Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 140, 45—57, 2021

UDC 582.303/.305(285.3)(497.11)
https://doi.org/10.2298/ZMSPN2140045C
ORIGINAL ARTICLE

Miloš ĆIRIĆ[1], *Jelena KRIZMANIĆ*[2], *Biljana P. DOJČINOVIĆ*[1], *Bojan GAVRILOVIĆ*[3*], *Dalibor M. MARINKOVIĆ*[1]

[1] University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, Belgrade 11000, Serbia
[2] University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Takovska 43, Belgrade 11000, Serbia
[3] Serbian Academy of Sciences and Arts, Geographical Institute "Jovan Cvijić", Department of Physical Geography, Đure Jakšića 9, Belgrade 11000, Serbia

OCCURRENCE OF *Botryococcus terribilis* Komárek & Marvan IN A SMALL SANDPIT LAKE – THE FIRST REPORT FROM SERBIA

ABSTRACT: Alkaline saline ponds and lakes are habitats with unique biodiversity. The green alga of the genus *Botryococcus* was accidentally discovered during a micro-algal survey of these habitats in Serbia. Species *B. terribilis* was found and identified for the first time in Serbia in the small sandpit lake of the Rusanda Nature Park. The first sampling was incomplete due to the unknown origin of the orange-red surface scum. After the identity of the species had been confirmed, three additional samplings were conducted aiming to collect phytoplankton and water samples for physical and chemical analyses. Major anions and cations were analysed in order to characterize the chemical type of sandpit lake, as well as nutrient content. Sandpit lake is an alkaline water body that belongs to sodium bicarbonate chemical type. Morphometric analysis of *B. terribilis* was performed using light and transmission electron microscopy. *B. terribilis* was found to be numerous in the phytoplankton community in late autumn and winter. Species from the genus *Botryococcus* are known as a rich source of different lipids and this discovery can be the first step in the further biotechnological application of this species in sustainable biofuel production.

KEYWORDS: Peskara, *Botryococcus terribilis*, phytoplankton

[*] Corresponding author. E-mail: b.gavrilovic@gi.sanu.ac.rs

INTRODUCTION

Algae from the genus *Botryococcus* (Trebouxiophyceae, Chlorophyta) are an interesting group of organisms in terms of their ecology, physiology and their application in biotechnology. Beside cyanobacteria, there are not so many photosynthetic microorganisms which can dominate or bloom in hypertrophic lakes and *Botryococcus* species are one of them (Jeppesen et al., 2007). Moreover, cells of their colonies are surrounded by mucilage containing numerous oil globules (John et al., 2011). Due to the high content of different hydrocarbons some species of the genus have the potential for use in biotechnology (Rai et al., 2007) and sustainable biofuel production.

Classification of *Botryococcus* taxa into several species or sub-species is still under debate (Metzger and Largeau, 2005). Komárek and Marvan (1992) analysed morphological variability of 47 populations, defined five new species (*B. comperei*, *B. australis*, *B. fernandoi*, *B. terribilis* and *B. pila*), and proposed a key for determination of 13 morphological types with the status of species. On the other hand, some authors in their studies have continued to refer to a single species, *B. braunii* Kützing, but there is molecular evidence based on the nuclear 18S rDNA that different *B. braunii* strains probably belong to more than one species (Senousy et al., 2004). In addition, there were some doubts about the taxonomic position of *Botryococcus* taxa, but nowadays it is generally accepted that *B. braunii* belongs to class Trebouxiophyceae, Chlorophyta (Senousy et al., 2004).

Botryococcus species occur in various aquatic habitats from ponds, oligotrophic and mesotrophic lakes to reservoirs with higher electrolyte content. They are generally euryhaline, tolerating salinities ranging from fresh, brackish to saline water (Hammer, 1986; John et al., 2011). In addition, there are strains that are eurythermal, such as two *B. braunii* strains (BOD-NG17 and BOD-GJ2) that can survive temperatures between -20 °C and 40 °C, and tolerate desiccation for over six months (Demura et al., 2014). Experiments with species *Botryococcus protuberans* West & G. S. West revealed that this organism can tolerate pH levels ranging from 7.0 to 9.5, with an optimum at about 8.5 (Rai et al., 2007). In spite of its slow growth rate, some *Botryococcus* species can form a dense bloom in subtropical and tropical regions (Shimamura et al., 2012; Janse van Vuuren and Levanets, 2019) or form benthic mats in shallow salt lakes (Hammer, 1986). The red and green blooms of *B. braunii* are reported in Australian freshwater lakes (Wake and Hillen, 1981). In some water bodies, such as Darwin River Reservoir in Australia Townsend (2001) recorded persistent dominance of *B. braunii* as a result of stable physical conditions. During the massive development, it is not rare to see colonies of *Botryococcus* floating on the surface and forming coloured scum. The higher density of this planktic microalga is usually associated with lower diversity of phytoplankton, zooplankton and even fish community. It is possible that some *Botryococcus* taxa, such as strains of *B. braunii*, produce different extracellular fatty acids with allelopathic effects on other organisms (Chiang et al., 2004).

