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Spatial Patterns in the Distribution and Diversity of *Diploneis* Genus-Level Diatoms in the Podlasie Springs (North-Eastern Poland)

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Abstract: This research is focused on the presence of the genus *Diploneis* (Bacillariopyta) in small lowland springs of north-eastern Poland. This study presents a biogeographic overview of the genus from 2 urban and 12 forest springs in the Podlasie area. Seven species were identified, some of which have been described recently and whose distribution is not well-known (*D. burgitensis*, *D. fontium*). The presence of *D. burgitensis* and *D. parapetersenii*, both rare taxa, are the first recorded for Poland. Their presence was confirmed using both LM and SEM micrographs. The presence of much more frequently noted European species (*D. elliptica*, *D. fontanella*, *D. krammeri*, and *D. separanda*) was also found. Greater knowledge of the *Diploneis* genus opens the way towards better and more comprehensive approaches to uncovering biological diversity and biogeographical patterns on the European continent and among the springs. The distribution of all recorded *Diploneis* species and their habitat preferences are briefly given.

Keywords: diatoms; Bacillariophyta; diversity; lowland springs; water quality

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1. Introduction

Springs create unique/specific biotopes for hydrobionts as underground aquifers and surface waters merge, and their possession of both subterranean and surface-water environmental features make them "double ecotones". Moreover, they are biodiversity refuges even in anthropogenically influenced areas. In phycological studies, the most important factors are the type of niche, water quality, and catchment area [1]. The high diversity of these factors is reflected in the broad range of spring habitats and the development of different alga communities [2,3]. Springs offer relatively high-stability environmental conditions for hydrobionts compared with other inland water bodies [4]. Springs are highly individual, which is also reflected in their alga communities. The very diverse composition of diatom assemblages is related to the substrate, chemistry of water, and human impact [5–8]. The diatom flora of springs in Poland has been studied for over 150 years, e.g., in [9,10]. Diatoms are among the most common and abundant organisms in Polish springs [7,8]. Most studies on diatoms occurring in Polish springs have focused on the uplands and mountains [7,8].

Benthic diatoms of lowland springs are still among the least recognized organisms in aquatic habitats. The latest information about diatom diversity in the lowland forest [11,12] and urban springs of north-eastern Poland [13] indicate that springs are refuges for many species, including rare and still poorly known ones.

Diatoms can colonize various substrates in spring niches, such as stones, sand, mosses, filamentous algae, or fallen tree branches. The largest number of poorly known taxa can be found in springs located in the areas least exposed to pollution [7].

A significant minority of the known *Diploneis* species inhabit freshwater habitats. During the 20th century, European freshwaters suffered from strong eutrophication and organic pollution. As a result, in eutrophic conditions, the huge rivers in Germany, Poland, and France are widely devoid of *Diploneis* species [14]. Identification difficulties related to the fine ornamentation and morphological variability of *Diploneis* are reflected in their nomenclature and taxonomy. As a result of numerous recent detailed biodiversity surveys, as well as taxonomic studies of material deposited in diatom collections, several new species of *Diploneis* have recently been described [14–16] or re-established [15]. Many *Diploneis* species are only known from restricted geographical areas (e.g., *D. burgitensis*, *D. fontium*) [15,16], whereas others, such as *D. elliptica*, *D. fontanella*, *D. krammeri*, *D. parapetersenii,* and *D. separanda*, are widely distributed and have defined environmental requirements [16,17]. *Diploneis* is a common and widespread diatom group in springs, streams, rivers, and lakes in Poland [7,8,18,19].

The aim of this paper is to provide basic information on seven *Diploneis* species identified from springs in Podlasie (NE Poland) and present a detailed distribution of individual species and their habitat preferences. All populations described and discussed in this paper originate from oligo- to slightly eutrophic spring waters (Podlasie, NE Poland).

2. Materials and Methods

2.1. Study Area

The research area was located in north-eastern Poland. The crenological studies covered 2 urban springs from the city of Białystok and 12 springs from the area of the Knyszyn Forest (Figure 1, Table 1). The Knyszyn Forest is the second-largest (after the Białowieża Forest) forest complex in the Podlaskie Voivodeship. Lowland springs are one of the most precious natural resources of the Knyszyn Forest.

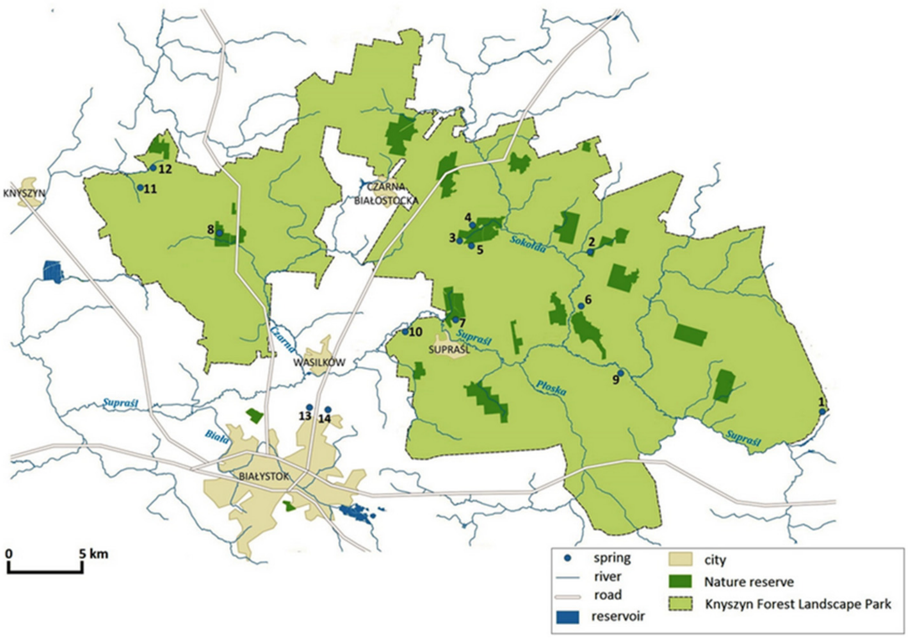

Figure 1. Location of springs in the Knyszyn Forest and Białystok [after Puczko et al. [20], modified]; 1—Piłatowszczyzna, 2—Woronicze, 3— Budzisk reserve I, 4—Budzisk reserve II, 5 –Pstrągownia, 6—Łaźnie, 7—Jałówka reserve, 8— Krzemianka reserve, 9—Nowosiółki, 10—Pólko, 11—Krynice, 12—Hatka, 13—Jaroszówka, 14—Pietrasze.

Table 1. Geographical characteristics of the springs in the Knyszyn Forest and in Białystok; $Q_{av.}$—average discharge.

