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PRIMARY RESEARCH PAPER

Haloleptolyngbya alcalis gen. et sp. nov., a new filamentous cyanobacterium from the soda lake Nakuru, Kenya

Pawan K. Dadheech · Huda Mahmoud · Kiplagat Kotut · Lothar Krienitz

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Abstract The food web of the saline-alkaline Lake Nakuru is dominated by the cyanobacterium *Arthrospira fusiformis* as the primary producer and a huge population of Lesser Flamingos as direct consumers. However, the dense blooms of *Arthrospira* are not stable, and collapse irregularly and unpredictably. During such periods they are replaced by other algae or cyanobacteria. The wide fluctuation in the cyanobacterial and algal populations of Lake Nakuru has a great influence on food availability for Lesser Flamingos, and is therefore of high ecological importance. To support the descriptive work on these phenomena, we describe here a new cyanobacterial taxon from this soda lake: *Haloleptolyngbya alcalis* Dadheech, Mahmoud, Kotut et Krienitz gen. et sp. nov. The study was based on multilocus molecular analyses of 16S rRNA gene, 16S-23S internal transcribed spacer, partial sequences of beta and alpha subunits including intergenic spacer (*cpc*BA-IGS) of phycocyanin operon, phenotypic features using light microscopy, scanning electron microscopy, transmission electron microscopy, and ecology. The new taxon established a separate lineage within the family of Peudanabaenaceae (Oscillatoriales).

Keywords *Haloleptolyngbya* · Cyanobacteria · Molecular phylogeny · Lake Nakuru · *Leptolyngbya* · Lesser Flamingo · Soda lakes

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P. K. Dadheech (✉)
Department of Botany, Government College, Ajmer 305001, Rajasthan, India
e-mail: pdadheech@igb-berlin.de; pdadheech@yahoo.com

P. K. Dadheech · L. Krienitz
Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Alte Fischerhütte 2, 16775 Stechlin, Germany

H. Mahmoud
Department of Biological Sciences, Kuwait University, P.O. Box 5969, Safat 13060, Kuwait

K. Kotut
Department of Plant and Microbial Sciences, Kenyatta University Nairobi, P.O. Box 43844, Nairobi GPO 00100, Kenya

Introduction

Lake Nakuru is an alkaline-saline lake in the Gregory Rift Valley in Kenya. It is home to a huge number of Lesser Flamingos (*Phoeniconaias minor* Geoffrey). Frequently, more than 1 million of these spectacular birds have been observed at the lake (Brown, 1973; Mari & Collar, 2000). The dominant phytoplankton is the cyanobacterium *Arthrospira fusiformis* (Voronichin) Komárek et Lund, which serves as the preferred food source for the Lesser Flamingos—the main primary consumer at the soda lakes of East Africa (Vareschi, 1978; Vareschi & Jacobs, 1985). Despite the existence of different morphotypes of *Arthrospira*

in East African saline lakes, the phylotype is identical and belongs to one and the same species *A. fusiformis* (Dadheech et al., 2010). The dense populations of *Arthrospira* are not stable and collapse irregularly and unpredictably (Vareschi, 1978; Tuite, 1981; Melack, 1988). This results in flamingos migrating to other lakes (Childress et al., 2004, 2008) or switching to a diet of benthic diatoms which are of lower productivity and nutritional value (Tuite, 2000).

Being a very shallow lake [mean depth 110 cm, maximum depth 450 cm, according to Schagerl & Oduor (2008)] Nakuru experiences a more frequent collapse of *Arthrospira* populations than the neighboring deeper soda lake Bogoria (Harper et al., 2003). During such periods, *Arthrospira* is usually replaced by other cyanobacteria or eukaryotic algae (Vareschi, 1978; Schagerl & Oduor, 2008; Krienitz & Kotut, 2010; Krienitz et al., 2011, 2012). Lake Nakuru is strongly influenced by weather conditions, and its water level fluctuates dramatically due to precipitation or drying up events. This results in considerable changes in the physico-chemical properties of the water body. The plankton community is often recruited from taxa which are hidden in the "ecological memory" of the lake. Such taxa were present in the phytoplankton in the past (may be unobserved) and are hidden in the sediments or other niches. In response to changes in environmental conditions, these species can establish new populations within a short period, which consequently changes the plankton community structure and succession pattern. Hence, the taxa play an important role of influencing the present or future responses of the community. This phenomenon has been observed in different lakes in Europe (Padisák, 1992; Padisák et al., 2010). Dadheech et al. (2009) detected DNA of several dormant taxa in the plankton and sediment of Kenyan lakes, such as *Chroococcidiopsis* which were not detectable by microscopy.

Over long periods in the last decade, *Arthrospira* populations in Nakuru were associated with heterocyst-forming nostocalean cyanobacteria of the genus *Anabaenopsis* (*A. abijatae*, and *A. arnoldii*) (Ballot et al., 2004a, 2008; Oduor & Schagerl, 2007; Schagerl & Oduor, 2008; Kotut & Krienitz, 2011), and *Cyanospira* (Sili et al., 2011). In periods of low water level, mats of cyanobacteria with tiny filaments ($\sim$2 μm in diameter) of uncertain taxonomic designation have been observed to rise from the sediments and establish on the water surface (Kotut, unpubl. observations). These cyanobacteria do not form heterocysts, and belong to the Oscillatoriales. The wide fluctuations in the cyanobacterial and algal population of Lake Nakuru have a great influence on the food availability for Lesser Flamingos, and are therefore of high ecological importance. Hence, to support the descriptive work on these phenomena, the systematics of organisms that regularly outcompete the populations of *Arthrospira* should be studied carefully.

In this paper, we provide the description of a new filamentous cyanobacterium, *Haloleptolyngbya alcalis* gen. et sp. nov., from Lake Nakuru, which has the potential to establish tychoplanktonic mats in the lake at the end of its life cycle.

