Chakia (cyanobacteria), a new heterocytous genus from Belizean marshes identified on the basis of the 16S rRNA gene

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Abstract: A new genus *Chakia* has been described on the basis of 16S rRNA sequences of four strains isolated from two localities in alkaline marshes in Belize (Central America). Characteristic morphological features of thalli both from natural environment and from cultivated strains were documented to complete the description of this new genus.

The type strain has been named *Ch.ciliosa* sp. nov. Previous findings and up to now performed samplings and studies of relative material have indicated that the occurrence of the genus in all Belizean alkaline marshes, Everglades (USA), Cuban and other tropical Caribbean alkaline marshes is evident or highly probable.

Key words: *Chakia,* Scytonemataceae, central America, alkaline marshes, taxonomy, ecology

Introduction

Recent situation in taxonomy of cyanobacteria is rather complicated as morphology of the thalli was ceased to be considered unique distinguishing feature for identification of taxonomic units.

Modern methods developed in study of genome enabled to investigate the genetic information of cells and molecular criteria became to be the most important. Sequences of the 16S rRNA gene serve especially for delimitation of genera inside groups of cyanobacteria (Wilmotte & Herdman 2001; Sihvonen et al. 2007; Komárková et al. 2010; Komárek & Mareš 2012). Heterocytous cyanobacteria (Komárek & Hauer 2012) from the group relative to *Scytonema* are characterized by a complicated thallus branching in a false mode. Morphological variability of both filaments and trichomes is wide, however, in spite of this the appearance of thallus can be distinguished from other genera.

Alkaline marshes in Belize have been submitted to intense scientific research during the last 15 years (Rejmánková et al. 2004; Komárek & Komárková–Legnerová 2007; Komárek et al. 2005; Turicchia et al. 2009; etc.). The most particular feature of marshes is appearance of so called “algal” (in fact cyanobacterial) mats. They are originally formed at the bottom of loose *Eleocharis, Cladium* and *Typha* stands as a thick mucilaginous blue–green layer during long part of the season. At the end of the season, the layers are removing due to a high production of gases and floating at the surface of water. Whole shallow pools are then covered with a carpet of mats. The main carrier of the mat is a net of fine filamentous species of Oscillatoriales (*Leptolyngbya, Schizothrix*, narrow species of *Phormidesmis* etc.). Inside of this net we can find many species of Chroococcales and heterocytous cyanobacteria. The unique character of this strictly oligotrophic, both saline and freshwater natural complex of shallow pools is recently endangered by increasing agricultural activity and human water pollution (Rejmánková & Komárková 2005).

Material and Methods

Material from two localities was collected at calcareous marshes of northern Belize during a detailed study of algal mats in the 2006–2007 period.

1. BV – locality Buena Vista situated 15 km north of Orange Walk, central Belize (strains 3,5 and 8)
2. LB – locality Little Belize situated 25 km from Orange Walk to the north (strain 27)

Detailed description, chemical composition of water, physical data and stands of vegetation have been published by Rejmánková & Komárková 2000, Rejmánková et al. 2004 and Komárek et al. 2005.

Material collected both from the bottom and from the surface mats was studied fresh or preserved by 2% formaline. Portions of the fresh algal mat were transported to the laboratory and the newly recognized heterocytous genus was isolated from the material firstly into a semi–culture with a small addition of cultivation media. Later we prepared monospecific and clonal cultures from one filament (hormogonia) using micropipettes and microtitration plates (12 wells, 4 ml each). The strains were cultivated under 22 °C and radiationof 35 μmol.m^{-2}.s^{-1} in following media: liquid and agarized WC (Guillard & Lorenzen 1972) and BG11 (Stanier et al. 1971). Photographs of thalli were taken both in fresh and preserved state under a microscope Olympus BX51 using a digital camera DP 70.

