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Diatom assemblages of the brackish Bolshaya Samoroda River (Russia) studied via light microscopy and DNA metabarcoding

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Summary

Diatoms are highly diverse and widely spread aquatic photosynthetic protists. Studies of regional patterns of diatom diversity are substantial for understanding taxonomy and biogeography of diatoms, as well as for ecological perspectives and applied purposes. DNA barcoding is a modern approach, which can resolve many problems of diatoms identification and can provide valuable information about their diversity in different ecosystems. However, only few studies focused on diatom assemblages of brackish rivers and none of them applied the genetic tools. Herein, we analyzed taxonomic composition and abundance of diatom assemblages in the brackish mixohaline Bolshaya Samoroda River flowing into the Elton Lake (Volgograd region, Russia) using light microscopy and high-throughput sequencing of the V4 region of the 18S rDNA gene amplicons. In total, light microscopy of the samples taken in 2011–2014 and 2018 allowed to distinguish 39 diatom genera, represented by 76 species and infraspecies taxa. Twenty three species of diatoms were recorded in the river for the first time. Next-generation sequencing revealed a larger number of diatom taxa (26 genera and 47 OTUs in two samples vs. 20 genera and 37 species estimated by light microscopy). As a result, sequences of *Haslea, Fistulifera, Gedaniella* were recorded in the river for the first time. Significant differences in the data obtained with molecular and light microscopy approaches are discussed. Some V4 18S rDNA sequences were characterized by a low similarity with homologues from the reference database. We revealed high spatial-temporal heterogeneity of the diatom assemblages, occurrence of freshwater species together with brackish and marine ones, and predominance of benthic and plankto-benthic species. Thus, investigations of diatoms in brackish rivers based on both morphological and molecular approaches provide a good chance of improving an understanding of diversity, ecology and biogeography of Bacillariophyta.

Key words: Bacillariophyta, brackish river, diatoms, diversity, metabarcoding, NGS, 18S rDNA

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Introduction

Diatoms (Bacillariophyta) are numerous, highly diverse and ubiquitous photosynthetic protists. The number of diatom species varies from 20,000 to 200,000 (Yi et al., 2017). Bacillariophyta inhabit fresh, brackish and saline inland water bodies, seas and oceans, soils, and wet substrates (Guo et al., 2015). They serve as the base of food webs in the water ecosystems and are responsible for most part of primary production in reservoirs of various types (Siqueiros-Beltrones et al., 2017; An et al., 2018). In addition, diatoms are considered to be environmentally and economically significant microorganisms (Pniewski et al., 2010).

Species diversity of Bacillariophyta is greatly influenced by environmental conditions. For this reason, diatoms are used as suitable bioindicators in ecological studies and water monitoring assessments (Barinova et al., 2006; Zimmermann et al., 2011, 2015; Pinseel et al., 2019). Diatoms are widely used in paleoecological reconstruction, forensic science, as well as oil and gas exploration, due to long-term preservation of their siliceous frustules in marine, lake and peat sediments (Bertrand, 2010; Kulikovskiy, 2016; Pinseel et al., 2019). Many diatom species produce carotenoids and polyunsaturated fatty acids, therefore, they are promising objects for biotechnology (Bertrand, 2010; Shishlyannikov et al., 2014; Petrushkina et al., 2017; Yi et al., 2017).

Diatoms attract an increased interest of researchers (Krivosheia and Vlasiuk, 2016; Siqueiros-Beltrones et al., 2017; An et al., 2018; Komulaynen, 2018). A large number of new species and genera of diatoms have been described during the last 30 years (Kulikovskiy, 2016). Diatoms distribution studied with molecular-based methods in different regions of the world demonstrates that many diatom genera and species, which were considered ubiquitous previously, consist of a number of cryptic or pseudo-cryptic species (Stepanek and Kociolek, 2014; Pinseel et al., 2019). There is a need for studies of regional diatom diversity and compilation of species lists including rare and endemic taxa, to develop a better understanding of taxonomy and biogeography of diatoms and to ensure the use of this knowledge for applied purposes including environmental management. Nevertheless, diversity of diatoms has not yet been studied in many regions of Russia and only few reports on the floristic diversity and distribution of Bacillariophyta contain microphotographs and genetic data (Kulikovskiy, 2016).

The hypersaline lake Elton with the inflowing saline rivers is one of the most unique natural aquatic systems of Russia (Kalyuzhnaya, 2007; Kalyuzhnaya et al., 2011). The Elton Nature Park including the Elton Lake with saline rivers was created in 2001 to preserve the unique saline ecosystems. In 2019, the Elton Nature Park was added to the World Network of Biosphere Reserves by UNESCO's Man and the Biosphere (MAB) programme. Unlike other brackish and saline habitats, rivers with elevated salinity are scarce on the Earth. Some of them are characterized by a wide salinity gradient and a variable hydrological regime both serving as the structure-forming factors for communities of the saline rivers (Zinchenko et al., 2017). Since 2006, researchers have been studying the ecological status and biological diversity of the saline rivers in the Elton region (Zinchenko et al., 2010; Kalyuzhnaya et al., 2011; Nomokonova et al., 2013; Zinchenko and Golovatyuk, 2013; Yatsenko-Stepanova et al., 2015; Burkova, 2016; Gorokhova and Zinchenko, 2016; Gusakov, 2019). One of the longest Elton rivers, the brackish Bolshaya Samoroda River, contains rich and unique biota, and plays a crucial role in stabilizing the natural environment and forming a biodiversity hotspot (Shubin et al., 2000). All previous studies of algal diversity in the Elton region have been carried out using only morphology-based approaches without genetic tools (Yatsenko-Stepanova et al., 2015; Burkova, 2016; Gorokhova and Zinchenko, 2016). Besides, identifications of diatoms in the previous studies of the Elton rivers have not been supported by microphotographs. DNA barcoding is a modern approach, which can resolve many problems of diatoms' identification (Mann et al., 2010; Guo et al., 2015; Rivera et al., 2018). Molecular-based methods that use the techniques of next-generation sequencing (NGS) provide a much more comprehensive insight into the taxonomic diversity of diatoms in the environmental samples (Zimmermann et al., 2015). Therefore, in this study we aimed to characterize the taxonomic composition and abundance of diatom assemblages in the brackish mixohaline Bolshaya Samoroda River flowing into the Elton Lake, using light microscopy (LM) and high-throughput sequencing of the 18S rRNA gene amplicons.

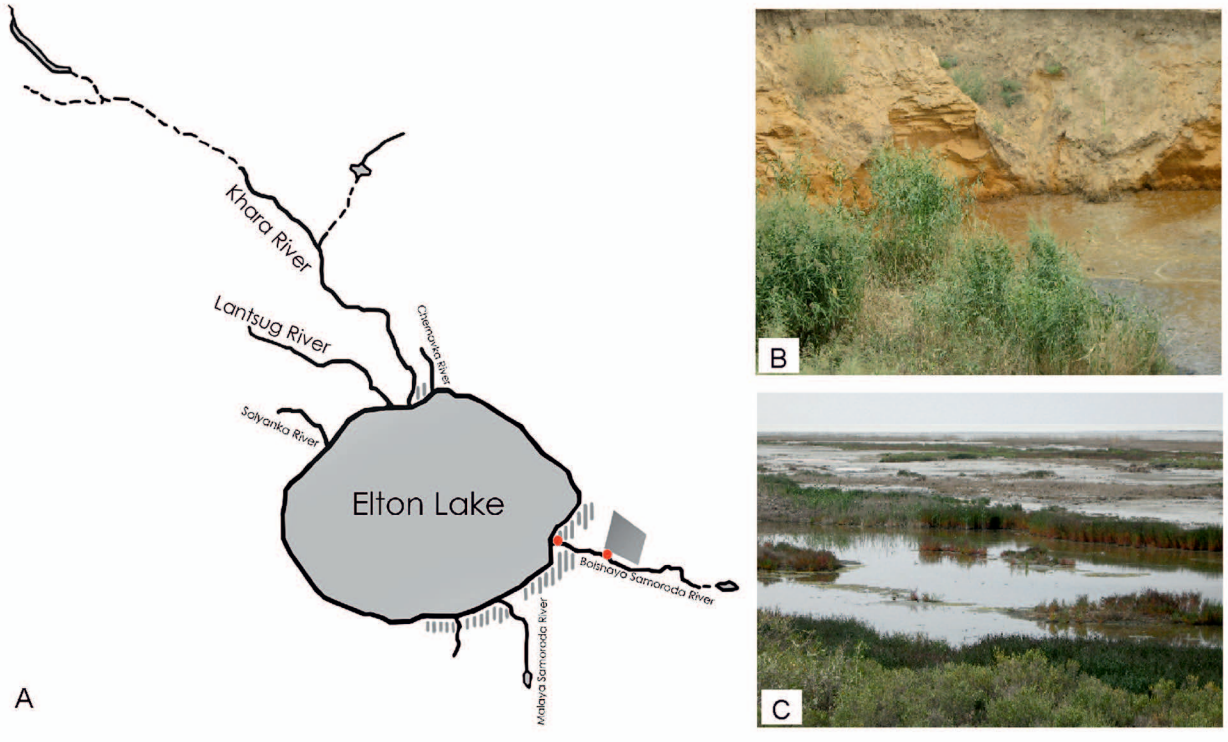

Fig. 1. A – Scheme of Elton region with sampling sites marked with red dots. B, C – Photos of the Bolshaya Samoroda River (B – middle course, C – mouth).