Spring No.	Spring Name	Geographical Coordinates	Hydrological Location	$Q_{av.}$ [L s^{-1}]	Geomorphological Type of Spring	Substrate	Land Use
			KNYSZYN FOREST				
1.	Piłatowszczyzna	N: 53°8′58.19″ E: 23°42′56.39″	right-side direct tributary of the Supraśl river → Breszczeczka catchment	1.5	hillslope	epiliton	forest
2.	Woronicze	N: 53°16′5.05″ E: 23°31′1.89″	Sokołda catchment → Łanga catchment	2.3	hillslope	epiliton	rural area
3.	Budzisk reserve I	N: 53°16′53.67″ E: 23°22′15.75″	Sokołda catchment → Migówka catchment	6.8	hillslope	mud	forest
4.	Budzisk reserve II	N: 53°16′54.62″ E: 23°22′27.36″	Sokołda catchment → Migówka catchment	4.5	hillslope	mud	forest
5.	Pstrągownia	N: 53°16′26.7″ E: 23°21′24.73″	Sokołda catchment → Migówka catchment	5.4	valley	epiliton	forest
6.	Łaźnie	N: 53°14′53.01″ E: 23°28′59.46″	direct tributary of the Sokołda river → Sokołda catchment	4.8	hillslope	epiliton	forest
7.	Jałówka reserve	N: 53°14′4.58″ E: 23°20′41.57″	Sokołda catchment → Jałówka catchment	2.3	valley	epiliton	forest
8.	Krzemianka reserve	N: 53°16′55.79″ E: 23°7′5.98″	Czarna catchment → Krzemianka catchment	8.6	valley	epiliton/moss	forest
9.	Nowosiółki	N: 53°10′21.9″ E: 23°31′2.85″	left-side direct tributary of the Supraśl river → Supraśl catchment	0.5	hillslope	mud	forest
10.	Pólko	N: 53°13′13.9″ E: 23°18′5.54″	left-side direct tributary of the Supraśl river → Supraśl catchment	1.5	valley	mud	forest/grassland
11.	Krynice	N: 53°18′19.94″ E: 23°1′55.69″	Narew catchment → Jaskranka catchment	3.2	hillslope	psammon	rural area
12.	Hatka	N: 53°19′12.49″ E: 23°3′23.81″	Narew catchment → Jaskranka catchment	1.1	hillslope	mud	rural area
			BIAŁYSTOK				
13.	Jaroszówka	N: 53°10′38.80″ E: 23°11′59.10″	Supraśl catchment → Jaroszówka catchment	5.2	hillslope	sand/mud	forest
14.	Pietrasze	N: 53°09′15.41″ E: 23°04′23.61″	Supraśl catchment	4.9	hillslope	sand	forest

In the Białystok region, situated in the catchments of Narew and Supraśl rivers of the Polish Lowland (North Podlasie Lowland), there are many different springs [13,21,22]. Twelve springs are located in the Supraśl basin, two springs in the direct basin of the Narew river. In terms of hydrobiology, rheocrene springs (Figure 2) dominate; there are fewer helocrenes and limnocrenes (Figure 3). The predominant share of rheocrenes results from the varied topography of the Knyszyn Forest (ground level differences reach several dozen meters). The springs' discharge in Knyszyn Forest and Białystok is various, from 0.5 to 50.5 L s^{-1} [13]. During low water levels in the summer of 2014 and 2015, the outflow yields most often fluctuated within the range of 0.5–2.0 L s^{-1} [13]. For our research, we selected springs with the highest discharge in the region (Table 1). The spring with the highest discharge in the Knyszyn Forest, which we covered in this study, is the spring in the Krzemianka valley (the Krzemianka reserve). Its discharge is over 8 L s^{-1} (Table 1). Its discharge is stable over time [13,21].

Figure 2. Rheocrene in Migówka catchment (Pstrągownia—spring No. 5; *photo. Jekatierynczuk-Rudczyk E.*).

Figure 3. Limnocrene in Sokołda catchment (Łaźnie, No. 6; *photo. Jekatierynczuk-Rudczyk E.*).

Most of the springs are located in the forest (Figures 2 and 3). Even the springs in Białystok are located in a forest area (Pietrasze Forest, forest in the Jaroszówka catchment) (Table 1). The development of the spring catchment determines the water quality and affects the biodiversity in the niche [20,23].

Springs are rather stable regarding their temperature and conductivity [13,22]. In most of the studied springs, the EC did not exceed 500 µS L^{-1}. Higher values were found in the spring in the Krzemianka nature reserve (source located near the national road S8) and the spring in the Jaroszówka catchment in Białystok (located in the vicinity of a former landfill) [13].

2.2. Sampling and Measurements

Samples for water and diatom analyses were taken once from springs (Figure 1, Tables 2 and 3) on one of the following dates: August 2014, August 2015, April 2017, May 2019, or June 2021. In Pstrągownia, Łaźnie, and Jałówka springs, the research was carried out twice (Tables 1–3).

Table 2. Hydrochemical characteristics of the springs in the Knyszyn Forest and in Białystok; * 2014, ** 2015, 2017 ***, 2019 ****, 2021 *****, n.a.—not analyzed.

Spring No.	Spring Name	Temperature [°C]	Reaction [pH]	Electrolytic Conductivity [µS L^{-1}]	Oxygen [mg L^{-1}]	Oxygen Saturation [%]	Hydrochemistry Type of Water
				KNYSZYN FOREST			
1 *	Piłatowszczyzna	11.1	7.27	354	7.63	63.3	HCO_3-Ca
2 *	Woronicze	15.4	8.16	321	8.02	80.7	HCO_3-Ca-Mg
3 *****	Budzisk reserve I	13.3	7.67	386	9.97	97.1	HCO_3-Ca
4 *****	Budzisk reserve II	12.6	7.74	379	9.83	94.1	HCO_3-Ca-Mg
5 **	Pstragownia	8.30	7.77	395	10.95	95.1	
5 *****	Pstragownia	13.2	8.07	401	9.36	91.0	HCO_3-Ca-Mg
6 *	Łaźnie	12.0	7.46	395	9.12	88.2	
6 *****	Łaźnie	11.9	8.30	n.a.	6.45	60.8	HCO_3-Ca-Mg
7 **	Jałówka reserve	15.1	7.99	424	8.48	86.9	
7 *****	Jałówka reserve	15.1	7.99	424	8.48	86.9	HCO_3-Ca-Mg
8 ***	Krzemianka reserve	8.30	7.85	590	10.14	86.7	HCO_3-Ca-Mg
9 *	Nowosiółki	11.5	8.21	261	9.74	89.4	HCO_3-Ca-Mg
10 *****	Pólko	11.0	8.03	425	8.3	76.6	HCO_3-Ca-Mg
11 **	Krynice	19.7	8.13	465	7.05	78.6	HCO_3-Ca-Mg
12 **	Hatka	17.9	7.81	484	6.03	64.9	HCO_3-Ca
				BIAŁYSTOK			
13 *****	Jaroszówka	14.9	8.08	412	8.9	90.3	HCO_3-Ca
14 ****	Pietrasze	12.5	8.14	853	7.7	97.3	HCO_3-Ca

Table 3. Hydrochemical characteristics of the springs in the Knyszyn Forest and in Białystok; concentration [mg L^{-1}]; * 2014, ** 2015, 2017 ***, 2019 ****, 2021 *****, n.a.—not analyzed.

