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DIVERSITY OF FRESHWATER BRYOZOA IN A DANUBE FLOODPLAIN AREA (KOPAČKI RIT NATURE PARK, CROATIA)

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After a four-year-long investigation, we present the finding of five bryozoan species in a eutrophic lake, situated in a natural floodplain of the Danube. Of the species found, three belong to the family Plumatellidae (*Plumatella emarginata*, *P. fungosa* and *P. repens*), one to the family Paludicellidae (*Paludicella articulata*), and one to the family Cristatellidae (*Cristatella mucedo*). Only *C. mucedo* was identified based on floatoblast appearance, while the identification of other species was based on colony fragments and dormant bodies. Scanning electron microscopy was applied to show the fine morphological structure of dormant bodies. In the present research, which covered a relatively small area, we discovered almost half of the bryozoan species listed for Croatia. Our results present the great diversity of Bryozoa in a floodplain system, indicating that water bodies such as Lake Sakadaš constitute a suitable habitat for bryozoan development, due to the favourable environmental conditions and the large amount and diversity of firm substrates in the water.

Keywords: bryozoans, biodiversity, statoblasts, wetland

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U eutrofnom jezeru smještenom u prirodnom poplavnom području Dunava pronađeno je pet vrsta mahovnjaka tijekom četiri godine istraživanja. Od pronađenih pet vrsta, tri pripadaju porodici Plumatellidae (*Plumatella emarginata*, *P. fungosa* i *P. repens*), jedna vrsta porodici Paludicellidae (*Paludicella articulata*) i jedna porodici Cristatellidae (*Cristatella mucedo*). Vrsta *C. mucedo* određena je samo na osnovu prisustva floatoblasta, dok se determinacija ostalih zabilježenih vrsta temeljila na građi zadruge i statoblasta. Kako bi se odredile fine morfološke strukture statoblasta, korištena je skenirajuća elektronska mikroskopija. U ovom istraživanju, koje je obuhvatilo relativno malo područje, pronađena je gotovo polovica vrsta mahovnjaka zabilježenih u Hrvatskoj. Rezultati ukazuju na veliku raznolikost mahovnjaka u ovom poplavnom području. Jezero Sakadaš predstavlja pogodno stanište za razvoj mahovnjaka zbog povoljnih okolišnih uvjeta te velikog broja različitih tipova čvrstih supstrata u vodi.

Ključne riječi: mahovnjaci, bioraznolikost, statoblasti, vlažno područje

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INTRODUCTION

Bryozoans are aquatic sessile invertebrates widely distributed in freshwater and marine habitats. Their colonies built of zooids can be found on various submerged substrates, including natural (e.g. branches, stones, aquatic plants, underwater root systems) and artificial firm substrates (e.g. pipes, bottles, floats and tires) (Wood & Okamura, 2005; Hartikainen *et al.*, 2009). Although each zooid is only about 1 mm long, the size of bryozoan colonies can be measured in centimetres, with great biomass acquired (Wood & Okamura, 2005; Wood, 2015). Bryozoans are suspension feeding animals, every zooid has a lophophore with ciliated tentacles which create feeding currents and capture a wide variety of suspended food such as organic and inorganic particles, bacteria, microscopic algae, protozoans and smaller invertebrates like rotifers, nematodes and microcrustaceans (Wood, 2015). From the extracted particles, bryozoans produce faecal pellets which are of high nutritive value for benthic meiofauna (Bushnell & Rao, 1974; Šatkauskiene *et al.*, 2018; Wood, 2019). In bryozoan colonies, the small interspaces between the zooids are microhabitats attractive to various aquatic organisms (e.g. protozoans, rotifers, nematodes and chironomids), serving as a shelter or important feeding area (Wood & Okamura, 2005; Vidaković *et al.*, 2011; 2012; Vlaičević *et al.*, 2017).

