Phycologia (2010) Volume 49 (4), 361–372 Published 2 July 2010

Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Oscillatoriales, Cyanobacteria) of African, Asian and American origin deduced by 16S–23S ITS and phycocyanin operon sequences

Pawan K. Dadheech[1,2], Andreas Ballot[2], Peter Casper[2], Kiplagat Kotut[3], Eberto Novelo[4], Brook Lemma[5], Thomas Pröschold[6] and Lothar Krienitz[2]*

[1]*Government College, Department of Botany, Ajmer 305001 (Rajasthan), India*
[2]*Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Limnology of Stratified Lakes, Alte Fischerhütte 2, 16775 Stechlin, Germany*
[3]*Kenyatta University, Plant and Microbial Sciences Department, P.O. Box 43844, Nairobi, Kenya*
[4]*National Autonomous University of México, Department of Comparative Biology, School of Sciences, A.P. 70 – 474, CU, Coyoacán, 04510, D.F. México*
[5]*Addis Ababa University, College of Education, Department of Biology, PO Box 1913, 1110 Addis Ababa, Ethiopia*
[6]*Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Dunbeg by Oban, Argyll, PA37 1QA, United Kingdom*

Dadheech P.K., Ballot A., Casper P., Kotut K., Novelo E., Lemma B., Pröschold T. and Krienitz L. 2010. Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Oscillatoriales, Cyanobacteria) of African, Asian and American origin deduced by 16S–23S ITS and phycocyanin operon sequences. *Phycologia* 49: 361–372. DOI: 10.2216/09-71.1

Arthrospira comprises multicellular, cylindrical, usually screwlike coiled trichomes and is cultivated commercially. In this study, 33 new strains of *Arthrospira* isolated from plankton samples collected in Mexico, East Africa and India were investigated and compared with 53 strains or samples of earlier considerations. The study included observations of morphological features and molecular phylogenetic analyses on the basis of nucleotide sequences of internal transcribed spacer (ITS) between 16S rRNA and 23S rRNA genes and partial sequences of beta and alpha subunits including intergenic spacer (*cpc*BA-IGS) of phycocyanin operon. Morphological traits of *Arthrospira* such as trichome width, type of coiling and apical cell were not always consistent in culture conditions. It was revealed that *Arthrospira* phylogeny on the basis of *cpc*BA-IGS locus was broadly comparable with the ITS region as both phylogenetic trees derived from nucleotide sequences could be divided into two main clusters. Cluster I comprised sequences from American strains mainly, whereas cluster II contained the sequences of the strains originating from Africa and Asia chiefly. Both genetic regions of the strains investigated in the present study coincidently showed a significant sequence divergence among *Arthrospira* strains from East Africa, India and Mexico indicating possible distinct evolutionary lineages.

Key Words: Cyanobacteria, Oscillatoriales, *Arthrospira*, Phylogeny, Phycocyanin operon, ITS, Mexico, Africa, India

INTRODUCTION

The *Arthrospira* Stizenberger (commercially known as *Spirulina*), is widely cultivated in various ways or harvested from natural environments worldwide (Fox 1996; Pushparaj *et al.* 1997; Jimenez *et al.* 2003; Shimamatsu 2004; Cogne *et al.* 2005; Ugwu *et al.* 2008) for use as dietary supplement (Belay *et al.* 1993; Gantar & Svirčev 2008) and as a source of pharmaceutical products (Ble-Castillo *et al.* 2002; Torres-Duran *et al.* 2007).

Conventionally, members of the genus *Arthrospira* are microscopically characterized by regularly coiled trichomes with visible cross-walls. The tightness of the spirals, arrangement of spirals, cell and trichome dimension, and apices of the trichome were considered as main taxonomic criteria for species differentiation (Hindák 1985; Anagnostidis & Komárek 1988; Guglielmi *et al.* 1993; Desikachary & JeejiBai 1996; Tomaselli *et al.* 1996; Vonshak & Tomaselli 2000). More than 20 species of *Arthrospira* were compiled on the basis of several conventional criteria (Komárek & Lund 1990; Komárek & Hauer 2009). Most common are the benthic species *Arthrospira jenneri* (Hass.) Stiz. and *Arthrospira platensis* (Nordst.) Gom., and the planktonic species *Arthrospira maxima* Setch. & Gard. and *Arthrospira fusiformis* (Woron.) Kom. & Lund. Benthic species were separated from planktonic taxa on the basis of aerotopes (*sensu* Komárek & Anagnostidis 2005; previously designated as gas vesicles). The benthic *A. jenneri* Stizenberger ex Gomont has been denoted as the type species of this genus (Geitler 1942; Komárek & Hauer 2009). In this study we focus on planktonic strains of *Arthrospira*. From planktonic species, the geographical distribution of *A. maxima* is considered to be mainly in the American continent, whereas *A. fusiformis* is widely distributed in Africa and Asia (Komárek & Lund 1990; Komárek *et al.* 2003). Both species are differentiated only by the type of coiling of the trichome. Molecular evidences are not available to differentiate these morphospecies of *Arthrospira*. Phenotypic analyses of *Arthrospira* strains obtained

* Corresponding author (krie@igb-berlin.de).

from various culture collections have been carried out on the basis of morphological features, particularly helical structure of the trichome, and correlation between two clusters attained from these phenotypic characters was determined (Mühling *et al.* 2006). However, variation and overlapping of criteria in trichome contour from spiral to straight and *vice versa* have been observed in natural environmental conditions and experimental studies (Lewin 1980; Hindák 1985; Desikachary & JeejiBai 1996; Mühling *et al.* 2003; Wang & Zhao 2005).

The genus and species concept of cyanobacteria is changing from the conventional morphological classification to a concept using genotypic features (Rippka *et al.* 1979; Turner 1997; Wilmotte & Herdman 2001; Hoffmann *et al.* 2004; Oren 2004). A polyphasic approach has been used to characterize cyanobacterial taxa (Castenholz *et al.* 2001; Komárek & Anagnostidis 2005; Casamatta *et al.* 2005; Comte *et al.* 2007; Palinska & Marquardt 2008). 16S rRNA gene sequence has been commonly used for characterization of interspecific variability and phylogenetic analysis among cyanobacterial genera (Castenholz 1992; Suda *et al.* 2002), including *Arthrospira* (Nelissen *et al.* 1994; Li *et al.* 2001; Ballot *et al.* 2004). The conserved nature and less variable position in the 16S ribosomal rRNA gene do not allow the distinction of intragenic taxa of cyanobacteria and also the conduction of phylogenetic studies (Nelissen *et al.* 1996). After introducing sequencing of the internally transcribed spacer between 16S rRNA and 23S rRNA genes (16S–23S ITS), it became possible to discriminate species and strains of bacteria because of variation in their nucleotide sequences (Gürtler 1993; Gürtler & Stanisich 1996). This method is now predominantly used in characterization and phylogenetic studies of cyanobacteria (Boyer *et al.* 2001, 2002; Iteman *et al.* 2000, 2002; Taton *et al.* 2003; Chen *et al.* 2006; Haverkamp *et al.* 2008). Similarly, other more variable regions like intergenic spacer of the phycocyanin operon and flanking regions (PC-IGS) have mostly been used for characterization of cyanobacterial strains (Neilan *et al.* 1995; Bittencourt-Oliveira *et al.* 2001; Dyble *et al.* 2002; Ballot *et al.* 2008; Berrendero *et al.* 2008).

Genetic variation of *Arthrospira* strains from an alkaline pond near Cluj-Napoca, Romania, has been assessed by polymerase chain reaction (PCR) technique based on genomic repetitive sequences (Aldea *et al.* 2002). *Arthrospira* strains were analyzed using amplified ribosomal DNA restriction analysis of total DNA (Viti *et al.* 1997) and the ITS region (Scheldeman *et al.* 1999). Isolates of *Arthrospira* from four continents, available in different culture collections, were characterized using sequences of the ITS region (Baurain *et al.* 2002) and *cpc*B–*cpc*A DNA fragment (Manen & Falquet 2002). These studies revealed a high phylogenetic similarity between the *Arthrospira* strains from the different continents. A clear connection was not established between the phylogenetic clusters and the geographic origin of strains. The phylogenetic relationship of *Arthrospira* cultures from Kenyan and Indian water bodies has been examined using sequences of the PC-IGS locus and the ITS (Ballot *et al.* 2004).

In the present study, 33 new *Arthrospira* strains from various aquatic habitats of three continents (Africa, Asia and America) were examined morphologically and genetically. Their phylogenetic relatedness and correlation between geographical origins were determined by nucleotide sequence analysis of the 16S–23S ITS region and the *cpc*BA-IGS locus. This is the first study in which an apparent phylogenetic distinction between African, Asian and American strains is documented. A few controversies of our findings with formerly published results will be discussed.

