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EXPANSION OF *DIDYMOSPHENIA GEMINATA* (LYNGBE) M. SCHMIDT (BACILLARIOPHYCEAE) IN RUNNING WATERS IN S-E POLAND: NEW RECORDS IN THE PODKARPACIE REGION

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ABSTRACT

Nowadays *Didymosphenia geminata* is one of the most frequently studied diatoms in the world, due to its massive development in most continents. In Poland, up to the 1990's only a small number of specimens were recorded, from streams in the Tatra Mountains and the Dunajec River. *D. geminata* was found in 2007–2008 in the middle and upper sections of the Wisłok River, while in 2009 and 2011 it was also found in the Wisłoka, Ropa, Bielcza and San. The cells belonged to one morphotype "geminata" and were characterized by high variability of size. It develops in mesotrophic, well-oxygenated, upper parts of flowing waters with rapid currents. It was most abundant below reservoirs on the San, Wisłok and Wisłoka.

Keywords: *Didymosphenia geminata*, running waters, ecology.

INTRODUCTION

Until the 1990's *Didymosphenia geminata* (Lyngbe) M. Schmidt was a species known mainly from the boreal and alpine regions of Europe, Asia (Himalayas), and North America. In Central Europe, until recently, it was very rare and found only in cold, oligotrophic waters, with moderate or high electrolyte content, in the Alps [Krammer, Lange-Bertalot 1986]. The above features confirm the presence of this diatom in unpolluted waters in Scandinavia [Cleve-Euler 1955]. It has been reported in Finland [Kawecka, Eloranta 1987], Sweden [Johansson 1980] and also in Norway, where it was found, amongst other places, in the river Glåma, which is considered to be its natural environment [Skulberg, Lillehammer 1984].

D. geminata was recorded in Poland in the 1960s, but only in oligotrophic streams in the Tatra Mts. [Siemińska 1964] and some other places, e.g. in the Białka River [Kawecka 1965]. In the 1990's this species started to spread to other Carpathian rivers: San, Soła, Skawa, and Raba [WIOŚ 1996, 2000]. Massive development, in a form of mucilaginous colonies, of *D. geminata* was observed below the Solina and Myczkowce reservoirs [Kawecka, Sanecki 2003]. At that time, it was also found in the seston of the Vistula [Bucka 2000, Kasza, Galas 2000]. In 2002 it was reported in the Czarna Orawa River and its tributaries in the Orawa region [Noga 2003]. The species is more and more frequently found in the running waters of SE Poland [Kawecka, Sanecki 2003, Mrozińska-Broda, Czerwik-Marcinkowska 2004, Mrozińska-Broda et al. 2006, Noga et al. 2012].

D. geminata is currently one of the most frequently studied diatom species, and it is spreading, often in large numbers, not only in Central

Europe but also on other continents [Bhatt et al. 2008, Blanco, Ector 2009, Whitton et al. 2009].

The aims of this study were: to investigate the distribution of *Didymosphenia geminata* in the Wisłok River and other rivers and streams in the Podkarpacie region, to make a taxonomic analysis of the population, and to determine its living conditions.

STUDY AREA

The Wisłok River is the longest left-bank tributary of the San, 228.5 km long, with 3516 km^2 of catchment area. The river rises at Mount Kanasiówka (823 m a.s.l.) in the Beskid Niski Mts., near the border between Slovakia and Poland. The catchment of the Wisłok is characterized by a varied landscape, as the river flows through seven geographic mesoregions distinguished within the western part of the Polish Carpathians: starting from the Beskid Niski Mts., through the Bukowskie Foothills (Pogórze Bukowskie) and the Jasielsko-Krośnieńska Basin (Kotlina Jasielsko-Krośnieńska), then forming a border between the Strzyżowskie Foothills (Pogórze Strzyżowskie) and the Dynowskie Foothills (Pogórze Dynowskie), while in its lower section the river crosses the Rzeszowskie Foothills (Podgórze Rzeszowskie) and finally meets the San River in the Podkarpacie Proglacial Valley (Pradolina Podkarpacka) at the altitude of 182 m a.s.l. In its upper section, the Wisłok flows through wooded, mountainous areas (relatively undisturbed by human activity), whereas in the middle and lower sections, it drains farmlands and industrial areas [Kondracki 2001, Dąbal 2005].

