Polyphasic evaluation of *Limnoraphis robusta*, a water–bloom forming cyanobacterium from Lake Atitlán, Guatemala, with a description of *Limnoraphis* gen. nov.

Jiří Komárek[1]*, Eliška Zapomělová[2], Jan Šmarda[3], Jiří Kopecký[4], Eliška Rejmánková[5], Jason Woodhouse[6], Brett A. Neilan[6] & Jaroslava Komárková[2]

[1] *Institute of Botany AS CR and University of South Bohemia, Dukelská 135, CZ – 379 82 Třeboň, Czech Republic*

[2] *Biology Centre of AS CR, Institute of Hydrobiology, Na Sádkách 7, CZ 37005 České Budějovice, Czech Republic*

[3] *Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, CZ– 625 00 Brno – Bohunice, Czech Republic*

[4] *Institute of Microbiology AS CR, Opatovický mlýn, CZ–379 81 Třeboň, Czech Republic*

[5] *University of California, Davis, One Shields Ave., Davis, CA 95616, USA*

[6] *Centre for Cyanobacteria and Astrobiology, University of New South Wales, Sydney, NSW 2052, Australia*

Abstract: A tropical planktic filamentous cyanobacterium, tentatively identified as *Lyngbya robusta*, recently increased in abundance in Lake Atitlán, Guatemala, and since 2008 annual water–blooms occurred. This was one from the first known cases of *L. robusta* water–blooms worldwide. A polyphasic evaluation of *L. robusta* using 16S rRNA gene sequencing, cytomorphological markers, and ecological characteristics was made. This species had several unique features. It produced aerotopes that were irregularly spaced in cells; cyanotoxins were not found, and it fixed nitrogen in spite of the lack of heterocytes. It contained a high amount of carotenoids, which caused an unusual brown color of the macroscopic scum on the water level. Molecular phylogenetic analyses using the 16S rRNA gene showed that *L. robusta*, together with few other planktic species, formed a clade, separated from typical *Lyngbya* species. The main diacritical markers of this clade were the planktic type of life and formation of gas vesicles in cells. Based upon molecular, morphological and ecological data, a new genus *Limnoraphis* was proposed with four species.

Key words: carotenoids, ecology, Guatemala, Lake Atitlán, *Limnoraphis,* planktic *Lyngbya*, taxonomy, ultrastructure, water–blooms

Introduction

Planktic, water–bloom forming cyanobacteria are known from numerous freshwater and marine locations worldwide, and their ecology, distribution, and taxonomy have been documented numerous times. An intense, seasonally occurring water–bloom of the traditional filamentous genus *Lyngbya* C. Agardh ex Gomont (cf. Komárek & Anagnostidis 2005) has appeared annually since 2008 in the large, tropical, volcanic Lake Atitlán, located in the highlands of Guatemala. This is the first case of a typical water–bloom produced by a *Lyngbya*–like organism from tropical reservoirs. Apparently, the water–blooms have been caused by increasing input of nutrients, particularly phosphorus, into the lake (Rejmánková et al. 2011; comp. also Mioni et al. 2011–2012).

The traditional genus *Lyngbya*, based on the type species *L. confervoides*, is characterized by relatively wide filaments (8–35 μm) composed of cylindrical and uniseriate trichomes with short cells (usually several times shorter than wide), and firm, relatively narrow gelatinous and colorless or (rarely) yellowish sheaths. The heterocytes, akinetes and branching of any type are lacking. Only four freshwater aerotopate planktic morphospecies are known in *Lyngbya,* which differ in morphology (mainly in dimensions) and phytogeography. Their main vegetation phase

exists in plankton in lakes (KOMÁREK 2003; KOMÁREK & ANAGNOSTIDIS 2005). Unlike other *Lyngbya* species, they contain reversible gas–vesicles in cells concentrated in bundles termed aerotopes. The aerotopes can be irregularly distributed within the cells, or they may develop in segments of the filaments. The species forming the water–blooms in Lake Atitlán corresponded morphologically to *Lyngbya robusta.*

The phylogenetic relations and the taxonomic position of the planktic *Lyngbya* species have been studied using molecular analyses to now only of the Mongolian populations of *L. hieronymusii* (SUDA et al. 1998). More information is known about non planktic members of the genus *Lyngbya,* which can occur in mass developments in benthic habitats, e.g., the marine *L. majuscula* (ALBERT et al. 2005) and the freshwater *L. wollei* sensu American authors (e.g., BRIDGEMAN & PENAMON 2010) and their phylogenetic relationships were partly clarified by ENGENE et al. (2012). We transferred *Lyngbya robusta* from Atitlán into a monospecific culture and, according to our results (based on 16S rRNA gene sequences), all planktic and aerotopated *Lyngbya* species must be classified as a new genus *Limnoraphis.*

Material and Methods

Site description. Lake Atitlán is a volcanic crater lake located in the highlands of southwestern Guatemala, Central America (Figs S1–3), and more than 50% of the watershed consists of steep slopes. A detailed hydrological and hydrobiological description of the lake are found in REJMÁNKOVÁ et al. (2011); (cf. Table S1). The streams around the lake are easily erodible because the original forests have been replaced with agricultural crops and urban areas in many places. Erosion, combined with uncontrolled wastewater input, has caused increasing eutrophication over the last several decades (DIX et al. 2003). The lake has been recently changed mainly as a consequence of the increase of the P concentrations from oligotrophic to mesotrophic state. The first water–blooms occurred in 2008 and a major bloom followed in 2009 (Figs 1, 2). The water–blooms had a distinct seasonality. They usually followed the rainy season, which was characterized by the mean air temperature of 18 °C. The temperature of water in epilimnion was relatively uniform during the development of *Lyngbya* water–blooms and ranged mostly from 19.5 to 22 °C. In the 2008/2009 season, the water–bloom occurred in December and a part of January, in 2009/2010

it lasted from October to December. In 2010 the massive development of *Lyngbya* increased in August following tropical storm Agatha, but then slowed down and did not form full macroscopic water–blooms. In 2011, a high density of *Lyngbya* was recorded in March and lasted to May. At other times, the hormogonia of *Lyngbya* occurred sporadically and irregularly in epilimnion and in sediments near the shores of the wind exposed parts of the lake or in shallow bays.

Material. Field samples were collected from Lake Atitlán from 2008 to 2011 and partly preserved. The living filaments were transferred onto agar plates and isolated in solid BG11 Medium. Isolated strains (reference strain = CCALA 966) were then grown in BG 11 Medium at a temperature of 22 °C and a light intensity of 70 $\mu mol.m^{-2}.s^{-1}$ (16:8 L:D cycle).

In this study three strains were evaluated: *Lyngbya robusta* CCALA 966 (isolated from Lake Atitlán in 2009), and other related planktic species *Planktothrix cryptovaginata* "KUTNAR 3" (isolated from the Czech Republic) and *Lyngbya hieronymusii* N–929 (isolated from Mongolia). First two are deposited in the strain collection of the Institute of Hydrobiology of the Biology Centre of AS CR in České Budějovice, the last strain is deposited in NIES collection, Tsukuba, Japan.