MATERIAL AND METHODS

Strains and culture conditions

We isolated 33 original, nonaxenic strains of *Arthrospira* from plankton samples of different water bodies located in East Africa (Kenya, Tanzania and Uganda), India and Mexico (Table 1). Presently Lake Texcoco (Mexico) is represented by several separated water bodies (Caracol/Lago recreativo/Nabor Carillo). In addition, 10 strains that we isolated and studied earlier (Ballot *et al.* 2004) were also considered for analysis of data. During sample collection, salinity and pH values were measured with a WTW Multiline P4 meter (Wissenschaftlich Technische Werkstätten Weilheim, Germany) in triplicate and mean values are presented (Table 1). Single filaments from the plankton samples were isolated using microcapillary procedure for clonal propagation and strain establishment (Pringsheim 1946). The Kenyan and Mexican strains were cultivated in Bourrelly medium (Hegewald *et al.* 1994) modified with an addition of Na_2CO_3 (0.3 g l^{-1}) and NaCl (15 g l^{-1}). The Indian strains were cultured in *Spirulina* medium (Schlösser 1994) modified from Aiba & Ogawa (1977). All strains were maintained at 18°C with photon flux density of 20 µmol of photons m^2 s^{-1} (equivalent to 1500 lx). For study of the morphology, the cultivation temperature was increased to 25°C. Photographs were taken with an Olympus BX51 system microscope at ×400 and ×1000 magnification using the Cell D software from Olympus Soft Imaging Solutions GmbH, Münster, Germany. Identification of the strains was determined according to Komárek & Anagnostidis (2005) and Komárek & Hauer (2009).

Genomic DNA extraction, PCR amplification, sequencing and phylogenetic analysis

The genomic DNA was extracted using Dynabeads DNA DIRECT System I (Invitrogen/Dynal Biotech, Oslo, Norway) according to the manufacturer's manual. PCR was performed in a Peltier Thermal Cycler PTC 200 (MJ Research Inc., San Francisco, CA, USA) using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany). The reaction mixture contained 14.4 µl of sterile water, 2.0 µl of 10× Qiagen PCR buffer, 0.5 µl of deoxynucleoside triphosphate mix (10 mM), 1 µl of forward primer (10 µM), 1 µl of reverse primer (10 µM), 0.1 µl of *Taq* DNA polymerase enzyme (5 U/µl; 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 final concentrations in a volume of 20 µl are as follows: Taq DNA polymerase (0.05 U), 1× Qiagen PCR buffer, 250 µM

Table 1. *Arthrospira* strain identification (ID), geographical origin and habitat characters.

Strain ID	Habitat	Country of origin	Salinity (ppt)[1]	pH
AB2006/01	Lake Bogoria^{Sa}	Kenya	55.1	9.8
AB2006/02	Lake Bogoria^{Sa}	Kenya	55.1	9.8
AB2006/03	Lake Bogoria ^{Sa}	Kenya	55.1	9.8
AB2006/04	Lake Elmenteita^{Sa}	Kenya	32.7	9.7
AB2006/05	Lake Bogoria^{Sa}	Kenya	55.1	9.8
AB2006/06	Lake Oloidien^{Sa}	Kenya	3.6	9.6
AB2006/08	Lake Texcoco^{Sa} (Lago recreativo)	Mexico	11.5	10.6
AB2006/09	Lake Texcoco^{Sa} (Caracol)	Mexico	35	10.2
AB2006/10	Lake Texcoco^{Sa} (Nabor Carillo)	Mexico	3.6	10.4
AB2006/11	Lake Texcoco^{Sa} (Lago recreativo)	Mexico	11.5	10.6
AB2006/12	Lake Texcoco^{Sa} (Caracol)	Mexico	35	10.2
AB2006/13	Lake Texcoco^{Sa} (Lago recreativo)	Mexico	11.5	10.6
AB2006/14	Lake Texcoco^{Sa} (Nabor Carillo)	Mexico	3.6	10.4
AB2006/15	Lake Texcoco^{Sa} (Nabor Carillo)	Mexico	3.6	10.4
AB2006/16	Lake Texcoco^{Sa} (Caracol)	Mexico	35	10.2
KR2004/02	Lake Texcoco^{Sa} (Nabor Carillo)	Mexico	11.5	10.4
KR2004/04	Lake Texcoco^{Sa} (Nabor Carillo)	Mexico	35	10.2
KR2004/05	Lake Texcoco^{Sa} (Caracol)	Mexico	35	10.2
KR2004/06	Lake Texcoco^{Sa} (Caracol)	Mexico	35	10.2
KR2004/09	Lake Texcoco^{Sa} (Nabor Carillo)	Mexico	3.6	10.4
KR2004/10	Lake Texcoco^{Sa} (Nabor Carillo)	Mexico	3.6	10.4
KR2004/12	Lake Texcoco^{Sa} (Nabor Carillo)	Mexico	3.6	10.4
KR2005/113	Lake Nakuru^{Sa}	Kenya	24.8	10.2
KR2005/115	Lake Nakuru^{Sa}	Kenya	24.8	10.2
KR2005/117	Lake Nakuru^{Sa}	Kenya	24.8	10.2
KR2005/118	Lake Kadwe^{Sa}	Uganda	300	9.7
PD2002/02	Lake Shambhar^{Sa}	India	40	9.8
PD2002/03	Lake Shambhar^{Sa}	India	40	9.8
PD2002/04	Lake Shambhar^{Sa}	India	40	9.8
PD2002/06	Lake Mansagar^{Fa}	India	1.1	9.2
PD2002/07	Lake Shambhar^{Sa}	India	40	9.8
PD2002/08	Lake Shambhar^{Sa}	India	40	9.8
AB2002/38	Lake Big Momella^{Sa}	Tanzania	28.5[2]	na
PD2002/ana	*Lake Anasagar^{Fa} Ajmer*	*India*	1.9	9.6
PD1998/pus	*Pushkar Pond^{Fa}, Pushkar*	*India*	< 1.0	7.6
PD1997/ram	*Vicinity of Lake Ramgarh^{Fa}, Jaipur*	*India*	< 1.0	8.0
AB2002/01	*Lake Elmenteita^{Sa}*	*Kenya*	27.7	10.4
AB2002/02	*Lake Sonachi^{Sa}*	*Kenya*	10.4	10.4
AB2002/03	*Lake Magadi^{Sa}*	*Kenya*	46.7	9.9
AB2002/04	*Lake Nakuru^{Sa}*	*Kenya*	30.1	10.5
AB2002/05	*Lake Bogoria^{Sa}*	*Kenya*	53.1	9.9
AB2002/10	*Lake Bogoria^{Sa}*	*Kenya*	53.1	9.9
AB2002/11	*Lake Simbi^{Sa}*	*Kenya*	9.7	10.1

Italic strain studied earlier by authors (Ballot *et al.* 2004) of this paper and included in phylogenetic analysis.
[1] ppt, parts per thousand; Sa, saline-alkaline; Fa, freshwater-alkaline.
[2] According to Lugomela *et al.* (2006).

each deoxynucleotide, *c.* 60–75 ng of each oligonucleotide primer and 1 µl of sample DNA (DNA amount not measured).

The primers 16S3′F and 23S5′R (Baurain *et al.* 2002) were used for amplification of ITS between 16S rRNA and 23S rRNA genes and the primers cpc_arF and cpc_arR (Ballot *et al.* 2004) were used to obtain partial sequences of *cpc*BA-IGS. The thermal cycling program applied for amplification of the ITS region was as follows: initial 3 min at 94°C, 30 cycles of 30 s at 94°C, 30 s at 50°C, 45 s at 70°C and a final elongation step at 72°C for 3 min. The PCR amplification of PC-IGS was performed with an initial denaturation step of 94°C for 3 min, followed by 30 cycles of 94°C for 20 s, 55°C for 30 s and 72°C for 1 min with a final extension step of 72°C for 5 min.

PCR products were visualized by 1.5% agarose gel electrophoresis with ethidium bromide staining and ultraviolet illumination. PCR products of the ITS region and the *cpc*BA-IGS locus were purified through Qiaquick PCR purification columns (Qiagen, Hilden, Germany) and DNA was redissolved in elution buffer according to the manufacturer's protocol. The purified products of both DNA fragments (ITS and *cpc*BA-IGS) were sequenced using the same primers as used for the PCR. Both strands of purified DNA were sequenced on ABI 3100 Avant Genetic Analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Applera, Deutschland, GmbH, Darmstadt, Germany) according to the manufacturer's manual.