Phylogenetic analyses. The biomass of the studied strains was harvested in the exponential phase of growth by repeated centrifugation, during which the trichomes were washed several times by NaCl solution (concentration 1 g.l^{-1}) to remove mucilaginous substances. The biomass samples were stored at –20 °C until DNA extractions. DNA was extracted using UltraClean^{TM} Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA). The 16S rRNA gene and partial ITS region were amplified with primers 16S27F and 23S30R (Taton et al., 2003). Amplification was carried out as follows: one cycle of 5 min at 94 °C; 10 cycles of 45 s at 94 °C, 45 s at 57 °C, and 2 min at 72 °C; 25 cycles of 45 s at 94 °C, 45 s at 54 °C, and 2 min at 72 °C; and a final elongation step of 7 min at 72 °C. PCR product was used as a template for sequencing with primers 16S27F (Taton et al. 2003), CYA781F(a) (Nübel et al. 1997), and the reverse complement of Primer 14 (Wilmotte et al. 1993).

Partial sequences of the 16S rRNA gene (1249 bp) were aligned using the program BioEdit version 7.0.9.0 (Hall, 1999) and the alignment was edited manually. Phylogenetic trees were constructed by maximum–likelihood (ML) maximum parsimony (MP) and neighbour–joining (NJ) (Saitou & Nei 1987) algorithms in the program PAUP* version 4.0b10 (Swofford 2003). The topology for the phylogenetic tree was derived from ML. The GTR+I+G evolutionary model of substitution was found for the best fit to the data using ModelTest 3.7 based on the Akaike information criterion (AIC) (Posada 2008). The parameters (base frequencies, rate matrix of substitution types and shape of gamma distribution) were estimated from the data. 100, 1000 and 1000 bootstrap replicates were performed for ML, MP and NJ analysis, respectively. Gaps were coded as missing data. The first step in selection of the scytonematacean representatives was BLAST. Nevertheless, only those sequences from the closest matches were selected whose taxonomic affiliation was reliably determined (based on published papers, or a clear documentation of morphology was available at the original authors). Nucleotide sequences were deposited at GenBank under the accession numbers KC875343, KC875344, KC875345, and KC875346.

RESULTS

The Belizean strains formed a pronounced phylogenetic cluster, receiving 100% bootstrap support of all phylogenetic analyses employed (ML, MP, NJ). This clade is situated at the base of nostocalean cyanobacteria and does not significantly cluster with any other heterocytous genera. On the basis of phylogenetic analyses and morphological studies, a new heterocytous filamentous cyanobacterial genus and species from the family Scytonemataceae has been described. The new genus was established according to molecular sequencing (Fig.1 and Table 1) together with morphological characteristics (Figs 2 and 3). Description of new taxa (genus and Type species) has been done according to prescriptions of International code of nomenclature for algae, fungi and plants (Melbourn code) (McNEILL et al. 2012).

Chakia genus novum Komárková , Zapomělová et Komárek 2012 (Scytonemataceae)

Diagnosis: Thalli live solitary or in small clusters among other algae and cyanobacteria. Simple filaments are heteropolar with a basal heterocyte, later simply *Tolypothrix* type false branching, rarely binary falsely branched, up to forming creaping isopolar filaments with numerous types of false branching. Filaments cylindrical, usually slightly widened towards ends (also narrowed when forming hormogonia); in narrow parts only (9.8)13.6–16 µm wide, later, especially at the ends up to 30 µm wide. Almost cylindrical filaments can also occur. Sheaths are of different width. When thick, they are densely lamellate, colorless up to yellow–brown mostly in inner parts, outer layers remain often colorless, sometimes partly with calcareous incrustations. Trichomes are uniseriate, of variable width, composed of variously shaped cells. Cells in some parts clearly constricted at cross walls, in terminal parts usually not constricted, sometimes club–like widened, terminal cells rounded. The width of young trichomes ranges from 6 to 10.5 µm, in apical and club–shaped widened segments elder trichomes can be up to 12.4 µm wide. Cells in younger parts are usually barrel–shaped or almost spherical, isodiametric or slightly shorter or longer than wide, in middle parts cylindrical and up to twice as long as wide, and at the ends usually cylindrical and often shorter than wide, bright blue–green. Heterocytes are basal in young germinating filaments, hemispherical or almost spherical, later ovoid, oval or cylindrical, 7.4–22.3 × 5–12.4 µm in diameter. In well developed filaments the heterocytes are spread irregularly between vegetative cells. Akinetes missing; reproduction by hormogonia and usually 1–4 celled hormocytes with densely lamellate, yellow–brown sheaths. Germination of hormocytes proceeds without the heterocyte which appears later during growth of a new filament.

