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ORIGINAL RESEARCH PAPER

Valuable habitats of protected areas in southern Poland – a source of rare and poorly known diatom species

Teresa Noga*

Department of Soil Studies, Environmental Chemistry and Hydrology, Faculty of Biology and Agriculture, University of Rzeszów, Zelwerowicza 8B, 35-601 Rzeszów, Poland

* Email: teresa.noga@interia.pl

Abstract

The peatbogs and natural upper sections of streams in national parks in south-eastern Poland represent unique study areas for research on freshwater diatom diversity. During studies conducted on diatoms in three Polish national parks, many little-known, very rare, and endangered species were noted. For most of the taxa presented in this article, especially from the *Adlafia*, *Eunotia*, and *Placogeia* genera, only single individuals have been observed, and only from a few localities worldwide. Moreover, this is the first presentation of SEM photodocumentation and descriptions for *Eunotia minutula* Grunow and *Fallacia sublucidula* (Hustedt) D. G. Mann. Based on both light and scanning electron microscopy, detailed descriptions of morphological characteristics, ecological notes, and new localities are presented for the following species: *Adlafia langebertalotii* Monnier & Ector, *Caloneis undulata* (Gregory) Krammer, *Eunotia fennica* (Hustedt) Lange-Bertalot, *E. glacialifalsa* Lange-Bertalot, *E. groenlandica* (Grunow) Nörpel-Schempp & Lange-Bertalot, *E. minutula* Grunow, *E. neocompacta* Mayama var. *neocompacta*, *E. superpaludosa* Lange-Bertalot, *Fallacia sublucidula* (Hustedt) D. G. Mann, *Pinnularia rhombarea* Krammer, *P. similiformis* Krammer, *Placogeia gereckei* (Cantonati & Lange-Bertalot) Bukhtiyarova, and *Sellaphora vitabunda* (Hustedt) D. G. Mann.

Keywords

rare diatoms; streams; peatbogs; morphology; ecology; SEM description

Introduction

Traditionally, most diatoms have been described as cosmopolitan species [1], although the biogeographic distribution of several species has been defined [2,3]. Recently, however, many new rare, invasive, exotic species with very limited distribution were described not only in Europe, but also globally [4–7]. Based on the niche theory, several groups have assumed that rare species have narrow environmental requirements and could, therefore, be used as indicators of environmental conditions [8,9].

Rare and endangered species are placed on algal red lists, including in several Central European countries such as Germany [10], Slovakia [11], Hungary [12], Bulgaria [13], and Poland [14].

Southeastern Poland comprises different geographical regions: lowlands in the north, low hills in the central region, and mountainous areas in the south. This part of Poland is characterized by a large variety of habitats, i.e., mountain and submountain valleys with streams (often forested), medium and large rivers (meandering, oxbow lakes), lakes, ponds, peatbogs, and swamps. The high proportion of forest areas, with their natural character and low level of anthropogenic impact on a national scale, makes this area unique. Many protected areas are often home to rare and endangered species of algae, including diatoms [15–19]. This is true even of urban and near-urban areas, especially the upper (often forested) sections of small rivers and streams [20].

The aim of this study was to present detailed morphological and ecological characteristics of several poorly studied and rare diatoms observed in different habitats of three Polish national parks. Some of the listed species have been recently described as new to science; they are known to exist only in a few locations, and only single individuals were observed. Information on these species is limited and they are usually listed without a detailed description of morphological and ecological characteristics. This applies particularly to species of the genus *Eunotia*. Therefore, this work will be a valuable complement to knowledge of new locations and environmental conditions related to these rare and variably endangered species. This work also contains detailed morphological descriptions of two species, *Eunotia minutula* and *Fallacia sublucidula*, based on SEM observations, for which there were no data in the literature.

Material and methods

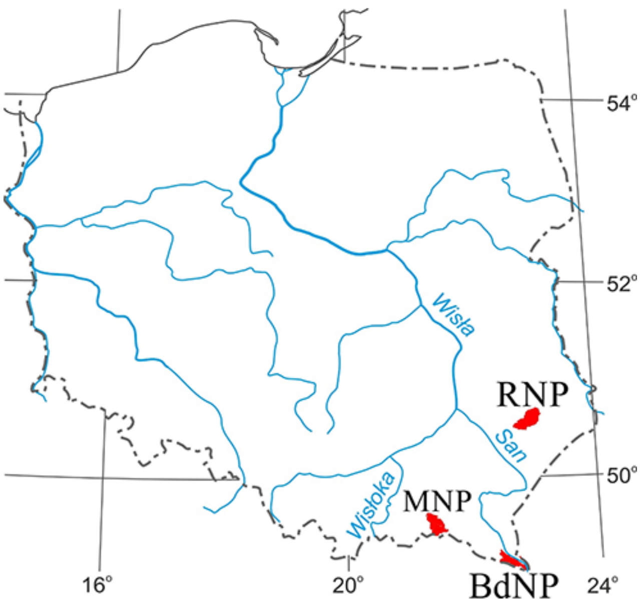

Fig. 1 Location of the study area: RNP – Roztocze National Park; MNP – Magura National Park; BdNP – Bieszczady National Park.

The studies were conducted within the boundaries of three national parks (Fig. 1): the Bieszczady National Park (BdNP), Magura National Park (MNP), and Roztocze National Park (RNP).

The Bieszczady National Park is located in the Western Bieszczady Mountains, which form part of the Eastern (Flysch) Carpathians, located in the European watershed area and separating the catchment areas of the Baltic and the Black seas [21,22]. The designated study sites were the Wołosaty stream (one of the largest left-bank tributaries of the upper San River) and its tributaries – the Terebowiec and Rzeczyca streams, as well as the Wołosate peatbog. This is a high ombrotrophic peatbog, formed on the terraces of the upper section of the Wołosatka stream (the upper part of the Wołosaty), on the slightly permeable slate layers of the Carpathian flysch [23].

The Magura National Park is located in the central part of the Beskid Niski, the largest mesoregion of the Polish Carpathians. The area of the Magura National Park is classified as low and middle mountains (650–700 m a.s.l.), and approximately 90% of its area is occupied by forests and shrubs. The designated study sites were the Wisłoka River (a right-bank tributary of the Vistula River) and its tributaries – the Kłopotnica, Krempna, Baranie, Rzeszówka, and two unnamed tributaries. The upper and middle parts of the basin are composed mainly of the Cretaceous and Tertiary flysch [24–26].

The Roztocze National Park is located in the middle part of the Roztocze, which is a belt of elevations lying between the Lublin and Podolska uplands. The Roztocze is a water divide between the lower San River and upper Bug River basins. The Polish section of the Roztocze occupies an area of approximately 2,200 km^2 and is divided into three mesoregions: western, middle, and eastern. The Międzyrzeki Reserve is situated in the southern part of the Roztocze National Park, and its absolute heights range between 246 and 253 m a.s.l. [25,27]. The studies were conducted at three designated sites in raised and transitional bogs of the Międzyrzeki Reserve, and in the Krupiec stream. The stream is periodic in its upper section, while its middle and lower sections are constant.

The physicochemical properties of the studied waters most often arise from natural processes like geochemical features and hydrometeorological conditions, whereas in the Magura and the Roztocze national parks, they also arise from the development and use of the land and surface runoffs. River water in studied national parks is normally characterized as neutral or weakly alkaline, of medium mineralization, and of low nutrient concentrations. The streams (especially in the Magura and the Bieszczady national parks) are representative of a predominantly bicarbonate-calcidic type. However, spring flooding may increase the concentration of nitrate ions. Generally, the waters of the studied areas can be considered clean, and the adverse water quality changes observed in some seasons are caused by small water flows during rainless periods and a large supply of organic matter in fall (fallen leaves). Additionally, in periods of little atmospheric precipitation, water quality in peatlands may deteriorate because of the mineralization of organic matter [22,26,28,29].

