Algal and cyanobacterial diversity in saline rivers of the Elton Lake Basin (Russia) studied via light microscopy and next-generation sequencing

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Introduction

Water bodies with naturally high levels of salinity are widely distributed on all continents of the globe and are the objects of numerous scientific studies (Heidelberg et al., 2013; Afonina & Tashlykova, 2016). The increased attention paid to saline waterbodies is primarily due to the development of specific biota in them, represented by halotolerant and halophilic species, many of which are of biotechnological importance as producers of biologically active substances (Sokolchenko et al., 2015; Moharrami et al., 2015; Almuhairi & Touilbahi, 2017).

Among the various types of mineralized water bodies, saline rivers are by far the least studied (Orfeo, 1999; Burbkova, 2015; Gutiérrez-Cánovas et al., 2019). Saline rivers with a salinity gradient from the source to the mouth are particularly interesting, because the range of salinity is the structure-forming factor of the hydrobiot, whereas such rivers are represented by saline rivers of the Elton Lake Basin in Volgograd region of Russia (the Bolsheya Samoroda River and the Malaya Samoroda River). Herein, we analyzed taxonomic structure and species diversity of microalgae and Cyanobacteria of the saline rivers flowing into the Elton Lake by light microscopy and next-generation sequencing. The differences and possible causes of inconsistencies in the results obtained by these methods are discussed. In total, 91 taxa of microorganisms were identified by integrated approach in the assemblages of microalgae and Cyanobacteria in the middle course of the Bolsheya Samoroda River, and 60 taxa – in the river mouth. The diversity of these assemblages in the hypersaline Malaya Samoroda River was lower: 27 taxa from the middle course and 23 taxa from the mouth. Next-generation sequencing allowed us to refine and expand the list of microalgae taxa in the studied saline rivers due to detection of species which were hard to identify, low-abundance taxa, as well as extremely small-cell forms. Some discrepancies between the data obtained by light microscopy and next-generation sequencing indicate the advantage of simultaneous use of both methods for study of the algal communities. Such a comprehensive approach provides the most accurate and correct list of taxa added with the morphological descriptions and 18S rRNA and 16S rRNA partial sequences. Generally, 18 taxa have been recorded for the first time in the Bolsheya Samoroda River, belonging to the phyla Chlorophyta (Pororocellispsis sp., Chlorochytrium lemnae Cohn, Caespitella sp., Halochlorococcus sp., Tetraselmis cordiformis (H. J. Carter F. Stein), Ochrophota (Pseudochrysochloris ovulis (Chodat) D. J. Hibberd, Characiopsis sp., Poterioochromonas stipitata Scherffel, Chryseisindea papillosa sp., Euglenozoa (Euglena burcharica I. Kisselev, Lepocinclis tringariae (Dujardin) B. Marin & Melkonian, Phaeocystis orbicularis K. Hübner, P. parva G. A. Klebs), Cryptophyta (Hemiselmis cryptochromatica C. E. Lane & J. M. Archibald, Rhodomonas sp., H. phila J. A. Deane), Haptochrota (Pavlova sp.), Cyanobacteria (Jo- hanesenienia construimt (Sezela) Haster, Dvorak & Psalkova) Seven taxa have been detected for the first time in the algal and cyanobacterial assemblages of the Malaya Samoroda River from the phyla Chlorophyta (Tetraselmis cordiformis, T. arnoldii Porochloris-Lavenko) R. E. Norris, Hor & Chilana, C. tetrahedra (West Butcher, Pyrobolus elongatus Korsikov), Cryptophyta (Hymasia phila), and Cyanobacteria (Synechococcus elongatus (Nägeli) Nägeli, Oscillatoria simplicissima Gomont).

Keywords: microalgae; Cyanobacteria; saline rivers; Elton; light microscopy; next-generation sequencing.
roda River, Bolshaya Samoroda River) by light microscopy (LM) and next-generation sequencing (NGS).

