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Phylogenetic relationship of *Arthrospira*, *Phormidium*, and *Spirulina* strains from Kenyan and Indian waterbodies

Phylogenetic relationship of *Arthrospira, Phormidium* and *Spirulina* strains from Kenyan and Indian waterbodies

Abstract

Morphological and genetic diversity of cultured cynobacterial strains of species of the genera *Arthrospira*, *Spirulina* and *Phormidium* from two geographically different regions and habitats (Kenyan saline-alkaline lakes and Indian freshwater bodies) were investigated. Light microscopy observations were used to determine morphological diversity of the cyanobacteria. Three independent molecular techniques, sequencing of 16S rRNA gene, internally transcribed spacer region between 16S and 23S rDNA (ITS) and the phycocyanin locus (PC-IGS) were conducted for the examination of phylogenetic relationship. Despite differences in morphology and habitats the Kenyan and Indian *Arthrospira* strains belong to the same cluster in phylogenetic trees of the 16S rDNA (AY575923-AY575932) or PC-IGS (AY575937-AY575946). The DNA similarity in both methods was 100%. In the ITS tree, the two Indian *Arthrospira* strains PD1998/pus (AY575930) and PD2002/ana (AY575932) form their own sub-cluster. The *Phormidium* strain AB2002/07 (AY575933) from Lake Nakuru, Kenya is included in the *Arthrospira* cluster in the ITS tree and very closely related in the 16S and PC-IGS trees. Based on 16S rDNA and PC-IGS phylogeny the sequences of the *Spirulina* strains form a separate cluster distinct from the *Arthrospira* cluster. The Kenyan and Indian *Spirulina subsalsa* strains show a considerable genetic variability as similarities in 16S rRNA gene sequence is 91.5% only. Molecular characterizations of cyanobacterial strains in the present study demonstrate that several distinct morphotypes may be genetically similar and vice versa.

Key words: *Arthrospira*, Cyanophyceae, morphology, *Phormidium*, phylogeny, *Spirulina*

Introduction

The species and genus concept of cyanobacteria is changing from the traditional morphological classification to a concept using different phenotypic features (e.g. biochemical and ultrastructural characteristics) and genotypic features (ANAGNOSTIDIS & KOMÁREK 1985, 1988, CASTENHOLZ et al. 2001, WILMOTTE & HERDMAN 2001). For phylogenetic analyses of bacteria and cyanobacteria one of the most informative genes is the 16S rRNA gene (WOESE 1987, OLSEN & WOESE 1993, LUDWIG & SCHLEIFER, 1994, LUDWIG

& KLENK 2001). The 16S rRNA gene has a conserved function and is universally present in bacteria and cyanobacteria. The phylogenetic investigations using 16S sequences have shown that many unicellular and filamentous non heterocysteous cyanobacterial genera are probably polyphyletic and can not be grouped as natural taxa, whereas heterocysteous strains form a monophyletic group (GIOVANNONI et al. 1988, WILMOTTE 1994, CASTENHOLZ et al. 2001, RIPPKA et al. 2001, WILMOTTE & HERDMAN 2001). As the number of variable positions is low in the 16S rDNA, it is not very useful for studying the relationship on the species level or below it. For this purpose other more variable non-coding sequences like the internal transcribed spacer region of the 16S-23S region (ITS) or the intergenic spacer of the phycocyanin operon between cpcB and cpcA subunit (PC-IGS) have been introduced (NEILAN et al. 1995, BOYER et al. 2001, BAURAIN et al. 2002, MANEN & FALQUET 2002).

In the case of the genera *Arthrospira* and *Spirulina*, which were merged by GEITLER (1932), several morphological studies have shown differences between the genera e.g. in helicity, trichome size, cross wall, cell wall structure, pore pattern and gas vesicles (DESIKACHARY 1959, HINDÁK 1985, GUGLIELMI and COHEN-BAZIRE 1982a, 1982b, GUGLIELMI et al. 1993, TOMASELLI et al. 1996, 1997). Investigations of the conserved 16S rRNA gene and the more variable PC-IGS revealed that *Arthrospira* is not closely related to *Spirulina* (NELISSEN et al. 1994, NELISSEN et al. 1996, MANEN & FALQUET 2002, LITVAITIS 2002). The separation of these two genera has been accepted in Bergey´s Manual of Systematic Bacteriology (CASTENHOLZ 1989, CASTENHOLZ et al. 2001).

Using amplified ribosomal DNA restriction analysis (ARDRA), ITS analysis and the PC-IGS several *Arthrospira* strains from four continents assigned to four different species (*Arthrospira fusiformis* (VOROCHININ) KOMÁREK & LUND, *A. platensis* (NORDSTEDT) GOMONT, *A. maxima* SETCHEL & GARDNER, and *A. indica* DESIKACHARY & JEEJI-BAI could be distinguished in different clusters (SCHELDEMAN et al. 1999, BAURAIN et al. 2002, MANEN & FALQUET 2002). No relationship was detected between the clusters and the geographic origin of the strains and their morphology.

The aim of this study is to examine the phylogenetic relationship among *Arthrospira*, *Spirulina* and *Phormidium* strains from seven Kenyan alkaline-saline habitats and four Indian freshwater habitats. For the investigation a polyphasic approach including morphological characters and sequence analysis of 16S rRNA gene, ITS region and PC-IGS locus is applied.

Materials and Methods

Organisms and culture conditions

Strains of *Arthrospira*, *Phormidium*, and *Spirulina* were isolated from water samples from Kenyan and Indian water bodies. The strains of cyanobacteria investigated in this study and their origin are listed in Table 1. They were determined according to HINDÁK (1985, 2001) and DESIKACHARY & JEEJI BAI (1992, 1996).

