Taxonomic revision of water-bloom-forming species of oscillatoriod cyanobacteria

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A polyphasic approach was used to clarify the taxonomy of the water-bloom-forming oscillatoriod cyanobacteria. Seventy-five strains of oscillatoriod cyanobacteria were characterized by 16S rDNA sequence analysis, DNA base composition, DNA–DNA hybridization, fatty acid composition, phycobilin pigment composition, complementary chromatic adaptation, morphological characters, growth temperature and salinity tolerance. Phylogenetic analysis based on 16S rDNA sequences divided the strains into six groups, all of which were clearly separated from the type species of the genus *Oscillatoria*, *Oscillatoria princeps* Gomont NIVA CYA 150. Therefore, these strains should be classified into genera other than *Oscillatoria*. Groups I–III were closely related to one another and groups IV–VI were distinct from one another and from groups I to III. Group I was further divided into two subgroups, group I-pc, which includes strains containing only phycocyanin (PC), and group I-pe, which includes strains containing large amounts of phycoerythrin (PE) in addition to PC. This phenotypic distinction was supported by DNA–DNA hybridization studies. Based on the properties examined herein and data from traditional, botanical taxonomic studies, the groups and subgroups were classified into single species and we propose either emended or new taxonomic descriptions for *Planktothrix agardhii* (type strain NIES 204^T), *Planktothrix rubescens* (type strain CCAP 1459/22^T), *Planktothrix pseudagardhii* sp. nov. (type strain T1-8-4^T), *Planktothrix mougeotii* (type strain TR1-5^T), *Planktothricoides raciborskii* gen. nov., comb. nov. (type strain NIES 207^T), *Tychonema bourrellyi* (type strain CCAP 1459/11B^T) and *Limnothrix redekei* (type strain NIVA CYA 277/1^T).

Keywords: cyanobacteria, water bloom, oscillatoriod, nomenclature, taxonomy

INTRODUCTION

Historically, the taxonomy of the Cyanophyceae (cyanobacteria) has been formulated under the International Code of Botanical Nomenclature and based primarily on phenotypic characters (Bourrelly, 1985; Desikachary, 1959; Geitler, 1932). However, more recently, the stability of these phenotypic characters and their use in taxonomy have been

questioned by a number of authors (reviewed by Anagnostidis & Komárek, 1988). The cell organization of the Cyanophyceae is obviously prokaryotic; therefore, bacteriological taxonomic approaches have been suggested for the Cyanophyceae (see Stanier *et al.*, 1978). In fact, the cyanobacteria are treated as one branch of the oxygenic photosynthetic bacteria in *Bergey's Manual of Systematic Bacteriology* (Castenholz & Waterbury, 1989), which is restricted to the genus and order levels and includes guidelines useful for describing novel species and redescribing species poorly characterized under the Botanical Code. These guidelines include the use of morphological, biochemical, genetic, physiological and ecological characteristics.

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Abbreviations: PC, phycocyanin; PE, phycoerythrin.

The GenBank/EMBL/DBJ accession numbers for the 16S rDNA sequences obtained in this study are listed in Table 1.

Table 1. Strains investigated in this study

Culture collections are abbreviated as CCAP (Culture Collection of Algae and Protozoa, UK), NIES (National Institute for Environmental Studies, Japan) and NIVA (Norwegian Institute for Water Research). Other strains were originally isolated from PR China, Japan and Thailand. Scientific names are given according to culture collection catalogues (CCAP, NIES and NIVA). NK, Not known.

Strain	Location of isolation	Collection date	16S rDNA accession no.
<i>Oscillatoria agardhii</i> Gomont			
CCAP 1459/11A	Windermere, Cumbria, UK	1975	AB045896
CCAP 1459/11B ^T	Loughgall, Northern Ireland, UK	1971	AB045897
CCAP 1459/15	Lough Neagh, Northern Ireland, UK	1979	AB045898
CCAP 1459/16	Blelham Tarn, Cumbria, UK	1979	AB045899
CCAP 1459/22 ^T	Lake Gjersjøen, Norway (= NIVA CYA 18 ^T)	1971	AB045901
CCAP 1459/36	Lake Gjersjøen, Norway (= NIVA CYA 29)	1968	AB045903
NIES 204 ^T	Lake Kasumigaura, Ibaraki, Japan	Aug. 1983	AB045954
NIES 205	Lake Kasumigaura, Ibaraki, Japan	Sep. 1982	AB045955
NIES 594	Northern Ireland, UK	NK	AB045956
NIES 595	Northern Ireland, UK	NK	AB045957
NIES 596	Veluwemeer, The Netherlands	NK	AB045958
NIES 610 ^T	Lake Gjersjøen, Norway (= NIVA CYA 18 ^T)	1971	AB045959
NIVA CYA 15	Lake Kolbotnvatnet, Akershus, Norway	1976	AB045923
NIVA CYA 21	Gulf of Finland, Helsinki, Finland	1973	AB045927
NIVA CYA 29	Lake Gjersjøen, Akershus, Norway	1968	AB045931
NIVA CYA 34	Lake Kolbotnvatnet, Akershus, Norway	1976	AB045935
NIVA CYA 59	Lake Jarevatnet, Oppland, Norway	1978	AB045939
NIVA CYA 61/1	Lake Kolbotnvatnet, Akershus, Norway	1978	AB045940
NIVA CYA 64/6	Lake Helgetjern, Østfold, Norway	1979	AB045941
NIVA CYA 68	Lake Veluwemeer, The Netherlands	ca. 1977	AB045943
NIVA CYA 105	Lake Brunnsjön, Hedemora, Dalarne, Sweden	1982	AB045908
NIVA CYA 116	Lake Årungen, Akershus, Norway	1983	AB045911
NIVA CYA 126	Lake Långsjön, Åland, Finland	1984	AB045914
NIVA CYA 127	Lake Vesijärvi, Lahti, Finland	1984	AB045915
NIVA CYA 153	Chao Phya, Bangkok, Thailand	1984	AB045922
NIVA CYA 229	Lake Östra, Kyrksundet, Åland, Finland	1987	AB045928
<i>O. agardhii</i> Gomont var.			
NIVA CYA 18 ^T	Lake Gjersjøen, Akershus, Norway	1971	AB045925
NIVA CYA 35	Lake Kolbotnvatnet, Akershus, Norway	1976	AB045937
NIVA CYA 73	Lake Kolbotnvatnet, Akershus, Norway	1980	AB045944
NIVA CYA 85	Lake Gjersjøen, Akershus, Norway	1981	AB048945
NIVA CYA 87	Lake Levasjön, Skåne, Sweden	1981	AB045946
NIVA CYA 90	Lilla Ullfjärden, Lake Mälaren, Uppland, Sweden	1982	AB045948
NIVA CYA 98	Lake Steinsfjorden, Buskerud, Norway	1982	AB045951
NIVA CYA 108	Stora Ullfjärden, Lake Mälaren, Uppland, Sweden	1982	AB045909
NIVA CYA 128	Lake Vesijärvi, Lahti, Finland	1984	AB045916
NIVA CYA 129	Lake Långsjön, Åland, Finland	1984	AB045917
NIVA CYA 151	Lake Almind sø, Denmark	1984	AB045921
NIVA CYA 320	Lake Kolbotnvatnet, Akershus, Norway	1993	AB045934
<i>O. agardhii</i> var. <i>isothrix</i> Skuja			
CCAP 1459/21	Esthwaite Water, Cumbria, UK	1985	AB045900
CCAP 1459/23	Esthwaite Water, Cumbria, UK	1985	AB045902
NIVA CYA 9	Lake Frøylandsvatn, Rogaland, Norway	1964	AB045952
NIVA CYA 10	Lake Gjersjøen, Akershus, Norway	1964	AB045910
NIVA CYA 11	Lake Akersvatn, Vestfold, Norway	1964	AB045913
NIVA CYA 30	Lake Frøylandsvatnet, Norway	1968	AB045932
NIVA CYA 56/3	Lake Steinsfjorden, Buskerud, Norway	1978	AB045938
NIVA CYA 65	Lake Vansjø, Østfold, Norway	1979	AB045942

Table 1 (cont.)