Materials and methods

Lake and phytoplankton characteristics at sampling time

Detailed characteristics of Lake Nakuru are provided by Vareschi (1978) and Ballot et al. (2004a). During a sampling exercise carried out on February 2, 2005, we measured the electrical conductivity, salinity, and pH of the lake water using a WTW Multiline P4 meter (Wissenschaftlich Technische Werkstätten, Weilheim, Germany) at two sampling points (Table 1). During this period, the phytoplankton of Lake Nakuru was heterogeneously distributed. At the sampling point located next to the Baboon Cliffs, a dense *Arthrospira*-population that occurred in association with *Anabaenopsis abijatae* and *Anabaenopsis arnoldii*, few cryptomonads and chlamydomonads were observed. At the Cormorant Point sampling site, near the inflow of the Njoro River, only a few individuals of *Arthrospira* and *Anabaenopsis* were observed in a sample dominated by different flagellates (chlamydomonads, cryptomonads,

Table 1 Physico-chemical properties of two sampling points in Lake Nakuru on February 2, 2005

Parameter	Baboon Cliffs	Cormorant Point
Conductivity (mS cm^{-1})	38.9	40.1
Salinity (ppt)	24.8	25.9
pH	10.24	10.08

and euglenoids). Furthermore, few filaments of *Haloleptolyngbya* were observed at both sampling points.

Culture and light microscopy

Filaments of *Haloleptolyngbya* were isolated by micropipettes and transferred to 15-ml culture tubes for the establishment of clonal cultures. The strain KR 2005/106, which is the subject of this investigation, was maintained at the algal culture collection of the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in suspension using a modified Bourrelly medium (Krienitz & Wirth, 2006), which was enriched with 0.3 g Na_2CO_3 and 15 g NaCl per liter. The strain was grown at room temperature under a 14 h:10 h light–dark regime. The strain was deposited under the designation UTEX B ZZ879 in collection UTEX (The Culture Collection of Algae at The University of Texas at Austin, USA). An air-dried as well as a formaldehyde-fixed sample was deposited at the Botanical Museum at Berlin-Dahlem, Germany, under the designation B 40 0040749. The cyanobacterium was studied under a Nikon Eclipse E600 light microscope with differential interference contrast (Nikon Corporation, Tokyo, Japan). Microphotographs were taken using a Nikon digital camera DS-Fi1 and Nikon software NIS-Elements D. The drawing of the iconotype of *H. alcalis* was prepared using a graphics editor application "ThumbsPlus 7" (Cerious Software Inc., USA).

Scanning and transmission electron microscopy (SEM and TEM)

Cyanobacterial samples were fixed with 3% glutaraldehyde in Millonigs (Millonig, 1964) phosphate buffer (MPB) (pH 7.2) for 24 h. The specimens were washed three times with the same buffer before they were post fixed with 2% osmium tetraoxide in MPB (pH 7.2) for 2 h. Finally, they were washed three times with MPB before being dehydrated in a graded ethanol series (i.e., ascending concentration series of ethanol, starting with 30% and ending with 100% ethanol). The above mentioned steps were performed at room temperature inside a fume cupboard on a slow-speed rotator (INFIL-Tissue Rotator, USA). The samples were dehydrated by critical point dryer (Bal-TEC, CPD 030, Australia) and mounted on aluminum stub, and then light gold-coated for 2 min in a Sputter Coater (BAL-TEC, SCD050, Australia). Coated samples were examined directly under the Leo Supra 50 V Variable Pressure Field Emission Scanning Microscope (Zeiss, Germany).

For TEM, the cyanobacterial samples were fixed, washed, postfixed, and dehydrated as described above for SEM procedure. The dehydration step was carried out in Leica Automatic Processor (Reicheart-Lynx, Australia). The samples were embedded in Epon resin and sectioned at 90 nm using ultra-microtome (LEICA Ultracut-UCT, Austria) with a diamond knife before being transferred onto 150-mesh copper grids. The prepared sections were stained with 2% uranyl acetate and lead citrate using LEICA EM staining instrument, and examined using the transmission electron microscope (TEM, JEOL's JEM-1200 EX II, Japan). Pictures were taken using digital camera (Gatan CCD Camera, USA).

Molecular methods: genomic DNA extraction and PCR amplification

The genomic DNA of the strain was extracted using Dynabeads DNA DIRECT System I (Invitrogen/Dynal Biotech, Oslo, Norway) following the steps outlined in the manufacturer's manual. The polymerase chain reaction (PCR) of 16S rRNA gene, 16S-23S internal transcribed spacer (ITS) region, and *cpc*BA-IGS locus was performed in a Peltier Thermal Cycler PTC 200 (MJ Research Inc., San Francisco, USA). Each 20 µl PCR cocktail contained 14.4 µl of sterile water, 2.0 µl of 10× PCR buffer A (Qiagen, Hilden, Germany), 0.5 µl of 20 mM dNTPs (Qiagen) in an equimolar ratio, 1 µl of a 10 µM concentration of the forward primer, 1 µl of a 10 µM concentration of the reverse primer, 0.1 µl of *Taq* DNA polymerase enzyme (5 U/µl from Qiagen), and 1.0 µl of sample DNA. The amount of template DNA was adjusted when necessary to generate sufficient PCR products for DNA sequencing. The primers pA and B23S (cyanobacteria specific) were used for the amplification of 16S rRNA gene (Edwards et al., 1989; Gkelis et al., 2005) with an annealing temperature of 50°C. The primers 322 and 340 (Iteman et al., 2002) were used for the amplification of ITS between 16S rRNA and 23S rRNA genes, and the primers cpc_arF and cpc_arR (Ballot et al., 2004b) were employed to obtain a partial sequence of *cpc*BA-IGS. The PCR protocols for the amplification of both regions were used as

described by Dadheech et al. (2010). Amplified products were purified through Qiaquick PCR purification columns (Qiagen) according to manufacturer's protocol.

The genetic potential of the studied *Haloleptolyngbya* taxon 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.

DNA sequencing and phylogenetic analyses

The amplified fragments of 16S rRNA gene were sequenced using the primers pA, pC, pE, pDr, pFr, and pHr (Edwards et al., 1989) to retrieve complete sequence. The purified products of ITS and *cpc*BA-IGS were sequenced using the same primers as used for the PCR. Both strands were sequenced on ABI 3100 Avant Genetic Analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Applera, Deutschland, GmbH, Darmstadt, Germany) as described in the manufacturer's manual.

