

## A new cyanobacterial species of *Anabaena* genus (Nostocales, Cyanobacteria) from Bulgaria

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### Resumen

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*Una nueva especie de cianobacteria del género Anabaena (Nostocales, Cyanobacteria) de Bulgaria*

Se describe una nueva especie del género de Cyanobacterias, *Anabaena* Bory ex Born. et Flah. (Nostocales) de las montañas Ródope de Bulgaria. *Anabaena rhodopensis* sp. nova. tiene acinetas con paredes celulares esculpidas. Se dan los datos biométricos para el tamaño de las células vegetativas, heterocistos y acinetos.

**Palabras clave:** *Anabaena*, Evaluación biométrica, Bulgaria, Cyanobacteria, Nueva especie.

### Abstract

A new species of cyanobacterial genus *Anabaena* Bory ex Born. et Flah. (Nostocales) from Rhodope Mountains in Bulgaria is described. *Anabaena rhodopensis* sp. nova. has akinetes with sculptured cell walls. Biometrical data for size of vegetative cells, heterocytes and akinetes are given.

**Key words:** *Anabaena*, Biometric evaluation, Bulgaria, Cyanobacteria, new species.

### Introduction

The cyanobacterial genus *Anabaena* Bory ex Born. et Flah. 1888 contains several morphologically different clusters (Komárek & Zapomělová 2007). More than 100 species have been described within the cyanobacterial genus *Anabaena* (Hollerbach *et al.* 1953, Desikachary 1959, Star-mach 1966, Kondrateva 1968, Guiry & Guiry 2014). Thirty one species of the traditional *Anabaena* with apoheterocytically developing akinetes are separated by Komarek & Anagnostidis (1989) in the genus *Trichormus* (Ralfs ex Born. et Flah.) Kom. et Anagn.

The systematic classification of Cyanobacteria

has changed significantly during the last decades. The modern classification of cyanoprokaryotes is based on a combined assessment of morphological and molecular analyses (Komárek 2012). The morphological description of specimens from natural populations is very important for determining phenotypic variations and studying the ecology of a specific type.

Different morphological changes of cyanobacterial strains can be observed early after their isolation (Komárek & Zapomělová 2008). The morphological studies depend on the samples directly collected from the field. The shape and size of vegetative cells and akinetes as well as the relative location of akinetes to heterocytes are impor-

tant for distinguishing *Anabaena* species (Tuji & Niiyama 2010).

The aim of this study was the description of the morphological and ecological characteristics of a newly found *Anabaena* species from Bulgaria.

## Material and methods

Our material was collected on 25-V-2010, in the central region of Rhodope Mountains, near the Madan town, Bulgaria (41°48'209"N, 24°93'358" E). The locality was a rock from which water constantly trickles. The altitude of the locality was over 800 AMSL. Morphological observations such as the shape and dimensions of vegetative cells, heterocytes, akinetes as well as the location of akinetes to heterocytes were conducted under light microscope Olympus CX31 in the Department of Biology and Aquaculture at the Trakia University. The population was statistically described for size of vegetative cells, heterocytes and akinetes in filaments (n=50).

## Description

### *Anabaena rhodopensis* sp. nova.

Trichomes solitary, straight or somewhat curved. Vegetative cells cylindrical with rounded edges without gas-vacuoles, blue green 4.4-5.1-5.7 µm wide, and 3.3-4.6-6.4 µm long (Table 1). Heterocytes spherical 5.0-6.2-7.2 µm in diameter (n=50). Akinetes solitary or in groups of 2,, not adjacent to heterocytes. They were cylindrical with parallel or very slightly swollen walls and rounded edges 7.7-9.8-12.2 µm wide and 19.8-28.6-37.6 µm long. The akinete wall was yellowish. The exospores were covered with coarse verrucae, which were rectangular in cross section and with irregular contours when observed from above. Verrucae were 0.6-1.1 µm high (Fig. 1).

**Ecology:** together with *Tribonema* Derbès & Soller, *Cosmarium* Corda, Bacillariophyta in fur on a rock stone down which water constantly trickles, Rhodope Mountains, Madan town, May 25 2010. pH 6.9, Temperature -20 °C.

**Type locality:** Rhodope Mountains, Madan town, Bulgaria.

**Iconotype:** Shown in figure 1.

**Etymology:** This species was named after the region of its occurrence-Rhodope Mountains, Bulgaria.

## Diagnosis

Trichoma recta aut leviter circumflexa. Cellulae vegetativae cylindricae, usque breviter cylindricae cum extremitatibus rotundatis, sine plenis aere vacuoles, caeruleo viridi, 4.4-5.1-5.7 µm latae et 3.3-4.6-6.4 µm longae. Heterocytae sphaericae, 5.0-6.2-7.2 µm diametro. Sporae elongatecylindricae cum parietibus parallelis aut leviter inflates et angulis obtusis. Tunica earum pallid flava. Exosporum cum verrucis rudibus quae in sectione in longitudinam rectangulare, sed in sectione transversa cum confiniis irregularibus, 0.6-1.1 µm latae et 0.8-1.5 µm aetae. Sporae singulae aut raro binae, non ligatae cum heterocytis, 7.7-9.8-12.2 µm latae et 19.8-28.6-37.6 µm longae.

