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New records of *Navicula* sensu stricto from Serbia with taxonomic notes and autecological characterization of *Navicula splendicula* and *N. moskalii*

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Abstract

A total of 15 *Navicula* taxa were recorded in epilithic communities occurring in seven rivers of Serbia, all of which are new to the diatom flora of Serbia. The most interesting of them are *N. splendicula* and *N. moskalii*. When observing *N. splendicula* specimens under SEM, we noticed a characteristic silicate tongue in the center of raphe ends, which had not been previously reported in the available literature. The insufficiently researched distribution of *N. moskalii* and scarce autecological information on the taxon prompted us to provide new details about the species in this study. These findings clearly indicate the need for further comprehensive research that would provide new information on rare taxa.

Key words: diatoms, ecological preferences, *Navicula splendicula*, *Navicula moskalii*, SEM, silicate tongue

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Introduction

Navicula, one of the most species-rich freshwater genera, was originally described in 1822 by Bory de St. Vincent. Its typification has been changed several times (Patrick 1959; Cox 1979) and species were organized into 15 sections (Hustedt 1961–1966; Van-Landingham 1975). Only members of the section *Lineolatae* Cleve (1895: 10), which comprises the neotypus generis *Navicula tripunctata* (O.F. Müller) Bory, were included in *Navicula* sensu stricto (Cox 1979). *Navicula* sensu stricto includes a group of species with boat-shaped valves characterized by different shapes of apices, uniseriate striae composed of slit-like areolae and two plate-like, girdle-appressed plastids (Round et al. 1990; Lange-Bertalot 2001). Central pores of the raphe can be deflected toward the primary side (section Alinea) or toward the secondary side, where the Voigt fault is located (section Navicula).

To date, the order Naviculales includes 5003 taxa, the family Naviculaceae – 1944 species, and the genus *Navicula* – 1344 species (Guiry 2018). *Navicula* sensu stricto has been recorded throughout Europe from different types of freshwater habitats, e.g. springs, rivers, lakes (Lange-Bertalot 2001; Miho et al. 2004; Werum, Lange-Bertalot 2004; Levkov et al. 2007; Van de Vijver et al. 2010; 2011; Beauger et al. 2015; Cantonati et al. 2016). In Serbia, the genus is also widespread and has so far been recorded in rivers (Laušević 1993; Andrejić et al. 2012a,b; Vidakovic et al. 2014; 2015a; Krizmanić et al. 2015a; Jakovljević et al. 2016a,b), peat bogs (Vidakovic et al. 2015b), salt marshes (Krizmanić et al. 2008), lakes (Zlatković et al. 2010; Trbojević et al. 2017) and reservoirs (Cvijan & Laušević 1997; Simić 2004; Gavrilović et al. 2016).

The main objectives of this study were as follows: (1) to describe *Navicula* taxa new to the territory of Serbia, (2) to describe in detail morphological characteristics of *N. splendicula* and *N. moskalii*, and (3) to provide ecological preferences of *N. splendicula* and *N. moskalii*.

Materials and methods

Epilithic diatom samples were collected from seven rivers in Serbia: the Rasina, the Rača, the Raška, the Studenica, the Mlava, the Vrla, and the Radovanska Reka. The Rača, the Raška and the Studenica are located in the southwestern part of Serbia, the Rasina is located in the central part, while the Mlava, the Vrla and the Radovanska Reka are located in the eastern part of Serbia (Fig. 1). The rivers have a carbonate bedrock substrate and run through trout farms.

Figure 1

Map of Serbia with an indication of the surveyed rivers. RČ – Rača; ST – Studenica; RŠ – Raška; RA – Rasina; ML – Mlava; RD – Radovanska Reka; VR – Vrla

Samples were collected in 2011 and 2012 by scraping the upper surface of rocks with a stiff brush (a total of 234 samples were collected). The analyzed chemical and physical factors include water temperature, pH, conductivity, oxygen, total phosphorus, ammonium and nitrate ions. Water temperature, pH, conductivity and oxygen were measured at each sampling site using a PCE-PHD device. Concentrations of total phosphorus, ammonium ions and nitrate ions were determined at the Institute of General and Physical Chemistry, University of Belgrade. D*iatom samples were processed* in the laboratory *according to the permanent slide preparation method described by* Krammer, Lange-Bertalot (1986*).*

A Zeiss AxioImagerM.1 microscope with DIC optics and AxioVision 4.8 software were used to carry out light microscope observations and to prepare micrographs. Abundance was estimated by counting 400 valves of each taxon present on a slide. SEM observations were made at the Institute of

Physics, University of Belgrade, using a TESCAN MIRA 3 scanning electron microscope with a maximum accelerating voltage of 30 kV. Surfaces of samples were sputtered with gold using a Quorum Technologies SC7620 Mini Sputter Coater for enhanced conductivity.

