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Diversity of the Euglenophyta division in the Zasavica River, Serbia

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ABSTRACT: The Zasavica River has an extraordinary richness of Euglenophyta algae. In general, those algae are cosmopolitan and very common. They usually inhabit stagnant and slow-flowing waters with high content of organic matter during the summer months. Euglenophyta of the Zasavica River in Serbia were studied at two localities ("Molo" and "Mostić") from December 2012 to November 2013. A total of 89 taxa were recorded. The genus with the highest diversity was *Euglena* Ehr.(28), followed by *Trachelomonas* Ehr. (25), *Phacus* Duj. (24), *Lepocinclis* Perty (11) and *Strombomonas* Defl. (1). The highest number of taxa (27) was recorded at "Molo" during August 2013. Nine Euglenophyta taxa found in this research are new for algal flora of Serbia.

Key words: Euglenophyta, Zasavica, Serbia

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INTRODUCTION

The Zasavica River is located in the municipality of Sremska Mitrovica and Bogatić and surrounding area occupies an area of 109 km^2. It is a part of the Special Nature Reserve "Zasavica", which covers the area of northern Mačva, east of the Drina River, and south of the Sava River. Zasavica River basin is five to six kilometers wide and consists of the Zasavica River and its tributaries Batara and Žurava. The river is 33.1 km long, with a maximum width that reaches up to 80 m. Its depth at medium water level is 2.5 m (Stanković 2006). The river is mostly surrounded by marshes which indicates that the Zasavica River is powered by groundwater from the Drina and Sava Rivers and for that reason Zasavica can be classified both as a flowing and a standing water.

This area was put under protection in 1997, which was proposed by the Institute for Protection of Nature of Serbia, to preserve the river and its coastal areas, as a natural asset of greatest importance. This calm flatland river provides conditions for survival of numerous and diverse wildlife. The reserve is characterized with very high biodiversity.

Photoautotrophic euglenophites are cosmopolitan and very common. Most of them are free-swimming and unicellular. They are most frequently found in slow-flowing and stagnant waters, rich with organic substances, during the summer months when the water temperature is raised and the light levels are high (Subakov-Simić 2006).

There are numerous papers from the previous floristic, taxonomic and ecological studies in Serbia that contain information about taxa from Euglenophyta division. However, only a few of them deal with this division specifically. The first papers in which the presence of Euglenophyta in Serbia were noted are a hydrobiology study of Daićko Lake and a hydrobiology study of Vlasinsko blato by Košanin (1908a,b).

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MATERIALS AND METHODS

The samples for physicochemical and phytoplankton analyses from the Zasavica River were taken once a month from December 2012 to November 2013 at two study sites, "Molo" (44°57'26.14" N and 19°31'37.58" E) and "Mostić" (44°56'59.26" N and 19°29'38.84" E).

The main physical parameters were measured using standard analytical methods and instruments: temperature with a multi digital thermometer, electrical conductivity with ECTestr 11+ multi range and pH value and dissolved oxygen with a WTW multi 3430 multiparameter meter. The transparency of the river was measured by Secchi disc.

The samples for quality analysis were collected at "Molo" and "Mostić" study sites by towing a plankton net (pore diameter of 22 μm) through the open water. In the field, phytoplankton samples from each study site were placed into two plastic bottles (100 ml). The sample in the first plastic bottle was preserved at once with Lugol's solution, while the sample in the second plastic bottle was not preserved immediately, but after the exploration. So, for algal research there were both preserved and non preserved samples. The metaphyton samples were collected by towing a plastic bottle (100 ml) through the water near the bank amidst the submerged vegetation. These samples were fixed with Lugol's solution immediately.

Algological material was analyzed using a Carl Zeiss AxioImager M1 microscope and digital camera AxioCam MRc5 with AxioVision 4.8 software, so some of the taxa were photographed. Taxonomic identifications of Euglenophyta were made according to widely used taxonomic keys: Starmach (1983), Popova (1966), Nemeth (1997a, 1997b), and Wolowski (1998).