The cyanobacterial sequences of 16S rRNA were retrieved from nucleotide database SILVA (Pruesse et al., 2007) and aligned by software CLUSTALX ver. 2.0 (Larkin et al., 2007). Alignment was checked visually using the Manual Sequence Alignment Editor Align v05/2008 (Hepperle, 2008). Sequence similarity (identity matrix) of the closest strains was calculated from all positions of the alignment including gaps using the program Align. The closest relatives to our strain were detected using the BLAST software (www.ncbi.nlm.nih.gov/blast). For the phylogenetic analyses, we initially selected a large group of sequences (>900 bp) belonging to non-heterocystous taxa to examine phylogenetic position of our newly sequenced strains. Later, a smaller subset with 104 taxa including our strain was selected from the large data set based on the relatedness of the sequences and excluding taxa of uncertain affiliation. To gain a synoptic view of the phylogenetic tree, only sequences of oscillatorian members were chosen as specimens. Phylogenetic analysis was carried out using 16S rRNA gene fragment of 1,000 nucleotides. The tree presented was constructed by the maximum likelihood (ML) method using the program TREEFINDER (Jobb et al., 2004) with default settings, applying a GTR+GI+5 model of nucleotide substitution because its fit to the present dataset was superior to other models according to the Akaike information criterion (AICc). Confidence values for the edges of the maximum-likelihood tree were computed by bootstrapping of 1,000 replications. Alternatively, we built parsimony, NJ trees using the program MEGA 5 (Tamura et al., 2011), and compared the topologies of the obtained trees to establish and validate the phylogenetic position of the studied strain (data not shown). *Gloeobacter violaceus* was chosen as the out-group.

The pattern of ITS sequences and their various regions were determined with the reference sequences (Iteman et al., 2000; Boyer et al., 2001). Secondary structures of the 16S-23S rRNA ITS regions were determined using RNA Mfold version 3.5 (Zuker, 2003) with default settings. The overview graphics of the secondary structures of ITS regions were performed in Pseudo Viewer ver. 2.5 (Byun & Han, 2003).

The nucleotide sequences reported in this study have been deposited in the NCBI database under the GenBank accession numbers JN712770 (16S rRNA gene) and JN712771 (16S-23S ITS) and JN712769 (*cpc*BA-IGS).

Results

Description

Haloleptolyngbya Dadheech, Mahmoud, Kotut et Krienitz gen. nov.

Diagnosis: Thallus constans ex tege tenui plana, aeruginosus. Filamenta solitaria vel dense tomentosa, tychoplanctica vel ad substrata affixa. Filamenta longa, recta vel undulata, ad apices rotundata, 1.2–1.9 μm lata, ad septa constricta. Vagina incolorata, mucilaginosa, trichomata involvens. Cellulae cylindricae, elongatae vel isodiametricae, sine aerotopis. A generibus ceteris familiae Pseudanabaenaceae morphologia et ordine nucleotidorum in 16S rRNA, PC-IGS et ITS differt.

Thallus thin, pale to bright blue: Filaments solitary or densely entangled to floating mats, tychoplanktonic or attached to the substrate. Filaments long, straight or wavy, with rounded ends, 1.2–1.9-μm wide,

constricted at the cross-walls. A colorless sheath covers the trichomes. Cells cylindrical, elongated or isodiametric, without aerotopes: the genus differs from other members of cyanobacteria in sequence of 16S rRNA gene, PC-IGS locus, and secondary structure of ITS regions.

Typus generis: *H. alcalis* Dadheech, Mahmoud, Kotut et Krienitz, sp. nov.

Etymology: the name of the genus refers to the saline habitat of the cyanobacterium and to the morphological similarity to members of the genus *Leptolyngbya*.

Haloleptolyngbya alcalis Dadheech, Mahmoud, Kotut et Krienitz, sp. nov.

Diagnosis: Sicut pro ordine. Thallus constans ex tege tenui plana, aeruginosus. Filamenta solitaria vel dense tomentosa, tychoplanctonica vel ad substrata affixa. Filamenta longa, recta vel undulata, ad apices rotundata, 1.2–1.9 μm lata, ad Septa constricta. Vagina incolorata, trichomata involvens. Cellulae cylindricae, elongatae vel isodiametricae, sine aerotopis.

Thallus thin, pale to bright blue. Filaments solitary or densely entangled to floating mats, tychoplanktonic or attached to the substrate. Filaments long, straight or wavy, with rounded ends, 1.2–1.9-μm wide, constricted at the cross-walls. A colorless sheath covers the trichomes. Cells cylindrical, elongated, or isodiametric, without aerotopes.

Holotype (designated here): a dried sample of the culture strain KR 2005/106 under the designation B 40 0040749 in the Herbarium at the Botanical Museum at Berlin Dahlem, Germany.

Cultura typica: a living culture deposited at UTEX, The Culture Collection of Algae at the University of Texas at Austin under the designation UTEX B ZZ879.

Type locality: Lake Nakuru, Kenya. GPS Data: S 00°19.583′; E 36°05.325′

Etymology: the specific epithet refers to the alkaline conditions at the locus classicus.

Icona typica: a drawing of *H. alcalis* strain KR2005/106 (Fig. 1).

Phenotypic characterization

The tropical tychoplanktonic cyanobacterial strain, herein described as a new genus *H. alcalis* from saline-alkaline Lake Nakuru (Table 1), was found as free floating filaments or mats or attached to substratum of the lake. The shape of the filaments was variable from straight to wavy (Fig. 2a–c). The motility of the filaments was not observed. Trichomes were enclosed by a firm, lucid, hyaline sheath, and an empty sheath was clearly visible in intact filaments (Fig. 2c) as well as in those breaking up (Fig. 2a). Multiplication of filaments proceeded by fragmentation. Although the trichome cells were usually longer, isodiametric shapes were observed during the division of cells. Cells were found to be 1.2–2.1-μm long and 1.2–1.9-μm wide. The constriction at the cross-walls of the cells was visible (Fig. 2a–c). Attenuation in the filaments was not recorded, and the end was rounded without a calyptra (Fig. 2b). The cell contents were heterogeneous, and appeared dense toward periphery and light in the centre (Fig. 2a–c).

