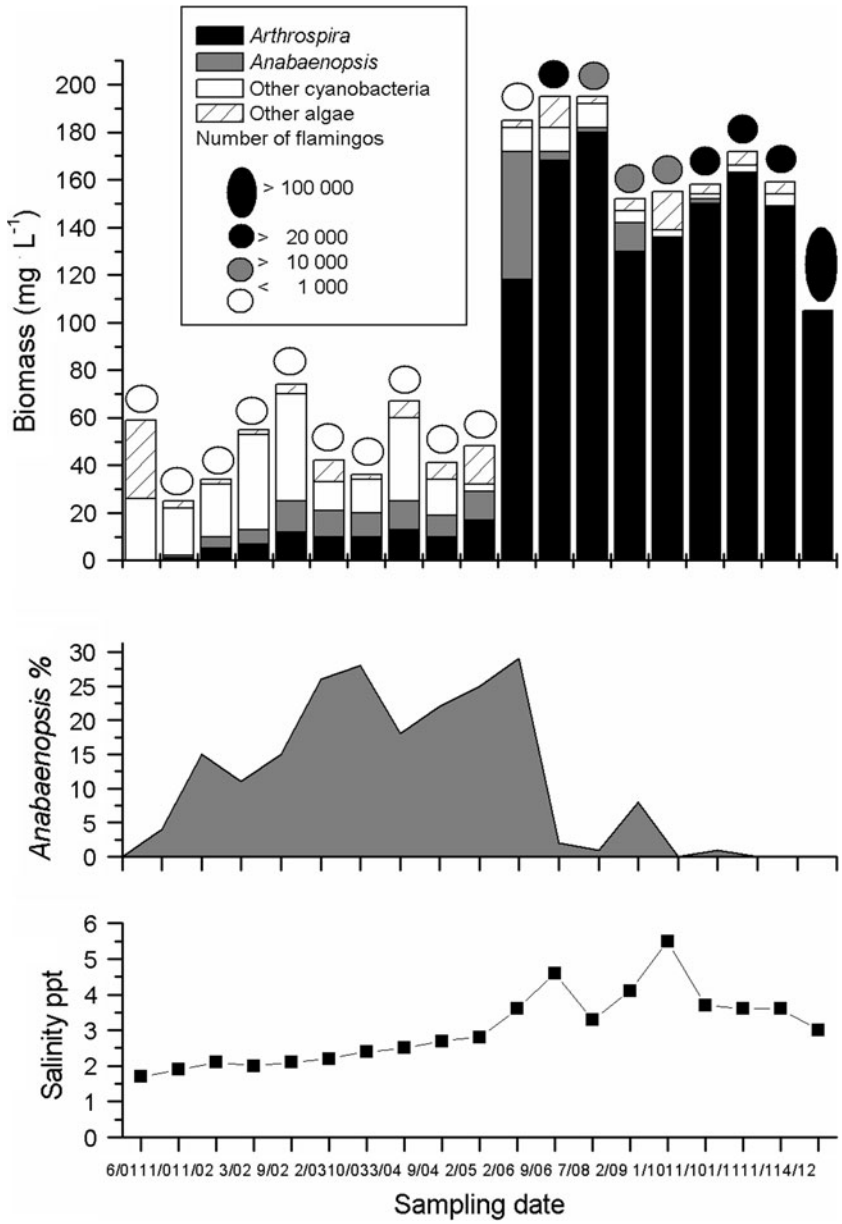

Fig. 4 Phytoplankton succession, contribution of the dominant taxa to phytoplankton biomass, number of flamingos, percentage contribution of *Anabaenopsis* to total phytoplankton biomass, and salinity levels in Lake Oloidien during the period 2001–2012

characterised by dominance changes between three main groups: (i) the oscillatorian cyanobacterium *A. fusiformis*, (ii) different members of the nostocalean genus *Anabaenopsis* and (iii) eukaryotic algae represented mainly by the prasinophyte *Picocystis*. *Arthrospira* is the main food for Lesser Flamingos, fish (*Tilapia grahami* Boulenger) and zooplankton such as copepods and rotifers (Vareschi, 1978, 1979; Vareschi & Vareschi, 1984). The entire functioning of the food web of saline–alkaline lake ecosystems in the African Rift Valley is solely based on *Arthrospira* as the principal primary producer. Every time this key species is outcompeted by other species, the food web is disturbed to a certain degree. *Picocystis* is too small to be ingested by the flamingos; however, it can be consumed by zooplankton (Krienitz et al., 2012). The impact of *Anabaenopsis* and *Cyanospira* on food consumption of the Lesser Flamingos is species specific. *C. capsulata* with its large mucilaginous colonies cannot be easily ingested by Lesser