ITS sequences of 16S–23S and *cpc*BA-IGS locus were analyzed for all the studied strains. Nucleotide sequences were aligned visually using Manual Sequence Alignment Editor Align v05/2008 (Hepperle 2008). For phylogenetic study, in case of the ITS region, a set containing 410 positions was used, and the *cpc*BA-IGS alignment comprised 347 positions. Our ITS alignment corresponds to position 40–449 of the alignment used by Baurain *et al.*

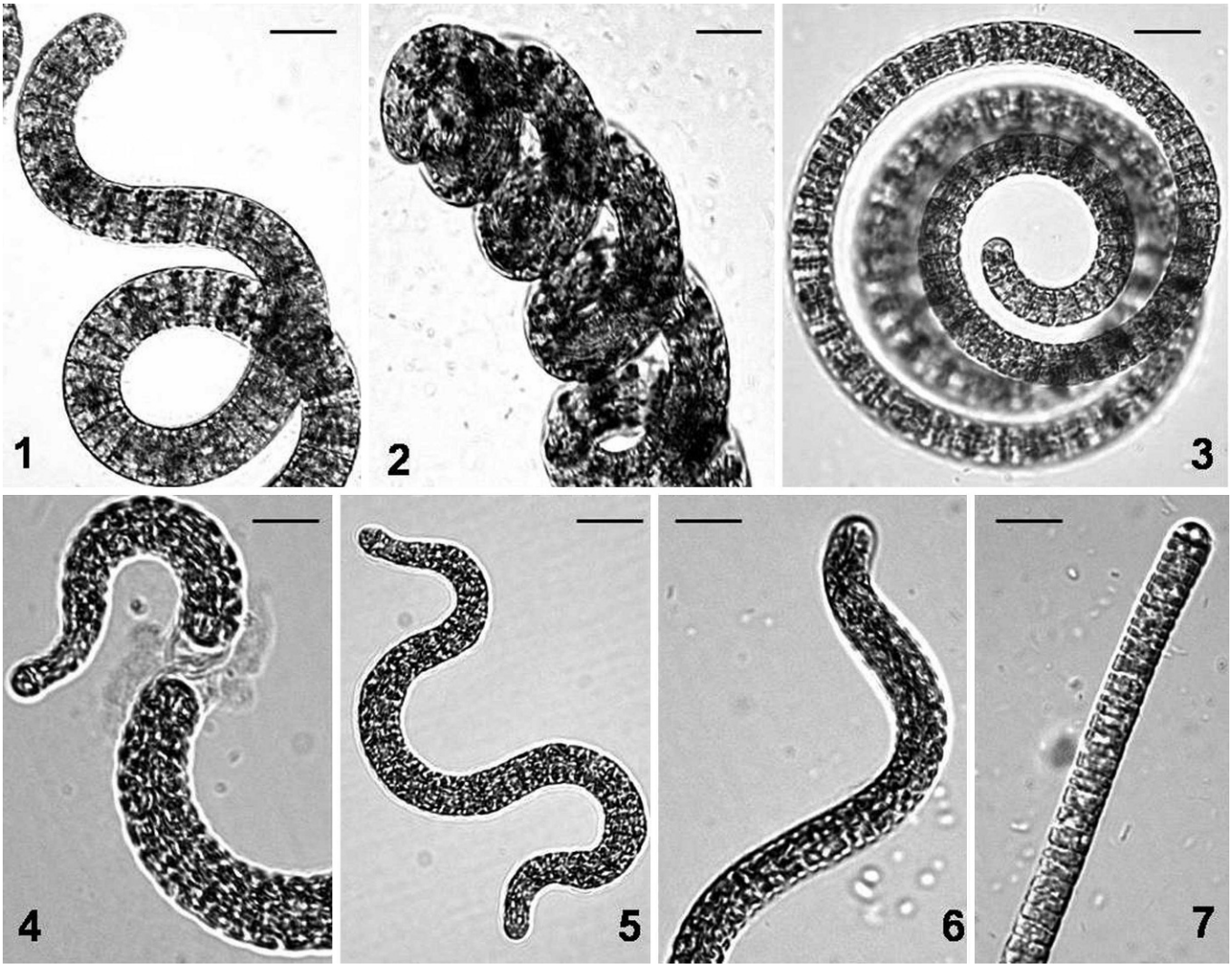
Table 2. GenBank accession numbers of *Arthrospira* sequences used for phylogenetic analysis. Strains investigated in this study are indicated in bold font; strains in normal font were studied earlier by authors of this paper. GenBank sequences obtained by other workers are indicated in italic font.

GenBank accession number				GenBank accession number	
Strain ID[1]	16S–23S ITS	*cpc*BA-IGS	Strain ID	16S-23S ITS	*cpc*BA-IGS
AB2006/01	**FJ001876**	**FJ001909**	*CCAP 1475/8 (Ethiopia)*●	*(AJ292321)*	na
AB2006/02	**FJ001877**	**FJ001910**	*Lefevre 1963/M-132-1 (Chad)*	*(FJ798612)*	na
AB2006/03	**FJ001878**	**FJ001911**	*DIHE1 (Nat. sample) (Chad)* © ●	*(AJ292338)*	na
AB2006/04	**FJ001879**	**FJ001912**	*3832 (Natural sample) (Chad)* ●	*(AJ292339)*	na
AB2006/05	**FJ001880**	**FJ001913**	*DIHE Q+ (Chad)***?** © ●	*(AJ292340)*	na
AB2006/06	**FJ001881**	**FJ001914**	*SP-10 (Madagascar)*●	*(AJ292325)*	na
AB2006/08	**FJ001882**	**FJ001915**	*SAC SP-16 (Thailand)***?** © ●	*(AJ292326)*	na
AB2006/09	**FJ001883**	**FJ001916**	*PCC 9223 (Spain)*●	*(AJ292335)*	na
AB2006/10	**FJ001884**	**FJ001917**	*SAG 84.79 (Chad)*●	*(AJ292323)*	na
AB2006/11	**FJ001885**	**FJ001918**	*EF-18A (USA)***?** © ●	*(AJ292333)*	na
AB2006/12	**FJ001886**	**FJ001919**	*C1 (Chad)*●	*(AJ292330)*	na
AB2006/13	**FJ001887**	**FJ001920**	*MCRC isolate straight (India)***?** © ●	*(AJ292336)*	na
AB2006/14	**FJ001888**	**FJ001921**	*PCC8005 (India, Kenya, Mexico or Peru)***?** ●	*(X70769)*	na
AB2006/15	**FJ001889**	**FJ001922**	*EF-2 (USA)***?** © ●	*(AJ292332)*	na
AB2006/16	**FJ001890**	**FJ001923**	*SAG 257.80 (Peru)*●	*(AJ292337)*	na
KR2004/02	**FJ001891**	**FJ001924**	*SP-7 (Mexico)*	*(EF432313)*	na
KR2004/04	**FJ001892**	**FJ001925**	*HEGEWALD 1976/83 (Kenya)*●	*(AJ292328)*	na
KR2004/05	**FJ001893**	**FJ001926**	*UTEX 1928 (USA)*●	*(AJ292322)*	na
KR2004/06	**FJ001894**	**FJ001927**	*PCC 9108***?** © ●	*(AJ292329)*	na
KR2004/09	**FJ001895**	**FJ001928**	*FACHB 439***?**	*(AF329391)*	na
KR2004/10	**FJ001896**	**FJ001929**	*MCRC isolate spiral (India)***?** © ●	*(AJ292334)*	na
KR2004/12	**FJ001897**	**FJ001930**	*SAC SP-17 (Thailand)***?** ©	*(EF432319)*	na
KR2005/113	**FJ001898**	**FJ001931**	*PCC 7345 (USA)*●	*(X75044)*	*(AJ401178)* ○†
KR2005/115	**FJ001899**	**FJ001932**	*OUQDS6***?**	*(AF329393)*	na
KR2005/117	**FJ001900**	**FJ001933**	*Compère 86.79 (Chad)*●	*(AJ292327)*	na
KR2005/118	**FJ001901**	**FJ001934**	*FACHB 438***?**	*(AF329392)*	na
PD2002/02	**FJ001902**	**FJ001935**	*BU2 (Burma)* ○	na	*(AJ401181)*†
PD2002/03	**FJ001907**	**FJ001936**	*Lonar-LoH7 (India)* ○	na	*(AJ401177)*†
PD2002/04	**FJ001903**	**FJ001937**	*M1 (Chad)* ○	na	*(AJ401169)*
PD2002/06	**FJ001904**	**FJ001938**	*Titi (Peru)* ○	na	*(AJ401172)*†
PD2002/07	**FJ001905**	**FJ001939**	*Mad (India)* ○	na	*(AJ401180)*†
PD2002/08	**FJ001906**	**FJ001940**	*C1***?**	na	*(Y09074)*
AB2002/38	**FJ001875**	**FJ001908**	*Sark***?** ○	na	*(AJ310555)*
PD2002/ana	AY575932	AY575944	*E1***?** ○	na	*(AJ310554)*
PD1997/ram	AY575931	AY575945	na**?** *China*	na	*(AF441177)*
PD1998/pus	AY575930	AY575946	*Dihé (Chad)* ○	na	*(AJ401176)*†
AB2002/01	AY575923	AY575937	*BU1 (Burma)* ○	na	*(AJ401167)*
AB2002/02	AY575924	AY575938	*Mix (India)***?** ○	na	*(AJ401182)*†
AB2002/03	AY575925	AY575939	*ThD***?** ○	na	*(AJ401171)*†
AB2002/04	AY575926	AY575940	*M2 (Chad)* ○	na	*(AJ401173)*†
AB2002/05	AY575927	AY575941	*PK***?** ○	na	*(AJ401179)*†
AB2002/10	AY575928	AY575942	*Maxima (Mexico)* ○	na	*(AJ401168)*
AB2002/11	AY575929	AY575943	*Fv-8***?** ○	na	*(AJ401174)*†
Sp-2 (Chad)	*(DQ279768)*	na	*Nord Geitl***?** ○	na	*(AJ401184)*†
*TKF1 (Taiwan)***?**	*(AY101599)*	na	*Paracas GE98 (Peru)* ○	na	*(AJ401175)*†
*Titicaca (Peru)***?** ●	*(AJ292331)*	na	*Paracas P2 (Peru)* ○	na	*(AJ401166)*
*ROHRER (Pill)***?** © ●	*(AJ292341)*	na	*Paracas E2 (Peru)* ○	na	*(AJ310553)*
SP-1 (Mexico)	*(AJ292324)*	na	*Paracas P0 (Peru)* ○	na	*(AJ401170)*†