Etymology: The genus has been named after Chak, the Mayan God of rain.

Typus generis: *Chakia ciliosa* sp. nov.

Type strain: *Chakia ciliosa* Komárková 2009/3, CCALA No. 1015; SAG (Göttingen) No. NA 2013.004. The sequence of the type species was assigned by the Genbank accession number KC875343. The Type strain is cultivated in two official collections (CCALA culture collection of the Botanical Institute AS CR at Třeboň, Czech Republic, www.cas.ccala.cz, and SAG

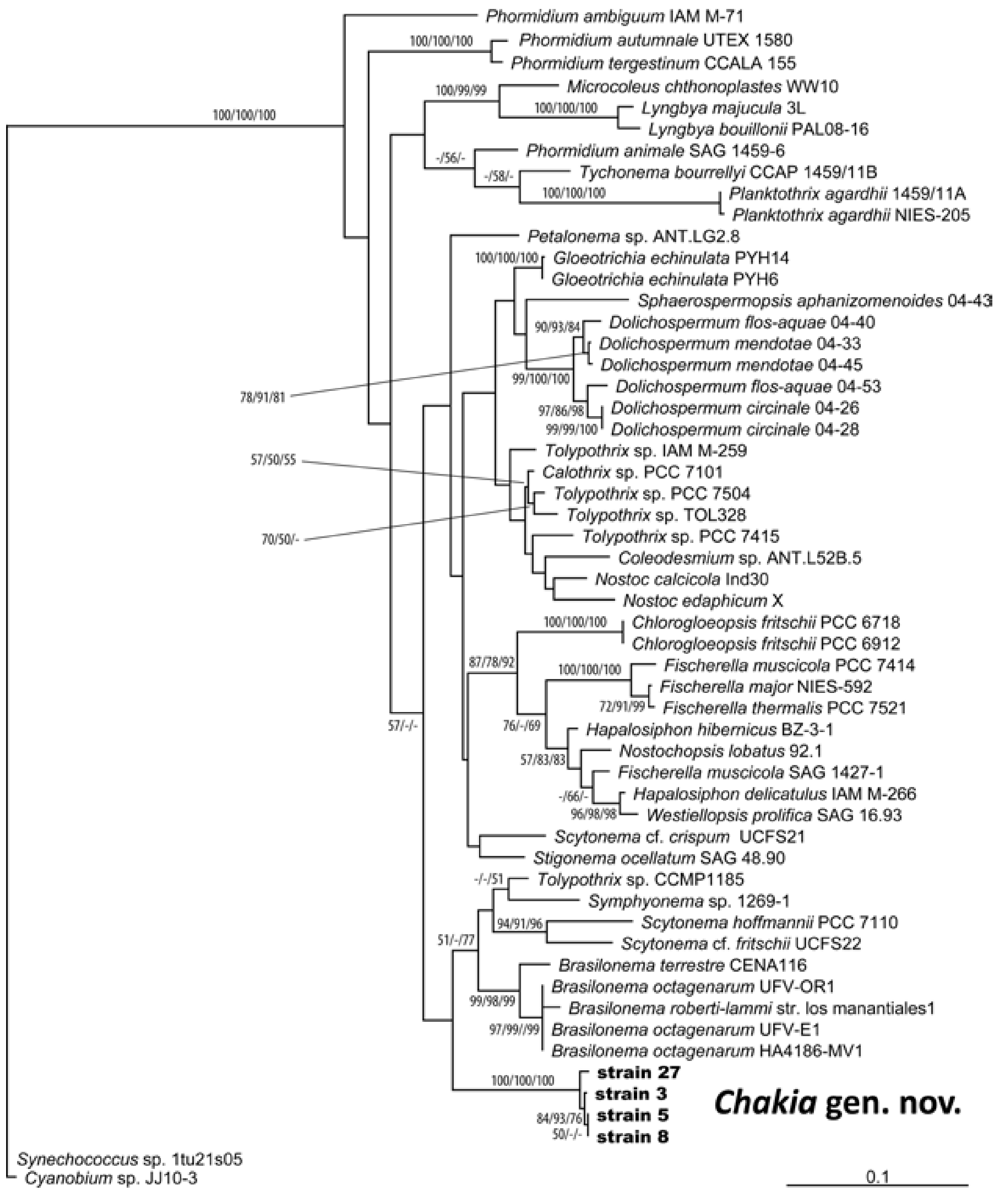

Fig. 1. Maximum likelihood phylogenetic tree with a position of *Chakia* genus novum. Bootstrap values are indicated in the following order: ML/MP/NJ.

Göttingen, www.uni–goettingen.de). Moreover, all four sequenced strains are available at the Cyano–collection of the Institute of Hydrobiology