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Phylogeography and local endemism of *Artemia salina* (Cruatacea, Anostraca) in the hypersaline Lake Sasyk-Sivash (Crimea)

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Abstract: For the first time, the molecular genetics study and phylogenetic analysis of the bisexual *Artemia* population were carried out in the hypersaline lake Sasyk-Sivash (Crimea, Russia). A comparative analysis of the lake population and 20 different populations of *A. salina* studied earlier was carried out. The *A. salina* population from Lake Sasyk Sivash holds substantial genetic diversity, have a pronounced phylogeographic structure and high regional endemism.

Motivation: The brine shrimp genus *Artemia* (*Crustacea, Anostraca*) are widely used in aquaculture and biotechnology as a live feed for fish and shrimps larvae (cysts) and its biomass as source of fatty acids, antioxidants and other biologically active substances. The amount and type of secondary metabolites synthesized by *Artemia* strongly depends on the species genetic peculiarities. Previously, genetic screening of the genus *Artemia* in the hypersaline Sasyk-Sivash lake (Crimea) was not carried out. In this work, the *Artemia* specimens collected in the summer of 2021 has been studied.

Methods: 7 bisexual (4 male and 3 female) individuals were used. Total DNA was extracted from individual specimens using the DNA extraction kit (Syntol, Russia) after dissection for gut removal. Quantitative determination of the obtained genomic DNA and evaluation of its purity was carried out on an Inplen nanophotometer (Germany) and using gel electrophoresis in 1 % agarose gel. The PCR reaction was performed using pairs of primers jgLCOI490 and jgHCO2198 for the mitochondrial *COI* gene [1]. The PCR reaction was carried out in a volume of 25 µl using ScreenMix reagents (Eurogen, Russia). Sequencing of the obtained fragments was carried out on the NANOPHOR-05 sequencer (Syntol, Russia) at the CCU "Molecular Structure of Matter" of Sevastopol State University. The obtained *COI* gene sequences were compared with those available in the National Center for Biotechnological Information (NCBI) database. All previously studied populations of *A. salina* were used in the work [2, 3]. Phylogenetic reconstruction was performed using a Bayesian Inference approach implemented in MrBayes version 3.2.6 [4]. MrBayes analyses consisted of two simultaneous runs of 100 million generations each, sampling trees every 10,000 generations. Mixing and convergence among runs were evaluated by checking the average standard deviation of split frequencies, the EES values and the Potential Scale Reduction Factor (PSRF) for each parameter. A majority consensus tree was reconstructed after discarding the first 2,000 sampled trees as burn-in. The *Daphnia Tenebrosa* sequence (GenBank accession number HQ972028) was used as an external group. Relationships among mitochondrial haplotypes were reconstructed based on the TCS method implemented in the PopART [5].

Results: Haplotype diversity (HD), nucleotide diversity (π), number of polymorphic sites (V), and number of nucleotide substitution (M) were calculated for each species using DnaSP 6.0 [6]. The analysis was carried out on 21 different populations of *Artemia Salina*, the total number of sequences used in the analysis was 115, the length of the sequences was 528 bp. No insertions, deletions or stop codons were present. The *COI* alignment consisted of 197 variable sites and 117 parsimony informative sites. The studied *A. salina* populations formed 72 haplotypes, the Sasyk-Sivash population forms 4 separate haplotypes. A comparative analysis of *A. salina* populations studied earlier and isolated in this work, based on estimates of evolutionary divergence over sequence pairs between groups, is carried out in MEGA X [7]. So, phylogeography and local endemism of *A. salina* from Sasyk Sivash Lake were compared with the results for *A. salina* in the Mediterranean basin [3], and its populations in Algeria and Tunisia, obtained by Eimanifar [2]. The Sasyk-Sivash population is most genetically close to CYP (Cyprus: Larnaca salt lake) and EBR (Spain: Tarragona) populations.

Conclusion: Thus, the *A. salina* population from Sasyk Sivash Lake holds substantial genetic diversity and, like the previously studied *A. salina* populations, have a strong phylogeographic structure and high regional endemism. It is important to note that the world's most widespread population of *Artemia* bisexual species *A. monica* (= *franciscana*) does not have a clearly defined phylogeographic structure.

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