Materials and methods

Water samples were taken in the middle course and mouth of Malaya Samoroda River and Bolshaya Samoroda River in August 2014. Due to the significant shallowing in summer, no samples were taken in the upper course of the rivers. The Malaya Samoroda and Bolshaya Samoroda Rivers flow into the largest hypersaline lake in Europe – Lake Elton (Lake Elton Biosphere Reserve, Russia, a UNESCO World Heritage site) (Fig. 1). These rivers are shallow lowland watercourses with slow current (0.01 < Di < 0.1). The Bolshaya Samoroda River has a length of 21–24 km, the catchment area is 130.0 km², the channel is 6.0–35.0 m wide, the depth varies from 0.10 to 0.70 m (Gusakov, 2019). The salinity level of the river (at the time of sampling) increased from the middle course (10 ppt) to the mouth (19 ppt). The length of the Malaya Samoroda River is 10.3 km, the catchment area is 48.7 km², the channel is 15.0–50.0 m wide, and the depth is 0.05–0.25 m (Gusakov, 2019). The salinity level (at the time of sampling) both in the middle course and in the mouth was constant and reached 85 ppt. Salinity was measured using a Master S–28a portable refractometer (Atago, Japan). The Bolshaya Samoroda River was classified as mixohaline (middle course – mesohaline, mouth – polyhaline), the Malaya Samoroda River is hypersaline according to the Venice system (1958).

Water samples of 0.5 L were 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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. filters by a combined method, including mechanical homogenization and Brans 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.

DNA libraries were prepared according to Illumina workflow (http://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) with primers targeting the V4 region of the 16S rRNA gene: forward TATACGCAAGCGCTCTTCTAAT and reverse TACTACGACGCTCTTCTAAT and with primers to the variable region V3–V4 of the 16S rRNA gene S-D-BCI-A-21 b (Klindworth et al., 2013).
Sequencing was performed on a MiSeq sequencer (Illumina, USA) using MiSeq Reagent v3 reagent kit for paired-end sequencing 2×300 bp in the Center of Shared Scientific Equipment “Persistence of Microorganisms” of the Institute for Cellular and Intracellular Symbiosis of the Ural Branch of the Russian Academy of Sciences.

Paired-end reads were merged with a minimal overlap of 40 bp and a p-value of 0.0001 using PEAR v. 0.9.10 (Zhang et al., 2014). Evaluation of the filtering quality was carried out with FastQC v.0.11.7. Quality filtering and amplion size selection (300 bp – minimal size for the 18S rRNA gene, 420 bp – minimal size for the 16S rRNA gene) were conducted using USEARCH v.9 (Edgar, 2013). During the filtering reads with Ns or an overall mean, Q-score <20 were discarded. As a result of dereplication and clustering with USEARCH, operational taxonomic units (OTUs) were formed, while singlets and doubletons were removed. OTUs were clustered using a similarity threshold of 97% between sequences to classify microorganisms at the species level. Chimeric sequences were detected and removed using USEARCH during the clustering phase.

Results

Morphological and genetic diversity of microalgae and Cyanobacteria of the Bolshaya Samoroda River. 91 taxa of microorganisms were identified in the assemblage of microalgae and Cyanobacteria of the middle course of the Bolshaya Samoroda River while using an integrated approach. Of this number, 87 taxa were autotrophic protists belonging to the phyla Bacillariophyta (24 OTUs, similarity 99.8% from database GenBank (NCBI) is identified as Chaetoceros sp. (JQ315647.1). They were not detected microscopically. Among the subdominants, Oscillatoria sp. (KT386323.1; similarity 99.8%), Chlorococcum oleofaciens (JR0977821; similarity 97.0%), Chlorococcum oleofaciens Trainor & Bold (MT9216291; similarity 99.8%), Ulva sp. (LC1313391; similarity 99.8%).

It should be noted that the representatives of only few taxa were found by both NGS and LM. These include Surirella strigilata Turtuin, S. ovalis Brebisson, Tabularia fasciculata (C. Agardh) D. M. Williams & Round, Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot, Cyclotella meneghiniana Kützing, Anaphora commutata Grunow, Halamphora conoides (C. Agardh) Levkov, Tryblionella apiculata W. Gregory (C. Agardh) Lange-Bertalot, Gomphonema parvulum (Kützing) Kützing, Pleurosigma sp., Tetraselmis sp.

The complex of dominant species identified by the LM and NGS differed significantly. Thus, according to the LM data, Hippodonta hungarica (Grunow) Lange-Bertalot, Metezchin & Wilkowski (= Navicula capitata var. hungarica (Grunow) R. Ross) (64.7% of the total abundance) and Tryblionella hungarica (Grunow) Freguellii (= Nitzschia hungarica Grunow) (12.0%) were predominant (Fig. 4b–d). The group of subdominants included T. fasciculata, Fragilaria sp., R. abbreviata, Navicula sp., Navi-

Fig. 2. Taxonomic structure of microalgal assemblages in the Bolshaya Samoroda River: LM – light microscopy, NGS – next-generation sequencing.