Material and methods

Water sampling

The Bolshaya Samoroda River is located in the Elton Nature Park (Fig. 1). The river flows through a wide valley with gentle slopes. It has a meandering channel and slow current (less than 0.2 m/s). The total length of the river is 21-24 km; the catchment area is 130 km^2. The channel is 6–35 m wide, and the depth is 0.1–0.7 m (Gusakov, 2019). The river is fed mainly by groundwater and precipitation (Brylev and Pryakhin, 2011; Burkova, 2015). The Bolshaya Samoroda River is mixohaline according to the Venice system (1958), with salinity ranging from 6.5 g/L in the middle course to 19 g/L in the mouth. A single observation of 118.8 g/L salinity in the mouth of this river as a result of a brine influx from the Elton Lake was recorded in May 2012. A wide range of salinity is formed due to salt and carbonates sedimentary rocks, salt marshes, and mineral springs in the floodplain terrace, including the Smorogdinsky mineral spring with sulfate-chloride-sodium water.

Water samples were taken in the middle course (49.208889°N, 46.941111°E) and in the river mouth (49.283333°N, 47.036944°E) during vegetative seasons of 2011-2014 and 2018. Salinity was measured using a Master S-28α portable refractometer (Atago, Japan).

Light microscopy observation

Water samples of 0.5 L were fixed with 4% formaldehyde immediately after sampling. Algae were concentrated by sedimentation method. Algal cells were counted in a Nageotte Counting Chamber (Assistent, Germany) at 400× magnification. Organic content of diatom cells was destroyed by the method of cold burning (Balonov, 1975). Then empty diatom frustules were embedded in the Canada balsam. Permanent slides were examined by phase contrast microscopy under an «Axioskop» microscope, equipped with 60× objective, 100× oil objective, and an «Axiocam» digital camera (Carl Zeiss, Germany). For diatom species identification the qualifiers *Süßwasserflora von Mitteleuropa* were used (Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b). Taxonomy and nomenclature of Bacillariophyta is given according to the on-line AlgaeBase database https://www.algaebase.org/ (Guiry and Guiry, 2019).

Ecological analysis

Salinity and habitat preferences of the revealed diatom species were assessed according to Barinova et al. (2006). Thus, all species were referred to one of four salinity indicator groups: oligohalobes (0–5 g/L), mesohalobes (5–20 g/L), euhalobes (20–40 g/L), and polyhalobes (40–300 g/L). Oligohalobes included oligohalobes-halophobes (typically freshwater avoiding brackish waters), oligohalobes-indifferent (typically freshwater, sometimes found in slightly brackish waters), oligohalobes-halophiles (mostly freshwater, also common in brackish waters). Comparison of the species composition was performed using Sörensen similarity coefficient (Sörensen, 1948). The similarity coefficient is 1 when the compared species sets are completely identical; it decreases when their differences increase; it is 0 when the species sets are completely different. In terms of their occurrence, diatom species were referred to constant (more than 70%), additional (20–70%) and rare (less than 20%), according to Kosolapova, 2005.

DNA extraction

Water samples of 500 mL were taken and filtered through membranes with 0.45 μm pore size. Total genomic DNA was isolated from the filters by a combined method, including mechanical homogenization and chemical extraction (Liu et al., 2009) in modification of Bel'kova et al. (2008). A lysing matrix E (MP Biomedicals, USA) and 400 μL of Tris-salt buffer (20 mM EDTA, 750 mM NaCl, 100 mM Tris-HCl, pH 8.0) were added in every sample. The samples were homogenized in Tissue Lyser LT (QIAGEN, Germany) for 1 min at 50 Hz. Then 50 μL of a sterile lysis buffer with lysozyme (50 μg/mL) were added and the samples were incubated for 60 min at 37 °C, followed by addition of 10 μL proteinase K (10 mg/mL) and 10% sodium dodecyl sulfate up to 1% in a final volume. The mixtures were incubated for 60 min at 60 °C. After extraction with phenol–chloroform–isoamyl alcohol (25:24:1) and chloroform–isoamyl alcohol (24:1), DNA in the aqueous phase was precipitated overnight at -20 °C with threefold volume of anhydrous ethanol and 10 M ammonium acetate added up to 10% of a final volume. After centrifuging and double washing with 80% ethanol, DNA was dried on air and dissolved in autoclaved MQ water. To assess contamination during DNA extracting, a negative control containing 100 μL of autoclaved MQ water was subjected to the same procedure. The quality of extracted DNA was checked with electrophoresis in 1.5% agarose gel. The DNA concentration was quantified using Qubit 2.0 Fluorometer (Life Technologies, USA) with dsDNA High Sensitivity Assay (Life Technologies, USA).

Preparing of DNA libraries and NGS

DNA libraries were prepared according to the Illumina workflow (Illumina protocol, part no. 15044223, Rev. B) (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). DNA amplification was performed using primers targeting the hypervariable V4 region of the 18S rRNA gene: forward TAReuk454FWD1 and reverse TAReukRev3 (Stoeck et al., 2010), producing amplicon with length about 500 bp. The polymerase chain reaction (PCR) mixture of volume 30 μL contained 0.25 mM of each primer, 0.125 mM of dNTP, PCR buffer and 0.15 U of Q5 DNA polymerase (New England Biolabs, Ipswich, MA, USA). Amplification was performed according to a PCR protocol applied by Stoeck et al. (2010). Size of the obtained amplicons was verified using electrophoresis in 1% agarose gel. Neither procedures to reduce artificial dominance of some PCR products nor mock communities were used. The following steps of the DNA-library preparation were carried out in full accordance with the Illumina workflow (Illumina protocol, part no. 15044223, Rev. B) and included clean-up of the amplicons obtained, index PCR, clean-up of the DNA libraries obtained, their quantification, normalization and pooling. Clean-up of amplicons and indexed DNA-libraries was performed with Agencourt AMPure XP magnetic beads (Beckman Coulter, USA). Index PCR with amplicons was carried out according to the Illumina protocol (part no. 15044223, Rev. B, p. 10-12) using dual indices from Nextera XT Index kit (Illumina, USA) and Q5 DNA polymerase (New England Biolabs, Ipswich, MA, USA). DNA libraries were quantified using Qubit 2.0 Fluorometer (Life Technologies, USA) with dsDNA High Sensitivity Assay (Life Technologies, USA). The DNA libraries were normalized by dilution up to 10 nM and pooled. The concentrated pooled library was diluted finally to 4 nM.

