

**Taxonomic evaluation of cyanobacterial microflora
from alkaline marshes of northern Belize.
2. Diversity of oscillatoriacean genera**

by

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With 26 figures and 2 tables

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Abstract: This article is the second part of the analysis of phenotype diversity of cyanobacterial assemblages in alkaline marshes in northern Belize. The cyanobacterial diversity of the marshes was studied in the frame of a long-term project focused on anthropogenic influences in this tropical country. The study was focused on the oscillatoriacean morphotypes, which are dominant in the studied ecosystem and form the main components of the cyanobacterial mats. Following the first part (Komárek & Komárková-Legnerová 2007 - coccoid types) of the characterization of the marshes, this study was also based on the phenotype analysis of natural samples. However, because several oscillatoriacean populations could be successfully brought into pure culture, the results of morphological and ecological diversity were supported by the 16S rRNA gene sequence analysis of the isolated strains. In the phenotype study of natural samples, 29 traditional morphospecies were detected. Of these, 31 strains were isolated in monospecific culture, and their 16S rRNA gene was sequenced. Separated clusters on the generic level with 13 subclusters were confirmed by the phylogenetic evaluation, which corresponded to the phenotype taxonomy. The morphology of all taxa which were recognized by combined molecular and phenotype evaluation are described, morphospecies which have never been transferred in cultures were mentioned. In the special habitats of Belizean alkaline marshes, we have found 9 new species, and one generic entity (*Phormidesmis*) was defined on the basis of combined molecular and phenotype analyses. Descriptions of two characteristic morphospecies, which were not isolated in monospecific cultures have been added and defined only

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according to phenotypic features. All new taxa were formally described following the Botanical Code. In the case of sequenced strains, a supplementary description following bacteriological nomenclatoric rules has been given.

Introduction

Alkaline marshes of northern Belize are characterized by the presence of cyanobacterial communities as their important ecological components. They have been studied in the context of a long-term project focused on anthropogenic changes to marsh ecosystems (Rejmánková & Komárková 2000, 2005). A wide cyanobacterial diversity was recognized, comprising over 90 morphospecies occurring exclusively in the studied marsh ecosystem (Rejmánková & Komárková 2000, 2005, Rejmánková et al. 2004, Komárek et al. 2005). The cyanobacterial mats (CBM) were studied primarily using traditional microscopy. We also tried to isolate all the observed types into unicyanobacterial culture. A wide spectrum of unicellular and colonial types was recognized in natural samples (45 morphospecies), but they only scarcely occurred as predominant in the ecosystem (e.g., *Aphanothece bacteriosa* Komárek et Komárková-Legnerová in oligotrophic marshes) and only few simple and not particularly important types from this group were successfully transferred into monospecific culture. Therefore, the review of diversity of unicellular types was published on the basis of mere natural populations (Komárek & Komárková-Legnerová 2007) and the support and revision of this review by molecular methods will have to be conducted in the future. A similar situation was found in heterocytous types (24 characteristic morphospecies), which, however, play an important role in the ecology of Belizean marsh ecosystems as subdominants in the mats in several seasonal periods. The group of heterocytous cyanobacteria will be reviewed in a separate study.

The predominant group of cyanobacterial assemblages in Belizean marshes is represented by oscillatoriacean types, i.e., by filamentous morphospecies without heterocytes and akinetes (especially by very thin types of the genus *Leptolyngbya*). While our work has been generally focused on the detailed phenotype analysis of cyanobacterial communities, in the case of oscillatoriacean morphotypes we tried to provide the synthetic review of their biodiversity using combined results from all available methodological procedures. This was possible due to the largely successful isolation of oscillatoriacean strains.

The modern taxonomy of oscillatoriacean cyanobacteria is complicated in light of the application of molecular methods. Following studies of molecular phylogenesis, many genera need to be revised and the delimitation of almost all species should be confirmed. The sequence analysis of the 16S rRNA gene is commonly used for the revision of cyanobacterial genera, but it is unsuitable for a clear and undoubted assessment of the subgeneric categories (similarly as it happens for many other prokaryotes and for cyanobacteria in the genera *Microcystis*, *Planktothrix*, *Anabaena* and many others, e.g. see also Johansen & Casamatta 2005). Consequently we have used the 16S rRNA sequencing to evaluate generic taxonomy in cases where we had obtained cultured strains, but for the species identification, we considered the concept

Tab. 1. Data about the main chemical characteristics of selected marshes (from Rejmánková & Komárková 2000).

Location	Size [ha]	Water Dept Mean/Range [cm]	Conduc- tivity (mScm ⁻¹)	Cl ⁻¹ (ppm)	SO ₄ ⁻² (ppm)	DIC (ppm)	DOC (ppm)	NH ₄ -N (ppb)	SRP (ppb)	Fe (ppb)	N:P
Big Snail	38	42/0–89	0.45	36	14	36	18	163	<2	-	
Buena Vista	75	22/0–49	2.3	66–297	44–90	42	32	33–189	<2–4.6	18.9	40
Chan Chen	140	34/0–90	4.6	85–432	464–1920	31	20	37–1149	<2–5.2	12.8	24
Doublon	65	17/0–85	5.5	621– 1969	562– 1708	36	25	76–186	<2–5.3	10.4	18

based on comments of Saez & Lozano (2005) and Johansen & Casamatta (2005) as the most appropriate. This was the only choice to characterize the cyanobacterial microvegetation from those very little known tropical habitats, also because knowledge of tropical cyanobacterial populations is particularly little known.

The present article includes taxonomic descriptions and characters of all studied oscillatorial taxa evaluated by morphological methods. The types, which were cultured, were studied by combined methods including 16S rRNA molecular phylogeny. Descriptions of morphospecies which were not transferred in cultures have been added.

Materials and methods

The results from 16S rRNA gene sequencing were used as a basic criterion for the final taxonomic evaluation and the resulting taxonomic units were compared with natural populations. We tried to provide a complete review of the oscillatorial cyanobacterial diversity from the studied alkaline marshes, including the morphospecies which did not grow in culture, but they were clearly recognizable in natural samples.

Samples were collected from alkaline marshes in Belize, Central America, in both the dry and wet seasons, in January and February 2001, January 2003, January 2004, and August 2006. Detailed marsh characteristics were provided in the introductory papers of Rejmánková et al. (2004) and in Komárek & Komárková-Legnerová (2007). The collected material was studied in the living state under optical microscopes (Nikon and Olympus), and observed populations were documented by drawings and microphotographs. The phenotype features were described in details, and special care was devoted to the phenotype variability, morphological changes during the vegetation season, and variation in dimensions. Samples were preserved in 2% formaldehyde solution for further studies.

The ecological data of studied marshes were published in previous papers and they are not the main point of this study. Particularly, the trophic level, high conductivity, salinity and changes in pH are described in detail in special articles (Rejmánková & Komárková 2000, 2005, Rejmánková 2001, Rejmánková et al. 2004, Komárek et al. 2005; compare Table 1). The microflora in the studied marshes was special and unique and differences were found only in dependence from different levels of conductivity and salinity. These differences are illustrated in Table 2, where the species preferring higher conductivity form a special ecological cluster. Where it was necessary, special ecological notes were mentioned in the descriptions of different species.

Because of the enormous variability of the mat structure, quantitative studies were not carried out. The term “dominant” is therefore used in a common sense (see Webster Dictionary) and represents only the estimated species richness, which was derived from several years of previous studies (see

Tab. 2. Oscillatorialean morphospecies from the marshes of different water conductivity ranges. Hydrochemical characteristics of marshes designated by black points (•) are included in Table 1.

Species (* = species known only from Belizean marshes)	Conductivity [$\mu\text{S}\cdot\text{cm}^{-1}$]		100-250					900-2500				3000-6000		
	Subcluster		Frank	Big Snail South•	Deep	Hidden	Cane	Buena Vista •	New	Quiet	Ellis's	Doubloon •	Little Belize	Chan Chen •
<i>Geitlerinema serpens</i> sp. nov. *			x	xx	x	x	x	xx	x	x	x	xx	xx	Xx
<i>G. splendidum</i> (Grev. ex Gomont) Anagn.								x		x				
<i>Geitlerinema</i> sp.						x								
<i>Komvophoron apiculatum</i> sp. nov. *	5			xx	x		x	xx		xx	xx	xx	x	
<i>K. rostratum</i> sp. nov.	5			x				xx			x	xx	xx	x
<i>Leibleinia</i> sp.							x							
<i>Leptolyngbya angustissima</i> (W. et G.S. West) Anagn. et Komárek	7	x	xxx	xx	x	x	xxx	xxx	xx	xx	xx	xx	xx	xx
<i>L. mucosa</i> (N.L. Gardner) Anagn. et Komárek	7	xxx	xxx	xxx	xx	xx	xxx	xxx	xxx	xxx	xx	xxx	xx	xx
<i>L. eliskae</i> sp. nov. *	7	xxx	xx	xx	xx	xx	xxx	xx	xxx	xx	xxx	xxx	x	x
<i>L. cf. perelegans</i> (Lemmerm.) Anagn. et Komárek								x	x	xx	xx	xx	xx	xx
<i>L. subsalina</i> sp. nov. *	7											xx	xxx	xxx
<i>Limnothrix</i> sp.?	3					x	x				x	x	x	x
<i>Lyngbya cf. intermedia</i> N.L. Gardner	9						x						x	
<i>L. cf. martensiana</i> Menegh. ex Gomont	9						x	x	x	x				
<i>L. minor</i> (N.L. Gardner) comb. nov.	9					xx	xx	xx	xx	xx	xx	xx	xx	xx
<i>L. cf. ocreata</i> N.L. Gardner	9								x				xx	
<i>L. cf. splendens</i> N.L. Gardner	9					x	x						x	
<i>Oscillatoria jenensis</i> G. Schmid							x		x		x	x	xx	
<i>O. miniata</i> [Zanardini] Hauck ex Gomont												xx	xx	x
<i>Phormidesmis molle</i> (Gomont) comb. nov.	6		x					x		x				x
<i>Phormidium granulatum</i> (N.L. Gardner) Anagn.		x	xx	xx	xx	x	xx	x	xx	xx	xx	xx	xx	xx
<i>P. pseudo-okenii</i> sp. nov. *	8		x	x		x	x		xx	xx	xx	xx	xx	xx
<i>Planktothrix</i> sp. *				x	x			x		xx	xx	xx	x	x
<i>Pseudanabaena apiculato-flexuosa</i> sp. nov. *	2	x	x	x	x	x	xx	x	xx	x	xx	x	xx	xx
<i>P. belizensis</i> sp. nov.	1		x	x	x	x	xx		xx	xx	xx	xx	xx	xx
<i>P. sp.</i> ("galeata" – type)	4												x	
<i>Schizothrix tenebrosa</i> sp. nov. *		x	xx	xx	x	x	x		xx	x	x	x	x	
<i>Schizothrix</i> sp. *			x					x		xx	x			
<i>Spirulina tenerrima</i> Kütz. ex Gomont			x									xx	xx	xx

Notes:

- Species not supported by molecular analyses should be considered ad interim as phenotypic morphospecies (not isolated in monospecific strains and not determined in molecular evaluation).
- The numbers of subclusters correspond with the phylogenetic tree (Fig. 1).

Legend:

Cross marks in the table express the estimated quantitative occurrence at the location according to semiquantitative scale: xxx = predominant or common species (in all samples from the corresponding marsh in a distinct quantity); xx = common occurrence (almost in all samples from the locality sometimes only sporadically); x = sparsely (only in a few samples from the locality, never in mass development).

Rejmánková & Komárková 2000, 2005, Rejmánková & al. 2004 and others). The dominant type of each functional group of cyanobacteria was estimated as follows: Twenty fields were evaluated to estimate the percent cover using an eye-piece graticle (10x10) under 200x magnification (objective 20x, eye-piece 10x). The semi-quantitative estimation scale used in Table 2 is explained in the legend to this table.

CULTIVATION, IDENTIFICATION: BG 11 cultivation medium (Allen & Stanier 1968), with pH modified to correspond to the original natural localities, was used for isolation and following cultivation of strains in both solid (agarized) substrates and in liquid media, but the cultivation was not successful in all cases. 31 strains were isolated from natural populations and kept in monospecific culture. They were analysed again from the morphological point of view and their phenotype was compared with observed natural populations. Cultures were kept parallelly in solid and liquid media BG11 and BG11₀ (Rippka et al. 1979) with half salt concentration using agarose (0.65%, w/v) instead of agar. Cycloheximide (100 ml.l⁻¹) was added to avoid the growth of eukaryotic contaminants. The cultures were incubated at room temperature under natural light with daily light periodicity. pH was set to alkaline values (Komárek et al. 2005). All strains were further studied by 16S rRNA gene sequence analysis. For the identification of taxa, papers from the Caribbean region (Gardner 1927, Schiller 1956, Komárek 1984, Mareš 2006) and the main identification manuals (Geitler 1932, Desikachary 1959, Komárek & Anagnostidis 2005) were used. The most modern classification system of Hoffmann et al. (2005a, b) was accepted.

