

The Aerophytic Diatom Assemblages Developed on Mosses Covering the Bark of *Populus alba* L.

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ABSTRACT

The study was conducted in an old, historical park, in the northern part of Stalowa Wola city (south-eastern Poland). The aim of the study was to investigate the diversity of moss-inhabiting diatoms of the white poplar (*Populus alba* L.) bark. During the study, a total of 47 diatom taxa were found, three out of which were considered as dominant. Three other species are mentioned in the Red List of the Algae in Poland: *Achnanthes coarctata* (Brébisson) Grunow, *Luticola acidoclinata* Lange-Bertalot and *Stauroneis thermicola* (Petersen) Lund. For three species: *Luticola sparsipunctata* Levkov, Metzeltin & Pavlov, *L. vanheurckii* Van de Vijver & Levkov and *Hantzschia subrupestris* Lange-Bertalot, this is the first report from Poland.

Keywords: diatoms, tree bark, mosses, diversity, ecology

INTRODUCTION

Many algae species can survive and develop outside of the aquatic habitats – on a trees bark, on leaves, on wet walls and rocks, or on wet to semi-wet soils. They occur in continuously or only periodically wet habitats, using water in the form of atmospheric precipitation, dew, fog and moisture contained in the air. The aerophytic algae develop most abundantly in a warm and humid climate (Podbielkowski 1996).

The arboreal algae find a specific and favourable microclimate on the bark of trees that provide shade, nutrients and shelter against wind. In Europe, the trees bark is usually covered with green algae (mostly *Pleurococcus vulgaris* Meneghini, *Protococcus viridis* C. Agardh and *Chlorella vulgaris* Beyerinck), rarely with blue-green algae and diatoms (the latter presumably due to the absence of silica in the environment). The arboreal algae have wide ecological amplitude. They are known for their nitrogenous compounds toler-

ance, so they can live even on the trees growing in cities (Kawecka, Eloranta 1994).

The mosses growing on the tree bark use it only as a substrate, while all nutrients are taken by mosses from an atmospheric precipitation or from the air humidity. The diversity of epiphytic bryophytes is affected by both the chemical (pH) and physical (including structure of surface and water absorption capacity) properties of the bark. In numerous ecosystems, bryophytes form an expansive cover that accumulates water and organic particles, providing a favourable environment for many organisms, from bacteria, algae and fungi to small animals (Lindo, Gonzales 2010, Plášek 2013, Glime 2017).

The studies on the moss-inhabiting diatoms (bryophytic diatoms) are most often related to peatland areas, mainly including the mosses from the genus *Sphagnum* spp. (Nováková, Pouličková 2004, Pouličková et al. 2004). The diatoms developing among the typical terrestrial bryophytes are very rarely studied, mainly on the mosses growing

on rocks (Round 1957) and on terrestrial mosses occurring in the polar regions (e.g. Hickman, Vitt 1973, Van de Vijver, Beyens 1997, Gremmen et al. 2007). Until now, the algological studies of tree barks have focused mainly on the green and blue-green algae in tropical zones (Foerester 1971, Mrozińska 1990, Thompson, Wujek 1997, Salleh, Milow 1999, Neustupa 2003, 2005, Neustupa, Škaloud 2008, 2010, Kharkongor, Ramanujam 2014, Štifterová, Neustupa 2015), while diatoms were noted rarely, usually mentioned only in the lists of species (Lakatos et al. 2004, Geissler et al. 2006, Neustupa, Škaloud 2010, Kharkongor, Ramanujam 2014, Štifterová, Neustupa 2015, Qin et al. 2016). In Poland, no studies on the diatom assemblages developing among mosses on the tree barks have been conducted so far.

The aim of the study was to investigate the diversity of diatom assemblages among the mosses growing on the bark of white poplar (*Populus alba* L.) in relation to chemical parameters (pH, conductivity, anions and cations) measured in the filtrates obtained from the tree bark.

METHODS

The conducted research concerns the mosses growing on white poplar tree trunk (*Populus alba*) in an old park, in the northern part of the Stalowa Wola city. The tree from which the research material was taken (50°36'01.1"N 22°01'53.3"E) grew in an exposed place (about 30 m from the nearest tree), which resulted in the trunk being directly illuminated. Mosses covered the base of the trunk in almost 100%, while on the trunk up to the height of 150 cm above the ground level, they formed small clumps (less than 5% of the bark surface).

