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Study of the COI gene fitness for a population-genetic analysis of endemic Baikal Sponges L. Baikalensis

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Abstract — **Freshwater sponges play an important role as filtrators in lake ecosystems. Endemic Baikal sponges make up the bulk of the lake benthos biomass. Events of mass diseases and death of sponges occur on Lake Baikal for the last decade. Due to high morphological plasticity, there is a lack of clear criteria for the species identification of Baikal sponges. However, the development of such criteria is very important for population structure studying purposes and determination of the recoverability of populations. In this work, we first assessed the suitability of the 5'-terminal fragment and the I3M11 fragment of the COI gene for population studies of endemic Baikal sponges, and also examined alternative markers for such studies. It is shown that in the Lubomirskia baikalensis samples, collected in different basins of Lake Baikal, only two different haplotypes were found, which indicates the unsuitability of this fragment for population studies. As an alternative, we propose to use microsatellite markers, which have successfully shown themselves both in studying the structure of populations of marine and freshwater sponges. Currently, work is underway to study the population structure of Baikal endemic sponges using microsatellite markers.**

Keywords — Sponges, population genetics, molecular markers, Baikal endemics, Porifera

Motivation and aim

Motivation

Sponges are ancient multicellular animals leading an attached lifestyle. They are widespread throughout the planet, inhabiting marine and freshwater bodies. According to the method of nutrition, the sponges are filtrators, which contribute to the increased sensitivity of the sponges to contaminants. Thus, sponges are bioindicators of the state of aquatic ecosystems. Freshwater sponges that live in the ancient rift lake Baikal, formed a bouquet of endemic species that have features that clearly distinguish them among freshwater cosmopolitans [1; 2]. Such features include the absence of gemmules and a long life cycle, which, undoubtedly, should affect the structure of populations of endemic species. Unfortunately, genetic structure of Baikal sponges still remains unknown.

Aim

A study of the population structure of sponges will make it possible to understand the causes of their mass disease and death; for marine sponges, such studies have been successfully conducted. In recent years, a process of mass disease and death of endemic sponges occurs on the lake Baikal [3]. Similar events were noted in some marine ecosystems. As shown [4], changes in the sponge population structure are observed precisely in places where their mass death was previously noted. Thus, the study of the population structure of Baikal endemic sponges will allow us to study the influence of sponge diseases on their population structure at the genetic level. Genetic markers successfully used for population genetic studies of sponges are the COI gene and microsatellite markers [5; 6]. Moreover, population analysis using COI is successfully used in the study of sea sponges [7], but there is no such data on freshwater sponges, and microsatellite analysis is used both in the study of sea sponges [8] and freshwater sponges [9, 10].

Methods

Baikal sponge samples were collected by SCUBA diving during expedition in 2018 in the southern, middle and northern basins of Lake Baikal. Samples were fixed in 70% ethanol for morphological and molecular analysis. Total genomic DNA extraction was performed using the CTAB method. The standard 5'-end fragment of COI was amplified with primers previously described by Folmer et al. (1994) (C_1538F: 5'-GGTCAACAAATCATAAAGATATTGG-3') and (C_1539R:5'-TAAACTTCAGGGTGACCAAAAATC A-3′), which amplifed a fragment of 632 base pairs (bp). The I3-M11 fragment was amplifed using primers developed by us (forward primer 5'-GCTGGAGGAGGAGACCCAAT-3') and (reverse primer 5'-TGGAAATCCGAATACCGTCTCG-3'). PCR was carried out in a 20-µl volume of the reaction mixture under the following conditions: preheating to activate the polymerase 2 min at 94 °C, followed by 35 cycles of DNA denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 45 s, followed by final elongation of 8 min at 72 °C. Each PCR reaction was purifed by electrophoresis in a 0.8% agarose gel, followed by extraction by freezing and thawing. Sequencing of the PCR products was carried out at the service laboratory "Syntol" (Moscow, Russia).

Results

In this work we analyzed 8 samples L.baikalensis species, collected in the Northern, Middle, and Southern hollows of Lake Baikal. During the sequence analysis for the 5'-terminal fragment of the COI, no differences were found among 8 samples, and for the I3M11 fragment, 2 haplotypes differing by 1 nucleotide were detected. The maximum p-distance value was 0.2%, the average value was 0.04%. Values obtained for intraspecific variability indicate the unsuitability of the COI gene for analysis of the population structure of Baikal endemic sponges. Although this gene has been successfully used for population-genetic studies of sea sponges [7]. The reason for the lower variability of the COI gene in Baikal endemic sponges compared with marine sponges could be the later formation of the *L.baikalensis* species. An alternative way to study the population structure of freshwater sponges is microsatellite analysis since it is successfully used both for studying marine [4; 8] and freshwater sponges (*Ephydatia fluviatilis*) [9; 10]. Analysis of the population structure of endemic Baikal sponges using microsatellite markers is being carried out for the first time now.

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REFERENCES

[1] Itskovich V.B. et al. (2006) Monophyletic origin of freshwater sponges in ancient lakes based on partial structures of COXI gene. Hydrobiologia. 568: 155–159.

[2] Itskovich V.B. et al. (2008) Ribosomal ITS sequences allow resolution of freshwater sponge phylogeny with alignments guided by secondary structure prediction. J. Mol. Evol. 67(6): 608–620.

[3] Khanaev I.V. et al. (2018) Current state of the sponge fauna (Porifera: Lubomirskiidae) of Lake Baikal: Sponge disease and the problem of conservation of diversity. J. Great Lakes Res. 44(1): 318-332.

[4] Riesgo A. et al. (2019) Genetic diversity, connectivity and gene flow along the distribution of the emblematic AtlantoMediterranean sponge Petrosia ficiformis (Haplosclerida, Demospongiae). BMC Evol. Biol. 19(1): 24-39.

[5] Gui J. F. Et al. (2010) Genetic basis and breeding application of clonal diversity and dual reproduction modes in polyploid Carassius auratus gibelio. Sci. China Life Sci. 53(4): 409–415.

[6] Vieira M.L.C. et al. (2016) Microsatellite markers: What they mean and why they are so useful. Genetics and Molecular Biology. 39(3): 312–28.

[7] López-Legentil S. et al. (2009) Genetic structure of the Caribbean giant barrel sponge Xestospongia muta using the I3-M11 partition of COI. Coral Reefs. 28(1): 157–165.

[8] Blanquer A. Et al. (2010) Population genetics at three spatial scales of a rare sponge living in fragmented habitats. BMC Evol. Biol. 28(1): 157–165.

[9] Lucentini L. et al. (2013) Spatially explicit genetic structure in the freshwater sponge Ephydatia fluviatilis (Linnaeus, 1759) within the framework of the monopolisation hypothesis. J. Limnol. 72: 172–181.

[10] Li R. et al. (2018) Mesoscale investigations based on microsatellite analysis of the freshwater sponge Ephydatia fluviatilis in the River-Sieg system (Germany) reveal a genetic divergence. Conserv. Genet. 19: 959–968.

[11] Efremova S.M. (2004) New Genus and New Species of Sponges from Family Lubomirskiidae Rezvoj, 1936. Index of animal species inhabiting Lake Baikal and its catchment area. 1(2): 182–192.

[12] Folmer O. et al. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3(5): 294–299.