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New records and rare taxa of the genus *Eunotia* Ehrenberg (Bacillariophyceae) for the diatom flora of Serbia

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ABSTRACT: Diatoms inhabiting different types of substrate including stone surfaces, sand, mud, filamentous algae and submerged mosses were studied in the Dojkinci River (Stara planina mountain, Serbia). In total 18 *Eunotia* taxa have been observed, among which three are recorded for the first time in Serbia (*Eunotia circumborealis*, *E. paludosa* and *E. boreoalpina*) and six are rarely recorded taxa for the Serbian diatom flora (*E. diodon*, *E. exigua*, *E. praerupta*, *E. subherkiniensis*, *E. tetraodon* and *E. flexuosa*). The morphological features, as observed by light microscopy (LM), are described for each taxon, as well as distribution, locality, altitude, substrate and physico-chemical parameters of water samples.

Key words: *Eunotia*, diatoms, distribution, Dojkinci River, autecology

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INTRODUCTION

It is considered that the most diverse group of algae, in lotic environments, are diatoms (Round *et al*. 1990). Common diatom species, which are regularly found in European freshwater habitats, have well known ecological preferences (Van Dam *et al.*1994; Coste *et al*. 2009). However, diatom species that are rarely observed may have incomplete data about their distribution and ecology (e.g. Wojtal 2001, 2004; Szabo *et al*. 2007; Van de Vijver & Lange-Bertalot 2009; Kelly *et al.* 2009; Andrejić *et al.* 2012). As diatoms are very important indicators of environmental changes and biological monitoring of lotic ecosystems (Stevenson & Pan 1999; Bere & Tundisi 2010), it is important that their prevalence and ecology are described as well as possible.

This paper presents results of a floristic study of the Dojkinci River (mountain Stara planina, Serbia), focusing on taxa of the genus *Eunotia*. Ehrenberg was the first to describe the genus *Eunotia* in 1837 (Ehrenberg 1837). Up to now, more than 150 *Eunotia* taxa have been observed in freshwater habitats of Europe (Krammer & Lange-Bertalot 2004; Lange-Bertalot *et al*. 2011). Restriction to the freshwater oligotrophic and oligosaprobic habitats is a unique ecological feature of this genus, enabling it to play an important role as an indicator of water quality monitoring (Mayama & Kobayasi 1991; Alles *et al.* 1991; Levkov *et al.* 2000, 2005; Kwandrans 2007; Pavlov & Levkov 2013).

Mountain Stara planina (Balkan mountain) belongs to the system of Balkan mountains which ranges from the Black Sea in the East to Vrška Čuka in the

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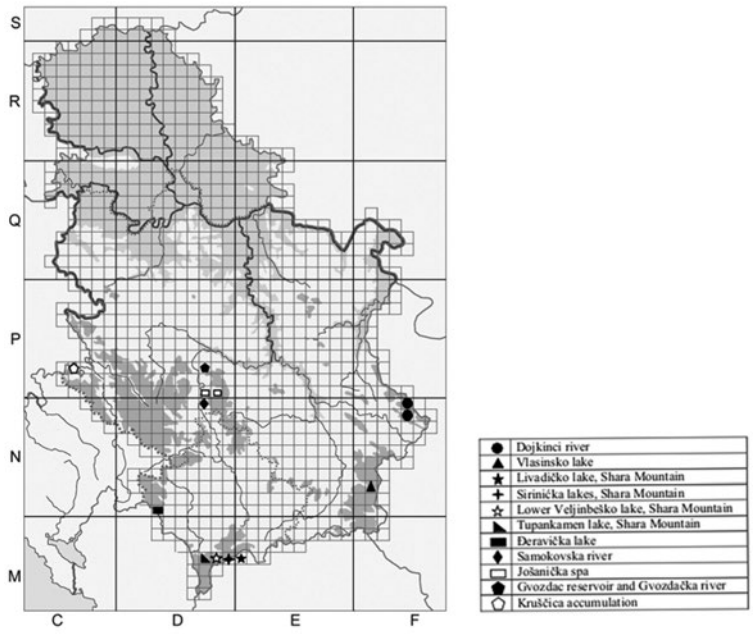

Figure 1. UTM map of Serbia with location of the habitats of newly recorded and rare taxa from the genus *Eunotia* for the diatom flora of Serbia.

