



# Epilithic diatom flora from sub-Mediterranean intermittent rivers in Bulgaria during two hydrological periods

Tsvetelina ISHEVA<sup>1\*</sup> and Plamen IVANOV<sup>2</sup>

1 Department of Aquatic Ecosystems, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria

2 Department of Botany, Faculty of Biology, "St. Kliment Ohridski" Sofia University, 8 Dragan Tzankov Blvd., Sofia 1164, Bulgaria

**ABSTRACT:** The paper presents the first floristic data on diatoms from sub-Mediterranean intermittent rivers in Bulgaria, located within the four largest river basins (Struma, Arda, Maritsa and Tundzha), which drain into the Aegean Sea. A total of 90 epilithic diatom samples were collected from 37 rivers at 50 sites during normal-flow and low-flow seasons. Altogether, 281 species, varieties and forms from 71 genera were identified, almost half of which (138 taxa) were recorded with relative abundance below 1%. Two hundred and forty-six taxa (87% of the total number) were identified during normal-flow periods, while 195 taxa (69%) were identified during low-flow periods. Diatoms recorded only during normal-flow periods were ones common for riverine environments, while in low-flow periods taxa characteristic of stagnant waters, intermittently wet habitats or such habitats with high electrolyte content (brackish habitats) were present. Four taxonomically unclear taxa (*Achanthidium cf. affine*, *Achnantheidium cf. nanum*, *Psammothidium cf. grischunum* and *Psammothidium cf. rossii*) are discussed in terms of their morphometrics, distribution and autecology; LM micrographs of these species are provided. Eleven taxa are new records for the Bulgarian diatom flora.

**KEYWORDS:** Bacillariophyta, Mediterranean rivers, phytobenthos, river type R14

Received: 30 May 2016

Revision accepted: 03 October 2016

UDC: 582.261.1(282)(497.2)  
DOI: 10.5281/zenodo.162213

## INTRODUCTION

Sub-Mediterranean rivers, classified according to Bulgarian legislation as national river type R14, are characterised by extremely high fluctuations of water flow and a strong torrential nature. In winter and spring they may cause extensive floodings, while in summer a transition from lotic to shallow lentic conditions is observed, and in some reaches, especially during dry years, surface water may cease to flow. The result is a hydrological mosaic and these rivers can therefore be more precisely defined as spatially intermittent. The main factors causing seasonal discontinuity of the river flow are the strong Mediterranean climate influence and natural drainage of the terrain,

which result in a lack of sufficient underwater reserves (CHESHMEDJIEV *et al.* 2013). A transitional continental-Mediterranean (sub-Mediterranean) climate is typical for vast parts of Southern Bulgaria and is characterised by mild winters and hot dry summers, with 500-600 mm of annual rainfall and mean annual temperature of 13-14°C. These regions have in recent years experienced more frequently than ever extensive flooding events, followed by harsh arid conditions with long periods of drought.

Although rivers with an intermittent water regime represent a dominant river type in Southern Europe, they are not solely restricted to arid/semi-arid regions or such regions with a Mediterranean climate, but are found all over the world (DATRY *et al.* 2011). Estimates suggest

\*correspondence: tsvetelina.isheva@abv.bg

that rivers which periodically stop flowing may be more common than perennial ones, and their number is expected to increase substantially in the near future due to socio-economic factors and climate change, as intermittency patterns become broader in space and time (LARNED *et al.* 2010; DATRY *et al.* 2014; NOVAIS *et al.* 2014).

Over the past two decades in Bulgaria, the composition of diatoms in perennial rivers, especially anthropogenically affected ones, has been relatively well studied (PASSY-TOLAR *et al.* 1999; IVANOV *et al.* 2003a, 2003b; IVANOV & KIRILOVA 2006; IVANOV *et al.* 2006a, 2006b; IVANOV *et al.* 2007; STANCHEVA *et al.* 2007; IVANOV 2013). However, little scientific attention has been paid to rivers with an intermittent water regime. The following paper represents the first study of the taxonomic composition and distribution of epilithic diatoms from intermittent rivers in Bulgaria during two hydrological periods.

## MATERIALS AND METHODS

**Study area.** The studied sub-Mediterranean rivers are small (catchment area <100 km<sup>2</sup>) to medium sized (100–1200 km<sup>2</sup>) rivers, which are common in SW and SE Bulgaria. They are heterogenic in regard to hydrological, morphological and biological characteristics, e.g., length, catchment area, geology, dominant substrate, flow characteristics, riparian vegetation, etc. The investigated intermittent rivers are located within the basins of the four largest rivers in Southern Bulgaria, which drain into the Aegean Sea (Fig. 1): the Struma River (total length 415 km, catchment area 17300 km<sup>2</sup>), Arda River (total length 272 km, catchment area 5795 km<sup>2</sup>), Maritsa River (total length 472 km, catchment area 53000 km<sup>2</sup>) and Tundzha River (total length 390 km, catchment area 8430 km<sup>2</sup>). The Arda and Tundzha Rivers are major tributaries of the Maritsa,



**Figure 1.** Map of the studied river basins (Struma, Arda, Maritsa and Tundzha). Black dots indicate the approximate localities of the sampling sites within each basin. Grey squares mark the general distribution of sub-Mediterranean intermittent rivers in Bulgaria.

making it the longest river with the largest basin on the Balkan Peninsula. The Maritsa flows through Bulgaria (66.4%), then forms the border between Turkey (27.2%) and Greece (6.4%) and finally empties into the Aegean Sea (SKOULIKIDIS *et al.* 2009).

