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## EXPRESSION OF LECTINS IN *PLANORBARIUS CORNEUS* SNAILS INFECTED BY TREMATODES

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Pulmonate freshwater molluscs *Planorbarius corneus* (Planorbidae) are widespread in Eurasia. They serve as intermediate hosts for trematodes of several species. Therefore, this species is the object in parasitological and comparative immunological studies. Previously, a wide range of transcripts of immuno-relevant genes was found in the transcriptome of *P. corneus* hemocytes, among which a significant variety of lectins was identified (Prokhorova et al., 2024). Lectins are considered to be one of the main humoral factors in the innate immune system: they are involved in the recognition, opsonization, and facilitation of pathogen phagocytosis.

In this study, C- type lectin expression was studied in uninfected *P. corneus* molluscs (n=15) and molluscs infected with trematodes *Bilharziella polonica* (n=5), *Cotylurus brevis* (n=5) and *Notocotylus ephemer*a (n=5). Tissues of the hepatopancreas, albumen gland, heart region, foot, and hemocytes were used to isolate RNA. Real-Time PCR was performed using specific primers for C-type lectin transcripts. The  $\beta$ -actin and GAPDH genes served as reference genes. Relative expression levels were calculated using the  $2^{-\Delta\Delta C_t}$  method.

In infected snails, C-type lectin gene expression increased in hemocytes, the hepatopancreas, and the heart. In contrast, no significant changes were observed in the foot and albumen gland compared to uninfected individuals. Interestingly, *B. polonica* causes the greatest increase in C-lectin gene expression in hemolymph (by more than twofold). At the same time, in molluscs infected with *C. brevis* and *N. ephemer*a, C-lectin expression is significantly increased in the hepatopancreas (2–5 fold). These differences may reflect the distinct biological characteristics and parasitism strategies of each trematode species. The most pronounced increase was observed in the heart tissue of snails infected with all three trematode species (10–26 fold). The heart tissue of molluscs is in constant contact with hemolymph, so it can be assumed that the heart increases C-lectin expression in reaction to pathogen molecules carried by the hemolymph.

The work was supported by a grant to carry out research in the framework of the main scientific directions by scientific and pedagogical staff of Herzen University No. 42–VG "Study of cellular immunity of gastropods".

**POPULATION GENETIC RESEARCH OF SABLE  
(*MARTES ZIBELLINA* LINNAEUS, 1758) IN THE PRIAMURYE REGION**

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The sable is a valuable fur-bearing species, whose range at 95% is located in Russia and partially occupying Mongolia, China, Korea and Japan. In the Priamurye region in the first half of the 20th century, autochthonous sables were preserved only in remote mountainous areas of Sikhote-Alin and in the north of the Bureinsky range, where the Verkhnebureinsky sable breeding ground was established in 1952. From there, the animals were dispersed to various regions of Russia, including the Bureinsky Highlands and Sikhote-Alin. In addition, the introduction of sables from Baikal Region happens to Sikhote-Alin.

This study is aimed at assessing the allelic diversity and the level of genetic differentiation of the Priamurye sable. To characterize populations on the left bank of the Amur River, samples of hunting collections from the southern part of the Bureinsky range in the Middle Priamurye region and from the vicinity of Lake Udy in the Lower Priamurye region were studied. A sample of sables from the middle Sikhote-Alin (the area of the middle reaches of the Bikin River) is considered along the right bank. The analysis of 8 microsatellites loci was carried out: Ma1, Ma3, Mer041, Mzf51, Mzf56, Mf8.7, Ma8, Ma15. The evaluation of genetic variability was carried out using the Arlequin 3.5.2.2 software.

An analysis of the segregation of the 8 loci under consideration showed their independent inheritance. No null-allele homozygotes were found. In a total sample of 82 sables 69 microsatellite alleles were found. Most of the alleles were found in the samples of all three regions under consideration. Ten alleles mark the middle Sikhote-Alin population. On the left bank, 1 allele that marking it was found in the population of the southern part of the Bureinsky range, and 5 such alleles were found in the samples of the Lower Priamurye region. However, most of the population-marking alleles occur with a frequency not exceeding 0.05. The average effective number of alleles ( $n_e$ ) in the populations under consideration ranged from 4.22 to 5.19.

The obtained pairwise  $F_{ST}$  values reflect the level of weak genetic differentiation of the populations under consideration. At the same time, the samples of sable from the left bank are closer to each other ( $F_{ST} = 0.03$ ) than to the Sikhote-Alin population ( $F_{ST}$  vary from 0.04 to 0.06). These differences are statistically significant.

Sables of Priamurye retain a relatively high level of population genetic diversity. The level of genetic differentiation observed in populations on the left and right banks of the Amur River and differences in allelic composition may be related to the history of settlement and different sources of sable introductions to the areas under consideration.

## PHYLOGENETIC RELATIONSHIPS OF THE WATER VOLE IN AZERBAIJAN BASED ON *CYT B* GENE SEQUENCES

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The genus *Arvicola* is a widely distributed Palaearctic taxon, occupying a range from the Atlantic coast of Europe to northwestern China from west to east, and from the shores of the Arctic Ocean to the Mediterranean and Caspian regions from north to south. Despite its widespread use as a model organism in ecological, evolutionary, and paleontological studies, the systematics of the genus *Arvicola* remains controversial.

Currently, the genus *Arvicola* is thought from 2-3 (Abramson, Lisovsky, 2012) to 4 species (Kryštufek, Shenbrot, 2022). The taxonomic status of two species—*Arvicola amphibius* and *Arvicola sapidus*—has been established based on a combination of genetic and morphological characters. However, the status of the persian (*A. persicus*) and italian (*A. italicus*) water voles remains under discussion.

A recent study based on *cyt b* gene variation demonstrated the distinctiveness of *A. persicus* as an independent species, with a distribution limited to the Alborz mountain region (Mahmoudi et al., 2019, 2022). However, an analysis of morphological variability in the cheek teeth of specimens from Western Asia suggests that this region could be included in the distribution range of *A. persicus* (Maul et al., 2021; Markova et al., 2024). Nevertheless, molecular genetic data are needed to resolve the taxonomic status of these water voles.

This study presents an analysis of the phylogenetic relationships of water voles in Azerbaijan based on *cyt b* gene sequences. The research utilized osteological collections stored in the archives of the Institute of Zoology of the Ministry of Science and Education of Azerbaijan. A morphological analysis of this material revealed that the dental characters of water voles in Azerbaijan differ from those of the eurasian *A. amphibius* and are closer to *A. persicus*—the persian water vole (Markova et al., 2024). The results of our genetic research align with the findings of the morphological variability analysis. Phylogenetic reconstructions showed that water voles from Azerbaijan are significantly differentiated from *A. amphibius* and more closely related to *A. persicus* from Iran. However, further studies involving material from other regions of Western Asia and using genetic markers from both mitochondrial and nuclear genomes are necessary to conclusively determine the taxonomic status and phylogenetic relationships of water voles in Azerbaijan.

This study was supported by the Russian Science Foundation (grant no. 22-14-00332-II).

## STUDY OF THE STRUCTURE OF GONADOTROPIC AXIS GENES IN MAMMALS WITH DIFFERENT LITTER SIZES

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Folliculogenesis is a key mechanism of reproductive function, responsible for the maturation of follicles in the ovaries. It is regulated by hormonal and genetic factors, including hormones of the gonadotropic axis, which determine the number of simultaneously maturing follicles. The aim of this bioinformatics study was to investigate structural features of gonadotropic axis genes in mammals with different litter sizes.

The methodology is based on the development of custom software solutions using the Python programming language and the Biopython, Pandas, and Matplotlib libraries. Automated algorithms were implemented for retrieving genetic data from the NCBI database, predicting introns based on consensus splice sites, assessing sequence conservation using pairwise alignment with a sliding window approach.

The resulting data were visualized as heatmaps and conservation distribution plots. The structures of the CGA, LHB, TSHB, and FSHB genes were analyzed in several mammalian species grouped by litter size in a single offspring (e.g., *Bos taurus*, *Homo sapiens*), 2–6 offspring (e.g., *Ovis aries*, *Capra hircus*), more than 6 offspring (e.g., *Sus scrofa*, *Mustela erminea*). Pairwise alignments and conservation analysis were carried out within each group. Intron sequences of each gene were compared using a custom-developed tool. As a result, a number of structural features were identified in the organization of gonadotropic axis genes that are associated with differences in the number of dominant follicles formed in various mammalian species.

This research was conducted as part of the state assignment of the Ministry of Science and Higher Education of the Russian Federation, project number **FENW-2023-0018**.

## Ka/Ks REVEALED GENES INVOLVED IN ADAPTIVE EVOLUTION OF *AEGILOPS TAUSCHII*

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*Aegilops tauschii* is a wild diploid, mostly self-pollinating goat-grass (genome DD,  $2n = 14$ ), a relative of common wheat, *T. aestivum* (genome AABBDD,  $2n = 42$ ). It inhabits a vast area in Central Eurasia, from Turkey to Kirghizstan. *Ae. tauschii* is presented by its two subspecies: subsp. *stragulata* (ancient, relict gene-pool TauL3 + major gene-pool TauL2) and subsp. *tauschii* (gene-pool TauL1) which essentially differ genetically and ecologically. The species had a complex evolutionary history. Its intraspecies divergence was mostly adaptive, and peculiarities of *Ae. tauschii* phylogenetic tree are the result of a long history of competition between its different lineages. That is why this research aimed to understand, polymorphism of what genes was involved in adaptive evolution of *Ae. tauschii*?

72 *Ae. tauschii* accessions (genetic lines) representing all the species range were used for the study, and transcriptome sequences of 27448 genes were obtained for each accession. Ka/Ks value for each gene was calculated. Ka/Ks values higher than 0.8 were considered as an evidence of positive natural selection acting on genes in the course of *Ae. tauschii* intraspecies divergence. The genes studied were known to have 4336 different GO-terms. We pointed out that 193 of them statistically significantly occurred more frequently among genes with high Ka/Ks, indicating what functions, processes and cellular components were of key importance for adaptive evolution of *Ae. tauschii*.

It was found that essential role in the species evolution played resistance genes (R-genes) protecting plant from different pathogens, the genes involved in photosynthesis, the genes controlling response to light stimulus (and by this helping plant to “tune” perfectly its flowering time), the genes involved in respiratory electron transport chain, regulation of transcription, microtubule motor activity, regulation of jasmonic acid mediated signaling pathway, and some others.

## EFFECTS OF THERMAL ADAPTATION AND ACUTE STRESS IN WHITEFISH AT THE TRANSCRIPTOMIC AND EPIGENETIC LEVEL

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Temperature stress significantly affects cold-water fish species and disrupts their physiology, spawning behavior and reproductive success. Deviations from the temperature optimum lead to oxidative stress and protein denaturation, which alters cellular processes. Although endogenous defense mechanisms (including the heat shock response) have been studied, the effects of severe temperature fluctuations on cold-water species in aquaculture need to be further investigated. A promising solution to increase stress resistance could be temperature preadaptation at early developmental stages.

