ISSN 2658-3518

# LIMNOLOGY & FRESHWATER BIOLOGY

2021, № 6

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

Limnology and Freshwater Biology 2021 (6): 1195-1198 DOI:10.31951/2658-3518-2021-A-6-1195

Short communication

ISSN 2658-3518
LIMNOLOGY FRESHWATER BIOLOGY
www.limnolfwbiol.com

# New records of *Vaucheria* (Xanthophyceae) from the Lake Baikal region

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**ABSTRACT.** As a result of the field studies (2019-2021), new data were obtained on the *Vaucheria*, a genus of yellow-green algae, of the Lake Baikal region. After the studying of 30 fertile specimens from new localities, most of them from close vicinities of Lake Baikal, we identified 10 species. The new records improve the species distributional ranges within the Lake Baikal region, which is particularly important for rare species that were previously known from a few localities, e.g. *V. alaskana*, *V. birostris*, *V. cruciata*, *V. megalaversa*, and *V. prona*. *Vaucheria uncinata* was first recorded from Lake Baikal being occurred in the Pokoinitski bay. A check-list is established for all *Vaucheria* species, ever discovered in the Lake Baikal region.

***Keywords***: *Vaucheria*, Siberia, Lake Baikal, new species records, check-list

## 1. Introduction

*Vaucheria* DC. is among the richest genera of yellow-green algae (Xanthophyceae, *Vaucheria*ceae) that can be delineated through filamentous coenocytic thalli and oogamous reproduction. The species of *Vaucheria* can be easily discovered by the naked eye; however, precise species identification requires thorough microscopic observations to detect reproductive structures. The species of *Vaucheria* occur over a wide range of marine, freshwater, and moist terrestrial habitats worldwide. In the Lake Baikal region (LBR) in Siberia, the studies of *Vaucheria* have a very brief history. There were only a few studies where these algae are identified at a species rank. Dorogostaisky (1904) first reported 3 species, *V. terrestris* Lyngb., *V. racemosa* (Vauch.) DC. sensu Götz and *V. sessilis* (Vauch.) DC., from some tributaries of Lake Baikal and vicinities of Irkutsk. Many years later, Bochka (2000) additionally published new records of *V. terrestris* and *V. aversa* Hass. from small waterbodies of Barguzinsky biosphere reserve, eastern shore of Lake Baikal. All these records lack descriptions or pictures that could confirm identifications, and the voucher specimens have not survived. The author of the present report has studied the genus *Vaucheria* in LBR. As a result, 15 species were identified, including *V. megalaversa* Vishnyakov, a new species described from the region (Vishnyakov, 2019a; 2019b; 2021; Vishnyakov et al., 2020; for more references see these publications). Although species of *Vaucheria* were revealed among the commonest algae in small lakes, rivers, streams, springs, and wet soils of LBR, none registered in Lake Baikal (cf. Izhboldina, 2007), which is the world's largest freshwater lake. Very little is known yet on *Vaucheria* species inhabiting tributaries of Lake Baikal and various waterbodies of the nearshore zone.

This report presents new records of *Vaucheria* from LBR, mostly from close vicinities of Lake Baikal, with a special focus on *V. uncinata* Kütz., a species first recorded from the lake.

## 2. Materials and methods

The specimens originate from various aquatic, semi-terrestrial, and terrestrial habitats. These were collected during the fieldwork in the Irkutsk region and the Republic of Buryatia in 2019-2021. In particular, for the first time, Barguzinski, Eravninski, and Khorinski districts were surveyed. The specimens were studied alive, where possible, or preserved in 95% alcohol or 4% formalin in vials. Some parts of abundant specimens were air-dried on cardboard sheets. For light microscopy (LM), several small pieces of each specimen were rinsed with tap water in Petri dishes and then transferred to microscope slides. LM was done using a CNOEC laboratory microscope (Opto-Edu Co. Ltd, Beijing, China) equipped with a digital camera. The voucher specimens were deposited in *Vaucheria*

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***Received:*** December 22, 2021; ***Accepted:*** December 27, 2021; ***Available online:*** December 30, 2021

collection, which is currently housed at the Papanin Institute for Biology of Inland Waters, RAS (Borok). A single access number V-n was affixed to each specimen. In total, 35 specimens have been studied, of which 30 specimens consist of fertile filaments and are listed below.