Table 2 Phenotypic characteristics and abundance of five morphospecies of *Anabaenopsis* and *Cyanospira* in soda lakes of Kenya

	A. abijatae Kebede et Willén	*A. arnoldii* Aptekarj	*A. elenkinii* Miller	*C. capsulata* Florenzano, Sili, Pelosi et Vincenzini	*C. rippkae* Florenzano, Sili, Pelosi et Vincenzini
Vegetative cells	Asymmetric barrel-shaped 7–10 x 5-9 µm	Widely barrel-shaped 9–10 × 6–9 µm	Ellipsoid, sometimes slightly sausage-shaped 4–9 × 3–6 µm	Barrel-shaped 6–7.5 µm	Barrel-shaped 4.5–6 µm
Heterocytes	Spherical or asymmetric ovoid 6–12 × 6–11 µm	Spherical or broad oval 6–9 × 6–10 µm	Spherical 3–7 µm	Spherical 7–10.5 µm	Spherical 7–9 µm
Akinetes	Spherical, solitary 9–13 µm	Broad oval, solitary or in pairs 10 × 12 µm	Spherical, solitary or in pairs 10–12 µm	Spherical or asymmetrical flattened, often in long chains 8.5–10.5 µm	Spherical, often in chains 8–10 µm
Remarks	Dense, botryoid-shaped colonies	Trichomes always in wide helices	Trichomes normally in 1–2 coils, but in Oloidien frequently in 3–5 coils	Wide and dense mucilage	No mucilage
Abundances					
Bogoria				xxx	
Elmenteita	xx	xxx	x		
Nakuru	xxx	xx	x		x
Olodien			xxx		

Abundance: xxx, sometimes mass developments; xx, frequent; x, abundant

Flamingos, whilst species of *Anabaenopsis* are easily ingested. The nutritional value of *Arthrospira* is very high (Tokuşoglu & Ünal, 2003; Mühling et al., 2005; Zieliňska & Chojnacka, 2009). However, information on the nutritional value of *Anabaenopsis* or *Cyanospira* is not available. A study by Kaggwa et al. (2012) indicates a lower food quality of other phytoplankton species when compared to *Arthrospira* in Lake Nakuru.

Many members of Nostocales including species of *Anabaenopsis* (Lanares & Cook, 1994) are known to produce cyanotoxins (Carmichael et al., 2001; Codd et al., 2005). A high concentration of cyanotoxins (microcystin and anatoxin-a) was detected in water samples from Lake Nakuru (2001 and 2002) colonized by *Anabaenopsis* (Ballot et al., 2004). Interestingly, cyanotoxins were not detected in samples from Lake Elmenteita collected at the same date and containing comparable or even higher amounts of *Anabaenopsis* (Ballot et al., 2004). Our search for the toxin genes (*mcy*B, *mcy*E, *ana*C and *sxt*A) in the three field samples from Lakes Bogoria, Elmenteita and Nakuru included in the molecular analyses did not reveal the presence of cyanotoxin genes. However, a recent PCR-based study established the presence of amino-transferase domains and confirmed the presence of potential toxin-producing cyanobacteria closely related to *Anabaena* or *Anabaenopsis* in the sediment of Lake Nakuru (Dadheech et al., 2009). Aminotransferase domains were also detected in samples from Lakes Bogoria and Elmenteita; however, the nearest taxonomic entity present was related to *Microcystis*. According to Kotut & Krienitz (2011), the survival of *Microcystis* in the plankton of the highly saline–alkaline soda lakes of Kenya is very unlikely. Hence, these domains may belong to a yet to be discovered microcystin producer. Alternatively, some close relatives of *Microcystis* could have been introduced into the lake during the phases of low salinity and survived in the sediment.

Oduor & Schagerl (2007b) and Schagerl & Oduor (2008) found a negative correlation between abundance of *Anabaenopsis* spp. and conductivity. We confirmed this observation on several occasions.