Strain	Location of isolation	Collection date	16S rDNA accession no.
NIVA CYA 88/3	Lake Ören, Åtvidanberg, Östergötland, Sweden	1981	AB045947
NIVA CYA 91	Lake Mälaren, Sweden	1982	AB045949
NIVA CYA 117/3	Lake Gjersjøen, Akershus, Norway	1983	AB045912
NIVA CYA 133	Lake Øgderen, Akershus, Norway	1984	AB045918
NIVA CYA 137	Lake Steinsfjorden, Buskerud, Norway	1984	AB045919
NIVA CYA 168	Windermere, Cumbria, UK	1975	AB045924
NIVA CYA 297	Lake Frøylandsvatnet, Norway	1990	AB045930
NIVA CYA 313	River Oder-Talsperre, Germany	1992	AB045933
<i>Oscillatoria raciborskii</i> Woloszyńska			
NIES 207 ^T	Lake Kasumigaura, Ibaraki, Japan	Jun. 1983	AB045960
<i>Oscillatoria rubescens</i> DC. ex Gomont			
NIVA CYA 1	Zürichsee, Switzerland	1958	AB045926
<i>O. rubescens</i> DC. ex Gomont var.			
NIVA CYA 13	Lake Steinsfjorden, Buskerud, Norway	1965	AB045920
NIVA CYA 55	Lake Steinsfjorden, Buskerud, Norway	1978	AB045937
NIVA CYA 97/5	Lake Steinsfjorden, Buskerud, Norway	1982	AB045950
<i>Oscillatoria redekei</i> Van Goor			
NIVA CYA 277/1 ^T	Lake Mälaren, Sweden	1990	AB045929
<i>Oscillatoria</i> sp.			
C1-12	Lake Dian-chi, Kunming, Yunan, PR China	Feb. 1996	AB045904
C1-17	Lake Dian-chi, Kunming, Yunan, PR China	Feb. 1996	AB045905
CW4-5	Lake Dalai, Inner Mongolia, PR China	Jul. 1996	AB045907
T1-8-4 ^T	Bangkok, Thailand	Jan. 1996	AB045968
T19-6'-6	Moat of King's Palace, Bangkok, Thailand	Mar. 1996	AB045965
T19-6'-8	Moat of King's Palace, Bangkok, Thailand	Mar. 1996	AB045966
INBaOR	Lake Inbanuma, Chiba, Japan	Aug. 1995	AB045953
T1-6-2	Bangkok, Thailand	Mar. 1996	AB045967
OR1-1	Bangkok, Thailand	Mar. 1996	AB045964
NSLAØ3*	Lake Alexandrina, New South Wales, Australia	1990	AB045962
NSLAØ4*	Lake Alexandrina, New South Wales, Australia	1990	AB045963
TK4-5	Bangkok, Thailand	Mar. 1996	AB045969
TK5-1	Bangkok, Thailand	Mar. 1996	AB045970
TR1-5 ^T	Nakhon Pathon, Thailand	Mar. 1996	AB045971
TR2-4	Nakhon Pathon, Thailand	Mar. 1996	AB045972
<i>Oscillatoria princeps</i> Gomont			
NIVA CYA 150	Chao Phya, Bangkok, Thailand	1984	AB045961
<i>Lynghya hieronymusii</i> Lemmermann			
CN4-3	Lake Dalai, Inner Mongolia, PR China	Jul. 1996	AB045906

* Strains were obtained from CSIRO, Australia.

Although cyanobacterial taxonomy should be studied using bacteriological approaches, species-name priority rests with the Botanical Code (Castenholz & Waterbury, 1989). Therefore, it is necessary to develop a bacteriological classification system from the existing botanical assemblage of names so that compatibility and continuity with the traditional botanical classification system are preserved. For this reason, the characteristics of axenic clones of each cyanobacterial morphospecies (botanical species) should be investigated according to the guidelines (Castenholz & Waterbury, 1989) for bacteriological approaches.

In this paper, the taxonomy of water-bloom-forming

species of freshwater oscillatoriod cyanobacteria is re-examined by employing the multidisciplinary criteria recommended by Castenholz & Waterbury (1989). *Oscillatoria agardhii* and related planktonic species such as *Oscillatoria rubescens*, *Oscillatoria mougeotii*, *Oscillatoria raciborskii* and *Oscillatoria redekei* commonly form water-blooms and, in some cases, produce toxins or excrete geosmin-like substances that reduce water quality and prevent the use of water-bodies for drinking and recreation (Skulberg & Skulberg, 1985; Persson, 1996). These water-bloom-forming species share common morphological characters, such as the presence of gas vacuoles and similar cell dimensions. The taxonomic delimitation of each taxon is difficult to

achieve. In addition, the heterogeneous characters of *O. agardhii* have caused confusion in its intra-specific taxonomy (Elenkin, 1949; Skuja, 1948; Pringsheim, 1965). The above factors have stimulated interest in both the identification and classification of *O. agardhii* and related species. Although taxonomic confusion still exists at the species level (Kützing, 1849; Lemmermann, 1907 or 1910; Geitler, 1932; Elenkin, 1949), *O. agardhii*, *O. rubescens*, *O. mougeotii* and *O. raciborskii* were included in a new genus, *Planktothrix*, with the type species *Planktothrix agardhii* by Anagnostidis & Komárek (1988). *O. redekei*, which is distinguished from the other oscillatorioids mentioned above by having very narrow cells and two large, polar gas vacuoles, was considered to belong to a different genus (*Limnothrix*) by Meffert (1988).

In this study, 75 strains that were, or may be, assigned to the above five taxa of water-bloom-forming oscillatorioid cyanobacteria were obtained from culture collections or were freshly isolated. The phylogeny of these 75 strains was analysed from their 16S rDNA sequences. In addition, DNA base composition, fatty acid profiles, phycobilin pigment content, morphology and growth at different temperatures and salinities were investigated. Furthermore, DNA–DNA hybridization experiments were performed to define the taxonomic relationship between closely related groups. This paper presents suggested taxonomic revisions of the water-bloom-forming oscillatorioid cyanobacteria based on data derived from this polyphasic taxonomic study.

METHODS

Strains and growth conditions. Seventy five strains of water-bloom-forming oscillatorioid cyanobacteria assigned to *O. agardhii*, *O. agardhii* var., *O. agardhii* var. *isothrix*, *O. raciborskii*, *O. rubescens*, *O. rubescens* var. and *Oscillatoria* sp. and one strain each of *Oscillatoria princeps* and *Lyngbya hieronymusii* var. *hieronymusii* were obtained from NIES (National Institute for Environmental Studies, Japan) (Watanabe & Hiroki, 1997), CCAP (Culture Collection of Algae and Protozoa, UK) (Tompkins *et al.*, 1995) and NIVA (Norwegian Institute of Water Research) (Skulberg, 1990) or isolated from lakes and reservoirs in Japan, PR China and Thailand (Table 1). Strains that were not axenic and/or clonal were purified and cloned by the micropipette-washing method (Hoshaw & Rosowski, 1973). The purity of the strains was checked with bacterial check media (Watanabe & Hiroki, 1997) and light microscopy. Stock cultures were maintained in screw-capped test tubes (18 × 150 mm) with 10 ml CT medium (Watanabe & Hiroki, 1997) and incubated at 20 ± 1 °C, under a 12 h:12 h light/dark cycle at 30 µmol photons m⁻² s⁻¹ provided by day-light fluorescent lamps (National Co. Ltd, Japan).

16S rDNA sequence analyses. Each strain was grown in a 2 l Erlenmeyer flask containing 1.5 l medium with aeration (1.5 l min⁻¹). After 4 weeks of cultivation, cells were harvested by centrifugation (10 min, 5 °C, 5000 g) and washed with distilled water. DNA for PCR templates was extracted from the cells according to Palinska *et al.* (1996). Primers for amplification and sequencing were designed

Table 2. PCR primer names, sequences and target sites

Positions of target sites refer to the *Escherichia coli* sequence.

Primer	Sequence (5' → 3')	Target site
16SF1	TAACACATGCAAGTCGAACG	49–68
16SR1	GCCGATTGAGGCACGGTCGT	523–504
16SF2	CTACGGGAGGCAGCAGTGGGGA	342–363
16SR2	CATCAGGATGCCATTTGCTA	821–800
16SF3	GTGTAGCGGTGAAATGCGTAG	683–703
16SR3	CCTCCTTCCACTCCTACTGCA	1194–1174
16SF4	GCATGGCTGTCGTACGCTCG	1053–1072
16SR4	CTTCAGCATTGTTCCATCGGCA	1512–1491

based on cyanobacterial 16S rDNA sequences (Table 2) and 16S rDNA sequences were amplified from the extracted DNA. PCR was performed in a 50–100 µl reaction mixture using 0.05 U AmpliTaq DNA polymerase µl⁻¹ (Perkin Elmer), buffer with 1.5 mM MgCl₂ supplied by Perkin Elmer, 0.2 mM dNTPs, 0.5 µM primers and 0.01–0.05 µl genomic DNA solution (100–500 ng µl⁻¹). The reactions were run in a Thermal Cycler (Takara Biomedicals) with one cycle of 92 °C for 2 min, 56 °C for 2 min and 70 °C for 3 min and then 29 cycles of 92 °C for 1.5 min, 56 °C for 2 min and 70 °C for 3 min, followed by 72 °C for 15 min. The amplification products were purified by using MicroSpin S-400 HR columns (Pharmacia LKB) and sequencing reactions were performed using a Thermo Sequenase fluorescent-labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham). The reaction products were run on a 4% Long Ranger gel (FMC Bio Products) using an ALF DNA sequencer (Pharmacia LKB) with autofluorescence primers, the sequences of which were the same as those of the primers used for PCR (Table 2). Both strands were sequenced.

