

## Mining salinisation of rivers: its impact on diatom (Bacillariophyta) assemblages

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**Abstract:** The composition of the diatom assemblages was analysed in four rivers of Upper Silesia, Poland in 2017. The diatom assemblages studied were found to reflect anthropogenic salinization caused by mining activities. The assemblages in those rivers characterised by the highest salinity (Bolina and Mleczna) showed a relatively low taxonomic richness. The diatom assemblages were dominated by species typical of brackish or marine waters. The rivers with a minimal or weak anthropogenic impact (Centuria and Mitręga) supported taxonomically richer diatom assemblages typical of mid–altitude siliceous or calcareous streams (respectively), that have a fine particulate substratum. The presence of a new species, *Planothidium nanum* sp. nov., was revealed. The new species shows a unique set of morphological characters, including small size; its elliptical outline as well as very widely–spaced central striae on the sternum valve (sinus) and widely–spaced central striae on the raphe valve allow to separate it from other similar *Planothidium*.

**Key words:** diatom, mining activities, new species, river salinisation, Upper Silesia, Poland

## INTRODUCTION

Aquatic organisms are the first environmental component that reacts to any hydromorphological and physico–chemical modifications of riverine water quality. Studies of aquatic organisms are therefore very useful in assessing the human impact and detecting any changes in lotic ecosystems. The use of various organisms that react to long– or short–term changes in aquatic environments gives a precise outlook on the health of these ecosystems (ECTOR & RIMET 2005; KELLY et al. 2009; LI et al. 2010; SOLAK et al. 2012; POIKANE et al. 2016). Additionally, the EU Water Framework Directive (WFD 2000) recommended that all biological components be used in order to have a full view of the ecological status of flowing waters. The diatoms used to monitor waters are particularly good indicators (e.g. KELLY 2002; BĄK et al. 2004; VILBASTE et al. 2004; BĄK & SZLAUER–ŁUKASZEWSKA 2012). They can be found in every aquatic environment in both clean springs and in heavily polluted environments. They settle on various substrates such as stones, macrophytes or on anthropogenic substrates. In addition, diatoms form a large part of the benthos, often reaching a value of 90–95% (ÁCS et al. 2004). However, the strong water pollution that is caused by, for example, salinisation is

the main factor that limits the occurrence of fauna and flora (WILLIAMS 1987). Therefore, in heavily polluted water environments in which it is impossible to examine all of the recommended biological elements, diatoms are very good indicators, especially when there is a lack of invertebrates and ichthyofauna.

The central part of Southern Poland is the most urbanised and at the same time the most polluted region. Upper Silesia, which is located in this part of the country, is a historic area in Poland and the Czech Republic (the upper Odra basin and the initial course of the Vistula) and is also one of the most industrialised and urbanised regions in Europe. Upper Silesia has no natural water reservoirs, and it has only artificial reservoirs (LEWIN et al. 2015). The degradation of many of the rivers in this region is primarily connected with hydromorphological transformations, the inflow of domestic sewage from household sewers and salt water from coal mines. These rivers are characterised by an increased concentration of nutrients and chlorides, water hardness and often a very high conductivity (e.g. HALABOWSKI et al. 2019a, 2019b; SPYRA et al. 2015; LEWIN et al. 2018; SOWA et al. 2018). The high level of urbanisation of the Upper Silesian region and the use of salts as de–icing agents for roads also contributes to the degradation of the

surrounding aquatic environments (including the spread of alien and invasive species; HALABOWSKI et al. 2019b; LEWIN et al. 2015).

The algae of the region were studied in a few surveys and primarily concerned phytoplankton and to a lesser extent microphytobenthos (STARMACH 1939; BUCKA 1960, 1964, 1966; HANAK-SCHMAGER 1974; KWANDRANS 1998, 2002; WILK-WOŹNIAK et al. 2011). It came to our attention that specific research on diatoms (Bacillariophyta) in the Polish part of Upper Silesia had not yet been performed, although quite a number of papers have been published on the diatoms that inhabit the waters from neighbouring areas. Most of the published results concerned the diatoms in springs and streams (e.g. KWANDRANS 2007; WOJTAŁ 2013) and rivers (fewer in water reservoirs), e.g. WOJTAŁ & KWANDRANS 2006; CICHÓN 2016). According to the EU Water Framework Directive (WFD 2000), the biomonitoring that is carried out in Poland is based on macrophytes, ichthyofauna, benthic macroinvertebrates and phytobenthos, including rivers that are located in the studied area. However, accessible data of the state monitoring lack identified taxa lists and detailed data on species are not usually

published.

The aims of the presented research were to compare the biodiversity of the diatom assemblages in rivers that are located in the industrial and urban area of Upper Silesia and adjacent regions that are affected by various degrees of anthropogenic pressure and to determine the most important environmental factors that have a significant impact on their structure. Another aim of the study was to assess the ecological condition of the investigated riverine courses based on microphytobenthos.

## MATERIALS AND METHODS

**Study area.** The study was carried out in four rivers that are located in southern Poland, all of which are part of the Upper Vistula River. Two of the rivers that were studied (the Mleczna and Bolina Rivers) are affected by the coal mining activity in Upper Silesia (Southern Poland). The two remaining study sites are located outside of area of the mining activity (the Centuria and the Mitręga Rivers; Fig. 1). The anthropogenic effects were primarily observed as changes in the physical and chemical parameters of the water and the morphological transformation

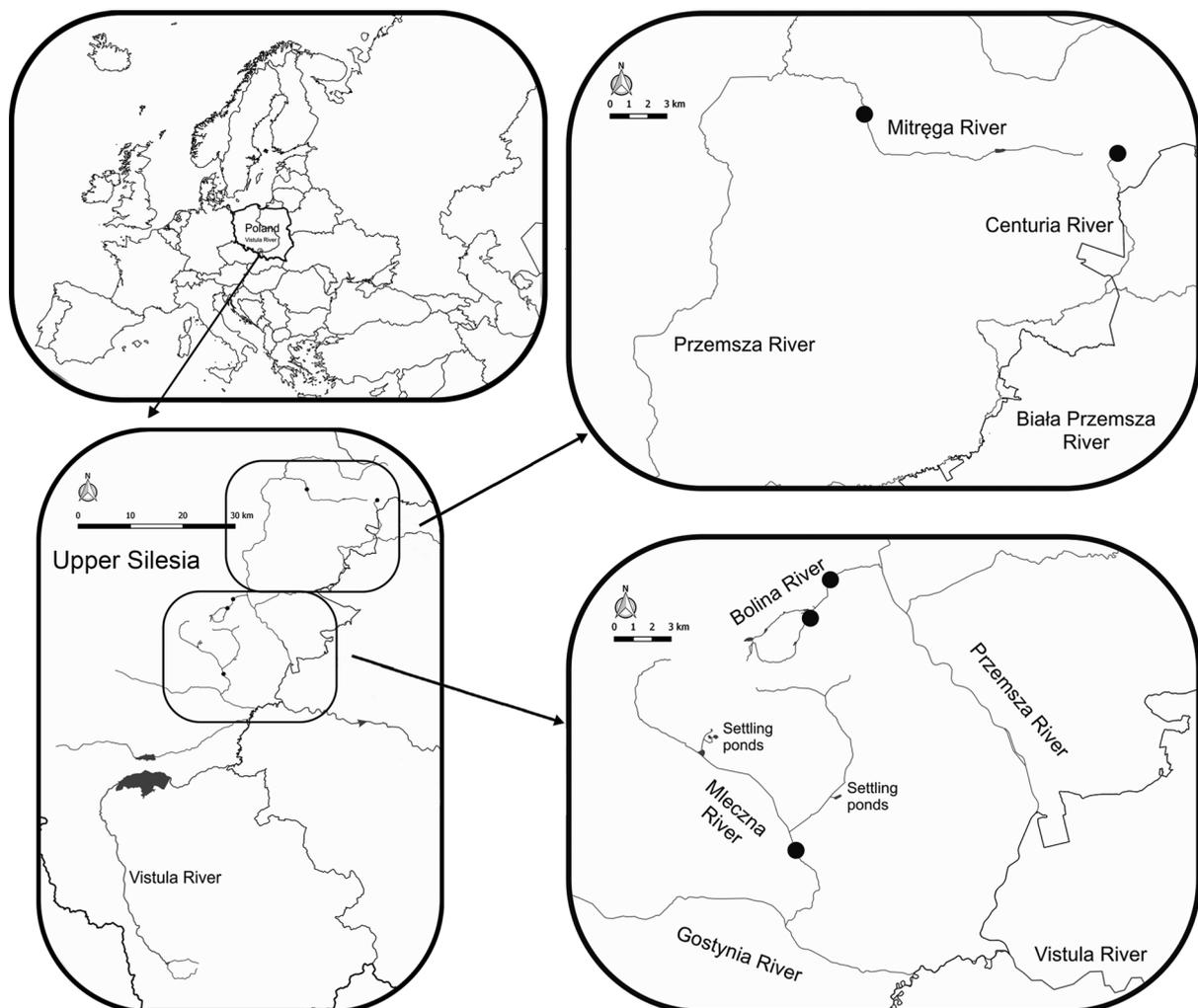


Fig. 1. Location of the study area in Southern Poland and the diatom sampling sites in rivers that were studied.