Using a microcapillary a single trichome was isolated, washed and placed in capped tubes containing 10 mL culture medium. The Kenyan cyanobacterial strains were cultivated in Bourrelly medium (HEGEWALD et al. 1994) modified with an addition of 0.3 g L^{-1} Na_2CO_3 and 15 g L^{-1} NaCl. The Indian *Arthrospira* strains were grown in *Spirulina* medium (SCHLÖSSER 1994) modified from AIBA & OGAWA (1977). The Indian *Spirulina* strain was cultured in BG-11 medium (RIPPKA et al. 1979). All strains investigated in this study were maintained at 18 °C with a photon flux density of 20 µmol of photon $m^{-2}s^{-1}$ (equivalent to 1500 lux). Morphological observations were made using a Nikon Optiphot 2 light microscope (Nikon, Tokyo, Japan).

Genomic DNA Extraction

Fresh cell material was centrifuged. After discarding the supernatant, the genomic DNA was extracted and purified using Dynabeads DNA DIRECT System I (Deutsche Dynal GmbH, Hamburg, Germany).

PCR Amplification and Sequencing

PCR amplification was done using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) and a Peltier Thermal Cycler PTC 200 (MJ Research, Inc., San Francisco, USA). The reaction mixture contained 0.1 µl Taq DNA polymerase (concentration 5 units per µL), 0.6 µL dNTP Mix (10 mM), 2 µL buffer (10x concentrated, containing Tris Cl, KCl, $(NH_4)_2SO_4$ and 15 mM $MgCl_2$, 1 µL forward primer (10 pmol μL^{-1}), 1 µL reverse primer (10 pmol μL^{-1}) and 1 µL genomic DNA in a total volume of 20 µL. Primers used for amplification of the 16S rRNA gene, the ITS region and PC-IGS locus are listed in Table 2.

The following program was used for amplification of 16S-23S rDNA fragment, employing cyanobacterial specific forward primer CYA106F and 23S5'R reverse primer: 5 min at 94 °C for 1 cycle; 30 cycles of 30 s at 94 °C; 30 s at 50 °C; 1 min at 70°C and a final elongation step

of 72 °C for 3 min. For the PCR of the PC-IGS the primers cpc_arF and cpc_arR and the following program were used: 3 min at 94 °C for 1 cycle; 30 cycles of 20 s at 94 °C; 30s at 55 °C; 1 min at 72°C with final elongation step of 72 °C for 5 min. PCR products were visualised by standard agarose gel electrophoresis and ethidium bromide staining.

Amplified products of 16S rDNA, ITS, and PC-IGS were purified through GFX spin columns (Amersham Pharmacia Biotech, Freiburg, Germany). The purified product of 16S-23S rDNA was sequenced using CYA106F, 23S5'R and the internal primers F3, 683R = F3reverse, 1045F, 1066R, R4R, 16S3'F. Sequencing of the purified PC-IGS locus was done employing the same primers used for PCR amplification. For each PCR product both strands were sequenced on ABI 3100 Avant Genetic Analyzer using BigDye Terminator V.3.1 Cycle Sequencing Kit (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany).

The 16S rDNA, ITS, PC-IGS sequences obtained were submitted to GenBank (NCBI). The accession numbers are depicted in Table 1.

Phylogenetic analysis

Sequences of partial 16S rRNA gene, the ITS region (including parts of 16S rDNA, tRNA Ile, ITS, tRNA Ala and parts of 23S region) and parts of the PC-IGS locus (including parts of cpcB subunit, IGS and cpcA subunit) were analysed for all the strains, except KR2003/25 where only 16S rDNA was analysed. The sequences were submitted to the GenBank (NCBI) (Table 1). The sequence parts of all strains with highly variable and ambiguous regions and gaps, where a proper alignment was impossible, were excluded from the alignment. In the case of 16S rDNA a set containing 1258 positions was used. ITS comprised 686 and PC-IGS 309 positions. In the 16S tree *Gloeobacter violaceaus* (AF132790) was used as an outgroup, in the ITS tree the cyanelle of *Cyanophora paradoxa* KORSH. (M19493) and in the PC-IGS tree *Cyanobacterium* sp. (AJ401183). The sequences (16S rDNA, ITS, PC-IGS) were aligned with other cyanobacterial sequences using MS Windows based Manual Sequence Alignment Editor (HEPPERLE 2002). The alignments are available from the authors on request. Fifty two (16S ribosomal DNA), 41 (PC-IGS locus) and 38 (ITS) sequences were considered for alignment and comparison including sequences taken from the National Center of Biotechnology Information, Rockeville (NCBI 2003). Phylogenetic analyses of aligned sequences were conducted using the ARB software package (http://www.arb-home.de). Phylogenetic trees were constructed by means of the maximum-likelihood algorithm using the fastDNAml program (FELSENSTEIN 1981, OLSEN et al. 1994).

Table 1. Isolated cyanobacterial strains from different Kenyan and Indian.alkaline and freshwater lakes

