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The Biodiversity of Shrimp Genus *Artemia* from Russian Lakes: Morphometric, Cytogenetics and DNA-analysis

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Shrimps of genus *Artemia* are the inhabitants of continental and marine waters with salinity of 70 to 350 g/l and above. *Artemia* is able to survive in the conditions in which other animals cannot exist. This is due to adaptations: effective osmoregulation system, the ability to synthesize of respiratory pigment (hemoglobin) and diapauses cysts (Litvinenko et al., 2009). Cysts of this Crustacean are important commercial object, produced in Russia on an industrial scale. Nauplii are used as start feed for fish and other crustaceans. Due to adapt to extreme and changing environment shrimps are able to vary the shape and size. This fact led to much confusion in the systematics of the genus *Artemia*. By modern taxonomy genus includes seven species: *A. salina* Linnaeus, 1758 synonym *A. tunisiana* Bowen and Sterling 1978; *A. urmiana* Gunther 1900; *A. sinica* Yaneng 1989; *A. persimilis* Picinelli and Prosdociami 1968; *A. franciscana* with two subspecies *A. franciscana franciscana* Kellogg 1906 and *A. franciscana monica* Verrill 1869; *A. species* Pilla and Beardmore 1994; *A. tibetiana* Abatropoulos, Zhang and Sorgeloos 1998 and mixed group of shrimps, breeding parthenogenesis *A. parthenogenetica* Barigozzi 1974; Bowen and Sterling 1978 (Gajardo et al., 2004). Currently, the problem of specific differentiation of genus *Artemia* is solving using a whole arsenal of methods and techniques, which indicates the importance of the various local classification problems crustaceans both in terms of fundamental, and from the point of view of applied science. The Russian populations are not identified to species. Currently, all the resources of modern science, including morphometric, biochemical, cytogenetic studies and DNA analysis have been using for identification of Russian populations (Boyko, et al., 2002, 2004; Boyko E.G., Muge N.S., 2009, 2011 et al.). The purpose of this study is to identify the *Artemia* shrimps from Russia on

the basis of morphometric, cytogenetic and DNA analyzes.

For many years scientists of FSUE «Gosrybcenter» have been carrying out morphometric analyzes of shrimps from lakes of Tyumen, Kurgan, Novosibirsk, Chelyabinsk and other areas on the following criteria: body length (tl), the length of the abdomen (al), abdomen width (aw), the distance between the eyes (de), eye diameter (ed), furca length (fl), length of the first antenna (la), the head width (hw), the ratio of body length to the overall length of abdomen (ra,%), the number of setae (sf) on the right and left lobes furca. Clustered and discriminant analysis have been done on the basis of these data, which revealed the presence of differentiation between studied populations. The salinity of lakes is the main differentiating factor. Correlation between the values of morphometric characteristics and total mineralization during the study period has found. The different degree of correlation between the analyzed traits has revealed. Size of mature individuals is inversely proportional to salinity. The last segment of the body due to high salinity becomes smaller and with fewer setae. As a rule, with increasing of salt concentration in the medium the size of crustaceans decreases the relative length of the abdomen increases and its width decreases (Boyko, et al., 2011; 2012; Boyko, 2013). Based on years of data, we can conclude that paratypic component is the main at the formation of morphotype of shrimp of Russian population but genotypic component is insignificant.

Cytogenetic analysis of nauplii was performed by pipetting the cell suspension and staining acetic orsein. Determination of the number of chromosomes in somatic cells was performed. A large number of prophase and interphase was cells revealed on preparations along with metaphase cells. The most metaphase contains 42 chromosomes, rarely 84. Some cells contain chromosome

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number, not a multiple of 42. Aneuploidy was detected for Russian *Artemia* populations ($2n=41$). All chromosomes are telocentric (Litvinenko et.al., 2009). These data indicate on the variability of chromosome numbers and the presence of aneuploidy in studied crustaceans of Russian populations.

Material for *Artemia* DNA analysis is cysts of the lakes from the Urals, Western and Eastern Siberia, Mongolia, Tuva, as well as cysts and unidentified species known to form populations by Artemia Reference Center (Ghent, Belgium). We used different methods of genetic analysis: RFLP-PCR mtDNA, RAPD-PCR of total DNA sequencing of cytochrome oxidase I (COI) mtDNA (Litvinenko et.al., 2009).

DNA fragment of shrimps from Russian lakes and *A. franciscana*, comprising approximately 1500 bp was isolated and amplified by using primers 12S-R and 16S-R. Restriction analysis of amplified DNA fragment using the five enzymes (HaeIII, TaqI, Tsp, HpaII, NdeII) allowed to identify fragments of different lengths and found no differences between the studied Russian populations of *Artemia*. RFLP-PCR revealed the presence of differentiation between the studied samples of Russian populations of shrimps and *A. franciscana*, dwelling in the United States.