[1] ID, identification; na, not available; **?**, doubtful origin of strain; ©, strains from commercial facility; ●, strains studied by Baurain *et al.* (2002); ○, strains studied by Manen and Falquet (2002); †, correct accession number that was mentioned incorrectly in the publication of Manen & Falquet (2002).

(2002). For the *cpc*BA-IGS alignment, 139 nucleotides (nt) of *cpc*B, 111 nt of spacer region and 97 nt of the *cpc*A were used. Phylogenetic analyses of the ITS and *cpc*BA-IGS regions were performed by MEGA software v4.0 (Tamura *et al.* 2007), and trees were constructed using a neighbor-joining (NJ) algorithm, Tamura–Nei model for nucleotide sequences available in the MEGA phylogenetic software. *Phormidium autumale* (AM778721) and *Spirulina subsalsa* (AY575949) were used as outgroup in the ITS and the *cpc*BA-IGS tree, respectively. The phylogenetic tree of concatenated ITS and *cpc*BA-IGS combined sequences was constructed following the same method used for an individual tree. The nodes of the tree were tested by bootstrap analysis of 1000 replicates using NJ method. The nucleotide sequences used to construct the phylogenetic trees were a combination of sequences achieved in the present study (33) plus those (10) we obtained in an earlier investigation (Ballot *et al.* 2004) and those (31 ITS, 23 *cpc*BA-IGS) provided by other researchers in the GenBank (Table 2).

The new nucleotide sequences have been deposited in the GenBank database under the accession numbers FJ001875-



Figs 1–7. Microphotographs of *Arthrospira* strains investigated in this study. Scale bars = 10 μm.
Fig. 1. Loosely coiled trichome (AB2006/01, Kenya).
Figs 2, 3. Tightly coiled trichome with different shape (KR2005/113; KR2005/117, Kenya).
Fig. 4. Trichome with attenuation at end and one calyptra (PD2002/05, India).
Fig. 5. Trichome with two calyptra at the tip (PD2002/06, India).
Fig. 6. Trichome with rounded end cell (KR2004/03, Mexico).
Fig. 7. Straight trichome (KR2004/09, Mexico).

FJ001907 (ITS) and FJ001908-FJ001940 (*cpc*BA-IGS). The alignment is available at TreeBase under the study accession number 52594.

RESULTS

The pH values of studied *Arthrospira* habitats varied between 8 and 11, whereas salinity ranged from 1.1 to 300 parts per trillion (ppt) (Table 1). One strain was isolated from the freshwater Lake Mansagar in India (PD2002/06) with a salinity of 1.1 ppt, whereas others were obtained from saline–alkaline lakes where salinity ranged from 3.6 to 55.1 ppt. One strain (KR2005/118) was isolated from the hypersaline Lake Katwe (Uganda) where a salinity of 300 ppt was recorded. The salinity of Lake Texcoco (Mexico) ranged from 3.6 ppt to 35 ppt, depending on the sampling site.

Morphological features of *Arthrospira* like width of trichome, types of helix and the apical shape of the trichomes were considered for phenotypic characterization of the studied strains. The trichome width of all strains was between 7 and 12 μm. The length of the trichomes varied widely. All strains except one (KR2005/09) were helical and maintained a spiral shape during successive culturing in laboratory. Strains isolated from East Africa and India exhibited a trichome shape that varied from loosely (Fig. 1) to tightly coiled (Figs 2, 3). Most strains of Mexican origin were found to be loosely twisted (Figs 4–6). The trichome of the strain KR2005/09 was straight in a natural sample collected from Lake Texcoco (Nabor Carillo) and maintained a similar morphology in culture (Fig. 7). The majority of the strains isolated from various habitats of

Fig. 8. Neighbour-joining tree of *Arthrospira* strains on the basis of 16S–23S ITS gene sequences as inferred with the MEGA v4.0 software. *Arthrospira* strains examined in this study (33) are in bold font, strains (10) studied earlier by the authors of this paper are in normal font, and strains (31) used from GenBank are in italic font. Only support values > 50% are shown. **?** = strain with doubtful origin; © = commercial product; ● = strains from reference Baurain *et al.* (2002); ◄ = contradictory strain with certain origin.

India exhibited a noticeable calyptra at the tip of the trichome (Fig. 4). This feature was also observed in few strains from African water bodies. The trichome was invariably attenuated at the end in the strains where a calyptra was present (Figs 4, 5). The calyptra was seldom observed at both ends of the trichome with a noticeable attenuation (Fig. 5). The attenuation at the end of the trichome was not apparent in Mexican strains. The trichomes possessed a rounded end cell without a calyptra (Figs 6, 7). Aerotopes were present in all investigated strains.

The NJ phylogenetic tree of the ITS sequence of *Arthrospira* strains showed two major clusters, designated as cluster I and cluster II (Fig. 8). Cluster I contained 18 strains of Mexican origin, two of US origin, one from Peru, one from Asia and seven of doubtful origin. Cluster II contained 33 strains from Africa and Asia, one from Spain and 10 strains of uncertain origin. The *p*-distances between sequences of the strains of cluster I and cluster II were 90.0–91.2% (Table 3). The *p*-distance value of sequences included in cluster I was between 98.8 and 100%. The calculated DNA sequence similarities of the strains of cluster II ranged from 99.5 to 100%. Clusters I and II were supported by bootstrap values of 99%.

The *Arthrospira* strains included in the phylogenetic tree of *cpc*BA-IGS locus were mainly distributed in a pattern similar to the ITS tree and featured two main clusters: cluster I and cluster II (Fig. 9). Cluster I contained all new Mexican strains together with earlier GenBank sequences obtained from strains of the American continent except one strain from Chad and three strains of uncertain origin. Cluster II was composed of 27 strains of African or Indian origin, and seven earlier GenBank sequences of strains with African/Asian origin (India, Burma, China, Chad). Furthermore, this cluster contained five strains of doubtful origin and one strain from Peru. The *p*-distances among the strains of cluster I and cluster II ranged from 95.6 to 97.6% (Table 3). Cluster I was supported by bootstrap value of 75% and cluster II by a bootstrap value of 77%.

The phylogenetic tree created from concatenated ITS + *cpc*BA-IGS sequences of the new strains showed a similar phylogenetic relationship between Indian and East African strains and a divergence to Mexican strains (Fig. 10).

Table 3. Calculated percentage DNA sequence similarities between cluster I and cluster II (see Figs 8 and 9).