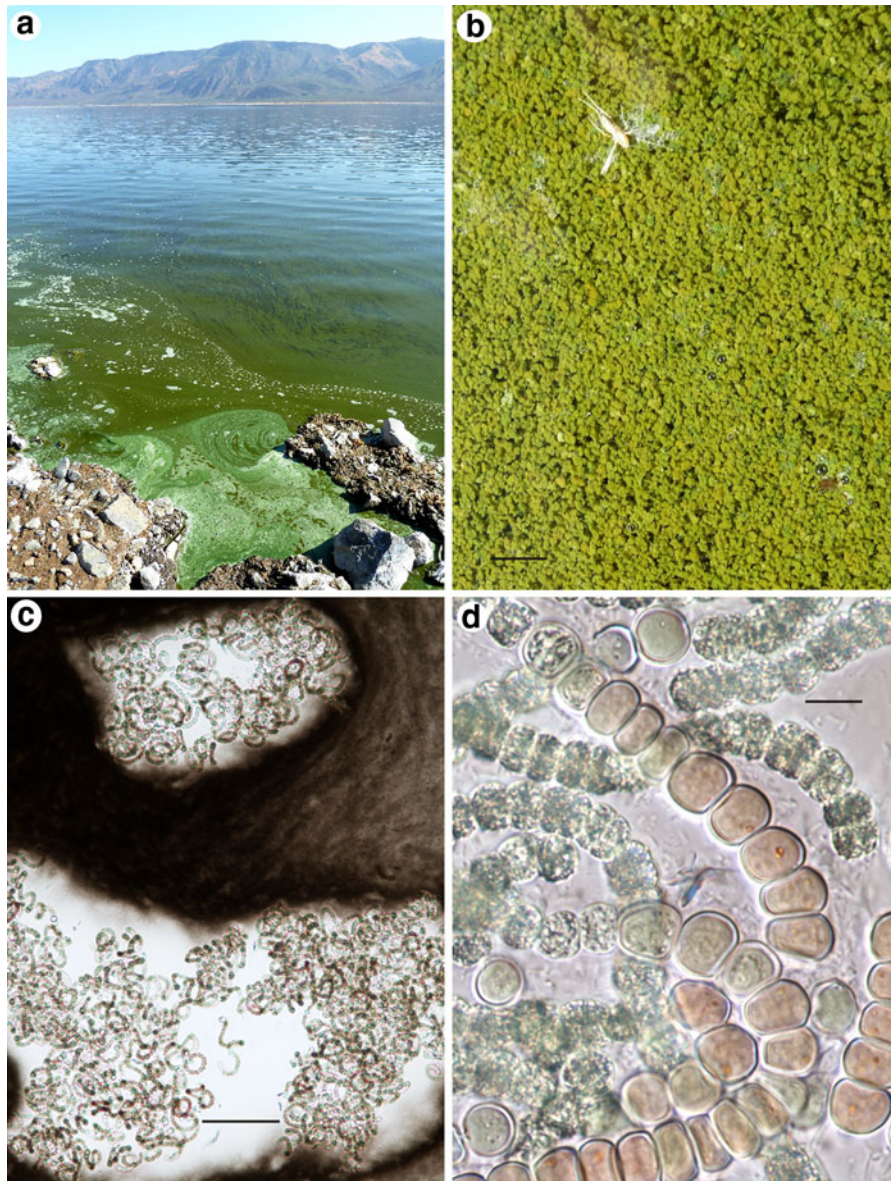

Fig. 5 Mass development of *Cyanospira capsulata* in Lake Bogoria in January 2011. **a** Scums on the shoreline. The pale material in foreground is *Arthrospira*, whereas the dark scums are formed by *Cyanospira*. **b** Visual appearance of scums of *Cyanospira*. *Scale bar* 1 cm. **c** Microscopic appearance of colonies under low magnification. Negative staining with Indian ink reveals the wide mucilaginous envelope. *Scale bar* 100 μm. **d** High magnification micrograph showing vegetative cells, heterocytes and chains of akinetes. *Scale bar* 10 μm

In Lake Bogoria, the two occasions characterised by mass development of *C. capsulata* coincided with a decrease in lake water salinity. Nevertheless, the different species of *Anabaenopsis* and *Cyanospira* exhibited preferences to different salinity ranges: the highest salinity values of between 30 and 40 ppt supported *C. capsulata* in Lake Bogoria. *C. rippkae* was observed to grow well at a salinity range of between 25 and 35 ppt in Lake Nakuru. *A. arnoldii* and *A. abijatae* preferred a salinity range of between 15 and 30 ppt in Lakes Elmenteita and Nakuru, but disappeared when salinity fell below 10 ppt. *A. elenkinii* preferred the more or less freshwater conditions in Lake Oloidien and were displaced by *Arthrospira* when salinity values rose above 3 ppt (Ballot et al., 2009). Interestingly, the same species (*A. elenkinii*) was able to survive at a salinity range from 5 to 15 ppt in Lakes Elmenteita and Nakuru.

