

First record of the species *Encyonema bonapartei* Heudre, C.E.Wetzel & Ector (Cymbellales, Bacillariophyceae) in Serbia

by

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Abstract

Encyonema bonapartei Heudre, C.E.Wetzel & Ector is a diatom species originally documented in 2016, following its discovery in three canals in the eastern region of France. In 2021, an extensive hydrobiological survey in a mountainous part of southwestern Serbia led to the first finding of this taxon outside of France, i.e. in the Uvac Special Nature Reserve – in Lake Zlatar (Zlatarsko jezero) and in the Marić River (Marića reka). Detailed light microscopy and scanning electron microscopy observations of a large population recorded in Lake Zlatar confirmed the unique morphological characteristics of *E. bonapartei*, which is also the first record for the diatom flora of Serbia. The present work provides new information on the distribution and ecological preferences of *E. bonapartei*, taking into account physical and chemical characteristics of its habitats in Serbia.

Key words: Diatoms, *Encyonema* sp., distribution, ecology, protected area, Europe

1. Introduction

Kützing (1833: 589) initially distinguished cymbelloid freshwater diatoms that live in mucilage tubes and those that are free-living or grow on mucilage stalks. The first group of diatoms was included in the genus *Encyonema*. At the beginning of the 20th century, the growth habit was not considered a constant characteristic and was therefore considered inadequate for distinguishing genera (Hustedt 1930). Until the end of the last century, numerous species of the genus *Encyonema* were treated as members of the genus *Cymbella* (i.e. Krammer, Lange-Bertalot 1986), mainly because both are characterized by dorsiventral valves (Silva, Souza 2015). In the revision of cymbelloid taxa by Krammer (1997a, b), the genus *Encyonema* was separated from *Cymbella* C.Agardh to include cymbelloid taxa with distal raphe ends that curve toward the ventral margin. In addition, in the same revision, Krammer proposed six other genera, of which the genus *Encyonopsis* shared the most similarities to the genus *Encyonema*. Indeed, the slightly dorsiventral outline of the valve and the terminal raphe fissures gently bent to the ventral margin are features of *Encyonopsis* species (Krammer 1997b; Potapova 2014; Kapustin 2021). After this revision, Krammer (2003) proposed an additional four new genera. While these genera include cymbelloid diatoms with dorsally bent terminal raphe fissures, species of the genus *Encyonopsis* still share most similar characteristics with the species of the genus *Encyonema*. In his first revision, Krammer (1997a, b) proposed about 150 *Encyonema* taxa, and currently around 300 taxa are recognized in different aquatic ecosystems worldwide (Guiry, Guiry 2023). According to the available data, 26 taxa belonging to *Encyonema* (Appendix) have been recorded in freshwater habitats in Serbia (Krizmanić, unpublished database of Serbian diatom flora, June 2023).

Encyonema bonapartei is a species described by Heudre et al. (2016). It was found in three canals in the Great East region of France, initially in 2014 in two benthic samples collected from the East Canal, followed by observations in 2015 in the Rhône-to-Rhine Canal and in the Champagne and Burgundy Canal. In this paper, we present new records of *E. bonapartei* in the Uvac Special Nature Reserve in southwestern Serbia. In 2021, a survey of benthic diatoms was conducted as part of the water quality monitoring (Official Gazette of the Republic of Serbia, No. 74/2011). Sampling included tributaries of the Uvac River and three reservoirs along the Uvac River. Previous data on diatoms in the Uvac reservoirs are scarce (Mandić et al. 1997; Čađo et al. 2003; Denić et al.

2015; Čađo et al. 2015; Subakov Simić et al. 2018; Pečić et al. 2018) and are mainly derived from phytoplankton analysis. Regarding the genus *Encyonema*, Čađo et al. (2015) documented the occurrence of *E. minutum* (Hilse) D.G.Mann and *E. leibleinii* (C.Agardh) Silva, Jahn, Ludwig, & Menezes in Lake Sjenica. Records of the diatom microflora of the Uvac tributaries are similarly limited (Obušković 2002).

Given that *E. bonapartei* has so far been found in France, the results presented herein provide new information on the distribution and ecological preferences of this species.