**V-584** 24.08.2019 Irkutsk region. Slyudyanski district, 123 km of the Circum-Baikal Railway, 51.776023°N, 104.180452°E, waterfall on the Kirkirey stream, on wet soil. **V-585** 24.08.2019 Irkutsk region. Slyudyanski district. 123 km of the Circum-Baikal Railway, tunnel of Kirkirey, 51.776067°N, 104.180327°E, on wet soil. **V-586** 24.08.2019 Irkutsk region. Slyudyanski district, the Medlyanka River downstream in Kultuk, 51.725567°N, 103.713176°E, in water on mosses and *Montia fontana*, abundantly, 60 ppm, pH 8.5. **V-588** 28.08.2019 Republic of Buryatia. Pribaikalski district, Goryachinsk, 52.987237°N, 108.307435°E, bank of the thermal spring, on wet soil. **V-589** 29.08.2019 Republic of Buryatia. Barguzinski district, the Maksimikha River downstream, 53.263396°N, 108.741017°E, rapids near the rock, on mosses, water depth 0–5 cm, abundantly. **V-592** 04.09.2019 Irkutsk region. Ekhirit-Bulagatski district, karst lake near Gushit, 52.888631°N, 104.975398°E, on wet soil. **V-593** 04.09.2019 Irkutsk, flowerbed near “Angara” hotel, on wet soil. **V-594** 06.09.2019 Irkutsk, 52.291696°N, 104.280019°E, flowerbed near Beloborodov’s monument. **V-595** 04.09.2019 Irkutsk region. Ekhirit-Bulagatski district, reservoir on the Ordushka River, 52.816101°N, 104.801734°E, on wet soil in *Typha laxmannii* community. **V-876** 04.07.2020 Irkutsk region. Bayandaevski district, ephemeral pond in Bayandai, 53°03’13.4”N 105°30’33.5”E, in water with *Zannichellia* and *Halerpestes salsuginosa*, electrical conductivity 2.4 mS, abundantly. **V-877** 01.08.2020 Irkutsk, bank of the Angara River channel, along Polarnaya Street, 52.354473°N, 104.267525°E, on wet soil. **V-878** 26.07.2020 Irkutsk region. Irkutski district, bank of the Krestovka River in Listvyanka, 51°51’21.6”N 104°51’39.9”E. **V-879** 12.08.2020 Irkutsk region. Shelekhovski district, right channel of the Olkha River in Olkha, 52°09’27.6”N 104°06’12.6”E, springs. **V-881** 06.07.2020 Irkutsk region. Olkhonski district, Olkhon Island (Lake Baikal), small lake near Shebetski Bay, 53°08’37.1”N 107°07’02.4”E, on depth 30 cm, electrical conductivity 1.5 mS. **V-883** 05.07.2020 Irkutsk region. Olkhonski district, Sakhyurta, wet meadow near Bazarnaya Bay, 53°01’05.9”N 106°53’04.6”E, ephemeral lake, in water of hoofprints and on wet soil. **V-884** 04.07.2020 Irkutsk region. Olkhonski district, oxbow of the Anga River near Ust-Anga, 52°46’28.2”N 106°33’24.0”E, electrical conductivity 446 μS. **V-885** 12.07.2020 Republic of Buryatia. Eravninski district, the Domnaya River, 52°37’10.1”N 111°40’18.1”E, in water under the bridge, electrical conductivity 258 μS. **V-886** 13.07.2020 Republic of Buryatia. Eravninski district, oxbow of the Egita River near Mozhaika, 52°23’58.4”N 110°45’58.6”E, on wet soil trodden up by cattle. **V-887** 11.07.2020 Republic of Buryatia. Khorinski district, oxbow in right floodplain of the Uda River, 52°21’14.8”N 110°23’26.6”E, on wet soil trodden up by cattle. **V-888** 08.08.2020 Republic of Buryatia. Tunkinski district, oxbow of the Irkut River in Tory, 51°47’15.8”N 103°00’03.0”E, on wet soil trodden up by cattle. **V-890** 07.08.2020 Republic of Buryatia. Tunkinski district, left branch of the Kyngarga River in Arshan, 51°55’20.9”N 102°25’32.6”E, in slow water, electrical conductivity 226 μS, t 8.2°C, abundantly. **V-904** 12.06.2021 Irkutsk region. Irkutski district, Bolshoe Goloustnoe, 52.027611°N, 105.413775°E, dried ephemeral lake, on wet soil and *Carex* leaves. **V-905** 09.06.2021 Irkutsk, floodplain of the Angara River, near “Polyana” park, 52.253184°N, 104.276419°E, on wet soil. **V-906** 12.06.2021 Irkutsk region. Irkutski district, right tributary of the Pravaya Ushakovka River, 52.290°N, 104.962°E, near road, in water, electrical conductivity 72 μS. **V-907** 20.06.2021 Irkutsk, Selivanikha, 52.293871°N, 104.234588°E, oxbow of the Irkut River, electrical conductivity 1085 μS, t 17.8°C. **V-911** 03.08.2021 Irkutsk region. Olkhonski district, Baikal, Zunduk cape, 53.392259°N, 107.426535°E, bank of small lake. **V-912** 04.08.2021 Irkutsk region. Olkhonski district, Lake Baikal, Pokoiniki cape, bay (Pokoinitski bay), 54.011713°N 108.241753°E, on 20-30 depth, gravel, electrical conductivity 168 *μ*S. **V-913** 08.08.2021 Republic of Buryatia. Severo-Baikalski district, bank of the Ayaya River, 55.456668°N, 109.915777°E, on sand. **V-914** 08.08.2021 Republic of Buryatia. Severo-Baikalski district, bog in the Ayaya River valley, 55.456359°N 109.9231°E, water pool. **V-916** 13.08.2021 Irkutsk region. Irkutski district, spring near the Bolshaya Kotinka River downstream, 51.904698°N, 105.073809°E, electrical conductivity 0.1 mS, t 11.5°C.

## 3. Results and discussion

In total, 10 species were identified.

***V. alaskana*** Blum

Specimens: V-884, 887. Rare species, mostly distributed within the Holarctic. In LBR, the species is known from a few localities in the vicinities of Irkutsk, in rivers of the Eastern Sayan Mountains, and Olkhon Island of Lake Baikal (Vishnyakov, 2019a; 2019b). The species primarily occurs in semi-terrestrial habitats, i.e. riverbank or damp soil, become exposed to the air when the water level recedes. However, specimen V-884 originates from aquatic habitat, being collected near the shore.

***V. birostris*** Simons

Specimens: V-888. Rare species with a Holarctic distribution. In LBR, the species is known from a few floodplain localities of the Angara, Kuda, Bolshaya Goloustnaya, and Selenga rivers (Vishnyakov, 2019a; 2019b). The species prefers semi-terrestrial habitats.

***V. bursata*** (O.F. Müll.) C. Agardh

Specimens: V-584, 586, 589, 595, 879, 884, 890, 904, 907, 913, 914, 916. Species with a cosmopolitan distribution, the most common representative of the genus in LBR (Vishnyakov, 2019a; 2019b). This amphibious species can occur in aquatic, semi-terrestrial, and terrestrial habitats.

***V. canalicularis*** (L.) T.A. Chr.

Specimens: 585, 588, 592, 595, 877, 883, 886, 888, 905, 907, 911. Cosmopolitan species, which is one of the commonest in LBR. However, most of the specimens originate from the Irkutsk region, and very rarely from the Republic of Buryatia (Vishnyakov, 2019a; 2019b). The species primarily occurs in aquatic and semi-terrestrial habitats.

***V. cruciata*** (Vauch.) DC.

Specimens: V-905. Cosmopolitan amphibious species, which was rarely recorded from LBR (Vishnyakov, 2019a; 2019b). The species mostly occurs in aquatic and semi-terrestrial habitats of floodplains.

***V. frigida*** (Roth) C. Agardh

Specimens: V-905, 906, 914. Cosmopolitan amphibious species. In LBR, it is widely distributed in various aquatic, semi-terrestrial, and terrestrial habitats (Vishnyakov, 2019a; 2019b).

