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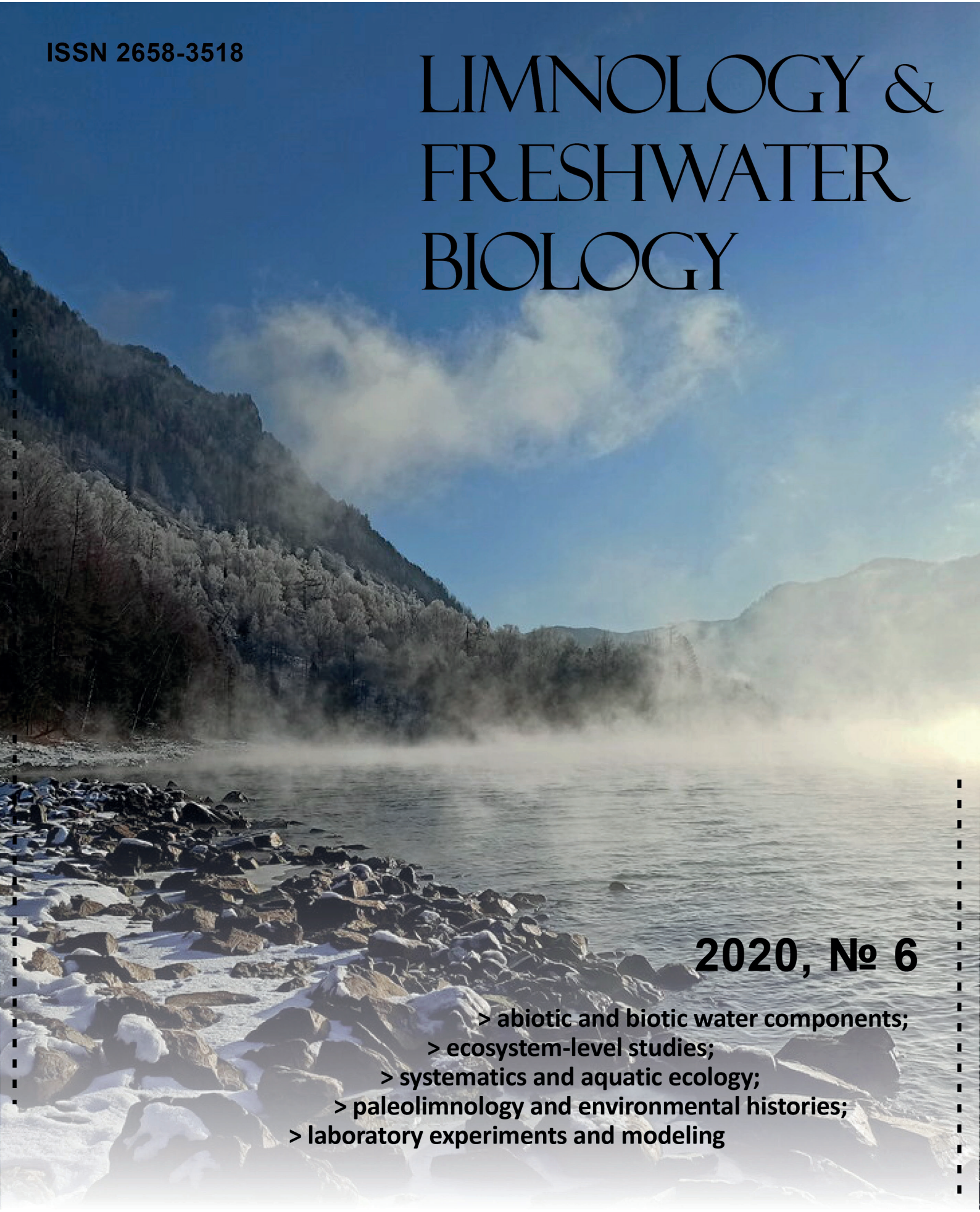

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> ecosystem-level studies;
> systematics and aquatic ecology;
> paleolimnology and environmental histories;
> laboratory experiments and modeling

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Short communication

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Development and deployment of autonomous water level monitoring system in the lower and upper sections of the Slyudyanka River

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ABSTRACT. The article presents the autonomous hydrometeorological station for organizing networks of monitoring the hydrological conditions in water bodies and collecting the related information, such as meteorological, hydrophysical, etc. The station showed high autonomy and accuracy in measuring water level in a water body. It also has an integrated GSM module for remote data transmission. It was successfully tested during a trial installation on the Slyudyanka River (Lake Baikal basin) in the autumn of 2020, which allowed us to characterize the behaviour of the main hydrological parameters of the watercourse.

Keywords: automated hydrometeorological station, river runoff, water level, Slyudyanka River, monitoring, Lake Baikal

1. Introduction

The problem of creating an integrated monitoring system for inland catastrophic hydrological phenomena is longstanding, and recent years have only confirmed it. The frequency of floods increases from century to century (Avakyan and Istomina, 2013), and due to the reduction of observation sites and limited instrumentation, the forecast of hazardous phenomena associated with a rise in the level of river waters becomes even more difficult. A comprehensive characterization of the flood hazard in the rivers of the Baikal region was described in (Kichigina, 2018). The catastrophic floods of 2019 in East Siberia is direct evidence of the severe negative consequences of such phenomena (Shalikovsky et al., 2019). Despite the obvious risks, coastal areas are still the most attractive for development in terms of economic activity.

In this situation, the development of the station that would be able to predict the occurrence of hazardous phenomena and characterize the behaviour of the main hydrological parameters of the investigated watercourses in an autonomous mode is the optimal solution of the problem of monitoring the hydrological conditions. To develop the algorithm for obtaining data on the water level regime in rivers and their further application for mathematical modelling, we organized the automated monitoring of water level in the upper and lower sections of the Slyudyanka River (Southern Baikal) using our automated stations designed at Limnological Institute SB RAS.

2. Materials and methods

To solve the problem of monitoring the hydrological conditions in rivers, researchers from Laboratory of Hydrology and Hydrophysics at Limnological Institute SB RAS developed an automated hydrometeorological station (AHMS) (Makarov et al., 2018). The station is designed to organize network for monitoring the hydrological conditions in water bodies and collect related information: meteorological, hydrophysical, etc. The measured environmental parameters are transmitted in real time or for certain periods via wireless communication channels to a remote Internet server. Functionally, this server with an external IP address is a data collection and data processing centre (data centre). Tasks of the data centre include receiving data from the network of monitoring stations, primary processing, storage and provision of the access through the web page (https://hlserver.lin.irk.ru/shs/monitor/).

A Microchip microcontroller with a 16-bit architecture, which is the link connecting all elements, sets the logic of the AHMS operation. A GSM terminal is integrated into the device for data transmission to the Internet. In the absence of cellular networks in the area of AHMS setup, it is possible to connect GlobalStar or Iridium satellite terminals or operate in fully autonomous mode, saving the collected data at the removable non-volatile memory SD card. Since the memory card is formatted in FAT32, the recorded data can be read from any operating system without

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using specialized software. To ensure the unity of time between stations and measurements synchronization, the device has an integrated ML8088s receiver of geographical coordinates (manufactured by NAVIA company, Russia), which provides a binding to the atomic clock of the GLONASS and GPS satellites. The station is powered by 12V lead-acid battery and charged from a 30W solar panel. A charge controller is integrated into the power supply circuit, which maintains the optimal charging voltage depending on the temperature of the batteries and protects them from overcharge and deep discharge, which significantly extends the service life. The presence of external I2C and COM ports in the device, as well as additional analogue input channels, provide the possibilities of its adaptable expansion: equipping with additional sensors and connecting external equipment.

The water level is determined by hydrostatic pressure measurement with software atmosphere pressure compensation. A high-precision digital pressure sensor developed in Laboratory of Hydrology and Hydrophysics at Limnological Institute SB RAS is a sensitive element for measuring the water level. The resolution of the sensor is about 0.2 mm of water column.

Meteorological parameters are measured with a set of Vantage Pro 2 sensors, Davis Instruments, USA (Vantage Pro2 Specification Sheets). The basic set includes the following sensors: air temperature, air humidity, atmospheric pressure, wind speed and direction, and precipitation sensor. The extended set is complemented with solar radiation and ultraviolet radiation sensors.

With hydrostatic pressure level sensors, the developed AHMS yields much more accurate (up to 0.2 mm) and high frequency measurements (with up to 5 Hz sampling frequency) of water level and atmospheric pressure with a precise timing received from GLONASS and GPS satellites. In terms of water level measurement accuracy, AHMS corresponds to the world's best analogues (e.g. the Orpheus Mini sensor manufactured by OTT Hydromet GmbH or the EHP-CMC system manufactured by EHP-Tekniikka Ltd.), and in terms of recording high frequency oscillations (with periods of less than a minute), it surpasses them. The results of measurements from the network of such stations and their integration into a single system for monitoring the ecological condition in Baikal natural territory will allow us to receive up-to-date information in real time and perform a reliable interpretation of the results.

The economic advantage of AHMS is its unpretentiousness, without additional maintenance by a person. Power from solar panels eliminates the cost of energy supply, and the cost of data transmission via cellular communication channels, as a rule, does not exceed one thousand rubles per year.

Study site

The Slyudyanka River is one of the many tributaries of South Baikal, runs down from the northern slope of the Khamar-Daban ridge and has a mountainous nature. Due to the small distance of the ridge axis to the lake coast (about 25-30 km), Slyudyanka is characterized by a small drainage area - 73.3 km^2. The difference in elevation between the source and the mouth of the river is about 1100 m, with the length of the main channel of 21 km. Its average flow rate is 0.71 m^3/s.

On 19 September 2020, we installed AHMS at two sections of the Slyudyanka River. The first station (Lower section) was located at a distance of 5 km from the site where the river inflows to Lake Baikal, the coordinate of 51.62939N 103.67485E (Fig. 1). The second station (Upper section) was installed upstream, at a distance of 8.5 km from the first station, the coordinate of 51.57230N 103.62080E. Height above sea level in the Baltic height system was 589 m for the lower station and 954 m for the upper station. On 22 October 2020, we removed the stations after two cases of freezing of the level sensors. The stations were powered only from lead batteries as a testing regime.

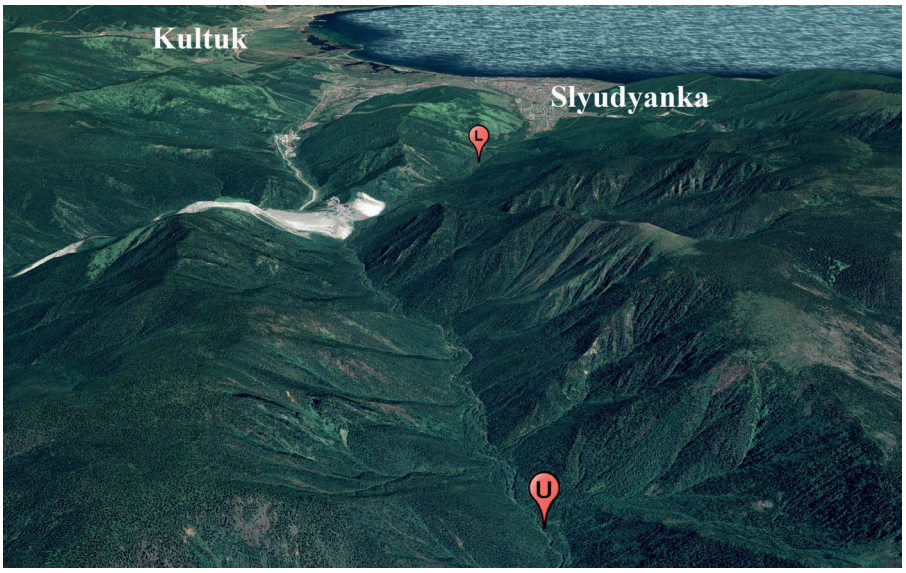

Fig.1. Sites of AHMS installation on the Slyudyanka River.

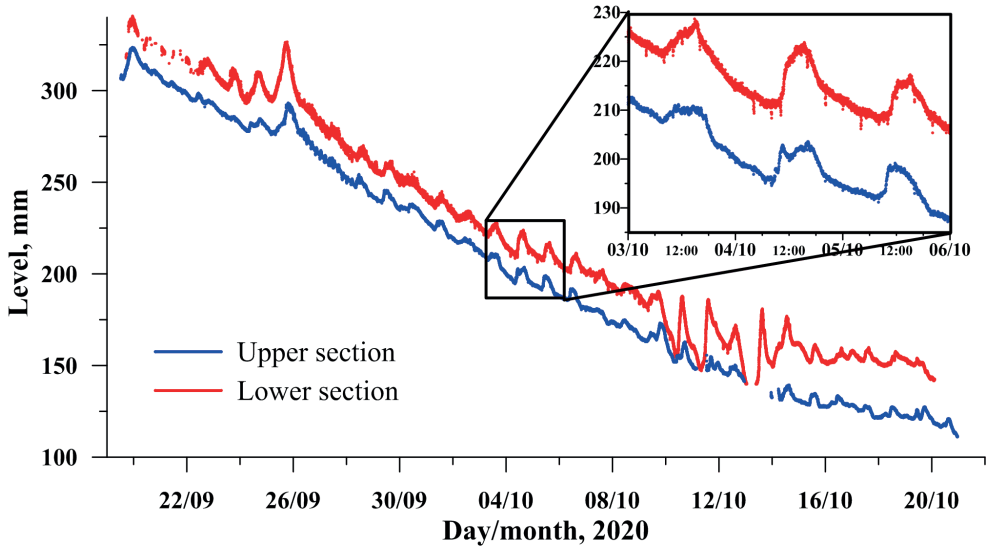

Fig.2. Comparison of data on the water level in the Slyudyanka River at two sections.