Phytoplankton samples for quantitative analysis were collected by using a Ruttner's bottle (1 l volume). Samples were fixed at once with Lugol's solution in 1 l plastic bottles. Phytoplankton quantitative analysis was made using the Utermöhl method (Utermöhl 1958) with a Leica inverted microscope. The results were expressed as number of cells per l and number of individuals per l.

All samples were analyzed and stored in the Institute of Botany and Jevremovac Botanical Garden, Faculty of Biology, University of Belgrade, Belgrade.

RESULTS AND DISCUSSION

Physicochemical analysis. During the study period the water level was variable, mostly low. From December 2012 to June 2013 the water level increased and then decreased from July to November 2013. The highest transparency and the maximum depth were recorded in May (170 cm) at both study sites. The lowest transparency (20 cm) at the "Mostić" site was detected in July while its depth was the least (40 cm) in October. At the "Molo" site, the lowest transparency of 90 cm was recorded in February, while the depth was the least (95 cm) in October and November.

Water temperature varied from 0.6°C in December 2012 to 28.3°C in July 2013 at the "Molo" site, while the "Mostić" site showed fewer temperature fluctuations during the study period (from 2.1°C in December 2012 to 25.0°C in July 2013). The higher the temperature, the greater the diversity of Euglenophyta taxa detected. Ice cover was detected at both study sites in December 2012. The thickness of ice was 7 cm at "Molo" and 10 cm at "Mostić".

The water pH varied from 7.6 to 8.82, so the water of the Zasavica River was slightly alkaline. Water conductivities of the Zasavica River could be characterized as medium-high (from 250 μS/cm to 820 μS/cm). Water conductivity increased from December 2012 to June 2013 and then decreased from July 2013 to November 2013.

Dissolved O_2 varied from 0.24 mg/l in August 2013 to 15.1 mg/l in December 2012 at "Molo" and from 1.59 mg/l in June 2013 to 15.2 mg/l in January 2013 at "Mostić". Lower dissolved oxygen concentrations indicate an increased organic load which favours development of algae from the Euglenophyta division.

Phytoplankton analysis. A total of 89 taxa from the Euglenophyta division was identified in the algological samples from the Zasavica River. The list of all identified Euglenophyta taxa in the Zasavica River is given in Table 1 and photographs of some taxa are in Figure 1. The genus *Euglena* had the highest number of taxa (28 taxa), followed by the genus *Trachelomonas* (25 taxa). The highest diversity of this division per water sample was recorded at "Molo" in August 2013, with 27 taxa from which the highest percent (48.1 %) belonged to the genus *Euglena*. Of these 89 taxa found in the Zasavica River, nine were new to the algal flora of Serbia (marked with an asterisk in Table 1). The most frequently found taxon was *Trachelomonas volvocina* var. *volvocina* (present in all samples except those from January).

Protić (1933, 1935, 1936, 1939), Milovanović & Živković (1953a, 1953b, 1956, 1963), Obušković (1977, 1982, 1986, 1992, 1993, 1994), and Urošević (1989, 1990a, 1990b, 1994, 1996, 1997a, 1997b, 1998a, 1998b, 1998c) have contributed the most to research of Euglenophyta in Serbia.

From 391 taxa of the Euglenophyta division which have been recorded in previous studies (Subakov-Simić 2006), only 20 taxa have been recorded previously in the Zasavica River (Branković *et al.* 1996). In our research, 14 of these 20 taxa recorded earlier were found, while *E. intermedia, E. proxima, L. fusiformis* var. *podolica* as *L. sphagnophila* var. *podolica, Ph. helicoides, T. ensifera and T. granulata* were not found. The number of new taxa for Serbia with these findings raises the total number of Euglenophyta taxa recorded in Serbia to 400. Thus, 75 taxa of the 89 taxa found in this research have not previously been recorded for the Zasavica River. So, the total number of Euglenophyta taxa recorded in the Zasavica River is 95.