Ultrastructure. For transmission electron microscopy, filaments of *Lyngbya robusta* were collected from agar cultures from natural material and fixed in two ways: (a) for three hours using 1% (w/v) osmium tetroxide in 0.7% (w/v) veronal–acetate buffer (pH 6.5) with traces of natrium chloride and calcium chloride; (b) for four hours using 3% (w/v) glutaraldehyde in 66 mM citrate–phosphate buffer (pH 7.3), applied at 4 °C, followed by post–fixation with 1% (w/v) osmium tetroxide for 1 hour. Fixed material was washed three times (10 min each) with the fixation buffer, instilled into 2% (w/v) agar and dehydrated with an ethanol gradient with concentrations increasing from 70% (50 min) to 100% (w/v) (30 min) followed with absolute acetone. Material was infiltrated with Durcupan ACN Fluka Nr. 1 (Buchs, Switzerland) resin in absolute acetone at room temperature overnight. Thereafter, the acetone concentration was gradually lowered at room temperature over four days, heated to 50 °C for 4 hours and kept in fresh Durcupan Nr. 2 at 50 °C for 2 hours. The resin was polymerized in the Beems flat embedding mould by increasing temperatures from 60 °C to 80 °C, one day each. Sections were cut using a Reichert–Jung ultramicrotome Ultracut E. Ready placed upon supporting grids, and stained with 2.5% (w/v) uranyl acetate and alkaline Reynolds solution (3%, w/v, lead nitrate with 3%, w/v, natrium citrate). Sections were examined on FEI Morgagni 268D transmission electron microscope (FEI Inc., Hillsboro, Oregon USA) and photographed with a digital camera.
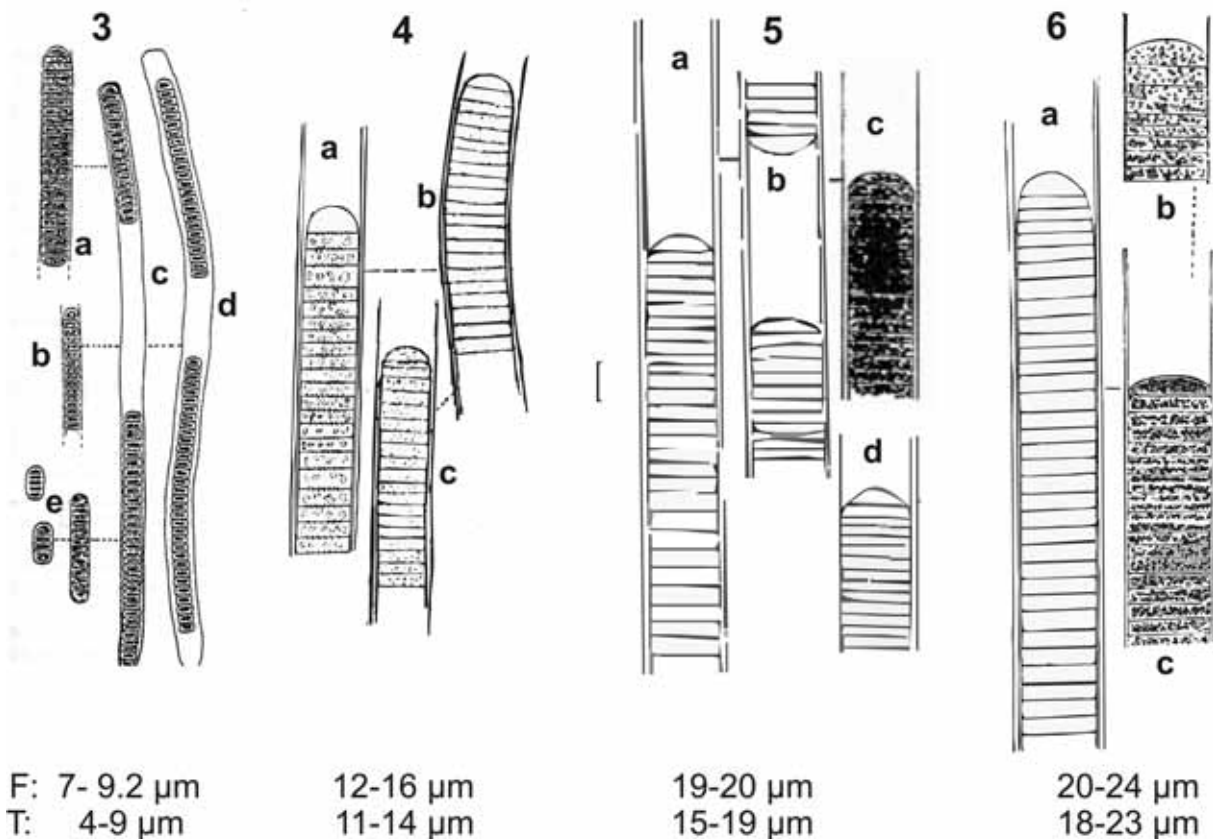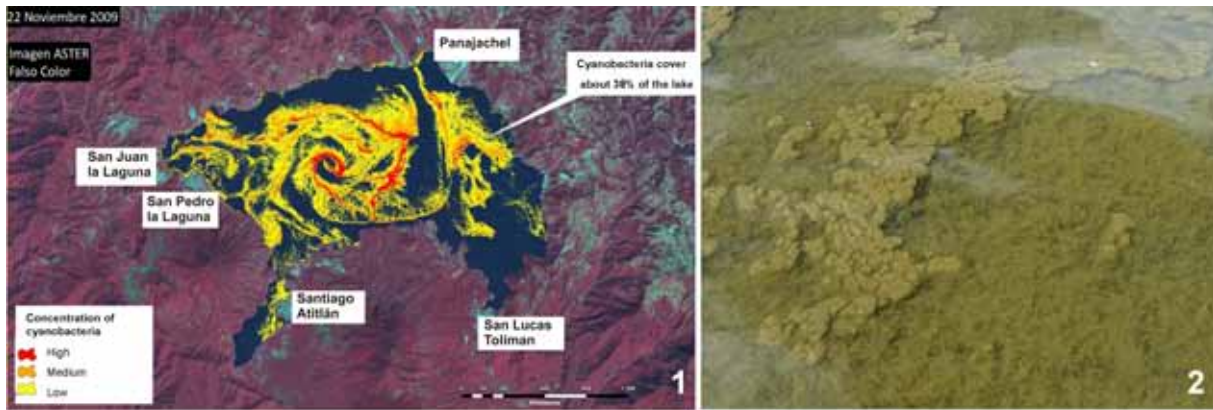
Fig. 1. ASTER satellite image of the cyanobacterial bloom in Lake Atitlán, Guatemala, November 22, 2009 (modified from the CATHALAC information material).

Fig. 2. Dense water–bloom of *Lyngbya robusta* in Lake Atitlán, November 2009 (photo REJMÁNKOVÁ).

Figs 3–6. Morphology of planktic *Lyngbya* (= *Limnoraphis*) species with gas vesicles within cells and with dimensions (widths) of filaments (F) and trichomes (T): (3) *L. cryptovaginata* after ŠKORBATOV in STARMACH 1966; (4) *L. hieronymusii* after PRESCOTT 1962 and LEMMERMANN 1905; (5) *L.robusta* after KOMÁREK & KOMÁRKOVÁ–LEGNEROVÁ 2007; (6) *L. birgei* after PRESCOTT 1962 and G.M. SMITH 1920.

Pigment analyses. For carotenoid analyses, cells of *L. robusta* (strain CCALA 966) were removed from liquid culture medium by centrifugation for 10 min at 4.500×g. Cells were extracted twice for 15 min at room temperature using 100% acetone. Extracts were clarified using 0.2 µm nylon filters (Micro–spin centrifuge filter, Alltech, Deerfield, IL, USA) and analyzed by high performance liquid chromatography (HPLC) using the Agilent 1100 Series system (Agilent Technologies Inc., Palo Alto, California, USA) equipped with the UV–VIS diode–array detector (Agilent DAD 61315B). Pigments were separated using a modified method of VAN HEUKELEM & THOMAS (2001) on a 35 °C Phenomenex Luna 3µ C8(2) 100 Å column with binary solvent system (0 min 100% A, 20 min 100% B, 25 min 100% B, 27 min 100% A, 30 1 min 100% A; A: 70% methanol + 28 mM ammonium acetate, B: methanol). The peak assignment was based
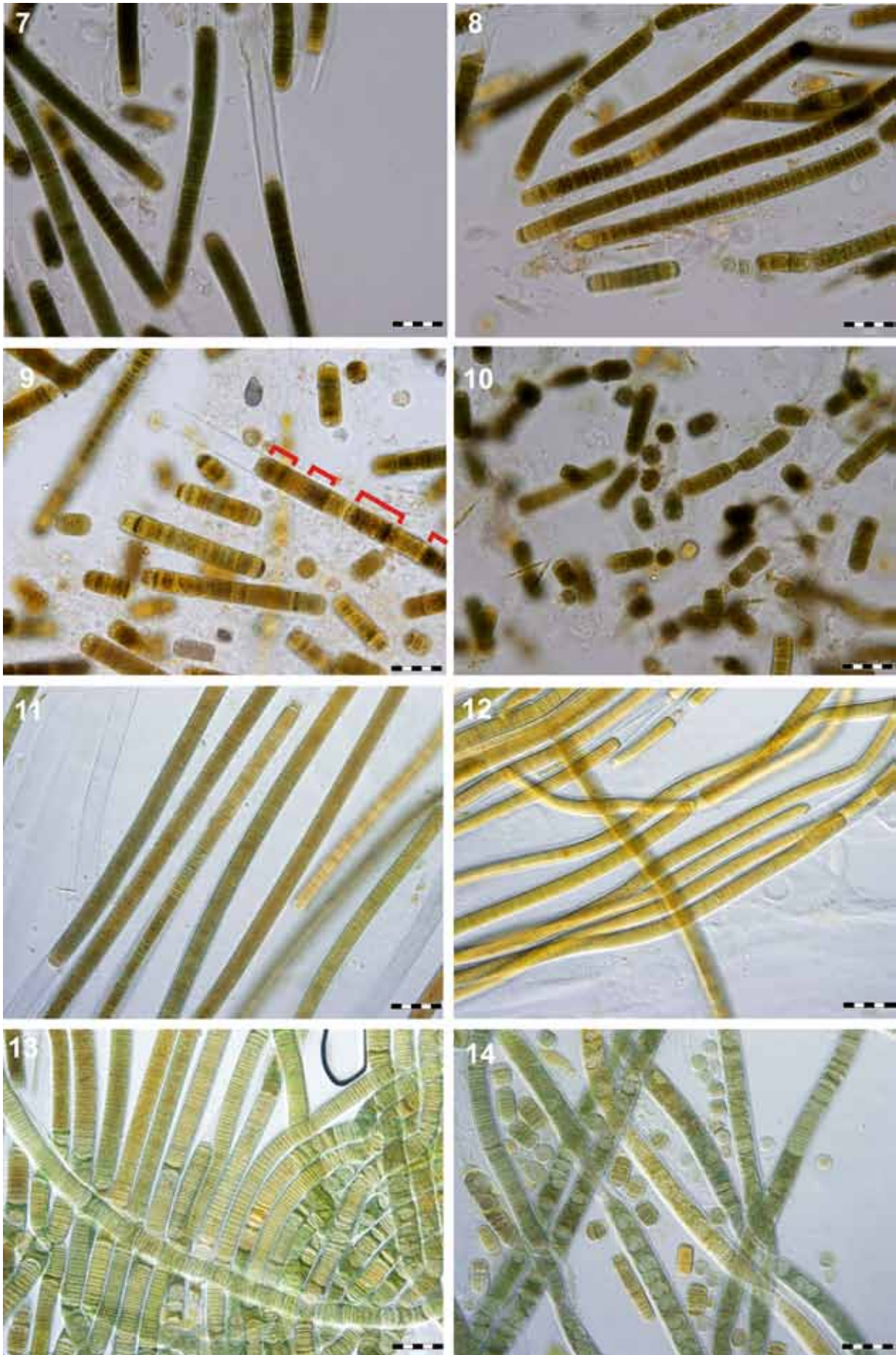
on obtained absorption spectra and retention behavior.