Results

Navicula is one of the most species-rich genera in Serbian rivers. To date, 61 *Navicula* taxa have been recorded in Serbia (Table 1, Supplementary material). During the field research conducted in seven rivers, we recorded 15 *Navicula* taxa new to the diatom flora of Serbia (Table 1, Fig. 2).

Water in these rivers is alkaline, oligo- to α-mesosaprobic, characterized by low to moderate electrolyte content, poor in total phosphorus, rich in ammonium and nitrate ions (Table 2).

Of all the recorded taxa, *N. splendicula* and *N. moskalii* are the most interesting ones. Their detailed morphological characteristics with ecological preferences are presented below.

Navicula splendicula Van Landingham (Fig. 3. 1–17; Fig. 4. 1–6)

Reference. Lange-Bertalot 2001 (p. 6, Figs 1–7; p. 65, Fig. 4; p. 69, Fig. 1)

Morphological characteristics (LM). Valve outline narrowly to broadly lanceolate. Ends broadly protracted and obtusely rounded. Length 23.2–45.9 µm, breadth 6.8–9.0 µm. Raphe filiform; axial area narrow, central area small, characterized by longer and shorter striae. Striae radiate in the middle and parallel at the ends, 14–17/10 µm (Fig. 3. 1–17).

Morphological characteristics (SEM). Axial and central areas flat without ornamentation (Fig. 4. 1, 3, 5). Raphe branches straight with deflected central pores toward the secondary side and elongated, drop-like (Fig. 4. 1–5). A characteristic silicate tongue appears in the center of raphe ends (Fig. 4. 2, 4). Terminal raphe fissures arising from the terminal pores running almost straight to the valve mantle (Fig. 4. 6). Striae composed of slit-like lineolae, 25–29/10 µm.

Distribution in Serbia. *Navicula splendicula* was recorded in the Rasina, the Rača, the Studenica and the Vrla. In the Rasina River, it occurred with relative abundance of 0.2–1.73%, in the Rača River – 0.44–0.99% and in the Vrla River – 0.3%; whereas in the Studenica River only the presence of the species was noted, without estimating its abundance.

Ecology: *Navicula splendicula* was recorded in epilithic diatom communities in small rivers, at altitudes between 273 and 1142 m a.s.l. The substrate consisted of carbonate bedrock, which is consistent with conductivity values. The proximity of sampling sites to trout farms explains the elevated concentrations of ammonium and nitrate ions. Waters were alkaline, oligo- to α-mesosaprobic (Table 2).

Navicula moskalii Metzeltin, Witkowski & Lange-Bertalot (Fig. 2. 1–10; Fig. 5. 1–5)

Reference. Lange-Bertalot 2001 (p. 14, Figs 1–14; p. 64, Fig. 8)

Table 1

List of Navicula species new to the diatom flora of Serbia

Taxon	River	Figure
N. aquaedurae Lange-Bertalot	ML	Fig. 2. 18
N. associata Lange-Bertalot	ML, RD	Fig. 2. 26, 27
N. cariocincta Lange-Bertalot	ML	Fig. 3. 11
N. cataracta-rheni Lange-Bertalot	VR	Fig. 2. 22, 23
N. cryptotenelloides Lange-Bertalot	RA, RČ, ST, ML, RD, VR	Fig. 2. 29–35
N. germainii J.H.Wallace	VR	Fig. 2. 20
N. libonensis Schoeman	RA, RŠ	Fig. 2. 25
N. moskalii Metzeltin, Witkowski & Lange-Bertalot	RA, RŠ, RČ, ST, ML, RD,	Fig. 2. 1–10, Fig. 5. 1–5
N. oppugnata Hustedt	RA, RŠ, ML, RD	Fig. 2. 12, 13
N. praeterita Hustedt	ST, ML	Fig. 2. 14–17
N. splendicula VanLandingham	RA, RČ, ST, VR	Fig. 3. 1–17, Fig. 4. 1–6
N. stankovicii Hustedt	RA	Fig. 2. 21
N. subalpina Reichardt	RŠ, ML	Fig. 2. 28
N. vandamii Schoeman & Archibald	ML	Fig. 2. 24
N. wiesneri Lange-Bertalot	RA, RŠ, VR	Fig. 2. 19

RČ – Rača; ST – Studenica; RŠ – Raška; RA – Rasina; ML – Mlava; RD – Radovanska Reka; VR – Vrla

