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Original Scientific Paper

First finding of a genus *Haslea* Simonsen in Serbia and new diatom taxa for the country's flora in extreme and unique habitats in the Vojvodina Province

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ABSTRACT:
Salt habitats are extreme and unique environments found from Austria through Hungary to the Vojvodina Province in Serbia. In our study, we investigated eight saline habitats (channeled salt marshes, moist salt meadows and saline ponds), from which we collectedplankton, epipelic and epiphytic diatoms. Seventeen diatom taxa new for the Serbian diatom flora were recorded. In addition, the genus *Haslea*, with one species (*H. spicula*), was recorded for the first time in Serbia. The identified taxa were sporadic or rare in the samples.

Keywords:
halophilic species, *Haslea spicula*, saline habitats

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INTRODUCTION

The Carpathian Basin is a region well-known for its biodiversity in both terrestrial and aquatic ecosystems. Among them, salt habitats such as shallow soda lakes and salt marshes are extreme environments with unique organisms. They are found from the Seewinkel region in Austria, through the Great Hungarian Plain in Hungary to the Vojvodina Province in Serbia (Boros *et al.* 2014). Unfortunately, according to some estimates, only 10% of the natural saline lakes have been preserved in this region (Boros *et al.* 2013). Intensive anthropogenic activities such as expansion of human settlements into semi-arid regions, drainage, irrigation and new ways of land use, together with accompanying climate change, have caused degradation and loss of these vulnerable habitats worldwide (Williams 2002).

An intrinsic feature of saline aquatic habitats is high daily and seasonal fluctuation of different abiotic factors such as temperature, pH, water level and salinity. Organisms capable of surviving in these environments developed some adaptations which help them to overcome challenges caused by extreme factors like osmotic stress or desiccation in temporary ponds. Thus, mainly alkaliphilous and salt-tolerant species can be found in salt ponds and marshes (Stenger-Kovács & Lengyel 2015; Żelazna-Wieczorek *et al.* 2015; Ács *et al.* 2017; Gavrilović *et al.* 2018). Moreover, it is known that the specific nature of these habitats can lead to regional endemicity in some taxonomic groups (Williams 2002).

Photoautotrophic picoplankton mainly comprised of coccoid cyanobacteria and some eukaryotic algae are common in athalassohaline environments (Boros *et al.* 2013). These waters usually have higher turbidity, and the light-limiting conditions create a competitive advantage for small-sized organisms (Földi *et al.* 2018). However, athalassohaline waters can also support diverse diatom communities, in both the benthos and the epiphyton (Vidaković *et al.* 2019). Even though some investigations have dealt with diatom diversity in salt ponds and marshes in Serbia (Gavrilović *et al.* 2018), there is an urgent need for one comprehensive study that will include all salt habitats in Vojvodina. Vidaković *et al.* (2019) recently published the first diatom list for Lake Velika Rusanda, one of the largest saline lakes in Serbia. There is evidence that diatoms can be used as bioindicators in assessment of the ecological status of saline lakes (Lengyel *et al.* 2016), so a better understanding of diatom community structure can support our efforts to protect these habitats from their further degradation and loss (Ács *et al.* 2019).

The main goal of our study was to analyse diatom taxa new for Serbia in several salt ponds and marshes located in the eastern part of Vojvodina.

MATERIAL AND METHODS

Algological samples were collected during various field campaigns, from 2003 until the present time. The study included eight saline habitats consisting of channeled salt marshes, moist salt meadows and saline ponds (Fig. 1, Table 1).

Phytoplankton samples were collected by towing a plankton net (Ø 22 μm) through the open water and stored in 80-ml plastic bottles. Epipelic diatoms were collected with a pipette from the muddy surface or by scraping with a knife and toothbrush from stones and gravel. Epiphytic diatoms were collected by squeezing or scraping dominant submerged macrophytes. All samples were fixed with formaldehyde to a final concentration of about 4%.