Spring No.	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^{-}	SO_4^{2-}	Cl^{-}	TFe	DOC	TN	N-NH_4^{+}	N-NO_3^{-}	N-NO_2^{-}	TP	SRP
								[mg L^{-1}]							
								KNYSZYN FOREST							
1 *	70.6	9.1	2.95	1.38	251	35.6	8.00	0.614	4.01	2.068	0.246	0.352	0.002	0.071	0.070
2 *	62.5	16.0	4.37	0.20	238	20.6	6.00	2.207	1.66	0.789	0.493	0.188	0.001	0.305	0.060
3 *****	65.1	8.33	2.56	0.40	275	21.3	2.70	0.051	2.88	0.788	0.006	0.327	0.001	0.309	0.044
4 *****	62.7	8.45	2.38	0.57	269	20.3	2.74	0.088	2.59	0.682	0.00	0.323	0.001	0.349	0.049
5 **	69.9	17.5	3.66	0.64	277	29.9	5.50	1.60	1.28	0.588	0.201	0.618	0.001	0.304	0.077
5 *****	62.8	9.40	2.45	0.46	268	20.4	2.41	0.044	2.94	1.254	0.006	0.618	0.0002	0.422	0.030
6 *	74.7	13.5	4.33	1.39	n.a.	24.0	11.30	0.809	3.21	1.667	0.210	0.066	0.001	0.028	0.013
6 *****	58.4	10.1	4.18	0.75	259	21.0	4.09	0.037	2.35	0.723	0.004	0.314	0.001	0.433	0.025
7 **	68.5	21.6	5.84	1.30	281	26.2	10.57	1.841	2.81	1.890	0.327	1.330	0.003	0.154	0.062
7 *****	67.6	9.40	2.86	0.55	278	32.4	4.68	0.037	4.17	1.166	0.010	0.487	0.001	0.390	0.036
8 ***	91.4	17.9	14.98	1.16	319	38.9	41.50	0.726	4.05	1.442	0.314	0.310	0.001	0.112	0.024
9 *	60.5	10.1	2.19	0.27	162	25.7	8.40	0.979	3.43	1.223	0.197	0.186	0.002	0.156	0.077
10 *****	71.1	10.2	4.24	0.61	275	39.4	4.79	0.029	2.79	3.276	0.012	1.891	0.003	0.321	0.027
11 **	59.5	16.8	4.00	0.42	290	42.3	8.02	1.580	2.70	0.203	0.242	0.242	0.001	0.178	0.097
12 **	70.4	11.6	4.46	1.96	299	30.3	12.18	1.561	2.22	1.975	0.266	1.572	0.003	0.216	0.127
								BIAŁYSTOK							
13 ****	123.4	12.63	31.23	12.27	476	79.66	54.26	0.037	3.04	3.211	0.014	1.970	0.002	0.397	0.096
14 ***	67.2	7.13	3.06	0.48	280	43.03	5.54	0.037	2.97	0.504	0.017	0.181	0.006	0.431	0.078

2.3. Physical and Chemical Water Analyses

In the field water temperature, pH, electrolytic conductivity (EC), oxygen concentration, and saturation were measured in situ using an HQ40D Multi Meter (Hach-Lange GmbH, Berlin, Germany). The samples for laboratory tests were collected in plastic bottles with a capacity of 0.5 L. Chemical water analyses were carried out in accordance with ISO standards by means of methods described by APHA [24]. Bicarbonates (HCO_3^-) were determined through titration with hydrochloric acid in the presence of methyl orange. Ammonium nitrogen (N-NH_4^+), nitrate nitrogen (N-NO_3^-), nitrite nitrogen (N-NO_2^-), soluble reactive phosphorus (SRP), sulfates ($SO_4{}^2$), chlorides (Cl^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), and potassium ions (K^+) were analyzed using the high-performance liquid chromatography technique with conductivity detection, based on the ICS-1100 compact ion chromatograph system (Dionex, CA, USA) with a double-piston pump isocratic, vacuum degassing of the eluent, thermostatic control of the columns and the detector cell, electronic suppression, and a Dionex AS-DV automatic sample feeder, controlled with the Chromeleon 7 software. Dissolved organic carbon (DOC) and total nitrogen (TN) were determined using high-temperature catalytic combustion using a TOC-L Series (Shimadzu, Kyoto, Japan).

The analyses of total phosphorus (TP) were conducted in the laboratory according to the conventional photocolorimetric method [25]. Iron ions were determined using the ortho-phenanthroline method. The total phosphorus (TP) and iron (TFe) fractions were determined in non-filtered water after mineralization.

2.4. Diatom Analyses

Relationships between diatom assemblages and measured water chemistry variables were examined for 14 springs. Samples were collected in the years 2014–2021 from several aquatic microhabitats in an area covering approximately 1050 km^2 and located at 91–211 m a.s.l. in north-eastern Poland. The samples were boiled in concentrated H_2O_2, treated with 10% HCl, and washed several times with distilled water in order to remove organic matter. The cleaned diatom material was air-dried on cover slips and mounted on Naphrax Mountant, Brunel Microscopes Ltd., Chippenham, UK, (refractive index, 1.74). Observations of the diatoms were performed with a Nikon Eclipse *80i* microscope equipped with oil immersion and differential interference contrast at 1000× magnification. Light micrographs were taken with a Nikon DS–Fi 1 camera. For SEM analysis, several drops of the cleaned material were pipetted onto coverglasses, air-dried, and affixed to an aluminum stub with double-sided transparent tape. The stubs were sputter-coated with gold and analyzed with a Hitachi S–4700 scanning electron microscope at 20 kV. SEM micrographs were taken in the Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences, Jagiellonian University, Poland. The identification of diatoms was based mainly on Lange-Bertalot et al.'s [16] and Jovanovska and Levkov's [15] taxonomic publications. Distribution data are provided for each species.

3. Results

3.1. Physical and Chemical Water Parameters

The concentrations of the main macroelements allowed for the classification of spring water into two-ion (HCO_3-Ca) or three-ionic waters (HCO_3-Ca-Mg) (Table 2). The water in the springs under study was well-oxygenated and had a reaction close to neutral or slightly alkaline (Table 2). The conductivity ranged between 261 and 853 µS cm $^{-1}$, and the contents of major ions (mg L^{-1}) varied; anions: Cl^- 2.41–54.26, SO_4^{2-} 20.3–79.66, HCO_3^- 162–476; cations: Mg^{2+} 7.13–21.6, Ca^{2+} 58.4–123.4 (Table 3). Spring waters in lowland areas are rich in biogenic substances (nitrogen, phosphorus, and organic carbon compounds), which was confirmed by our research (Table 3). The average TN content in the tested samples was 1.38 mg L^{-1}. In two springs, the TN concentration exceeded 3 mg L^{-1}. Among the analyzed mineral forms of nitrogen, nitrate nitrogen most often dominated. Ammonium nitrogen predominated in several samples (Table 3). The average TP concentration in the analyzed waters was 0.269 mg L^{-1}. The average concentration of DOC in the tested waters was 2.89 mg L^{-1}. Only in two outflows was it lower than 2.00 mg L^{-1} (Table 3).

3.2. Diatoms

3.2.1. *Diploneis burgitensis* Prudent 1905, Figures 4a and 5

Synonym: *D. alpina* W. Meister.

Ref. Jovanovska et Levkov [15], 532 p., figs 1–3; Lange-Bertalot et al. [16], 25 p., plate 3.

The valves were linear to linear-elliptic with slightly concave margins and bluntly rounded ends (Figures 4a and 5). The valve length was 48.6–74.2 µm and valve width was 18.3–25.3 µm (length-to-breadth ratio was 3.1–3.4). The striae were slightly radiate, 9–10 in 10 µm, built of rectangular areolae, 7 in 10 µm (Figures 4a and 5).

Distribution and Ecology.

