

The problem of species delimitation within the endemic Lake Baikal sponges Lubomirskiidae

LIMNOLOGY
FRESHWATER
BIOLOGY

www.limnolfwbiol.com

Itskovich V.B.* , Kaluzhnaya O.V., Glyzina O.Y.

Limnological Institute of the Siberian Branch of the Russian Academy of Sciences, 3 Ulan-Batorskaya Str., 664033, Irkutsk, Russia

ABSTRACT. The delimitation of species within the endemic sponge family Lubomirskiidae is difficult. Along with specimens falling within the range of morphological variability of previously described specimens, we found specimens with intermediate morphological characteristics. The largest number of such specimens had intermediate characteristics between representatives of the genera *Lubomirskia* and *Baikalospongia*. For some species of these genera, a common feature is the structure of megasclera in the form of oxeads of a similar size. At the same time, the genera *Swartschewskia* and *Rezinkovia* have less variable morphological characteristics, so the identification of species belonging to these genera based on morphology is not difficult. The results of our study indicate the need for delimitation of Lubomirskiidae species based on molecular data.

Keywords: Porifera, Baikal, Lubomirskiidae, biodiversity, species delimitation

1. Introduction

Sponges (Porifera) are important component of marine and freshwater ecosystems due to their species richness and widespread abundance (Diaz and Rützler, 2001; Bell, 2008; Van Soest et al., 2012). Baikal sponges make up the bulk of the benthic biomass in Lake Baikal (Timoshkin, 2001) and play an important ecological role as biofilters. Endemic sponge family Lubomirskiidae includes today 18 accepted species (Rezvoi, 1936; Efremova, 2004; Itskovich et al., 2015; Bukshuk and Maikova, 2020).

For sponges, the insufficiency of morphological data alone was noted for the development of taxonomy and the study of phylogeny (Van Soest et al., 2012). Molecular studies provide additional data to address these issues in the case of marine sponges (Morrow et al., 2012; Boury-Esnault et al., 2013). Molecular studies have also helped to determine the phylogenetic position of a number of cosmopolitan freshwater sponges Spongillidae (Addis and Peterson, 2005; Itskovich et al., 2008; Carballo et al., 2018; Erpenbeck et al., 2019; Sokolova et al., 2021). The use of molecular markers with different evolutionary rates also made it possible to identify founder species of endemic families in ancient lakes (Meixner et al., 2007; Erpenbeck et al., 2011; Itskovich et al., 2013a; Sokolova et al., 2020).

Species identification of Lubomirskiidae is difficult both on the basis of morphological and molecular methods. To date all molecular analyzes

based on the protein coding (COXI, silicatein) and intron (ITS region rDNA and intergenic spacer region mtDNA) sequences have not supported the current morphology-based taxonomy of Lubomirskiidae (Meixner et al., 2007; Itskovich et al., 2008; 2013b).

Taxonomy and the definition of species criteria are the fundamental basis for theoretical and applied biological research. Recently, there has been an increase in the number of publications devoted to the issue of species boundaries in marine sponges, most of which indicate an underestimation of the existing biodiversity (Cruz-Barraza et al., 2012; Boury-Esnault et al., 2013; DeBiaise and Hellberg, 2015). Sponges present a good model for testing hypotheses of species delimitation by study of molecular evolution and morphological variability.

The problem of species differentiation is especially relevant in Lake Baikal, which is a natural laboratory for the study of evolutionary processes. In Baikal along with other ancient lakes there is a process of accelerated evolution driven by the extreme and unique environmental conditions of the lake. Rapid radiation processes operate at all levels from cell to species and affect both genetic and anatomical characteristics. The high intraspecific morphological variability of Lubomirskiidae is probably the result of their morphofunctional and anatomical adaptations.

In this work, we analyzed the variability of morphological characteristics in representatives of 4 Lubomirskiidae genera.

*Corresponding author.

E-mail address: itskovich@mail.ru (V.B. Itskovich)

Received: November 25, 2022; **Accepted:** December 09, 2022;

Available online: December 13, 2022

© Author(s) 2022. This work is distributed under the Creative Commons Attribution-NonCommercial 4.0 International License.