For phycobilin analysis, pelleted cells were either stored at −80 °C or immediately resuspended to the same final volume in 10 mM phosphate buffer (pH 6.8). Pellets were subjected to freeze–thaw cycles, i.e. immersed in 5 ml of 10 mM phosphate buffer (pH 6.8) and frozen at −20 °C, thawed, and extracted at 5 °C for 24 h. This freeze–thaw cycle was repeated three times. All supernatants were pooled, centrifuged at 12,000 rpm for 15 min at 4 °C, and the blue supernatant was analyzed. Spectrophotometric quantification was measured from 400 to 750 nm in a UV–VIS 3000 Shimadzu dual wavelength dual beam spectrophotometer using a 1 cm quartz glass cuvette against a phosphate buffer blank. Absorbance values were scatter–corrected by subtracting the absorbance at 750 nm from the absorbance maximum of the phycobilin peak [565 nm for phycoerythrin (PE); 620 nm for phycocyanin (PC); 650 nm for allophycocyanin (AC)]. The amounts of PE, PC, and AP were calculated by using the simultaneous equations of Bennett & Bogorad (1973) and the extinction coefficients from Bryant et al. (1979).

Toxicity. DNA was extracted from 100 mg of dried water–bloom material using the MP Biosystems Soil DNA Extraction kit according to manufacturers specifications. To test DNA quality, extractions were pre–screened using 16S rRNA gene PCRs from two duplicate DNA extractions using the cyanobacterial–specific primers 27F1 and 809R (Jungblut et al. 2005). Toxins were assessed individually, for each toxin, using the primer pairs DQmcyF/DQmcyR for microcystin/nodularin; sxtA–F/sxtA–R for saxitoxin; and cyrF/cyrR for cylindrospermopsin (Al–Tebrineh et al. 2001). As controls, *mcyE* positive *Microcystis* sp. PCC 7806, *sxtA* positive *Cylindrospermopsis raciborskii* T3 and *cyrA* positive *Cylindrospermopsis raciborskii* AWT205, were used. When primers amplified the positive control but not the sample DNA, 1 ng of positive control DNA was used to spike the sample to ensure no PCR inhibitors were present. PCR was performed using 0.2 U of *Taq* DNA polymerase (Bioline, A Meridian Life Science® Company, UK) in a 20 µl reaction mix containing 2.5 mM MgCl$_2$,

PCR buffer (Bioline), 0.2 mM dNTPs (Bioline), 10 pmol (or 25 pmol for DQmcyF/DQmcyR) of each primer (Sigma–Genosys, Sigma® Life Science Custom Products, Canada) and 1 ng of template DNA. Thermal cycling was performed in a Mastercycler® (Eppendorf, Germany). PCR conditions for 16S rRNA gene amplification were performed as previously described (Jungblut et al. 2005). PCR conditions for toxin–specific primer pairs were: initial denaturation step of four min at 94 °C was followed by 45 cycles involving a 10 s denaturation step at 94 °C, a 20 s annealing step at 60 °C and a 25 s elongation step at 72 °C, followed by a final extension step of 5 min at 72 °C. The amplicons were electrophoresed through a 1% agarose gel, stained with 0.5 µg.ml^{-1} ethidium bromide and photographed with a Gel Doc XR camera using Quantity one 4.6.1 software (Bio–Rad, Bio–Rad Laboratories, Inc. Berkeley, California, USA).

DNA extraction, PCR, 16S rDNA sequencing. The biomass for the phylogenetic analyses was harvested in the exponential growth phase by repeated centrifugation, during which the filaments were washed several times with a NaCl aqueous solution of concentration 1 g.l^{-1} to remove or reduce mucilaginous substances. The final biomass pellets were stored at –20 °C. For the16S rRNA gene sequencing, DNA was extracted using UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, California, USA). The 16S rRNA gene and ITS region were amplified with primers 16S27F, 23S30R (Taton et al. 2003) and CYA359F (Nübel et al. 1997). The 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; the final elongation step was 7 min at 72 °C. PCR products were cleaned using NucleoSpin ExtractII kit (Macherey–Nagel GmbH & Co. KG, Düren, Germany). PCR products were then used as templates for sequencing with primers 16S27F, 23S30R (Taton et al. 2003), primer cAlaR (Wilmotte et al. 1994), primers WAW1486R and Primer 14 (Wilmotte et al. 1993), and primer CYA781F (a) and CYA359F (Nübel et al. 1997). The sequencing was performed at a commercial facility

Figs 7–10. Trichome morphology of *Limnoraphis robusta* changes in natural population from Lake Atitlán: (7) young filaments developing from hormogonia, intensely growing with common aerotopes in cells; (8) vegetative filaments from starting vegetation of water bloom; (9) start of the disintegration of trichomes in hormogonia at the end of the vegetation period, in the apical part of one filament are marked segments with common aerotopes in cells; (10) disintegration of filaments in short hormogonia. Scale bars 50 µm.

Figs 11–14. Trichomes of *Limnoraphis robusta* from culture CCALA 966. Successive stages during the development of the *Limnoraphis* population in culture and the disappearing of gas vesicles (disappearing of "black segments" in trichomes) is well visible. Sheaths and aerotopes in cells are slightly reduced in the material from cultures: (11) filaments from the strain CCALA 966 after reinoculation, in young trichomes are parts with developed aerotopes (blackish segments); (12–13) aerotopes disappear during longer cultivation on agar plates (12) as well as in liquid medium (13); (14) disintegration of trichomes in short hormogonia and monocytes. Scale bars 50 µm.

(Laboratory of Genomics, Biology Centre of AS CR, České Budějovice, Czech Republic).

Phylogenetic analyses. Partial 16S rRNA sequences (1211 bp) were aligned using BioEdit version 7.0.9.0 (Hall 1999) and this alignment was then edited manually. Phylogenetic trees were constructed using maximum likelyhood (ML) and neighbour–joining (NJ) (Saitou & Nei 1987) algorithms in the program PAUP* version 4.0b10 (Swofford 2003). For ML, the GTR+I+G evolutionary model of substitution was found for the best fit to the data using ModelTest 3.7 (Posada 2008). The parameters (base frequencies, rate matrix of substitution types and shape of gamma distribution) were estimated from the data. One hundred and 1,000 bootstrap replicates were performed for ML and NJ analysis, respectively. Nucleotide sequences of studied planktic strains were deposited at GenBank under the accession numbers JN854138–JN854140.

Taxonomic evaluation. The separated phylogenetic position of *Lyngbya robusta* and four related strains of other planktic "*Lyngbya*" species (*L. hieronymusii, L. cryptovaginata*), following from the 16S rRNA gene sequencing was accepted as the basis of their taxonomic classification. They belong morphologically to the traditional family Oscillatoriaceae, genus *Lyngbya* sensu lato (Anagnostidis & Komárek 1988; Komárek & Anagnostidis 2005). For the final identification, all available criteria for polyphasic evaluation were used. Their phylogenetic, morphological and ecological position is isolated in such a degree, that the genus *Limnoraphis* was validly established from this cluster on the basis of Botanical nomenclatoric rules (ICBN; McNeill et al. 2006). The isolated strain of *Lyngbya* (=*Limnoraphis*) *robusta* was deposited in the collections CCALA (Třeboň) and PCC (Paris). The exsiccated type material is deposited in the herbaria of Moravian Museum in Brno, Czech Republic, under the number BRNM–HY 2365.

Results

Lyngbya robusta (= *Limnoraphis robusta* comb. nov.) is a novel water–bloom forming cyanobacterium from mesotrophic waters in the Lake Atitlán. As well as other related planktic *Lyngbya*–species (Figs 3–6), it lives in stagnant water reservoirs in solitary, ± straight or slightly curved filaments, which make free and irregular clusters only in mass development. They have more or less uniform morphology: trichomes are 15–19 μm wide, straight, composed of short cells, which only occasionally can reach up to semi–quadratic shape. The cells are usually 2–4 times shorter than wide. The common cytomorphological feature of planktic *Lyngbya* species, which separates them from other *Lyngbya* species, is the ability to produce gas vesicles in cells (gathered to aerotopes); this character does not occur in any other type of *Lyngbya*. Gas vesicles and aerotopes are not developed regularly along the whole trichome and their frequency changes during the vegetation period (cf. Figs 7–10 and 11–14). The production of aerotopes was facultative when grown in culture. Trichomes are cylindrical, usually not constricted at cross–walls, never distinctly narrowed towards ends, end cells are widely rounded, without calyptra. The cross–walls can be only exceptionally and slightly constricted. The sheaths are firm, clearly visible, relatively thick (4–12 μm), colorless, sometimes more widened, variable and gelatinized in comparison with other *Limnoraphis* species. They surpass the trichomes in form of a firm tube. Hormogonia vary in length and cell numbers, sometimes reduced to few–celled segments or even monocytes (cf. Figs 10, 14).

Molecular phylogenetic analyses of the 16S rRNA gene showed that the Atitlán planktic and aerotopated strain of *Lyngbya* (=*Limnoraphis*) *robusta* forms a discrete phylogenetic clade with 100% and 99% bootstrap supports (Fig. 15, clade A; Table S2) with *Limnoraphis hieronymusii* comb. nov. from Mongolian lakes (strains N–929, CN4–3) and *Limnoraphis cryptovaginata* comb. nov. (strain "KUTNAR 3") from an oxbow lake Kutnar, SE Czech Republic. *Limnoraphis* gen. nov. was a sister clade to B (Fig. 15), identified as "*Lyngbya aestuarii*" with 96.3% 16S rRNA gene sequence similarity.

The original planktic *Lyngbya* species *L. robusta, L. birgei, L. hieronymusii* and *Planktothrix/Lyngbya cryptovaginata* must therefore be classified as different species of the same, newly established generic entity (*Limnoraphis*). These taxa differ from each other morphologically (width of trichomes) and by geographic distribution. The position of planktic *Limnoraphis*–cluster (Fig. 15, clade A, Table 1) is separated from the typical line of *Lyngbya* with the marine type–species *L. confervoides* (Fig. 15, clade C), which occurred in a distinct clade (Fig. 15, clade C). The genus *Moorea*, recently separated from *Lyngbya* by Engene et al. (2012), formed a second marine clade (Fig. 15, clade D), and freshwater *Lyngbya martensiana* formed the fifth clade of the original *Lyngbya sensu lato* (Fig.