Therefore, to save battery power, the obtained data were transmitted every four hours. The upper station was equipped with a set of meteorological sensors, which allowed us to track the main meteorological parameters. The stations were installed without tide gauge wells, therefore, water level fluctuations were filtered by a digital method, through averaging measurements of level during 30-second period, which were carried out every two minutes to save electricity.

3. Results and discussion

The obtained data on the water level at both stations for the entire observation period are shown on Fig. 2. The stations were installed after heavy rains in the first half of September, and during their operation, the water level in the river gradually decreased. On average, during a month, both in the upper and lower sections, the water level decreased by approximately 200 mm.

A detailed analysis of the data on the water level at the two stations has revealed that in both sections, the water level experiences diurnal fluctuations with an amplitude from 5 to 10 mm, which are not associated with precipitation. The daily maximum of the water level lags behind the noon of the local time and occurs approximately at 14 or 15 o'clock (see inset in Fig 2). Analysis of the data did not allow us to estimate the delay time between stations because the daily rise in the water level at the lower station usually began earlier than at the upper one, but, at the same time, it was more extended in time. At the upper station, respectively, the water level rise took place later. We think that this is due to a huge number of streams flowing into the river in the area from the upper station to the lower one. Consequently, the streams flowing into the Slyudyanka River downstream thaw out and affect the lower station earlier than the upper station.

Unfortunately, there was no significant precipitation during the operation of AHMS, and at the upper station, precipitation fell in the form of snow. In 2021, we plan to install the stations at the onset of the spring season, after snowmelt and breakup of river ice, to study the period of filling the river bed with water.

4. Conclusions

The automated hydrometeorological station (AHMS) developed at Limnological Institute SB RAS showed high autonomy and accuracy in measuring the water level. It proved its worth at Lake Baikal, working throughout the year and receiving energy from 30W solar panels. And it was successfully tested during a trial installation on the Slyudyanka River in the autumn of 2020, but it had to be removed with the onset of cold weather. The hydrostatic method and a highly sensitive pressure sensor ensure high water level measurement accuracy. However, as wee see, this method is not suitable for rivers. Many rivers freeze completely in the winter and have flood events, during which water carries fragments of trees, stones, etc. Such debris will inevitably damage the sensor installed at the river bottom or underwater communication cable. Taking into account the above specifics of the station installation on the river, it is necessary to modernize it, in particular, to replace the sensor with a non-contact radiowave radar level meter of the ULM-31A1-HF-LC type (manufactured by LiMako company, Russia).

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest.

References

Avakyan A.B., Istomina M.N. 2013. Floods as a global problem. Strategiya Grazhdanskoy Zashchity: Problemy i Issledovaniya. [Civil Protection Strategy: Problems and Research] 1: 180-193. (in Russian)

Kichigina N.V. 2018. Flood hazard on the rivers of the Baikal region. Geography and Natural Resources 39: 120-129. DOI: 10.1134/S187537281802004X

Makarov M.M., Kucher K.M., Aslamov I.A. et al. 2018. The monitoring system of hydrophysical and hydrochemical parameters of Lake Baikal. Mezhdunarodnyy Zhurnal Prikladnykh i Fundamental'nykh Issledovaniy [Journal of Applied and Fundamental Sciences] 12(1): 120-124. DOI: 10.17513/mjpfi.12533 (in Russian)

Shalikovsky A.V., Lepikhin A.P., Tiunov A.A. et al. 2019. The 2019 floods in Irkutsk region. Vodnoye Khozyajstvo Rossii: Problemy, Tekhnologii, Upravleniye [Water Sector in Russia: Problems, Technologies, Management] 6: 48-65. (in Russian)

Vantage Pro2 Specification Sheets // Davis Instruments. URL: https://www.davisinstruments.com/product_documents/weather/spec_sheets/6152_62_53_63_SS.pdf

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Original Article

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Study of microsatellite cross-species specificity in freshwater sponge families Lubomirskiidae and Spongillidae

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ABSTRACT. The endemic Baikal sponges of the Lubomirskiidae family are a unique bouquet of closely related species formed from a common ancestor with the present-day cosmopolitans, *Ephydatia muelleri*, facing today are big ecological problems that require careful study. It is necessary to analyze the genetic structure of endemic freshwater sponge populations for a better understanding of the influence of such adaptive features on permanent habitat conditions as the loss of the ability to form gemmules. Microsatellite markers are best suited for analyzing population structure. The closest species to them, for which microsatellite markers have been developed to date, is *Ephydatia fluviatilis*. In this article, we check the suitability of these markers for population genetic analysis of *Lubomirskia baikalensis* and *E.muelleri* species using bioinformatic and molecular genetic methods of analysis, since the cross-species specificity of microsatellite markers has been shown for many closely related species. Despite the revealed 45.5% cross-species specificity for both *L.baikalensis* and *E.muelleri* at the level of genomic data, qualitative population genetic analysis requires the development of specific microsatellite markers *de novo* based on the genomic data of *L.baikalensis* and *E.muelleri*.

Keywords: Genetic markers, microsatellites, interspecies specificity, sponges, Porifera

1. Introduction

Lake Baikal is a unique ancient rift lake, the deepest on the planet (Kozhov, 1962; Jaguś et al., 2015). These features contributed to the formation of endemic species, which currently make up approximately 70% of the species inhabiting Baikal. The Baikal sponges are no exception. The ancestral species of endemic Baikal sponges colonized the lake millions of years ago and formed a bouquet of closely related endemic species (Efremova, 2004; Itskovich et al., 2006; 2008; Meixner et al., 2007; Maikova et al., 2015). Overall, 19 species of sponges live in the lake today, 15 of which are endemic (Itskovich et al., 2015; Manconi and Pronzato, 2019; Bukshuk and Maikova, 2020).

During the formation of endemic species of Baikal sponges from the cosmopolitan genera *Ephydatia* (Itskovich et al., 2008), Baikal sponges have lost the ability to form gemmules as an adaptation to permanent habitat conditions. Due to the loss of this method of asexual reproduction, a significant decrease in the representation of clones in the populations of Baikal sponges and a change in the population structure are expected. Research on the population genetic structure of freshwater sponges is limited to a few studies of *Ephydatia fluviatilis* (Lucentini et al., 2013; Li et al., 2018). In this regard, the study of the population structure of *Lubomirskia baikalensis* and *E.muelleri* is highly relevant.

The study of Lubomirskiidae and Spongillidae at the molecular genetic level has been actively pursued in recent years. At the moment, the draft genome of *L.baikalensis* and four transcriptomes from the species *L.baikalensis*, *L.abietina*, *B.bacillifera*,(Kenny et al., 2019), and *Sw.papyracea* (Kenny and Itskovich, 2021) have been discovered. For cosmopolitan freshwater sponges, the transcriptome of *E.muelleri* at the chromosomal level genome was discovered (Kenny et al., 2020). Despite the great success in the study of endemic Baikal sponges at the level of genomes and transcriptomes, the issue of molecular marker development for studying the population structure of Lubomirskiidae remains uncovered.

Microsatellite markers are widely used for study the population structure of marine (Blanquer and Uriz, 2010; Dailianis et al., 2011; Pérez-Portela et al., 2015; Riesgo et al., 2019) and freshwater (Lucentini et al., 2013; Li et al., 2018) sponges. The study of the genetic diversity of endemic Baikal sponges at the population level is fundamentally important for the conservation of species, especially in the conditions of mass mortality observed in Lake Baikal during the past decade (Kaluzhnaya and Itskovich, 2015; Denikina et al., 2016; Itskovich et al., 2018; Khanaev et al., 2018; Kulakova et al., 2018; Belikov et al., 2019).

Several approaches can be used in choosing microsatellite markers for the analysis of population structure. These can be the development of markers

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de novo for a target species or testing of previously developed markers for closely related species. The second approach is the cheapest and shows good efficiency. The analysis of published data on the development of microsatellite markers (Barbará et al., 2007) revealed that for invertebrates, on average, 72% of markers were successfully amplified from the DNA of closely related species, 77% of which were polymorphic. Moreover, the use of cross-species microsatellite markers facilitates the comparison of closely related taxa in the study of the mechanisms involved in population divergence and speciation (Noor and Feder, 2006), which makes the approach for identifying universal microsatellite markers more attractive. Among freshwater sponges, microsatellite markers are currently developed only for the species *Ephydatia fluviatilis* (Anderson et al., 2010), which is closely related to the Baikal endemic sponges.

In this work, we investigated the cross-species specificity of microsatellite markers developed for the cosmopolitan freshwater sponge, *E. fluviatilis*, within the population genetic studies of the closely related sponge *E. muelleri* and the endemic Baikal sponge *L. baikalensis* using bioinformatic and molecular genetic methods.

2. Methods

2.1 Sampling

Specimens of *L. baikalensis* sponges were collected during the 2018 expeditions by SCUBA divers in the northern basin of Lake Baikal (55°17.067' N; 109°45.401' E) from a depth of 10 - 17 m; immediately after collection, they were fixed and stored in 70% ethanol at a temperature of +4 C°. The species were identified by morphological characteristics such as body shape and size.

2.2. DNA isolation, PCR analysis and fragment analysis

Total genomic DNA was extracted using the CTAB solution (Gustincich et al., 1991).

Microsatellite markers were published previously (Anderson et al., 2010); forward primers were marked with a fluorescent label (Table 1).

PCR amplification of gene fragments was performed in a thermal cycler Techne TC 5000 (UK) using the Encyclo Plus PCR kit (Eurogen, Russia). The PCR protocol published for these primer pairs (Li et al., 2018) did not yield PCR products for Baikal sponges; therefore, the PCR protocol was optimized:

Initial denaturation for 2 min at 94 C°, followed by 11 cycles of denaturation for 30 sec at 94 C°, annealing for 30 sec at 65-55 C° (1-degree reduction every cycle), the extension for 30 sec at 72 C°, followed by 24 cycles of denaturation for 30 sec at 94 C°, annealing for 30 sec at 55 C°, the extension for 30 sec at 72 C°, then the final extension for 8 min at 72 C°.

PCR products were visualized by electrophoresis in 2% agarose gel for 40 minutes. Fragment analysis was performed for two loci that gave clear single bands on the agarose gel. The exact length of the loci was determined using fragment analysis on an ABI 3130xl Genetic Analyzer (Syntol, Moskow Russia). The six obtained fragments were analyzed using GeneMarker 3.01 (Hulce et al., 2011).

2.3. Genome data analysis

To study the suitability of the *E.fluviatilis* microsatellite markers for population genetic analysis of the Baikal endemic sponges of the *L.baikalensis* species, we searched for the flanking regions of microsatellite markers in the draft genome of *L.baikalensis* (Kenny et al., 2019) (Table 2). To assess the level of cross-species specificity of microsatellite markers among freshwater sponges, we additionally searched for flanking regions of the *E.fluviatilis* microsatellite markers in the genome assembly of the *E.muelleri* chromosomal level containing assemblies in the form of a scaffold for each of 22 chromosomes and 2 scaffolds for 23 chromosomes likely to connect by a centromere (Kenny et al., 2020) (Table 3). In each genome, flanking sequences of microsatellite markers (left and right separately) were searched using the BLAST + software package (Camacho et al., 2009); for matches greater than 25 base pairs long, the aligned sequences plus 500 base pairs on each side were extracted using the SeqinR package in the R programming language. The resulting sequences for both species were aligned to the original sequence of the microsatellite with flanking regions of

Table 1. Fluorescent labels for primers and repeat type for the *E.fluviatilis* microsatellite markers

GenBank Accession no.	Locus	fluorescent label for forward primer	
FJ752588	Efi-3	FAM	(CA)9
GQ476799	Efi-4	R6G	(CA)22
FJ752589	Efi-5	TAMRA	(ATT)8
FJ752590	Efi-7	FAM	(TGT)5
FJ752591	Efi-9	R6G	(TATG)4 (TG)15 C(GT)11
FJ752592	Efi-10	TAMRA	[(GAAT)4 (GAA)2TT]2(GATT)5
FJ752593	Efi-12	FAM	(CA)8T(CA)3
FJ752594	Efi-14	R6G	(TG)13
FJ752595	Efi-17	TAMRA	(CA)5TGCG(CA)8TGTG(CA)6TGCG (CA)6
GQ476801	Efi-20	FAM	[(CA)2/4/6T]4CTA(CA)4A4(CA)2TCAATA(CA)3TAT(CA)3)
GQ476800	Efi-22	R6G	(TG)23(AG)4 (TG)8

Table 2. Hits found in the *L.baikalensis* draft genome for microsatellite markers Efl3 – Efl22

				Coordinates in the genome assembly (Kenny et al., 2019)			
Locus	Alignment on flanking region	Presence of microsatellite	Number of copies	Sequence name	Sequence start	Sequence end	Amplification
Efi-3	+	+	1	NODE_133600_length_504_cov_14.1171	263	1	No amplification product
Efi-4	+	+	1	NODE_15577_length_2618_cov_33.3609	269	478	Multiple non-specific amplification
Efi-5	+	-	2+	NODE_3929_length_6454_cov_36.6491	453	883	Multiple non-specific amplification
				NODE_50989_length_1056_cov_44.9704	548	977	
Efi-7	-	-	-	-	-	-	**One clear band**
Efi-9	+	+	1	NODE_5049_length_5597_cov_19.842	5186	4893	Multiple non-specific amplification
Efi-10	-	-	-	-	-	-	Two bands
Efi-12	-	-	-	-	-	-	No amplification product
Efi-14	+	-	1	NODE_100388_length_621_cov_27.2923	434	288	Multiple non-specific amplification
Efi-17	+	+	3+	NODE_4777_length_5775_cov_22.5239	5469	5732	Multiple non-specific amplification / no amplification product
				NODE_4777_length_5775_cov_22.5239	5469	5775	
				NODE_59274_length_939_cov_10.8979	645	936	
Efi-20	+	+	1	NODE_68985_length_832_cov_30.9788	219	643	**One clear band**
Efi-22	-	-	-	-	-	-	Two bands

E.fluviatilis (Anderson et al., 2010) and on the primer sequences using the BioEdit 7.0 software package (Hall, 1999) and the MAFFT v 7 online service (Katoh et al., 2018). We also carried out an analysis of the matching of the *E.fluviatilis* primer sequences with similar regions in the genomes of *L.baikalensis* and *E.muelleri* (Table 4).