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According to the NGS data, as well as the LM results, the DNA libraries of the water samples of the mouth of the river was distinguished by a large number of OTUs. 8 OTUs were identified. Five of them were classified as representatives from the order Synechococcales of the family Synechococaceae and three sequences were identified only at the phylum level. The DNA libraries of the water samples of the middle course included only two unclassified representatives of Cyanobacteria.

Morphological and genetic diversity of microalgae and Cyanobacteria of the Malaya Samoroda River. The algal assemblage of the middle course of the Malaya Samoroda River was represented by 27 taxa, 23 of which were autotrophic protists and 4 – Cyanobacteria. 10 taxa ranked below the genus from the phyla Chlorophyta (8 species) and Bacillariophyta (2), as well as 4 taxa of Cyanobacteria were found by LM method. The genetic diversity of microalgae of the middle course of the Malaya Samoroda River was slightly higher. The DNA libraries of the water samples of the middle course included 20 OTUs belonging to the phyla Chlorophyta (17 OTUs, 99.9% of the total number of reads), Bacillariophyta (2 OTUs) and Cryptophyta (1 OUT, Fig. 5). Only one sequence corresponding to the phylum Cyanobacteria was found with NGS. The closest homologues of this OTU with a similarity 99.0% in the GenBank database were the sequences deposited: Geitlerinema sp. SAS11146 (KX359357.1) and Phormidium sp. GLBOAO (KY363612.1).

It should be noted that the species of algae of the genus Tetraselmis F. Stein, Asteromonas A. Artari, Dunaliella Teodoresco and also Anagnostidinema amphibium (C. Agardh ex Gomont) Stručeky, Bohanicka, J. R. Johansen (=Phormidium amphibium (C. Agardh ex Gomont) Anagnostidis & Kornárek, Geitlerinema amphibium (C. Agardh ex Gomont) Anagnostidis) were found simultaneously by both methods.

Representatives of Chlorophyta were the most abundant in the algal assemblage of the middle course of the Malaya Samoroda River (Fig. 4a). The dominant complex, according to LM data, included Tetraselmis arnoldii (Proskhina-Lavrenko) R. E. Norris, Hori & Chihara, T. tetrathele (West) Butcher, Tetraselmis sp., which together made up 89.4% of the total abundance of the algal assemblage. The group of subdominants was formed by T. cordiformis (H. J. Carter) F. Stein and T. contracta (N. Carter) Butcher. Similar results were obtained by the NGS method. More than a half (12 OTUs) of the total number of OTUs belonged to the genus Tetraselmis (Chlorophyta). At the same time, the absolute predominance (96.9% of the total number of series) of the sequence whose closest homologue in the GenBank database (NCBI) is T. indica Arora & Anil (HQ651184.3, 100% identity) was noted.

The assemblage of microalgae and Cyanobacteria of the mouth of the river didn’t differ significantly from the middle course. 24 taxa of micro-organisms were found in the algae flora of the river mouth. Among them, 20 taxa were accounted by microalgae of the phyla Chlorophyta and Bacillariophyta and 4 – Cyanobacteria. Here, the representatives of Chlorophyta that belonged to the genus Tetraselmis, as in the algal assemblage of the middle course of the river, were the most abundant. According to LM data, the dominant complex included T. cordiformis, T. arnoldii, T. tetrathele. Chlamydononas sp. is noted as a subdominant. According to the NGS, 96.0% of the total number of reads accounted for the sequence whose closest homologue is T. indica (HQ651184.3).
Fig. 6. Venn’s diagram showing common and specific taxa of microalgae and Cyanobacteria revealed by light microscopy and next-generation sequencing in the Malaya Samoroda River and Bolshaya Samoroda River: 1 – assemblages of microalgae and Cyanobacteria in the middle course of the Bolsyha Samoroda River, 2 – assemblages of microalgae and Cyanobacteria in the mouth of the Bolsyha Samoroda River, 3 – assemblages of microalgae and Cyanobacteria in the middle course of the Malaya Samoroda River, 4 – assemblages of microalgae and Cyanobacteria in the mouth of the Malaya Samoroda River.