**Sampling.** A total of 90 epilithic diatom samples were collected at 50 sampling sites from 37 sub-Mediterranean rivers during periods of normal-flow (spring) and low-flow (summer) between 2012 and 2015. During normal water flow, in contrast to high-flow, all river habitats could be accessed and sampled. This period was identified as the time between May and mid-June (end of June). During low-flow, most of the riverbed is dry, low water flow persists in some reaches and stagnant pools are present, either isolated ones or with insufficient surface flow connecting them. This period was identified as the time between July and the end of August (mid-September). Eighteen samples (10 under normal-flow conditions and eight under conditions of low-flow) were collected from five rivers (five sampling sites) within Struma's basin, in addition to 27 samples (20 under normal-flow conditions, seven under conditions of low-flow) from 11 rivers (18 sites) within the Arda's basin; 21 samples (16 under normal-flow conditions, five under conditions of low-flow) from nine rivers (12 sites) within the Maritsa's basin; and 24 samples (19 under normal-flow conditions, five under conditions of low-flow) from 12 rivers (16 sites) within the Tundzha's basin. The relatively lower number of samples collected under conditions of low-flow is due to the lack of surface water during the sampling period.

General physico-chemical variables (presented in Table 1) were measured *in situ* with portable Hanna instruments (HI98129) and WTW equipment (Profiline Oxi 3310) calibrated in the field. Quantitative and qualitative hydromorphological and biological observations were also recorded, e.g. river width and depth, dominant substrates and characteristics of riparian vegetation (Table 1), which provide additional information for a more precise characterisation of these rivers.

Diatom sampling and laboratory pretreatment were carried out according to the European guidance standard for routine sampling and pretreatment of benthic diatoms from rivers (EN 13946:2003). Epilithic diatoms were brushed from at least five boulders, cobbles or other available stone substrates, situated in the main water flow. Samples were directly fixed with 4% formaldehyde. In the laboratory, the diatom samples were treated with cold sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and potassium permanganate (KMnO<sub>4</sub>) to get a clean diatom suspension and then mounted on permanent microscope slides using Naphrax®. Light microscopy and identification were carried out according to the European guidance standard for identification, enumeration and interpretation of benthic diatom samples from rivers (EN 14407:2004), taxa being identified to the lowest taxonomic level possible. At least 400 diatom valves were counted on

**Table 1.** Range (minimum-maximum) and average values of measured environmental variables, together with quantitative and qualitative hydromorphological and biological characteristics of the studied rivers.

	<u>Struma (n=18)</u>		<u>Arda (n=27)</u>		<u>Maritsa (n=21)</u>		<u>Tundzha (n=24)</u>	
	Range	Average	Range	Average	Range	Average	Range	Average
<b>Water temperature, °C</b>	15-25	20	16-30	22	17-30	21	15-26	19
<b>pH</b>	8-8.7	8.4	8.1-8.8	8.5	7.7-9	8.3	7.5-8.5	8
<b>Conductivity, <math>\mu\text{S cm}^{-1}</math></b>	240-580	370	106-590	405	170-935	480	270-1200	560
<b>Dissolved O<sub>2</sub>, mg l<sup>-1</sup></b>	9-11	10	9-15	11	5-15	9	7-12	9
<b>O<sub>2</sub> saturation, %</b>	90-130	105	90-175	115	53-175	100	75-125	93
<b>Length (km)</b>	10-31	22	16-98	40	19-70	39	15-72	30
<b>Catchment size (km<sup>2</sup>)</b>	40-100	78	41-1200	355	18-570	195	61-533	169
<b>Riverbed width (m)</b>	5-13	6	10-150	40	5- 25	9	5-20	8
<b>River width (m)</b>	3-8	4	5-40	15	3-12	6	3-8	5
<b>River depth (m)</b>	0.2-0.5	0.3	0.1-0.6	0.2	0.2-0.6	0.4	0.2-0.6	0.3
<b>Channel shading (%)</b>	5-30	10	1-20	5	20-60	40	10-50	30
<b>Geology</b>	Mixed siliceous+calcareous		Predominantly calcareous		Mixed calcareous+siliceous		Predominantly calcareous	
<b>Flow characteristics</b>	Fast-Medium		Medium-Slow		Slow-Medium		Slow-Medium	
<b>Dominant substrates</b>	Stones		Gravel		Gravel/Stone		Sand/Mud	
	Gravel		Sand		Sand/Mud		Gravel/Stones	
	Sand		Stones		Bedrock		Bedrock	
<b>Riparian vegetation</b>	Sparse		Very sparse		Semi-continuous			
	(e.g., <i>Alnus glutinosa</i> , <i>Platanus orientalis</i> , <i>Salix</i> spp., rarely <i>Tamarix</i> spp.)		(e.g., <i>Salix</i> spp., rarely <i>Alnus glutinosa</i> )		(e.g., <i>Alnus glutinosa</i> , <i>Populus alba</i> , <i>P.nigra</i> , <i>Robinia pseudoacacia</i> , <i>Salix</i> spp.)			
<b>Algal communities</b>	Diatoms		Diatoms		Diatoms			
	rarely <i>Bangia</i> sp. (Rhodophyta)		<i>Cladophora</i> spp. (Chlorophyta)		Cyanoprokaryota (e.g., <i>Lyngbya</i> spp., <i>Nostoc verrucosum</i> , <i>Phormidium</i> spp.)			
	rarely <i>Cladophora</i> sp. (Chlorophyta)		rarely <i>Monostroma bullosum</i> (Chlorophyta)		Rhodophyta (e.g., <i>Batrachospermum</i> spp., <i>Hildenbrandia rivularis</i> , <i>Lemanea</i> spp.)			
	rarely <i>Vaucheria</i> sp. (Xanthophyceae)		rarely <i>Vaucheria</i> sp. (Xanthophyceae)		Chlorophyta (e.g., <i>Cladophora</i> spp., <i>Draparnaldia</i> spp., <i>Tetraspora</i> spp., <i>Ulothrix</i> spp.); Streptophyta ( <i>Charales</i> , <i>Zygnematales</i> )			
<b>Submerged macrophytes</b>	Absent		Absent (rarely <i>Myriophyllum spicatum</i> )		Diverse (e.g., <i>Callitriche platycarpa</i> , <i>Ceratophyllum demersum</i> , <i>Groenlandia densa</i> , <i>Myriophyllum spicatum</i> , <i>Potamogeton crispus</i> , <i>P. nodosus</i> , <i>Ranunculus aquatilis</i> , <i>R. trichophyllus</i> , <i>Stuckenia pectinata</i> , <i>Zannichellia palustris</i> )			
<b>Habitat diversity</b>	Low		Very low		Medium (High)		High (Medium)	