The *Botryococcus* taxa are known as a group of algae that have an ability to produce and accumulate different hydrocarbon molecules and even some of those molecules, such as botryococcenes, are named after them (Metzger and Largeau, 2005). Moreover, within the same species, there can be several chemical races. For instance, different *B. braunii* strains can be divided into three chemical races, called A, B and L (Metzger and Largeau, 2005). Since some *Botryococcus* cells can have a very high percentage of oil-related molecules (up to 75% of the dry weight), these microalgae can be used as a potent feedstock for renewable liquid fuel production (Demura et al., 2014). Interestingly, species *B. sudeticus* Lemmermann is able to produce and accumulate lipids with oil composition that is similar to the content of olive oil (Senousy et al., 2004).

Distribution of colony-forming planktic algae of the genus *Botryococcus* is most likely cosmopolitan (Komárek and Marvan, 1992; John et al., 2011). For instance, *B. terribilis* Komárek & Marvan is observed in slightly alkaline waters in temperate and tropical regions. The species was found in southern Sweden, Czechoslovakia, Austria, southern Spain, Chad and Cuba (Komárek and Marvan, 1992; Fanés Treviño et al., 2009). In Serbia, *Botryococcus* species have been found in the northern part of the country, in the Vojvodina province (Milovanović and Živković, 1953; Szemes, 1967; Guelmino, 1973; Seleši, 1981, 1982; Obušković, 1982; Đukić et al., 1991a, 1991b; Maletin et al., 1994; Pujin et al., 1996; Pujin, 1998; Trbojević, 2018). Most findings are related to species *B. braunii* that was recorded in lakes and reservoirs (Sava Lake, Lake Palić, Lake Ludaš, and Borkovac reservoir), in rivers and channels (Danube River, Tisa River, Jegrička, Mostonga, and Bajski channel), and different ponds that are a part of Ečka fishpond system. It is interesting that Obušković (1982) reported summer maxima of *B. braunii* in July and August in Sava Lake. Finally, in Tisa River besides *B. braunii* Guelmino (1973) observed one more species of this genus, *B. micromorus* W. West & G. S. West.

The main aims of this study were: (1) to describe the green alga that was found and identified for the first time in Serbia in the small sandpit lake and (2) to analyse the relationship between the chemical composition of the water and the occurrence of the recorded *Botryococcus* species. The morphological and ecological characterisation of this micro-organism should be the first step in the realisation of its biotechnological potential in our country.

MATERIAL AND METHODS

Description of the study site

Water and plankton sampling as well as *in situ* measurements of physical and physico-chemical parameters were conducted on the shore of the sandpit lake known as Peskara (N45°31’06” E20°17’54”). This waterbody is located in the protected zone of the Rusanda Nature Park, between two lakes – Lake Velika