West (Marković 1980). Because of the very rich and valuable flora and fauna and their value for biodiversity conservation, the mountain Stara planina was declared as a Nature park in 1997 (Mijović 2001). The Dojkinci River is formed by merging two streams: Belčin Dol and Tri kladenca. It is about 25 km long and ends by merging with the Jelovička River.The Visočica River is formed that way, and it belongs to the Black Sea basin. The substrate through the longest part of the Dojkinci River runs is red fine-grained sandstone, whose color comes from a large amount of Fe_2O_3 and is extremely rich in quartz sand (Andjelković 1958). This kind of bottom supstrate is very unusual for Serbia because most rivers have predominantly carbonate riverbeds (Marković 1980). These siliceous, low-conductivity aqueous mountain habitats are being shown to host benthic diatom communities of high species richness (Cantonati & Lange-Bertalot 2011; Cantonati *et al.* 2011, Liu *et al.* 2011).

There is relatively little information on algae of mountain Stara planina (Simić 1995, 1996, 2002), especially for diatoms from the Dojkinci River (Obušković 1993; Obušković *et al*. 1994). Here we describe three new records and six rare taxa of the genus *Eunotia* for Serbian diatom flora. Their morphology, distribution and ecology are presented here.

MATERIALS AND METHODS

The material used in the present study was collected during 2010 from 15 localities along the Dojkinci River. Epilithic samples were scraped from the surface of gravel and boulders using a scalpel blade and brush. Epipsamic and epipelic samples were collected from the surface of sand and mud substrates, while the epiphyton was taken from mosses *Plagiomnium* sp. and *Philonotis* sp. and liverwort *Marchantia polymorpha* L. subsp. *polymorpha*. Samples were fixed immediately with formaldehyde to a final concentration of 4%. In the laboratory, the field samples were treated with concentrated acid (H_2SO_4) and $KMnO_4$ to remove organic matter, and then washed several times with distilled water. After this process the material was airdried on coverglasses and mounted in Naphrax®. Permanent slides, prepared material and aliquots of the samples were deposited in the diatom collection of the University of Belgrade, Faculty of Biology. Light microscope observations and micrographs were made using a Zeiss AxioImagerM.1 microscope with DIC optics and AxioVision4.8 software. Conductivity, oxygen, pH and water temperature were measured with a Lovibond Multimeter WTW 340i at each sampling site. Ammonium ions, nitrates, phosphates, alkalinity and total hardness were measured using a Lovibond MultiDirect Photometer.

Terminology of valve morphology is based according to Barber & Haworth (1981) and Round *et al.* (1990). Abundance was estimated by counting 400 valves of each taxa present on the slide.

Location of the habitats of new and rare diatom taxa from the genus *Eunotia* in Serbia were presented on the UTM map of Sebia (10 × 10 km) (Fig. 1).

RESULTS

A total of 18 *Eunotia* species were indentified in the study area. Among them we observed three newly-recorded

Table 1. Physico-chemical parameters of the water during sampling period.

Variable	Values
Water temperature (°C)	6.4-12.5
Altitude (m)	924-1723.5
Conductivity (μS cm^{-1})	213-302
pH	5.46-6.5
Hardness (mg l^{-1})	25-67
Total alkalinity (mg l^{-1})	0.1
Alkalinity P (mg l^{-1})	0.1
NO_3-N (μg l^{-1})	0.635
PO_4-P (μg l^{-1})	1.45

taxa for the Serbian diatom flora (*Eunotia circumborealis, E. boreoalpina* and *E. paludosa*) and six rarely-recorded taxa in Serbia (*E. diodon*, *E. exigua*, *E. praerupta*, *E. subherkiniensis*, *E. tetraodon* and *E. flexuosa*).

Physico-chemical analyses of the Dojkinci River during our research are presented in Table 1.

Description of the new taxa for Serbian diatom flora:
Eunotia boreoalpina Lange-Bertalot & Nörpel-Schempp (1998: 52, figs 163: 1-7) (Metzeltin & Lange-Bertalot 1998)
(Pl. 1 figs 20-24)

Valve length is 18.58-29.82 μm, breadth 3.78-5.83 μm, 15-17 striae in 10 μm at the middle and 16-18 striae in 10 μm at the ends. *E. boreoalpina* was found along the entire course of the Dojkinci River, on all types of surfaces.