The most abundant and the most numerous *Diploneis* species in the material were found in different springs (Łaźnie, Piłatowszczyzna, and Woronicze in 2014; Jałówka, Krynice and Hatka in 2015; Krzemianka in 2017; Pietrasze in 2019; Budzisk I, Pstrągownia, Łaźnie, Pólko and Jaroszówka in 2021 (Table 4). *Diploneis burgitensis* shows a wide range of water chemistry values, including nutrients, which present mostly in circumneutral and slightly alkaline waters, especially in the epipsammon, with relative abundances reaching 56% in Jałówka in 2015) (in counts of at least 400 valves). *Diploneis burgitensis* is a new species for Polish flora.

Table 4. Occurrence of diatoms of the *Diploneis* genus recorded at 14 localities (1–14 as in Figure 1, Table 1) in springs in the Knyszyn Forest and in Białystok; presence marked +.

Species/Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diploneis burgitensis	+	+	+		+	+	+	+		+	+	+	+	+
Diploneis elliptica		+			+					+			+	
Diploneis fontanella		+								+				+
Diploneis fontium										+			+	
Diploneis krammeri			+	+			+		+	+				+
Diploneis parapetersenii							+							
Diploneis separanda										+				

According to Lange-Bertalot et al. [16], *D. burgitensis* is an infrequent, Nordic-alpine diatom. In Sweden and Finland, it occurs in several alkaline, oligotrophic pre-alpine, and alpine lakes [16]. This diatom (as *D. alpina*) is known from Great Britain [26], Germany [27], Slovakia [28], and Macedonia [29]—from Lake Ohrid and a spring near St. Naum [15]. Our results correspond with Jovanovska and Levkov [15], who characterize the taxon as developing large psammon populations in circumneutral, calcium-rich waters.

3.2.2. *Diploneis elliptica* (Kützing) Cleve 1891, Figure 4b,c

Ref. Jovanovska et Levkov [15], 535 p., figs 11–13; Lange-Bertalot et al. [16], 41 p., plates 7–9.

The valves were rhombic-elliptic with obtusely rounded ends. The length of valves was 26.2–34.6 µm, and the width of the valves was 14.5–18.0 µm (length-to-breadth ratio was 1.6–2.15). The striae were parallel in the middle, becoming radiate towards the valve ends, 8–10 in 10 µm, built of eight areolae in 10 µm (Figure 4b,c).

Distribution and Ecology.

Diploneis elliptica was found in Woronicze in 2014; Jaroszówka in 2019, Jaroszówka, Pólko and Pstrągownia in 2021 (Table 4).

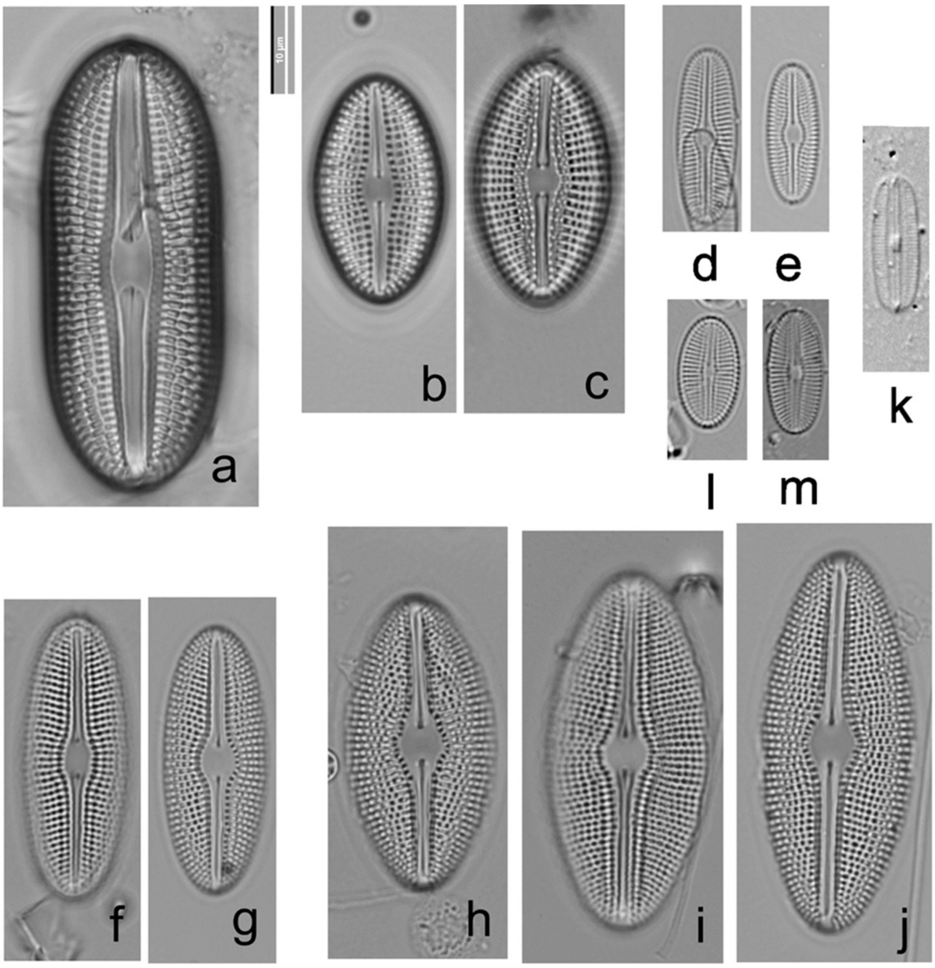

Figure 4. (**a**)—*Diploneis burgitensis* Prudent; (**b**,**c**)—*D. elliptica* (Kützing) Cleve; (**d**,**e**)—*D. fontanella* Lange-Bertalot; (**f**,**g**)—*D. fontium* Reichardt and Lange-Bertalot; (**h**–**j**)—*D. krammeri* Lange-Bertalot and Reichardt; (**k**)—*D. parapetersenii* Lange-Bertalot; (**l**,**m**)—*D. separanda* Lange-Bertalot. Scale bar = 10 µm.

This diatom is known from a few sites in Poland [8,30,31]. The older literature indicates the occurrence of this species in a range of conditions from nutrient-poor to eutrophic waters. This diatom was regarded as the most frequently reported species throughout the world, found in different waterbodies, showing a wide range of water chemistry values, including nutrients, and mostly present in circumneutral and slightly alkaline waters. However, *D. elliptica* is similar to other common *Diploneis*, i.e., *D. krammeri*, with which it can be misidentified. According to Cantonati et al. [17], *D. elliptica* occurs in very few populations in low numbers. This diatom is distributed in the Alps, alpine foothills, and post-glacial lakes of the northern lowlands of Germany. It occurs mainly in oligotrophic and mesotrophic standing waters [17]. Similar data published by Lange-Bertalot et al. [16] indicate the preference of stagnant *D. elliptica* oligotrophic waters to mesotrophic ones, where it is scarce. The distribution of this species was given the same as that of Cantonati et al. [17].