Freshwater bryozoan species have been found in ponds, lakes, rivers and estuaries (Økland & Økland, 2005; Wöss & Walzl, 2006; Massard & Geimer, 2008). More than 80 freshwater bryozoan species are estimated to be distributed worldwide, 19 of which have been documented in European countries (Massard & Geimer, 2005; 2008; 2008a). Despite their sessile lifestyle, bryozoans have a great potential for spreading, mainly because of the ability to produce statoblasts. Statoblasts are very small and numerous dormant asexual bodies (Wood & Okamura, 2005) that are highly resistant to unfavourable environmental conditions. For example, statoblasts of most *Plumatella* and *Fredericella* species can survive 1-2 years in extreme conditions of desiccation and freezing (Bushnell & Rao, 1974). Brown (1933) documented the recovery of statoblasts of the *Fredericella*, *Plumatella* and *Pectinatella* species after they had passed through the digestive systems of vertebrates. The percentage of *Pectinatella magnifica* statoblast germination passing through salamander was the highest (3-73%), followed by statoblast germination from frog (0-69%), turtle (0-32%) and duck (0-4%) (Brown, 1933). Statoblasts can be spread by water currents, floods or via different animal vectors, primarily waterfowl, fish, amphibians and reptiles. Animals may carry statoblasts externally on their body or internally inside the digestive tract (Brown, 1933; Wood, 2002). Some bryozoan species, like *Cristatella mucedo* Cuvier, 1798, produce free statoblasts, which have hooks and spines that promote entanglement in animal fur and feathers (Bilton *et al.*, 2001). Humans may also participate in the spreading of bryozoan statoblasts, through ballast waters and sediment transport (Wood, 2015). Except via statoblasts, bryozoans can be transported over smaller or larger distances in the larval stage or as colony fragment, and even entire colonies can be transferred attached to a floating substrate (Wood, 2015).

According to the morphological characteristics there are three types of statoblasts. Floatoblasts, free statoblasts characterized by chambers filled with gas, are usually dispersed by water currents. Sessoblasts are sessile statoblasts which are not released from the colonies and remain cemented to the substrate surface. Piptoblasts stay inside the colony, though not cemented to the substrate surface, and are only known in *Fre-*

dericella species (Wood & Okamura, 2005). Because of their distinctive appearance, statoblasts are used for species identification (Reynolds, 2000; Wood *et al.*, 2006; Masard & Geimer, 2008; Šatkauskiene *et al.*, 2018).

The species *Paludicella articulata* (Ehrenberg, 1831) does not have statoblasts, but instead produces over-wintering hibernacles that have a function in dispersal similar to that of statoblasts (Økland & Økland, 2000).

Given the scarcity of the research and publication on Bryozoa species diversity and distribution, and a substantial, regular development of its colonies on various substrates in the water bodies of Kopački Rit Nature Park, we wanted to confirm which species are present in this Danube floodplain.

MATERIALS AND METHODS

Study area

The study on bryozoan fauna was carried out in Lake Sakadaš which is situated in the western part of the Kopački Rit Nature Park. Kopački Rit is an inner delta situated in north-east Croatia, between the rivers Drava and Danube (Fig. 1). The basic characteristic of this floodplain area is the dynamics of inundation, which is mainly associated with the Danube water level oscillations, and to a lesser extent with the changes in Drava water levels (Schwarz, 2005).

Lake Sakadaš, with a surface area of about 0.15 km^2 is connected with the Danube through the Čonakut and Hulovo channels (Schwarz, 2005). The lakeshore is covered with the emerged *Phragmites*, *Typha* and *Carex* macrophyte species, combined with *Populus nigra* and *Salix alba* trees. Sporadically, *Myriophyllum spicatum* and *Ceratophyllum demersum* beds were recorded during earlier surveys (Čerba *et al.*, 2009, 2010; Bogut *et al.*, 2010). Also, *Lemna* sp., *Nymphoides peltata, Polygonum amphibium, Potamogeton gramineus, Spirodela* sp. and *Trapa natans* were found in the lake (Bogut *et al.*, 2010).