Diatom samples were treated with concentrated acid (H_2SO_4) and $KMnO_4$ to remove organic matter according to the method described by Krammer & Lange-Bertalot (1986). The acid was then removed through a series of water washes. After this process, the material was air-dried on coverglasses and mounted in Naphrax®. Photomicrographs were taken with a Carl Zeiss AxioImager.M1 microscope with DIC optics. Morphological characteristics (length, breadth and stria number) of the taxa presented in this paper were obtained by measuring with AxioVision 4.8 software. As these taxa were rarely found on the slide, the number of measured valves ranged from 1 to 15.

RESULTS

The presence of 17 diatom taxa new for the Serbian diatom flora is established in eight studied saline habitats in the Vojvodina Province. The recorded taxa are classified into 10 genera, among which the genus *Haslea* Simonsen is recorded for the first time in Serbia.

Morphological characteristics and distribution in Serbia of the recorded taxa are presented below.

Achnanthidium saprophilum (Kobayashi & Mayama) Round & Bukhtiyarova (Fig. 2: 7-9)
Basionym: *Achnanthes minutissima* var. *saprophila* Kobayasi & Mayama
Reference: Lange-Bertalot *et al.* 2017 (p. 24, figs. 53-57)
Valve morphology: Valve linear-elliptic with broadly rounded and weakly drawn-out ends. Valve length 8.6-12.2 μm, breadth 3.2-3.78 μm.
Distribution in Serbia: Novo Ilje I (Fig. 1, Table 1), in phytobenthos.

Caloneis permagna (Bailey) Cleve (Fig. 2: 3)
Basionym: *Pinnularia permagna* Bailey
Reference: Krammer & Lange-Bertalot 1986 (p. 168, figs. 1-3; p. 169, fig. 4)
Valve morphology: Valve rhombic to elliptic-lanceolate, with tapering and rounded ends. Valve length 66.54-127.33 μm, breadth 28.09-36.38 μm. Striae radiate in the middle, parallel toward the ends, 13-14/10 μm.
Distribution in Serbia: Velika Slatina, Slatina (Fig. 1, Table 1), in phytobenthos and phytoplankton.

Gyrosigma macrum (W.Smith) J.W.Griffith & Henfrey (Fig. 2: 1)
Basionym: *Pleurosigma macrum* W.Smith
Reference: Krammer & Lange-Bertalot 1986 (p. 116, fig. 5)
Valve morphology: Valve moderately sigmoid with strongly attenuated apices. Valve length 200 μm, breadth 11.5 μm.
Distribution in Serbia: Velika Slatina (Fig. 1, Table 1), in the epiphytic community.

Haslea spicula (Hickie) Bukhtiyarova (Fig. 2: 6)
Basionym: *Stauroneis spicula* Hickie
Reference: Lange-Bertalot 2001 (p. 125, figs. 3-10)
Valve morphology: Valve narrowly lanceolate, concave in the middle with acutely rounded ends. Valve length 59.26-71.74 μm, breadth 8.2-9.07 μm.
Distribution in Serbia: Slatina (Fig. 1, Table 1), in the epiphytic community in low relative abundances (0.21%).

Mastogloia elliptica (C.Agardh) Cleve (Fig. 2: 18, 19)
Basionym: *Frustulia elliptica* C.Agardh
Reference: Lange-Bertalot *et al.* 2017 (p. 53, figs. 17, 18)
Valve morphology: Valve elliptic to linear-lanceolate with wedge-shaped ends. Valve length 20.2-38.38 μm, breadth 8.9-12 μm. Striae weakly to moderately radiate, 14-16/10 μm.
Distribution in Serbia: Slatina, Okanj bara (Fig. 1, Table 1), in the epiphytic community in low relative abundances (2.93%).

Nitzschia elegantula Grunow (Fig. 2: 13, 14)
Basionym: *Nitzschia microcephala* var. *elegantula* (Grunow) van Heurck
Reference: Krammer & Lange-Bertalot 1988 (p. 83, figs. 20-24, ?25, 26)
Valve morphology: Valve linear to lanceolate, in the center concave with sub-rostrate to sub-capitate ends. Valve length 12.74-18.46 μm, breadth 2.48-3.24 μm. Fibulae square- or circle-like and the central ones not farther apart than the others, 15/10 μm.
Distribution in Serbia: Slatina (Fig. 1, Table 1), in the epiphytic community in low relative abundances (2.03%).

