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ANABAENA BERGII OSTENF. [F. *MINOR* (KISSELEV) KOSSINSK.] (CYANOPROKARYOTA) – THE FIRST RECORD IN SERBIA, ITS TAXONOMIC STATUS, AND THAT OF THE GENUS *ANABAENA* BORY EX BORN. & FLAH.

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Abstract — Within the framework of a detailed survey of the algal community in salt marshes of the Vojvodina Province (Northern Serbia), we rather unexpectedly found the blue-green alga *Anabaena bergii* Ostenf. [forma *minor* (Kisselev) Kossinsk.] in water samples from Slatina Pond near Opovo. Our finding represents its first record in Serbia. The present paper gives general characteristics of this alga and of the habitat in which it was found. Based on analysis of a large number of works dealing with characteristics and the taxonomic status of the genus *Anabaena*, the species *A. bergii,* and its forma *minor*, it is concluded that there are numerous problems in taxonomy of the given genus, with no consensus among researchers. In light of the available data, the authors retain the name of the species *A. bergii*, but accept forma *minor* with some reserve.

Key words: *Anabaena bergii* (forma *minor*), Cyanoprokaryota, Slatina Pond, salt marshes, Serbia

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INTRODUCTION

Salt marshes for many reasons are very specific freshwater habitats. In the Vojvodina Province (Northern Serbia), they are characterized by exceptionally high mineralization of their water, which consequently has high electro-conductivity and pH values.

As salt marshes in Vojvodina are characterized by shallow depth (seldom over 0.5 m), they are most often transparent to the bottom, and day-night fluctuations of water temperature are more marked compared to other aquatic ecosystems.

In addition to this, the effect of air currents is considerable, so that even weaker winds cause water movements that raise sediment from the bottom of these salt marshes, making their water muddy to an extent that water transparency on such occasions drops below 0.05 m.

It is important to point out that they relatively seldom dry up even though their depth is slight. This is a consequence of their location on the Pannonian Plain (in depressions) and the proximity of groundwater. Continual percolation from the raised surrounding terrain fills salt marshes with water, and continuous evaporation and retention of mineral substances in the remaining water cause their high mineralization.

Bearing in mind all of the above, we can expect salt marshes to be distinguished by a particular biocenotic structure. For this reason, the Institute of Botany, Belgrade, has launched a project concerned with research on algae in the salt marshes of Vojvodina.

MATERIAL AND METHODS

A detailed survey of salt marshes was carried out during 2003, 2004, and 2006, when a great number of samples were collected on five occasions from 14 salt marshes in the Vojvodina Province (Northern Serbia). One of them was Slatina Pond near Opovo, from which samples were collected on three occasions, on one occasion in each research year in March, April, and July, respectively.

For the qualitative and quantitative analyses

of algae and physicochemical analyses of water of interest to us currently, samples were collected from Slatina Pond on 18 July 2006.

Water temperature (by mercury thermometer, accuracy of 0.1ºC) and transparency (by Secci-disc) were measured at the time of sampling. Determination of other physicochemical parameters was done at the Institute of Public Health of Serbia in Belgrade.

Samples of phytoplankton for qualitative analyses were collected by filtering water through a plankton net (mesh size of 25 μm).

Samples of epiphytes were collected from *Phragmites australis* (Cav.) Trin. & Steud. (= *Ph. communis* Trin.) and *Typha latifolia* L. by scraping from the plants or together with parts of the plants themselves.

Microscopic processing of algal samples was done at the Institute of Botany of the Faculty of Biology on a Zeiss AxioImager.M1 microscope using AxioVision 4.6 software.

Quantitative analysis was done according to Utermöhl (1958).

The habitat in which *Anabaena bergii* Ostenf.[forma *minor* (Kisselev) Kossinsk.] was found was marked on a UTM map grid with cells of 10 x 10 sq. km, on which the territory of the Republic of Serbia encompasses 986 squares and belongs to the 34 T UTM world section.

Records of certain taxa of the division Cyanoprokaryota on the territory of Serbia, including also data on *A. bergii* (forma *minor*) were analyzed according to Blaženčić and Cvijan (1988) and Cvijan and Blaženčić (1996), as well as on the basis of reviewing all works published from 1996 to date on algae occurring in Serbia and information in the database of the Chair of Algology, Mycology, and Lichenology of the Faculty of Biology in Belgarde.

RESULTS AND DISCUSSION

I. Location and general characteristics of Slatina Pond

Slatina Pond is situated in the vicinity of the village of Opovo, about 30 km north of Belgrade, in the valley (S 1).