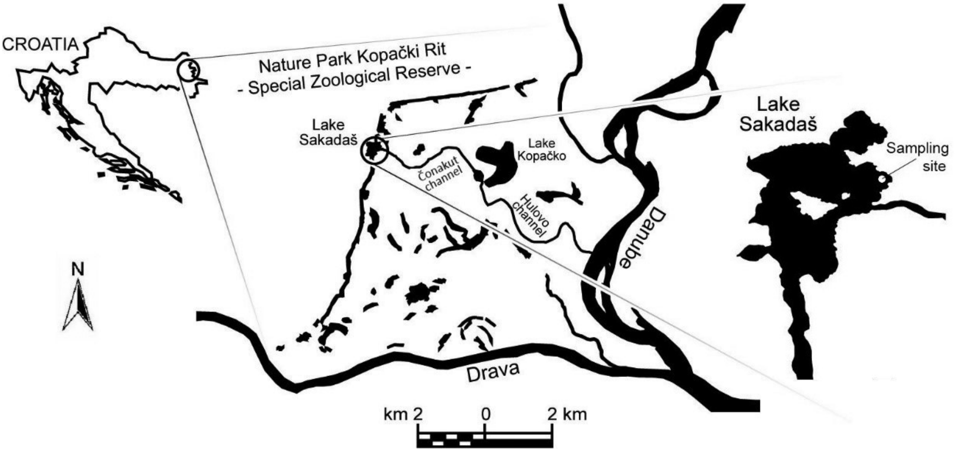

Fig. 1. Position of Lake Sakadaš in the southern part of the Kopački Rit floodplain (Special Zoological Reserve) and the sampling site located in the eastern part of the lake (white dot – picture on the right).

Bryozoa sampling

Presented data on the bryozoan diversity in Lake Sakadaš were gathered from 2007 to 2010. At the sampling site located about 10 m from the lake shoreline, modified plastic slide boxes with microscopic glass slides were immersed in the water. The glass slides were placed at a depth of 25 cm. They served as a firm substrate for bryozoan attachment and development. Slide boxes were fastened to a Styrofoam float for the first three years of the investigation, or to a wooden frame in 2010. Both the float and the frame were connected with a rope to stone blocks set on the lake bottom, thus maintaining the same position in the lake. In 2007 and 2008 only glass slides were collected. The sampling was performed in the period from May (when bryozoan colonies started to develop) till August (after that period colonies started to decay). In 2009 and 2010, in addition to glass slides, we sampled bryozoan colonies developed on various other artificial substrates present at the sampling site, including the plastic slide boxes, rope, Styrofoam float, plastic buoys and bottles, which were introduced as a part of the experimental design. The colonies that developed on natural substrates (branches, wooden debris and macrophytes) at the same sampling location were also sampled. Approaching by boat, we also collected bryozoan colonies along the shoreline, where the colonies were removed from the various substrates (artificial and natural) in the littoral zone using scissors or a knife. Sampled bryozoans were stored in the glass jars filled with lake water and transported to the laboratory.

Colonies were analysed under the stereoscopic microscope Olympus SZX9 using different magnifications and photographed with the Olympus CAMEDIA C-4040z. All material was preserved in 4 % formaldehyde. Statoblasts were identified under the light microscope Olympus BX51. *Cristatella mucedo* floatoblast was photographed with Motic Moticam 5 camera under Motic BA310 microscope. Statoblasts were first washed in distilled water and cleaned in aqueous solution of bleach (Martinović-Vitanović *et al.*, 2010). The following keys were used for species identification: Geimer & Massard (1986), Reynolds (2000), and Wood & Okamura (2005). Professor Timothy S. Wood confirmed our identification, inspecting separated segments of the sampled colonies, as well as isolated statoblasts, which were analysed under scanning electron microscope.

Environmental parameters

At the sampling site, water transparency was determined using a Secchi disc, while other water parameters including dissolved oxygen concentration, electrical conductivity, water temperature and pH were measured with the portable multimeter (WTW Multi set/340i). Water samples were collected for analyses of nutrient (ammonium, nitrates, nitrites, total nitrogen and total phosphorus) and chlorophyll *a* concentration. The nutrients' concentration was determined according to APHA (1985). Chlorophyll *a* concentration was determined according to Strickland & Parsons (1968) and SCOR-Unesco (1966). TSI (trophic state index) was calculated according to Carlson & Simpson (1996) using water transparency, chlorophyll *a* concentration and total phosphorous concentration as parameters.

RESULTS

In the study area, during the four-year research period, in total five bryozoan species were identified (Tab. 1), of which three species belong to the family Plumatellidae (*Plumatella emarginata* Allman, 1844, *P. fungosa* (Pallas, 1768) and *P. repens* (Linnaeus,