The Wisłok River and its major tributaries: Morwawa, Pielnica, Stobnica, Strug and Mleczka belong to the catchment of the San River, which is the left tributary of the Vistula. The Wisłok is a relatively shallow river (especially in its upper course), and it has a rocky bottom, overgrown in some places by aquatic plants. Mleczka River is of different character, its bottom in the middle and lower reaches is silted and the current is very slow. A detailed description of the sampling sites on the Wisłok River is shown in Table 1.

Because of its varied landscape and high conservation value, much of the catchment area is protected by law within the Jaśliski Landscape Park and the Czarnorzecko-Strzyżowski Landscape Park.

The upper section of the river ends in the Besko Reservoir, at the concrete dam in Sieniawa (Figure 1, site 3). The Rzeszów Reservoir (Zbiornik Rzeszowski) near the city of Rzeszów was created to ensure water intake for the city, to protect it against floods, and for recreation. The waters of the Wisłok River are used for industrial and communal purposes. The river receives urban sewage, industrial

Table 1. Morphological and physico-chemical profile of Wisłok River on chosen sites (2007–2008)

Site	1. Wisłok Wielki	2. Rudawka Rymanowska	3. Besko	4. Żarnowa
Section of river	upper			middle
Insolation	average	large	large	large
Bottom	stony, inshore muddy	stony, inshore muddy	stony, overgrown with algae	stony, overgrown with algae and plants
Width [m]	2–4	20–25	20–22	22–25
Depth [m]	0.1–0.2	0.1– 0.5	0.3– 0.5	0.2–1.0
Temperature [°C]	4.8–19.7	5.3–27	4.2–26	3.8–24.2
pH	7.3–8.1	7.4–8.4	7.5–8.2	8.0–8.4
Conductivity [μS cm^{-1}]	212–327	165–410	299–322	460–500
Oxygen dissolve [mg O_2 L^{-1}]	–	7.4–12.4	8–10.5	7.85–11.18
BOD_5 [mg O_2 L^{-1}]	0.9–1.5	0.7–2.1	0.9–2.3	1.1–2.3
COD_{Cr} [mg O_2 L^{-1}]	<10–12.6	<10–22.1	10.6–13.4	13.7–28.7
PO_4 – P [mg L^{-1}]	< 0.05–0.058	< 0.05	< 0.05	0.099–0.392
Total P [mg L^{-1}]	<0.015–0.055	< 0.05	< 0.05	0.068–0.172
Ca [mg L^{-1}]	35–54	50–62.7	47.7–50	69–76
N_{NH4} – N [mg L^{-1}]	<0.1–0.366	0.076–0.106	0.093–0.14	<0.163–0.7
N_{NO2} – N [mg L^{-1}]	0.0031–0.0118	< 0. 003–0.0045	0.006–0.015	0.0169–0.0426
N_{NO3} – N [mg L^{-1}]	<0.2–0.711	0.124–0.928	0.532–1.23	1.31–2.02