The samples were collected in 2012, 2013, and 2016 within the boundaries of the Roztocze National Park, in 2013 and 2014 in the Magura National Park, and from 2013 to 2015 in peatbogs and small streams and rivers in the Bieszczady National Park. Sampling for diatom and water analysis was always carried out in spring (March–May) and fall (September–October). In the streams and rivers, the samples were collected from stones and submerged mosses (if present at the sampling site). In the peatbogs, the samples were collected from small depressions with water or from mosses. Diatom samples were preserved in a 4% formalin solution. To obtain pure diatom valves, part of the collected material was digested in a chromic acid cleaning mixture (a mixture of sulfuric acid and potassium dichromate at a ratio of 3:1) and then washed in a centrifuge at 2,500 rpm. Diatoms were mounted in Pleurax resin (refractive index 1.75). Laboratory processing of diatoms was carried out using Kawecka [30] methods. All slides and material are stored at the Podkarpackie Innovative Research Center of the Environment (PIRCE) at the University of Rzeszów, Poland.

Chemical analyses were performed using a Thermo Scientific DIONEX ICS-5000+DC device in the Faculty Laboratory for Analysis of Environmental Health and Materials of Agricultural Origin at the University of Rzeszów. Diatoms were identified using both Nikon ECLIPSE 80i and Carl Zeiss Axio Imager A2 light microscopes (LM) with a Plan Apochromatic ×100 objective with differential interference contrast (DIC) for oil immersion. The diatoms were identified from the following available literature: Krammer and Lange-Bertalot [31–34], Krammer [35], Hofmann et al. [36], Lange-Bertalot et al. [37], Bąk et al. [38], and Monnier et al. [39].

For SEM observations, the samples were coated with gold using a Turbo-Pumped Sputter Coater Quorum Q 150OT ES and observed under a Hitachi SU 8010 at the PIRCE, University of Rzeszów.

The diatom species composition of the samples was determined by counting specimens in randomly selected fields of view under a light microscope. The number of valves counted was 400. Species comprising 5% or more of each assemblage were defined as abundant.

Results

The studies were conducted in two different oligotrophic habitats in southern Poland – the upper sections of watercourses and shallow depressions in peatbogs. Studied watercourses were characterized by usually alkaline to circumneutral pH (6.4–8.8) and low to slightly high conductivity (78–530 μS cm^{-1}) (Tab. 1 and Tab. 2). Acidic pH (<4) was noted only in the peatbogs (Site 1 and 7). The ion content was usually low or very low, often below the limit of quantification, indicating pure, oligotrophic waters. Elevated biogenic values (mostly nitrates) were found mainly in the spring (4.76–10.0 mg L^{-1}) (Tab. 1, Tab. 2).

During this survey, 13 rare diatom taxa were observed, of which eight were new to Poland. Detailed ecological and morphological notes for these taxa (which are not provided in the literature) are presented below and in Tab. 3.

Below are listed diatom species rare and new to Poland noted in upper sections of watercourses in southern Poland.

Adlafia langebertalotii Monnier & Ector (Fig. 2A–K, Fig. 4G–I)

Type of substrate. Stones, sand, rarely among mosses.

Tab. 1 The physicochemical parameters measured on peatbogs (1, 7) and streams (2–6) of Roztocze (RNP) and Bieszczady (BdNP) National Parks.

	RNP		BdNP				
	1	2	3	4	5	6	7
Temperature (°C)	6.5–28.6	4.1–14.7	5.6–12.2	7.4–10.0	6.1–9.3	5.6–9.0	11.5–12.5
pH	3.2–4.2	5.5–8.2	6.4–8.8	6.4–8.4	6.4–8.4	7.9–8.1	3.8–4.2
Conductivity (μS cm^{-1})	16–265	91–306	78–278	97–246	86–284	106–140	35–168
O_2 (mg L^{-1})	-	-	10.2–11.4	10.6–10.2	10.7–11.1	10.9–11.0	10.0
Cl^- (mg L^{-1})	1.14–2.12	4.6–38.52	0.43–5.35	0.6–5.31	0.41–5.59	0.47–1.03	0.74–3.8
SO_4^{2-} (mg L^{-1})	8.73–79.2	14.4–56.1	11.0–26.8	16.3–33.3	12.5–27.2	11.1–14.1	0.87–10.38
NO_3^- (mg L^{-1})	<0.01–1.2	<0.01–9.4	1.67–3.15	1.78–3.2	2.01–2.86	2.89–4.76	<0.01–0.08
NH_4^+ (mg L^{-1})	0.07–2.44	0.03–0.08	0.06–0.88	0.04–0.06	0.06–0.13	0.06–0.09	0.74
PO_4^{3-} (mg L^{-1})	<0.02–0.9	0.04–0.12	<0.01–0.6	<0.01	<0.01	<0.01	<0.01
Mg^{2+} (mg L^{-1})	0.56–4.99	1.06–2.37	5.26–10.1	5.33–9.32	5.62–10.1	8.32–9.83	0.20
Ca^{2+} (mg L^{-1})	2.62–17.9	16.2–23.0	33.7–41.6	38.8–39.7	32.4–35.3	36.2–40.3	0.97

1 – Międzyrzeki peatbog; 2 – Krupiec; 3 – Wołosaty; 4 – Rzeczyca; 5 – Terebowiec; 6 – Terebowiec tributary; 7 – Wołosate peatbog.

Tab. 2 The physicochemical parameters of investigated waters measured in the Magura National Park (MNP).

	MNP							
	8	9	10	11	12	13	14	15
Temperature (°C)	8.8–21.4	10.7–13.3	9.8–14.8	11–17.9	10.3–18	8.5–16.6	11.0–23.8	11.1–16.5
pH	5.5–8.8	7.3–7.9	6.3–8.6	6.0–8.4	6.3–8.5	6.4–8.3	6.0–8.7	6.3–8.3
Conductivity (μS cm^{-1})	73–402	136–341	161–530	103–315	78–307	273–431	119–420	88–332
O_2 (mg L^{-1})	6.7–12.3	10.2–10.9	7.4–10.5	8.0–11.0	8.8–9.7	9.6–9.7	7.8–10.2	9.4–10.1
Cl^- (mg L^{-1})	0.42–7.29	0.69–5.2	0.63–1.57	0.85–1.14	0.69–4.85	0.57–5.63	0.07–1.62	1.07–5.13
SO_4^{2-} (mg L^{-1})	9.8–29.97	18.2–21.3	5.1–46.78	10.1–27.3	13.5–22.4	7.7–41.54	5.8–28.35	14.8–30.0
NO_3^- (mg L^{-1})	<0.01–5.3	0.94–5.76	<0.01–0.5	<0.01–4.2	1.08–10.0	2.13–9.66	0.09–5.83	1.38–7.19
NH_4^+ (mg L^{-1})	0.004–0.3	0.3–0.59	<0.001–0.5	<0.001–0.5	<0.001–0.6	0.01–0.59	<0.001–0.6	<0.001–0.5
PO_4^{3-} (mg L^{-1})	0.01–4.17	<0.01–0.03	<0.01–0.07	<0.01–0.09	<0.01–0.02	<0.01–0.06	<0.01–0.03	<0.01–0.03
Mg^{2+} (mg L^{-1})	2.51–7.4	9.68	6.46–11.0	2.38–7.13	1.45–5.8	4.70	3.86–7.26	4.63–8.37
Ca^{2+} (mg L^{-1})	25.6–65.3	44.45	49.2–76.4	18.9–43.9	12.4–45.7	51.28	27.9–58.2	34.6–50.6

8 – Wisłoka; 9 – Wisłoka tributary; 10 – Krempna; 11 – Rzeszówka; 12 – Baranie; 13 – Zimna Woda tributary; 14 – Wilsznia; 15 – Kłopotnica.