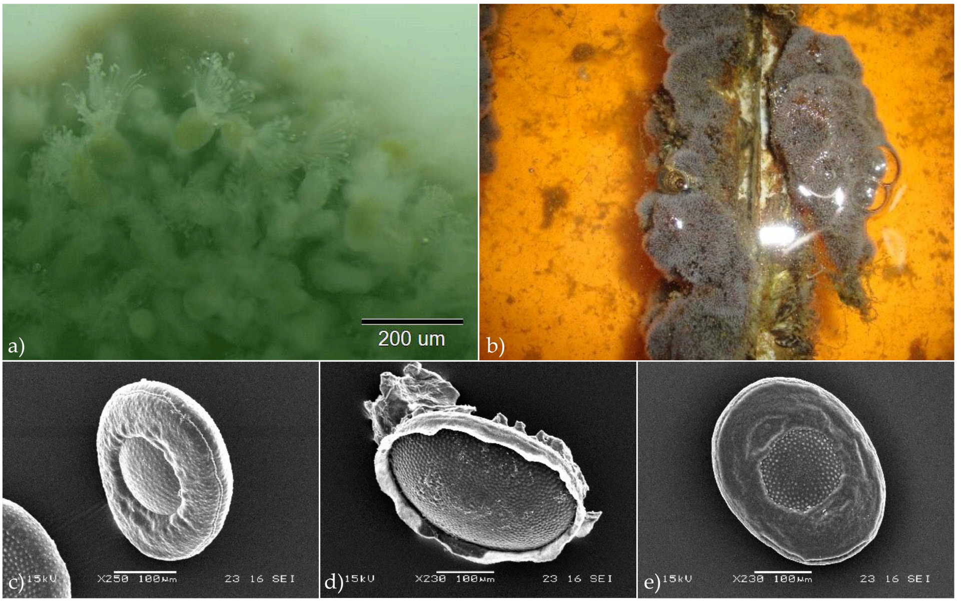

Fig. 2. Family Plumatellidae – (a) zooids with U-shaped lophophore, (b) *Plumatella fungosa* colonies, (c) SEM photo showing tubercles on the floatoblast annulus of *P. fungosa* (d) SEM photo showing serrated annulus of *P. fungosa* sessoblast and (e) SEM photo showing dorsal view of *Plumatella repens* floatoblast. SEM photos provided by Professor Timothy S. Wood.

1758) (Fig. 2), one to the family Cristatellidae (*Cristatella mucedo*) (Fig. 3) and one to the family Paludicellidae (*Paludicella articulata*) (Fig. 4). In 2007 and 2008 only *P. emarginata* was found on microscopic glass slides, while in 2009 all five bryozoan species were

Tab. 1. List of freshwater bryozoan species recorded in Lake Sakadaš (Kopački Rit floodplain) during the research period 2007 to 2010.

Phylum: Bryozoa Ehrenberg, 1831
Class: Phylactolaemata Allman, 1856
Order: Plumatellida Pennak, 1953
Family: Cristatellidae Allman, 1856
Genus: *Cristatella* Cuvier,1798
Cristatella mucedo Cuvier, 1798
Family: Plumatellidae Allman, 1856
Genus: *Plumatella* Lamarck, 1816
Pumatella emarginata Allman, 1844
Plumatella fungosa (Pallas, 1768)
Plumatella repens (Linnaeus, 1758)
Class: Gymnolaemata Allman, 1856
Order: Ctenostomata Busk, 1852
Family: Paludicellidae Allman, 1885
Genus: *Paludicella* Gervais, 1836
Paludicella articulata (Ehrenberg, 1831)

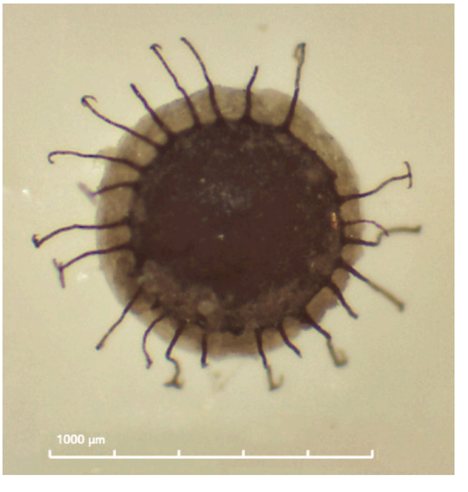

Fig. 3. Family Cristatellidae – dorsal view of *Cristatella mucedo* floatoblast showing spines radiating from margins of the fenestra.

Tab. 2. Environmental parameters measured at the sampling station in Lake Sakadaš during the research period 2007 to 2010.

