The Species of Diversity of Sredniy Kaban Lake by 18s Rrna of Hydrobionts on next-Generation Sequencing Method

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Abstract
The paper presents the freshwater Sredniy Kaban Lake species diversity study results, (Kazan, Russia) by the 18S rRNA gene based on the next-generation sequencing method. Lakes Sredniy Kaban, Verhniy Kaban, and Nizhniy Kaban are included in the system of urban lakes Kaban and are polluted lakes according to ecological studies. Sequences of 18S rRNA gene fragment of freshwater Sredniy Kaban lake hydrobionts in FASTQ format were submitted to international SRA database on the NCBI website SRR7510987, SRR7465570, SRR7516496. A total of 44292 (2016) and 36631 (2017) high-quality reads were obtained; 13.1% (2016) and 58% (2017) of the hydrobionts was classified to the kingdom, while 12.75% (2016) and 57.6% (2017) was classified to the phylum level, 12.7% (2016) and 56.6% (2017) was classified to the class level, 7.9% (2016) and 56.2% (2017) was classified to the order level, 56.1% (2016) was classified to the family level, 56% (2017) was classified to the genus level, and 54.7% (2017) was classified to the species level. The analysis of the metagenomic data for Sredniy Kaban Lake shows that indicator organisms identified by the 18S rRNA gene are grouped between o-saprobity and b-mesosaprobity. Sredniy Kaban Lake can be characterized by water quality as a transition from a polluted to a clean state.

Keywords: Saprobity, gene 18S rRNA, Next-generation sequencing, Freshwater lake, Ecology

1 Introduction
Sredniy Kaban Lake is included in the system of city lakes Kaban along with lakes Verhniy Kaban and Nizhniy Kaban. On shore of Sredniy Kaban Lake, there is a rowing center, which is a place for training athletes in all types of rowing. According to ecological studies, Sredniy Kaban Lake is a polluted lake.

One of the methods for assessing the ecological state of water reservoirs is the bioindication method (1). Traditionally, the identification of species in a water sample is carried out visually using a microscope. The development of modern methods of molecular genetics and bioinformatics allows identifying organisms by marker genes. Currently, next-generation sequencing methods have gained rapid development. Metagenomics can provide valuable information on the functional ecology of environmental communities (2). We previously used metagenomic DNA sequencing to analyze species diversity by COI marker gene of animal organisms to assess the ecological status of freshwater Sredniy Kaban Lake by bioindication (3). In contrast to the marker genes COI and rbcL, 18S rRNA gene is present in all eukaryotes. This is one of the most conserved genes that is used to determine the systematic position of the body and the time of discrepancy with similar species based on the analysis of similarities and differences in rRNA sequences (4). For eukaryotes, it is most convenient to analyze 18S rRNA of the three types of rRNA. The paper presents the results of the study of the species diversity of the freshwater Sredniy Kaban Lake (Kazan, Russia) by 18S rRNA gene of freshwater organisms based on the next-generation sequencing method. Lakes Sredniy Kaban, Verhniy Kaban, and Nizhniy Kaban are included in the system of urban lakes Kaban. According to ecological studies, Kaban Lakes are polluted lakes.

2 Methods
Sampling from Sredniy Kaban Lake (Kazan, Russia) was carried out in 2016-2017 in accordance with the standard hydrobiological methods (5). DNA isolation from the sediment obtained by centrifugation of 50 ml of the sample at a speed of 10000 g for 15 min was carried out using a set of FAST DNA Kit (MP biomedicals) according to the manufacturer's Protocol. Amplification of the extracted DNA was carried out by the Phusion High-Fidelity DNA polymerase (Thermo Fisher) using primers (Table 1).

The second PCR cycle was performed to index samples (Nextera XT indices). Purification of PCR products was

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performed using Agencourt AMPure XP beads (Beckman Coulter). The obtained DNA libraries were sequenced on an Illumina MiSeq equipment (MiSeq Reagent kit v3). Metagenomic data were submitted to the international SRA database on the NCBI website with the numbers SRR7510987, SRR7465570, SRR7516496 (6). After filtering reads by quality, trimming of service sequences, and removing chimeric sequences, obtained nucleotide sequences of the hydrobionts’ 18S rRNA gene were aligned with the BLAST program to determine the taxonomic composition. The Krona chart (7) and GraphPad (8) software are used for charting.