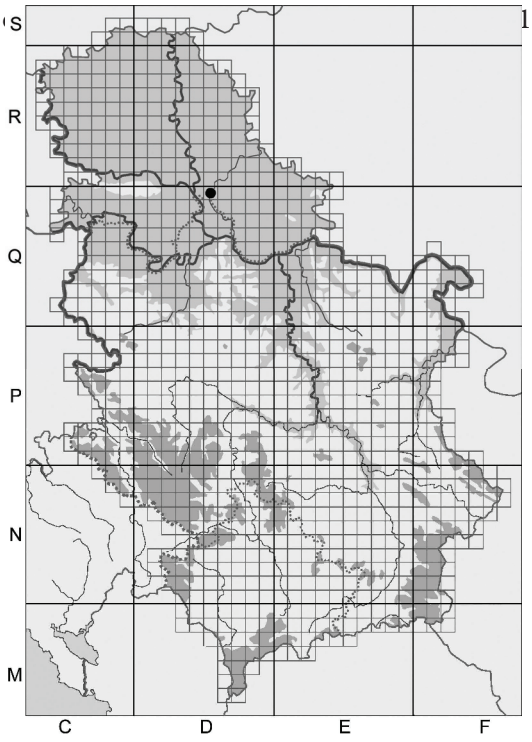

Fig. 1. Map of the Vojvodina Province (Northern Serbia) with location of Slatina Pond (solid circle).

The climate of this region is temperate-continental. Precipitation during the year is unevenly distributed, with two distinct maximums (primary in June, secondary in November and December) and two minimums (primary in October, secondary in March) (Ćurčić, 1996). Winds are an important climatic factor, the most frequent one being that from the southeast.

Waters in the environs of Opovo consist of underground waters of the Tamiš River, river lakes, ponds, and swampy terrains. In depressions of diluvial terraces, salt marshes also occur (Ćurčić, 1996).

Slatina Pond is a natural fluvial lake located on a diluvial plain. This is a fossil riverbed showing traces of varying phases of shifting of the Tamiš River course. Marsh humus occurs within arcs of the old meander of Slatina Pond, whereas the bottom is salted humus. The water level in Slatina Pond depends on the level of the Tamiš River and that of phreatic waters, whose mid-depth near Slatina Pond amounts to 0.8 m (Ćurčić, 1996). For all these

reasons, Slatina Pond is distinguished by an unstable water level.

The banks of Slatina Pond are overgrown with reed and cattail, whereas its central part constitutes free water.

II. Characteristics of A. bergii Ostenf. [forma minor (Kisselev) Kossinsk.] and habitats in which it was recorded

Analysis of algal samples collected from the water of Slatina Pond revealed the presence of a great number of algal taxa. Out of 10 taxa of the division Cyanoprokaryota (the division's name is according to Komárek and Anagnostidis, 1998) found in Slatina Pond, particularly interesting and unexpected is the record of *A. bergii* Ostenf. [forma *minor* (Kisselev) Kossinsk.], which is the subject of the present paper.

Detailed analysis of other algae from Slatina Pond will be presented in future papers.

Bearing in mind the original description of *A. bergii*. Ostenf. forma *minor* (Kisselev) Kossinsk. (Gollerbakh et al., 1953), we note that algal individuals found in water samples from Slatina Pond fully correspond to the original description of this taxon (Table 1).

Table 1. General characteristics of *Anabaena bergii* Ostenf. forma *minor* (Kisselev) Kossinsk. A – according to Gollerbakh et al. (1953). B – according to Cvijan and Krizmanić.

	Trichome width (μm)	Cell length (μm)	Size (length x width) (μm)		Habitats
			-heterocysts-	-akinetes-	
A	4.5-6.5	2.5-6.0	5-6.5 x 4.5-6.5	13-16 x 11-12	Plankton of salt waters and relict lakes, the Caspian and Aral Seas, Central Asia.
B	5.2-6,5	3.6-5.8	6-6.4 x 5.7-6.5	absent	Slatina Pond (salt marsh) Vojvodina, Serbia

Table 2. Physicochemical properties of the water of Slatina Pond near Opovo.