2. Materials and methods

Samples of sponges were collected by SCUBA diving at two collection points in Central Baikal: Maloe more strait, Kurma village (53°11'496"N 106.59'752"E), Maloe more strait, Zama village (53°28'275"N 107°32'022"E), Cape Izhimei on Olkhon Island (53°13'444"N 107°44'052"E) during expeditions performed in 2022. During the collection of samples, ecological parameters such as temperature, water quality and mineral composition, and substrate type were recorded, which will reveal the links between taxonomy and ecology, which is important for species delimitation. Underwater photo and video surveys were carried out and for all samples in vivo images were captured (Fig. 1, Fig. 2). Spicule and skeleton preparation were performed as previously described (Efremova, 2004) and examined using an Olympus CX22 microscope. The shape and consistency of sponges, osculum structure, skeletal characteristics, the form and size of spicules, the presence and shape of spicule spines, the nature of their distribution along the spicule and their variability in each sample were analyzed. Spicule sizes were determined by the results of measurements of 50 spicules within each sample.

3. Results and discussion

We collected and performed morphological analysis of 208 *Lubomirskiidae* specimens. Most of them were identified as *Lubomirskia baikalensis*, *L. fusifera*, *L. abietina*, *L. incrustans*, *Baikalospongia intermedia*, *B. bacillifera*, *B. recta*, *B. martinsony*, *Rezinkovia echinata*, *Swartschewskia papyracea* (Fig. 1). About 20 samples had difficult species identification (Fig. 2). Some of them had spicule sizes beyond the previously described species. Some samples had combinations of features of different species.

The largest number of such specimens had intermediate characteristics between representatives of the genera *Lubomirskia* and *Baikalospongia*, but at the same time, we did not encounter any difficulties in identifying such a species as *Lubomirskia baikalensis* (in the case of adult branched specimens). For some species of these genera (for example, *Lubomirskia baikalensis*, *Lubomirskia incrustans*, *Baikalospongia intermedia*), the structure of megasclera in the form of oxeas of similar size is common. Important characteristics for the identification of these species are also the structure of the skeleton, the shape and size of the skeletal bundles, and the nature of their branching, however, these characters vary significantly. Oxeas are also present in Spongillidae, which lose gemmules when living in constant conditions in Baikal, and this sometimes makes it difficult to identify Baikal sponges even to the family level (Itskovich et al., 2015).

At the same time, the genera *Swartschewskia* and *Rezinkovia* have less variable morphological characteristics within the genus, so the identification of species belonging to these genera based on morphology is not difficult. *Swartschewskia papyracea* also differs in ecological features. This species lives on negative slopes, in places with low amount of light, that also affects the composition of its symbiotic community (Kaluzhnaya and Itskovich, 2016).

Our data are consistent with the data of other researchers, who also noted the blurring of morphological features and overlapping of species boundaries in *Lubomirskiidae* (Efremova, 2001; Masuda, 2009; Maikova et al., 2017). Some genetic markers also indicated the proximity of the genera *Lubomirskia* and *Baikalospongia* (Itskovich et al., 2013b; Maikova et al., 2017), however, the lack of separation of other *Lubomirskiidae* species using intergenic mtDNA regions indicates the need for additional studies. Rare deep-water species (*R. arbuscula*, *B. abyssalis*) and three