each slide in random transects in order to calculate the relative abundance (%) of each taxon. Light microscopy was performed with Amplival (Carl Zeiss Jena) and Olympus BX51 microscopes equipped with 100× oil-immersion objectives and the latter with a digital camera for light micrographs. The materials are stored in the algal collection of the Department of Botany, Faculty of Biology, "St. Kliment Ohridski" Sofia University.

Diatoms were determined mainly according to KRAMMER & LANGE-BERTALOT (1986-1991), LANGE-BERTALOT & KRAMMER (1989), LANGE-BERTALOT (1993, 2001), LANGE-BERTALOT & METZELTIN (1996), KRAMMER (1997a, 1997b, 2000, 2002, 2003), REICHARDT (1999, 2004), WERUM & LANGE-BERTALOT (2004), HOFMANN *et al.* (2013) with some additions by LIU *et al.* (2015), NOVAIS *et al.* (2014) and WETZEL *et al.* (2015).

## RESULTS

The epilithic diatom flora was represented by 281 species, varieties and forms from 71 genera (Appendix, available online). One-hundred and thirty-eight (49% of all) taxa were recorded with a relative abundance below 1%. Two hundred and forty-six taxa (87% of the total number) were identified during normal-flow periods, while 195 taxa (69%) were identified during low-flow periods. The raphid pennate diatoms constituted 86.2% of all identified taxa, and the taxon richest in genera were *Navicula* Bory (40 taxa), *Nitzschia* Hassall (32), *Gomphonema* Agardh (21) and *Achnantheidium* Kützing (15). The araphid pennate diatoms represented 8.8% of all identified taxa and *Fragilaria* Lyngbye (10 taxa) and *Diatoma* Bory (four) were the genera with the most taxa. The centric diatoms constituted 5% of all identified taxa, with a relatively low number of taxa within each genus.

The investigated rivers within the basin of the Tundzha had the highest diatom diversity (207 taxa, 73% of the total number), 182 taxa (64%) being recorded for normal-flow and 127 taxa (45%) for low-flow periods. Rivers within the basin of the Maritsa had the second highest species richness: 199 (70% of all) taxa, with 167 taxa (59%) recorded in normal-flow and 134 taxa (47%) in low-flow periods, followed by rivers within the Arda's basin (155 taxa, 55% of the total number) with 127 taxa (45%) in normal-flow and 102 taxa (36%) in low-flow periods. The Struma's basin had the lowest diatom diversity (111 taxa, 39% of the total number), with 92 taxa (32%) in normal-flow and 78 taxa (27%) in low-flow periods.

Twenty-eight taxa were present in over 50% of the samples in both sampling periods. Some of the diatoms most frequently observed during normal-flow were: *Achnantheidium minutissimum* (Kützing) Czarnecki (in 91% of all samples), *Ulnaria ulna* (Nitzsch) Compère (74%), *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot (71%), *Nitzschia inconspicua* Grunow (68%), *Navicula antonii* Lange-Bertalot (65%), *Melosira*

*varians* Agardh (63%) and *Cymbella excisa* Kützing (60%). In contrast, the diatoms most frequently observed during low-flow periods were: *Cocconeis euglypta* Ehrenberg (in 96% of all samples), *Nitzschia paleacea* (Grunow) Grunow (88%), *Navicula cryptotenella* Lange-Bertalot (84%), *Nitzschia fonticola* Grunow (80%), *Amphora pediculus* (Kützing) Grunow (80%), *Sellaphora nigri* (De Notaris) Wetzel & Ector (80%) and *Navicula reichardtiana* Lange-Bertalot (72%).

