

Anabaena eucompacta sp. nov. (Nostocales, Cyanobacteria),
a New Planktonic Species with Tightly Spiraled Filaments from Japan

Renhui Li and Makoto M. Watanabe*

National Institute for Environmental Studies,
Onogawa 16–2, Tsukuba, Ibaraki, 305–0035 Japan

Abstract A clonal strain of a planktonic species of *Anabaena* was isolated from an eutrophic lake in Chiba prefecture, Japan, and its taxonomic evaluation was made following morphological observations. This strain was characterized by regularly and compactly spiraled filaments with akinetes adjacent to heterocysts. It differs from *Anabaena compacta* (Nygaard) Hickel and *Anabaena pseudocompacta* M. Watanabe by the relative position of akinetes to heterocysts, and differs from another related species *Anabaena reniformis* Lemmermann by akinete shape and coil diameter. It is described here botanically as a new species of *Anabaena*, *Anabaena eucompacta* Li et M. M. Watanabe, based on morphological characters, but requires further verification by physiological, biochemical and genetic characters in future studies.

Key words: *Anabaena eucompacta* sp. nov., Cyanobacteria, Planktonic species, Nostocales, Water bloom

Introduction

It is generally accepted that the current system of taxonomy of cyanobacteria should use the Bacteriological Code of Nomenclature (Stanier *et al.*, 1971; Waterbury and Stanier, 1978; Rippka *et al.*, 1979; Rippka and Cohen-Bazier, 1983; Holt *et al.*, 1994). “The Guideline for Characterization of the Cyanobacteria” was proposed, in which the criteria for the classification of cyanobacteria include morphological, cytological, physiological, chemical and genetic characteristics (Castenholz and Waterbury, 1989). However, the development of this bacteriological system must be based on the existing botanical assemblage of name, so that both bacteriological and botanical systems do not diverge without control. Hence, the botanical name based on morphological characteristics remains the initial step in the new bacteriological approach.

During our taxonomic studies on waterbloom-forming cyanobacteria, we isolated a clonal strain of *Anabaena* from a sample of water bloom in Chiba Prefecture, Japan, and found that this alga possessed filaments with very compact, regular coils, which is a typical feature of *Anabaena compacta* and *A. pseudocompacta*. However, by the following detailed morphological examination, especially after formation of

*Author for reprint request

akinetes, the alga was shown to be different from *A. compacta* and other related taxa. In this paper, a new species of *Anabaena*, *A. eucompacta* is described under the code of Botanical Nomenclature.