Sequencing was performed on a MiSeq sequencer (Illumina, USA) using MiSeq Reagent Kit v3 (600 cycle) (Illumina, USA) for paired-end sequencing 2×300 bp in the Center of Shared Scientific Equipment "Persistence of Microorganisms" of the

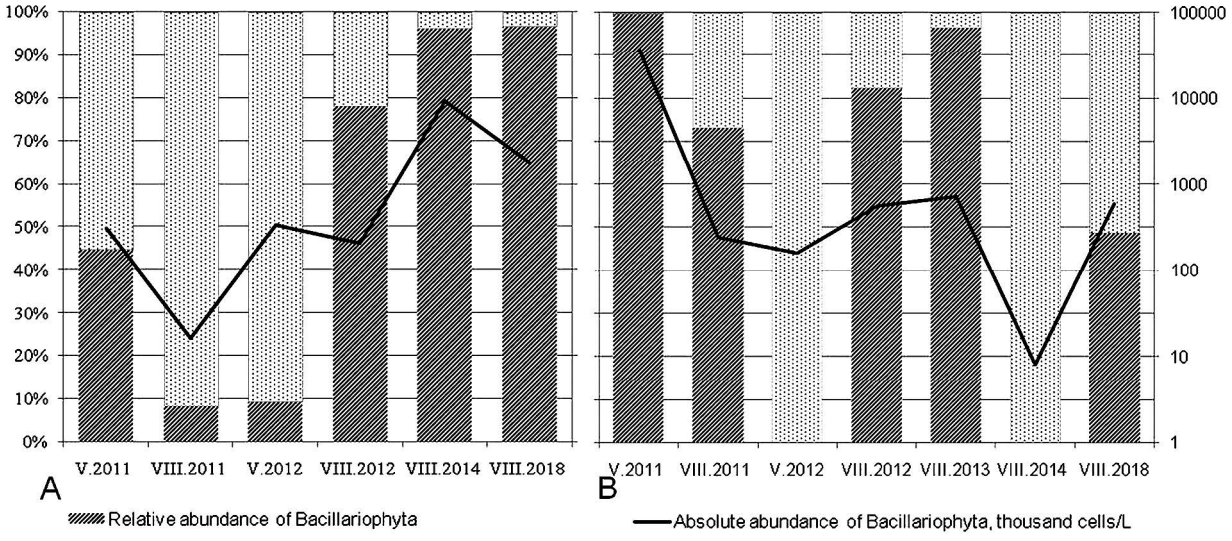

Fig. 2. Absolute and relative abundances of Bacillariophyta in the Bolshaya Samoroda River. A – middle course, B – mouth.

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Bioinformatic analysis

Bioinformatic analyses were conducted using several tools. Paired-end reads were merged with PEAR v.0.9.10 (Zhang et al., 2014). Evaluation of the filtering quality was carried out with FastQC v.0.11.3. Quality filtering and amplicon size selection (350 bp minimal size) were conducted using USEARCH 10.0.240_i86linux32 (Edgar, 2013). During the filtering reads with Ns or an overall mean, Q-score <15 were discarded. As a result of dereplication and clustering with USEARCH, operational taxonomic units (OTUs) were formed at 97% level of similarity, while singletons were removed. The most common sequence was selected as representative in each OTU. Each OTU was formed by 2 to 18,295 reads. The similarity of reads with the most common sequence was in the range of 97-100%, but more than 90% of sequences had similarity of 99.5–100%. Chimera detection and removal was conducted via UCHIME (Edgar et al., 2011) using USEARCH 10.0.240_i86linux32.

For taxonomic classification all OTUs that belonged to diatoms were aligned using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the nr/nt database of nucleotide sequences of the National Center for Biotechnology Information (NCBI). The OTUs obtained in this study were deposited to the GenBank (NCBI) under accession numbers MK626723 – MK626753 and MK656291 – MK656308.

Results and discussion

Light microscopy data

The phytoplankton of the Bolshaya Samoroda River is composed of the phyla Bacillariophyta, Chlorophyta, Euglenophyta, Cryptohpyta, Dinophyta, and Chrysophyta. Diatoms were present in all samples unlike other algal phyla. Relative abundances of Bacillariophyta in the algal communities ranged greatly from 0.1 to 99.8%, and their absolute abundances varied from 8×10^3 to 3.7×10^7 cells/L (Fig. 2). Richness of diatom species and infraspecies taxa ranged from 4 to 30 per sample which drastically exceeded richness of other algal phyla. Similar high levels of diatoms relative abundance and diversity in the phytoplankton have been previously reported in many other inland brackish and saline lakes (Naumenko, 2001; Ovchinnikov et al., 2015; Makeeva and Naumenko, 2016), as well as lagoons (Siqueiros-Beltrones et al., 2017), bays (An et al., 2018), and estuaries (Begyn, 2017).

In this study, a total of 76 diatom species and infraspecies taxa belonging to 39 genera, 26 families, 14 orders, 4 subclasses, and 2 classes, were revealed by light microscopy in the middle course and in the mouth of the Bolshaya Samoroda River

Table 1. List of Bacillariophyta species and infraspecies taxa found in the Bolshaya Samoroda River via light microscopy.

Site (m. – mouth; m.c. – middle course)		m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	S.t.	G.e.
Month		V	V	VIII	VIII	V	V	VIII	VIII	VIII	VIII	VIII	VIII	VIII	VIII		
Year		11	11	11	11	12	12	12	12	13	13	14	14	18	18		
Salinity, g/L		6.5	19	9.6	8.9	8.4	119	11.5	14.3	13	16	10	19	10	14		
	Bacillariophyceae, Bacillariophycidae																
	Mastogloiales, Mastogloiaceae																
1	*Mastogloia pumila* (Grunow) Cleve*									+	+					mh	m
	Mastogloiales, Achnanthaceae																
2	*Achnanthes armillaris* (O.F. Müller) Guiry						+		+	+						hl	m
3	*A. brevipes* C. Agardh var. *brevipes*		+		+		+		+		+			+		hl	
4	*A. brevipes* var. *intermedia* (Kützing) Cleve						+		+	+		+		+	+	mh	b
5	*A. parvula* Kützing*									+						hl	m/f
6	*Platessa salinarum* (Grunow) Lange-Bertalot					+						+		+	+	mh	f
	Cocconeidales, Achnanthidiaceae																
7	*Planothidium delicatulum* (Kützing) Round & Bukhtiyarova								+							hl	u
	Cocconeidales, Cocconeidaceae																
8	*Cocconeis placentula* Ehrenberg											+		+	+	i	f
9	*Cocconeis lineata* Ehrenberg								+			+				i	f
	Cymbellales, Anomoeoneidaceae																
10	*Anomoeoneis sphaerophora* (Ehrenberg) Pfitzer		+		+						+					hl	m/f
11	*A. sphaerophora* var. *sculpta* (Ehrenberg) O. Müller*												+		+	mh	f
	Cymbellales, Cymbellaceae																
12	*Cymbella* sp.		+													-	
	Cymbellales, Gomphonemataceae																
13	*Encyonema silesiacum* (Bleisch) D.G. Mann*											+				i	f
14	*Gomphonema parvulum* (Kützing) Kützing											+		+		i	f
	Cymbellales, Rhoicospheniaceae																
15	*Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot											+		+		i	f
	Bacillariales, Bacillariaceae																
16	*Bacillaria paxillifera* (O.F. Müller) T. Marsson		+											+		mh	b
17	*Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin												+		+	mh	m

Table 1. Continuation.

Site (m. – mouth; m.c. – middle course)		m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	S.t.	G.e.
Month		V	V	VIII	VIII	V	V	VIII	VIII	VIII	VIII	VIII	VIII	VIII	VIII		
Year		11	11	11	11	12	12	12	12	13	13	14	14	18	18		
Salinity, g/L		6.5	19	9.6	8.9	8.4	119	11.5	14.3	13	16	10	19	10	14		
18	*Nitzschia acicularis* (Kützing) W. Smith										+		+		+	i	m/f
19	*N. communis* Rabenhorst					+	+		+	+	+	+		+		i	f
20	*N. obtusa* W. Smith*									+	+					mh	m
21	*N. reversa* W. Smith*														+	hl	f
22	*N. scalpelliformis* Grunow*													+		hl	b
23	*N. sigma* (Kützing) W. Smith*													+		mh	b
24	*Tryblionella apiculata* W. Gregory					+		+				+		+	+	mh	m
25	*T. compressa* (Bailey) Poulin								+	+	+					mh	m
26	*T. hungarica* (Grunow) Frenguelli		+		+				+	+		+		+		mh	b
27	*T. hantzschiana* Grunow*											+				i	m
	Naviculales, Naviculineae, Naviculaceae																
28	*Caloneis amphisbaena* (Bory) Cleve*													+		hl	f
29	*Gyrosigma acuminatum* (Kützing) Rabenhorst													+		i	f
30	*Gyrosigma sp.*								+							-	
31	*Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski							+				+		+		i	f
32	*Navicula capitatoradiata* Germain				+									+		i	b
33	*N. cryptocephala* Kützing								+							-	m/f
34	*N. radiosa* Kützing					+						+		+	+	i	f
35	*N. subrhynchocephala* Hustedt		+													i	f
36	*N. veneta* Kützing		+		+					+						hl	b
37	*Navicula* sp.	+	+		+		+						+	+	+	-	
	Naviculales, Naviculineae, Pleurosigmataceae																
38	*Pleurosigma elongatum* W. Smith*							+		+	+	+		+		mh	m
39	*Pleurosigma* sp.					+										-	
	Naviculales, Naviculineae, Stauroneidaceae																
40	*Stauroneis anceps* Ehrenberg		+													i	f
41	*Stauroneis* sp.					+										-	

Table 1. Continuation.

