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Cylindrospermopsis raciborskii (Cyanoprokaryota) – potential invasive and toxic species in Serbia

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ABSTRACT: In water samples of salt marsh Slatina in 2006 we found *Cylindrospermopsis raciborskii* (Woloszińska) Seenayya et Subba Raju. The findings of *C. raciborskii* are of particular importance given that this species produces cyanotoxins and that it may be considered as potentially invasive and harmful to waters in Serbia. The present paper is concerned with both general and specific characteristics of *C. raciborskii* and with specific characteristics of the habitats in which the species was found considering its invasive and harmful potential.

Key words: *Cylindrospermopsis raciborskii*, Cyanoprokaryota, potential invasive species, Serbia

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INTRODUCTION

In the last thirty-odd years blooming of Cyanoprokaryota in the rivers and lakes, and notably in accumulations for water supply has been observed throughout Serbia. The dominant blooming Cyanoprokaryota ("cyanobacterial blooms") belong to the genera *Microcystis*, *Anabaena* and *Planktothrix* (Svirčev *et al.* 2007).

However, during the last decade the species *Cylindrospermopsis raciborskii* has been found in three localities in Serbia. Of particular interest is the fact that *C. raciborskii* is known as a producer of cyanotoxins which may significantly affect the health of humans and animals due to its hepatotoxic effect.

As part of a detailed survey of salt marsh planktons in Vojvodina (a province in northern Serbia) during 2003, 2004 and 2006 (Cvijan and Krizmanić 2009, Ćirić *et al.* 2010, Cvijan & Fužinato 2011), the saline Slatina pond was examined for the presence of Cyanoprokaryota in 2006. Here we report the finding of *C. raciborskii*, a producer of cyanotoxins, in Slatina pond and discuss the importance of *C. raciborskii* in Serbia.

MATERIAL AND METHODS

Samples for qualitative and quantitative analyses of Cyanoprokaryota and for the physicochemical analyses of water were collected from Slatina pond, situated in the vicinity of the village of Opovo, about 30 km north of Belgrade (Fig. 1), on 18 July 2006.

Water temperature was measured by mercury thermometer (accuracy of 0.1°C) and transparency by Secci-disc, at the time of sampling. Other physicochemical parameters were determined at the Institute of Public Health of Serbia "Dr Milan Jovanović - Batut" by means of standard analytical methods.

For qualitative analysis, plankton samples were collected by filtering water through a plankton net (mesh size of 25 mm).

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 examination of Cyanoprokaryota from Slatina pond, including quantitative analysis (according to

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Utermöhl 1958) was done at the Institute of Botany.

The habitats in which *C. raciborskii* was found were marked on a UTM map grid with cells of 10 x 10 sq. km of Serbia (34T UTM world section).

RESULTS AN DISCUSSION

As for habitats in which *C. raciborskii* was found, of interests are salt marsh Slatina pond, the Ponjavica river and two fish ponds of Kapetanski Rit fish farm. All of them are located in Vojvodina (Fig. 1.).

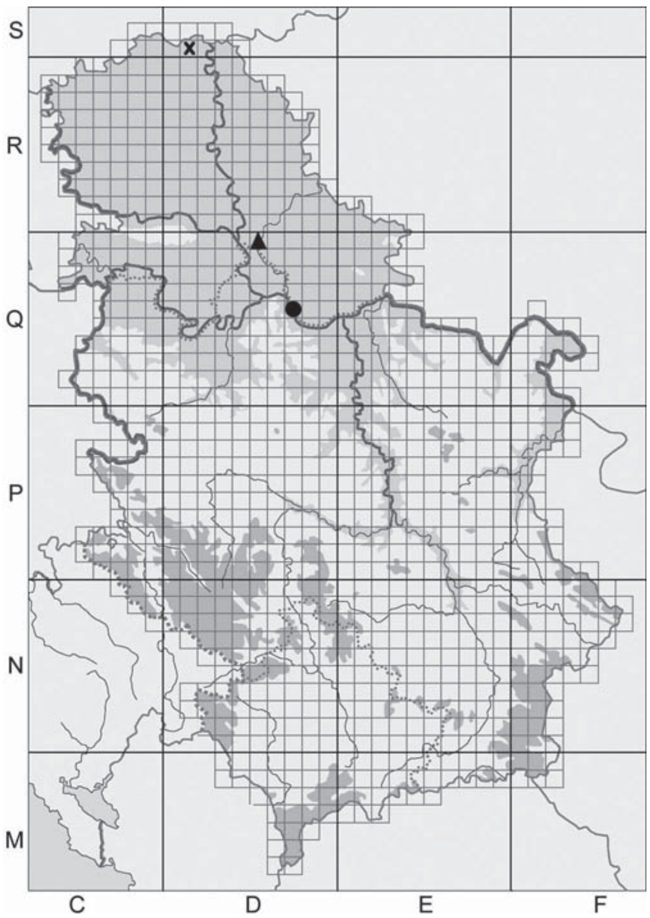

Fig. 1. Map of Vojvodina (Province in northern Serbia) with locations of Slatina pond (▲), Ponjavica river (●) and Kapetanski Rit fish farm (**X**).