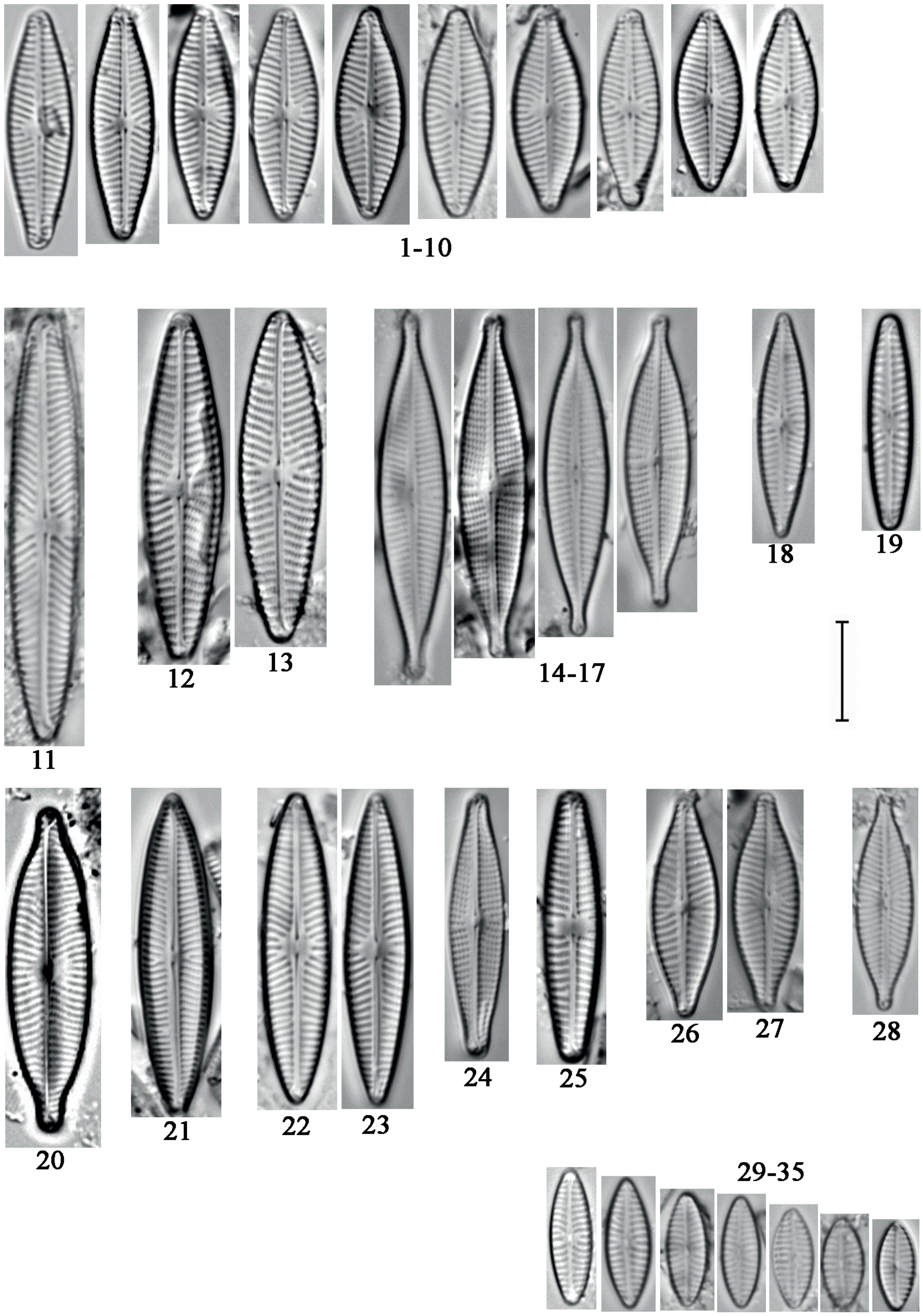

Figure 2

Light microscopy (LM) micrographs. **1–10** *N. moskalii*; **11** *N. cariocincta*; **12, 13** *N. oppugnata*; **14–17** *N. praeterita*; **18** *N. aquaedurae*; **19** *N. wiesneri*; **20** *N. germainii*; **21** *N. stankovicii*; **22, 23** *N. cataracta-rheni*; **24** *N. vandamii*; **25** *N. libonensis*; **26, 27** *N. associata*; **28** *N. subalpina*; **29–35** *N. cryptotenelloides*. **Scale bar = 10 µm**