of the riverbeds at the research locations. The Mleczna River is characterised by a high mineralisation of the water due to the discharge of underground salt water from four coal mines, i.e. ‘KWK Boże Dary’, ‘KWK Mysłowice–Wesoła’, ‘KWK Ziemowit’ and Experimental Mine ‘Barbara’. The water from the ‘KWK Boże Dary’ coal mine flows through a system of reservoirs before discharging into the river, while the water from the ‘KWK Mysłowice–Wesoła’ coal mine flows through the Przyrwa River, which is a tributary of the Mleczna River. Two sites (at the upper and lower courses) that have different degrees of water pollution from the coal mines were selected at the Bolina River for the study. The upper course is contaminated by salt water from the ‘KWK Murcki–Staszic’ coal mine. By contrast, the lower course of the Bolina River is affected by the discharge of underground salt water from the ‘KWK Wieczorek’ coal mine. The anthropopressure of the study sites on the Mitęga River is caused by a dam reservoir that was constructed a few kilometres away. The sampling site of the Centuria River is not affected by anthropopressure. This site is located near a river spring. The general characteristics of the rivers and the sampling sites are summarised in Tables 1 and 2.

**Sampling procedure and taxonomic analyses.** The samples were collected four times per sampling site in 2017 (spring, early and late summer, autumn and winter) in order to determine the physical and chemical parameters of the water.

Conductivity, total dissolved solids, pH and the temperature of the water were measured in the field using a HI 9811–5 pH/EC/TDS/°C meter (Hanna Instruments) and dissolved oxygen was measured using a CO–401 oxygen meter (Elmetron). Salinity was measured using a WTW meter and the result was expressed as the total dissolved solids that had been converted from the measurements of electrical conductivity. For the high anthropogenic impact sites (conductivity > 1000  $\mu\text{S}\cdot\text{cm}^{-1}$ ), a conversion factor of  $0.72 \times$  conductivity was applied according to PISCART et al. (2005), while for the low anthropogenic impact sites (conductivity < 1000  $\mu\text{S}\cdot\text{cm}^{-1}$ ), the conversion factor was  $0.77 \times$  conductivity. Analyses of the concentrations of chlorides, sulfates, iron, nutrients, calcium and magnesium as well as total hardness and alkalinity were performed in the laboratory according to the standard methods of HERMANOWICZ et al. (1999). The morphometry of the riverbed, i.e. the average width of the channel, the depth of the river and the flow velocity were also performed in the field.

For the taxonomic analyses, diatoms were taken from a 25 cm<sup>2</sup> surface of the bottom sediments or concrete slabs (at each of the study sites). Permanent slides for the light microscopy were prepared following the standard protocol (BATTARBEE 1986; BODÉN 1991). The samples were treated with 10% hydrochloric acid (HCl) to remove any calcium carbonate and after a thorough washing, they were boiled in 37% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to eliminate any organic

Table 1. General characteristics of the sites that were investigated.

Characteristic	The Bolina River, lower course	The Bolina River, upper course	The Centuria River	The Mitęga River	The Mleczna River
Geographical coordinates	N 50° 14' 43"; E 19° 06' 02"	N 50° 13' 48"; E 19° 05' 08"	N 50° 24' 52"; E 19° 29' 12"	N 50° 26' 02.9"; E 19° 17' 58.3"	N 50° 06' 58.4"; E 19° 04' 30.2"
Elevation of sampling Sites (m a.s.l.)	257	262	343	300	236
Environment	Build-up area / wasteland	Wasteland / woodland	Woodland	Wasteland	Farmlands
Location / region	Upper Silesia / Silesian Upland	Upper Silesia / Silesian Upland	Nature monuments „Źródła Centurii”/ Kraków–Częstochowa Upland	Silesian Upland	Upper Silesia / Silesian Upland
The part of the river studied	Downstream	Upstream	Upstream / Spring	Downstream	Downstream
Human pressure	Extreme	Very high	No impact	Low	High
Hydromorphological transformations and development	Concrete channelled, very heavy pressure of mine water supply	Channelled with concrete plate; heavy pressure of mine water supply	Unaltered site	Channelled, with fascine-reinforced banks and a dam reservoir	Channelled, with fascine-reinforced banks and indirect supply of mine waters
Type of river / geology	(5) Mid-altitude siliceous streams that have a fine-particulate substratum	(5) Mid-altitude siliceous streams that have a fine-particulate substratum	(5) Mid-altitude siliceous streams that have a fine-particulate substratum	(6) Mid-altitude calcareous streams that have a fine-particulate substratum on loess	(6) Mid-altitude calcareous streams that have a fine-particulate substratum on loess
Type of bottom sediments	Silt and sand	Coal mine sludge	Sand and silt	Sand and silt	Silt and sand

matter. After washing four times with distilled water, the final suspension was pipetted onto cover slips, left to evaporate and mounted on glass slides using Naphrax® diatom mountant. The slides were examined using Zeiss Axio Scope A1 and Nikon Eclipse E600 light microscopes. The measurements and photographic documentation were performed using AxioVision Rel. 4.8 software. In all of the samples that were studied, a minimum of 400 valves were identified to the species or variety level and their relative abundance was determined. A number of ecological metrics were used to analyse the diatoms – the percentages of particular taxa:

$$D_i = \frac{n_i}{N} \cdot 100 [\%],$$

where  $D_i$  – percentages of particular taxon 'i',  $n_i$  – the abundance of a particular taxon 'i',  $N$  – the sum of all of the taxa abundances in a sample (TÜMPLING & FRIEDRICH 1999), the frequency coefficient:

$$C_i = \frac{k_i}{k} \cdot 100 [\%],$$

where  $C_i$  – the frequency coefficient of particular taxon 'i' [%],  $k_i$  – the number of samples with taxon 'i',  $k$  – the number of all of the samples (species were grouped according to their frequency coefficient as euconstants = 75–100%, constants = 75–50%, accessory = 25–50%, incidental = 1–25%; TROJAN 1975), and biodiversity indices (i.e. species richness, Shannon index:

$$H' = \sum_{i=1}^S \left( \frac{n_i}{N} \log_2 \frac{N}{n_i} \right),$$

where  $S$  – the number of species (species richness),  $n$  – the abundance of a taxon,  $N$  – the sum of all of the taxa abundances and the Evenness index:

$$E = \frac{H'}{H'_{\max}},$$

where  $H'$  – the Shannon index,  $H'_{\max}$  – the maximum possible value of the index  $H'$  were calculated (SHANNON 1948). OMNIDIA v5 software was used to calculate the percentages of diatoms in specific ecological groups. For scanning electron microscope (SEM) observations, cleaned material was pipetted onto a 25 mm diameter polycarbonate membrane Whatman® Nuclepore filter with a 2 µm mesh, attached to aluminium stubs and sputtered with 20 nm of gold using a Quorum Q 150OT ES Turbo-Pumped Sputter Coater. The diatoms were observed using a Hitachi SU 8010 SEM at the Podkarpacie Innovative Research Center of the Environment (PIRCE) at the University of Rzeszów, Poland.

The diatoms that were identified served as the basis for assessing the ecological status of the rivers with the diatom index IO according to the methodology used by the national monitoring system (PICIŃSKA-FALTYNOWICZ et al. 2006; PICIŃSKA-FALTYNOWICZ & BŁACHUTA 2010; PN-EN 13946 2014; PN-EN 14407 2014; ZGRUNDO et al. 2018).

The identifications were based on the following

Table 2. The physical and chemical parameters of the water and the morphology of the riverbed.

