A new red colonial *Pseudanabaena* (Cyanoprokaryota, Oscillatoriales) from North American large lakes

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Abstract: A new morphotype of the genus *Pseudanabaena* (Cyanoprokaryota, Oscillatoriales) was identified from bloom samples of *Aphanizomenon flos–aquae* Ralfs ex Bornet et Flahault taken from large central North American water bodies, Lake Winnipeg (LWPG) and Lake of the Woods (LOW), which drains into Lake Winnipeg (Fig. 1) and a strain of this morphotype was isolated from LOW in the fall of 2009. Here we describe the morphology and basic ecology, cytology and phylogenetic position of this new chromatic adaptive and colony forming planktonic species and propose a new species *Pseudanabaena rutilus–viridis* Kling et al. Preliminary research has indicated that it has the ability to produce the toxin microcystin and further research is under way to assess its ability to produce taste–odour and other toxins and nitrogen–fixing capacity.

Key words: Cyanoprokaryotes, Oscillatoriales, *Pseudanabaena*, taxonomy, morphology, cytology, genetics, ecology, toxins

Introduction

The morphologically variable (Romo & Miracle 1994) genus *Pseudanabaena,* established in 1915 by Lauterborn, revised by Anagnostidis & Komárek (1988; Komárek 2003) is closely related genetically and morphologically to the genus *Limnothrix* Meffert. Further molecular work by Acinias et al. (2009), using the 16S–23S internal transcribed unit (ITS) confirmed that some of the morphotypes assigned to *Pseudanabaena* belong to the *Pseudanabaena* cluster. Acinias et al. (2009) also noted a special group of complementary chromatic adaptive types, while Rudiger et al. (2007) described the adaptive phycoerythrin capacity of a strain of *Pseudanabaena* from their lakes. Hindák (2008) in his Atlas of Cyanophytes shows a photo of the species identified as *P. galeata* Böcher with both reddish–violet and blue–green filaments.

Recently we identified a new morphotype of the genus *Pseudanabaena* (Cyanoprokaryota, Oscillatoriales) in *Aphanizomenon flos–aquae* Ralfs ex Bornet et Flahault bloom samples from large central North American water bodies, Lake Winnipeg (LWPG) and Lake of the Woods (LOW), which drains into Lake Winnipeg (Fig. 1) and isolated a strain of this morphotype from LOW in the fall of 2009. Here we describe the morphology and basic ecology, cytology and

phylogenetic position of this new chromatic adaptive and colony forming planktonic species, proposing a new species *Pseudanabaena rutilus–viridis*. Preliminary research has indicated that it has the ability to produce the toxin microcystin and further research is under way to assess its ability to produce taste–odour and other toxins and nitrogen–fixing capacity.

Material and Methods

Material. *Pseudanabaena rutilus–viridis* was initially found during routine examination of live bloom samples of the *Aphanizomenon flos–aquae* complex from Lake Winnipeg (2005), Lake of the Woods (2009), and more recently in Killarney Lake, Manitoba 2010. The main characters of the lakes can be seen in Table 1.

A subsample from Lake of the Woods was sent to the Canadian Phycological Culture Collection (CPCC) at the Biology Department, University of Waterloo for isolation in late October 2009. It was isolated into CYANO growth media (Jüttner). The isolate CPCC697 was transferred and grown in BG–11 and CYANO media at 10°C and 20°C in low (3–13 µmol(photons)/m².s) and high light (25–30 µmol(photons)/m².s). Photographs were taken of the strain CPCC under both growth conditions (Fig 2 A & B). Width and length measurements were taken from random trichomes for over 100 cells from each treatment, and the data tested for significant differences among population means using proc UNIVARIATE and NPAR1WAY (SAS©). Cell length data showed a distribution that was not significantly different from normality ($p>0.05$) but cell width showed significant skewness and kurtosis ($p<0.01$). Differences among means were therefore tested using non–parametric comparisons (Wilcoxon rank sum test (Mann–Whitney U test).

Microcystins and general toxicity were tested using ELISA and PPIA (expressed as total microcystin) on subsamples of culture CPCC697 at Environment Canada (EC), Burlington, Ontario.

Total chlorophyll and break down of the chlorophyll as measured using a Fluoroprobe (to be verified by HPLC) was undertaken on two subsamples (CPCC 697) at the Freshwater Institute Winnipeg, MB.

Electron microscopy. A subsample of CPCC697 was grown on agar plates at the Institute of Botany, Třeboň, Czech Republic, and filaments from these agar cultures were fixed in two fixatives:

a) osmium fixation: 1% (w/v) osmium tetroxide in 0.7% (w/v) veronal–acetate buffer, pH 6.5, with traces of natrium chloride and calcium chloride, was applied for 3 hours, followed by postfixation with 0.5% (w/v) uranyl acetate in the same buffer;
b) glutaraldehyde fixation: 3% (w/v) glutaraldehyde in 100 mM cacodylate buffer, pH 7.3, was applied