Materials and Methods

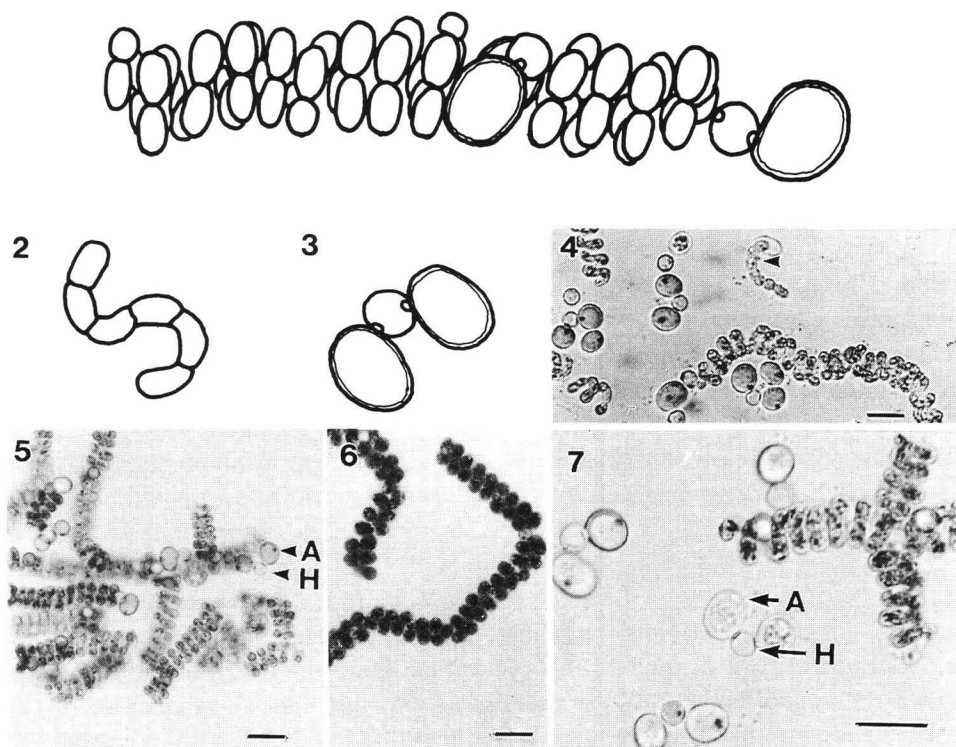
A natural sample was collected from lake Yatsuru in August 1996 and isolated using the micropipette washing method (Watanabe *et al.*, 1985) into unialgal cultures. Among the cultures, a strain of *Anabaena eucompacta* (strain Ana chiba, NIES culture collection) was grown and maintained in screw-capped tubes (18×150 mm; Fujimoto Rika, Tokyo, Japan) containing 10 mL of CT medium (Watanabe and Nozaki, 1994), at 20°C under a 12:12 LD cycle with a photon flux density of $40 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ provided by daylight fluorescent lamps. The formation of akinetes was induced by the low temperature treatment method developed by Li *et al.* (1997). The sample for morphological observation was fixed with 3% formalin, and examined using an Olympus Vanox photomicroscope. Cell dimensions were measured on 50 individual filaments of the strain. The fixed sample was preserved in the herbarium of the National Science Museum, Tokyo (TNS).

Results and Discussions

Trichomes free-floating, solitary, and regularly tightly coiled (Figs. 1, 5, 6 and 7), without mucilaginous sheath. Coil 9.3–13.4 μm in diameter, 3.1–4.7 μm in distance. Vegetative cells with gas vesicles, ellipsoidal or elongate, slightly curved (Fig. 2), 3.0–5.6 μm in diameter, 4.1–6.3 μm in length. Heterocysts spherical (Figs. 3 and 7), 4.8–6.3 μm in diameter. Akinetes broadly ovoid or kidney-shaped (Figs. 1, 3, 4 and 7), solitaria, 6.3–9.6 μm in diameter, 6.6–12.0 μm in length, locating at both sides of heterocysts (Figs. 3, 4 and 7). The ratio of length to diameter of akinetes 1.1–1.4.

This species superficially appears most similar to *Anabaena compacta* (Nygaard) Hickel and *Anabaena pseudocompacta* M. Watanabe in the form of filaments with very tight and regular coils. *A. compacta* was established as a new combined species from *A. spiroides* var. *minima* f. *compacta* Nygaard by Hickel (1982, 1985) after she examined natural and cultured material of *A. spiroides* var. *minima* f. *compacta* and revealed that this taxon warrants a separate status from *Anabaena spiroides* Klebahn because of the small cell size, amplitude and period of the helix. The related species, *Anabaena pseudocompacta*, was established by M. Watanabe (1997) based on examination of samples from eutrophic lakes in central Japan and was characterized by longer akinetes and larger vegetative cells than *Anabaena compacta*. Both *A. compacta* and *A. pseudocompacta* have akinetes situated distant from heterocysts, which distinguishes them from the present alga. *Anabaena reniformis* Lemmermann is also another similar species to the present alga in terms of akinete position and shape

1



Figs. 1-3. Sketches of *Anabaena eucompacta* sp. nov. 1. A filament with akinetes and heterocysts. 2. Part of filament showing the shape of vegetative cells. 3. Separated akinetes and heterocyst showing akinetes adjacent position to heterocyst. All: $\times 1000$

Figs. 4-7. *Anabaena eucompacta* sp. nov. 4. Part of filament. Arrow shows germination of akinete. 5-7. Filaments with akinetes and heterocyst. A: Akinete H: Heterocyst. All bars represent $20\text{ }\mu\text{m}$.

of vegetative cells (Elenkin, 1938), however, the spherical akinetes and larger diameters and length of coils of *A. reniformis* separate it from the present alga.