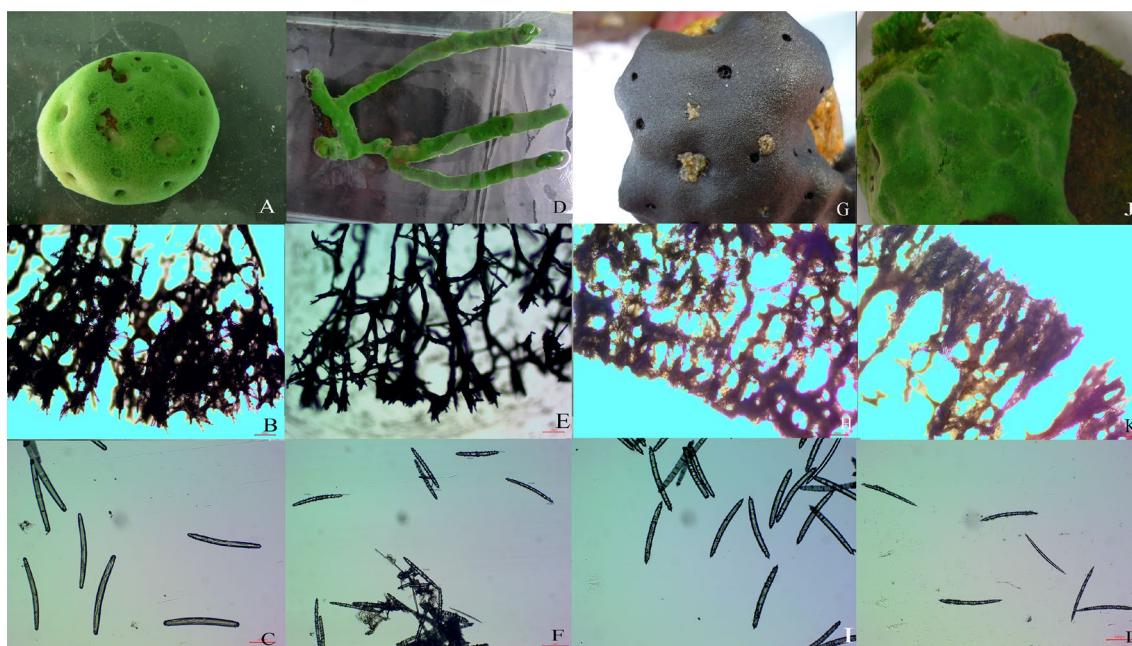


Fig.1. Sponge body, the skeleton and microscopy of megascleres, respectively, of (A–C) *Baikalospongia bacillifera*; (D–F) *Lubomirskia baikalensis*; (G–I) *B. intermedia*; and (J–L) *L. abietina*.

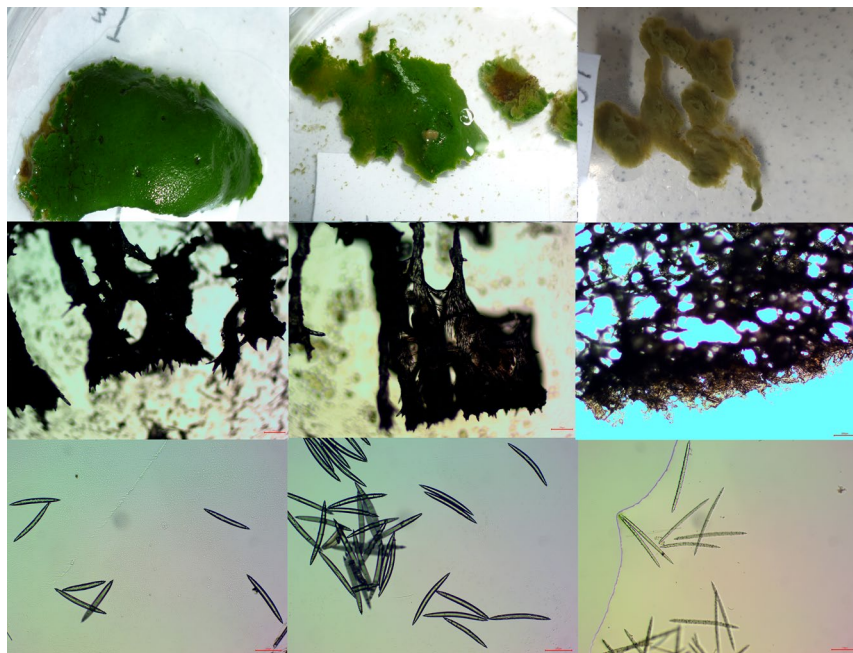


Fig.2. Sponge body, the skeleton and microscopy of megascleres of unidentified samples of Lubomirskiidae.

closely related species of the genus *Swartschewskia* should also become the object of close attention for species delimitation.

Marine sponges also have a phenotypic plasticity that can lead to misidentification of specimens and a problem to determine their low level relationships. For example, morphological studies of sponges of the genus *Callyspongia* have shown that some species are actually growth forms (Zea et al., 2014). However, molecular analysis revealed evolutionarily distinct lineages that were not concordant with current species designations in *Callyspongia* (DeBiasse and Hellberg, 2015).

The morphologically divergent specimens identified by us in this work may be either new species of Lubomirskiidae or indicate that the real number of sponge species in Baikal is overestimated. Molecular studies based on genomic data will help resolve this issue. A large-scale ddRAD analysis of the Lubomirskiidae specimens collected and analyzed by us is currently in progress.

4. Conclusions

The results of morphological analysis made it possible to identify samples with intermediate characteristics between representatives of the genera *Lubomirskia* and *Baikalospongia*. The delimitation of Lubomirskiidae species including these specimens, as well as a number of closely related and deepwater species, should be carried out using morphological, ecological and genomic data.