15, clade E). The percent similarity between different *Lyngbya* clades was calculated (Table 1).

Limnoraphis robusta has a characteristic ultrastructure. Cell walls are thick, distinct, and cross–walls are slightly undulate (Figs 16–22). Walls of necridic cells form typical morphological rests (nc) at the ends of the disintegrated filaments. The sheath is composed of several layers of the same structure (ms). Numerous short thylakoids (th) are situated ± near walls and cross–walls and agglomerated in very dense, irregular fascicles. Numerous pores penetrating the cross–walls between the cells are clearly visible (Fig. 21). Gas vesicles (gv) are parallel arranged in aerotopes and situated more or less in whole cell volume. Irregularly scattered polyphosphate granules, cyanophycin granules, carboxysomes, lipidic droplets and ribosomes are visible. Some thylakoids can be slightly widened, especially near cross walls, although majority of thylakoids are densely arranged and short.

The filaments of *Limnoraphis robusta* are brownish colored. The color is well recognizable in natural samples (in massive water–blooms, Fig. 2) and particularly in cultures, when gas vesicles are absent (Figs 9, 11–13). Pigment analyses showed the presence of chlorophyll–*a,* the carotenoids myxoxanthophyll, ß–carotene and, in less quantity, canthaxanthin, echinenon and polar xanthophylles (Figs 23, S4). Phycobilins were consistent with other cyanobacteria (Fig. 24). The complex of carotenoids covers evidently other pigments and causes the atypical brown coloration of biomass both in culture and natural water–bloom.

Limnoraphis robusta belongs to oscilla-torialean cyanobacteria without heterocytes, which are capable of nitrogen fixation. The fixation proceeds during the night and the nitrogenase activity is enhanced by the addition of phosphorus and iron (REJMÁNKOVÁ et al. 2011 and unpublished data). The presence of firm sheaths and clustering in mats evidently helps to induce favorable conditions for nitrogen fixation, which is intensive particularly during the night. The presence of dinitrogenase reductase gene, *nifH* has been confirmed (comp. also WOODHOUSE et al., pers. comm.).

Genomic DNA extracted from *Limnoraphis robusta* water–bloom did not yield a positive result for any of the toxin gene primer assays. The toxic screening by PCR returned negative results for the 4 major cyanotoxins (SXT, MCY, CYN, NOD).

Duplicate DNA samples positively amplified the 16S rRNA gene and all primer pairs successfully amplified spiked positive control.

Taxonomic solution. The phylogenetic cluster of *Limnoraphis* (planktic *Lyngbya*–strains, Fig. 15A) contains two strains of *Lyngbya hieronymusii* (cf. SUDA et al. 1998 from lakes of Mongolia; strains CN4–3 and N–929), a strain of *L. robusta* isolated from the Lake Atitlán, Guatemala, and a strain of *Planktothrix cryptovaginata*, isolated from central Europe (an old oxbow lake Kutnar in SE Czech Republic). The morphology of all these types is similar (cf. Figs 3–6). *Planktothrix cryptovaginata* (originally described also as *Lyngbya*) differs from other *Planktothrix* species by the presence of sheaths and by distinct phylogenetic affiliation. This whole *Limnoraphis* cluster (originally *Lyngbya hieronymusii, Lyngbya robusta, Planktothrix cryptovaginata*) should therefore be characterized as a special generic entity, with three species differing morphologically, by ecology and geographic distribution. *Lyngbya birgei* from North Ameri-can mesotrophic lakes must be included also to this cluster according to the typical morphology and ecology. The *Limnoraphis* clade, based upon molecular, morphological and ecological data, is distinct from other clusters of *Lyngbya sensu lato*.

Limnoraphis gen. nov.

Filamenta cyanoprocaryotica, simplicia, libera, solitaria, 5–25 µm lata, vel in fasciculis parvis, fatiscentis, aggregata, recta vel paucim flexuosa, vaginata. Vaginae plus minusve firmae, achroae, hyalinae, tenues vel paucim incrassatae. Trichoma uniseriata, ad dissepimenta non constricta vel paucim constricta, cylindrica, ad apices non attenuata, non distincte calyptrata. Cellulae cylindricae, curtae, semper brevior quam latae, cum vesiculis gaseosis (aerotopis) impletae. Heterocyta akinetesque carentes. Reproductio hormogoniis cylindraceis. – Praecipue natantes in plancto aquis dulcis.

Limnoraphis hieronymusii (LEMMERMANN) comb. nov., type species

(basionym: *Lyngbya hieronymusii* LEMMERMANN, Forschungsber. biol. Stat. Plőn 12: 146, 1905); this taxon was selected as the type species, because it is the oldest and best known species from this cluster, with type material deposited in collection BREM (Bremen, Germany).

Filaments cyanoprokaryotic, simple, solitary, free–floating, 5–25 µm wide, or growing in small

Planktothricoides raciborskii NIES-207

A — *Limnoraphis robusta* ATITLAN, *L. cryptovaginata* KUTNAR, *L. hieronymusii* N-929, CN4-3 (4 OTUs) 100/99

79/98

95/95

B — *Lyngbya aestuarii* PCC7419 (2 OTUs) 100/100

99/100 — *Arthrospira fusiformis* AB2002/01, *A. indica* PD1997/ram, *A. platensis* AICB 49, *Phormidium* cf. *terebriformis* AB 2002/07 (6 OTUs)

100/100 — *Phormidium* spp., *Oscillatoria acuminata* PCC 6304 (4 OTUs)

98/95 — *Phormidium* cf. *aerugineo-coeruleum* R-aq

C — *Lyngbya* cf. *confervoides* VP0401

73/81 — *Pseudoscillatoria coralii* BgP10_4S, *Oscillatoria spongeliae* 34P8, *Oscillatoria* cf. *corallinae* PCC 6304 (3 OTUs)

100/100 — *Hydrocoleum lyngbyaceum* HBC7, *Oscillatoria* cf. *curviceps* Fkv-3, *margaritifera* NAC8-54, *O. miniata* NAC8-50, *O. nigroviridis* 3LOSC, *O. sancta*, *Trichodesmium* spp. (11 OTUs)

86/100 — *Microcoleus* spp., *Tychonema bourrellyi* HAB663, *Phormidium* cf. *amoenum* BW-1, *P. autumnale* Ant-Ph68, *P. corium* PMC299.07,, *P.* cf. *subfuscum* I-Roc (8 OTUs)

62/73 — 100/100 — *Oscillatoria lutea* SAG 1459-3 / *Phormidium animale* SAG1459-6

Microcoleus chthonoplastes WW10

D — marine *Lyngbya* spp. = *Moorea* gen. nov. (Engene *et al.* 2011) (former *Lyngbya bouillonii*, *L. majuscula*, *L. polychroa*, *L. sordida*) (21 OTUs) 100/100

Geitlerinema carotinosum AICB 37

Symploca atlantica CCY9617

66/71 — 99/100 — *Microcoleus paludosus* SAG 1449-1a / *Microcoleus glaciei* UTCC 475 / *Phormidium murrayi* Ant-Ph58

–/60 — *Leptolyngbya mycoidea* LEGE07157

–/65 — *Oscillatoria rosea* IAM M-220

88/80 — *Halospirulina tapeticola* CCC Baja-95 / *Spirulina subsalsa*

E — *Lyngbya martensiana* H3b/7, H3b/33 (2 OTUs) 100/100

64/68 — *Phormidium ambiguum* IAM M-71

72/64 — *Pseudanabaena minima* GSE-PSE20-05C, *P. mucicola* IAM M-221, PMC269.06, *Oscillatoria limnetica* MR1 (4 OTUs)

Arthronema africanum SAG 12.89

100/100 — *Leptolyngbya angustata* UTCC 473 / *Leptolyngbya foveolarum*

73/66 — *Phormidium priestleyi* ANT.L61.2

Planktolyngbya limnetica PMC271.06

90/97 — *Pseudanabaena constantiae* KM1c, *Pseudanabaena tremula* UTCC 471, *Leptolyngbya tenuis* PMC304.07 (3 OTUs)

89/79 — *Leptolyngbya* spp. (3 OTUs)

71/64

100/100 — *Phormidium chlorinum* PMC274.06, *Jaaginema homogeneum* PMC252.05, *Limnothrix redekei* 2LT25s01 (3 OTUs)

92/95 — *Phormidium persicinum* SAG 80.79, *Spirulina laxissima* SAG 256.80, *Oscillatoria neglecta* IAM M-82, *Leptolyngbya* cf. *halophila* LEGE 06152, *L. nodulosa* UTEX 2910 (5 OTUs)

100/100 — *Oscillatoria duplisecta* ETS-06, *O. kawamurae*, *O. princeps* NIVA-CYA 150 (3 OTUs)

Phormidium uncinatum SAG 81.79

98/100

100/99 — *Plectonema wollei* Carmichael/Alabama, NO99 (2 OTUs)

–/57 — *Phormidium* cf. *irriguum* CCALA 759 / *Phormidium tergestinum* CCALA 155

Microcoleus steenstrupii FI-LIZ3B clone B

100/100 — *Crinalium epipsammum* SAG 22.89, *C. magnum* SAG 34.87, *Starria zimbabweensis* SAG 74.90 (3 OTUs)

81/95 — *Planktothrix* spp. - planktonic strains (4 OTUs)

99/100

Cuspidothrix issatschenkoi C5

Aphanizomenon flos-aquae 1478

Aphanizomenon gracile 6

0.1

cluster A:

100/99 — *Lyngbya robusta* ATITLAN / *Lyngbya hieronymusii* N-929 / *Planktothrix cryptovaginata* KUTNAR / *Lyngbya hieronymusii* var. *hieronymusii* CN4-3

cluster D:

64/– — *Lyngbya bouillonii* PAL08-16 / *Lyngbya bouillonii* PNG7-4 / *Lyngbya bouillonii* PNG5-198

Lyngbya polychroa PNG6-54

87/96 — *Lyngbya majuscula* PNG6-24 / *Lyngbya majuscula* PAL-8-17-08-2 / *Lyngbya majuscula* HECT / *Lyngbya majuscula* 19L

–/89 — *Lyngbya majuscula* PAB / *Lyngbya majuscula* 3L / *Lyngbya polychroa* LP5

55/98 — *Lyngbya sordida* NAC8-48 / *Lyngbya sordida* NAC8-52 / *Lyngbya sordida* NAC8-53

100/100 — *Lyngbya polychroa* PNG7-19 (FJ / *Lyngbya majuscula* JHB / *Lyngbya majuscula* NAC8-47

52/69 — *Lyngbya majuscula* PNG6-221 / *Lyngbya polychroa* PNG5-192 / *Lyngbya polychroa* PNG6-48 / *Lyngbya bouillonii* PNG6-41
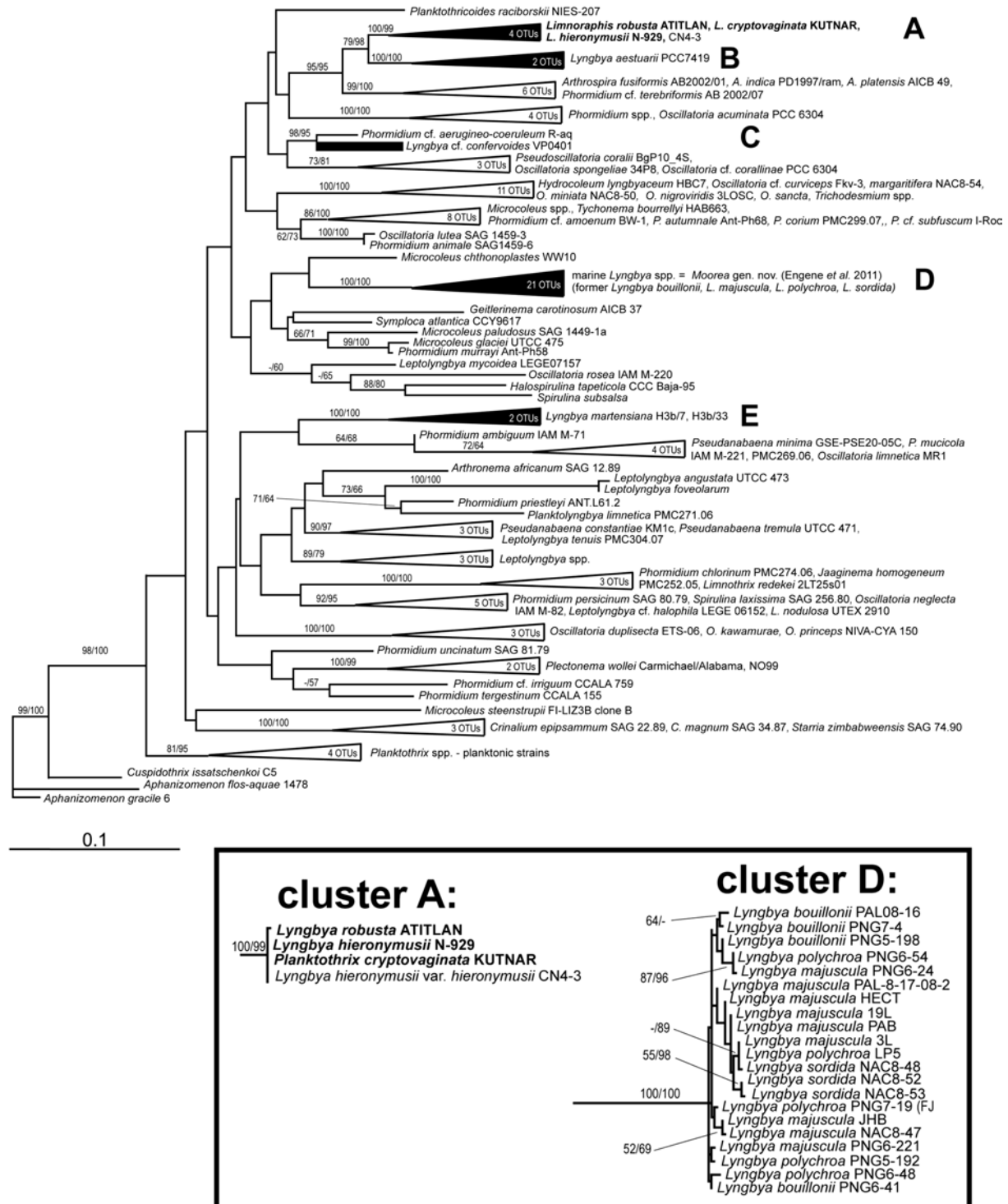
Fig. 15. Maximum Likelihood tree based on the 16S rRNA gene sequences (1211 bp) showing the clustering of strains from the traditional genus Lyngbya. Numbers near nodes indicate bootstrap values of ML/NJ over 50%. Cluster A contains the planktic types with aerotopes in cells, corresponding to *Limnoraphis* gen. nov. The strains whose 16S rRNA gene sequences were acquired within this study are in bold. Clusters containing cyanobacteria that were originally classified into the genus *Lyngbya* sensu lato are marked with black colour. Description of clusters B–E see in text. Cluster D is derived from Engene et al. 2012. Genbank Accession numbers are in Supplementary Table S2.

aggregations, straight or slightly curved, with distinct sheaths. Sheaths firm, colorless, hyaline, thin or slightly widened. Trichomes uniseriate, not or slightly constricted at the cross–walls, cylindrical, not narrowed towards ends, without clear calyptra. Cells cylindrical, short, always wider than long, with facultative gas vesicles (and aerotopes), present in distinct segments of a trichome. Heterocytes and akinetes missing. Reproduction by intense hormogonia production. – Mainly free–floating in freshwater plankton.

***Limnoraphis birgei* (G.M.Smith) comb. nov.**
Basionym: *Lyngbya birgei* G.M.Smith, Bull. Torrey Bot. Club 43(9): 482, 1916.

***Limnoraphis cryptovaginata* (Škorbatov) comb. nov.**
Basionym: *Lyngbya cryptovaginata* Škorbatov, Not. Syst. Inst. Crypt. Horti Petrop. 2(6): 88, 1923.
Synonym: *Planktothrix cryptovaginata* (Škorbatov) Anagnostidis et Komárek, Algol. Stud. 50–53: 416, 1988.

***Limnoraphis robusta* (Parakutty) comb. nov.**
Basionym: *Lyngbya hieronymusii* f. *robusta* Parakutty, Proc. Ind. Acad. Sci. 11: 123, 1940.
Synonym: *Lyngbya robusta* (Parakutty) Komárek et Komárková–Legnerová, Hoehnea 34(1): 55, 2007.

Reference strain: CCALA 966. The reference strain is deposited in the collection CCALA at the Institute of Botany of the Academy of Sciences of the Czech Republic.
Type specimen: BRNM HY 2365; Moravian Museum in Brno, Czech Republic.

Discussion

Based on the presented results from phylogenetic analyses of the 16S rRNA gene sequences, the traditional genus *Lyngbya* C. Agardh ex Gomont 1892 has to be divided into at least five genetic clades, which display less than 95% of the 16S rRNA gene sequence similarity one to another and the genus *Limnoraphis* (with the type *L. hieronymusii*) should be taxonomically defined (Fig. 15, clade A). The only exception are two strains, identified as halophilic *Lyngbya aestuarii* (strains with accession nos. AB 075989 and AB 039013) (Fig. 15, clade B). The strain PCC 7419 of *Lyngbya aestuarii* has 96.3% of sequence similarity to the *Limnoraphis* cluster (Table 1).

However, the identification of these strains is not yet confirmed and this cluster needs further morphological and molecular studies as a whole. The cytomorphology and ecology of *Lyngbya aestuarii* is very different from the planktic cluster A, and their taxonomic separation is highly probable.

The next two clusters comprise marine species: The type species of the genus *Lyngbya* (*L. confervoides*) has not yet been well identified (Fig. 15, clade C; "cf."). However, only the species (strains) belonging to this phylogenetic clade should be classified as typical *Lyngbya* species, but the cluster designated by this name is clearly distinguished from *Limnoraphis* taxonomically and ecologically. It is a marine species, it grows on rocks in the supralitoral zone, and it completely lacks aerotopes.

Another compact marine group (Fig. 15, clade D), comprising mostly the robust strains from tropical seas (mostly from Pacific ocean), was studied in detail by Engene et al. (2012) and classified into the specific genus *Moorea,* with the type *M. producens.*

The last cluster comprises benthic and periphytic freshwater *Lyngbya*–strains ("*Eulyngbya*"). It is represented especially by the morphospecies *Lyngbya martensiana* (strains H3b/7, H3b/33; Fig. 15, clade E). A more or less homogeneous group of strains and species of identical morphological characters from freshwater alkaline marshes in Belize were studied by Turicchia et al. (2009) (*Lyngbya minor, Lyngbya* cf. *martensiana, Lyngbya* cf. *ocreata, Lyngbya* cf. *splendens, Lyngbya* sp.). The authors recognized a specific cluster (cluster 9 in Turicchia et al. 2009) of these species based on their 16S rRNA gene sequences, receiving a high bootstrap support. We have not included these strains in the phylogenetic tree as they are not available in GenBank. Judging by the high morphological similarity with our strains from cluster E and also by the presence of the strain identified as *Lyngbya* cf. *martensiana* (3CC03S04) in the tree by Turicchia et al. (2009), we suppose, however, that all these species probably belong to the same phylogenetic clade. Such types correspond morphologically to the traditional *Lyngbya* species, but the type species *L. confervoides* is marine and belongs to another phylogenetic clade according to the 16S rRNA gene sequences (Fig. 15, clade C). The taxonomic status of this cluster (Fig. 15, clade E) must be resolved in future.