Parameter	The Bolina River, lower course	The Bolina River, upper course	The Centuria River	The Miłża River	The Mleczna River
Width of the river bed (m)	4.41–4.53	7.24–7.78	4.47–5.95	2.87–3.39	6.26–9.36
Depth of the river bed (m)	0.21–0.25	0.25–0.34	0.10–0.23	0.40–0.59	0.81–1.09
Flow velocity (m.s <sup>-1</sup> )	0.40–0.51	0.06–0.42	0.07–0.17	0.01–0.29	0.16–0.21
Temperature (°C)	15.5–29.1	15.7–28.6	7.5–13.6	10.4–18.4	14.8–25.1
pH	7.5–7.9	7.5–7.8	7.5–7.8	7.3–8.1	7.4–8.0
Salinity (PSU)	16.34–33.55	6.57–12.25	0.19–0.21	0.29–0.35	2.97–5.16
Dissolved oxygen (mg.dm <sup>-3</sup> )	5.13–9.69	4.65–7.09	4.89–6.61	4.62–6.12	0.69–4.82
Conductivity (µS.cm <sup>-1</sup> )	22700–44600	9130–17020	250–270	380–460	4120–7160
Total dissolved solids (mg.dm <sup>-3</sup> )	11360–23300	4570–8510	110–140	180–220	2050–3570
Chlorides (mg.dm <sup>-3</sup> )	7528–17028	2823–5590	8–20	18–26	1340–1970
Total hardness (mg <sub>CaCO<sub>3</sub></sub> .dm <sup>-3</sup> )	2268–4858	1072–1920	160–225	175–330	405–560
Calcium (mg.dm <sup>-3</sup> )	548–1310	328–687	55–60	69–94	101–158
Magnesium (mg.dm <sup>-3</sup> )	225–670	124–270	4–22	1–30	38–60
Alkalinity (mg <sub>CaCO<sub>3</sub></sub> .dm <sup>-3</sup> )	230–320	275–380	75–110	165–250	240–275
Phosphates (mg.dm <sup>-3</sup> )	0.05–0.10	0.02–0.14	0.00–0.11	0.08–0.24	0.16–3.84
Ammonium (mg.dm <sup>-3</sup> )	1.25–12.12	0.62–1.00	0.00–0.23	0.26–0.36	0.23–1.21
Nitrates (mg.dm <sup>-3</sup> )	4.43–10.63	0.00–79.74	0.00–11.96	0.89–15.95	5.32–10.19
Nitrites (mg.dm <sup>-3</sup> )	2.49–9.96	0.68–1.44	0.00–0.01	0.11–0.24	0.20–0.73
Iron (mg.dm <sup>-3</sup> )	0.12–0.39	0.13–0.88	0.03–0.65	0.25–0.78	0.26–1.46
Sulfates (mg.dm <sup>-3</sup> )	320–550	352–770	35–44	49–66	200–272

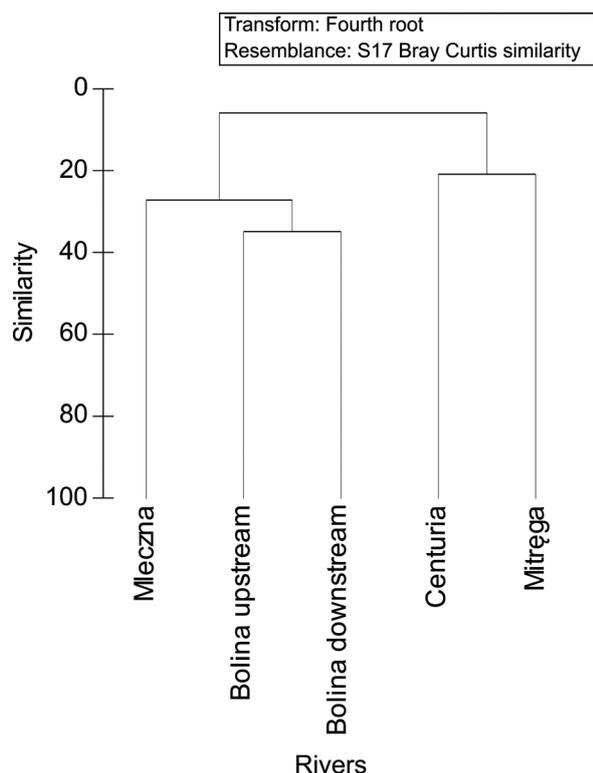


Fig. 2. A Bray–Curtis similarities dendrogram of the sampling sites with respect to the diatom flora and the abundance of taxa.

references: KRAMMER & LANGE–BERTALOT 1986, 1988, 1991a, 1991b; LANGE–BERTALOT & METZELTIN 1996; WITKOWSKI et al. 2000; KRAMMER 2000, 2002, 2003; LANGE–BERTALOT 2001; LEVKOV 2009; LEVKOV et al. 2013, 2016; LANGE–BERTALOT et al. 2011; HOFMANN et al. 2011; BAŁ et al. 2012; CANTONATI et al. 2017.

**Data analysis.** The significance of the differences in the median values of the environmental variables among the sampling sites in the rivers was calculated using the Kruskal–Wallis one–way ANOVA and the multiple comparison post hoc tests using Statistica version 13.1. The value of the environmental variables did not reveal a normal distribution according to the Lilliefors test of normality, which justified the use of a non–parametric test. Canonical ordination analyses for relating diatom species composition to environmental variables were carried out using CANOCO for Windows version 4.5 (TER BRAAK & ŠMILAUER 2002). The chosen system for species examination used detrended correspondence analysis (DCA) and the gradient length, which exceeded 4 standard deviations. Therefore, a unimodal direct ordination canonical correspondence analysis (CCA) with a forward selection was used to reduce the large set of environmental variables. The relationship between diatom species and environmental variables was evaluated using the Monte Carlo permutation test (499 permutations). The CCA was performed based on both the log–transformed species and environmental data.

The structures of the diatom flora and the relationships between their assemblages in the rivers that were studied were analysed using the statistical multivariate methods provided by the PRIMER software (Plymouth Routines in Multivariate Ecological Research; CLARKE & WARWICK 2001). Discrimination

between the groups of sites with similar diatom populations was aided by cluster analysis. The ranked similarity matrices were constructed using the Bray–Curtis similarity measure with a fourth root transform and group–average sorting, i.e. the average similarity indices for successively created group of sites (LANCE & WILLIAMS 1967).

## RESULTS

### Environmental conditions of the investigated rivers

The salinity, conductivity, total dissolved solids, total hardness, concentration of chlorides, calcium, magnesium, ammonium and nitrites in the water were extremely high at the sampling site with the highest degree of anthropogenic pressure, i.e. in the lower course of the Bolina River (Tables 1 and 2). However, the concentration of sulfates, nitrates and alkalinity were higher in the upper course of the river. Because of the discharge of the coal mine waters into the Bolina River, the salinity ranged from 16.34 to 33.55 PSU (Table 2). The salinity values of the lower course of the Bolina River exceeded the maximum salinity that was recorded for the Baltic Sea (SATBAŁTYK 2018). By contrast, the lowest values of most of the physical and chemical parameters of the water were recorded in the site that is not impacted by anthropogenic activity (the Centuria River) except for the dissolved oxygen or pH. Both rivers (Bolina and Centuria) represent the abiotic type 5 – mid–altitude siliceous streams that have a fine–particulate substratum. When the two other rivers of the same abiotic type (type 6 – mid–altitude calcareous streams that have a fine–particulate substratum on loess, PICIŃSKA–FAŁTYNOWICZ & BŁACHUTA 2010) but with different anthropogenic pressure were compared, lower values of the physical and chemical parameters were obtained in the Mitřęga River (low anthropogenic pressure) than in the Mleczna River (high anthropogenic pressure).

### Diatom assemblages in the rivers that were studied

The diatom taxa (species and varieties) that were identified in the microphytobenthos of the rivers that were studied were represented by 55 genera. The centrics were represented by six genera with eight species and varieties, while the 49 pennate genera comprised 150 species and varieties. Only two of the identified taxa were euconstants (taxa occurring in 75–100% of the samples that were analysed) and these were *Navicula gregaria* Donkin and *Planothidium frequentissimum* (Lange–Bertalot) Lange–Bertalot. The constants (occurrences in 50–75% of the samples) included seven taxa: *Achnanthydium minutissimum* (Kützing) Czarnecki, *Ctenophora pulchella* (Ralfs ex Kützing) D.M. Williams et Round, *Cyclotella meneghiniana* Kützing, *Gyrosigma attenuatum* (Kützing) Rabenhorst, *Hippodonta capitata* (Ehrenberg) Lange–Bertalot, Metzeltin et Witkowski, *Planothidium delicatulum* (Kützing) Round et Bukhtiyarova, *Pleurosigma salinarum* Grunow in Cleve et Grunow. The