Site (m. – mouth; m.c. – middle course)		m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	S.t.	G.e.
Month		V	V	VIII	VIII	V	V	VIII	VIII	VIII	VIII	VIII	VIII	VIII	VIII		
Year		11	11	11	11	12	12	12	12	13	13	14	14	18	18		
Salinity, g/L		6.5	19	9.6	8.9	8.4	119	11.5	14.3	13	16	10	19	10	14		
	Naviculales, Neidiineae, Amphipleuraceae																
42	*Halamphora coffeiformis* (C.Agardh) Mereschkowsky		+	+	+		+		+	+	+	+		+	+	mh	b
43	*H. holsatica* (Hustedt) Levkov								+		+					mh	b
	Naviculales, Sellaphorineae, Sellaphoraceae																
44	*Fallacia pygmaea* (Kützing) Stickle & D.G. Mann		+		+				+	+	+			+	+	mh	f
	Naviculales, Sellaphorineae, Pinnulariaceae																
45	*Pinnularia viridis* (Nitzsch) Ehrenberg	+														i	f
	Rhopalodiales, Rhopalodiaceae																
46	*Epithemia adnata* (Kützing) Brebisson*			+												i	f
47	*E. operculata* (C. Agardh) Ruck & Nakov*											+				-	f
48	*Rhopalodia gibberula* (Ehrenberg) O. Müller*											+				mh	m/f
49	*R. musculus* (Kützing) O. Müller *									+	+					mh	b
	Surirellales, Entomoneidaceae																
50	*Entomoneis paludosa* var. *subsalina* (Cleve) Krammer*									+				+		hl	m
	Surirellales, Surirellaceae																
51	*Campylodiscus clypeus* (Ehrenberg) Ehrenberg ex Kützing		+		+										+	mh	m/f
52	*C.bicostatus* W. Smith ex Roper*											+				hl	f
53	*Surirella brebissonii* Krammer and Lange-Bertalot		+											+		i	b
54	*S. ovalis* Brèbisson*						+					+		+		mh	m/f
55	*S. striatula* Turpin				+				+	+	+	+	+	+	+	mh	m
	Thalassiophysales, Catenulaceae																
56	*Amphora commutata* Grunow				+				+	+		+			+	mh	b
57	*A. libyca* Ehrenberg*						+				+					hl	m
	A. ovalis (Kützing) Kützing								+							i	m/f

Table 1. Continuation.

Site (m. – mouth; m.c. – middle course)		m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	S.t.	G.e.
Month		V	V	VIII	VIII	V	V	VIII	VIII	VIII	VIII	VIII	VIII	VIII	VIII		
Year		11	11	11	11	12	12	12	12	13	13	14	14	18	18		
Salinity, g/L		6.5	19	9.6	8.9	8.4	119	11.5	14.3	13	16	10	19	10	14		
	Bacillariophyceae, Fragilariophycidae																
	Fragilariales, Fragilariaceae																
59	*Fragilaria mesolepta* Rabenhorst*													+		i	f
60	*F. crotonensis* Kitton				+											hl	m/f
61	*Fragilaria sp.*													+		-	
	Fragilariales, Staurosiraceae																
62	*Opephora mutabilis* (Grunow) Sabbe & Wyverman								+							-	m
63	*Staurosira construens* Ehrenberg	+							+		+					i	f
	Licmophorales, Ulnariaceae																
64	*Ctenophora pulchella* (Ralfs ex Kützing) D.M.Williams & Round						+				+	+		+		hl	f
65	*Tabularia fasciculata* (C.Agardh) D.M. Williams & Round		+		+	+	+	+	+	+	+	+		+	+	hl	m
	Tabellariales, Tabellariaceae																
66	*Diatoma moniliformis* (Kützing) D.M. Williams*			+												-	f
67	D. vulgaris Bory*			+												i	f
	Mediophyceae, Chaetocerotophycidae																
	Chaetocerotales, Chaetocerotaceae																
68	*Chaetoceros mulleri* Lemmermann		+													hl	m/f
69	*Chaetoceros* sp. 1		+												+	-	
70	*Chaetoceros* sp. 2		+													-	
	Mediophyceae, Thalassiosirophycidae																
	Stephanodiscales, Stephanodiscaceae																
71	*Cyclostephanos dubius* (Hustedt) Round		+		+											-	f
72	*Cyclotella distinguenda* Hustedt*									+	+					hl	f
73	*Cyclotella meneghiniana* Kützing			+		+	+	+	+	+	+	+	+	+	+	hl	m/f

Table 1. Continuation.

	Site (m. – mouth; m.c. – middle course)	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	m.c.	m.	S.t.	G.e.
	Month	V	V	VIII	VIII	V	V	VIII	VIII	VIII	VIII	VIII	VIII		
	Year	11	11	11	11	12	12	12	12	13	13	14	18		
	Salinity, g/L	6.5	19	9.6	8.9	8.4	119	11.5	14.3	13	16	10	14		
74	*Cyclotella* sp.		+											-	
75	*Stephanodiscus* sp.						+		+					-	
	Thalassiosirales, Thalassiosiraceae														
76	*Thallasiosira* sp.	+		+						+	+			-	
	Species number	4	19	6	14	8	12	5	21	20	20	24	6	30	18	

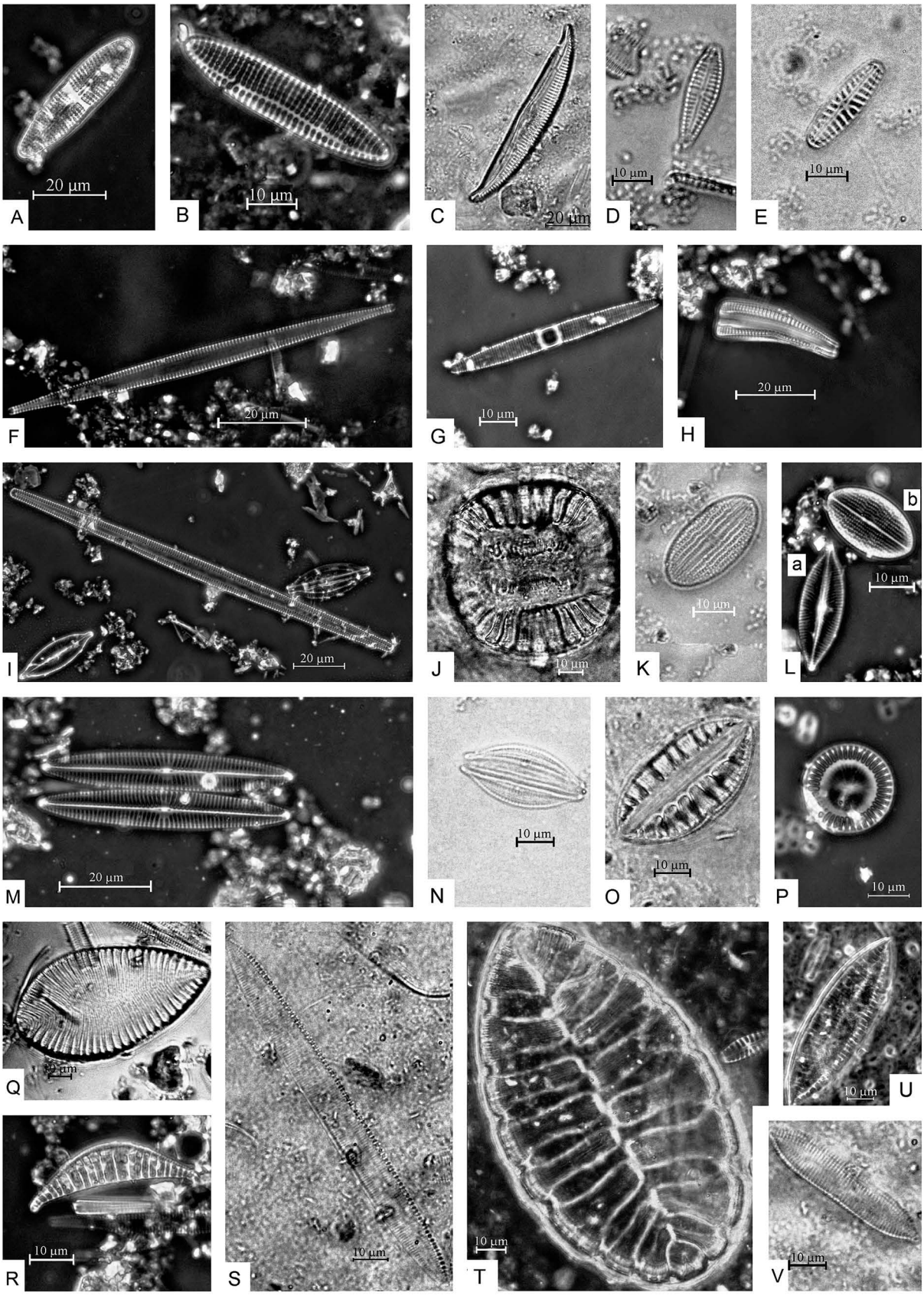
Designations: S.t. – salinity tolerance (according to Barinova et al., 2006): mh - mesohalobes; hl – oligohalobes-halophiles, i – oligohalobes-indifferent; G.e. – general environment (according to Algaebase): m – marine, f – freshwater, b – brackish, u – ubiquitous; (*) – new diatom taxa for the river.