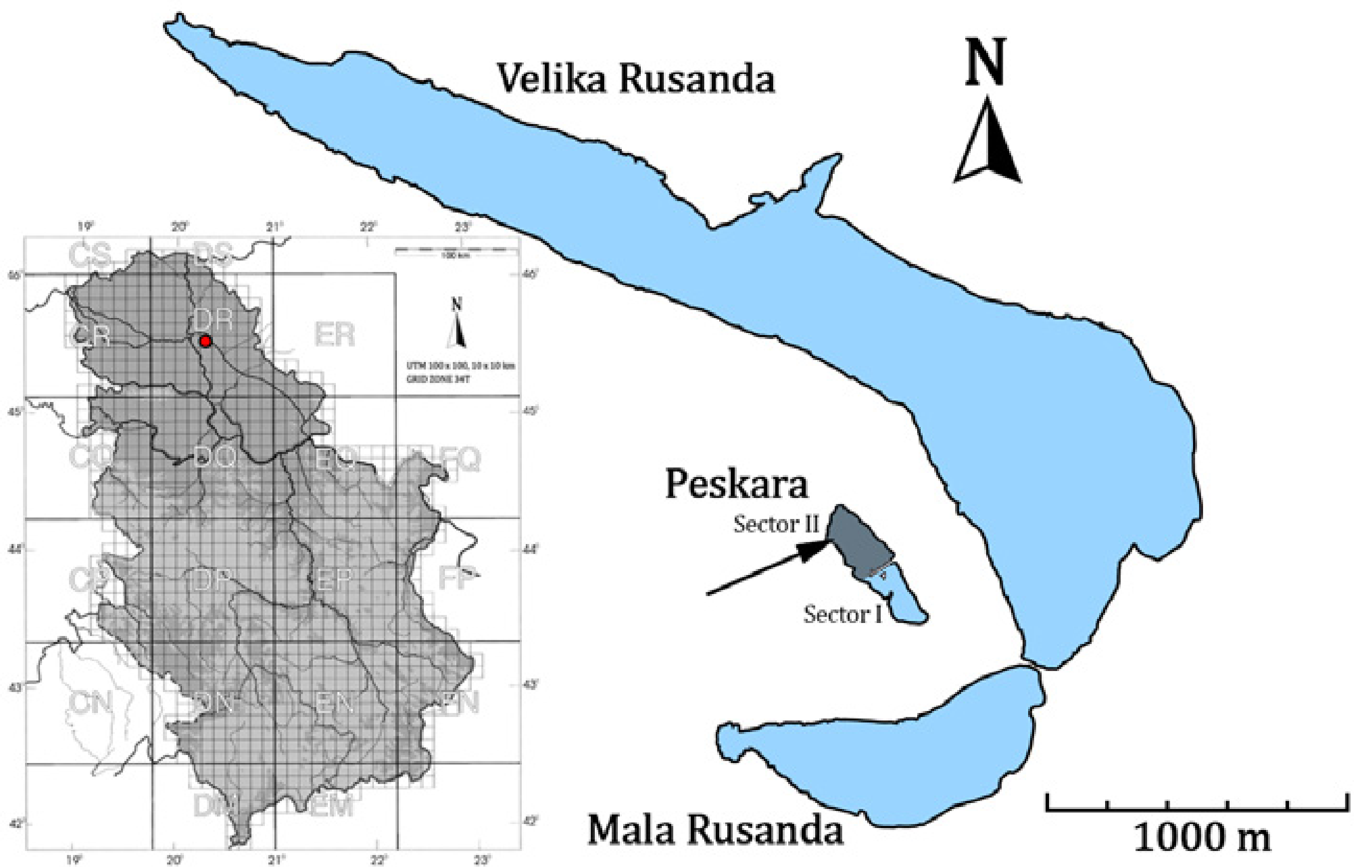

Figure 1. Location of the sampling site. Black dot denotes position of the study site on UTM map. Black arrow indicates the deeper part of the sandpit lake "Peskara" where the sampling was conducted.

Rusanda and Lake Mala Rusanda (Figure 1). Description of the sandpit lake origin is given in the document published by the Institute for Nature Conservation of the Vojvodina Province (2011). Since the data on the sandpit lake morphometry were not available, we conducted measurements of depth at several sites along two axes inside the lake (Figure 1). The deeper part of the lake (Sector II) is 205 m long, approximately 107 m wide, with the maximum and average depth of 6.6 m and 4.4 m respectively.

Physical and physico-chemical parameters

Several water quality parameters were measured *in situ*: water temperature (T), pH, conductivity (COND), and dissolved oxygen (DO). T, pH, and COND were monitored using Water Multimeter 18.52.01 (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) and DO was measured with DO meter HI9147 (Hanna Instruments, Woonsocket, USA). Samples for chemical analyses of different cations and anions were collected with two 0.5-L plastic bottles. The concentrations of different metals (Na^+, K^+, Ca^{2+} and Mg^{2+}) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK) according to the procedure described in Vidaković et al. (2019). The water NH_4^+ content was measured using 930 Compact IC Flex ion chromatograph (Metrohm, Herisau, Switzerland) following SRPS EN ISO 14911:2009. The determination of NO_2^-, NO_3^- and PO_4^{3-} was done using the same ion chromatograph according to U.S. EPA (1997). The total phosphorus (TP) concentration was measured with UV/Vis spectrophotometer Specord 50 (Analytic Jena, Jena, Germany) following SRPS EN ISO 6878:2008. The calculation of CO_3^{2-} and HCO_3^- was based on p– and m–alkalinity that was determined by the titration method (APHA, AWWA & WPCF 1995a). Cl^- content was determined by the argentometric method following APHA, AWWA & WPCF (1995b). Finally, the determination of SO_4^{2-} concentration was done using the gravimetric method with the ignition of residue according to APHA, AWWA & WPCF (1995c).