This study investigated the effects of temperature preliminary adaptation during the egg stage on the stress resistance of juvenile Baikal whitefish by transcriptomic and epigenetic analyses. Fertilized eggs were incubated under two conditions: control (3-6°C) and pre-adaptation (periodic temperature increase by 6°C followed by a decrease to the initial temperature, twice a week for three months). After hatching, the larvae were kept at 10-12°C and then exposed to thermal stress (+12°C above control). The study included 53 one-month-old juveniles (12-16 fish per group), including both pre-adapted and non-adapted subgroups.

Transcriptomic profiling of Baikal whitefish identified three functionally distinct gene clusters that show different responses to thermal stress and pre-adaptation. The stress-responsive genes (*HSP-30*, *HSP-40*, *HSP-47*, *HSP-70*, *HSP-90*, *TRIM16*) showed significant up-regulation after heat exposure, independent of pre-adaptation. Metabolic regulatory genes (*MtCK*, *ARF*, *aFGF*, *D-DT*, *CRYGB*) showed increased activity only in non-adapted individuals, while genes related to oxygen transport, growth and immune function (*HBB*, *MHC*, *plumieribetin*, *TnI*, *HBA*, *myosin VI/VII*, *CYP450*, *LDB3*) showed increased expression patterns only in pre-adapted individuals. These results indicate the formation of specific transcriptional responses depending on prior thermal adaptation.

Bisulfite sequencing revealed distinct DNA methylation profiles between experimental groups. Temperature-adapted fish exhibited predominant gene hypomethylation in intron and promoter regions, coupled with exon hypermethylation. In non-adapted fish, acute stress led to genome-wide hypo methylation (affecting both introns and exons), resulting in genome activation. Conversely, pre-adapted fish showed stress-induced intron hypermethylation and exon hypomethylation that modulated cellular processes. These results demonstrate temperature-dependent adaptive epigenetic modifications.

The results obtained show that pre-adaptation to thermal stress at early developmental stages in Baikal whitefish promotes stress resistance (hormesis effect) by modulating specific gene expression patterns and their epigenetic regulation, thereby improving the ability to cope with later temperature fluctuations.

This study was conducted at the Large-Scale Research Facilities "Experimental Freshwater Aquarium Complex for Baikal Hydrobionts" and supported by the Russian Science Foundation grant no. RSF 25-24-00634.

## FIRST REPORT ON PRION PROTEIN GENE VARIATION AMONG RUSSIAN RED DEER *CERVUS ELAPHUS SENSU LATO*

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Chronic Wasting Disease (CWD) belong to transmissible spongiform encephalopathies (TSEs). It is a prion disease that affects members of the Cervidae family. The development of TSEs is directly related to the expression of the PrP protein, encoded by the *PRNP* gene, and the transformation of its tertiary structure from the normal PrP<sup>c</sup> form to the infectious isoform PrP<sup>Sc</sup>. CWD is a fatal disease, which is characterized by a long incubation period and extremely high stability of the infectious agent. For a long time, CWD was only found in North America. However, in 2016, the first cases of CWD were detected in Norway, and to date, 42 cases have been reported, all within Scandinavia. The origin of this disease in Europe remains unknown and was most likely not related to translocations of infected animals from North America.

The red deer which inhabit the territory of Russia, belong to two species within the *Cervus elaphus sensu lato* complex: the European *C. elaphus* and the Asian *C. canadensis*, which is closely related to North American wapiti. Like the other cervids, these species are susceptible to CWD, with its pathogenesis being strongly influenced by polymorphisms in the *PRNP* gene. The potential resistance or susceptibility to CWD of red deer *s.l.* populations inhabiting Russia remain poorly studied. The aim of this research was to analyze the *PRNP* gene variation in red deer *C. elaphus s.l.* from different regions of Russia.

The analysis included 105 specimens: 54 from *C. canadensis*, (Siberian *C. c. sibiricus* and Manchurian *C. c. xanthopygus* wapiti) and 51 from *C. elaphus* from various parts of their range. Complete sequences of the open reading frame (ORF) of the *PRNP* gene (771 base pairs) were obtained. As a result, 17 different genotypes were identified, seven of these were shared between *C. elaphus* and *C. canadensis* from Russia. Four single nucleotide polymorphisms (SNPs) were detected across both species at nucleotide positions 63 (C/T), 312 (G/A), 408 (T/C), and 676 (C/G). Only SNP at position 676 was nonsynonymous, corresponding to a previously described amino acid substitution from glutamine to glutamic acid (Q226/E226) in PrP protein. The mutation at codon 226 is common for European red deer; in contrast, North American wapiti typically carry a methionine (M) to leucine (L) substitution at codon 132 (M132/L132), which is associated with increased resistance to CWD and a longer incubation period. All studied individuals were homozygous for methionine at codon 132 and glycine at codon 96 which is common for European red deer and, at the same time, is associated with lower resistance to CWD in American cervids.

Our findings indicate that *PRNP* genotypes in Siberian *C. c. sibiricus* and Manchurian *C. c. xanthopygus* wapiti are more similar to those found in European red deer than to those common for North American wapiti. This pattern may be explained by selective pressures acting on *PRNP* variation in American cervids due to prolonged exposure to extensive CWD outbreaks in North America over many years.

This research was supported by RFBR grant No. 25-24-0038.

## INTRASPECIFIC STRUCTURE OF *MYOTIS MACRODACTYLUS* BASED ON *CYTb* mtDNA SEQUENCES

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The Japanese big-footed bat, *Myotis macrodactylus* (Temminck, 1840) is a Far Eastern bat species, protected and vulnerable throughout its range, which limited to the Japanese islands, Kunashir Island, the Korean Peninsula, Jeju Island, the southern Primorsky Krai and adjacent areas of northeastern China. Three morphological subspecies have been noted for the Japanese big-footed bat: the nominotypical one, distributed on the islands of Hokkaido, Honshu, Kyushu, Tsushima and Yakushima, *M. m. continentalis* Tiunov, 1997, described from southern Primorye, and *M. m. insularis* Tiunov, 1997, presumably living on Kunashir Island. Nevertheless, intraspecific variability in the mainland part of the range, as well as genetic differences between subspecies, remains unknown. Therefore, this work aimed to study *M. macrodactylus* intraspecific structure based on mtDNA variability. A total of 14 *cytB* gene sequences obtained for specimens from Primorsky Krai (n = 9) and Kunashir Island (n = 5) were analyzed and compared with all available data from GenBank.

This allowed us to identify two highly divergent genetic lineages in *M. macrodactylus* with an average *p*-distance between them of 3.94%, indicating subspecific level of differences. The island lineage "insularis" is distributed on the Japanese Islands, Kunashir Island and South Korea, the mainland lineage "continentalis" is revealed exceptionally in the southern Primorye and adjacent territories of China. In contrast to the mainland lineage, the island one is more differentiated and contains at least four subgroups with unclear distribution. The most common subgroups is detected on Honshu, Hokkaido and Kunashir, the second is observed just on Hokkaido, and the other two are found only either in South Korea (Gangwon-do) and Tsushima Island, or on Honshu and Jeju-do. Although the mainland lineage appears slightly homogeneous, the individuals from Primorsky Krai are more variable than Chinese specimens, which are represented by a single central haplotype for the lineage.

Thus, a new data on the *M. macrodactylus* intraspecific structure show that bats living in Primorsky Krai and China differ significantly from specimens in the rest of the range, which may confirm the subspecific status of *M. m. continentalis*. No reliable differences were found between individuals from Kunashir Island and the Japanese Islands, or between Honshu and Hokkaido.

**COMPARATIVE ANALYSIS OF THE GENETIC VARIABILITY OF TWO SPECIES OF TREMATODES FROM *CARASSIUS GIBELIO* (BLOCH, 1782) IN THE RUSSIAN FAR EAST**

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The comparative analysis of interspecific genetic diversity was obtained for two species of intestinal trematodes, *Parasymphylodora ussuriensis* Atopkin, Ivashko, Zhokhov, Besprozvannykh, 2023 and *Carassotrema koreanum* Park, 1938 from *Carassius gibelio* from the different reservoirs in the Primorsky Territory. The study based on the data from the fragments of the nucleotide sequence of cytochrome *c* oxidase subunit 1 (*cox1*) of the mitochondrial genome and the first internal transcribed spacer (*ITS1*) of ribosomal DNA.

It was revealed that gene *cox1* is informative and shows the differences in the genetic variability and differentiation. Thus, by this marker species *C. koreanum* proved to be genetically more heterogeneous than *P. ussuriensis* that shows values of parameters haplotype (Hd) and nucleotide diversity (Pi): Hd = 0.873 vs 0.419; Pi = 0.012 vs 0.00088, and the score of *p*-distance equals to: 1.63 vs 0.08%. The range of values of the index of differentiation G<sub>st</sub> is vary in the limit from 0.13 to 0.29 for *C. koreanum*. The parameter G<sub>st</sub> varies from 0 to 1 for *P. ussuriensis*. The observed variation indicates a significant impact of the intrapopulation component on the differentiation of all local samples for *P. ussuriensis*. Considering this, the species is genetically homogeneous as a whole in contrast to *C. koreanum*.

The examination of mismatch distribution and haplotype network indicated that the local population of *C. koreanum* displayed differing levels of isolation and potential of past reconnection among the studied individuals. The same analyses indicated a decrease in the population of trematodes *P. ussuriensis* across the Primorye region in the past, following a sudden expansion. This hypothesis is supported by studies of geological and climatic changes in the Khanka Lake and river systems and the Japanese Sea, which were characterized by transgressions and regressions.

# FIRST MOLECULAR DATA FOR TREMATODES OF THE FAMILY PARAMPHISTOMIDAE FISCHOEDER, 1901 FROM THE RUSSIAN SOUTHERN FAR EAST

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Among the numerous Paramphistomidae, a significant number of species are parasites of wild and domestic ungulates. Many of these species are the causative agents of intestinal paramphistomiasis, a severe disease of ruminants that, in cases of high intensity of infection, leads to emaciation of the host. Species identification for most species is based on the morphology of mature individuals. At the same time, the number of species for which molecular data were obtained is minimal compared to the number of known species of Paramphistomidae infecting ungulates. Given the increasing frequency of cryptic species among trematodes, without consolidated morphological and molecular data, it is difficult to determine the number of paramphistomid species recorded in the East Asian region.