The samples were collected in March 2016 and in March, June and August 2017. Small pieces of bark (approx. 8×8 cm) covered by mosses were collected from two heights: 20 cm and 150 cm above the ground level. The collected material was placed in paper envelopes. In August 2017, the material was collected only at the base of the trunk, because at the height of 150 cm, mosses were absent. During the each sampling, a piece of bark from height of 150 cm was collected for chemical analyses.

The filtrates for chemical analyses were prepared according to Schmidt et al. (2001). The filtrates were obtained by soaking pieces of bark in

deionized water (in weight ratio 1:10) for 24 hours. For the preparation of filtrates, the intact pieces of bark were used to obtain a solution similar to that forming on the surface of the bark that is a source of water and nutrients for epiphytic organisms.

The pH and electrolytic conductivity were measured using a MARTINI pH56 pH meter and MARTINI EC59 conductivity meter. The ions content was determined using a Thermo scientific DIONEX ICS-5000+DC device in the Departmental Laboratory of Analysis of Environmental Health and Materials of Agricultural Origin at the University of Rzeszów.

In order to obtain cleaned diatom material, a modified Qin et al. (2016) method was used:

1. For the purpose of separating mosses and diatoms from the bark surface, a part of the collected material was placed in beakers and 50 ml of 30% hydrogen peroxide (H₂O₂) was added, and left at room temperature for 48 hours.
2. In the next step, the bark fragments were rinsed with deionized water, and the formed solution was collected into the same beaker, in which the bark was digested.
3. In order to obtain clean diatom valves, the solution was centrifuged to remove the excess of hydrogen peroxide and again digested in the mixture of sulfuric acid and potassium dichromate, until organic matter was completely dissolved.
4. In the last step the chromic mixture was removed by centrifugation with distilled water (at 2 500 rpm).

For LM (light microscope) slides, pure diatom valves were mounted in Pleurax resin (refractive index 1.75). Diatoms were identified under a Carl Zeiss Axio Imager.A2 light microscope (LM) with a Zeiss AxioCam ICc 5 camera at 1000 × magnification. For Scanning Electron Microscope (SEM), the observations samples were coated at Turbo-Pumped Sputter Coater Quorum Q 1500T ES with a 20 nm layer of gold and observed under a Hitachi SU 8010 microscope.

The diatoms identification was conducted in accordance with the following references: Krammer and Lange-Bertalot (1986, 1988, 1991a,b), Krammer (2000), Lange-Bertalot et al. (2003), Hofmann et al. (2011) and Levkov et al. (2013). The nomenclature of mosses is according to Ochyra et al. (2003). The habitat and substrate preferences of mosses were determined according to Dierßen (2001), Smith (2004) and Ellenberg, Leuschner (2010).

The species composition of the samples was determined by counting specimens on randomly selected fields of view under light microscope according to a modified method described by Lakatos et al. (2004). There were about 300 valves. In the samples with low number of valves on slide, all specimens were counted. The species with a share more than 50 valves were defined as dominant.

The categories of endangered for diatom taxa were distinguished using the Red List of the Algae in Poland (Siemińska et al. 2006).

The statistical analyses with graphical interpretation were performed using the Canoco software (version 5.03). In order to analyse the differentiation of individual samples collected at different heights above ground level and in different seasons, the Principal Component Analysis (PCA) was used (Ter Braak, Šmilauer 2012).

RESULTS

The chemical analysis of filtrates obtained from soaking the bark showed that the solutions had a slightly acidic to neutral pH (5.3–6.9), while the conductivity values ranged from 43 to 427 $\mu\text{S cm}^{-1}$. The values of ions were significantly higher in the early spring periods compared to the summer season, when the concentration of nutrients decreased even below the limit of quantification (Table 1).