Sampling and identification of *Botryococcus terribilis*

For the purpose of alga identification, a 10-L water sample was taken and filtered through a plankton net (mesh size of 25 μm, HYDRO-BIOS Apparatebau GmbH, Altenholz, Germany). Identification of *B. terribilis* was done using Leica DM750 microscope (Leica Microsystems) with objective HI PLAN 40/0.65 (at 40× magnification) according to the following literature: John et al. (2011) and Komárek and Marvan (1992). Microphotographs of *B. terribilis* were made using Zeiss Axioimager.M1 microscope with AxioVision 4.9 software at 400× magnification.

Counting of *B. terribilis* colonies was performed according to Utermöhl (1958). Firstly, 10 mL subsample was taken from 0.5-L unfiltered water sample and left in the HYDRO-BIOS sedimentation chamber for 48 hours. Calculation of *B. terribilis* abundance was done after counting its colonies using the inverted microscope INVE 500T (COLO Lab Experts, Novo Mesto, Slovenia) with objectives 20× and 40×. The abundance of *B. terribilis* was expressed as a number of colonies per ml.

Algal cells preparation for transmission electron microscopy

The liquid algal sample was fixed in 5% glutaraldehyde, rinsed toughly with phosphate buffer, embedded in agar and postfixed in osmium tetroxide in the same buffer for 2h. Afterwards, samples were dehydrated through ethanol (30–100%) and routinely embedded in Araldite. Ultrathin sections (100 nm thickness) were obtained using a Leica UC6 ultramicrotome (Leica Microsystems, Germany), mounted on copper grids, contrasted in uranyl acetate and lead citrate using Leica EM STAIN (Leica Microsystems), and examined on a Philips CM12 transmission electron microscope (Philips/FEI, Eindhoven, The Netherlands) equipped with the digital camera SIS MegaView III (Olympus Soft Imaging Solutions, Münster, Germany).

RESULTS

Physical and chemical analysis of water

The sandpit lake "Peskara" is subsaline (according to classification given by Hammer, 1986) and alkaline pond filled with water that can be classified into sodium bicarbonate chemical type. The first sampling, when only a few water quality parameters were determined, was conducted in October 2019 when the temperature was higher (17 °C) (Table 1). A more detailed water analysis conducted in December 2019, and May and July 2020 revealed that among cations and anions sodium and bicarbonate were dominant, respectively. In all samples, pH values were high indicating alkaline conditions. When it comes to the nutrient content, all nitrogen compounds (NH_4^+, NO_2^-, NO_3^-) and PO_4^{3-} were under the detection limit. TP level indicated mesotrophic conditions according to OECD (1982).

Table 1. Physical and chemical characteristics of water in the small sand pit lake "Peskara" during the sampling in 2019 and 2020 (TRANS Transmission; T Water temperature; COND Conductivity; DO Dissolved oxygen; TP Total phosphorus)

Parameter	Unit	October 2019	December 2019	May 2020	July 2020
TRANS	m	–	–	0.85	0.89
T	°C	17.0	6.7	20.9	25.1
pH		8.95	8.99	8.97	9.06
COND	µS/cm	1860	1895	1947	1952
DO	mg/L	12.2	16.8	9.1	10.5
Na^+	mg/L	–	173.30	428.3	–
K^+	mg/L	–	3.76	8.60	–
Ca^{2+}	mg/L	–	5.58	13.75	–
Mg^{2+}	mg/L	–	17.09	32.52	–
NH_4^+	mg/L	–	<0.05	<0.05	–
NO_2^-	mg/L	–	<0.02	<0.02	–
NO_3^-	mg/L	–	<0.5	<0.5	–
PO_4^{3-}	mg P/L	–	<0.02	<0.02	–
TP	mg P/L	–	0.027	0.024	–
CO_3^{2-}	mg/L	–	118.2	174.0	–
HCO_3^-	mg/L	–	723.5	750.3	–
Cl^-	mg/L	–	99.1	102.0	–
SO_4^{2-}	mg/L	–	60.0	70.6	–

Morphometric analysis and abundance of *B. terribilis*

The young colonies were more or less spherical. Older ones, with an irregularly ovoid shape, were 17.5–115.0 µm long and 17.5–77.5 µm wide (n=30), and composed of several sub-colonies that are joined with short mucilaginous connections. Cells were completely embedded within the wrinkled mucilage with numerous oil droplets that helped a colony to float (Figure 2. A, B).

The mucilage was indistinctly layered, yellow or orange, with short and simple gelatinous processes on its margin. Cells had obovoid shape and were usually radially oriented (Figure 2. C). Cells were 5.5–8.0 µm long and 3.5–5.0 µm wide (n=15). The abundance of *B. terribilis* in December 2019, May 2020 and July 2020 was 132, 45, and 20 colonies per ml, respectively.