(Table 1). The orders Naviculales (18 species), Bacillariales (12), and Surirellales (6 species and infraspecies taxa) were the most diverse. The most species-rich families were Bacillariaceae (12 species), Naviculaceae (10 species), Surirellaceae (5 species), Stephanodiscaceae (5 species), Achnanthaceae (5 species and infraspecies taxa), and Rhopalodiaceae (4 species). These families included 41 species and infraspecies taxa, which corresponded to 53.9% of the total Bacillariophyta species and infraspecies taxa. Most families comprised of 2-3 species only, whereas eight families were represented by only one species, such as Mastogloiaceae, Achnanthidiaceae, Cymbellaceae, Rhoicospheniaceae, Sellaphoraceaea, Pinnulariaceaea, Entomoneidaceae, and Thalassiosiraceae.

Compared to the previously reported data (Yatsenko-Stepanova et al., 2015; Burkova, 2016; Gorokhova and Zinchenko, 2016) our study revealed 23 new species and infraspecies taxa of Bacillariophyta, which have never been recorded in the Bolshaya Samoroda River before, such as *Surirella ovalis* Brèbisson, *Mastogloia pumila* (Grunow) Cleve, *Epithemia adnata* (Kützing) Brebisson, *Campylodiscus bicostatus* W. Smith, *Diatoma moniliformis* Kützing, *Entomoneis paludosa* var. *subsalina* (Cleve) Krammer, etc. (Figs 3, 4; Table 1).

The analysis of diatoms occurrence showed that only three diatom species were estimated to be permanent in all samples studied, namely *Halamphora coffeiformis* (C. Agardh) Mereschkowsky, *Tabularia fasciculata* (C. Agardh) D.M. Williams et Round, *Cyclotella meneghiniana* Kützing. Twenty four species and infraspecies taxa (31.6%) were referred to additional, whereas 49 species and infraspecies taxa (64.5%) were revealed to be rare. Such variability of community composition may be rela-

Fig. 3. Light and phase-contrast microphotographs of diatom valves recorded in the Bolshaya Samoroda River in August 2014. A, B – *Achnanthes brevipes var. intermedia*, C – *Amphora commutata*, D – *Gomphonema parvulum*, E – *Hippodonta capitata*, F, I – *Tabularia fasciculata*, G – *Ctenophora pulchella*, H – *Rhoicosphenia abbreviata*, J – *Campylodiscus bicostatus*, K – *Cocconeis lineata*, L (a) – *Platessa salinarum*, L (b) – *Cocconeis placentula*, M – *Navicula radiosa*, N – *Halamphora coffeiformis*, O – R*hopalodia gibberula*, P – *Cyclotella meneghiniana*, Q – *Surirella ovalis*, R – *Epithemia operculata*, S – *Tryblionella hungarica*, T – *Surirella striatula*, U – *Tryblionella hantzschiana*, V – *Tryblionella apiculata*.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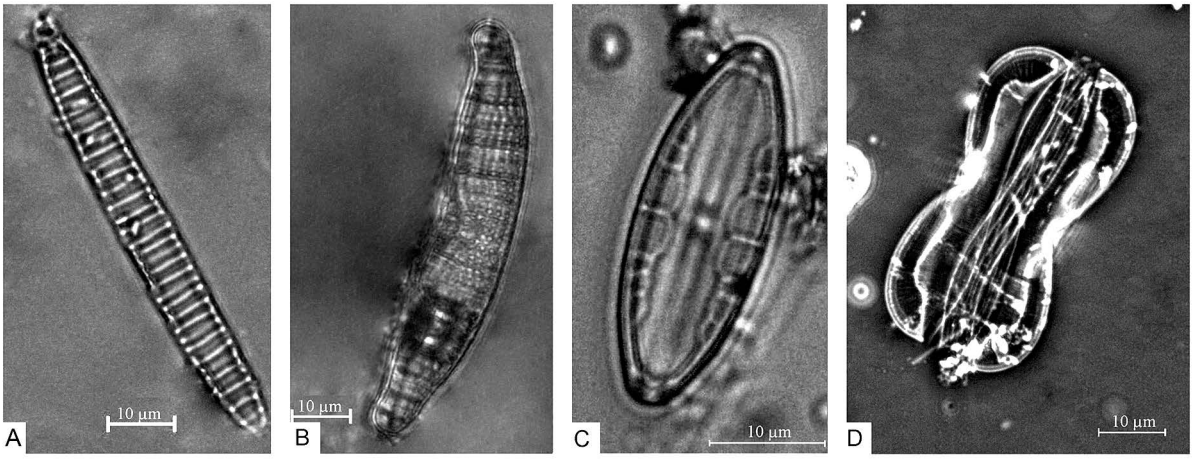

Fig. 4. Light and phase-contrast microphotographs of diatom valves newly recorded in the Bolshaya Samoroda River. A – *Diatoma moniliformi*s, B – *Epithemia adnata*, C – *Mastogloia pumila*, D – *Entomoneis paludosa* var. *subsalina*.

ted to salinity fluctuations (Table 1) or instability of other hydrochemical parameters in the river (Zinchenko et al., 2017). Significant ecological plasticity of *H. coffeiformis, T. fasciculata*, and *C. meneghiniana* most likely determines their survival in a wide range of salinity proved by their presence in most samples. This observation is in a good agreement with other reports that noted these species to have cosmopolitan distribution at different salinities including both fresh and marine waters (Krammer and Lange-Bertalot, 1986, 1991a). *H. coffeiformis* and *C. meneghiniana* are considered typical inhabitants of freshwater bo-dies in the "Roztocze" International Biosphere Reserve, Ukraine (Krivosheia and Vlasiuk, 2016). *C. meneghiniana* was reported in the Lake Baikal (Genkal et al., 2013). *H. coffeiformis* and *C. meneghiniana* were registered in the Ubsu-Nur Lake (Tyva, Russia) at salinity of 18.7 g/L (Naumenko, 2001) and the Ulugkol Lake (Khakassia, Russia) at salinity range of 18.7–21.7 g/L (Makeeva and Naumenko, 2016). Litvinenko et al. (2013) indicated the constant presence of *H. coffeiformis* in the algal assemblages of meso- and hypersaline lakes in the south of Western Siberia, where salinity ranged from 28 to 417 g/L. *T. fasciculata* and *H. coffeiformis* dominated in the benthic samples of saline lakes in the Republic of Kalmykia (Russia), where salinity varied from 156 to 252 g/L (Ovchinnikov et al., 2015).