Table 1

| Taxa                        | Locality       | LM | NGS            | Similarity (%) |
|-----------------------------|----------------|----|----------------|----------------|
| Euglena buchanica I. K. Kozelev | B.S.R., m.c.   | +  | NGS            |                |
| Lepocinclis tripitris (Dujardin) B. Marin & Melkonian | B.S.R., m.c.   | +  | NGS            |                |
| Phacodictyon thomasi K. Hünne | B.S.R., m.c.   | +  | NGS            |                |
| Ph. parvus G. A. Kolbs       | B.S.R., m.c.   | +  | NGS            |                |
| Pseudochromatocystis ovata (Chodat) D. J. Hibberd | B.S.R., m.c.   | +  | NGS            |                |
| Characopsis sp.              | B.S.R., m.c.   | +  | NGS            |                |
| Poterioreochromonas stipitata Scherfli | B.S.R., m.c.   | +  | NGS            |                |
| Chrysosolenmonas sp.         | B.S.R., m.c.   | +  | NGS            |                |
| Pavlova sp.                  | B.S.R., m.c.   | +  | NGS            |                |
| Hamiselmis cryptochromatica C. E. Lane & J. M. Archibald | B.S.R., m.c.   | +  | NGS            |                |
| Rhodomonas sp.               | B.S.R., m.c.   | +  | NGS            |                |
| H. phil J. A. Deane          | B.S.R., m.c.   | +  | H. phil (M31321.1) | 99.8 |
| Borodinella sp.              | B.S.R., m.c.   | +  | Borodinella taxon(KM202019.1) | 98.1 |
| Chlorochytrium lemnue Cohn   | B.S.R., m.c.   | +  | Chlorochytrium lemnue (HE860281.1) | 98.8 |
| Caesitella sp.               | B.S.R., m.c.   | +  | Caesitella pescheri (LN870281.1) | 99.5 |
| Halochlorococcus sp.         | B.S.R., m.c.   | +  | Halochlorococcus porphyrae (DQ621520.2) | 99.3 |
| Tetraselmis cordiformis (H. J. Carter) F. Stein | B.S.R., m.c.   | +  | Tetraselmis sp. MA-2011 (T. indica) (H6515184.1) | 100.0 |
| T. arschnii (Proshkina-Lavrenko) R. E. Norris, Hori & Chihara | M.S.R., m.c.   | +  | Tetraselmis sp. MA-2011 (T. indica) (H6515184.1) | 100.0 |
| T. thalassae (Bothe) Bouchan | M.S.R., m.c.   | +  | Tetraselmis sp. MA-2011 (T. indica) (H6515184.1) | 100.0 |
| Pyrobrya elongata Koosikov    | M.S.R., m.c.   | +  | Pyrobrya elongata (LC0934681.1) | 99.0 |
| Oscillatoria simplicissima Gomont | M.S.R., m.c.   | +  | Oscillatoria simplicissima (KM202019.1) | 99.0 |
| Synochococcus elongatus (Nageli) Nageli | M.S.R., m.c.   | +  | Synochococcus elongatus (Nageli) Nageli | 99.0 |
| Achnanthidium constrictum (Santer) Hasler, Dvorak & Pouliková | B.S.R., m.c.   | +  | Achnanthidium constrictum (Santer) Hasler, Dvorak & Pouliková | 99.0 |

Comparison of the species diversity of algae flora of the Malaya Samoroda River and Bolsyha Samoroda River. Despite the territorial proximity and similar climatic conditions, significant differences in the mineralization level probably determined the specificity of the taxonomic structure and species diversity of the algal communities of the studied watercourses. In general, less species diversity with a pronounced dominance of halophilic species was characterized by the assemblage of microalgae and Cyanobacteria of the Malaya Samoroda River. The comparative analysis showed that only six taxa (Bolamnemonas rauniaensis Ettl, Chaetoceros sp., H. phi, Dunaliella sp., Nannochloris sp., T. cordiformis (LM) / T. indica (NGS)) were common to the algae flora of the studied rivers, and only three of them (Dunaliella sp., Nannochloris sp., T. cordiformis (LM) / T. indica (NGS)) were registered at each point of sampling (Fig. 6). Compared to the previously reported data (Burkova, 2012, 2015; Gorokhova & Zinchenko, 2014; Yatsenko-Stepanova et al., 2015; Selivanova et al., 2019) our study revealed new taxa for the algae flora of the saline Elton rivers. Thus, 18 taxa belonging to the phyla Chlorophyta (5), Ochrophyta (4), Euglenozoa (4), Cryptophyta (3), Haptophyta (1), Cyanobacteria (1) have been recorded in the Bolsyha Samoroda River for the first time. Seven taxa have been detected for the first time in the algal and cyanobacterial assemblages of the Malaya Samoroda River from the phyla Chlorophyta (4), Cryptophyta (1), and Cyanobacteria (2) (Table 1).