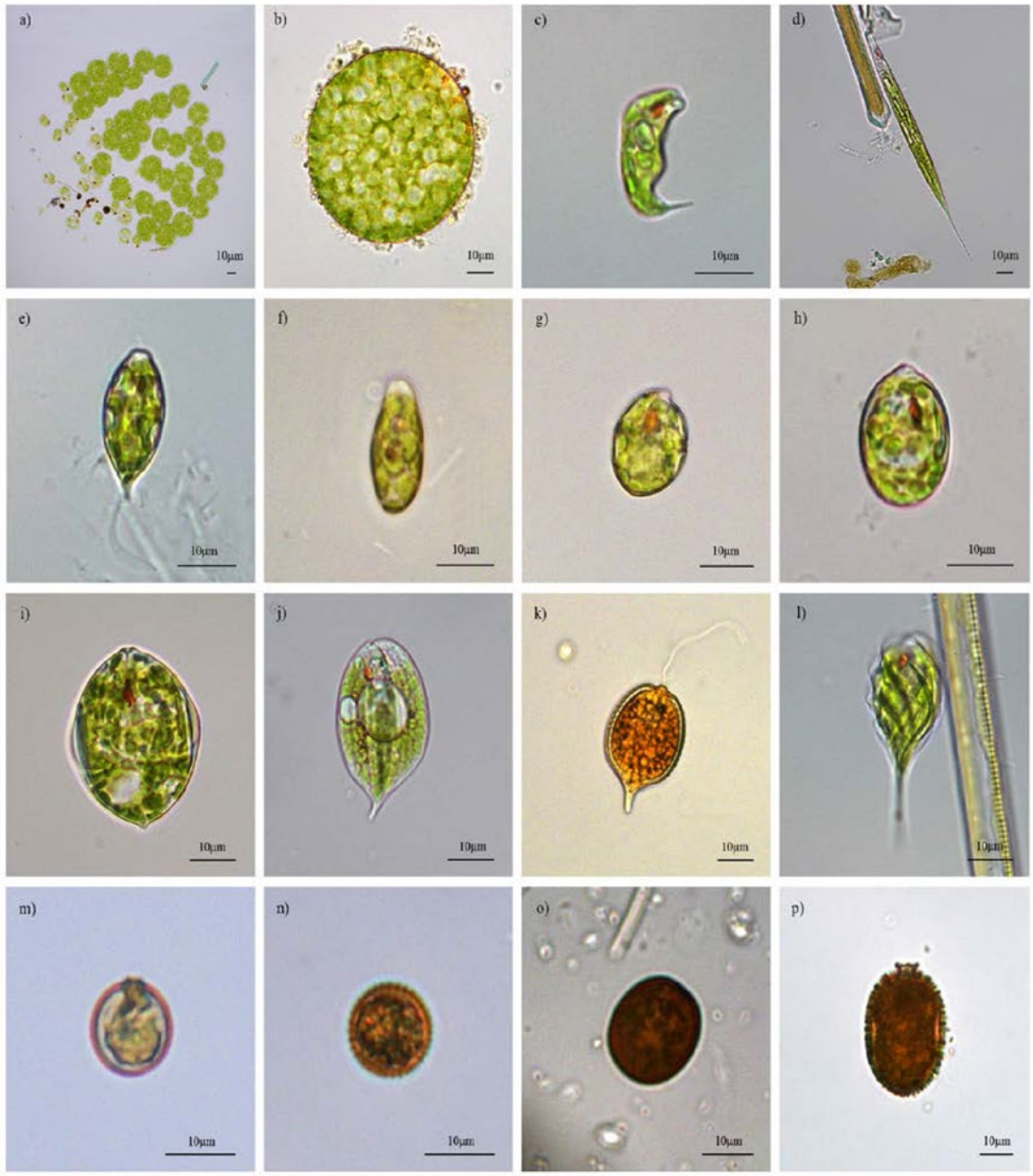

Figure 1. Pictures of some recorded Euglenophyta taxa in the Zasavica River during the studied period: a) reproductive cyst of Euglenophyta b) protective cyst of Euglenophyta c) *Euglena excavata* d) *Euglena acus* var. *acus* e) *Lepocinclis acicularis* f) *Euglena bellovacensis* g) *Phacus corculum* h) *Lepocinclis globosa* i) *Lepocinclis ovum* var. *ovum* j) *Phacus triqueter* k) *Phacus monilatus* l) *Phacus pyrum* m) *Trachelomonas volvocina* var. *volvocina* n) *Trachelomonas woycickii* fo. *pusilla* o) *Trachelomonas dybowskii* var. *dybowskii* p) *Trachelomonas sydneyensis* var. *sydneyensis*