Clusters	Cluster I (Mexican)	Cluster II (East African, India)
ITS (423 bp)		
Cluster I (Mexican)	98.8–100	
Cluster II (East African, Indian)	90.0–91.2	99.5–100
*cpc*BA-*IGS (348 bp)*		
Cluster I (Mexican)	97.4–100	
Cluster II (East African, Indian)	95.6–97.6	100

DISCUSSION

The presence of *Arthrospira* strains in varied range of saline habitats shows the ability of *Arthrospira* to adapt to freshwater–alkaline conditions as well as saline–alkaline and even hypersaline environments. In this study, we did not find a habitat-dependent phylogenetic grouping among *Arthrospira* strains. The strains studied clustered in phylogenetic trees of ITS and *cpc*BA-IGS that were not related to habitat salinity. Mussagy *et al.* (2006) compared an *Arthrospira* strain from freshwater (a wastewater treatment pond in Maputo, Mozambique) with a strain from the saline–alkaline Lake Nakuru, Kenya and found identical ITS sequences in both strains. These findings closely agree with an earlier study in which it was found that *Arthrospira* strains from the saline–alkaline habitats in Kenya and freshwater habitats in India were identical (Ballot *et al.* 2004). In contrast, studies on other oscillatoriacean such as *Spirulina* Turp. ex Gom. and *Geitlerinema* (Anagnost. & Kom.) Anagnost. distinguished separate clusters on the basis of their salinity or alkalinity tolerance by amplified ribosomal DNA restriction analysis (ARDRA), in accordance with the respective environmental origin (Margheri *et al.* 2003). Phylogenetic analysis of the 16S rRNA gene of three *Spirulina*-like isolates from hypersaline habitats produced a tightly monophyletic cluster that has been described as a new genus *Halospirulina* Nübel, Garcia-Pichel & Muyzer (Nübel *et al.* 2000).

Arthrospira strains investigated in this study were not determined at morphospecies level because they depict high morphological variability under both natural and culture conditions (Hindák 1985; Desikachary & JeejiBai 1996; Ballot *et al.* 2004; Mühling *et al.* 2003; Wang & Zhao 2005). We recorded variability in coiling of trichomes of *Arthrospira* as tightly coiled trichomes became loose or at times straight in culture. According to the conventional approach to species differentiation, the tightly coiled strains are identified as *A. fusiformis*, whereas the loosely coiled and broad trichomes are *A. maxima* (Komárek & Lund 1990; Li *et al.* 2001; Mussagy *et al.* 2006). Because of the plasticity in the coiling of trichomes, this is not a suitable feature to differentiate between two species. A variation in the degree of trichome coiling and the spontaneous emergence of straight trichomes of *Arthrospira* has also been recognized in natural conditions (Tomaselli *et al.* 1997). In contrast, the reversion of *'Spirulina platensis'* (taxonomic designation not clear) from linear to helical shape has also been documented during culturing and found the same genetic background in both forms of the trichomes with random amplified polymorphic DNA analysis technique (Wang & Zhao 2005). In our cultures a change from coiled to straight filament morphology was observed in few strains; however, no reversal of the straight to helical phenotype could be documented. It is noteworthy that the strain KR2005/09 from Lake Texcoco was straight in its natural habitat; hence, it was not easy to identify it as *Arthrospira* under low magnification. The arrangement and morphology of cells under higher magnification provided some resemblances to *Arthrospira*, which we later confirmed using molecular tools. This observation points out that occasionally morphological features of a cyanobacterium in its natural environment and in culture can lead to false identification even at genus level. The finding also supports an earlier opinion (Palinska *et al.* 1996) attesting to a need to include

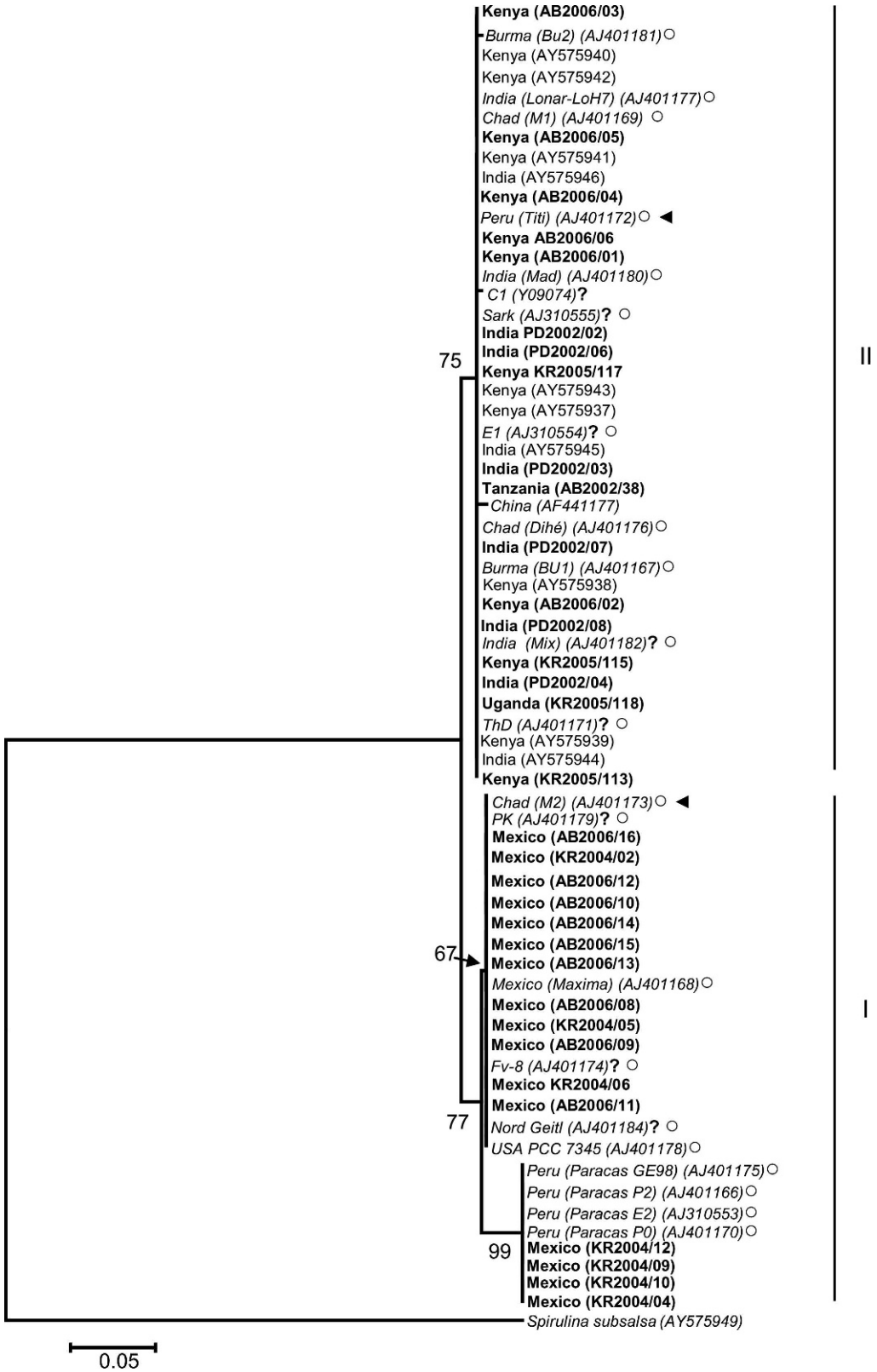


Fig. 9. Neighbour-joining tree of *Arthrospira* strains on the basis of *cpc*BA-IGS region sequences of phycocyanin operon as inferred with the MEGA v4.0 software. *Arthrospira* strains examined in this study (33) are in bold font, the strains (10) studied earlier by the authors of this paper are in normal font and the strains (23) used from GenBank are in italic font. Only support values > 50% are shown. **?** = strain with doubtful origin; ○ = strains from reference Manen & Falquet *et al.* (2002); ◄ = contradictory strain with certain origin.

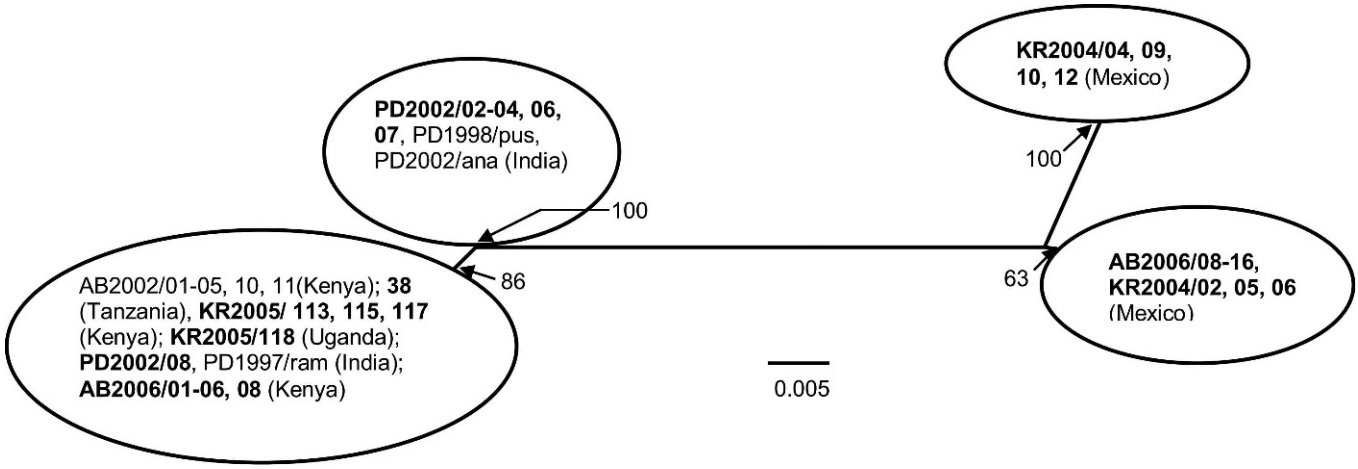


Fig. 10. Neighbor-joining radiation style tree of new *Arthrospira* strains only, constructed with MEGA v4.0 software from concatenated sequences (ITS region + *cpc*BA-IGS locus). Only support values > 50% are shown.