2. Materials and methods

2.1. Study area and sample collection

The Uvac River is a mountain river in the Inner Dinarides in southwestern Serbia. It carves a canyon-type valley in the limestone terrain, with several entrenched meanders. With a total length of 119 km, it is the largest tributary of the Lim River. Three reservoirs, Lake Sjenica, Lake Zlatar and Lake Radoinja, were created by damming the Uvac River and occupy a large part of the gorge. Due to its specific geomorphological features and importance for biodiversity conservation, part of the Uvac gorge has been designated as the Uvac Special Nature Reserve (Official Gazette of the Republic of Serbia, No. 25/2006, 110/2006).

Periphyton sampling in the Uvac Special Nature Reserve included seven rivers (Uvac, Vapa, Zložnica, Marić River, Veljušnica, Kladnica, and Tisovica) and three reservoirs (Lake Sjenica, Lake Zlatar and Lake Radoinja; Fig. 1). Sampling sites important for our findings were located at Lake Zlatar and the Marić River. Lake Zlatar was created in the 1960s after the construction of a dam and hydropower plant in the middle stretch of the Uvac River. The dam is 83 m high and 1264 m long, and its foundations are situated at an altitude of 850 m (Petraš, Grubač 2019). Lake Zlatar is about 23 km long and covers an area of 7.25 km² with a maximum depth of 75 m (Mičković et al. 2013). It is characterized by a significant annual water level amplitude of up to 45 m (Sunjog 2016). Typically, the maximum is reached in May and June, and the minimum in November and February, primarily due to increased electricity production (Petraš, Grubač 2019). The Marić River is a small upland-mountain river classified as a salmonid watercourse (Petraš, Grubač 2019). It is a right-bank tributary of the Uvac River, with a confluence located 1.2 km downstream of the dam on Lake Zlatar.