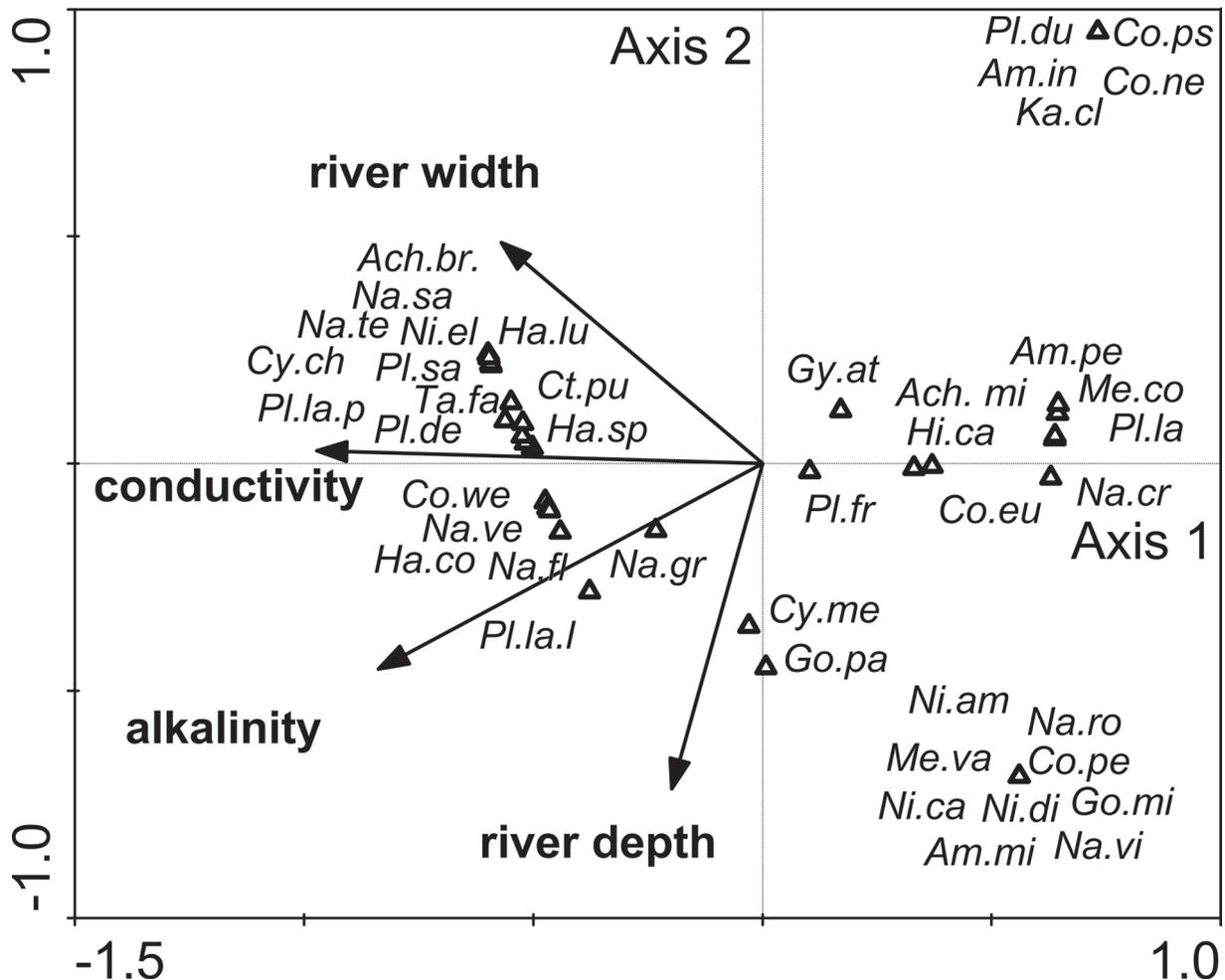


Fig. 3. Ordination diagram (biplot) based on the canonical correspondence analysis (CCA) of diatom data and environmental variables of the studied rivers. Abbreviations: (*Ach.br.*) *Achnanthes brevipes* var. *intermedia*, (*Ach.mi*) *Achnantheidium minutissimum*, (*Am.in*) *Amphora inariensis*, (*Am.mi*) *Amphora minutissima*, (*Am.pe*) *Amphora pediculus*, (*Co.eu*) *Cocconeis euglypta*, (*Co.ne*) *Cocconeis neothumensis*, (*Co.pe*) *Cocconeis pediculus*, (*Co.ps*) *Cocconeis pseudothumensis*, (*Co.we*) *Conticribra weissflogii*, (*Ct.pu*) *Ctenophora pulchella*, (*Cy.ch*) *Cyclotella choctawhatcheeana*, (*Cy.me*) *Cyclotella meneghiniana*, (*Go.mi*) *Gomphonema minutum*, (*Go.pa*) *Gomphonema parvulum*, (*Gy.at*) *Gyrosigma attenuatum*, (*Ha.co*) *Halamphora coffeiformis*, (*Ha.lu*) *Halamphora luciae*, (*Ha.sp*) *Haslea spicula*, (*Ka.cl*) *Karayevia clevei*, (*Me.co*) *Meridion constrictum*, (*Me.va*) *Melosira varians*, (*Na.cr*) *Navicula cryptotenella*, (*Na.fl*) *Navicula flandriae*, (*Na.gr*) *Navicula gregaria*, (*Na.ro*) *Navicula rostellata*, (*Na.sa*) *Navicula salinarum* var. *minima*, (*Na.te*) *Navicula tenelloides*, (*Na.ve*) *Navicula veneta*, (*Na.vi*) *Navicula viridula*, (*Ni.am*) *Nitzschia amphibia*, (*Ni.ca*) *Nitzschia capitellata*, (*Ni.di*) *Nitzschia dissipata*, (*Ni.el*) *Nitzschia elegantula*, (*Pl.de*) *Planothidium delicatulum*, (*Pl.du*) *Planothidium dubium*, (*Pl.fr*) *Planothidium frequentissimum*, (*Pl.la*) *Planothidium lanceolatum*, (*Pl.la.l*) *Pleurosira laevis* var. *laevis*, (*Pl.la.p*) *Pleurosira laevis* var. *polymorpha*, (*Pl.sa*) *Pleurosira salinarum*, (*Ta.fa*) *Tabularia fasciculata*.

accessory species (occurrences in 25–50% of the samples) comprised 29 taxa; the largest number of taxa (120) was classified as incidental. Their frequency coefficient was less than 25% of the samples that were analysed.

#### Similarities between the rivers with respect to the diatom flora

An analysis of the similarities between the sampling sites with respect to the diatom assemblages and the abundance of taxa was aided by using the Bray–Curtis similarity measure. The resulting dendrogram shows two groups of rivers with an approximately 30% similarity (Fig. 2).

Although the similarity of diatom assemblages was not very high, the rivers that were studied seem to cluster together predominantly due to anthropogenic pressure. The first group comprised sites with a strong

impact – the salinised rivers (Mleczna and Bolina). The diatom assemblages from these sites were predominantly composed of taxa that prefer brackish or brackish–marine water: *Pleurosira laevis* var. *laevis* (Ehrenberg) Compère and var. *polymorpha* Compère, *Ctenophora pulchella*, *Achnanthes brevipes* var. *intermedia* (Kützing) Cleve, *Halamphora coffeiformis* (C.Agardh) Levkov, *H. luciae* (Cholnoky) Levkov, *Navicula salinarum* f. *minima* Kolbe, *Gyrosigma attenuatum*, *Haslea spicula* (Hickie) Bukhtiyarova and *Pleurosira salinarum*. The second distinct group comprised the sites with a minimal or weak anthropogenic impact (Centuria and Mitreğa). The taxa that distinguished this group from the previous one primarily prefer fresh–brackish water. The other ecological preferences of the taxa do not significantly differentiate the rivers that were studied and were clustered in two

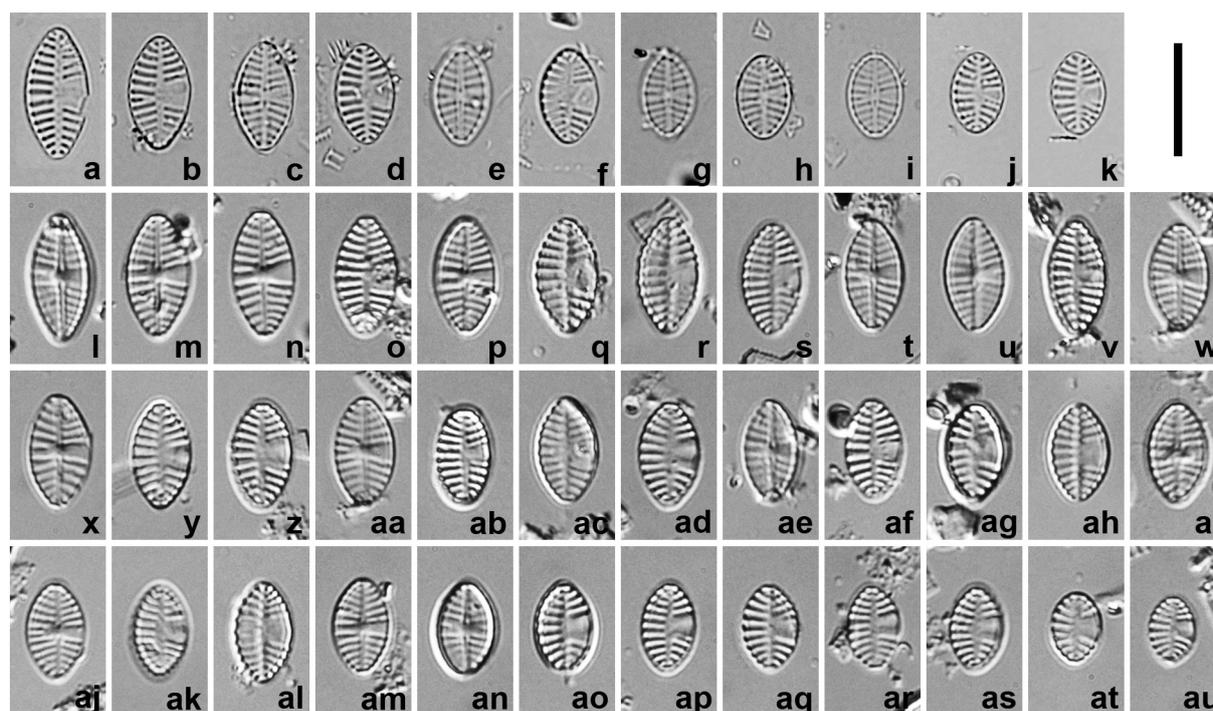


Fig. 4. *Planothidium nanum* sp. nov. LM images. Size diminution series. In the valve views, the variations in the size of the type population are shown. Fig. 4d and 4e are of the holotype. Scale bar 10  $\mu$ m.