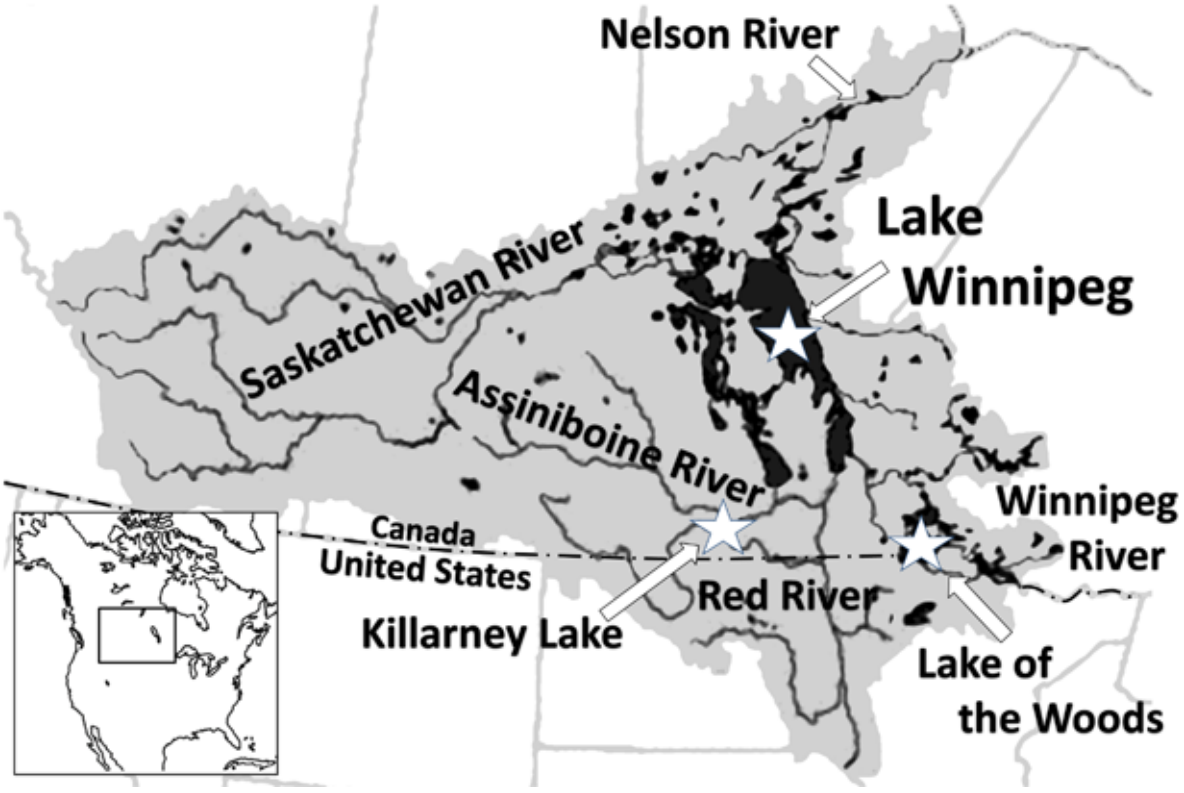

Fig. 1. Map of regions and stars on lakes indicate lake and region from where the samples were taken.

at 4 °C over night, washed 3× (10 min each) with the same buffer, followed by postfixation with 2% (w/v) osmium tetroxide in the same buffer for 2 hours.

The fixed material was washed 3× (10 min each) with the fixation buffer above, instilled into 2% (w/v) agar and dehydrated by a gradual series of ethanol baths with concentrations increasing from 30% to 100% (w/v), at 3–hour intervals. Following this, the material was infiltrated with the metacrylate LR White Hard, encapsulated and polymerized by 60 °C temperature for 2 days. Sectioning was undertaken using a Reichert–Jung ultramicrotome Ultracut E. The final sections placed on supporting grids were contrasted with 2.5% (w/v) uranyl acetate and with the alkaline Reynolds solution (3%, w/v, lead nitrate with 3%, w/v, Sodium citrate). The final sections were photographed at various magnifications in a digital transmission electron microscope FEI Morgagni 268D (Figs 5–6).

Genomic DNA Extraction, PCR Amplification, and Sequencing. Total genomic DNA was isolated from fresh culture material (CPCC697) at the Molecular Microbial Ecology Laboratory (IMET–UMCES) by the following method: 2 ml of fresh culture material was washed 3× with TE buffer, centrifuged at 10,000 rpm for 10 min at 4 ºC. The supernatant was discarded, keeping the cyanobacterial pellet. This pellet was macerated for 8 min and boiled for 12 min, releasing the genomic DNA. The boiled material was centrifuged at 5,000 rpm for 10min, and the supernatant was used as the template for polymerase chain reaction (PCR), in 3 concentrations (1x, 1/3×, and 1/10× original conc.), to decrease the effect of other organic components.

Partial 16S rRNA was amplified by using the oligonucleotide primers CYA106F (5'–CGG ACG GGT GAG TAA CGC GTG–3') and CYA781R (5'–GAC TAC AGG GGT ATC TAA TCC–3') (Nübel et al. 1997) and the almost complete 16S–23S internal transcribed spacer (ITS) of the rRNA operon, including the $tRNA^{Ala}$ and $tRNA^{Ile}$, was amplified using the primers Picocya 16S–F (5'–TGG ATC ACC TCC TAA CAG GG–3') and Picocyano 23S–R (5'–CCT TCA TCG CCT CTG TGT GCC–3') (Cai et al. 2010)). A 50 µl reaction for each sample was performed on a MJ Research PYC–200 Thermo Cycler, with the following conditions: 1) 94 °C for 5 min, 2) 94 °C for 60 sec, 3) 55 °C for 60 sec, 4) 72 °C for 60 sec, 5) repeat steps 2–4 for 35 cycles, 6) 72 °C for 10 min, then 7) 4 °C, indefinitely. The concentration of the amplified products was verified on a 1% agarose gel. Direct sequencing of PCR products, with primers CYA106F and Picocya 16S–F, for both the 16SrRNA gene and the ITS, respectively, were undertaken using Big Dye Terminator Chemistry and an ABI 3130 XL Genetic Analyzer (Applied Biosystems, Foster City, CA) at the BioAnalytical Services Laboratory (BASLab) of the Institute of Marine Technology, University of Maryland Center for Environmental Sciences (IMET–UMCES).

The Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) was utilized for locating strains/sequences similar to our proposed new taxon. The phylogenetic analysis of 16S rRNA was based on 811 sites and 46 sequences; the ITS was based on 813 sites and 41 sequences. We obtained 45 and 40 reference sequences for the 16S rRNA and ITS, respectively, from NCBI after performing BLAST and identifying similar sequences (see Supplement Table S2).

Sequences were aligned using MAFFT version 6 (Katoh & Toh, 2008), then manually refined considering conserved regions using SeaView version 4 (Gouy et al., 2010). For construction of the phylogenetic trees, substitution models for nucleotide evolution for both the 16S rRNA and ITS was determined using jModeltest (Guindon & Gascuel 2003; Posada 2008). Under the Bayesian Information Criterion (BIC), the GTR+gamma model was utilized for the 16S rRNA and HKY+I+gamma for the ITS. Three trees were constructed on these two markers. The software MrBayes v3.2 (Ronquist & Huelsenbeck 2003) was used for determining Bayesian inference. For priors, we assumed no prior knowledge on the data, thus a Dirichlet (1,1,1,1,1,1) prior for substitution rate parameters was estimated, in addition to, a uniform prior (0,1) for the pinvar parameter. A uniform (0, 200) prior was set on the gamma shape parameters and for branch lengths, an unconstrained:Exponential (10) prior. Two runs of four chains (three heated and one cold) were run for 1.5×10^6 generations, sampling every 100 trees. In each run, the first 25% of samples were discarded as the burn–in phase. Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses were conducted using MEGA 5.0 (Tamura et al. 2011). Parsimony was run considering all sites informative and using the Close–Neighbor–Interchange algorithm. Maximum likelihood was run using the GTR+gamma model (for 16S rRNA) and HKY+I+gamma (for ITS). Statistical analyses of tree topologies were undertaken by performing bootstrap analysis with 1,000 pseudoreplicates. After comparison of different tree topologies, the Bayesian tree was prepared for publication for the 16S rRNA and the ML tree was used for the 16S–23S internal transcribed unit.

The DNA sequences from both genes of *Pseudanabaena rutilus–viridis* CPCC 697 are deposited in the NCBI GenBank under the accession numbers JN641732 and JN641733.