We found cercariae of three species of Paramphistomoidea emitted from the naturally infected freshwater snails *Bithynia fuchsiana* (Möllendorff, 1888) collected in Vietnam, as well as *Helicorbis suffunensis* Starobogatov, 1957 and *Gyraulax centrifugops* (Prozorova & Starobogatov, 1997) found in the Russian southern Far East. The analysis of morphological data of the detected cercariae showed that the cercariae obtained in *G. centrifugops* were the closest to three species, *Paramphistomum ichikawai* Fukui, 1922, *Paramphistomum petrowi* (Davydova, 1961), and *Calicophoron ijimai* (Fukui, 1922). Cercariae from *H. suffunensis* have a structure of the collecting channels of the excretory system which is identical to those of cercariae of *Fischoederius elongatus* (Mukherjee, 1986). As for the cercariae collected from Vietnamese snails, the structure of the collecting ducts is most similar to those of *Ceylonocotyle scolicoelium* (Fischoeder, 1904), *Calicophoron dicranocoelium* (Fischoeder, 1901), *Gastrothylax crumenifer* Creplin, 1847, *Cercaria kareilliensis* Mukherjee, 1972 and *Cercariae indicae* XXXII Sewell, 1922 (Mukherjee, 1986). At the same time, there are also some morphological differences. Thus, morphological analysis of two of the three species under study does not reveal species and even genus affiliation of the detected paramphistomids. The analysis of phylogenetic reconstructions based on nuclear (the 28S rRNA) and mitochondrial (the *cox1*) gene markers showed that nucleotide sequences for cercariae from *G. centrifugops* are united in a clade with samples for which only sequences designated as *Paramphistomum leydeni* Näsmark, 1937 are accompanied by information on the morphology of the worms. Based on the above, we suggest that the cercariae we found should be assigned to the genus *Paramphistomum* Fischoeder, 1901. The nucleotide sequence for the second cercarial type obtained from Russia, based on phylogenetic reconstruction for the mitochondrial marker *cox1*, forms a clade with trematodes from the genera *Gastrothylax* (Creplin, 1847) and *Fischoederius* Stiles & Goldberger, 1910. In this clade, only the samples of *F. elongatus* have a morphological description. Based on this and data on the morphology of the cercariae found in this research, we assign them to the genus *Fischoederius*. Unfortunately, for Paramphistomidae obtained in snails from Vietnam, both morphological and molecular data did not give a definite answer about their taxonomic affiliation; for this reason, until data on the morphology of sexually mature individuals are obtained, we designate them as Paramphistomidae gen. sp. In general, the results of our studies showed that the assessment of phylogenetic relationships of the family Paramphistomoidea is complicated by the fact that, in the presence of molecular data for many species, there is no confirmation of their genus and species affiliation at the morphological level.

**NEW MOLECULAR GENETIC ANALYSIS DATA FOR IDENTIFICATION OF  
DIATOM SPECIES *SKELETONEMA MENZELII* FROM THE SEA OF JAPAN**

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*Skeletonema* species are the most common marine planktonic diatoms of the temperate zone of the world ocean. In warm seasons along with other genera of the order Thalassiosirales (Bacillariophyta) they represent a major part of the phytoplankton biomass and cause "blooming" of the coastal waters. In the Sea of Japan on the Far East of Russia 5 species of the genus *Skeletonema* are occurred, while the species *S. menzeli* have discovered relatively recently and data about its structure, features and genetic variability is scattered. Due to the difficulty in the identification *S. menzeli* more complete and accurate analysis requires an integrated approach combining morphological and molecular genetic methods. This study shows the distinctive features of species genetic variability of *S. menzeli* found in the waters of the Sea of Japan from other representatives of the species described in world literature.

In this research we continue our former work on molecular genetic identification of the species *S. menzeli*. Three new clones collected in 2021 on the coast of the Sea of Japan in Russia were added to this work. Molecular genetic analysis of the samples was carried out on the 18S rDNA and 28S rDNA regions. To compile a matrix of nucleotide sequences and construct phylogenetic trees we used data of the other *S. menzeli* from the GenBank. The sequences obtained in our study had high divergence values and formed separate clusters on phylogenetic trees from the *S. menzeli* taken from the GenBank, and also had differences in genetic distances (>0.008). According to the obtained data on morphological (provided by the co-author) and phylogenetic analysis we can confirm the differences between our samples and other *S. menzeli* described in the literature.

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**THE *ITS2* rDNA AS A SUITABLE MARKER FOR PHYLOGENETIC ANALYSIS OF SUPERFAMILY ECHINOSTOMATOIDEA**

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The Echinostomatoidea are a large, cosmopolitan group of digeneans. The vast majority of species trematodes of this group infected birds, mammals, reptiles, and fish. Some parasites have been recorded in humans. Therefore, it is necessary to consider all aspects of parasite survival. It is also important to study the *ITS2* rRNA for understanding processing and the biogenesis of active ribosomal subunits. Obtaining and analyzing the secondary structures of the *ITS2* region is in demand for understanding the biological role of these molecules.

In this study, complete nucleotide sequences of the *ITS2* rRNA region were obtained for 12 species from the superfamily Echinostomatoidea. The remaining representatives of the superfamily were taken from the GenBank database. Alignment of the nucleotide sequences was done through ClustalW, R-coffee, and MAFFT. Phylogenetic trees were constructed using Bayesian inference (BI) and Maximum likelihood (ML). We created secondary structures of the *ITS2* spacer region using three different web servers.

Phylogenetic analysis showed that the positions of all families in the obtained reconstructions are similar to those from the previous study based on the 28S rRNA gene (Tkach et al., 2016). According to previously published data, the spatial structure of the *ITS2* region for most trematodes has a typical four-domain structure. However, we found that in some cases the structures are variable. This depends on the length of the sequences: the longer ones have more loop domains. A general secondary structure has been designed for the superfamily Echinostomatoidea, despite only the first and second domains being conserved. Moreover, although the *ITS2* rRNA region is not suitable for phylogenetic analysis in many trematode groups, it was successful in assessing phylogenetic relationships within the superfamily Echinostomatoidea.

## EXAMPLES OF THE USE OF MOLECULAR MARKERS IN EVOLUTIONARY BIOLOGY AND FOR NEEDS OF MODERN SOCIETY

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**Introduction:** The application of molecular genetic markers is very fruitful in many ways. One is the validation of modern paradigms, the Synthetic Theory of Evolution (STE) and the Biological Species Concept (BSC), and another is a biodiversity estimation for the variety of social needs, including the evaluation of seafood mislabeling of marine species.

**Methods:** Vast sequence data on the single genes of nDNA and mtDNA and complete mitogenomes have been gathered through our own experiments and from literature, analyzed by a variety of software tools, and provided in the presentation.

**Results & Conclusions:** The main issues of the report are focused on 5 points. (1) A combination of nDNA and mtDNA markers best suits the hybrid identification and estimates of genetic introgression. (2) The available facts on nDNA and mtDNA diversity seemingly make the introgression among many taxa obvious, although it is evident that introgression may be quite restricted or asymmetric, thus holding at least the “source” taxon (taxa) intact. (3) If we accept that sexually reproducing species in the marine and terrestrial realms are introgressed, as is still evident in many cases, then we should recognize that the BSC, in terms of the complete lack of gene flow among species, is inadequate due to the fact that many zoological species are not biological species yet. However, the modern molecular data proved that they definitely should become biological species sooner or later (Kartavtsev, 2013, 2018, 2021; Hedges et al., 2015). (4) The recent studies involving the fish and shellfish taxa divergence (Kartavtsev, 2017, 2018, 2021; Redin, Kartavtsev, 2022; Kartavtsev, Masalkova, 2024) and the comprehensive usage of the BOLD database show that the gene trees are basically monophyletic, with the interspecies reticulations that are rare. (5) A variety of evidence is available globally that proves the seafood fraud; thus, scientists must develop molecular control for this and other similar industries.

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## MITOGENOME OF WATER ORGANISMS: STRUCTURE, EVOLUTION AND IMPLICATIONS IN MOLECULAR PHYLOGENETICS

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Recent advances in genomics, particularly mitochondrial genomics (mitogenomics), have established this field as a transformative frontier in molecular sciences with profound scientific, societal, and economic impacts (Kartavtsev, 2025). The special issue (SI) of the *International Journal of Molecular Sciences* (IJMS) highlights the crucial role of aquatic organisms by focusing on the structure, evolution, and diversity of their mitogenomes. The collection of SI comprises 12 original research articles published between 2023-2024 ([https://www.mdpi.com/journal/ijms/special\\_issues/P9J4DJGUON](https://www.mdpi.com/journal/ijms/special_issues/P9J4DJGUON)), demonstrating applications of mitogenomes and mitochondrial DNA (mtDNA) markers in both basic biology and practical applications.

The SI addresses five key aspects of mitogenomics:

1. Structural organization,
2. Evolutionary patterns,
3. Phylogenetic utility,
4. Biological diversity,
5. Mitonuclear interactions.

Three major research themes emerged as the real outcome of SI:

1. **Molecular Phylogenetics** - Multiple studies advanced fundamental concepts in evolutionary relationships;
2. **Structural Genomics** - Detailed analyses of mitogenome organization and variability;
3. **Adaptive Evolution** - Investigations of selection pressures shaping mitochondrial DNA.

Critical research gaps identified include:

- The need for deeper exploration of mitonuclear interactions
- Challenges in accurately detecting natural selection at single-marker resolution, where current methods may overestimate signals.

While several studies report putative selection events, we emphasize (as noted in the editorial and supporting papers) the importance of cautious interpretation. The field would benefit from more balanced discussions of both findings and methodological limitations.

The summary in the editorial anticipate this SI will:

- Showcase significant achievements in mitogenomic methodologies;
- Stimulate future research addressing the identified knowledge gaps;
- Promote continued advancement of high-quality mitogenomic studies in IJMS.

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## MITOCHONDRIAL GENOME ANALYSIS OF MUSSEL SPECIES (BIVALVIA, MYTILIDAE)

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This investigation provides original experimental data and wide variety of viewpoints on mitogenome structure, evolution, natural selection influence on molecular polymorphism in nature and diverse phylogenetic and taxonomic impacts of mitogenome data on one of the most abundant bivalves, the mussel family Mytilidae. Authors investigated the evolutionary dynamics of Mytilidae mussels through comparative mitogenomic analysis of *Arcuatula senhousia* and *Mytilus coruscus*, combining GenBank NCBI data in genomic, phylogenetic, and evolutionary genetic approaches. We have discussing three main subject area of mitogenome analysis belonging to: Structural variability, Molecular evolution, and Phylogenetic resolution. It is obtained: 1. Mytilid mitogenomes exhibit remarkable structural plasticity, featuring: frequent gene rearrangements (particularly in tRNAs), significant size variation between species, and pronounced female vs. male (F/M) lineage differentiation. 2. Hydrophobicity patterns in polypeptides correlate with purine/pyrimidine ratios while purifying selection is expected to maintain protein function, our data provide ambiguous support for the late hypothesis. 3. Analysis of 26-27 curated mitogenomes (selected from 100 GenBank entries with verified F/M origin) yields: a revised Mytilidae phylogeny with divergence dating to ~293 Mya (Silurian origin), strong evidence against Mytilinae monophyly, consistent topologies across multiple data matrices (PCGs, rRNA+tRNA, and combined genes). Presented evidence has newly obtained fundamental significance. This study: (i) provides the most comprehensive mitogenomic framework for Mytilidae systematics to date, (ii) resolves long-standing controversies about Mytilinae and Mytilidae monophyly, (iii) highlights unique evolutionary constraints in bivalve mitochondrial genomes. (iv) establishes a robust foundation for future comparative genomic studies in marine mollusks.