groups, but some differences were still visible. In the Bolina and Mleczna Rivers, species that are characteristic of eutrophicated waters and for  $\alpha$ -mesosaprobic zone dominated. The species that were present in the Centuria River were typical of those that prefer oligotrophic and oligosaprobic waters as well as those that are highly tolerant to trophic conditions, the saprobic status and nutrient content (the dominants included *Planothidium dubium* (Grunow) Round et Bukhtiyarova, *Cocconeis pseudothumensis* Reichardt, *C. neothumensis* Krammer, *Karayevia clevei* (Grunow) Bukhtiyarova, *Amphora inariensis* Krammer and *Achnanthydium minutissimum* var. *minutissimum*). The taxa that were present in the Mitřęga River were typical cosmopolitan forms that are found in various types of water, but that quite frequently occur in waters with an elevated trophic and saprobic status (no distinct dominants).

#### Biodiversity of the diatom flora in the rivers that were studied

The biodiversity of the flora in the rivers with a minimal or weak anthropogenic impact (Centuria and Mitřęga) was high, whereas in those that were strongly affected – the salinised rivers (Mleczna and Bolina) – it was quite low. The Bolina and Mleczna sites, which were characterised by the highest salinity (7–12 PSU in the upstream and 16–34 PSU downstream of the Bolina River and the 3–5 PSU of the Mleczna River), had a relatively low taxonomic richness (23 and 32 taxa in the upstream and downstream courses of the Bolina River, respectively, and 23 taxa in the Mleczna River). The initial stretch of the Centuria River and the downstream section of the

Mitřęga River had a much higher taxonomic richness (55 and 74 taxa, respectively).

The Shannon ( $H'$  for the logs to the base 2) and the Evenness indices were almost two-fold higher in the rivers that were not salinised (Centuria 4.26 and 0.74, Mitřęga 5.36 and 0.87) than in the salinised rivers (2.23 and 0.49 in the upstream and 2.04 and 0.41 in the downstream of the Bolina River and 2.84 and 0.63 in the Mleczna River).

The diatoms that were identified served as the basis for assessing the ecological status of the rivers with the diatom index IO according to the methodology that is used by the state monitoring system. The status of the Bolina River, in both the downstream and upstream course was considered to be poor, whereas the state monitoring system classified the Bolina (both ecological and chemical) as bad. The diatom index that was applied to the remaining rivers confirmed the ecological status classification from the state monitoring – the Mleczna, Mitřęga, and Centuria Rivers were classified as representing a poor, moderate and good ecological status, respectively. The lists of the indicator species that were used by the state monitoring system until 2017 did not include the brackish species *Pleurosira laevis*, which was dominant in the Bolina and Mleczna Rivers. After adding *P. laevis* to the list of indicator species in 2018, the results of the assessment of the ecological status of the Bolina and Mleczna Rivers was improved by one level. According to the classification that was developed by DENYS (1991) and VAN DAM et al. (1994), this species is eutrophic and oligosaprobic. However, as was shown by results of our studies, the species is able to tolerate

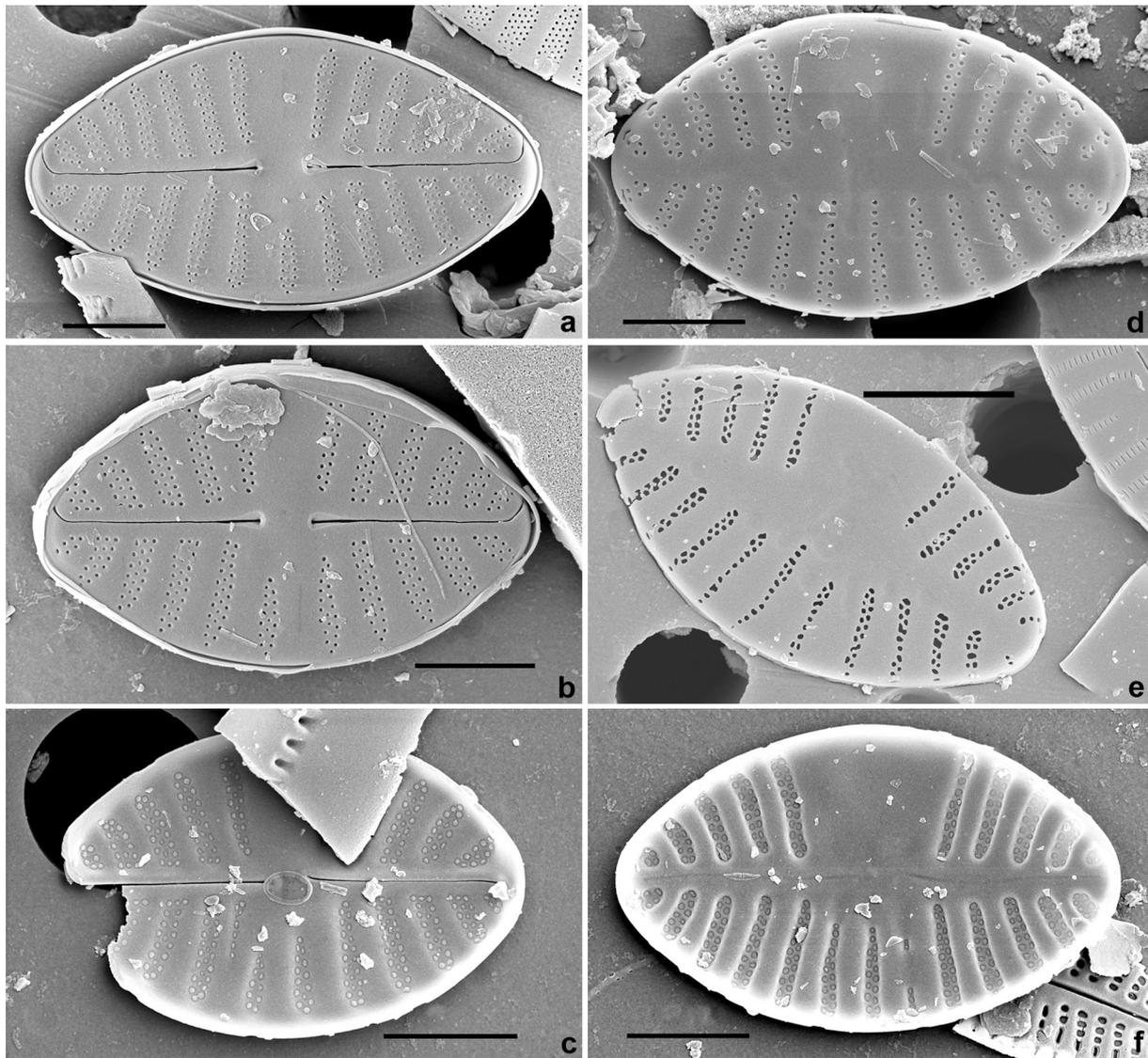


Fig. 5. *Planothidium nanum* sp. nov. SEM images. (a–c) raphe valves: (a–b) external view, note the somewhat expanded external proximal central raphe endings; (c) internal view, note the internal proximal raphe endings that are bent in the opposite directions. (d–f) sternum valves: (d–e) external view, (f) internal view, note the presence of a unilateral central area. Scale bars 2  $\mu$ m.

considerably elevated levels of nutrients and organic pollutants and is capable of forming very abundant populations in rivers with a periodically, and significantly, elevated salinity due to discharges of saline mine water.