Results and Discussion

Ecology and morphology. This new species of *Pseudanabaena* has been found in Lake of the Woods, Lake Winnipeg and more recently in

Table 1. Summary values of major physical and chemical parameters for study lakes.

Parameters	Lake of the Woods	Lake Winnipeg	Killarney Lake
Coordinates (degrees; longitude, latitude)	49–50°N; 95°W	96º20' – 99º20' W.; 50º20' – 53º51' N.	49 °11'N ;98 °42'W [6]
Surface Area (km^2) Whole Lake	3,630[2]	23,750[3]	1.64 [6]
Max depth Z_{max} (m) Whole Lake	54.3[2]	36 [7]	6[6]
Mean depth Z_m (m) Whole Lake	6.3[2]	12.0[3]	
Mixing regime	Dimictic/Polymictic	Polymictic/weakly stratified	Polymictic
Residence time (yr) Whole Lake	1.7[2]	4.4[3]	Indefinite (no out flow)
Secchi depth (m)	1.2–3.3[8]	0.8 – 1.9[4]	0.8 – 2.85[6]
pH	8.0[8]	8.3[11]	8.2[6]
Conductivity (µmhos. cm^{-1})	82–125[8]	200–318[11]	753[6]
Avg. summer epilimnetic temperature (°C)	19–20[8]	20–25[4]	20–25 (estimate)
DOC ($mg.l^{-1}$)	9.7 [8]	7.3–10.2	
DIC ($mg.l^{-1}$)	9.7 [8]	17.4–26.4	176–206 [6]
Alkalinity ($mg.l^{-1}$)	38–56[9]	43–178	195
TN ($\mu g.l^{-1}$)	414–475[8]	459–823[11]	0.750–1.5[6a] (as TKN)
TP ($\mu g.l^{-1}$)	19–25[8]	25–126[11]	30–275[6], 12–516[13], 35–185[6a]
Mean chla ($\mu g .l^{-1}$) Whole Lake	4.1–8.7[8]	3.3–9.7[11]	11–32 [6], .7–94[13]
Algal bloom biomass (range; $\mu g.l^{-1}$)	5000–45000 [10]	7000–87000[10]	7500–100000[10]
Late summer cyanobacteria bloom species	*Aphanizomenon flos–aquae* complex (several morphotypes)	*Aphanizomenon flos–aquae* complex (several morphotypes)	*Aphanizomenon flos–aquae* v. *flos–aquae* complex

[1] Yang & Teller (2005); [2]Zhang et al. (in prep.);[3]Zhang & Rao (2010); [4]Salki (2007); [5] McCullough et al. (in press.); [6]Richmond (1997); [7]Brunskill et al. (1980); [8]Watson, Pascoe et al. (unpublished, 2008–09); [9] Lake of the Woods – Status Report (2006 wq–lar39–0002);[10] Kling (unpublished); [11]McCullough (unpublished); [12]Goldborough unpublished (1991);[13]Manitoba Conservation Water Quality Data unpublished; blank = no data found

Killarney Lake (Fig. 1). All three lakes situated in the very large drainage basin of Lake Winnipeg are shallow prairie or partially prairie lakes dominated in the late summer and fall with cyanobacterial blooms consisting of one – several morphotypes of the *Aphanizomenon flos–aquae* complex. Lake Winnipeg and Lake of the Woods are lakes with shallow areas where the turbid nutrient rich, high pH and conductivity prairie waters flow into regions of clear water with low nutrient, low pH and low turbidity water from the Canadian Shield. Killarney Lake, a shallow (6 meters maximum

depth) turbid eutrophic prairie riverine lake also with periods or areas of very low visibility, has generally higher conductivity and alkalinity. A range of chemical and physical parameters for the three localities is given in Table 1. This table has been included to give the readers a very general impression of the types of lakes with in which this species occurs. The habitat for this species covers a wide range of both physical and chemical conditions since the two larger lakes both border two very distinct geological regions.

The sample from which this species was isolated, was collect, by J. Toogood from Lake of the Woods, Whyle Point in September 2009. This was a sample dominated by *Aphanizomenon flos–aquae* complex, which was accompanied in the background levels of *Pseudanabaena rutilus–viridis*, found either in small flakes or entwined in the loose bundles of *Aphanizomenon*. This sample was sent to J. Acreman at the University of Waterloo Canadian Phycological Culture Centre for isolation of cyanobacteria in particular *Aphanizomenon* morphotypes. However, by November 2009 a nice population of a *P. rutilus–viridis* single trichomes and flakes was growing in some of the cultures. Further isolation and experimentation at low and high light yielded information on its ability to chromatic adapt (Supplement Fig. S1). Figures 2 A–E show the variation in morphology and pigmentation of trichomes under low (3–13 µmol (photons)/m².s) and high light (25–30 µmol(photons)/m².s) at 10 °C and 20 °C resp. Light also affected the pigmentation and the red cultures grown in low light after exposure to higher light began to shift their dominant pigment from reddish to green within 4–5 days of exposure to the new condition Cells appeared much healthier and less fragmented in the low temperature low light conditions (Fig. 2E) where the filaments under low light were found in greater frequency in flakes (Fig. 2F) while those in the high light even at the low temperature were more fragmented and shorter (Fig. 2D). Figure 3 shows a graph of measurements from cultures grown at 10 °C under high and low light. There is some slight degree of the variation in cell dimensions between the culture conditions with a small but significant difference in width but not in length (Figs 3a, b, S3).

Under natural conditions *P. rutilus–viridis* is primarily found in single trichomes except in circumstances where filaments sometimes entwine themselves between the flake filaments

Table 2. Comparison of *Pseudanabaena* species (subg. *Pseudanabaena).**(Komárek & Kling (1991) all taxa except *P. rutilus–viridis*).

	Ps. catenata	*Ps. articulata*	*Ps. limnetica*	*Ps. tenuis*	*Ps. moniliformis*	*Ps. galeata*	*Ps. rutilus–viridis*
Length of filaments (µm)	–200	40–120(300)	120(–300)			80–500 (1000)	8 – 200 (600)
Length of cells (µm)	2–4(7)	5–11	(1.2)4–12	6–8	2.5–5.6	(1.2)2–7 (10–15)	2–4.4 (8)
Width of cells (µm)	1.3–1.5–2	1–1.3	1–1.5(2)	1–1.5	± 2	.8–2.4 (2.7)	(1.4)1.6–2.5(3.2)
Number of cells in trichomes		4–40			–18(–26)	50–150 (200–350)	2 – >100
Trichomes							bundles (flakes)
Cell content	Pale blue–green	Pale grayish–blue	Pale blue–green	Pale blue–green	Pale blue–green or olive green		pale blue–green or greyish to reddish violet occasional granules
Ecology	benthic– or tychoplanktonic; slightly eutrophic waterbodies	Planktonic in lakes	Planktonic; slightly eutrophic lakes and reservoirs	Benthic; lakes	Planktonic; large lakes	benthic– or tychoplanktonic	planktonic in meso to eutrophic large lakes with *Aphanizomenon flos–aquae* complex
Distribution	Temperate zone?	N. Europe	Temperate zone; India?	N. Europe	E. African	Temperate; Europe	Temperate; central N. America