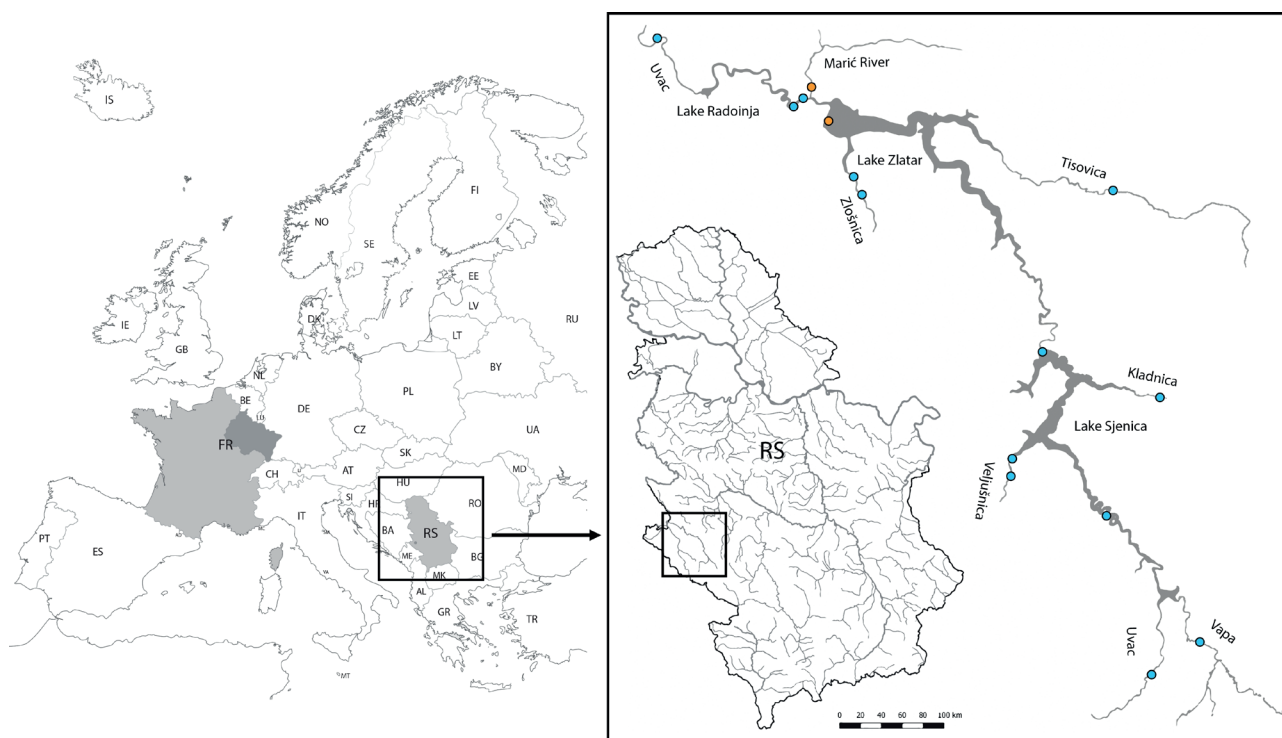


Figure 1

Distribution of *Encyonema bonapartei* Heudre, C.E.Wetzel & Ector: first record in France (left, dark gray area), and new records in Serbia: in Lake Zlatar and the Marić River (right, orange circles). The blue and orange circles represent all sampling sites during the survey in the Uvac Special Nature Reserve.

Samples were collected according to the standard procedure (Institute for Standardization of Serbia 2015a). An area of about 10 cm² of each of the five stones selected from the euphotic zone was brushed and rinsed into a sampling bottle. The samples were fixed in formaldehyde with a final concentration of 4%. During three field surveys in early August, late August, and late October 2021, a total of 30 epilithic diatom samples were collected from 15 sampling sites (Fig. 1). Due to monitoring requirements and observed field conditions, sampling dynamics varied from site to site. One sample was collected in Lake Zlatar in late August 2021. Periphyton sampling was not conducted during the October survey due to very low water level. In contrast, three samples were collected from the Marić River, one during each field survey.

Physicochemical parameters were assessed *in situ* during each sampling. These included temperature (T, °C), dissolved oxygen (O₂, mg l⁻¹), oxygen saturation (O₂, %), and conductivity (EC, μS cm⁻¹), which were measured using a WTW MultiLine® Multi 3630 IDS multiparameter probe, and ammonia (NH₃, mg l⁻¹), orthophosphate (PO₄³⁻, mg l⁻¹), nitrite (NO₂⁻, mg l⁻¹) and nitrates (NO₃⁻, mg l⁻¹) measured with a Lovibond® MD 600 multiparameter photometer.

2.2. Sample processing and microscopic observations

In the laboratory, samples were cleaned of organic matter using the hot HCl and KMnO₄ method (Taylor et al. 2007). Permanent diatom slides were prepared by mounting the cleaned diatom frustules in Naphrax® synthetic medium. Permanent slides and micrographs were examined by light microscopy (LM) according to the standard procedure (Institute for Standardization of Serbia 2015b) using a Carl Zeiss Axiolmager.M1 microscope, equipped with an AxioCam MRc5 camera and AxioVision 4.9 software. The relative abundance of diatoms in the samples was determined as the percentage of taxa relative to 400 counted valves on each permanent slide. For scanning electron microscopy (SEM), a portion of the oxidized diatom suspension was filtered and washed through a 3-μm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were fixed on SEM stubs and coated with gold using a SEM Autocoating Unit E5200 (Polaron Equipment, Ltd.) sputter coater. Micrographs were acquired using a FEI Scios 2 DualBeam field emission scanning electron microscope (FE-SEM) operating at 10 kV and 7 mm working distance. Identification of



E. bonapartei was based on Heudre et al. (2016). Samples and slides are deposited at the University of Belgrade, Institute for Biological Research “Siniša Stanković” – National Institute of the Republic of Serbia, Department of Hydroecology and Water Protection.

3. Results

Microscopic observations of benthic algae revealed the presence of *Encyonema bonapartei*, previously unknown in the diatom flora of Serbia.

Encyonema bonapartei Heudre, C.E.Wetzel & Ector (Fig. 2, Fig. 3)

Light microscopy (Fig. 2, a-p): The length of the 31 measured valves varies in the range of 12.0–15.0 μm (13.6 ± 0.7), the width 4.5–5.0 μm (4.8 ± 0.3), the length/width ratio 2.4–3.1 (2.9 ± 0.2), the dorsal stria density 15–18 per 10 μm (16.4 ± 0.7), and the ventral stria density 16–18 per 10 μm (16.1 ± 0.8). The valves are asymmetrical, semi-elliptical-lanceolate, with rounded to slightly protracted apices, a narrow axial area, and a tiny central area. A shorter stria or striation interruption on the ventral side is a characteristic feature of this species.