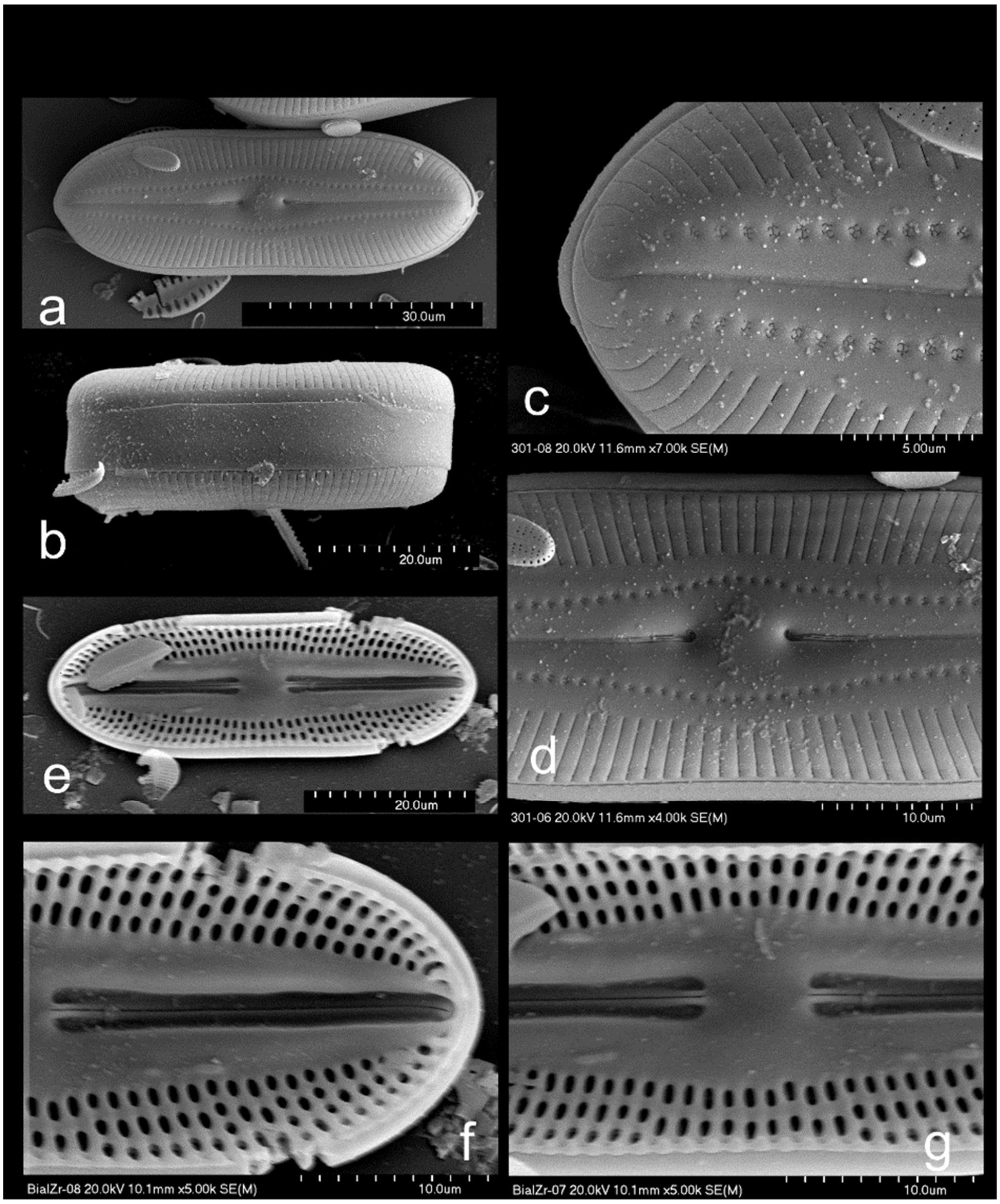

Figure 5. Valve morphology of *Diploneis burgitensis*, SEM. (**a**–**d**)—external valve view; (**e**–**g**)—internal valve view. (**a**)—linear-elliptic valve with slightly concave margins and bluntly rounded ends; (**b**)—side view of the intact frustule showing smooth surface; (**c**)—the most prominent features being the raphe slit, hooked, and continue to the valve mantle; (**d**)—the proximal raphe ends are positioned in a depression and bent to the same side of the valve; (**e**)—the whole valve view showing a thick silica plate near longitudinal canal throughout its whole length; (**f**)—distal raphe end in slightly raised and thickened helictoglossa; (**f**,**g**)—detailed internal view with simple proximal and distal raphe ends.

3.2.3. *Diploneis fontanella*, Lange-Bertalot in Werum and Lange-Bertalot 2004, Figure 4d,e

Ref. Jovanovska et Levkov [15], 538 p., figs 48–50; Lange-Bertalot et al. [16], 47 p., plates 137–140.

The valves were elliptical-lanceolate with narrowly rounded ends. The valve length was 16.6–20.4 µm, and the valve width was 6.1–6.8 µm (length-to-breadth ratio was 2.7–3.0). The striae were radiate throughout the valve, 18–19 in 10 µm (Figure 4d,e).

Distribution and Ecology.

Sparse, in samples with mosses from Pólko (2021), sand from Woronicze (2014) and Pietrasze (2019) (Table 4).

In Poland, it is known from small streams near Krakow [18] and the rivers and ponds of southern Poland [19].

Diploneis fontanella belong to widespread taxa in Europe and the Northern Hemisphere, where this species infrequently occurs in low abundance [16]. The range of its occurrence is wide, as *D. fontanella* is known from electrolyte-poor circumneutral waters, is associated with acidophilous or acid-tolerant species, and from calcium-bicarbonate-rich waters [16,17]. Our results correspond with these data, as the taxon develops very small populations in circumneutral, calcium-rich waters, with a variable range of other environmental factors.

3.2.4. *Diploneis fontium*, Reichardt and Lange-Bertalot 2004, Figure 4f,g and Figure 6a–f

Ref. Jovanovska et Levkov [15], 539 p., figs 35, 36; Lange-Bertalot et al. [16] p. 49, plate 160.

The valves were elliptical-lanceolate with bluntly rounded ends. The valve length was 30.4–35.1 µm, and the valve width was 11.4–12.9 µm (length-to-breadth ratio was 2.1–2.5). The striae were radiate throughout the valve, 13–14 in 10 µm (Figures 4f,g and 6a–f).

Distribution and Ecology.

Sparse, in samples with mosses in Pólko and Jaroszówka springs (Table 4). In Poland, it is known from one stream [32].

Diploneis fontium belong to a rare taxa group of Europe. This species was found in North Macedonia in material from subaerial habitats and small pools [15], the Austrian Alps, and is also known from southern Germany [16]. Data on the occurrence of this species and its environmental preferences are currently unclear. The confusion comes from the description of separate, though similar, species (*D. calcifuga* and *D. mollenhaueri*) that prefer low-pH water with low conductivity [16]. So far, the range of tolerance of habitat conditions has been similarly wide as it is now in the case of *D. fontanella*. *Diploneis fontium* occurs in alkaline, calcium-rich waters of moderately low conductivity [16].

3.2.5. *Diploneis krammeri*, Lange-Bertalot and Reichardt 2004, Figure 4h–j

Ref. Lange-Bertalot et al. [16], 69 p., plates 44–47; Jovanovska et Levkov [15], 542 p., figs 27, 28.

The valves were elliptical to linear-elliptical with bluntly rounded ends. The valve length was 27.2–58.1 µm and the valve width was 14.7–25.0 µm (length-to-breadth ratio was 2.2–2.6). The striae were radiate throughout the valve, 10–14 in 10 µm, built of 10–12 areolae in 10 µm (Figure 4h–j).

Distribution and Ecology.