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**STUDY OF MOLECULAR PHYLOGENICS AND GENETIC DIVERGENCE OF MUSSELS (MOLLUSCA, MYTILIDAE) BASED ON THREE NUCLEAR DNA (18S RRNA, 28S RRNA, HISTONE H3) AND ONE mtDNA (CO-I) GENE SEQUENCES**

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Based on nucleotide sequences of three nuclear genes (nDNA) and *Co-I* gene (mtDNA) the molecular phylogenetic and evolutionary genetic research was performed. The molecular phylogeny of the sampled representatives for one of the largest taxa among bivalve mollusks, the family Mytilidae, was studied with inferences in the systematics of the family and with the focus on subfamily Mytilinae. A molecular phylogenetic relationship for the family Mytilidae and nearest taxon Modiolidae of the order Mytilida, which currently has no consensus among experts based on traditional characters, have tested at single-locus level and at combined sequence set at three genes. Using nucleotide sequences of the 28S rRNA, 18S rRNA, and *H3* genes, a consensus representation on the system was established by the study of specimens from Mytilidae-Modiolidae data matrix. Some concerns of mussel systematics were resolved. In particular, the monophyly of the subfamily Mytilinae Rafinesque, 1815 after recent revision ([WoRMS - World Register of Marine Species - Mytilidae Rafinesque, 1815](#)) was supported. The data obtained proved former conclusions on polyphyly-paraphyly of the family Mytilidae Rafinesque, 1815. Isolation of the taxon in the rank of the Modiolidae G. Termier & H. Termier, 1950 from the family Mytilidae was preliminary confirmed as well. Our own and recent literature sources well support monophyly of subfamily Mytilinae in revised version [that allows](#) inclusion at least genera [Mytilus Linnaeus, 1758](#), [Pacifimytilus Kafanov, 1984](#), and [Crenomytilus T. Soot-Ryen, 1955](#) ([WoRMS - World Register of Marine Species - Mytilidae Rafinesque, 1815](#)) available in our collection. As to the family Mytilidae data obtained in the present study agree with inclusion of several proved lineages in the family: (1) Mytilinae, (2) Crenellinae, (3) Mytiliseptinae, and (4) Septiferinae ([WoRMS - World Register of Marine Species - Mytilidae Rafinesque, 1815](#)). [Comparative data obtained on genetic distance increase with taxa tank](#) support the generalization that phyletic evolution prevails at the molecular level in the mussels and animal kingdom in general, and that speciation occurs mainly on the basis of a geographic model.

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## MOLECULAR OR CHROMOSOMAL SPECIATION IN THE “MAXIMOWICZII” SPECIES GROUP OF THE GENUS *ALEXANDROMYS*?

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Speciation often occurs during periods of isolation. Isolation itself is not the cause of speciation. Mutations in DNA and chromosomes, as well as isolation of gene flow, can be the causes of reproductive isolation and speciation. The type of differentiation (genetic or chromosomal) determines the nature of speciation. As a rule, species or chromosomal races with a short period of isolation are distinguished by insignificant genetic and morphological differentiation or its complete absence (King, 1995). Such species are an excellent model for studying microevolutionary processes at the stage of speciation.

The species of the “maximowiczii” group of the genus *Alexandromys* - *A. maximowiczii* (2n=36-44) and *A. evoronensis* (2n=34-41), *A. mujanensis* (2n=38) - inhabit various geographical regions of the southern Russian Far East. These species are ideal for studying microevolutionary processes at the initial stages of speciation. If the first species (*A. maximowiczii*) enters the territory of Russia with the northeastern part of its range (Buryatia, Zabaikalsky Krai, Amur Oblast, Jewish Autonomous Oblast, Khabarovsk Krai), then the other two species are located entirely on the territory of Russia: *A. mujanensis* inhabits the intermountain basins of northern Buryatia; *A. evoronensis* inhabits three isolated mountain basins of Amur Oblast and Khabarovsk Krai. The karyotypes of these species differ in the number and morphology of chromosomes. For *A. mujanensis*, multiple chromosomal polymorphisms by pericentric inversions have been described. For *A. maximowiczii* and *A. evoronensis*, in addition to chromosomal inversions, intra- and interpopulation polymorphism by multiple chromosomal rearrangements (inversions, Robertsonian and tandem fusions) has been identified. While polymorphism by Robertsonian fusions is relatively common in mammal populations, tandem fusions are rare. Five chromosomal forms have been described for *A. maximowiczii*, and two chromosomal races for *A. evoronensis*. Despite the fact that, according to mt DNA data, these species have distances at the level of interpopulation, structural rearrangements are different for each species, and first-generation hybrids are sterile. Morphologically, the species are almost indistinguishable, which may indicate rapid speciation.

In this report I will provide an overview of chromosomal, molecular and morphological data showing the taxonomic independence of the three species and the leading role of chromosomal rearrangements in the speciation of the three species under consideration.

**POPULATION STRUCTURE OF *KOREOLEPTOXIS AMURENSIS*, THE FIRST INTERMEDIATE HOST OF TREMATODES**

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Freshwater mollusks are important benthic animals that dominate in many rivers and lakes in terms of abundance and biomass. They play a role as aquatic biofilters, take part in the decomposition of organic matter, and are also a source of food for many birds and mammals. No less importantly, that these mollusks are also the first intermediate hosts of various parasites and participate in the circulation of epidemiologically significant trematodes such as *Nanophyetus salmincola* and *Metagonimus suifunensis*.

In 2009, using molecular analysis, Strong and Köhler indicated that “*Parajuga*” is an invalid species name. Later, Köhler, based on molecular data for one specimen of “*Parajuga*”, collected in the Russian Far East, suggested that it belongs to *Koreoleptoxis amurensis*. Results of this study confirm data of our previous study that combined four other species of the genus “*Parajuga*” into a single species, *Koreoleptoxis amurensis*.

In this study, partial nucleotide sequences of the mitochondrial DNA *cox1* gene were used to describe the population structure of the species *Koreoleptoxis amurensis* from four regions of the Russian Far East, as well as from one region of China (NCBI data). Based on the analysis of *p*-distances, we reaffirmed that all the specimens obtained belong to one species, *Koreoleptoxis amurensis*, despite the wide variability of their conchological features. Although a simple star-like structure of the MST reconstruction was obtained, we have identified unique haplotypes and haplogroups for each region. It was also revealed a haplogroup with specimens from different geographic regions, which had numerous mutation steps from most samples. The values of genetic distances do not reach the values known for interspecies differentiation. Still, it is possible to assume the existence of isolation and minimal genetic exchange among settlements, which can comprise the basis for processes of speciation.

## ASSESSING TAXONOMIC DIVERSITY OF FISH ACROSS RIVER SYSTEMS IN INDIA: INTER AND INTRA-BASIN CALIBRATION

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India's river systems harbour a vast diversity of freshwater fish, yet comprehensive taxonomic assessments remain challenging due to morphological similarities among species. This study employs DNA barcoding to assess taxonomic diversity across major Indian river basins, including the Godavari, Krishna, Narmada, Mahanadi, Tapi, etc. Mitochondrial cytochrome c oxidase subunit I (*COI*) sequences were analyzed to resolve species identification and assess inter- and intra-basin diversity. Species were found to be part of a large no of barcode bins suggesting incomplete taxonomic efforts. Genetic divergence, species delimitation methods, and phylogenetic analyses were used to evaluate taxonomic structure and basin-wise differentiation. The results reveal significant cryptic diversity and instances of genetic admixture, highlighting the complex biogeographical histories of Indian riverine ichthyofauna. Additionally, intra-basin comparisons indicate localized genetic variations influenced by environmental factors and anthropogenic pressures. The study also supports the acceptance of speciation based on deep genetic divergence, reinforcing the role of molecular thresholds in delineating fish taxa. These findings underscore the importance of DNA barcoding in uncovering hidden diversity and refining species classifications, offering valuable insights for conservation planning and sustainable fisheries management in India's river ecosystems.

**Key words:** river, fish diversity, inter-basin, intra-basin, speciation.

**NEW DATA ON MITOCHONDRIAL GENOME INTROGRESSION FROM THE NORTHERN RED-BACKED VOLE *CLETHRIONOMYS RUTILUS* (PALLAS 1779) TO THE BANK VOLE *CLETHRIONOMYS GLAREOLUS* (SCHREBER 1780) (ARVICOLINAE, RODENTIA) ON THE SOUTHERN URALS**

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Introgression of *Clethrionomys rutilus* (Pallas, 1779) mtDNA into the genome of *Clethrionomys glareolus* (Schreber, 1780) has long been known (Tegelström, 1987), and its spatial distribution and possible mechanisms of development have been widely studied (Melnikova et al., 2012). However, the relationship between mitochondrial introgression (as evidence of ancient hybridization) and the potential interspecific transfer of morphological traits between the northern red-backed vole and the bank vole remains poorly understood. This study aims to explore that relationship.

Vole specimens for this study were collected from the South Ural, an area where mtDNA introgression has previously been reported, but material was collected in the new localities. To rapidly identify introgressed mtDNA among voles, a real-time PCR system was employed. Utilizing species-specific SNPs, this system allows assignment of mitochondrial (*COI*) and one nuclear (*LCAT*) marker to one of the species. The *MT-CYB* gene of specimens from populations with mtDNA introgression was sequenced and used for phylogenetic reconstruction. Morphological traits were assessed without prior knowledge of the genotyping results, using general morphometric and dental features typically employed to distinguish *Clethrionomys* species.

As a result, a bank vole population with 100% introgression of *C. rutilus* mtDNA—but lacking nuclear alleles of *C. rutilus*—was discovered in the South Ural Nature Reserve. Their mitochondrial haplotypes were not identical to those of sympatric red-backed voles and clustered with the Western *C. rutilus* phylogroup (Kohli et al., 2015), which is associated with a possible Eurasian LGM refugium. The dental and external traits of the studied specimens were consistent with the nuclear DNA marker: features such as the anteroconid shape, molar proportions, and tail length were typical for the respective species and did not show mixed characteristics.

Together with previous findings, this study suggests that the refugial hypothesis of mtDNA introgression from *C. rutilus* to *C. glareolus* is less reliable than the hypothesis that the current mtDNA introgression zone developed in the process of the formation of a contemporary zone of sympatry.

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## **INTEGRATIVE ANALYSIS OF OMICS BIG DATA AND WEB APPLICATION DEVELOPMENT FOR THE IDENTIFICATION OF DIAGNOSTIC TARGETS**

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age. The molecular mechanisms underlying the condition remain poorly understood, complicating early diagnosis and the development of effective treatment strategies. This study presents an integrative analysis of transcriptomic, metabolomic, and genetic data from open-access sources (GEO, MetaboLights, GWAS Catalog, etc.) to identify potential diagnostic targets. Using data derived from various tissues (ovarian, adipose, blood), we performed differential gene expression analysis, functional annotation, and pathway enrichment to uncover molecular signatures associated with PCOS. Bioinformatics tools such as DAVID, Reactome, and MetaboAnalyst were used to systematize and interpret the results. A key component of this project is the development of a web-based application designed to facilitate interactive omics data analysis, visualization of molecular alterations, and automation of standard workflows. The platform is implemented using modern technologies and can serve both scientific and educational purposes. This work demonstrates the potential of public Big Data and digital tools in systems biology and highlights the role of computational solutions in biomarker discovery and personalized medicine approaches for PCOS.

**RAG1 AS A MARKER FOR GENETIC DIFFERENTIATION OF EUROPEAN CISCO  
(*COREGONUS ALBULA* LINNAEUS) AND SARDINE CISCO  
(*COREGONUS SARDINELLA* VALENCIENNES)**

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Protection of rare fish species and conservation of endemic populations have become critical issues in recent decades. Reintroduction of species into the wild during breeding in fish farms may be the reason for the loss of unique adaptations acquired by individuals raised in the natural habitat. Therefore, the study of genetic differences between closely related species plays a critical role in the field of conservation of biological resources.