For the phytoplankton community in the Zasavica River, the Euglenophyta division is not quantitatively very important. This division was the most abundant in May 2013 at "Molo" when it occupied 35 % (Figure 2) of the total number of cell per liter of phytoplankton, but most of the time that percentage was much lower. The highest abundance of taxa belonging to this division was in the summer months due to physicochemical conditions, which coincide with their ecology (Starmach 1983).

The maximal abundance (254000 cell/l) of Euglenophyta in the Zasavica River was recorded in August 2013 at the "Molo" site. The abundance of Euglenophyta (Figure 3) ranged from a minima of 400 cell/l in November 2013 at "Molo" and 2800 cell/l in October at "Mostić" to maxima of 254000 cell/l in August 2013 at "Molo" and 92000 cell/l in July 2013 at "Mostić".

The presence of reproductive cysts (palmeloid stadium) of taxa belonging to this division in August 2013 indicates

Table 1. The list of all identified Euglenophyta taxa from December 2012 to November 2013 in the Zasavica River. The new taxa for Serbia are marked with an asterisk.

Taxa	Taxa
Euglena acus var. *acus* Ehr.	* *Ph. corculum* Pochm.
E. acus var. *hyalina* Klebs	*Ph. inconspicuus* Def.
E. agilis Carter	*Ph. longicauda* var. *insecta* Koczw.
E. anabaena Mainx	*Ph. longicauda* var. *tortus* Lemm.
E. anabaena var. *minima* Mainx	*Ph. megalopsis* Pochm.
* *E. bellovacensis* Chad. & Gojd.	* *Ph. monilatus* Stokes
E. caudata var. *caudata* Hüb.	*Ph. orbicularis* fo. *orbicularis* Hüb.
E. caudata var. *minor* Def.	*Ph. orbicularis* fo. *communis* Pop.
E. clara Skuja	*Ph. orbicularis* fo. *gigas* (da Cun.) Pop.
E. clavata Skuja	*Ph. parvulus* Klebs
E. deses fo. *klebsii* (Lemm.) Pop.	*Ph. pleuronectes* var. *hamelii* (Allo. & Lef.) Pop.
E. ehrenbergii Klebs	*Ph. pleuronectes* var. *pleuronectes* (Müll.) Duj.
* *E. excavata* Schill.	*Ph. pyrum* (Ehr.) Stein.
E. gymnodinioides Zakrys	*Ph. raciborskii* Drez.
E. gracilis fo. *gracilis* Klebs	*Ph. tortuosus* Roll
E. gracilis fo. *hiemalis* (Matv.) Pop.	*Ph. triqueter* (Ehr.) Duj.
E. hemichromata Skuja	*Ph. undulatus* (Skvor.) Pochm.
E. limnophila var. *limnofila* Lemm.	*Ph. unguis* Pochm.
E. minima Francé	*Strombomonas acuminata* (Schmar.) Def.
E. oblonga Schm.	*Trachelomonas armata* var. *sparsigranosa* Play.
E. oxyuris fo. *oxyuris* Schm.	*T. bacillifera* Play.
E. pascheri Swir.	*T. dybowskii* var. *dybowskii* Drez.
Euglena Ehr. Sp.	*T. globularis* fo. *globularis* (Awer.) Lemm.
E. srinagari (Bhatia) Hüb.-Pest.	*T. granulosa* var. *granulosa* Play.
E. texta var. *texta* (Duj.) Hüb.	*T. hispida* var. *crenulatocollis* (Maskell) Lemm.
E. thinophila Skuja	*T. hispida* var. *granulata* Play.
E. variabilis Klebs	*T. hispida* var. *hispida* (Perty) Stein
E. viridis fo. *viridis* Ehr.	*T. intermedia* fo. *crenulatocollis* (Szab.) Pop.
* *Lepocinclis acicularis* Francé	*T. intermedia* fo. *intermedia* Dang.
L. elongata (Swir.) Conr.	*T. irregularis* var. *irregularis* Swir.
L. fusiformis var. *fusiformis* (Carter) Lemm.	*T. lefevrei* fo. *lefevrei* Def.
* *L. globosa* Francé	*T. nigra* Swir.
L. globula fo. *globula* Perty	*T. oblonga* var. *australica* Play.
L. marssonii var. *marrsonii* Lemm.	*T. oblonga* var. *oblonga* Lemm.
L. ovum fo. *dimidio-minor* Def.	*T. obovata* fo. *klebsiana* (Def.) Pop.
* *L. ovum* var. *maior* (Hüb.-Pest.) Conrad	*T. planctonica* fo. *oblonga* (Drez.) Pop.