***V. megalaversa*** Vishnyakov

Specimens: V-914, 916. Rare Asian aquatic species. The distribution of *V. megalaversa* appears to be disjunctive between LBR and Ola plateau at the Russian Far East. This was identified previously as a "giant forma of *Vaucheria aversa*" (Vishnyakov, 2019a; 2019b) and accepted as a distinct species only recently (Vishnyakov, 2021). In LBR, the species was previously known from a few riverine localities surrounding Southern Baikal and Tunka rift valley. One new specimen (V-916) originates from a spring associated with *locus typicus*, the Bolshaya Kotinka River, a small tributary of Lake Baikal, another one (V-914) comes from a small minerotrophic bog situated nearby northeastern shore of Lake Baikal. The species prefers clean habitats and is currently known from both stagnant and flowing waters.

***V. prona*** T.A. Chr.

Specimens: V-593, 594. Semi-cosmopolitan species. In LBR, it was previously known from a few localities in Irkutsk and its vicinities, in quarries near Cheremkhovo and in the Selenga River delta (Vishnyakov, 2019a; 2019b). The species prefers terrestrial or semi-terrestrial habitats, which are frequently disturbed by human activity.

***V. racemosa*** (Vauch.) DC.

Specimens: 589, 876, 878, 881, 883, 885, 888, 914, 916. Semi-cosmopolitan species, one of the commonest in LBR (Vishnyakov, 2019a; 2019b). The species primarily occurs in aquatic habitats, both stagnant and flowing, becoming rare in semi-terrestrial habitats.

***V. uncinata*** Kütz.

Specimens: V-912. Semi-cosmopolitan amphibious species, which was recorded 5 times from LBR. Previously known localities belong to small tributaries of the Irkutsk reservoir on the Angara, Bolshaya and Burduguz rivers (Vishnyakov, 2019a; 2019b). *V. uncinata* was first discovered in Lake Baikal in 2021. The locality belongs to Pokoinitski bay, the northwestern shore of the lake, where the species was locally abundant in a sparse community of *Persicaria* and *Carex* at the shallowest part (Fig. 1A). The locality is in sharp contrast with an open shore of Lake Baikal, which is exposed to wave activity, deep, and cold, and yet connected directly with it. As is currently known, the coastal zone of Lake Baikal serves as a home for many hydrobionts, both endemics and immigrants from the Holarctic waters, and the latter are commonly restricted to semi-isolated shallowest parts of the lake (Izhboldina, 2007; Timoshkin et al., 2012). In this regard, *V. uncinata* is a species of the Siberian floristic complex that entered the shallow bay. Thorough future investigations of the Lake Baikal coastal zone are needed to determine whether the *Vaucheria* species is more widely distributed or limited to the known locality.

There are only four species among identified, which can be considered common in LBR: *V. bursata*, *V. canalicularis*, *V. frigida*, and *V. racemosa*. The rest are sporadically occurred throughout the region.

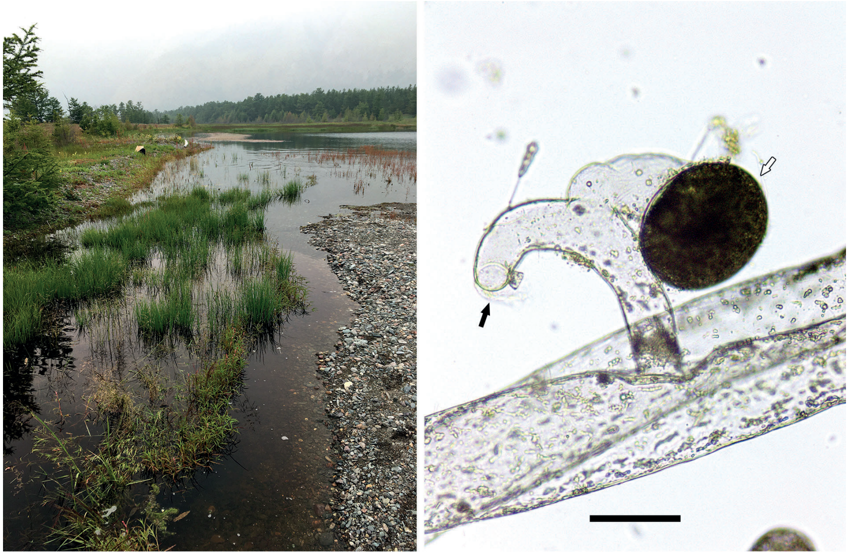

**Fig.1.** Locality of V*aucheria uncinata* in Lake Baikal (A) and LM micrograph (B) showing fruiting branch bearing stalked antheridium (disintegrated wall is indicated by black arrow) and oogonium without distinctive fertilization pore (white arrow). Scale bar: 100 μm.

The most of species show broad geographical ranges that often span more than one continent, and there is a single species, *V. megalaversa*, restricted to local habitats in northeastern Asia. The morphology of all studied species was in good agreement with that of previously studied (Vishnyakov, 2019a; 2021). As a new member of the Lake Baikal algal flora, *V. uncinata* is additionally described here. Filaments are 65–140 µm in diameter. Gametangia born on short lateral gametophores. Each gametophore consists of 1 or 2 pendent oogonia and 1 antheridium (Fig. 1B). Oogonia are ellipsoidal, 130–170 µm in diameter. Antheridia are circinate-cylindrical, 32–45 µm in diameter. Asexual reproduction is unknown. An absence of distinctive oogonial fertilization pore allows distinguishing easily *V. uncinata* from all other species in LBR.

Based on the results of our previous studies, we propose here a conspectus of 15 species of the genus *Vaucheria* in LBR. These belong to 6 sections. I. Section *Woroninia* (Solms-Laub.) Heer.: *V. schleicheri* De Wild. (**IR**). II. Section *Corniculatae* (Walz) Heer.: *V. bursata* (O.F. Müll.) C. Agardh (**IR, RB**). III. Section *Tubuligerae* (Walz) Heer.: *V. fontinalis* (L.) T.A. Chr. (**IR**), *V. megalaversa* Vishnyakov (**IR, RB**), *V. pseudaversa* Vishnyakov (**RB**). IV. Section *Vaucheria*: *V. birostris* Simons (**IR, RB**), *V. canalicularis* (L.) T.A. Chr. (**IR, RB**), *V. cruciata* (Vauch.) DC. (**IR, RB**). V. Section *Racemosae* (Walz) Entwisle: *V. alaskana* Blum (**IR, RB**), *V. frigida* (Roth) C. Agardh (**IR, RB**), *V. nuoljae* (Skuja) Vishnyakov (**IR**), *V. prona* T.A. Chr. (**IR, RB**), *V. racemosa* (Vauch.) DC. (**IR, RB**), *V. taylorii* Blum (**RB**). VI. Section *Heeringia* Blum: *V. uncinata* Kütz. (**IR**). Abbreviations: IR – Irkutsk Region, RB – Republic of Buryatia.