molecular characterization for correct identification of a cyanobacterial taxon. Mühling *et al.* (2006) established two clusters on the basis of phenotypic characters, mainly the helical shape of trichomes of *Arthrospira* strains obtained from various culture collections. They also correlated these clusters with two other clusters previously obtained using ARDRA (Scheldeman *et al.* 1999) and sequencing of ITS (Baurain *et al.* 2002). The helix feature in *Arthrospira* shows high plasticity (Lewin 1980; Hindák 1985; Desikachary & JeejiBai 1996; Mühling *et al.* 2003; Li *et al.* 2001; Wang & Zhao 2005) and could not be considered a reliable character for describing a species.

A conspicuous difference was observed in the morphology of the apical cell of Indian *Arthrospira* strains and the strains from East Africa and Mexico. Mexican strains deviated in their phenotypic trait from East African/Indian strains by having a rounded cell end and the absence of a calyptra. The presence of a calyptra had also been recorded in an earlier study on *Arthrospira* strains from India (Ballot *et al.* 2004). Considering the importance of this morphological feature, a new morphospecies, *Arthrospira indica*, was established from *A. fusiformis* (Desikachary & JeejiBai 1992, 1996). On the basis of molecular study using 16S rRNA gene (Ballot *et al.* 2004) and sequence analysis of ITS region and *cpc*BA-IGS locus in the present investigation, we did not find phylogenetic divergence between calyptrate and noncalyptrate strains.

The present knowledge on the molecular phylogeny of *Arthrospira* was mainly based on strains and their details available in various culture collections (Scheldeman *et al.* 1999; Baurain *et al.* 2002; Manen & Falquet 2002). In these preceding studies, geographical origins and habitats of most of the strains were unknown or uncertain. Studies on molecular phylogeny of *Arthrospira* using strains isolated by authors themselves from natural habitats using 16S rRNA gene (Li *et al.* 2001; Ballot *et al.* 2004; Mussagy *et al.* 2006), ITS and *cpc*BA-IGS locus (Ballot *et al.* 2004) are scarce. We suggest that the certainty of geographical location and type of habitat should be established to allow for a logical phylogenetic relationship in accordance with geographic peculiarities.

ITS sequence analysis of *Arthrospira* strains obtained from different sources and belonging to four continents showed remarkable sequence conservation and were included in two clusters (Baurain *et al.* 2002). However, each cluster contained sequences of strains from all origins and a clear phylogenetic lineage pattern that relates to the geographic data could not be established. In contrast, our studies achieved a conspicuous divergent cluster from the ITS sequences of Mexican *Arthrospira* strains. Of 74 strains included in the analysis, sequences of only six strains seem to be in contradiction to a hypothesis of coincidence of distinct evolutionary lineages with geographic origin. Fourteen strains have an ambiguous origin. The origin of those 10 strains used in commercial cultivation facilities remains especially uncertain because it cannot be confirmed whether the strains were collected and isolated elsewere before procured for mass culture. Regarding the origin of strain 'Titicaca', Baurain *et al.* (2002) already expressed doubt. Finally, only two of six conflicting strains (Hegewald 1976/83, Compére 86.79) remain of unambiguous origin. All other sequences support the hypothesis of divergent evolution. Probably, a genetic demarcation separating isolates from East African/Asian and Mexican origins has taken place; however, this was interfered with by processes of distribution already discussed by Baurain *et al.* (2002) such as the transportation of cyanobacterial propagation units by vectors, e.g. migratory birds and the wind (summarized by Kristiansen 1996 and Padisák 2009). The speed of such propagation can be high. This was demonstrated on the expansion of the nostocalean cyanobacterium *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya & Subba Raju from its place of origin in tropical Africa to areas in the temperate zone of northern Europe within a period of less than a century (Padisák 1997).

The overall topology of the phylogenetic tree constructed with sequences of *cpc*BA-IGS locus corresponds to the ITS tree. The African/Asian *Arthrospira* strains formed a coherent group in the phylogenetic tree of *cpc*BA-IGS sequences showing significant sequence dissimilarity from isolates of Mexico. A previous report demonstrated two clusters in the phylogenetic analysis of *cpc*BA-IGS locus of

Arthrospira (Manen & Falquet 2002). Each cluster included a mix of strains from different continents. These differences in findings emphasize the necessity of clear geographic designations of strains. Excluding the strains with doubtful source, only two strains remained in our analyses that did not coincide with our hypothesis of distinct evolutionary lineages in the African/Asian vs American geographic region, the strains Titi (Peru) and Ms (Chad). Strain Titi is suspected to be identical with strain Titicaca, already found of doubtful origin (Baurain *et al.* 2002). Our findings revealed similar mosaic-like distribution of variable sites at the *cpc*B–*cpc*A locus of *Arthrospira* found by Manen & Falquet (2002, Fig. 2). The recombination pattern indicates possible evidence of horizontal gene transfer.

The three phylogenetic approaches (ITS, *cpc*BA-IGS and concatenated) produced similar tree topology. The concatenated approach has been suggested as a stronger tool in differentiating strains isolated from different geographical locations (Haande *et al.* 2008). The minor topology can be dissimilar because of different evolutionary histories of different genes within the same group of organisms (Haverkamp *et al.* 2008).

The present study carried out by molecular approach indicates that the phylogeny of members of this genus could be closely related to their geographical origin. Organisms that live in different habitats and are geographically separated could acquire specific metabolic adaptations that are reflected in genotypic divergence (Nübel *et al.* 2000; Margheri *et al.* 2003) as it was shown exemplarily for extreme halotolerant unicellular cyanobacteria of the genus *Halothece* (Garcia-Pichel et al. 1998) and polar Oscillatoriales (Casamatta et al. 2005). According to the conventional approach, considering geographical distribution and slight morphological deviation, *Arthrospira* strains of neotropical regions were considered as *A. maxima* species, whereas the tropical strains were assigned to *A. fusiformis* species (Komárek & Lund 1990; Mussagy *et al.* 2006).

In this study, molecular cladistic evidence of deep genotypic divergences among Mexican and African/Indian strains of *Arthrospira* is presented. It is apparent that Mexican strains and East African/Indian strains represent distinct genotypes. Phenotypic observations reveal that planktonic species of *Arthrospira* may be distinguished on the basis of morphology of trichome toward the end and its apical cell. Principally, the existence of the two tropical main planktonic species *A. fusiformis* and *A. maxima* were confirmed by the present study. We are of the opinion that sequence analysis of 16S–23S ITS region and the *cpc*BA-IGS locus of phycocyanin operon together could be used to assign a molecular species concept in the genus *Arthrospira*.

ACKNOWLEDGEMENTS

The study was made possible by funding support from the German Federal Ministry of Education and Research (grant no. BIOLOG 01LC0001). Two scientific meetings were supported by UNESCO and TWAS in the frame of the UNESCO–International Basic Sciences Programme: IBSP 1-DE-09. Permission to carry out the study was granted by the Kenya Goverment (no. MOEST 13/001/31C90). Our deep gratitude goes to Kenya Wildlife Services (KWS) and County Council of Koibatek for granting us access to protected areas within their jurisdiction. We are thankful to Gerencia del Lago de Texcoco-CONAGUA, Mexico for the permission to collect material for this study. PKD is grateful to Principal, Government College, Ajmer and Department of Higher Education, Government of Rajasthan, Jaipur for granting permission to participate in UNESCO-TWAS project. We are thankful to Monika Papke for maintaining the cultures and Monika Degebrodt for the technical assistance with DNA sequencing.

REFERENCES

Aiba S. & Ogawa T. 1977. Assessment of growth yield of a blue green alga: *Spirulina platensis*, in axenic and continuous culture. *Journal of General Microbiology* 102: 179–182.

Aldea R., Guţu A., Nicoara A. & Dragoş N. 2002. Genetic variation of *Arthrospira* (*Spirulina*) strains assessed by PCR technique based on genomic repetitive sequences. *Contribuţii Botanice* XXXVII: 127–136.