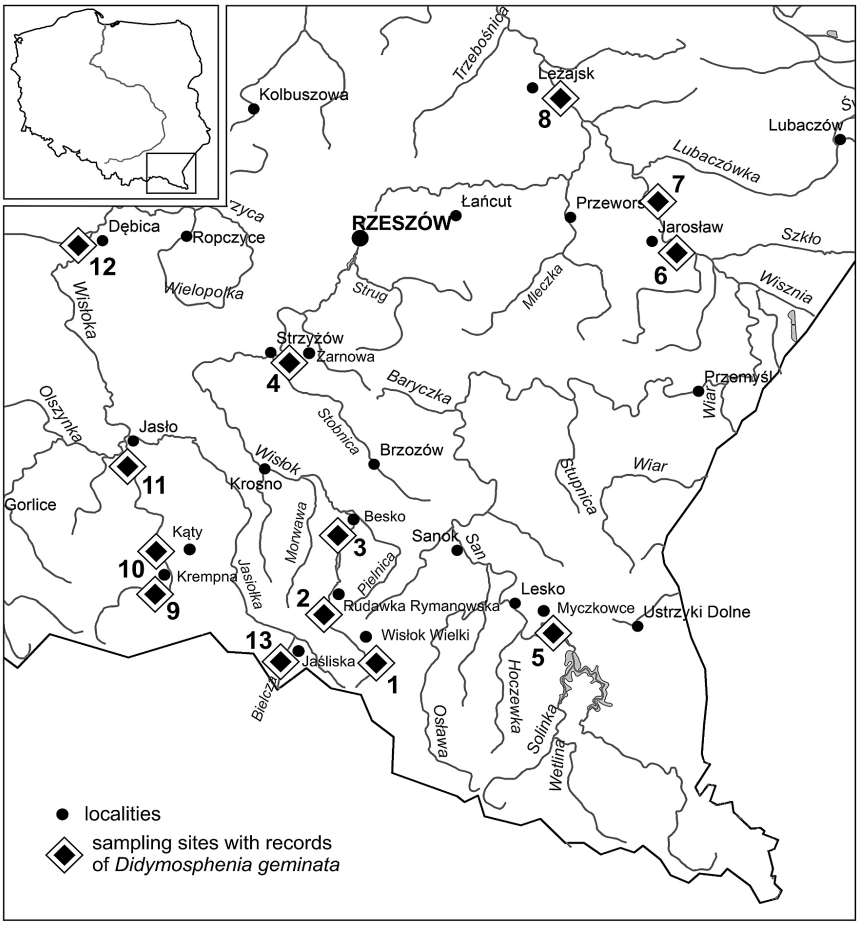

Figure 1. The distribution of sites with records of *Didymosphenia geminata* in Wisłok River and others rivers in the Subcarpatian region

waste, and wastewater from rural areas, discharged directly or indirectly by way of tributaries.

The catchment of the Wisłok is characterized by many large sources of pollution, both communal and industrial. As a result, the Wisłok is distinguished by higher concentrations of organic pollution and nutrients, when compared with other large rivers of the Podkarpacie Region [WIOŚ 2009].

The Wisłoka River is a right bank tributary of the Vistula River, flowing through the area of the Beskid Niski, Jasielskie Foothills (Pogórze Jasielskie), Jasielsko-Krośnieńska Valley (Kotlina Jasielsko-Krośnieńska), Strzyżowskie Foothills (Pogórze Strzyżowskie), Lower Wisłoka Valley (Dolina Dolnej Wisłoki) and Nadwiślańska Lowland. Wisłoka River is 164 km long, with a catchment area of 4110 km^2. It drains half of the area of the Magura National Park, and its sources are near the Dębi Wierch Peak. A small dam was built in 1972 in Krempna on the Wisłoka River, forming a reservoir with the area of 3.2 ha. The initial capacity of the reservoir was 112 000 m^3 (Figure 1, site 9), which at present is over 50% full [Bednarczyk, Michalec 1997]. In its upper reaches the Wisłoka River is a mountainous river, shallow, with a rocky bottom, with a predominance of small and medium-sized stones in the riverbed. All sampling sites were characterized by high insolation and a rapid current (Figure 1, sites 9–12).

The Bielcza River is a left-bank tributary of the Jasiołka (a right-bank tributary of the Wisłok River). On the sampling site (Figure 1, site 13), it was about 3–4 m wide and 30 cm deep. It was characterized by a rocky bottom with a predominance of small and medium-sized stones and a rapid current. The site was well insolated.

The San River is the largest river of the Carpathian Region, it is 443 km long with a 16 861 km^2 catchment area. It flows from Ukraine, from the Pinaszkowski Slope, at the altitude of 961 m a.s.l. [Michałowicz-Kubal, Kubal 2001]. The first sampling site was under the dam in Myczkowce (Figure 1, site 5). The river was about 4–5 m wide and 30–60 cm deep, flowing over quite a wide riverbed, in some places growing clumps of grass vegetation. The river bottom was rocky,

with small amounts of mud on the shore and well insolated. The San River (Figure 1, sites 6–8) was about 80 m wide and over 2 m deep in its middle reaches on the other sites. The sites were well insolated, the bottom in the middle was stony, with a predominance of silt and sand at the shore.

MATERIALS AND METHODS

Material for this study was collected from the summer of 2007 to the spring of 2008, in four seasons (07.2007, 10.2007, 02.2008, 05.2008), in the Wisłok River and its major tributaries. In 2009 and 2011 (from May to October, San – 05.2010, Bielcza – 07.2010, Wisłoka – 09.2019 – upper, middle section, and 05.2011 – site in Dębica city), single samples were taken from various sites in the Podkarpacie region, to check if the species was also present in other rivers and streams.