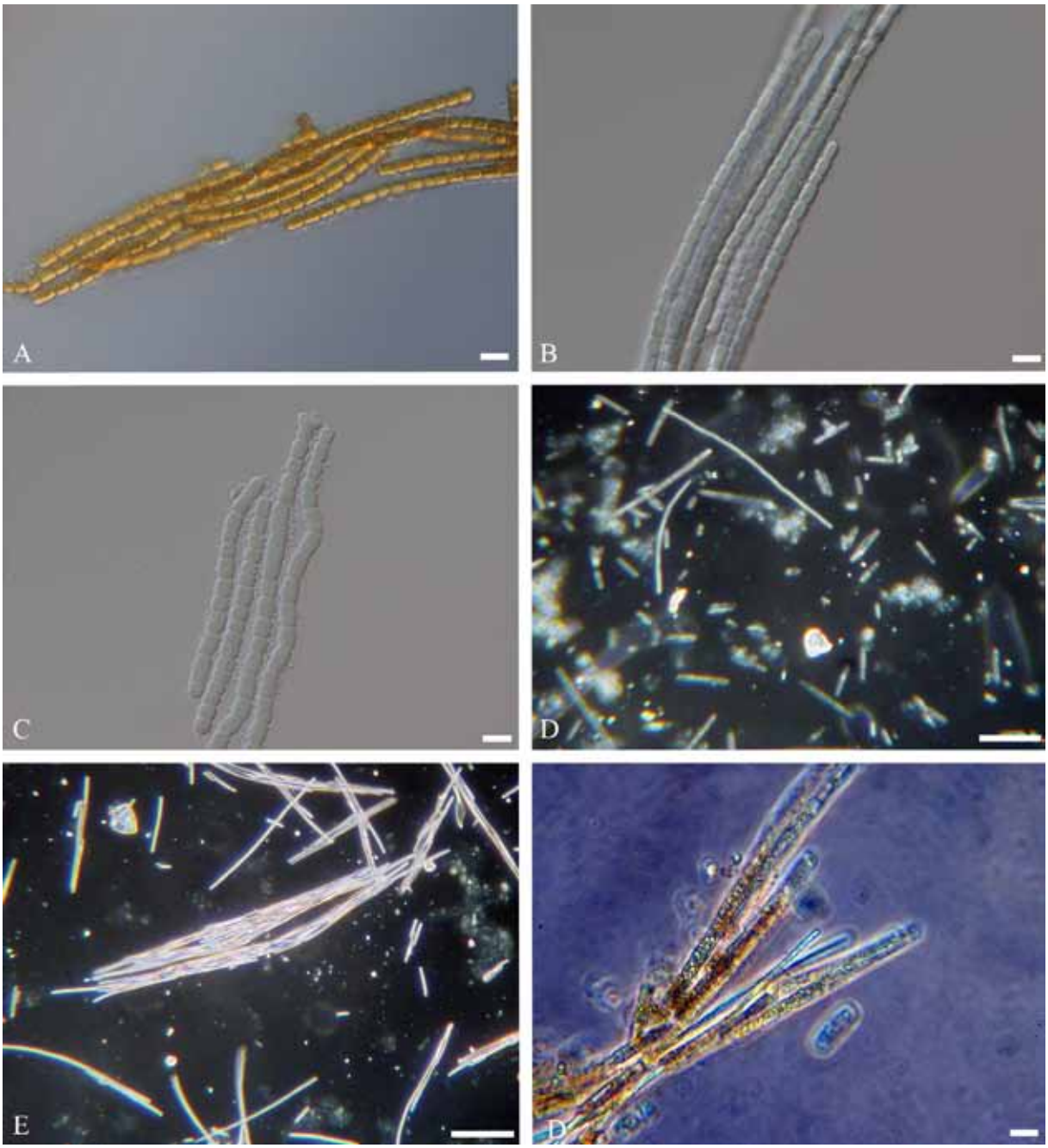

Fig. 2. (A) filaments and cell morphology in *P. rutilus–viridis* cultures at 10 °C and low light. In color photo has a reddish coloration (See Supplementary Fig. 1); (B, C) filaments which were cultured in high light and 10 °C; (D) morphology of *P. rutilus–viridis* in culture grown at 10 °C in high light showing a lower frequency of flakes and greater fragmentation, and shorter length of filaments; (E) morphology of *P. rutilus–viridis* in culture grown at 10 °C in low light showing high frequency of flake formation and reddish purple pigmentation(in colored photo); (F) an example of *P. rutilus–viridis* single filaments and filaments entwined in *Aphanizomenon flos–aquae* complex colonies in sample from Lake of the Woods, Lily Pad Bay, June 2010. Scale bar 10 µm (A – C, F), 50 µm (D, E).

of (Fig. 2F) morphotypes of the *Aphanizomenon flos–aquae* complex, which often come together in a mixed population in the regions where the turbid nutrient–rich water enters the more light–replete regions of the north basins of both Lake Winnipeg and Lake of the Woods. The *P. rutilus–viridis* flake formation seems to occur primarily when the *Aphanizomenon* population is beginning to collapse or in a state of collapse. This became more apparent when a sample of a good healthy population was brought to the lab and kept for several days in the refrigerator. An interesting phenomenon was observed in that recent bloom sample of *Aphanizomenon flos–aquae* complex