The morphological features and dimensions of *E. bonapartei* in the samples from Serbia correspond to the species description presented by Heudre et al. (2016). A slight difference was observed only in the

dorsal stria density: 15–18 per 10 μm in the samples from Serbia, compared to 17–22 per 10 μm in the samples from France.

Scanning electron microscopy (Fig. 3, a-f): The valve in external view shows rows of rounded lineolae forming uniseriate striae (Fig. 3a), a slightly sinuous raphe bent to the dorsal side at the valve center (Fig. 3a, Fig. 3c), and terminal fissures hooked to the ventral margin (Fig. 3e). The valve in internal view shows uniseriate striae settled between the virgae (Fig. 3b), with a pair of short stubs on each areola protruding from the opposite positions of lateral virgae (Fig. 3d). The raphe fissure is straight, with proximal ends close to each other (Fig. 3d) and distal ends terminating in helictoglossae (Fig. 3f). An isolated pore is not present. There are 40 areolae per 10 μm on the dorsal side of the valve and 50 per 10 μm on the ventral side.

SEM observations are consistent with the original description of *E. bonapartei* (Heudre et al. 2016).

Distribution in Serbia: *E. bonapartei* was identified in the material collected from two localities in a mountainous area of southwestern Serbia – Lake Zlatar near the dam (43°30'36.162"N; 19°48'42.0372"E; UTM – DP01), in a sample collected in late August 2021 (slide HED0052), and the Marić River near its confluence with the Uvac River (43°31'12.2664"N; 19°48'4.0176"E; UTM – DP01), in a sample collected in October 2021 (slide HED0072) (Fig. 1). The taxon was not found in two samples collected in the Marić River in August 2021.

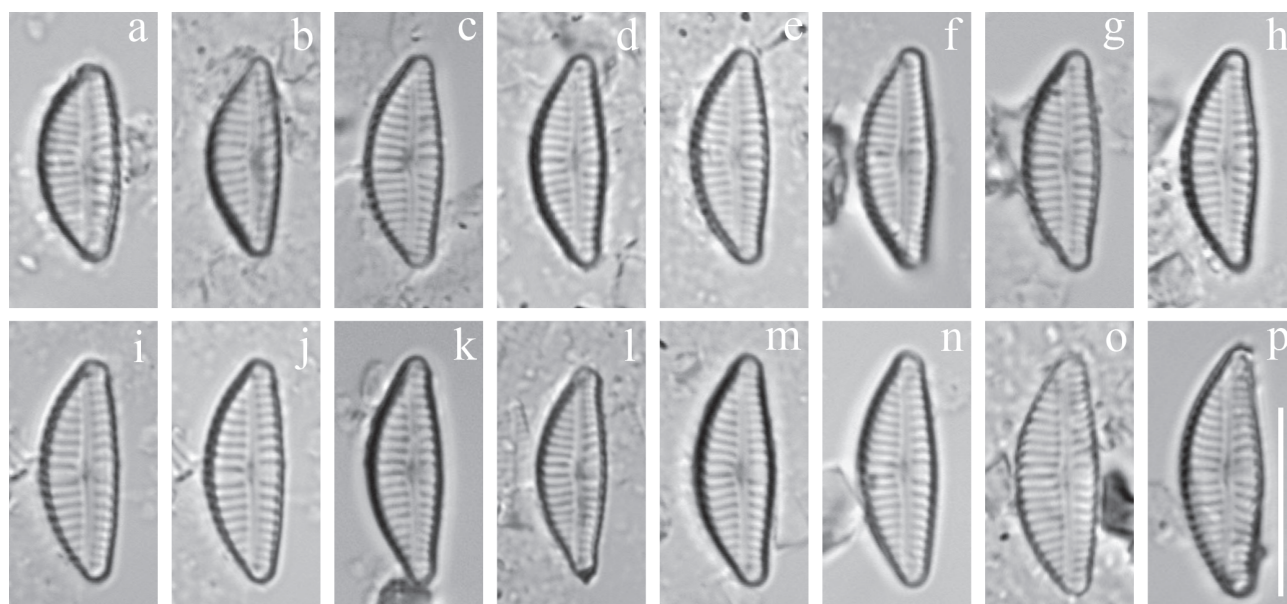


Figure 2

Encyonema bonapartei Heudre, C.E.Wetzel & Ector LM valvae views (material from Lake Zlatar). Scale bar = 10 μm .