Distribution in Poland. *Adlafia langebertalotii* is a new species for Poland.

Notes. Knowledge about the ecological preferences of this species is limited. Our studies have shown that the species tolerates a wide range of pH (5.5–8.2) but is also abundant at pH < 6 and medium electrolyte content (254–302 μS cm^{-1}). More valves (2–3% of the assemblage) were observed during autumn in the Krupiec stream, on a sandy substrate with high water nitrate content (9.4 mg L^{-1}). The bottom of the stream was covered in a large amount of organic matter, especially fallen leaves. At the other sites where *A. langebertalotii* was found (Tab. 3), the nutrient content was low and the calcium content often exceeded 40 mg L^{-1}.

Tab. 3 Morphological characteristic and distribution of rare diatom taxa occurred in SE Poland with co-occurrence of dominant taxa (*n* – number of measured valves; *m* – mean).

Taxa	Length (µm)	Width (µm)	Striae (in 10 µm)	Distribution	Dominant taxa
Adlafia langebertalotii (*n* = 10)	6.6–13.6 *m* = 8.9	2.1–3.0 *m* = 2.5	30–40 *m* = 37	MNP: on most streams (single specimens) BdNP: Terebowiec stream (not often) RNP: Krupiec stream (2–3%)	RNP: *Achnanthidium bioretii*, *Psammothidium rechtense* (20–40%), *Achnanthes ventralis*, *Achnanthidium minutissimum*, *A. subatomoides* (5–20%) BNP: *Meridion circulare* (5–60%), *Achnanthidium minutissimum* (5–20%), *Planothidium lanceolatum* (6–8%), *Cocconeis pseudolineata*, *C. placentula* var. *euglypta*, and *C. placentula* var. *lineata* (10–15%) MNP: *Achnanthidium pyrenaicum*, *A. minutissimum* (often more than 50%), *Gomphonema micropus*, *G. olivaceum* (5–25%)
Caloneis undulata (*n* = 4)	31–36.2 *m* = 32.8	4.7–5.5 *m* = 5.1	20–22 *m* = 20.7	RNP: Międzyrzeki peatbog (not often)	*Eunotia exigua* (10–70%), *E. paludosa* (5–70%), *Brachysira brebissonii* (10–30%), and *B. serians* (10–50%)
Eunotia fennica (*n* = 12)	16.8–50 *m* = 31	3.7–5.0 *m* = 4.4	17–20 *m* = 18.3		
E. glacialifalsa (*n* = 2)	85.5–87.3 *m* = 86.4	6.6–6.7 *m* = 6.65	11		
E. groenlandica (*n* = 4)	18.5–37.5 *m* = 30.2	2.8–3.2 *m* = 3.1	14–15 *m* = 14.5	RNP: Międzyrzeki peatbog (not often) BdNP: Wołosate peatbog (not often) MNP: Krempna stream and tributary of Zimna Woda stream (not often)	RNP: *Brachysira serians* (60–70%), *Frustulia saxonica* (25–30%), *Eunotia paludosa* (5–50%), and *E. exigua* (5–20%) BNP: *Kobayasiella parasubtilissima* (near 90%) MNP: *Achnanthidium pyrenaicum* (50–75%), *A. minutissimum* (30–50%)
E. minutula (*n* = 4)	10.7–14.0 *m* = 12.6	2.6–3.5 *m* = 2.8	20–22 *m* = 21.3	RNP: Międzyrzeki peatbog (not often)	*Eunotia paludosa* (5–90%), *E. exigua* (5–80%), *Brachysira serians* (7–70%), *Frustulia saxonica* (5–40%)
E. neocompacta (*n* = 6)	23.5–32 *m* = 26.5	2.6–4.4 *m* = 3.3	17–20 *m* = 19		
E. superpaludosa (*n* = 3)	32.5–35.6 *m* = 33.7	2.9–3.9 *m* = 3.4	19		
Fallacia sublucidula (*n* = 6)	7.7–9.0 *m* = 8.4	3.8–4.0 *m* = 3.7	26–28 *m* = 27.7	BdNP: Wołosaty stream and Terebowiec tributary stream (not often) MNP: Wisłoka and Wisłoka tributary (not often)	BPN: *Achnanthidium pyrenaicum* (20–90%), *A. thienemannii*, *Cymbella parva*, *Diatoma ehrenbergii*, *D. mesodon*, *Meridion circulare* var. *circulare* (5–20%) MNP: *Achnanthidium pyrenaicum* (5–60%), *A. minutissimum* (5–40%)
Pinnularia rhombarea (*n* = 10)	56–103 *m* = 78.8	11.7–12.8 *m* = 12.2	9–11 *m* = 10.4	RNP: Międzyrzeki peatbog (to 5%) BdNP: Wołosate peatbog (not often)	RNP: *Eunotia exigua* (40–80%), *Frustulia saxonica* (20–50%) BNP: *Kobayasiella parasubtilissima* (70–90%), *Eunotia paludosa* (5–7%)
P. similiformis (*n* = 6)	36.8–51.5 *m* = 44.3	5.5–6.8 *m* = 6.2	11–12 *m* = 11.3	RNP: Międzyrzeki peatbog (not often)	*Brachysira serians* (5–60%), *Eunotia exigua* (15–40%), *E. paludosa* (5–30%), *Frustulia saxonica* (5–40%)
Placogeia gereckei (*n* = 6)	11.3–14.7 *m* = 12.9	5.0–5.8 *m* = 5.4	14–18 *m* = 15.7	BdNP: Wołosaty, Terebowiec and Terebowiec tributary (not often)	*Achnanthidium pyrenaicum* (50–70%), *Diatoma ehrenbergii* (5–30%)
Sellaphora vitabunda (*n* = 15)	11.8–18.6 *m* = 16.1	5.2–5.9 *m* = 5.6	26–30 *m* = 27.3	MNP: Kłopotnica (not often) Wisłoka (to 2% at one sampling site)	Wisłoka River: *Achnanthidium pyrenaicum* (5–70%), *A. minutissimum* (5–60%), *Encyonopsis subminuta* (5–50%) Kłopotnica River: *Melosira varians* and *Rhoicosphenia abbreviata* (5–20%)