The dimensions of vegetative cells, heterocysts, akinetes and coils of the present new species and related species were shown in Table 1.

Komárek (1973) indicated that investigations of cultured material is an indispensable part of taxonomic revisions and the data obtained from cultures are of great importance for taxonomic evaluations. It is shown in the present study that cultured materials provided many advantages. Firstly, akinete was successfully induced at low temperature culture conditions, which made able to compare the material with the related species by key characters, such as relative position of akinetes to heterocysts and akinetes shape and size. Secondly, in contrast to the result of Gorham *et al.*

Table 1. Differences in dimensions of vegetative cells, heterocysts, akinetes and coils among the related taxa.

Species	Vegetative cells		Heterocysts		Akinetes			Coils		Akinete position to heterocyst	Reference
	Diameter	Length	Diameter	Length	Diameter	Lenght	L/D ratios	Diameter	Distance		
<i>Anabaena eucompacta</i>	3.0–5.6	4.1–6.3	4.8–6.3		6.3–9.6	6.6–12.0	1.1–1.4	9.2–13.4	3.1–4.7	adjacent	Present study
<i>A. compacta</i>	4.0–5.0		5.5–6.0		8.0–10.5	11.0–12.5	1.2–1.4	11.0–16.0	4.0–12.0	remote	Nygaard 1949
<i>A. pseudocompacta</i>	5.2–7.0	3.0–6.8	5.5–7.5		7.5–11.3	16.8–21.3	1.8–5.6	18.0–20.0	5.8–7.5	remote	Watanabe 1997
<i>A. reniformis</i>	4.0–5.5	6.0–8.0	4.2–7.0	4.2–8.0	8.5–11.0			12.0–23.0	10.0–12.0	adjacent	Elenkin 1938

(1964), who reported a gradual loss of filament coiling after a few week to months in culture of *Anabaena flos-aquae*, the present alga showed stable helical shape and dimensions at the culture conditions. Finally, the establishment of the present alga will allow the morphological identification for planktonic species of *Anabaena* to be much clearer, especially for species with very compact coils.

As we described above, the new bacteriological approach to cyanobacterial taxonomy combining morphological, physiological, biochemical and genetic characteristics should be based on the existing botanical assemblage of name, but it also poses the question of how should we treat the case when a proposed new species is described by morphological observation. Although there are not yet legalized, it is better to give a new species name and latin description by the botanical nomenclature initially in order to distinguish it from other species without any described name (e.g. *Anabaena* sp.). It is emphasized here that we believe that morphological observations of a new species should be verified by physiological, biochemical and genetic characters. Therefore, in the present study, a new species name (*Anabaena eucompacta*) and latin description (see below) are given, and the further studies on other features are planned for future work.

***Anabaena eucompacta* Li et M. M. Watanabe sp. nov.**

Trichomata libere natantia, solitaria, regulariter circinata, sine vaginis mucosis. Spirae regulares, generaliter arte contractae, 9.3–13.4 μm in diametro, 3.1–4.7 μm distantes. Cellulae vacuolis gaseosis includentes, cupiformes vel longus, 3.0–5.6 μm in diametro, 4.1–6.3 μm longae. Heterocytae sphaericae, 4.8–6.3 μm in diametro, 4.1–6.3 μm longae. Akineta late ovoidae vel nephroideae, 6.3–9.6 μm in diametro, 6.6–12.0 μm longae, 1.1–1.4 plolongiora quam latiora, ad heterocytae utrinque affixa.