The examined tree was abundantly covered with mosses at the trunk base, while at a height of 150 cm, the mosses were less numerous. The base of the trunk was overgrown by typical terrestrial species: *Amblystegium serpens* Schimp, *Brachythecium salebrosum* (Web. Mohr) Schimp., *Herzogiella seligeri* (Brid.) Z. Iwats., *Rosulabryum capillare* (Hedw.) and epiphytic: *Hypnum cupres-*

siforme Hedw. and *Platygyrium repens* (Brid.) Schimp., J.R. Spence. The mosses formed a uniform, thick layer of about 5 cm, completely surrounding the base of the trunk. At the height of 150 cm, there were only single, small clumps of two species *Platygyrium repens* (Brid.) Schimp. and *Orthotrichum speciosum* Nees.

During the research carried out in 2016–2017, 47 diatom taxa from 23 genera were observed (Table 2). The most numerous genera were *Luticola* (9 taxa), followed by *Hantzschia* (4 taxa). The selected diatom taxa are presented in Figures 1 and 2.

Diatoms were numerous only at the base of the trunk. At the height of 150 cm above the ground level, only single valves were observed (Table 3). The PCA analysis also split the samples collected at different heights above the ground into two groups. The samples taken from a height of 20 cm were more varied, compared to those collected from a height of 150 cm, which formed a more compact group (Fig. 3).

Among all the observed diatoms, the dominant ones were: *Hantzschia amphioxys*, *Luticola acidoclinata* and *Pinnularia borealis*. In early spring (both in 2016 and 2017), only one species prevailed in the diatom assemblage – *Luticola acidoclinata* in 2016 and *Hantzschia amphioxys* in 2017. In the summer (both in June and August), all three species dominated the diatom flora (Table 3).

In the studied assemblages, three diatom species from Red List of the Algae in Poland were recorded (Siemińska et al. 2006). All of them are classified in the R category (rare): *Luticola acidoclinata* (which is also the dominant species), *Achnanthes coarctata* and *Stauroneis thermicola* (both observed as single specimens). In addition, three species have not been reported

Table 1. Values of chemical parameters measured in filtrates obtained from poplar bark in years 2016–2017

Date	03.2016	03.2017	06.2017	08.2017
pH	6.8	6.9	5.8	5.3
Conductivity [$\mu\text{S cm}^{-1}$]	397	427	145	43
Cl ⁻ [mg l ⁻¹]	12.55	14.43	1.19	0.33
NO ₂ ⁻ [mg l ⁻¹]	1.14	2.91	<0.001	<0.001
NO ₃ ⁻ [mg l ⁻¹]	3.63	1.37	<0.001	<0.001
PO ₄ ³⁻ [mg l ⁻¹]	3.93	3.29	<0.001	<0.001
SO ₄ ²⁻ [mg l ⁻¹]	23.45	42.06	13.35	0.18
Na ⁺ [mg l ⁻¹]	8.80	8.44	0.40	0.21
NH ₄ ⁺ [mg l ⁻¹]	0.43	2.23	1.10	0.08
K ⁺ [mg l ⁻¹]	25.03	61.69	35.22	10.88
Mg ²⁺ [mg l ⁻¹]	23.24	20.34	1.73	0.64
Ca ²⁺ [mg l ⁻¹]	23.14	20.04	4.94	1.91

Table 2. List of diatom taxa found in epiphytic mosses overgrowing the bark of white poplar, at different highs above soil level in years: 2016–2017