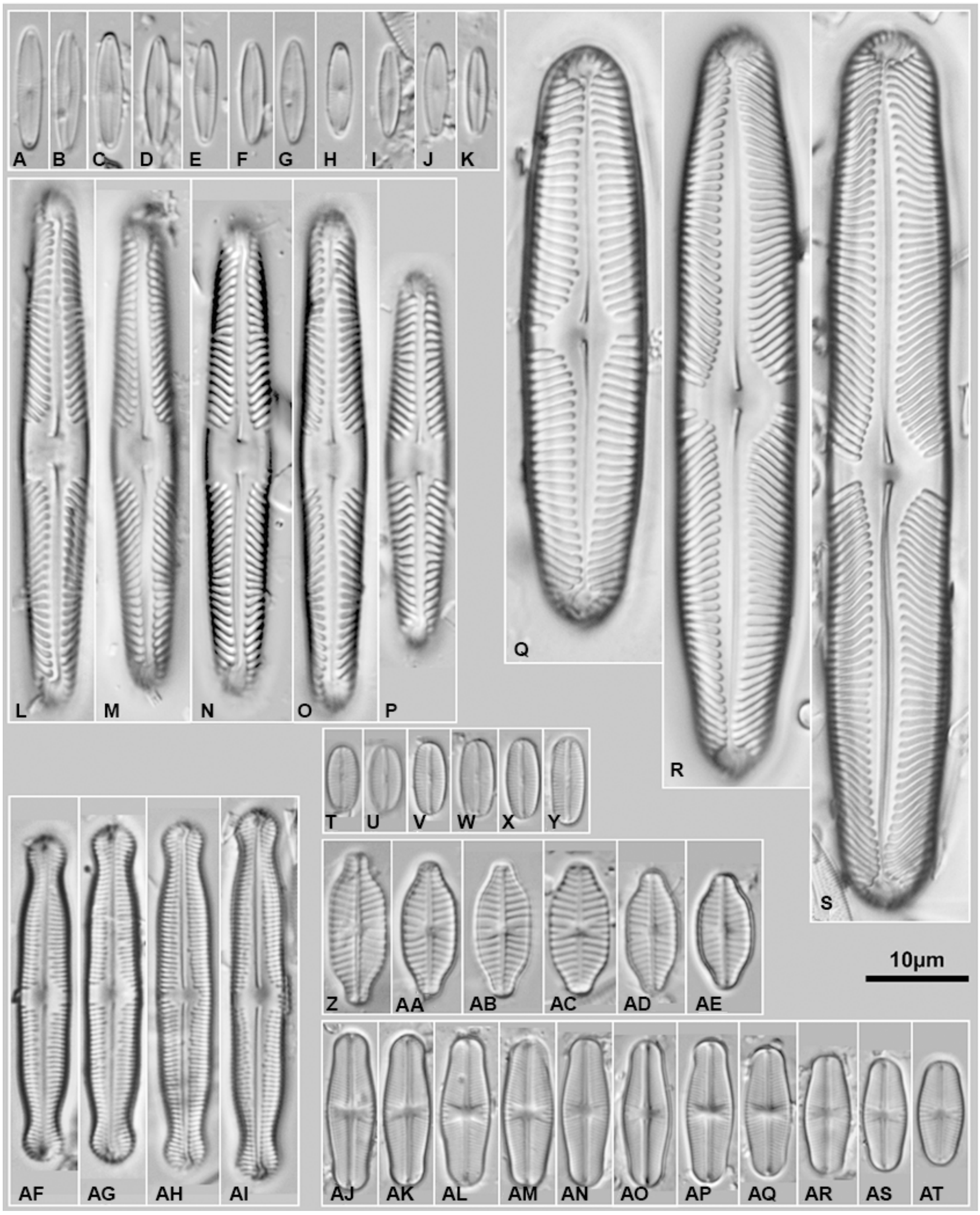

Fig. 2 Light micrographs. (**A–K**) *Adlafia langebertalotii*; (**L–P**) *Pinnularia similiformis*; (**Q–S**) *Pinnularia rhombarea*; (**T–Y**) *Fallacia sublucidula*; (**Z–AE**) *Placogeia gereckei*; (**AF–AI**) *Caloneis undulata*; (**AJ–AT**) *Sellaphora vitabunda*.

Placogeia gereckei (Cantonati & Lange-Bertalot) Bukhtiyarova (Fig. 2Z–AE, Fig. 4D–F)

Basionym: *Geissleria gereckei* Cantonati & Lange-Bertalot.

Type of substrate. Species developed among mosses covering submerged or emerged stones.

Distribution in Poland. *Placogeia gereckei* is a new species for Poland.

Notes. *Placogeia gereckei* was only observed in the upper, shaded parts of small streams with rapid currents, among mosses, in oligotrophic conditions, most often when the

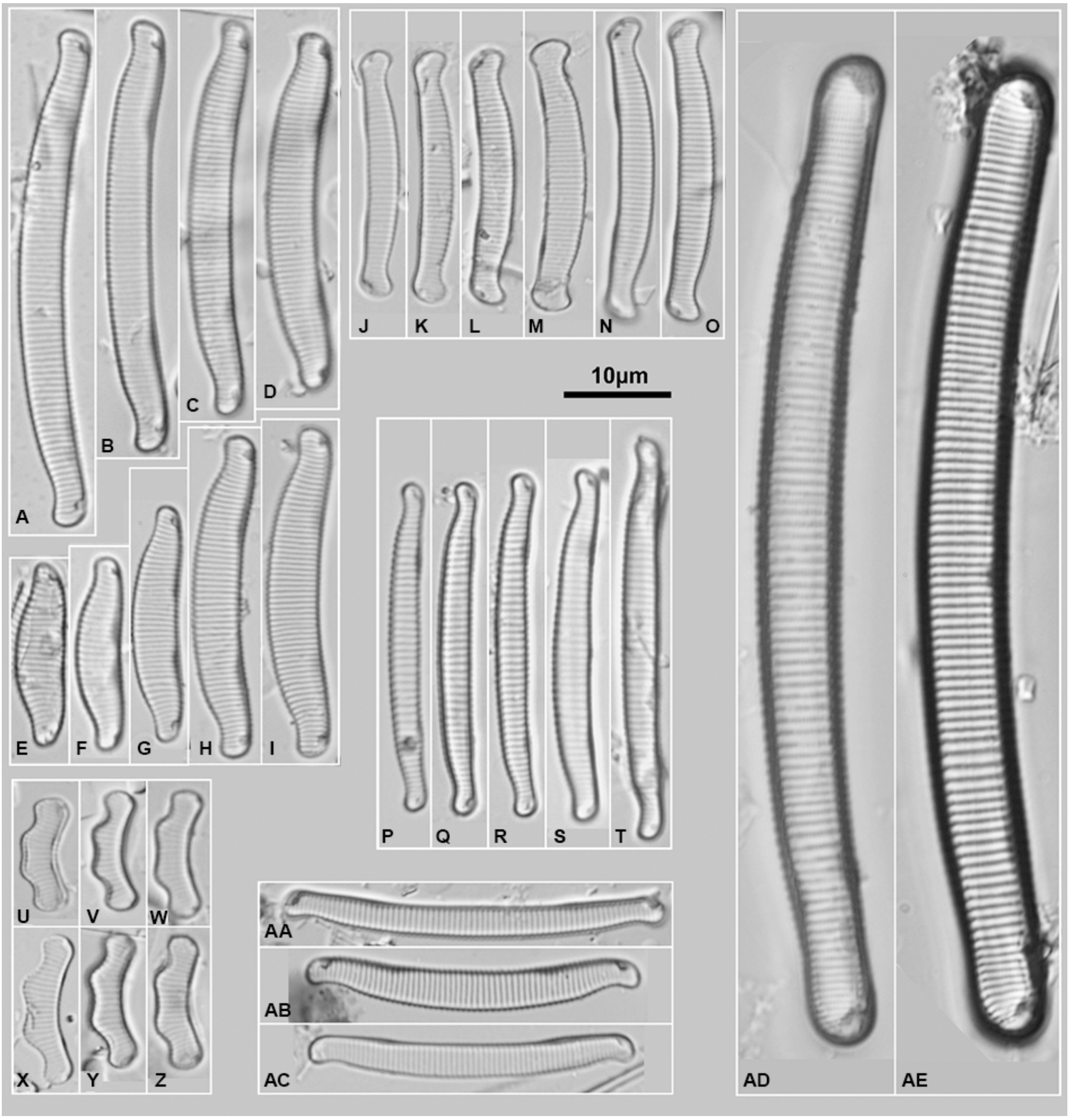

Fig. 3 *Eunotia* species light micrographs. (**A–I**) *Eunotia fennica*; (**J–O**) *E. neocompacta* var. *neocompacta*; (**P–T**) *E. groenlandica*; (**U–Z**) *E. minutula*; (**AA–AC**) *E. superpaludosa*; (**AD–AE**) *E. glacialifalsa*.

pH was close to neutral and with medium conductivity. Fallen leaves in the streams resulted in an increase in organic matter at these sampling sites.

Sellaphora vitabunda (Hustedt) D. G. Mann (Fig. 2AJ–AT, Fig. 4A–C)

Synonyms: *Navicula vitabunda* Hustedt, *Naviculadicta vitabunda* (Hustedt) Lange-Bertalot.

Type of substrate. Always developed on stones.