Prevalent in springs samples, especially from Jałówka in 2015 (Table 4). This is interesting, because *D. krammeri* then reached a 4% abundance accompanied by a 56% presence of *D. burgitensis*. Moreover, it appeared very rarely in Pietrasze (2019), Budzisk I, Budzisk II, Pólko and Nowosiółki (2021). The species was the second most widespread *Diploneis* in the material, though generally sparse specimens were found, showing a wide range of water chemistry values, including nutrients, mostly in circumneutral and slightly alkaline running waters.

In Poland, it is known from several localities of a broad range of environmental variables. However, *D. krammeri* occurred abundantly in calcium-rich waters of high specific conductivity [7].

Diploneis krammeri is widespread in central Europe, albeit not abundantly. This diatom is well-known from well-aerated lakes and intermittently wet habitats, e.g., from wetted bryophyte carpets [17]. Usually, this diatom inhabits calcium-bicarbonate-rich, oligo- to mesotrophic waters [16].

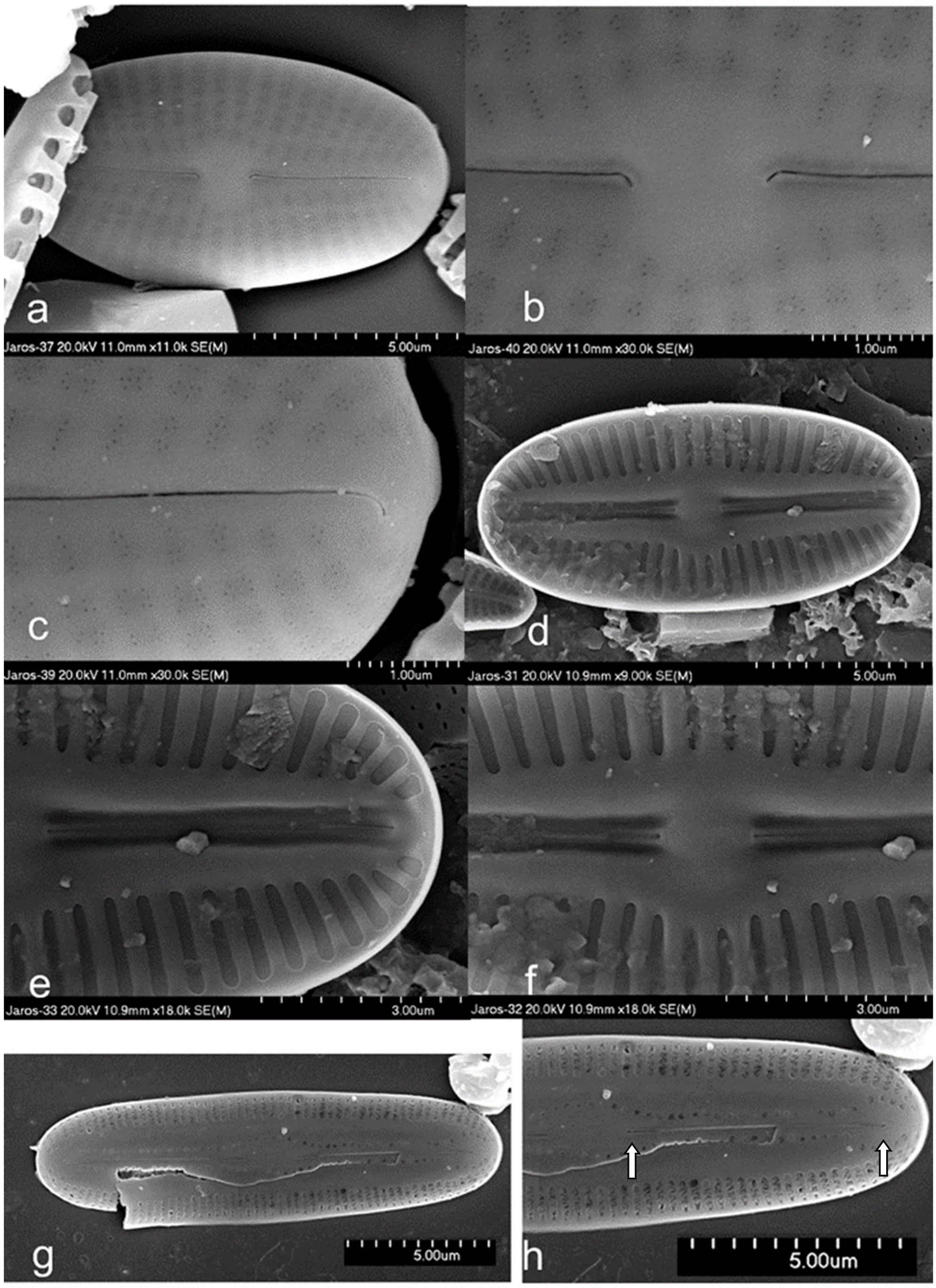

Figure 6. Valve morphology of *Diploneis fontium* (**a**–**f**) and *D. parapetersenii* (**g**,**h**), SEM. (**a**–**c**,**g**,**h**)—external valve view; (**d**–**f**)—internal valve view. (**a**)—elliptical-lanceolate valve with bluntly rounded ends; (**b**)—proximal raphe ends bent to the same side of the valve; (**c**)—distal raphe end bent to the same side of the valve as proximal raphe ends; (**d**)—internal valve view with simple proximal and distal raphe ends; (**e**)—distal raphe end in slightly raised and thickened helictoglossa; (**f**)—the mid-valve view showing a moderately thick silica plate near longitudinal canal and simple proximal raphe ends. *D. parapetersenii*: (**g**)—oblong-elliptical valve with striae interrupted by broad hyaline area above internal canal; reniform and apically elongated areolae forming striations, (**h**)—raphe ends terminate in delicate proximal and distal raphe ends (arrows).

3.2.6. *Diploneis parapetersenii* Lange-Bertalot and Fuhrmann in Lange-Bertalot 2021, Figures 4k and 6g,h

Synonym: *Diploneis pseudopetersenii* Lange-Bertalot & Fuhrmann (in Lange-Bertalot, Fuhrmann and Werum) 2020: 494, pl. 174: 1–38, nom. illeg., non *Diploneis pseudopetersenii* Cholnoky 1963.

Ref. Lange-Bertalot et al. [16], 112 p., plate 174: 1–38.

The valves were elliptical to slightly linear-elliptical with bluntly rounded ends. The valve length was 12.5–16.2 µm and the valve width was 5.4–5.8 µm (length-to-breadth ratio was 2.3–2.8). The striae were parallel, becoming slightly radiate towards the apices, 26 in 10 µm (Figure 4k). In contrast to *D. petersenii*, having a volate areolae, *D. parapetersenii* has a reniform areolae (Figure 6g,h).

Distribution and Ecology.

Rarely in spring samples, especially from Jałówka in 2020 (Table 4).

Diploneis parapetersenii is given as new for the flora of Poland.

This diatom is known from Germany, Austria, France, Croatia, and Albania [16]. According to Lange-Bertalot et al. [16], *D. parapetersenii* is a diatom of distribution in the Holarctic in the broad ecological range. The species is mostly noted in alkaline calcium-carbonate-rich lakes and slowly flowing water of oligo- to eutrophic conditions. *Diploneis parapetersenii* is given as new for the flora of Poland.