Eunotia circumborealis Lange-Bertalot & Norpel (1993: 143, fig. 27: 30) (Lange-Bertalot 1993)
(Pl. 1 fig. 13)

Valve is 25.69 μm long, 6.26 μm wide, 16 striae in 10 μm. *E. circumborealis* was found in the upper course of the Dojkinci River at only one locality, on the surface of boulders.

Eunotia paludosa Grunow (1862: 336, fig. 6: 10) (Grunow 1862)
(Pl. 1 figs 3-10)

Valve is 10.79-28.38 μm long, 2.1-3.92 μm wide and 18-24 striae in 10 μm. *E. paludosa* was found along the entire flow of the Dojkinci River, on all types of surfaces. It was the dominant taxon in samples, in the upper course up to even 80%.

Description of rare taxa for Serbian diatom flora:
Eunotia diodon Ehrenberg (1837: 45) (Ehrenberg 1837)
(Pl. 1 fig. 12)
Valve is 35.29 μm long, 6.51 μm wide, 15 striae in 10 μm. *E. diodon* was found in the upper course of the Dojkinci River at only one locality, on the surface of boulders.
Distribution in Serbia: DN30 Đeravica Lake (Urošević 1996); FN12 Vlasina Lake (Laušević 1995; Cvijan & Laušević 1997); DM74 Tupankamen Lake, Shara mountain (Urošević 1997a, 1997b, 1998).

Eunotia exigua (Bréb. Ex Kütz.) Rabenhorst (1864: 73) (Rabenhorst 1864)
(Pl. 1 figs 25-30)
Valve is 10.46-27.74 μm long, 2.56-3.89 μm wide, 19-24 striae in 10 μm. *E. exigua* was found along the entire course of the Dojkinci River, on all types of surfaces.
Distribution in Serbia: River Pek and its tributaries (Obušković 1984); DN79/70 River Samokovska (Laušević 1992, 1993; Laušević & Cvijan 1996); FN12 Vlasina Lake (Laušević 1995; Cvijan & Laušević 1997); DN30 Đeravica Lake (Urošević 1996); DP72 Gvozdac reservoir and River Gvozdačka (Obušković & Obušković 1998); CP63 Kruščica reservoir (Karadžić *et al.* 2008).

Eunotia flexuosa (Bréb. ex Kütz.) Kützing (1849: 6) (Kützing 1849)
(Pl. 1 figs 1-2)
Valve is 97.25-192.42 μm long, 7.3-8.05 μm wide, 11-13 striae in 10 μm at mid-valve and 13-14 striae in 10 μm at valve apices. *E. flexuosa* was found in the upper course of the Dojkinci River at three localities, on all type of surfaces.
Distribution in Serbia: lakes of Shara mountain (DM96 Big Vir) (Urošević 1987, 1994a, 1998); DN30 Đeravica Lake (Urošević 1996); DM86 Middle Defsko Lake, Shara mountain (Urošević 1997b, 1998).

Eunotia praerupta Ehrenberg (1843: 41) (Ehrenberg 1843)
(Pl. 1 fig. 11)
Valve is 27.11 μm long, 7.34 μm wide, 10 striae in 10 μm. *E. praerupta* was found in the upper course of the Dojkinci River in only one locality, on a mud surface.
Distribution in Serbia: River Pek and its tributaries (Obušković 1984); DN79/70 River Samokovska (Laušević 1992, 1993; Laušević & Cvijan 1996); FN12 Vlasina Lake (Laušević 1995; Cvijan & Laušević 1991, 1997); lakes of Shara mountain (DM96 Big Jažinačko, Small Vir I, Small Vir II, Small Jažinačko) (Urošević 1987, 1994a, 1998); DM86 Lower Veljinbeško Lake, Shara mountain (Urošević 1997b, 1998); DM74 Ginevodna lakes, Shara mountain (Urošević 1997c, 1998).

Eunotia subherkiniensis Lange-Bertalot (2011: 224, figs 83: 16-17) (Lange-Bertalot 2011)
(Pl. 1 figs 14-17)

Valve is 17.38-24.39 µm long, 4.75-6.05 µm wide, 13-15 striae in 10 µm. *E. subherkiniensis* was found in the upper flow of the Dojkinci River, on a mud surface and as an epiphyte on moss.

Distribution in Serbia: lakes of Shara mountain (DM96 Small Vir I, Small Vir II, Small Jažinačko, Big Vir) (Urošević 1987, 1994a, 1998); EM06 Livadičko Lake (Urošević 1994b, 1998); DN30 Đeravica Lake (Urošević 1996).