Distribution in Poland. Species known only from Kortowskie Lake in northern Poland [40] and Eemian freshwater sediments near Wroclaw [41,42].

Notes. *Sellaphora vitabunda* was identified individually from both the Wisłoka and Kłopotnica sites. It only developed abundantly (1.7%) at one site on the Wisłoka River,

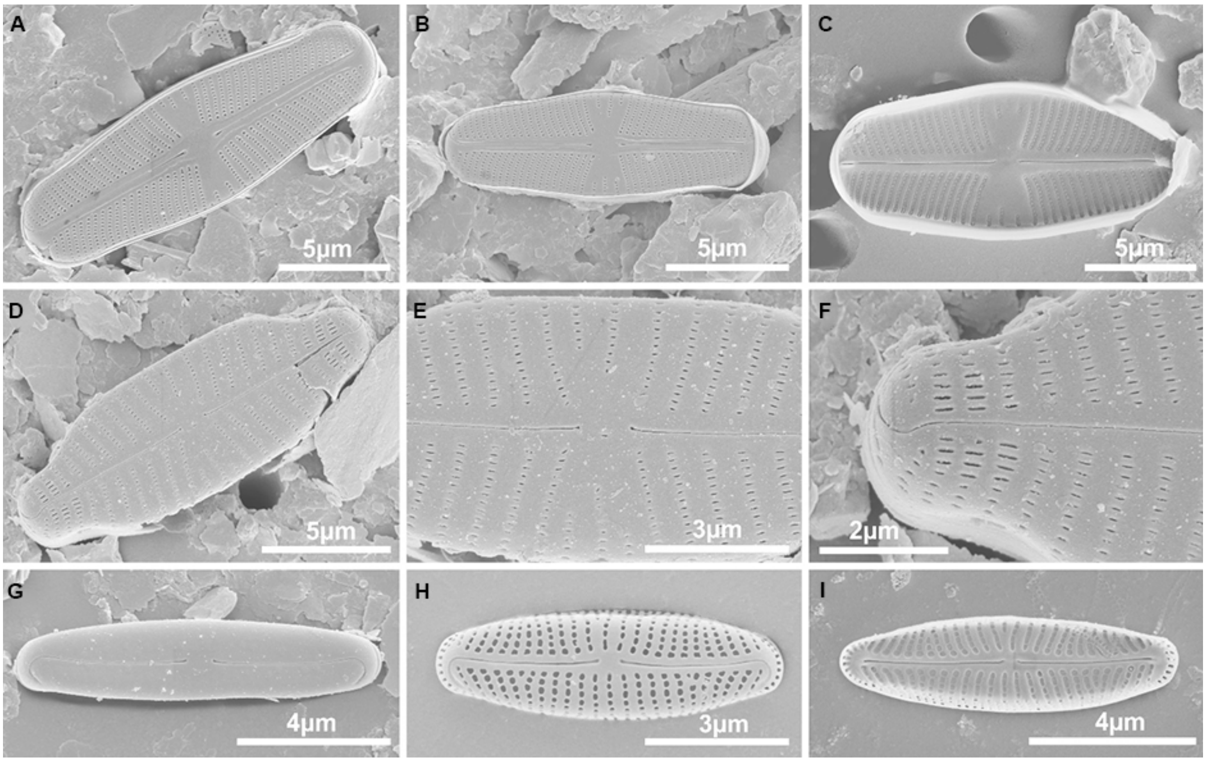

Fig. 4 SEM micrographs. (**A,B**) External valve view of *Sellpahora vitabunda*. (**C**) Internal valve view of *S. vitabunda*. (**D**) External view of *Placogeia gereckei*. (**E**) Central area detailed view of *P. gereckei*. (**F**)Valve apex details of *P. gereckei*. (**G**) External valve view of *Adlafia langebertalotii*. (**H**) External, not fully silicate valve view of *A. langebertalotii*. (**I**) Internal valve view of *A. langebertalotii*.

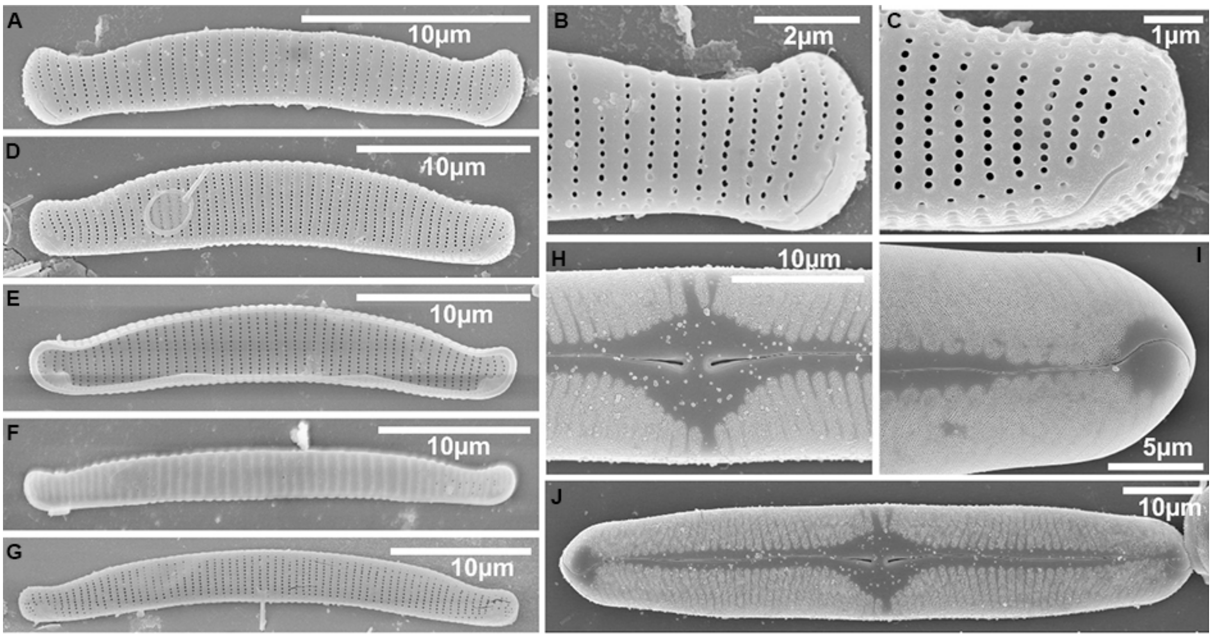

Fig. 5 SEM micrographs. (**A**) External valve view of *Eunotia neocompacta* var. *neocompacta*. (**B**) Details of valve apex of *E. neocompacta* var. *neocompacta*. (**C**) *Eunotia fennica* – details of valve apex. (**D**) External valve view of *E. fennica*. (**E**) Internal valve view of *E. fennica*. (**F**) *Eutonia groenlandica* – external view of entire valve. (**G**) *Eunotia superpaludosa* – external view of entire valve. (**H–J**) *Pinnularia rhombarea* external view. (**H**) Details of central area. (**I**) Details of valve apex. (**J**) Image of entire valve.