A comparison of the species lists in the middle course and the mouth of the river showed that 31 species and infraspecies taxa were common for both sampling points, which corresponded to 40.8% of total diatom species revealed. Twenty two (28.9%) species and infraspecies taxa were specific for the middle course while 23 (30.3%) species were specific for the mouth of the river. Sörensen coefficients (SC) were rather low for diatom assemblages revealed in different sampling sites and periods of time. The SCs varied between the diatom assemblages sampled in different years from 0 to 0.63 (mean – 0.23) in the middle course; and from 0.08 to 0.61 (mean – 0.34) in the mouth. The SCs between the diatoms sampled at the same time in the middle course and the mouth were also low ranging from 0.07 to 0.65 (mean – 0.24). Thus, the obtained results demonstrate high spatial-temporal heterogeneity and specificity of the diatom assemblages in the Bolshaya Samoroda River.

The analysis of the taxonomic composition of the diatom species lists in terms of salinity tolerance revealed the presence of mesohalobes, oligohalobes-halophiles, oligohalobe-indifferent taxa, and the absence of oligohalobes-halophobes (Fig. 5, A). In terms of general environments from the Algaebase database (Fig. 5, B) the identified diatom taxa belonged to brackish, marine, freshwater, and ubiquitous groups. Interestingly, the proportion of freshwater taxa in the species lists was rather large reaching almost 50% in the middle river course (Fig. 5, B). These findings show high adaptive ability of many diatom taxa to the conditions of varying salinity. Unquestionably, in further studies traditional preferences of many diatom taxa to salinity should be revised. Proportion of oligohalobes-halophiles together with mesohalobes increased from 60% in the middle course to 74%

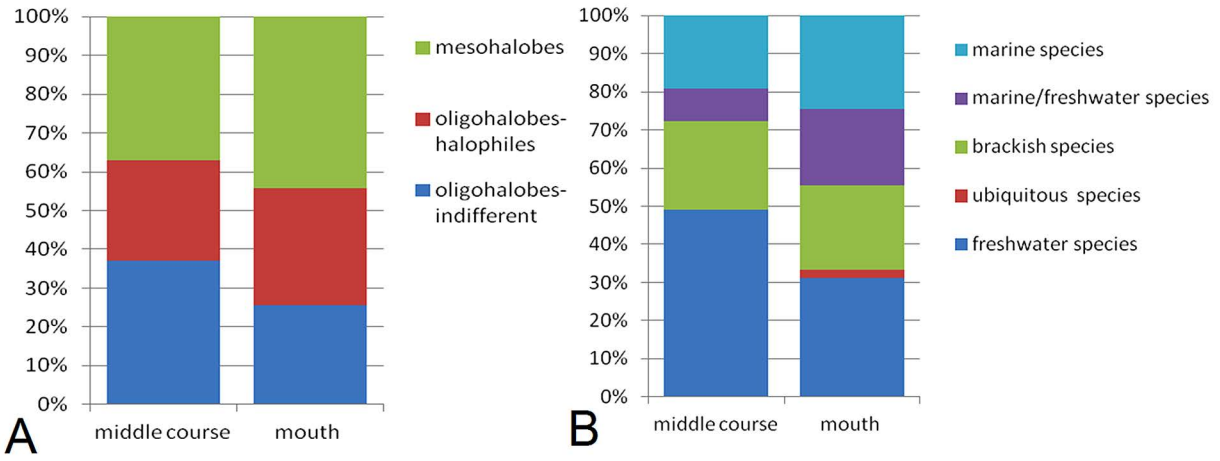

Fig. 5. Number of diatom species with different salinity preferences in the species lists according to Barinova et al., 2006 (A) and Algaebase (B).

in the river mouth vs. proportion of oligohalobe-indifferent taxa (Fig. 5, A). These data corresponded to a smaller proportion (1.6 times) of freshwater species in the mouth compared to the middle course of the river (Fig. 5, B). The observed shifts in the diatom species composition are in good agreement with elevated level of salinity in the river mouth vs. the middle course.

The analysis of the habitat preferences showed that most of the diatom species (34) were benthic, 22 species were plankto-benthic, 1 species was soil and plankto-benthic, and 5 species were planktonic. Probably, the predominance of benthic and plankto-benthic diatoms is determined by a shallow depth of the river. These observations are in good agreement with the similarity and close linkage of plankton and bottom communities of invertebrates described recently in the Elton saline rivers (Zinchenko et al., 2018).

NGS data and their comparison with LM data

Samples taken in August 2014 were analyzed by NGS and LM. A library from the mouth sample of the Bolshaya Samoroda River contained 1,541 V4 SSU rDNA assembled reads of diatoms that were equal to 15% of total microalgae reads. A library from the middle course sample included 22,691 reads (80.3% of total microalgae reads). The assembled reads had the length of 410-446 bp and an average overlap of 182 bp. The relative abundances of diatoms estimated by LM were in similar ratio between the river mouth (0.1%) and the middle course (96.2%) samples.

The overall genetic diversity of diatoms found in our study was higher than morphological diversity. In total, 47 different OTUs referred to 26 genera were revealed in two samples in contrast to 37 species from 20 genera estimated using light microscopy. The genera *Haslea, Fistulifera*, and *Gedaniella* were detected in the Bolshaya Samoroda River by NGS for the first time. In addition, more OTUs that belonged to the genera *Halamphora, Navicula, Nitzschia*, and *Cyclotella* were found with NGS compared to the number of morphologically identified species. For example in the middle course sample only one representative of the genus *Halamphora*, *H. coffeiformis*, was recorded using LM (Table 2). In the same sample NGS revealed 3 OTUs that belonged to the *Halamphora* genus. The closest homologues of these OTUs in the GenBank database were the sequences deposited: *Halamphora americana* MG027295, *Halamphora coffeiformis* (deposited as *coffeaeformis*) KX257363, and *Halamphora terroris* KC222330 (Table 3). Besides, *Nitzschia communis* and *Navicula radiosa* were detected by LM vs. three *Nitzschia* and two *Navicula* OTUs recorded with NGS (Table 3). The reason of the observed differences may be a greater sensitivity of metabarcoding allowing detection of rare and not numerous species (Groendahl et al., 2017). Perhaps, our NGS data underestimate genetic diversity of diatoms, due to a low resolution of the 18S rDNA gene insufficient for species discrimination of diatoms (An et al., 2017). In our study many OTUs were aligned at high similarity level (more than 99.0%) to several closely related sequences belonging to different species, e.g. representatives

Table 2. Heatmap of diatom taxa revealed by LM (number of species) and NGS (number of OTUs) in plankton samples of the Bolshaya Samoroda River.

Class	Order	Family	Genus	Middle course		Mouth	
				LM	NGS	LM	NGS
Bacillariophyceae	Bacillariales	Bacillariaceae	*Cylindrotheca*	0	1	1	1
			Nitzschia	1	3	1	1
			Tryblionella	3	1	0	1
	Cocconeidales	Cocconeidaceae	*Cocconeis*	2	0	0	0
			UI Cocconeidaceae	0	1	0	0
	Cymbellales	Anomoeoneidaceae	*Anomoeoneis*	0	0	1	0
			UI Anomoeoneidaceae	0	0	0	1
		Gomphonemataceae	*Encyonema*	1	0	0	0
			Gomphonema	1	1	0	0
		Rhoicospheniaceae	*Rhoicosphenia*	1	1	0	0
	Mastogloiales	Achnanthaceae	*Achnanthes*	1	0	0	0
			Platessa	1	0	0	0
	Naviculales	Naviculaceae	*Hippodonta*	1	1	0	0
			Navicula	1	2	1	0
			Haslea	0	0	0	1
			UI Naviculaceae	0	0	0	1
		Pleurosigmataceae	*Pleurosigma*	1	1	0	0
		Stauroneidaceae	UI Stauroneidaceae	0	0	0	1
		Amphipleuraceae	*Halamphora*	1	3	0	2
		Stauroneidaceae	*Fistulifera*	0	1	0	0
	Rhopalodiales	Rhopalodiaceae	*Rhopalodia*	2	0	0	0
	Surirellales	Surirellaceae	*Campylodiscus*	1	0	0	0
			Surirella	2	2	1	1
	UI Bacillariophycidae			0	2	0	0
	Thalassiophysales	Catenulaceae	*Amphora*	1	1	0	1
	Fragilariales	Fragilariaceae	*Gedaniella*	0	0	0	1
			UI Fragilariophycidae	0	0	0	1
	Licmophorales	Ulnariaceae	*Ctenophora*	1	0	0	0
			Tabularia	1	1	0	0
	UI Bacillariophyceae			0	1	0	0
Mediophyceae	Chaetocerotales	Chaetocerotaceae	*Chaetoceros*	0	0	0	3
	Stephanodiscales	Stephanodiscaceae	*Cyclotella*	1	2	1	1
	Thalassiosirales	Thalassiosiraceae	*Thalassiosira*	0	2	0	1
		UI Thalassiosirales		0	1	0	0
	UI Mediophyceae			0	1	0	0
UI Bacillariophyta				0	2	0	0

Designation: UI – unidentified member of certain taxon.

of the genera *Thalassiosira, Cyclotella, Fistulifera, Gomphonema, Nitzschia, Tabularia, Surirella, Navicula, Halamphora, Cylindrotheca, Gedaniella*, and *Chaetoceros* (Table 3). That is why identification of most diatom OTUs was possible at the genus level only.