Eunotia tetraodon Ehrenberg (1838: 192, fig. 21: 25) (Ehrenberg 1838)

(Pl. 1 Figs 18-19)

Valve is 43.4-48.9 µm long, 12.7-14.14 µm wide, striae at mid-valve 8-10 in 10 µm, at the ends 11-14 in 10 µm, areolae clearly discernible in LM view, 24-25 in 10 µm. *E. tetraodon* was found at one locality in the upper course of the Dojkinci River, as well as one in the lower course, as an epiphyte on mosses.

Distribution in Serbia: DP70/80 Jošanička Spa (Cvijan 1986); lakes of Shara mountain (DM96 Small Vir II, EM06 Upper Blateštičko) (Urošević 1987, 1994a, 1998); DN79/70 Samokovska River (Laušević 1992, 1993; Laušević & Cvijan 1996); DN30 Đeravica Lake (Urošević 1996); DM86 Middle Defsko Lake, Shara mountain (Urošević 1997b, 1998); DM74 Ginevodna lakes, Shara mountain (Urošević 1997c, 1998).

DISCUSSION

The genus *Eunotia* does not have a wide distribution in Serbia (Krizmanić 2009). It is assumed that the reason for this could be the small number of habitats (springs, streams, rivers) with a silica substrate (Marković 1980). Waters containing species of the genus *Eunotia* have been characterized by low to moderate concentrations of electrolytes and pH ranges from acidic to neutral (Wojtal *et al.* 1999; Krammer & Lange-Bertalot 2004; Hofmann *et al.* 2013). Ortiz-Lerin & Cambra (2007) state that pH and electrolyte content are major factors in the widespread species of this genus, while temperature does not have a major impact.

The genus *Eunotia* was very common in the Dojkinci River. One part of the riverbed was covered by red sandstone. Physico-chemical analysis of water showed that the pH was between 5.46-6.5 and conductivity was 213-302 µS cm^{-1}, which confirms conditions for presence of the genus *Eunotia*.

E. boreoalpina, E. circumborealis and *E. paludosa* are new taxa for the Serbian diatom flora.

Eunotia boreoalpina can be found in waters of mountain regions (springs, streams and lakes) from different parts of Europe (France, Italy, Finland, Poland), Canada and the USA (Lange-Bertalot *et al.* 2011). It has also been found on Jablanica, Kozuf and Shara mountains in Macedonia (Pavlov & Levkov 2013). The taxon is characteristic for moderately acidic waters with low electrolyte content (Hofmman *et al.* 2013). We found that water of the Dojkinci River is moderately acidic on sites at which this alga was recorded, at an altitude range of 1015-1579 m a.s.l. Hofmann *et al.* (2013) stated that the valve is 12-50 µm long, 4-6 µm wide, 13-17 striae in 10 µm. According to Pavlov & Levkov (2013), valve length is 11.5-45.6 µm, valve breadth is 4.5-6.0 µm, striae 15-17 in 10 µm at the mid-valve and 13-17 in 10 µm at valve apices. Valve measurements of the population from the Dojkinci River were similar, as well as valves according to Hofmann *et al.* (2013), but comparing to Pavlov & Levkov (2013) the breadths of our valves were smaller.

Eunotia circumborealis is widespread in northern and Central Europe in oligo- to dystrophic freshwaters. Krammer & Lange-Bertalot (2004) stated that the valve is 13-45 µm long, breadth 6-8 µm and 13-17 striae in 10 µm, which was consistent with our findings. It has been recorded in a sample of pH 6.5, at an altitude of 1503 m a.s.l.

The optimal distribution of *Eunotia paludosa* is in acidic waters (Van Damm *et al.* 1994). This species is often in bogs and small streams with low electrolyte content (Patrick & Reimer 1966; Wojtal *et al.* 1999). A study by Wojtal *et al.* (1999) conducted in southern Poland established the dominant presence of *E. paludosa* in samples taken from mud substrates and from moss. Pavlov & Levkov (2013) found the species only in the periphyton of a small fountain near the city of Kumanovo (Macedonia). In our samples, *E. paludosa* was the dominant taxon even up to 80% and was found on all types of substrates, at an altitude range of 924-1723 m a.s.l. Hofmann *et al.* (2013) stated that the valve is 6-45 µm long, 1.8-3.5 µm wide, 18-25 striae in 10 µm. Valve measurements according to Pavlov & Levkov (2013) are: 17.0-42.0 µm long, 3.3-4.6 µm wide, 15-18 striae in 10 µm. Our results were consistent with valve dimensions according to Hofmann *et al.* (2013).