	2007				2008						2009						2010					
	May	June	July	August	June		July		August		June		July		August		June		July		August	
	29.05.	28.06.	30.07.	30.08.	10.06.	24.06.	08.07.	22.07.	04.08.	19.08.	16.06.	30.06.	14.07.	28.07.	11.08.	25.08.	14.06.	28.06.	12.07.	26.07.	09.08.	23.08.
Water transparency (cm)	72	69	68	59	114	122	72	108	154	72	133	203	288	188	144	99	150.5	271	82	91	107	107
Water temperature (°C)	23.6	25.5	24.9	23.9	23.8	28.8	25.7	22.1	26.4	23.6	26.6	23.7	23.5	24	23.2	24.1	21.9	20.4	25	23.8	23.5	24.3
Dissolved oxygen (mg L^{-1})	8.05	8.21	11.63	6.43	11.63	7.63	9.37	8.58	10.36	7.52	6.87	6.5	9.22	9.69	6.65	5.86	7.4	6.12	3.19	2.34	8.67	3.32
O2 (%)	96.4	100.8	144.8	76.2	137.6	98.9	114.9	98.3	128.7	88.7	85.8	76.9	108	115.7	77.6	70.1	85.8	68.2	39.4	27.3	102.3	40.1
pH	7.99	8.05	8.48	8.05	8.70	7.83	8.29	7.89	8.25	7.75	10.1	7.37	8.08	8	7.6	7.42	7.73	7.65	7.56	7.38	8.08	7.45
Conductivity (µScm^{-1})	630	533	346	338	349	420	475	378	332	454	423	362	325	331	360	394	368	429	685	556	426	422
Water chlorophyll *a* (µg L^{-1})	31.46	53.15	54.88	71.26	35.94	17.54	42.43	46.04	17.71	31.02	11.46	17.40	13.04	31.12	38.36	33.69	29.99	13.04	22.04	61.21	47.40	53.89
NH_4 (mg L^{-1})	0.014	0.012	0.028	0.033	0.011	0.004	0.015	0.041	0.004	0.261	0.009	0.044	0.092	0.027	<0.005	<0.005	0.143	0.077	0.069	0.131	0.028	0.129
NO_2^- (mg L^{-1})	0.002	0.012	0.016	0.008	0.049	0.040	0.010	0.768	0.017	0.010	0.063	0.054	0.774	0.085	0.635	0.112	1.785	0.305	0.264	0.211	0.392	0.153
NO_3^- (mg L^{-1})	0.038	0.147	0.044	0.050	0.009	0.005	0.007	0.024	0.001	0.011	0.006	0.019	0.039	0.009	0.009	0.007	0.051	0.053	0.007	0.004	0.017	0.012
KjdN (mg L^{-1})	0.540	0.621	1.246	1.344	0.469	0.155	0.082	0.836	0.183	1.345	0.038	0.149	0.494	0.281	0.162	0.159	0.815	0.566	1.211	1.186	0.564	0.379
TN (mg L^{-1})	0.593	0.792	1.333	1.436	0.538	0.197	0.104	1.669	0.200	1.616	0.116	0.266	1.399	0.402	0.816	0.278	2.794	1.001	1.550	1.534	1.000	0.674
TP (mg L^{-1})	0.138	0.055	0.054	0.227	0.139	0.039	0.181	0.286	0.133	0.325	0.014	0.537	0.121	0.236	0.146	0.103	0.517	0.090	0.382	0.303	0.111	0.074

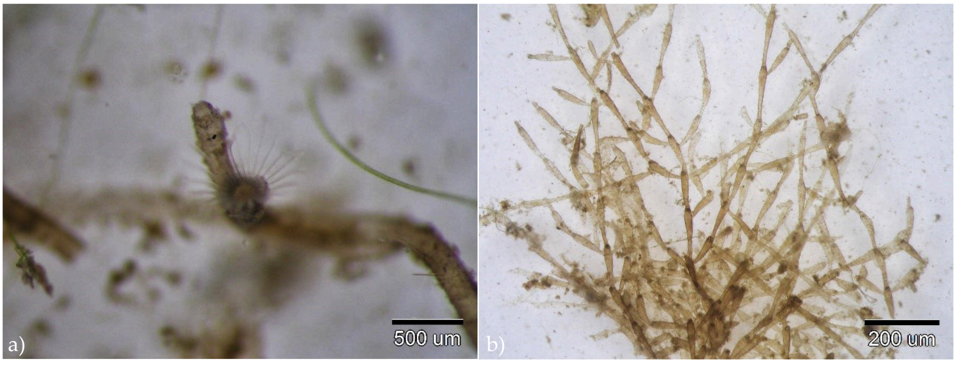

Fig. 4. Family Paludicellidae – (a) zooid with circular lophophore and (b) colony of *Paludicella articulata.*