All OTUs of diatoms were represented by 2 classes, 13 orders, 16 families, and 18 genera. Thirteen OTUs could not be identified at the genus level. *Cylindrotheca, Nitzschia, Tryblionella, Halamphora, Surirella, Amphora, Cyclotella*, and *Thalassiosira* were shared genera for both river mouth and middle course samples. Nevertheless, major part of diatom genera was specific for each sampling site (Table 2). In the middle course sample 31 OTUs were represented by 15 genera while 8 OTUs remained unidentified at the genus level. At the same time, in this sample 24 species belonging to 19 genera

Table 3. Closest homologues of 18S rRNA gene diatom sequences from the NCBI GenBank database.

OTU (accession no.)	Identified as	Closest homologue (accession no.)	Similarity (%)	Query Cover (%)
ID-19-1 (MK626723)	*Thalassiosira* sp.	*Thalassiosira weissflogii* (HM991702)	99.76	100
ID-19-7 (MK626724)	*Cyclotella* sp.	*Cyclotella meneghiniana* (KT386323)	99.76	100
ID-19-23 (MK626725)	*Halamphora coffeiformis*	*Halamphora coffeaeformis* (KX257363)	99.76	100
ID-19-25 (MK626726)	*Hippodonta capitata*	*Hippodonta capitata* (AM501966)	99.76	100
ID-19-26 (MK626727)	*Tryblionella apiculata*	*Tryblionella apiculata* (HQ912600)	99.57	100
ID-19-31 (MK626728)	Bacillariophyceae sp.	*Mayamaea fossalis* var. *fossalis* (KF959655)	91.41	100
ID-19-36 (MK626729)	*Surirella striatula*	*Surirella striatula* (KX120757)	99.52	100
ID-19-40 (MK626730)	*Fistulifera* sp.	*Fistulifera saprophila* (AB769958)	99.28	100
ID-19-45 (MK626731)	*Pleurosigma* sp.	*Pleurosigma intermedium* (AY485489)	98.34	100
ID-19-48 (MK626732)	UI Cocconeidaceae	*Cocconeis placentula* (AM502013)	95.63	100
ID-19-49 (MK626733)	*Halamphora* sp.	*Halamphora americana* (MG027295)	98.78	100
ID-19-57 (MK626734)	*Gomphonema* sp.	*Gomphonema parvulum* (KF959660)	99.76	100
ID-19-79 (MK626735)	*Nitzschia* sp.	*Nitzschia microcephala* (KC759159)	99.76	100
ID-19-89 (MK626736)	*Tabularia* sp.	*Tabularia fasciculata* (EF423417)	99.76	100
ID-19-98 (MK626737)	*Nitzschia* sp.	*Nitzschia* sp. (FJ546709)	99.53	100
ID-19-106 (MK626738)	*Nitzschia* sp.	*Nitzschia supralitorea* (AJ867019)	99.76	100
ID-19-112 (MK626739)	UI Mediophyceae	*Minutocellus polymorphus* (KY980146)	82.62	100
ID-19-119 (MK626740)	*Amphora commutata*	*Amphora commutata* (KX120667)	99.52	100
ID-19-138 (MK626741)	*Rhoicosphenia abbreviata*	*Rhoicosphenia* cf. *abbreviata* (KU965565)	100	100
ID-19-139 (MK626742)	*Surirella* sp.	*Surirella minuta* (KX120726)	99.76	100
ID-19-151 (MK626743)	*Navicula* sp.	*Navicula perminuta* (KY320361)	99.04	100
ID-19-158 (MK626744)	*Thalassiosira* sp.	*Thalassiosira weissflogii* (HM991702)	99.74	100
ID-19-160 (MK626745)	*Halamphora* sp.	*Amphora terroris* (KC222330)	99.04	100
ID-19-210 (MK626746)	UI Bacillariophyta	*Thalassiosira weissflogii* (HM991702)	96.71	72
ID-19-214 (MK626747)	UI Thalassiosirales	*Thalassiosira pseudonana* (KU900218)	96.92	100
ID-19-248 (MK626748)	UI Bacillariophycidae	*Nitzschia supralitorea* (KU341756)	95.04	100
ID-19-254 (MK626749)	*Cylindrotheca* sp.	*Cylindrotheca closterium* (KY045848)	99.52	100
ID-19-262 (MK626750)	UI Bacillariophyta	*Thalassiosira weissflogii* (HM991702)	93.38	100
ID-19-317 (MK626751)	*Navicula* sp.	*Navicula phyllepta* (FJ624231)	99.05	100
ID-19-320 (MK626752)	*Cyclotella* sp.	*Cyclotella meneghiniana* (KY364696)	97.87	100
ID-19-334 (MK626753)	UI Bacillariophycidae	*Achnanthidium daonense* (KJ658413)	96.37	100
ID-20-19 (MK656293)	*Cylindrotheca closterium*	*Cylindrotheca closterium* (GQ468535)	99.76	100
ID-20-55 (MK656301)	*Nitzschia* sp.	*Nitzschia microcephala* (KC759159)	100	100
ID-20-95 (MK656305)	*Tryblionella* sp.	*Tryblionella apiculata* (HQ912600)	98.33	100
ID-20-89 (MK656304)	UI Anomoeoneidaceae	*Dickieia ulvacea* (AY485462)	97.81	100
ID-20-54 (MK656300)	*Halamphora* sp.	*Halamphora subtropica* (KY054941)	99.76	100
ID-20-137 (MK656307)	*Halamphora* sp.	*Halamphora aponina* (MG027296)	99.51	100
ID-20-27 (MK656294)	*Haslea spicula*	*Haslea spicula* (HM805034)	99.52	100

Table 3. Continuation.

OTU (accession no.)	Identified as	Closest homologue (accession no.)	Similarity (%)	Query Cover (%)
ID-20-136 (MK656306)	UI Naviculaceae	*Navicula* sp. (KF791556)	97.60	100
ID-20-49 (MK656299)	UI Stauroneidaceae	*Stauroneis latistauros* (KM116114)	97.12	100
ID-20-74 (MK656303)	*Surirella* sp.	*Surirella striatula* (KX120757)	98.52	100
ID-20-138 (MK656308)	*Amphora* sp.	*Amphora commutata* (KX120667)	98.80	100
ID-20-44 (MK656297)	*Gedaniella* sp.	*Gedaniella boltonii* (MF093083)	99.76	100
ID-20-47 (MK656298)	UI Fragilariophycidae	*Tabularia* sp. (KT860991)	97.86	100
ID-20-14 (MK656292)	*Chaetoceros* sp.	*Chaetoceros muellerii* (KX609786)	99.76	100
ID-20-7 (MK656291)	*Chaetoceros* sp.	*Chaetoceros* sp. (EF473734)	99.76	100
ID-20-34 (MK656296)	*Chaetoceros* sp.	*Chaetoceros* sp. (HM106503)	98.57	100
ID-20-59 (MK656302)	*Cyclotella meneghiniana*	*Cyclotella meneghiniana* (KY364696)	99.76	100
ID-20-32 (MK656295)	*Thalassiosira* sp.	*Thalassiosira weissflogii* (HM991702)	99.76	100

Designation: UI – unidentified member of certain taxon.

were revealed by LM (Fig. 4). Among 18 diatom OTUs from the river mouth sample 14 OTUs were attributed to 11 genera, whereas 4 OTUs remained unidentified at the genus level. Only 6 species belonging to 6 genera were revealed by LM there. Thus, taxonomic richness of diatoms in the middle course of the river was higher than in the mouth based on the results of both NGS and LM methods.