During the species identification, one new species for Poland – *Navicula flandriae* Van de Vijver et Mertens in BEAUGER et al. (2015) and one species that is new to science were found – *Planothidium nanum* sp. nov., a description of which follows. In recent years, the genus *Planothidium* has been in the focus of both morphological and molecular studies. Currently, it comprises about 60 accepted species. Over the last five years (2014–2019), 32 new species were described, of which 31 are freshwater and only one is marine.

#### The results of the canonical correspondence analysis (CCA)

A canonical correspondence analysis (CCA) revealed

that the first and second axes explain 70.0% of species variance and 78.5% of the variance in the species and environment relationship. Conductivity, alkalinity, river width and depth were most associated (statistically significant according to the forward selection results) with the diatom taxa distribution (Fig. 3). The CCA analysis was significant (Monte Carlo test on the first canonical axis, F-ratio = 11.757, P-value = 0.002; test of significance of all of the canonical axes, F-ratio = 30.897, P-value = 0.002). The CCA analysis showed that *Achnanthes brevipes* var. *intermedia*, *Halamphora coffeiformis*, *Halamphora luciae*, *Haslea spicula*, *Navicula salinarum* var. *minima*, *Pleurosigma salinarum*, *Pleurosira laevis* var. *laevis*, and *P. laevis* var. *polymorpha* were associated with higher riverine conductivity and alkalinity (Fig. 3). By contrast, *Achnantheidium minutissimum*, *Amphora inariensis*, *Cocconeis euglypta* Ehrenberg, *Hippodonta*

*capitata*, *Meridion constrictum* Ralfs, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange–Bertalot, and *Navicula cryptotenella* Lange–Bertalot in Krammer & Lange–Bertalot were associated with lower conductivity and alkalinity. *Achnantheidium minutissimum*, *Cocconeis pediculus* Ehrenberg, *Gomphonema minutum* (C.Agardh) C.Agardh, *Melosira varians* C.Agardh, *Nitzschia amphibia* Grunow, *N. dissipata* (Kützing) Rabenhorst, and *Navicula viridula* (Kützing) Ehrenberg were associated with lower water conductivity and river width (Fig. 3).

### Description of the new species

#### *Planothidium nanum* sp. nov. Bąk, Kryk et Halabowski (Figs 4–5)

**Description:** Light microscopy, Figs 4a–au – Valves consistently elliptical with obtusely rounded ends that were never protracted. Length 5.5–11.5 µm, breadth 4.0–5.5 µm.

Rapheless valve: Raphe filiform, straight with slightly expanded central pores and distinctly curved distal endings. Axial area very narrow, straight. Central area is asymmetrical due to the two more widely spaced central striae on the primary side than on the secondary side of the valves, where one or two shortened striae are present. Striae slightly radiate throughout the valves, 12–14 in 10 µm.

Scanning electron microscopy, external view (Figs 5a–b): Raphe straight with slightly expanded central ends, terminal fissures unilaterally bent to the mantle. Striae moderately radiate becoming strongly radiate near the ends. Transapical striae composed of 2–3 series of round areolae. Areola density counted in a single series is ca. 55–65 in 10 µm.

SEM internal view (Fig. 5c): Central raphe ends not coaxial but deflected to the opposite sides. Distal ends with small helictoglossae. Central nodule together with the virgae features a high profile raised above the troughs containing the bi- or triseriate occluded areolae.

Rapheless valve: A depression is visible in LM as a broad space between two central striae on primary side. Cavum structure is absent. Axial area narrow, straight or slightly broader towards the central area.

SEM external view (Figs 5d–e): Striae consisting of 1–2 series of areolae; virgae considerably broader than striae. The surface on the valve is entirely smooth. SEM internal view, see Fig. 5f.

**Type:** Poland, Upper Silesia, Centuria River, 50°24'52"N, 19°29'12"E, 11 July 2017.

**Holotype** (assigned here): Slide no. 25760 in Coll. Andrzej Witkowski, Institute of Marine and Environmental Sciences, University of Szczecin (SZCZ), the holotype is represented by Figs 4d and 4e.

**Type locality:** Centuria River located in southern Poland that belongs to the Upper Vistula River system; epipsammic sample from bottom sediment.

**Etymology:** The Latin epithet means dwarf.

**Distribution:** Known only from the type locality.

**Ecology:** Freshwater, slightly alkaline with low conductivity, oligotrophic and oligosaprobic waters. The species co-occurring with species that are typical of oligotrophic and oligosaprobic waters as well as those that are highly tolerant of trophic conditions, saprobic status and nutrient content (in the order of domination): *Karayevia clevei*, *Cocconeis pseudothumensis*, *Planothidium dubium*, *Cocconeis neothumensis*, *Amphora inariensis*, *Achnantheidium minutissimum*, *Amphora pediculus* (Kützing) Grunow in Schmidt, *Ellerbeckia arenaria* (G.Moore ex Ralfs) R.M. Crawford, *Reimeria sinuata* (W.Gregory) Kociolek et Stoermer, *Staurosirella lapponica* (Grunow) D.M. Williams et Round, *Staurosirella pinnata* (Ehrenberg) D.M. Williams et Round, *Cocconeis pseudolineata* (Geitler) Lange–Bertalot in Werum et Lange–Bertalot, *Hippodonta capitata*, *Navicula cryptotenella*, *Platessa conspicua* (Ant.Mayer) Lange–Bertalot in Krammer et Lange–Bertalot, *Geissleria acceptata* (Hustedt) Lange–Bertalot et Metzeltin, *Gomphonema clavatum* Reichardt, *Gomphonema subclavatum* (Grunow) Grunow, *Meridion constrictum*, *Odontidium mesodon* (Kützing) Kützing, *Planothidium lanceolatum*, *Psammothidium bioretii* (H.Germain) Bukhtiyarova et Round, *Reimeria uniseriata* S.E.Sala, J.M.Guerrero et M.E.Ferrario, *Sellaphora bacillum* (Ehrenberg) D.G.Mann, *Ulnaria ulna* (Nitzsch) Compère and other species.

### Comparison the new species with morphologically related taxa

The small size of *Planothidium nanum* and its elliptical outline combined with the very widely spaced central striae on the sternum valve (sinus) and the widely spaced central striae on the raphe valve should clear up any confusion with other species. However, the taxa that were most similar to the new species are *P. frequentissimum*, *P. dubium*, *P. rostratoholarcticum* Lange–Bertalot et Bąk in Bąk et Lange–Bertalot, *P. minutissimum* (Krasske) E.A.Morales, *P. granum* (M.H.Hohn et Hellerman) Lange–Bertalot and *P. reichardtii* Lange–Bertalot et Werum. The new species differs from *P. frequentissimum* and *P. rostratoholarcticum* by the absence of a cavum (*P. nanum* has a sinus). It differs from *P. reichardtii*, *P. granum* and *P. dubium* by the shape of its ends—subrostrate vs rounded in *P. nanum*. In addition, *P. dubium* has larger valves (length 10–20 µm, width 5.0–7.5 µm) and *P. granum* is generally narrower, 3.6–4.2 µm (vs length 5.5–11.5 and width 4.0–5.5 µm in *P. nanum*). New species differs from *P. minutissimum* in its valve outline – rhombic– to elliptic–lanceolate in *P. minutissimum* and elliptical in *P. nanum* and by being smaller in size than *P. minutissimum*, i.e. length 6.0–8.5 µm and width 3.0–3.5 µm, and also by the striae density – 16–18/10 µm (vs 12–14/10 µm in *P. nanum*).