recorded on glass slides and on various other artificial and natural substrata. All species were found as live colonies, except *C. mucedo*, whose presence was confirmed only from the sampled floatoblasts. Therefore, in order to find the colony of *C. mucedo,* sampling of different substrate types was repeated in 2010, but neither colony nor statoblasts of *C. mucedo* were found.

During the research, young colonies with only few zooids were usually found in May, while colonies found in June were well-developed and noticeable. Most colonies found in the late autumn and in early winter were degraded, and occasionally we found only sessoblasts attached to different substrates, or floatoblasts entangled in aquatic plants.

Environmental parameters measured at the sampling site during the research period are summarized in Tab. 2. Some parameters oscillated substantially during the investigation period, however this was in accordance with the season. Total nitrogen concentration varied from 0.104 mg L^{-1} to 2.794 mg L^{-1}, and total phosphorus concentration varied from 0.014 mg L^{-1} to 0.537 mg L^{-1}. Chlorophyll *a* concentration in water varied from minimal value of 11.46 μg L^{-1} to maximal value of 71.26 μg L^{-1}. pH values (7.37 – 10.10) indicated that the lake was slightly alkaline. The lowest measured water temperature was 20.4 °C and the highest was 28.8 °C. The surface lake water was well-oxygenated. Conductivity fluctuated from 325 μS cm^{-1} to 685 μS cm^{-1}.

The trophic state index (TSI) based on the water transparency measurements indicated that the lake was in a eutrophic state during the whole investigation period, while TSI based on the chlorophyll *a* concentration and total phosphorous indicated the eutrophic/hypertrophic state of Lake Sakadaš.

DISCUSSION

Freshwater Bryozoa are often neglected as taxonomic group since they are not necessary for the water quality assessment and frequently are only mentioned as present or absent in the ecological research of macrozoobenthos or periphyton. Therefore, any new information on the diversity and distribution of these colonial invertebrates is considered important, especially in the aquatic ecosystems connected to large rivers such as the Danube, which is the main dispersal route for aquatic organisms across

Europe (Sommerwerk *et al.*, 2021). Although the present research was not extensive, it resulted with some interesting and valuable data on the freshwater bryozoan diversity. Of the five bryozoan species recorded, *Plumatella repens*, *Paludicella articulata* and *Cristatella mucedo* represent new species for the Kopački Rit floodplain area. The presence of *Plumatella emarginata* and *P. fungosa* in the Kopački Rit was confirmed earlier during molecular analyses of the bryozoan fauna of Croatia (Franjević *et al.*, 2015). Interestingly, at this one location within the floodplain alone, we recorded almost half of the species listed for Croatia. So far, a total of 12 freshwater and brackish bryozoan species have been documented (Wöss & Novosel, 2013; Franjević *et al.*, 2015).

Bryozoans are most likely introduced in the Kopački Rit floodplain via Danube waters, since all species so far recorded in Kopački Rit have also been found upstream in the Austrian part of the Danube (river main channel, backwaters and floodplains), where, in total, ten bryozoan species have been recorded (Fesl *et al.*, 2005; Wöss, 2002; Wöss & Walzl, 2006). In the Slovakian part of the Danube, nine bryozoan species have been reported (Šporka, 2003), five of which have also been found in our research. The available literature data discloses that *Paludicella articulata* (Pécsi & Erdelics, 1970) and the non-native *Pectinatella magnifica* were recorded in the Hungarian part of the Danube River (Szekeres *et al.*, 2013; Zorić *et al.*, 2015). The first record of *P. magnifica* in Hungary was established by Szekeres *et al.* (2013), who found the species in a side arm of the Danube (Rackeve-Soroksar) in summer of 2011. In the following years colonies were found at the same site and in two additional sites. During the JDS3 (Joint Danube Survey 3), *P. magnifica* was observed in the main river channel of the Danube, at nine sites along the 900 km long section from Hungary to Romania. Additionally, it was found at one site downstream from Belgrade in Serbia (Zorić *et al.*, 2015). In the lower part of the Danube, Martinović-Vitanović *et al.* (2010) registered five species of Bryozoa in Serbia. Contrary to our expectations, considering the direct connection of Kopački Rit to the Danube, only two species (*P. emarginata* and *C. mucedo*) were present in both countries. We believe that further research within the Kopački Rit floodplain area, conducted in all types of water bodies (ponds, lakes and channels), would provide a more complete list of bryozoan species. Even though there are some differences in the species composition between Croatia and its neighbouring countries, the connection between them by waterways or some other means is very important for the dispersal of bryozoans. This includes the possibility of the appearance of above-mentioned invasive bryozoan species in the Croatian part of the Danube and consequently the Nature Park.