Samples were taken at regular intervals along the Wisłok in the lower, middle and upper parts of its course. In other rivers the sampling came from different places, mainly below major towns and villages along the rivers. The map shows only the localities where the occurrence of *D. geminata* was confirmed (Figure 1).

Samples were collected from stones, sediments, and aquatic plants. All the samples were preserved in 4% formalin, with application of methods used in research on river algae by Kawecka [1980, 2012]. To obtain clean diatom valves, part of each sample was macerated in a mixture of sulphuric acid and potassium dichromate at a ratio of 3:1. Next, the material was washed in a centrifuge (2500 rpm). Finally, the valves were embedded in the synthetic resin Pleurax (refractive index 1.75).

The diatoms were identified under a light microscope (Nikon ECLIPSE 80i), using the keys: Krammer, Lange-Bertalot [1986, 1988, 1991a,b], Lange-Bertalot [1993, 2001], and Krammer [2000].

The abundance of species was estimated by counting the individuals of all diatom species in a randomly selected microscopic field of view, up to a total of 400 cells. The species whose contributions exceeded 5% of the total were regarded as the most common.

RESULTS

The sites where *Didymosphenia geminata* was found in the Wisłok River had alkaline water (pH: 7.3–8.4), and conductivity ranging from 165 to 500 $\mu S \times cm^{-1}$ (Table 1). The water had a mainly mesotrophic character, also eutrophic in its middle and lower courses, whereas in its upper course it was oligotrophic or ranging between oligo- and mesotrophic (Table 1).

1. Distribution of *Didymosphenia geminata* in the Wisłok River and its tributaries

The studies conducted in 2007–2008 showed the presence of *D. geminata* in the upper and middle course of Wisłok River (Figure 1, sites 1–4). It was not found in the studied tributaries (Morwawa, Pielnica, Stobnica, Strug, Mleczka). *D. geminata* developed on rocks in rapid currents. Single deciduous trees were growing along the river. Site 1 was slightly shaded, others were in full sun (Table 1).

In the Wisłok, the species was represented by single individuals, and while it was more abundant at site 3, below the Besko Reservoir (Figure 1), it was still not a dominant species there. At site 4, located in Żarnowa, only a few cells were found in samples, and only in winter (02.2008) and spring (05.2008). At the sites where this species was recorded, the most common diatom species were: *Achnanthidium pyrenaicum, A. minutissimum* var. *minutissimum, Navicula lanceolata, Diatoma moniliformis, Fragilaria vaucheriae* and *Encyonema minutum* (Table 2).

2. Distribution of *Didymosphenia geminata* in other Carpathian rivers

From spring to autumn 2010, the species was found also in the San, Wisłoka, and Bielcza (Figure 1).

Catchment of the river Wisłoka

In the Wisłoka, this species was recorded on four sites (Figure 1, sites 9–12). The diatom was very abundant on site 9, in the village of Myscowa, in September (Plate 1: 7). Mats were observed only on the rocks where the river current was rapid. It was less numerous on two sites (10 and 11), where it formed mucilaginous stalks. It was recorded on site 4 in Dębica, as single cells. All the localities were fully insolated for most of the day, with only single trees scattered along the edges (water temperature 19–20°C, pH 7.5–7.8, conductivity 305–320 $\mu S \times cm^{-1}$).

D. geminata was also found in the Bielcza stream (a small left-bank tributary of the Jasiołka River, site 13). It developed in large numbers in July and formed gelatinous stalks.

Table 2. The most frequent diatom species coexisting with *Didymosphaenia geminata* and them proportional share on individual sites (2007–2008): 1: <5%; 2: 5–10%; 3: 10–30%; 4: 30–50%

Site / Taxa	1. Wisłok Wielki	2. Rudawka Rymanowska	3. Besko	4. Żarnowa
Achnanthidium pyrenaicum (Hust.) Kobayasi	3	2	3	1
A. minutissimum (Kütz.) Czarnecki var. *minutissimum*	3	3	4	1
Amphora pediculus (Kütz.) Grunow	1	1	1	3
Cocconeis pediculus Ehrenb.	2	1	2	1
C. placentula var. lineata (Ehrenb.) Van Heurck	1	1	1	2
Cymbella excisa Kütz.	1	2	1	1
Diatoma moniliformis Kütz.	2	2	1	1
Didymosphenia geminata (Lyngbe) M. Schmidt	1	1	1	1
Encyonopsis microcephala (Grunow) Krammer	1	2	1	1
Encyonema minutum Hilse	1	2	2	1
Fragilaria vaucheriae (Kütz.) Petersen	1	2	1	1
Navicula lanceolata (Ag.) Ehrenb.	2	1	1	3
N.reichardttiana Lange-Bert.	1	1	1	2