AS CR, České Budějovice, Czech Republic. Preserved sample of all four studied strains are maintained in Moravian Museum in Brno (BRNM HY2367), Czech Republic.

Ecology: Freshwater, originally periphytic, growing among cyanobacteria and algae forming mats at the bottom of oligotrophic tropical and subtropical alkaline marshes. Simple filaments of *Chakia* grow entangled in upper parts of mats. At the end of vegetation at the bottom they form longer, more diversified, isopolar and more branched filaments. Known mostly from Belizean wetlands, but it occurs probably also in other localities in Caribbean region (Everglades, Portorico, Cuba).

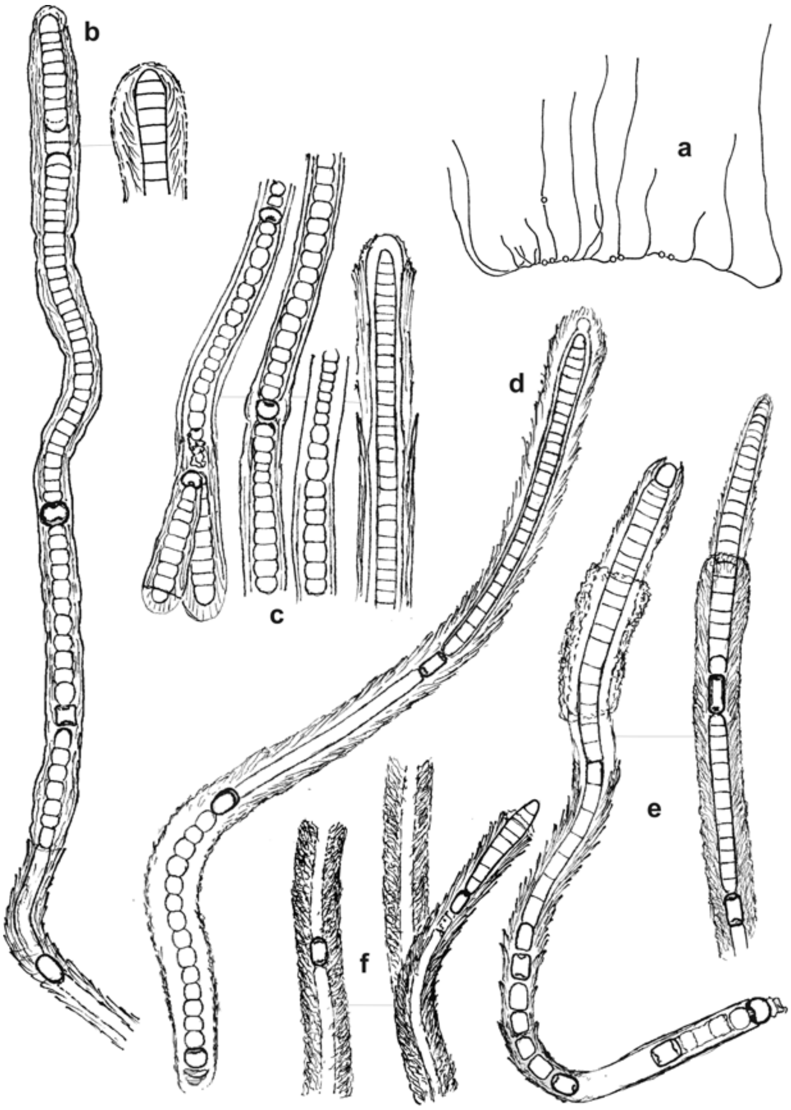

Fig. 2. *Chakia ciliosa*: (a) scheme of old thalus of *Chakia;* (b–e) details of trichomes; (f) details of old sheaths.