MOLECULAR METHODS: Phenol-chloroform extraction of DNA from biomasses of freshly grown cyanobacterial cultures and selective PCR amplification of the cyanobacterial 16S rRNA gene were performed as described in Papaefthimiou et al. (2008). Aliquots of PCR products were sent for full-length sequencing to BMR Genomics (Padua, Italy). Sequencing primers were 16S979F, 16S544R and 16S1092R (Hrouzek et al. 2005). The chromatograms were analysed by the gauntlet PHRED/PHRAP/CONSED (Gordon 2004) and consensus sequences imported in ARB (Ludwig et al. 2004) and merged to the SILVA 95 SSU Ref rRNA database (Pruesse et al. 2007). Nearest neighbour sequences to the Belizean strains, longer than 1200 bp, were selected from the SILVA 95 SSU Ref database and aligned with the SINA Webaligner, available from the SILVA website <http://www.arb-silva.de/aligner/>. Several other sequences, relevant as references, were obtained from the NCBI Entrez Nucleotide Database <http://www.ncbi.nlm.nih.gov/>. With ARB, the alignment was used to generate preliminary phylogenetic trees including 201 sequences. The number of sequences in the final tree was reduced taking only one or two reference sequences from each 95% similarity cluster, plus all the Belizean strain sequences. The distance tree was constructed with the Neighbour-joining method for the phylogenetic analysis (Saitou & Nei 1987) and was bootstrapped 1000 times. *Gloeobacter violaceus* Rippka, Waterbury et Cohen-Bazire strain PCC 7421 was used as outgroup.

Results

All recognized 29 oscillatorialean morphospecies from natural samples are listed in Table 2. The distance tree shown in Fig. 1 shows the phylogenetic relationships among sequenced strains from Belizean marshes. Our strains were divided in two distinct main clusters (A and B), that were also well separated morphologically and cytologically. Our results from phenotype and molecular analyses were in agreement, and compatible with the modern systematic classification (Hoffmann et al. 2005a, 2005b). Cluster A (subclusters 1–7) corresponds to the subclass Synechococcineae, while cluster B (subclusters 8–9) to the subclass Oscillatoriineae.

The sequence analysis of 32 strains was mainly used for confirmation of the phenotype analysis. As follows from Fig. 1, in a number of cases we have used more than one strain of the same morphotype for the sequence analysis. A number of clearly characterized species from natural samples were not successfully transferred to culture and therefore their description is based on these natural populations. The phylogenetic tree confirmed the phenotype analysis particularly in the following genera:

- i) The heterogeneity of the present genus *Leptolyngbya* is well illustrated. The separation of the morphologically similar genus *Halomicronema* from *Leptolyngbya*, identified by Abed et al. (2002), was supported by our analyses. Our



Fig. 1. Phylogenetic tree containing oscillatoriacean strains isolated from alkaline marshes in northern Belize (in bold). See comments in text.

morphotypes from Belize (strains 3CC04S02 and 3CC01S01) correspond with *Halomicronema* phenotypically, but do not belong to this cluster (cf. subcluster 1 and 3). Therefore, they were designated in our tree as “*Limnothrix* sp.” (subcluster 3), which is another similar and ecologically variable genus. This classification should be considered as provisional.

ii) Two isolated strains of *Komvophoron* were identified according to morphological features. They represent a special subcluster (5), which also includes the only typical *Komvophoron* species [*K. schmidlei* (Jaag) Anagnostidis et Komárek] currently sequenced. This position indicates the genetic coherency of this whole *Komvophoron* cluster.

- iii) Several cryptospecies of the morphologically uniform genus *Pseudanabaena* were found with wide phylogenetic diversity. The taxonomy of this genus is complicated (see also Casamatta et al. 2005 and Johansen & Casamatta 2005). The various types (genotypes) differ slightly morphologically, but evidently ecologically and genetically, and therefore new species were described from Belizean marshes.
- iv) The cluster of *Lyngbya* strains from the family Oscillatoriaceae (Komárek & Anagnostidis 2005) is sharply delimited. There is also a certain genetic agreement with morphological species.
- v) The heterogeneity of the genus *Phormidium* was again confirmed and the position of *Ph. pseudo-okenii* sp. nov. was recognized as a member of group VII in the sense of Komárek & Anagnostidis (2005). This cluster contains *Phormidium autumnale* [C.Agardh] Trevisan ex Gomont complex and is related to *Microcoleus* and *Symploca*.
- vi) The location of the cluster of *Phormidium molle* Gomont among pseudanabaenacean types is important. The phenotype analysis confirmed the specificity of this type and *Ph. molle* was therefore defined as a special genus, *Phormidesmis*.

Taxonomic description of Belizean strains and populations (cf. Fig. 1):

Subcluster 1 (Fig. 1) corresponded phenotypically to the form-genus *Pseudanabaena*. Very fine, colourless sheaths occurred only in culture. Because our morphotype did not correspond to any other *Pseudanabaena*-species and represented a genetically and ecologically special cluster, we described it as a special species, *Pseudanabaena belizensis* (cf. Table 2). In the tree, it was well separated at the species level from all other related *Pseudanabaena* types, but morphologically it was not clearly distinguishable from the other species of the genus *Pseudanabaena*. Natural populations from ten marshes in northern Belize, and the strains 3LM05S05 (as a reference strain) and the morphologically similar 3LB05S05 and 3BV01S13 were used for the description. The type locality of the reference strain is the Little Belize marsh (LM).

Pseudanabaena belizensis sp. nov.

Fig. 2

TYPE AND REFERENCE STRAIN: 3LM05S05 (deposited in collections CNR, Firenze, Italy, and CCALA, Třeboň, Czech Republic).

Pseudanabaena belizensis sp. nov.: Filamenta longa, solitaria vel in strata parva, irregularia, aggregata, sine vaginis; vaginae tantum rare in culturis, tenuissimae, diffluentes, incolores. Trichomata aeruginosa, cylindrica, plus minusve irregulariter contorta, ad dissepimenta clare constricta, ad apices haud attenuata, 1.0–2.4 µm lata, subinde parum repens. Cellulae longior quam latae, cylindricae, interdum aliter longae, 6–15 µm longae, contentu homogeneo, pallide aerugineo; cellula terminalis cylindrica, apice rotundata. - Habitatio: In paludis alcalinis et paucim salinis; locus classicus: Belize, prope oppido Orange Walk dicto, palus Buena Vista, coll. 26.1.2003. - Holotypus: BRNM 1450, iconotypus figura nostra 2 c.

DESCRIPTION: Long, solitary trichomes without sheaths, or joined in irregular clusters, slightly to intensely coiled, cylindrical, distinctly constricted at cross-walls, not attenuated towards ends, 1–2(–2.4) µm wide, slightly motile. Sheaths occur only

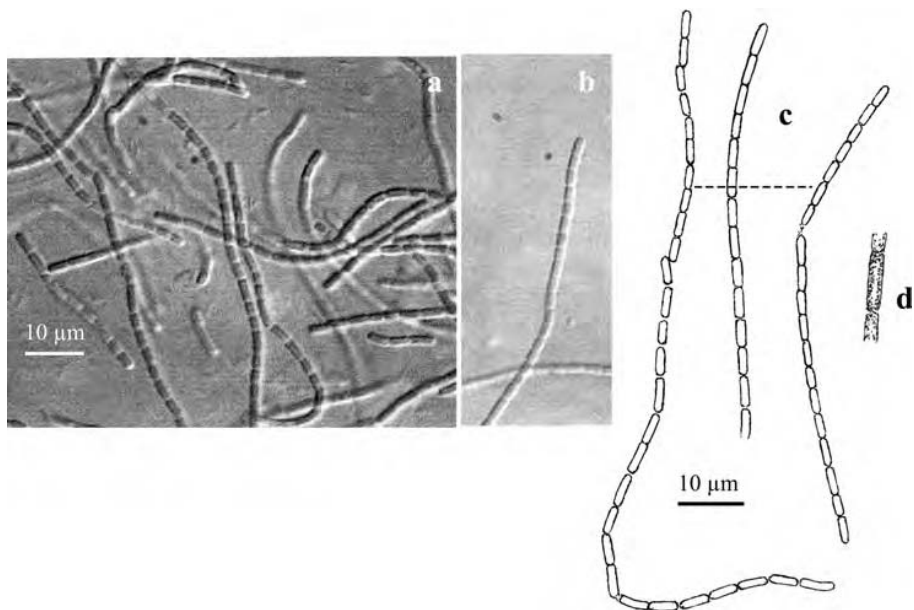


Fig. 2. Subcluster 1 (*Pseudanabaena belizensis*): a–b. strain 3LM05S05, c–d. specimens from natural samples (Buena Vista 26.1.2003).

exceptionally in cultures, very fine, thin, diffuse, colourless. Cells longer than wide, cylindrical, sometimes of different length (in one and the same trichome), 6 up to 15 μm long, with \pm homogeneous, pale blue-green content; end cells cylindrical and rounded. - Never in large biomass, but quite common, particularly in marshes with higher conductivity. - Differential features from related species: Position in phylogenetic tree; small morphological deviations (form of cells, contorted trichomes) connected with unique ecology.

Subcluster 2 included two morphologically similar strains corresponding also to the form-genus *Pseudanabaena*. Similar morphotypes were identified in natural samples from several localities in the marshes. They corresponded to typical *Pseudanabaena*-species (structure of trichomes, cell morphology), but they were characterized by conical apical cells and differed by the rare and facultative presence of very fine, colourless, diffluent sheaths. Both these features were recognizable in natural populations as well as in culture. The differences between natural populations and cultured strains were the larger frequency of sheaths, and the not so distinct conical narrowed apical cells in culture. The position of this subcluster in the tree was sufficiently isolated, its ecology was characteristic and the whole type did not correspond to any other described *Pseudanabaena* species. Therefore, a new species is defined, *P. apiculato-flexuosa* (cf. Fig. 1, Table 2). This morphotype occurs sparsely in mats in marshes over the whole area, but more frequently in sites with higher conductivity (higher than 1000 $\mu\text{S}\cdot\text{cm}^{-1}$). The strain 3CC04S01 was used for

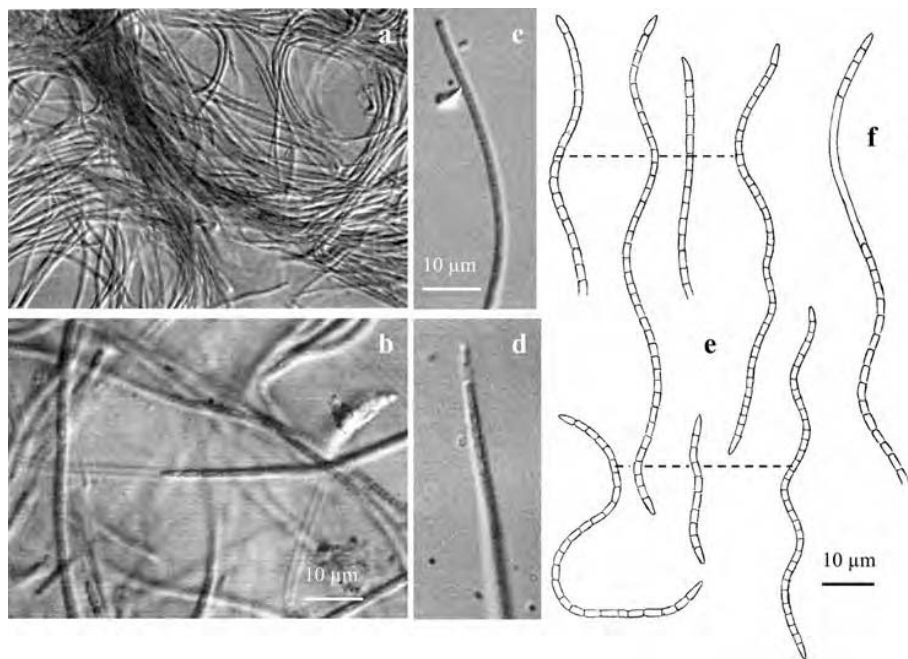


Fig. 3. Subcluster 2 (*Pseudanabaena apiculato-flexuosa*): a–d. strain 3CC04S01, e–f. specimens from natural samples (Buena Vista 26.1.2003).

the taxonomic description (as a reference strain), together with a further strain from the same genetic cluster (3CC04S04), and several natural populations, from which the marsh Buena Vista was selected as a type locality (locus classicus).

***Pseudanabaena apiculato-flexuosa* sp. nov.**

Fig. 3

TYPE AND REFERENCE STRAIN: 3CC04S01 (deposited in collections CNR, Firenze, Italy, and CCALA, Třeboň, Czech Republic).

Pseudanabaena apiculato-flexuosa sp. nov.: Filamenta solitaria vel in strata irregularia aggregata, sine vaginis; vaginae tantum rare in culturis, tenuissimae, incolores. Trichomata aeruginosa, cylindrica, flexuosa, rare spiraliter contorta, ad dissepimenta paucim constricta, ad apices haud attenuata, 1.0–2.5 µm lata, subinde parum repens. Cellulae longior quam latae, 4.8–6.2 µm longae, cellula terminalis conica, apiculato-rotundata. - Habitatio: In paludibus alcalinis et salinis; locus classicus: Belize, prope oppido Orange Walk dicto, palus Buena Vista, coll. 26.1.2003. - Holotypus: BRNM 1451, iconotypus figura nostra 3 e.