**Habitatio-locus classicus:** simui cum *Tribonema*, *Cosmarium*, Bacillariophyta in tegumento saxa aqua fluente continenter. Montes Rhodope, prope Madan, Bulgaria, Anno 2010, 25 Majo.

**Iconotypus:** Figura nostra 1.

## Remarks

The newly found species from Bulgaria had characteristic sculpture on the akinete exospores. The sculpture on akinete wall is considered to be a good feature of *Anabaena* species (Elenkin 1938, Bourrelly 1970). The existence of sculptures is a rarity in the genus. The species this genus having

| <i>Anabaena rhodopensis</i> (n=50)        | Vegetative cells |         | Akinetes |          | Heterocytes |
|---|------------------|---------|----------|----------|-------------|
|   | Length           | Width   | Length   | Width    | Diameter    |
| min                                       | 3.3              | 4.4     | 19.8     | 7.7      | 5.0         |
| max                                       | 6.4              | 5.7     | 37.6     | 12.2     | 7.2         |
| $\bar{x} \pm m_x$ (arithmetic mean value) | 4.6±0.3          | 5.1±0.1 | 28.6±0.4 | 9.8±0.4  | 6.2±0.2     |
| $\sigma \pm m_\sigma$ (root mean square)  | 2.0±0.2          | 0.9±0.1 | 13.5±1.0 | 3.7±0.3  | 1.3±0.1     |
| $v \pm m_v$ (coefficient of variation)    | 43.5±4.4         | 1.8±0.2 | 47.2±3.3 | 37.8±2.7 | 20.9±2.1    |

**Tabla 1.** Características biométricas de *Anabaena rhodopensis* sp. nova (n=50).

**Table 1.** Biometrical characteristics for *Anabaena rhodopensis* sp. nova (n=50).

akinetes with different sculptured wall are *Anabaena verrucosa* B. Peters. and its f. *major* Kosinsk., *Anabaena tatarica* Kosinsk. (Kondratieva 1968), *Anabaena batophora* Frémy, *Anabaena aspera* Frémy, *Anabaena fuellebornii* Scmidle (Geitler 1932) and *Anabaena echinospora* Skuja (Elenkin 1938) (Table 2).

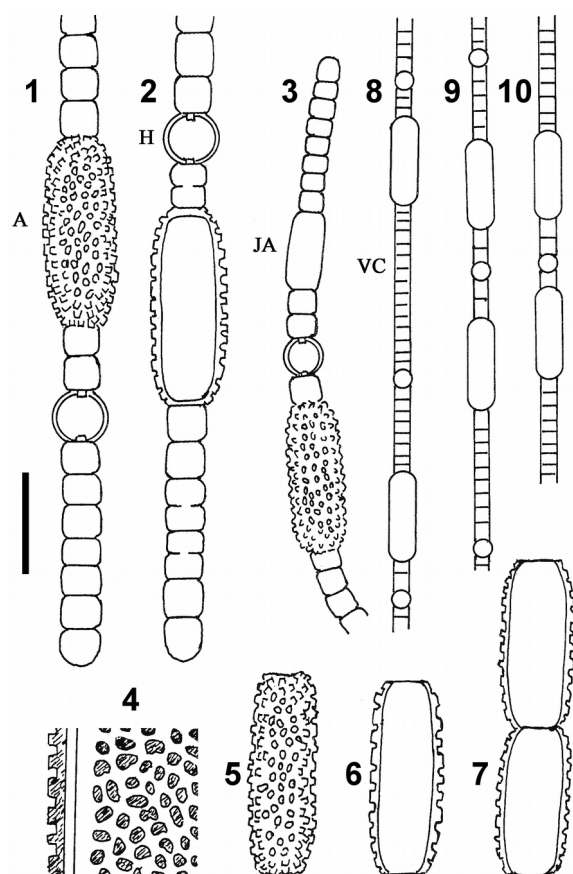
The species *A. verrucosa*, *A. batophora* and *A. aspera* are the closest to the newly found *Anabaena* sp. from Bulgaria by the sculptured wall. The species described here differed distinctly from all three species mentioned above (Table 2). *A. rhodopensis* sp. nova. had spherical heterocysts and sculptural formations at the akinete surface, which were rectangular in cross section and with irregular contours (Fig. 4). *A.*

*batophora* is characterized particularly by cylindrical or ellipsoidal heterocysts and triangular papillae on akinetes. The heterocysts are cylindrical and the akinetes are with broadly triangular papillae in *A. aspera*. *A. verrucosa* and f. *major* differ clearly from the mentioned species by their cylindrical heterocysts and their narrow trichomes and akinetes.