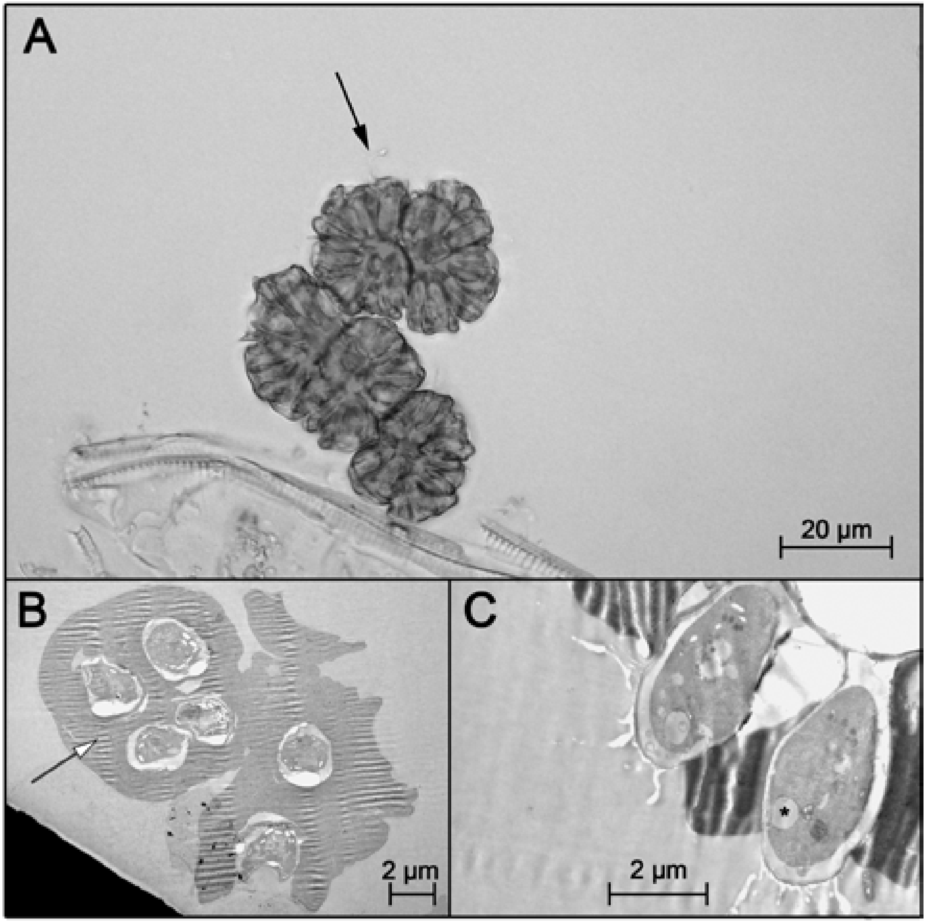

Figure 2. Microphotographs of *B. terribilis*: **A** Light microscopy of colony collected in July 2020, branched gelatinous processes at the periphery (black arrow); **B**, **C** Transmission electron microscopy (TEM) of colonies collected in July 2020. **B** showing colony in cross section with cells completely embedded within the wrinkled mucilage (white arrow); **C** cells in longitudinal section with lipid bodies (asterisk)

DISCUSSION

The species that we found during our long-term algal survey of salt alkaline ponds in the Vojvodina Province, had different morphological and ecological features than *B. braunii*. The colonies found in the sandpit lake Peskara appear to belong to species *B. terribilis* since the cup-shaped, mucilaginous sheath around cells, a characteristic of *B. braunii*, was absent and cells were completely hidden within extracellular matrix. In their study of eight *B. terribilis* strains isolated from freshwaters of Romania, Hegedűs et al. (2015) also noticed colonies composed of tight subcolonies with cells completely or partially embedded in the hydrocarbon matrix. Moreover, in our study short, branched mucilaginous processes were observed on the colony margin. *B. neglectus* is another species that forms similar gelatinous processes at the periphery, but compared to *B. terribilis* it has smaller colonies and cells (Komárek and Marvan, 1992; De Queiroz Mendes et al., 2012). Finally, chemical analysis of water in Peskara revealed the existence of alkaline conditions that are favourable for the development of *B. terribilis.* It is possible that the species of the genus *Botryococcus* found earlier in some alkaline lakes in Serbia and designated as *B. braunii* was, in fact, *B. terribilis*. Therefore, only a comparative analysis based on molecular and ultrastructural evidence of strains isolated from different lakes in Serbia can give an unambiguous answer to this question.