Date	03.2016	03.2017	06.2017	08.2017
<i>Achnanthes coarctata</i> (Brébisson) Grunow			+ o	
<i>Aulacoseira distans</i> (Ehrenberg) Simonsen	+			
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	+	o	+	
<i>Aulacoseira muzzanensis</i> (Meister) Krammer			o	+
<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg) Van Heurck	+			
<i>Cocconeis pseudolineata</i> (Geitler) Lange-Bertalot	o			
<i>Cyclostephanos dubius</i> (Hustedt) Round		+	+	+
<i>Epithemia adnata</i> (Kützing) Brébisson	+			
<i>Eunotia bilunaris</i> (Ehrenberg) Schaarschmidt	+	+		
<i>Fallacia insociabilis</i> (Krasske) D.G. Mann		+		
<i>Frustulia saxonica</i> Rabenhorst	+			
<i>Halamphora montana</i> (Krasske) Levkov	+ o			+
<i>Hantzschia abundans</i> Lange-Bertalot	+	+ o	+	+
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	+ o	+ o	+ o	+
<i>Hantzschia calcifuga</i> Reichardt & Lange-Bertalot	+	+	+	+
<i>Hantzschia subrupestris</i> Lange-Bertalot	+ o			
<i>Humidophila contenta</i> (Grunow) Lowe, et al.	+	+	+ o	+
<i>Humidophila gallica</i> (W. Smith) Lowe, et al.	+			
<i>Luticola acidoclinata</i> Lange-Bertalot	+	+	+ o	+
<i>Luticola sparsipunctata</i> Levkov, Metzeltin & A. Pavlov	+	+	+ o	+
<i>Luticola vanheurckii</i> Van de Vijver & Levkov		o		
<i>Luticola ventricosa</i> (Kützing) D.G. Mann, Morphotyp I		+		
<i>Luticola ventricosa</i> (Kützing) D.G. Mann, Morphotyp II	+	+ o		+
<i>Luticola</i> cf. <i>nivalis</i> (Ehrenberg) D.G. Mann	+	+ o		
<i>Luticola</i> sp. 1	+	+		+
<i>Luticola</i> sp. 2	+ o	+ o	+	+
<i>Luticola</i> sp. 3	+	+	+	+
<i>Mayamaea atomus</i> (Kützing) Lange-Bertalot	+		+	+
<i>Mayamaea excelsa</i> (Krasske) Lange-Bertalot			+	
<i>Mayamaea permitis</i> (Hustedt) K. Bruder & Medlin			+	
<i>Meridion circulare</i> (Greville) C. Agardh		o		
<i>Muelleria gibbula</i> (Cleve) Spaulding & Stoermer			o	
<i>Navicula wiesneri</i> Lange-Bertalot	+	o	o	
<i>Nitzschia pusilla</i> Grunow			+	+
<i>Nitzschia solgensis</i> Cleve-Euler			o	
<i>Orthoseira dendroteres</i> (Ehrenberg) Genkal & Kulikovskiy	+		+ o	+
<i>Pantocsekiella</i> sp.	+	o	o	
<i>Pinnularia borealis</i> Ehrenberg	+ o	+ o	+ o	+
<i>Pinnularia issealana</i> Krammer	o			
<i>Pinnularia obscura</i> Krasske	+	+ o	+	+
<i>Sellaphora atomoides</i> (Grunow) Wetzel & Van de Vijver	+	+		+
<i>Sellaphora saugerresii</i> (Desmazières) Wetzel & D.G. Mann		+		
<i>Stauroneis borrichii</i> (Petersen) Lund				+
<i>Stauroneis lundii</i> Hustedt				+
<i>Stauroneis thermicola</i> (Petersen) Lund		+		+
<i>Surirella minuta</i> Brébisson	+			+
<i>Surirella terricola</i> Lange-Bertalot & Alles	+ o			