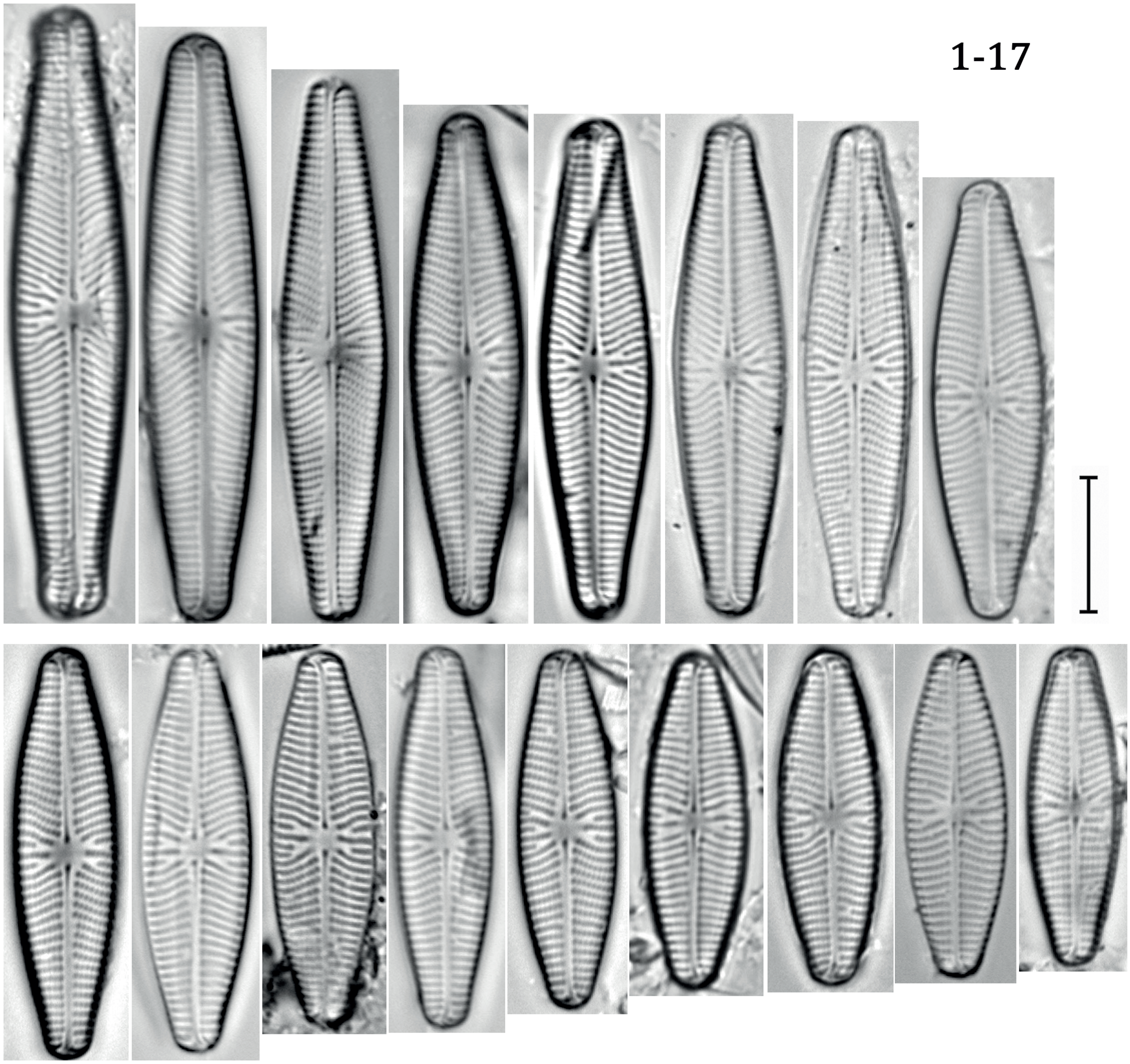

Figure 3

Light microscopy (LM) micrographs. **1–17** *N. splendicula*. **Scale bar = 10 µm**

Table 2

Mean values of physical and chemical parameters of water from seven rivers in Serbia

Parameters	Unit	Rivers						
		Rasina	Rača	Studenica	Raška	Mlava	Vrla	Radovanska
T	°C	2.5–18.7	5.7–17.3	1.4–19.1	9.9–13.5	6.4–16.8	2–15	6.4–15.4
pH		7.32–8.41	7.9–8.68	7.6–8.72	7.02–8.23	7.14–8.12	7.5–8.43	7.18–8.34
DO	$mg\ l^{-1}$	9.2–14.4	9.5–12.5	9.2–16.4	7–12.6	7.5–12.2	8.8–15.1	7.5–12.8
BOD		1.3–8.8	2.2–7.5	2.45–9	2.05–8.3	1.4–7.8	< 0.2–6.7	1.25–7.2
$N\text{-}NH_4^+$		0.02–0.33	0.04–0.41	0.02–0.30	0.01–0.52	0.04–0.56	0.005–0.26	0.02–1.1
$N\text{-}NO_3^-$		1.41–6.7	1.39–7.3	0.93–2.21	6.3–7.9	3.62–7.6	0.60–3.4	4.23–9
Con.	$\mu S\ cm^{-1}$	120–345	247–384	146–257	305–423	340–506	58–115.6	278–540
TP	$\mu g\ P\ l^{-1}$	11–61.6	6.7–86.5	11.5–73	13.5–97.6	8.7–115.1	22.1–91	16–66.5

T – Temperature, DO – Dissolved oxygen, TP – Total phosphorus, Con. – Conductivity