In the course of our study, we regularly reported the presence of *B. terribilis* in the sandpit lake Peskara. In October 2019, *B. terribilis* formed an orange-red surface scum, but later, especially in summer 2020, the number of colonies decreased. It is known that buoyant colonies of *Botryococcus* may stay in the water column long after the maximum growth period and even be abundant under conditions which are no longer optimal for them (Tyson, 1995). Thus, it is possible that *Botryococcus* bloom was an accidental event, not so frequent in this lake.

A recent study focused on hydrocarbon biosynthesis in *B. terribilis* strain collected in adjacent Romania revealed that this taxon has similar properties as the most extensively studied *B. braunii* (Szöke-Nagy et al., 2020). Since our finding of *B. terribilis* is the first report of this alga in Serbia, further work should be focused on the isolation of *B. terribilis* strain from the sandpit lake "Peskara" and characterisation of its lipid molecules.

CONCLUSION

The green alga *B. terribilis* was discovered and identified for the first time in Serbia in the small sandpit lake in the Rusanda Nature Park. Its ecology and potential application in biotechnology is still insufficiently known and should be further investigated.

ACKNOWLEDGEMENTS

This study was financially supported by the Serbian Ministry of Education, Science, and Technological Development (Grant No. OI172001, Grants No. 451-03-68/2020-14/200026 and 451-03-68/2020-14/200178). Transmission electron microscope analysis was done in the Center for electron microscopy at the University of Belgrade – Faculty of Biology. Finally, we would like to thank professor Zlatko Levkov for his valuable advice, and professor Lothar Krienitz for his help in confirming the identity of the species.

REFERENCES

APHA, AWWA, WPCF (1995a): Method 2320 B. In: Eation AD, Clesceri LS & Greenberg AE. (eds.), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 2–26.

APHA, AWWA, WPCF (1995b): Method 4500-Cl^- B. In: AD Eation, LS Clesceri & AE Greenberg (eds.), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 4–49.

APHA, AWWA, WPCF (1995c): Method 4500-SO_4^{2-} C. In: AD Eation, LS Clesceri & AE Greenberg (eds.), *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 4–135.

Chiang IZ, Huang WY, Wu JT (2004): Allelochemicals of *Botryococcus braunii* (Chlorophyceae). *J. Phycol*. 40: 474–480.

De Queiroz Mendes MC, González AAC, Moreno MLV, Figueira CP, De Castro Nunes JM (2012): Morphological and ultrastructural features of a strain of *Botryococcus terribilis* (Trebouxiophyceae) from Brazil. *J. Phycology* 48: 1099–1106. [DOI: 10.1111/j.1529-8817.2012.01181.x]

Demura M, Ioki M, Kawachi M, Nakajima N, Watanabe MM (2014): Desiccation tolerance of *Botryococcus braunii* (Trebouxiophyceae, Chlorophyta) and extreme temperature tolerance of dehydrated cells. *J. Appl. Phycol*. 26: 49–53.

Đukić N, Pujin V, Maletin S, Gajin S, Gantar M, Petrović O, Ratajac R, Seleši Đ, Matavulj M (1991a): Eutrofizacija stajaćih voda Vojvodine – "Borkovac" (Lentic waters eutrophication in Vojvodina – "Borkovac"). *Godišnjak Vodoprivrede Vojvodine* I: 4–6.

Đukić N, Pujin V, Maletin S, Gajin S, Gantar M, Petrović O, Ratajac R, Seleši Đ, Matavulj M (1991b): Eutrofizacija stajaćih voda Vojvodine – "Palić" (Lentic waters eutrophication in Vojvodina – "Palić"). *Godišnjak Vodoprivrede Vojvodine* I, 52–57.

Fanés Treviño I, Sánchez Castillo P, Comas González A (2009): Contribution to the taxonomic study of the family Botryococcaceae (Trebouxiophyceae, Chlorophyta) in southern Spain. *Cryptogam. Algol*. 30: 17–30.

Guelmino J (1973): Zenta és környékének növényei II. Virágtalanok (Plants of Senta and its surroundings II. Non-flowering plants). *Građa za monografiju Sente* 13: 39–102.

Hammer UT (1986): *Saline lake ecosystems of the world*. Springer Science & Business Media.

Hegedűs A, Mocan A, Barbu-Tudoran L, Comon C, Drugă B, Sicora C, Dragos N (2015): Morphological, biochemical, and phylogenetic assessments of eight *Botryococcus terribilis* strains collected from freshwaters of Transylvania. *J. Appl. Phycol*. 27: 865–878. [DOI: 10.1007/s10811-014-0387-2]

Institute for Nature Conservation of Vojvodina Province (2011): *Studija zaštite: Park Prirode "Rusanda" – Predlog za stavljanje pod zaštitu kao zaštićenog područja II kategorije*. Novi Sad, Srbija.