A number of studies support the thesis that salinity is one of the driving forces behind evolution and diversification (Kirkwood et al., 2008). Cyanobacterial systematics have displayed several examples of convergent evolution of morphotypes in freshwater or low salinity habitats and extreme saline environments

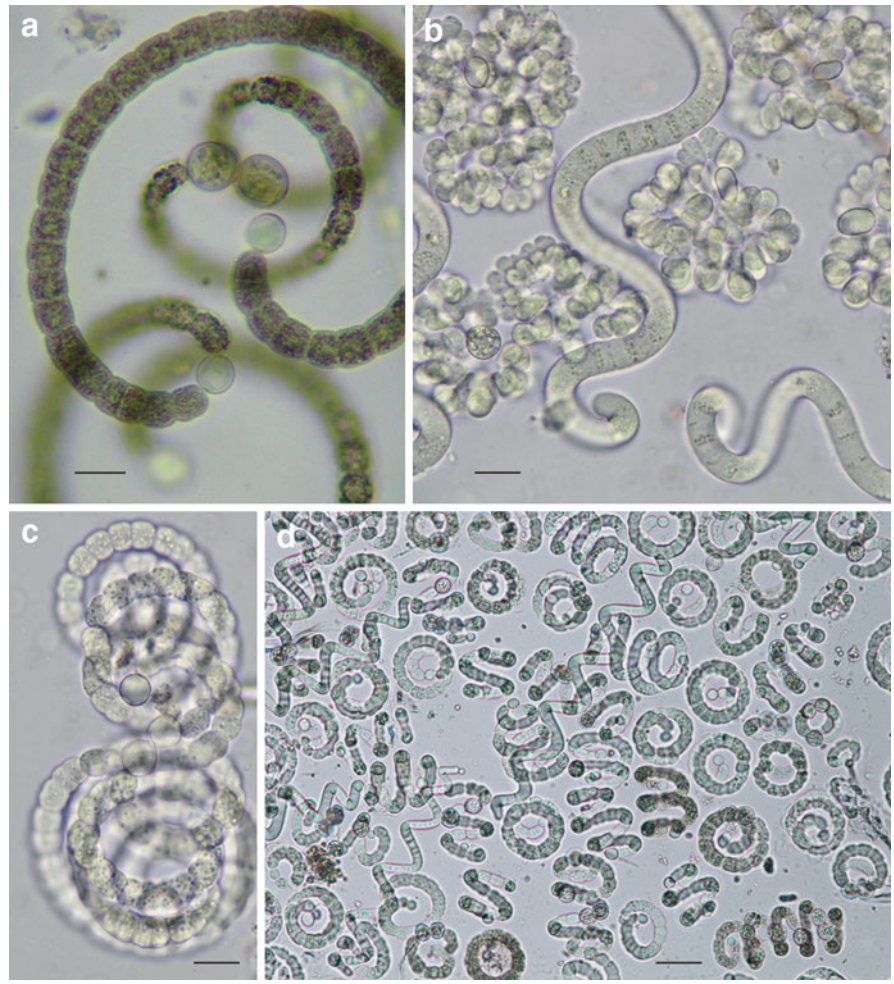

Fig. 6 Different species of *Anabaenopsis* and *Cyanospira*. *Scale bar* in **a–c** 10 μm, **d** 25 μm. **a** *A. arnoldii* in Lake Elmenteita with heterocytes and a pair of akinetes. **b** *A. abijatae* in Lake Nakuru, accompanied by wavelike filaments of *Arthrospira*. **c** *C. rippkae* in Lake Nakuru. **d** *A. elenkinii* accompanied by *Arthrospira* in Lake Oloidien