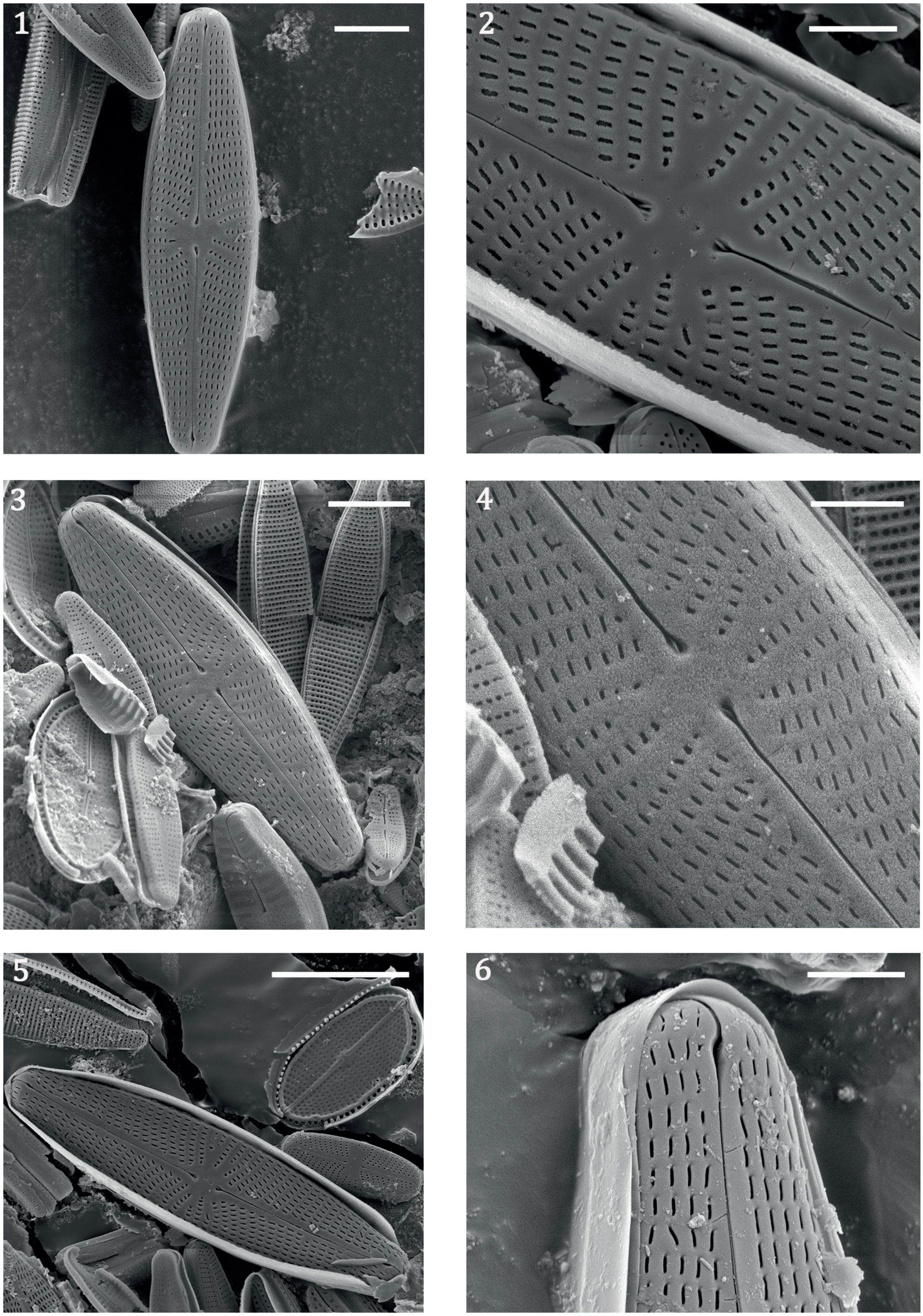

Figure 4

Scanning electron microscopy (SEM) micrographs of *N. splendicula* (1–6). **1–6** External valve view of the frustule; **6** Terminal raphe fissures; **2, 4** Center of raphe ends with the silicate tongue. **Scale bar = 5 μm for 1, 3; 2 μm for 2, 4, 6; 10 μm for 5**