DESCRIPTION: Long, solitary or clustered trichomes without sheaths (in nature), usually coiled (sometimes regularly in \pm free screws), cylindrical, slightly constricted at cross-walls, not attenuated towards ends, 1.0–2.5 µm wide, slightly motile. Sheaths occur facultatively (mainly in cultures), very fine, thin, colourless. Cells longer than wide, 4.8–6.2 µm long, with blue-green cell content and sometimes visible chromatoplasmic area; end cells conical, rounded-pointed. - Commonly distributed

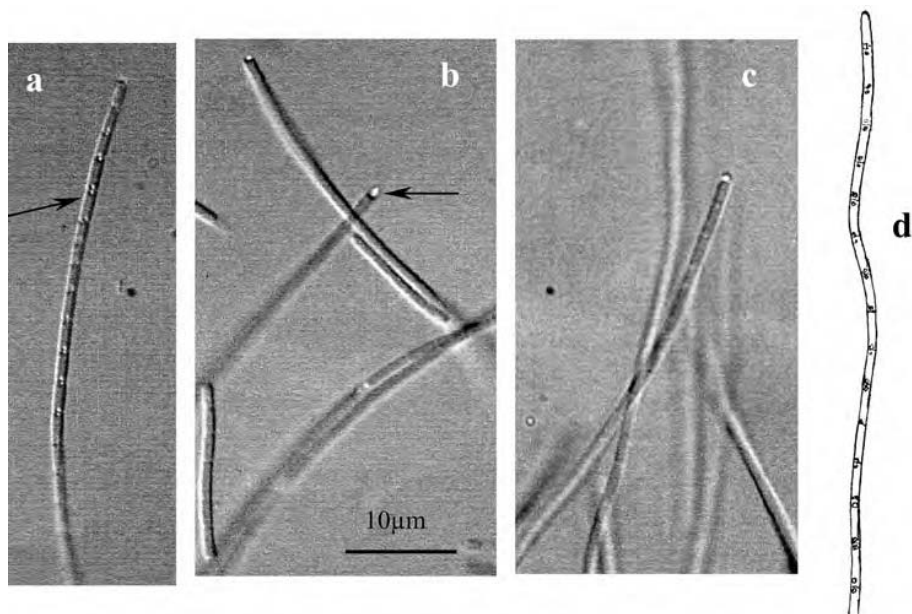


Fig. 4. Subcluster 3 (*Limnothrix* sp.): a–c. strain 3CC01S01, d. natural population (Buena Vista 26.1.2003).

in all marshes, especially at higher conductivity, but never dominant. - Differential features: Position in phylogenetic tree; facultative occurrence of sheaths in cultures, flexuous trichomes, morphology of terminal cells.

Subcluster 3 contains the morphotype similar to the genus *Halomicronema*, which was separated from the morphologically similar genus *Limnothrix* by Abed et al. (2002) and described from saline habitats in Spain. Trichomes corresponding with *Halomicronema* occurred sporadically in our CBM samples, particularly in marshes with higher salinity (cf. Table 2). However, the position of two our strains (subcluster 3) is far from the type strain of *Halomicronema excentricum* and therefore, we designate them provisionally as *Limnothrix* sp. The genus *Limnothrix* is morphologically also similar to the genus *Halomicronema* and ecologically very diverse. The generic and specific status of our specimens must be determined by further studies of the whole complex *Limnothrix/Halomicronema/Leptolyngbya*.

DESCRIPTION (our populations):

Limnothrix sp. (Fig. 4): Solitary filaments mixed with other cyanobacteria in mats, or in small, irregular fascicles with few trichomes. Trichomes cylindrical, more or less straight, arcuated or slightly coiled, without sheaths, cylindrical, slightly constricted at cross-walls, 1.0–1.8 µm wide. Cells cylindrical with homogeneous, pale blue-green or yellowish content and with facultative polar aerotopes, always

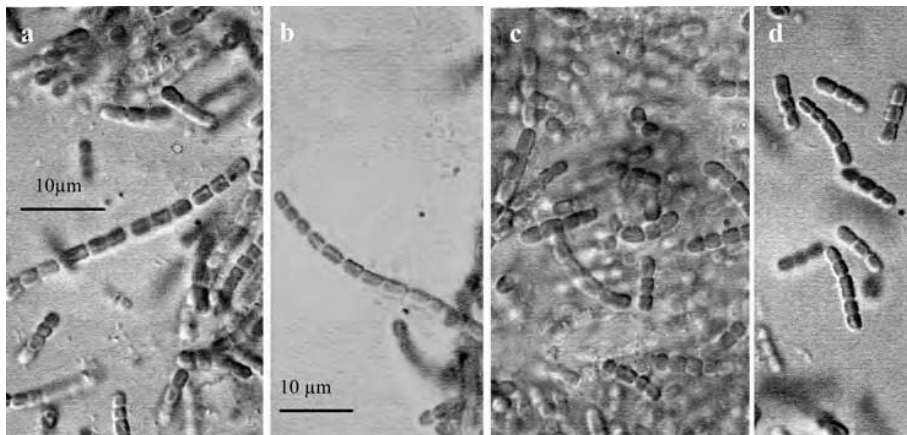


Fig. 5. Subcluster 4 (*Pseudanabaena* III - from the subgenus *Ilyonema*): a–d. strain 3LMS02.

longer than wide (up to 4-times); end cells cylindrical and rounded, usually with distinct polar, refractive aerotopes (granules?; polar aerotopes sometimes lacking). Fine, indistinct sheaths rarely occurred in cultured material.

Subcluster 4 was related to *Pseudanabaena galeata* Böcher (subg. *Ilyonema*). Similar types occurred rarely and sparsely in various marshes. One strain from this group was isolated (3LMS02), and its position near other *Pseudanabaena* morphotypes was confirmed by sequence analysis (Fig. 1). A close relationship of this strain to commonly and widely distributed *Pseudanabaena/Ilyonema* types is possible and it would indicate the separated status of *Ilyonema* at the generic level.

DESCRIPTION (our populations):

***Pseudanabaena* sp.** (Fig. 5): Short trichomes agglomerated in small, irregular clusters, usually \pm parallelly arranged, sometimes only few-celled, less frequently longer, slightly coiled, cylindrical, without sheaths, distinctly constricted at cross-walls, 1.8–2.0 μm wide. Cells cylindrical, bright blue-green, with homogeneous content with distinct chromatoplasmic region, isodiametric, or slightly (up to 2-times) longer than wide. End cells rounded. Very fine sheaths occur facultatively in culture.

Subcluster 5 represented a special generic entity according to genetic criteria. Morphologically it corresponded mainly to the traditional genus *Komvophoron* (sensu lato). The only partially sequenced strain of the genus *Komvophoron* available from GenBank (accession code AF355398, type species *K. schmidlei*) was also closely aligned with our strains. However, this genus is evidently heterogeneous in respect to detailed morphological and cytological features. Cluster 5 is included within the pseudanabaenacean group, which showed the characteristic parietal position of thylakoids (visible parietal chromatoplasmic region in cells, visible also in our strains). Our material contained two distinct morphotypes, which we classified into the genus

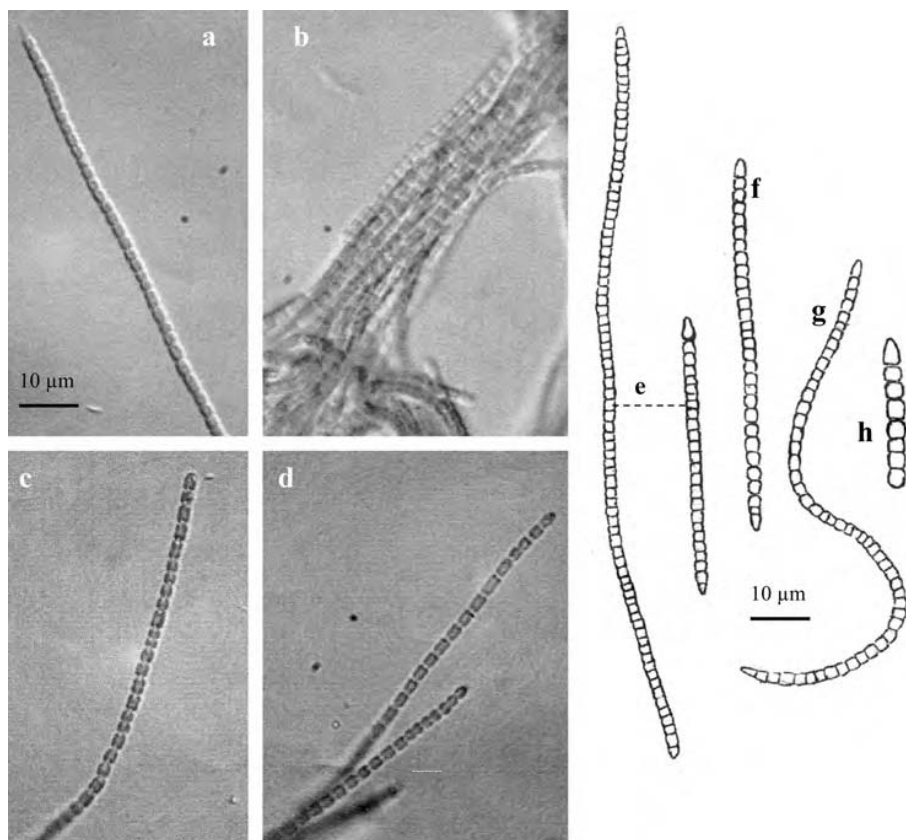


Fig. 6. Subcluster 5 (*Komvophoron apiculatum*): a–d: strain 3BV01S04, f. natural population (Buena Vista 26.1.2003), e, g–h. natural population (Little Belize 2.2.2003).

Komvophoron according to present taxonomy. Both strains that were included in genus *Komvophoron*, 3BV01S04 and 3LM05S06, evidently differed on the specific level from one another, both according to the distance in the tree (Fig. 1; 71%) and morphological characters (Fig. 6). Because they did not correspond to any *Komvophoron* species currently known, we described them ad interim as two new species of the genus *Komvophoron*. The studied natural populations corresponded morphologically well to the cultured strains. The only exception was the facultative presence of very fine, thin, colourless sheaths in both strains and less frequent occurrence of the apical, slightly elongated, conical and bent end cells in the strain 3LM05S06. Both species were not dominant, but widely spread in the majority of Belizean marshes (particularly the smaller *Komvophoron apiculatum*, while *K. rostratum* is more common in marshes with higher salinity). It is also possible, that both types belong to a special generic entity (rare presence of fine sheaths in culture).

Komvophoron apiculatum sp. nov.

Fig. 6

TYPE AND REFERENCE STRAIN: 3BV01S04 (deposited in collections CNR, Firenze, Italy, and CCALA, Třeboň, Czech Republic).

Komvophoron apiculatum sp. nov.: Filamenta cylindrica, plus minusve curta, praecipue ad 100 µm longa (longior in culturis), solitaria vel in strata parva fasciculatim et plus minusve paralleliter ordinata, praecipue sine vaginis; vaginae rare in culturis, tenuissimae, incolores. Trichomata pallide aeruginea, plus minusve recta vel paucim irregulariter contorta, paucim sed distincte constricta ad dissepimenta, ad apices non attenuata. Cellulae plus minusve isodiametricae vel paucim longior or brevior quam latae, paucim barriliformes; cellula terminalis conice attenuata vel curta et plus minusve rotundata. - Habitatio: Plerumque bentice in paludis alcalinis sed non in massis; locus classicus: Belize, prope oppido Orange Walk dicto, palus Buena Vista, coll. 26.1.2003. - Holotypus: BRNM 1452, iconotypus figura nostra 6 d,e.

DESCRIPTION: Filaments solitary, rarely ± parallely organized in fascicle-like clusters, mainly without sheaths (exceptionally in cultures with sheaths), ± straight or slightly irregularly coiled, short (usually less than 100 µm long, but in culture longer). Trichomes cylindrical, distinctly constricted at cross walls, 1.5–2.0(–2.6) µm wide, pale blue-green. Sheaths very fine, thin, indistinct, colourless. Cells ± isodiametric or slightly longer or shorter than wide, ± barrel-shaped; apical cells conical or narrow and rounded. - Not dominant, but spread over all marshes. - Differential features: Dimensions, morphology of cells, morphology of end cells, ecology.

Komvophoron rostratum sp. nov.

Fig. 7

TYPE AND REFERENCE STRAIN: 3LM05S06 (deposited in collections CNR, Firenze, Italy, and CCALA, Třeboň, Czech Republic).

Komvophoron rostratum sp. nov.: Filamenta cylindrica, plus minusve curta, recta vel paucim contorta, solitaria vel plus minusve in fasciculis irregularibus, liberis ordinata, praecipue sine vaginis; vaginae solo in culturis evolutae, tenues, incolores. Trichomata praecipue vivide aeruginea, cylindrica, ad dissepimenta clare constricta, ad apices interdum paucim attenuata et paucim flexa (praecipue in populationibus naturalis), 2.5–3.5 µm lata, interdum repens. Cellulae barriliformes, brevior quam latae, raro ad isodiametricae, cum chromatoplasmate distincto; cellula apicalis longior quam lata, conica, apiculato-rotundata, flexuosa in populationibus naturalibus. - Habitatio: Plerumque bentice in paludis alcalinis, praecipue salinis; locus classicus: Belize, prope oppido Orange Walk dicto, palus Little Belize, coll. 12.1.2003. - Holotypus: BRNM 1453, iconotypus figura nostra 7 a,e,g.

DESCRIPTION: Trichomes solitary, rarely in free clusters, mainly without sheaths in nature (with facultative sheaths in culture), ± straight, bent or slightly coiled, usually short, distinctly constricted at cross-walls, towards ends shortly narrowed and sometimes bent (mainly in nature), 2.5–3.5 µm wide, bright blue-green, slightly motile; sheaths fine, thin, colourless. Cells barrel-shaped, usually shorter than wide, rarely up to ± isodiametric, with distinct chromatoplasmic region; end cells (in nature) elongated (clearly longer than wide), conical rounded and bent, in culture usually only slightly conical and rounded. - Solitary in marshes, more common at sites with higher salinity. - Differential features: Morphology of cells, morphology of apical cells, dimensions, ecology.