## 4. Conclusions

The present report improves significantly the distributional ranges of 10 previously reported species within the Lake Baikal region, which is particularly important for rare species that were previously known from a few localities. One such species is *V. uncinata*, which was first discovered in Lake Baikal.

## Acknowledgments

The study was performed as a part of the state assignment of the Russian Federation to IBIW RAS, no. 121051100099-5. Thanks to the crews of “Titov” and “Professor Kozhov” research vessels, E.A. Volkova, E.V. Mincheva, T.E. Peretolchina (Limnological Institute of SB RAS, Irkutsk) for comprehensive assistance in field studies.

## Conflict of interests

The author declares no conflict of interests

## References

Bochka A.B. 2000. Vodorosli [Algae]. In: Flora i fauna vodoemov i vodotokov Barguzinskogo zapovednika. Flora i fauna zapovednikov. Annotirovannye spiski vidov. Vyp. 91 [Flora and fauna of waterbodies and streams of Barguzin Reserve. Flora and fauna of reserves. Annotated lists of species. Vol. 91]. Moscow, pp. 8-123. (in Russian)

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

Izhboldina L.A. 2007. Atlas i opredelitel’ vodorosley bentosa i perifitona ozera Baikal (meyo- i makrofity) s kratkimi ocherkami po ikh ekologii [Guide and key to benthonic and periphyton algae of Lake Baikal (meio- and macrophytes) with short notes of their ecology]. Novosibirsk: Nauka-Center. (in Russian)

Timoshkin O.A., Suturin A.N., Bondarenko N.A. et al. 2012. Introduction into biology of the coastal zone of Lake Baikal. 1. Splash zone: first results of interdisciplinary investigations and its role for the lake ecosystem monitoring. Izvestiya Irkutskogo Gosudarstvennogo Universiteta [The Bulletin of Irkutsk State University] 5(3): 33-46. (in Russian)

Vishnyakov V.S. 2019a. Representatives of genera *Botrydium* Wallroth and *Vaucheria* De Candolle (Xanthophyceae) in the South of Baikal Region (Russia). International Journal on Algae 21(1): 25-42. DOI: 10.1615/InterJAlgae.v21.i1.20

Vishnyakov V.S. 2019b. The localities of *Vaucheria* (Xanthophyceae) in Irkutsk Region and Republic of Buryatia. Trudy Instituta Biologii Vnutrennikh Vod im. Papanina [Transactions of Papanin Institute for Biology of Inland Waters] 85(88): 44-58. (in Russian)

Vishnyakov V.S. 2021. Revision of *Vaucheria* sect. *Tubuligerae* (Xanthophyceae) in Russia. Botanicheskii Zhurnal [Botanical Journal] 106(7): 703-723. (in Russian)

Vishnyakov V.S., Romanov R.E., Chemeris E.V. et al. 2020. New records of *Vaucheria* (Ochrophyta, Xanthophyceae) in Russia. Novosti Sistematiki Nizshikh Rastenii [Non-vascular Plants Taxonomy News] 54(1): 7-41. (in Russian)

Limnology and Freshwater Biology 2021 (6): 1199-1204 DOI:10.31951/2658-3518-2021-A-6-1199

**Original Article**

ISSN 2658-3518
LIMNOLOGY FRESHWATER BIOLOGY
www.limnolfwbiol.com

# Prospects for population genetic studies of cosmopolitan freshwater sponges of the Spongillidae family in Lake Baikal

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**ABSTRACT.** Cosmopolitan freshwater sponges inhabit Lake Baikal. They are of great interest for carrying out population genetic studies. Microsatellite markers are best suited for population genetic studies of sponges. To date, no markers have been developed for the species *Ephydatia muelleri* that is widespread in Lake Baikal and found across the northern hemisphere. In the course of this study, a search was carried out for microsatellite markers in the previously published complete genome of *E.muelleri*. The most promising microsatellite loci were selected among those found in the genome data. Selected loci were tested on *E.muelleri* DNA samples. A set of 11 specific variable microsatellite markers was developed and tested for further population genetic studies of *E.muelleri*. Also, the Maloye More Strait area of Lake Baikal was surveyed to determine the sites of mass accumulation of the Spongilliadae family representatives. An analysis of the species composition of cosmopolitan sponges was carried out for Site 1 (Olkhon Island). Two species were identified: *E.muelleri* (72%) and *Spongilla lacusris* (18%).

***Keywords***: Genetic markers, microsatellites, population genetics, sponges, Porifera, Lake Baikal

## 1. Introduction

Sponges are one of the oldest multicellular organisms that have survived and thrive today (Philippe et al., 2009). More than 8500 sponge species were described and accepted worldwide (van Soest et al., 2012). Sponges lead an attached lifestyle and have a filtration type of nutrition (van Soest et al., 2012). Most freshwater sponge species are capable of asexual reproduction (Maldonado and Riesgo, 2008) and can survive under unfavorable conditions, for example, desiccation (Manconi and Pronzato, 2008), in the form of resting stages, gemmules. Due to the limited swimming ability of the larvae, most sponge species are found only in a limited habitat or are endemic. However, there are cosmopolitan sponge genera that spread through gemmules (Bilton et al., 2001; Manconi and Pronzato, 2008). Sponges make a significant contribution to the ecology of both marine and freshwater ecosystems (Dröscher and Waringer, 2007; Bell, 2008; Vohmann et al., 2009). Due to the filtration type of nutrition, sponges serve as sensitive bioindicators of the pollution of aquatic ecosystems (Roveta et al., 2021).

Several studies of the population structure were conducted for marine sponges (Duran et al., 2004; Calderón et al., 2007; Hoshino et al., 2008; Blanquer et al., 2009; Blanquer and Uriz, 2010; 2011; Dailianis et al., 2011; Guardiola et al., 2012; Noyer and Becerro, 2012; Riesgo et al., 2016); however, data for freshwater sponges are very scarce.