It should be noted that the sculptural formation occurred only in fully mature akinetes since the very young akinetes have smooth walls. As the size of the akinetes increased, a certain roughness appeared. Akinetes of different ages could be seen on one and the same filament.

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**Figura 1.** *Anabaena rhodopensis* sp. nova. 1-3: Trichomas con teterocitos (H), acinetos viejos (A) y jóvenes (JA); 4: Detalle de la vaina y ornamentación de la pared celular de los acinetos; 5-7: Acinetos viejos. 8-10: Ejemplos (esquemas) de los principales tipos estructurales de heterocistos (H), acinetos (A) y células vegetativas (VC). Barra de escala=5  $\mu$ m.

**Figure 1.** *Anabaena rhodopensis* sp. nova. 1-3: Trichomes with heterocysts (H), old akinetes (A) and young akinetes (JA); 4: Detail of sheath and sculpture cell wall of akinetes; 5-7: Old akinetes; 8-10: Examples (schemes) of main types trichome structure-heterocysts (H), akinetes (A) and vegetative cells (VC). Scale bar=5  $\mu$ m.

| Species   | Trichomes Diameter | Cells form Length                        | Heterocytes Form & dimensions                  | Akinetes   |   |  |
|---|--------------------|--|--|--|---|--|
|   |                    |  |  | Form & dimensions  | Position  | Ornaments  |
| <i>A. rhodopensis</i> sp.nov.                                       | 4.4-5.7            | Cylindrical with rounded ends<br>3.3-6.4 | Spherical<br>5.0-7.2                           | Long cylindrical or with slightly swelled walls and rounded ends<br>7.7-12.1X19.8-36.4 | Solitary or by 2, not adjacent to the heterocysts | Yellowish with rectangular cross section warts           |
| <i>A. verrucosa</i> J.B.Petersen by Kondrateva, 1968                | 2.7-4.0            | Cylindrical with rounded ends<br>4-8     | Cylindrical till elliptical<br>3-4X5-8         | Cylindrical<br>6-7.2X12-18   | Solitary or by 2, not adjacent to the heterocysts | Warts  |
| <i>A. verrucosa</i> f. <i>major</i> Kossinskaya by Kondrateva, 1968 | 4-5                | Cylindrical with rounded ends<br>4-8     | Cylindrical till elliptical<br>4.5-5.5X5.5-7.5 | Cylindrical<br>7-8X20-28   | Solitary or by 2, not adjacent to the heterocysts | Warts  |
| <i>A. batophora</i> Freymy by Geitler, 1932                         | 4-5                | Barrel shaped=diam. till or shorter      | Cylindrical or elliptical<br>5-6X6-7.5         | Cylindrical or with slightly swelled walls and rounded ends<br>10-12X24-30             | Solitary, not adjacent to the heterocysts         | Colorless or yellowish, triangular papilla 2-2.5 µm high |
| <i>A. aspera</i> Freymy by Geitler, 1932                            | 5                  | Rectangular=diam. till 1.5X longer       | Cylindrical<br>6X6.9                           | Cylindrical with rounded ends<br>8-9 till 45   | Solitary or by 2, not adjacent to the heterocysts | Brownish broad triangular warts                          |
| <i>A. fuellebornii</i> Schmidle by Geitler, 1932; Desikachary, 1959 | 5                  | Cylindrical or rounded cylindrical       | Barrel-shaped or round cylindrical<br>7X10     | Cylindrical<br>10X20   | Solitary or by 2, adjacent to the heterocysts     | Yellowish, tine spikes                                   |
| <i>A. tatarica</i> Kossinsk. by Kondrateva, 1968                    | 4.8-6.0            | Barrel shaped<br>3.6-6.0                 | Cylindrical or elliptical<br>5.4-7.2X13.2-16.8 | Elliptical<br>13.2-16.8X30-36  | Solitary, adjacent to the heterocysts             | Yellowish, brownish, small warts                         |
| <i>A. echinospora</i> Skuja by Elenkin, 1938                        | 6-11               | Spherical<br>(6)-8-11                    | Spherical= veget.cells                         | Cylindrical with rounded ends<br>16-18X55  | Solitary or by 2, adjacent to the heterocysts     | Colorless, papillae                                      |

**Tabla 2.** Revisión de los taxones de *Anabaena* con acinetos ornamentados. Las especies *A. hallensis* (Janecz.) Born. et Flach., *A. pseudovariables* Woronich. y *A. stelooides* Canab., que también presentan acinetos ornamentados, han sido incluidos por Komárek & Anagnostidis (1989) en el género *Trichormus* (Ralfs ex Born. et Flah.) Kom. et Anagn. Las medidas están expresadas en µm.

**Table 2.** Review of *Anabaena* taxa with sculptured akinetes. Species *A. hallensis* (Janecz.) Born. et Flach., *A. pseudovariables* Woronich. and *A. stelooides* Canab., which have also sculptured akinetes, are included by Komárek & Anagnostidis (1989) in genus *Trichormus* (Ralfs ex Born. et Flah.) Kom. et Anagn. All measurements are in µm.