Janse van Vuuren S, Levanets A (2019): First record of *Botryococcus braunii* Kützing from Namibia. *Bothalia* 49: 1–5.

Jeppesen E, Meerhoff M, Jacobsen BA, Hansen RS, Søndergaard M, Jensen JP, Lauridsen TL, Mazzeo N, Branco CWC (2007): Restoration of shallow lakes by nutrient control and biomanipulation–the successful strategy varies with lake size and climate. *Hydrobiologia* 581: 269–285. [DOI: 10.1007/978-1-4020-6158-5_28]

John DM, Whitton BA, Brook AJ (2011): *The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae.* Cambridge: Cambridge University Press.

Komárek J, Marvan P (1992): Morphological differences in natural populations of the genus *Botryococcus* (Chlorophyceae). *Arch. Protistenkunde* 141: 65–100. [10.1016/S0003-9365(11)80049-7]

Maletin S, Pujin V, Kostić D (1994): The diet, growth and fecundity of the allochtonous planktivorous fish in the Danube. In: DV Janković, MŽ Jovičić (eds.), *The Danube in Yugoslavia – contamination, protection and exploitation*. Belgrade: Institute for Biological Research "Siniša Stanković", Institute for Development of Water Resources "Jaroslav Černi", 174–184.

Metzger P, Largeau C (2005): *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl. Microbiol. Biotechnol.* 66: 486–496. [DOI: 10.1007/s00253-004-1779-z]

Milovanović D, Živković A (1953): Ispitivanja planktonske produkcije u ribnjacima Ečke, prilog regionalnoj limnologiji stajaćih voda Panonske nizije [Plankton production investigations in Ečka fishponds, contribution to regional limnology of stagnat waters in the Pannonian plain]. *Zbornik radova* XXIX, Institut za ekologiju i biogeografiju SAN, 3: 197–264.

Obušković Lj (1982): Dinamika fitoplanktona i nekih ekoloških faktora kao odraz eutrofizacije u Savskom jezeru kod Beograda [Dynamics of phytoplankton and some ecological factors as an eutrophication effect in the Sava Lake near Beograd]. *Vodoprivreda* 14: 123–128.

OECD (Organization for Economic Cooperation and Development) (1982): Eutrophication of waters. Monitoring, assessment and control. Paris, France: OECD.

Pujin V, Đukić N, Maletin S, Miljanović B, Ivanc A (1996): Hidrobionti kao pokazatelji kvaliteta vode Jegričke [Hydrobionts as indicators of water quality of Jegrička water]. In: L. Lazić (ed.), *Jegrička*, Edicija Tija Voda I, PČESA, Novi Sad, Serbia, 69–78.

Pujin V (1998): Sastav planktona u nekim kanalima hidrosistema DTD [Plankton composition of some channels in DTD hydrosystem]. In: L. Lazić (ed.), *Mostonga i vode Zapadne* Bačke, Edicija Tija Voda II, PČESA, Novi Sad, Serbia, 54–57.

Rai UN, Dwivedi S, Baghel VS, Tripathi RD, Shukla OP, Shukla MK (2007): Morphology and cultural behavior of *Botryococcus protuberans* with notes on the genus. *J. Environ. Biol.* 28: 181–184.

Seleši Đ (1981): Limnološka istraživanja Ludoškog jezera [Limnological investigations of Lake Ludoško]. *Vode Vojvodine* 9: 333–352.

Seleši Đ (1982): Limnološka istraživanja Palićkog jezera [Limnological studies on Lake Palić]. *Vode Vojvodine* 10: 345–368.

Senousy HH, Beakes GW, Hack E (2004): Phylogenetic placement of *Botryococcus braunii* (Trebouxiophyceae) and *Botryococcus sudeticus* isolate UTEX 2629 (Chlorophyceae). *J. Phycol.* 40: 412–423. [10.1046/j.1529-8817.2004.03173.x]

Shimamura R, Watanabe S, Sakakura Y, Shiho M, Kaya K, Watanabe MM (2012): Development of *Botryococcus* seed culture system for future mass culture. *Procedia Environ. Sci.* 15: 80–89. [DOI: 10.1016/j.proenv.2012.05.013]

SRPS EN ISO 6878:2008: *Water Quality – Determination of phosphorus – Ammonium molybdate spectrometric method (ISO 6878:2004)*. Institute for Standardization of Serbia, Belgrade.

SRPS EN ISO 14911:2009: *Water Quality – Determination of dissolved* Li^+, Na^+, NH_4^+, K^+, Mn^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+} *and* Ba^{2+} *using ion chromatography – Method for water and waste water*. Institute for Standardization of Serbia, Belgrade.