Discussion

According to modern phylogenetic and combined polyphasic assessment, the family Scytonemataceae is well defined and distinguishable (Flechtner et al. 2002; Hoffmann et al. 2005; Fiore et al. 2007) even if it belongs to monophyletic group of heterocytous cyanobacteria (Wilmotte & Herdman 2001). The difference is apparent between this family and the family Rivulariaceae which used to be unified also on the basis of appearance of the type of false branching, but the trichomes are distinctly heteropolar during the whole life cycle and many genera have the filaments elongated into hair–like ends. The only distinguishing morphological feature between the families is polarity of filaments: all the genera belonging to Rivulariaceae have got only heteropolar filaments while filaments of scytonematacean genera are mainly isopolar. Besides, branching of rivulariacean genera is not as rich as branching of the genera belonging to Scytonemaceae.

Richly and repeatedly branched thalli occur especially in genera *Scytonema, Scytonematopsis,* or *Chakia*. However, *Chakia* also forms heteropolar simple filaments (Fig. 3b), while fully developed thalli form heterocytes at all possible positions throughout the filaments (Fig. 3f).

There are not many morphological features differentiating *Chakia* from other genera belonging to Scytonemataceae. The ends of vegetative filaments are never as narrow as they are at *Scytonematopsis*. Whole trichomes are narrow in comparison with the most relative species of *Scytonema.* Cells are very variable in shape, they can be shortly cylindrical, elongated or barrel shaped. Type species *Chakia ciliosa* found in natural samples formed often hormocytes, what has not been found at other species of Scytonemataceae (Komárek 2013).