Thirty-three taxa were present in all investigated river basins in both sampling periods, e.g., *Achnantheidium minutissimum*, *A. eutrophilum* (Lange-Bertalot) Lange-Bertalot, *A. saphophilum* (Kobayashi & Mayama) Round & Bukhtiyarova, *Caloneis lancettula* (Schulz) Lange-Bertalot et Witkowski, *Cocconeis euglypta*, *Cymbella excisa*, etc. The diatoms with the greatest relative abundances were as follows: *Achnantheidium pyrenaicum* (Hustedt) Kobayasi (88%) and *Nitzschia fonticola* (52%) within the Struma's basin; *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot (84%) and *Gomphonema tergestinum* (Grunow) Fricke (83%) within the Arda's basin; *Achnantheidium minutissimum* (58%) and *Craticula subminuscula* (Manguin) Wetzel et Ector (54%) within the Maritsa's basin; and *Achnantheidium minutissimum* (56%) and *Amphora pediculus* (48%) within the Tundzha's basin.

Diatoms with high values of relative abundance in normal-flow periods but substantially lower values of this index during low-flow periods were: *Achnantheidium pyrenaicum* (from the maximum value of 88% in normal-flow periods to the minimum value of 66% in low-flow periods), *Gomphonema tergestinum* (83% to 11%), *Nitzschia paleacea* (58% to 19%), *Achnantheidium minutissimum* (58% to 39%), *Nitzschia fonticola* (52% to 23%) and *Cymbella excisa* (52% to 36%). On the other hand, species whose relative abundances increased in low-flow periods were: *Amphora pediculus* (from the minimum value of 39% in normal-flow periods to the maximum value of 62% in low-flow periods), *Reimeria sinuate* (Gregory) Kociolek et Stoermer (16% to 46%), *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot (7% to 32%), *Gomphonema minutum* (Agardh) Agardh (6% to 28%) and *Epithemia adnata* (Kützing) Brébisson (6% to 28%).

Diatoms recorded only during normal-flow periods were as follows: *Achnantheidium subatomus* (Hustedt) Lange-Bertalot, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot, *Diatoma mesodon* (Ehrenberg) Kützing, *Encyonema silesiacum* (Bleisch) Mann, *Eucoconeis laevis* (Østrup) Lange-Bertalot, *Fragilaria nanana* Lange-Bertalot, *Nitzschia dissipata* var. *media* (Hantzsch) Grunow and *Sellaphora atomoides* (Grunow) Wetzel et Van de Vijver. On the other hand, species occurring only during low-flow periods were: *Achnantheidium atomoides* Monnier, Lange-Bertalot et Ector, *A. catenatum* (Bily et Marvan) Lange-Bertalot, *Adlafia bryophila* (Petersen) Lange-Bertalot, *Diademsis confervacea* Kützing, *Hippodonta costulata* (Grunow) Lange-Bertalot, Metzeltin et Witkowski,

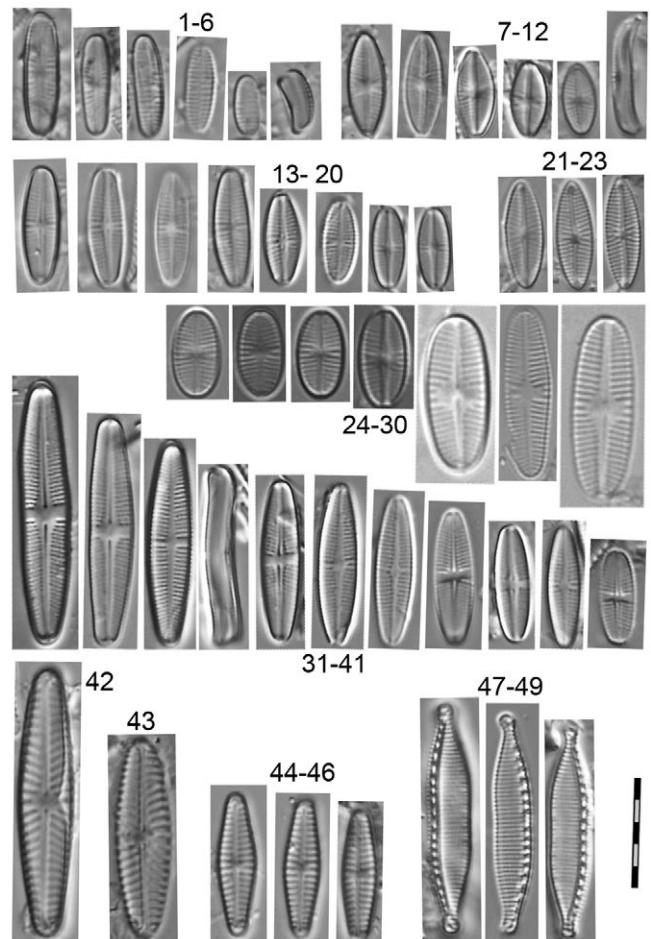
*Karayevia ploenensis* (Hustedt) Bukhtiyarova, *Mayamaea agrestis* (Hustedt) Lange-Bertalot (Fig. 2: 21-23), *Nitzschia elegantula* Grunow (Fig. 2: 40-42), *Placoneis gastrum* (Ehrenberg) Mereschkowsky, *Planothidium minutissimum* (Krasske) Morales, *Rhopalodia parallela* (Grunow) Müller and *Tryblionella calida* (Grunow) Mann.