Nitzschia incognita Legler & Krasske (Fig. 2: 12)
Reference: Krammer & Lange-Bertalot 1988 (p. 77, figs. 1-5, ?6)

Valve morphology: Valve narrow lanceolate with sub-capitate ends. Valve length 24.13-56.77 µm, breadth 2.27-3.4 µm. Fibulae small, rectangle- or circle-like with a bigger gap between central fibulae, 11-13/10 µm.
Distribution in Serbia: Velika Slatina (Fig. 1, Table 1), in the epiphytic community.

Nitzschia pellucida Grunow (Fig. 2: 2)
Reference: KRAMMER & LANGE-BERTALOT 1988 (p. 47, figs. 4-6; ?p. 48, figs. 1-9)
Valve morphology: Valve narrow, linear-lanceolate, in the centre concave with capitate ends. Valve length 125 µm, frustule breadth 17 µm. A bigger gap between central fibulae, 9/10 µm.
Distribution in Serbia: Velika Slatina (Fig. 1, Table 1), in the epiphytic community.

Nitzschia vitrea* var. *salinarum Grunow (Fig. 2: 23)
Reference: KRAMMER & LANGE-BERTALOT 1988 (p. 56, figs. 3-5)
Valve morphology: Valve linear with sub-capitate to capitate ends. Valve length 46 µm, breadth 8 µm. Fibulae square- or circle-like and the central ones not farther apart than the others, 6/10 µm.
Distribution in Serbia: Slatina, Okanj bara (Fig. 1, Table 1), in the epiphytic community.

Pinnularia ammerensis Kulikovskiy, Lange-Bertalot & Metzeltin (Fig. 2: 10)
Reference: KRAMMER 2000 (p. 47, figs. 7-9)
Valve morphology: Valve linear to linear-elliptic with wedge-shaped and broad ends. Valve length 22 µm, breadth 6.5 µm Striae parallel throughout the valve, 11/10 µm.
Distribution in Serbia: Novo Ilje II, Jaruge (Fig. 1, Table 1), in the epiphytic community.

Pinnularia eifelana (Krammer) Krammer (Fig. 2: 5)
Basionym: *Pinnularia esoxiformis* var. *eifelana* Krammer
Reference: KRAMMER 2000 (p. 104, figs. 9-12; p. 105, figs. 1-10; p. 106, figs. 1-10)
Valve morphology: Valve linear with narrowing cuneate rounded ends. Valve length 61.33 µm, breadth 9.93 µm. Striae parallel to slightly radiate in the middle, parallel to slightly convergent at the ends, 9/10 µm.
Distribution in Serbia: Aleksića Slatina (Fig. 1, Table 1), in phytobenthos.

Pinnularia schimanskii Krammer (Fig 2: 11)
Reference: KRAMMER 2000 (p. 9, figs. 10-13)
Valve morphology: Valve linear-elliptic with broadly cuneiform rounded ends. Valve length 29.26 µm, breadth 5.7 µm. Striae parallel to weakly radiate at the centre, slightly convergent toward the ends, 9-10/10 µm.
Distribution in Serbia: Okanj bara (Fig. 1, Table 1), in phytoplankton.

Pinnularia subgibba* var. *undulata Krammer (Fig 2: 4)
Reference: KRAMMER 2000 (p. 64, figs. 4-8, 10, 11; p. 66, figs. 3-7)
Valve morphology: Valve linear with weakly undulate margins. Ends slightly capitate and broadly rounded. Valve length 62.83-72.75 µm, breadth 8.75-10.58 µm. Striae radiate in the middle, convergent at the ends, 9-10/10 µm.
Distribution in Serbia: Aleksića Slatina (Fig. 1, Table 1), in phytobenthos.