For the freshwater sponge, *Ephydatia fluviatilis*, an analysis for compliance with the hypothesis of monopolization, which is confirmed for crustaceans (De Gelas and De Meester, 2005; Muñoz and Pacios, 2010), bryozoans (Hoare et al., 2001; Massard and Geimer, 2008) and rotifers (Mills et al., 2007; Fontaneto et al., 2008) was carried out. For samples from Central Italy located within 100 km, no genetic differentiation was found. Differentiation was found between populations from Italy and Hungary, located more than 600 km apart. Sponges in terms of their genetic relationships between populations are similar to rotifers and crustaceans and can be used in combination with these organisms for further studies of the monopolization hypothesis (Lucentini et al., 2013). Additionally, the population structure of sponges inhabiting the River-Sieg system was studied for the *E.fluviatilis* freshwater sponge. That study revealed a clear genetic differentiation between populations living at a distance of up to 50 kilometers (Li et al., 2018). The population structure of freshwater sponges has not previously been studied for large

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***Received:*** December 14, 2021; ***Accepted:*** December 26, 2021;
***Available online:*** February 15, 2022

ancient lakes. However, the relevance of such studies is undoubtable because, for example, Lake Baikal is a unique ecosystem that has existed for millions of years (Kozhov, 1962; Jaguś et al., 2015), and is mainly represented by endemic flora and fauna.

Cosmopolitan sponges live only in shallow waters; therefore, their settlement and distribution pattern in Lake Baikal is not obvious. According to paleontological data, sponges have lived in Baikal for at least 10 Ma (Veynberg, 2009). Since the formation of Lake Baikal, catastrophic events have occurred several times, causing a dramatic change in the level of the lake (Arzhannikov et al., 2017; 2021). There is no evidence that the episodes of the water levels changes led to the disappearance and subsequent recolonization by cosmopolitan sponge species of Lake Baikal or they developed continuously in the water area of the lake. The level of migration of cosmopolitan sponges between the bays separated from the main water area of Lake Baikal is also not obvious. The search for the answers to these questions is of interest and requires the study of the population genetic structure of Baikal cosmopolitan sponges. Also, endemic sponges inhabit Lake Baikal. Baikal endemic sponges have a common ancestor with the cosmopolitan genus *Ephydatia* (Itskovich et al., 2008). During the formation of an endemic family, Baikal endemic sponges lost their ability to form gemmules and acquired a long-term life cycle. The question remains open of how such changes affected the ability of endemic sponges to settle and migrate within Lake Baikal. It is necessary to carry out a comparative analysis between the population structures of the cosmopolitan sponges of the genus *Ephydatia* inhabiting Lake Baikal and the endemic ones.

According to the data from the recent revision of Baikal sponges (Efremova, 2004), there are members of four genera of the cosmopolitan family Spongillidae: *Ephydatia* Lamouroux, 1816, including the species *Ephydatia muelleri*, *Spongilla* Lamarck, 1816, *Eunapius* Gray, 1867, and *Trochospongilla* Vejdovsky, 1888. We found massive concentrations of individuals of this species in the Maloye More Strait. Therefore, this species was chosen as a promising one for carrying out population genetic studies of cosmopolitan freshwater sponges in Lake Baikal.

Molecular genetic markers were used for carrying out population genetic studies of sponges. The low resolution of mitochondrial markers such as COI was indicated for sponges (Yakhnenko and Itskovich, 2020a); however, microsatellite markers were used quite successfully. Several sets of microsatellite markers were published for marine sponge species (Duran et al., 2002; Knowlton et al., 2003; Blanquer et al., 2005; Hoshino and Fujita, 2006; Noyer et al., 2009; Anderson et al., 2010; Dailianis and Tsigenopoulos, 2010; Guardiola et al., 2012; Giles et al., 2013; Taboada et al., 2018). For freshwater sponges, microsatellite markers were developed only for *E.fluviatilis* (Anderson et al., 2010). As we have shown previously (Yakhnenko and Itskovich, 2020b), these markers are not suitable for population genetic studies of other species of the Spongillidae family, specifically for *E.muelleri*, although these species are closely related. Thus, it is necessary to develop and test a set of microsatellite markers specific for *E.muelleri*. To solve this problem, an analysis of the chromosomal level genomic data of *E.muelleri* published previously (Kenny et al., 2020) will be carried out.

To assess the prospects for analyzing the genetic distances between the populations of cosmopolitan freshwater sponges in Lake Baikal, it is also necessary to conduct fieldwork to survey the coves and bays of the Maloye More Strait at Lake Baikal to select the site of mass accumulation of the Spongillidae members.

## 2. Materials and methods

### 2.1. Sampling

Sponges were collected in November 2019, July and November 2020 in four bays and lagoons in the area of the Maloye More Strait at Lake Baikal (Fig. 1; Fig. 2; Table 1) at depths of 0 to 1.5 meters. Most of the sponge samples were collected from the back surface of the stones.

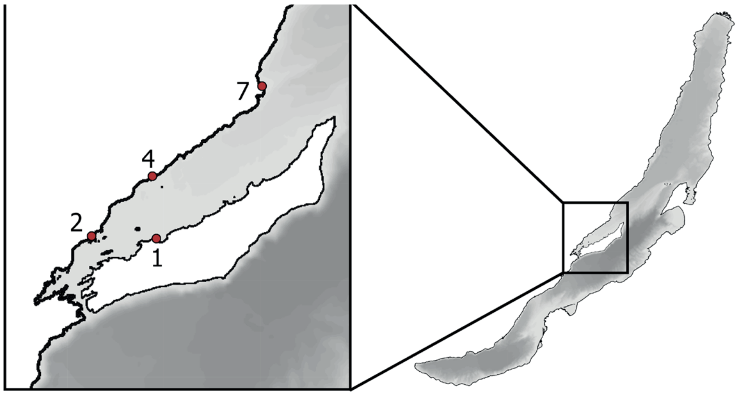

**Fig.1.** Spongillidae mass accumulation sites near the Maloye More Strait.

**Fig.2.** Sponge sample of the genus *Ephydatia* from Site 1.

The samples were fixed in 70% ethanol immediately after collection. After 24 hours ethanol was replaced with a new one for long-term storage at +4C°. Some samples in the form of gemmules were washed from the remains of the skeleton and were placed in a refrigerator at +4C° in a humid state without access to sunlight.