3. Results and discussion

Based on the results of bioinformatic analysis of genomic data of *L.baikalensis* and *E.muelleri*, we identified and analyzed hits with flanking regions of microsatellite markers Efl3 - Efl22. For *E.muelleri*, the published genome of 1490 times total coverage (Kenny et al., 2020) allows us to assess the real picture of the representation of microsatellite markers Efl3 - Efl22 based only on bioinformatic analysis, without testing in the laboratory. When analyzing the genome, hits were found for seven markers (Table 2), while microsatellite sequences were present only in five of them. For the two markers, more than one coincidence was found in different regions of the genome.

For *L.baikalensis*, the published draft genome is incomplete. Therefore, in addition to bioinformatic analysis, we also assessed the cross-species specificity of Efl3 - Efl22 microsatellite markers using standard laboratory methods (see the Methods section). During genome analysis, we detected hits for seven markers, two of which did not match with the markers identified in the *E.muelleri* genome (Table 2). Microsatellite sequences were present only in five of seven markers identified, one of which did not coincide with those identified in the *E.muelleri* genome. More than one match was found for two markers in different regions of the genome. Each marker Efl3 - Efl22 was amplified with three samples of *L.baikalensis* and only for two markers out of 11 (Efl7 and Efl20); clear single bands were obtained on gel electrophoresis (Table 2). Based on the results of the fragment analysis, the length of the Efl7 fragment was 337 nucleotides. The lack of matches in the *L.baikalensis* draft genome may be caused by incomplete genome sequence. The Efl20 locus length was158 base pairs, although the expected fragment length was approximately 213 base pairs. All three samples at both loci were homozygous and had the same length. The rest of the markers did not produce a PCR product, or a multiple PCR product was amplified.

The analysis of the matching of the *E.fluviatilis* primer sequences with similar regions in the genomes of *L.baikalensis* and *E.muelleri* revealed that the pairs of primers published for microsatellite markers of *E.fluviatilis* (Efl3 - Efl22) are not suitable for specific

Table 3. Hits found in the *E.muelleri* genome assembly for microsatellite markers Efl3 – Efl22

				Coordinates in the genome assembly (Kenny et al., 2020)		
Locus	Alignment on flanking region	Presence of microsatellite	Number of copies	Sequence name	Sequence start	Sequence end
Efi-3	+	+	1	Scaffold 0005	13008353	13008032
Efi-4	+	+	1	Scaffold 0006	8489737	8489969
Efi-5	+	-	4+	Scaffold 0590	24234	24672
				Scaffold 0022	739868	740297
				Scaffold 0431	21422	21075
				Scaffold 0019	333069	332719
Efi-7	Low quality alignment	-	1	Scaffold 0015	4326639	4327264
Efi-9	+	+	1	Scaffold 0014	6568680	6568945
Efi-10	+	+	1	Scaffold 110	11587	11767
Efi-12	-	-	-	-	-	-
Efi-14	-	-	-	-	-	-
Efi-17	+	+	2+	Scaffold 0366	10252	10568
				Scaffold 0006	12667029	12667346
Efi-20	-	-	-	-	-	-
Efi-22	-	-	-	-	-	-

Table 4. Cross-species specificity of primer pairs

Fw 3' - 5'		Rev 3' - 5'
Efi3	CCAC - - - - - AGGACACAACT - - - - - - - - ACCACA	ACCGAGCAGACCGTTGTATT
E.muelleri	CCACAGTGGTAGCAAACACTTTCTTTTAGTGCCA	ACGGAGCAGACTGTTGTGTT
Efi3	CCAC - - AGGACAC - - - - - - AACTACCACA	ACCGAGCAGACCGTTGTATT
L.baikalensis	CCATAGTGGTCACTGTGGTGACTAACAGG	TCGGAGCAGACTGTGGTGTT
Efi4	GAAGCAGCTACGGCACTACC	TTCACACCTCACGATAAGACAAA
E.muelleri	GAAGCAGTTACGACACTACC	TGTACATATGTGTATGTGTGTGT
L.baikalensis	AAAGCAGCTAAGGCACTACC	TTCACCAGACATG-TAC - AT AAT
Efi5	AGTAA – GCCACGAAGCA - GCAT	GTGGCGA - - CATCATGCAAGTA
E.muelleri	AGTAACGATGCAAAATGTGAAG	GTGGCTAATCTTCCTGCAAGTC
L.baikalensis	AGTAATGATGCAAAATGTGGAG	GTGGCTAGTCTTCCAGCAAGTC
Efi9	GGAATGGTAAGGTTCCTGCAT	GCCATACTA CTT TCTCT CTTGTGC
E.muelleri	GGAATGGTAG G - - - -- TGTGT	CACTCAAAGCTATACTAGCTGTAC
Efi9	GGAATGGTAAGGTTCCTGCAT	GCCATACTACT - TTCTCTC - - - - - - TTGTGC
L.baikalensis	GGAATGGTAAGGACCCTGTGT	GCCACTCAACTCTTCTATCACAACATGTGT
Efi10	GGAGAAAACATATGCAAGCAA	CGTGCTATTACTTGCCTTCTAGC
E.muelleri	GGAAT CACCTGAAGATGGCAC	CGTGCCACTACTTGCCTTCTTGC
Efi14	CTGCACGTATAGGGA - ATGGA	TGATGAGATGCTTGACACACA
L.baikalensis	CTGCACGTGTAGGGATATGGA	TGCCAAGTCCTCAGCAACACA
Efi17	CCATGTGTGTGC - TCA -TGAAA	TCACACACTTGACGT - TGGAGA
E.muelleri	CCAAGTGTGCGCATCAGTGAAA	CCACACACTAGACGCGGATGTGCGTGTCTCTGCGATGGAGA
L.baikalensis	CCATGTGTGTATATAAGTGAAT	CCACACACTAGACGCGGATGTGTGTGTCTCTGCGATGGAGA
Efi20	GGTTGATGGGCAATTTAGGA	CTCCCAAACTCCAGAAGCAG
L.baikalensis	TAATAATTGGAAGTGTTGGA	CTGCCAAACTCCAGAAGCAG

amplification of markers for *L.baikalensis* and *E.muelleri* species, since the genome regions containing primer sequences contain a large number of substitutions (Table 4). This explains the lack of specific amplification for *L.baikalensis* samples.

Thus, markers Efl3, Efl4, Efl9, Efl17, and Efl20 are cross-specific for species *L.baikalensis*: 45.5% of the total number of tested markers, and for species *E.muelleri*, Efl3, Efl4, Efl9, Efl10, and Efl17 are also 45.5% of those tested. This is 10% lower than the average value of cross-specific polymorphic microsatellite markers for invertebrates (Barbará et al., 2007)

Despite the presence of microsatellites and matches in the flanking regions of these loci, all loci require the development of new specific primer pairs for population genetic analysis of *E.muelleri* and *L.baikalensis* (Table 4). Markers Efl9, Efl10, Efl17, and Efl20 contain imperfect microsatellite repeats, and their use for population genetic studies can lead to erroneous identification of alleles because microsatellite elongation can occur in different parts of the imperfect repeat; thus, PCR products of the same length will have different sequences and, hence, will be different alleles. The flanking regions of markers Efl3 and Efl4 differ significantly in *E.muelleri* and *L.baikalensis*, which indicates a high variability of this genome region.

4. Conclusions

Microsatellite markers developed and successfully used for population genetic studies of *E.fluviatilis* (Anderson et al., 2010; Lucentini et al., 2013; Li et al., 2018) are not suitable for population genetic studies of the *E.muelleri* and *L.baikalensis* species.

The *de novo* development of microsatellite markers based on the genomic data of *E.muelleri* and *L.baikalensis* is more promising. Universal microsatellite sequences with conserved flanking regions have already been identified in *E.muelleri* and *L.baikalensis* genomes (Yakhnenko and Itskovich, 2020), and work on the development and testing of specific primers is underway.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

Anderson C., Gallego M., Aparicio J. et al. 2010. Permanent genetic resources added to molecular ecology resources database 1 December 2009 – 31 January 2010. Molecular Ecology Resources 10: 576-579. DOI: 10.1111/j.1755-0998.2010.02851.x

Barbará T., Palma-Silva C., Paggi G.M. et al. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. Molecular Ecology 16(18): 3759-3767. DOI: 10.1111/j.1365-294X.2007.03439.x

Belikov S., Belkova N., Butina T. et al. 2019. Diversity and shifts of the bacterial community associated with Baikal sponge mass mortalities. PLoS ONE 14(3). DOI: 10.1371/journal.pone.0213926

Blanquer A., Uriz M.J. 2010. Population genetics at three spatial scales of a rare sponge living in fragmented habitats. BMC Evolutionary Biology 10. DOI: 10.1186/1471-2148-10-13

Bukshuk N.A, Maikova O.O. 2020. A new species of Baikal endemic sponges (Porifera, Demospongiae, Spongillida, Lubomirskiidae). ZooKeys 906: 113-130. DOI: 10.3897/zookeys.906.39534

Camacho C., Coulouris G., Avagyan V. et al. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10. DOI: 10.1186/1471-2105-10-421

Dailianis T., Tsigenopoulos C.S., Dounas C. et al. 2011. Genetic diversity of the imperilled bath sponge *Spongia officinalis* Linnaeus, 1759 across the Mediterranean Sea: patterns of population differentiation and implications for taxonomy and conservation. Molecular Ecology 20(18): 3757-3772. DOI: 10.1111/j.1365-294X.2011.05222.x

Denikina N.N., Dzyuba E.V., Belkova N.L. et al. 2016. The first case of disease of the sponge *Lubomirskia baicalensis*: investigation of its microbiome. Biology Bulletin 43(3): 263-270. DOI: 10.1134/S106235901603002X

Efremova S.M. 2004. New genus and new species of sponges from family Lubomirskiidae Rezvoj, 1936. In: Timoshkin O.A. (Ed.), Index of animal species inhabiting Lake Baikal and its catchment area. Novosibirsk: Nauka, pp. 1261-1278. (in Russian)

Gustincich S., Manfioletti G., Del Sal G. et al. 1991. A fast method for high-quality genomic DNA extraction from whole human blood. BioTechniques 11(3): 298-300, 302. PMID: 1931026

Hall T.A. 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symposium Series 41: 95-98.

Hulce D., Li X., Snyder-Leiby T. et al. 2011. GeneMarker® genotyping software: tools to increase the statistical power of DNA fragment analysis. Journal of Biomolecular Techniques : JBT 22: S35-S36. PMCID: PMC3186482

Itskovich V.B., Belikov S.I., Efremova S.M. et al. 2006. Monophyletic origin of freshwater sponges in ancient lakes based on partial structures of COXI gene. Hydrobiologia 568: 155-159. DOI: 10.1007/s10750-006-0320-z

Itskovich V.B., Shigarova A.M, Glyzina O.Y. et al. 2018. Heat shock protein 70 (Hsp70) response to elevated temperatures in the endemic Baikal sponge *Lubomirskia baicalensis*. Ecological Indicators 88: 1-7. DOI: 10.1016/j.ecolind.2017.12.055

Itskovich V.B., Kaluzhnaya O.V, Veynberg E. et al. 2015. Endemic Lake Baikal sponges from deep water. 1: Potential cryptic speciation and discovery of living species known only from fossils. Zootaxa 3990(1): 123-137. DOI: 10.11646/zootaxa.3990.1.7

Itskovich V.B., Gontcharov A., Masuda Y. et al. 2008. Ribosomal ITS sequences allow resolution of freshwater sponge phylogeny with alignments guided by secondary structure prediction. Journal of Molecular Evolution 67. DOI: 10.1007/s00239-008-9158-5

Jaguś A., Rzętała M.A., Rzętała M. 2015. Water storage possibilities in Lake Baikal and in reservoirs impounded by the dams of the Angara River cascade. Environmental Earth Sciences 73(2): 621-628. DOI: 10.1007/s12665-014-3166-0

Kaluzhnaya O.V., Itskovich V.B. 2015. Bleaching of Baikalian sponge affects the taxonomic composition of symbiotic microorganisms. Genetika 51: 1153-1157. DOI: 10.7868/s0016675815110077

Katoh K., Rozewicki J., Yamada K.D. 2018. MAFFT online service: multiple sequence alignment, interactive sequence

choice and visualization. Briefings in Bioinformatics 20(4): 1160-1166. DOI: 10.1093/bib/bbx108

Kenny N., Plese J.B., Riesgo A. et al. 2019. Symbiosis, selection, and novelty: freshwater adaptation in the unique sponges of Lake Baikal. Molecular Biology and Evolution 36(11): 2462-2480. DOI: 10.1093/molbev/msz151

Kenny N., Francis J., Rivera-Vicéns R.E. et al. 2020. Tracing animal genomic evolution with the chromosomal-level assembly of the freshwater sponge *Ephydatia muelleri*. Nature Communications 11(1). DOI: 10.1038/s41467-020-17397-w

Kenny N., Itskovich V.B. 2021. Phylogenomic inference of the interrelationships of Lake Baikal sponges. Systematics and Biodiversity 19(2): 209-217. DOI: 10.1080/14772000.2020.1827077

Khanaev I.V., Kravtsova L.S., Maikova O.O. et al. 2018. Current state of the sponge fauna (Porifera: Lubomirskiidae) of Lake Baikal: sponge disease and the problem of conservation of diversity. Journal of Great Lakes Research 44. DOI: 10.1016/j.jglr.2017.10.004

Kozhov M.M. 1962. Biologiya ozera Baikal [Biology of Lake Baikal]. Moscow: Akad. Nauk SSSR press.