The isolated subcluster 6 contained four strains, which were morphologically similar, and which corresponded morphologically to the traditional species *Phormidium molle*, identified from several samples from Belizean marshes. A similar morphospecies is the pantropical *Phormidium hamelii* (Frémy) Anagnostidis et Komárek with more

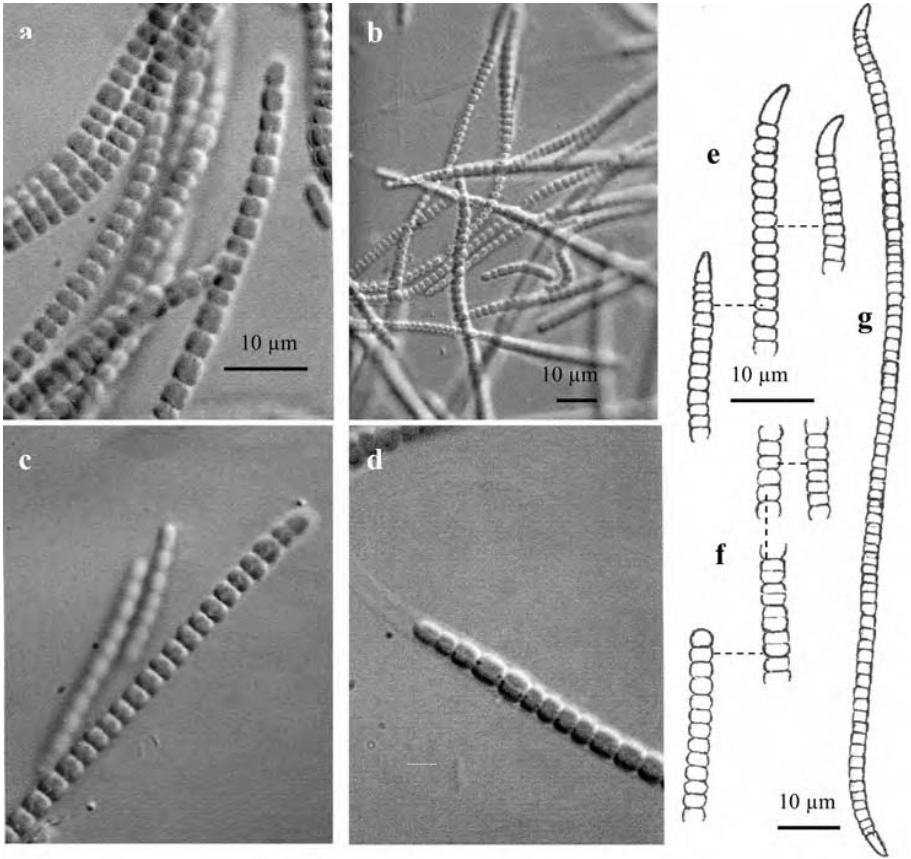


Fig. 7. Subcluster 5 (*Komvophoron rostratum*): a–d. strain 3LM05S06, e–g. natural population (Buena Vista 26.I.2003).

elongated cells. Our morphotype did not occur in large quantities in marshes, but it was spread over the whole area. *Ph. molle* is considered as a cosmopolitan species mainly distributed in tropical regions. It was classified in the genus *Phormidium* according to simple cylindrical trichomes without heterocytes, with facultative, firm, thin, colourless sheaths. The successful isolation of several strains from our samples supported the notion of its wider ecological valence. However, the molecular evaluation proved that a group of four relevant strains represented a special cluster, indicating a special generic entity (60% similarity with the nearest evaluated types), clearly distant from the typical *Phormidium* (cf. subcluster 8). We therefore described a new generic entity *Phormidesmis*, which could also be satisfactorily defined according to phenotype characters (structure of trichomes, constrictions at cross-walls, not differentiated apical cells). The type species, *Ph. molle* was recorded also from other similar localities in the Caribbean district, and probably other similar

morphotypes from the vicinity of the present widely conceived genus *Phormidium* belong into this cluster.

Phormidesmis genus novum:

Phormidesmis genus novum - Genetic code: Fig. 1, cl.6; 8a. Filamenta cylindrica, solitaria vel in strata intricata, plus minusve recta vel flexuosa, facultative cum vaginis tenuis, firmis, rarius latis, ambitu plus minusve inaequalibus, apice apertis, hyalinis et incoloris vel paucim coloratis, luteo-brunescens vel. Trichomata cylindrica, ad dissepimenta clare constricta, ad apices non attenuata. Cellulae plus minusve isodiametricae vel paucim longior vel brevior quam latae, saepe barriliformes, plerumque cum chromatoplasma visibili; cellula terminalis cum cellulis vegetativis similis, rotundata, plerumque non attenuata, sine calyptra. - Typus generis: *Phormidesmis molle* (Gomont) comb. nova.

DESCRIPTION: Filaments aggregated in mats or clusters, rarely solitary, cylindrical, ± straight or slightly coiled, with facultative sheaths. Trichomes cylindrical, constricted at cross-walls, not attenuated towards ends. Sheaths firm, thin, colourless or slightly yellowish-brownish. Cells isodiametric or slightly longer or shorter than wide, sometimes with visible chromatoplasma, cylindrical to barrel-shaped, end cells rounded, without typical calyptra. - Type species: *Phormidesmis molle* (Gomont) comb. nova (type strain: 3CC04S05, deposited in collections CNR, Firenze, Italy, and CCALA, Třeboň, Czech Republic). - Main generic features (in comparison with most related genera): Genetic position among pseudanabaenacean genera (isolated subcluster); autapomorphic marker: cells barrel-shaped to spherical forming moniliform cylindrical trichomes in facultative sheaths, without differentiated apical cells (which is able to divide).

***Phormidesmis molle* (Gomont) comb. nov.** [basionym: *Phormidium molle* Gomont - Ann. Sci. Nat., Bot. (Sér. 7) 16: 163, 1892] Fig. 8

DESCRIPTION: Filaments short or long, irregularly clustered or solitary among other cyanobacteria in mats, slightly coiled, cylindrical, slightly, but distinctly constricted at cross-walls, (2.5–)3–4(–6) µm wide, not attenuated at the ends, immotile. Cells shortly barrel-shaped, with homogeneous blue-green content, usually with visible chromatoplasmic region. Sheaths facultatively developed, fine or firm, thin, colourless. End cells do not differ from other vegetative cells, rounded at the end, rarely slightly conical. Rarely plectonematoid branching in cultures. Necridic cells are present.

Subcluster 7 contained five strains, corresponding to the heterogeneous and wide genus *Leptolyngbya*. Their position in the tree indicated their differentiation in several types at the subgeneric level. However, their morphology was uniform in culture and the differences between morphospecies recognized in nature were unclear. *Leptolyngbya* species were dominant in natural mats, where five morphospecies were distinguishable. Among them, three could be identified according to their phenotype and ecology with already described taxa (*L. angustissima*, *L. mucosa*, *L. perelegans*), two other are described as new specific units (*L. eliskae*, *L. subsalina*). Their identification with sequenced strains is difficult, strain 3CC01S07 is probably identical with *L. eliskae*, but it can hardly be selected as the reference strain. *Leptolyngbya perelegans* occurred less frequently. In the whole marsh ecosystem other modifications of cyanobacteria of the *Leptolyngbya*-type could occur. Few other modifications were recognized less frequently in natural samples, but their

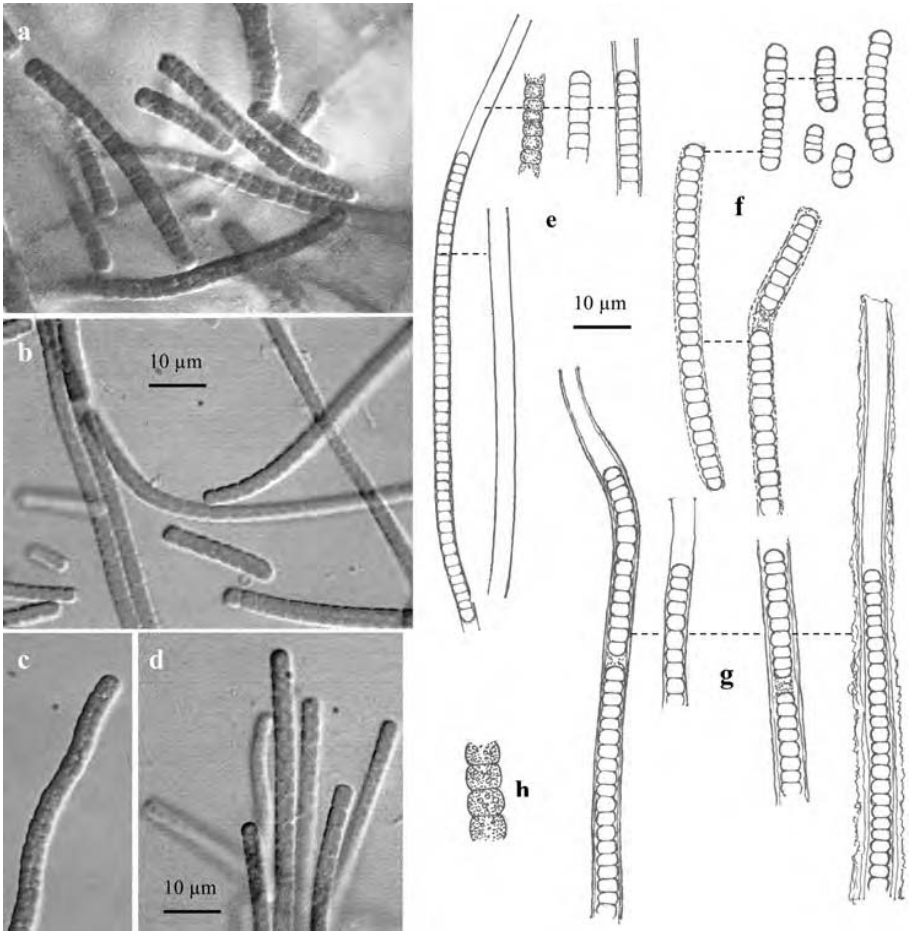
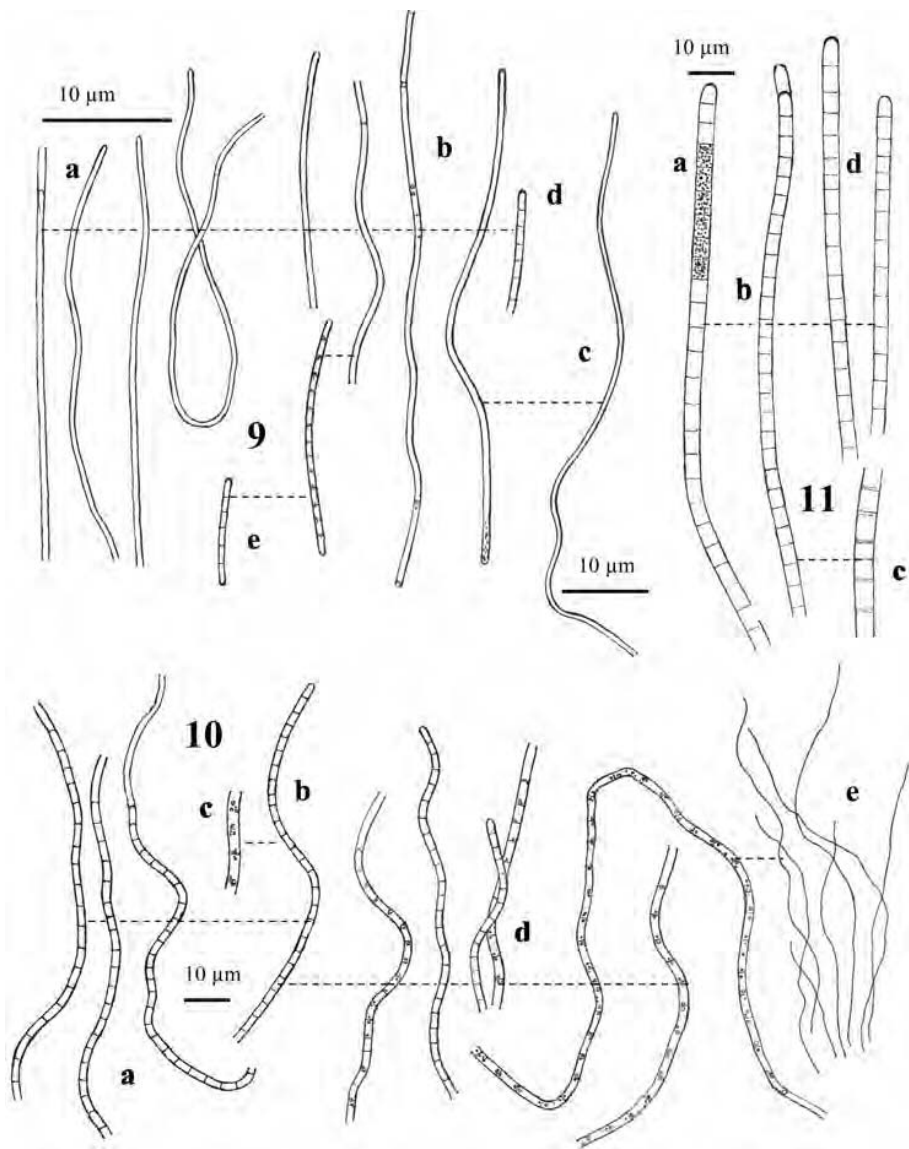


Fig. 8. Subcluster 6 (*Phormidesmis molle*): a,c. strain 3CC04S05, b,d. strain 3CC01S08, e. natural population (Quiet 7.8.2006), f-h. natural population (Little Belize 17.8.2006).

identification was difficult even according to traditional criteria. It is now impossible to recognize whether they were modifications of other dominant species, or whether they represented special genotypes. The false branching occurred in cultured strains quite commonly, but it could have been a modification caused by cultivation.