Species were identified based on morphological characteristics such as appearance, length and shape of spicules and gemmosclera using a light microscope. Spicules were isolated from a small fragment of the sample. The organic part was dissolved by decolorant; then, the decolorant was washed off with distilled water.

### 2.2. Microsatellite markers development

Tandem repeats with 7 to 30 blocks in the complete genome data on *E.muelleri* (Kenny et al., 2020) were searched for using the STR detection tool in Galaxy software (Fungtammasan et al., 2015). The most suitable microsatellites with flanking regions were selected among the identified ones. For the set of 28 microsatellites, primer pairs were designed and tested. Primers were searched for using Primer-BLAST NCBI (Ye et al., 2012). Primers were purchased from Evrogen (Moscow, Russia) and Syntol (Moscow, Russia).

### 2.3. Microsatellite amplification and genotyping

DNA from sponge samples was isolated using the CTAB method (Gustincich et al., 1991). PCR was performed in a Peltier Thermal Cycler (MJ Research, USA) using a ScreenMix-HS kit (Evrogen), Russia. The PCR program was optimized. Initial denaturation carried out for 2 min at 94C° followed by 11 cycles of denaturation for 30 sec at 94C°, annealing for 30 sec at 65-55C° (one-degree reduction every cycle), the extension for 30 sec at 72C° followed by 24 cycles of denaturation for 30 sec at 94C°, annealing for 30 sec at 55C°, the extension for 30 sec at 72C°, and then the final extension for 8 min at 72C°.

**Table 1.** Coordinates of sampling sites

| Sampling site No | Coordinates |
|---|---|
| 1 | 53°09’10.4»N 107°10’11.4»E |
| 2 | 53°09’32.5»N 106°56’56.9»E |
| 4 | 53°16’59.1»N 107°09’21.4»E |
| 7 | 53°28’11.1»N 107°32’02.5»E |

PCR products were visualized in a 2% agarose gel with Syber Green dye. The exact size of PCR products was estimated using fragment analysis on an ABI 3130xl Genetic Analyzer (Syntol, Moscow) and analyzed with the GenMarker 3.01 software.

## 3. Results and discussion

During the fieldwork, seven bays and lagoons were surveyed in the area of the Maloye More Strait at Lake Baikal. Mass accumulations of freshwater cosmopolitan sponges were found at four out of seven locations (Fig. 1).

We analyzed species composition for Site 1. The collections contained samples of two species: *Spongilla lacustris* (18%) and *Ephydatia muelleri* (72%).

Based on the analysis of the *E.muelleri* genomic data, we selected 28 promising microsatellite loci with flanking regions suitable for the development of primers. A pair of primers for each locus was developed. Each locus was tested on four DNA samples of *E.muelleri.* Fragment analysis was carried out for 17 loci, giving clear bands in the gel electrophoresis. Among the analyzed loci, 11 were variable, which were included in the set of microsatellite markers (Table 2). For fragment analysis, fluorescent labels were attached to the forward primers. (Table 3).

**Table 2.** Coordinates of microsatellite markers in genomic data on *E.muelleri* (Kenny et al., 2020)

| Locus Name | Scaffold No* | Query start* | Query end* | Sequence length | Repeat type | n of alleles |
|---|---|---|---|---|---|---|
| Emu_241 | scaffold_0019 | 1357021 | 1357338 | 318 | (CT)14 | 4 |
| Emu_249 | scaffold_0019 | 1810514 | 1810717 | 204 | (CA)13 | 2 |
| Emu _257 | scaffold_0019 | 1386901 | 1387195 | 295 | (CG)6(CA)21 | 2 |
| Emu_187 | scaffold_0016 | 868299 | 867998 | 302 | (CA)18 | 2 |
| Emu_291 | scaffold_0020 | 6431917 | 6432205 | 289 | (TG)9 | 3 |
| Emu_124 | scaffold_0012 | 8003328 | 8003132 | 197 | (GTG)5 | 2 |
| Emu_369 | scaffold_0023 | 541061 | 540749 | 313 | (GT)27 | 3 |
| Emu_266 | scaffold_0019 | 6087901 | 6087619 | 283 | (TGG)7 | 3 |
| Emu_260 | scaffold_0019 | 1043658 | 1043935 | 278 | (CA)28 | 2 |
| Emu_217 | scaffold_0018 | 7273471 | 7273864 | 394 | (AC)9 | 3 |
| Emu_367 | scaffold_0023 | 2188522 | 2188841 | 320 | (AC)23 | 2 |

*In genome assembly from (Kenny et al., 2020)

**Table 3.** Primer pairs for the set of microsatellite markers

| Locus Name | Fw. primer | Rev. Primer | Tm | Fluorescent label |
|---|---|---|---|---|
| Emu_241 | GCTCACTACTCCAACCCGAC | CAAAGCATGGCGTGTTGTGT | 59-61 | Tamra |
| Emu_249 | CATCTCTGGTGAAGTACACAGGTG | CAGAGTGCTCCAGCTGCT | 59-61 | Fam |
| Emu _257 | GAGTCCGTCCTCCTGTTCAC | TGCCAGCAGAGGATATAGCATTC | 59-61 | R6G |
| Emu_187 | AGCACAGCATAGCAACGATTG | ATGTGGACTTCAGGCACCTG | 59-61 | Fam |
| Emu_291 | ACGTACCTCAAACACCGTAGTAC | CCCGGCTGTCTGTAAGTCAT | 59-61 | Tamra |
| Emu_124 | ACTGCCACTCAGGCTCAAG | TTATCCTCAGCGAGAACGTAGTC | 59-61 | Rox |
| Emu_369 | ACAAGGGTTAGTTAGGAGGCAG | GAGTACTAGGAGGATGAACCAGTG | 59-61 | Rox |
| Emu_266 | GCCTGTGGTGTAAACAGTGG | CCAAGCGTCCCAGCTAAGAG | 59-61 | Fam |
| Emu_260 | CCTTCGTCCACAATGGCTTG | ACCTTGGACCAGATTACTCCAAC | 59-61 | Fam |
| Emu_217 | GTGTCATGGAAGACCAATGAGC | CCTTCAGGCAGGACATCAATACT | 59-61 | Fam |
| Emu_367 | CCACTGTTCCTTGCCAGACA | CCAGAGGGTGTCAGGATTGAG | 59-61 | Rox |

As shown by González-Ramos et al. (2015), a set of nine microsatellite markers is sufficient to conduct a population genetic study of sponges. It was also revealed that with 12 loci or more, the analysis resolution does not change. Blanquer and Uriz (2010) showed the presence of genetic structure within and between populations of marine sponges as well as between different geographic areas using seven microsatellite markers. Duran et al. (2004) identified the genetic structure between geographically distant locations for the *Crambe Crambe* marine sponge using six microsatellite loci. Both population genetic studies of freshwater sponges used the same set of 11 microsatellite markers (Lucentini et al., 2013; Li et al., 2018).