This problem directly relates to such phylogenetically closed species as the European cisco (*Coregonus albula* Linnaeus) and Siberian cisco (*Coregonus sardinella* Valenciennes). In most cases, they are differentiated only on the basis of geographical criteria. The habitat of the European cisco does not extend east of the Urals, while the Siberian cisco inhabits rivers from the Urals to the Bering Sea. Species also have a zone of overlapping habitats and can form hybrids. Morphological traits are highly variable in both species, making them unreliable for taxonomic classification. The solution to this problem is the use of molecular genetic approaches to species differentiation.

Molecular genetic analysis allows the creation of genetic markers suitable for the rational use of water resources and conservation of species diversity in natural basins, as well as for screening breeding stocks in fish farms, followed by the selection of reintroduction locations.

Previous research has focused on allozyme analysis and mitochondrial DNA analysis of these species. Nuclear genome research is not of extensive usage currently. The single-copy recombination activation gene 1 (*RAG1*) contains a slowly evolving, highly conserved 3'-region of the coding sequence and has shown representative results in studies related to the origin of char belonging to the family *Salmonidae*, along with cisco. Our work presents the use of *RAG1* as the nuclear marker for genetic differentiation of European and Siberian cisco. There is also a comparison of the data we have experimentally obtained with the data from the GenBank.

**FIRST GENETIC DIVERSITY ASSESSMENT OF *SYNCHAETA* EHRENBURG, 1832  
(ROTIFERA: MONOGONONTA: SYNCHAETIDAE) FROM LAKE BAIKAL**

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*Synchaeta* Ehrenberg, 1832 comprises predominantly planktonic species, which are distributed widely in fresh and brackish waters worldwide. *Synchaeta* is characterized by a high species diversity which, in combination with its high adaptability, makes this genus a suitable model for biodiversity and evolution studying of microscopic invertebrates. The traditional morphological identification of *Synchaeta* species is associated with a number of difficulties, including the loss of morphological features during the preservation of organisms (Wilke et al., 2018). Nine species of *Synchaeta* have been recorded in Lake Baikal, of which three species are endemic. The aim of this study was to assess the diversity of *Synchaeta* in Lake Baikal using genetic methods.

The material was collected in March and September 2024, 2025 from the littoral and pelagic zones of the Southern Basin of Lake Baikal. DNA was isolated from living specimens of *Synchaeta*, which were had previously been photographed under a light microscope. We obtained *COI* and *ITS1* sequences for the first time of endemic species *S. pachypoda* Jashnov, 1922 and *S. prominula* Kutikova et Vassiljeva, 1982. These species are among the dominant rotifers in the winter-spring zooplankton of Baikal. On the *COI* tree *S. pachypoda* and *S. prominula* are genetically distinct from other members of the genus. *S. grandis*, a Holarctic species, is one of the dominant species in the summer-autumn zooplankton of Baikal. On the *COI* tree it clusters with representatives of the same species from Germany, however forms a distant genetic lineage. The genetic K2P distances (*COI*) between *S. grandis* from Baikal and Germany are about 11%. Probably, specimens from Baikal, which morphologically close to *S. grandis* is a potentially new species, which is sister to *S. grandis*. In the coastal zone of Lake Baikal, the cold-loving species *S. lakowitziana* Lucks 1912 was discovered for the first time in March. This species belongs to the northern invaders and has been recorded in recent years from European Russia and Siberia (Slynko et al., 2002; Zhdanova, 2023). Thus, the initial genetic data of *Synchaeta* from Lake Baikal demonstrate that the species composition of this genus in the lake is more abundant than previously assumed and requires revision with the integration of intravital microscopy and genetic methods.

We are grateful to A.P. Fedotov, P.N. Anoshko, I.N. Smolin, S.A. Potapov (LIN SB RAS) for helping us collect the material. Sequencing and microscopy were performed at the Instrumentation Center "Electronic Microscopy" of the Collective Instrumental Center "Ultramicroanalysis" (LIN SB RAS). The work was supported by state assignment No. 0279-2021-0005 (121032300224-8).

## NEW INFORMATION ON THE SMELT IN THE LAPTEV SEA BASIN WITH PHYLOGENETIC CONSIDERATION

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We analyzed the genetic diversity and biological characteristics of a previously unstudied smelt population from the Lena River delta (Laptev Sea basin). A comparative analysis was performed based on a large number of sequences of two individual mitochondrial genes encoding cytochrome *c* oxidase-I (*COI*) and cytochrome *b* (*Cytb*). In addition to the newly obtained sequences, public data available from GenBank were used which included *Osmerus dentex* (Steindachner et Kner, 1870) sequences from different localities in the Pacific and Arctic Ocean basins. For more accurate identification and determination of interspecific divergence within the genus *Osmerus*, sequences of *O. mordax* and *O. eperlanus* were included in the analysis. The capelin *Mallotus villosus* was considered as an outgroup. The length of the analyzed sequences after alignment was 665 and 606 bp for the *Cytb* and *COI* gene fragments, respectively.

Phylogenetic reconstructions based on the selected mitochondrial markers revealed four monophyletic groups taxonomically corresponding to the species *O. dentex*, *O. mordax*, *O. eperlanus*, and *M. villosus*. The gene trees differ in the position of the branches of *O. mordax* and *O. eperlanus*, which occupy a basal position relative to *O. dentex*. Molecular data indicate that *O. dentex* and *O. eperlanus* are sister lineages. The monophyletic group of the genus *Osmerus* is strongly supported in all variants of phylogenetic analysis. The tested sample of smelts from the Lena River basin belongs to *O. dentex*, and does not change its position in the gene trees.

Nucleotide sequence divergence and genealogical networks confirm the belonging of the tested sample to *O. dentex* and its phylogenetic closeness to *O. dentex* from the Pacific part of the range. Thus, we confirm the phylogeographic hypothesis on the dispersal of *O. dentex* from the main refugium in the East Pacific along the eastern and Arctic coasts of Eurasia during the periods of world ocean transgressions.

Molecular identification is consistent with the data of comparative biological analysis. The analyzed characteristics of the Lena River delta population correspond to the average ones for *O. dentex*. New data allowed us to clarify the patterns of changes in biological parameters of *O. dentex* across its range. A tendency is observed towards an increase in the length and weight of smelts in the Arctic Ocean basin and a slight decrease in the Pacific Ocean basin, as well as towards a significant decrease in the age of attaining sexual maturity in Pacific populations, which may be due to fishing pressure. The natural population from the Laptev Sea basin retains the optimal age composition and optimal size and weight traits of the smelts.

We are grateful to our colleague, and friend Lubov Skurikhina for encouragement and consultations and sequencing assistance. The study was performed under research project no. 124021900010-2 (Ministry of Science and Higher Education of the Russian Federation and A.V. Zhirmunsky National Scientific Center of Marine Biology, FEB RAS, Vladivostok, Russia).

## THE DIVERSITY AND EVOLUTION OF LIGHT-SENSITIVE OPSINS IN CTENOPHORES

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Opsins are a large family of light-sensitive proteins that bind retinal as a chromophore and play a key role in the visual systems of most modern animals. Ctenophores belong to the oldest evolutionary lineage of multicellular animals, in which opsin-based photosensitivity may have first emerged. Despite their evolutionary significance, ctenophore opsins remain poorly characterized. This study investigates their diversity, structural evolution, and phylogenetic relationships to reconstruct the earliest stages of the evolution of animal visual systems.

We retrieved 41 transcriptome samples from the NCBI SRA database and assembled them following the approach described in Krivenko et al. (2024). Our dataset includes opsin-coding sequences from 31 planktonic and benthic ctenophore species across the five main orders of Tentaculata and Nuda. For this set, we analyzed the structure and phylogeny of the protein-coding sequences.

We reconstructed a phylogenetic tree from the opsin data and compared it with the mitochondrial *COI* phylogeny of ctenophores. We used AlphaFold3 to build 3D protein models and analyzed opsin-retinal binding with CB-DOCK2, focusing on key amino acids near the  $\beta$ -ionone ring, Schiff base, and counterions.

Within the general phylogenetic tree of visual opsins in Metazoa, ctenophore opsins form an independent branch, which is a daughter clade of the most known animal opsin types. This branch is divided into two monophyletic lineages: Ctenopsin 1 and Ctenopsins 2. The structure of these clusters generally reflects the present-day phylogeny of ctenophores as reconstructed from transcriptomic data. Ctenopsins 1 and 2 likely diverged following a gene duplication in the common ancestor of Lobata, Cydippida, and Beroida. An additional expansion of the opsin protein family in the evolutionary history of ctenophores was found only in the Ctenopsin 2 lineage. Gene duplication may also have occurred at the last common ancestor of modern Ctenophora with both functional copies being preserved in benthic Coelopanidae and planktonic *Lampea pancerina* which belongs to an early branch of modern comb-jellies. Planktonic species retain only one copy of this gene associated with a specific subtype with the exception of *Lampea pancerina* which belongs to an early branch of modern comb-jellies that has Ctenopsin 2 in another subclade.

Comparative analysis of amino acid sequences and structural models of Ctenopsins 1 and 2 revealed key differences likely reflecting their functional diversification. They differ notably in conserved regions involved in G-protein interactions, suggesting distinct intracellular signaling. Ctenopsin 2 has a more stable hydrophobic retinal binding site, while Ctenopsin 1 contains more polar stabilizing residues. Obtained structural differences result in distinct spectral properties.

Ctenophore opsins show a dynamic evolutionary history involving gene duplication, structural changes, and adaptation to different environments. The retention of dual opsins in benthic taxa versus reduction in planktonic groups underscores the role of environmental pressures in sensory system evolution. These findings illuminate the early emergence of opsin diversity and provide a framework for studying vision origins in ancient animal lineages.

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## INTRAGENOMIC POLYMORPHISM AS A POSSIBLE MOLECULAR ADAPTATION OF TREMATODE *ISOPARORCHIS* SPP.

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Trematodes of the genus *Isoparorchis* are predominantly parasites of catfish. An important stage in their life cycle is considered to be the transmission from the third intermediate host, a freshwater non-catfish fish, to a definitive host, catfish. To accomplish this, the parasite weakens the intermediate host, causing various diseases, or it leaves tissues and swims actively in search of the definitive host. Thus, trematodes of the genus *Isoparorchis* are a danger to various species of freshwater fish and can lead to mass death of fish in rivers. In addition, just as importantly, infected fish are often exported to various countries, which increases the likelihood of spreading these trematodes.

The sequences of the 28S rRNA gene and the ITS2 region of nuclear DNA were used to clarify the phylogenetic position of the representatives of the genus *Isoparorchis* collected in the south of the Russian Far East. The trematodes of the genus *Isoparorchis* from this region were previously classified as *Isoparorchis eurytremus*, but we found nucleotide differences for samples from Russia and Japan. In this study, we also revealed a high frequency of intragenomic variations for samples of *Isoparorchis* from different localities of the Russian Far East. This may be related to recent migration and crossing between isolated populations of the parasite being studied. At the same time, this trait may be a result of the adaptation to a complex life cycle and the possibility of long-term free-living stages in the environment.