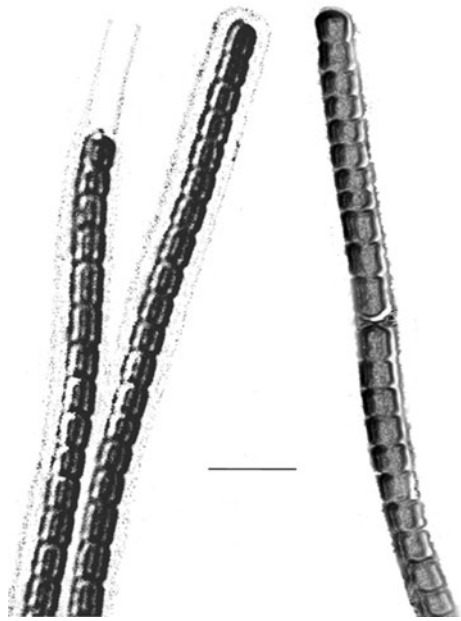

Fig. 1 Iconotype of *Haloleptolyngbya alcalis* strain (KR2005/106) prepared with ThumbsPlus 7 (graphics editor application). Scale bar = 5 μm

Ultrastructural characterization

SEM showed that filaments have a distinct sheath, which is sometimes absent in some individuals, with bacteria often adhering to surfaces of the cell wall and the sheath (Fig. 3a). Distinct constriction at the cross-wall of cells and rounded apical cells were clearly visible under SEM examination (Fig. 3a). TEM observations (Fig. 3b–d) supported SEM results. The

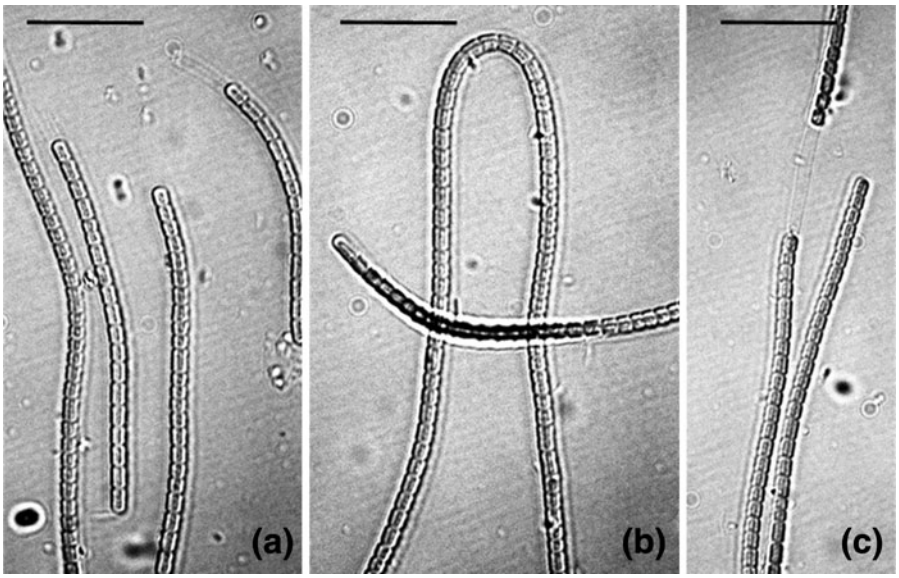

Fig. 2 *Haloletolyngbya alcalis*. **a–c** Straight and wavy filaments; **b** rounded end cell without calyptra; and **c**, **a** sheath during and after breakage of filaments. Scale bars 10 μm

trichomes were confirmed to be uniseriate with cylindrical cells; mostly longer than broad with constriction at the cross-walls, and with rounded apical cells. They lacked heterocysts and akinetes, and were surrounded by persistent, fibrous, visible multi-layered sheath (Fig. 3b, c) that was separated from the cell wall by a transparent zone. The thickness and density of the fibrous layer was variable. Cell walls and cross-walls were distinct under TEM observations. No visible intracellular connection between vegetative cells was observed (Fig. 3d). Thylakoids showed a parietal arrangement in the longitudinal and cross section of cells (Fig. 3b–d) with a parallel arrangement at the inner periphery of the cell, where it forms concentric rings around the nucleoid (Fig. 3b, c). A tangential section for the thylakoids showed the rows of closely packed phycobilisomes running at an angle to the long axis of the cell (Fig. 3d). Large carboxysomes, polyphosphate bodies, and small lipid droplets were present in the cytoplasm of most cells and sometimes granules could be found attached to the cell wall (Fig. 3c, d).

Molecular phylogenetics

The 16S rRNA gene sequence (1,432 bp) was obtained for the novel strain. BLASTN searches against the non-redundant nucleotide sequence database (NCBI) revealed that the sequence of novel taxon is mostly similar to 16S rRNA gene sequences which correspond to four top hits (accession numbers AJ639894, FR798934, EF654067, and AJ639895). Sequence similarity with these sequences was 94% only. To ascertain the precise phylogenetic position of *H. alcalis*, we conducted a detailed phylogenetic analysis employing a comprehensive selection of 16S rRNA gene sequences from different members of Oscillatoriales particularly Pseudanabaenaceae. The resulting tree (Fig. 4) indicated that *H. alcalis* is phylogenetically close to species identified as *Leptolyngbya*. However, our strain formed a separate lineage showing significant divergence from closely related taxa with 99% bootstrap value.

A sequence of 523 bp of the ITS region was obtained by PCR amplification of the 16S-23S ITS region. In BLAST analysis, the identity of ITS region of our strain and sequences available in GenBank was lower than 82%, and was difficult to align. The ribosomal operon of our strain possesses an ITS region with both $tRNA^{Ile}$ and the $tRNA^{Ala}$ genes. In addition to these genes, the ITS region also contained other conserved and variable domains (data not shown). The secondary structures of D1–D1′, Box B, and V3 regions of the novel strain, and morphologically