Kulakova N.V., Sakirko M.V., Adelshin R.V. et al. 2018. Brown rot syndrome and changes in the bacterial community of the Baikal sponge *Lubomirskia baicalensis*. Microbial Ecology 75(4): 1024-1034. DOI: 10.1007/s00248-017-1097-5

Li R., Nitsche F., Arndt H. 2018. Mesoscale investigations based on microsatellite analysis of the freshwater sponge *Ephydatia fluviatilis* in the River-Sieg system (Germany) reveal a genetic divergence. Conservation Genetics 19: 959-968. DOI: 10.1007/s10592-018-1069-4

Lucentini L., Gigliarelli L., Puletti M.E. et al. 2013. Spatially explicit genetic structure in the freshwater sponge *Ephydatia fluviatilis* (Linnaeus, 1759) within the framework of the monopolisation hypothesis. Journal of Limnology 72(1)-. DOI: 10.4081/jlimnol.2013.e14

Maikova O.O., Khanaev I.V., Belikov S.I. et al. 2015. Two hypotheses of the evolution of endemic sponges in Lake Baikal (Lubomirskiidae). Journal of Zoological Systematics and Evolutionary Research 53(2): 175-179. DOI: 10.1111/jzs.12086

Manconi R., Pronzato R. 2019. Phylum Porifera. In: Damborenea C. (Ed.), Thorp and Covich's freshwater invertebrates. Academic Press, pp. 43-92. DOI: 10.1016/b978-0-12-385024-9.00003-4

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New taxonomic records of *Zygnemataceae* (Charophyta) from the Lake Baikal region

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ABSTRACT. *Spirogyra*, *Mougeotia*, and *Zygnema*, the most species-rich genera of green filamentous algae family *Zygnemataceae*, are globally distributed. However, in many regions of the world, including the Lake Baikal region, they remain poorly studied taxonomically. The traditional species identification of zygnemataleans is based on the morphology of asexual and sexual stages of their life cycle. During the study conducted in 2020, we identified 18 taxa of *Spirogyra*, *Mougeotia*, and *Zygnema* from 24 new locations, including Lake Baikal, the Angara River, the Irkut River, and small rivers and water bodies in the lake's surrounding area. Fertile stages were observed only in five *Spirogyra* species, including one variety. *S. circumlineata* is first reported for Lake Baikal. Eight morphotypes of *Spirogyra*, three of *Zygnema*, and two of *Mougeotia* are likely new species for the region. *Spirogyra* is more widespread than *Zygnema* and *Mougeotia* in the region. The taxa of all three genera are dynamic components of periphyton, metaphyton, and aquatic macrophyte communities in the studied area and regularly occur together. Their ability to develop both attached and unattached communities facilitates their distribution, particularly in Lake Baikal's coastal zone.

Keywords: morphotaxonomy, *Spirogyra*, *Mougeotia*, *Zygnema*, Lake Baikal, algal bloom

1. Introduction

Filamentous green algae of the family *Zygnemataceae* (Charophyta), and particularly its most species-rich genera *Spirogyra* (535 valid species), *Zygnema* (210), and *Mougeotia* (173) are globally distributed and abundant in various fresh and brackish waters (Guiry and Guiry, 2020). However, neither family member can be identified at the species level without observations on fertile material, i.e., conjugating filaments and zygospores, whose morphological features are crucial in species delimitation. Thus, in many parts of the world, particularly in Siberia, these algae remain poorly studied taxonomically. Dorogostaisky (1904) first reported eight taxa of *Zygnemataceae*, i.e., three species and one forma of *Mougeotia*, four species of *Spirogyra*, and one species of *Zygnema*, from the Lake Baikal region. Some of them were discovered in several small tributaries of the lake, i.e., Turka, Bolshaya Kotinka, and Chernaya. Meyer (Meyer and Reinhardt, 1925; Meyer, 1927; 1930) was the first who provided records of *Zygnemataceae* from Lake Baikal, including five *Spirogyra*, two *Mougeotia* species, and one *Zygnema* species. These were the only data on *Zygnemataceae* from the lake for an extended period (cf. Volkova et al., 2018). A few additional records appeared as a result of algological studies of the Angara River reservoirs (Zagorenko, 1971; Vorob'eva, 1987), small waterbodies of the Kitoy River, which is a left tributary of the Angara (Egorova et al., 2001), Alla thermal springs (Takhteev et al., 2006), lakes and tributaries of the Selenga River and the Amur River basins in Zabaikalye (Kachaeva, 1974; 1980, Kuklin, 2002; Landscape …, 2002). In total, 30 species of *Zygnemataceae* were reported from the Lake Baikal region, including *Sirogonium sticticum* (Engl. Bot.) Kützing. However, most of the records had no descriptions or pictures of the collected specimens and environmental data on sampling sites. Many other surveys (Votyakova, 1981; Izhboldina, 2007) reported sterile members of *Zygnemataceae* in Lake Baikal and its surrounding area. Over the last decade, filamentous zygnemataleans, especially *Spirogyra*, have become a significant component of the Lake Baikal shallow-water communities (Kravtsova et al., 2014; Timoshkin et al., 2015; Khanaev et al.; 2016; Volkova et al., 2018). Their mass proliferation manifested the serious ecological crisis of the unique shallow-water ecosystem (Timoshkin et al., 2016). In this regard, special studies of the family *Zygnemataceae*, including their taxonomy, peculiarities of their development, and distribution over the lake water area, are relevant.

In our previous work (Volkova et al., 2018), we discovered 15 *Spirogyra* taxa based on thorough

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morphological observations. Eight were new for Lake Baikal and its tributaries, and eight were new for East Siberia. In this paper, we report new records of taxa of the family *Zygnemataceae* in Lake Baikal and its surrounding area based on materials collected during the summer of 2020.

2. Materials and methods

We collected 60 qualitative samples of filamentous zygnemataleans from 26 locations, including Lake Baikal, Lake Bolshoe Eravnoe, the Angara River, the Irkut River, and small rivers and water bodies in the lake's surrounding area during June-September 2020.

List of the studied locations:

1. the bay opposite Bolshoye Goloustnoye village, Lake Baikal, 52°01'36.5"N 105°24'46.8"E;
2. Barguzinskyi Bay, Lake Baikal, opposite Maksimikha village, 53°16'16.2"N 108°45'07.9"E;
3. Listvennichny Bay, Lake Baikal, 51°51'01.5"N 104°52'04.8"E;
4. Cape Berezovy, Lake Baikal, 51°50'78.4"N 104°54'00.8"E;
5. Bolshie Koty Bay, Lake Baikal, 51°54'11.4"N 105°03'84.2"E;
6. Senogda Bay, Lake Baikal, 55°36'48'7"N 109°14'09'1"E;
7. Sakhurta Bay, Maloe More Strait, Lake Baikal, 53°01'08.5"N 106°54'00.7"E;
8. the bay opposite Kultuk village, Lake Baikal, 51°43'01.12"N 103°43'47.5"E;
9. the bay opposite Zarechnoye village, Lake Baikal, 55°35'20'2"N 109°18'19'3"E;
10. Anga Bay, Lake Baikal, 52°46'57.6"N 106°34'29.7"E;
11. the Irkutsk reservoir, the bay near Bolshaya Rechka village, 51°56'31.2"N 104°43'45.5"E;
12. the bay opposite Slyudyanka town, Lake Baikal, 51°39'57.8"N 103°13'09.2"E;
13. Lake Bolshoe Eravnoe, 52°34'13.8"N 111°27'46.9"E;
14. the Egia River, near the Mozhai village, 52°23'58.3"N 110°45'55.0"E;
15. the Medlyanka River, Kultuk village, 51°43'33.1"N 103°43'08.6"E;
16. the Bolshaya Kotinka River, 51°54'12.1"N 105°04'26.8"E;
17. the Malaya Kotinka River, 51°54'20.9"N 105°05'05.7"E;
18. small ponds nearby Solontsovyi Bay of Lake Baikal, 54°13'00.7"N 108°24'43.1"E;
19. swampy ponds nearby the Irkut River, 51°46'57.2"N 102°58'30.9"E;
20. the mouth of the Frolikha River, 55°30'59.5"N 109°52'16.8"E;
21. the Chernaya River, 51°53'29.2"N 105°02'31.6"E;
22. backwater of the Angara River, 52°22'08.6"N 104°13'44.8"E;
23. in the coast of the Angara River, 52°21'50.7"N 104°14'50.7"E;
24. the floodplain of the Angara River, 52°22'16.8"N, 104°13'34.8"E.

The samples were collected at depths up to 1.5-2 m by hands or using a perforated shovel, plankton nets, and knifes. The projective cover (PC) of algae and macrophytes was assessed by eye on a scale in steps of 5%. The water temperature, pH, and electrical conductivity (EC) were measured during sampling using portable device HI 98501 Checktemp (Hanna Instruments Ltd., USA). The samples containing alive specimens were delivered to the laboratory in vials placed in refrigerant. The specimens were further stored in Petri dishes on the north window at a room temperature (20°C) and a natural light source. For the identification and standard morphometric measurements, we used the Olympus CX 21 light microscope equipped with a digital camera and the ToupView 3.7 software. If there was no sexual reproduction, we described sterile filaments, which differed from taxa already reported in the region in at least one of the following characteristics: the type of chloroplast (spiral chloroplast(s) in *Spirogyra*, stellate chloroplast in *Zygnema*, lamellar chloroplast in *Mougeotia*), the number of chloroplasts in a cell, the type of a cell septum, the width and length of the cells. Overall, 357 specimens were analyzed and photographed. In addition, co-occurring macroalgae and aquatic plants were identified. After microscopical observations, the specimens were fixed with 70% ethanol or 4% formalin. The collection of labeled samples and micrographs are stored in the Limnological Institute SB RAS, Irkutsk, Russia. The taxonomic sources on *Zygnemataceae* included Kolkwitz and Krieger (1941), Transeau (1951), Kadlubowska (1984), Rundina (1998), Johnson (2011), Stancheva et al. (2013). The nomenclature of the studied taxa is according to *Algae Base* (Guiry and Guiry, 2020).

3. Results

We identified 18 taxa of the family *Zygnemataceae* in the studied area. Thirteen taxa belong to the genus *Spirogyra*, two – *Mougeotia*, three – *Zygnema*. In total, 23 species and one variety of macroscopic algae (thallus size ≥ 2 mm) and three species of higher aquatic plants belonging to 11 genera, 9 families, 7 orders were identified in plant-aggregations with zygnemataleans. Here, we provide morphological accounts of the discovered members of the family *Zygnemataceae*, new geographical locations together with environmental characteristics, co-occurring macrophytes and algae.

Phylum CHAROPHYTA Migula
Class *Zygnematophyceae* Round ex Guiry
Order *Zygnematales* C.E. Bessey
Family *Zygnemataceae* Kützing
Genus *Spirogyra* Link
***Spirogyra circumlineata* Transeau**
(Fig. 1A, Fig. 1B, Fig. 1C, Fig. 1D)

Vegetative cells 38–49 μm wide, 38–110 μm long; transverse walls plane; chloroplast single with 3–5 turns per cell. Conjugation scalariform, tubes formed by both gametangia. Donor gametangium not inflated, 38–49 μm wide, 32–114 μm long; recipient gametangium slightly inflated, 44–50 μm wide, 65–140 μm long; cells without conjugation pair not inflated or somehow inflated, 46–54 μm wide, 73–128 μm long. Zygospores ellipsoid, 39–48 μm wide, 47–90 μm long; mesospore yellow-brown, smooth.

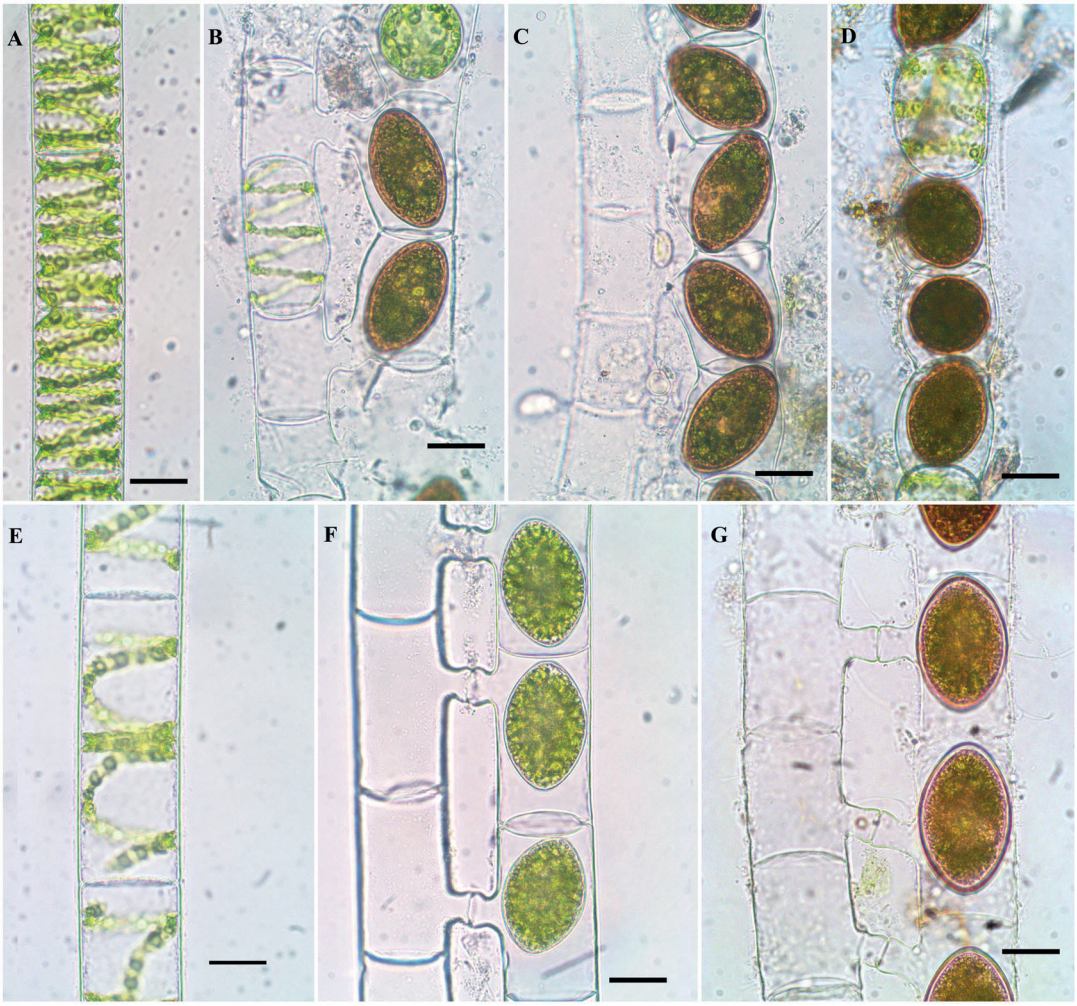

Fig.1. Light microscopic images of *Spirogyra* species from the Lake Baikal region: (A, B, C, D) *S. circumlineata*; (E, F, G) *S. condensata*. Scale bar, 30 μm.