Leptolyngbya angustissima (W. et G.S.West) Anagnostidis et Komárek - Arch. Hydrobiol. Suppl. 80 (Algol. Stud. 50-53): 390, 1988 Fig. 9

DESCRIPTION: Filaments more or less straight or coiled, solitary among other cyanobacteria in mats or forming irregular free clusters, sometimes dominant.



Figs 9–11. Subcluster 7 (*Leptolynghya*): 9. *L. angustissima* - natural population, a. Big Snail South (13.2.2003), b–e. Buena Vista (26.1.2003). - 10. *L. mucosa* – natural population, a–c. Big Snail South (13.2.2003), d–e. Buena Vista (26.1.2003). - 11. *L. cf. perelegans* - natural population, a. Little Belize (2.2.2003), b–c. Big Snail South (6.2.2003), d. Big Snail South (13.2.2003).

Trichomes cylindrical, very thin, pale grey to blue-green, without constrictions at almost invisible cross-walls, not attenuated at the ends, (0.4–)0.5–0.8(–1) μm wide. Sheaths thin, firm, colourless, common. Cells longer than wide, without granulation or rarely with small, solitary, indistinct granules. End cells cylindrical and rounded. - Co-dominant species in several marshes.

Leptolyngbya mucosa (N.L.Gardner) Anagnostidis et Komárek - Arch. Hydrobiol. Suppl. 80 (Algol. Stud. 50–53): 392, 1988 Fig. 10

DESCRIPTION: Filaments intensely coiled, sometimes forming massive and macroscopic clusters and mats, cylindrical, pale blue-green, without constrictions at cross-walls, not attenuated at the ends, (1.2–)2.0–2.4(–3) μm wide. Sheaths colourless, firm, distinct. Cells cylindrical, always longer than wide, often with distinct, solitary granules, mainly at cross-walls. End cells cylindrical, rounded. - One of the dominant species in CBM.

Leptolyngbya cf. perelegans (Lemmermann) Anagnostidis et Komárek - Arch. Hydrobiol. Suppl. 80 (Algol. Stud. 50–53): 392, 1988 Fig. 11

DESCRIPTION: Filaments solitary among other cyanobacteria in mats, coiled. Trichomes cylindrical, pale blue-green, not constricted at cross-walls, not attenuated at the ends, 3.6–4.5(–5) μm wide. Sheaths thin, firm, colourless, distinct, frequent. Cells cylindrical, up to 2-times longer than wide, with homogeneous or finely granular content. End cells cylindrical, rounded. Trichomes motile. - Solitary among other cyanobacteria.

Leptolyngbya eliskae sp. nov. Fig. 12

Reference strain: 3CC01S07 (deposited in collections CNR, Firenze, Italy, and CCALA, Třeboň, Czech Republic).

Leptolyngbya eliskae sp. nov.: Filamenta cylindrica, recta vel paucim irregulariter flexuosa, rare solitaria, praecipue in strata macroscopica intricata, saepe plus minusve paralleliter in coloniis ordinata, saepe cum vaginis tenuis, firmis, hyalinis. Trichomata cylindrica, pallide griseo-aeruginosa, rare in protoplasmate cum granulis parvis, irregularibus, ad dissepimenta non constricta, ad apices non attenuata (vel paucim attenuata in culturis), cum articulis paulo visibilis, plus minusve 2.4 μm lata, subinde repens. Cellulae longior quam latae, cylindricae, cellulae terminales cylindricae, apice complanate rotundatae et cum membrana exteriore paucim incrassata. Hormogonia curta, cylindrica. - Habitatio: Communis dominansque (strata macroscopica formans), in paludibus alcalinis, bentice et despuis superficie fluitans; locus classicus: Belize septentrionalis, prope oppido Orange Walk dicto, palus Buena Vista, coll. 23.1.2003. - Holotypus: BRNM 1454, iconotypus figura nostra 12 g-i.

DESCRIPTION: Filaments straight or slightly irregularly flexuous forming macroscopic mats, often in more or less parallel oriented fascicles. Trichomes cylindrical, pale grey or greyish-green, not constricted at cross-walls, not attenuated towards ends (in cultures sometimes slightly attenuated), more or less 2.4 μm wide, with slightly visible cross-walls (staining!), slightly motile. Sheaths facultative, thin, smooth, firm, colourless. Cells longer than wide, sometimes with solitary, small irregular granules, end cells cylindrical, rounded, with thickened outer cell wall. Hormogonia cylindrical with indistinct cross walls, short. – Dominant and mat-forming particularly in upper parts of greyish mats. - Differential features: Dimensions, morphology of straight filaments and trichomes, ecology.

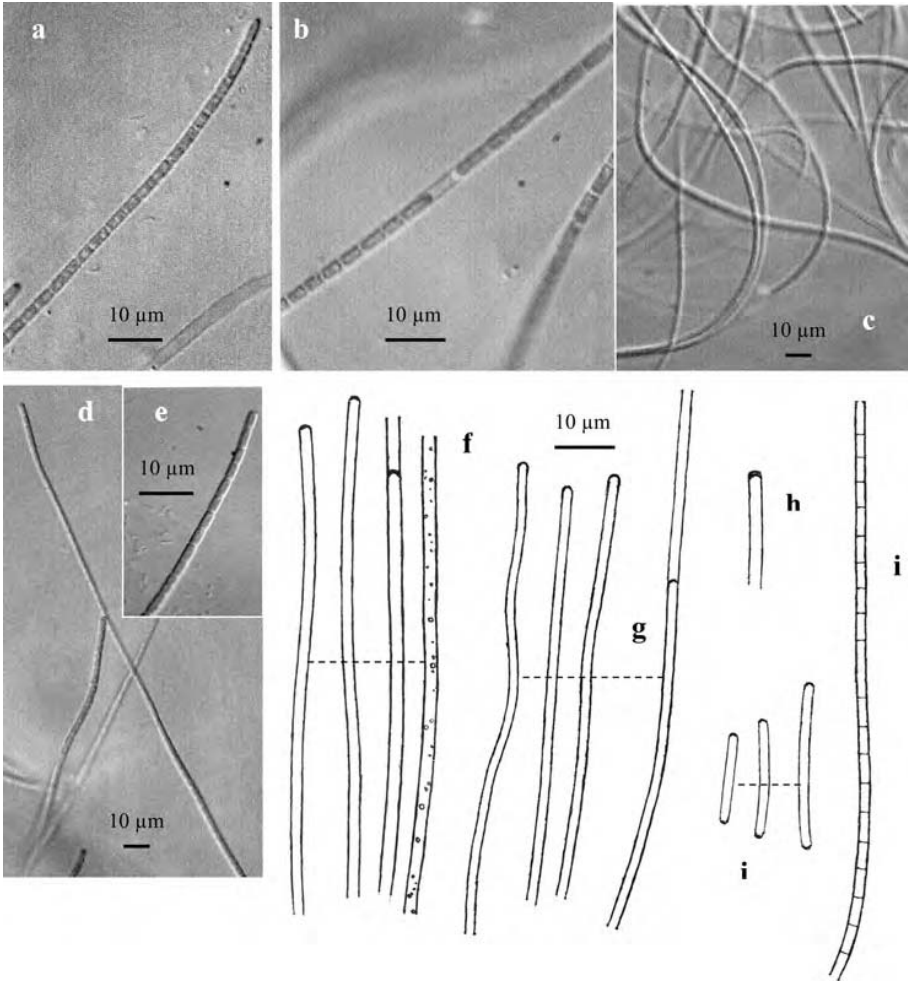


Fig. 12. Subcluster 7 (*Leptolyngbya eliskae*): a–c. strain 3CC01S07, d–e. strain 3CC01S11, f–j. natural population (Buena Vista 26.1.2003).

***Leptolyngbya subsalina* sp. nov.**

Fig. 13

REFERENCE STRAIN: 3LM05S03 (deposited in collections CNR, Firenze, Italy, and CCALA, Třeboň, Czech Republic).

Leptolyngbya subsalina sp. nov.: Filamenta plus minusve recta vel flexuosa, longa, rare solitaria, plerumque in strata macroscopica, dense paralleliter vel irregulariter contorta, cum vaginis tenuis, incoloris, diffluentibus. Trichomata cylindrica, pallide virides vel aeruginosae, ad dissepimenta non, rare paucim constricta, ad apices non attenuata, cum articulis paulo visibilis, 1.5–2.0 µm lata. Cellulae plus minusve isodiametricae, interdum paulo longiores quam latae, contentu homogeneo; cellulae terminales cylindricae, rotundatae, cum membrana exterior paucim incrassata. Hormogonia curta,

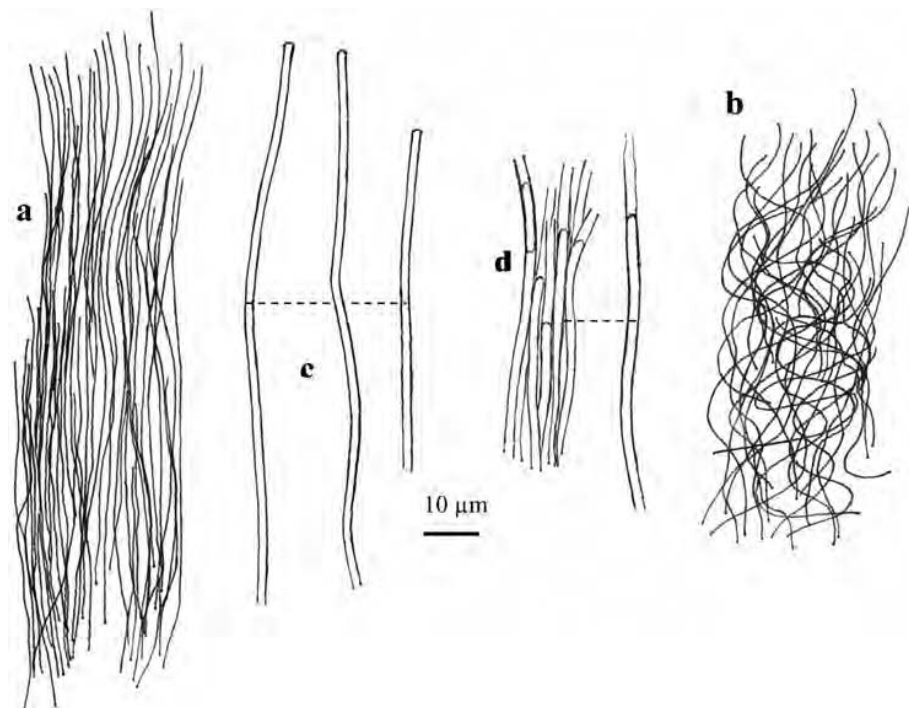


Fig. 13. Subcluster 7 (*Leptolyngbya subsalina*): various natural populations from Little Belize (1.2.2003). Correspond to the strain 3LM05S03.

cum cellulis paucis. - Habitatio: Strata communes in paludis alcalinis salinisque, bentice dominans et fluitantes; locus classicus: Belize septentrionalis, prope oppido Orange Walk dicto, palus Little Belize, coll. 1.2.2003. - Holotypus BRNM 1455, iconotypus figura nostra 13 a–d.

DESCRIPTION: Filaments straight or slightly flexuous, usually more or less parallel arranged, forming massive macroscopic mats, trichomes cylindrical, light green or olive-green, not constricted or slightly constricted at cross-walls, not attenuated towards ends, 1.5–2.0 µm wide, slightly visible cross-walls. Sheaths common, thin, colourless, gelatinizing and confluent. Cells more or less isodiametric, sometimes slightly longer than wide with homogeneous content, end cells cylindrical, rounded. Fragmentation in short, few-celled segments. - Dominant in mats in marshes with higher salinity. - Differential features: Dimensions, morphology of cells and flexuous filaments, form of colonies, ecology.

The genus *Phormidium* belonged to subcluster 8, according to both the position in the phylogenetic tree and to morphological characters. In samples from all the marshes, most common types from the genus *Phormidium* belonged to an atypical, but common species, *P. granulatum*, but its isolation in culture was unsuccessful. Another *Phormidium* morphospecies, morphologically similar to *P. okenii* (C.Agardh ex Gomont) Anagnostidis

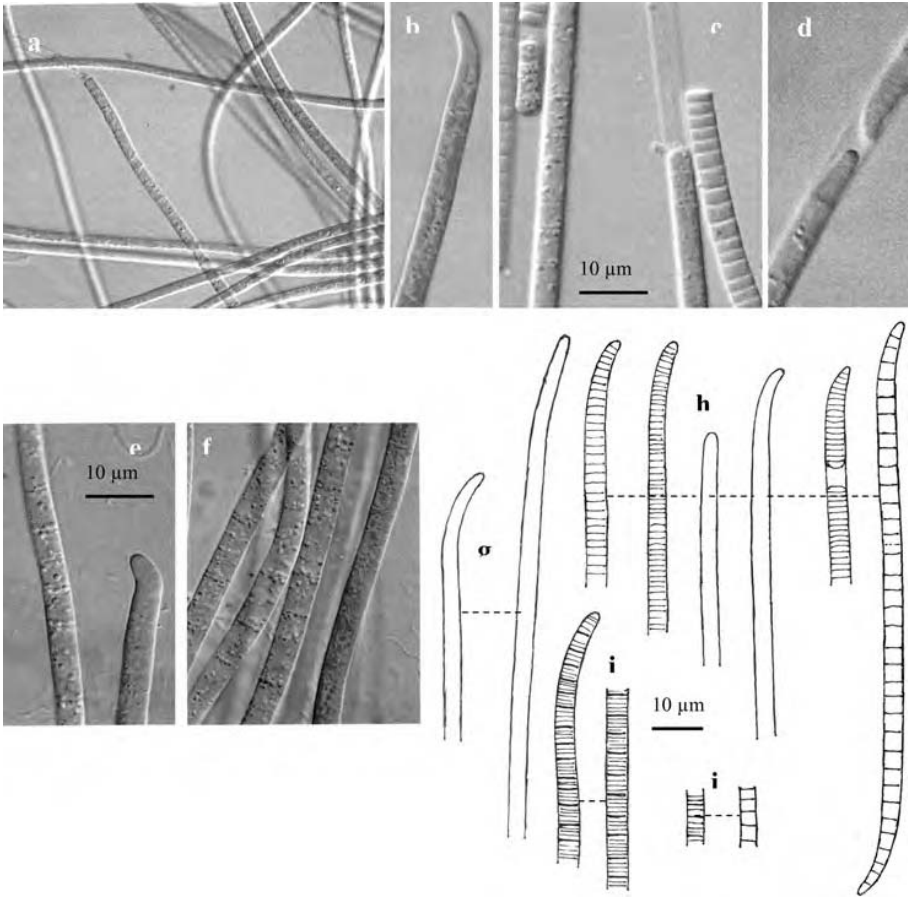


Fig. 14. Subcluster 8 (*Phormidium pseudo-okenii*): a–d. strain 3LM05S01, e–f. strain 3CCS03, g–j. specimens from natural population (Buena Vista 26.1.2003).

et Komárek, occurred sporadically and solitary in all marshes, but more commonly at sites with higher salinity. Three strains of this morphotype represented a special entity in the frame of the genus *Phormidium*, but they could not be identified with any known species of this genus. It was interesting that the length of the cells in our specimens varied from very short ones up to those longer than wide (in different trichomes). This phenomenon appeared also in culture (Fig. 14h). We therefore described this type as a special species *P. pseudo-okenii* from the type locality Buena Vista.