Catchment of the river San

D. geminata was found in the San, along almost the whole length of the river. Single cells were recorded as far downstream as near the town of Leżajsk. However, the best conditions for it were to be found in the upper section (05.2010), at a site below the dam at Myczkowce (water temperature 8°C, pH 7.5, conductivity 165 $\mu S \times cm^{-1}$), where numerous individuals were observed and the cells formed mucilaginous stalks (Plate 1: 5–6). No mucilaginous mats were observed on pebbles, unlike in the upper section of the Wisłoka.

3. Taxonomy of *Didymosphenia geminata* (Lyngbe) M. Schmidt

Morphological variables of 100 randomly selected cells of *D. geminata* were compared from all sampling sites in the Wisłok River (Plate 1). Their cells were 47.0–107.5 µm long and 20.2–30.0 µm wide. The number of striae per 10 µm ranged from 9 to 13, while the number of stigmata in the central area, from 1 to 3.

With respect to morphology, the individuals from the Wisłok River are slightly different from the diagnosis of this species found in the identification keys (length: 48–140 µm; width: 25–45 µm; number of striae per 10 µm: 8–10, according to Krammer, Lange-Bertalot 1986 and Hofmann et al. 2011), single cells were observed that were shorter and narrower (Plate 1: 1).

DISCUSSION

The Podkarpacie region is still poorly studied with respect to phycology. The diversity of diatoms has been monitored for a few years, mostly in the catchment of the Wisłok River, including observations on the distribution of *Didymosphenia geminata* [Noga 2012, Pajączek et al. 2012]. Single studies were also carried out in the upper section of the San, River, because of the high abundance of the diatom *D. geminata* there [Kawecka, Sanecki 2003]. In 2010–2011 *D. geminata* was found in the rivers Ropa and Biała Tarnowska, over their entire length. On most sampling sites it was mainly recorded in a form of single specimens, on stones. Larger populations were observed only in the Ropa River below the "Klimkówka" dam reservoir, during the production of gelatinous stalks in the spring and autumn. In autumn 2010, a brown mat with a thickness of 2 cm was also observed on stones, covering the bottom of the river by about 40% [Noga et al. 2012]. The present study provides the first data on the occurrence of *D. geminata* in the Wisłok and other Carpathian rivers (except for the Wisłoka, Bielcza and San – and with the exception of the upper section).

Until the 1990's *Didymosphenia geminata* had been regarded as a northern alpine species [Krammer, Lange-Bertalot 1986] and an indicator of xenosaprobic, unpolluted waters [Sladeček 1986]. *D. geminata* was rare in Poland until recently, represented by a small number of individuals, mostly in streams in the Tatra Mountains and the Dunajec River [Siemińska 1964]. How-