+ 20 cm above soil level

o 150 cm above soil level

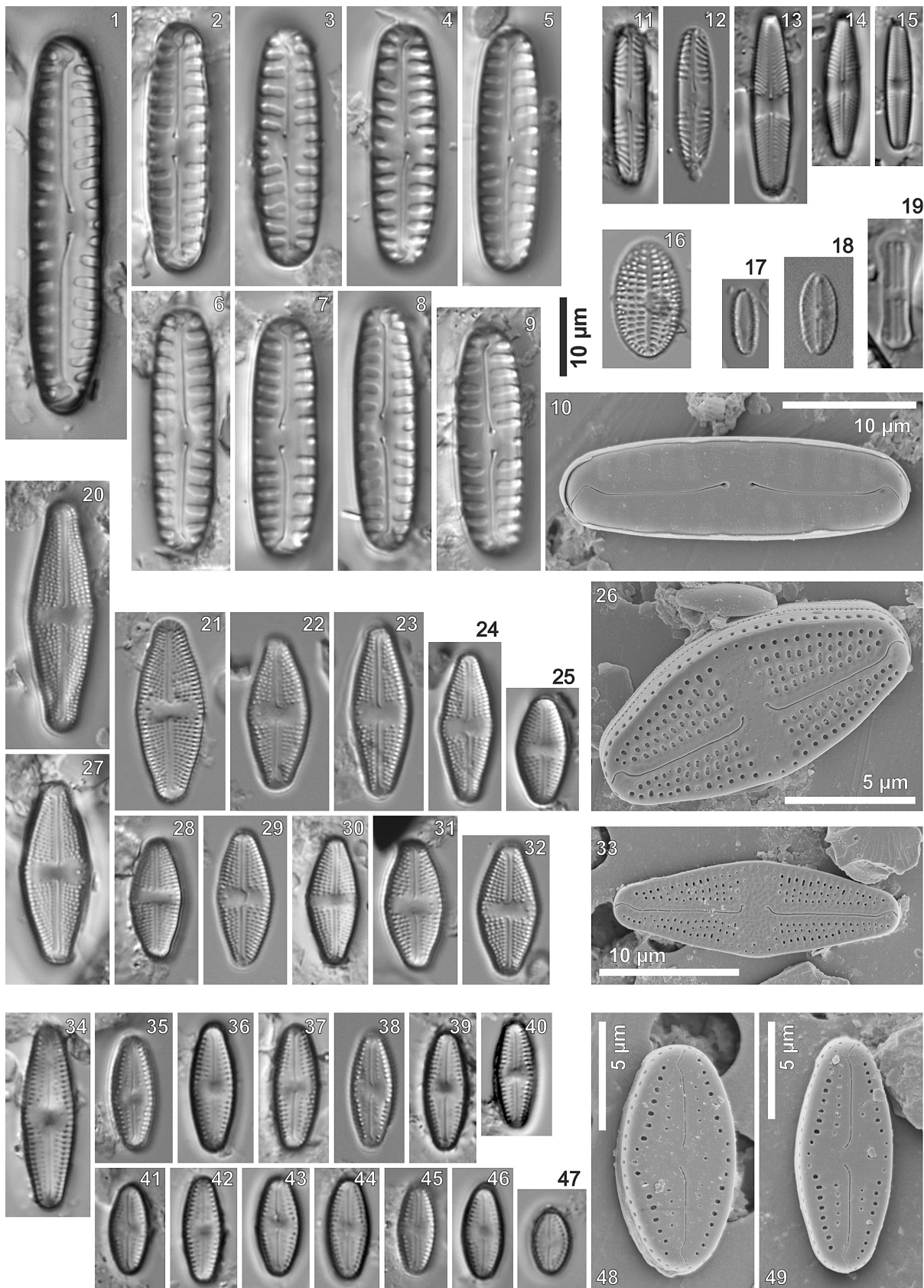


Fig. 1. Selected diatom taxa: 1–10 – *Pinnularia borealis*, 11–12 – *P. obscura*, 13 – *Stauroneis borrichii*, 14 – *S. lundii*, 15 – *Sellaphora atomoides*, 16 – *Cocconeis pseudolineata*, 17 – *Humidophila gallica*, 18 – *Mayamaea atomus*, 19 – *Humidophila contenta*, 20–33 – *Luticola acidoclinata*, 34–49 – *L. sparsipunctata*