Discussion

This study represents one of the first comparative analyses of taxonomic structure and species diversity of microalgae and Cyanobacteria of the saline Elton rivers employing both morphology-based and molecular methods. It should be noted that in all the samples that we studied the genetic diversity significantly exceeded the diversity estimated by LM method. This is also indicated by other authors who conducted studies using the traditional morphological method and high-throughput sequencing (Groendahl et al., 2017; Rivera et al., 2018a, b; Gao et al., 2018). One of the reasons for the observed differences may be the high sensitivity of the NGS method, which allows detection of low-abundance taxa (Groendahl et al., 2017). Thus, only four genera of microorganisms were registered by both methods in the assemblage of microalgae and Cyanobacteria of the middle course of the Malaya Samoroda River, while Achnanthidium sp,
microscopically. At the same time, it should be noted that the Euglenozoa registered in the assemblage of microalgae of the middle course of the Bolshaya Samoroda River by morphological analysis were not detected by high-throughput sequencing. This discrepancy may be due to the use of universal primers, which (compared to selective primer pairs) due to insufficient coverage reveal only half of the OTUs (Lentenda et al., 2018; Selivanova et al., 2019). In addition, the use of universal primers does not allow for a clear differentiation of species within the genus (Arora et al., 2013). So, for example, DNA libraries of the water samples of the middle course and the mouth of the Malaya Samoroda River included a sequence identified as Dunaliella sp. The closest homologues of this OTU with a similarity 99.0% in the GenBank database were the sequences such as Dunaliella primolecta strain SAG 183.80 (KJ460749.1), D. salina strain SAG 184.80 (KJ460749.1), D. tertiolecta strain SAG 13.86 (EF473737.1), D. parva strain SAG 19-1 (DQ009763.1). For the same reason, 12 OTUs in the DNA libraries of the water samples of the middle course and the mouth of the Malaya Samoroda River were identified only as Tetraedrum sp.

It is also noteworthy that the complex of dominants and subdominants of the algal assemblage of the middle course of the Bolshaya Samoroda River, characterized by the NGS, significantly differed from the similar one estimated by the LM. Thalassiosira sp. predominated in the algae assemblage according to the results of the NGS. Whereas according to the LM data, pennate diatoms (H. capitata, T. hungarica) dominated, and centric forms were represented by single specimens. The revealed differences probably may be related with the detection of "extracellular" DNA extracted from dead cells and capable of persisting for a long time in aquatic ecosystems (Pawlowski et al., 2018). On the other hand, the LM method is also not devoid of error. The diatom analysis could take into account the forms represented by frustules of dead diatoms, which, due to their siliceous composition, can be retained for a long time in the reservoir (Rivera et al., 2018b; Selivanova et al., 2019). This can be confirmed by the fact that, for example, Navicula sp. and Nitzschia sp. were detected in the assemblage of microalgae of the middle course of the Malaya Samoroda River only by the LM method, but they were not registered with NGS.

At the same time, it should be noted that the dominant species of the mouth of the Bolshaya Samoroda River identified by us as Cryptophyceae sp., according to the LM data, has a morphological similarity with the predominant according to the NGS data Hanausia phi J. A. Deane (U531261.1). The first description of the H. phi is given in Deane et al. (1998). Thus, the length of the algae cells which we found varies from 7 to 10 µm, and the width is 5 µm. There are two flagella at the anterior end of the cell at the base of the reservoir (funnel). The reservoir (funnel) is lined with ejectosomes and reaches to the middle of the cell. The shape of the cell is obovate with a truncated anterior end. The posterior end of the cell is somewhat drawn back, forming a narrow tail, which according to Deane et al. (1998) was observed in H. phi during the period of intensive growth of the culture. Morphological similarity between species which we found and H. phi allows us to conclude that the data about a dominant species estimated by the LM and NGS methods are comparable.