Species	**Strain**	**GenBank acc. no.** 16S-23S (16S, ITS)	**GenBank acc. no.** PC-IGS (cpcB-cpcA)	**Habitat**	**Country of origin**
Arthrospira fusiformis	AB2002/01	AY575923	AY575937	Lake Elmenteita [a]	Kenya
Arthrospira fusiformis	AB2002/02	AY575924	AY575938	Lake Sonachi [a]	Kenya
Arthrospira fusiformis	AB2002/03	AY575925	AY575939	Lake Magadi [a]	Kenya
Arthrospira fusiformis	AB2002/04	AY575926	AY575940	Lake Nakuru [a]	Kenya
Arthrospira fusiformis	AB2002/05	AY575927	AY575941	Lake Bogoria [a]	Kenya
Arthrospira fusiformis	AB2002/10	AY575928	AY575942	Lake Bogoria [a]	Kenya
Arthrospira fusiformis	AB2002/11	AY575929	AY575943	Lake Simbi [a]	Kenya
Arthrospira indica	PD2002/ana	AY575932	AY575944	LakeAnasagar [f], Ajmer	India
Arthrospira indica	PD1997/ram	AY575931	AY575945	Vicinity of Lake Ramgarh [f], Jaipur	India
Arthrospira indica	PD1998/pus	AY575930	AY575946	Lake Pushkar [f], Pushkar	India
Phormidium cf. *terebriformis*	AB2002/07	AY575933	AY575947	Lake Nakuru [a]	Kenya
Phormidium cf. *terebriformis*	KR2003/25	AY575936*	-	Hot spring at Lake Bogoria	Kenya
Spirulina subsalsa	AB2002/06	AY575934	AY575948	Lake Nakuru [a]	Kenya
Spirulina subsalsa	PD2002/gca	AY575935	AY575949	Pond[f], Govt. College, Ajmer	India

[a] alkaline-saline habitat

[f] freshwater habitat

*= 16S rDNA only

Table 2. Primers employed in the present study.

Primers	Sequence (5'-3')	Reference
16S rRNA gene and ITS region		
CYA106F	TAACACATGCAAGTCGAA	NÜBEL et al. 1997, LI et al. 2001
F3	GTGTAGCGGTGAAATGCGTAGA	LI et al. 2001
1045F	TGCCATCATTCAGTTGGGCAC	Newly designed
16S3'F	TGTGGCTGGATCACCTCCTT	BAURAIN et al. 2002
683R = F3reverse	TCTACGCATTTCACCGCTACAC	LI et al. 2001
1066R	GTGCCCAACTGAATGATGGCA	Newly designed
R4R	TACGGCTACCTTGTTACGAC	LI et al. 2001
23S5'R	TCTGTGTGCCTAGGTATCCACCGTT	BAURAIN et al. 2002
Phycocyanin Locus (PC-IGS)		
cpc_arF	TCGAAGATCGTTGCTTGAACG	Newly designed
cpc_arR	TTAGGTCCCTGCATT TGGGTG	Newly designed

Results

Ten strains of *Arthrospira* spp., two strains of *Spirulina* spp. and two strains of *Phormidium* sp. isolated from seven Kenyan alkaline and four Indian freshwater habitats were cultivated and investigated for their morphological characteristics and their phylogenetic relationship. The morphological characteristics of the cultivated cyanobacterial strains are depicted in Table 3. For the phycological identification of *Arthrospira*, morphological features mainly width of trichome, cell size, nature and shape of helix, gas vesicles and calyptra on the end cells were taken into account. On the basis of morphological features observed in light microscope, the Kenyan *Arthrospira* strains were identified as *A. fusiformis* and the *Phormidium* strains were designated as *P.* cf. *terebriformis* (AGHARDH ex GOMONT) ANAGNOSTIDIS & KOMÁREK. All Indian *Arthrospira* strains corresponded to *A. indica.* Kenyan and Indian *Spirulina* strains were assigned to *S. subsalsa* Oersted. Species that were distinguished in the present study are illustrated in Fig. 1.

Table 3. Morphological characteristics of different *Arthrospira*, *Spirulina* and *Phormidium* strains from Kenya and India.

Strain	Characteristics					
	Width of trichomes (µm)	**Cell length** (µm)	**Calyptra on end cells**	**Gas vesicles**	**Type of coiling**	**Shape of helix end and end cell morphology**
Arthrospira fusiformis						
AB2002/01	8-9	4-6	Absent	Present	Variable	Both ends rounded
AB2002/02	8-15	4-6	Absent	Present	Variable, straight	One end of trichome slightly diminished
AB2002/03	8	4-6	Absent	Present	Variable	One end of trichome slightly diminished
AB2002/04	4-10	4-8	Absent	Present	Very variable	Both ends rounded
AB2002/05	10-11	4-8	Absent	Present	Variable	Both ends rounded
AB2002/10	8-12	4-6	Absent	Present	Variable	Both ends rounded
AB2002/11	8-12	4-5	Absent	Present	Tightly coiled	Both ends rounded
Arthrospira indica						
PD2002/ana	8-9	6-8	Present	Present	Variable	Slightly diminished at one end
PD1998/pus	6-8	6-8	Present	Present	Straight	Slightly diminished at one end
PD1997/ram	6-8	4-5	Present	Present	Variable	Slightly diminished at one end
Phormidium* cf. *terebriformis						
AB2002/07	6-7	3-5	Absent	Absent	Slightly undulating	Slightly diminished at one end
KR2003/25	5-6	2-4	Absent	Absent	Straight to slightly undulating	Slightly diminished at both ends
Spirulina subsalsa						
AB2002/06	2-2.5	Septa not visible	Absent	Absent	Regularly coiled	Both ends rounded
PD 2002/gca	1.5-2.0	Septa not visible	Absent	Absent	Regularly coiled	Both ends rounded