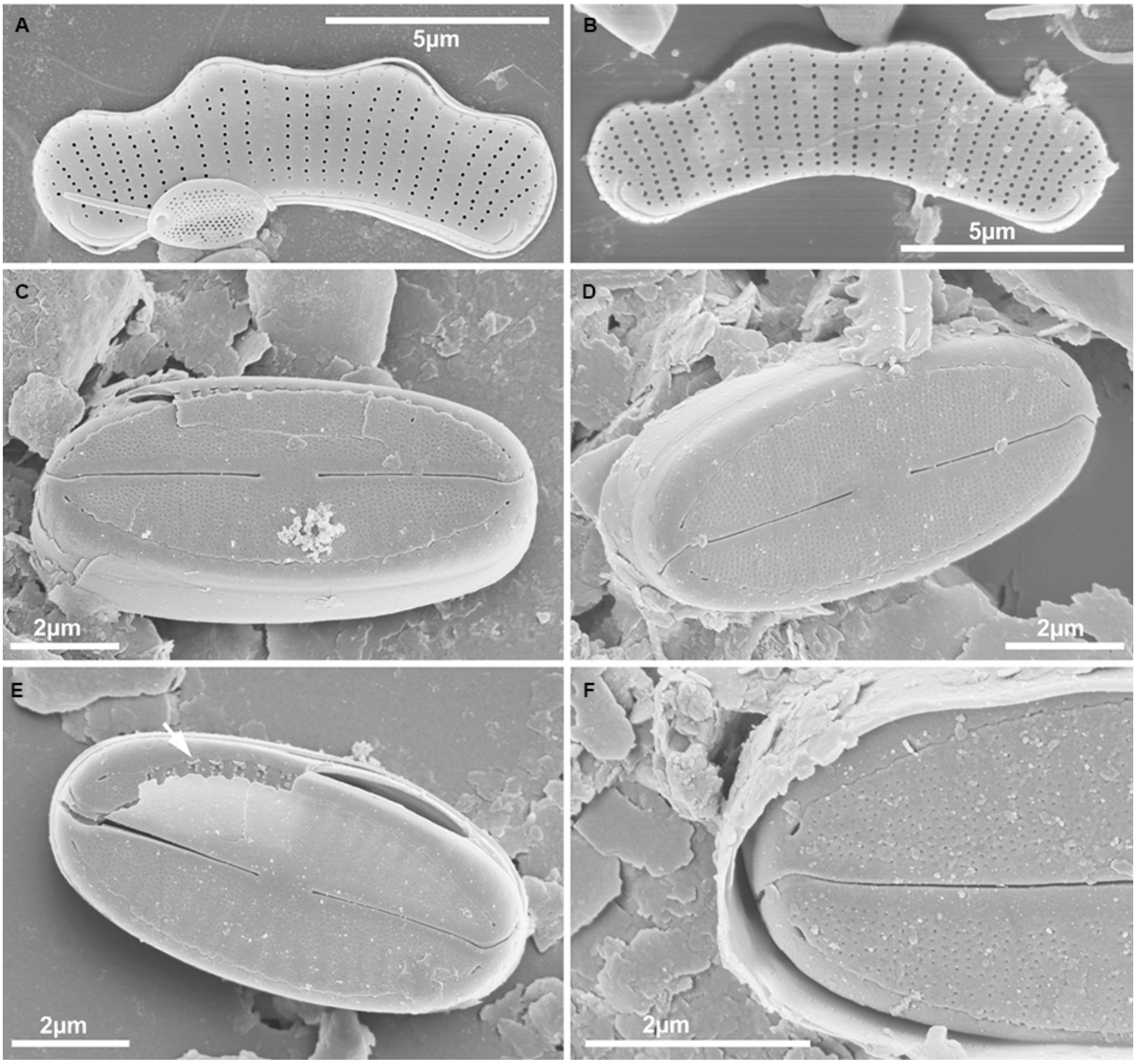

Fig. 6 SEM micrographs. (**A,B**) External valve view of entire valve of *Eunotia ninutula*. (**C–F**) *Fallacia sublucidula*. (**C,D**) Entire valve view. (**E**) View of broken valve with visible of internal striae surrounded by silicate plates (see arrow). (**F**) Detailed view of valve apex.

below a dam reservoir, in August 2013. It was always found in water with an alkaline pH and high electrolyte content in oligo- to mesotrophic conditions. All sites where *S. vitabunda* was found were located in flowing waters.

Fallacia sublucidula (Hustedt) D. G. Mann (Fig. 2T–Y, Fig. 6C–F)

Synonym: *Navicula sublucidula* Hustedt.

Type of substrate. The species developed mainly among mosses growing on stones, very rarely it occurred on stones.

Distribution in Poland. Species occurred in small numbers in periphyton on the Kraków-Częstochowska Upland [43,44] and in the Gulf of Gdańsk [45].

Notes. Single valves of *F. sublucidula* were observed only in the upper shaded sections of small streams, most commonly at alkaline pH (7.5–8.4) and medium conductivity under oligo- to mesotrophic conditions. Most of the cells were found among mosses growing on stones, partly or completely submerged in water.

Fallacia sublucidula was formerly documented by SEM micrographs, but without a detailed description of the species. Wojtal and Sobczyk [43] presented SEM image of only one cell, but did not make a description.

SEM description. Valves ovate to elliptic, with broadly rounded ends, small to 10 µm long. Valve face flat. Striae weakly radiated and curved, covered externally by finely porous conopea. At the valve margin near to apices, few (usually 5–6) striae occluded by hymen are present. Areolae not visible in LM. In internal view areolae surrounded by irregular plates (see arrow in Fig. 6E). Filiform, straight raphe with proximal raphe endings very weakly widened. Distal raphe endings curved in to the same direction. Longitudinal lines (breaks) exist in the conopeum, near its margins, creating two small openings at each apex. The central and axial area small.

Caloneis undulata (Gregory) Krammer, nom. illeg., non Skvortsov & K. I. Meyer 1928 (Fig. 2AF–AI)

Basionym: *Pinnularia undulata* Gregory.

Type of substrate. Organic sediments in small, shallow depressions on peatbogs, and among mosses.

Distribution in Poland. Species were observed in Poland as *Pinnularia undulata*, from fossil deposits [46,47], the Tatra Mountains [48,49], and from peatbogs [50,51].

Notes. *Caloneis undulata* was found only in the Międzyrzeki peatbog (RNP), with very low pH (3.2–4.4) and biogenic content and low to medium conductivity.

Eunotia fennica (Hustedt) Lange-Bertalot (Fig. 3A–I, Fig. 5C–E)

Basionym: *Eunotia denticulata* var. *fennica* Hustedt.

Type of substrate. Organic sediments in shallow, small depressions in peatbogs.

Distribution in Poland. *Eunotia fennica* is a new species for Polish diatom flora.

Remarks. During the studies, single specimens were found in the oligotrophic, acidic waters of the Międzyrzeki peatbog, under conditions similar to those described in the literature [36,37,52].

Eunotia glacialifalsa Lange-Bertalot (Fig. 3AD,AE)

Type of substrate. Organic sediments in small and shallow depressions in the peatbogs.

Distribution in Poland. *Eunotia glacialifalsa* is known only from a few localities in the East Tatra Mountains, where it developed in waters with a pH from slightly acidic to slightly alkaline, low nitrate content, and very low conductivity [53].

Notes. *Eunotia glacialifalsa* was recorded very rarely only at one site on the peatbog Międzyrzeki in acidic, oligotrophic waters, in fall.

Eunotia groenlandica (Grunow) Nörpel-Schempp & Lange-Bertalot (Fig. 3P–T, Fig. 5F)

Basionym: *Eunotia paludosa* var. *groenlandica* Grunow.

Synonyms: *Eunotia fallax* var. *gracillima* Krasske, *E. fallax* var. *groenlandica* (Grunow) Lange-Bertalot & Nörpel-Schempp.

Type of substrate. This species occurred in organic sediments in small and shallow depressions and among the mosses in peatbogs. It was noted in streams among mosses growing on stones partly or completely submerged in water.

Distribution in Poland. *Eunotia groenlandica* has been so far listed only from the Roztoka stream, from the Tatra Mountains as *Eunotia fallax* var. *groenlandica* [54].

Notes. The species was found rarely both in peatbogs and in the upper sections of small streams. In the streams, single valves were most often observed on moss covered stones partially or completely submerged in water. In the peatbogs the species was observed in oligotrophic waters with low pH (3.0–4.5), while in streams, it was found in circumneutral or slightly alkaline pH (6.3–8.6). The conductivity value was low or average.