It should also be noted that in all previous studies performed using the LM method (Burkova, 2012, 2016; Yatsenko-Stepanova et al., 2015; Gorokhova, 2018), representatives of golden algae (Cryptophyceae) were not identified in the algae flora of saline Elton lakes, with the exception of Salpingoeca fregentissima (Zacharias) Lemmermann (Burkova, 2016). But, now, according to modern taxonomy, S. fregentissima is classified as a heterotrophic flagellate of the class Chaoallflagellatae (Protozoa) (Guiry & Guiry, 2021). The integrated approach used by us allowed us to characterize the diversity of Cryptophyceae of the studied watercourses. Microorganisms of this class were registered only in the algal communities of the Bolshaya Samoroda River, which probably indicates their limited range of halotolerance and their inability to exist in hyperhaline conditions. The main part of the Cryptophyceae was identified by the NGS method (total 8 sequences, six of which were identified at the genus level as Ochromonas sp., and also Chrysoleidomonas sp. and Chromulina sp.) and only one taxon (Pseudokeliothrix entii W. Conrad) has been detected by LM. Similarly, for the first time the representatives of Xanthophyceae (Characiopsis sp.) and Haptophyta (Pavlova sp.) were found in communities of the mouth and middle course of the Bolshaya Samoroda River by NGS. Thus, it should be emphasized that each of the methods under consideration has its own specific disadvantages. At the same time, the use of the NGS method in combination with the traditional morphological method in the analysis of algae biodiversity increases the reliability of the results obtained.

A comparison of the species diversity of the algae flora of the Bolshaya Samoroda and Malaya Samoroda Rivers revealed an extremely low level of similarity (6 common taxa). Communities of microalgae and Cyanobacteria of the Bolshaya Samoroda River (mixohaline river) were characterized by high species diversity, whereas the algae flora of the hyperhaline Malaya Samoroda River was represented by only three phyla of microalgae and Cyanobacteria. Our results are in good agreement with the data of other authors, which also indicate decrease in species richness and simplification of the structure of algae flora in hyperhaline conditions (Gorokhova & Zinchenko, 2014; Afonina & Tashlykova, 2016; Skarlato & Telesh, 2017). Only three taxa were identified in each point of sampling (in the range of salinity from 10 to 85 ppt). These are Dunaliella sp., Nanochloris sp., T. cordiformis (LM)/T. indicus (NGS). Algae of the above genera are well studied, as they are considered today as promising sources of carotenoids and polysaturated fatty acids (Solovchenko et al., 2015; Mohammadi et al., 2015; Almutairi & Toulibah, 2017). In particular, extensive material has been accumulated indicating a wide range of halotolerances of these algae (Masuy et al., 2007; Saadhoui et al., 2016; Ishika et al., 2018), which explains their detection at each point of sampling.

Conclusion

Thus, for the first time, an assessment was made of the species diversity of the assemblages of microalgae and Cyanobacteria of the saline rivers of the Elton Lake Basin (Malaya Samoroda River and Bolshaya Samoroda River) by a combined approach using morphological analysis and high-throughput sequencing. The species diversity revealed with NGS was higher compared to that estimated by the morphological method. Next-generation sequencing allowed us to refine and expand the list of microalgae taxa in the studied saline rivers due to detection of difficult species to identify, low-abundance taxa, as well as extremely small-cell forms. Generally, 91 taxa of microorganisms were identified by integrated approach in the assemblages of microalgae and Cyanobacteria in the middle course of the Bolshaya Samoroda River, and 60 taxa—in the river mouth. The species diversity of those assemblages in the hyper saline Malaya Samoroda River was lower: 27 taxa from the middle course and 23 taxa from the mouth. Eighteen taxa have been recorded for the first time in the Bolshaya Samoroda River, belonging to the phyla Chlorophyta (5), Ochrophyta (4), Euglenozoa (4), Cryptophyta (3), Haptophyta (1), Cyanobacteria (1). Seven taxa have been detected for the first time in the algal and cyanobacterial assemblages of the Malaya Samoroda River from the phyla Chlorophyta (4), Cryptophyta (1), and Cyanobacteria (2). Some discrepancies between the data obtained by light microscopy and next-generation sequencing indicate the advantage of simultaneous use of both methods for study of algal communities.

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References

Afonina, E. Y., & Tashlykova, N. A. (2018). Plankton community and the relationship with the environment in saline lakes of Onon-Torey plain, Northeastern Mongolia. Saudi Journal of Biological Sciences, 25, 399–408. Almutairi, A. W., & Toulibah, H. E. (2017). Effect of salinity and pH on fatty acid profile of the green algae Tetraselmis suecica. Journal of Petroleum and Environmental Biotechnology, 8(3), 1–6.
Rivera, S. F., Vasselon, V., Jacquet, S., Bouchez, A., Ariztegui, D., & Rimet, F. (2018b). Metabarcoding of lake benthic diatoms: From structure assemblages to ecological assessment. Hydrobiologia, 807, 37–51.