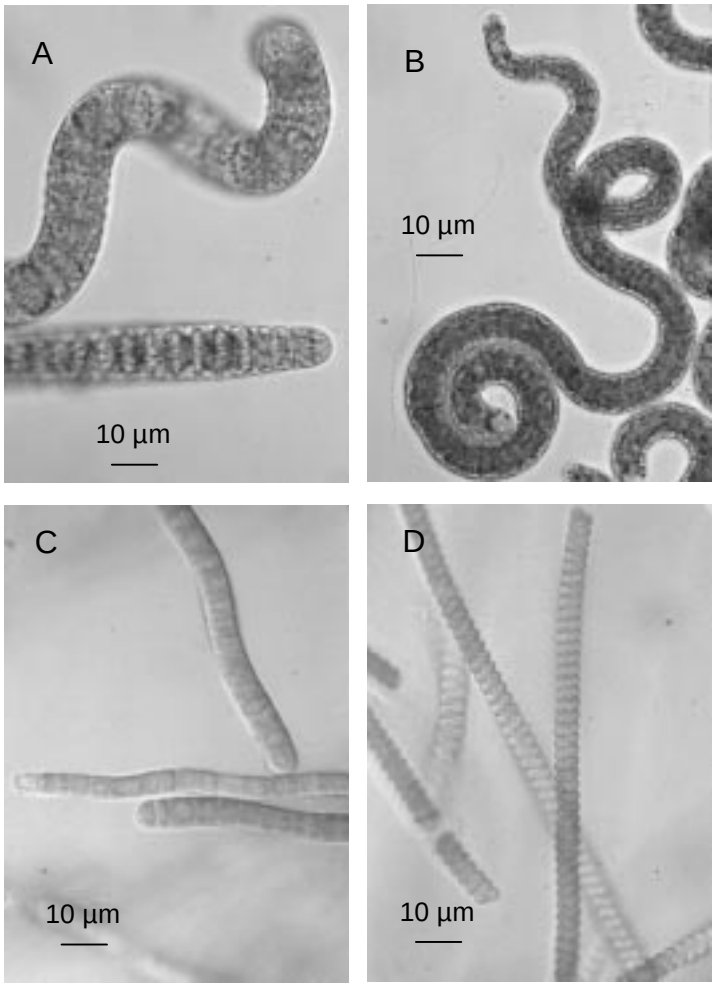

Fig. 1. A) *Arthrospira fusiformis*, B) *Arthrospira indica*, C) *Phormidium cf. terebriformis*, D) *Spirulina subsalsa.*

A phylogenetic tree of 16S rDNA sequences of the Kenyan and Indian strains within a larger group of cyanobacteria is presented in Fig. 2. The 16S tree showed a tight cluster of the *Arthrospira* strains (AY575923-AY575932) and a closely related *Phormidium* cf. *terebriformis* strain (AY575933). The whole cluster was supported by a bootstrap value of 99%. *Phormidium* cf. *terebriformis* (AY575936) from the hot springs near Lake Bogoria was clearly separated from *P.* cf. *terebriformis* (AY575933) from Lake Nakuru. A closer relationship of the whole cluster to a cluster of *Planktothrix* and *Oscillatoria* spp. could be observed. This cluster was supported by a bootstrap value of 77%. The *Spirulina* cluster was clearly separated from the *Arthrospira* cluster and was more related to *Synechocystis* sp. and *Microcystis* sp. (Fig 2). A comparison for the 16S rDNA nucleotides of the investigated strains in pairs is shown in Table 4. The seven Kenyan strains of *Arthrospira fusiformis* and the three Indian strains of *A. indica* had identical 16S rDNA sequences at 100% similarity.

The 16S rDNA sequence of *Phormidium* cf. *terebriformis* from Lake Nakuru (AY575933) had similarity of 99.6% with the *Arthrospira* sequences (Table 4). The 16S rDNA similarity between the two *Phormidium* sequences was only 92.0% (Table 4).