## POPULATION GENETIC STRUCTURE OF *BOS TAURUS* INFERRED FROM THE MHC CLASS II *DRB3* GENE

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Cattle (*Bos taurus*) are among the most important domesticated animals, bred on all continents. Their global distribution, large population size, and high economic value have led to intense artificial selection for productivity traits. However, this artificial selection, acting alongside natural selection, often results in reduced overall genetic diversity, which may limit adaptive potential, including resistance to infectious diseases.

Genes of the major histocompatibility complex (MHC), particularly *DRB3*, play a key role in immune responses and are known for their exceptionally high polymorphism. This makes them useful markers for assessing immunogenetic status as well as for population genetic studies. According to current data, the number of alleles in exon 2 of the *DRB3* gene, which encodes the peptide-binding region of the MHC class II heterodimer, ranges from 54 (using PCR-RFLP) to over 300 (using sequence-based typing, SBT). The aim of this study was to assess intraspecific variability of the *DRB3* gene across a broad dataset.

We analyzed *DRB3* gene polymorphism based on published data from 18 cattle breeds (42 samples totaling over 4,000 animals) of diverse geographic origin and breeding history. The dataset included representatives of both the taurine (*B. taurus taurus*) and indicine (*B. taurus indicus*) subspecies, comprising dairy, beef, and dual-purpose breeds.

Alleles with frequencies above 5% were identified: 8–9 alleles were detected in some Creole breeds from Colombia, Mongolian cattle, and Japanese Shorthorn; slightly fewer in regional taurine breeds such as Kostroma, Yaroslavl, Kalmyk, and Russian Black-and-White. The lowest diversity (4 alleles) was observed in the Yakut breed, which is highly adapted to the extreme conditions of the continental subarctic climate. Expected heterozygosity values ranged from 0.94 (in some Creole and the Kalmyk breeds) to 0.73 in the Yakut cattle.

Principal coordinate analysis based on pairwise Wright's  $F_{ST}$  values (calculated from all alleles regardless of frequency) revealed clear within-breed differentiation ( $F_{ST}$  values between 2.1–4.6%) as well as inter-breed distances: approximately 5.7% among Creole breeds and over 20% between some Creole and Yakut samples.

Differences in allele spectra among breeds reflect both historical breed formation processes and the impact of directional selection, and may be applied for monitoring overall genetic diversity and evaluating the immunological potential of *Bos taurus* populations.

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**GENOME-WIDE DNA METHYLATION LANDSCAPE OF MANILA CLAM  
(*RUDITAPES PHILIPPINARUM*) AND THE EPIGENETIC REGULATION  
MECHANISM OF IMMUNE RESPONSE INDUCED BY PATHOGENS**

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We jointly analyzed whole-genome bisulfite sequencing (WGBS) and RNA-seq results and revealed the immune response mechanism of *R. philippinarum* challenged by *V. anguillarum* from the perspective of epigenetics. The results showed that DNA methylation in *R. philippinarum* primarily occurs in the CpG context. The average genome-wide methylation level of *R. philippinarum* was 9.99%. The average methylation level of resistant group was significantly higher than that of control group and susceptible groups after *V. anguillarum* challenged. A total of 5,355 differentially methylated regions (DMRs) were identified, including 2,827 differentially methylated genes (DMGs), which were significantly enriched in the Notch signaling pathway, mTOR signaling pathway, FoxO signaling pathway, fatty acid metabolism, and glutathione metabolism. A total of 794 differentially methylated and expressed genes were identified in gene body regions, and 177 DMEGs were identified in promoter regions. The overlapping differential genes of DMGs and DEGs are significantly enriched in the immune and metabolic pathways such as AGE-RAGE signaling pathway, ubiquitin-mediated proteolysis, phagocyte, Notch, Foxo, Wnt and mTOR. DMGs with different functions, such as apoptosis, autophagy, antiviral and inflammation, were screened between VaR and VaS groups, including *MRC1*, *Tnfrsf10*, *Pik3cb*, *TRAF3*, *STAT5B*, *AP-1*, *notch1a*, and *CASP6*, *MIF*, *nfkb2* genes located in the promoter region. The macrophage migration factor *MIF* gene with significantly different expression was selected for BSP verification. The results showed that the methylation degree of Con and VaS groups of *MIF* was higher than that of VaR group, and the CpG island with 165 bp gene promoter region had a high level of negative regulation gene expression of DNA methylation. DNA methylation plays an important epigenetic role in the immune response of *R. philippinarum* to pathogen stress by regulating the expression of immune genes. These results provide new insights for the role of DNA methylation in the immune response of invertebrates.

**Keywords:** *Ruditapes philippinarum*; DNA methylation; *Vibrio anguillarum*; Whole Genome Bisulfite-Seq

**VARIATIONS IN CELLULAR AND HUMORAL IMMUNE PARAMETERS IN  
*RUDITAPES PHILIPPINARUM* FOLLOWING *VIBRIO ANGUILLARUM* INFECTION**

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This study investigated the immune response mechanisms of *Ruditapes philippinarum* against *Vibrio anguillarum* infection under controlled environmental conditions. Experimental infection was induced by introducing *V. anguillarum* into the aquatic environment of the clams. Hemolymph, hepatopancreas, and gill tissues were sampled at designated intervals post-infection for immunological analyses. Key parameters assessed included hemocyte density, antibacterial/lytic activities ( $0.33 \pm 0.07$  and  $0.24 \pm 0.12$  U/mg prot at 6 h, respectively), acid phosphatase (ACP), and lysozyme levels. Quantitative analysis revealed significant upregulation of immune-related genes (defensin and lectin), peaking at 12 h post-infection (hpi). Concurrently, ACP activity in hepatopancreatic and branchial tissues showed rapid elevation, reaching maximal levels at 12 hpi before subsequent decline. Lysozyme activity exhibited a similar temporal pattern, peaking at 12 hpi. These findings demonstrate that *V. anguillarum* infection triggers both cellular and humoral immune responses in *R. philippinarum*, characterized by hemocyte proliferation and time-dependent activation of immune enzymes/gene expression. The 12 hpi timepoint represents a critical juncture in the host-pathogen interaction. This study provides fundamental data for understanding bivalve immunology and supports disease-resistant breeding strategies for Manila clams.

**Keyword:** *Ruditapes philippinarum*, *Vibrio anguillarum*, Cellular immune response, Humoral immune response

**PHYLOGENY, DIVERSIFICATION, AND DEMOGRAPHIC HISTORY OF DUCK MUSSEL (*ANODONTA ANATINA*) SHED LIGHT ON PALEO GEOGRAPHIC EVENTS DURING THE NEOGENE–QUATERNARY**

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Phylogenetic modelling revealed the diversification of major *Anodonta anatina* clades in Eurasia during the period from the Late Pliocene to the Early Pleistocene. Comparative analysis of divergence time inferred from phylogenetic and demographic reconstructions indicates that the diversification of duck mussel populations in Northern Eurasia coincided with multiple episodes of Ponto-Caspian transgression. These paleogeographic events likely altered hydrological networks across the region, thereby driving the diversification of freshwater fauna, including widespread mussel species. This study estimates the divergence time between major genetic groups of *A. anatina* in Northern Eurasia in the Early Pleistocene. The median age of the observed Eurasian lineage split was estimated at 1.78 Ma using the DIYABC approach, corresponding to the Apsheronian transgression of the Ponto-Caspian basin. In contrast, the BEAST phylogenetic model dated this divergence event to 3.08 Ma (95% HPD: 1.94–4.46 Ma), highlighting a significant discrepancy in divergence time estimation between these two methodologies. This disparity underscores the influence of analytical frameworks (demographic vs. phylogenetic) on temporal inferences and emphasizes the need for methodological caution when interpreting biogeographic and evolutionary timelines.

Based on ABC modeling, we determined that a significant evolutionary event occurred during the divergence of the Italian Peninsula population from the continental population (mean age 6.29 Ma (95% CI: 3.33–9.27 Ma), possibly linked to the formation of a major geographic barrier or a significant climatic shift. This event was likely related to the Messinian Salinity Crisis and the associated marine regression, which caused the fragmentation of the duck mussel distribution area during the Pliocene.

This study was supported by the Ministry of Science and Higher Education of Russia (project № FUUW-2025-0022).

## SEQUENCING OF WHOLE OR PARTIAL MITOCHONDRIAL GENOMES AS A TOOL FOR STUDYING POPULATION STRUCTURE

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The partial or full sequencing of mitochondrial genomes allows us to study the precise structures of populations. To carry such research out, one must sequence, collect, and annotate a reference mitogenome of a species specimen, then design primers based on the observed order to amplify its mitochondrial ring fragments. Thanks to this method, one can collect and compare mitogenome sequences of many individuals. Family and closely related groups using their structures together is a common natural phenomenon, whereas non-kin specimens using shared structures is rarer, and generates a line of evolutionary questions around costs, cooperation, and cheating. In this study, we aimed to investigate the degree of kinship on the mast structures of *Dyopedom bispinis* (Gurjanova, 1930) around the Solovetsky Islands, located in the White Sea. We were interested whether mast inhabitants are always siblings, meaning the children of one female, or whether they represent a more complex social structure. We sequenced the entire *D. bispinis* mitochondrial genome alongside genome fragments of 59 specimens from four masts. Two of the masts each hosted a single female and several juveniles; our data confirmed that these were classic family groups by their identical partial mitogenomes. On each of the two other masts, which we label collective masts, were several adult females with marsupial embryos – on one, they all had identical partial mitogenomes, meaning they were all offsprings of one female. The other, however, was host to several independent non-kin groups – internally, these groups' studied fragments were identical, but between groups they were as distant from each other as groups inhabiting different masts. This means that a mast can be jointly used by non-kin individuals and allows us to propose the possibility of non-kin cooperation among amphipods. One way or another, such social structures in amphipod builders are described here for the first time and can be a good model for future research in cooperative structure use by distantly related specimens.

The study was supported by RSF. Project #21-74-20028

**APPLICATION OF AUTOENCODER FOR THE ANALYSIS OF GENETIC SEQUENCES OF DAPHNIA: CLUSTERING POPULATION STRUCTURES OF BAIKAL *DAPHNIA***

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In recent decades, the population genetics of aquatic organisms, such as *Daphnia*, has become a subject of intensive study due to their key role in ecosystems and sensitivity to environmental changes. *Daphnia*, as model organisms, provide a unique opportunity for investigating genetic diversity and adaptive mechanisms in the environment of the Baikal Lake ecosystem—one of the largest and deepest freshwater bodies in the world.

In this work, we present the results of our research dedicated to the development and application of an autoencoder for clustering and analysis of microsatellite DNA genetic sequences collected from Baikal *Daphnia*. Samples were collected during an August 2024 expedition aboard the research vessel “Titov” on the territory of Lake Baikal (Core Facility “Center for Comprehensive Shipboard Research of Lake Baikal”).

An autoencoder is a type of neural network consisting of an encoder and a decoder, used for compressing data into a compact representation and subsequently reconstructing the original data. The autoencoder, utilizing a multilayer neural network, includes both encoder and decoder components, enabling effective learning from complex data and its reproduction. We chose this architecture due to its ability to identify nonlinear dependencies and extract key features from complex data. One of the most popular dimensionality reduction methods today is Principal Component Analysis (PCA), defined by the formula  $x_k = \sum_{i=1}^N a_i x_i$  where  $N$  is the number of variables. In contrast to PCA, the autoencoder uses the function  $x_k = f(x_i)$ , obtained through deep learning, to determine the values. The advantage of the autoencoder over PCA is that, thanks to deep learning, it provides less data distortion and higher resolution as a result of dimensionality reduction.