Phylogenetic analysis. The 16S rDNA sequences from 77 strains, including *L. hieronymusii* var. *hieronymusii* and *O. princeps*, were aligned and analysed by comparison with the aligned dataset from the rRNA WWW Server at the University of Antwerp (Van de Peer *et al.*, 1997). Sequences determined in this study, or not recorded in this database, were added individually to the aligned dataset through a profile alignment process using CLUSTAL W version 1.71 (Thompson *et al.*, 1994). Finally, the alignment was refined manually. The dataset contained 1361 positions with hyper-variable regions, ambiguous sequences and gaps. This set was used subsequently for phylogenetic analysis among closely related strains. *Synechocystis* sp. PCC 6803 and *Gloeobacter violaceus* PCC 7421 were used as outgroups for the phylogenetic analysis. Hypervariable regions, ambiguous sequences and gaps were then removed as suggested by Turner (1997) to obtain a set containing 1182 positions that was used for phylogenetic analysis of 39 cyanobacteria strains, *Rhodospirillum rubrum* ATCC 11170^T and *Agrobacterium tumefaciens* K-Hg-3. Phylogenetic trees were generated on the basis of the neighbour-joining method (Saitou & Nei, 1987). Neighbour-joining analysis was performed using PAUP* 4.0 β version, running on a Macintosh computer (Swofford, 1999), with each topology checked using 1000 bootstrap replications (Kimura's two-parameter correction). Accession numbers are given in Table 1. The accession numbers of the reference sequences from

Fig. 1. Phylogenetic tree based on 16S rDNA sequences of water-bloom-forming oscillatoriods and related cyanobacteria displaying the positions of strains of groups I–VI. The neighbour-joining tree was based on 1361 nucleotide positions, including variable regions and gaps. Numbers at the branching points are bootstrap values expressed as percentages of 1000 replications; values < 50% were omitted. Strains in brackets are representatives of strains showing identical sequences: [NIES 204^T] represents the sequences of NIES 204^T, CCAP 1349/15, C1-12, C1-17, NIVA CYA 21, 64, 68, 105, 116 and 126 and NIES 205, 594 and 595; [CCAP 1459/36] represents the sequences of CCAP 1459/36 and NIVA CYA 29, 59 and 137; [NIVA CYA 127] represents the sequences of NIVA CYA 127, NIVA CYA 56 and 313; [NIVA CYA 18^T] represents the sequences of NIVA CYA 18^T, CCAP 1459/11A, 1459/16, 1459/22 and 1459/23, NIVA CYA 1, 13, 35, 55, 61, 65, 73, 85, 87, 88, 90, 91, 97, 98, 108, 117, 128, 129, 133, 168, 297 and 320 and NIES 610^T; [NIVA CYA 229] represents the sequences of NIVA CYA 229 and NIVA CYA 34; [NIVA CYA 9] represents the sequences of NIVA CYA 9, NIVA CYA 11 and 15; [CW4-5] represents the sequences of CW4-5 and T19-6-8; and [NIES 207^T] represents the sequences of NIES 207^T, INBaOR and T1-6-2.

EMBL/GenBank/DBJ are listed on the phylogenetic trees (Figs 1 and 2).

DNA base composition. Approximately 10 mg lyophilized cells or 0.5 ml cell pellet from fresh cultures was used for the analysis. Isolation and enzymic hydrolysis of DNA were performed according to Tamaoka & Komagata (1984) and analyses were performed using reverse-phase HPLC (Shimadzu) equipped with an SPD-10A UV/VIS spectrophotometric detector and a Cosmosil 5C18 column (4.6 mm i.d. × 150 mm; Nakarai). Nucleosides were eluted with a mixture of 0.4 M NH₄H₂PO₄ and acetonitrile (40:1, v/v) at a flow rate of 1 ml min⁻¹ at 40 °C controlled by a column

oven (Shimadzu CTO-6A). Each nucleoside was detected by absorbance at 270 nm. Each sample was analysed at least three times from the DNA isolation procedure.

Fatty acid composition. Samples (5–10 mg lyophilized cells) were transmethylated with 2 ml 5% methanol/HCl (TCI-SUTH, Japan) at 100 °C for 3 h. After cooling to room temperature, the methylated acids were extracted with 1.5 ml *n*-hexane and washed with 0.5 ml distilled water. The hexane layer was transferred to a new glass tube and concentrated to a volume of approximately 100 µl by vacuum evaporation. A 1 µl aliquot of the concentrated sample was injected into a Shimadzu GC-17A gas chromatograph equipped with a hydrogen flame-ionization detector and a capillary column (25 m × 0.25 mm × 0.25 µm) coated with methyl silicon as the polar stationary phase (007-FFAP; Tokyo Kasei). The flow of carrier gas (helium) was 40 ml min⁻¹ and the column temperature was programmed to increase from 130 to 220 °C at 5 °C min⁻¹. Methyl esters were identified by co-chromatography of authentic standards (Sigma) and by mass spectroscopy. The relative concentrations of fatty acids were calculated by comparing the areas under the chromatographic peaks with a data processor (C-R6A; Shimadzu).

In vivo and phycobiliprotein absorption spectra. Exponential-phase cells were used for determination of *in vivo* and phycobiliprotein absorption spectra. An aliquot of culture was transferred to a spectrophotometric cuvette (4 ml) and the *in vivo* absorption spectrum was measured from 350 to 850 nm by using a Hitachi type U-3000 spectrophotometer equipped with a 60 mm-diameter integrating sphere device. For phycobiliprotein absorption spectra, an aliquot of 5–15 ml culture was centrifuged at 500 *g* for 15 min at room temperature. The pellet was suspended in potassium phosphate buffer (pH 6.5) and the cells were broken in a bead beater (5000 r.p.m., two periods of 30 s) at about 5 °C. The broken cell suspension was centrifuged (500 *g*, 15 min) to remove unbroken cells. The crude supernatant was centrifuged (80000 *g*, 1 h) in an ultracentrifuge (Beckman L7-55) and the absorbance of the supernatant was measured from 350 to 750 nm as described above.

Complementary chromatic adaptation. Each strain was cultured separately under continuous light with pure green (20-S-G-F, National Co. Ltd) or pure red (20-S-R-F, National Co. Ltd) fluorescent lamps (20 µE m⁻² s⁻¹) at 20 ± 1 °C. Phycobiliprotein absorption spectra were then determined spectrophotometrically for each strain as described above.

Morphology. Morphological observations were made using an Olympus Vanox photomicroscope equipped with phase-contrast and Nomarski optics systems. The mean cell size and standard deviation of 50 cells were measured for each strain.

Temperature and salinity experiments. Experimental cultures inoculated with one or two filaments from the stock culture were grown at five different temperatures, 10, 20, 30, 40 and 50 °C (±1 °C), and at eight different salinities, approximately 0.08 (original CT medium), 0.17, 0.26, 0.43, 0.78, 1.28, 1.83 and 3.58% NaCl (w/v). Growth was checked visually after 14 and 28 days. Each experiment was done in triplicate.

DNA–DNA hybridization. DNA extraction and purification were done according to Tamaoka & Komagata (1984). Microplate hybridization procedures were performed according to Ezaki *et al.* (1989) with extracted DNA further purified by ultracentrifugation (Hamamoto & Nakase, 1995).

Fig. 2. Phylogenetic tree based on 16S rDNA sequence of water-bloom-forming oscillatorioids and related cyanobacteria displaying the positions of strains of groups I–VI, *O. princeps* NIVA CYA 150 and *L. hieronymusii* CN4-3 with other cyanobacterial taxa. The neighbour-joining tree was based on 1182 nucleotide positions that excluded hypervariable regions. Numbers at the branching points are bootstrap values expressed as percentages of 1000 replications; values < 50 % were omitted. Strains in brackets are representative of the groups.

and measured by a CytoFluor 4000 (Perseptive Biosystems). Biotin-labelled DNA was prepared from *O. agardhii* NIES 204^T and *O. rubescens* CCAP 1459/22^T.

RESULTS

Phylogenetic analysis of the conserved regions of 16S rDNA

All the oscillatorioid strains sequenced in this study were found to have identical 16S rDNA lengths,

1361 bp, corresponding to positions 101–1469 of the *Escherichia coli* numbering system. Although some of the 75 strains of water-bloom-forming oscillatorioids had identical sequences, six distinct groups were recognized (Fig. 1). Group I consisted of 58 strains sharing more than 99.2 % similarity of 16S rDNA, group II contained five strains sharing more than 98.4 % similarity, group III contained four strains sharing more than 99.8 % similarity and group IV contained six strains sharing more than 99.1 % simi-

Table 3. Fatty acid composition and DNA G + C content of 16S rDNA groups

Fatty acid	I	II	III	IV	V	VI
14:0	2.0	2.3	2.7	2.1	2.5	32.8
16:0	27.0	33.3	36.4	55.1	31.7	11.7
16:1*					0.9	5.0
16:1 _c	16.1	24.4	17.1	1.0		1.0
16:2	1.9	0.6			4.9	
16:3					12.0	
18:0	2.6	0.8	1.3	1.2	0.9	1.3
18:1	2.7	2.8	5.3	2.5	0.7	4.5
18:2	9.1	10.9	14.1	1.7	10.6	7.8
18:3 _γ				1.3		
18:3 _α	38.7	24.9	23.2	9.8	35.8	36.0
18:4				25.2		
Fatty acid type†	2B	2B	2C	4	2A	2C'
DNA G + C content (mol %)‡	39.5 ± 0.4	39.5 ± 0.2	40.0 ± 0.2	44.0 ± 0.4	44.4 ± 0.6	40.9 ± 0.3

* Double-bond position not determined.

† Fatty acid composition types of cyanobacteria according to Kenyon (1972), Kenyon *et al.* (1972), Murata *et al.* (1992) and Li *et al.* (1998).

‡ Means ± standard deviation of triplicate measurements.

larity. Groups I–III had less than 96.8% similarity to one another and group IV had less than 89.7% similarity to groups I–III. Groups V and VI contained single strains, CCAP 1459/11B^T (V) and NIVA CYA 277/1^T (VI). These strains respectively had less than 91.9 and 86.4% similarity to the other groups.

A phylogenetic tree based on 1361-bp 16S rDNA sequences revealed that the six groups were divided into four large clusters (Fig. 1). The first cluster contained groups I–III, the second contained group IV, the third contained group V and the fourth contained group VI. Groups I, II and III formed independent subclusters, each with high bootstrap values. This result suggests that each group is an independent taxon.