### *Navicula flandriae* Van de Vijver et A.Mertens – first record for Poland (Figs 6a–p)

LM and SEM examinations of the samples that had been

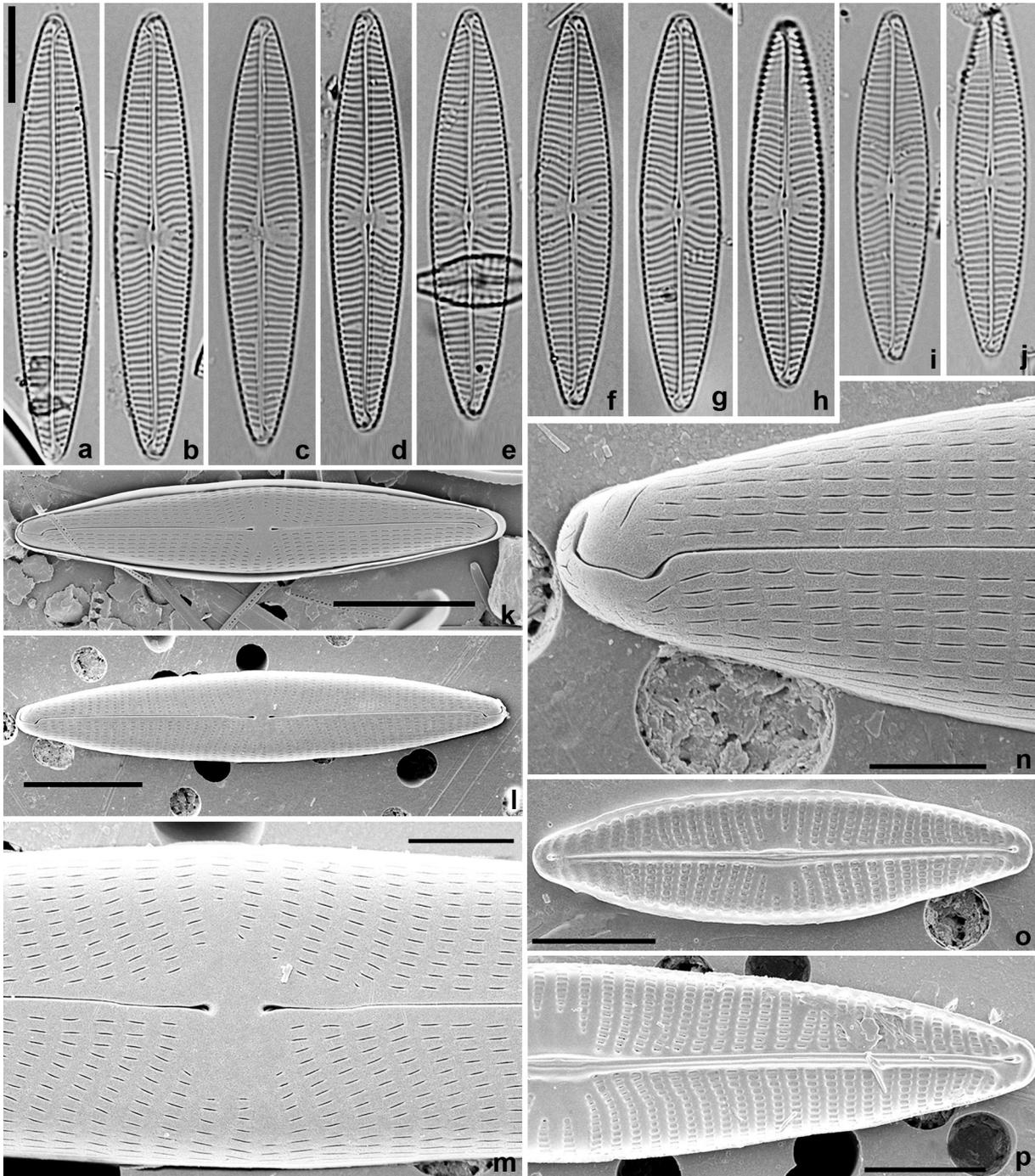


Fig. 6. *Navicula flandriae* Van de Vijver & A.Mertens. (a–j) Size diminution series in the LM images. Scale bar 10  $\mu\text{m}$ . (k–p) SEM images: (k–l) external view of an entire valve; (m) close-up of the central area; (n) detail of a valve apex showing the curved terminal raphe endings; (o) internal view of an entire valve; (p) close-up of the central area and valve apex. Scale bars (k, l) 10  $\mu\text{m}$ , (m, n) 2  $\mu\text{m}$ , (o, p) 5  $\mu\text{m}$ .

collected from the Mlecza and Bolina Rivers revealed the occurrence of *N. flandriae*. The species was described in 2015 from Flanders (Flandria), the northern region in Belgium where this species was observed in several rivers and canals (i.e. Leopoldkanaal, Oostpolderkreek) in alkaline waters with a high conductivity. *Navicula flandriae* was very abundant in the Mlecza River (38% relative abundance) and a few specimens occurred in the downstream of the Bolina River (1.2%). The valves were 35–46  $\mu\text{m}$  long, 7–8  $\mu\text{m}$  wide with 13–14 striae

per 10  $\mu\text{m}$  (Fig. 6a–j). According to VAN DE VIJVER and MERTENS in BEAUGER et al. (2015), the valves are 35–65  $\mu\text{m}$  long, 7.0–9.5  $\mu\text{m}$  wide with 12–13 striae per 10  $\mu\text{m}$ . The specimens that were found in Poland were characterised by a higher stria density in both the smaller and larger specimens. The LM and SEM observations (Fig. 6k–p) were consistent with those given by BEAUGER et al. (2015). *Navicula flandriae* was accompanied by *Pleurosira laevis* var. *laevis*, *Gomphonema parvulum* (Kützing) Kützing, *Cyclotella meneghiniana*,

*Halamphora coffeiformis*, *Cocconeis placentula* var. *placentula* Ehrenberg, *Navicula perminuta* Grunow in Van Heurck, *Planothidium delicatum*, *Conticribra weissflogii* (Grunow) Stachura–Suchoples et D.M. Williams, *Navicula gregaria*, *Navicula veneta* Kützing, *Ctenophora pulchella*, *Haslea spicula*, *Nitzschia palea* var. *palea* (Kützing) W. Smith – as the most abundant taxa in the Mleczna River.

*Navicula flandriae* was recently observed during routine monitoring in the province of Zuid–Holland (in: Hoofdwatergang Boutweg and Gemaal de Biersum Schuddebeursedijk) and the province of Zeeland (in: Boezem K van Steelandpolder and Goese Polder; ADRIENNE MERTENS, personal communication) in the Netherlands. In 2015 and 2016, *N. flandriae* was recorded in Egyptian inland waters (the Damietta Branch of the Nile River), which area N and P enriched and eutrophic (CANTONATI et al. 2016). These data indicate that it may be a widely distributed species that occurs in nutrient–rich waters that are usually accompanied by a high–conductivity. *Navicula flandriae* has similarities to several quite commonly occurring taxa such as *N. tripunctata* (O.F. Müller) Bory in Bory de Saint–Vincent, *N. recens* (Lange–Bertalot) Lange–Bertalot in Krammer et Lange–Bertalot, *N. margalithii* Lange–Bertalot in Krammer et Lange–Bertalot, *N. korzeniewskii* Witkowski, Lange–Bertalot et Metzeltin or *N. radiosa* Kützing. It is highly probable that it was wrongly identified in Polish waters and confused with the above–mentioned species. Detailed descriptions of its morphological differences are provided by BEAUGER et al. 2015.

## DISCUSSION

### Decrease in diatom biodiversity and taxonomic richness

The diatom flora at all the five sampling sites closely reflected the extent of the anthropogenic alteration of the environment and habitat. Our results show a decrease in both the biodiversity and taxonomic richness of the diatom assemblages that occurred in the rivers that are affected by mining activity, which was more than two–fold lower than in the rivers with a weak activity. Although it is well known that salinity can have a strong influence on the distribution of diatom species (CHOLNOKY 1968), the actual importance of this factor has not yet been precisely circumscribed at regional scales with a sufficient number of samples (POTAPOVA & CHARLES 2003). To date, there have been no studies that compare diatom assemblages and their biodiversity in salt–polluted rivers with the more natural rivers in the region of Upper Silesia. However, some research was recently conducted in adjacent regions. In the industrial water biotopes of three small water reservoirs that have a high salinity in southern Poland that are connected to the Bobrza River, Kielce Upland, that had conductivity that ranged from 711 to 865  $\mu\text{S}\cdot\text{cm}^{-1}$  (seven samples),

only 36 diatom taxa from 24 genera were identified, of which only 16 taxa were recognised as being species that are common in central Europe inland waters and the assemblages were characteristic for alkaliphilous, mesotraphentic and eutraphentic and  $\beta$ –mesosaprobous waters (MALINOWSKA–GNIĘWOSZ et al. 2018). In our study, the salt–polluted rivers were characterised by a similarly low number of identified diatom taxa (23 to 32 taxa in the upstream and downstream sites of the Bolina River, respectively), and 23 taxa in the Mleczna River and the diatom flora was characteristic for eutrophicated and  $\alpha$ –mesosaprobic waters, whereas the Centuria and Mitręga Rivers were characterised by a higher taxonomic richness (55 and 74 taxa, respectively). It is worth mentioning that our research showed a similar decreasing trend in the biodiversity of the salt–polluted rivers compared to the rivers with weak pollution, i.e. the Shannon index decreased from 4.26 and 5.36 (Centuria River and Mitręga River) to only 2.23 and 2.84 (Bolina River and Mleczna River). Based on this, elevated salinity in rivers can cause a decrease in the aquatic biodiversity of diatom assemblages. Like Poland, the Czech part of Upper Silesia has a long mining history. ČECHÁKOVÁ et al. (2014) analysed selected groups of plants and animals such as amphibians, aquatic molluscs, other macroinvertebrates, diatoms and aquatic plants in polluted (including post–industrial salinisation) subsidence reservoirs. The conductivity of the reservoirs that were studied at specific sites was significantly higher than in our studies (exceeding 1500  $\mu\text{S}\cdot\text{cm}^{-1}$ ) and the conclusion was that increased levels of salts in the affected areas result in a decrease in aquatic biodiversity and have a negative effect on different groups of plants and animals (ČECHÁKOVÁ et al. 2014). More studies that compared changes in diatom assemblages in terms of land use were carried out in South Africa (PAN et al. 2004; TAYLOR et al. 2005, 2007a, 2007b). The most comprehensive results, which were published by WALSH & WEPENER (2009), showed that 72% of the diatom taxa that were identified in streams with a significant agricultural impact were salt tolerant. The study was conducted in the Crocodile and Magalies Rivers in South Africa, where in some of the samples (conductivity over 600  $\mu\text{S}\cdot\text{cm}^{-1}$ ), the diatom index scores had a low to moderate integrity. Among the impacted sites, agricultural sites had a somewhat worse ecological status than urban sites according to the diatom indices. The reference sites were dominated by diatom species that preferred clean, freshwater and the indices that were calculated indicated a good ecological status, while the sites that had been impacted by an agricultural or urban influence had a poor to moderate status (WALSH & WEPENER 2009). LAVOIE et al. (2018) analysed the diatom assemblages from several streams and creeks of the Greater Sudbury River (Ontario, Canada). The results of these studies are another piece of evidence for the decrease in diatom biodiversity in water bodies that have been affected by an anthropogenic impact. The calculated Eastern Canadian Diatom Index (IDECE) had a