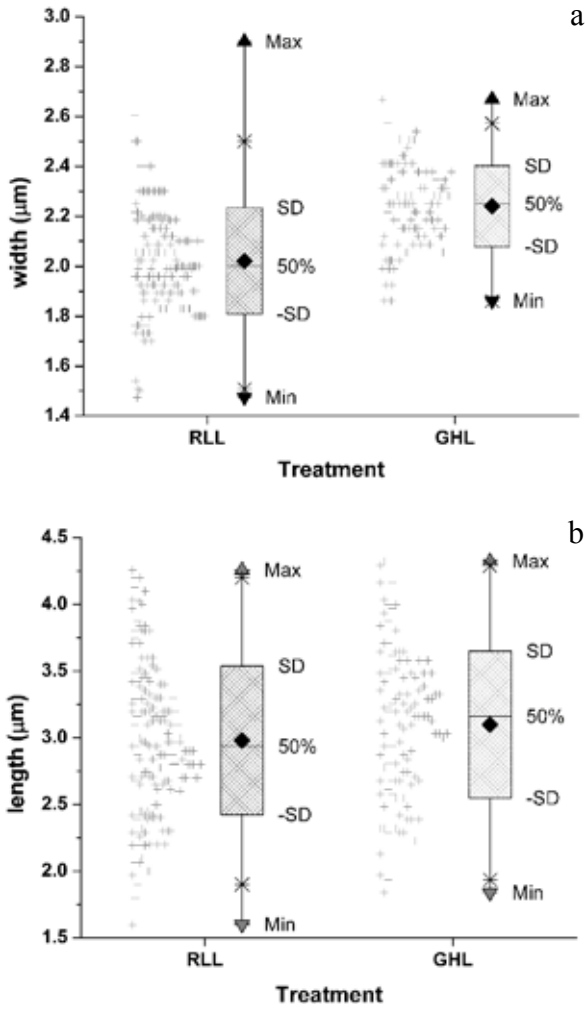

Fig. 3. Box and whisker plots of *Pseudanabaena rutilus–viridis* cell width (3a) and length (3b) measurement statistics for red phenotype, grown under low light (RLL) and green phenotype, grown under high light (GHL). Plots show data (crosses), offset to the left. Legend: mean (diamond), median (50%), standard deviation (SD), 1–99% percentiles (star), minima and maxima (black triangles).

taken from Lake Winnipeg August 9, 2010. In the original preserved sample *Pseudanabaena rutilus–viridis* existed either as separate single filaments with a few flakes in the background of the *Aphanizomenon* bloom or entwined within the *Aphanizomenon* flakes. Re–examination of this sample following 3 weeks in the refrigerator (5 °C) showed healthy *Pseudanabaena rutilus–viridis* in the sample and primarily forming flakes. The flakes and still some single trichomes dominated the sample along with amoeba and phagotrophic flagellates and the *Aphanizomenon* had disappeared except for akinetes and heterocytes. It seems from this observation that the *Pseudanabaena* may potentially have some heterotrophic capacity and is not harmed by the predators which consumed *Aphanizomenon.* This of course needs further research and is beyond the scope of this paper. Re–examination of old bloom samples also showed the presence of this species accompanying blooms dominated by *Aphanizomenon flos–aquae* complex in Lake Winnipeg. It is not new to the lakes but was not recognized as a unique or new species until this present study.

Comparisons of cell morphology, filament length and structure, etc., are presented in Table 2. The dimensions of *P. rutilus–viridis* over–lap with several other *Pseudanabaena* species. However, it is unique in its association with the *Aphanizomenon flos–aquae* complex, ecology, ability for chromatic adaptation and the formation of flakes. Red pigments (PE) predominated under low light and is quite noticeable in the culture vials (Supplement Fig. S1). Fluoroprobe analysis of older cultures showed the red pigments indicating phycoerythrin were high and variable. The ratios of pigments present under the different conditions still need to be confirmed using HPLC and/or the absorption spectra (400–700nm) of culture under different light regimes (ie white, green and red light) to confirm the group of CCA defined by Kehoe & Gutu (2006). The initial screening for microcystin production indicated below detection using ELISA and low–level toxicity using PPIA screening. This indicates the possibility for potential toxins, and needs further research for confirmation.

***Pseudanabaena rutilus–viridis* Kling, Laughinghouse et Komárek sp. nov. (Figs 2, 4)**

Diagnosis: trichoma plus minusve recta vel paucim curvata, libere natantia, solitaria, mobilia (tremula repentiaque), 20–600 µm longa, 1.4–3.2 µm lata, sine muco vel vagina mucilaginosa, interdum ad fasciculos parvos coniuncta. Trichoma cylindracea, ad dissepimenta constricta. Cellulae isodiametricae vel leviter longior (1–2×) quam latae, cylindricae, barriliformes ad ovales, apice rotundatae vel rotundato–acutae, contentu homogeneo, pallide aerugineo vel rubescente, interdum cum granulis solitariis prope dissepimenta, 2–8 µm longae, 1.4–3.2 µm latae. Divisio cellularum perpendicullariter; trichoma phragmentatione dividuntur. Heterocytae, akineta vel ramificatio carentes. – Habitatio: Planktice in lacubus mesotrophicis ad eutrophicis in Canada meridionali. – Typus: Exsiccatum No 2335 in herbario BRNM/HY depositum et CANA 84235 in herbario Ottawa; cultura typica No CPCC 697 in collection CPCC (University of Waterloo) deposita; icona typica: figurae nostrae 2 et 4.