Six strains, NIES 204^T, T1-8-4^T, TR1-5^T, NIES 207^T, CCAP 1459/11B^T and NIVA CYA 277/1^T, were selected as representatives of each group. A phylogenetic tree based on the 1182-bp 16S rDNA sequences is shown in Fig. 2. In the phylogenetic tree, the type species of *Oscillatoria*, *O. princeps*, appeared as a sister group of the Nostocales/Stigonematales clade (Fig. 2). This topology was not stable but different operational taxonomic units and algorithms used for phylogenetic analyses revealed that *O. princeps* NIVA CYA 150, NIES 204^T (group I), T1-8-4^T (group II), TR1-5^T (group III), NIES 207^T (group IV), CCAP 1459/11B^T (group V) and NIVA CYA 277/1^T (group VI) were separated from each other and formed independent clusters (data not shown). Other strains assigned to the genus *Oscillatoria* on the phylogenetic tree (Fig. 2), *Oscillatoria sancta* PCC 7515; *Oscillatoria corallinae* CJ1 SAG8.92, *Oscillatoria rosea* M220, *Oscillatoria limnetica* MR1 and *Oscillatoria neglecta* M82, were

placed in clusters different from the type species of *Oscillatoria*, *O. princeps*.

DNA base composition

The DNA base compositions of the 75 strains were summarized for each group recognized by 16S rDNA sequences (Table 3). Group I (58 strains) had a mean G + C content of 39.5 ± 0.4 mol %. The highest value obtained was 40.8 ± 0.4 mol % (NIVA CYA 30) and the lowest was 38.6 ± 0.4 mol % (NIVA CYA 108). Group II, including five strains, showed a mean of 39.5 ± 0.2 mol %. The highest value was 40.6 ± 0.1 mol % (T1-8-4^T) and the lowest was 38.3 ± 0.2 mol % (CW4-5). Group III, including four strains, showed a mean of 40.0 ± 0.2 mol %, with a maximum of 41.4 ± 0.2 mol % (TK5-1) and a minimum of 39.5 ± 0.2 mol % (TR2-4, TK4-1). Group IV includes six strains and the mean was 44.0 ± 0.4 mol %. Group V, including the single strain CCAP 1459/11B^T, showed a value of 44.4 ± 0.6 mol %. Group VI, including the single strain NIVA CYA 277/1^T, showed a value of 40.9 ± 0.3 mol %.

Fatty acid composition

Of the 75 strains of water-bloom-forming oscillatoriod cyanobacteria studied, fatty acid profiles were obtained for 73 strains (Table 3). All strains contained 14:0, 16:0, 16:1_c, 18:0, 18:1, 18:2 and 18:3_α as major fatty acids. Strains of groups I, II, III, V and VI were characterized by having 18:0, 18:1, 18:2 and 18:3_α but no 18:3_γ or 18:4, and strains of group IV were characterized by having all of these fatty acids. On the basis of previous work (Kenyon, 1972; Kenyon *et al.*, 1972; Murata *et al.*, 1992), cyanobacterial strains were

Fig. 3. *In vivo* absorption spectra (a) and phycobiliprotein absorption spectra (b) of strains C1-12 (group I-pc), CCAP 1459/22^T (group I-pe) and TR1-5^T (group III).

categorized as type 1, possessing saturated (18:0) and monounsaturated (18:1) fatty acids, type 2, possessing 18:0 to 18:3 α but no 18:3 γ and 18:4, type 3, possessing 18:0 to 18:3 α and 18:3 γ but no 18:4, and type 4, possessing 18:0 to 18:4. According to this classification system, group I, II, III, V and VI strains were classified as type 2 and group IV as type 4. Type 2 could be further separated into two subtypes based on the species of 16-carbon fatty acids; type 2A (*Anabaena* type) contained 16:0 to 16:3 and type 2B (*Oscillatoria* type) contained 16:0 to 16:2 (Li *et al.*, 1998). Group V was defined as type 2A and strains of groups I and II were type 2B (Table 3). Groups III and VI were assigned to type 2 and had no 16:2 (Table 3); they were defined as representing a new subtype, type 2C. In

addition to definitions of these subtypes, the fatty acid composition of group VI was characterized further as containing a 16:1 fatty acid with the double bond in a different position from 16:1*c* and large amounts of 14:0, thereby being defined as type 2C'.

Phycobilin pigment composition and chromatic adaptation

The 58 strains of group I contained two types of phycobilin pigment composition. One type had a large valley in the *in vivo* absorption spectrum around 550 nm (Fig. 3a) and a large absorption peak around 630 nm of the phycobiliprotein absorption spectrum (Fig. 3b), which indicated that they contain phyco-cyanin (PC) but no phycoerythrin (PE). Forty strains of group I showed this type of phycobilin pigment composition and were designated as group I-pc. The same patterns were indicated in strains of groups II and IV. The other strains in group I had no valley in the absorption spectrum around 550 nm (Fig. 3a) and possessed large and small absorption peaks, respectively, around 570 and 630 nm (Fig. 3b). Thus, they contained both PE and PC and had a high PE:PC ratio. In total, 18 strains of group I showed this type of phycobilin pigment composition and were designated as group I-pe. The same patterns were indicated in groups V and VI. Strains of group III had a small valley in their *in vivo* absorption spectrum around 550 nm (Fig. 3a) and small and relatively large peaks, respectively, around 570 and 630 nm of the phycobiliprotein absorption spectrum (Fig. 3b). This indicated that they contained both PE and PC and had a low PE:PC ratio.

When the strains of groups I-pc, I-pe, II, III and IV were grown under red or green light, their trichome colour did not change and the *in vivo* and phycobiliprotein absorption spectra showed the same patterns as those obtained when cultures were incubated under standard light conditions. When the group V strain CCAP 1459/11B^T was grown under green light, trichomes were reddish purple or reddish brown in colour and the absorption peak around 570 nm became much larger (Fig. 4a). In contrast, when it was grown under red light, the trichome colour was pale blue-green and the absorption peak around 570 nm was conspicuously smaller (Fig. 4a). These data suggest that the strain has the ability to undergo complementary chromatic adaptation by altering the PE content. When the group IV strain NIVA CYA 277/1^T was grown under red light, the trichome mass colour was pale blue-green with no peak of *in vivo* absorption around 570 nm but a large peak around 630 nm (Fig. 4b). In contrast, when it was grown under green light, the trichome mass colour was reddish purple or reddish brown with large and small peaks, respectively, around 570 and 630 nm of the *in vivo* absorption spectrum (Fig. 4b). These data suggest that the strain has the ability to undergo complementary chromatic adaptation by altering both PC and PE contents.

Fig. 4. *In vivo* absorption spectra of strains CCAP (1459)/11B^T (group V) (a) and NIVA CYA 277/1^T (group VI) (b) under red, white [CCAP (1459)/11B^T only] and green light.

Growth at different temperatures and salinities

On the basis of molecular and chemotaxonomic characteristics, the strains used in this study were separated into seven different groups and subgroups, groups I-pc, I-pe, II, III, IV, V and VI. The temperature ranges of growth of groups I–IV are shown in

Fig. 5. Growth at different temperatures and salinities. Percentages of strains capable of growth are shown. Groups V and VI, which each include only one strain, were omitted.

Fig. 5. Group I-pc grew mainly in the range 10–20 °C (some strains also grew at 30 °C), most strains of group I-pe grew at 10–20 °C, group II grew at 20–30 °C (some also grew at 40 °C), group III grew at 20–30 °C (some also grew at 10 and/or 40 °C), group IV grew at 20–30 °C (half of this group also grew at 40 °C) and groups V and VI grew at 20–30 °C. From these data, the strains could be separated into two groups, those that grew at 10–20 °C (groups I-pc, I-pe, V and VI) and those that grew at 20–30 °C (groups II, III and IV).

Salinity tolerances of groups I–IV are also shown in Fig. 5. Many strains of groups I-pc and II grew in medium with up to 0.78 ‰ NaCl (some also grew at up to 1.28 ‰), many strains of groups I-pe and IV grew at up to 0.78 ‰ (none up to 1.28 ‰) and all strains of group III grew at up to 1.28 ‰ (half of them also grew at up to 1.83 ‰). Groups V (CCAP 1459/11B^T) and VI (NIVA CYA 277/1^T) respectively grew in media with up to 0.43 and 0.26 ‰ NaCl (data not shown). From the data obtained, the cyanobacterial strains could be separated into three groups on the basis of their

Fig. 6. Light micrographs of *Planktothrix agardhii* strains CCAP 1459/15 (a) and CCAP 1459/21 (b) (group I-pc), *Planktothrix rubescens* NIVA CAY 108 (c) (group I-pe), *Planktothrix pseudagardhii* T1-8-4^T (d) (group II), *Planktothrix mougeotii* TK5-1 (e) (group III), *Planktothricoides raciborskii* OR1-1 (f) (group IV), *Tychonema bourrellyi* CCAP 1459/11B^T (g) (group V) and *Limnithrix redekei* NIVA CYA 277/1^T (h) (group VI). Panels (a), (c), (d) and (h) are phase-contrast micrographs; panels (b) and (e-g) are Nomarski micrographs. Strains CCAP 1459/15 (a) and CCAP 1459/21 (b) are respectively representative of group I-pc strains with narrow and wide cells (see Fig. 7). The arrow in (c) indicates a calyptra. Bars, 10 µm.

salinity tolerance; wide (group III), medium (groups I-pc, I-pe, II and IV) and narrow (groups V and VI).