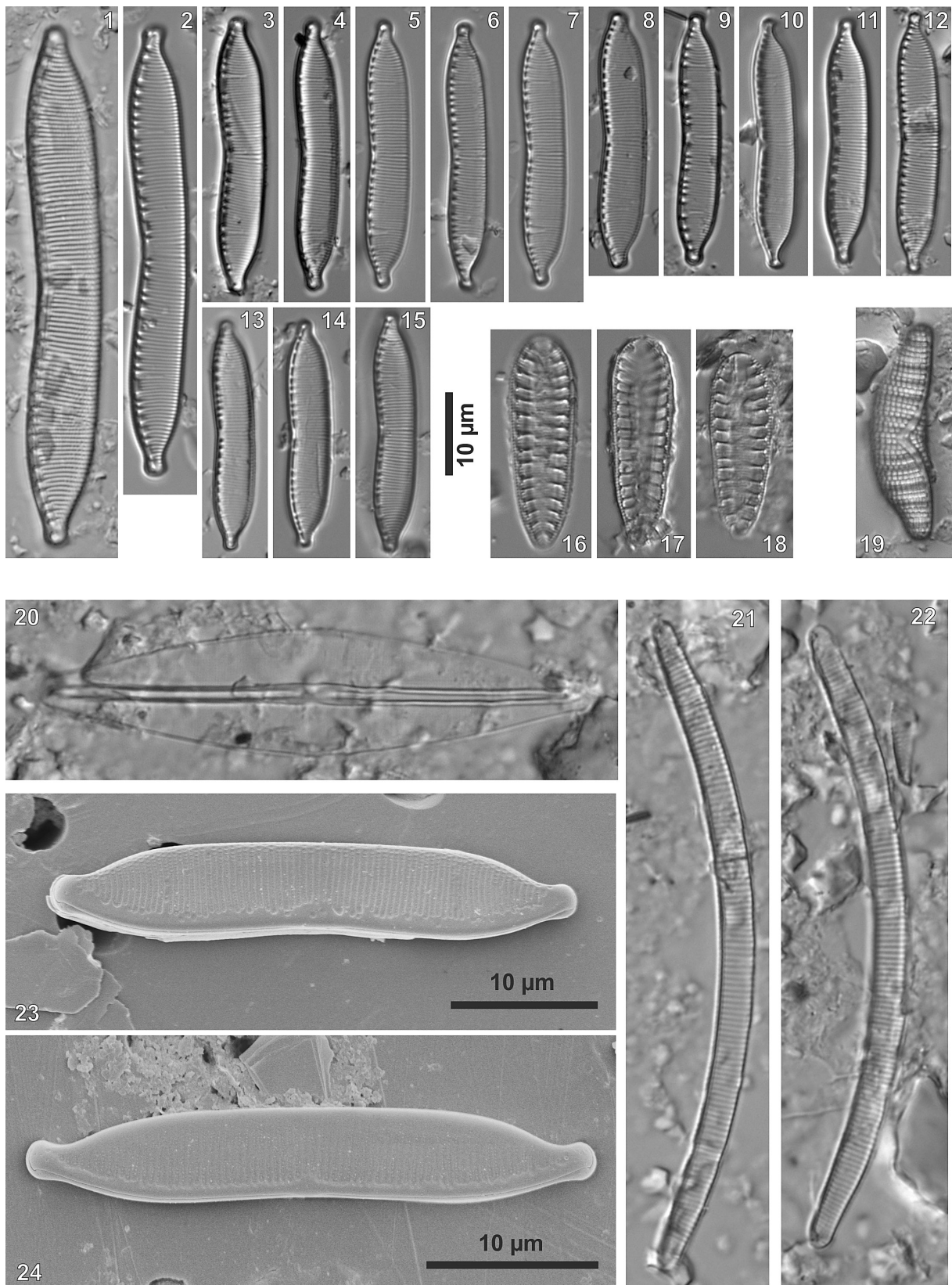


Fig. 2. Selected diatom taxa: 1 – *Hantzschia subrupestris*, 2 – *H. calcifuga*, 3–15 and 23–24 – *H. amphioxys*, 16–18 – *Surirella minuta*, 19 – *Epithemia adnata*, 20 – *Frustulia saxonica*, 21–22 – *Eunotia bilunaris*

Table 3. The number of diatoms frustules with the number of taxa and dominants found at different heights above ground level in particular research seasons. for the dominants, the total number of frustules counted in the sample was given

Date	03.2016		03.2017		06.2017		08.2017
High above soil level	20 cm	150 cm	20 cm	150 cm	20 cm	150 cm	20 cm
Number of all frustules counted in sample	224	22	292	31	247	21	293
Total number of taxa	29	6	20	12	19	10	23
Number of dominant taxa in sample	1	0	1	0	3	0	3
<i>Hantzschia amphioxys</i>	43	9	144	13	54	3	103
<i>Luticola acidoclinata</i>	97	0	22	0	79	10	70
<i>Pinnularia borealis</i>	43	7	39	1	78	1	68