The genus *Limnoraphis* has several specific characters. The intracellular structures display an unusual pattern. The gas vesicles are agglomerated as in other planktic cyanobacteria, however, the distribution of aerotopes along the filaments is irregular. This phenomenon often supports an irregularly changing color of the filament with "dark" segments with cells full of aerotopes, which are irregularly distributed along the filament (Figs 5,9,11). Similar "dark" segments are found also in *L. hieronymusii* (fig. 2 in SUDA et al. 1998). Gas vesicles are a diagnostic morphological feature for *Limnoraphis* coinciding with genetic position of this whole cluster; they are closely connected with the ecology. The composition of pigments is also different from the common water–bloom forming cyanobacterial species. The relatively high amount of xanthophylls and β–carotene, combined with lower amounts of phycobilins causes the unusual yellow–brown coloration of the macroscopic clusters and mats floating on the water level. No tox–genes have been detected in water–blooms of *Limnoraphis*. The apparent absence of toxic compounds suggests that *L. robusta* does not manipulate the community with toxins.

The four planktic species of *Limnoraphis* (*L. hieronymusii, L. birgei, L. robusta, L. cryptovaginata*) are widely separated from *Lyng-bya* in our molecular phylogenetic analysis, they possess obligatory aerotopes, and they occur only in the plankton and less frequently in metaphyton (*L. cryptovaginata*) of freshwater reservoirs. The best–known "*Lyngbya*" species, described from plankton of mesotrophic lakes in northern Germany is *L. hieronymusii* (LEMMERMANN 1905). This species has not been confirmed during recent decades in Europe, but it sporadically occurs in other locations within the northern temperate zone of Eurasia. Populations and small water–blooms of this species were recently described in detail by SUDA et al. (1998) from the Mongolian Lake Dalai and from lakes in South Korea (LEE & BAE 2001). Both cited papers contain valuable data on the ecophysiology and biochemistry of this species.

Interestingly, the report about harmful cyanobacteria blooms in Clear Lake and the Sacramento San Joaquin Delta (California) was presented quite recently, prepared by MIONI et al. (2011–2012), in which the planktic, aerotopated "*Lyngbya* sp.", corresponding evidently to *Limnoraphis robusta* is presented and documented. It would be a second record of this species in

water blooms from western America.

Limnoraphis robusta was originally described from eutrophic, large water–bodies in India (originally as *Lyngbya hieronymusii* var. *robusta)* and later also recorded sporadically from Africa and Brazil (cf. PARAKUTTY 1940; DESIKACHARY 1959; KOMÁREK & KOMÁRKOVÁ–LEGNEROVÁ 2007). Some changes in morphology of *Limnoraphis robusta* after the transfer into agarized culture media are also remarkable (cf. Figs 7–10 and 11–14). The gas vesicles are mostly reduced and mucilaginous sheaths are distinctly thinner and less developed in comparison with natural material. It is an example of morphological changes of cyanobacterial taxa after transfer into the culture conditions.

Limnoraphis birgei G. M. SMITH with relatively short, but wide solitary filaments (20–26 μm wide) occurs in plankton of large mesotrophic to oligotrophic lakes in North America (northern USA, Canada) (G.M. SMITH 1916, 1920; PRESCOTT 1966; KLING in litt.). The species was recorded several times and seems to be common, but it has not yet been registered as forming dense populations, and data about the ecology are still scarce. The floristic data of this species from other regions are unconfirmed and problematic. This species was transferred in the genus *Limnoraphis* only according to the cytomorphological and ecological similarity (cf. Fig. 6).

Limnoraphis cryptovaginata is a metaphytic species of oxbows or small lakes with macrophytes and occurs in central and eastern Europe, and central and southern Asia (cf. ANAGNOSTIDIS & KOMÁREK 1988). It belongs to the same cluster as *Limnoraphis robusta* according to phylogenetic relations (Fig. 15). It differs from other *Limnoraphis* species by smaller dimensions (7–9.2 μm wide filaments), facultative presence of sheaths and constrictions at cross–walls. It occurs in metaphyton of smaller water bodies rather than in plankton.

The genus *Limnoraphis*, particularly the tropical *L. robusta,* differs from all other water–bloom forming cyanobacterial species from other genera also by specific ecological characteristics. The high production of *"Lyngbya robusta"* (= *Limnoraphis robusta*) in Lake Atitlán is the first case of this type of cyanobacterial "water–bloom" in freshwaters worldwide. However, the development of this "*Lyngbya*" (= *Limnoraphis*) water–blooms is apparently not a case of phytogeographic separation, or the consequence

Table 1. Comparison of percentage sequence similarities of partial 16S rRNA gene (1212 bn) of selected traditional *Lyngbya* and *Limnoraphis* strains, derived from analysis in Fig. 15.

		1	2	3	4	5	6	7	8	9	10
1	*Lyngbya robusta* ATITLÁN, CCALA 966				*Limnoraphis*						
2	*Planktothrix cryptovaginata* KUTNAR, strain 3	99.9									
3	*Lyngbya hieronymusii* CN4-3	99.9	100.0								
4	*Lyngbya martensiana* H3b/7	90.7	90.5	90.5							
5	*Lyngbya martensiana* H3b/33	91.0	90.8	90.8	99.4						
6	*Lyngbya bouillonii* PAL08-16	90.6	90.7	90.7	88.8	89.0					
7	*Lyngbya majuscula* 19L	90.9	91.0	91.0	89.3	89.6	99.2				
8	*Lyngbya majuscula* PNG6-24	90.7	90.8	90.8	89.2	89.4	99.1	99.2			
9	*Lyngbya sordida* NAC8-52	90.8	90.9	90.9	89.3	89.6	99.0	99.9	98.9		
10	*Lyngbya polychroa* LP5	90.8	90.9	90.9	89.1	89.3	99.1	99.9	99.2	99.6	
11	*Lyngbya* cf. *confervoides* VP0401	93.0	92.9	92.9	88.9	88.9	90.2	90.9	90.4	90.9	90.8

of simple increase of nutrients, because in the same region heavy water–blooms of *Microcystis* develop in the eutrophic Lake Amatitlán, which is supplied with nitrogen–rich waste waters. According to our results, *L. robusta* develops here in large, oligo– to slightly mesotrophic reservoir with increased content of phosphorus. Concentration of nitrogen in the epilimnion is typically quite low. Therefore, it seems that in the case of Atitlán, *Limnoraphis robusta* substitutes other species due to specific mesotrophic conditions with remarkable increase of phosphorus. It is also an interesting evidence of the potential development and vitality of any cyanobacteria as dominant microorganisms in various habitats and their quick adaptation to changing ecological conditions. Rare cyanobacterial genotypes can display enhanced vitality and rapid development, then gradually dominate various habitats.

Acknowledgements

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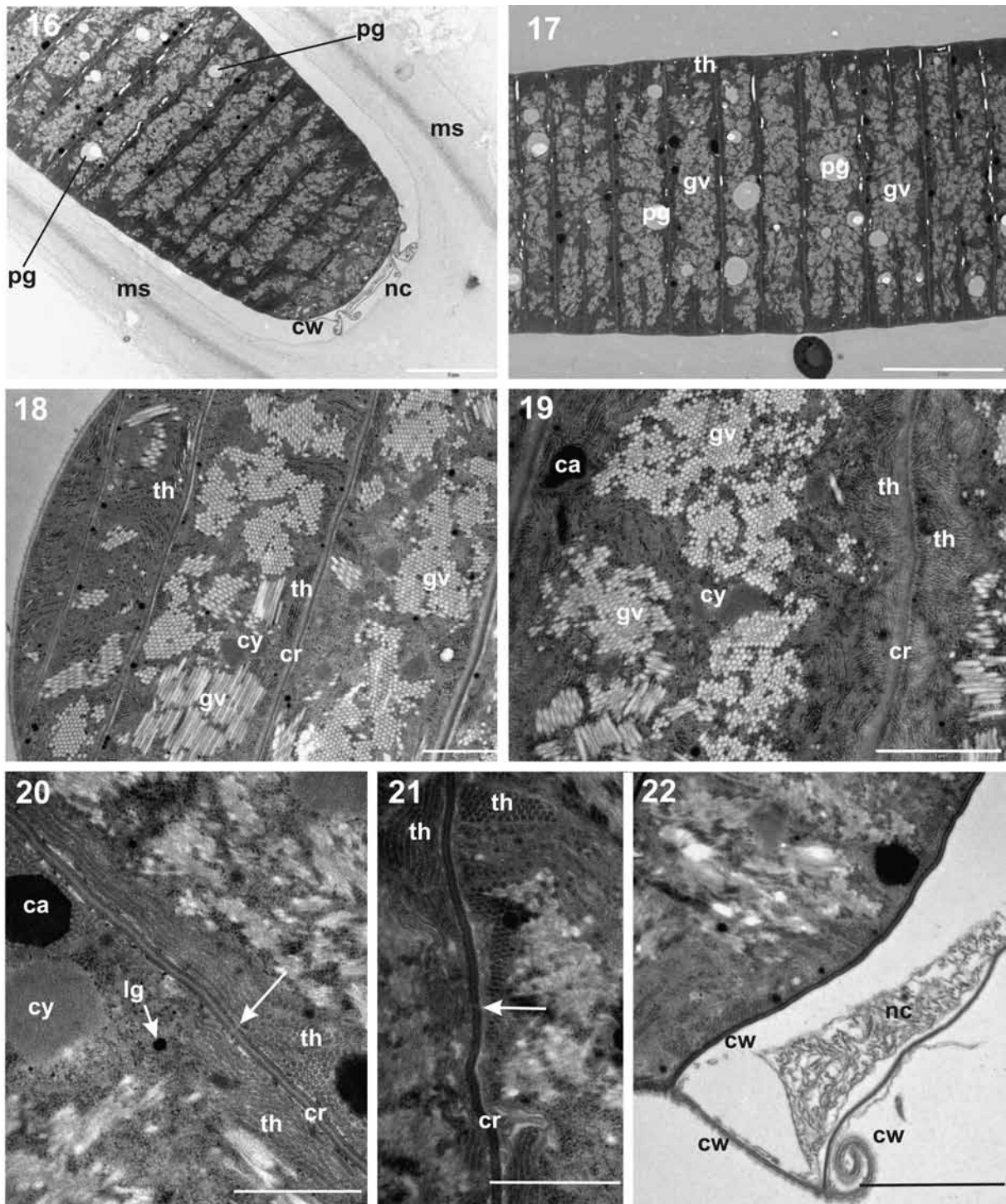
Figs 16–22. Ultrastructural sections of trichomes of *Limnoraphis robusta* strain CCALA 966 with details of cell walls and cross–walls. In the Fig. 22 are visible typical rests of necridic cells at the ends of disintegrated trichomes [(ca) carboxysomes; (cr) cross–walls; (cw) cell walls; (gv) gas vesicles; (ld) lipidic droplets; (ms) mucilaginous sheath; (pg) polyphosphate granules; (th) thylakoids; (cy) cyanophycin granules; (nc) rests of necridic cells; (white arrows) pores in cross–walls]. Scale bars (16–17) 5 μm, (18–19) 1 μm, (20–21) 0.5 μm, (22) 1μm.