Parameter (in)	Result
Water temperature (°C)	28
Depth (m)	0.9
Transparency (m)	0.35
pH	8.4
Electro-conductivity at 20°C (μS/cm)	1900±100
Ammonia, NH_4 (mg/l)	0.76±0.05
Nitrites, NO_2 (mg/l)	0.008±0.001
Nitrates, NO_3 (mg/l)	<0.5
Chlorides, Cl (mg/l)	200±10
Sulfates, SO_4 (mg/l)	22±2
$KMnO_4$ consumption (mg/l)	90±2
Orthophosphates, PO_4-P (mg/l)	<0.01
Total phosphorus, P (mg/l)	0.10±0.01
Oxygen, O_2 (mg/l)	8.6±0.5
Chlorophyll *a* (mg/l)	93
Calcium, Ca (mg/l)	29±2
Magnesium, Mg (mg/l)	75±2
Sodium, Na (mg/l)	490
Potassium, K (mg/l)	5.1

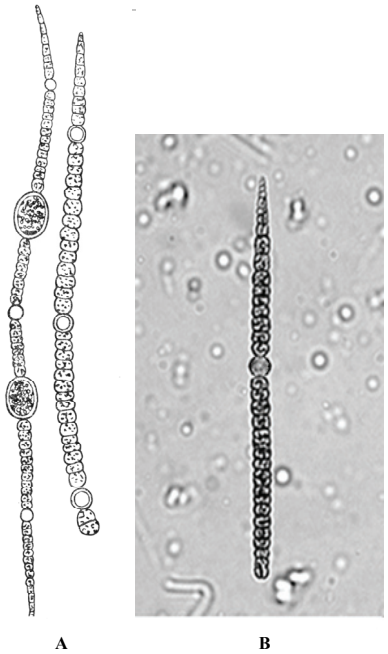

Fig. 2. *Anabaena bergii* Ostenf. forma minor (Kisselev) Kossinsk. A – according to Gollerbakh et al. (1953). B – according to Cvijan and Krizmanić.

The general characteristics are very similar. In addition, comparison of the original drawing of forma *minor* (Fig. 2A) with micrograms of algae from Slatina Pond (Fig. 2B) indicates without any dilemma that the pond is inhabited by *A. bergii* Ostenf. forma *minor* (Kissel.) Kossinsk. according to Gollerbakh et al. (1953), except that akinetes were not found in the Slatina algae.

According to Gollerbakh et al. (1953), *A. bergii* is found in plankton of salt waters, whereas forma *minor* occurs in plankton of salt waters and relict lakes. In recent literature (Komárek and Komárkova, 2006), it is stated that *A. bergii* Ostenf. occurs in the plankton of salt lakes of the temperate Eurasian zone (which is not quite correct – for details see III). We note that *A. bergii*, and particularly its forma *minor*, have been relatively rarely recorded in the world.

In view of the above, the record of *A. bergii* (forma *minor*) in Slatina Pond might be considered to be rather unexpected. However, water of the pond in its physicochemical properties (Table 2) basically corresponds to characteristics of the habitat from which the taxon was originally described, as well as to properties of many habitats in which it was later found.

To be specific, as can be seen from Table 2, the concentrations of sulfate, calcium, magnesium, potassium, and (especially) chloride and sodium in Slatina Pond are very high. As a consequence, values of water electro conductivity and pH are also remarkably high.

In addition, it should be noted that Slatina Pond at the time of sampling was characterized by relatively great depth (0.9 m), but also by significantly decreased water transparency (0.35 m), given that water movements caused by air currents readily swept very fine deposit upwards to the free water. Naturally, the presence of a significant number of algae in the water also contributed to decrease of water transparency, but certainly to a lesser extent than the air current.

It is necessary to mention the comparatively high oxygen content in the water of Slatina Pond, especially in the light of high water temperature.

Quantitative analysis of algae from the water of Slatina Pond showed that *A. bergii* (forma *minor*) in the plankton was represented by a relatively small number of individuals (about 1720 trichomes/l), from which it might be concluded that it developed under conditions which did not fully suit it. However, the absence of akinetes suggests the opposite conclusion. Besides, in the material collected from other plants, individuals of *A. bergii* (forma *minor*) were present in somewhat higher numbers.

It is therefore certain that physicochemical properties of the water were favorable for the development of *A. bergii* (forma *minor*), the low abundance of its individuals being conditioned instead by competition with other algae, also well adapted to the specific physicochemical conditions of their environment.

On the other hand, *A. bergii* (forma *minor*) is a multicellular, filamentous alga, so comparison of its abundance with that of the large number of unicellular algae present would not be correct. To be specific, in 18 analyzed individuals of *A. bergii*, the number of cells in the filament ranged between 22 and 53, the average being 40.25.