***Phormidium pseudo-okenii* sp. nov.**

Fig. 14

TYPE AND REFERENCE STRAIN: 3LM05S01 (deposited in collections CNR, Firenze, Italy, and CCALA, Třeboň, Czech Republic).

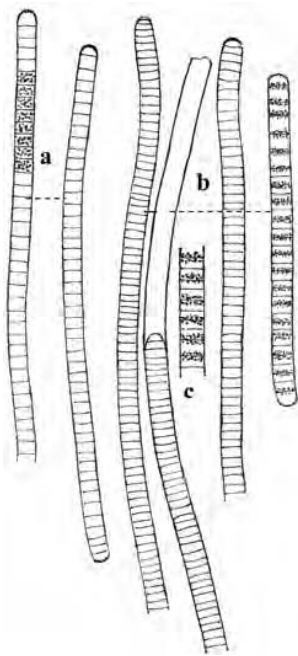


Fig. 15. Subcluster 8 (*Phormidium granulatum*): natural population, a. (Cane 7.2.2003), b–c. (Little Belize 2.2.2003).

Phormidium pseudo-okenii sp. nov.: Filamenta cylindrica, solitaria vel rare in fasciculis irregularibus, plus minusve paralleliter ordinata, recta vel paucim flexuosa, interdum cum vaginis tenuis, firmis, hyalinis. Trichomata cylindrica, non vel paucim constricta ad dissepimenta, ad apices breve attenuata et paucim flexuosa, 3.8–5.5 μm lata (ad 6 μm in culturis, 3.4–3.6 μm ad apices), interdum motilia. Cellulae cylindricae, plus minusve isodiametricae vel longior quam latae in trichomatibus adultis, vel distincte brevior quam latae in partes cum cellulis dividendis, contentu olivaceo-aeruginoso vel aerugineo, interdum cum granulis solitariis; cellula terminalis conically rotundata, flexuosa, sine calyptra. - Habitatio: Benthice metaphyticeque in paludis alkalinis; locus classicus: Belize septentrionalis, prope oppido Orange Walk dicto, palus Buena Vista, coll. 26.1.2003. - Holotypus: BRNM 1456, iconotypus figura nostra 14 h.

DESCRIPTION: Trichomes solitary or in small clusters, \pm parallelly arranged, straight or slightly coiled. Trichomes cylindrical, not or very slightly constricted at cross-walls, shortly attenuated towards ends and usually bent, 3.8–5.5(–6 in cultures) μm wide (only 3.4–3.6 μm wide at the end), olive-green or blue-green, motile. Sheaths rare, facultative, thin, firm, colourless, cylindrical. Cells \pm isodiametric or longer or shorter than wide, cell content sometimes with solitary granules; end cells conical-rounded, without calyptra. - Common, but not in masses, among other cyanobacteria, more common in marshes with higher salinity. - *Phormidium pseudo-okenii* belongs in the group VII according to Komárek & Anagnostidis (2005). - Differential features: Morphology of terminal parts of trichomes, morphology of terminal cells, frequency of sheaths, dimensions, ecology, life form.

Phormidium granulatum (N.L.Gardner) Anagnostidis - Preslia 73: 370, 2001
Fig. 15

DESCRIPTION: Solitary filaments or in small clusters as a part of typical CBM. Trichomes cylindrical, slightly coiled, not constricted at cross-walls, not attenuated towards ends, with blue-green or little brownish, usually distinctly granular cell content, 5.0–5.2(–6) µm wide; granulation particularly at cross-walls. Sheaths facultative, sometimes rare, colourless, firm, distinct. Cells shorter than wide up to isodiametric, cylindrical. End cells rounded. - Not dominant, but common and widely distributed in all marshes, in summer periods at higher quantities.

The well delimited subcluster 9 comprised all types from the traditional family Oscillatoriaceae, particularly the members of the genera *Lyngbya* and *Oscillatoria* (in the revised concept of Anagnostidis & Komárek 1988 and Hoffmann et al. 2005b). According to their position in the phylogenetic tree (Fig. 1), seven sequenced strains from this group could be separated into five specific entities corresponding to the traditional species *Lyngbya* cf. *intermedia* N.L.Gardner, *L. minor* (N.L.Gardner) comb. nova, *L. martensiana* Meneghini ex Gomont, *L. cf. ocreata* N.L.Gardner and *L. splendens* N.L.Gardner. They were not dominant in natural samples, but well recognizable and could be easily compared with relevant isolated strains. Their taxonomic value was therefore proven. Several other very characteristic types, not successfully transferred in culture, were recognized in natural marshy habitats (*Oscillatoria jenensis* and *O. miniata*). Short descriptions and documentation of these types have been added to our review.

Lyngbya minor (N.L.Gardner) **comb. nov.** [Basionym: *Lyngbya martensiana* var. *minor* Gardner - Mem. New York Bot. Gard. 7: 41, 1927] Fig. 16

DESCRIPTION: Filaments slightly coiled, mixed with other cyanobacteria in mats, up to 7.2 µm wide. Trichomes cylindrical, not attenuated towards ends, not constricted or very slightly constricted at cross-walls, bright blue-green or olive-green, (4–) 4.8–6.0(–7) µm wide, immotile. Sheaths firm, distinct, smooth, sometimes slightly widened, colourless. Cells always shorter than wide, usually only 2–3 µm long, end cells widely rounded. - It is a characteristic and common species in marshes, well distinguishable from the larger *L. martensiana*. Because separation of this type from other *Lyngbya* species was supported also by molecular criteria, we considered the morphotype, described originally as var. *minor*, as a distinct species with special ecology (calcareous habitats in Caribbean region).

Lyngbya martensiana Meneghini ex Gomont - Ann. Sci. Nat., Bot. (Sér. 7) 16: 145, 1892 Fig. 17

DESCRIPTION: Filaments straight or slightly bent, coiled, or screw-like coiled, solitary among other cyanobacteria in mats, up to 9 µm wide. Trichomes cylindrical, not attenuated towards ends, occasionally slightly constricted at cross-walls, bright blue-green, (6.2–)7.0–7.5(–8.7) µm wide, immotile. The facultative slight constrictions at cross-walls do not correspond to original description. In our populations were found all transitions between constricted and not constricted

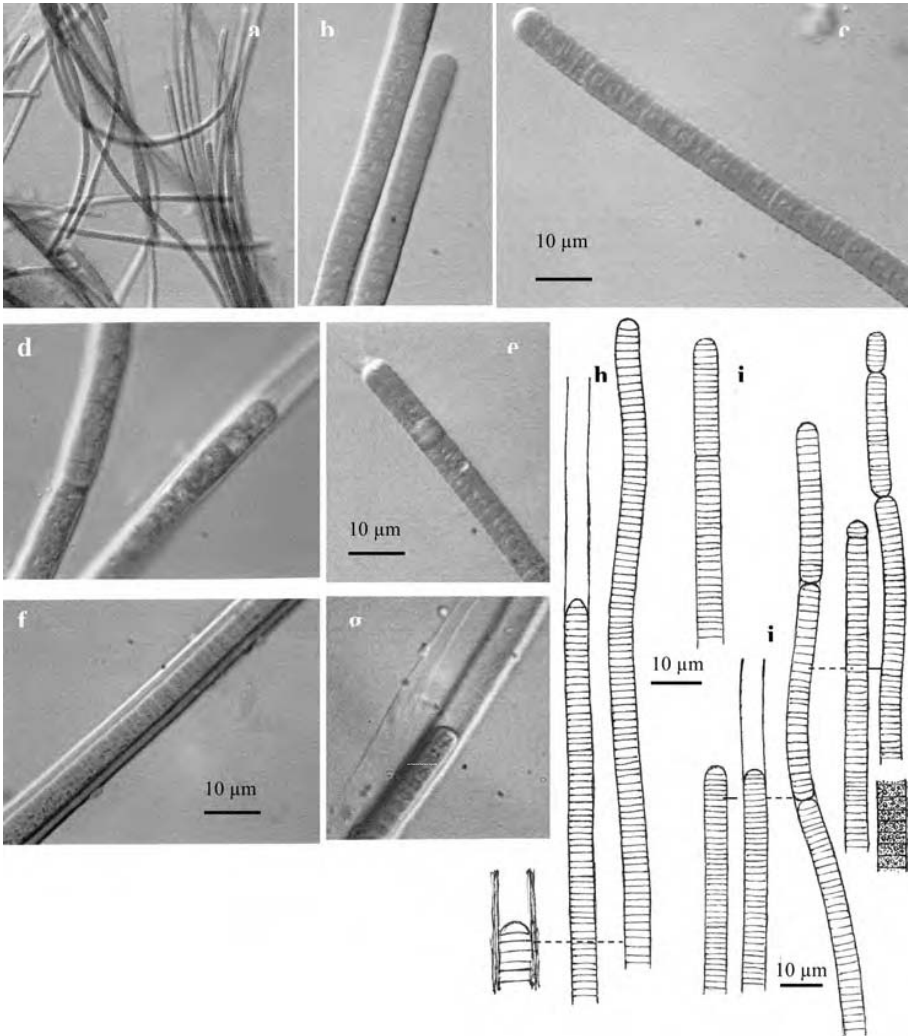


Fig. 16. Subcluster 9 (*Lyngbya minor*): a–c. strain 3CC01S06, d–e. strain 3LM05S04, f–g. strain 3BV01S06, h–i. natural population (Little Belize 2.2.2003), j. natural population (Cane 7.2.2003).

trichomes. This feature belongs evidently to the natural variability of this species. Sheaths firm, smooth from outside, usually widened (up to 15 μm wide), colourless. Cells commonly only about 1.4 μm long, end cells widely rounded. - Rarely among other cyanobacteria in CBM.

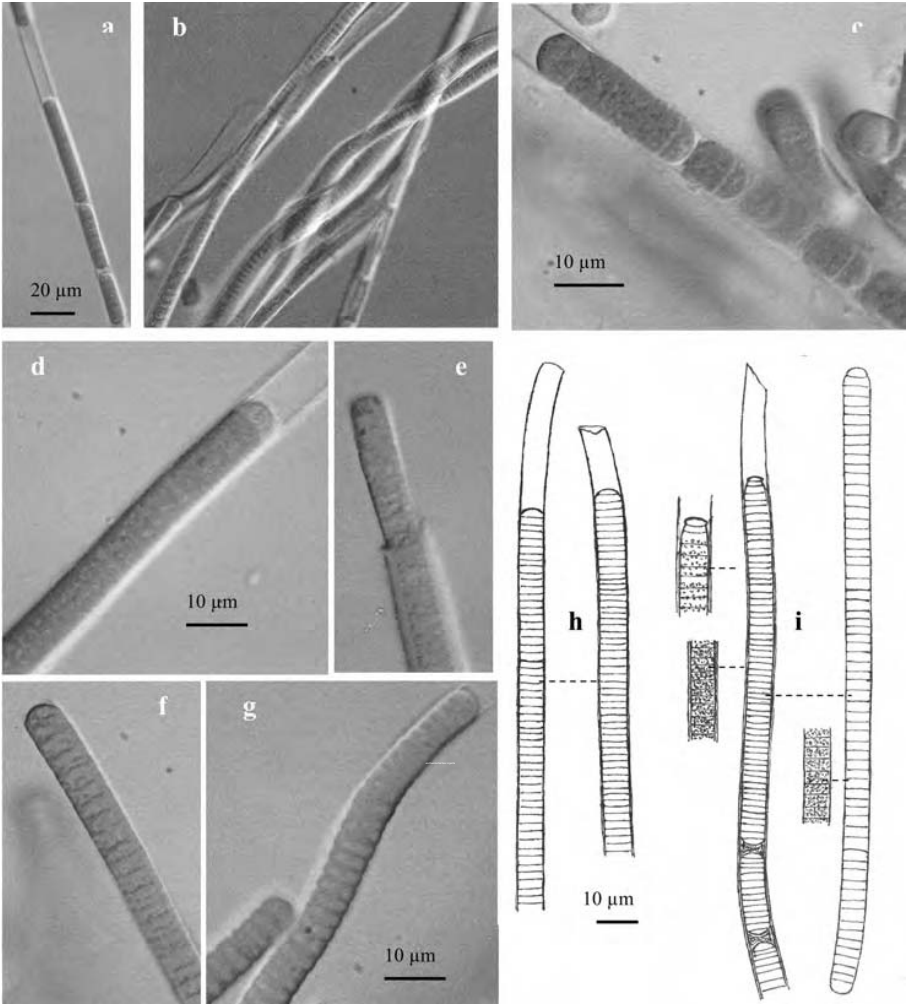


Fig. 17. Subcluster 9 (*Lyngbya martensiana*): a–d. strain 3CC03S04, e–g. strain 3CC03S02, h. natural population (Little Belize 2.2.2003), i. natural population (Buena Vista 26.1.2003).