Thus, the most appropriate number of microsatellite loci for sponges can be from 9 to 12, taking into account that with a smaller number of microsatellite markers, it is also possible to reveal the population genetic structure, albeit with a lower resolution. Our set of microsatellite markers includes 11 loci and meets all the requirements for high-quality population genetic analysis.

## 4. Conclusions

Based on the results of the fieldwork, the sites of mass accumulations of Spongillidae representatives were identified. We analyzed species diversity for Site 1. The species *E.muelleri* was the predominant species. A set of 11 specific and variable microsatellite markers was successfully developed and tested for the *E.muelleri*. Thus, we prepared a basis for population genetic studies of cosmopolitan freshwater sponges in Lake Baikal and further possible studies of the population structure of *E.muelleri* around the world.

## Acknowledgements

The authors thank Mikhail Yakhnenko, Sergey Arzhannikov, Anastasia Arzhannikova, Ksenia Danilova, Danil Kazakov, Pavel Kosarev, Ksenia Derevtsova, and Vladimir Vasiliev for assistance in fieldwork and sampling.

This study was funded by RFBR and the Government of the Irkutsk Region, project number 20-44-383010 as well as by the State Project number 0279-2021-0011

## Conflict of interests

The authors declare no conflict of interests.

## References

Anderson C.M., Aparicio G.J., Atangana A.R. et al. 2010. Permanent genetic resources added to molecular ecology resources database 1 December 2009-31 January 2010. Molecular Ecology Resources 10(3): 576-579. DOI: 10.1111/j.1755-0998.2010.02851.x

Arzhannikov S.G., Ivanov A., Arzhannikova A. et al. 2017. Catastrophic events in the Quaternary outflow history of Lake Baikal. Earth-Science Reviews 177: 76-113. DOI: 10.1016/j.earscirev.2017.11.011

Arzhannikov S., Arzhannikova A., Ivanov A. et al. 2021. Lake Baikal highstand during MIS 3 recorded by palaeo-shorelines on Bolshoi Ushkanii Island. Boreas 50: 101-113. DOI: 10.1111/bor.12464

Bell J.J. 2008. The functional roles of marine sponges. Estuarine, Coastal and Shelf Science 79(3): 341-353. DOI: 10.1016/j.ecss.2008.05.002

Bilton D.T., Freeland J.R., Okamura B. 2001. Dispersal in freshwater invertebrates. Annual Review of Ecology and Systematics 32: 159-181. DOI: 10.1146/annurev.ecolsys.32.081501.114016

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

Blanquer A., Uriz M.J. 2011. Living together apart: the hidden genetic diversity of sponge populations. Molecular Biology and Evolution 28(9). DOI: 10.1093/molbev/msr096

Blanquer A., Uriz M.J., Caujapé-Castells J. 2009. Small-scale spatial genetic structure in *Scopalina lophyropoda*, an encrusting sponge with philopatric larval dispersal and frequent fission and fusion events. Marine Ecology Progress Series 380: 95-102. DOI: 10.3354/meps07931

Blanquer A., Uriz M.J., Pascual M. 2005. Polymorphic microsatellite loci isolated from the marine sponge *Scopalina lophyropoda* (Demospongiae: Halichondrida). Molecular Ecology Notes 5(3). DOI: 10.1111/j.1471-8286.2005.00927.x

Calderón I., Ortega N., Durán S.A. et al. 2007. Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera). Molecular Ecology 16(9). DOI: 10.1111/j.1365-294X.2007.03276.x

Dailianis T., Tsigenopoulos C., 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

Dailianis T., Tsigenopoulos C.S. 2010. Characterization of polymorphic microsatellite markers for the endangered Mediterranean bath sponge *Spongia officinalis* L. Conservation Genetics 11(3): 1155-1158. DOI: 10.1007/s10592-009-9906-0

Dröscher I., Waringer J. 2007. Abundance and microhabitats of freshwater sponges (Spongillidae) in a Danubean floodplain in Austria. Freshwater Biology 52(6). DOI: 10.1111/j.1365-2427.2007.01747.x

Duran S. et al. 2002. Polymorphic microsatellite loci in the sponge *Crambe crambe* (Porifera: Poecilosclerida) and their variation in two distant populations. Molecular Ecology Notes 2(4). DOI: 10.1046/j.1471-8286.2002.00285.x

Duran S., Pascual M., Estoup A. et al. 2004. Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. Molecular Ecology 13(3). DOI: 10.1046/j.1365-294X.2004.2080.x

Efremova S.M. 2004. New genus and new species of sponges from family Lubomirskiidae Rezvoj, 1936. In: Timoshkin O.A. (Ed.), Opredelitel’ presnovodnykh bespozvonochnykh Rossii i sopredel’nykh territoriy [Index of animal species inhabiting Lake Baikal and its catchment area]. Novosibirsk, pp. 1261-1278. (in Russian)

Fontaneto D., Barraclough T.G., Chen K. et al. 2008. Molecular evidence for broad-scale distributions in bdelloid rotifers: everything is not everywhere but most things are very widespread. Molecular Ecology 17(13). DOI: 10.1111/j.1365-294X.2008.03806.x

Fungtammasan A., Ananda G., Eile S.H. et al. 2015. Accurate typing of short tandem repeats from genome-wide sequencing data and its applications 25(5): 736-749. DOI: 10.1101/gr.185892.114

De Gelas K., De Meester L. 2005. Phylogeography of *Daphnia magna* in Europe. Molecular Ecology 14(3). DOI: 10.1111/j.1365-294X.2004.02434.x

Giles E.C., Saenz-Agudelo P., Berumen M.L. et al. 2013. Novel polymorphic microsatellite markers developed for a common reef sponge, *Stylissa carteri*. Marine Biodiversity 43(3). DOI: 10.1007/s12526-013-0151-x

González-Ramos J., Agell G., Uriz M.J. 2015. Microsatellites from sponge genomes: the number necessary for detecting genetic structure in Hemimycale columella populations. Aquatic Biology 24(1). DOI: 10.3354/ab00630

Guardiola M., Frotscher J., Uriz M.J. 2012. Genetic structure and differentiation at a short-time scale of the introduced calcarean sponge *Paraleucilla magna* to the western Mediterranean. Hydrobiologia 687(1). DOI: 10.1007/s10750-011-0948-1

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.