Six rarely-recorded taxa for the Serbian diatom flora were identified: *E. diodon*, *E. exigua*, *E. flexuosa*, *E. praerupta*, *E. subherkiniensis* and *E. tetraodon*.

The cosmopolitan species, *Eunotia diodon* is often found in northern Europe, as well as in the waters of high mountainous regions of Central Europe, which are characterized by low to moderate concentrations of electrolytes (Krammer & Lange-Bertalot 2004). Van Damm *et al.* (1994) stated that the taxon is common in waters with pH <7. According to Krammer & Lange-Bertalot (2004), this taxon could be found on wet rocks or between mosses, while the study of Wojtal *et al.* (1999) showed that *E. diodon* could be found on mud substrate. In our investigation, this taxon was found on a rock surface, with pH of 6.5, at an altitude of 1503 m a.s.l. Krammer & Lange-Bertalot (2004) stated that the valve is 10-65 µm long, 5-14 µm wide, 12-16 striae in 10 µm. Our valve measurements were consistent with literature data.

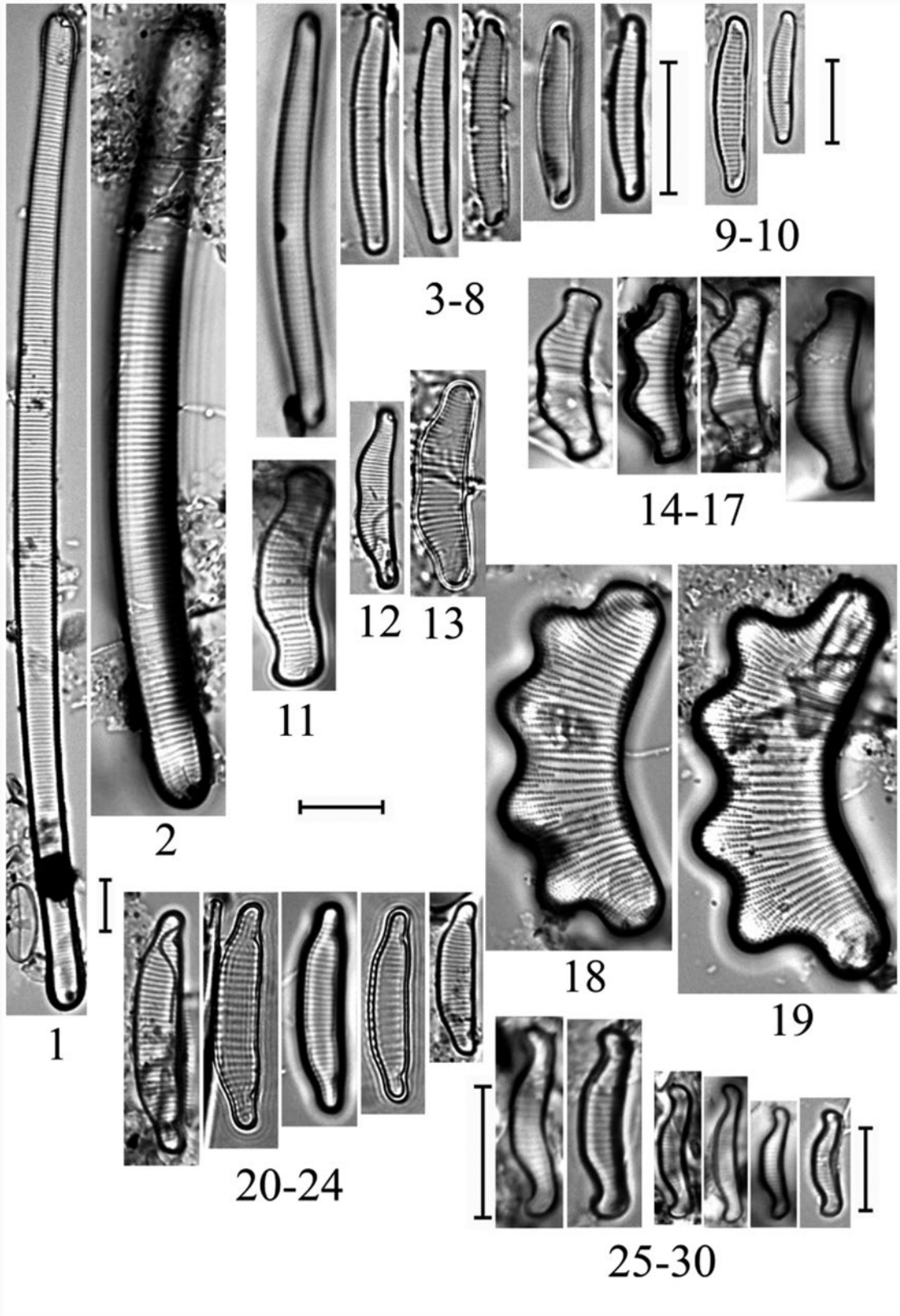

Plate 1. LM micrographs. Figs 1-2. *Eunotia flexuosa*; Figs 3-10 *E. paludosa*; Fig. 11 *E .praerupta*; Fig. 12 *E. diodon*; Fig. 13 *E. circumborealis*; Figs 14-17 *E. subherkiniensis*; Figs 18-19 *E. tetraodon*; Figs 20-24 *E. boreoalpina*; Figs 25-30 *E. exigua*. Scale bar = 10 μm.

Eunotia exigua has a cosmopolitan distribution in freshwaters (springheads, fast streams and bogs) that are rich in minerals and humic acids. This taxon is a good indicator of anthropogenic acidification in small lakes and rivers (Hofmman *et al*. 2013). According to Van Damm *et al.* (1994), this taxon is located in water with pH <5.5. Hofmann *et al*. (2013) stated that *E. exigua* is located in waters where the pH ranged between 2 and 7. According to Ortiz-Lerin & Cambra (2007), *E. exigua* was found in samples where the pH was between 4.4 and 6.4 and conductivity was 17.4-518 μS cm^{-1}. In addition, Lessmann *et al.* (2000) have found the presence of the taxon in lakes of the lignite area in Germany, in which pH was between 2 and 3. Kamijo *et al.