3.2.7. *Diploneis separanda*, Lange-Bertalot in Werum and Lange-Bertalot 2004, Figure 4l,m

Ref. Jovanovska et Levkov [15], Lange-Bertalot et al. [16]; 126 p., plates 65–67; 542 p., figs 27, 28.

The valves were elliptical to linear-elliptical with broadly rounded ends. The valve length was 13.4–14.4 µm and the valve width was 6.4–7.0 µm (length-to-breadth ratio was 2.0–2.6). The striae were radiate throughout the valve, 18–21 in 10 µm (Figure 4l,m).

Distribution and Ecology.

Sparse, in samples with mosses in Pólko (Table 4).

In Poland, it is known from several localities of a broad range of environmental variables. *Diploneis separanda* was particularly common in the carbonated spring waters in Tatra Mts, Krakowsko-Częstochowska Upland, and Beskid Sądecki [7].

This diatom belongs to widespread taxa in Europe and the Northern Hemisphere [16]. *Diploneis separanda* occurs in calcium-bicarbonate-rich, oligosaprobic waters [16,17]. They also stated that, due to common misidentifications in the past, it is difficult to determine the rank of the preferred trophy.

In our results, the taxon develops very small populations in circumneutral, calcium-rich spring waters. Our data only indicate the possibility of the occurrence of this diatom in the spring waters of the Knyszyn Forest, as only single specimens have been recorded.

4. Discussion

A spring occurs when groundwater appears at the land surface. The spring flows because the pressure in the aquifer (water bearing soil or rock) is greater than the atmospheric pressure on the land. Springs are hotspots of biodiversity as well as systems particularly sensitive to environmental change [2–4,7,8]. One of the most important functions of springs is to provide habitat conditions (refuges) for freshwater algae appropriate for a given area [7,8]. Springs, as with many other inland waterbodies, are threatened by direct—nutrient enrichment, forestry practices, agriculture pressure, and indirect factors—climate change [4,5,7,33]. Recently observed environmental changes of anthropogenic origin are associated with the steady establishment of new relationships between the environment and ecological communities. Groundwater pollution results in the deterioration of environmental conditions in springs. However, slow-flowing water can also improve in quality. It takes place in large spring areas, where one spring has outflows over several hundred meters. Springs are rather stable regarding their temperature and conductivity [4,13,22,34]. In most of the studied springs, the EC did not exceed 500 µS L^{-1}. Higher values were found in the spring in the Krzemianka nature reserve (source located on the national road S8) and the spring in the Jaroszówka catchment in Białystok (located in the vicinity of a former landfill) [13]. Interdisciplinary studies have shown the increasing impact of climate change on springs' water chemistry, resulting in an uncommon dangerous decline in biodiversity [33]. The diversity and distribution of diatoms (Bacillariophyta) is mainly driven by environmental conditions, and their assemblages are particularly interesting for considering the scale of the geographical distribution of individual species [7].

Alongside cosmopolitan and widespread diatoms, many *Diploneis* species have specific ranges of occurrence. We studied the group of freshwater *Diploneis* species, of which the vast majority of found taxa appeared in the study material only in the form of single individuals. In having such data, we can say only that spring waters in Knyszyńska Forest are in a range of preferred/tolerated factors of the observed *Diploneis* species. Attention is drawn to the possibility of confusing the identification of many *Diploneis* taxa (e.g., *D. fontium, D. elliptica, D. fontanella, D. krammeri*, and *D. parapetersenii*). Therefore, our data have been illustrated with LM photographs. Noticed here, *D. burgitensis* and *D. separanda* belong to the most taxonomically indisputable species. *Diploneis burgitensis* was the most common species in the Knyszyn Forest and Białystok springs. This species was not found only in Budzisk reserve II and the Nowosiółki springs. In the Nowosiółki spring, specific water conductivity was the lowest (261 µS). Moreover, it was the most numerous *Diploneis*, having relative abundances reaching 56% in Jałówka and 19% in Pstrągownia in 2015. The water here was alkaline (Table 2) and of higher Mg^{2+}, TFe, and N-NH_4^+ concentration (Table 3). Finding *D. burgitensis* in 2015, not reported from Poland yet, prompted us to study several springs and features of the environment. Unfortunately, in the research carried out in 2020, *D. burgitensis* was in the springs, but in a much smaller number. Therefore, we can actually say (like Jovanovska and Levkov [15]) that this species is found in circumneutral, calcium-rich waters and is very common in the north-eastern part of Poland. The most rich in various *Diploneis* species was the spring in Pólko. The spring is, as the only one, in forest/grassland use and is periodically flooded by the waters of the Supraśl River. The large spring area provides diverse habitats with shallower and deeper water, covered with moss and grasses with varying insolation. Probably due to grassland usage, TN was the highest here—3.276 mg L^{-1} (Table 3). The concentration of TN was close to the Jaroszówka spring in Białystok. The second new species in the flora of Poland was *D. parapetersenii*, found in the oligo-mesotrophic Jałówka spring. Our results correspond with Lange-Bertalot et al. [16], who characterize the taxon as developing populations in alkaline, calcium-rich, and slowly moving water.

5. Conclusions

Fourteen springs of the Podlasie (NE Poland) are biodiversity refuges, even in anthropogenically influenced areas. The genus *Diploneis* is found here in great species diversity. All seven recorded species from Podlasie springs are well-documented with photos, characterized by distribution and habitat preferences, and compared with a general biogeographical patterns. In the case of *Diploneis burgitensis* and *D. parapetersenii*, first recorded in Poland, both light and scanning micrographs are presented.

Author Contributions: All authors contributed to the study's conception and design. Sample collection and physical and chemical water analyses were done by M.G., A.Z.W. and E.J.-R. Diatom preparation and analyses were performed by M.G. and A.Z.W. Microphotographs were prepared by A.Z.W. The first draft of the manuscript was written by M.G., A.Z.W. and O.K.-Z. All authors have read and agreed to the published version of the manuscript.