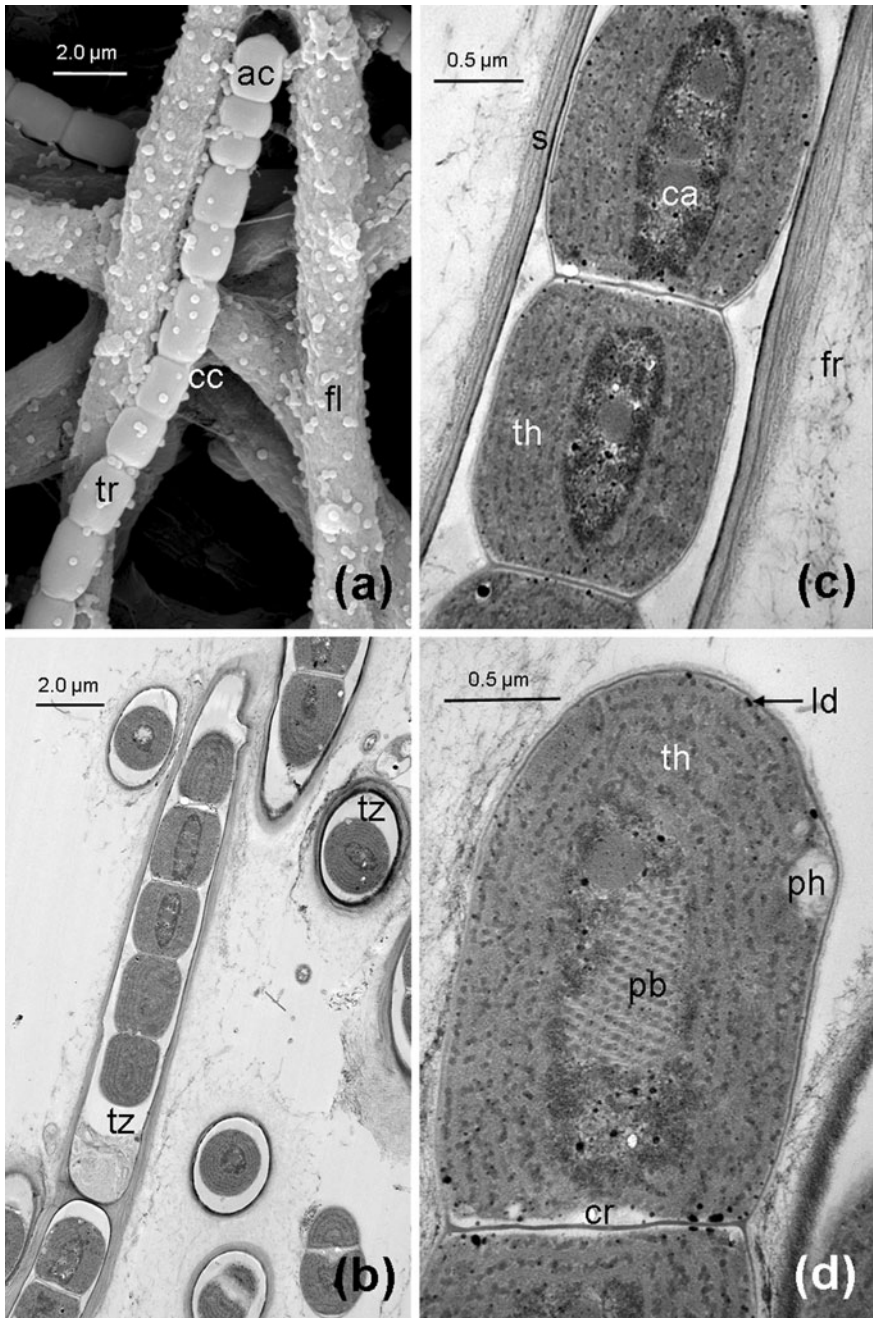

Fig. 3 SEM and TEM images of *Haloleptolyngbya alcalis*. **a** SEM image showing trichomes with or without sheath and shape of apical cell; **b–d)** TEM of sectioned filaments in longitudinal view and cross-sectional view showing ultrastructural details. *ac* apical cell, *cc* constriction at the cross-wall, *tr* trichome without sheath, *fl* filament with sheath, *tz* transparent zone between cell wall and sheath, *s* sheath, *th* thylakoids, *ca* carboxysome, *fr* microfibrillar structures, *ld* lipid droplet, *ph* phosphate body, *pb* closely packed phycobilisomes, *cr* cross-wall

related type species of *Leptolyngbya* (*L. boryana*; EF429290), and available sequence of most close in 16S rRNA gene similarity (Pseudanabaenacea cyanobacterium: FR798943) were constructed for comparison (Fig. 5a–i). The D1–D1′ helix of the novel taxon contained 61 nucleotides (nt), and in