* (1974) reported this taxon as very rare in the naturally-acidic Iwo-gawa River and Su-kawa River in Japan at pH 2.4-2.8. It is quite abundant as an epiphyte on Osogovo Mountain, at an altitude of about 2000 m a.s.l. (Pavlov & Levkov 2013). *E. exigua* was found in samples where pH varied between 6.5 and 6.4, at an altitude of 1115-1723 m a.s.l. Hofmann *et al*. (2013) states that the valve is 6-30 μm long, 3-4 μm wide, 19-24 striae in 10 μm. According to Pavlov & Levkov (2013), valve length is 15.8-35.0 μm, valve breadth is 4.0-5.2 μm, striae 22-24 in 10 μm. Valve measurements of the population from the Dojkinci River were similar, as well as valves according to Hofmann *et al.* (2013), but compared with Pavlov & Levkov (2013) our valves were smaller and narrower.

In Central Europe, *Eunotia flexuosa* is very rarely found, though it has been frequently recorded in western, northern and eastern Europe (Hofmman *et al.* 2013). It was quite abundant in mixed substrate samples on Kozuf Mountain, Macedonia, but periodically found in central and western Macedonian mountains (Pavlov & Levkov 2013). According to Krammer & Lange-Bertalot (2004), this taxon is widespread in dystrophic, anthropological intact habitats (springs, streams, wetlands) with low to moderate concentrations of electrolytes. Van Damm *et al.* (1994) reported that this taxon is found in waters with pH <7. *E. flexuosa* was found at three localities in the Dojkinci River with pH 6.5, at an altitude of 1183-1432 m a.s.l. Hofmann *et al.* (2013) stated that the valve is 80-270 μm long, 5-6.5 μm wide (at the ends 6-8 μm), 10-12 striae in 10 μm, at the ends up to 15-18 in 10 μm. According to Pavlov & Levkov (2013), valve length is 127.2-216.0 μm, valve breadth 6.9-8.7 μm, striae 11-12 in 10 μm at mid-valve and 11-14 in 10 μm at valve apices. Valve measurements from our samples were similar to these, as well as to valves according to Hofmann *et al.* (2013), but compared with Pavlov & Levkov (2013), our valves were smaller and narrower.