Fertile thalli of *S. circumlineata* have been encountered in 2 new locations: **1** - June 2020, in the water column, water temperature 14.0-15.2°C, pH 7.6, EC 129.3 μS cm^{-1}, metaphyton (*Ulothrix zonata* (F. Weber & Mohr) Kützing, *S. fluviatilis*, *S. condensata*, *S. decimina* var. *juergensii*, *S. circumlineata*, *S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Spirogyra* sp. ster. 4, *Spirogyra* sp. ster. 5); **2** - June 2020, on the water surface, water temperature 19°C, pH 7.6, synusia of unattached free-floating algae, metaphyton (*Spirogyra* spp. ster., *S. fluviatilis*, *S. condensata, S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 6, *Spirogyra* sp. ster. 7, *Spirogyra* sp. ster. 8, *Mougeotia* sp. ster. 1) (PC 5-10%) in macrophyte communities dominated by *Elodea canadensis* Michx. (40%) + *Myriophyllum spicatum* L. (30%) + *Potamogeton* sp. (10%).

Rare species in Europe, Asia, and North America (Transeau, 1951; Kadlubowska, 1984; Rundina, 1998). Previously, we discovered this species in the Bolshaya Kotinka River (Volkova et al., 2018). This is the first report of this species for Lake Baikal. *S. circumlineata* is morphologically similar to *S. varians* (Hassall) Kützing, however differs in larger cell size, constancy of the conjugation form and zygospores, and a lower degree of swelling of the cells without conjugating pair.

Spirogyra condensata (Vaucher) Dumortier (Fig. 1E, Fig. 1F, Fig. 1G)

Vegetative cells 44–55 μm wide, 30–118 μm long; transverse walls plane; chloroplast single with 1.5–4 turns per cell. Conjugation scalariform, tubes formed by both gametangia. Donor gametangium not inflated, 44–60 μm wide, 30–180 μm long; recipient gametangium not inflated, 46–60 μm wide, 46–105 μm long; cells without conjugation pair not inflated; 46.4–62.1 μm wide, 44–160 μm long. Zygospores ellipsoid, often with pointed tops, 41–54 μm wide, 47–96 μm long; exospore thin, smooth; mesospore thick, thick, yellow-brown, smooth.

Fertile thalli of *S. condensata* have been encountered in 5 new locations: **1** - June-July 2020, in the water column, water temperature 14.0-15.2°C, pH 7.6, EC 129.3 μS cm^{-1}, metaphyton (*Ulothrix zonata*, *S. fluviatilis*, *S. condensata*, *S. decimina* var. *juergensii*, *S. circumlineata*, *S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Spirogyra* sp. ster. 4, *Spirogyra* sp. ster. 5); **2** - June 2020, in the water column and on the water surface, water temperature 18.3°C, pH 7.2, EC 213 μS cm^{-1}, synusia of unattached free-floating algae, or metaphyton (*S. fluviatilis*, *S. condensata, S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 6, *Spirogyra* sp. ster. 7, *Spirogyra* sp.

ster. 8, *Mougeotia* sp. ster. 1) (5-15%) in macrophytes community dominated by *Elodea canadensis* (40%) + *Myriophyllum spicatum* (30%) + *Chara globularis* Thuill. (5-10%); **3** - September 2020, depth < 2 m, stony substrate, water temperature 16°C, pH 7.1, EC 118.7–122.4 μS cm^{-1}, periphyton (*S. fluviatilis*, *S. condensata*, *Ulothrix zonata*, *Tetraspora cylindrica* var. *bullosa* K.I. Meyer, *Didymosphenia* sp.); **4** - September 2020, depth < 2 m, stony substrate, water temperature 16.5°C, pH 7.0, EC 122.4 μS cm^{-1}, periphyton (*S. condensata* ; *Ulothrix zonata*); **5** - June 2020, in the water column and on the water surface, water temperature 17°C, pH 7.2, EC 118.6 μS cm^{-1}, metaphyton (*S. condensata, Ulothrix zonata*, *Cladophora floccosa* K.I. Meyer, *Zygnema* sp. ster. 1, *Mougeotia* sp. ster.1).

Widespread species (Kadlubowska, 1984; Rundina, 1998). In previous surveys, we first identified *S. condensata* from 3 sites of Lake Baikal (Volkova et al., 2018).

Spirogyra decimina var. juergensii (Kützing) O.V. Petlovany (Fig. 2A, Fig. 2B, Fig. 2C)

Vegetative cells 27–34 μm wide, 52–72 μm long; transverse walls plane; chloroplast single, 2–4 turns per cell. Conjugation scalariform, tubes formed by both gametangia with slight predominance by the male gametangium. Donor gametangium not inflated, 30–33 μm wide, 37–67 μm long; recipient gametangium not inflated or slightly inflated on the conjugating side, 27–30 μm wide, 66–97 μm long. Zygospores ellipsoid, sometimes with rounded tops, 26–32 μm wide, 57–72 μm long; mesospore yellow-brown, smooth.

Fertile thalli of *S. decimina* var. *juergensii* have been encountered in 2 new locations: **1** - June 2020, in the water column, water temperature 14.0-15.2°C, pH 7.6, EC 129.3 μS cm^{-1}, metaphyton (*Ulothrix zonata*, *S. fluviatilis*, *S. condensata*, *S. decimina* var. *juergensii*, *S. circumlineata*, *S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Spirogyra* sp. ster. 4, *Spirogyra* sp. ster. 5); **11** - July 2020, depth < 0.5 m, sand, water temperature 24.1°C, pH 7.3, EC 167 μS cm^{-1}, periphyton (*S. decimina* var. *juergensii* , *Ulothrix zonata*).

Widespread species and variety (Kadlubowska, 1984; Rundina, 1998). In Lake Baikal, it was first found by Meyer and Reinhardt (1925) in Istoksky Bay. In previous surveys, we identified *S. decimina* var. *juergensii* from 6 other sites of Lake Baikal (Volkova et al., 2018).

Spirogyra fluviatilis Hilse (Fig 2D, Fig 2F, Fig 2G)

Vegetative cells 34–47 μm wide, 84–285 μm long; transverse walls plane; 3–4 chloroplasts with 5–14 turns per cell. Vegetative filaments often with multicellular branched rhizoids. Conjugation scalariform, tubes formed by both gametangia, sometimes predominantly by male gametangia. Donor gametangium not inflated or slightly cylindrically inflated, 35–45 μm wide, 66–225 μm long; recipient gametangium more or less inflated mainly on conjugation side or both sides, 33–60 μm wide, 70–194 μm long; cells without conjugation pair not swollen, rarely slightly cylindrically inflated, sometimes form rhizoids. Zygospores ellipsoid, 44–70 μm wide, 68–180 μm long; exospore smooth, colorless; mesospore brown, thick, distinctly multilayered, reticulate, sometimes wrinkled.

Fertile thalli of *S. fluviatilis* have been encountered in a single location: **7** - June 2020, depth 1.5-2 m, stony substrate, water temperature 16.2°C, pH 6.9, periphyton (*Ulothrix zonata*, *Tetraspora cylindrica* var. *bullosa, Draparnaldioides simplex* (K.I. Meyer) Vishnyakov, *S. fluviatilis*).

Widespread species (Kadlubowska, 1984; Rundina, 1998; Stancheva et al., 2013; Sherwood et al., 2018). In previous surveys, we first identified *S. fluviatilis* from 25 sites of Lake Baikal and in the Angara River (Volkova et al., 2018).

Spirogyra varians (Hassall) Kützing (Fig. 2H, Fig. 2I, Fig. 2J)

Vegetative cells 38–41 μm wide, 63–78 μm long; transverse walls plane; chloroplast single with 1.5–3.5 turns per cell. Conjugation scalariform, tubes formed by both gametangia with predominance by the male gametangium; conjugation often occurs among three filaments. Donor gametangium sometimes inflated, 41–50 μm wide, 38–82 μm long; recipient gametangium strongly inflated on the conjugating side, 41–50 μm wide, 49–75 μm long; cells without conjugating pair swollen, 53–64 μm wide, 60–90 μm long. Zygospores ellipsoid, 45–57 μm wide, 50–81 μm long; mesospore yellow-brown, thick, smooth.

Fertile thalli of *S. varians* have been encountered in 3 new locations: **1** - June-July 2020, in the water column, water temperature 14.0-15.2°C, pH 7.6, EC 129.3 μS cm^{-1}, metaphyton (*Ulothrix zonata*, *S. fluviatilis*, *S. condensata*, *S. decimina* var. *juergensii*, *S. circumlineata*, *S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Spirogyra* sp. ster. 4, *Spirogyra* sp. ster. 5); **2** - June 2020, depth < 2 m and on the water surface, water temperature 18.3-19°C, pH 7.2, EC 213 μS cm^{-1}, synusia of unattached algae, or metaphyton (*S. fluviatilis*, *S. condensata, S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 6, *Spirogyra* sp. ster. 7, *Spirogyra* sp. ster. 8, *Mougeotia* sp. ster. 1) (5-15%) in macrophytes community dominated by *Elodea canadensis* (40%) + *Myriophyllum spicatum* (30%) + *Chara globularis* (5-10%); **6** - June 2020, in the water column and on the water surface, water temperature 18°C, pH 7.3, EC 184 μS cm^{-1}, metaphyton (*Spirogyra varians*, *Ulothrix zonata*).

The first findings of this species were reported from two sites of Lake Baikal and two of its tributaries (Volkova et al., 2018). *S. varians* is among the commonest in Lake Baikal and its surroundings, which corresponds to the cosmopolitan status of the species (Transeau, 1951; Kadlubowska, 1984; Rundina, 1998; Stancheva et al., 2013; Sherwood et al., 2018).

Spirogyra sp. ster. 1 (Fig. 3A)

Vegetative cells 54-67 μm wide, 132-340 μm long; transverse walls plane; 5-6 chloroplasts with 6-12 turns per cell.

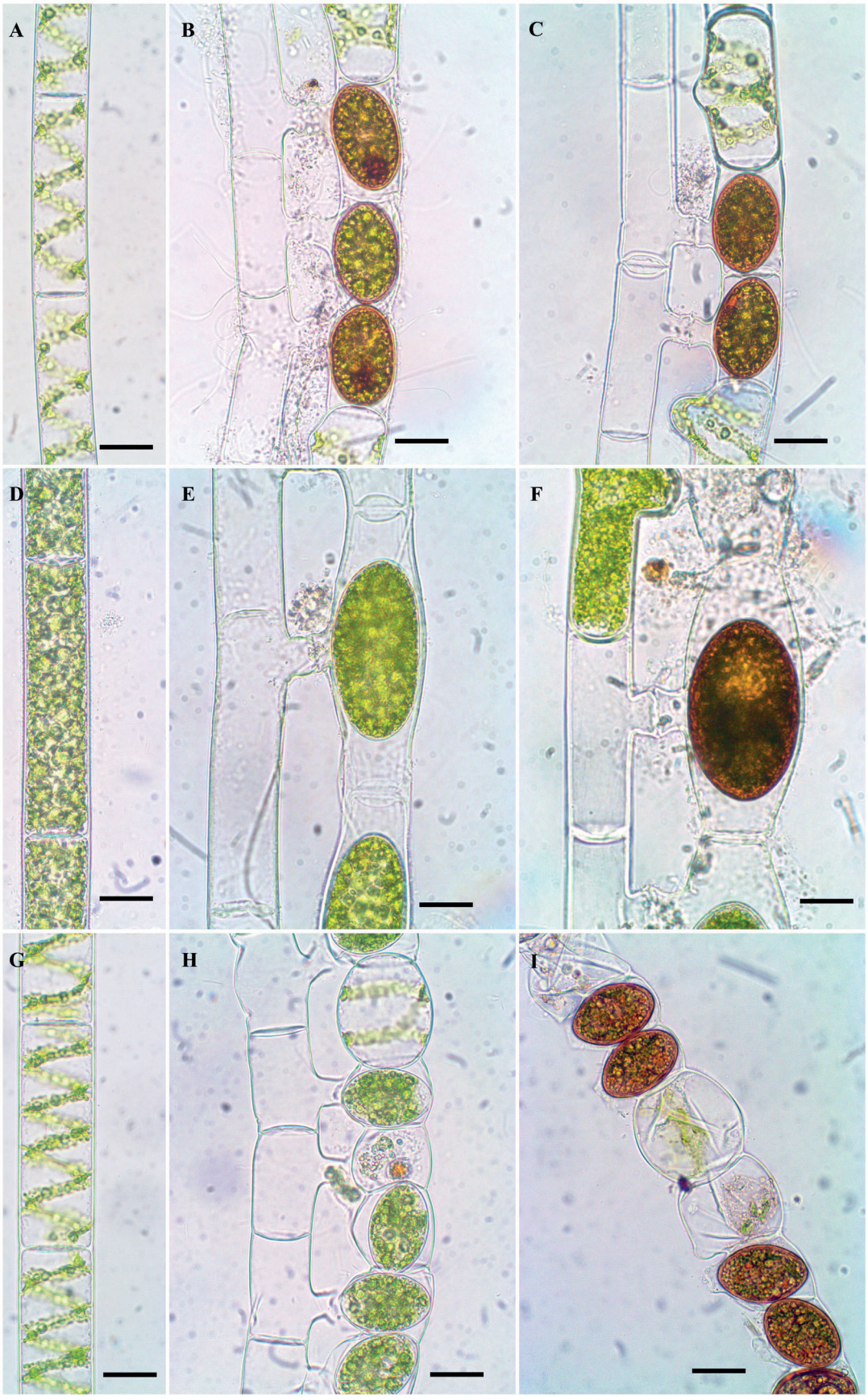

Fig.2. Light microscopic images of *Spirogyra* species from the Lake Baikal region: (A, B, C) *S. decimina* var. *juergensii*; (D, E, F) *S. fluviatilis*; (G, H, I) *S. varians*. Scale bar, 30 μm.