As a result of our work, we were able to identify several clusters reflecting the diverse population structures of Baikal *Daphnia*. This opens new avenues for exploring the relationships between ecological conditions and genetic diversity in this unique ecosystem. The specific patterns identified during the clustering also suggest possible adaptive strategies of *Daphnia* in response to environmental changes, including climatic fluctuations and anthropogenic factors.

Thus, our research findings not only confirm the effectiveness of applying autoencoders in biological data analysis but also enrich our understanding of the structural features of *Daphnia* populations in Lake Baikal. These discoveries may serve as a foundation for further studies aimed at understanding the mechanisms of adaptation and evolution of aquatic organisms, as well as developing effective strategies for their conservation and management in the ecosystems they inhabit. The methodology described in this work can also be applied to the study of other aquatic organisms, opening up prospects for a comprehensive analysis of biological diversity in various water bodies.

The work was carried out within the framework of the state project No. 0279-2021-0010 "Genetics of communities of Baikal organisms: gene pool structure, conservation strategies." Samples were collected during expeditionary research in June 2024 from the board of the research vessel "Titov" (Center for Comprehensive Baikal Ship Research).

**THE USE OF COMPLETE MITOCHONDRIAL GENOMES FOR IDENTIFICATION  
AND CLARIFYING THE TAXONOMIC STATUS OF ENDEMIC SPECIES:  
IMPLICATIONS FOR UNDERSTANDING OF PREVIOUSLY RECOGNIZED  
DIVERSITY**

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Gene fragments on which databases usually rely are not always suited for identification of endemic species of a disputable origin. Clarification of the unresolved issues requires more comprehensive mitochondrial genomes-based phylogenetics to enhance understanding of the relationship and delineate species. However, current databases offering limited information on the mitogenomes of rare, narrow-range, or hard-to-access species. The present study examines the charrs (*Salvelinus*, Salmonidae) with specific focus on unique, endemic taxa from northeastern Asia, thus expanding the mitogenome data set for this important group of salmonids. For these purposes, we used 36 mitogenomes as a basic set to identify the mitogenome structure of endemic charrs and the factors that influence the variability, phylogenetic relationships, and divergence within the lineages. Moreover, based on the current and previously analyzed mitogenomes, we are discussing whether particular endemic or unidentified taxa of charrs deserve the species status, taking into account their genetic and phenotypic divergence.

We evaluated the variability and suitability of mitogenomes at multiple levels of divergence among charr taxa. Answering the posed question, we could say that intensive taxonomic sampling of rare, narrow-range/endemic charrs allows us to reconstruct a more detailed picture of the evolutionary relationships with more accurate estimates of the species variability. Mitogenomes data can reliably establish the relationships between ancestral lineages and within distinct lineages of charrs. The comparative analysis of mitogenomes has shown, firstly, the presence of a statistically significant gap in the distribution of divergence values at different levels of taxonomic hierarchy and, secondly, a tendency for the divergence to increase simultaneously with the hierarchical level of the compared groups. Furthermore, mitogenomes proved to be a useful tool for clarification of the taxonomic status of narrow-range endemics or disputable charr taxa. Comparisons of mitogenomes of several endemic charrs showed a lower level of diversification than had been described previously. Nonetheless, our data suggest that the isolated charr populations under discussion, even if morphologically different and diagnosable, are not necessary species.

It is important to note that expanding genetic resources is necessary for biodiversity monitoring. Therefore, the conclusions of our study may be useful for future genetic investigations of charrs of the genus *Salvelinus* and other highly diversified fishes toward proper assessment of the status of the controversial taxa and better understanding of the relationship and delineation of species.

We are grateful to our colleague, and friend Lubov Skurikhina for encouragement and consultations and sequencing assistance. The study was performed under research project no. 124021900010-2 (Ministry of Science and Higher Education of the Russian Federation and A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS, Vladivostok, Russia).

**EVOLUTION, SYSTEMATICS AND ADAPTATION OF ZOKORS - MYOSPALACINAE  
LILLJEBORG, 1866 (RODENTIA, MAMMALIA): SHOT REVIEW ON CONTEMPORARY  
INVESTIGATIONS OF THE TAXONOMY, PHYLOGENY, GENETIC DIVERSITY AND  
ECOLOGY**

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Zokors Myospalacinae Lilljeborg, 1866 are a group of highly specialized underground rodents, endemic of East Asia. Based on molecular genetic markers, zokors are considered to be part of the mole rat family Spalacidae as a subfamily Myospalacinae (Norris et al., 2004), represented by one (*Myospalax*), or two (*Myospalax* and *Eospalax*), or even three (*Myospalax*, *Eospalax*, *Siphneus*) genera (Golovanov, Zazhigin, 2023). The group is poorly studied in all respects, including no consensus on the number of species and their phylogenetic relationships. Several species, between 5 and 11, are recognized in the group (Wilson, Reader, 2005; Norris, 2017), while in Russia up to 5 mentioned (Puzachenko et al, 2009; 2014; Pavlinov, 2012). Most of the ranges of these species, except for the Altai zokors, lie within the territory of China. There are two marginal populations of North Cina zokor in Russia: first, in South-East Transbaikalia, and second one in Khanka plain region in Primorye (Ussuriland). Systematic status of these forms is now debated (Puzachenko et al, 2014; Pavlenko et al., 2014). The most complex group of zokors in subfamily is the most southern representatives that distributed in China within the Loess Plateau and Tibetan Plateau and distinguished as a separate genus (*Eospalax*) (Liu. et al., 2022; Zhang et al., 2022).

Five main directions of researches of zokors can be identified. First one is taxonomy, based on genetics and morphological differentiation; than phylogenetic relationships based on molecular data and paleontology; some researches are devoted to study of adaptation to hypoxia and evolutionary significant genes. Traditional species ecology studies, such as life style, habitat adaptations, daily and seasonal activity, main reproductive characteristics, such as breeding, mating and pregnancy time, litter size are clearly insufficient. Low level of reproductive features and their habitat reduction make zokors potentially vulnerable to anthropogenic impact.

Most interesting results were obtained with the development of high-throughput sequencing technologies. For instance, SNPs (Single nucleotide polymorphisms) markers have been applied into researches in taxonomy and phylogeny of *Eospalax* in Myospalacinae (Liu et al., 2022). The contribution of Russian researchers belongs to the genetic and morphological analysis of the populations inhabiting country, discovering new species for Russia in Transbaikalia (Martynova et al., 1977; Puzachenko et al., 2009, 2014, Tsvirka et al., 2011; Pavlenko, et al., 2014). The parapatric habitat of three species of zokors in the southeastern Transbaikalia is shown (Bazhenov, Pavlenko 2020). For the first time, a comparative study of the reproductive characteristics of zokor's species living in the east of Russia was conducted (Bazhenov, Pavlenko, 2025). Recent paleontological studies have shown strong difference in the evolution of West Siberian zokors and zokors from other regions, which allowed to identify a separate phyletic line that existed in West Siberia at least since the Early Pleistocene and to propose a revision for taxonomy of Myospalacinae (Golovanov, Zazhigin, 2023). Pleistocene and modern distribution of the Altai zokors in response to environmental factors were described as well (Golovanov et al., 2024).

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**NEW DATA ON GENETIC DIVERSITY OF THE URAL FIELD MOUSE  
(*SYLVAEMUS URALENSIS* P., 1881) IN THE NORTHERN PART OF THE EASTERN  
CAUCASUS**

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Analysis of genetic diversity and phylogeography of wide-ranging species is successfully used to solve issues related to the reconstruction of the past landscapes and climate, as well as to construct the evolutionary scenarios for individual taxa and whole ecosystems (Riddle, 1999; Avise, 2000). One of such species is the Ural field mouse *Sylvaemus uralensis* (Pallas, 1881), whose range covers significant territories of the temperate zone of Eurasia — from Central Europe to the south of Western Siberia, Altai, Tian Shan and Pamir (Gromov, Erbaeva, 1995; Chelomina, Atopkin, 2010; Kryštufek et al., 2017). The species *S. uralensis* inhabits both plains and mountainous areas, particularly, the Caucasus Mountains, where it is found at altitudes up to 3500 m. The geographical location (the border of Europe and Asia) and the geological history of the Caucasus determine its importance for the formation of many taxa as an isolation factor, refugial zone or migration route (Tembotova, 2015; Yalkovskaya et al., 2022). Despite the genetic studies conducted on *S. uralensis* in the Caucasus (Tembotova et al., 2023), a significant area of the region remains unexplored.

This study presents the results of phylogenetic reconstructions of the Ural field mouse based on the cytochrome *b* gene sequences (569 bp) with the inclusion of new data from the northern part of the Eastern Caucasus (Republic of Dagestan) and also the southeastern foothills of the Greater Caucasus (Azerbaijan). In addition, the analysis included new data from the territories of the Southern Urals and the southeast of the East European Plain.

For 13 obtained *cyt b* sequences of *S. uralensis*, 10 new haplotypes were described, six of which were found in the Caucasus territories. The phylogenetic reconstructions conducted using the Bayesian analysis showed differentiation of *S. uralensis* into two large haplogroups. All haplotypes sequenced by us from the Eastern Caucasus, the Southern Urals and the East European Plain were included in haplogroup I (Western haplogroup) together with haplotypes from the western and central parts of the Northern Caucasus, Central Europe and the East European Plain, Western Siberia, and Western Asia. Haplogroup II (Eastern haplogroup) included haplotypes from Central Asia, Altai and China. Division of *S. uralensis* into two haplogroups with no clear differentiation within of each is confirmed by the results of the median network analysis. The results of phylogenetic reconstructions, genetic diversity and demography analyses are consistent with data of the previous studies in the western and central parts of the North Caucasus (Tembotova et al., 2023) and indicate that the formation of the modern genetic diversity of *S. uralensis* in the North Caucasus is the result of a recent (most likely at the turn of the late Pleistocene and Holocene) and rapid expansion from the remote or relatively isolated local populations.

This study was supported by the Russian Science Foundation (grant no. 22-14-00332-II).

## GENETIC STRUCTURE OF POPULATIONS AND ECOLOGICAL FEATURES OF *APOSTICHOPUS JAPONICUS* IN THE KUNASHIR ISLAND AREA

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An analysis of the genetic structure of the sea cucumber *Apostichopus japonicus* inhabiting five geographic locations around Kunashir Island (Sea of Okhotsk) was conducted. Specimens were collected by divers during the spring-summer periods of 2023–2024 for this study.

Ten microsatellite loci of nuclear DNA were used in the study. All loci exhibited a high level of polymorphism, with the number of alleles per locus ranging from 6 to 15 and allelic richness from 4,2 to 7,8. The greatest allelic diversity was observed in samples from Spokoyny Bay (South Kuril Strait), which may be attributed to more stable environmental conditions. Observed heterozygosity ranged from 0.65 to 0.78, while expected heterozygosity ranged from 0,72 to 0,84. The inbreeding coefficient was positive in all samples (0,05–0,12), indicating possible inbreeding or the Wahlund effect.