Pseudofallacia tenera (Hustedt) Y.Liu, Kociolek & Q.Wang (Fig. 2: 15, 16)
Basionym: *Navicula tenera* Hustedt
Reference: LANGE-BERTALOT *et al.* 2017 (p. 47, figs. 35-38)
Valve morphology: Valve elliptic, linear-elliptic to linear with broadly rounded ends. Valve length 12.84-14.57 µm, breadth 5.07-5.83 µm. Striae weakly radiate to almost parallel, 18-19/10 µm.
Distribution in Serbia: Velika Slatina (Fig. 1, Table 1), in the epiphytic community in low relative abundances (1.6%).

Rhopalodia constricta (W.Smith) Krammer (Fig. 2:20)
Basionym: *Epithemia constricta* W.Smith
Reference: KRAMMER & LANGE-BERTALOT 1988 (p. 110, fig. 3; p. 113A, figs. 1-6)
Valve morphology: Frustules isopolar. Width in girdle view, 15-48 µm. Fibulae elongated to form fibular ribs, 3.5-6/10 µm. Striae resolvable between the fibular ribs, 15-20/10 µm.
Distribution in Serbia: Slatina (Fig. 1, Table 1), in the epiphytic community.

Rhopalodia gibba* var. *minuta Krammer (Fig. 2: 21, 22)
Reference: KRAMMER & LANGE-BERTALOT 1988 (p. 111A, figs. 2-7)
Valve morphology: Frustules roundish, rhombic to linear with convex margins and swollen in the centre. Ends broadly or obtusely rounded. Valve strongly dorsiventral with a moderately inflated centre. Ends acutely rounded and turned to the ventral side. Valve length 33.03-39.19 µm, breadth 9.5-10.26 µm, frustule breadth 22.2 µm. Fibulae elongated to form fibular ribs, 5-9/10 µm.
Distribution in Serbia: Velika Slatina, Pečena Slatina (Fig. 1, Table 1), in the epiphytic community in low relative abundances (0.46%).

Sellaphora harderi (Hustedt) J.Foets & C.E.Wetzel (Fig. 2: 17)
Basionym: *Navicula harderi* Hustedt
Reference: FOETS & WETZEL 2018 (figs. 1-42)
Valve morphology: Valve rhombic-lanceolate with moderately pointed ends. Valve length 10.47 µm, breadth 3.56 µm.
Distribution in Serbia: Aleksića Slatina, Okanj bara (Fig. 1, Table 1), in phytobenthos.