Anagnostidis K. & Komárek J. 1988. Modern approaches to the classification system of cyanophytes 3. Oscillatoriales. *Algological Studies* 80: 327–472.

Ballot A., Dadheech P.K. & Krienitz L. 2004. Phylogenetic relationship of *Arthrospira*, *Phormidium* and *Spirulina* strains from Kenyan and Indian waterbodies. *Algological Studies* 113: 37–56.

Ballot A., Dadheech P.K., Haande S. & Krienitz L. 2008. Morphological and phylogenetic analysis of *Anabaenopsis abijatae* and *Anabaenopsis elenkinii* (Nostocales, Cyanobacteria) from tropical inland water bodies. *Microbial Ecology* 55: 608–618.

Baurain D., Renquin L., Grubisic S., Scheldeman P., Belay A. & Wilmotte A. 2002. Remarkable conservation of internally transcribed spacer sequences of *Arthrospira* (''*Spirulina*'') (Cyanophyceae, Cyanobacteria) strains from four continents and of recent and 30-year-old dried samples from Africa. *Journal of Phycology* 38: 384–393.

Belay A., Ota Y., Miyakawa K. & Shimamatsu H. 1993. Current knowledge on potential health benefits of *Spirulina*. *Journal of Applied Phycology* 5: 235–241.

Berrendero E., Perona E. & Mateo P. 2008. Genetic and morphological characterization of *Rivularia* and *Calothrix* (Nostocales, Cyanobacteria) from running water. *International Journal of Systematic and Evolutionary Microbiology* 58: 447–460.

Bittencourt-Oliveira M.D., de Oliveira, M.C. & Bolch C.J.S. 2001. Genetic variability of Brazilian strains of the *Microcystis aeruginosa* complex (Cyanobacteria/Cyanophyceae) using the phycocyanin intergenic spacer and flanking regions (*cpc*BA). *Journal of Phycology* 37: 810–818.

Ble-Castillo J.L., Rodriguez-Hernandez A., Miranda-Zamora R., Juarez-Oropez M.A. & Diaz-Zagoya J.C. 2002. *Arthrospira maxima* prevents the acute fatty liver induced by the administration of simvastatin, ethanol and a hypercholesterolemic diet to mice. *Life Sciences* 70: 2665–2673.

Boyer S.L., Flechtner V.R. & Johansen J.R. 2001. Is the 16S–23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in cyanobacteria. *Molecular Biology and Evolution* 18: 1057–1069.

Boyer S.L., Johansen J.R., Flechtner V.R. & Howard G.L. 2002. Phylogeny and genetic variance in terrestrial *Microcoleus* (Cyanophyceae) species based on sequence analysis of the 16s rRNA gene and associated 16S–23S ITS region. *Journal of Phycology* 38: 1222–1235.

Casamatta D.A., Johansen J.R., Vis M.L. & Broadwater S.T. 2005. Molecular and morphological characterization of ten polar and near-polar strains within the Oscillatoriales (Cyanobacteria). *Journal of Phycology* 41: 421–438.

Castenholz R.W. 1992. Species usage, concept, and evolution in the cyanobacteria (blue-green-algae). *Journal of Phycology* 28: 737–745.

Castenholz R.W., Rippka R., Herdman M. & Wilmotte A. 2001. Subsection III. (Formally Oscillatoriales Elenkin 1934.) In: *Bergey's manual of systematic bacteriology* (Ed. by D.R. Boone & R.W. Castenholz), pp. 539–562. Springer, New York.

Chen F., Wang K., Kan J.J., Suzuki M.T. & Wommack K.E. 2006. Diverse and unique picocyanobacteria in Chesapeake Bay, revealed by 16S–23S rRNA internal transcribed spacer sequences. *Applied and Environmental Microbiology* 72: 2239–2243.

Cogne G., Cornet J.F. & Gros J.B. 2005. Design, operation, and modeling of a membrane photobioreactor to study the growth of the cyanobacterium *Arthrospira platensis* in space conditions. *Biotechnology Progress* 21: 741–750.

Comte K., Sabacka M., Carre-Mlouka A., Elster J. & Komárek J. 2007. Relationships between the Arctic and the Antarctic cyanobacteria; three *Phormidium*-like strains evaluated by a polyphasic approach. *FEMS Microbiology Ecology* 59: 366–376.

Desikachary T.V. & Jeejibai N. 1992. Taxonomic studies in *Spirulina*. In: *Spirulina ETTA National Symposium* (Ed. by C.V. Sheshadri & N. JeejiBai), pp. 12–21. MCRC, Madras, India.

Desikachary T.V. & Jeejibai N. 1996. Taxonomic studies in *Spirulina*. II. The identification of *Arthrospira* (''*Spirulina*'') strains and natural samples of different geographical regions. *Algological Studies* 83: 163–178.

Dyble J., Paerl H.W. & Neilan B.A. 2002. Genetic characterization of *Cylindrospermopsis raciborskii* (Cyanobacteria) isolates from diverse geographic origins based on nifH and cpcBA-IGS nucleotide sequence analysis. *Applied and Environmental Microbiology* 68: 2567–2571.

Fox R.D. 1996. Spirulina *production and potential*. Edisud, Aix en Provence, France.

Gantar M. & Svirčev Z. 2008. Microalgae and cyanobacteria: food for thought. *Journal of Phycology* 44: 260–268.

Garcia-Pichel F., Nübel U. & Muyzer G. 1998. The phylogeny of unicellular, extremely halotolerant cyanobacteria. *Archives of Microbiology* 169: 469–482.

Geitler L. 1942. *Schizophyta: Klasse Schizophyceae*. In: *Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigen Arten, insbesondere den Nutzpflanzen* (Ed. by A. Engler & K. Prantl), vol. 1b. Verlag von Wilhelm Engelmann, Leipzig. 232 pp.

Gürtler V. 1993. Typing of *Clostridium difficile* strains by PCR-amplification on variable length 16S–23S rDNA spacer regions. *Journal of General Microbiology* 139: 3089–3097.

Gürtler V. & Stanisich V.A. 1996. New approaches to typing and identification of bacteria using 16S–23S rDNA spacer region. *Microbiology* 142: 3–16.

Gugliemi G., Rippka R. & Tandeau De Marsac N. 1993. Main properties that justify the different taxonomic position of *Spirulina* spp. and *Arthrospira* spp. among cyanobacteria. *Bulletin de l'Iinstitut Oceanographique Monaco* 12: 13–23.

Haande S., Rohrlack T., Ballot A., Røberg K., Skulberg R., Beck M. & Wiedner C. 2008. Genetic characterization of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) isolates from Africa and Europe. *Harmful Algae* 7: 692–701.

Haverkamp T., Acinas S.G., Doeleman M., Stomp M., Huisman J. & Stal L.J. 2008. Diversity and phylogeny of Baltic Sea picocyanobacteria inferred from their ITS and phycobiliprotein operons. *Environmental Microbiology* 10: 174–188.

Hegewald E., Krienitz K. & Schnepf E. 1994. Studies on *Scenedesmus costatogranulatus* Skuja. *Nova Hedwigia* 59: 97–127.

Hepperle D. 2008. Align, Multisequence Alignment Editor ver. 05/2008 SequentiX – Digital DNA Processing, Klein Raden, Germany, www.sequentix.de.

Hindák F. 1985. Morphology trichomes in *Spirulina fusiformis* Voronichin from Lake Bogoria, Kenya. *Archiv für Hydrobiologie* 38/39: 201–218.

Hoffmann L., Komárek J. & Kaštovský J. 2004. System of cyanoprokaryotes (cyanobacteria) state 2004. *Algological Studies* 117: 95–115.

Iteman I., Rippka R., de Marsac N.T. & Herdman M. 2000. Comparison of conserved structural and regulatory domains within divergent 16S rRNA–23S rRNA spacer sequences of cyanobacteria. *Microbiology-Sgm* 146: 1275–1286.

Iteman I., Rippka R., de Marsac, N.T. & Herdman M. 2002. rDNA analyses of planktonic heterocystous cyanobacteria, including members of the genera *Anabaenopsis* and *Cyanospira*. *Microbiology-Sgm* 148: 481–496.

Jimenez C., Cossio B.R. & Niell F.X. 2003. Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: a predictive model of algal yield. *Aquaculture* 221: 331–345.

Komárek J. & Anagnostidis K. 2005. Cyanophyta, 2, Oscillatoriales. In: *Süsswasserflora von Mitteleuropa,* vol. 19/2 (Ed. by B. Büdel, L. Gärtner Krienitz & M. Shagerl), Spectrum, Academischer Verlag, Elsevier, München.

Komárek J. & Hauer T. 2009. CyanoDB.cz – on-line database of cyanobacterial genera. http://www.cyanodb.cz.