I. Distribution of *C. raciborskii* in Serbia and its habitat properties.

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 minima (primary in March, secondary in October) (Ćurčić 1996). Winds are important climatic factors, the most frequent direction being from the southeast.

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 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.

Physicochemical properties of the water of Slatina pond are presented in Table 1.

As can be seen from Table 1, the concentrations of sulphate, calcium, magnesium, potassium and especially chloride and sodium are very high. As a consequence, water electro-conductivity and pH are also remarkably high.

In addition, it should be noted that at the time of sampling,Slatina pond was characterized by a depth of 0.9 m, and also by significantly decreased water transparency (0.35 m) given that water movements, caused by air

Tab. 1. Physicochemical properties of the water of Slatina pond.

Parameter (in)	Result
Water temperature (°C)	28
Depth (m)	0,9
Transparency (m)	0,35
pH	8,4
Electro-conductivity at 20°C (m	1900±100
Ammonia, NH_4 (mg/l)	0,76±0,05
Nitrities, NO_2 (mg/l)	0,008±0,001
Nitrates, NO_3 (mg/l)	<0,5
Chlorides, Cl (mg/l)	200±10
Sulphates, 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

currents, readily swept very fine deposit upwards to the free water. Naturally, the presence of a significant number of algae in the water also resulted in the decrease of water transparency, but to a lesser extent than did the air current. The oxygen concentration in the water of Slatina pond was comparatively high, especially in the light of the high water temperature.

The Ponjavica river is situated on the left bank of the lower course of the Danube through Serbia (Fig. 1.). It is a natural heritage classified as a nature park. The length of the river course within the nature park "Ponjavica" is 7.2 km.

Unfortunately, though it is natural heritage, classified as nature park, many factors contribute to deterioration of the river state. Thus, waste waters, from the surrounding villages and from the weekend zone along one part of the watercourses, reach the Ponjavica bringing with them nutrients, causing a marked eutrophication of water and worsening of its quality. The decrease of reed belt, as an important phyto-filtering element, also contributes to this phenomenon. For this reason the quality of river water is variable during the years and ranges from β- to a-mesosaprobic, and even to polysaprobic.

Kapetanski Rit fish farm, located in the far north of Serbia (Fig. 1.), was established in 1953. Its total area is 642 ha and it consists of a number of small carp ponds. *C. raciborski* has previously been found in Pond 1 and Pond 2 (Ćirić *et al.* 2010).

Although the presence of *C. raciborski* was previously established at these locations by Ćirić *et al.* (2010), no data were available regarding either general characteristics or physicochemical properties of the water of the Ponjavica river and Kapetanski Rit fish farm. Given that these habitats are situated in the same area of Serbia as Slatina pond the general climatic characteristics for both habitats would be expected to be very similar to those in Slatina pond.

II: General characteristics of *C. raciborskii*

Cylindrospermopsis raciborskii is a trichous, unbranched species. Trichomes are single and according to Saker *et al.* (1999) there are two distinct morphotypes, straight and open coiled. Trichomes are usually short though they may be as long as 300 µm.

The cells are elongated-cylindrical, seldom almost square. A specific feature of the species is heterocysts, which are elongated-conical, elongated-egg-like and sometimes bent lengthwise. They are on the trichome tips. Akinetes are cylindrical or oval in shape, single or in pairs, located in the vicinity of terminal heterocysts. In natural conditions they are rarely formed (Padisák 2003). The akinetes have the capacity to survive in sediments for a very long period of time (Moore *et al.* 2003) and usually germinate when water temperature reaches 22-23°C (Briand *et al.* 2004).

Otherwise, morphological variations of different trichome features, even in genetically very similar isolates, greatly depend on various environmental factors, primarily abiotic (Saker *et al.* 1999; Saker & Neilan 2001; Shafik *et al.* 2003).

C. raciborskii usually inhabits tropic and subtropic regions around the world. However, it has been found lately as an invasive species also in moderate regions worldwide. Many hypotheses have been proposed to explain its expansion towards cooler regions (Vidal & Kruk 2008). It is certain that climatic changes affect the expansion of this species range also to moderate latitudes (Briand *et al.* 2004). Thus, in the last thirty years, *C. raciborskii* has been found in temperate regions of Asia, Africa, Australia, Europe, North and South America (Branco & Senna 1991; Chapman & Schelske 1997; Padisák 1997; Moore *et al.* 2003; Dvořák & Hašler 2007; Kling 2009).

Of particular interest is the fact that the species *C. raciborskii* is known as a producer of cyanotoxins, primarily of hepatotoxic cylindrospermopsin (CYN), as well as anatoxin and saxitoxin, which both act as paralytic shellfish neurotoxins (Schrembri *et al.* 2001). Therefore, this species is recognized as especially important given that by its development in freshwaters, especially if used for water supply, it may significantly affect the health of humans and animals due to its hepatotoxic effect. However, non toxin-producing strains are also documented (Stucken *et al.* 2009).