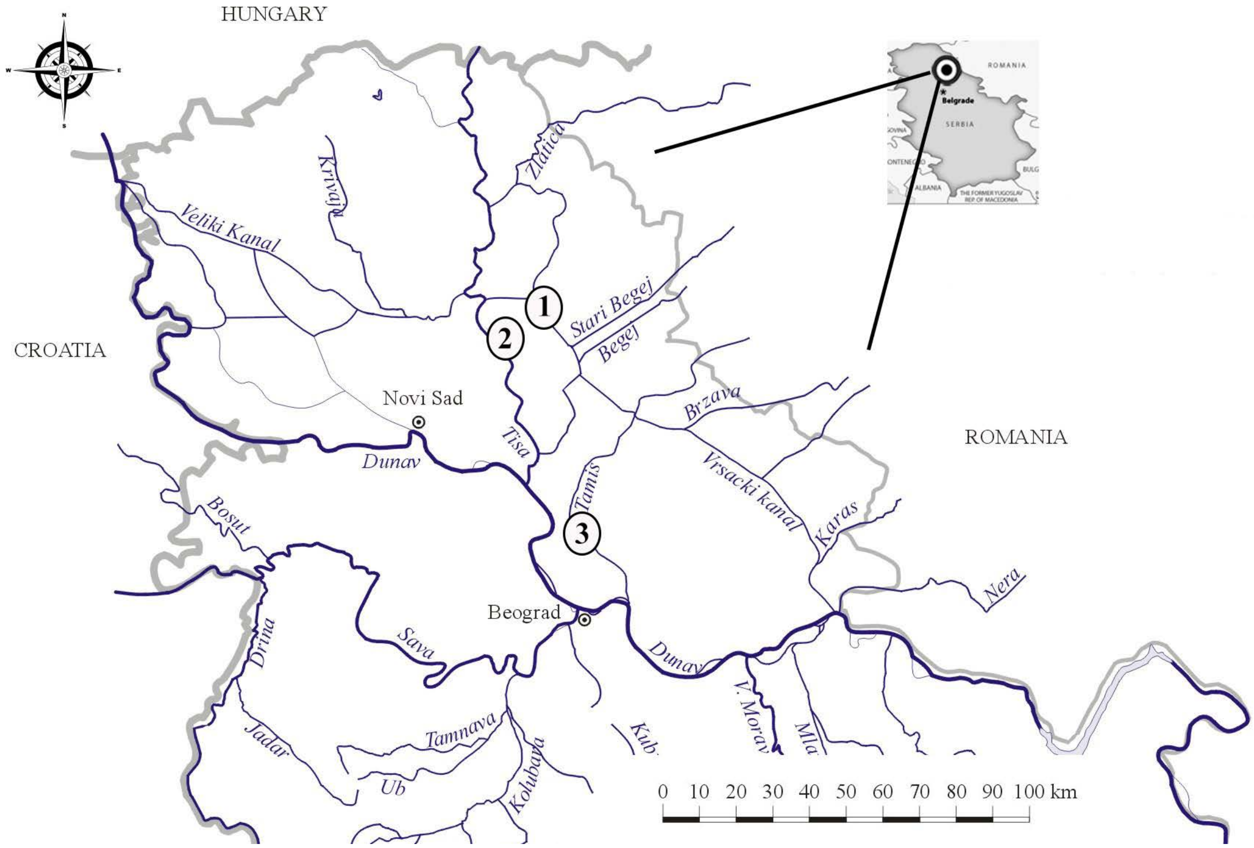

Fig. 1. Location of sampling sites in Vojvodina. 1 - Jaruge; 2 - Novo Ilje I, Novo Ilje II, Okanj bara; 3 – Slatina, Pečena Slatina, Aleksića Slatina.

DISCUSSION

Diatoms are widespread organisms and can be found in a different types of water, as well as out of water (Van de Vijver *et al.* 2002; Bellinger & Sigee 2010). Also, they are one of the most dominant groups in saline habitats (Ács *et al.* 1994; Stenger-Kovács *et al.* 2014). Investigations of diatoms in saline habitats have been sporadic, not only in Serbia, but also in Europe. Gavrilović *et al.* (2018) provide an overview of diatom research in this type of habitat in Serbia. However, a detailed study has been done only on Lake Velika Rusanda, an alkaline soda lake, where 27 diatom taxa were recorded (Vidaković *et al.* 2019). Similar investigations were carried out in Romania on the saline lakes Lacul Dulce and Lacul Sulfuros, where 80 and 97 diatom taxa were found, respectively (Nagy *et al.* 2006, 2008). Żelazna-Wieczorek *et al.* (2015) recorded 179 diatom taxa in salt marshes near Łęczyca (Central Poland). In the Chícamo Brook, a semiarid Mediterranean stream in southeastern Spain, 133 diatom taxa were observed (Ros *et al.* 2009). However, the most numerous and comprehensive investigations of this habitat type have been done in Hungary [Buczkó & Ács (1996–1997), Stenger-Kovács *et al.* (2014), Stenger-Kovács & Lengyel (2015)].

The taxa presented in this paper are most common on marine coasts, in brackish habitats or freshwater ones with high electrolyte content (Krammer & Lange Bertalot 1986, 1988; Krammer 2000; Lange-Bertalot 2001; Lange-Bertalot *et al.* 2017). Generally, the dominant genus in saline habitats is most often *Nitzschia* Hassall, followed by *Navicula* Bory (Stenger-Kovács & Lengyel

Table 1. List of studied saline habitats.