Morphological observations of each group and subgroup

With the exception of cell width, most morphological characters were common to strains of group I-pc and II (Fig. 6a, b, d), as outlined below in the descriptions of *Planktothrix agardhii* (group I-pc strains) and *Planktothrix pseudagardhii* sp. nov. (group II strains). Cells of group I-pc strains were 2.3–9.8 µm wide and 1.2–5.3 µm long and the ratio of cell length to cell width was 1:3–1:1. The minimum cell width observed

was 3.0 ± 0.7 µm (NIES 205) and the maximum was 8.7 ± 1.3 µm (CCAP 1459/21; Fig. 6b). In contrast, the mean and standard deviation for cell length ranged from 2.2 ± 0.5 µm (NIVA CYA 91) to 3.5 ± 1.3 µm (CCAP 1459/16). Since the standard deviation ranges of strains overlapped continuously (Fig. 7), clear divisions cannot be made among the group I-pc strains on the basis of cell size. Cells of group II strains were 3.0–6.4 µm wide and 1.2–4.2 µm long and the ratio of cell length to cell width was about 1:3–1:1. The range of cell dimensions of group I-pc strains included that of group II strains. Hence, groups I-pc and II could not be distinguished/separated by macroscopic and light-microscopic, morphological characters.

Fig. 7. Cell dimensions of strains of groups I–IV. Groups V and VI, which each include only one strain, were omitted. Narrow lines indicate the range; thick lines indicate the standard deviation.

With the exception of trichome colour, the morphological characters of group I-pe were similar to those of strains of groups I-pc and II (Fig. 6c). Cells of all group I-pe strains were $3.9\text{--}9.4\text{ }\mu\text{m}$ wide and $1.1\text{--}5.2\text{ }\mu\text{m}$ long and the ratio of cell length to cell width was about 1:

$3\text{--}1:1$ (Fig. 7). The minimum cell width observed was $4.2 \pm 0.2\text{ }\mu\text{m}$ (NIVA CYA 151) and the maximum was $8.6 \pm 0.5\text{ }\mu\text{m}$ (NIVA CYA 13). In contrast, the mean and standard deviation of cell length ranged from $2.1 \pm 0.5\text{ }\mu\text{m}$ (NIES 610^T) to $3.3 \pm 0.6\text{ }\mu\text{m}$ (NIVA CYA

35). The standard-deviation ranges for the strains overlapped (Fig. 7), indicating that clear divisions cannot be made among the group I-pe strains on the basis of morphology.

The morphological characters of the four strains of group III (TR1-5^T, TR2-4, TK4-5 and TK5-1) (Fig. 6e) are outlined below in the description of *Planktothrix mougeotii*. Cells of group III strains were 5.9–7.8 µm wide and 1.5–3.6 µm long and the ratio of cell length to cell width was about 1.3–2.3. The minimum cell width observed was 6.5 ± 0.2 µm (TK5-1) and the maximum was 7.5 ± 0.2 µm (TR1-5^T). The mean and standard deviation of the smallest and largest cell lengths were respectively 2.3 ± 0.4 µm (TK5-1) and 2.6 ± 0.6 µm (TR1-5^T) (Table 3). As the standard-deviation ranges overlapped (Fig. 7), clear divisions cannot be made among these strains on the basis of cell size.

The morphological characters of the six strains of group IV (Fig. 6f) are given below in the description of *Planktothricoides raciborskii* gen. nov., comb. nov. Cells of the group IV strains were 5.4–12.2 µm wide and 1.7–6.7 µm long and the ratio of cell length to cell width was about 2.7–1.1. On occasion, wide sections were observed in a single trichome, in both lab cultures and field samples. The minimum cell width was 7.4 ± 1.2 µm (NIES 207^T) and the maximum was 9.6 ± 1.0 µm (T1-6-2). The mean and standard deviation of cell length ranged from 3.3 ± 0.6 µm (OR1-1) to 4.9 ± 0.9 µm (NSLAØ3). The standard deviation ranges of each strain overlapped (Fig. 7), indicating that clear division cannot be made among these strains on the basis of cell size.

The morphological properties of the group V strain, CCAP 1459/11B^T (Fig. 6g), and the group VI strain, NIVA CYA 277/1^T (Fig. 6h), are listed below in the descriptions of *Tychonema bourrellyi* (group V) and *Limnothrix redekei* (group VI).

DNA–DNA relatedness between groups I-pc and I-pe

With the exception of strains belonging to groups I-pc and I-pe, the groups were well characterized genotypically and phenotypically. In contrast, groups I-pc and I-pe had the same 16S rDNA sequences and DNA base composition (Fig. 1, Table 3) but were phenotypically different (PE absent or present). DNA–DNA hybridization experiments were performed to clarify these subgroups further. Labelled DNA derived from the group I-pc strain NIES 204^T was hybridized to DNA of group I-pc strains C1-12, CCAP 1459/11A, NIVA CYA 10, NIVA CYA 11, NIVA CYA 21, NIVA CYA 88, NIVA CYA 116, NIVA CYA 126, NIVA CYA 229 and NIVA CYA 313 and their relative binding ratios (RBRs) were respectively 71, 73, 89, 77, 86, 83, 92, 74, 85 and 80 %. In addition, labelled DNA of the group I-pc strain NIES 204^T and group I-pe strain CCAP 1459/22^T (= NIVA CYA 18^T = NIES 610^T) was hybridized to DNA of group I-pe strains

NIVA CYA 35, NIVA CYA 85, NIVA CYA 90, NIVA CYA 98 and NIVA CYA 129. RBRs between NIES 204^T and the strains of group I-pe were 54, 49, 53, 44 and 49 % and those between CCAP 1459/22^T and the other group I-pe strains were 81, 97, 98, 95 and 79 %.

DISCUSSION

Compatibility with the traditional, botanical taxonomy

The 75 strains of water-bloom-forming species of freshwater, oscillatoroid cyanobacteria in this study were separated into the six phylogenetic groups, I–VI, based on 16S rDNA sequences. Group I was further divided into two groups based on PC/PE composition, which was supported by total genome DNA–DNA hybridization. Strains of groups I-pc and I-pe shared RBRs of more than 70 % within each group but less than 55 % between groups. Since it has been widely accepted that the same species should share more than 70 % DNA–DNA binding (Wayne *et al.*, 1987), the results indicate clearly that these two groups correspond to different taxa. The seven recognized groups were also characterized by genetic and phenotypic properties such as DNA base composition, fatty acid composition, phycobilin pigment composition, chromatic adaptation, growth at different temperatures and salinities and morphology.

Groups I-pc and II corresponded to *O. agardhii* and its intraspecific taxa (Table 4). Elenkin (1949) described five intraspecific taxa in *O. agardhii* (Table 4). These intraspecific taxa seem to be morphological variations within *O. agardhii*, because the 41 strains showed the following common features; (i) trichomes with no or small constrictions at the cross-walls were found in the same strain, (ii) a gelatinous sheath was occasionally observed under unfavourable conditions and (iii) calyptra and attenuation towards the ends of trichomes were variable characters on different trichomes within a single clone. These results agree with the suggestions of Komárek (1958). Furthermore, Skuja (1948) described *O. agardhii* var. *isothrix*, which is characterized by cell widths of 6–9.5 µm and no attenuation towards the ends of trichomes (Table 4). Later, however, he revised his original description of this intraspecific taxon and concluded that the difference between *O. agardhii* and *O. agardhii* var. *isothrix* was only cell width, which was respectively 4–6 µm and 6–9.5 µm (Skuja, 1956). These differences seem to be clear, but our actual results indicated that it is difficult to define precise morphological criteria to delineate these taxa. The same is true for *O. agardhii* var. *suspensa*, which was characterized by a cell width of 3.8–4 µm (Pringsheim, 1965). In conclusion, on the basis of morphological features, there are no criteria for distinguishing these intraspecific taxa from *O. agardhii*. Furthermore, group II strains could not be separated from strains of group I-pc on phenotypic/morphological criteria. Hence, group II strains can

Table 4. Characters of water-bloom-forming *Oscillatoria* species

Taxa are listed as: 1, *O. agardhii* Gomont 1892; 2, *O. agardhii* f. *aequicrassa* Elenkin 1949; 3, *O. agardhii* f. *gomontii* Elenkin 1949; 4, *O. agardhii* f. *moebiusii* Elenkin 1949; 5, *O. agardhii* f. *lemmermannii* Elenkin 1949; 6, *O. agardhii* f. *wislouchii* Elenkin 1949; 7, *O. agardhii* var. *isothrix* Skuja 1948; 8, *O. agardhii* var. *suspensa* Pringsheim 1965; 9, *O. prolifica* (Greville) Gomont 1892; 10, *O. rubescens* D.C. ex Gomont 1892; 11, *O. rubescens* var. *caucasica* Schmidle 1897; 12, *O. mougeotii* Kützing ex Lemmermann 1907; 13, *O. raciborskii* Woloszyńska 1912; 14, *O. redekei* Van Goor 1918.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cell width (µm)	4–6	4–6	4–6	3–4	4–6	4–6	6–9.5	3.8–4	2.2–5.5	6–8	8–9	5.5–7.5	8–9	1.3–2.3
Length:width	1:2	1:2	1:2	1:2	1:2	1:2	1:3–3:4	1:2–1:1	1:1	1:3–1:2	1:2–1:1	1:2	1:2	3:1–8:1
Apical cell type*	V	R	V	V	V	R	C	C	CC	CC	C	BR	TB	R
Calyptra	+	–	+	+	+	–	–	+	+	+	+	–	–	–
Gelatinous sheath	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Attenuation at the end	+	–	+	+	+	+	–	+	+	+	+	–	+	–
Trichome colour†	PBG	PBG	PBG	PBG	PBG	PBG	PBG	PBG	RP	RP	G	DBG	YG	PBG

* BR, Broadly rounded; C, conical; CC, conical, capitate; R, rounded; TB, tapered, bent; V, variable.