Several studies indicated the important role of migratory animals in the transport of biotic components such as seeds, aquatic plants, molluscs, parasites and pathogens between disparate locations, consequently influencing nutrients and energy balance within the system (Brochet *et al.*, 2010; Bauer & Hoye, 2014). Brown (1933) was the first to demonstrate the possibility of bryozoan statoblast transport by waterfowl on their feathers and feet or in their gut, as well as statoblast germination after release from a vector's digestive system. Wood (2002) confirmed the importance of waterfowl in the dispersal of freshwater bryozoans. Therefore, the migratory routes of these birds can also serve as corridors for statoblast dispersal (Wood *et al.*, 2006). The area of Kopački Rit supports over 30000 water birds throughout the year. Apart for breeding, this area is used as a stopover during the migration of many waterfowl species and a wintering site for about 10000 to 15000 birds (https://rsis.ramsar.org/ris/583; https://pp-kopacki-rit.hr/). Such diverse and abundant ornithofauna could be one of the reasons why this many species of Bryozoa have been found in such a small area as Lake Sakadaš.

Another probable reason for the diversity of the bryozoan community found in this research is the favourable environmental conditions in the lake. Freshwater habitats with warm, eutrophic, well-oxygenated water, support bryozoan diversity and growth potential, while in aquatic systems with low oxygen concentration, pH below 6, clear and cold water and no suitable firm substrata, bryozoans are usually not present (Wood, 2005). According to the same author the optimal water temperature range for most freshwater bryozoans is 14 – 28 °C (Wood, 2015), yet Riccardi (1994) found species *Cristatella mucedo* at a temperature of 4 °C and *Paludicella articulata* at a temperature of 5 °C. He characterized these two species as eurytopic (water temperature and pH conditions), because, while most bryozoan species prefer higher temperature values and neutral or slightly alkaline waters, *C. mucedo* and *P. articulata* are found in waters with lower temperature and pH 5.9 (Riccardi, 1994; Økland & Økland, 2000). Productivity of Lake Sakadaš is high, and a considerable amount of food is available for filter-feeding animals like bryozoans (Peršić *et al*., 2010; Čerba *et al*., 2011; Vidaković *et al*., 2012). The results of research conducted in Norway indicate that the species *Plumatella fungosa* and *P. repens* prefer eutrophic waters, *P. emarginata* eutrophic or mesotrophic waters (Økland & Økland, 2000), while *P. articulata* avoids eutrophic conditions and prefers oligotrophic waters (Økland *et al*., 2003). *C. mucedo* is characterized as a species that exhibits no specific preference for any trophic status (Økland & Økland, 2003). Hartikainen *et al.* (2009) observed that rivers characterized by higher nutrient concentrations, especially high phosphorus, also had higher *Plumatella* and *Lophopus crystallinus* statoblast concentration. The results of an experiment in which alaboratory microcosm was created indicated that bryozoan growth rate, in terms of higher biomass values, increases with nutrient enrichment (Hartikainen *et al*., 2009).

In conclusion, although Bryozoa are very common in freshwater habitats, they are often overlooked and left out from the limnological investigations. Probably, the main reasons for that is their distinguishing moss-like appearance and periodical absence of live colonies during a year. We found that the Kopački Rit floodplain represents a favourable habitat for bryozoan development mainly due to the advantageous environmental conditions (high water temperature, slightly alkaline conditions, well-oxygenated water and high food availability), as well as large amount and diversity of firm substrates in the water. We believe that our results contribute to the current knowledge on the geographical distribution and diversity of bryozoans in Europe, particularly in the Danube watershed. Further research, which would cover a wider area and different water bodies within Kopački Rit, would possibly result in the findings of new bryozoan species.

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