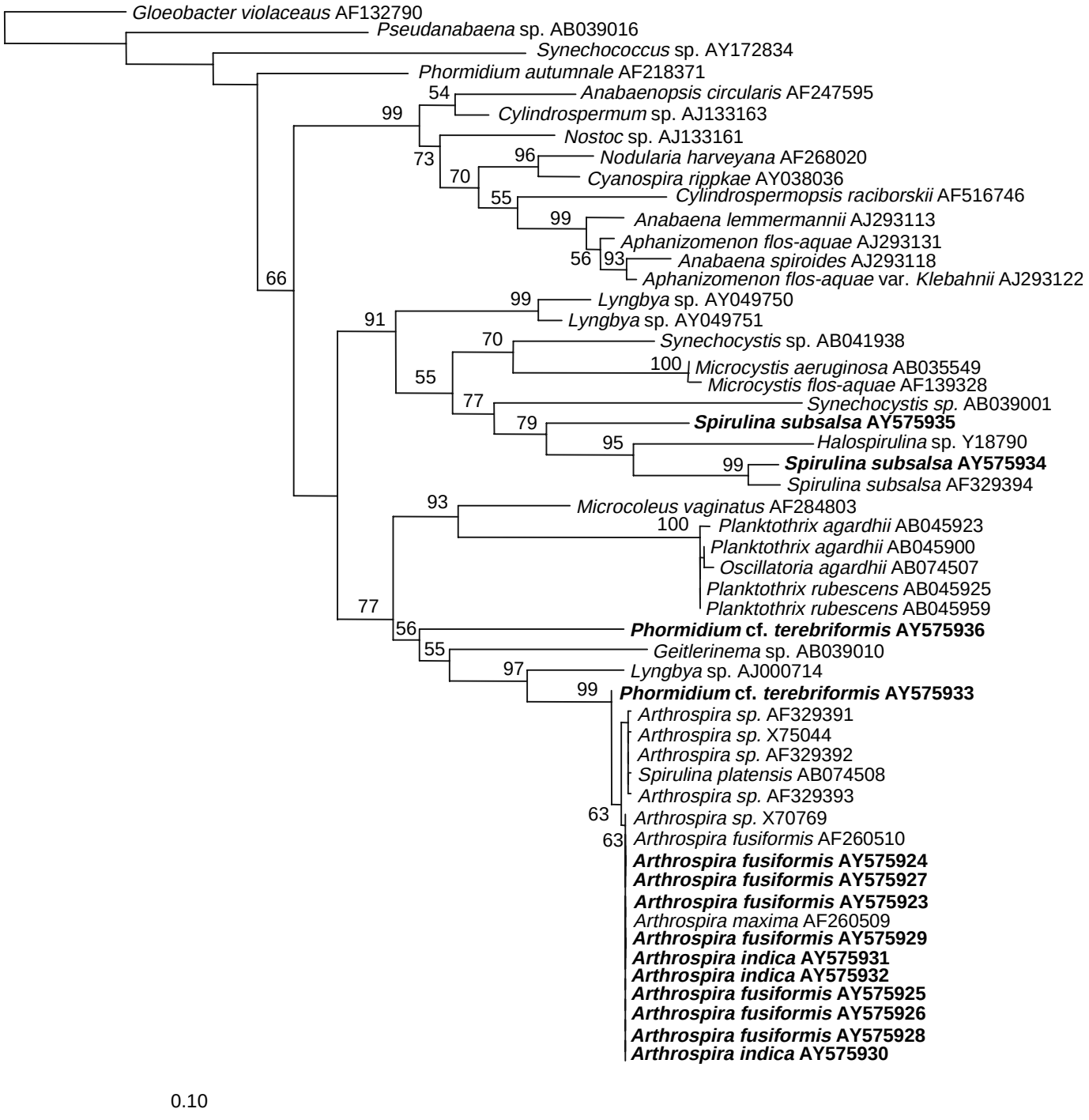

Fig. 2. Phylogenetic tree based on the alignment of partial 16S sequences of 51 cyanobacterial strains. The tree was calculated using the maximum likelihood algorithm. Bootstrap values above 50% are included. Bar indicates 10% sequence divergence.

Table 4. Calculated similarities between 16S rDNA sequences (1282 bp) of *Arthrospira*-, *Phormidium*- and *Spirulina* strains. The values in the upper right triangle are the percent identities between a pair of sequences. The values in the lower left triangle are the numbers of dissimilar nucleotides. For the related species name, see Table 1, AF260510 = *Arthrospira fusiformis.*

	AY575923	AY575924	AY575925	AY575926	AY575927	AY575928	AY575929	AY575932	AY575930	AY575931	AY575934	AY575935	AF260510	AY575933	AY575936
AY575923	-	100	100	100	100	100	100	100	100	100	89.9	90.5	100	99.6	92.3
AY575924	0	-	100	100	100	100	100	100	100	100	89.9	90.5	100	99.6	92.3
AY575925	0	0	-	100	100	100	100	100	100	100	89.9	90.5	100	99.6	92.3
AY575926	0	0	0	-	100	100	100	100	100	100	89.9	90.5	100	99.6	92.3
AY575927	0	0	0	0	-	100	100	100	100	100	89.9	90.5	100	99.6	92.3
AY575928	0	0	0	0	0	-	100	100	100	100	89.9	90.5	100	99.6	92.3
AY575929	0	0	0	0	0	0	-	100	100	100	89.9	90.5	100	99.6	92.3
AY575932	0	0	0	0	0	0	0	-	100	100	89.9	90.5	100	99.6	92.3
AY575930	0	0	0	0	0	0	0	0	-	100	89.9	90.5	100	99.6	92.3
AY575931	0	0	0	0	0	0	0	0	0	-	89.9	90.5	100	99.6	92.3
AY575934	129	129	129	129	129	129	129	129	129	129	-	91.5	89.9	89.9	89.2
AY575935	122	122	122	122	122	122	122	122	122	122	109	-	90.5	90.3	88.4
AF260510	0	0	0	0	0	0	0	0	0	0	129	122	-	99.6	92.3
AY575933	5	5	5	5	5	5	5	5	5	5	130	124	5	-	92.0
AY575936	99	99	99	99	99	99	99	99	99	99	139	149	99	103	-

A phylogenetic tree for the PC-IGS region is represented mainly focusing on the sequences of *Arthrospira* and *Spirulina* strains (Fig. 3). A tight *Arthrospira* cluster was obtained, where *Phormidium* cf. *terebriformis* (AY575947) was closely related to. This was supported by a bootstrap value of 99%. The *Arthrospira* cluster was divided into three subclusters. All Kenyan and Indian *Arthrospira* strains (AY575937-AY5759346) were located in one subcluster with a bootstrap value of 95%. The similarity (455 bp) in the Kenyan and Indian

Arthrospira sequences was 100% (Table 5). The *Spirulina* strains formed their own cluster relatively distant from the *Arthrospira* cluster.