Table 1: Primers for PCR of 18S rRNA gene

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR1_Illumina</td>
<td>5’- tcgtcgccagtcagatgtgataaagacagattaccggtctgc-3’</td>
</tr>
<tr>
<td>SR1r_Illumina</td>
<td>5’- gtctcgtggtcggatagatgtgataaagacagattacgggt tgcgtgcacctgtgc-3’</td>
</tr>
</tbody>
</table>

3 Results and Discussion

In 2016-2017 the next-generation sequencing method was used to identify hydrobionts by 18S rRNA gene from Sredniy Kaban Lake. It is a lake about 3 km in length and 0.6 km in wide and has the geographical coordinates - 55.750352°N and 49.146739°E (Fig. 1). Sredniy Kaban Lake is located in the center of an industrial city, thus experiencing anthropogenic stress. On the shore of Sredniy Kaban Lake, there is a large sports complex - the Rowing Sports Center. According to ecological studies, Sredniy Kaban Lake is a polluted lake.

Figure 1: Sredniy Kaban Lake (Kazan city, Russia) [https://www.sport-line.ru/objects/grebyne-kanaly/76/]

3.1 Krona chart of the hydrobionts represented by 18S rRNA gene amplicon-based species diversity

The percentage distribution of hydrobionts of Sredniy Kaban Lake by reads for 2016, (Unclassified 87%) is shown in Fig. 2. Percentage distribution of hydrobionts of Sredniy Kaban Lake by reads for 2017, (Unclassified 42%) is shown in Fig. 3. Each circle represents the kingdom, phylum, class, order, family, genus, and species from the inside to the outside of the circle, respectively, indicated by percentage of diversity.

Figure 2: The percentage of 18S rRNA hydrobionts by reads of Sredniy Kaban Lake (2016)
3.2 The percentage of 18S rRNA hydrobionts by reads of Sredniy Kaban Lake (2016)

The percentage of species diversity of 18S rRNA hydrobionts of Sredniy Kaban Lake by kingdom (2016-2017), shown in Figure 4. As can be seen from Fig. 4, the most numerous at the kingdom level, respectively, by year are Chromista (90.48%/51.90%), Metazoa (4.76%/29.52%) and Viridiplantae – 9.52% (2017) by species diversity.

The percentage of species diversity of 18S rRNA hydrobionts of Sredniy Kaban Lake by kingdom (2016-2017) shown in Figure 5. As can be seen in Fig. 5 among the hydrobionts, the most numerous according to the reads are Chromista (96.31% / 55.76%) and Metazoa - 43.6% (2017).

3.3 The percentage of 18S rRNA hydrobionts by reads of Sredniy Kaban Lake (2016)

The percentage of species diversity of 18S rRNA hydrobionts of Sredniy Kaban Lake by kingdom (2016-2017), shown in Figure 5. As can be seen from Fig. 6, the most numerous at the phylum level for 2016 are Cryptophyta (8%), Ciliophora (2.5%), Cercozoa (1.2%), and Unclassified (87%); for 2017 - Dinoflagellata (30%),...
Arthropoda (23.3%), Ciliophora (1.64%), Rotifera (1.54%) and Unclassified (42.4%).

### 3.4 The percentage of 18S rRNA hydrobionts of Sredniy Kaban Lake by species

The species diversity of hydrobionts in 2017 is 54.7% in reads of the total number of organisms. Ten hydrobiont species are included on top by reads - Ceratium hirundinella (52.7%), Bythotrephes cederstroemi (15.0%), Cyclops insignis (3.9%), Chydorus sphaericus (3.3%), Cyclops kolensis (2.0%), Keratella cochlearis (2.0%), Acanthocyclops viridis (1.5%), Daphnia pulex (0.5%), Filinia longiseta (0.5%), Erpobdella obscura (0.4%) (Fig 7).

140 species from Sredniy Kaban Lake were identified by 18S rRNA gene. Among them, there are 22 hydrobionts with saprobities in Sladechek’s list (9). Table 2 shows the saprobionts of Sredniy Kaban Lake.