Saadaoui, I., Ghazal, G. A., Boumint, T., Khlaifi, F. A., Jabri, H. A., & Potts, M. (2016). Evidence of thermo and halotolerant *Nannochloris* isolate suitable for biodiesel production in Qatar Culture Collection of Cyanobacteria and Microalgae. Algal Research, 14, 39–47.

Selivanova, E. A., Ignatenko, M. E., Yatsenko-Stepanova, T. N., & Plotnikov, A. O. (2019). Diatom assemblages of the brackish Bolshaya Samoroda River (Russia) studied via light microscopy and DNA metabarcoding. Protistology, 13(4), 215–235.

Shitikov, V. K., Rosenberg, G. S., & Zinchenko, T. D. (2003). Kolichestvennaya gidroekologiya: Metody sistemnoj identifikacii [Quantitative hydroecology: Methods of system identification]. IEWB RAS, Togliatti (in Russian).

Skarlato, S. O., & Telesh, I. V. (2017). Razvitie koncepcii maksimal’nogo raznoobraziya protistov v zone kriticheskoj solenosti vody [Development of the concept of maximum diversity of protists in the zone of critical salinity of water]. Biology of the Sea, 43(1), 3–14 (in Russian).

Solovenko, A. E., Selivanova, E. A., Chakanov, K. A., Sidorov, R. A., Nemtseva, N. V., & Lobokova, E. S. (2015). Indukciya vtorichnogo karotinogeneza u novyh galofil’nyh mikrovodoroslej iz roda *Dunaliella* (Chlorophyceae) [Induction of secondary carotenogenesis in new halophile microalgae from genus *Dunaliella* (Chlorophyceae)]. Biochemistry, 80, 1508–1513 (in Russian).

Telesh, I., Schubert, H., & Skarlato, S. (2013). Life in the salinity gradient: Discovering mechanisms behind a new biodiversity pattern. Estuarine, Coastal and Shelf Science, 135, 317–327.

Tsarenko, P. M. (1990). Opredelitel’ hlorokokkovyh vodoroslej Ukrainskoj SSR [Determinant of Chlorococcal algae of the Ukrainian SSR]. Naukova Dumka, Kiev (in Russian).

Vasser, S. P., Kondratyeva, N. V., Masyuk, N. P., Palamar-Mordvintseva, G. M., Vetrova, Z. I., Kordyum, E. L., Moshkova, N. A., Prakhodkova, L. P., Kovalenko, O. V., Stupina, V. V., Tsarenko, P. M., Junger, V. P., Radchenko, M. I., Vinogradova, O. N., Bulkhityanov, L. N., & Razumina, L. F. (1989). Vodorosli. Spravochnik [Algae. Guide]. Naukova Dumka, Kiev (in Russian).

Wangensteen, O. S., Palacin, C., Giardolila, M., & Turon, X. (2018). DNA metabarcoding of littoral hardbottom communities: High diversity and database gaps revealed by two molecular markers. PeerJ, 6, e4705.

Yatsenko-Stepanova, T. N., Ignatenko, M. E., Nemtseva, N. V., & Goredkhova, O. G. (2015). Avtotrofnye mikroorganizmy ust’evyh uchastkov vodotokov sistemy ozero El’ton [Autotrophic microorganisms of the mouth parts of the watercourses in the system of Lake Elton]. Arid Ecosystems, 21(2), 47–54 (in Russian).

Zhan, A., Hulak, M., Sylvester, F., Huang, X., Adeshayo, A. A., Abbott, C. L., Adamowicz, S. J., Heath, D. D., Cristescu, M. E., & Muclisac, H. J. (2013). High sensitivity of 454 pyrosequencing for detection of rare species in aquatic communities. Methods in Ecology and Evolution, 4, 558–565.

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina paired-end read merger. Bioinformatics, 30(5), 614–620.

Zinchenko, T. D., Golovatyuk, L. V., Abrosimova, E. V., & Popchenko, T. V. (2017). Macrozoo-benthos in saline rivers in the Lake Elton Basin: Spatial and temporal dynamics. Inland Water Biology, 10(4), 384–398.