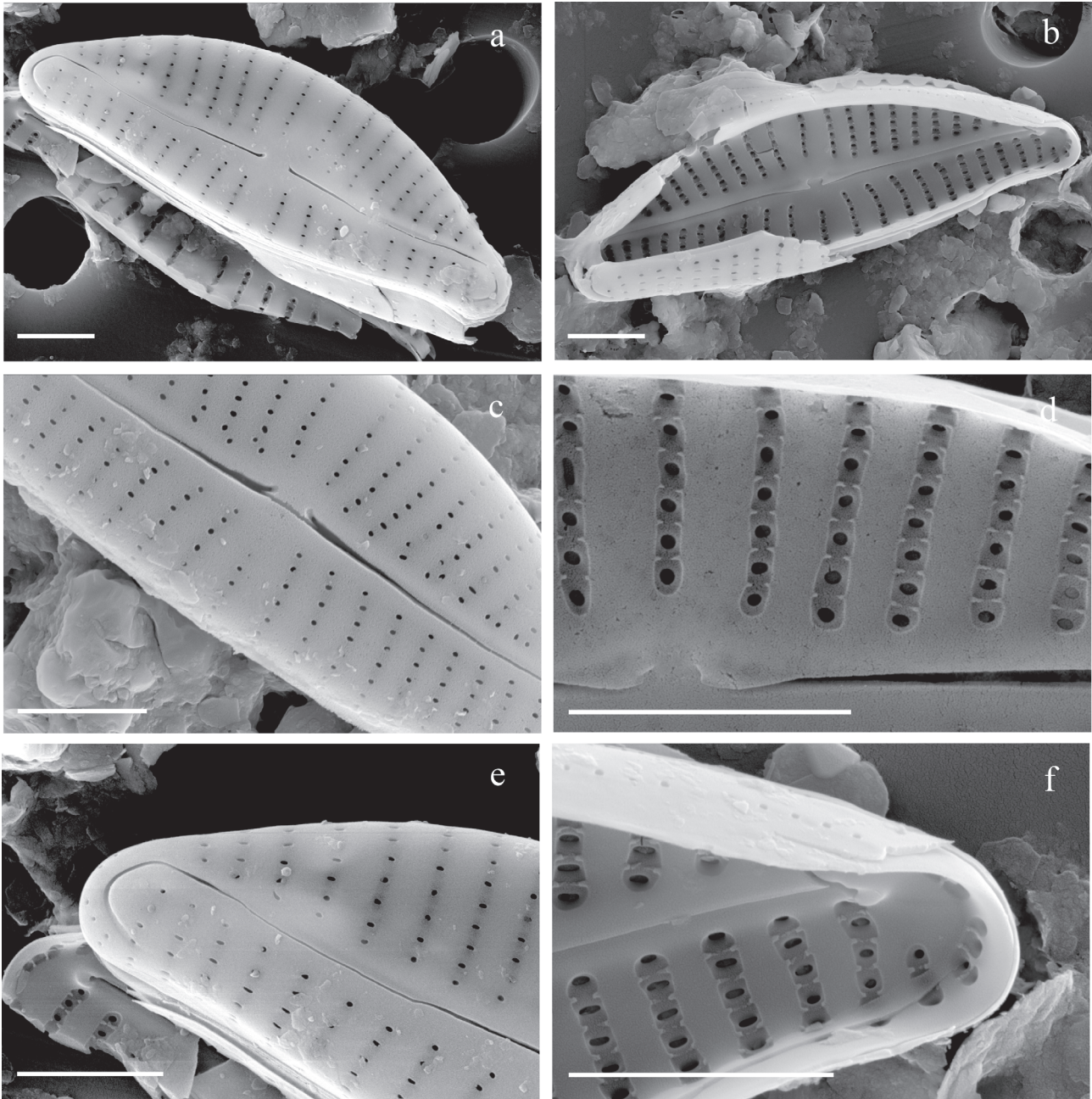


Figure 3

SEM images of *Encyonema bonapartei* Heudre, C.E.Wetzel & Ector (material from Lake Zlatar). External view: (a) valve, (c) proximal raphe ends, (e) valve apex and terminal fissure. Internal view: (b) valve, (d) proximal raphe ends and areolae, (f) distal raphe end terminating in helictoglossae. Scale bars = 2 μm .