Acknowledgements

The reported study was funded by the Russian Science Foundation, grant number 22-24-01037. We thank Yuri Yushchuk for the help with samples collection. The present study was conducted at the

“Experimental freshwater aquarium complex of Baikal hydrobionts” of the Limnological Institute SB RAS, Irkutsk, Russia.

Conflict of interest

The authors declare no conflict of interest.

References

- Addis J.S., Peterson K.J. 2005. Phylogenetic relationships of freshwater sponges (Porifera, Spongillina) inferred from analyses of 18S rDNA, COI mtDNA, and ITS2 rDNA sequences. *Zoologica Scripta* 34: 549-557. DOI: [10.1111/j.1463-6409.2005.00211.x](https://doi.org/10.1111/j.1463-6409.2005.00211.x)
- Bell J.J. 2008. The functional roles of marine sponges. *Estuarine Coastal and Shelf Science* 79: 341-352. DOI: [10.1007/s10750-013-1799-8](https://doi.org/10.1007/s10750-013-1799-8)
- Boury-Esnault N., Lavrov D.V., Ruiz C.A. et al. 2013. The integrative taxonomic approach applied to porifera: a case study of the homoscleromorpha. *Integrative and Comparative Biology* 53: 416-427. DOI: [10.1093/icb/ict042](https://doi.org/10.1093/icb/ict042).
- Carballo J.L., Cruz-Barraza J.A., Yáñez B. et al. 2018. Taxonomy and molecular systematic position of freshwater genus *Racekiela* (Porifera: Spongillida) with the description of a new species from North-west Mexico. *Systematics and Biodiversity* 16: 160-170. DOI: [10.1080/14772000.2017.1359216](https://doi.org/10.1080/14772000.2017.1359216)
- Cruz-Barraza J.A., Carballo J.L., Rocha-Olivares A. et al. 2012. Integrative taxonomy and molecular phylogeny of genus *Aplysina* (Demospongiae: Verongida) from Mexican Pacific. *PLoS One* 7: e42049. DOI: [10.1371/journal.pone.0042049](https://doi.org/10.1371/journal.pone.0042049)
- 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](https://doi.org/10.3897/zookeys.906.39534)
- DeBiasse M.B., Hellberg M.E. 2015. Discordance between morphological and molecular species boundaries among Caribbean species of the reef sponge *Callyspongia*. *Ecology and Evolution* 5: 663-675. DOI: [10.1002/ece3.1381](https://doi.org/10.1002/ece3.1381)
- Diaz M.C., Rützler K. 2001. Sponges: an essential

component of Caribbean coral reefs. *Bulletin of Marine Science* 69: 535-546. DOI: [10.1371/journal.pone.0098181](https://doi.org/10.1371/journal.pone.0098181)

Efremova S.M. 2001. Sponges (Porifera). In: Timoshkin O.A. (Ed.), Index of animal species inhabiting Lake Baikal and its catchment area. Vol. 1. Lake Baikal. Book 1. Novosibirsk: Nauka, pp. 182-192. (in Russian)

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. Vol. 1. Lake Baikal. Book 2. Novosibirsk: Nauka, pp. 1261-1278. (in Russian)

Erpenbeck D., Weier T., de Voogd N.J. et al. 2011. Insights into the evolution of freshwater sponges (Porifera: Demospongiae: Spongillina): barcoding and phylogenetic data from Lake Tanganyika endemics indicate multiple invasions and unsettle existing taxonomy. *Molecular Phylogenetics and Evolution* 61: 231-236. DOI: [10.1016/j.ympev.2011.05.021](https://doi.org/10.1016/j.ympev.2011.05.021)

Erpenbeck D., Steiner M., Schuster A. et al. 2019. Minimalist barcodes for sponges: a case study classifying African freshwater Spongillida. *Genome* 62: 1-10. DOI: [10.1139/gen-2018-0098](https://doi.org/10.1139/gen-2018-0098)

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: 608-620. DOI: [10.1007/s00239-008-9158-5](https://doi.org/10.1007/s00239-008-9158-5)

Itskovich V., Kaluzhnaya O., Ostrovsky I. et al. 2013a. The number of endemic species of freshwater sponges (Malawispongiidae; Spongillina; Porifera) from Lake Kinneret is overestimated. *Journal of Zoological Systematics and Evolutionary Research* 51: 252-257. DOI: [10.1111/jzs.12022](https://doi.org/10.1111/jzs.12022)