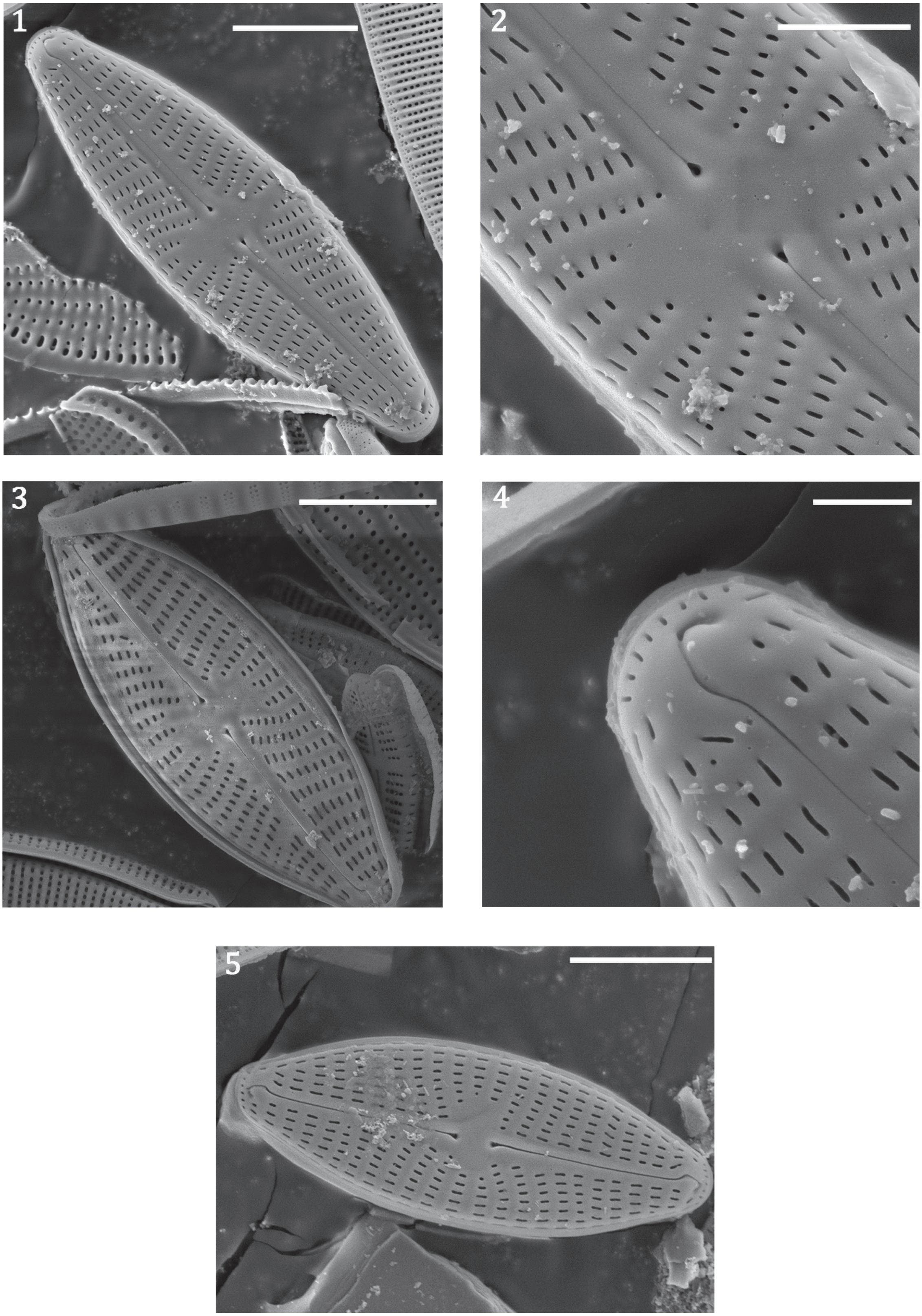

Figure 5

Scanning electron microscopy (SEM) micrographs of *N. moskalii* (1–5). **Scale bar = 5 µm for 1, 3, 5; 2 µm for 2; 1 µm for 4**

Morphological characteristics (LM). Valve outline broadly lanceolate. Ends slightly protracted and obtusely rounded. Length 16.1–31.7 µm, breadth 5.8–8.5 µm. Raphe filiform, axial area linear, central area moderately large, rectangular to elliptic and asymmetric. Striae radiate and sometimes curved at the center, parallel to slightly convergent at the ends, 13–16/10 µm; lineolae 29–34/10 µm (Fig. 2. 1–10).

Morphological characteristics (SEM). Sternum elevated relief-like, interrupted in the center (Fig. 5. 1, 2, 5). On the strongly tilted valve, the sternum appears scarcely elevated (Fig. 5. 3). Central pores expanded drop-like and the central area almost asymmetric (Fig. 5. 2). Terminal raphe fissures slightly curved, with one row of terminal pores visible (Fig. 5. 4).

Distribution in Serbia. *Navicula moskalii* was recorded in the Rača, the Raška, the Rasina, the Studenica, the Mlava and the Radovanska Reka. In the Rača River, it occurred with relative abundance of 0.42–0.49%, in the Rasina River – 0.49%, in the Studenica River – 0.5% and in the Radovanksa Reka – 0.24%; whereas in the Raška and the Mlava only the presence of *N. moskalii* was noted, without estimating its abundance.