**Table 2: Hydrobionts with saprobities in Sladechek’s list of Sredniy Kaban Lake**

<table>
<thead>
<tr>
<th>Species</th>
<th>Saprobity</th>
<th>Reads %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acroperus harpae, Amphora ovalis</td>
<td>o-b</td>
<td>3.993</td>
</tr>
<tr>
<td>Cyclops insignis, Leptodora kindtii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tintinnidium fluviatile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinocephalus vetulus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chydorus sphaericus, Cryptomonas curvata</td>
<td>b</td>
<td>3.515</td>
</tr>
<tr>
<td>Cocconeis placentula, Cymbella cistula</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vorticella mayeri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratella cochlearis</td>
<td>b-o</td>
<td>1.954</td>
</tr>
<tr>
<td>Daphnia pulex</td>
<td>a</td>
<td>0.469</td>
</tr>
<tr>
<td>Brachionus calyciflorus, Coleps hirtus</td>
<td>b-a</td>
<td>0.254</td>
</tr>
<tr>
<td>Acanthotheberis curvostris</td>
<td>o</td>
<td>0.140</td>
</tr>
<tr>
<td>Alomopsis elongata, Lepadella rhomboides</td>
<td>p</td>
<td>0.140</td>
</tr>
<tr>
<td>Acineria incurvata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclotella meneghiniana</td>
<td>a-b</td>
<td>0.005</td>
</tr>
</tbody>
</table>

o – oligosaprobity (clean), o-b, b-o – between oligosaprobity and b-mesosaprobity, b-mesosaprobity (polluted), a-b – between a- and b-mesosaprobity, a-mesosaprobity (very polluted), p-saprobity (dirty)
produce any toxins (17). Cryptomonas are also photolithotrophs that contribute to oxygenic carbon fixation making them highly critical to the carbon levels of freshwater reservoirs (18).

13 Freshwater species of Ciliata. Vorticella habitats may include moist soil, mud and plant roots. They are known to feed on bacteria and can also form extracellular associations with mosquitoes, nematodes, prawns, and tadpoles (19).

12 Keratella cochlearis is a rotifer (20). The planktonic animal occurs worldwide in freshwater and marine habitats.

11 The planktonic animal occurs in freshwaters. It is commonly used as a model organism in toxicology, ecology and evolutionary biology (22).

16 The C. hirtus does not possess algal symbionts and feeds directly on other microbes (23). Coleps feeds on bacteria, algae, flagellates, living and dead ciliates, animal and plant tissues (24,29).

18 A. curvirostris is a littoral macrofiltrator. Since it occurs with the highest frequency in acid water, it is used as an indicator of acid water (pH <5.0). It is most common in ponds and small lakes, often with a high content of humus (15).

19 A. elongata is one of the most common littoral species. It occurs in waters of different pH from 3.9 to 8.2. A. elongata is slightly more common in electrolyte poor water than in electrolyte-rich water (15).

20 Lepadella rhomboidis and Acineria incurvata are species of Ciliata. The ciliates are a group of protozoans characterized by the presence of hair-like organelles called cilia (25,30,31).

22 Cyclotella is a centric diatom. Cyclotella occurs in stagnant waters. The nutrient content does not matter. They live both floating (plankton) and on the ground (benthos). C. meneghiniana was firstly described by Kützing (26-28,33).

Fig. 8 shows the percentage of saprobionts of Sredniy Kaban Lake by reads for 2017.

As can be seen from Fig. 8, most indicator organisms are grouped between o-saprobity and beta-mesosaprobity. The water quality of Sredniy Kaban Lake is characterized as a transition from a polluted to a clean state.

4 Summary

Based on the results of the study, the next-generation sequencing method was used to identify hydrobionts by the gene 18S rDNA for Sredniy Kaban Lake for 2016-2017.

A total of 44292 (2016) and 36631 (2017) high-quality reads were obtained; 13.1% (2016) and 58% (2017) of the hydrobionts was classified to the kingdom, while 12.75% (2016) and 57.6% (2017) was classified to the phylum level, 12.7% (2016) and 56.6% (2017) was classified to the class level, 7.9% (2016) and 56.2% (2017) was classified to the order level, 56.1% (2017) was classified to the family level, 56% (2017) was classified to the genus level, and 54.7% (2017) was classified to the species level.

Altogether, 140 species of hydrobionts were identified, among which Sladechek saprobity was determined for 22 species. Indicator hydrobionts are of the greatest importance for reads - Cyclops insignis, o-b (4%), Chydrorus sphaericus, b (3%), Keratella cochlearis, b-o (2%). Thus, the quality of the water of Sredniy Kaban Lake is between a clean and polluted.

5 Conclusions

The use of the next-generation sequencing method allows the identification of a large number of hydrobionts. The method can be used for bioindication in assessing the ecological status of water reservoirs. The results obtained are of great practical interest in the field of monitoring of water objects.

Acknowledgments

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