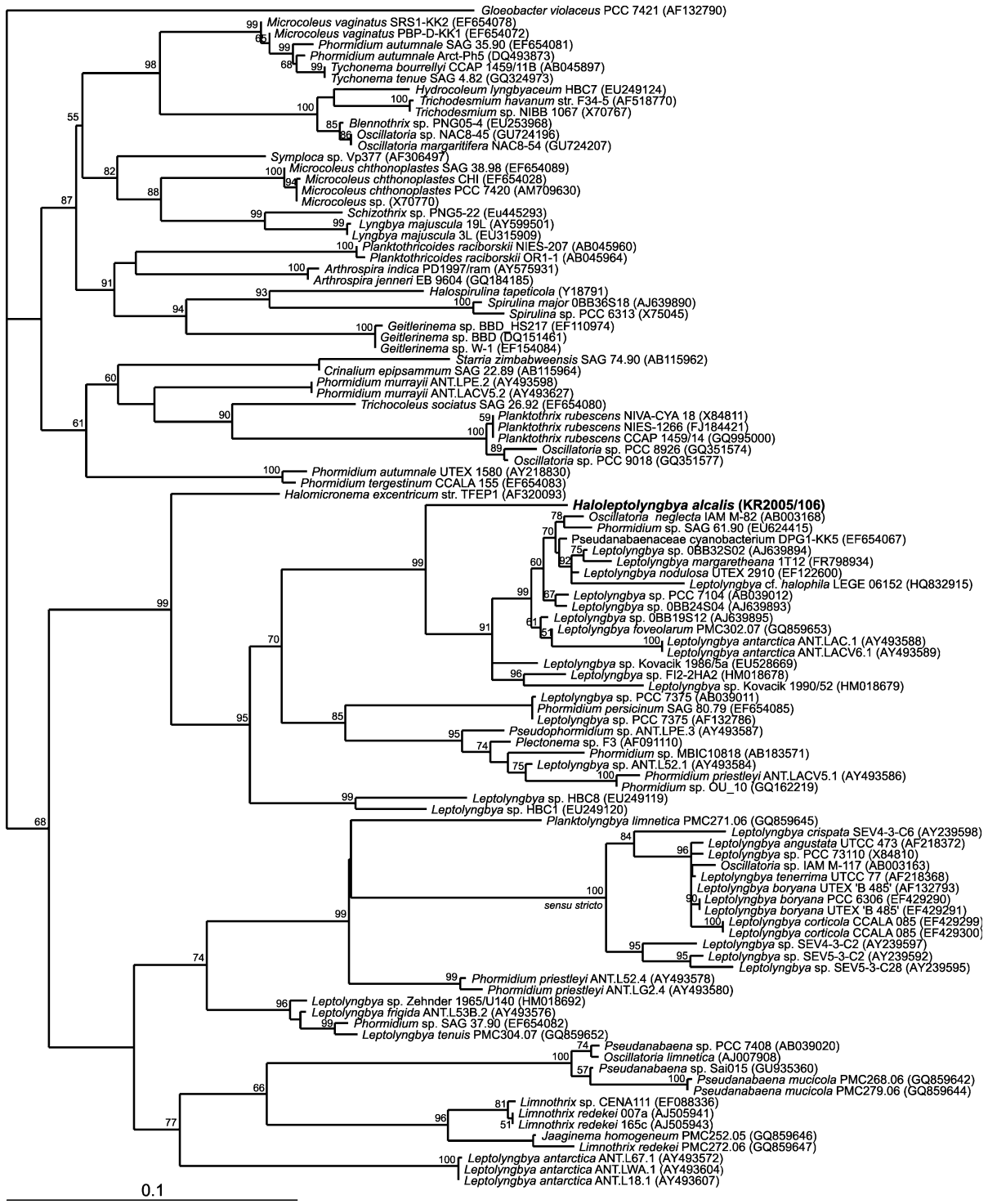

Fig. 4 Maximum-likelihood phylogenetic tree based on partial sequence of 16S rRNA gene. Numbers above branches indicate bootstrap support (>50%) from maximum likelihood (1,000 replicates). The novel taxon is in larger bold font. *Gloeobacter violaceus* was used as the out-group

the secondary structure it possessed four loops and two bubbles (Fig. 5a). The secondary structure of Box B helix also showed four loops with a length of 49 nt (Fig. 5d). The V3 region had 23 nt and formed a loop and an unfolded sequence of 9 nt (Fig. 5g).

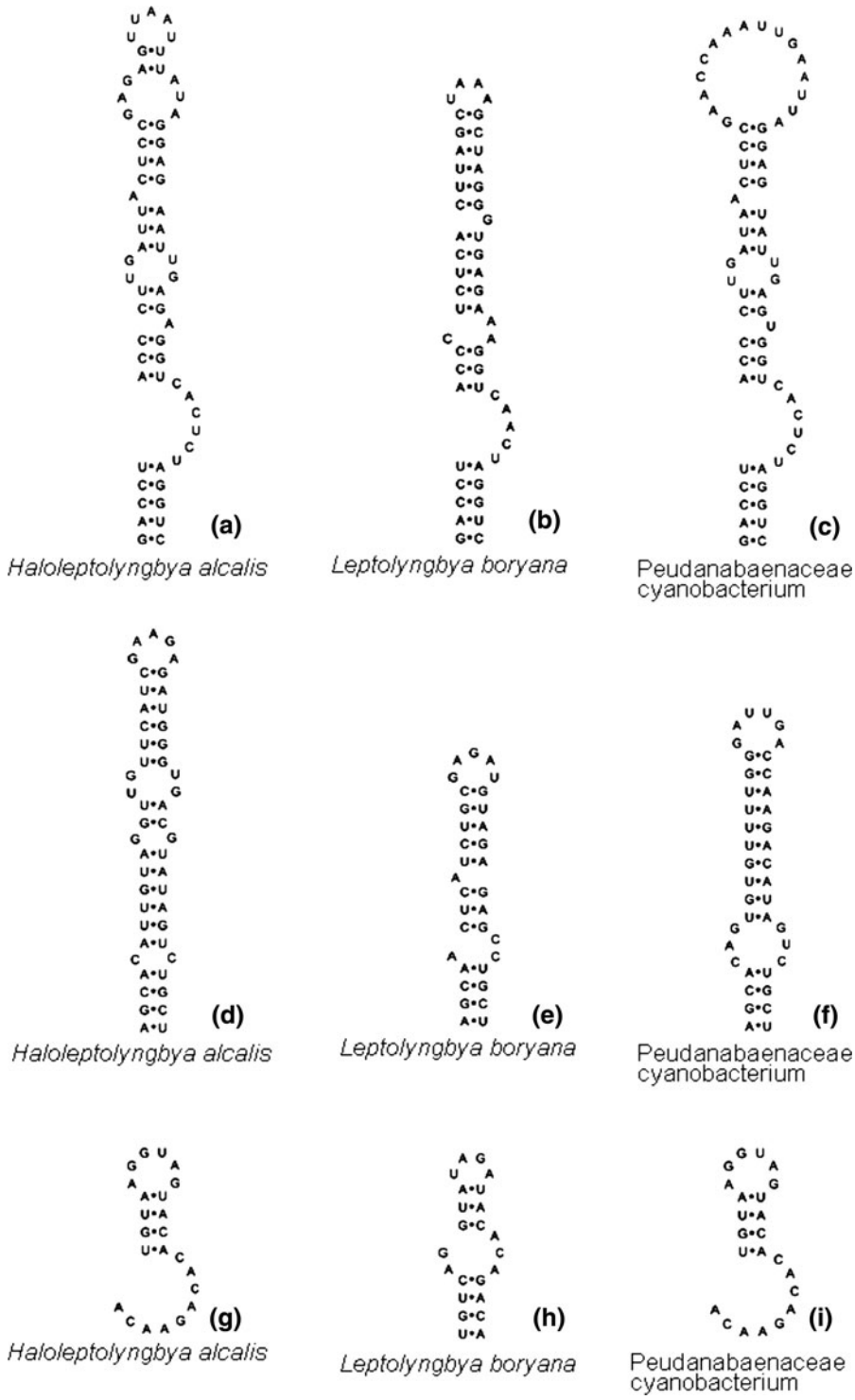

Fig. 5 16S-23S ITS regions secondary structure of *Haloleptolyngbya alcalis*, type species of *Leptolyngbya* (*L. boryana*) and, a taxon (Peudanabaenaceae cyanobacterium) close in sequence similarity as well as phylogenetically. **a–c** D1–D1′ helix; **d–f** Box-B helix; **g–i** V3 region

We obtained a sequence (366 bp) of *cpc*BA-IGS that contained a full portion of intergenic spacer and a partial sequence of beta and alpha subunit of the phycocyanin locus. To evaluate the sequence similarity with other cyanobacterial taxa, the *cpc*BA-IGS sequence of *Haloleptolyngbya* was compared with available sequences in GenBank using BlastN (NCBI). Our sequence only matched to a sequence (AJ401183) related to an unidentified cyanobacterial taxon of unknown origin.