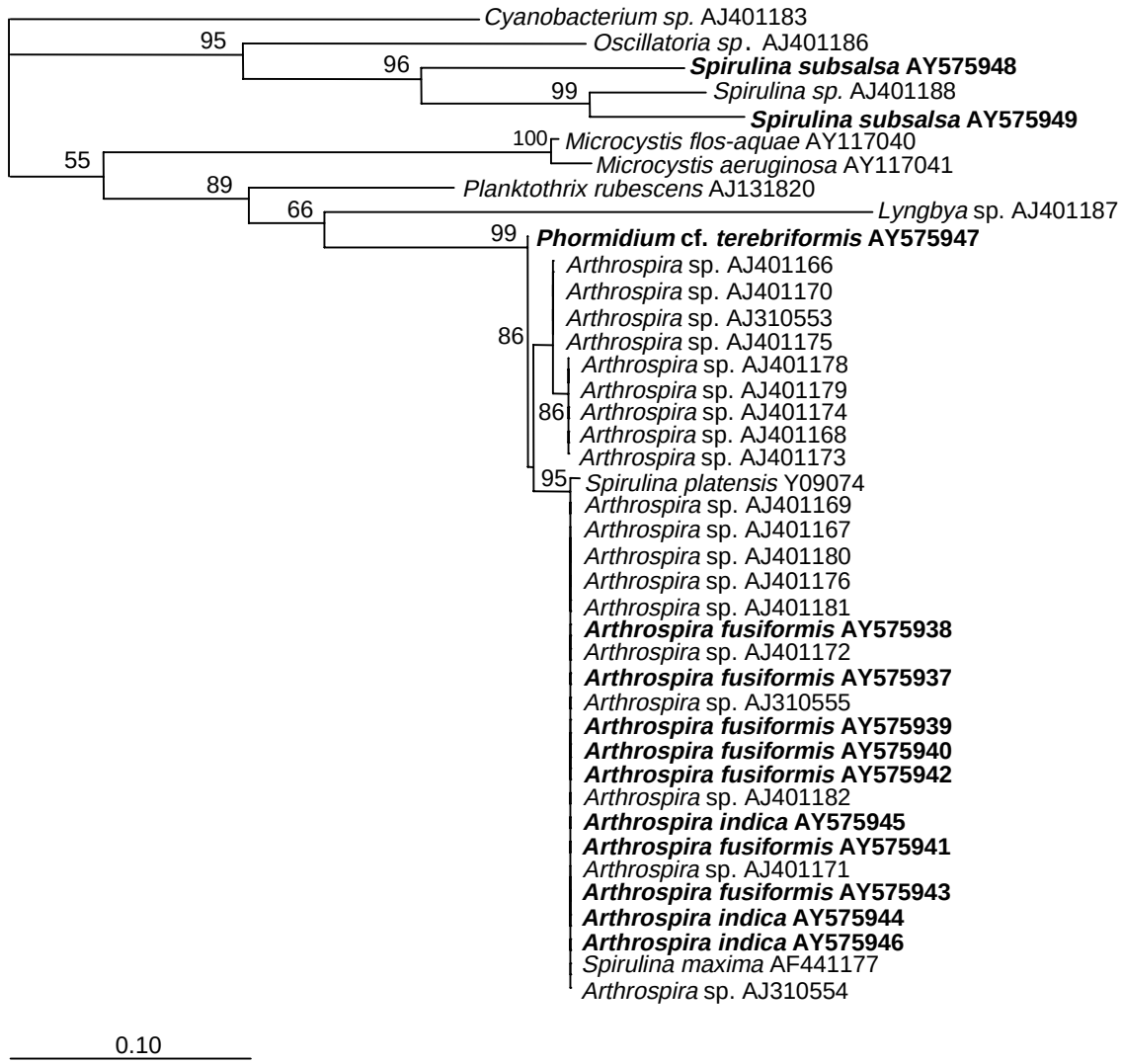

Fig. 3. Phylogenetic tree based on the alignment of sequences of the phycocyanin locus (PC-IGS) of 41 cyanobacterial strains. The tree was calculated using the maximum likelihood algorithm. Bootstrap values > 50% are included. Bar indicates 10% sequence divergence.

Table 5. Calculated DNA (455 bp) similarities in the phycocyanin operon (PC-IGS) between *Arthrospira*- and *Phormidium* strains. The values in the upper right triangle are the percent identities between a pair of sequences. The values in the lower left triangle are the numbers of dissimilar nucleotides. For the related species name, see Table 1.

	AY575937	AY575938	AY575939	AY575940	AY575941	AY575942	AY575943	AY575944	AY575946	AY575945	AY575947
AY575937	-	100	100	100	100	100	100	100	100	100	98.0
AY575938	0	-	100	100	100	100	100	100	100	100	98.0
AY575939	0	0	-	100	100	100	100	100	100	100	98.0
AY575940	0	0	0	-	100	100	100	100	100	100	98.0
AY575941	0	0	0	0	-	100	100	100	100	100	98.0
AY575942	0	0	0	0	0	-	100	100	100	100	98.0
AY575943	0	0	0	0	0	0	-	100	100	100	98.0
AY575944	0	0	0	0	0	0	0	-	100	100	98.0
AY575946	0	0	0	0	0	0	0	0	-	100	98.0
AY575945	0	0	0	0	0	0	0	0	0	-	98.0
AY575947	9	9	9	9	9	9	9	9	9	9	-

The phylogenetic tree for the ITS focused only on strains of *Arthropira* spp. and *Phormidium* cf. *terebriformis* (Fig. 4). Four distinct subclusters were identified. The Kenyan *Arthrospira* (AY575923-AY575929) and *Phormidium* (AY575933) strains and the Indian *A. indica* strain PD1997/ram (AY575931) were collectively in one subcluster. The two Indian *A. indica* strains PD1998/pus (AY575930), PD2002/ana (AY575932) and *A. indica* (AJ292336) formed a second subcluster. The sequences of the remaining other *Arthrospira* strains constituted the two other subclusters. The similarity of the ITS sequences (470 bp) for the Kenyan *Arthrospira* strains and the Indian strain PD1997/ram was 100%. The ITS sequences of the Indian strains PD1998/pus (AY575930) and PD2002/ana (AY575932) had similarity of 99.6% to sequences of the other investigated strains (Table 6).