Iconotypus: Figura 1

Locus typicus: in lacu Yatsuru Chiba Praefecturae Japoniae.

Acknowledgments

The authors thank Mr. H. Sekiuchi and Mr. S. Sakuma, University of Tsukuba, Japan, for providing the water sample. This work was supported by the Japanese Government's Special Coordination Funds by the Science and Technology Agency (STA).

References

- Castenholz, R. W. and J. B. Waterbury, 1989. Cyanobacteria. In Staley, J. T., M. P. Bryant, N. Pfennig and J. G. Holt (Eds.), *Bergey's Manual of Systematic Bacteriology*, Vol. 3. pp. 1710–1727, Williams & Wilkins, Baltimore.
- Elenkin, A. A. 1938. Monographia Algarum Cyanophycearum aquidulcium et terrestrium in finibus URSS inventarum. Fasc., I: 1–984.

- Gorham, P. R., J. S. McLachlan, U. T. Hammer and W. K. Kim, 1964. Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) De Bréb. *Verh. Internat. Verein. Limnol.*, **15**: 796–804.
- Hickel, B. 1982. A helical, bloom-forming *Anabaena*-like blue-green alga (Cyanophyta) from hypertrophic lakes. *Arch. Hydrobiol.*, **95**: 115–124.
- Hickel, B. 1985. Observation on *Anabaena compacta* (Nygaard) nov. comb. (Cyanophyta) with helical, planktonic filaments and macroscopic aggregates. *Arch. Hydrobiol. (Suppl. 71) Algol. Studies*, **38/39**: 269–270.
- Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley and S. T. Williams, 1994. *Bergey's Manual of Determinative Bacteriology*, 9th edition, 787 pp., Williams & Wilkins. Baltimore, Philadelphia, Hong Kong, London, Munich, Sydney, Tokyo.
- Komárek, J. 1973. Prospects for Taxonomic Developments. In Carr, N. G. and B. A. Whitton (Eds.), *The Biology of Blue-green Algae*, pp. 482–486. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.
- Li, R., M. Watanabe and M. M. Watanabe, 1997. Akinete formation of planktonic *Anabaena* spp. by low temperature treatment. *J. Phycol.*, **33**: 576–584.
- Nygaard, G. 1949. Hydrological studies on some Danish ponds and lakes. *Kongl. Danske Vidensk. Selskab., Biol. Skr.*, **7**: 1–293.
- Rippka, R. and G. Cohen-Barzire, 1983. The Cyanobacteriales: a legitimate order based on type strain *Cyanobacterium stanieri*? *Ann. Microbiol. (Inst. Pasteur)* **134B**: 21–36.
- Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman and R. Y. Stanier, 1979. Generic assignments, strain histories and properties of pure culture of cyanobacteria. *J. gen. Microbiol.*, **111**: 1–61.
- Stainer, R. Y., R. Kunisawa, M. Mandel and G. Cohen-Bazire, 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). *Bact. Rev.*, **35**: 171–205.
- Watanabe, M. 1997. Studies on planktonic blue-green algae 7. *Anabaena pseudocompacta* sp. nov. from eutrophic lakes in central Japan. *Bull. Natn. Sci. Mus., Tokyo*, Ser. B, **22**: 1–10.
- Watanabe, M. M. and H. Nozaki, 1994. *NIES-Collection, List of Strains, Algae and Protozoa*, 4th edition, 127 pp., National Institute for Environmental Studies, Environment Agency, Japan.
- Watanabe, M. M., S. Suda, F. Kasai and T. Sawaguchi 1985. Axenic culture of three species of *Microcystis* (Cyanophyta=Cyanobacteria). *Bull. JFCC*, **1**: 57–63.
- Waterbury, J. B. and R. Y. Stanier, 1978. Patterns of growth and development in pleurocapsalean cyanobacteria. *Microbiol. Rev.*, **42**: 2–44.