leading to the establishment of 'sister genera' (Sili et al., 2011). For example, *Spirulina* has a 'sister genus' in saline habitats, described as *Halospirulina* by Nübel et al. (2000). *Cyanothece* has two halophytic 'sisters' in *Halothece* and *Euhalothece* (Garzia-Pichel et al. 1998; Margheri et al., 2008). *Leptolyngbya* has a 'sister' genus in the soda lake Nakuru, which was recently described as *Haloleptolynbya* (Dadheech et al., 2012b). The 'sister genera' that are of interest to our present study are represented by *Anabaenopsis* and *Cyanospira* (Florenzano et al., 1985; Sili et al., 2011). Molecular data available until now for differentiating between *Anabaenopsis* and *Cyanospira* are not adequate. The two genera exhibit a very high percentage similarity in 16S rRNA gene sequences of >98%. Strains of the two type species, *A. elenkinii* and *C. rippkae*, showed a similarity of 98.20%. Conventionally, to differentiate between genera, a 95% similarity or lower in 16S rRNA gene sequence has been suggested for prokaryotes (Stackebrandt & Goebel, 1994). The use of 16S rRNA gene sequence similarity data in cyanobacterial systematics has been on the increase in the recent past (Nübel et al., 2000; Abed et al., 2002, Dadheech et al., 2012a, b). Komárek (2010) has suggested that the characterisation of genera should be based on 95% or less genetic similarity combined with at least one diacritical autapomorphic character. All the 'sister genera' listed using percent sequence similarity fulfil the minimum noticeable percentage difference of 5%. However, *Anabaenopsis* and *Cyanospira* only differed by less than 2% in 16S rRNA gene sequence similarity. Recent studies have referred to remarkable differences in relative binding, ribotypes or ITS sequences despite the very high 16S rRNA gene similarity (Rosselló-Mora & Amann, 2001; Iteman et al. 2000). However,