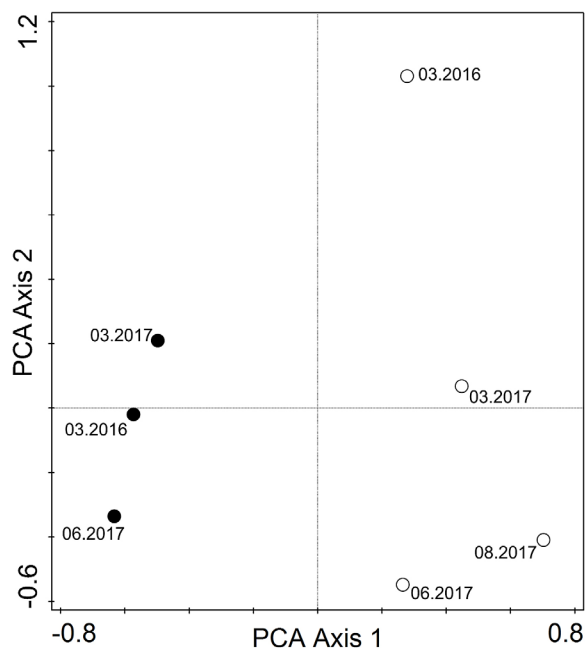


Fig 3. PCA ordination of sampling sites determined by relative community composition. Clusters represent sites of similarity based on height of sampling on three bark (black points – 150 cm, white points – 20 cm)

from Poland: *Hantzschia subrupestris*, *Luticola sparsipunctata* and *L. vanheurckii*.

DISCUSSION

The values of chemical parameters measured in the filtrates of the white poplar bark showed large fluctuations throughout the year. The electrolytic conductivity, the ions (especially nutrients) and pH decreased significantly in summer in comparison to the early spring in 2016 and 2017. All chemical parameters had the lowest values in August. This is probably the result of nutrients leaching by rainwater (from retained dust and decay of the bark).

The bark of trees, due to its porosity, absorbs rainwater and in this way undergoes acidification. The degree of acidification depends on the bark structure, which is different depending on the tree species (Zimny 2006). The pH characterizing the bark of the *Acer platanoides* L., *Fraxinus excelsior* L. and *Tilia cordata* Mill. in clean areas is usually subneutral (4.9–7.5), while in the *Betula pendula* Roth, *Picea abies* (L.) H. Karst. and *Pinus sylvestris* L. is generally acidic (Wirth 1995). Štifterová and Neustupa (2015) measured the pH of the bark in KCl and obtained the highest values (about 5.5) for the maple (*A. platanoides*, *A. pseudoplatanus* L.), while Marmor and Randlane (2007) measured the bark pH of the *Tilia cordata* (also in KCL), which resulted in the pH range from 4.1 to 5.5.

Epicolous bryophytes also have specific preferences related to the chemical properties of a bark (Bates 2009). Typical epiphytic species inhabit the bark of deciduous trees, while the acidic bark of conifers is inhabited only at the base by forest floor species (Rydin 2009).

Only two species of mosses were found during the study, which can be considered as epiphytic. Both have overgrown bark at a height of 150 cm above soil level. *Platygyrium repens* (Brid.) Schimp. is a moss occurring mainly on the bark of trees and especially on rotting wood, while *Orthotrichum speciosum* Nees. is a typical epiphytic species associated with the bark of deciduous trees (Dierßen 2001, Ellenberg, Leuschner 2010).

The moss-inhabiting diatom assemblages were characterized by a high proportion of aerophytic species, which are able to grow outside the typical aquatic habitats. Many species from the genera: *Hantzschia*, *Luticola* and *Mayamaea*, as well as species: *Muelleria gibbula*, *Pinnularia borealis*, *P. obscura* or *Stauroneis thermicola* are usually noted in soils and very often domi-

nated the diatom flora (Stanek-Tarkowska, Noga 2012a,b, Levkov et al. 2013, Stanek-Tarkowska et al. 2013, 2015, 2016, Barragán et al. 2017).