† DBG, Dull blue-green; G, green; PBG, pale blue-green; RP, reddish purple; YG, yellow-green.

only be identified as *O. agardhii* under the traditional, botanical taxonomy.

The group I-pe strains were identified as *O. rubescens*. These strains had previously been named as *O. agardhii*, *O. agardhii* var., *O. rubescens* and *O. rubescens* var. in the NIVA and CCAP collections (Table 1). According to the original descriptions of *O. agardhii* and *O. rubescens*, except for trichome colour, differences between these species were restricted to cell width, which is respectively 4–6 µm and 6–8 µm. Because of the apparent instability of trichome colour, there has been a tendency to use cell width rather than trichome colour as the key diagnostic character for classification (e.g. Elenkin, 1949). Cell-size measurements in the present study indicated that cell width does not distinguish these species. However, for group I-pe strains and group I-pc and II strains, trichome colours were stable and corresponded to qualitative and quantitative differences in phycobilin pigments. The other gas-vacuolated, reddish-brown-coloured *Oscillatoria* species is *Oscillatoria prolifica* (Greville) Gomont (Table 4). Cells of this species are 2.2–5 µm wide and 4–6 µm long and cells are as long or longer than they are wide. The apical cells are capitate and have calyptra. These characters do not concur with the narrow width of the group I-pe strains.

Group III strains were identified as *O. mougeotii* Kützing ex Lemmermann. There is considerable confusion regarding the taxonomy and description of *O. mougeotii*. The first description of *O. mougeotii* was made by Bory (1827) (cited by Gomont, 1892). Later, Kützing (1849) published a more detailed description with figures, detailing that *O. mougeotii* has reddish purple trichomes, many small granules and a cell width of 6–7 µm. In the first comprehensive taxonomy of the Oscillatoriales, published by Gomont (1892), which is approved as a starting point of Oscillatoriales in the International Code of Botanical Nomenclature, the

type specimen of *O. mougeotii* Bory was described as the same as *Oscillatoria formosa* Bory and *O. mougeotii* Bory was therefore described as a synonym of *O. formosa* Bory. In addition, Gomont (1892) did not comment on *O. mougeotii* Kützing (1849). Therefore, it is possible to interpret that there was no valid *O. mougeotii* at the taxonomic starting point of the Oscillatoriales, although Gomont (1892) failed to include *O. mougeotii* Bory in the rejected species list (*species excludendae*). Accordingly, the description of *O. mougeotii* after Gomont (1892) could be recognized as valid. In 1907, Lemmermann and Forti independently described *O. mougeotii* Kützing. The description of Lemmermann (1907) differed from that of *O. mougeotii* Bory as follows. The cell mass is dark or dull blue-green, cells are 5.5–7.5 wide and 2–3 µm long and the trichome breaks easily. Trichomes are straight or slightly curved and not constricted at the cross-walls. The apical cells are broadly rounded and have no calyptra. In the early stages of cell growth, trichomes attach to substrates and then float freely. These characteristics agreed well with those of the strains in this study. In contrast, the description of *O. mougeotii* by Forti (1907) is the same as that of Kützing (1849) except that the cell width is 7.5 µm, and he also reported that *O. mougeotii* was similar to *O. rubescens*. The largest difference between Lemmermann's description of *O. mougeotii* and Forti's is the trichome colour; the former gives dark blue-green and the latter gives reddish purple. Lemmermann's paper was combined with other papers and republished in 1910. This republished paper was widely distributed and has been cited as 'Lemmermann (1910)' [here, we refer to this paper as Lemmermann (1907 or 1910)]. As a result, Lemmermann's description has understandably been misunderstood to have been published later than Forti's. This situation has probably given rise to confusion among later researchers. Geitler (1932) and Elenkin (1949) described *O. mougeotii* Kützing and *O.*

mougeotii Kützing ex Forti, respectively, but their descriptions were similar to those of Lemmermann (1907 or 1910). Furthermore, Elenkin (1949) added that the trichome colour was reddish purple in some cases, in accordance with Forti's description. More recently, Anagnostidis *et al.* (1985) emphasized that *O. mougeotii* was similar to *O. agardhii* var. *isothrix* Skuja. Later, when Anagnostidis & Komárek (1988) established the new genus *Planktothrix* for gas-vacuolate *Oscillatoria* species, *O. mougeotii* was changed to '*Planktothrix mougeotii* (Bory ex Gomont) Anagnostidis & Komárek'. However, their revision incurred the following contradiction in nomenclature; '*P. mougeotii* (Bory ex Gomont) Anagnostidis & Komárek', which has gas vacuoles and is planktonic, is a new combination of *O. mougeotii* Bory ex Gomont, which is the same specimen as that of *O. formosa* Bory ex Gomont, which has no gas vacuoles and is benthic. Thus, the new combination caused more chaos in the taxonomy and nomenclature of *O. mougeotii* and related species. In addition, although Anagnostidis & Komárek (1988) treated *O. agardhii* var. *isothrix* Skuja as a synonym of '*Planktothrix mougeotii*', *O. agardhii* var. *isothrix* should be included within the morphological variations displayed by *O. agardhii* detailed above.

Since it was observed repeatedly in our study that strains of *O. rubescens* have characteristics close to those of the reddish-purple-coloured *O. mougeotii* described by Kützing (1849) and Forti (1907), it is thought that granulated trichomes of *O. rubescens* were described as *O. mougeotii*. This view is supported by the fact that reddish-purple-coloured *O. mougeotii* that is clearly different from *O. rubescens* has never been isolated or reported. In contrast, there are cultures that are identified as dark blue-green-coloured *O. mougeotii*, as described by Lemmermann (1907). *O. mougeotii* Kützing ex Forti would be better treated as a synonym of *O. rubescens*.

The morphological characters of the group IV strains corresponded to the original description of *O. raciborskii*, isolated from ponds on the island of Java, Indonesia (Woloszynska, 1912). Although *O. raciborskii* was reported to have very long trichomes in the original description, this should not be regarded as a taxonomic characteristic, because the length of trichomes changes easily under different conditions and at different growth stages. In fact, the trichomes tended to be shorter in old cultures (data not shown).

The group V strain, CCAP 1459/11B^T, had previously been identified as *O. agardhii* in the CCAP (Table 1). However, morphologically, the strain corresponds to the original description of *Oscillatoria bourrellyi* Lund (1955), which has been revised as *Tychonema bourrellyi* (Skulberg & Skulberg, 1991). Although *O. bourrellyi* was reported to have calyptra in the original description, the strain in this study had no calyptra. However, this diagnostic feature may be variable, as the presence or absence of calyptra is due to the developmental stage of individual filaments in *Lyngbya*

hieronymusii var. *hieronymusii* and the character is variable under different environmental conditions (Suda *et al.*, 1998). Based on the above considerations, the group V strain was identified as *O. bourrellyi*.

The group VI strain, NIVA CYA 277/1^T, which is listed as *O. redekei* in the NIVA collection (Skulberg, 1990), has features quite different from the other water-bloom-forming *Oscillatoria*. They are characterized by having no attenuation towards the ends of trichomes, narrow, long cells, rounded apical cells and two large, polar gas vesicles. The only difference between the original description of *O. redekei* and the present strain is colour. However, Meffert (1987) reported that *O. redekei* showed complementary chromatic adaptation, which was also demonstrated in the present study.

Revision of the taxonomy of water-bloom-forming oscillatorioids

From the above discussion, it is proposed that groups I-pc and II correspond to *O. agardhii*, group I-pe to *O. rubescens*, group III to *O. mougeotii*, group IV to *O. raciborskii*, group V to *O. bourrellyi* and group VI to *O. redekei*. In this section, we outline the taxonomic relationships between the seven groups based on results obtained in this study and give new or emended descriptions of each taxon recognized.

In order to ascertain the taxonomic position of the seven groups recognized by the present study, it was necessary to define the phylogenetic position of the type species of the genus *Oscillatoria* (*O. princeps* Gomont). However, to date, the 16S rDNA sequence of this species has not been published. The morphology of *O. princeps* NIVA CYA 150 corresponded well with original description of Gomont (1892), except for trichome colour. This strain was dark brown-green, rather than blue-green as in the original description of *O. princeps* (Gomont, 1892). However, this difference was not considered phylogenetically significant enough to prevent the use of sequence data for *O. princeps* NIVA CYA 150 as that of the type species of the genus *Oscillatoria* (Gomont, 1892), because similar 16S rDNA sequences have previously been reported in different-coloured *Merismopedia* strains (Palinska *et al.*, 1996) and *Microcystis* strains (Otsuka *et al.*, 1998). From the phylogenetic tree based on 16S rDNA sequences, it was found that *O. princeps* Gomont (1892) was located as a sister group of *Scytonema hoffmanii* PCC 7110 and was not related to groups I–VI. This suggested that the bloom-forming oscillatorioid species should not be listed under the genus name *Oscillatoria* and that their generic name should be changed, as proposed previously by Anagnostidis & Komárek (1988), Meffert (1988) and Skulberg & Skulberg (1991) on the basis of purely phenotypic characteristics.