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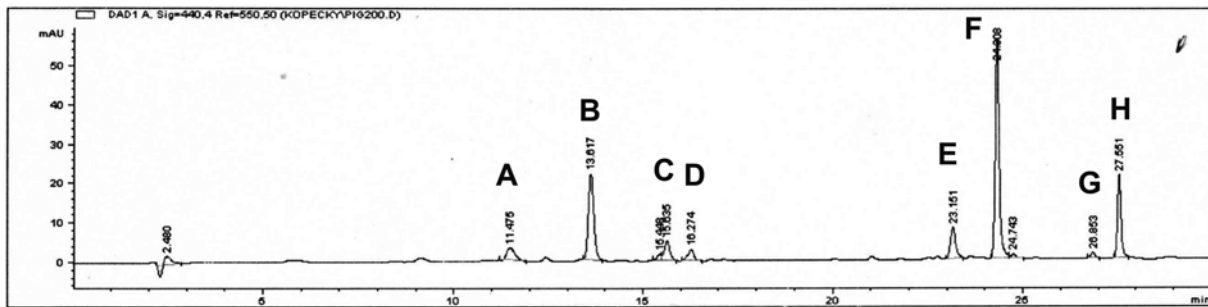
Fig. 23. Spectral analysis of chlorophylls and carotenoids from the strain CCALA 966 [(x) retention time (min.), (y) absorbance at 440 nm (nAU); in spectrograms A–H: (x) wavelength (nm), (y) absorbance (mAU); (A) polar xanthophyll I, (B) myxoxanthophyll, (C) polar xanthophyll II, (D) canthaxanthin, (E) echinenon, (F) chlorophylls, (G) cis–ß caroten, (H) ß carotene].
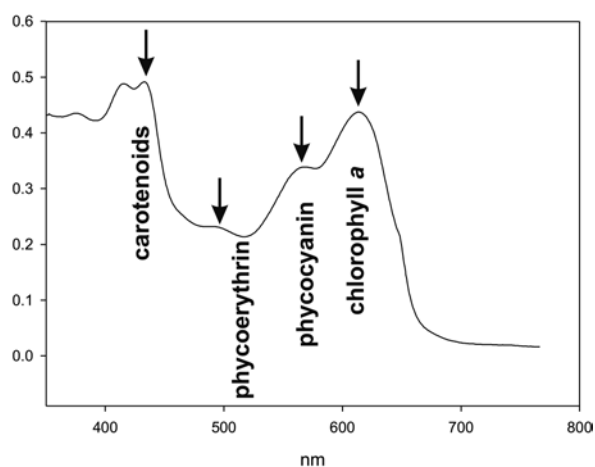

Fig. 24. Spectral analysis of chlorophyll–*a*, phycobilins and carotenoids from the strain CCALA 966 [(x axis) wavelength (nm), (y axis) absorbance (AU)].

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Note of authors:
This manuscript was prepared during the year 2011. However, the expedition in Atitlán 2012 found already the homogeneous distribution of *Limnoraphis robusta* over the whole lake. The development of this cyanobacterium follows evidently after the continual, intense eutrophication of the reservoir. In the year 2012 also other water–bloom cyanobacteria appeared in various locations of the lake, e.g. *Microcystis* cf. *aeruginosa* or *Aphanizomenon* sp.

Supplementary material

the following supplementary material is available for this article:

Table S1. Ecological characteristics of *Limnoraphis robusta* from the Lake Atitlán, Guatemala.
Table S2. Accession numbers of strains included in the phylogenetic tree.
Fig. S1. Lake Atitlán, Guatemala.
Fig. S2. Geographic location of Lake Atitlán.
Fig. S3. Bathymetric map of Lake Atitlán.

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

Table S1. Ecological characteristics of *Limnoraphis robusta* from the Lake Atitlán, Guatemala. Data on phosphorus (SRP), and nitrogen (NO_3–N) are from the central sampling station (DIX and REJMÁNKOVÁ, unpublished data).

Geographic position		91°07'01''– 91°16'08'' w.g.l. 14°37'00'' – 14°43'00'' n.g.w.
Altitude above see level		1555 m
Average area of the lake		137 km²
Maximal depth of the lake		341 m
Volume		24 km³
SRP, no bloom (April 2010)	Epilimnion	5 µg.l^{-1}
	Hypolimnion	25 µg.l^{-1}
NO_3-N, no bloom (April 2010)	Epilimnion	5 µg.l^{-1}
	Hypolimnion	80 µg.l^{-1}
SRP, onset of a bloom (June 2010)	Epilimnion	20 µg.l^{-1}
	Hypolimnion	50 µg.l^{-1}
NO_3-N, onset of a bloom (June 2010)	Epilimnion	ND
	Hypolimnion	46 µg.l^{-1}

Table S2. Accession numbers of strains included in the phylogenetic tree (Fig. 15).

Taxon	Strain code	Genbank accession number	Note
Planktothricoides raciborskii	NIES-207	AB045960.1	
Lyngbya robusta	ATITLAN	JN854138	*Limnoraphis robusta* comb. nov.
Planktothrix cryptovaginata	KUTNAR	JN854139	*Limnoraphis cryptovaginata* comb. nov.
Lyngbya hieronymusii	N-929,	JN854140	*Limnoraphis hieronymusii* comb. nov.
Lyngbya hieronymusii	CN4-3	AB045906.1	*Limnoraphis hieronymusii* comb. nov.
Lyngbya aestuarii	PCC7419	AB039013.1	
Lyngbya aestuarii		AB075989.1	
Arthrospira platensis	AICB 49	AY672713.1	
Arthrospira fusiformis	AB2002/01	AY575923.1	
Arthrospira indica	PD1997/ram	AY575931.1	
Arthrospira maxima	KCTC AG30054	DQ393281.1	
Arthrospira jenneri	EB 9604	GQ184185.1	
Phormidium cf. terebriformis	AB 2002/07	AY575933.1	
Phormidium lumbricale	UTCC 476	AF218375.1	
Phormidium pseudopriestleyi	ANT.LACV5.3	AY493600.1	
Phormidium cf. formosum	P-O	EU196640.1	
Oscillatoria acuminata	PCC 6304	AB039014.1	

Table S2 Cont.

Phormidium cf. aerugineo-coeruleum	R-aq	EU196641.1	
Lyngbya cf. confervoides	VP0401	AY599507.1	
Pseudoscillatoria coralii	BgP10_4S	FJ210722.2	
Oscillatoria spongeliae	34P8	AY615507.1	
Oscillatoria cf. corallinae	PCC 6304	X84812.1	
Hydrocoleum lyngbyaceum	HBC7	EU249124.1	
Oscillatoria sancta		AB039015.1	
Oscillatoria cf. curviceps	Fkv-3	EU196623.1	
Oscillatoria nigroviridis	3LOSC	EU244875.1	
Oscillatoria miniata	NAC8-50	GU724203.1	
Oscillatoria margaritifera	NAC8-54	GU724207.1	
Trichodesmium contortum		AF013028.1	
Trichodesmium tenue		AF013029.1	
Trichodesmium thiebautii		AF091321.1	
Trichodesmium hildebrandtii		AF091322.1	
Trichodesmium havanum	F34-5	AF518770.1	
Microcoleus antarcticus	UTCC 474	AF218373.1	
Microcoleus rushforthii	UTCC 296	AF218377.1	
Microcoleus vaginatus	CSU-U-KK1	EF667962.1	
Tychonema bourrellyi	HAB663	FJ184385.1	
Phormidium autumnale	Ant-Ph68	DQ493874.1	
Phormidium cf. subfuscum	I-Roc	EU196634.1	
Phormidium cf. amoenum	BW-1	EU196636.1	
Phormidium corium	PMC299.07	GQ859649.1	
Oscillatoria lutea	SAG 1459-3	AB115967.1	
Phormidium animale	SAG1459-6	EF654087.1	
Microcoleus chthonoplastes	WW10	EF654058.1	
Lyngbya bouillonii	PNG5-198	FJ041298	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya bouillonii	PNG6-41	FJ147302	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya bouillonii	PNG7-4	FJ151519	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya bouillonii	PAL08-16	GU111927	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya majuscula	19L	AY599501	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya majuscula	PAB	AY599502	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya majuscula	HECT	AY599503	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya majuscula	3L	EU315909	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya majuscula	PNG6-24	FJ041308	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya majuscula	JHB	FJ151521	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya majuscula	PNG6-221	FJ356669	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya majuscula	PAL-8-17-08-2	GQ231522	*Moorea* gen nov. (ENGENE et al. 2012)