Lyngbya ocreata N.L.Gardner - Mem. New York Bot. Gard. 7: 39, 1927 Fig. 18

DESCRIPTION: Filaments slightly coiled, solitary or in small irregular clusters, up to 16 μm wide. Trichomes cylindrical, not attenuated towards ends, slightly constricted at cross walls, bright blue-green or olive-green, (5.6–)7.4–10.0 μm wide, immotile. Sheaths firm, thick, in old filaments slightly lamellated, colourless or brownish in old filaments. Cells always shorter than wide; end cells widely rounded. - Species, occurring in marshes with higher conductivity, mostly in the surface layer of floating mats.

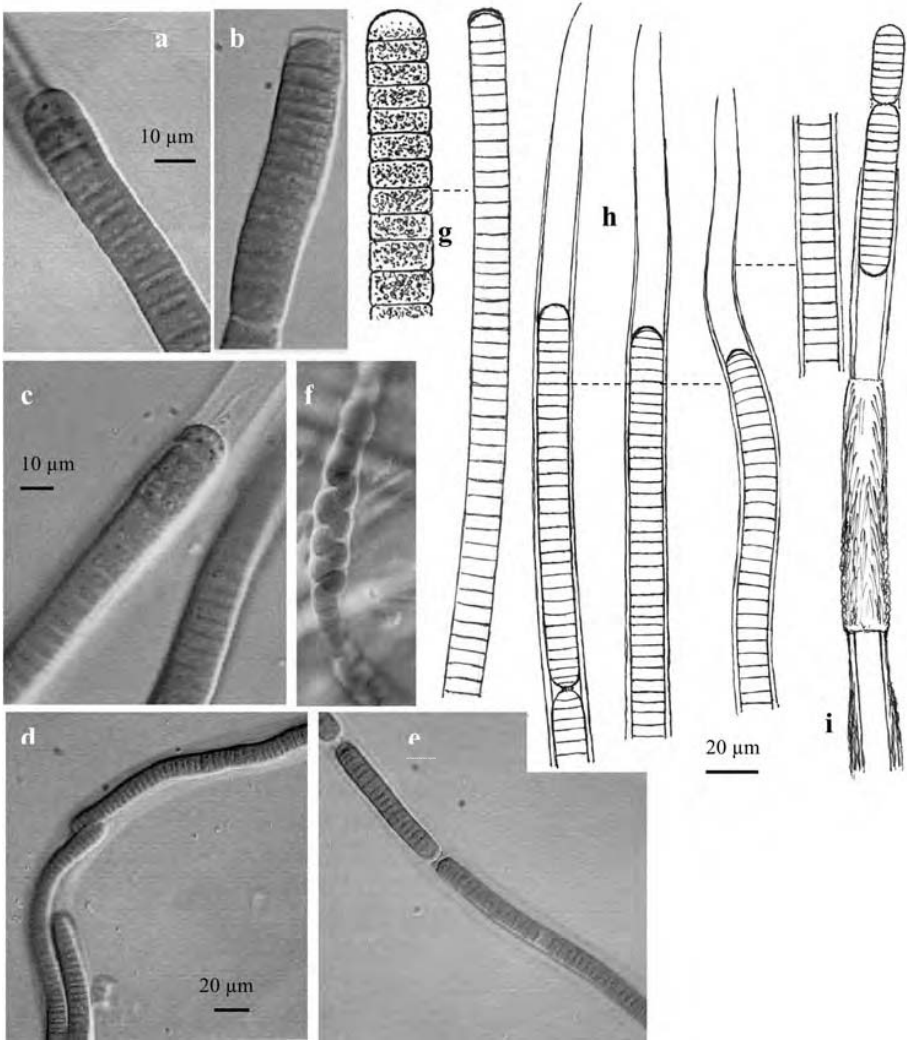


Fig. 18. Subcluster 9 (*Lyngbya ocreata*): a-f. strain 3CC03S01, g-i. natural population (Little Belize 2.2.2003).

Lyngbya* cf. *splendens N.L.Gardner - Mem. New York Bot. Gard. 7: 40, 1927

Fig. 19

DESCRIPTION: Filaments straight or slightly curved, usually solitary among other cyanobacteria, 27–30 μm wide, rarely narrower. Trichomes cylindrical, occasionally slightly narrower at the end, slightly constricted at cross-walls, with bright blue-green, finely granular content (granulation is more intense at cross walls),

14.5–15.2 μm wide, immotile. Sheaths firm, thick, particularly in old filaments rough on the surface and lamellated, towards ends thinner and narrower, colourless or slightly yellowish. Cells always distinctly shorter than wide, end cells widely rounded, sometimes hyaline or yellowish. - Rarely occurring species, mainly in places with higher input of nutrients.

Lyngbya cf. **intermedia** N.L.Gardner - Mem. New York Bot. Gard. 7: 41, 1927

DESCRIPTION: Filaments long, sometimes clustered, straight or arcuated, 14–20(–22) μm wide. Trichomes cylindrical, not constricted at cross walls, not attenuated towards ends, 12–17(–20) μm wide, dark blue-green. Sheaths thick (up to 2 μm), colourless or yellowish. Cells short, up to 4 μm long, with granular content, sometimes with aerotopes (?). - Rarely, only in sites with higher trophic level.

Description of non-sequenced morphospecies from the same morphological group, subcluster 9 (Oscillatoriaceae sensu stricto):

Oscillatoria **jenensis** G.Schmid - Jahrb. Wiss. Bot. 60: 572, 1921 Fig. 20

DESCRIPTION: Solitary trichomes without sheaths, cylindrical, not constricted at cross-walls, distinctly narrowed towards ends and usually slightly bent at the apex, 21–23(–27) μm wide in the middle, up to 12 μm narrowed at the ends, olive-green or blue-green. Cells distinctly shorter than wide, last few cells usually with yellowish up to hyaline content, end cells flat (“cut”). - Rarely in marshes with higher conductivity.

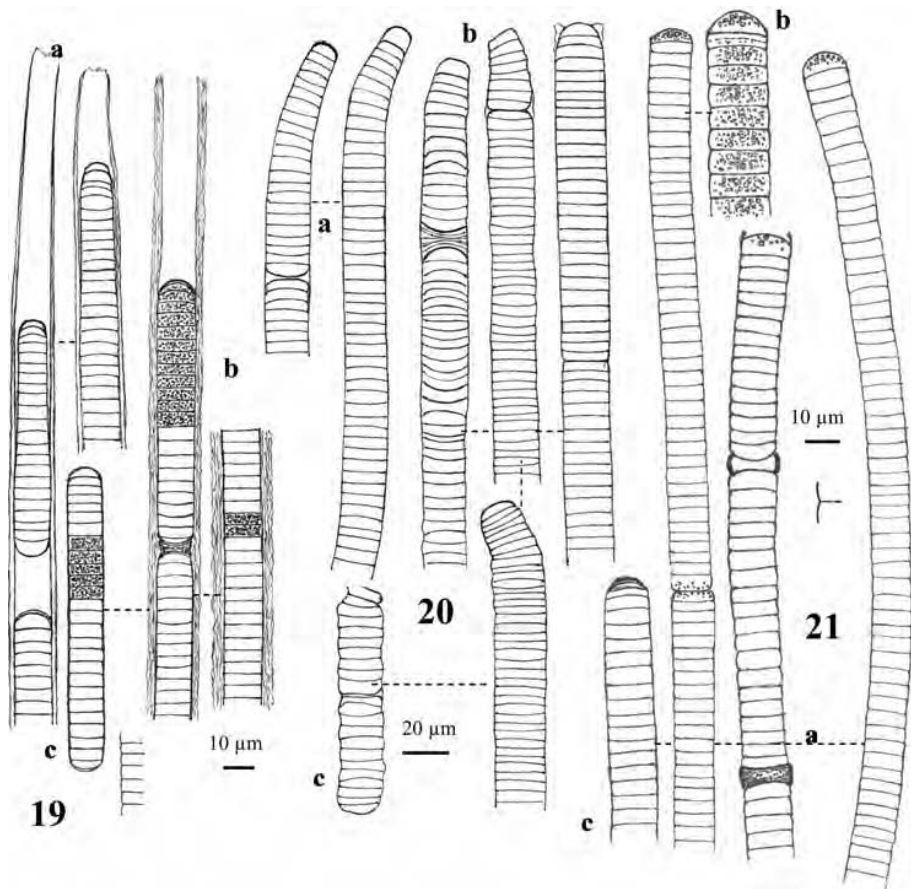
Oscillatoria cf. **miniata** [Zanardini] Hauck ex Gomont - Ann. Sci. Nat., Bot. (Sér. 7) 16: 216, 1892 Fig. 21

DESCRIPTION: Trichomes solitary or in small clusters, without sheaths, cylindrical, slightly constricted at cross-walls, not attenuated towards ends, 12.4–14.5 μm wide, reddish or pinkish-violet. Cells always shorter than wide, with granular content, end cells lighter or almost hyaline, with distinct granulation at the outer cell-wall. - More common in summer season in marshes with higher salinity.

Several other morphospecies that were neither cultured nor sequenced were found in natural samples. Six traditional morphospecies were found in typical mat communities in Belizean marshes, the isolation of which was unsuccessful. They appeared commonly and were characteristic for this habitat, therefore we decided to add their morphological characterization. Few of them were identifiable according to the currently known and described cyanobacterial diversity; two unidentified morphospecies (*Geitlerinema* cf. *splendidum* and *Leibleinia* sp.) occurred rarely, and therefore they are listed only in Tab. 1. Two distinct and not identifiable morphospecies have been described as new species according to Botanical nomenclatoric Code:

Geitlerinema **serpens** sp. nov. Fig. 22

Geitlerinema serpens sp. nov.: Trichomata cylindrica, solitaria, irregulariter vel spiraliter contorta, sine vaginis, ad dissepimenta non constricta, ad apices interdum breve attenuata et paulo flexuosa,



Figs 19–21. Not sequenced species corresponding morphologically to types from subcluster 9: 19. *Lyngbya* cf. *splendens* (Cane 7.2.2003), corresponds morphologically to the strain 3CAS01. - 20. *Oscillatoria jenensis* (a. Chan Chen 3.2.2006, b–c. Little Belize 1.2.2003). - 21. *Oscillatoria* cf. *miniata* (Little Belize 2.2.2003).

intense serpens, 2.5–3.2 µm lata, pallide aeruginosa. Cellulae brevior quam latae ad isodiametricae; cellula terminalis conice rotundata. – Habitatio: Metaphytice in paludis alkalinis; locus classicus: Belize septentrionalis, prope oppido Orange Walk dicto, palus Big Snail South, coll. 25.1.2003. - Holotypus: BRNM 1457, iconotypus figura nostra 22 a–e.

DESCRIPTION: Solitary trichomes without sheaths, among other cyanobacteria in mats, common, but never forming clusters or colonies; trichomes cylindrical, not constricted at cross-walls, at the ends shortly narrowed and sometimes shortly bent, along the whole length more or less regularly, freely coiled, intensely motile (creeping, waving); under less favourable conditions irregularly coiled or almost straight in some parts; trichomes 2.5–2.8(–3.2) µm wide, pale blue-green. Cells with slightly

visible cross-walls, slightly shorter than wide up to isodiametric, end cells conical-rounded. – Characteristic species in Belizean mats, corresponding mostly to the genus *Geitlerinema* (Anagnostidis 1989), but not identifiable with any known species (cf. Komárek & Anagnostidis 2005). Occurs almost in all studied marshes. - Differential features: Morphology of irregularly coiled trichomes, special type of intense motility (slithering motion), morphology of terminal parts of trichomes, ecology.

Geitlerinema aff. **splendidum** (Greville ex Gomont) Anagnostidis - Pl. Syst. Evol. 164: 43, 1989

This common and cosmopolitan species occurs in Belizean marshes only exceptionally and its identity with other populations is unclear. The width of trichomes in our specimens ranged from 2.4–3.7 μm . They occurred exceptionally among other cyanobacteria.

Planktothrix sp.

Fig. 23

DESCRIPTION: Solitary trichomes among other cyanobacteria in mats, not rare, but never in higher quantity. Trichomes cylindrical, not constricted at cross-walls, not attenuated towards ends, almost straight or slightly coiled, (2.4–)3.0–4.5 μm wide, pale blue-green, with many irregularly localized small aerotopes, sometimes (rarely) with solitary large aerotopes. The trichomes are sometimes slightly motile, and sometimes with pale segments in trichomes (diazocytes?). Sheaths occur very rarely, thin, colourless, slightly visible. Cells \pm isodiametric or shorter than wide; end cells conical-rounded, sometimes with thickened outer cell-wall. - Widespread in the whole area. This morphotype corresponds morphologically to the genetically uniform genus *Planktothrix*, but not to any morphospecies inside this genus.