Diatomstypical for stagnant waters and/or intermittently wet habitats or such habitats with high electrolyte content (brackish habitats) were recorded during low-flow periods. The following species were in this category: *Achnantheidium catenatum*, *Aneumastus stroesei* (Østrup) Mann, *Diademesmis confervacea*, *D. perpusilla* (Grunow) Mann, *Grunowia tabellaria* (Grunow) Rabenhorst, *G. solgensis* (Cleve) Aboal, *Hantzschia amphioxys* (Ehrenberg) Grunow, *Hippodonta pumila* Lange-Bertalot, Hofmann et Metzeltin (Fig. 2: 37-39), *Mayamaea agrestis*, *Navicula cataracta-rheni* Lange-Bertalot, *N. cincta* (Ehrenberg) Ralfs (Fig. 2: 43) and *N. kotschyi* Grunow.

Nine taxa were identified with a certain degree of uncertainty (noted as 'cf.' in Appendix, available online), since they didn't entirely fit the species diagnosis and/or were present with few individual valves. Although four unclear species were relatively abundant, the lack of sufficient and straightforward data on their taxonomy, morphology, ecology and distribution made their identification to a certain extent more difficult. Further microscopic investigations will be carried out to reveal their taxonomic identity. Data on their morphology, distribution and ecology are given below.

*Achnantheidium cf. affine* (Fig. 2: 31-41), valve length 7.2-24 µm, width 3-4.3 µm, stria density 25-29/10 µm (n=30). The species was recorded in one river within the Arda's basin (locality: Krumovitsa River N41°35'07.1", E25°40'08.6", at 69 m a.s.l.) with 42% relative abundance, occurring at 27°C water temperature, slightly alkaline pH (8.2), moderate specific conductivity (440 µS cm<sup>-1</sup>), dissolved oxygen content of 9.8 mg l<sup>-1</sup> and 109% oxygen saturation, on silicate substrate. The river is under moderate anthropogenic impact. Associated diatoms with the highest relative abundances were *Cymbella excisa* (13%), *Nitzschia amphibia* Grunow (12%) and *N. fonticola* (8%).

*Achnantheidium cf. nanum* (Fig. 2: 13-20), valve length 6-10 µm, width 2.5-3.4 µm, stria density 25-30/10 µm (n=30). The species was recorded with a maximum relative abundance of 7% within the Maritsa's basin (locality: Byala River, N41°22'47.2", E26°01'47.3", at 115 m a.s.l.), occurring at 26°C water temperature, slightly alkaline pH (8.1), moderate specific conductivity (478 µS cm<sup>-1</sup>), dissolved oxygen content of 7.8 mg l<sup>-1</sup> and 85% oxygen saturation, on a silicate substrate. The river is under low anthropogenic impact. The sample had a relatively high species richness (67 taxa), but none of the associated diatoms had a relative abundance above 10%, the most abundant ones being *Amphora pediculus* (9%), *Navicula cryptotenella* (7%), *N. cryptotenelloides* Lange-Bertalot



**Figure 2.** LM micrographs. 1-6 *Achnantheidium atomoides*; 7-12 *Psammothidium cf. rossii*; 13-20 *Achnantheidium cf. nanum*; 21-23 *Mayamaea agrestis*; 24-30 *Psammothidium cf. grischunum*; 31-41 *Achnantheidium cf. affine*; 42 *Navicula seibigiana*; 43 *Navicula cincta*; 44-46 *Hippodonta pumila*, 47-49 *Nitzschia elegantula*. Scale bar 10 µm.

(7%) and *Pseudostaurosira brevistriata* (Grunow) Williams et Round (5%).

*Psammothidium cf. grischunum* (Wuthrich) Bukhtiyarova et Round (Fig. 2: 24-30), valve length 6-16 µm, width 3-4.5 µm, stria density 18-24/10 µm (n=30). The species was recorded with a maximum relative abundance of 11% under conditions of normal-flow in four rivers within the Tundzha's basin (locality: Ahlatliiska River, N42°03'45.2", E26°57'02.9", at 324 m a.s.l.) occurring at 19°C water temperature, pH 7.8, specific conductivity of 268 µS cm<sup>-1</sup>, dissolved oxygen content of 8.1 mg l<sup>-1</sup> and 90% oxygen saturation, on a silicate substrate. The river is under low anthropogenic impact. Associated diatoms with the highest relative abundances were *Meridion circulare* (Greville) Agardh (35%), *Nitzschia paleacea* (14%) and *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot (11%).