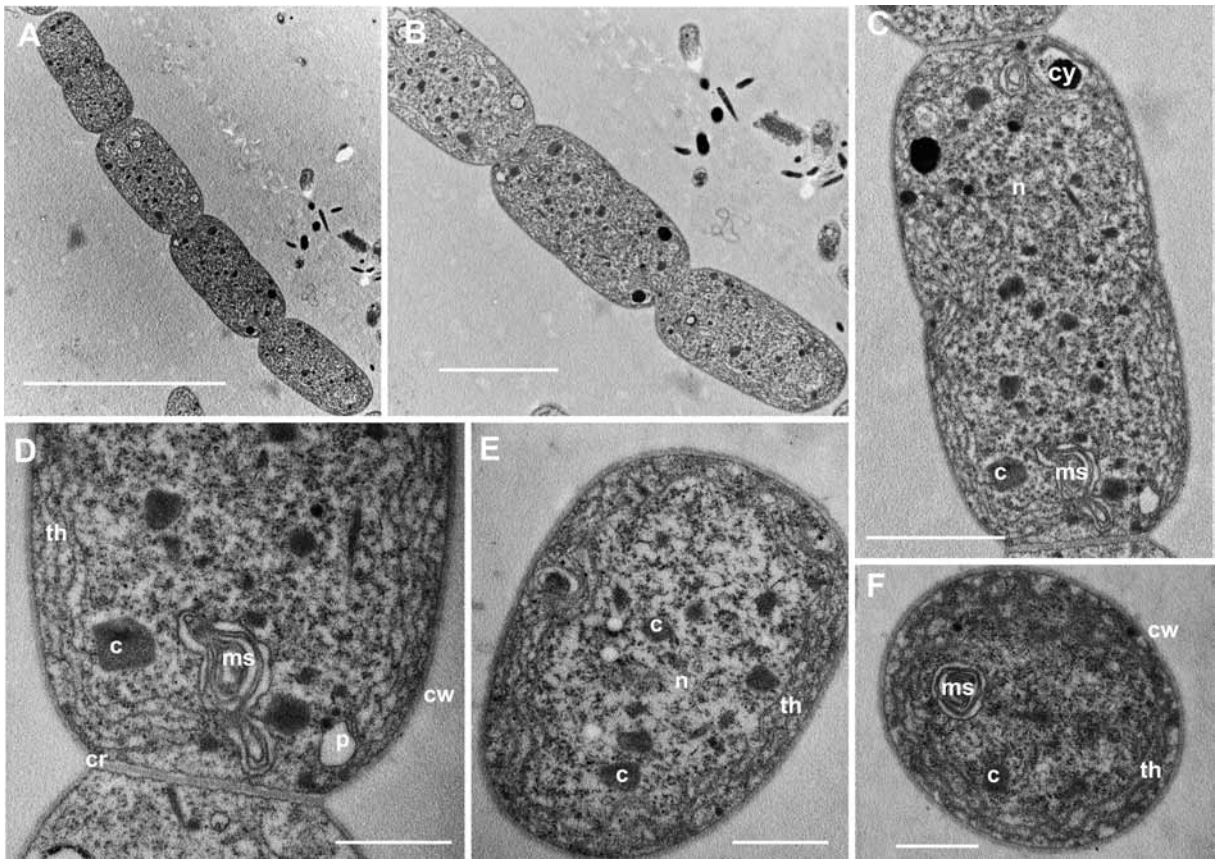

Fig. 4. Morphology and ultrastructure of *Pseudanabaena rutilus–viridis* . Thin sections showing general trichome or filament structure from ultrathin sections (TEM): (A–B) general view of longitudinal section of a cell, (C–D) detail of vegetative cells, (E–F) cross and oblique sections. Explanations: (cy) cyanophycin granules, (n) nucleoid, (c) carboxysomes, (ms) specific membrane system, (th) thyalokoids, (cr) crosswalls, (cw) cell wall, (p) polyphosphate bodies. Scale bars on each micrograph.

Description: trichomes solitary, planktonic, motile (trembling and creeping), <20 – >200 (600) µm long and usually (1.4)1.6–2.5 (3.2) µm wide, without mucilage or sheath, sometimes arranged in small flake–like colonies. Cells isodiametric or slightly longer than wide (1–2×), cylindrical, barrel shaped to oval with rounded ends or bluntly pointed, pale homogenous, reddish violet or blue–green contents (chromatic adaptive), constricted at the cross walls and sometimes with a granule (aerotopes?) at the cross–walls, 2–4.4 (up to 8) µm long × (1.4)1.6–2.5(3.2) µm wide. Cell division perpendicular, trichomes separate by cell disintegration, necridia or fragmentation. Heterocytes not known.

Type locality: large Central Canadian Lakes

Type strain and type material: Canadian Phycological Culture Collection CPCC 697 University of Waterloo, Waterloo Ontario Deposited in Canadian Museum of Nature, Ottawa: Assession number CANA 84325

Isotype: GenBank Accession Numbers: JN641732 and JN641733.

Ultrastructure. The ultrastructure (Figs 4–5) corresponds in principle to other *Pseudanabaena* studies (WHITTON & PEAT 1969; BOURRELLY & COUTÉ 1975; GUGLIELMI & COHEN–BAZIRE 1984; CHANG et al. 1985), but there are some specific differences. The thylakoids (th) are arranged more or less in parietal fascicles but more undulating, than has been found in other species. The nucleoplasma (n) is located in the centre of cells. The cell wall (cw) and cross walls (cr) are simple, similar to other *Pseudanabaena*–species, without prominent mucilaginous envelopes or sheaths. The cells show numerous carboxysomes (c), solitary cyanophycin (cy) and /or polyphosphate (p) granules. Polar gas vesicles were lacking, and were never observed. The thylakoids were occasionally enlarged in cells from older cultures (Fig. 5). More unique and interesting were the groups of membrane systems (ms) found situated near the poles of several cells (Fig. 5).

Phylogenetic Analysis. The three phylograms (Bayesian, ML, and MP) for each genetic marker

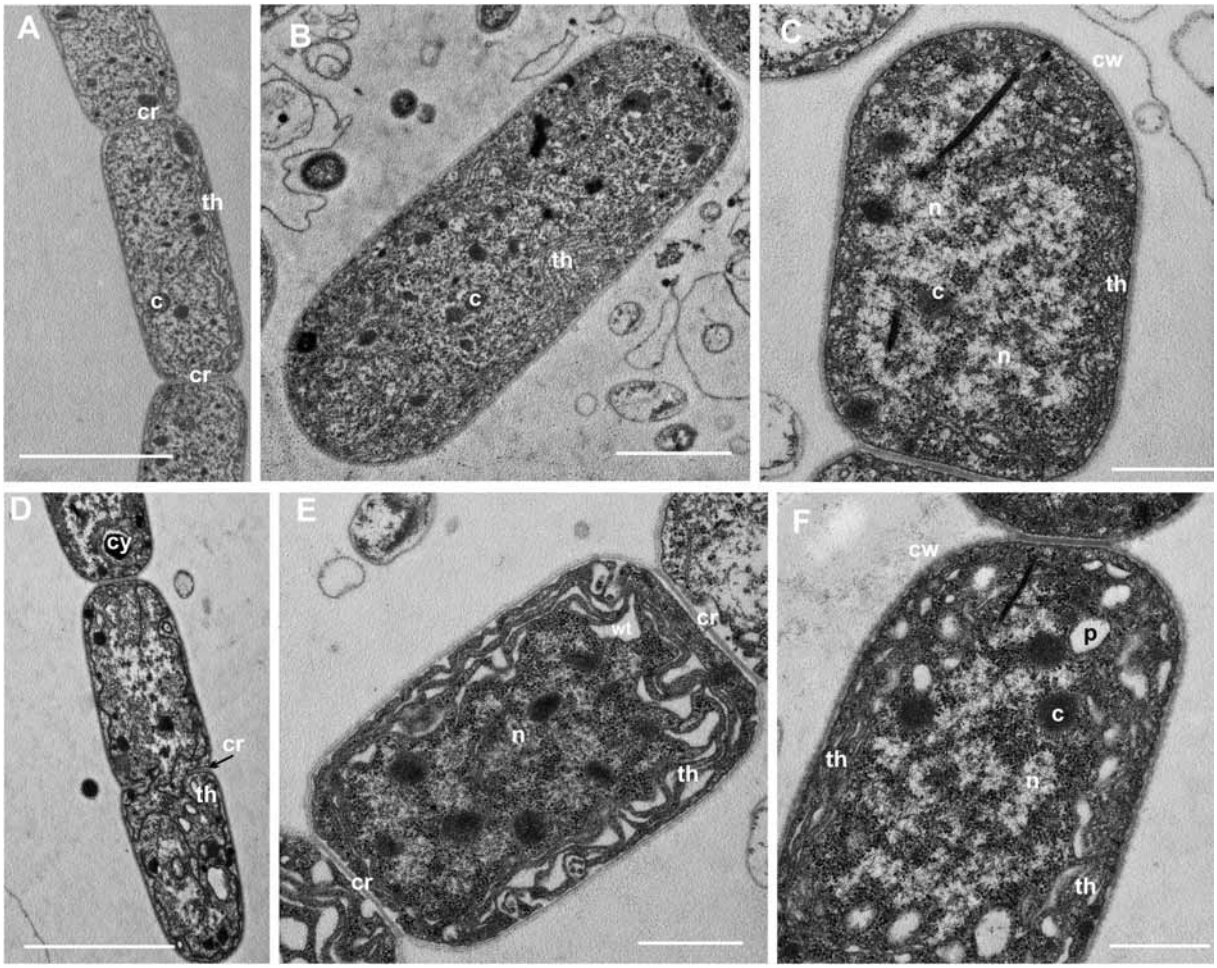

Fig. 5. Fine sections through cells of *Pseudanabaena rutilus–viridis*. In several cells occur widened thylakoids (wt). Explanations as in Fig. 5. Scale bars in micrographs.