Ecology: Twenty-six diatom taxa were identified in the sample from Lake Zlatar. The dominant taxon in the community was *Achnanthis minutissimum* (Kütz.) Czarn. (53.72%), followed by *E. bonapartei* (24.22%). In the sample from the Marić River, 39 diatom taxa were recorded. The most abundant were *A. minutissimum* (39.62%), *Achnanthis pyrenaicum* (Hust.) H.Kobayasi

(12.65%) and *Amphora pediculus* (Kütz.) Grunow (10.02%). *E. bonapartei* was recorded only in low abundance (2.39%).

Lake Zlatar and the Marić River showed some differences in terms of measured physical and chemical parameters (Table 1). The sampling site in Lake Zlatar with a large population of *E. bonapartei*



Table 1

Measured physicochemical parameters at the sampling sites where *Encyonema bonapartei* Heudre, C.E.Wetzel & Ector was identified.

Sampling site	Lake Zlatar (dam)		Marić River (near confluence with the Uvac River)		
Sampling date	25 Aug. 2021	28 Oct. 2021	2 Aug. 2021	24 Aug. 2021	26 Oct. 2021
Physicochemical parameters					
T (°C)	20.8	10.42	18	15.5	9
O ₂ (%)	107	81.9	82	129	31
O ₂ (mg l ⁻¹)	8.64	9.14	7.6	9.5	2.4
EC (µS cm ⁻¹)	229	177	357	325	284
pH	8.51	8.63	8.3	8.07	7.65
NH ₃ (mg l ⁻¹)	0.04	1.4	< 0.02	0.24	0.93
PO ₄ ³⁻ (mg l ⁻¹)	0.18	< 0.05	0.1	0.49	0.21
NO ₂ ⁻ (mg l ⁻¹)	< 0.01	< 0.01	0.01	< 0.01	0.02
NO ₃ ⁻ (mg l ⁻¹)	0.1	0.2	1.2	< 0.08	0.37

was characterized by higher water temperature, more alkaline pH and lower conductivity, compared to the Marić River. Saturation and dissolved oxygen concentration were high at both localities, with the exception of extremely low values in the Marić River in October (31% and 2.4 mg l⁻¹, respectively). Nonetheless, *E. bonapartei* was recorded there only during the October sampling. Regarding nutrient measurements, the concentration of orthophosphates was elevated at both sampling sites, with higher values in the Marić River. Ammonia concentrations at both sampling sites were higher in October compared to August, with an exception of the measurement at Lake Zlatar in October. Nitrate and nitrite concentrations remained low during all sampling events.

4. Discussion

Encyonema bonapartei Heudre, C.E.Wetzel & Ector is a new record for the diatom flora of Serbia. The reported localities in Lake Zlatar and the Marić River in the mountainous part of southwestern Serbia are so far the only localities of this taxon outside France (Heudre et al. 2016). The authors of the initial study hypothesized that the species was most likely imported to French biotopes, as it was not recorded in samples during a previous survey in 2008. It was subsequently found in three canals in 2014 and 2015 (Heudre et al. 2016). Our survey covered the main waterbodies of the Uvac River basin, where *E. bonapartei* was recorded in only two neighboring localities. The water bodies from the first report and those from our discovery are distant and unconnected, therefore neither the pathway nor the

vector of dispersal are obvious. This is to be expected given the size of the diatoms, which may lead to their inconspicuous and unintentional spreading (Spaulding et al. 2010).

The benthic diatom community in Lake Zlatar, besides highly abundant *Achnantheidium minutissimum* (Kütz.) Czarn, included taxa typically associated with the littoral zone of lakes and with substrates characterized by the dominance of larger fractions (Trbojević et al. 2021). The benthic diatom community recorded in the Marić River, common to small and medium-sized watercourses in the mountainous region of Serbia (Vasiljević et al. 2014; Jakovljević et al. 2016; Vidaković et al. 2018), was dominated by *A. minutissimum*, *A. pyrenaicum* (Hust.) H.Kobayasi, and *Amphora pediculus* (Kütz.) Grunow. In the diatom community from the East Canal in France, where *E. bonapartei* was recorded, *A. minutissimum* (Heudre et al. 2016) was also one of the most frequent taxa. The relative abundance of *E. bonapartei* in our samples was similar to that in samples from France. A large population was found in Lake Zlatar, with a relative abundance of 24.22%, comparable to the population in the East Canal at Sampigny (30%; Heudre et al. 2016). A small population was observed in the Marić River (2.39%), similar to the population found in the Rhône-to-Rhine Canal at Mackenheim (2%; Heudre et al. 2016).