*Psammothidium* cf. *rossii* (Hustedt) Bukhtiyarova et Round (Fig. 2: 7-12), valve length 5-11  $\mu\text{m}$ , width 3-3.8  $\mu\text{m}$ , stria density 28-30/10  $\mu\text{m}$  (n=20), recorded only from one river in the Maritsa's basin (locality: Luda River N41° 24'14.7", E26°09'40.6", at 64 m a.s.l.) with a relative abundance of 1.1% under low-flow conditions, occurring at 30°C water temperature, slightly alkaline pH (8.3), relative low specific conductivity (179  $\mu\text{S cm}^{-1}$ ), dissolved oxygen content of 8.2  $\text{mg l}^{-1}$  and 94% oxygensaturation, on a silicate substrate. The river is under very low anthropogenic impact (near reference conditions), but the environmental conditions change during low-flow periods due to significantly lower levels of surface water verging on an almost complete lack of flow, with the presence of stagnant pools. As a result, the associated taxa with the highest relative abundances [*Epithemia sorex* Kützing (21%), *Rhopalodia gibba* (Ehrenberg) Müller (8%), *Nitzschia inconspicua* (6%) and *N. elegantula* (5%)], were diatoms common for lentic environments and/or such environments with moderate (high) trophic levels.

Eleven taxa are new for the Bulgarian diatom flora. Indicated with an asteriks in Appendix (available online), they are as follows: *Achnantheidium atomoides* (Fig. 2: 1-6), *Achnantheidium catenatum*, *Hippodonta pumila*, *Mayamaea agrestis*, *Navicula densilineolata*, *Navicula gerloffii*, *Navicula seibigiana* (Fig. 2: 42), *Navicula tenelloides*, *Navicula wiesneri*, *Nitzschia elegantula* and *Planothidium minutissimum*.

## DISCUSSION

The aim of the present study was to understand more about the diversity and distribution of diatoms in non-perennial rivers in Bulgaria, since globally they represent a major yet still understudied and particularly vulnerable portion of river networks (McDONOUGH *et al.* 2011). The diversity of diatoms in the studied rivers was relatively high, with characteristic taxa present during normal-flow and low-flow periods. During the latter, diatom diversity was lower in all of the investigated river basins and there was an increase of taxa typically occurring in lentic, aerophilic or brackish environments, whereas in normal-flow periods diatoms typical for lotic habitats were present. Comparing our results with those of a study exploring permanent and temporary watercourses in Portugal (NOVAIS *et al.* 2014), we see that the taxonomic composition is similar in that the diatoms with maximum relative abundances and frequency of occurrence in both studies were *Achnantheidium minutissimum*, *Amphora pediculus*, *Cocconeis euglypta*, *Nitzschia inconspicua*, *Planothidium frequentissimum* and *Reimeria sinuata*. It is interesting to note that diatoms identified from permanent watercourses in Portugal, e.g., *Pinnularia microstauron* and *Fragilaria nanana*, were present in Bulgarian intermittent rivers only during normal-flow periods, while diatoms characteristic of Portuguese temporary watercourses, e.g.,

*Navicula cataracta-rheni*, was recorded only during low-flow periods in Bulgaria. Thus, the diatom composition of Bulgarian intermittent rivers during normal-flow periods resembles that of perennial rivers in Portugal, whereas during low-flow periods it resembles that of Portuguese temporary rivers. Hence, hydrology is perhaps one of the most significant drivers of community structure.

A study of Mediterranean rivers by GOMA *et al.* (2004) showed that rivers with larger catchments are richer in diatom species (due to the higher diversity of river ecotypes in the watershed) in comparison with smaller catchments, where species diversity is lower. The present study showed that catchment size doesn't influence diatom diversity, at least for Bulgarian intermittent rivers. We believe that local habitat heterogeneity plays a much more crucial role for the diversity of epilithic diatom assemblages. The rivers with the highest diatom diversity (ones in the basins of the Tundzha and Maritsa) were more diverse in terms of the range of measured environmental variables, substrate and vegetation (riparian and aquatic), than rivers having lower diatom diversity (those in the basins of the Struma and Arda) where the local habitat diversity was significantly lower and uniform (Table 1).

## CONCLUSIONS

This paper presents the first study of the taxonomic composition and distribution of epilithic diatoms from intermittent rivers in Bulgaria, and it contributes to the broader and regional knowledge of diatom diversity in these hydrologically challenged freshwater environments. Furthermore, the study can be used in future investigations and comparisons of diatom communities from intermittent rivers. Inasmuch as the important role of diatoms as ecological indicators is recognized worldwide, the diatom taxa characteristic of Bulgarian sub-Mediterranean rivers will be further analysed in order to explore the possibility of predicting flow intermittency based on species composition and abundance. A further step will be to evaluate the ecological status of these rivers based on the qualitative and quantitative diatom data from the present study. The results of such evaluation will be discussed in a future paper.

**Acknowledgements** — The authors are grateful to the anonymous reviewers for their comments and suggestions, which improved the manuscript.

## REFERENCES

- CHESHMEDJIEV S, MARINOV M & KARAGYOZOVA T. 2013. Characterization and definition of the ecological goals for the types of surface water bodies In: BELKINOVA D & GECHIEVA G (eds.), *Biological analysis and ecological assessment of the surface water types in Bulgaria*, pp. 12–52, Plovdiv University Press, Plovdiv. (In Bulgarian).