After consulting Blaženčić and Cvijan (1988), Cvijan and Blaženčić (1996), and all published works treating the algae of Serbia from 1996 to date, we established that *Anabaena bergii* Ostenf. [forma *minor* (Kisselev) Kossinsk.] is here recorded for the first time on the territory of Serbia.

III. Analysis of taxonomic status of the genus Anabaena Bory ex Born. & Flah., the species Anabaena bergii Ostenf., and its forma minor (Kisselev) Kossinsk.

According to Elenkin (1938), *Anabaena bergii* Ostenf. is found in the plankton of salt waters and was recorded and described as early as the beginning of the 20th century in plankton of the Aral Sea (Ostenfeld, 1908, according to Elenkin, 1938).

Somewhat later, a separate variety, *A. bergei* var. *minor* (Kiselev, 1927, according to Elenkin, 1938), was also described from the Aral Sea.

Gollerbakh et al. (1953) reduced *A. bergei* var.

minor Kisselev to the rank of a forma under the name of *A. bergii* forma *minor* (Kisselev) Kossinsk.

However, problems regarding the taxonomic status of the genus *Anabaena* Bory ex Born. & Flah. are remarkably great, with the result that the taxonomic status of individual species within this genus is in many cases unclear and their classification difficult.

In many contemporary scientific works and algological studies carried out in Europe and beyond (e.g., in Slovakia – Hindák, 1992, 2000; the Ukrainian part of the Black Sea – Brjanceva et al., 2005; Venezuela – Delgado and Sánches, 2005; New Zealand – Wood et al., 2005; Germany – Stüken et al., 2006; Egypt – Hamed, 2008), all three names [*Anabaena bergii* Ostenf.; *A. bergei* var. *minor* Kisselev; and *A. bergii* forma *minor* (Kisselev) Kossinsk.] are used, sometimes with different synonymy.

The extent to which the taxonomy of *A. bergii* is still unclear is evident from the fact that Hindák in one work considers that *A. bergii* var. *minor* Kisselev sensu Hindák is a synonym of the species *A. minderi* (Hindák, 1992), whereas in another he treats *A. bergii* var. *minor* Kisselev as a synonym of the species *A. bergii* (Hindák, 2000).

On the other hand, many authors deny existence of the genus *Anabaena* as a fully independent entity.

Thus, 20 years ago Komárek and Anagnostidis (1989) proposed including the so-called "traditional species of the genus *Anabaena* with apoheterocystic trichomes" in the genus *Trichomus* (Ralfs ex Born. & Flah.) Komárek & Anagnostidis.

Separation of planktonic from benthic *Anabaena* species was also justified by molecular sequencing (Gugger et al., 2002; Iteman et al., 2002; Hofmann et al., 2005; Rajaniemi et al., 2005a, 2005b; Willame et al., 2006).

According to Komárek and Kováčik (1989), some *Aphanizomenon* and *Anabaena* species (including *A. bergii*) „belong to a group of species characterized by solitary trichomes which are narrowed towards the ends and in which elongated terminal cells are absent". Thus, the position of this group within the genus *Anabaena* or *Aphanizomenon* or even as a separate genus is still open to question (Komárek and Kováčik, 1989).

It has been further suggested that *Aphanizomenon ovalisporum* and *Anabaena bergii* are morphological variants of the same cyanobacterium (Fergusson and Saint, 2000).

According to Bazzichelli and Abdelahad (1994), Pollingher et al. (1998), Shaw et al. (1999), and Gkelis et al. (2005) (see Gkelis et al., 2005), it appears that all populations described as *Aphanizomenon ovalisporum* exhibit a morphological variability which ranges from typical *Anabaena bergii* to *Aphanizomenon ovalisporum* to *Anabaena minderi*.

On the other hand, in analyzing the diversity of *Aphanizomenon*-like cyanobacteria, Komárek and Komárkova (2006) separate a morphotype („morphotype of *Aphanizomenon gracile*") as a heterogenous group possibly encompassing some *Anabaena*-morphotype such as *A. bergii*, which they maintain lives in the plankton of salt lakes of the temperate Eurasian zone (see Delgado and Sánches, 2005; Wood et al., 2005; Hamed, 2008).