Szemes G (1967): Systematisches Verzeichnis der Pflanzenwelt der Donau mit einer zusammenfassenden Erläuterung. In: R. Liepolt (ed.), *Limnologle der Donau*, Stuttgart, Germany: E. Schweizerbart'sche Verlagsbuchhandlung, 70–131.

Szöke-Nagy T, Porav SA, Dragoș N (2020): In silico modeling and analysis of squalene synthase-like 1 (SSL-1) enzyme from green microalga *Botryococcus terribilis* AICB 872. *Studia UBB Biologia* 65: 5–18.

Townsend SA (2001): Perennial domination of phytoplankton by *Botryococcus* and *Peridinium* in a discontinuously polymictic reservoir (tropical Australia). *Arch. Hydrobiol.* 151: 529–548. [DOI: 10.1127/archiv-hydrobiol/151/2001/529]

Trbojević I (2018): *Analiza perifitona sa veštačkih podloga iz Savskog jezera i jezera Vrutci* [Analysis of periphyton developed on artificial substrates in the Sava lake and the Vrutci lake]. PhD thesis. Faculty of Biology, University of Belgrade, Serbia.

Tyson RV (1995): Distribution of the Palynomorph Group: Phytoplankton Subgroup, Chlorococcale Algae. In: RV Tyson (ed.), *Sedimentary Organic Matter*, Springer, Dordrecht, 309–317.

U.S. EPA 1997: *Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography*. Revision 1.0, Cincinnati, OH.

Utermöhl H (1958): Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel [Methods of collecting plankton for various purposes are discussed]. *Mitt. – Int. Ver. Theor. Angew. Limnol.* 9: 1–38. [DOI: 10.1080/05384680.1958.11904091]

Vidaković D, Krizmanić J, Dojčinović B, Pantelić A, Gavrilović B, Živanović M, Novaković B, Ćirić M (2019): Alkaline soda Lake Velika Rusanda (Serbia): the first insight into diatom diversity of this extreme saline lake. *Extremophiles* 23: 347–357. [DOI: 10.1007/s00792-019-01088-6]

Wake LV, Hillen LW (1981): Nature and hydrocarbon content of blooms of the alga *Botryococcuc braunii* occuring in Australian freshwater lakes. *Aust. J. Mar. Freshw. Res.* 32: 353–367.

ПОЈАВА АЛГЕ *Botryococcus terribilis* Komárek & Marvan У ПЕСКАРИ – ПРВИ НАЛАЗ ЗА СРБИЈУ

Милош ЋИРИЋ[1], Јелена КРИЗМАНИЋ[2], Биљана П. ДОЈЧИНОВИЋ[1], Бојан ГАВРИЛОВИЋ[3], Далибор М. МАРИНКОВИЋ[1]

[1] Универзитет у Београду, Институт за хемију, технологију и металургију, Институт од националног значаја за Републику Србију, Његошева 12, Београд 11000, Србија
[2] Универзитет у Београду, Биолошки факултет, Институт за ботанику и ботаничка башта „Јевремовац”, Таковска 43, Београд 11000, Србија
[3] Српска академија наука и уметности, Географски институт „Јован Цвијић”, Департман за физичку географију, Ђуре Јакшића 9, Београд 11000, Србија

РЕЗИМЕ: Алкалне слане баре и језера су станишта са јединственим биодиверзитетом. Зелена алга из рода *Botryococcus* је случајно откривена током алголошких истраживања ових станишта у Србији. Врста *B. terribilis* је пронађена и по први пут идентификована у Србији у Пескари у Парку природе „Русанда”. Прво узорковање током кога је на површини воде забележена наранџасто-црвена скрама непознатог порекла било је непотпуно. Након што је врста идентификована спроведена су још три додатна узорковања с циљем сакупљања фитопланктона и воде за физичко-хемијске анализе. Анализирани су доминантни анјони и катјони како би се утврдио хемијски тип воде у Пескари, али и садржај нутријената. Утврђено је да је вода у Пескари алкална и да припада натријум-бикарбонатном хемијском типу. Морфометријска анализа *B. terribilis* је изведена светлосном и трансмисионо-електронском микроскопијом. *B. terribilis* је био нарочито бројан у фитопланктонској заједници током касне јесени и и току зиме. Врсте рода *Botryococcus* су познате као богати извори различитих липида, па ово откриће може бити први корак у примени ове врсте у одрживој производњи биогорива.

КЉУЧНЕ РЕЧИ: Пескара, *Botryococcus terribilis*, фитопланктон