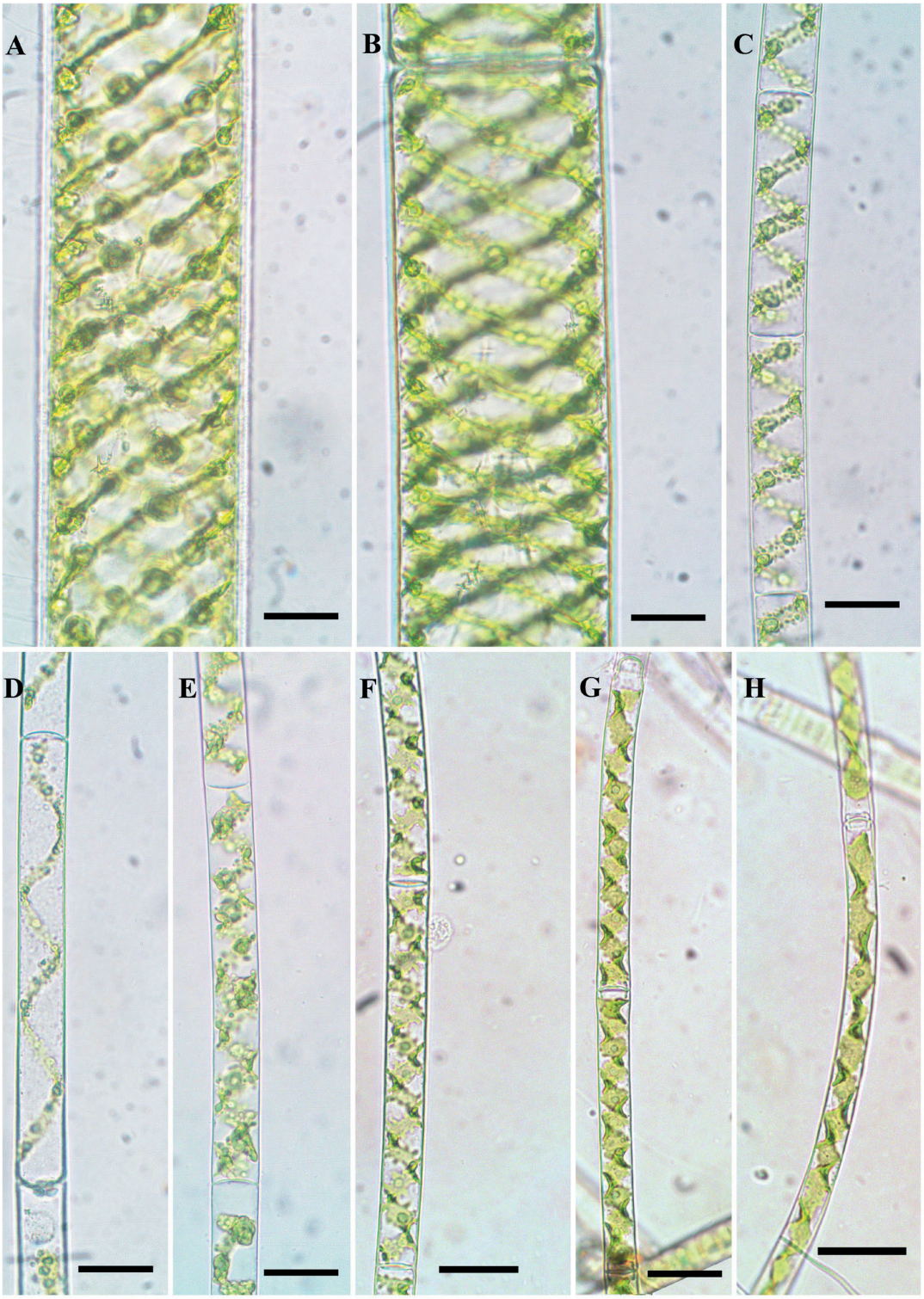

Fig.3. Light microscopic images of *Spirogyra* morphotypes from the Lake Baikal region: (A) *Spirogyra* sp. ster. 1; (B) *Spirogyra* sp. ster. 2; (C) *Spirogyra* sp. ster. 3; (D) *Spirogyra* sp. ster.4; (E) *Spirogyra* sp. ster. 5; (F) *Spirogyra* sp. ster. 6; (G) *Spirogyra* sp. ster. 7; (H) *Spirogyra* sp. ster. 8. Scale bar, 30 μm.

This is the first report of this *Spirogyra* morphotype for the region. *Spirogyra* sp. ster. 1 has been first encountered in 7 locations: **1** - June-July 2020, in the water column and on the water surface, water temperature 14.0-15.2°C, pH 7.6, EC 129.3 μS cm^{-1}, metaphyton (*Ulothrix zonata*, *S. fluviatilis*, *S. condensata*, *S. decimina* var. *juergensii*, *S. circumlineata*, *S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Spirogyra* sp. ster. 4, *Spirogyra* sp. ster. 5); **2** - June 2020, depth < 2 m and on the water surface, water temperature 18.3-19°C, pH 7.2, EC 213 μS cm^{-1}, synusia of unattached algae, or metaphyton (*S. fluviatilis*, *S. condensata, S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 6, *Spirogyra* sp. ster. 7, *Spirogyra* sp. ster. 8, *Mougeotia* sp. ster. 1) (5-15%) in macrophytes community dominated by *Elodea canadensis* (40%) + *Myriophyllum spicatum* (30%) + *Chara globularis* (5-10%); **3** - September 2020, depth ≥ 1.5-2 m, stony substrate, water temperature 9-16°C, pH 7.1, EC 118.7–122.4 μS cm^{-1}, periphyton

(*S. fluviatilis*, *S. condensata*, *Ulothrix zonata*, *Tetraspora cylindrica* var. *bullosa* K.I. Meyer, *Didymosphenia* sp.); **8** - June-July 2020, on the water surface an in the water column, water temperature 18-19°C, EC 224 μS cm^{-1}, metaphyton (*Spirogyra* sp. ster. 1., *Mougeotia* sp. ster. 1); **9** - June 2020, in the water column and on the water surface, water temperature 19.4-20°C, EC 210 μS cm^{-1}, metaphyton (*Spirogyra* sp. ster. 1, *Mougeotia* sp. ster. 1); **12** - June-July 2020, depth ~ 2 m, stones, water temperature 13°C, metaphyton (*Ulothrix zonata*, *Spirogyra* sp. ster. 1); **13** - July 2020, algal accumulations on the shore, water temperature 19.2°C, EC 403 μS cm^{-1}, synusia of *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2.

***Spirogyra* sp. ster. 2 (Fig. 3B)**

Vegetative cells 86-91 μm wide, 200-520 μm long; transverse walls plane; 4-5 chloroplasts with 6-12 turns per cell.

This is the first report of this *Spirogyra* morphotype for the region. *Spirogyra* sp. ster. 2 has been first encountered in 4 locations: **2** - June 2020, depth < 2 m and on the water surface, water temperature 18.3-19°C, pH 7.2, EC 213 μS cm^{-1}, synusia of unattached algae, or metaphyton (*S. fluviatilis*, *S. condensata, S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 6, *Spirogyra* sp. ster. 7, *Spirogyra* sp. ster. 8, *Mougeotia* sp. ster. 1) (5-15%) in macrophytes community dominated by *Elodea canadensis* (40%) + *Myriophyllum spicatum* (30%) + *Chara globularis* (5-10%); **13** - July 2020, algal accumulations on the shore, water temperature 19.2°C, EC 403 μS cm^{-1}, synusia of *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2; **14** - July 2020, water temperature 21.5°C, EC 944 μS cm^{-1}, metaphyton (*Spirogyra* sp.ster.2); **15** – August, 2020, water temperature 8.2°C, EC 102 μS cm^{-1}, metaphyton (*Spirogyra* sp. ster.2).

***Spirogyra* sp. ster. 3 (Fig. 3C)**

Vegetative cells 23-24 μm wide, 101-120 μm long; transverse walls plane; 1 chloroplast with 4 turns per cell.

This is the first report of this *Spirogyra* morphotype for the region. *Spirogyra* sp. ster. 3 has been first encountered in a single location: **1** - June-July 2020, in the water column and on the water surface, water temperature 14.0-15.2°C, pH 7.6, EC 129.3 μS cm^{-1}, metaphyton (*Ulothrix zonata*, *S. fluviatilis*, *S. condensata*, *S. decimina* var. *juergensii*, *S. circumlineata*, *S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Spirogyra* sp. ster. 4, *Spirogyra* sp. ster. 5).

***Spirogyra* sp. ster. 4 (Fig. 3D)**

Vegetative cells 19-20 μm wide, 190-199 μm long; transverse walls plane; 1 chloroplast with 3-3.5 turns per cell.

This is the first report of this *Spirogyra* morphotype for the region. *Spirogyra* sp. ster. 4 has been first encountered in a single location: **1** - June-July 2020, in the water column and on the water surface, water temperature 14.0-15.2°C, pH 7.6, EC 129.3 μS cm^{-1}, metaphyton (*Ulothrix zonata*, *S. fluviatilis*, *S. condensata*, *S. decimina* var. *juergensii*, *S. circumlineata*, *S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Spirogyra* sp. ster. 4, *Spirogyra* sp. ster. 5).

***Spirogyra* sp. ster. 5 (Fig. 3E)**

Vegetative cells 18-19 μm wide, 190-199 μm long; transverse walls plane; 1 chloroplast with 3-3.5 turns per cell.

This is the first report of this *Spirogyra* morphotype for the region. *Spirogyra* sp. ster. 5 has been first encountered in a single location: **1** - June-July 2020, in the water column and on the water surface, water temperature 14.0-15.2°C, pH 7.6, EC 129.3 μS cm^{-1}, metaphyton (*Ulothrix zonata* (*Ulothrix zonata*, *S. fluviatilis*, *S. condensata*, *S. decimina* var. *juergensii*, *S. circumlineata*, *S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 3, *Spirogyra* sp. ster. 4, *Spirogyra* sp. ster. 5).

***Spirogyra* sp. ster. 6 (Fig. 3F)**

Vegetative cells 14-16 μm wide, 112-195 μm long; transverse walls plane; 1 chloroplast with 2-12 turns per cell.

This is the first report of this *Spirogyra* morphotype for the region. *Spirogyra* sp. ster. 6 has been first encountered 3 locations: **2** - June 2020, depth < 2 m and on the water surface, water temperature 18.3-19°C, pH 7.2, EC 213 μS cm^{-1}, synusia of unattached algae, or metaphyton (*Spirogyra* spp. ster., *S. fluviatilis*, *S. condensata, S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 6, *Spirogyra* sp. ster. 7, *Spirogyra* sp. ster. 8, *Mougeotia* sp. ster. 1) (5-15%) in macrophytes community dominated by *Elodea canadensis* (40%) + *Myriophyllum spicatum* (30%) + *Chara globularis* (5-10%); **3** - September 2020, at depth ≥ 1.5-2 m, stony substrate, water temperature 9-16°C, pH 7.1, EC 118.7–122.4 μS cm^{-1}, metaphyton (*S. fluviatilis*, *S. condensata*, *Ulothrix zonata*, *Tetraspora cylindrica* var. *bullosa*, *Didymosphenia* sp., *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster.6, *Zygnema* sp.ster.1); **6** - June 2020, in the water column and on the water surface, water temperature 18°C, pH 7.3, EC 184 μS cm^{-1}, metaphyton (*Spirogyra varians*; *Spirogyra* sp. ster. 2).

***Spirogyra* sp. ster. 7 (Fig. 3G)**

Vegetative cells 12.5-13 μm wide, 104-125 μm long; transverse walls replicate; 1 chloroplast with 5-6 turns per cell.

First encountered in a single location: **2** - June 2020, depth < 2 m and on the water surface, water temperature 18.3-19°C, pH 7.2, EC 213 μS cm^{-1}, synusia of unattached algae, or metaphyton (*Spirogyra* spp. ster., *S. fluviatilis*, *S. condensata, S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 6, *Spirogyra* sp. ster. 7, *Spirogyra* sp. ster. 8, *Mougeotia* sp. ster. 1) (5-15%) in macrophytes community dominated by *Elodea canadensis* (40%) + *Myriophyllum spicatum* (30%) + *Chara globularis* (5-10%).