Spirulina tenerrima Kützing ex Gomont - Ann. Sci. Nat., Bot. (Sér. 7) 16: 252, 1892

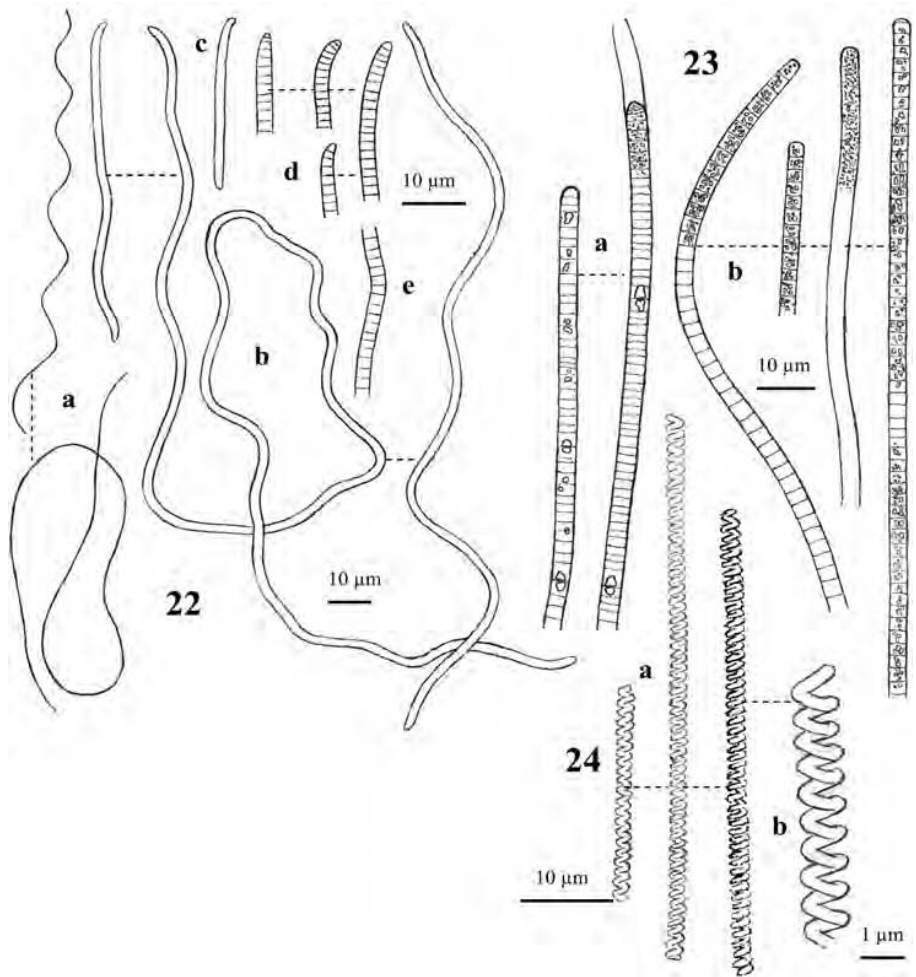
Fig. 24

DESCRIPTION: Solitary, \pm short trichomes, always shorter than 65 μm , mostly 30–40 μm long, among other cyanobacteria in mats and in benthos, without sheaths, intensely and regularly screw-like coiled, about 0.5 μm wide, coils 1.8–2.0 μm wide, \pm straight or slightly arcuated, pale blue-green, motile. Cells not recognizable in optical microscope, at the ends rounded. - Common, but never dominant, in sites with higher conductivity (salinity).

Schizothrix tenebrosa sp. nov.

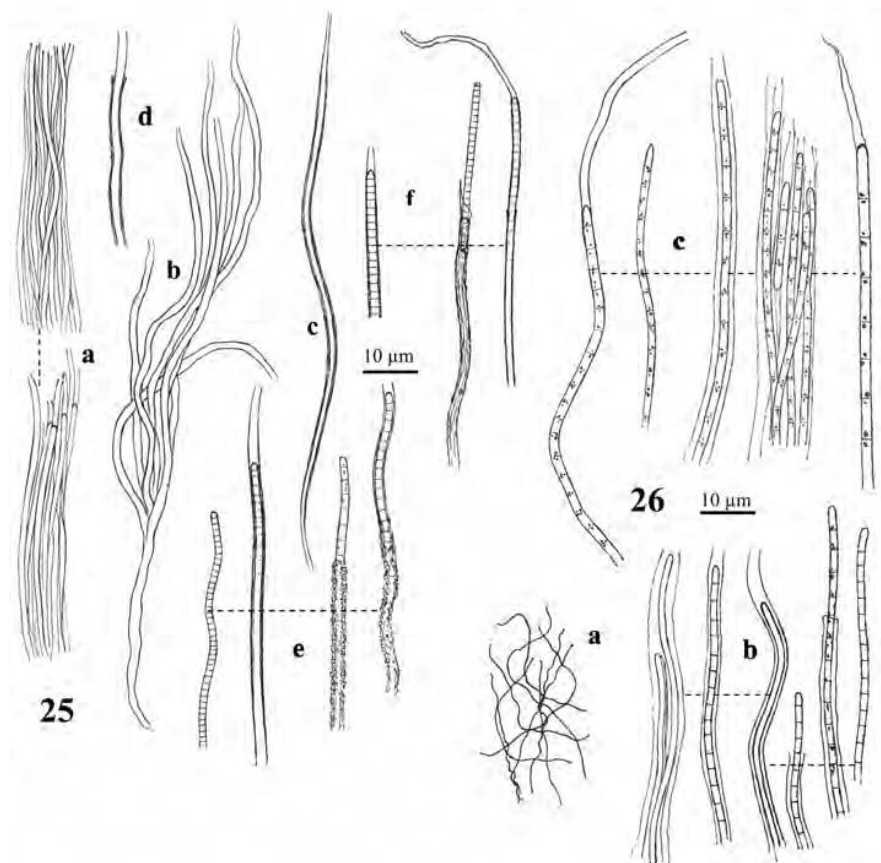
Fig. 25

Schizothrix tenebrosa sp. nov.: Filamenta solitaria vel plus minusve paralleliter fasciculata, non motilia, intricata, tortuosa, raro false pseudoramosa, ad apices attenuata, cum vaginis firmis, ambitu inaequalis, agglutinatis, achrois, despues granulosis, paucim stratosis et nigrescentis, 1.2–2.4 μm lata. Trichomata cylindrica, ad apices non attenuata, ad dissepimenta non constricta, pallide griseo-aeruginosa, 1.0–2.4 μm lata. Cellulae plus minusve isodiametricae vel paucim longior quam latae; cellula terminalis rotundata. - Habitatio: Metaphytice, inter algas cyanobacteriasque in stratis fluitantibus in paludis alcalinis; locus classicus: Belize septentrionalis, prope oppido Orange Walk dicto, palus Big Snail South, coll. 26.1.2003. – Holotypus: BRNM 1458, iconotypus figura nostra 25 b,e,f.



Figs 22–24. Not sequenced morphospecies (morphologically distinct morphotypes in natural samples): 22. *Geitlerinema serpens* (Big Snail South 25.1.2003). - 23. *Planktothrix* sp. (Cane 7.2.2003). - 24. *Spirulina tenerrima* (Buena Vista 26.1.2003).

DESCRIPTION: Filaments solitary or fasciculated, slightly coiled; sometimes falsely branched, narrowed towards ends (sheaths), 1.2–2.5 µm up to 5 µm wide. Trichomes thin, cylindrical, not attenuated towards ends, not constricted at cross-walls, 1.0–2.4 µm wide, pale greyish blue-green. Sheaths firm, sometimes telescopic, colourless, smooth, up to granular, dark blackish-brown. Cells ± isodiametric or slightly longer than wide; end cells rounded. - Characteristic species for the surface layer of floating mats. - Differential features: Morphology of filaments and trichomes, colouring of sheaths, life form, ecology.



Figs 25–26. Not sequenced morphospecies (morphologically distinct morphotypes corresponding to the genus *Schizothrix*): 25 *Schizothrix tenebrosa* (Big Snail South 26.1.2003). - 26. *Schizothrix* sp. a–b (Big Snail South 21.8.2006), c (Little Belize 1.2.2003).

Schizothrix sp.

Fig. 26

DESCRIPTION: Filaments solitary or in small (microscopic) fascicles, slightly or intensely coiled, rarely falsely branched, $\pm 3 \mu\text{m}$ wide, with more trichomes up to $6.2\text{--}32.0 \mu\text{m}$ wide. Trichomes thin, cylindrical, not attenuated towards ends, not constricted at cross-walls ($1.2\text{--}2.0\text{--}(2.5) \mu\text{m}$ wide, pale greyish, usually all trichomes in fascicles have their own sheath. Sheaths thick, colourless and hyaline, sometimes confluent, narrowed towards ends, outside smooth. Cells cylindrical, always clearly longer than wide (up to 4-times), with pale homogeneous content and irregular granules, particularly at cross-walls; end cells rounded or slightly conical-rounded. - In mats among other cyanobacteria, not in sites with high conductivity.

Discussion

The taxonomy of cyanobacterial diversity is now based on combined molecular and phenotype criteria. Therefore we tried to study the cyanobacterial assemblages of alkaline marshes of northern Belize applying this combined (molecular plus phenotype) evaluation. However, the biggest problem was that we were not able to transfer all the observed morphotypes from nature (which had the character of traditional species) into culture. The reason was the extreme character of the habitat, to which several morpho- or ecospecies were adapted and that therefore would have required to use of highly specific, but difficult to determine cultivation conditions. Despite this, 21 out of the observed 29 morphospecies were transferred in culture and their 16S rRNA genes sequenced. The clusters resulting from molecular evaluation were in good agreement with species identified according to phenotypic markers. The taxonomic status of several species was amended according to our phylogenetic analysis. According to the molecular evaluation, the cluster with the common tropical species *Phormidium* (= *Phormidesmis*) *molle* evidently did not belong to the group of typical *Phormidium* species. This cluster was also sufficiently phenotypically isolated and must be therefore described as a new genus. Its main characteristics have been given in the text, while more details of this new genus have been included in another article (Komárek et al., in press).

The floristic-taxonomic analysis based on traditional system yields a review of all present morphotypes in natural assemblages, but it is considered insufficient for a detailed and precise taxonomic evaluation. On the other hand, the application of only direct genetic methods, based on the analysis of the total community DNA (e.g., DGGE/TGGE analysis), can yield, in the best case, a review of the most represented genotypes, but a very restricted information about position of individual types in the ecosystem and their ecological significance. It is impossible to recognize the identity of clusters obtained from such analysis with different morpho- and ecospecies in the biocoenosis without a precise comparison, either at the morphological and genetic level, with isolated strains, particularly if more species of one genus are present.

The combined “polyphasic” approach was therefore preferred and used for the evaluation of cyanobacterial microflora as the best methodological way. We tried to fulfil the modern requirements for the characterization of cyanobacterial microflora of unusual alkaline Belizean marshes as much as possible. However, we were not successful in bringing to culture all morphotypes we observed in the natural samples. Therefore, for the identification of 8 morphospecies, we had to rely only on the traditional morphological characteristics.

Our present work has been focused on a very uniform and specialized ecosystem, which is known also from other alkaline marshes, located in limestone regions of the Caribbean district (Komárek et al. 2005). The papers from Central America (Gardner 1927, Schiller 1956, Komárek 1984, 1989) can be used for identification of different morphotypes, while the old monographs (Geitler 1932, Desikachary 1959) are usable only in a restricted manner. A comparison of our results with still unpublished, but careful floristic-taxonomic analyses of cyanobacterial microflora from marshes in

Everglades in Florida (Mareš 2006) was also important. However, several cyanobacterial types occur exclusively in this Belizean special habitat and must be taxonomically defined.

Our studies confirmed that the cyanobacterial microflora of various tropical habitats is specific and contains numerous special morpho- and ecotypes. Alkaline oligotrophic marshes of northern Belize with gradients of salinity in dependence on the distance from the sea and on the unique hydrology of the region, represent a specialized ecosystem, where various adapted forms prevail among the cyanobacterial assemblages, of which only few can be recognized as morphotypes described from other biotopes. The study of morphological variability in nature and culture, and in several cases the molecular support indicated the existence of special morpho- and genotypes in this ecosystem. The study of other similar biotopes from different tropical regions will be therefore important for the knowledge of cyanobacterial taxonomy (including simple typical oscillatorialean types). A similarly complicated and special diversity of cyanobacteria has been already published from other localities with extreme features, in particular from polar regions (e.g., Casamatta et al. 2005, Taton 2006 and others). It is a limitation that similar work using a polyphasic evaluations of complex oscillatorialean microflora from another tropical region is very rare (cf. also Garcia-Pichel et al. 2001, Thacker & Paul 2004, Richert et al. 2006).

The nomenclatoric principles for cyanobacterial taxonomy according to the Botanical and/or Bacteriological Codes are presently under discussion (cf. Oren 2004), and therefore we decided to apply both botanical criteria (in all new taxa) and bacteriological rules in the description of new taxa from cultured and sequenced types.

Our review included only filamentous cyanobacteria without heterocytes (*Oscillatoriales*), while coccoid and heterocytous types were included in other reviews (Komárek & Komárková-Legnerová 2007 and in prep.). All morphotypes, which were found in natural samples and isolated in monospecific culture have been mentioned. Of course, the diversity can be much higher, and the TGGE analyses of natural samples (Komárek et al. 2005) indicate wider diversity especially of very fine, morphologically simple filamentous types. However, their recognition needs further, more specialized studies. The comparison of results from genetic analyses (e.g., by TGGE or DGGE) with phenotype diversity is, of course, still more difficult. Therefore, we have used the combined molecular and phenotype evaluation as much as possible to our taxonomic review.

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