were similar. We show the Bayesian phylogram for the 16S rRNA gene (Fig. 6) and the ML phylogram for the ITS region (Fig.7), with the bootstrap support values and Bayesian posterior probability on the respective nodes.

Pseudanabaena rutilus–viridis falls within the clade of the genus *Pseudanabaena* in all analyses conducted using both genetic markers, with high support values. It is also a novel branch in both the 16S rRNA and ITS phylograms. The highest similarity for 16S rRNA was to *Pseudanabaena* sp. ABRG5–3 (96% identity, 87% coverage) and *Pseudanabaena* sp. dqh15 (97% identity, 87% coverage); for ITS, it was *Limnothrix redekei* CCAP 1443/1 (91% identity, 67% coverage) and *Pseudanabena minima* GSE–PSE20–05C (87% identity, 72% coverage).

Our analyses found that within the *Pseudanabaena* clade, there were some peculiar taxa. *Anabaena spiroides* NIES–78 was found to cluster together with *Pseudananabena*. Looking through GenBank, we noticed that this taxa had also been identified as *Anabaena solitaria* NIES–78 thus giving us the confidence to state that the same strain could potentially be forming straight filaments, however this still does not explain how a member of Nostocales is clustering with *Pseudanabaena*. Using BLAST, we searched the NCBI database for the sequences that were most closely related and its closest hits were *Anabaena solitaria* NIES–78, and other *Pseudanabaena* sp. strains, several which are used in this study. This led us to believe to the erroneous identification of this strain. We used this same hypothesis to test the strains of *Limnothrix*, *Oscillatoria*, and *Arthronema*, and came to the same conclusion. This demonstrates the importance of the correct identification of strains deposited in GenBank (and the use of binomial names), to be able to study the occurrence of polyphyletic genera or just trying to understand misidentifications carried out by previous works.

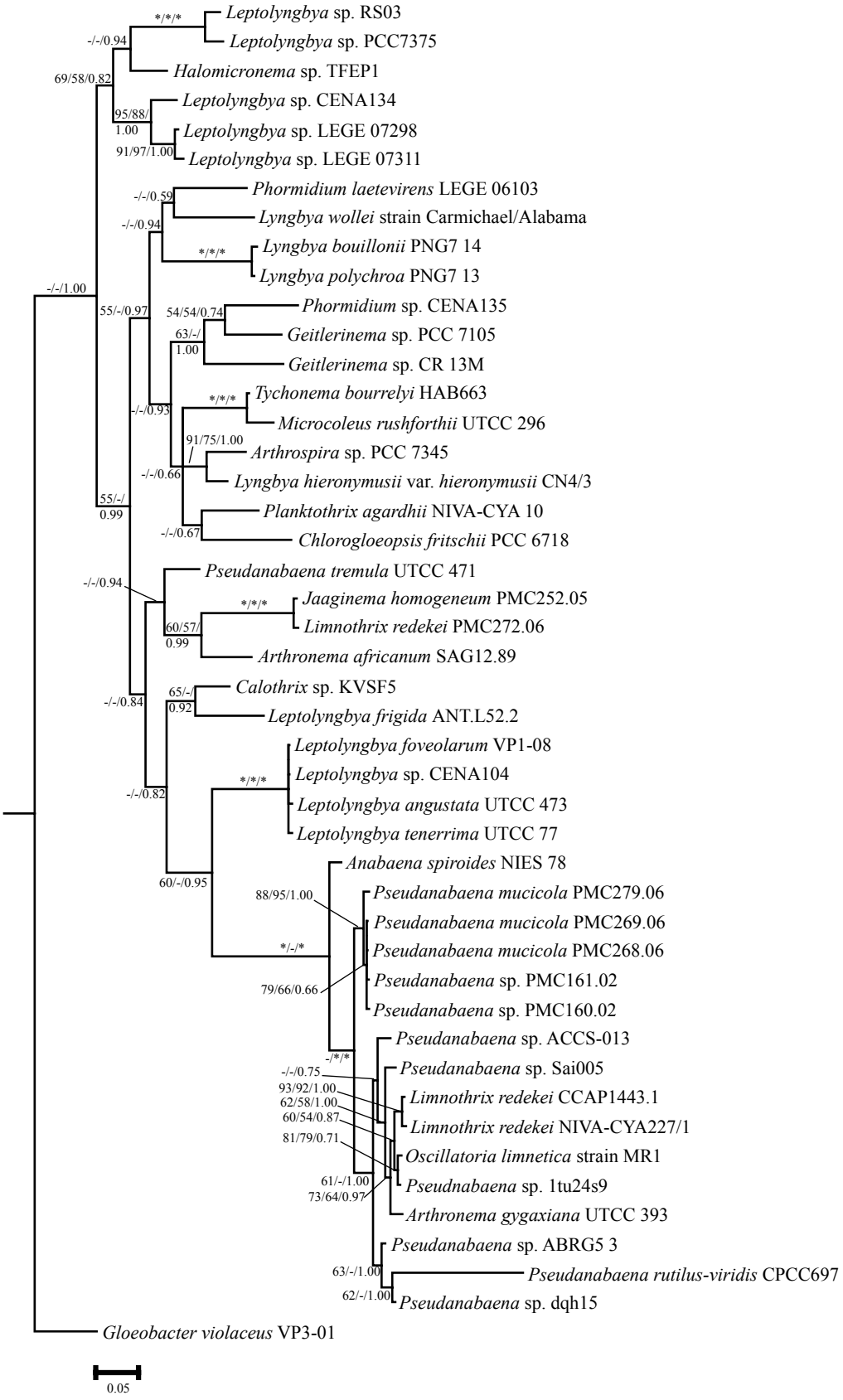

Fig. 6. (16S rRNA) Bayesian phylogram calculated from 46 taxa using the 16S rRNA gene marker. Node support is indicated as ML/MP bootstrap support values/Bayesian posterior probabilities. An */*/* means 100%/100%/1.00, a – means less than 0.50 or 50% support.