PCR analyses aimed at detecting potential cyanotoxin production by the taxon under investigation using several specific primer pairs mentioned earlier, did not lead to the amplification for any genetic locus responsible for production of cyanotoxins (data not shown).

Discussion

Oscillatorian cyanobacteria with unbranched (rarely pseudobranched) filaments less than 3.5-μm width, covered by a sheath, and exhibiting a wide range of phenotypic features are mainly included in the genus *Leptolyngbya* Anagnostidis et Komárek (Anagnostidis & Komárek, 1988; Komárek & Anagnostidis, 2005). Hence, it is difficult to come up with a morphological character that can distinguish phylogenetically distinct taxa from the group characterized by fine filaments. The observed morphological traits appear to be insufficient for identification of members of *Leptolyngbya* because some phylogenetically distinct groups also showed similar morphological features (Palinska et al., 2011). Therefore, in a polyphasic approach, the determination of the 16S rRNA gene is the first criterion for the classification process (Komárek, 2008; Strunecký et al., 2011).

The 16S rRNA gene sequence of the new strain was evaluated, taking into account the percentage similarity as well as the phylogeny. Most of the top hits against the 16S rRNA gene sequence of our strain retrieved nr BLASTN searches (data not shown) have previously been assigned to genera of family Pseudanabaenaceae of the order Oscillatoriales. Our strain covers only 87.40% 16S rRNA gene sequence similarity to the reference strain *Leptolyngbya boryana* (Gomont) Anagnostidis et Komárek PCC 6306 in pairwise global alignment of 1,115 bp. The novel strain showed 6% 16S rRNA gene sequence divergence in BLASTN searches of 1,432 bp (as of August 15, 2011) to its closest related taxa (Pseudanabaenaceae cyanobacterium DPG1-KK5, *Leptolyngbya* sp., 0BB32S02, *L. margaretheana* 1T12, and *Leptolyngbya* sp., 0BB19S12). A 16S rRNA gene sequence similarity of 97% has been suggested as a threshold for a congeneric prokaryotic genus (Stackebrandt & Goebel, 1994). Several cut off similarity levels have been suggested for bacteria, ranging from 97% for the genus level to 99% for the species level (Janda & Abbott, 2002; Stackebrandt et al., 2002; Harris & Hartley, 2003). Within the Oscillatoriales, different new genera have been described based on sequence dissimilarity to their closest relatives in public nucleotide databases. For example, the genus *Halospirulina* was established to include halotolerant cyanobacteria with *Spirulina*-like morphology that had $\geq$7.7% 16S rRNA gene sequence divergence to all other cyanobacteria (Nübel et al., 2000). Using a 16S rRNA gene sequence divergence of >7%, the new genus *Halomicronema* was proposed to describe four moderately halophilic and moderately thermophilic thin filamentous cyanobacteria isolated from a hypersaline microbial mat (Abed et al., 2002). Some studies have even accepted a phylogenetic divergence of less than 5% to describe new genera. For example, *Mojavia pulchra* Řeháková et Johansen isolated from desert soil has recently been proposed in the order Nostocales by considering <5% dissimilarity of 16S rRNA gene sequences as an evidence of evolutionary divergence (Řeháková et al., 2007). Recently, it was suggested that the characterization of genera should be based on 95% or less genetic similarity combined with at least one diacritical autapomorphic character (Komárek, 2008). A new genus *Halothece* has been described on the basis of 16S rRNA gene analyses and ecophysiological characters (Garcia-Pichel et al., 1998). The clade considered as *Leptolyngbya s. str.* (Johansen et al., 2011) forms a strongly supported lineage within the Oscillatoriales, with the type species *L. boryana* (Fig. 4). Our strain emerges a separate lineage that is very distant from the *Leptolyngbya s. str.* clade (Fig. 4). The lineage of *H. alcalis* is strongly supported by bootstrap value of 99% that indicates its precise distinctiveness from other cyanobacterial taxa. It has already been revealed that members of Oscillatoriales, particularly the *Leptolyngbya*-morphotype, evolved polyphyletically and therefore occur in different clades in the phylogenetic tree of 16S rRNA gene (Albertano & Kováčik, 1994; Nelissen et al., 1994;

Turner, 1997; Ishida et al., 2001; Casamatta et al., 2005; Komárek & Anagnostidis, 2005; Taton et al., 2006; Li & Brand, 2007; Johansen et al., 2008; Bohunická et al., 2011). Hence, taxa included in clades other than the clade with the type species require a revision based on a polyphasic approach.