The similarity of the 1361-bp 16S rDNA sequences was 95.4–96.7% between groups I and II, 95.9–96.8% between groups I and III and 96.4–96.8% between

Table 5. Diagnostic characters of taxonomic groups of water-bloom-forming oscillatoriod cyanobacteria and their type strains

Character	I-pc	I-pe	II	III	IV	V	VI
Type strain	<i>Planktothrix agardhii</i> NIES 204 [†]	<i>Planktothrix rubescens</i> CCAP 1459/22 [†]	<i>Planktothrix pseudagardhii</i> TI-8-4 [†]	<i>Planktothrix mougeotii</i> TRI-5 [†]	<i>Planktothricoides raciborskii</i> NIES 207 [†]	<i>Tychonema bourrellyi</i> CCAP 1459/11B [†]	<i>Limnolthrix redekei</i> NIVA CYA 277/1 [†]
Number of strains examined	40	22	5	4	6	1	1
G+C content* (mol%)	40.2 ± 0.4 (38.6–41.2)	39.6 ± 0.3 (38.2–40.8)	40.6 ± 0.1 (38.0–40.7)	39.6 ± 0.3 (39.3–41.6)	44.4 ± 0.5 (42.5–44.9)	44.4 ± 0.6	40.9 ± 0.3
Fatty acid type	2B	2B	2B	2C	4	2A	2C [†]
Phycobilin pigment composition	PC	PE > PC	PC	PE < PC	PC	PE, PC	PE, PC
Complementary chromatic adaptation	—	—	—	—	—	+	+
Temperature range for growth (°C)	10–30	10–20	20–40	10–30	20–40	10–20	10–20
Maximum salinity for growth (‰)	1.28	0.78	1.28	1.83	0.43	0.43	0.26
Cell width* (µm)	3.1–4.2 (2.3–9.8)	5.0–6.2 (3.9–9.4)	4.2–5.1 (3.0–6.4)	7.1–7.8 (5.9–7.8)	5.4–9.8 (5.4–12.2)	4.9–6.3	1.8–2.7
Length:width	1:3–1:1	1:3–1:1	1:3–1:1	1:3–2:3	2:7–1:1	3:5–3:2	3:1–7:1
Apical cell type [†]	V	V	V	BR	TB	R	R
Gas vacuoles	Large, scatter strong	Large, scatter strong	Large, scatter strong	Small, central	Small, scatter weak	None	Polar, central

* Value for type strain. Range for group given in parentheses.

† See Table 4 for definition of abbreviations.

groups II and III. These values are at the congeneric level according to Devereux *et al.* (1990). In addition, the fatty acid compositions and G+C contents of groups I–III were similar and they did not undergo complementary chromatic adaptation. Anagnostidis & Komárek (1988) have renamed gas-vacuolated *Oscillatoria* as genus *Planktothrix*, with the type species *Planktothrix agardhii*. Our results support the appropriateness of classifying the water-bloom-forming, gas-vacuolated *Oscillatoria* species in other genera. Group I was further divided into two sub-groups, groups I-pc and I-pe, by phycobilin pigment composition. These groups demonstrated little difference in growth temperature and salinity tolerance. However, DNA–DNA hybridization revealed that groups I-pc and I-pe were independent species. Combining these data and the traditional taxonomic conclusions outlined above, it is appropriate that strains of group I-pc should be treated as *Planktothrix agardhii* and those of group I-pe as *Planktothrix rubescens* (Table 5). As detailed above, we propose to treat *O. mougeotii* Kützinger ex Forti as a synonym of *Planktothrix rubescens* (= *O. rubescens*).

Group II strains were identical to group I-pc in morphology, fatty acid composition, G+C content and phycobilin pigment, but showed a clear difference in growth temperature range and, moreover, these two groups can be distinguished clearly from each other in phylogenetic trees. Hence, it is quite reasonable that group II should be treated as a novel species, and we propose the name *Planktothrix pseudagardhii* sp. nov. for this taxon because strains are similar in morphology to *Planktothrix agardhii*. It is appropriate that strains of group III, which were morphologically compatible with *O. mougeotii* Kützinger ex Lemmermann (different from *O. mougeotii* Bory ex Gomont), should be treated as a species within the genus *Planktothrix*.

We propose to reject '*Planktothrix mougeotii* (Bory ex Gomont) Anagnostidis et Komárek' for the following reasons (detailed above): (i) *O. mougeotii* Bory ex Gomont was changed to '*Planktothrix mougeotii* (Bory ex Gomont) Anagnostidis et Komárek'; (ii) *O. mougeotii* Bory ex Gomont is clearly described as a synonym of *O. formosa* Bory (Gomont, 1892); and (iii) *O. formosa* Bory was described as having no gas vacuoles and as a benthic species, which is not appropriate for a member of the genus *Planktothrix*.

We also propose to give the name *Planktothrix mougeotii* to group III in this study, since the group III strains were identified as *O. mougeotii* Kützinger ex Lemmermann, which is regarded as a valid name (as detailed above) and has been shown to be appropriate as a species in the genus *Planktothrix*.

The strains of group IV, which were morphologically similar to *O. raciborskii*, formed an independent phylogenetic cluster separated from the cluster of groups I–III, which were assigned to *Planktothrix*. This group showed less than 89.7% similarity of 16S

rDNA sequence to the other groups and the *O. princeps* and *L. hieronymusii* var. *hieronymusii* strains. According to Devereux *et al.* (1990), less than 94.6% similarity corresponds to the level of different genera. In addition, group IV was distinguished by fatty acid composition and G + C content from the *Planktothrix* groups, and we propose the name *Planktothricoides raciborskii* gen. nov., comb. nov. for group IV (*O. raciborskii*).

Group V, strain CCAP 1459/11B^T, identified as *O. bourrellyi*, has been renamed *Tychonema bourrellyi* by Anagnostidis & Komárek (1988) and Skulberg & Skulberg (1991). From the phylogenetic data obtained from 16S rRNA gene sequence analysis of this strain, it was found that this strain forms a cluster independent of the other groups and the type species of *Oscillatoria* (*O. princeps*). This strongly supports the view proposed by the above authors. Hence, it is appropriate that the group V (*O. bourrellyi*) should be treated as *Tychonema bourrellyi*.

Group VI, strain NIVA CYA 277/1^T, identified as *O. redekei*, has been renamed *Limnothrix redekei* by Meffert (1988). Phylogenetic trees revealed that the species was very far from *O. princeps* and the other groups. This species was further characterized by the ability to undergo complementary chromatic adaptation, fatty acid composition, growth temperature and salinity tolerance. Hence, it is appropriate that group VI (*O. redekei*) should be treated as *Limnothrix redekei*.

Phylogenetic analyses based on 16S rDNA sequences could draw distinctions among five taxa, *Planktothrix pseudagardhii*, *Planktothrix mougeotii*, *Planktothricoides raciborskii*, *Tychonema bourrellyi* and *Limnothrix redekei*. However, *Planktothrix agardhii* and *Planktothrix rubescens* could not be separated by 16S rDNA sequence analysis, morphology, fatty acid composition or G + C content (Table 5), but were distinguishable by phycobilin pigment composition, growth at different temperatures and salinities (Table 5) and DNA–DNA hybridization. These observations suggest that their differentiation may be a relatively recent event. Use of 16S rDNA sequence analysis is a powerful tool for taxonomy; however, characterization based on the sequence alone is not enough for classification of species of some organisms, especially for closely related species.

Planktothrix agardhii and *Planktothrix pseudagardhii* were well defined by 16S rDNA sequence analysis. They shared the same characters of fatty acid composition, phycobilin pigment, G + C content and morphology (Table 5). This suggests that their speciation may have occurred prior to that between *Planktothrix agardhii* and *Planktothrix rubescens*. Without doubt, a polyphasic approach will be needed to solve the confusion facing cyanobacteria taxonomy, as well as modern bacterial classification suggested by Vandamme *et al.* (1996).

Emended description of *Planktothrix* Anagnostidis & Komárek 1988

Water-bloom-forming, non-heterocystous and filamentous cyanobacteria bearing many gas vacuoles. Trichomes are straight or slightly curved, attenuated or not toward the ends and not surrounded by a clear sheath. Cross-walls are constricted or unconstricted and usually not granulated. DNA G + C content is about 40 mol %. Fatty acid composition is type 2. The type species is *Planktothrix agardhii* (Anagnostidis & Komárek 1988).

Emended description of *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek 1988

Basonym: *Oscillatoria agardhii* Gomont 1892.

Synonyms: *Oscillatoria agardhii* f. *aequicrassa* Elenkin 1949; *Oscillatoria agardhii* f. *moebiusii* Elenkin 1949; *Oscillatoria agardhii* f. *lemmermannii* Elenkin 1949; *Oscillatoria agardhii* f. *wislouchii* Elenkin 1949; *Oscillatoria agardhii* f. *gomontii* Elenkin 1949; *Oscillatoria agardhii* var. *isothrix* Skuja 1948; *Oscillatoria agardhii* var. *suspensa* Pringsheim 1965; *Planktothrix mougeotii* (Bory ex Gomont) Anagnostidis et Komárek 1988.