Locality	Type of saline habitat
Jaruge	channeled salt marsh
Novo Ilje I	channeled salt marsh
Novo Ilje II	moist salt meadows
Okanj bara	saline pond
Slatina	saline pond
Velika Slatina	saline pond
Pečena Slatina	saline pond
Aleksića Slatina	moist salt meadows

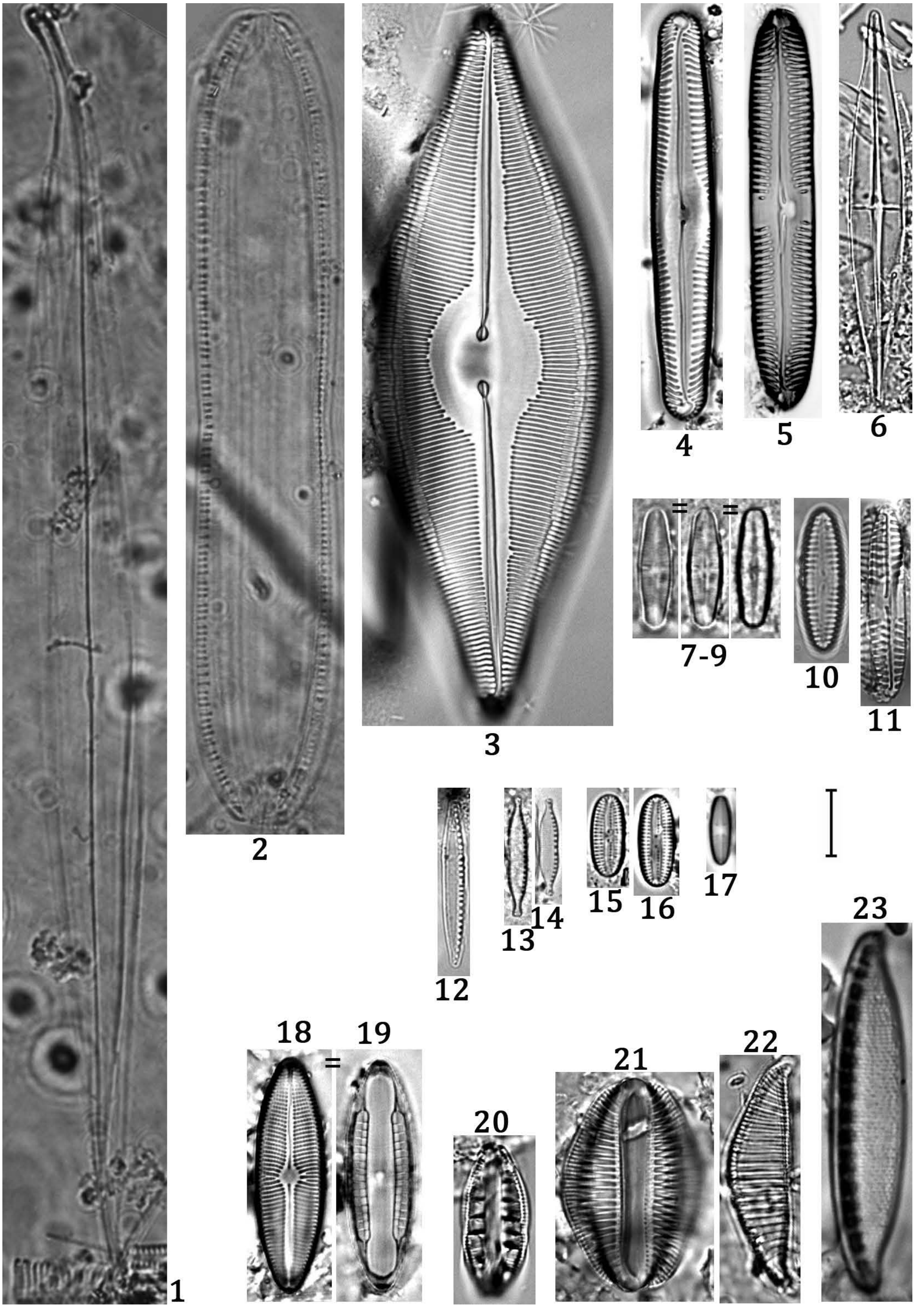

Fig. 2. Light microscopy (LM) micrographs. 1 - *Gyrosigma macrum*; 2 - *Nitzschia pellucida*; 3 - *Caloneis permagna*; 4 - *Pinnularia subgibba* var. *undulata*; 5 - *P. eifelana*; 6 - *Haslea spicula*; 7-9 - *Achnanthidium saprophilum*; 10 - *Pinnularia ammerensis*; 11 - *P. schimanskii*; 12 - *Nitzschia incognita*; 13, 14 - *N. elegantula*; 15, 16 - *Pseudofallacia tenera*; 17 - *Sellaphora harderi*; 18, 19 - *Mastogloia elliptica*; 20 - *Rhopalodia constricta*; 21, 22 - *R. gibba* var. *minuta*; 23 - *Nitzschia vitrea* var. *salinarum*. Scale bar = 10 µm.

2015; Vidaković *et al.* 2019). The majority of taxa in saline habitats represent sporadic or rare species with little floristic importance (Ros *et al.* 2009; Stenger-Kovács & Lengyel 2015). For example, *Nitzschia elegantula* was recorded at four saline habitats in Hungary with low abundance (Stenger-Kovács *et al.* 2014; Stenger-Kovács & Lengyel 2015), as well as in the saline lake Lacul Sulfuros in Romania and the Chícamo Brook in Spain (Nagy *et al.* 2008; Ros *et al.* 2009). *Nitzschia pellucida* was recorded in periphyton of Lake Lacul Sulfuros, *Rhopalodia constricta* in Lake Lacul Dulce and *Mastogloia elliptica* in both lakes. Ros *et al.* (2009) also recorded *M. elliptica*, with low abundance (0-0.1%), in the epilithon and epipelon. In our samples from Slatina and Okanj bara, *M. elliptica* was recorded in the epiphytic community with low abundance (2.93%).

The genus *Haslea* is here recorded for the first time in Serbia. So far, 29 taxa of *Haslea* are known (Guiry & Guiry 2019) and most are marine planktonic algae (Talgatti *et al.* 2014). A small number occur in electrolyte-rich inland waters in Europe (Lange-Bertalot 2001) and one of them is *H. spicula*, which is recorded in our samples from Slatina. According to Witkowski *et al.