Given the limited number of documented occurrences of *E. bonapartei* in both France and Serbia, as well as the measured physicochemical parameters, determining the ecological preferences of this species appears to be challenging. However, the environmental factors measured in Lake Zlatar and the Marić River provide new information on the ranges

of temperature, conductivity and orthophosphate concentration of the waters where *E. bonapartei* was found (Table 1). These data indicate that the taxon has the capacity to colonize waters with lower temperature and conductivity but higher orthophosphate concentration compared to data for French canals (Heudre et al. 2016). In addition, our study provides preliminary information on the ecological preferences of *E. bonapartei* in terms of oxygen, ammonia, nitrates and nitrites (Table 1). The largest population was found at a locality characterized by high oxygen saturation and dissolved oxygen concentration and low levels of ammonia, nitrates, and nitrites.

In terms of habitat, large populations of *E. bonapartei* have been found in both France and Serbia, in artificial or heavily modified water bodies such as canals and a reservoir. The sampling site in Lake Zlatar with the highest abundance of this species is located near a dam, in an area with developed tourism infrastructure, with various facilities, boats and platforms (Dobričić, Marjanović 2017). Further research in the Uvac River basin will provide insights into the potential of *E. bonapartei* to thrive in new habitats, as the species was also found in low abundance in the upland-mountain Marić River, at a sampling site near its confluence with the Uvac River, directly downstream of the dam.

More new records of *E. bonapartei* are expected in the future, which will contribute to a better overall understanding of the complex microalgae biogeography. The finding of a large population of *E. bonapartei* in Serbia was the result of monitoring requirements conducted in the Uvac River catchment. The necessity to identify diatoms to the finest taxonomic levels is widely acknowledged as essential for accurate bioassessment (Charles et al. 2021). Along with additional data on the characteristics and distribution of newly described species, understanding their ecological preferences is crucial for assessing ecological status, thereby addressing the discrepancy between the increasing number of newly described species and our knowledge of their indicator values.

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Appendix:

The check list of *Encyonema* taxa recorded in Serbia, with UTM coordinates of the localities (Krizmanić, unpublished database of Serbian diatom flora, June 2023).