Itskovich V.B., Kaluzhnaya O.V., Belikov S.I. 2013b. Investigation of nuclear and mitochondrial DNA polymorphism in closely related species of endemic Baikal sponges. *Russian Journal of Genetics* 49: 966-974. DOI: [10.7868/s0016675813080031](https://doi.org/10.7868/s0016675813080031)

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: 123-137. DOI: [10.11646/zootaxa.3990.1.7](https://doi.org/10.11646/zootaxa.3990.1.7)

Kaluzhnaya O.V., Itskovich V.B. 2016. Distinctive features of the microbial diversity and the polyketide synthase genes spectrum in the community of the endemic Baikal sponge *Swartschewskia papyracea*. *Russian Journal of Genetics* 52: 38-48. DOI: [10.1134/S1022795416010099](https://doi.org/10.1134/S1022795416010099)

Maikova O.O., Bukshuk N.A., Itskovich V.B. et al. 2017. Transformation of Baikal sponges (family Lubomirskiidae) after penetration into the Angara River. *Russian Journal of Genetics* 53: 1343-1349. DOI: [10.1134/S1022795417120092](https://doi.org/10.1134/S1022795417120092)

Masuda Y. 2009. Studies on the taxonomy and distribution of freshwater sponges in Lake Baikal. In: Müller W.E.G., Grachev M.A. (Eds.), *Biosilica in evolution, morphogenesis, and nanobiotechnology*. Berlin/Heidelberg, pp. 81-110. DOI: [10.1007/978-3-540-88552-8_4](https://doi.org/10.1007/978-3-540-88552-8_4)

Meixner M.J., Luter C., Eckert C. et al. 2007. Phylogenetic analysis of freshwater sponges provide evidence for endemism and radiation in ancient lakes. *Molecular Phylogenetics and Evolution* 45: 875-886. DOI: [10.1016/j.ympev.2007.09.007](https://doi.org/10.1016/j.ympev.2007.09.007)

Morrow C.C., Picton B.E., Erpenbeck D. et al. 2012. Congruence between nuclear and mitochondrial genes in Demospongiae: a new hypothesis for relationships within the G4 clade (Porifera: Demospongiae). *Molecular Phylogenetics and Evolution* 62: 174-190. DOI: [10.1016/j.ympev.2011.09.016](https://doi.org/10.1016/j.ympev.2011.09.016)

Rezvoi P.D. 1936. Fauna SSSR. Gubki. T. 2. Presnovodnyye gubki (sem. Spongillidae i Lubomirskiidae) [Fauna of the USSR. Porifera. Vol. 2. Freshwater sponges (Spongillidae and Lubomirskiidae)]. Moscow-Leningrad: Publishing house of the Academy of Sciences of the USSR. (in Russian with German summary)

Sokolova A.M., Palatov D.M., Itskovich V.B. 2020. Genetic analysis confirms the freshwater origin of the endemic Caspian sponges (Demospongiae, Spongillida, Metschnikowiidae). *ZooKeys* 915: 1-16. DOI: [10.3897/zookeys.915.47460](https://doi.org/10.3897/zookeys.915.47460)

Sokolova A.M., Palatov D.M., Masuda Y. et al. 2021. Investigation of the spongillid *Spongilla alba* Carter, 1849 reveals a new group of brackish water sponges. *Systematics and Biodiversity* 19: 976-992. DOI: [10.1080/14772000.2021.1958948](https://doi.org/10.1080/14772000.2021.1958948)

Timoshkin O.A. 2001. Lake Baikal: fauna diversity, problems of its immiscibility and origin, ecology and exotic communities. In: Timoshkin O.A. (Ed.), Index of animal species inhabiting Lake Baikal and its catchment area. Novosibirsk: Nauka, pp. 74-116. (in Russian)

Van Soest R.W.M., Boury-Esnault N., Vacelet J. et al. 2012. Global diversity of sponges (Porifera). *PLoS ONE* 7: e35105. DOI: [10.1371/journal.pone.0035105](https://doi.org/10.1371/journal.pone.0035105)

Zea S., Henkel T.P., Pawlik J.R. 2014. The sponge guide: a picture guide to Caribbean sponges. 3rd Edition. URL: www.spongeguide.org