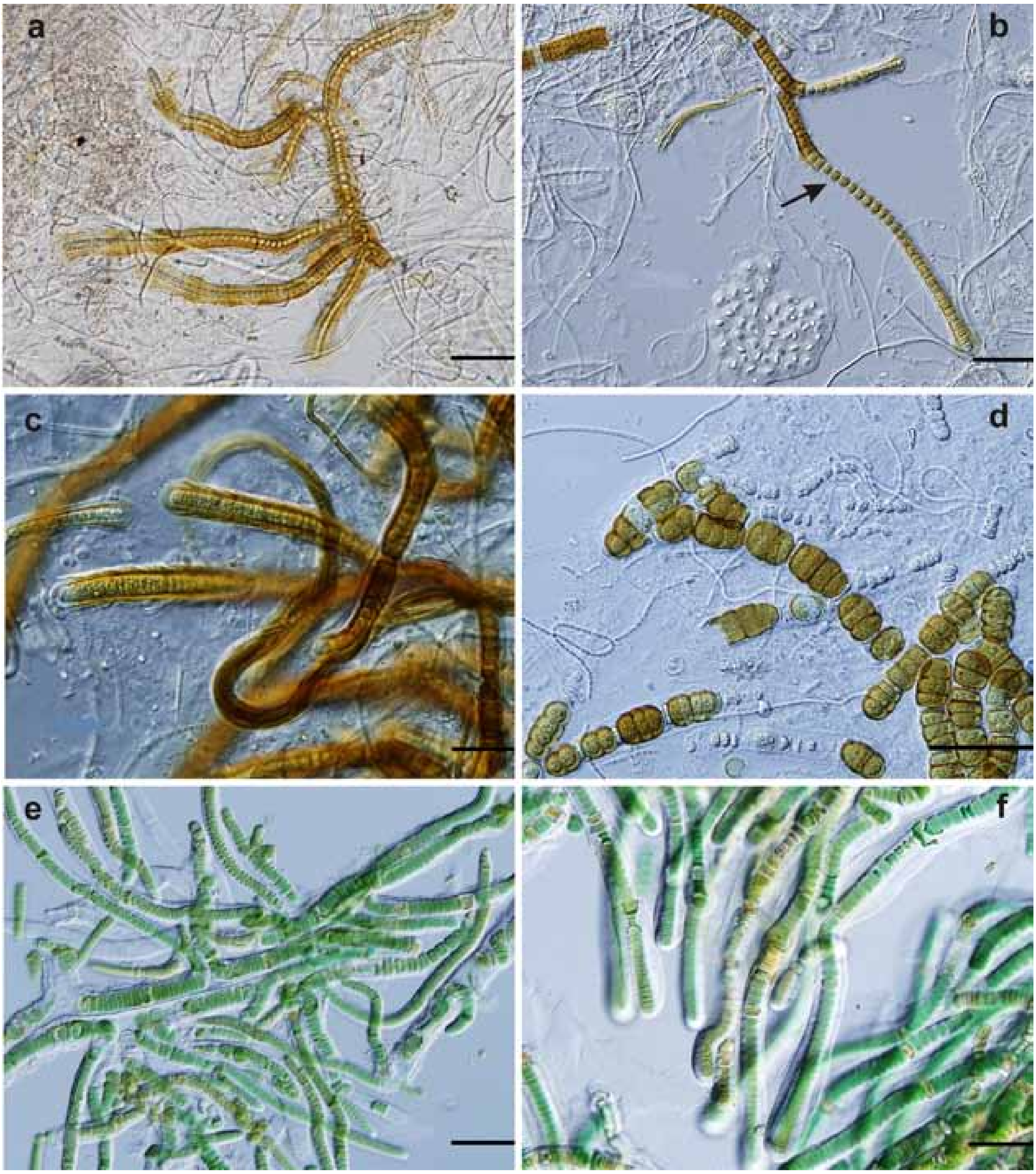

Fig. 3. *Chakia ciliosa*: (a) fully developed thallus from floating algal mat among the dominant population of *Leptolyngbya*; (b) detail of heteropolar branche, from natural material; (c) structure of ends of filaments with "ciliose" sheaths, from natural material; (d) hormocytes; (e–f) filaments from monospecific cultures (strain 3 – type material). Scale bars 50 µm.

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Table 1. Matrix showing similarities (%) between the sequence alignment (1451 bp) of four strains of *Chakia* and representants of relative genera based on the 16S rRNA gene.

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	***Chakia ciliosa*** **strain 3 (Buena Vista)**											
2	***Ch. ciliosa*** **strain 5 (Buena Vista)**	**99.8**										
3	***Ch. ciliosa*** **strain 8 (Buena Vista)**	**99.9**	**99.9**									
4	***Ch. ciliosa*** **strain 27 (Little Belize)**	**99.3**	**99.3**	**99.4**								
5	*Brasilonema octagenarum* UFV-OR1	91.7	91.7	91.8	91.5							
6	*B. terrestre* CENA116	91.7	91.7	91.8	91.7	97.4						
7	*Scytonema hoffmannii* PCC 7110	90.0	90.1	90.1	90.0	92.9	92.8					
8	*S.* cf. *crispum* UCFS21	91.2	91.3	91.3	91.0	91.7	92.4	91.3				
9	*Tolypothrix* sp. CCMP1185	93.1	93.1	93.2	92.9	95.6	95.4	93.4	92.4			
10	*Tolypothrix* sp. PCC 7504	91.3	91.4	91.4	91.0	92.6	93.4	91.1	94.0	94.4		
11	*Nostoc calcicola* Ind30	91.4	91.5	91.5	92.0	91.7	92.6	90.9	93.9	93.4	95.9	
12	*Petalonema* sp. ANT.LG2.8	91.0	91.1	91.1	91.1	92.6	93.3	93.3	93.7	93.6	93.2	94.5

sequence alignment editor and analysis program for Windows 95/98/NT. – Nucleic Acids Symposium Series 41: 95–98.

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Supplementary material

the following supplementary material is available for this article:

Table S1. List of strains.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)

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