Hoare K., Goldson A.J., Giannasi N. et al. 2001. Molecular phylogeography of the cosmopolitan bryozoan Celleporella hyalina: cryptic speciation? Molecular Phylogenetics and Evolution 18(3). DOI: 10.1006/mpev.2000.0892

Hoshino S., Fujita T. 2006. Isolation of polymorphic microsatellite markers from *Hymeniacidon sinapium* (Porifera: Demospongiae: Halichondrida). Molecular Ecology Notes 6(3). DOI: 10.1111/j.1471-8286.2006.01360.x

Hoshino S., Saito D.S., Fujita T. 2008. Contrasting genetic structure of two Pacific *Hymeniacidon* species. Hydrobiologia 603(1). DOI: 10.1007/s10750-008-9295-2

Itskovich V., 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(6): 608-620. 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

Kenny N.J., Francis W.R., 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

Knowlton A.L., Pierson B.J., Talbot S.L. et al. 2003. Isolation and characterization of microsatellite loci in the intertidal sponge Halichondria panacea. Molecular Ecology Notes 3(4). DOI: 10.1046/j.1471-8286.2003.00511.x

Kozhov M.M. 1962. Biologiya ozera Baikal [Biology of Lake Baikal]. Moscow: Akad. Nauk SSSR. (in Russian)

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(2). 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: 172-181. DOI: 10.4081/jlimnol.2013.e14

Maldonado M., Riesgo A. 2008. Reproduction in the phylum Porifera: a synoptic overview. Treballs de la Societat Catalana de Biologia [Works of the Catalan Society of Biology] 59: 29-49. DOI: 10.2436/20.1501.02.56

Manconi R., Pronzato R. 2008. Global diversity of sponges (Porifera: Spongillina) in freshwater. Hydrobiologia 595: 27-33. DOI: 10.1007/s10750-007-9000-x

Massard J.A., Geimer G. 2008. Global diversity of bryozoans (Bryozoa or Ectoprocta) in freshwater. Hydrobiologia 595(1). DOI: 10.1007/s10750-007-9007-3

Mills S., Lunt D.H., Gómez A. 2007. Global isolation by distance despite strong regional phylogeography in a small metazoan. BMC Evolutionary Biology 7(1). DOI: 10.1186/1471-2148-7-225

Muñoz J., Pacios F. 2010. Global biodiversity and geographical distribution of diapausing aquatic invertebrates: the case of the cosmopolitan brine shrimp, *Artemia* (Branchiopoda, Anostraca). Crustaceana 83(4). DOI: 10.1163/001121610X489449

Noyer C., Agell G., Pascual M. et al. 2009. Isolation and characterization of microsatellite loci from the endangered Mediterranean sponge *Spongia agaricina* (Demospongiae: Dictyoceratida). Conservation Genetics 10(6): 1895-1898. DOI: 10.1007/s10592-009-9848-6

Noyer C., Becerro M.A. 2012. Relationship between genetic, chemical, and bacterial diversity in the Atlanto-Mediterranean bath sponge *Spongia lamella*. Hydrobiologia 687(1). DOI: 10.1007/s10750-011-0884-0

Philippe H., Derelle R., Lopez P. et al. 2009. Phylogenomics revives traditional views on deep animal relationships. Current Biology 19(8): 706-712. DOI: 10.1016/j.cub.2009.02.052

Riesgo A., Pérez-Portela R., Pita L. et al. 2016. Population structure and connectivity in the Mediterranean sponge *Ircinia fasciculata* are affected by mass mortalities and hybridization.

Heredity 117: 427-439. DOI: 10.1038/hdy.2016.41

Roveta C., Annibaldi A., Afghan A. et al. 2021. Biomonitoring of heavy metals: the unexplored role of marine sessile taxa. Applied Sciences (Switzerland) 11(2). DOI: 10.3390/app11020580

van Soest R.W.M., Boury-Esnault N., Vacelet J. et al. 2012. Global diversity of sponges (Porifera). PLoS ONE 7(4). DOI: 10.1371/journal.pone.0035105

Taboada S., Kenny N.J., Riesgo A. et al. 2018. Mitochondrial genome and polymorphic microsatellite markers from the abyssal sponge *Plenaster craigi* Lim & Wiklund, 2017: tools for understanding the impact of deep-sea mining. Marine Biodiversity 48(1). DOI: 10.1007/s12526-017-0786-0

Veynberg E. 2009. Fossil sponge fauna in Lake Baikal region. In: Müller W.E.G., Grachev M.A. (Eds.), Biosilica in evolution, morphogenesis, and nanobiotechnology. Progress in molecular and subcellular biology. Vol. 47. Springer: Berlin, Heidelberg, pp. 185-205. DOI: 10.1007/978-3-540-88552-8 8

Vohmann A., Mutz M., Arndt H. et al. 2009. Grazing impact and phenology of the freshwater sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana* under experimental warming. Freshwater Biology 54(5). DOI: 10.1111/j.1365-2427.2008.02155.x

Yakhnenko A.S., Itskovich V.B. 2020a. Analysis of mtDNA variability in closely related Baikal sponge species for new barcoding marker development. Limnology 21(3). DOI: 10.1007/s10201-019-00599-7

Yakhnenko A.S., Itskovich V.B. 2020b. Study of microsatellite cross-species specificity in freshwater sponge families Lubomirskiidae and Spongillidae. Limnology and Freshwater Biology 6: 1084-1089. DOI: 10.31951/2658-3518-2020-a-6-1084

Ye J., Coulouris G., Zaretskaya I. et al. 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 13. DOI: 10.1186/1471-2105-13-134