Eunotia minutula Grunow (Fig. 3U–Z, Fig. 6A,B)

Type of substrate. Organic sediments in small and shallow peat pits.

Distribution in Poland. *Eunotia minutula* have not been reported from within Polish territory.

Notes. The species occurred only at several sites in the Międzyrzeki peatbog, under oligotrophic conditions with low pH (below 3.5) and low or medium conductivity.

SEM description. Dorsal edge biundulate (Lange-Bertalot et al. [37] reported triundualate forms also), ventral edge concave. Valve ends capitate and weakly recurved to dorsal site. Terminal raphe ends curved reaching about one third to one half of valve face. Striae composed of rounded areolae (44–50 in 10 µm). Individual striae running from dorsal site often shortened (do not reach ventral edge).

Eunotia neocompacta Mayama var. *neocompacta* (Fig. 3J–O, Fig. 5A,B)

Synonyms: *Eunotia exigua* var. *compacta* Hustedt, *E. compacta* (Hustedt) Mayama.

Type of substrate. Organic sediments in small and shallow peat pits.

Distribution in Poland. *Eunotia neocompacta* var. *neocompacta* is a new species for the Polish diatom flora.

Notes. The species was always found in acidic pH (below 3.5) and low to medium conductivity, only in the Międzyrzeki peatbog.

Eunotia superpaludosa Lange-Bertalot (Fig. 3AA–AC, Fig. 5G)

Type of substrate. Organic sediments in small and shallow peat pits.

Distribution in Poland. *Eunotia superpaludosa* has not have been reported from Poland.

Notes. Few frustules of *E. superpaludosa* were found only in two sites in the Międzyrzeki peatbog, so it is difficult on this basis to draw conclusions about the ecology and occurrence of this species. This taxon always occurred in low pH (under 3.5) and low to moderate conductivity.

Pinnularia rhombarea Krammer (Fig. 2Q–S, Fig. 5H–J)

Type of substrate. Organic sediments in small and shallow peat pits and among mosses.

Distribution in Poland. *Pinnularia rhombarea* was not reported for Polish territory.

Notes. The species was observed only in peatbogs, more often in the Międzyrzeki (up to 5% of all counted valves), under oligotrophic conditions, always at a pH below 4. It has not been found in flowing waters, even those flowing from peatlands.

Pinnularia similiformis Krammer (Fig. 2L–P)

Synonyms: *Pinnularia similis* Krasske, *P. subrostrata* var. *similis* Cleve-Euler, *P. similis* Hustedt.

Type of substrate. Organic sediments in small and shallow peat pits.

Distribution in Poland. *Pinnularia similiformis* is new to Poland.

Notes. Only a few specimens were stated on the Międzyrzeki peatbog in oligotrophic and acidic waters.

Discussion

The waters of the studied watercourses and peatbogs were usually oligotrophic, with a low ion content. Nutrient values were high only in spring (April 2013), which was related to the rapid melting of the snow cover and surface run-off from surrounding fields, meadows, and pastures that had been fertilized with manure the previous fall (especially in the Magura National Park). Within the area of the Roztocze National Park, high nutrient values are observed mainly in fall, when organic matter (mostly fallen leaves and branches) enriches the stream beds. Many rivers and streams in the Magura and the Bieszczady national parks are characterized by average and high concentrations of calcium ions, often >40 mg L^{-1}, due to the presence of Carpathian flysch in the bedrock [23,26]. Watercourse habitats were considerably more diverse (from more than 100 to more than 250 taxa per site in the studied streams) than peatbogs (50–100 taxa at the site) (T. Noga, unpublished data in preparation).

Four species were distinguished from the watercourses. Two of them, *Adlafia langebertalotii* and *Placogeia gereckei*, have only been reported from Europe. *Adlafia langebertalotii* was described from rivers in Luxembourg by Monnier et al. [39] as an aerophilous diatom, occurring mainly in small rivers with low mineralization and organic pollution levels. Recently, these taxa have also been noted in France [55] and Italy [56]. *Placogeia gereckei* was described from the Dolomiti Bellunesi National Park in the southeastern Alps, Italy. *Placogeia gereckei* was characteristically found only on wet stones (epilithic taxon) covered by leaf litter. The species was found in small mountain springs located at low elevations (<1,000 m a.s.l.), with an extremely low discharge and always in very shaded sites. The species occurred under oligotrophic conditions, where the pH was alkaline (8.0) and conductivity was low [57]. The species was later reported several times from sources in the Alps [58–60], and recently in Serbia in two small mountain rivers, in the epilithon and epibryon [61]. The species developed in oligotrophic conditions in the upper, shaded parts of small streams with a rapid current, and among mosses. *Placogeia gereckei* was reported by Cantonati and Lange-Bertalot [57] and Vidakovic et al. [61] under similar conditions. As the species usually develops in the upper reaches of streams or sources, it may be a good indicator species for unpolluted, oligotrophic waters.

In contrast, two other species, *Fallacia sublucidula* and *Sellaphora vitabunda*, are widespread, but often reported only in different checklists, meaning knowledge about their ecology is limited. Little is known about the ecology and occurrence of *Fallacia sublucidula*, which is present throughout both the lowlands and highlands [31,38]. *Fallacia sublucidula* is usually only mentioned in lists of species from Great Britain [62], Germany [10,63,64], Macedonia [65], the Netherlands [66], Romania [67,68], and North America [69]. *Fallacia sublucidula* probably has a wide development spectrum. Lange-Bertalot [10] described it as an eutraphentic species, which does not quite match the conditions in which it was observed in this study. It is also likely to be an aerophytic species, mainly because it was found on mosses growing on stones, often extending beyond the water surface, depending on water levels in the watercourses.

Krammer and Lange-Bertalot [31] reported that *Sellaphora vitabunda* has been reported from Europe and Northern America. This species is widespread and often grows in oligo- to mesotrophic lakes with varying amounts of electrolytes. According to Lange-Bertalot et al. [70] and Bąk et al. [38], it is a poorly known species and often mistaken with for *Sellaphora verecundiae*. It develops in alkaline lakes and lowlands and is relatively rare in the Alps. There is no evidence for the occurrence of this species in flowing waters. Hällfors [71] reported *S. vitabunda* from the Gulf of Finland (Baltic Sea) and characterized it as a freshwater species that does not tolerate full salinity. According to Lange-Bertalot [10], it is a very endangered (Category 2) species in Germany. *Sellaphora vitabunda* has a wide global distribution but is usually only mentioned in species lists. Only Kaczmarska [72] gives a detailed morphological description of the species, together with SEM micrographs with descriptions. *Sellaphora vitabunda* is reported from Europe including the Baltic Sea [71], Great Britain [62,73,74], Germany [10, 63,70], Ireland [75,77], the Netherlands [66], Macedonia [65], Romania [67,68,77], Slovakia [78], Spain [79,80], Turkey [81], and Iceland [82], and from other continents: North America [69,83–85], South America [86], Africa [87], Asia [88,89], and Australia (Tasmania) [90]. To date, the species has only been described from lakes [31,38,70], but recent studies have shown that *S. vitabunda* can also develop in flowing waters if the run of the water is slowed down by natural or artificial barriers (e.g., dams and beaver ponds).

Nine species were distinguished from the peatbog. Most rare or new species were found in the Międzyrzeki peatbog in the Roztocze National Park, showing that protected areas have proved to be a refuge for rare and poorly known diatom species.

Some species, especially from the genus *Eunotia*, *Pinnularia*, and *Caloneis undulata*, are associated with oligotrophic and acidic habitats, with a low electrolyte content [31,35,37]. These are often peatland areas that in many countries, including Poland, represent only a small percentage of the different types of habitat and are still poorly studied. Species of the genus *Eunotia* – typical of oligotrophic, low-mineral aquatic habitats – are especially rare and threatened to different degrees in Europe. Many of them prefer acidic water and are referred to as acidiobiontic or acidophilous [10,91–93].