Hantzschia amphioxys and *Pinnularia borealis* form the largest populations in terrestrial environments and have wide ecological preferences. They are typical aerophytic and soil species, which can also develop besides aquatic environment, i.e. on moist walls, in wet rock crevices and among mosses (Bağ et al. 2012, Hofmann et al. 2011, Lange-Bertalot et al. 2017). *Pinnularia borealis* var. *borealis* is a cosmopolitan species that can be often widely distributed by winds (Krammer 2000). In the studied material, *H. amphioxys* formed the most numerous populations and dominated the assemblages at the base of the trunk in almost every season, while *P. borealis* dominated the diatom flora only in the summer. In Podkarpacie region both species were noted in many rivers and streams, but always as single specimens (Noga et al. 2014), while on soils they are numerous and often dominant species (Stanek-Tarkowska, Noga 2012a,b, Stanek-Tarkowska et al. 2013, 2015, 2016).

The third dominant species – *Luticola acidoclinata* occurs in oligotrophic waters with circumneutral to slightly acidic. Formerly, it was reported as *Luticola mutica* var. *intermedia* (Hustedt) Hustedt (Hofmann et al. 2011, Bağ et al. 2012, Lange-Bertalot et al. 2017). Levkov et al. (2013) frequently reported populations of *L. acidoclinata* from oligotrophic, slightly acidic waters (springs, small rivers and peat bogs) and subaerial habitats, usually as epiphytic on mosses. *Luticola acidoclinata* is noted in the Red list of Algae in Poland as a rare species (R category) (Siemińska et al. 2006). In the Podkarpacie region, the species is found always as single specimens in the upper sections of small rivers and streams (Noga et al. 2014). The conducted study showed that in the aerophysical environment (among the mosses growing on the tree bark), *L. acidoclinata* developed in large numbers and dominated the diatom assemblages in three seasons (it was the only one dominant in March 2016). This species is also often observed on soils in southern Poland, especially on meadows, pastures, fallow lands, etc., which are overgrown with varying degrees by mosses (Poradowska, personal communication). All the information mentioned above suggests that *L. acidoclinata* is a rare species only in waters, but has the optimum occurrence in aerophysical environments, especially among mosses.

The remaining species from the R (rare) category were noted individually. Both *Achnanthes coarctata* and *Stauroneis thermicola* are aerophytic species that do not form numerous populations in aquatic environments. They are found in periodically dry habitats, especially on wet rocks and mosses (Hofmann et al. 2011, Bağ et al. 2012, Lange-Bertalot et al. 2017). In the Podkarpacie region, both species were reported individually in different types of waters (Noga et al. 2014), while *Stauroneis thermicola* developed numerous also in soils, where it was often one of the main dominant species. Therefore, it was considered as the soil species (Stanek-Tarkowska et al. 2013).

In the studied material, three species that have not previously been reported from Poland, were found. Among them, only *Luticola sparsipunctata* occurred in all of the studied seasons, but not as the dominant species. The other two species *Hantzschia subrupestris* and *Luticola vanheurckii* were found as single specimens only in the spring seasons.

So far, *Luticola sparsipunctata* is known only from type locality in the Czech Republic and Austria (Levkov et al. 2013) and from caves in Hawaii (Miscoe et al. 2016). It was described as a new species in 2013 (Levkov et al. 2013), therefore its ecology is still poorly known.

Luticola vanheurckii is also known only from the type locality in Belgium (Levkov et al. 2013). It was firstly observed in Poland in a small puddle in the city of Stalowa Wola – a few kilometres from the presented site (Noga, Rybak – in press). In the mosses collected from white poplar, only one valve was found.

In the spring of 2016, few *Hantzschia subrupestris* frustules were noted both at the base of the trunk and at a height of 150 cm. So far, *H. subrupestris* is known only from a few localities, mainly from Europe (Lange-Bertalot 1993, 1996, Lange-Bertalot et al. 2003, Denys, Oosterlynck 2015, Veen et al. 2015), less often from North America (Bahls 2009).

Epiculous mossy sites, as habitat diatoms, have so far been poorly studied. Both rare and well-known diatom species can develop in this habitat. Usually, this kind of habitat is dominated by the aerophytic diatoms, which are resistant to lack of water and changing nutrient levels. These species also often occur in soils and on wet rocks. For this reason, the studies on diatom assemblages from arboreal mosses can extend the knowledge about the ecology, distribution and adaptability of many diatom species.

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