Taxa	Taxa
L. ovum var. *ovum* (Ehr.) Lemm.	*T. planctonica* fo. *planctonica* Swir.
L. pseudonayalii Tell & Zalo.	*T. raciborskii* var. *incerta* Drez.
L. salina fo. *salina* Fritsch	*T. rotunda* var. *rotunda* Swir.
Phacus acuminatus var. *acuminatus* Stokes	*T. sydneyensis* fo. *sydneyensis* Play.
Ph. acuminatus var. *discifera* (Pochm.) Hüb.-Pest.	*T. verrucosa* var. *verrucosa* Stokes
Ph. ankylonoton Pochm.	*T. volvocina* var. *compressa* Drez.
Ph. brevicaudatus (Klebs) Lemm.	*T. volvocina* var. *volvocina* Ehr.
Ph. caudatus var. *caudatus* Hüb.	* *T. woycickii* fo. *pusilla* (Drez.) Pop.
* *Ph. caudatus* var. *minor* Drez.	

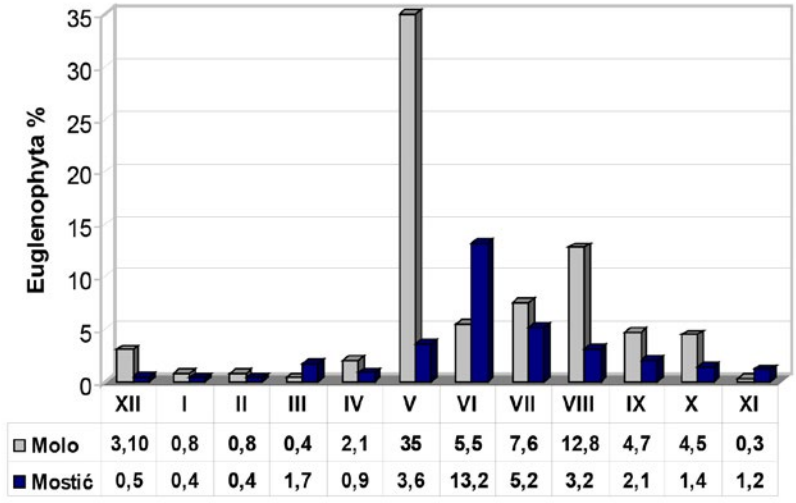

Figure 2. Percentage of the Euglenophyta division present in phytoplankton of the Zasavica River from December 2012 to November 2013.