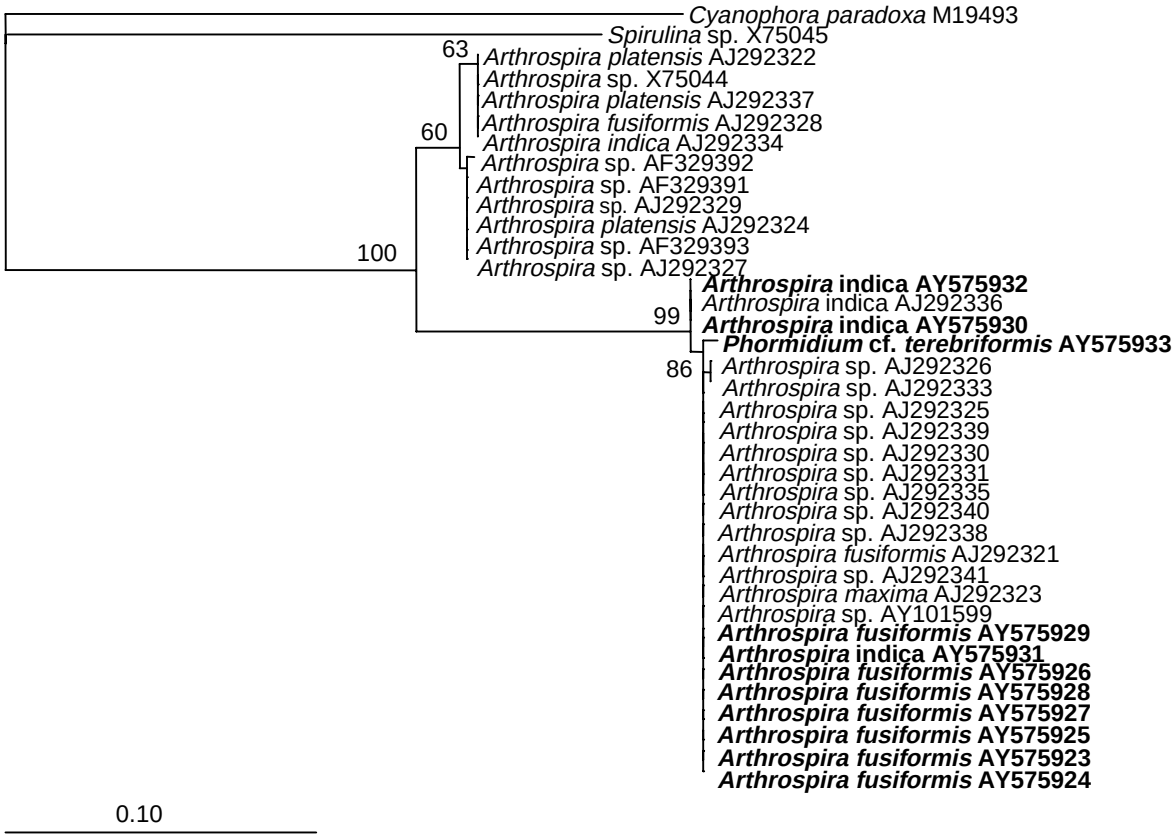

Fig. 4. Phylogenetic tree based on the alignment of sequences of the internally transcribed spacer region (ITS) of 38 cyanobacterial strains. The tree was calculated using the maximum likelihood algorithm. Bootstrap values > 50% are included. Bar indicates 10% sequence divergence.

Table 6. Calculated DNA similarities (470 bp) in the internally transcribed spacer region (ITS) between *Arthrospira* and *Phormidium* strains. The values in the upper right triangle are the percent identities between a pair of sequences. The values in the lower left triangle are the numbers of dissimilar nucleotides. For the related species name, see Table 1.

	AY575923	AY575924	AY575925	AY575926	AY575927	AY575928	AY575929	AY575932	AY575930	AY575931	AY575933
AY575923	-	100	100	100	100	100	100	99.6	99.6	100	99.6
AY575924	0	-	100	100	100	100	100	99.6	99.6	100	99.6
AY575925	0	0	-	100	100	100	100	99.6	99.6	100	99.6
AY575926	0	0	0	-	100	100	100	99.6	99.6	100	99.6
AY575927	0	0	0	0	-	100	100	99.6	99.6	100	99.6
AY575928	0	0	0	0	0	-	100	99.6	99.6	100	99.6
AY575929	0	0	0	0	0	0	-	99.6	99.6	100	99.6
AY575932	2	2	2	2	2	2	2	-	100	99.6	99.1
AY575930	2	2	2	2	2	2	2	0	-	99.6	99.1
AY575931	0	0	0	0	0	0	0	2	2	-	99.6
AY575933	2	2	2	2	2	2	2	4	4	2	-

Discussion

KOMÁREK & LUND (1990) and DESIKACHARY & JEEJI BAI (1992, 1996) have described different species of the genus *Arthrospira* (*A. platensis, A. fusiformis, A. jenneri, A. maxima* and *Arthrospira indica*) considering various morphological characters and habitats. According to these features, using light microscopic observations, the Kenyan *Arthrospira* strains have been recognized as *A. fusiformis*, whereas the calyptrated Indian strains were determined as *A. indica*. Coiling and pitch of the helix are highly variable in the Kenyan and in the Indian strains and are therefore not considered for the species identification of *Arthrospira*. Spiral trichomes of cyanobacteria can transform to straight filaments but this phenomenon can not be observed the other way round (BAKER 1997). The straight *Arthrospira* strain PD1998/pus remained unchanged even after several years of being placed in different physical conditions.

Both helical and straight filaments were observed in *Arthrospira* strain AB2002/02 during culturing in laboratory. Reversible change from loose helix to tight helix has also been reported (LI et al. 2001). Helix orientation in the cyanobacterium *Arthrospira* has extensively been studied and found that this feature is not stable and is controlled by environmental conditions, particularly temperature (MÜHLING et al. 2003).

Despite their differences in morphology and habitats, in the 16S and PC-IGS phylogenetic trees all sequences of Kenyan and Indian *Arthrospira* strains (AY575923-AY575932; AY575937-AY575946) are grouped in a uniform cluster each and in pairs show similarities of 100%. LI et al. (2001) also reported 100% sequence similarity when investigating the 16S rDNA sequences of an *Arthrospira maxima* strain (AF260509) and *Arthrospira fusiformis* strain (AF260510). The partial 16S rDNA sequences of both strains match to 100% with those of our strains. NELISSEN et al. (1994) found a 16S rRNA sequence similarity of two *Arthrospira* strains (PCC7345 and PCC8005) of 99.7%. According to CASTENHOLZ et al. (2001) such a high molecular similarity makes it likely that all *Arthrospira* strains are representatives of only one species. A 16S rRNA gene sequence similarity value of more than 97.5% is regarded the level at which two bacterial strains can be congeneric or belong to the same species (DEVEREUX et al. 1990, STACKEBRANDT & GOEBEL 1994). In contrast FOX et al. (1992) have mentioned that 16S rRNA sequences analysis is not necessarily a foolproof criterion to guarantee species identity, because closely related species may not be recognizable with this tool.