***Spirogyra* sp. ster. 8 (Fig. 3H)**

Vegetative cells 10-11 μm wide, 199-204 μm long; transverse walls replicate; 1 chloroplast with 8-12 turns per cell.

First encountered in a single location: **2** - June 2020, depth < 2 m and on the water surface, water temperature 18.3-19°C, pH 7.2, EC 213 μS cm^{-1}, synusia of unattached algae, or metaphyton (*Spirogyra* spp. ster., *S. fluviatilis*, *S. condensata, S. varians*, *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster. 2, *Spirogyra* sp. ster. 6, *Spirogyra* sp. ster. 7, *Spirogyra* sp. ster. 8, *Mougeotia* sp. ster. 1) (5-15%) in macrophytes community dominated by *Elodea canadensis* (40%) + *Myriophyllum spicatum* (30%) + *Chara globularis* (5-10%).

Genus *Mougeotia* C. Agardh

***Mougeotia* sp. ster. 1 (Fig. 4A)**

Filaments without rhizoids. Vegetative cells 18-23 μm wide, 40-270 μm long; transverse walls plane, with 1 axial lamellar chloroplast.

This is the first report of this *Mougeotia* morphotype for the region. *Mougeotia* sp. ster. 1 has been first encountered in 8 locations: **5** - June 2020, in the water column and on the water surface, water temperature 17°C, pH 7.2, EC 118.6 μS cm^{-1}, metaphyton (*S. condensata, Ulothrix zonata*, *Cladophora floccosa* K.I. Meyer, *Zygnema* sp. ster. 1, *Mougeotia* sp. ster.1); **8** - June-July 2020, on the water surface and in the water column, water temperature 18-18-19°C, EC 224 μS cm^{-1}, metaphyton (*Spirogyra* sp. ster. 1., *Mougeotia* sp. ster. 1); **9** - June 2020, in the water column and on the water surface, water temperature 19.4-20°C, EC 210 μS cm^{-1}, metaphyton (*Mougeotia* sp. ster. 1); **10** - June 2020, sandy substrate, water temperature 17°C EC 124 μS cm^{-1}, synusia of unattached algae, metaphyton (*Zygnema* sp. ster. 1, *Mougeotia* sp. ster. 1) (15%) in macrophytes community dominated by *Myriophyllum sibiricum* Kom. (30%) + *Elodea canadensis* (20%) + *Potamogeton* spp. (10%) + *Chara* sp. (10%); **16** - June 2020, depth < 0.5m, stony substrate, water temperature 16.5-17.5°C, pH 7.1, metaphyton (*Zygnema* sp. ster. 1, *Zygnema* sp. ster. 3, *Mougeotia* sp. ster.1); **17** - June 2020, depth < 0.5 m, sandy substrate, water temperature 15-25°C, pH 6.8, metaphyton (*Zygnema* sp. ster. 1, *Mougeotia* sp. ster. 1); **18** - July 2020, water temperature 15-23°C, synusia of *Mougeotia* sp. ster. 1. in macrophytes community dominated by *Potamogeton* sp. and *Myriophyllum* sp.; **19** - July 2020, water temperature 26.5°C, EC 334 μS cm^{-1}, algal mats (*Mougeotia* sp. ster. 1, *Zygnema* sp. ster.1).

***Mougeotia* sp. ster. 2 (Fig. 4B)**

Filaments without rhizoids. Vegetative cells 15-19 μm wide, 30-200 μm long; transverse walls plane, with 1 axial lamellar chloroplast.

This is the first report of this *Mougeotia* morphotype for the region. *Mougeotia* sp. ster. 2 has been first encountered in 4 locations: **19** - July 2020, sandy substrate, water temperature 11.5°C, EC 375 μS cm^{-1}, metaphyton (*Mougeotia* sp. ster. 2); **20** - June 2020, at depth < 0.5 m, sandy substrate, water temperature 12°C, metaphyton of *Mougeotia* sp. ster. 2, (15%) in macrophytes community dominated by *Potamogeton* sp. (30%) + *Myriophyllum* sp. (20%); **22** - July 2020, sandy substrate, water temperature 24.4°C, EC 603 μS cm^{-1}, in a large accumulation of filamentous algae on a flooded meadow, in shallow water in the flooded grass; **23** - July 2020, sandy substrate, water temperature 27.1°C, EC 555 cm^{-1}, metaphyton (*Mougeotia* sp. ster. 2).

Mougeotia sp. ster. found in Angaro-Kicherskoye Bay (Lake Baikal) (Meyer, 1930), Davsha Bay, Kotelnokovskyi Cape (Lake Baikal) (Izhboldina, 2007), the delta of the Selenga River (Votyakova, 1981), the Peremennaya River, and Krivoye Lake (Izhboldina, 2007). *Mougeotia genuflexa* (Dillw.) Ag. was found in Anga Bay, Proval Bay, Nizhneangarsky Bay (Lake Baikal) (Meyer, 1930). *M. laetevirens* (A. Br.) Wittr. was found in Anga Bay (Meyer, 1930).

Genus *Zygnema* C. Agardh (Fig. 4C)

***Zygnema* sp. ster. 1**

Filaments without basal rhizoids. Vegetative

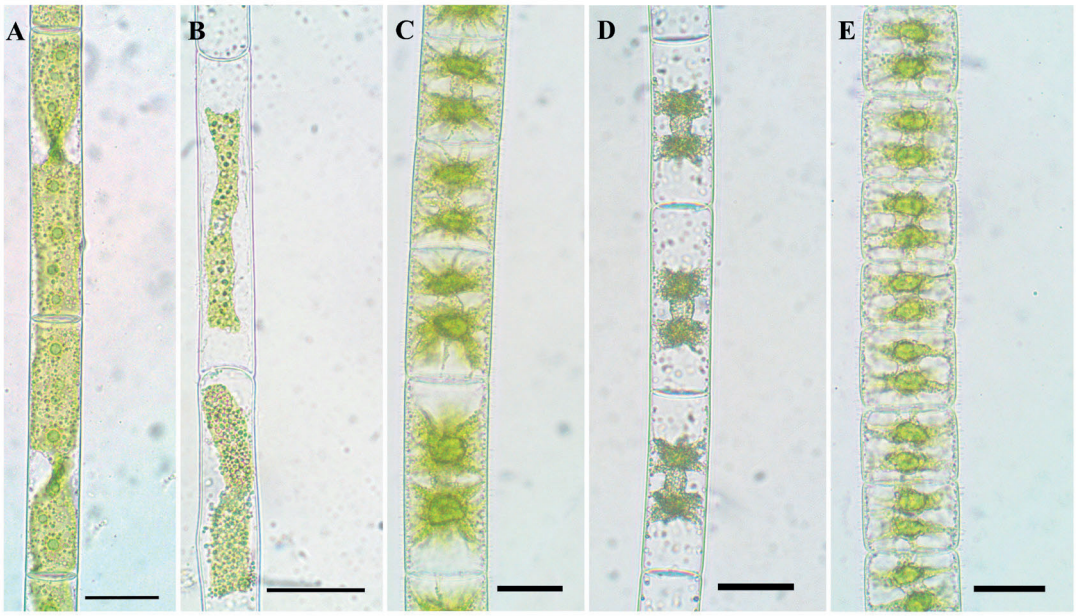

Fig.4. Light microscopic images of *Mougeotia* and *Zygnema* morphotypes from the Lake Baikal region: (A) *Mougeotia* sp. ster. 1; (B) *Mougeotia* sp. ster. 2; (C) *Zygnema* sp. ster. 1; *Zygnema* sp. ster. 2; *Zygnema* sp. ster. 3. Scale bar, 30 μm.

cells 32-38 μm wide, 35-98 μm long; transverse walls plane, 2 stellate chloroplasts in each cell.

This is the first report of this *Zygnema* morphotype for the region. *Zygnema* sp. ster. 1 has been first encountered in 6 locations: **3** - September 2020, depth ≥ 2 m, stony substrate, water temperature 9-14°C, pH 7.1, EC 118.7–122.4 μS cm^{-1}, metaphyton (*S. fluviatilis*, *S. condensata*, *Ulothrix zonata*, *Tetraspora cylindrica* var. *bullosa*, *Didymosphenia* sp., *Spirogyra* sp. ster. 1, *Spirogyra* sp. ster.6, *Zygnema* sp.ster.1); **5** - September 2020, depth < 1-1,5 m, in the water column and on the water surface, water temperature 17°C, pH 7.2, EC 118.6 μS cm^{-1}, metaphyton (*S. condensata, Ulothrix zonata*, *Cladophora floccosa* K.I. Meyer, *Zygnema* sp. ster. 1, *Mougeotia* sp. ster.1); **10** - June 2020, depth < 0.5 m, sandy substrate, water temperature 17°C, EC 124 μS cm^{-1}, among of unattached free-floating algae, or metaphyton (*Zygnema* sp. ster. 1, *Mougeotia* sp. ster. 1) (15%) in macrophytes community dominated by *Myriophyllum sibiricum* (30%) + *Elodea canadensis* (20%) + *Potamogeton* spp. (10%) + *Chara* sp. (10%); **16** - June 2020, depth < 0.5m, stony substrate, water temperature 16.5°C, pH 7.1, metaphyton (*Zygnema* sp. ster. 1, *Zygnema* sp. ster. 3, *Mougeotia* sp. ster.1); **17** - June 2020, depth < 0.5 m, sandy substrate, water temperature 15-25°C, pH 6.8, metaphyton (*Zygnema* sp. ster. 1, *Mougeotia* sp. ster. 1); **21** - June 2020, depth < 0.5 m, sandy substrate, in water column, water temperature 15°C, pH 7.2, among of unattached free-floating algae, or metaphyton (*Zygnema* sp. ster, *Mougeotia* sp. ster.1);

***Zygnema* sp. ster. 2 (Fig. 4D)**

Filaments without basal rhizoids. Vegetative cells 23-24 μm wide, 68-78 μm long; transverse walls plane, 2 stellate chloroplasts in each cell.

This is the first report of this *Zygnema* morphotype for the region. *Zygnema* sp. ster. 2 has been first encountered in the quarry lake in a single location: **24** - July 2020, in a large accumulation of filamentous algae on a flooded meadow, in shallow water in the flooded grass, water temperature 27.1˚C, EC 555 μS cm^{-1}.

***Zygnema* sp. ster. 3 (Fig. 4E)**

Filaments without basal rhizoids. Vegetative cells 40-43 μm wide, 31-40 μm long; transverse walls plane, 2 stellate chloroplasts in each cell.

This is the first report of this *Zygnema* morphotype for the region. *Zygnema* sp. ster. 3 has been first encountered in a single location: **16** - August 2020, depth < 0.5m, stony substrate, water temperature 16.5°C, pH 7.1, metaphyton (*Zygnema* sp. ster. 1, *Zygnema* sp. ster. 3, *Mougeotia* sp. ster.1).

In Lake Baikal, one *Zygnema* species was found in Istoksky Bay (Meyer, 1930). Filaments of *Zygnema* sp. ster. were found in Maloe More Bay, Lake Baikal (Izhboldina, 2007). In the Lake Baikal surrounding area, *Zygnema* sp. ster. were observed in the Osinovka River and in some small freshwater lakes (Izhboldina, 2007).

4. Discussion

Reliable taxonomic reports of *Zygnemataceae* are scarce, being commonly reduced to simply mentioning a generic name without the description of specimens. This because sexual reproduction, or conjugation, is a rarely observed phenomenon in nature. According to different estimates, fertile specimens may occur only in 10-20% of a large-scale field collection (McCourt and Hoshaw, 1986; Novis, 2004; Hainz et al., 2009; Stancheva et al., 2012; 2013; Volkova et al., 2018). Nevertheless, we believe it is essential not only to name the taxa but also to provide morphological descriptions, although not enough for precise identifications, even if fertile filaments were not found. Such an approach would make taxonomic surveys dealing with sterile zygnemataleans as biologically meaningful as possible and a practical basis for further studies. Detailed information on these algae would help assess their bio-indicating role and proliferation degree to respond to water pollution (Hainz et al., 2009) or trace the species origin (Volkova et al., 2018). For instance, when the massive proliferation of *Spirogyra* occurred in Lake Baikal ten years ago, it was uncertain whether native or invasive species caused it (Grachev, 2016). That was primarily since most of the records that mentioned zygnemataleans from this region earlier were not focused on describing either fertile or, even less, sterile filaments. Thus, in the case of the blooming of uncommon zygnemataleans in Lake Baikal, not only their precise identification was challenging, but also understanding their origin and life history. In addition, the description of morphology combined with DNA barcoding might help reveal the hot spots of their diversity and estimate the species' morphological plasticity. It is relevant, especially given the problems inherent in the existing species concept in *Zygnemataceae* (McCourt and Hoshaw, 1990).

In this survey, 18 taxa were identified using morphological characters and classified into three genera, *Spirogyra*, *Mougeotia*, and *Zygnema*. *Spirogyra* was the most diverse genus with thirteen taxa. Two and three taxa belong to *Mougeotia* and *Zygnema* genera, respectively. Fertile stages were in five *Spirogyra* species, including one variety. *S. circumlineata* is reported for Lake Baikal for the first time. Previously, this species was found only in the Bolshaya Kotinka River, one of the lake's southern tributaries (Volkova et al., 2018). Eight new morphotypes of *Spirogyra* were first discovered in the studied area. These do not correspond to descriptions of the already known species and likely represent eight species new for the region. We observed only sterile filaments of *Mougeotia* and *Zygnema*. The *Mougeotia* and *Zygnema* morphotypes we discovered are first reported for the region. The cell dimensions of *Mougeotia* sp. ster. 1, *Mougeotia* sp. ster. 2, and *Zygnema* sp. ster. 1, *Zygnema* sp. ster. 2 are close to those in *Mougeotia genuflexa*, *M. laetevirens*, and *Z. cruciatum*, the species already known from Lake Baikal (Izhboldina, 2007). However, these may also represent previously unknown species since the morphology of sterile filaments overlaps considerably in various taxa (Rundina, 1998). Nevertheless, as in *Spirogyra*, the lack

of conjugation or resting stages in the studied taxa of *Mougeotia* and *Zygnema* prevents their identification at the species level.