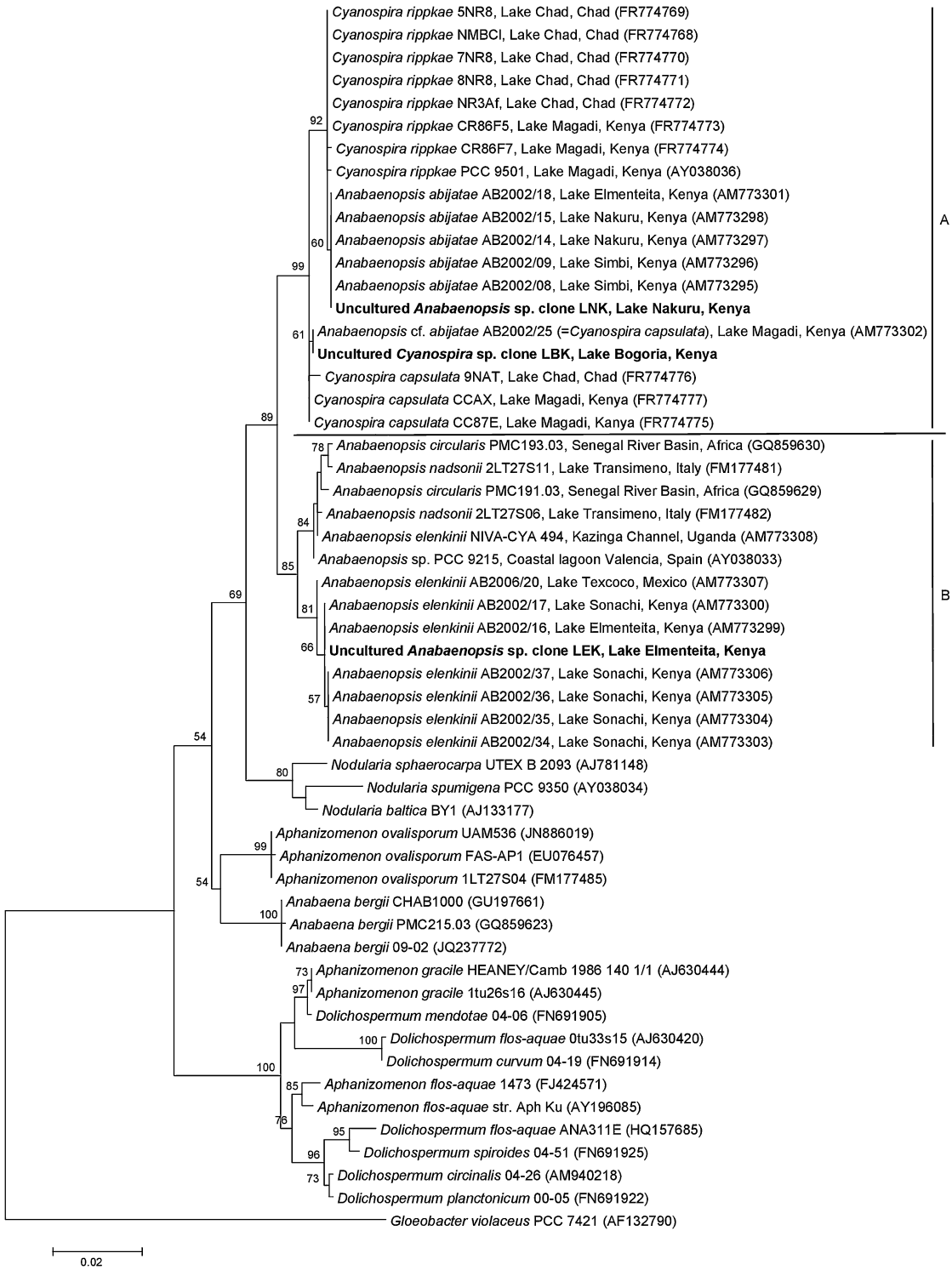

Fig. 7 Maximum likelihood phylogenetic tree of different species of *Anabaenopsis*, *Cyanospira* and related nostocalean cyanobacteria based on sequences of 16S rRNA gene. Numbers above branches indicate bootstrap support (50%) from maximum likelihood (1,000 replicates). Two uncultured clones of *Anabaenopsis* sp. and one clone of *Cyanospira* sp. from the three soda lakes are presented in larger bold font. *Gloeobacter violaceus* was selected as the out-group

these criteria are commonly used to differentiate species and not genera. Even Iteman et al. (2000), who strongly support the differentiation of *Anabaenopsis* and *Cyanospira*, expresses some doubt about this assignment. Komárek (2005) fused both genera and combined *Cyanospira rippkae* with *Anabaenopsis rippkae*. However, in a recent review of modern taxonomy of heterocytous cyanobacteria (Komárek & Mareš, 2012), the option of differentiating the two genera by other qualitative markers such as cytology and biochemical characteristics was left open. Sili et al. (2011) employed cytological criteria to differentiate *Cyanospira* and *Anabaenopsis*, such as the apoheterocytic development of akinetes, i.e. the ability to transform all vegetative cells between two heterocytes into akinetes. Finally, long chains of vegetative cells can develop to akinetes in these taxa. We also observed the chains of akinetes in our collections of *C. capsulata* and *C. rippkae*. However, this was not recorded in *A. abijatae,* which exhibits true branching as an exclusive character (Ballot et al., 2008). Common morphological features for all species of *Cyanospira* species included in the clade A are not available. Nevertheless, we leave the question open whether *Anabaenopsis* and *Cyanospira* genera should be fused or not, because available details are not sufficient at present.

We corroborate the observation by Sili et al. (2011) that comparatively, *C. capsulata* occurs in waters with the highest salinity followed by *C. rippkae*. In our study, *C. capsulata* was observed in Lake Bogoria under meso- to hypersaline conditions, whilst *C. rippkae* occurred in Lake Nakuru (see also Kotut & Krienitz, 2011, Fig. 1g) under a comparatively lower salinity condition. The presence of *Anabaenopsis* taxa in fresh and moderate saline waters, such as the occurrence *A. elenkinii* in Lakes Oloidien, Elmenteita and Nakuru, supports the observation by Komárek (2005) that *Anabaenopsis* taxa occur in both fresh and moderate salinity waters. Other species known to occur in fresh and saline waters include *A. arnoldii* (Taylor, 1932; Komárek, 2005), *A. doliiformis* Noda (Komárek 2005), and *A. circularis* (G.S. West) Wołoszynska et Miller (Rich, 1933; Komárek, 2005). For example, *A. circularis* was found by Rich (1933) in the large freshwater Lake Edward, Uganda, as well as in the saline Lake Nakuru, Kenya. In clade B of our phylogenetic tree (Fig. 7), *A. circularis*, *A. nadsonii* and *A. elenkinii* are closely related and need more consideration in order to confirm whether they are distinct species. According to Jeeji-Bai et al. (1980), *A. nadsonii* is synonymous with *A. elenkinii*. The phylogenetic position of *A. arnoldii* has remained unresolved because all our attempts to isolate it or to establish clones from the field samples that match this species have repeatedly failed.

Komárek (2005) has concluded that there are sufficient arguments to delineate the genus *Anabaenopsis* from other members of Nostocales. However, he takes cognizance of the high variability of *Anabaenopsis* morphospecies: 'Each population differs only slightly from other ones, and transitional forms occur between almost all described species' (loc. cit. p. 1). Consequently, we therefore used available tools of polyphasic approaches including morphology, ontogeny, ecology, physiology and molecular phylogeny to study members of this amazing genus and its relationship with *Cyanospira*.

Acknowledgments We thank the Government of Kenya for permission to carry out this research (No. MOEST 13/001/31 C 90, and NCST/RRI/12/1/BS/232).

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