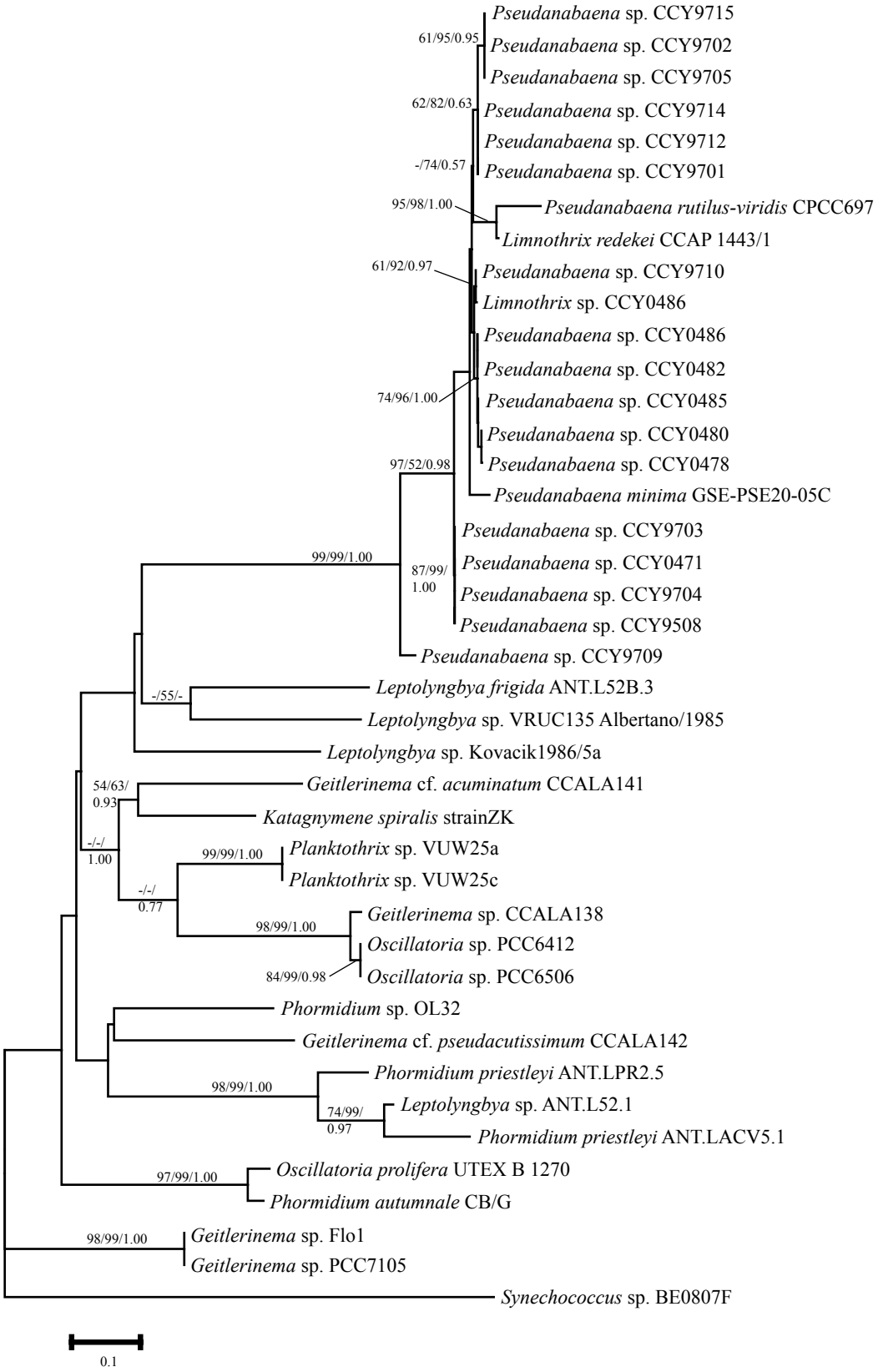

Fig. 7. (ITS) ML phylogram calculated from 41 taxa using the 16S–23S rRNA internal transcribed spacer region. Node support is indicated as ML/MP bootstrap support values/Bayesian posterior probabilities. An */*/* means 100%/100%/1.00, a – means less than 0.50 or 50% support.

Analyzing the ITS phylogram, we notice that *P. rutilus–viridis* is most closely related to *Limnothrix redekei* CCAP 1443/1 with high support. This species was probably identified incorrectly (ZWART et al. 2005), and is a species of *Pseudanabaena*, since we analyzed the photograph presented by them in their paper and it resembles a *Pseudanabaena* more than a *Limnothrix*.

In summary, this paper gives a morphological (including ultrastructure) description, phylogenetic placement and ecology of the new *P. rutilus–viridis* sp. nova documenting its ability to exist in the plankton in either solitary filaments or flake–like colony formation in low back ground (usually ~1% by biomass) levels among the blooms of flake forming *Aphanizomenon* of the *Aphanizomenon flos–aquae* complex. Some simple tests indicated that it has chromatic adaptive capacity. Cell dimensions, filament length, colony formation and pigment composition appear to be highly related to light and temperature conditions. Screening for microcystin production indicated below detection using ELISA and low–level toxicity using PPIA screening. Phycoerythrin (red pigment) content appears to be variable depending on the light regime. Initial taste–odour screen appears to be negative. Some observations have generated the hypothesis that the species may have some heterotrophic capacity, which would enable it to survive and form flakes in cold dark conditions following the collapse of an *Aphanizomenon* bloom.

According to the cytological studies, *P. rutilus–viridis* fits nicely with other members of the genus *Pseudanabaena* with unique membrane system present at the poles of some cells. Phylogenetically *Pseudanabaena rutilus–viridis* falls within the clade of the genus *Pseudanabaena* in all analyses conducted using both genetic markers, with high support values. It is also a novel branch in both the 16S rRNA and ITS phylograms. The ITS phylogram showed it related most closely the culture identified in ZWART et al 2005 as *Limnothrix redekei* CCAP 1443/1 which appears to have likely been misidentified.

Further research is required for details on the geographic distribution, genetic sequencing of other markers, detailed pigment analysis, taste–odour products, toxin types and N fixation and heterotrophic capacity. Some of this work is currently underway.

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Supplementary material

the following supplementary material is available for this article:

Fig. S1. (A) initial cultures November 2009 grown in Jüttner Media at 10 °C (B) cultures from July 2010 grown under 10 °C and 20 °C and low and high light. Notice visible pigment shift from red to green.

Table S2. Taxon, strain, accession number and genetic marker of strains used in this study.

Table S3. Summary statistics, *Pseudanabaena rutilus–viridis* cell dimensions for red phenotype, grown under low light (RLL) and green phenotype, grown under high light (GHL).

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