Lyngbya majuscula	NAC8-47	GU724198	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya polychroa	PNG5-192	FJ041300	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya polychroa	PNG6-48	FJ041301	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya polychroa	PNG7-19	FJ041307	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya polychroa	PNG6-54	FJ151522	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya polychroa	LP5	FJ602753	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya sordida	NAC8-48	GU724199	*Moorea* gen nov. (ENGENE et al. 2012)
Lyngbya sordida	NAC8-53	GU724206	*Moorea* gen nov. (ENGENE et al. 2012)
Geitlerinema carotinosum	AICB 37	AY423710.1	
Symploca atlantica	CCY9617	GQ402026.1	
Microcoleus paludosus	SAG 1449-1a	EF654090.1	
Microcoleus glaciei	UTCC 475	AF218374.1	
Phormidium murrayi	Ant-Ph58	DQ493872.1	
Leptolyngbya mycoidea	LEGE07157	HQ832916.1	
Oscillatoria rosea	IAM M-220	AB003164.1	
Halospirulina tapeticola	CCC Baja-95	NR_026510.1	
Spirulina subsalsa		AF329394.1	
Lyngbya martensiana	H3b/7	JN854141	
Lyngbya martensiana	H3b/33	JN854142	
Phormidium ambiguum	IAM M-71	AB003167.1	
Pseudanabaena mucicola	IAM M-221	AB003165.1	
Oscillatoria limnetica	MR1	AJ007908.1	
Pseudanabaena mucicola	PMC269.06	GQ859643.1	
Pseudanabaena minima	GSE-PSE20-05C	HQ132935.1	
Arthronema africanum	SAG 12.89	AB115966.1	
Leptolyngbya angustata	UTCC 473	AF218372.1	
Leptolyngbya foveolarum		X84808.1	
Phormidium priestleyi	ANT.L61.2	AY493582.1	
Planktolyngbya limnetica	PMC271.06	GQ859645.1	
Pseudanabaena constantiae	KM1c	DQ393595.1	
Pseudanabaena tremula	UTCC 471	AF218371.1	
Leptolyngbya tenuis	PMC304.07	GQ859652	
Leptolyngbya frigida	ANT.L70.1	AY493574.1	
Leptolyngbya antarctica	ANT.L18.2	AY493606.1	
Leptolyngbya laminosa	ETS-08	FM210757.1	
Phormidium chlorinum	PMC274.06	GQ859648.1	
Jaaginema homogeneum	PMC252.05	GQ859646.1	
Limnothrix redekei	2LT25s01	FM177493.1	
Phormidium persicinum	SAG 80.79	EF654085.1	

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Spirulina laxissima	SAG 256.80	DQ393278.1	
Oscillatoria neglecta	IAM M-82	AB003168.1	
Leptolyngbya cf. halophila	LEGE 06152	HQ832915.1	
Leptolyngbya nodulosa	UTEX 2910	EF122600.1	
Oscillatoria princeps	NIVA-CYA 150	AB045961.1	
Oscillatoria kawamurae		AB298443.1	
Oscillatoria duplisecta	ETS-06	AM398647.1	
Phormidium uncinatum	SAG 81.79	EF654086.1	
Plectonema wollei	Carmichael/ Alabama	EU439567.1	
Plectonema wollei	NO99	HQ419204.1	
Phormidium cf. irriguum	CCALA 759	EU196638.1	
Phormidium tergestinum	CCALA 155	EF654083.1	
Microcoleus steenstrupii	FI-LIZ3B clone B	EU586740.1	
Crinalium epipsammum	SAG 22.89	AB115964.1	
Crinalium magnum	SAG 34.87	AB115965.1	
Starria zimbabweensis	SAG 74.90	AB115962.1	
Planktothrix rubescens	CCAP 1459/14	FJ184422.1	planktic
Planktothrix mougeotii	HAB3343	FJ434250.1	planktic
Planktothrix pseudagardhii	2LT34S02	FM177501.1	planktic
Planktothrix agardhii	PMC253.05	GQ859639.1	planktic
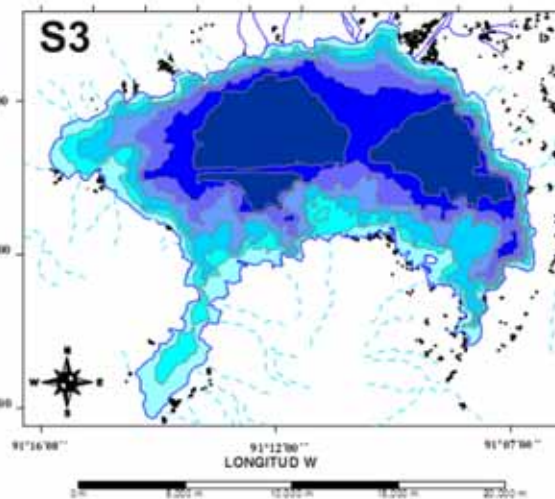
Fig. S1. Lake Atitlán, Guatemala.
Fig. S2. Geographic location of Lake Atitlán.
Fig. S3. Bathymetric map of Lake Atitlán. Blue colors indicate 50 m depth intervals; maximum depth nearly 350 m; from CASTELLANOS & DIX 2009.
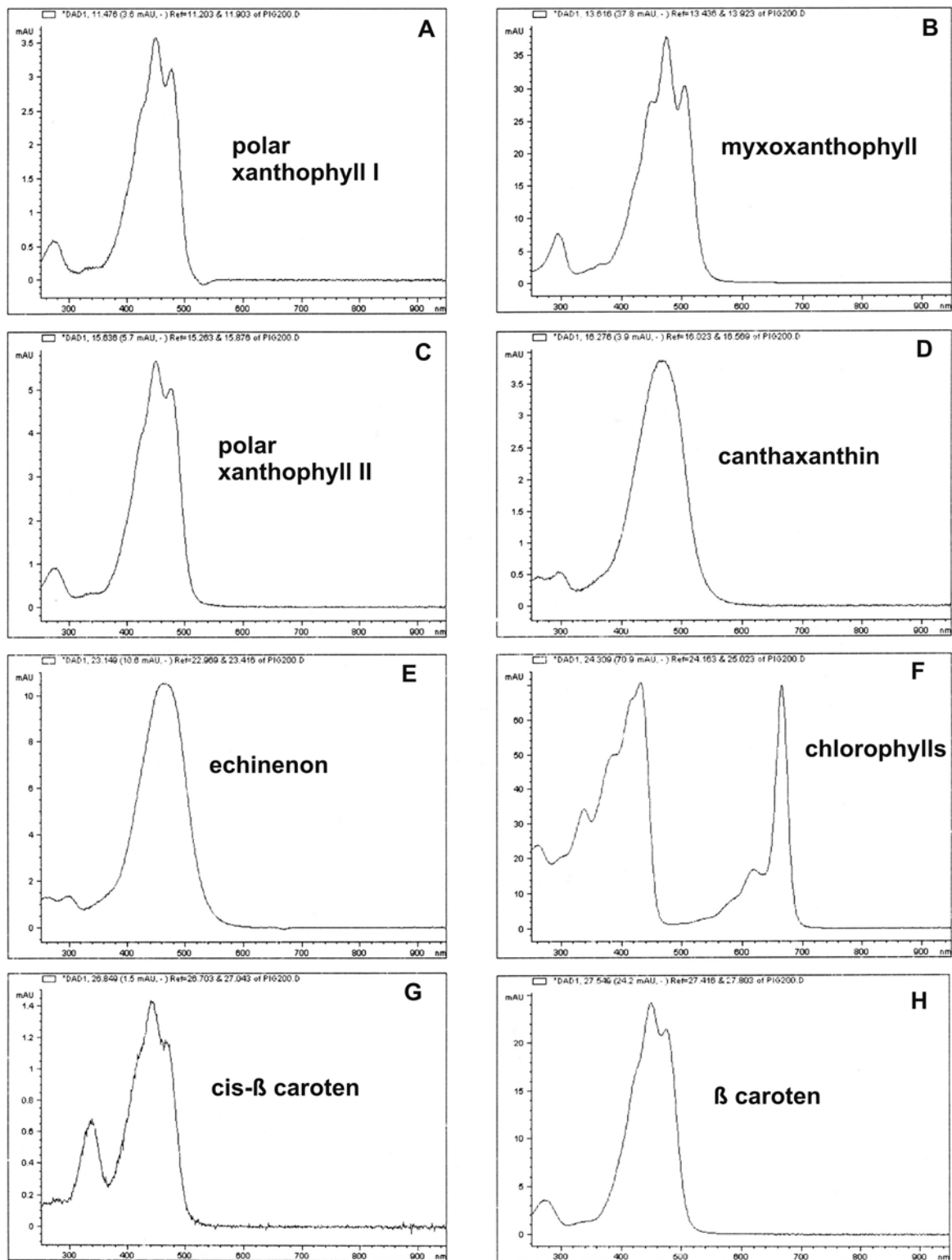
Fig. S4. Spectral analysis of chlorophylls and carotenoids from the strain CCALA 966 [(x) retention time (min.), (y) absorbance at 440 nm (nAU); in spectrograms A–H: (x) wavelength (nm), (y) absorbance (mAU)].