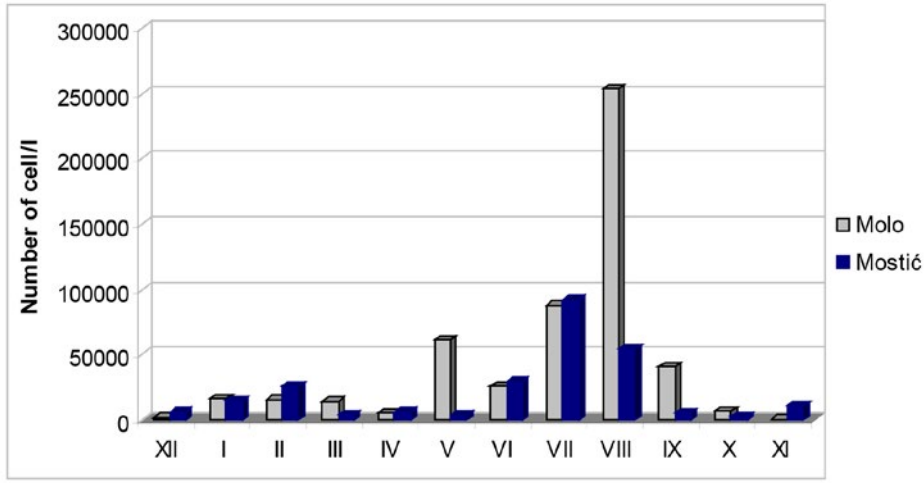

Figure 3. Quantitative presence of the Euglenophyta division in the phytoplankton of the Zasavica River from December 2012 to November 2013.

that it is the period of their reproduction. Reproductive cysts (Figure 1a) may contain a large number of cells and they are always without flagella (Buetow 1968). However, in the same month protective cysts (Figure 1b) were also recorded. It is possible that Euglenophyta taxa prepare for the forthcoming unfavourable conditions. With lower water temperature, diversity of this division declines, so only one taxon (*Trachelomonas volvocina* var. *volvocina*) was recorded in November at the "Mostić" site in a very low abundance. The most abundant taxon was *Lepocinclis ovum* var. *dimidio-minor* (120000 cell/l) in August 2013 at the "Molo" site, but *Trachelomonas volvocina* var. *volvocina* was the most frequently found taxon and quantitatively dominant among Euglenophyta taxa in many other samples from both sites (with abundance from 1280 to 66000 cell/l), followed by *Euglena hemichromata*.

Also, a significant number of Euglenophyta taxa was recorded in the metaphyton community from April to July 2013 at both study sites. The highest diversity (25 taxa) in a metaphyton was in June 2013 at "Molo".

However, taxa belonging to this division are known to be found in stagnant water, such as that of ponds, swamps, canals, ditches and lakes. Therefore, the huge number of Euglenophyta taxa found here is not surprising because the Zasavica River can be classified both as a stagnant and as a running water body.

CONCLUSION

The taxa of the Euglenophyta division are widespread. In general, they inhabit stagnant water with a high content of organic matter which coincides with our findings. They are quantitatively more abundant and have higher diversity in warmer months of the year, when the high water temperature and irradiance are suitable for their growth and development. In this period, they can be one of the primary sources of food for zooplankton.

In total, 89 taxa were recorded in this research. Together with six taxa found earlier, the total number of Euglenophyta taxa recorded in the Zasavica River is now raised to 95. Nearly a quarter of all taxa recorded in Serbia is present in the Zasavica River. Hence, it can be concluded that the Zasavica River is characterized by an extraordinary richness of algae from the Euglenophyta division.

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REZIME

Raznolikost razdela Euglenophyta u reci Zasavici (Srbija)

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Reka Zasavica odlikuje se ogromnim bogatstvom algi iz razdela Euglenophyta. Uopšteno, alge ove grupe su kosmopoliti i veoma su česte. Obično nastanjuju stajaće i sporotekuće vode sa visokim sadržajem organskih materija tokom letnjih meseci.

Alge iz razdela Euglenophyta u reci Zasavici istraživane su na dva lokaliteta („Molo" i „Mostić") od decembra 2012. godine do novembra 2013. godine. Ukupno 89 taksona je zabeleženo. Rod sa najvećim diverzitetom je *Euglena* Ehr. (28), a prate ga rodovi *Trachelomonas* Ehr. (25), *Phacus* Duj. (24), *Lepocinclis* Perty (11) i *Strombomonas* Defl. (1). Najveći broj taksona (27) zabeležen je na lokalitetu „Molo" tokom avgusta meseca 2013. godine. Sedam taksona Euglenophyta zabeleženih u ovom istraživanju je novo za floru algi u Srbiji.

Ključne reči: Euglenophyta, Zasavica, Srbija