Ecology: The species was recorded in epilithic diatom communities in small, alkaline, oligo- to α-mesosaprobic mountain rivers, at altitudes between 273 and 701 m a.s.l. The substrate was carbonate bedrock, which is consistent with conductivity values. The proximity of the sampling sites to trout farms explains the elevated concentrations of ammonium and nitrate ions (Table 2).

Discussion

The first diatom taxa were recorded in Serbia by Schaarschmidt (1883). However, extensive research on diatoms did not begin until the second half of the 20th century (Blaženčić et al. 1985; Blaženčić 1986). Several papers have been published so far (e.g. Andrejić et al. 2012a,b; Krizmanić et al. 2015a,b; 2016; Vidaković et al. 2014; 2015a,b; 2017; 2018; Jakovljević et al. 2016a,b), which have resulted in the extension of the diatom checklist to about 900 taxa (Krizmanic et al. unpubl. data). The main reason for the continuous increase in the number of new diatom taxa in the flora of Serbia is most likely insufficient research on different types of habitats and under-reporting.

Detailed descriptions of *Navicula splendicula* morphology are provided in Diatoms of Europe (Lange-Bertalot 2001) and Freshwater Benthic Diatoms of Central Europe (Cantonati et al. 2017). The only available SEM micrographs are presented in Diatoms of Europe, where Lange-Bertalot gives the following description: "central area almost symmetric; central pores apically elongated drop-like" (Lange-Bertalot 2001: 374, Fig. 1). The SEM micrographs presented in our paper clearly show a silicate tongue in the center of raphe ends. The silicate tongue was observed in other taxa, e.g. *N. tipunctata* (Lange-Bertalot 2001: 370, Figs 3, 4), *N. concentrica* (Lange-Bertalot 2001: 380, Fig. 6) and *N. jakovljevicii* (Lange-Bertalot 2001: 378, Fig. 6), which suggests that this may be an important taxonomic character. This character is not mentioned in the description of *N. splendicula* probably due to possible corrosion of the described material. In several papers, *N. splendicula* was just listed with information about the community in which it was found, without details about ecological preferences. It was recorded in small numbers in epipelic (Atici & Obali 2010), epiphytic (Millie, Lowe 1981; Atici and Obali 2010), epilithic (Battegazzore et al. 2003; Atici & Obali 2010; Noga et al. 2014) and plankton communities (Ziller, Economou-Amilli 1998; Bolgovics et al. 2015). Known facts about its ecological preferences indicate that the taxon prefers oligo-mesotrophic to eutrophic calcium-rich waters with average electrolyte content and oligo- to β-mesosaprobic characteristics (Lange-Bertalot 2001; Cantonati et al. 2017). *Navicula splendicula* belongs to the group of diatoms tolerant of a wide spectrum of oligo- to eutrophic conditions, but intolerant of higher saprobity levels (Lange-Bertalot 2001). According to our results, it occurs at sites with elevated concentrations of ammonium and nitrate ions. According to Lange-Bertalot (2001), *N. splendicula* was recorded in Southern and Central Europe. The exception is the type locality (Drinkuellen – St. Naum Springs), which is located near Lake Ohrid. In recent years, it was recorded in Italy in the Argentino and Abatemarco rivers (Battegazzore et al. 2003), in streams of the central Apennine mountains (Dell'Uomo & Torrisi 2009), in Lake Ohrid in Albania (Miho & Tase 2004), in the San River in southeastern Poland (Noga et al. 2014) and in the Sajó River in Slovakia and Hungary (Bolgovics et al. 2015). The taxon was recorded outside Europe, in the Asartepe Dam Lake in Turkey (Atici & Obali 2010), in Lake Erie and the Laurentian lakes in North America (Millie & Lowe 1981), and in the Niger Delta system in Nigeria (Ziller & Economou-Amilli 1998).

The distribution of *Navicula moskalii* is still insufficiently explored and autecological data on the species are still scarce, which prompted us to present details about this species. According to Cantonati et al. (2017), *N. moskalii* is more or less characteristic of calcium-rich and meso- to eutrophic lotic waters and lakes. Our results confirm that the taxon occurs in calcium-rich lotic waters, but also supplement the available ecological data with the information about its presence in oligo- to α-mesosaprobic waters with low to moderate electrolyte content, low concentrations of total phosphorus and elevated concentrations of ammonium and nitrate ions. According to Szczepocka & Rakowska (2015), *N. moskalii* is an indicator species characteristic of waters with good ecological status as well as calcareous and silicate waters, which contradicts the statement that it is "apparently absent from nutrient-poor freshwater habitats of siliceous regions" (Cantonati et al. 2017). After the first finding on the Arctic Bear Island, *N. moskalii* was also found in the Kinzig River in Germany (Lange-Bertalot 2001), in a peat bog in the Eastern Carpathians and in the Apuseni Mountains in Transylvania (Szigyártó et al. 2017, only mentioned in the text), in the Kobylanka stream and the Czarna Staszowska River in Poland (Wojtal 2009: 174, Fig. 30, 15; Szczepocka & Rakowska 2015, only mentioned in the text), and in a peat bog in the Novgorod region of Russia (Kulikovskiy 2009: 96, Fig. 1, 13). Wojtal (2009) and Szczepocka & Rakowska (2015) found *N. moskalii* only in the epipelic community, while in our samples it was present in the epilithic community. The valve dimensions reported by Lange-Bertalot (2001) are: length 24–27 µm, breadth 6.8–8 µm, with 11.5–15 striae per 10 µm. Kulikovskiy (2009) found a single specimen, which was 21.8 µm long, 6.4 µm wide, with 12 striae per 10 µm. Our population shows a broader dimension range, with a length of 16.1–31.7 µm and a breadth of 5.8–8.5 µm.