Komárek J. & Lund J.W.G. 1990. What is '*Spirulina platensis*' in fact? *Algological Studies* 58: 1–13.

Komárek J., Komárková J. & Kling H. 2003. Filamentous cyanobacteria. In: *Freshwater algae of North America: ecology and classification* (Ed. by J. Wehr & R. Sheth), pp. 117–196. Academic Press, San Diego.

Kristiansen J. 1996. Dispersal of freshwater algae – a review. *Hydrobiologia* 336: 151–157.

Lewin R.A. 1980. Uncoiled variants of *Spirulina platensis* (Cyanophyceae: Oscillatoriaceae). *Algological Studies* 26: 48–52.

Li R.H., Debella H.J. & Carmichael W.W. 2001. Isolates identifiable as *Arthrospira maxima* and *Arthrospira fusiformis* (Oscillatoriales, Cyanobacteria) appear identical on the basis of a morphological study in culture and 16S rRNA gene sequences. *Phycologia* 40: 367–371.

Lugomela C., Pratap H.B. & Mgaya Y.D. 2006. Cyanobacteria bloom – a possible cause of mass mortility of Lesser Flamingoes in Lake Manyara and Lake Big Momela, Tanzania. *Harmful Algae* 5: 534–541.

Manen J.F. & Falquet J. 2002. The *cpc*B–*cpc*A locus as a tool for the genetic characterization of the genus *Arthrospira* (Cyanobacteria): evidence for horizontal transfer. *International Journal of Systematic and Evolutionary Microbiology* 52: 861–867.

Margheri M.C., Piccardi R., Ventura S., Viti C. & Giovannetti L. 2003. Genotypic diversity of oscillatoriacean strains belonging to the genera *Geitlerinema* and *Spirulina* determined by 16S rDNA restriction analysis. *Current Microbiology* 46: 359–364.

Mühling M., Harris N., Belay A. & Whitton B.A. 2003. Reversal of helix orientation in the cyanobacterium *Arthrospira*. *Journal of Phycology* 39: 360–367.

Mühling M., Somerfield P.J., Harris N., Belay A. & Whitton B.A. 2006. Phenotypic analysis of *Arthrospira* (*Spirulina*) strains (cyanobacteria). *Phycologia* 45: 148–157.

Mussagy A., Wilmotte A. & Cronberg G. 2006. The cyanophyte *Arthrospira fusiformis* from Mozambique, Africa: morphological and molecular characterization. *Algological Studies* 121: 59–73.

Neilan B.A., Jacobs D. & Goodman A.E. 1995. Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. *Applied and Environmental Microbiology* 61: 3875–3883.

Nelissen B., Wilmotte A., Neefs J.M. & De Wachter R. 1994. Phylogenetic relationships among filamentous helical cyanobacteria investigated on the basis of 16S ribosomal-RNA gene sequence analysis. *Systematic and Applied Microbiology* 17: 206–210.

Nelissen B., De Beaere R., Wilmotte A. & De Wachter R. 1996. Phylogenetic relationships of nonaxenic filamentous cyanobacterial strains based on 16S rRNA sequence analysis. *Journal of Molecular Evolution* 42: 194–200.

Nübel U., Garcia-Pichel F. & Muyzer G. 2000. The halotolerance and phylogeny of cyanobacteria with tightly coiled trichomes (*Spirulina* Turpin) and the description of *Halospirulina tapeticola* gen. nov., sp nov. *International Journal of Systematic and Evolutionary Microbiology* 50: 1265–1277.

OREN A. 2004. A proposal for further integration of the cyanobacteria under the Bacteriological Code. *International Journal of Systematic and Evolutionary Microbiology* 54: 1895–1902.

PADISÁK J. 1997. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive blue-green algal species: geographic distribution and autecology. *Archiv für Hydrobiologie/Monographic Studies* 107: 563–593.

PADISÁK J. 2009. The phycogeography of freshwater algae. In: *Encyclopedia of inland waters* (Ed. by G.E. Likens), pp. 219–223. Elsevier, Oxford, UK.

PALINSKA K.A. & MARQUARDT J. 2008. Genotypic and phenotypic analysis of strains assigned to the widespread cyanobacterial morphospecies *Phormidium autumnale* (Oscillatoriales). *Archives of Microbiology* 189: 325–335.

PALINSKA K.A., LIESACK W., RHIEL E. & KRUMBEIN W.E. 1996. Phenotype variability of identical genotypes: the need for a combined approach in cyanobacterial taxonomy demonstrated on *Merismopedia*-like isolates. *Archives of Microbiology* 166: 224–233.

PRINGSHEIM E.G. 1946. *Pure cultures of algae, their preparation and maintenance*. Cambridge University Press, Cambridge, UK.

PUSHPARAJ B., PELOSI E., TREDICI M.R., PINZANI E. & MATERASSI R. 1997. An integrated culture system for outdoor production of microalgae and cyanobacteria. *Journal of Applied Phycology* 9: 113–119.

RIPPKA R., DERUELLES J., WATERBURY J.B., HERDMAN M. & STANIER R.Y. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* 111: 1–61.

SCHELDEMAN P., BAURAIN D., BOUHY R., SCOTT M., MÜHLING M., WHITTON B.A., BELAY A. & WILMOTTE A. 1999. *Arthrospira* ('*Spirulina*') strains from four continents are resolved into only two clusters, based on amplified ribosomal DNA restriction analysis of the internally transcribed spacer. *FEMS Microbiology Letters* 172: 213–222.

SCHLÖSSER U.G. 1994. SAG-Sammlung von Algenkulturen at the University of Göttingen. Catalogue of strains, 1994. *Botanica Acta* 107: 113–186.

SHIMAMATSU H. 2004. Mass production of *Spirulina*, an edible microalga. *Hydrobiologia* 512: 39–44.

SUDA S., WATANABE M., OTSUKA S., MAHAKAHANT A., YONGMANITCHAI W., NOPARTNARAPORN N., LIU Y. & DAY J.G. 2002. Taxonomic revision of water-bloom-forming species of oscillatorioid cyanobacteria. *International Journal of Systematic and Evolutionary Microbiology* 52: 1577–1595.

TAMURA K., DUDLEY J., NEI M. & KUMAR S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software v4.0. *Molecular Biology and Evolution* 24: 1596–1599.

TATON A., GRUBISIC S., BRAMBILLA E., DE WIT R. & WILMOTTE A. 2003. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo dry valleys, Antarctica): a morphological and molecular approach. *Applied and Environmental Microbiology* 69: 5157–5169.

TOMASELLI L., PALANDRI M.R. & TREDICI M.R. 1996. On the correct use of *Spirulina* designation. *Algological Studies* 83: 539–584.

TOMASELLI L., BOLDRINI G. & MARGHERI M.C. 1997. Physiological behaviour of *Arthrospira* (*Spirulina*) *maxima* during acclimation to changes in irradiance. *Journal of Applied Phycology* 9: 37–43.

TORRES-DURAN P.V., FERREIRA-HERMOSILLO A. & JUAREZ-OROPEZA, M.A. 2007. Antihyperlipemic and antihypertensive effects of *Spirulina maxima* in an open sample of Mexican population: a preliminary report. *Lipids in Health and Disease* 6: 33.

TURNER S. 1997. Molecular systematics of oxygenic photosynthetic bacteria. *Plant Systematics and Evolution* suppl. 11: 13–52.

UGWU C.U., AOYAGI H. & UCHIYAMA H. 2008. Photobioreactors for mass cultivation of algae. *Bioresource Technology* 99: 4021–4028.

VITI C., VENTURA S., LOTTI E., CAPOLINO E., TOMASELLI L. & GIOVANNETTI L. 1997. Genotypic diversity and typing of cyanobacterial strains of the genus *Arthrospira* by very sensitive total DNA restriction profile analysis. *Research in Microbiology* 148: 605–611.

VONSHAK A. & TOMASELLI L. 2000. *Arthrospira* (*Spirulina*): systematics and eco-physiology. In: *The ecology of Cyanobacteria* (Ed. by B.A. Whitton & M. Potts), pp. 505–522. Kluwer, Dordrecht, the Netherlands.

WANG Z.P. & ZHAO Y. 2005. Morphological reversion of *Spirulina* (*Arthrospira*) *platensis* (Cyanophyta): from linear to helical. *Journal of Phycology* 41: 622–628.

WILMOTTE A. & HERDMAN M. 2001. Phylogenetic relationships among the cyanobacteria based on 16S rRNA sequences. In: *Bergey's manual of systematic bacteriology* (Ed. by D.R. Boone & R.W. Castenholz), pp. 487–493. Springer, New York.

Received 4 September 2009; accepted 19 December 2009
Associate editor: Jiří Komárek