Micrographs are shown in Fig. 6(a, b). Trichomes are pale blue-green or yellow-green. Trichomes are solitary and float, although in the early stages of cultivation they sometimes become entangled and sink to the bottom of the culture tube; however, they never attach there. They are straight or slightly curved, usually attenuated towards the ends and not surrounded by a sheath under conditions favourable for growth. Under unfavourable conditions (e.g. nutrient depletion, heavy contamination by heterotrophic bacteria), trichomes may be surrounded by a gelatinous sheath and may attach to the walls of the culture tube. Cross-walls are slightly constricted or unconstricted and not granulated. Gas vacuoles are relatively large and scattered at the periphery of the cells. Apical cells show variable shapes: rounded, tapered, bluntly conical, occasionally capitate, with or without calyptra. Cell width is 2.3–9.8 µm, cell length is 1.3–5.1 µm and the ratio of cell length to width is about 1.3–1:1. PE is absent. Not capable of complementary chromatic adaptation. Fatty acid composition is type 2B. DNA G + C content is about 40 mol %. Growth temperature range is 10–30 °C. Salinity tolerance for growth is up to 0.78 ‰. The type strain is strain NIES 204^T (= CCAP 1460/5^T) (Table 5).

Emended description of *Planktothrix rubescens* (D.C. ex Gomont) Anagnostidis et Komárek 1988

Basonym: *Oscillatoria rubescens* D.C. ex Gomont 1892.

Synonym: *Oscillatoria mougeotii* Kützinger ex Forti 1907.

A micrograph is shown in Fig. 6(c). Trichomes are reddish purple or reddish brown. Trichomes are solitary and float, although sometimes they become entangled and sink to the bottom of the culture tube; however, they never attach there. Trichomes are straight or slightly curved and attenuated or not towards the ends. Cross-walls are slightly constricted or not constricted and not granulated. Gas vacuoles are relatively large and scattered at the periphery of the cells. Apical cells show variable shapes: rounded, tapered, bluntly conical, rarely capitate, with or without calyptra. The cell width is 3.9–9.4 µm, cell length is 1.1–4.9 µm and the ratio of cell length to cell width is about 1:3–1:1. A large amount of PE is present with a high PE:PC ratio. Not capable of complementary chromatic adaptation. Fatty acid composition is type 2B. DNA G + C content is about 40 mol%. Growth temperature range is 10–20 °C. Salinity up to 0.43 % is tolerated for growth. The presence of PE is a principal distinguishing diagnostic character from *P. agardhii*. The type strain is strain CCAP 1459/22^T (= NIVA CYA 18^T = NIES 610^T) (Table 5).

Description of *Planktothrix pseudagardhii* sp. nov.
Suda & Watanabe

Planktothrix pseudagardhii [pseu.da.gar'dhi.i. Gr. adj. *pseudos* false, N.L. fem. n. *pseudagardhii* false *agardhii*, referring to *Planktothrix agardhii* (Gomont) Anagnostidis et Komárek].

A micrograph is shown in Fig. 6(d). Trichomes are pale blue-green or yellow-green. Trichomes are solitary and they float, they sometimes become entangled and sink to the bottom of culture tubes, but they never attach there. They are straight or slightly curved, sometimes attenuated towards the ends and not surrounded by sheath under conditions favourable for growth. Cross-walls may be slightly constricted or not constricted and not granulated. Gas vesicles are relatively large and scattered at the periphery of the cells. Apical cells show variable shapes such as rounded, tapered, bluntly conical, occasionally capitate, with and without calyptra. Cell dimensions are 3.0–6.4 µm wide, 1.2–4.2 µm long and the ratio of cell length to width is about 1:3–1:1. PE is absent. Not capable of complementary chromatic adaptation. Fatty acid composition is type 2B. DNA G + C content is about 40 mol%. Growth temperature range is 20–30 °C. Salinity up to 0.78 % is tolerated for growth. 16S rDNA sequence is the only distinguishing character between *P. agardhii* and this species. The type strain is strain T1-8-4^T (= NIES 845^T = CCAP 1460/7^T) (Table 5).

Description of *Planktothrix mougeotii* (Kützing ex Lemmermann) comb. nov. non (Bory ex Gomont) Anagnostidis et Komárek 1988

Basonym: *Oscillatoria mougeotii* Kützing ex Lemmermann 1907, 1910.

A micrograph is shown in Fig. 6(e). Trichomes are dark blue-green to olive-green. Trichomes are initially attached to the bottom or side wall of culture tubes and then float in the upper to middle layer of the culture medium. Entangled trichomes sometimes cover the entire surface of the medium. Usually, the trichomes are straight, occasionally twisted and not attenuated towards the ends. Cross-walls are slightly constricted. Gas vacuoles are small and cluster in the equatorial part of the cells and they rarely rupture on placing a microscope cover slip on a drop of culture. Apical cells are broadly rounded and have no calyptra. Cell width is 5.9–7.8 µm, cell length is 1.5–3.6 µm and the ratio of cell length to cell width is about 1:3–2:3. A small amount of PE is present, with a low PE:PC ratio. Not capable of complementary chromatic adaptation. Fatty acid composition is type 2C. DNA G + C content is about 40 mol%. Growth temperature range is 20–30 °C. Salinity up to 1.28 % is tolerated for growth. The type strain is strain TR1-5^T (= NIES 844^T = CCAP1460/6^T) (Table 5).

Description of *Planktothricoides* gen. nov. Suda & Watanabe

Planktothricoides (Plank.to.thric.oi'des. N.L. n. *Planktothrix* a genus of cyanobacteria; Gr. comp. *oides* resembling; N.L. fem. n. *Planktothricoides* resembling *Planktothrix* Anagnostidis et Komárek).

Trichomes are solitary, float and are generally straight or slightly bent near the apex and attenuated towards the ends. Occasionally, a very thin, clear sheath is observed. Cross-walls are slightly constricted or not constricted and not granulated. Many small gas vacuoles are scattered at the periphery of the cells and they are easily ruptured during observation with a light microscope when a cover slip is placed on a drop of culture. Has no PE and no capacity to undergo complementary chromatic adaptation. Fatty acid composition is type 4. DNA G + C content is about 44 mol%. Forms a phylogenetic cluster independent from *Planktothrix* on the basis of 16S rDNA sequence analysis. The type species is *Planktothricoides raciborskii*.

Description of *Planktothricoides raciborskii* comb. nov.

Basonyms: *Planktothrix raciborskii* (Woloszynska) Anagnostidis et Komárek 1988; *Oscillatoria raciborskii* Woloszynska 1912.

A micrograph is shown in Fig. 6(f). In addition to the properties listed in the genus description, the following properties are observed. Trichomes are pale blue-green or yellow-green. Apical cells are rounded, more or less tapered, bent but not pointed, bluntly conical and have no calyptra. Cell width is 5.4–2.2 µm, cell length is 1.7–6.7 µm and the ratio of cell length to cell width is 2:7–1:1. On occasion, thick sections are found in a single trichome in both lab cultures and field samples.

Growth temperature range is 20–30 °C; some strains grow at 40 °C. Salinity up to 0.78 % is tolerated for growth. The type strain is strain NIES 207^T (= CCAP 1465/1^T) (Table 5).

Emended description of *Tychonema bourrellyi* (Lund) Anagnostidis & Komárek 1988

Basonym: *Oscillatoria bourrellyi* Lund 1955.

Synonyms: *Oscillatoria bornetii* f. *tenuis* Skuja 1930; *Tychonema tenue* (Skuja) Anagnostidis et Komárek 1988.

A micrograph is shown in Fig. 6(g). Trichomes are grey to greyish-brown under normal daylight. Trichomes are solitary or sometimes become entangled and sink to the bottom of the culture tubes, but never attach there. They are straight or slightly attenuated toward their ends. Cross-walls are not constricted and not granulated. Apical cells are rounded, not capitate and occasionally have calyptra. Cells have keritomized chromatoplasm (Geitler, 1932). Cells are 4.9–6.3 µm wide and 3.2–8.0 µm long and the ratio of cell length to cell width is approximately 3.5–3.2. PE is present. Capable of complementary chromatic adaptation, which causes a change to reddish-brown cell colour under green light; cell colour does not change significantly under red light. Fatty acid composition is type 2A. DNA G+C content is about 44 mol %. Growth temperature range is 10–20 °C. Salinity up to 0.43 % is tolerated for growth. The type strain is strain CCAP 1459/11B^T (= NIES 846^T) (Table 5).

Emended description of *Limnothrix redekei* (Van Goor) Meffert 1988

Basonym: *Oscillatoria redekei* Van Goor 1918.

A micrograph is shown in Fig. 6(h). The trichome mass is brownish-green to reddish-brown under normal daylight, but the colour of individual trichomes is not recognizable because of their slenderness. Trichomes are solitary or sometimes become entangled and sink to the bottom of the culture tubes, but never attach there. They are straight and not attenuated toward their ends. Cross-walls are not constricted and not granulated. Apical cells are rounded, not capitate and they have no calyptra. Two large gas vacuoles are distributed at the poles of the cells and occasionally in the centre of the cells. Vacuoles are rarely punctured on placing a microscope cover slip on a drop of culture. Cells are 1.8–2.7 µm wide and 5.4–14.3 µm long and the ratio of cell length to cell width is approximately 3:1–7:1. PE is present, with a high PE:PC ratio under normal daylight conditions. Capable of complementary chromatic adaptation, which causes a change to pale blue-green cell colour with a large amount of PC under red light and to reddish-brown cell-mass colour with a large amount of PE under green light. Fatty acid composition is type 2C'. DNA G+C content is about 41 mol %. Growth temperature range is 10–20 °C. Salinity up to 0.26 % is tolerated for

growth. The type strain is strain NIVA CYA 277/1^T (= NIES 847^T) (Table 5).

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