According to Komárek and Zapomělova (2007), the traditional cyanobacterial genus *Anabaena* Bory ex Born. & Flah. contains several groups of morphospecies that should be classified into different genera according to modern phenotypic and molecular criteria. Moreover, their separation into several clusters was confirmed by 16S rRNA sequencing. According to the cited authors, the generic name *Anabaena* must also be maintained for benthic species without gas vesicles. On the other hand, the planktonic types fall into several subclusters. However, it should be pointed out that *A. bergii* is a planktonic species and that Komárek and Zapomělova (2007) did not analyze its taxonomic status.

CONCLUSIONS

A detailed survey of salt marshes in the Vojvodina Province (Northern Serbia) was carried out during 2003, 2004, and 2006. One of them was Slatina Pond

near Opovo, from which samples were collected on three occasions, on one occasion in each research year in March, April, and July, respectively.

For the qualitative and quantitative analyses of algae and physicochemical analyses of water of interest to us currently, samples were collected from Slatina Pond on 18 July 2006.

Analysis of algal samples collected from the water of Slatina Pond revealed the presence of a great number of algal taxa. Out of 10 taxa of the division Cyanoprokaryota found in Slatina Pond, particularly interesting and unexpected is the record of *Anabaena bergii* Ostenf. [forma *minor* (Kisselev) Kossinsk.], its first record in Serbia. This record was rather unexpected in light of data on the distribution of *A. bergii* forma *minor* worldwide, as well as characteristics of the habitats from which it was originally described and those where it was found later.

Detailed analysis of physicochemical properties of the water in which this alga developed revealed high concentrations of sulfate, calcium, magnesium, potassium, and (particularly) chloride and sodium. As a consequence, water electro-conductivity and pH values were very high. Such physicochemical properties of the water in which this alga was found corresponded more or less considerably to those of water of the habitat from which the form was originally described and in which it was found later.

Based on analysis of a great number of works dealing with the characteristics and taxonomic status of the genus *Anabaena* Bory ex Born. & Flah., the species *A. bergii* (Kisselev) Kossinsk., and its forma *minor* (Kisselev) Kossinsk., it is concluded that there are numerous problems relating to taxonomy of the entire genus and no clear consensus among researchers about it. In addition, at the level of the Central European algal flora (see Süβwasserflora von Mitteleuropa), the revision of algae of the division Cyanoprokaryota (to which *Anabaena* belongs) has not been done as yet.

In light of the available data, the authors retain the name of the species *Anabaena bergii* Ostenf., but accept forma *minor* (Kisselev) Kossinsk. with some reserve.

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ANABAENA BERGII OSTENF. [ФОРМА *MINOR* (KISSELEV) KOSSINSK.] (CYANOPROKARYOTA) – ПРВИ НАЛАЗ У СРБИЈИ И АНАЛИЗА ЊЕНОГ, КАО И ТАКСОНОМСКОГ СТАТУСА РОДА *ANABAENA* BORY EX BORN. & FLAH.

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У оквиру детаљних истраживања слатина Војводине која су проведена 2003, 2004, и 2006. године, у узорцима воде из баре Слатине код Опова, северна Србија који су сакупљени јула месеца 2006. године, утврђено је присуство алге *Anabaena bergii* Ostenf. [форма *minor* (Kisselev) Kossinsk.] (Cyanoprokaryota). У раду су дате опште одлике *A. bergii* (форма *minor*).

На основу анализе свих објављених радова који се односе на алге у Србије, утврђено је да је *A. bergii* (форма *minor*) у Србији забележена по први пут.

При том је њен налаз из више разлога био неочекиван имајући у виду станишта на којима је она до сада налажена у свету – уобичајено у планктону слаих језера (па и мора), превасходно у умереној зони Евроазије.

Међутим, детаљном анализом физичко-хемијских својстава воде баре Слатине утврђене су високе концентрације сулфата, калцијума, магнезијума и калијума, а посебно су биле високе концентрације хлорида и натријума. Последично, вредности електропроводљивости воде, као и pH воде, биле су веома високе. Практично,

физичко-хемијска својства воде баре Слатина била су у значајној мери слична физичко-хемијским својствима воде станишта са којих је алга примарно описана, односно на којима је и касније налажена.

На основу анализе великог броја радова који се баве својствима и таксономским статусом рода *Anabaena* Bory ex Born. & Flah., врсте *A. bergii* Ostenf., као и њене форме *minor* (Kisselev) Kossinsk., утврђено је да су проблеми на нивоу таксономије рода *Anabaena* веома бројни и да у свету не постоји усаглашеност истраживача по том питању. Стога су аутори задржали назив *Anabaena bergii*, док се њена форма *minor* прихвата са одређеном резервом.