These findings clearly indicate the need for further extensive research to provide new information on insufficiently known and rare taxa.

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Supplementary material

Checklist of the recorded *Navicula* taxa in Serbia (from the unpublished database of Serbian diatom flora compiled by Dr Jelena Krizmanić, University of Belgrade, Faculty of Biology)

No.	Taxon
1.	*Navicula amphiceropsis* Lange-Bertalot & U.Rumrich
2.	*Navicula angusta* Grunow
3.	*Navicula antonii* Lange-Bertalot
4.	*Navicula aquaedurae* Lange-Bertalot
5.	*Navicula bourrellyivera* Lange-Bertalot, Witkowski & Stachura
6.	*Navicula broetzii* Lange-Bertalot & E.Reichardt
7.	*Navicula capitatoradiata* H.Germain ex Gasse
8	*Navicula cari* Ehrenberg
9.	*Navicula catalanogermanica* Lange-Bertalot & G.Hofmann
10.	*Navicula cincta* (Ehrenberg) Ralfs
11.	*Navicula cryptocephala* Kützing
12.	*Navicula cryptofallax* Lange-Bertalot & G.Hofmann
13.	*Navicula cryptotenella* Lange-Bertalot
14.	*Navicula dealpina* Lange-Bertalot
15.	*Navicula digitoradiata* (W.Gregory) Ralfs
16.	*Navicula erifuga* Lange-Bertalot
17.	*Navicula exilis* Kützing
18.	*Navicula exilissima Grunow*
19.	*Navicula gregaria* Donkin
20.	*Navicula jakovljevicii* Hustedt
21.	*Navicula lanceolate* Ehrenberg
22.	*Navicula lundii* E.Reichardt
23.	*Navicula hasta* Pantocsek
24.	*Navicula hintzii* Lange-Bertalot
25.	*Navicula margalithii* Lange-Bertalot
26.	*Navicula menisculus* Schumann
27.	*Navicula microdigitoradiata* Lange-Bertalot
28.	*Navicula novaesiberica* Lange-Bertalot
29.	*Navicula oblonga* (Kützing) Kützing
30.	*Navicula oligotraphenta* Lange-Bertalot & G.Hofmann
31.	*Navicula peregrine* (Ehrenberg) Kützing
32.	*Navicula perminuta* Grunow
33.	*Navicula phyllepta* Kützing
34.	*Navicula pseudolanceolata* Lange-Bertalot
35.	*Navicula pseudosilicula* Hustedt
36.	*Navicula pseudotenelloides* Krasske
37.	*Navicula radiosa* Kützing
38.	*Navicula recens* (Lange-Bertalot) Lange-Bertalot
39.	*Navicula reinhardtii* (Grunow) Grunow
40.	*Navicula reichardtiana* Lange-Bertalot
41.	*Navicula rhynchotella* Lange-Bertalot
42.	*Navicula rostellata* Kützing
43.	*Navicula salinarum* Grunow
44.	*Navicula salinarum* var. *rostrate* (Hustedt) Lange-Bertalot
45.	*Navicula semen* Ehrenberg
46.	*Navicula slesvicensis* Grunow
47.	*Navicula subrhynchocephala* Hustedt
48.	*Navicula symmetrica* R.M.Patrick
49.	*Navicula staffordiae* L.L.Bahls
50.	*Navicula streckerae* Lange-Bertalot & Witkowski
51.	*Navicula rhynchocephala* Kützing
52.	*Navicula tenelloides* Hustedt
53.	*Navicula tripunctata* (O.F.Müller) Bory
54.	*Navicula trivialis* Lange-Bertalot
55.	*Navicula trophicatrix* Lange-Bertalot
56.	*Navicula veneta* Kützing
57.	*Navicula viridula* (Kützing) Ehrenberg
58.	*Navicula viridulacalcis* Lange-Bertalot
59.	*Navicula vulpine* Kützing
60.	*Navicula upsaliensis* (Grunow) M.Peragallo
61.	*Navicula wildii* Lange-Bertalot