Four species of the genus *Eunotia* have never been reported from Poland: *Eunotia fennica*, *E. minutula*, *E. neocompacta* var. *neocompacta*, and *E. superpaludosa*.

Eunotia fennica normally develops in ombrotrophic peatbogs or dystrophic lakes associated with *Sphagnum* spp. [37]. In Central Europe, they are medium-dispersed species and develop on undisturbed peatbogs [36,70]. According to Foged [52], *E. denticulata* var. *fennica* is a halophobic and acidophilic species. When the differentiating characteristics were still unknown, *E. fennica* was recorded as *E. denticulata* (Brébisson) Rabenhorst [34]. Currently, *E. fennica* is one of the most commonly observed species, distinct from *E. denticulata* sensu lato, and often develops in places where *E. paludosa* is dominant [37]. This species was also observed in Great Britain [62,73], the Czech Republic [94], the Netherlands [66], Romania [68,77], North America (Alaska) [52], and South America [86].

In this study, single specimens were found in the oligotrophic, acidic waters of the Międzyrzeki peatbog, under conditions like those described in the literature [36,37,52].

Eunotia minutula is a rare species in Europe, occurring in oligotrophic or dystrophic habitats [37]. Among other places, it was reported from the Netherlands [66], Portugal [95], and Asia [96].

The third species, *E. superpaludosa*, was described recently by Lange-Bertalot et al. [37], but its occurrence in Europe and North America is not precisely defined due to its similarity to many species of the genus *Eunotia*, including *E. exigua*, *E. fallax*, *E. paludosa*, and *E. nymanniana*. *Eunotia superpaludosa* occurred in ombrotrophic peatbogs with *Sphagnum* spp. and with other acidobiontic diatoms, including *E. paludosa* and *E. fennica*. The species was reported from the Netherlands [66] and several other places.

The last species new to Poland is *E. neocompacta* var. *neocompacta*, a rare taxon, occurring mainly in peatbogs [36,37]. It can also develop in fens, lakes, springs, and streams on sandstones, mainly in oligotrophic, acidic waters with low conductivity [37]. According to Hofmann [97], it is an acidophilic species and a dystrophy indicator. The species is known from Georgia [98], Ireland [75,76], Iceland [82], Germany [97], the Netherlands [66], the alpine region in Italy [99], Romania [77], Russia [100], Mongolian peatbogs [101], Asia [96], and North America [52,83]. Studies have shown that *E. neocompacta* var. *neocompacta* developed under similar conditions to those reported by Lange-Bertalot et al. [37] and Hofmann et al. [36].

The other two taxa from the genus *Eunotia* (*E. glacialifalsa* and *E. groenlandica*) were previously reported from Poland, but usually from only a few sites.

Eunotia glacialifalsa is a very scattered species in Central Europe, never forming a large population. It develops in acidic waters, poor in electrolytes, as well as alkaline, oligotrophic waters buffered by carbonates. It is often found in standing waters. However, this species occurs not only in oligotrophic environments, but also tolerates mesotrophic conditions [36–38]. It is very rare in the Holarctic region, and has been reported from the Netherlands [66], Belgium [102], and peatbogs in northern Mongolia [101], the Czech Republic, and Denmark [103].

The distribution of *Eunotia groenlandica* is not precisely defined due to it being confused with *E. fallax* or *E. pseudogroenlandica*. Lange-Bertalot et al. [37] found *E. groenlandica* in various regions of Eurasia and North America. It prefers low pH, low conductivity, and semiaquatic habitats on intermittently wet silicate rocks and bryophytes. This species is known from Europe as *Eunotia fallax* var. *groenlandica*, mostly from Great Britain [62], the Netherlands [66], Germany [63], and Slovakia [78]. It was also recorded from North America [83,104], the Czech Republic [94], the Netherlands [66], and Asia [89]. *Eunotia groenlandica* seems to prefer semiaquatic habitats, as was reported by Lange-Bertalot et al. [37], especially habitats among mosses. It was found in both peatbogs and streams. It is classified as vulnerable (VU) in the "Red list of Bulgarian algae" [13].

Two species from the genus *Pinnularia*, *P. rhombarea* and *P. similiformis*, have also never been reported from Poland. Both taxa prefer cold, oligotrophic waters with a low electrolyte content [35]. *Pinnularia rhombarea* is more developed in the northern and subarctic regions than in Central Europe [35]. On the "Red list of Bulgarian algae", it is considered an endangered species (EN) [13]. The species is mentioned in the list of species of many European countries, including the Netherlands [66], Romania [67,68], and Bulgaria [13], as well as North America [69], South America [105,106], and Asia [88,89,96,101,107,108]. Oligotrophic waters with a low electrolyte content promote the growth of this species, as also reported by Krammer [35]. Research has shown that *P. rhombarea* is a peatbog species which prefers small ponds with stagnant water.

Pinnularia similiformis is a cosmopolitan and aerophilic species that develops in oligotrophic, electrolyte-poor, oxygen-rich moorland waters. It is common in Northern Europe, the Alps, and in northern Germany [10,35,109]. *Pinnularia similiformis* is also known from Chile [110], the Netherlands [66], and Russia [89], among other countries.

Caloneis undulata, the last of the discussed species, has been recorded mainly from the Northern Hemisphere and is considered nordic-alpine. It occurs in oligo-dystrophic waters with low electrolyte content. In Central Europe, *C. undulata* has been found in mountain areas, usually as single individuals [31,38]. According to Foged [52], it is a cosmopolitan species, oligohalobe and circumneutral. This species was identified as oligotrophic and classified as vulnerable (V) in the German red list of algae [10]. *Caloneis undulata* has been reported from many places worldwide, but usually it is only listed. It was identified in the Arctic [96] and Iceland [82] as *Pinnularia undulata*. It is often found in Europe, including in Great Britain [62,73,74], Germany [10], Ireland [75,76], the Netherlands [66], Romania [67,68,77], and Spain [79,111]. *Caloneis undulata* also occurs in other continents, including North America [52,69,83,112,113], Asia [88,89,96,107,114,115], and even in Australia [90]. This study and data from the literature confirm that the species develops in oligotrophic waters and often in peatbogs, but usually as single individuals.

This paper presents rare diatom species, which are often very small, making them difficult to identify. The discussed taxa occur very rarely in specific, uncommon, vulnerable habitats (i.e., unpolluted headwaters and peatbogs) which in many cases are endangered. Consequently, most of these diatom taxa can also be considered as endangered. Most of them, i.e., *Adlafia langebertalotii*, *Eunotia fennica*, *E. minutula*, *E. neocompacta* var. *neocompacta*, *E. superpaludosa*, *Pinnularia rhombarea*, *P. similiformis*, and *Placogeia gereckei*, have not previously been reported from Poland. Two of the mentioned species, *Fallacia sublucidula* or *Sellaphora vitabunda*, are often described from Europe and other continents; however, they are usually only mentioned on diatom lists, likely because of their small size and scattered, rare occurrences, as well as the difficulty associated with full SEM documentation. Here, a detailed description of the cell structure with SEM pictures is presented for *Fallacia sublucidula*. To date, only Wojtal and Sobczyk [43] have presented a specimen of *F. sublucidula* photographed under SEM in their work, but without visible details of the cell structure. Species like *Adlafia langebertalotii*, *Eunotia minutula*, *E. superpaludosa*, or *Placogeia gereckei* have only been reported from a few sites and their occurrence and ecology are still poorly known. They were recently described as new to science [37,39,57], and are therefore also not included in national red lists of algae. Every new report on the occurrence and ecological conditions where they develop allows the extension and valuable supplementation of autecological characteristics.

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