The Venn diagrams were created to compare common diatom genera identified by LM and NGS simultaneously, as well as specific genera found by each method separately. More than a half of all identified diatom genera were found by both LM and NGS in the middle course sample, whereas in the river mouth sample only 30.8% of those were shared (Fig. 6).

Representatives of only few genera were found simultaneously under light microscope and with NGS, such as *Nitzschia, Gomphonema, Rhoicosphenia, Hippodonta, Pleurosigma, Surirella, Tabularia*, and *Cyclotella*. The genera *Cylindrotheca, Tryblionella, Halamphora*, and *Amphora* were revealed in one of two samples by both NGS and LM (Table 2). At the same time their cells were not found with LM in another sample, whereas their sequences were distinguished. Furthermore, some other genera were recognized only with NGS, e.g. *Fistulifera, Gedaniella, Thalassiosira, Chaetoceros,* and *Haslea*. This fact suggests that NGS approach is more sensitive than LM. Sometimes light-microscopic identification of diatoms may be doubtful because of too small size of a cell or slight morphological differences between species (Rivera et al., 2018). Another reason may be detection of free DNA recovered from dead diatom cells and transported by the river flow.

The genera *Cocconeis, Anomoeoneis, Encyonema, Achnanthes, Platessa, Rhopalodia, Campylodiscus*, and *Ctenophora* were not supported by NGS and were revealed with LM only (Table 2). These genera might be identified at too low level as unidentified representatives of the appropriate families, orders, subclasses and even classes, due to insufficient data on the mentioned genera or their misidentification in the GenBank database. Also universal primers were shown to reveal only half of OTUs due to insufficient coverage compared to more selective primer pairs (Lentendu et al., 2014). In addition, some diatoms found with LM and not confirmed by the NGS may represent frustules of dead diatoms, which are able to retain for a long time due to highly resistant and strong siliceous composition. At last, erroneous morphological classification, misclassification of OTUs due to low variability within the metabarcoding marker could take place (Groendahl et al., 2017). Therefore, only eight diatom species identified by morphology were robustly supported by 18S rDNA metabarcoding, e.g. *Cyclotella meneghiniana* Kützing, *Surirella striatula* Turpin, *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin, *Amphora commutata* Grunow, *Halamphora coffeiformis, Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski, *Tryblionella apiculata* W. Gregory, and *Rho-*

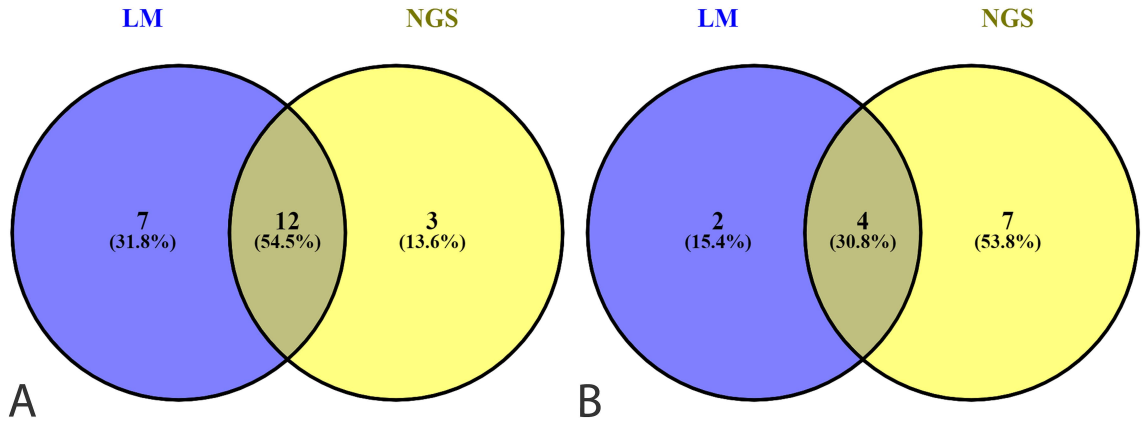

Fig. 6. Venn's diagrams showing common and specific diatom genera revealed with LM and NGS in the Bolshaya Samoroda River. A – middle course, B – mouth.

icosphenia abbreviata (C. Agardh) Lange-Bertalot (Table 3).

Alignment of the OTUs against nr database of GenBank (NCBI) (Table 3) retrieved some OTUs closely related to those diatom genera and species, for which LM identification had not been confirmed by NGS. These OTUs were identified at taxonomic levels of family or order. For example, *Cocconeis placentula* Ehrenberg was detected in the middle course sample by LM only, whereas an OTU of Cocconeidaceae sp. was found with NGS. The sequence closest to the OTU in GenBank is assigned to *C. placentula* (AM502013) with low similarity (95.63%), which is insufficient for diatom identification even at the genus level (An et al., 2017). Then, the sequence of *Anomoeoneis* was not found, whereas Anomoeoneidaceae sp. phylotype was identified. A phylotype of Naviculaceae sp. was similar at 97.60% or less with sequences of known *Navicula* strains. Such morphologically identified diatoms, which were not supported with respective 18S signatures, may belong to novel species and genera.

Study of diatom biodiversity in brackish rivers could provide valuable information about pseudocryptic or cryptic species, which are still undescribed, and have slight, or even do not have any morphological distinctions from the existing diatom species, respectively. For example, Vanelslander et al. (2009) revealed three pseudocryptic species of the widespread benthic diatom *Navicula phyllepta* in an estuary in the Netherlands, and described their distinct ecological niches characterized by different salinity tolerance ranges, preferred sediment types, and optimal ammonium concentrations. Estimation of diatom pseudocryptic or cryptic species in brackish rivers using metabarcoding will be successful, if it is supplemented by culture isolation and evaluation of their additional genetic markers, e.g. ITS2 secondary structure, as well as phenotypic properties, such as sexual compatibility, chemotaxonomic markers, and ecological features (Amato et al., 2019).

Conclusions

The Bacillariophyta was revealed to be a permanent, taxonomically diverse, and often the most abundant component of algal communities in the brackish mixohaline Bolshaya Samoroda River located in the Elton Nature Park. In total, light microscopy of the samples taken in 2011–2014 and 2018 allowed to distinguish 39 diatom genera, represented by 76 species and infraspecies taxa. Twenty three species of diatoms were recorded in the river for the first time.

The diatom assemblages showed high spatial-temporal heterogeneity in the Bolshaya Samoroda River, probably due to the influence of dynamically changing abiotic factors. Thus, differences in salinity influence the species composition and proportions of freshwater, marine and brackish species of diatoms. Detection of species known as freshwater together with brackish and marine ones suggests that adaptive capacities of diatoms to high salinity are underestimated.

Our study demonstrated the absence of a direct relationship of diatoms species richness and abundance with salinity in the river. We suggest that

drastic fluctuations in abundance and richness of diatoms in the river cannot be justified by only salinity fluctuations. Nevertheless, they may be determined by the combined influence of environmental factors including biotic interactions.

NGS was applied for the first time to characterize the taxonomic diversity of Bacillariophyta in the Bolshaya Samoroda River. As a result, sequences of the genera *Haslea, Fistulifera*, and *Gedaniella* have been recorded in the river for the first time. The data obtained with NGS and LM demonstrated pronounced differences. The diatoms taxonomic richness revealed with NGS was higher compared to that estimated by LM. Next-generation sequencing revealed 26 genera and 47 OTUs in two samples vs. 20 genera and 37 species estimated by light microscopy. However, DNA barcoding based on the V4 marker region of 18S rRNA gene did not allow distinguishing most diatom species reliably. In our study we discovered high genetic richness of diatoms and some V4 18S rDNA sequences characterized by a low similarity with homologues from the reference database. That is why we can expect that a large number of novel for science diatom taxa might be described in further studies of the saline Elton rivers. For this, future investigations of diatoms will require isolation of pure cultures and their thorough study. Results of our investigations of diatoms in the brackish Bolshaya Samoroda River using both morphology-based and molecular techniques open new perspectives for the in-depth understanding of the diversity patterns, ecology and biogeography of Bacillariophyta.

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