better biological integrity at the reference sites (LAVOIE et al. 2018) that had had weak anthropogenic influences that was similar to our results. HUSTEDT (1957) recorded that it is not the concentration of a particular salt in the water that influences freshwater diatoms the most but rather the osmotic pressure. Later experiments confirmed the fact that osmotic pressure is an important factor that limits the occurrence of sensitive freshwater diatom species (CLEAVE et al. 1981) by limiting their ability to absorb nutrients (TUCHMAN et al. 1984).

### Brackish and marine species in the analysed rivers

Based on the cluster and the canonical correspondence analysis (CCA) analyses, we were able to indicate the differences between the diatom assemblages in the rivers with a strong and weak anthropogenic impact. The first populations were rich in taxa that preferred brackish or brackish–marine water and the second populations were dominated by taxa that preferred more fresh–brackish water. In the rivers with a strong anthropogenic salinisation, *Pleurosira laevis* was the dominant species. It is typically a halophilic, sometimes rheophilic species, known for its ability to live in freshwater environments (COMPÈRE 1982). In the beginning, *P. laevis* was recorded in freshwater, oligohaline environments of North America, e.g. the Colorado River in the Grand Canyon region (CRAYTON & SOMMERFELD 1979), the Great Lakes (WUJEK & WELLING 1981) or the Maumee River in Ohio (KOCIOLEK et al. 1983). Later, the species was reported in Europe (SIMS 1996; GÓMEZ 2008; PEREZ et al. 2009), Asia (JOH 2010; KARTHICK & KOCIOLEK 2011; SHARIFINIA et al. 2016) and South America (METZELTIN & GARCÍA-RODRÍGUEZ 2003). Recently, *P. laevis* was found in the Zarafshan River, which is one of the largest rivers in Uzbekistan (MAMANAZAROVA & GOLOBOVA 2017). *Pleurosira laevis* is mainly associated with brackish or marine environments where its populations grow along with an increase in the chloride levels of the water (WUJEK & WELLING 1981; KOCIOLEK et al. 1983). In SHARIFINIA et al. (2016), this species occurred in sites that were characterised by conductivity that ranged from slightly over 600 to 1000  $\mu\text{S cm}^{-1}$ . In our study, *P. laevis* comprised 73% of all of the identified diatom taxa in the Bolina River and 21% in the Mleczna River, although the values of their salinity (periodically exceeded 40 000 and 7000  $\mu\text{S cm}^{-1}$ , respectively) and noticeable concentration of sulfates (550 and almost 300  $\text{mg dm}^{-3}$ , respectively). During a microscopic observation, we did not observe any significant teratologies of the *P. laevis* frustule, which usually suggests a response to ecological stress or metal pollution (LAVOIE et al. 2018). SCHRÖDER et al. (2015) analysed the benthic diatom assemblages of a Western Germany lowland river – the Lippe River. Its lower and middle courses had increased levels of salinity that were caused by coal mining activities (PETRUCK & STÖFFLER 2011). The authors found that 31% of all of the diatom species were reliable salinity indicators and that five species were characterised by a positive relationship to

increasing salinities: *Amphora libyca* Ehrenberg, *Bacillaria paxillifera* (O.F.Müller) T.Marsson, *Navicula subhamulata* Grunow in Van Heurck, *Nitzschia inconspicua* Grunow, *Rhoicosphenia abbreviata* (C.Agardh) Lange–Bertalot, of which *Bacillaria paxillifera* and *Rhoicosphenia abbreviata* also occurred in our studies. In the upper course of the Bolina River, *Ctenophora pulchella* constituted 63% of all of the identified taxa. This species primarily occurs in brackish waters in coastal regions but can also be found in freshwater bodies that have an increased conductivity, for example, in a polluted urban stream – Collins Channel, California, where the conductivity was almost 3000  $\mu\text{S cm}^{-1}$  (JONES 2013). Except for *P. laevis* and *C. pulchella* in the aforementioned rivers, we identified more diatoms that prefer brackish and marine environment such as *Achnanthes brevipes* var. *intermedia* (3% of all of the taxa in the Bolina River), *Halamphora coffeiformis* (2% of the taxa in the Mleczna River), *Halamphora luciae* (2% of the taxa in the Bolina River), *Navicula salinarum* (1% of the taxa in the Bolina River), *Gyrosigma attenuatum* (4% of the taxa in the Bolina River) and *Pleurosigma salinarum* (1% of the taxa in the Bolina River). In addition, *Tabularia fasciculata* (C.Agardh) D.M.Williams et Round comprised 6% of all of the identified species in the entire Bolina River diatom population. It is a cosmopolitan species that has a worldwide distribution and a tolerance for wide ranges of salinity (SNOEIJIS 1992). It has been found in marine, brackish as well as freshwater habitats (DAVIDOVICH et al. 2010). The relationship between benthic diatom assemblages and conductivity has been well studied in the USA where POTAPOVA & CHARLES (2003) have analysed 3239 diatom samples from 1109 river sites located in different parts of North America, which have conductivity that ranges from 10 to almost 15 000  $\mu\text{S m}^{-1}$ . The authors considered a value of 1000  $\mu\text{S cm}^{-1}$  as the threshold between brackish and freshwater habitats. However, the results may have been underestimated in terms of the lack of a brackish water dataset, which caused species that prefer brackish waters to have their optima set below the aforementioned threshold (e.g. *Ctenophora pulchella*). Compared to our study, *Pleurosira laevis*, which dominated the downstream sites of the Bolina and Mleczna Rivers occurred in a conductivity of 44 600  $\mu\text{S cm}^{-1}$  and 7090  $\mu\text{S cm}^{-1}$ , respectively, during the inflow of polluted post–mining waters, while POTAPOVA & CHARLES (2003) reported an optimum of 573  $\mu\text{S cm}^{-1}$  with a maximum conductivity value of 1122  $\mu\text{S cm}^{-1}$  for the same species. Our results show that *P. laevis* is able to withstand periodic higher conductivity levels and grow (it was the dominant species in the Bolina River). A similar conclusion was drawn for other species, e.g. *Ctenophora pulchella* or *Tabularia fasciculata*. Determining the salinity preferences of individual species seems to be a very difficult task taking into account the multitude of water factors that affect individual diatom populations besides salinity. The occurrence of halophilous species is not always associated with an

increased salinity. At the beginning of the 20<sup>th</sup> century, *Actinocyclus normanii* (W.Gregory ex Greville) Hustedt, which was considered to be a marine species, appeared in rivers and at the end of that century, it occurred in many European inland water bodies (KISS et al. 1990; GEISSLER et al. 2006; KAŠTOVSKÝ et al. 2010). Later, a similar spread of *Didymosphenia geminata* (Lyngbye) Mart.Schmidt in A.Schmidt as well as *Pleurosira laevis* and *Bacillaria paxillifera* (FRÁNKOVÁ–KOZÁKOVÁ et al. 2007; HINDAKOVÁ et al. 2010) was observed in the Czech Republic (GÁGYOROVÁ & MARVAN 2002). In terms of the periodic inflow of salinised mine waters into the Bolina and Mleczna Rivers, it is difficult to establish whether we observed a case of a marine species invasion or only examples of the short appearance of these organisms into unfavourable environmental conditions. There is a great need to carry out detailed studies on the diatom assemblages of saline rivers, especially on the ecological preferences of the dominant species in them.

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#### Supplementary material

The following supplementary material is available for this article:

Supplementary plates.

This material is available as part of the online article (<http://fottea.czechphycology.cz/contents>)

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