The investigation of the PC-IGS locus of *Arthrospira* strains from different continents has shown a separation in three clusters. No relation to morphology and geographical origin of the strains was found (MANEN & FALQUET 2002). They suggested horizontal transfers of parts of the sequence responsible for the different clusters. We have found the same division when using the PC-IGS locus for tree construction. The Kenyan and Indian *Arthrospira* strains (AY575937-AY575946) were included together in one subcluster showing 100% basepair similarities. The only variations between the studied strains were found in the ITS region. SCHELDEMAN et al. (1999) and BAURAIN et al. (2002) have investigated the ITS regions of several *Arthrospira* strains, assigned to the species *A. fusiformis*, *A. maxima*, *A. platensis* and *A. indica* from four continents. According to their results, the strains could be divided into two main clusters with two subclusters each. Like in the investigation of the PC-IGS locus by MANEN & FALQUET (2002) a clear relationship between ITS clusters and strain denomination,

morphology or the geographical origin was not found. In the study of BAURAIN et al. (2002), Kenyan *Arthrospira* strains from L. Nakuru and L. Sonachi could be found in both main clusters. In contradiction to their findings, all our 7 Kenyan *Arthrospira* strains taken out from 6 alkaline lakes are grouped in one cluster (AY575923-AY575929), that is subcluster IA according to BAURAIN et al. (2002). The Indian strain PD1997/ram (AY575931) also belonged to subcluster IA, whereas the two other Indian strains PD1998/pus and PD2002/ana (AY575930 and AY575932) belonged to subcluster IB with 100% base pair similarity. PD1998/pus has a straight and PD2002/ana a spiral morphotype.

Like the investigated *Arthrospira* strains the two *Spirulina* strains were isolated from an alkaline and a freshwater habitat. The Kenyan alkalitolerant *Spirulina* strain AB2002/06 and the Indian freshwater strain PD2002/gca have been recognized as *S. subsalsa.* In the16S and the PC-IGS trees, a S*pirulina* cluster could be clearly distinguished from the *Arthrospira* cluster. This supports the earlier findings of NELISSEN et al. (1994) who demonstrated using 16S rDNA sequences that *Spirulina* sp. PCC6313 is not closely related to *Arthrospira.* Similar results were obtained from analysis of the phycocyanin locus (MANEN & FALQUET 2002). The low similarity of only 91.5% (16S rDNA) between the Indian and Kenyan *Spirulina* strains shows, that the two strains are not closely related. Inside the *Spirulina* cluster a separation between the alkalitolerant *S. subsalsa* strain AB2002/06 (AY575934) from Kenya, the halotolerant *Halospirulina* (Y18790) and the Indian freshwater *S. subsalsa* strain PD2002/gca (AY575935) is obvious. MARGHERI et al. (2003) could clearly distinguish separate clusters correlated with habitats, when they investigated *Spirulina* and *Geitlerinema* strains from different alkaline, saline and freshwater habitats using ARDRA. They reported that hypersaline and alkaline strains are genetically distinct from the other isolates of marine and fresh water environments. In the present study, alkaline (Kenyan) and fresh water (Indian) strains of *Arthrospira* with different morphotypes are genetically identical and form a phylogenetically coherent group.

The *Phormidium* cf. *terebriformis* strain AB2002/07 was isolated from a phytoplankton sample from Lake Nakuru, the strain KR2003/25 from a less alkaline hot spring at the shore of Lake Bogoria. They are not planktic and grow in culture forming mats on the surface of the culture flask. Morphologically they can be clearly distinguished from the *Arthrospira* strains. The filaments are slightly undulated. HINDÁK (2001) and KRIENITZ et al. (2003) have described *P.* cf. *terebriformis* from hot springs at the shore of Lake Bogoria, where it is found

in mats on the substrate surface together with *Spirulina subsalsa*, *Oscillatoria willei* and *Synechococus bigranulatus*. In the 16S, the PC-IGS and the ITS trees the *P.* cf. *terebriformis* strain AB2002/07 (AY575933; AY575947) was always found close to or in the *Arthrospira* cluster. That makes it evident that it is closely related to *Arthrospira.* The high similarity values of 99.6% in the sequences of the 16S rDNA and ITS and 98.0% in the PC-IGS locus support this suggestion (DEVEREUX et al. 1990, STACKEBRANDT & GOEBEL 1994). However, this strain was lacking helicity, a major character of the genus *Arthrospira*. Because of frequent movements of flamingos between the alkaline lakes in the Rift Valley, it can be suggested that *Phormidium* cf. *terebriformis* strains are transported from the hot springs to other lakes. The Lesser Flamingos (*Phoeniconaias minor* GEOFFROY) use the less saline water of the hot springs for washing themselves and drinking (MARI & COLLAR 2000, Krienitz et al. 2003). During this procedure filaments and single cells of hot spring cyanobacteria can adhere to their bodies. However, in the 16S tree the *Phormidium* strain KR2003/25 (AY575936) is clearly separated from the strain AB2002/07 (AY575933), which is supported by a 16S similarity of only 92.0%. Both strains are living in habitats with differences in temperature and salinity conditions and most likely they are not members of the same species.

Molecular characterizations of *Arthrospira, Spirulina* and *Phormidium* strains in the present study demonstrate that different morphotypes may be genetically similar and vice versa.

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