Taxon	UTM coordinates
<i>Encyonema auerswaldii</i> Rabenh.	CP95; CR66; CR94; DQ31; DQ50; DQ55; DQ58; DP05; DR01; DR22; DR44; EM06; EN29; EP19; EP33; EP34; EP50; EP75
<i>Encyonema bipartitum</i> (A.Mayer) Krammer	CP93
<i>Encyonema brehmii</i> (Hust.) D.G.Mann	DP72
<i>Encyonema brevicapitatum</i> Krammer	EQ40; EP46; EP75; FN02
<i>Encyonema caespitosum</i> Kütz.	CP95; CR34; CR38; CR57; CR94; DQ55; DQ56; DR22; DP05; DR25; DP26; DP45; DP47; DP55; DP74; DP76/86; EN29; EP22; EP75; FN28
<i>Encyonema elginense</i> (Krammer) D.G.Mann	CQ90; CP99; DN79/70; DP18; DQ10; DQ20; DQ21; DQ31
<i>Encyonema hebridicum</i> Grunow ex Cleve	DM74; DM86; DM87; DN22; DN30; M96
<i>Encyonema lange-bertalotii</i> Krammer	CQ87; CP76; DN57; EP19; EP26; EP33; EP46; EQ03
<i>Encyonema leibleinii</i> (C.Agardh) Silva, Jahn, Ludwig, & Menezes	CP99; CQ47; CQ90; CR34; CR37; CR38; CP66/76; CR62; CR57; CR66; CR71; DN41; DN46/56; DN57; DN94; DP05; DP10; DP14; DP19; DP22; DP24; DP26; DP36; DP45; DP74; DP76/86; DQ10; DQ20; DQ21; DQ31; DR00; DR24; DR25; DR28; DR34; DR35; DR51; DR72/62; DR74; DR83; DQ10; DQ15; DQ31; DQ44/45; DQ46/56; DQ49; DQ56; DQ59; DQ65; DQ76; DQ76/77; DQ94; DS10; DS40; EM06; EN01; EN64; EN74; EN76; EN77; EN82; EP22; EP26; EP27; EP33; EP75; EP77; EP78; EP99; EQ15; EQ54; ER10; FN28; FN28; FN36; FN47; FP01; FP02; FP10
<i>Encyonema lunatum</i> (W.Sm.) Van Heurck	CP63; DM74; DM86; DM96; DN30; DN79/70; DP36; DP56; DQ10; DQ20; DQ21; DQ31; DR25; EM06; FN12; FN48; FN49
<i>Encyonema minutiforme</i> Krammer	CP93; DQ50; FN28
<i>Encyonema minutum</i> (Hilse) D.G.Mann	CP63; CP79; CP81; CQ47; CQ67; CQ77; CQ87; CQ88; CQ95; CQ97; CR37; CR71; CR80; CR96; DM74; DM86; DM87; DM96; DN19; DN21; DN30; DN46/56; DN57; DN75; DN79/70; DN84; DN85; DP10; DP24; DP25; DP25/35; DP26; DP36; DP45; DP46; DP47; DP55; DP56; DP74; DQ07; DQ10; DQ15; DQ20; DQ21; DQ31; DQ35; DQ44/45; DQ45; DQ46/56; DQ48; DQ49; DQ50; DQ55; DQ59; DQ60; DQ65; DQ66; DQ74; DQ76; DQ88; DQ94; DQ95; DR00; DR10; DR25; DR24; DR28; DR34; DR35; DR39; DR40; DR50; DR51; DR72/62; DR74; DR83; DS00; DS10; EM06; EN02; EN03; EN11; EN14; EN64; EN68; EN68/78; EN74; EN77; EN82; EN84; EP10; EP15; EP22; EP26; EP27; EP33; EP34; EP46; EP69; EP75; EP77; EP78; EP78/77; EP95/FP05; EP99; EQ03; EQ15; EQ54; ER10; FN03; FN04; FN05; FN12; FN29; FN39; FN47; FN48; FP20/30
<i>Encyonema neogracile</i> Krammer	DN27; DN26
<i>Encyonema neomesianum</i> Krammer	CQ87; CR57; CR92; DR25; FN12
<i>Encyonema norvegicum</i> (Grunow) A.Mayer	DN79
<i>Encyonema obscurum</i> (Krasske) D.G.Mann	DR25; EP22
<i>Encyonema perminutum</i> Krammer	DN27; DN26
<i>Encyonema perpusillum</i> (A.Cleve) D.G.Mann	DN30
<i>Encyonema persilesiacum</i> Krammer	DN27; DN26
<i>Encyonema procerum</i> Krammer	EN48
<i>Encyonema semilanceolatum</i> Krammer	CP93
<i>Encyonema silesiacum</i> (Bleisch) D.G.Mann	CP66/76; CP76; CP93; CP95; CQ64; CQ77; CQ87; CQ95; CQ97; CR57; CR62; DN57; DN79/70; DP05; DP14; DP24; DP25; DP26; DP45; DP56; DP74; DP72; DP76/86; DQ07; DQ45; DQ50; DQ55; DQ58; DR25; DR34; EN82; EN84; EP19; EP22; EP26; EP34; EP50; EP46; EP52; EP75; EQ03; EQ17; ER10; FN03; FN04; FN12; FN28; FN48; FN49; FP01; FP02; FP10; FP20/30
<i>Encyonema simile</i> Krammer	DP18; DQ50
<i>Encyonema subminutum</i> Krammer & Lange-Bert.	EN48; EP75; EQ40
<i>Encyonema ventricosum</i> (C.Agardh) Grunow	CP76; CP90; CP93; CQ64; CQ77; CQ87; CQ95; CQ97; CR57; CR66; CR92; CR94; DN57; DP05; DP18; DP22; DP26; DP36; DP56; DP74; DQ07; DQ31; DQ45; DQ50; DQ55; DQ60; DR22; DR24; DR44; DR46; EP17; EP19; EP26; EP33; EP34; EP35; EP46; EP50; EP69/79; EP75; EP79; EQ03; EQ70; FN03; FN28; FN36; FN48; FN49
<i>Encyonema vulgare</i> Krammer	CQ87; CR57; CR66; DM74; DR24; DR44; EQ15; FN02; EN64; EN74