* (2000), *H. spicula* is characteristic of brackish water or can be found in seawater. In Europe, this species was found in saline lakes of Romania in their periphyton and plankton (Nagy *et al.* 2006, 2008), as well as in the Chícamo Brook in Spain, with low abundance (0.0-0.56%) in the epilithon and epipelon. In our samples, *H. spicula* was recorded in the epiphytic community, also with very low relative abundance (0.21%). Although this species is an indicator of high levels of Cl^-, Rimet (2009) found it in several French rivers with high electrolyte content. Some are naturally salty, but a number of them are impacted by soda industries or coal mines during the high water level.

CONCLUSIONS

Saline habitats are unique ecosystems in Carpathian Basin. Due to anthropogenic activity, the area of saline habitats in the Vojvodina Province has shrunk drastically and we still do not know which species are specifically characteristic of these habitats. Our study revealed several new species that will be a part of complete diatom list for saline habitats that is necessary for the preservation of these unique ecosystems.

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REZIME

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Prvi nalaz roda *Haslea* Simonsen u Srbiji i novi taksoni silikatnih algi za floru države u ekstremnim i jedinstvenim staništima Vojvodine

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Alkalna slana jezera, bare i močvare su ekstremna i jedinstvena staništa rasprostranjena u Austriji, Mađarskoj i Srbiji (Vojvodina). Istraživanje je obuhvatilo osam slanih staništa različitog tipa: kanalizovane slatine, zabarena slana staništa i slane bare. Uzorci obuhvataju različite zajednice silikatnih algi: plankton, bentos i epifite. Utvrđeno je prisustvo 17 taksona silikatnih algi po prvi put zabeleženih na teritoriji Srbije, a među njima prvi put i rod *Haslea* Simonsen sa jednom vrstom, *H. spicula*. Identifikovani taksoni su retki i sporadično nalaženi u uzorcima.

Ključne reči: halofilne vrste, *Haslea spicula*, slana staništa.