- DATRY T, ARSCOTT D & SABATER S. 2011. Recent perspectives on temporary river ecology. *Aquatic Sciences* **73**:453–457.
- DATRY T, LARNED S & TOCKNER K. 2014. Intermittent Rivers: A Challenge for Freshwater Ecology. *BioScience* **64**:229–235.
- EN 13946. 2003. *Water quality – Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers*. European Committee for Standardization, Brussels.
- EN 14407. 2004. *Water quality – Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters*. European Committee for Standardization, Brussels.
- GOMA J, ORTIZ R, CAMBRA J & ECTOR L. 2004. Water quality evaluation in Catalanian Mediterranean rivers using epilithic diatoms as bioindicators. *Vie et Milieu* **54**: 81–90.
- HOFMANN G, WERUM M & LANGE-BERTALOT H. 2013. *Diatomeen im Süßwasser-Benthos von Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis*. Koeltz Scientific Books, Königstein.
- IVANOV P. 2013. Epilithic diatom flora and evaluation of ecological status of the Mesta river during 2000–2009. In: UZUNOV Y, PEHLIVANOV L, GEORGIEV BB & VARADINOVA E (eds.), *Mesta River: Biological quality elements and ecological status*, pp. 23–48, “Prof. Marin Drinov” Academic Publishing House, Sofia.
- IVANOV P, CHIPEV N & TEMNISKOVA D. 2003a. Diatoms of the river Iskur (Sofia Plain) and their implication for water quality assessment, Part 1. The diatom flora, ecology and community structure. *Journal of Environmental Protection and Ecology* **2**: 288–300.
- IVANOV P, CHIPEV N & TEMNISKOVA D. 2003b. Diatoms of the river Iskur (Sofia Plain) and their implication for water quality assessment, Part 2. Diatom indices and their implication for water quality monitoring. *Journal of Environmental Protection and Ecology* **2**: 301–310.
- IVANOV P & KIRILOVA E. 2006. Benthic diatom assemblages from different substrates of the Iskar River, Bulgaria. In: WITKOWSKI A (ed.), *Proceedings of the 18th International Diatom Symposium*, pp. 107–124, Biopress Limited, Bristol.
- IVANOV P, KIRILOVA E & ECTOR L. 2006a. Diatom taxonomic composition of rivers in South and West Bulgaria. *Phytologia Balcanica* **12**(3): 327–338.
- IVANOV P, KIRILOVA E & ECTOR L. 2006b. Diatom species composition from the River Iskur in the Sofia region, Bulgaria. In: OGNJANOVA-RUMENOVA N & MANOYLOV K (eds.), *Advances in Phycological Studies, Festschrift in honour of Prof. Dobrina Temniskova-Topalova*, pp. 167–190, Pensoft & University Publishing House, Sofia-Moscow.
- IVANOV P, KOURTEVA E & MANCHEVA A. 2007. Diatom taxonomic composition of streams and small rivers in the Strouma basin, SW Bulgaria. *Phytologia Balcanica* **13**(3): 293–305.
- KRAMMER K. 1997a. Die cymbelloiden Diatomeen. Teil 1. Allgemeines und *Encyonema* part. *Bibliotheca diatomologica* **36**: 1–382.
- KRAMMER K. 1997b. Die cymbelloiden Diatomeen. Teil 2. *Encyonema* part., *Encyonopsis* und *Cymellopsis*. *Bibliotheca diatomologica* **37**: 1–469.
- KRAMMER K. 2000. The genus *Pinnularia*. In: LANGE-BERTALOT H (ed.), *Diatoms of Europe* **1**, pp.1–703, A.R.G. Gantner-Verlag, Ruggell, Liechtenstein.
- KRAMMER K. 2002. The genus *Cymbella*. In: LANGE-BERTALOT H (ed.), *Diatoms of Europe* **3**, pp. 1–584, A.R.G. Gantner-Verlag, Ruggell, Liechtenstein.
- KRAMMER K. 2003. *Cymbopleura, Delicata, Navicymbula, Gomphocymbellopsis, Afrocybella*. In: LANGE-BERTALOT H (ed.), *Diatoms of Europe* **4**, pp. 1–530, A.R.G. Gantner-Verlag, Ruggell, Liechtenstein.
- KRAMMER K & LANGE-BERTALOT H. 1986–1991. Bacillariophyceae 1–4. In: Ettl H, Gerloff J, Heynig H & Mollenhauer D (eds.), *Süßwasserflora von Mitteleuropa* **2/1–4**, Gustav Fisher Verlag, Stuttgart.
- LANGE-BERTALOT H. 1993. 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süßwasserflora von Mitteleuropa. *Bibliotheca diatomologica* **27**: 1–164.
- LANGE-BERTALOT H. 2001. *Navicula* sensu stricto. 10 genera separated from *Navicula* sensu lato. *Frustulia*. In: LANGE-BERTALOT H (ed.), *Diatoms of Europe* **2**, pp. 1–526, A.R.G. Gantner-Verlag, Ruggell, Liechtenstein.
- LANGE-BERTALOT H & KRAMMER K. 1989. *Achnanthes* eine Monographie der Gattung mit Definition der Gattung *Cocconeis* und Nachtragen zu den Naviculaceae. *Bibliotheca diatomologica* **18**:1–393.
- LANGE-BERTALOT H & METZELTIN D. 1996. Indicators of oligotrophy. In: LANGE-BERTALOT H (ed.), *Iconographia Diatomologica* **2**, pp.1–390, Koeltz Scientific Books, Königstein.
- LARNED ST, DATRY T, ARSCOTT DB & TOCKNER K. 2010. Emerging concepts in temporary river ecology. *Freshwater Biology* **55**: 717–738.
- LIU Q, KOCIOLEK JP, WANG QX & FU CX. 2015. Two new *Prestauroneis* Bruder & Medlin (Bacillariophyceae) species from Zoige Wetland, Sichuan Provincem, China, and comparison with *Parlibellus* E.J. Cox. *Diatom Research* **30**(2): 133–139.
- MCDONOUGH OT, HOSEN JD & PALMER MA. 2011. Temporary streams: the hydrology, geography and ecology of non-perennially flowing waters. In: ELLIOT HS & MARTIN LE (eds.), *River Ecosystems: Dynamics, Management and Conservation*, pp. 259–289, Nova Science Publishers Inc., New York.
- NOVAIS MH, MORAIS MM, ROSADO J, DIAS LS, HOFFMANN L & ECTOR L. 2014. Diatoms of temporary and permanent watercourses in Southern Europe (Portugal). *River Research and Applications* **10**: 1216–1232.
- PASSY-TOLAR S, PAN R & LOWE R. 1999. Ecology of the major periphytic diatom communities from the Mesta River, Bulgaria. *International Review of Hydrobiology* **2**: 129–174.