It is noteworthy that it has not been confirmed that the strains, isolated till now from Europe, produce CYN. Thus, Fastner *et al.* (2003) reported that in the past decade *C. raciborskii* has been recorded in German water bodies, and that none of its seven isolates contained CYN.

C. raciborskii exhibits an array of characteristics enabling it to adapt to highly various environmental conditions:

- the species is a diazotroph, using ammonia as a source of nitrogen (in general, growth rates were lowest in the absence of a fixed nitrogen source, and highest with NH_4^+ as nitrogen source – Saker & Neilan 2001);
- it has a high affinity for phosphorus, being capable of absorbing it even when its concentrations are low (Shafik *et al.* 2001; Sprober *et al.*2003);
- relatively high phosphorus uptake affinity and storage capacity confer it a competitive advantage both in deep lakes with nutrient stratification and in lakes with no such nutrient gradation (Istvanovics *et al.* 2000);
- it may develop in conditions of low light intensity (Atenucci *et al.* 2005; Burford *et al.* 2006);
- it can tolerate higher light conditions and lower

temperatures by increasing its production of polyunsaturated fatty acids (Varkonyi *et al.* 2000);
– it possesses gas vacuoles which regulate its suspension and motion in the water column (Jones & Sauter 2005); etc.

III. Some peculiarities of *C. raciborskii* in Slatina pond.

Besides the presence of *C. raciborskii* in the water of Slatina pond (Cvijan & Fužinato 2011), analyses of samples in this pond revealed the presence of other taxa. Of ten taxa from the division of Cyanoprokaryota (the division's name is according to Komárek & Anagnostidis 1998), three of these taxa have now been found in Serbia: *Anabaena bergii* f. *minor* (Kisselev) Kossinsk. (Cvijan & Krizmanić 2009), *Arthrospira fusiformis* (Voronichin) Komárek et Lund (Fužinato *et al.* 2010) and *C. raciborskii* (Cvijan & Fužinato 2011).

Important morphological features of *C. raciborskii* from Slatina pond were presented in Cvijan & Fužinato (2011).

Quantitative analysis of water from Slatina pond showed that *C. raciborski* was present with 960 trichomes/L. Thus, it might be concluded that the species developed under conditions which did not fully suit it. However, the absence of akinetes suggests, perhaps, the opposite conclusion. Besides, in the material collected from other plants, individuals of *C. raciborskii* were present in somewhat higher numbers.

Ćirić *et al.* (2010) reported that *C. raciborskii* from two carp ponds of Kapetanski Rit fish farm had the maximum density, being 3.7×10^6 trichomes/L in Pond 1 (5 October) and 4.0×10^6 trichomes/L in Pond 2 (21 September).

The density of *C. raciborski*, however, in the water of Slatina pond may be considered as extremely low compared with its density in the carp ponds of Kapetanski Rit fish farm and bearing in mind the fact that trichome numbers during blooming of the species may be as high as 10^9/L (Padisák 1997).

CONCLUSIONS

Given that the physicochemical properties of the water in salt marsh Slatina pond are very specific, generally differing significantly from those in rivers and fish-ponds, *C. raciborskii* shows a high level of adaptability to various physicochemical factors in Serbia. This conclusion is in agreement with the fact that *C. raciborskii* is known to possess a generally high level of adaptability to widely various environmental conditions. Therefore, further extension of the distribution range of *C. raciborskii* could be expected in Serbia, owing to its high level of adaptability to widely various environmental conditions.

Potentially, *C. raciborskii* in Serbia might become a highly invasive and undesirable species.

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REZIME

Cylindrospermopsis raciborskii (Cyanoprokaryota) – potencijalno invazivna i toksična vrsta u Srbiji

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Analaizom uzoraka vode iz Slatine kod Opova, sakupljenih 18. jula 2006 godine utvrđeno je prisustvo 10 taksona iz razdela Cyanoprokaryota, među kojima i vrste *Cylindrospermopsis raciborskii* (Woloszińska) Seenayya et Subba Raju. *C. raciborskii* je u međuvremenu nađen i na drugim lokalitetim u Srbiji – u reci Ponjavici kod Pančeva i u dva mala ribnjaka u Kapetanskom ritu. To je od posebnog značaja ako se ima u vidu da je vrsta poznata kao producent cijanotoksina.

Poznato je da vrsta *C. raciborskii* poseduje niz svojstava koja joj pružaju mogućnost prilagođavanja na raznolike uslove životne sredine. Isto tako, fizičko-hemijska svojstva vode Slatine se značajno se razlikuju od istih u rekama i ribnjacima uopšte, pa tako i u reci Ponjavici i ribnjacima Kapetanskog rita. Posledično, vrsta poseduje značajnu sposobnost za daljim širenjem svog areala u Srbiji i potencijalno bi u Srbiji mogla da bude veoma invazivna, a krajnje nepoželjna.

Ključne reči: *Cylindrospermopsis raciborskii*, Cyanoprokaryota, potencijalno invazivna vrsta, Srbija