Eunotia praerupta is widespread in waters of mountain regions on silica surfaces with low electrolyte contents and in acidic to neutral oligotrophic waters up to dystrophic waters (Patrick & Reimer 1966; Hofmann *et al.* 2013). It was recorded as an epiphyte and in the epipelon in streams and glacial lakes on Shara and Nidze Mountain at an altitude of 1290-2300 m a.s.l. (Pavlov & Levkov 2013). According to Van Damm *et al.* (1994), this taxon was found at pH <7. In a Spanish study (Ortiz-Lerin & Cambra 2007), this taxon was found only on one locality as an epilitic alga at pH 6.47. *E. praerupta* was found at one locality in the Dojkinci River at pH 6.5, on a mud surface, at altitude 1442 m a.s.l. Hofmann *et al.* (2013) stated that the valve is 28-105 μm long, 10-18 μm wide and 5.5-8 striae in 10 μm, at the ends 12 striae in 10 μm. Valve measurements according to Pavlov & Levkov (2013) are: 36.8-73.3 μm long, 13.5-17.0 μm wide, striae at mid-valve 5-9 in 10 μm, at the ends 8-12 μm. Comparing literature data with our results, we can conclude that *E. praerupta* in our sample had smaller dimensions.

Eunotia subherkiniensis is very rare in Central Europe. It is characteristic for unpolluted waters of mountain regions that are rich in humic acids with low electrolyte content (Hofmann *et al.* 2013). According to Furey (2012), this taxon could be found in streams and lakes, on wet rocks, in waterfalls and as an epiphyte on moss. In our samples, *E. subherkiniensis* was found on a mud surface and as an epiphyte on moss, at an altitude of 1432-1723 m a.s.l. Hofmann *et al.* (2013) states that the valve is 10-30 μm long, 5-8 μm wide and has 12-15 striae in 10 μm, which was consistent with our findings. Valve breadth was slightly narrower, as well as valves according to Furey (2012).

A cosmopolitan species, *Eunotia tetraodon* is widespread in boreal and alpine regions, while it is seldom in Holarctic (Bahls 2012). This taxon is characteristic for streams, swamps and lakes with low electrolyte contents and with pH from 5.5 to 8.8 (Bahls 2012). *E. tetraodon* was found at only one locality in the Dojkinci River with pH 6.5, at an altitude of 1015 m a.s.l. Bahls (2012) stated that the valve is 37-51 μm long, 14.2-18.8 μm wide, striae at mid-valve 8-10 in 10 μm, at the ends 12-15 in 10 μm. According to Pavlov & Levkov (2013), dimensions are valve length 31.5-60.0 μm, valve breadth 9.0-13.0 μm, striae 8-10 in 10 μm at mid-valve and 13-15 in 10 μm at valve apices. Valve measurements in our samples were similar to those of literature data.

CONCLUSION

The substrate through the longest part of the Dojkinci River runs is red fine-grained sandstone, which is very unusual for Serbia because most riverbeds are predominantly of carbonate. Here, we have focused on taxa of the genus *Eunotia* that has rarely been recorded for diatom flora of Serbia. Until now, there had been few literature data on diatoms in the Dojkinci River. In Serbia, many aquatic ecosystems are poorly investigated, and because of this further evaluation of diatom floristic richness, distribution and biodiversity is needed.

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REZIME

Novi nalazi i retki taksoni roda *Eunotia* Ehrenberg (Bacillariophyceae) za floru dijatomeja Srbije

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Rad sadrži podatke o sastavu silikatnih algi Dojkinačke reke (Stara planina, Srbija). Uzorci su sakupljani sa različitih tipova podloga, kao što su: kamen, pesak, mulj, filamentozne alge i mahovine. Pregledom preparata identifikovano je 18 taksona silikatnih algi roda *Eunotia*. Od 18 identifikovanih taksona roda *Eunotia* zabeležena su tri nova taksona (*Eunotia circumborealis*, *E. paludosa* i *E. boreoalpina*) i šest retko zabeleženih taksona za floru silikatnih algi Srbije (*E. diodon*, *E. exigua*, *E. praerupta*, *E. subherkiniensis*, *E. tetraodon* i *E. flexuosa*). U radu su date morfološke karakteristike svakog taksona, kao i rasprostranjenje, lokalitet, nadmorska visina, tip substrata i fizičko-hemijske karakteristike vode.

Ključne reči: *Eunotia*, silikatne alge, rasprostranjenje, Dojkinačka reka, autekologija