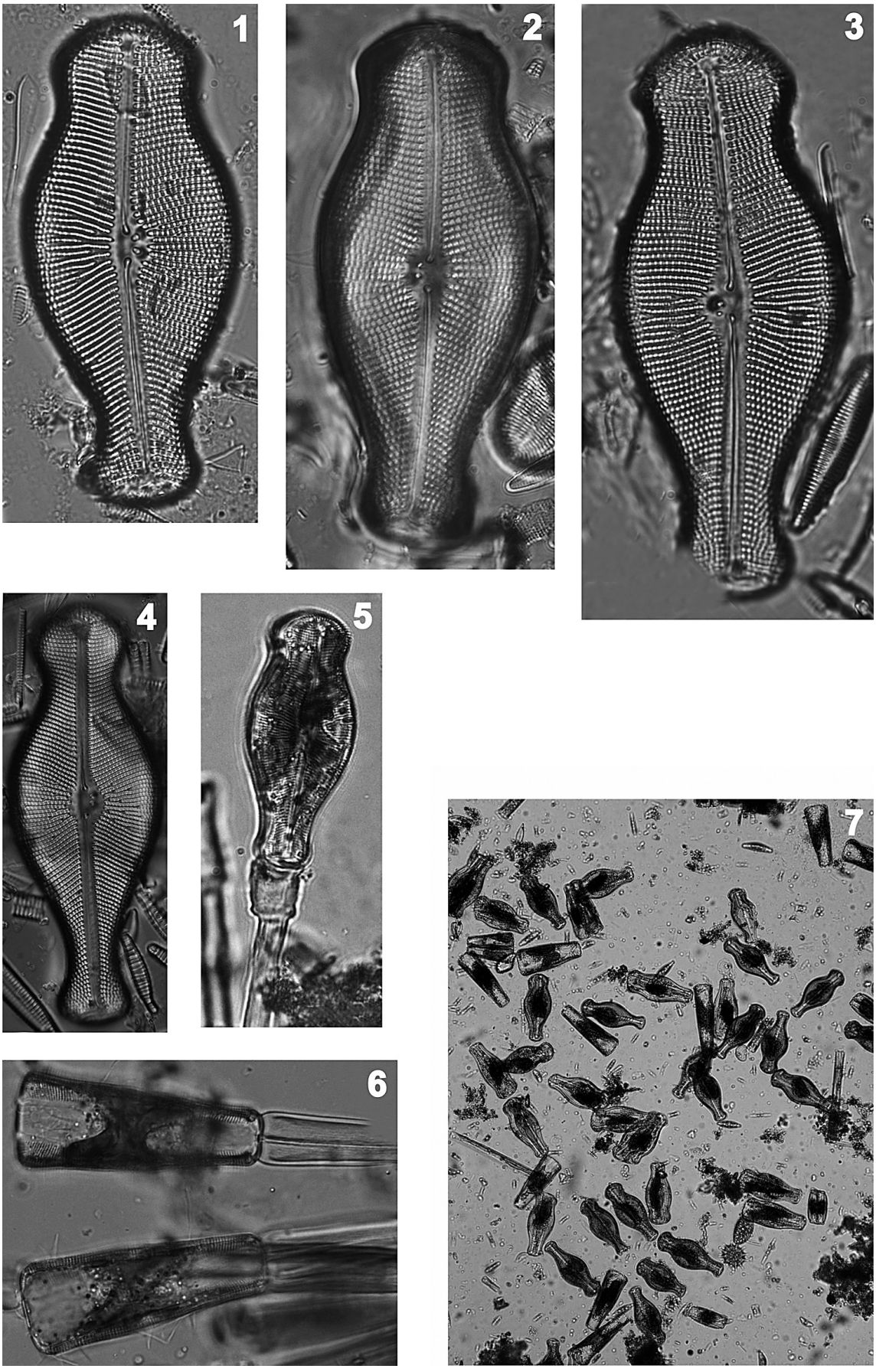

Plate 1. Morphology of *Didymosphenia geminata* (Lyngbe) M. Schmidt from Wisłok River and others subcarpatian rivers; 1–3 (1000×), 4 (400×), 5–6 (200×), 7 (100×)

ever, in recent years it has formed large populations in some rivers and streams of Europe, North America, and New Zealand [Biggs et al. 2006, Blanco, Ector 2009, Whitton et al. 2009]. It is also more and more frequent in the running waters of southern Poland [Kawecka, Sanecki 2003, Noga 2003, Mrozińska-Broda, Czerwik-Marcinkowska 2004, Mrozińska et al. 2006, Noga 2012, Noga et al. 2012]. Currently, it also occurs in the rivers of the Gorce Mountains: Ochotnica, Forendówka and Jaszcze [Noga, unpubl. data].

D. geminata cells identified in the Wisłok River mostly match the dimensions given in the keys [Krammer, Lange-Bertalot 1986, Hofmann et al. 2011]. Single specimens have different dimensions (they were smaller and narrower). One morphotype (”Morphotype geminata”) appeared in all the analyzed samples. Metzeltin and Lange Bertalot [1995] noted ”Morphotype geminata” from different parts of the northern hemisphere and showed large differences in the size of cells. The smallest specimens of *D. geminata* are recorded in Norway (48–97×26–35 μm) and Siberia (60–110×25–39 μm). In the Wisłok River single cells were observed, which were slightly smaller (47–107.5 μm) and narrower (20.2–30 μm) – Plate 1: 1.