Analysis of total DNA by RAPD-PCR allowed allocating for statistical evaluation of polymorphic fragments of 31 representatives from different sample shrimp habitat. Identification by RAPD-PCR profiles by direct comparison possible to establish the degree of similarity and differences of the populations studied *Artemia*. All samples of studied Russian populations of *Artemia* (including bisexual from Lake Tanatar) belong to one group. Based on the analysis of the frequency of obtained profiles in different populations of *Artemia* we should be noted genetic similarity of the investigated samples of Russia, Kazakhstan, Turkmenistan and Mongolia, as well as to *A. urmiana*, *A. sinica* and *A. tibetiana*. Despite of the lack of a clear correlation between the nature of the distribution of DNA fingerprints in the dendrogram and geographic location of reservoirs, as well as belonging to a certain type, there is a tendency to group of individuals united by geographical factor. The dendrogram of similarity was constructed based on the frequency of the selected fragments by using three different primers A-01, A-09 and A-14. Analysis of this dendrogram revealed a similar pattern of differentiated grouping test *Artemia*. samples. Outgroup was formed by populations from Europe (*A. tunisiana*), South America (*A. persimilis*) and North America (*A. franciscana*). All Russian parthenogenetic populations formed a single cluster. Bisexual population from Lake Svatikovo (Tuva)

has teamed up with *A. sinica* (fig.).

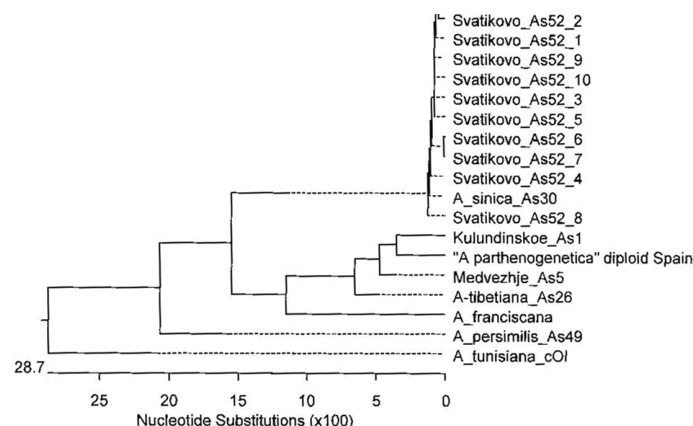


Fig. 1. Phylogenetic tree of *Artemia*, based on the analysis of cytochromeoxidase I (COI) mtDNA (Litvinenko et.al., 2009).

Based on the analysis of the nucleotide sequence fragment of cytochromeoxidase I mitochondrial DNA found that *Artemia* from Svatikovo Lake was absolutely identical to *A. sinica*, which formed a single cluster at the highest level of similarity. All studied Russian parthenogenetic populations belong to the same group. Males are rarely discovered in some populations. They have been analyzed. Absolute similarity of females and a few males in these populations were obtained. The received regularity is quite consistent with the geographic location of the analyzed lakes, as well as the biological features of the object, in particular with the type of reproduction (parthenogenetic or bisexual).

The obtained results on the identification of Russian *Artemia* populations indicate on need for further research to detect markers which allow dividing the Russian population of crustaceans breeding parthenogenetic.

In our opinion, the study of phylogenetic system of genus *Artemia* based on mitochondrial DNA sequences is the most promising.

Key words: *Artemia*, morphometric analysis, cytogenetic analysis, DNA analysis, salinity.

References

- Boyko E.G., Saukova N. and Sorgeloos P., 2002. Characterization of *Artemia* populations from Western Siberia: cytogenetica and morphometry 8th international conference on salt lakes (23-26 July 2002, Republic of Khakasia). Russia: 96 – 97
- Boyko E.G., Barmintsev A., Volkov A. 2004. Experimental development of molecular – genetic testing of different *Artemia* populations International Workshop on *Artemia*. INCO-DEV project on *Artemia* biodiversity. Iran international Workshop – Sept. 21 – 25, 2004, Urmia, Iran: 37
- Boyko E.G., Litvinenko L.I., Fomin A.L. and Kiskin I.A., 2011.

- The population differentiation of жаброногих shrimp of genus *Artemia* Leach, 1819. *Fisheries and aquaculture*, 2: 68 – 75.
- Litvinenko L.I., Kutsanov K.V. and Gabdullin M.A., 2012. Specific features of the biology of *Artemia* in lakes of the Urals and Western Siberia. *Ecology*, 4: 308 - 316
- Boyko E.G., 2013. Influence of ecological factors on the growth of Crustaceans from the genus *Artemia* in Ural and Siberian populations. *Contemporary problems of ecology*, 3: 333-339
- Boyko E.G. and Muge N.S., 2009. Species and population identification shrimp (*Artemia* sp.) in Lakes of the Russian Federation. X Congress of the Hydrobiological Society of RAS (Vladivostok, September 28 - October 2: 46 - 47
- Boyko E.G. and Muge N.S., 2011. The species identification of Russian populations of hypergallin shrimps *Artemia*, based on the sequencing of mitochondrial DNA. International Conference "Problems of population and general genetics" (14-16 November 2011). – Moscow: 53
- Gajardo G., Crespo J., Triantafyllidis A., Tzika A., Baxevanis A.D, Kappas I. and Abatzopoulos Th.J., 2004. Species identification of Chilean *Artemia* populations based on mitochondrial DNA RFLP analysis. *Journal of Biogeography* (J. Biogeogr.), 31: 547-555.
- Litvinenko L.I., Litvinenko A.I. and Boyko E.G. 2009. *Artemia* in Western Siberia Lakes. Novosibirsk: Science: 304