The regions of the 16S-23S ITS are difficult to align due to variability in sequences (Iteman et al., 2000). However, the ITS region of *Arthrospira* exhibits a remarkable sequence conservation (Baurain et al., 2002; Dadheech et al., 2010). Several semi-conserved secondary structures of helices (D1–D1′, Box B, and the V3) of the ITS have been considered for differentiating cyanobacterial taxa (Casamatta et al., 2006; Řeháková et al., 2007; Siegesmund et al., 2008; Bohunická et al., 2011; Johansen et al., 2011) and also as an apomorphic character (Johansen et al., 2011). The difference in length of D1–D1′ helix between the novel strain and closely related unidentified taxon (Pseudanabaenaceae cyanobacterium; EF 654067) is only one nt, but the helix is distinct in the number of loops, sequence, and size of terminus (Fig. 5a, c). We observed considerable variability in the secondary structure of Box-B helix of our *Haloleptolyngbya* isolate and Pseudanabaenaceae cyanobacterium as our strain has four loops, while only two loops occur in the helix sequence of the related taxa (EF 654067) (Fig. 5d, f). There are also nine base pair differences in the length of the helix and sequences of terminus loops. The length of V3 region and its pattern of helices are similar in both taxa (Fig. 5g, i). Our strain exhibits significant distinctiveness in the secondary structure of D1–D1′, Box-Bs and V3 helices from the type species of morphologically closest genus *Leptolyngbya*. We observed disparities in length, number of loops, and bubbles of D1–D1′ helix of the novel taxon and the type species *L. boryana* (Fig. 5a, b). Likewise, there are significant differences between the helix of Box-B (Fig. 5d, e) and V3 region (Fig. 5g, h) of *H. alcalis* and the type species of *Leptolyngbya*. Apart from this, several compensatory base pair changes are observed in central stem region of our strain when compared to the *L. boryana*. Owing to apparent differences in the ITS sequence as a whole and secondary structures of D1–D1′, Box-B, and V3 regions from available sequences in GenBank of known cyanobacterial taxa, we consider these features as apomorphies for defining *Haloleptolyngbya acalis* to a new species.

We did not find comparable sequences of *cpc*BA-IGS locus of our strain in BLASTN (NCBI) to sequences of other cyanobacterial taxa except the sequence (AJ401183) of an unidentified cyanobacterium txid129981 from an unknown origin (Manen & Falquet, 2002) that showed 99% similarity. The non-availability of the sequence of an identified cyanobacterial taxon to the sequence of our strain reveals its uniqueness as a new genus.

Morphological characters of the strain investigated in this study seems to somewhat fit into previously described species of *Leptolyngbya* as the genus *Leptolyngbya* (Komárek & Anagnostidis, 2005) has been described with wide range of different phenotypic features. Hence, we evaluated phenotypic features of our strain in contrast to *L. boryana* (Komárek & Anagnostidis, 2005) and other phylogenetically related taxa (Table 2). Our strain differs from type

Table 2 Morphological and habitat characters of *Haloleptolyngbya alcalis* and its phenotypically closely related taxa

Feature	*Haloleptolyngbya alcalis*	*Leptolyngbya boryana*[a]	*Leptolyngbya halophila*[a]
Filament	Solitary, unbranched	Solitary, pseudobranching	Solitary
Sheath	Hyaline, colorless	Hyaline, colorless	Hyaline, Colorless to golden to yellow-brownish
Necrotic cell formation	+	+	+
Cell width (μm)	1.2–1.9	1.0–3.0	1.0–2.0
Cell shape	Mainly longer than wide	Mainly isodiametric	Isodiametric or longer than wide
Apical cell type	Rounded	Rounded	Rounded
Constriction at cross-wall	Distinctly constricted	Strongly constricted	Constricted
Habitat	Saline-alkaline water	Freshwater	Coastal and inland saltwaters

[a] According to Komárek & Anagnostidis (2005) and Guiry & Guiry (2011)

species in absence of pseudobranching, cell shape and width, constriction at the cross-wall, and habitat. Like wise, considering the saline-alkaline habitat of our strain, we compared it to *L. halophila* (Hansgirg ex Gomont) Anagnostidis et Komárek as described (Komárek & Anagnostidis, 2005), and found that the novel strain could be differentiated from this species by absence of golden to yellow-brownish sheath. The occurrence of *L. halophila* has been recorded from Europe and central Asia only and not reported from the African continent (Komárek & Anagnostidis, 2005). Moreover, the novel strain is phylogenetically distant from *L. halophila* (Fig. 4).

The ultrastructural features, mainly the parietal arrangement of thylakoids (Komárek & Anagnostidis, 2005), observed in strain KR2005/106 (UTEX B ZZ879) suggest that it belongs to subsection III (formally Oscillatoriales) of the Cyanobacteria, family Pseudanabaenaceae (Boone & Castenholz, 2001). Lack of the intracellular pores in the cross-wall and the possession of a fibrous multilayered sheath by the novel taxon distinguish it from *Leptolyngbya*.

The strain investigated was isolated from a soda lake that had high salinity and alkalinity (Table 1). We therefore consider this specific habitat as an autapomorphic character for our taxon. Habitat specificity was suggested to be a taxonomic informative character for cyanobacteria (Řeháková et al., 2007). Ecologically distinct organisms thriving in different habitats have different evolutionary histories that are reflected in genetic divergence (Nübel et al., 2000). Evolutionary distinction has been documented in *Chroococcidiopsis* variants from hot and cold desert (Bahl et al., 2011). Tolerance of high salt concentration has been recognized as a significant phenotypic character that is closely correlated with the phylogeny of cyanobacteria (Garcia-Pichel et al., 1998; Nübel et al., 2000; Abed et al., 2002; Miyashita et al., 2003).

Obviously, our strain exhibits significant 16S rRNA gene sequence deviation from available sequences in GenBank, differences in secondary structures of ITS helices from the type and other phylogenetically close strains of *Leptolyngbya*, separate phylogenetic position in ML tree, and a typical saline-alkaline habitat. These are sufficient evidences to consider *H. alcalis* as a new taxon within the Oscillatoriales.

The role of such tiny filamentous cyanobacteria in the food web of Lake Nakuru should in future be studied in greater detail. Such organisms normally grow on the sediment surface; however, they are also able to colonize the open water as tychoplanktons at the end of their life cycles. In September and October 2009, a monospecific, thick mat forming bloom of an unknown filamentous cyanobacterium was observed and provisionally designated as *Pseudanabaena acicularis* Nygaard (Kaggwa, Gruber, Oduor & Schagerl, unpubl. observation). This organism is known from freshwaters in Denmark (Komárek & Anagnostidis, 2005), does not possess aerotopes, and establishes short filaments with pointed ends. Our taxon differs by its longer filaments with rounded apices. This shows that in such shallow saline lakes like Nakuru, the diversity of cyanobacteria recruited from the "ecological memory" responding to changed ecosystem conditions may have a higher diversity than expected. Probably, like benthic diatoms, they can act as alternative food resource for the Lesser Flamingos. In this context, it is notable that our molecular results revealed the non-toxic character of *Haloleptolyngbya*.

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