In the Wisłok River *D. geminata* was the most abundant on site 3, below the Besko Reservoir, in mesotrophic waters (Table 1). Nevertheless, it did not form water blooms and in none of the studied seasons was it a dominant species. Among the dominant species on that site, the most abundant were *Achnanthidium pyrenaicum*, *A. minutissimum* var. *minutissimum*, *Cocconeis pediculus* and *Encyonema minutum* (Table 2). On other sites on the Wisłok River *D. geminata* was mostly represented by single individuals during the whole study period. It was not detected in any of the tributaries of the Wisłok (Morwawa, Pielnica, Stobnica, Strug, Mleczka). Most of them are similar to lowland rivers and streams, with a slower water flow or even high turbidity (the Mleczka River).

The materials collected in 2009 from rivers and streams in the Podkarpacie Region showed that *D. geminata* is present also in the catchments of the Wisłoka and San (Figure 1). A very high abundance was observed in September in the upper section of the Wisłoka below the dam in Krempna, where mucilaginous mats (about 1 cm thick) were visible on stones. It was also abundant in May in the upper section of the San, below the Myczkowce Reservoir. In the river, on stones, no mats were noticeable, but under a microscope numerous and long mucilaginous stalks were visible.

The above data on site conditions suggest that a low water level, full light, and well-oxygenated water are favourable for this diatom species. Chemical analyses of water (Table 1) in the Wisłok River showed that it contains high concentrations of nutrients, and thus the river is mesotrophic or even eutrophic. This is also confirmed by the dominant diatom species, most frequently *Encyonema minutum* and *Cocconeis pediculus*. They are α-β-mesosaprobic species, found also in eutrophic waters at pH ≥ 7 [Van Dam et al. 1994]. A frequent dominant, particularly in winter and spring, was *Navicula lanceolata*, a cosmopolitan diatom, with a wide ecological spectrum: from springs and brackish waters. It prefers lower temperatures and forms large populations in the colder part of the year [Krammer, Lange-Bertalot 1986]. The species is eutrophic, α-mesosaprobous and alkaliphilous [Van Dam et al. 1994]. Among the dominants *Achnanthidium minutissimum* var. *minutisimum* was the most abundant. It is a cosmopolitan species, found in a wide range of pH, from 4.3 to 9.2 [Krammer, Lange-Bertalot 1991b], preferring well-oxygenated waters. *A. pyrenaicum* was also abundant in mesotrophic waters, rich in calcium, pH>7 [Van Dam et al. 1994].

D. geminata grows when there are no great floods, in full sun, with a pH close to neutral, and in the presence of specific nutrient chemistry. It is frequent, but not always, in waters where the N:P ratio is high for much of the year. However, the key factor is the ratio of organic to inorganic phosphate. *D. geminata* probably prefers low temperatures and full sun in the spring season [Whitton et al. 2009]. In such conditions it was also observed in the San, at Myczkowce.

Its mats were formed in later seasons, if the dominant form of phosphorus changed from organic to inorganic [Whitton et al. 2005]. In the Wisłoka River, mats were observed in September, i.e. at the end of summer, only on stones and in rapid currents. Mucilageous overgrowth was not observed on stones in places where the current was slow. Our results show that in the Podkarpacie Region *D. geminata* is always the most abundant below reservoirs, located on the San, Wisłok and Wisłoka. It was the most frequent on the Ropa River, also below the “Klimkówka” dam reservoir [Noga et al. 2012]. These sites were always fully insolated.

D. geminata grows in similar conditions in the San River, where it forms very large populations, covering up to 100% of the river bed [Kawecka, Sanecki 2003], and also in the Czarna Orawa River and its tributaries [Noga 2003].

The spreading of *D. geminata* in recent years in Europe, including the southern part of Poland and in other continents, indicates that this species could be expansive. Its required environmental conditions (alkaline pH, moderate values of electrolytic conductivity, well oxygenated, rapid current and mesotrophic character of water) indicate that this species has a greater adaptive potential than believed so far. Our preliminary research in the Podkarpacie Region suggests that *D. geminata* is probably also present in many other rivers and streams in that region, particularly in their upper sections, and its abundance varies between seasons. Further research is needed to determine a more detailed number of sites where the species is present, and the seasons and physicochemical conditions are favourable for its growth.

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