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References

1. Żelazna-Wieczorek, J.; Bik, A. Dynamics of diatom communities in springs of different hydrobiological types. *Fragm. Flor. Geobot. Pol.* **2009**, *16*, 155–167, (In Polish with English Summary)
2. Cantonati, M.; Komárek, J.; Montejano, G. Cyanobacteria in ambient springs. *Biodivers. Conserv.* **2015**, *24*, 865–888. [CrossRef]
3. Wojtal, A.; Sobczyk, Ł. The influence of substrates and physicochemical factors on the composition of diatom assemblages in karst springs and their applicability in water quality assessment. *Hydrobiologia* **2012**, *695*, 97–108. [CrossRef]
4. Cantonati, M.; Gerecke, R.; Bertuzzi, E. Springs of the Alps, sensitive ecosystems to environmental change: From biodiversity assessments to long–term studies. *Hydrobiologia* **2006**, *562*, 59–96. [CrossRef]
5. Angeli, N.; Cantonati, M.; Spitale, D.; Lange-Bertalot, H. A comparison between diatom assemblages in two groups of carbonate, low–altitude springs with different levels of anthropogenic disturbances. *Fottea* **2010**, *10*, 115–128. [CrossRef]
6. Knysak, P.J. Human Impact on Crenic Ecosystems Based on the Diversity of Diatoms and Their Autecology. Ph.D. Thesis, University of Lodz, Lodz, Poland, 2019. (In Polish with English Abstract)
7. Wojtal, A.Z. Species composition and distribution of diatom assemblages in spring waters from various geological formations in southern Poland. *Bibl. Diatomol.* **2013**, *59*, 1–436.
8. Żelazna-Wieczorek, J. *Diatom Flora in Springs of Łódź Hills (Central Poland): Biodiversity, Taxonomy, and Temporal Changes of Epipsammic Diatom Assemblages in Springs Affected by Human Impact*; A. R. G. Gantner: Liechtenstein, Germany, 2011; pp. 1–419.
9. Gutwiński, R. Materyjały do flory wodorostów Galicyi. *Spraw. Komis. Fizjograf.* **1884**, *18*, 127–138. (In Polish)
10. Schumann, J. *Die Diatomeen der Hohen Tatra*; Wien, W. Braumüller: Wien, Austria, 1867; 102p.
11. Grabowska, M. The role of springs in maintaining the biodiversity of freshwater algae. In Proceedings of the 1st International Electronic Conference on Biological Diversity, Ecology and Evolution, Online, 15–31 March 2021.
12. Grabowska, M.; Danilczyk, M.; Jekatierynczuk-Rudczyk, E.; Fieducik, J.; Jankowska, L. A preliminary study of benthic diatoms in Knyszyńska Forest springs. In Proceedings of the Book of Abstracts, XXXVth International Conference of the Polish Phycological Society "Algae in Anthropogenically Transformed Ecosystems", Łódź—Stryków, Poland, 1–4 June 2016; p. 45.
13. Jekatierynczuk-Rudczyk, E.; Zieliński, P.; Puczko, K.; Micun, K.; Puczyłowska, E. The Role of the Catchment Area in Shaping Water Quality in the Lowland Springs of the Knyszyn Forest (NE Poland). *Water* **2022**, *14*, 3202. [CrossRef]
14. Lange-Bertalot, H.; Fuhrmann, A. Contribution to the genus Diploneis (Bacillariophyta): Twelve species from Holarctic freshwater habitats proposed as new to science. *Fottea* **2016**, *16*, 157–183. [CrossRef]
15. Jovanovska, E.; Levkov, Z. The genus *Diploneis* in the Republic of North Macedonia. In *Diatoms of Europe*; Lange-Bertalot, H., Ed.; Koeltz Botanical Books: Glashütten, Germany, 2020; Volume 9, pp. 527–653.
16. Lange-Bertalot, H.; Fuhrmann, A.; Werum, M. Species Diversity in the Holarctic and Spot Checks from Elsewhere. In *Diatoms of Europe*; Lange-Bertalot, H., Ed.; Koeltz Botanical Books: Glashütten, Germany, 2020; Volume 9, pp. 1–526.
17. Cantonati, M.; Kelly, M.G.; Lange-Bertalot, H. *Freshwater Benthic Diatoms of Central Europe: Over 800 Common Species Used in Ecological Assessmen*; Koeltz Botanical Books: Glashütten, Germany, 2017; pp. 1–942.
18. Wojtal, A.Z. The diatoms of Kobylanka stream near Kraków (Wyżyna Krakowsko- Częstochowska, S Poland). *Pol. Bot. J.* **2009**, *54*, 129–330.
19. Pociecha, A.; Wojtal, A.Z.; Szarek-Gwiazda, E.; Cieplok, A.; Ciszewski, D.; Cichoń, S. Neo- and paleo-limnological studies on diatom and cladoceran communities of subsidence ponds affected by mine waters (S. Poland). *Water* **2020**, *12*, 1581. [CrossRef]
20. Puczko, K.; Zieliński, P.; Jusik, S.; Kołakowska, A.; Jekatierynczuk-Rudczyk, E. Vascular plant and bryophyte species richness in response to water quality in lowland spring niches with different anthropogenic impacts. *Environ. Monit. Assess* **2018**, *190*, 338. [CrossRef]
21. Jekatierynczuk-Rudczyk, E.; Zieliński, P.; Puczko, K. Is the protection of springs in Knyszyn Forest effective and satisfactory? *Prot. Nativ. Nat.* **2017**, *73*, 135–147, (In Polish with English Summary)
22. Puczko, K.; Jekatierynczuk-Rudczyk, E. Analysis of urban land cover influence to organic carbon and nutrients in surface water via impacted groundwater. *Environ. Monit. Assess* **2020**, *192*, 145. [CrossRef] [PubMed]
23. Zieliński, P.; Jekatierynczuk-Rudczyk, E.; Puczko, K. Factors affecting the abundance and activity of the bacterioplankton in lowland forest springs in northeastern Poland. *Ecohydrol. Hydrobiol.* **2020**, *20*, 675–686. [CrossRef]
24. APHA. *Standard Methods for Examination of Water and Waste Water*; APHA, AWWA and WPCF: Washington, DC, USA, 1992.
25. Neal, C.; Neal, M.; Wickham, H. Phosphate measurement in natural waters: Two examples of analytical problems associated with silica interference using phosphomolybdic acid methodologies. *Sci. Total Environ.* **2000**, *251–252*, 513–542. [CrossRef]
26. Hartley, B.; Ross, R.; Williams, D.M. A check-list of the freshwater, brackish and marine diatoms of the British Isles and adjoining coastal waters. *J. Mar. Biol. Assoc. UK* **1986**, *66*, 531–610. [CrossRef]
27. Ludwig, G.; Schnittler, M. Rote Liste gefährdeter Phlanzen Deutschlands. *Schriftenreihe Veg.* **1996**, *28*, 1–744.

28. Hindák, F.; Hindáková, A. Cyanobacteria and diatoms of cold mineral springs in the National Natural Landmark of Mičiná (Central Slovakia). *Bull. Slov. Bot. Spoločn.* **2016**, *38*, 13–19, (In Slovak with English Abstract)
29. Levkov, Z.; Williams, D.M. Checklist of diatoms (Bacillariophyta) from Lake Ohrid and Lake Prespa (Macedonia), and their watersheds. *Phytotaxa* **2012**, *45*, 1–76. [CrossRef]
30. Bąk, M.; Witkowski, A.; Żelazna-Wieczorek, J.; Wojtal, A.Z.; Szczepocka, E.; Szulc, K.; Szulc, B. *The Key to the Determination of Diatoms in Phytobenthos for the Assessment of the Ecological Status of Surface Waters in Poland*; Biblioteka Monitoringu Środowiska: Warszawa, Poland, 2012; pp. 1–452. (In Polish)
31. Pliński, M.; Witkowski, A. *Diatoms from the Gulf of Gdańsk and Surrounding Waters (the Southern Baltic Sea)*; Gdańsk University Press: Gdańsk, Poland, 2020; pp. 1–442. (In Polish)
32. Noga, T.; Stanek-Tarkowska, J.; Pajaczek, A.; Kochman, N.; Peszek, L. Ecological assessment of the San River water quality on the area of the San Valley Landscape Park. *J. Ecol. Eng.* **2014**, *15*, 1222.
33. Eliasz-Kowalska, M.; Wojtal, A.Z.; Barinova, S. Influence of Selected Environmental Factors on Diatom β Diversity (Bacillariophyta) and the Value of Diatom Indices and Sampling Issues. *Water* **2022**, *14*, 2315. [CrossRef]
34. Jekatierynczuk-Rudczyk, E.; Ejsmont-Karabin, J. Rotifers of Inter-Forest Springs. *Diversity* **2023**, *15*, 153. [CrossRef]