Based on presented data and previous taxonomic surveys (Meyer, 1930; Rundina, 1998; Volkova et al., 2018), *Spirogyra* appears more widely distributed than *Mougeotia* or *Zygnema* among the sites investigated. In this study, *Spirogyra*, *Mougeotia*, and *Zygnema* were found in 14, 12, and 7 out of 24 locations, respectively. The distributions of the taxa are likely broader than reported here since most of the species of all three genera are cosmopolitan (Kadlubowska, 1984; Rundina, 1998). Nevertheless, considering the ancientness and specific environmental features of Lake Baikal resulted in its exceptional biodiversity, one could also expect endemic or rare zygnemataleans, as in other unique regions of our planet (Novis, 2004; Sherwood et al., 2018).

Algae of the family *Zygnemataceae* often dominate periphyton or metaphyton assemblages (Hoshaw, 1968; Simons and Van Beem, 1990; Rundina, 1998). Furthermore, they can be a regular part of plant communities or act as an optional component, namely seasonal synusia (Sviridenko et al., 2012). In this study, zygnemataleans were part of 4 attached (periphyton) and 16 free-floating (metaphyton) algal communities. The first ones were observed in Lake Baikal and also consisted of green algae, such as *Ulothrix zonata*, *Tetraspora cylindrica* var. *bullosa*, *Draparnaldioides simplex*, and diatom *Didymosphenia* sp. These are typical species for the lake's open littoral zone with an active hydrodynamic regime (Izhboldina, 2007). The composition of metaphyton varied depending on location and biotope. In four investigated sites (Anga Bay, Barguzinskyi Bay, the Frolikha River's mouth, ponds near the Solontsoviy Cape of Lake Baikal), free-floating synusia of *Spirogyra*, *Zygnema*, and *Mougeotia* with the PC of 5–15% were part of macrophytes communities dominated by *Myriophyllum sibiricum*, *M. spicatum*, *Elodea canadensis*, *Potamogeton* spp., and *Chara globularis*. In seven Lake Baikal's sites (the bay opposite Bolhoye Goloustnoye village, Bolshiye Koty Bay, the bay opposite Kultuk, Listvennochniy Bay, Senogda Bay, the bay opposite Slyudyanka town, the bay opposite Zarechnoye village) and in the shallow, warmed-up areas of the studied rivers (the Chernaya River, the Bolshaya Kotinka River, the Malaya Kotinka River, the Angara River, the Irkut River, the Egia River, the Medlyanka River), metaphyton consisted of *Spirogyra* and/or *Mougeotia*, *Zygnema*, and occasionally included other green filamentous algae, *Ulothrix zonata*, and *Cladophora floccosa*.

5. Conclusion

This study is a new taxonomic report on *Zygnemataceae* from the Lake Baikal region with morphological descriptions of the discovered taxa, characteristics of their environment, and accounts of the co-occurring macrophytes and algae. We identified 18 taxa of the genera *Spirogyra*, *Zygnema*, and *Mougeotia* from 26 new locations, including the Angara River, the Irkut River, Lake Baikal, the five tributaries of the lake (the Chernaya River, the Bolshaya Kotinka River, the Malaya Kotinka River, the Frolikha River, the Medlyanka River), and the small ponds and swamps in the lake surrounding area. In addition to 19 taxa of the family already known from the area, we described thirteen potentially new species. *Spirogyra* is more diverse and widespread than *Zygnema* and *Mougeotia* in the Lake Baikal region. However, the taxa of all three genera regularly present in the communities. The predomination of sterile specimens complicates their precise taxonomic identification; however, it indicates optimal conditions for the vegetation of zygnemataleans and their biomass growth. The members of the family *Zygnemataceae* represent a dynamic component of periphyton, metaphyton, and aquatic macrophyte communities in the studied area. This, on the one hand, reveals them as a significant yet optional component of plant communities. On the other hand, this contributes to their actual distribution, in particular, in the littoral zone of Lake Baikal.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

Egorova I.N., Svetlichny R.V., Sudakova E.A. 2001. Some features of the algal flora of bogs and water bodies of the Kitoy River basin (Southern Cisbaikalia). In: Sovremenniye problemy baikalovedeniya: sbornik trudov molodykh uchenykh. [Modern problems of Baikal studies: collection of works of young scientists]. Irkutsk: Irkutsk State University Press, pp. 98-109. (in Russian)

Dorogostaisky V. 1904. Materiaux pour servir a l'algologie du lac Baikal et de son basin. Bulletin de la Societe Imperiale des Naturalistes de Moscou [Bulletin of the Imperial Society of Moscow Naturalists] 18(1): 229-265. (in French)

Grachev M.A. 2016 «Avoska and avos». The ecological crisis on Lake Baikal: the riddle of the century. Science First Hand 68(2). (in Russian)

Guiry M.D., Guiry G.M. 2020. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. URL: http://www.algaebase.org.

Hainz R., Wöber C., Schagerl M. 2009. The relationship between *Spirogyra* (Zygnematophyceae, Streptophyta) filament type groups and environmental conditions in Central Europe. Aquatic Botany 91: 173-180. DOI: 10.1016/j.aquabot.2009.05.004

Hoshaw R.W. 1968. Biology of filamentous conjugating

algae. In: Jackson D.F. (Ed.), Algae, man and the environment. Syracuse: Syracuse University Press, pp. 135-184.

Izhboldina L.A. 2007. Atlas i opredelitel' vodorosley bentosa i perifitona ozera Baykal (meyo- i makrofity) s kratkimi ocherkami po ikh ekologii [Atlas and key to benthic and periphyton of Lake Baikal (meyo- and macrophytes) with short essays on their ecology]. Novosibirsk: Science Center. (in Russian)

Johnson L.R. 2011. Family Zygnemataceae. In: John D.M., Whitton B.A., Brook A.J. (Eds.), The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae, 2nd ed. Cambridge: Cambridge University Press, pp. 576-607.

Kachaeva M.I. 1974. Bottom fouling algae in the Ingoda River (Transbaikalia). Gidrobiologicheskiy Zhurnal [Hydrobiological Journal] 12(5): 68-72. (in Russian)

Kachaeva M.I. 1980. Algal flora of the Ingoda River tributaries. In: Flora, rastitelnost I rastitelnye resursy Zabaikalya [Flora, vegetation and plant resources of Zabaikalya]. Irkutsk, pp. 30-31. (in Russian)

Kadlubowska J.Z. 1984. Conjugatophyceae I - Zygnemales. In: Ettl H., Gerloff H., Heynig H. et al. (Eds.), Süßwasserflora von Mitteleuropa, Chlorophyta VIII [Freshwater flora of Central Europe, Chlorophyta VIII]. Stuttgart, New York: Gustav Fischer Verlag, pp. 1-531. (in German)

Khanaev I.V., Dzyuba Ye.V., Kravtsova L.S. et al. 2016. The effect of bloom of filamentous green algae on the reproduction of yellowfin sculpin *Cottocomephorus grewingkii* (Dybowski, 1874) (Cottoidae) during ecological crisis in Lake Baikal. Doklady Biological Sciences 467(1): 63-64. DOI: 10.1134/S0012496616020022

Kolkwitz R., Krieger H. 1941. Zygnemales. In: Rabenhorst L. (Ed.), Kryptogamen-Flora von Deutschland, Osterreich und der Schweiz [Cryptogam flora of Germany, Austria and Switzerland], vol. 13. Leipzig: Akademische Verlagsgesellschaft, pp. 1-499. (in German)

Kravtsova L.S., Izhboldina L.A., Khanaev I.V. et al. 2014. Nearshore benthic blooms of filamentous green algae in Lake Baikal. Journal of Great Lakes Research 40: 441-448. DOI: 10.1016/j.jglr.2014.02.019

Kuklin A.P. 2002. Ecology of macrophytic algae of Eastern Zabaikalye (an example from waterbodies of Khilok River Basin). Cand. Sc. Dissertation, Buryat Scientific Center of SB RAS, Ulan-Ude. (in Russian)

Landscape and biological diversity of the Khilok River Basin: experience of the study and management. 2002. In: Malchikova I.Yu., Itigilova M.T., Makarov V.P. (Eds.) Novosibirsk: Publishing House of SB RAS. (in Russian)

McCourt R.M., Hoshaw R.W., Wang J.-C. 1986. Distribution, morphological diversity and evidence for polyploidy in North American Zygnemataceae (Chlorophyta). Journal of Phycology 22: 307-315. DOI: 10.1111/j.1529-8817.1986.tb00028.x

McCourt R.M., Hoshaw R.W. 1990. Noncorrespondence of breeding groups, morphology, and monophyletic groups in *Spirogyra* (Zygnemataceae: Chlorophyta) and the application of species concepts. Systematic Botany 15: 69-78. DOI: 10.2307/2419017

Meyer K.I., Reinhardt L.V. 1925. To the algae flora of Lake Baikal and Transbaikalia. Byulleten' Moskovskogo Obshchestva Ispytatelei Prirody [Bulletin of Moscow Society of Naturalists] 33(3-4): 201-243. (in Russian)

Meyer K.I. 1927. About algae of northern Baikal. Russkii arkhiv protistologii 4(1-4): 93-118. (in Russian)

Meyer K.I. 1930. Introduction to algal flora of Lake Baikal. Byulleten' Moskovskogo Obshchestva Ispytatelei Prirody [Bulletin of Moscow Society of Naturalists] 39: 179-396. (in Russian)

Novis P.M. 2004. New records of *Spirogyra* and *Zygnema* (Charophyceae, Chlorophyta) in New Zealand. New Zealand Journal of Botany 42. DOI: 10.1080/0028825X.2004.9512895

Rundina L.A. 1998. The Zygnematales of Russia (Chlorophyta: Zygnematophyceae, Zygnematales). St. Peterburg: Nauka. (in Russian)

Sherwood A.R., Neumann J.M., Dittbern-Wang M. et al. 2018. Diversity of the green algal genus *Spirogyra* (Conjugatophyceae) in the Hawaiian Islands. Phycology 57: 331-344. DOI: 10.2216/17-111.1

Stancheva R., Hall J. D., Sheath R. G. 2012. Systematics of the genus *Zygnema* (*Zygnematophyceae*, Charophyta) from Californian watersheds. Journal of Phycology 48: 409-422. DOI: 10.1111/j.1529-8817.2012.01127.x

Stancheva R., Hall J.D., McCourt R. et al. 2013. Identity and phylogenetic placement of *Spirogyra* species (Zygnematophyceae, Charophyta) from California streams and elsewhere. Journal of Phycology 49: 588-607. DOI: 10.1111/jpy.12070

Takhteev V.V., Sudakova E.A., Egorova I.N. et al. 2006. On the characterization of aquatic and terrestrial biocenoses in the places of outlets of thermal springs in the eastern Baikal region. In: Takhteev V.V. (Ed.), Hydrobiology of water bodies in the south of Eastern Siberia. Proceedings of the Faculty of Biology and Soil Science of Irkutsk State University. Irkutsk: Irkutsk State University Press. (in Russian)

Timoshkin O.A., Bondarenko N.A., Volkova E.A. et al. 2015. Mass development of green filamentous algae of the genera *Spirogyra* and *Stigeoclonium* (Chlorophyta) in the littoral zone of the Southern part of Lake Baikal. Hydrobiological Journal 51: 13-23. DOI: 10.1615/HydrobJ.v51.i1.20

Timoshkin O.A., Samsonov D.P., Yamamuro M. et al. 2016. Rapid ecological change in the coastal zone of Lake Baikal (East Siberia): is the site of the world's greatest freshwater biodiversity in danger? Journal of Great Lakes Research 42: 487-497. DOI: 10.1016/j.jglr.2016.02.011

Transeau E.N. 1951. The *Zygnemataceae*. Columbus: Ohio State University.

Volkova E.A., Bondarenko N.A., Timoshkin O.A. 2018. Morphotaxonomy, distribution and abundance of *Spirogyra* (Zygnematophyceae, Charophyta) in Lake Baikal, East Siberia. Phycologia 57: 298-308. DOI: 10.2216/17-69.1

Vorob'eva S.S. 1987. Phytoplankton. In: Biologiya Ust'-Ilimskogo vodokhranilischa [Biology of the Ust'-Ilimsk Reservoir]. Novosibirsk: Nauka, pp. 8-82. (in Russian)

Votyakova N.E. 1981. Systematic characterization of fouling algae. Ecology of the Selenga River Delta. Novosibirsk: Nauka. (in Russian)

Simons J., Van Beem A.P. 1990. *Spirogyra* species and accompanying algae from pools and ditches in the Netherlands. Aquatic Botany 37: 247-269. DOI: 10.1016/0304-3770(90)90073-T

Sviridenko B.F., Evzhenko K.S., Efremov A.N. et al. 2012. Phytocoenotic role of *Zygnematales* in the West Siberian Plain. Vestnik Tomskogo Gosudarstvennogo Pedagogicheskogo Universiteta [Tomsk State Pedagogical University Bulletin] 8: 36-42. (in Russian)

Zagorenko G.F. 1971. Ecology of mass algae of vegetation fouling of the Bratsk Reservoir. In: Issledovaniya gidrobiologicheskogo rezhima vodoemov Vostochnoi Sibiri [Studies of the hydrobiological regime of water bodies in Eastern Siberia]. Irkutsk, pp. 69-75. (in Russian)