- REICHARDT E. 1999. Zur Revision der Gattung *Gomphonema*. Die Arten um *G. affine/insigne*, *G. angustatum/micropus*, *G. acuminatum* sowie gomphonemoide Diatomeen aus dem Oberoligozän in Böhmen. In: LANGE-BERTALOT H (ed.), *Iconographia Diatomologica* **8**, pp.1-206, Koeltz Scientific Books, Königstein.
- REICHARDT E. 2004. Eine bemerkenswerte diatomeenassoziation in einem Quellhabitat im Grazer Bergland, Österreich. Ein Beitrag zur Kenntnis seltener und weing bekannter Diatomeen. In: LANGE-BERTALOT H (ed.), *Iconographia Diatomologica* **13**: 418-480, Koeltz Scientific Books, Königstein.
- SKOULIKIDIS NT, ECONOMOU AN, GRITZALIS KC & ZOGARIS S. 2009. Rivers of the Balkans. In: TOCKNER K, UEHLINGER U & ROBINSON CT (eds.), *Rivers of Europe*, pp. 421-466, Elsevier Academic Press, Amsterdam.
- STANCHEVA R, MANCHEVA A & IVANOV P. 2007. Taxonomic composition of the epilithic diatom flora from rivers Vit and Osum, Bulgaria. *Phytologia Balcanica* **1**: 53-64.
- WERUM M & LANGE-BERTALOT H. 2004. Diatoms in springs from Central Europe and elsewhere under the influence of hydrogeology and anthropogenic impacts. In: LANGE-BERTALOT H (ed.), *Iconographia Diatomologica* **13**, pp. 3-417, A.R.G. Gantner Verlag.
- WETZEL CE, ECTOR L, VAN DE VIJVER B, COMPÈRE P & MANN DG. 2015. Morphology, typification and critical analysis of some ecologically important small naviculoid species (Bacillariophyta). *Fottea* **2**: 203-234.

## Botánica SERBICA



## REZIME

## Flora epilitskih dijatomeja iz sub-mediteranskih intemitentnih reka u Bugarskoj tokom dva hidrološka perioda

Tsvetelina ISHEVA i Plamen IVANOV

U radu su predstavljene prvi podaci o dijatomejama iz submediteranskih intermitentnih reka u Bugarskoj, lokalizovanih unutar četiri najveća rečna sliva (Struma, Arda, Marica i Tundža) koji gravitiraju ka Egejskom moru. Ukupno je sakupljeno 90 uzoraka epilitskih dijatomeja iz 37 reka na 50 lokaliteta tokom sezona sa normalnim i niskim protokom. Identifikovan je 281 takson iz ukupno 71 roda, s tim da je gotovo polovina taksona (138) zabeležena sa relativnom gustomanjom od 1%. Tokom perioda normalnog protoka konstatovano je 246 taksona (87%), a tokom niskog protoka 195 (69%). Diatomeje konstatovane samo tokom perioda sa normalnim protokom su uobičajene za priobalje reka, dok su one koje se javljaju u periodu niskog protoka karakteristične za stajaće vode, povremeno vlažna staništa ili staništa sa brakičnom vodom. Četiri interesantne, a time i taksonomski nedovoljno jasne vrste (*Achanthidium* cf. *affine*, *Achnanthidium* cf. *nanum*, *Psammothidium* cf. *grischunum* i *Psammothidium* cf. *rossii*) su istražene u smislu morfometrije, distribucije i autekologije, a predstavljene su i njihove LM mikrofografije. Jedanaest taksona predstavljaju nove nalaze za floru dijatomeja Bugarske.

**KLJUČNE REČI:** Bacillariophyta, mediteranske reke, fitobentos, R14 tip reke