Population structure analysis revealed three clusters corresponding to the geographic distribution of the samples. A Hardy-Weinberg equilibrium test showed deviations in three loci, suggesting the influence of selection or other evolutionary forces. The Evans-Watterson test with Manly's algorithm confirmed the neutrality of most loci, except for two, which showed signs of disruptive selection. The Dirichlet polynomial test revealed no significant deviations from neutrality. Genetic differentiation among populations ranged from 0,08 to 0,15, indicating moderate genetic isolation.

A comparison of allele frequencies with phenotypic traits (body weight, color morph) and ecological factors (habitat depth) revealed significant correlations. Individuals with the red color morph exhibited higher allele frequencies at loci AJ20333 and Psj2464, while the black color morph was associated with loci AJ10675 and fcs9. Body weight was positively correlated with alleles at loci Psj2889 and 3S16. Habitat depth was statistically linked to alleles at loci PS205 and Psj2409, which may reflect adaptation to different environmental conditions.

Thus, the *Apostichopus japonicus* population around Kunashir Island exhibits moderate genetic differentiation associated with geographic isolation and ecological factors. The identified allele associations with morphological and ecological traits highlight the role of selection in shaping the genetic structure.

Analyzing the genetic status of wild populations is essential for maintaining the genetic diversity of *A. japonicus*. The data on genetic variability and differentiation obtained in this study can be applied for future genetic monitoring of *A. japonicus* populations, selecting individuals with high variability for broodstock formation in mariculture, and developing appropriate conservation measures for this species across its range.

## COMPARATIVE ANALYSIS OF REPRODUCTIVE STRATEGIES OF DAPHNIIIDAE USING INDIVIDUALLY ORIENTED MODELS AND MICROSATELLITE MARKERS

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This study investigates the reproductive strategies of Daphniidae species (*Daphnia sinensis*, *D. longispina*, *Simocephalus vetulus*, *S. mixus*, *S. expinosus*) using an integrative approach that combines molecular genetics, computational modeling, and machine learning techniques. Samples were collected during an August 2024 expedition aboard the research vessel "Titov" (Core Facility "Center for Comprehensive Shipboard Research of Lake Baikal") and analyzed for microsatellite polymorphism using universal primer sets developed and validated specifically for Daphniidae species. This enabled the first comprehensive genetic structure analysis of these understudied crustaceans, providing valuable insights into their reproductive strategies.

Primer analysis was conducted using capillary electrophoresis with fluorescently labeled primers (ROX, R6G, TAMRA, and FAM), which were compatible with the NANOFOR-05 genetic analyzer. Initially, fragment analysis was done using agarose gel electrophoresis, followed by confirmation through Sanger sequencing. The resulting .fas files were then used to generate microsatellite length frequency tables.

To test competing hypotheses about population establishment, the study utilized individual-based models. These models included parameters for genetic diversity, reproductive strategy switching (sexual vs. asexual), and environmental adaptation. The models tested whether a single colonization event with a founder effect or continuous migration was more likely to explain the observed genetic diversity in the population.

A significant innovation was the development of Syrin, a user-friendly software for microevolutionary analysis. This software overcomes previous script-based limitations and successfully reproduces empirical microsatellite variation patterns observed in Baikal populations. The integrated microsatellite-IBMs approach shows promise for invasive species monitoring and assessing climate change impacts on freshwater ecosystems.

Data processing was performed using Syrin to calculate key population parameters, such as  $H_o/H_e$ ,  $F_{st}$ , linkage disequilibrium and association index. These calculations reveal significant differences between obligate and facultative parthenogenesis strategies, allowing for a better understanding of population dynamics.

Neural network integration, specifically CNN for genotype classification and LSTM for population dynamics forecasting, significantly improved prediction accuracy, with  $MSE < 0.05$  in reproductive strategy modeling. Notably, some Daphniidae species exhibit complex mixed reproductive strategies that are highly sensitive to environmental conditions, explaining their resistance to laboratory cultivation. These methods will be adapted to study other aquatic invertebrates with similar reproductive strategies, potentially providing new insights into the evolutionary maintenance of sexual reproduction.

The work was carried out within the framework of the state project No. 0279-2021-0010 "Genetics of communities of Baikal organisms: gene pool structure, conservation strategies." Samples were collected during expeditionary research in June 2024 from the board of the research vessel "Titov" (Center for Comprehensive Baikal Ship Research).

## **AUTOMATING GENE ENRICHMENT ANALYSIS FOR ECOLOGICALLY RELEVANT BIOLOGICAL PROCESSES WITH A REST API**

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Ecological genetics, which studies the interaction of genetic factors with the environment, requires powerful bioinformatic tools to analyze the functional roles of genes in the adaptation of populations. Traditional methods of Gene Set Enrichment Analysis (GSEA), despite their effectiveness in detecting biological processes such as response to oxidative stress or toxin metabolism, remain time-consuming, requiring significant computational resources and specialized knowledge. The project has developed a REST API (Representational State Transfer Application Programming Interface), including endpoint/api/annotate, which automates GSEA, integrates results with a web interface, and provides visualized data, making the analysis accessible to environmental genetics researchers. This tool makes it possible not only to simplify the processing of gene lists, but also to link them to environmentally significant processes such as adaptation to environmental stresses, which previously required complex local calculations. The endpoint accepts CSV files with genes, performs enrichment using the gseapy libraries for the GO Biological Process and KEGG Pathways databases, generates scatter plots using seaborn, and returns JSON responses with image URLs integrated with the frontend. The application of the API to genes related to environmental stress, such as HSP70 and SOD1, revealed the enrichment of terms, including GO:0006979 (response to oxidative stress) and KEGG:hsa05208 (chemical carcinogenesis), which underlines its relevance for the study of genetic-ecological interactions. Unlike traditional approaches, the API eliminates the need for manual configuration of GSEA by providing unified web access to the analysis and visualization, which significantly reduces barriers for researchers. The tool complements the endpoint /api/filtrate, which filters genetic data for subsequent enrichment, forming a holistic bioinformatic cycle. Development prospects include support for additional databases such as Reactome, the introduction of filters for environmentally relevant terms, and performance optimization through asynchronous computing, which will expand API capabilities for analyzing large datasets. Deploying the API on a public server will ensure its accessibility to the international scientific community. Thus, it represents not only a technical achievement in bioinformatics, but also a conceptual bridge between computational methods and environmental genetics, allowing researchers to effectively study adaptive processes in populations in the context of a changing environment. The report will examine the technical aspects of the implementation, application examples, and the potential of the API for future research on genetic-ecological interactions.

**MOLECULAR SYSTEMATICS AND EVOLUTIONARY GENETICS OF FLATFISH  
(OSTEICHTHES: CARANGIFORMES: PLEURONECTOIDEI) IN THE FAR EASTERN  
SEAS OF RUSSIA**

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Research into the systematics and evolution of flatfish is relevant for two reasons. Firstly, these fish are important as food sources. Secondly, representatives of this taxon are widely used for scientific research. However, the proliferation of molecular phylogenetic studies based on non-representative taxonomic samples or insufficient molecular data can lead to contradictory results. This, in turn, leads to additional confusion in the development of taxonomy. This problem is exacerbated for taxonomic groups with historically complex evolutionary relationships, such as flatfish. Researchers are attracted to the taxonomy and evolution of flatfish both because of the importance of these fish for fishing and mariculture, and because of their widespread use in various scientific disciplines. Nevertheless, the taxonomic relationships of flatfish remain the subject of numerous studies and discussions. This study examined taxonomy based on the *16S*, *Cyt-b* and *Co-I* genes, as well as the complete mitochondrial genome.

This presentation mostly reports the first complete sequence of the mitochondrial genome (mitogenome) of the yellow-striped flounder *Pseudopleuronectes herzensteini* (Pleuronectoidei: Pleuronectidae). Mitogenome evolution, and molecular phylogenetic reconstruction based on four to six techniques, including coalescent analysis, were performed for flatfish. The genome size of the specimen sampled was 16,845 bp, including 13 protein-coding genes, 22 tRNA genes, *12S*, and *16S* rRNA genes, and the control region, CR. The composition and arrangement of the genes are similar to those in other teleost fish, including the second mitogenome reported in this paper. The frequency of A, C, G, and T nucleotides in the *P. herzensteini* mitogenome is 27%, 29.2%, 17.6%, and 26.2%, respectively. The ratio of complementary nucleotides in the mitogenome of this and other species of the family was A+T:G+C (53.2:46.8%) and do not deviate significantly from the expected equilibrium proportion. The submission to the global database (GenBank) of two new mitogenomes along with 106 analyzed GenBank sequences will contribute to phylogenetic studies of flounders at the family and suborder levels. Based on 26 and 108 nucleotide sequences of protein-coding genes (PCGs), we investigated the molecular phylogeny of flounders and performed analysis for two sets of sequences, including those of members of the family Pleuronectidae and the suborder Pleuronectoidei and estimated their importance in establishing the taxonomy at these two levels. Data obtained by up to six techniques of multigene phylogenetic reconstructions support monophyly within the family Pleuronectidae with high statistical confidence; however, conclusions regarding the phylogenetics at the suborder level require further investigation. Our results also revealed paraphyletic and weakly supported branches that are especially numerous at the suborder level; thus, there is a clear need for taxonomic revisions at the suborder, and possibly family levels. Genetic distance analysis reveals the suitability for DNA barcoding of species specimens at single genes as well as at whole mitogenome data.

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## EVOLUTIONARY PROCESSES IN THE POPULATION DYNAMICS OF A SEX-STRUCTURED POPULATION WITH NON-OVERLAPPING GENERATIONS

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The present work is devoted to the development of an eco-genetic approach to modeling the dynamics of limited sex-structured populations with non-overlapping generations within mathematical biology. We propose a dynamic model of a population structured by sex, which allows for studying evolutionary processes. Note that adding the sex structure to the model allows us to explain some phenomena of population dynamics with non-overlapping generations. At the same time, sex reproduction associated with gene recombination is an important mechanism of microevolutionary transformations and animal adaptation to the environment. This paper studies a discrete-time model of population dynamics for a sex-structured population with non-overlapping generations in which density-dependent factors limit survival. The population is assumed to have genetic variety among individuals in reproductive potential, controlled by a single autosomal diallelic locus. We consider a panmictic population with Mendelian inheritance rules. This paper aims to study the evolutionary processes of such populations with seasonal breeding. In exploring the model, we used the analytical and numerical methods of dynamic systems studies. The genetic composition of the population, namely monomorphic genotype fixation or coexistence of all genotypes in polymorphism, is shown to be determined by the values of the reproductive potentials of heterozygotes and homozygotes, initial conditions, and a parameter describing the ratio of newborn females to males. At the same time, population dynamics, both regular and irregular, depend on the average value of the birth rate and the intensity of self-regulation processes. Successful reproduction providing survival and growth of the population is shown to be determined by the genotype fitness and the secondary sex ratio. Despite its simplicity, the proposed model of a sex-structured population demonstrates the possibility of bottleneck effects and evolutionary rescue. This model reveals two variants of evolutionary rescue when the population recovers due to the existence of rare alleles with higher fitness or the emergence of new alleles caused by mutations.

**DIVERSITY OF B CHROMOSOME MORPHOTYPES FOR *APODEMUS PENINSULAE* (RODENTIA) IN GEOGRAPHICAL POPULATIONS OF THE RUSSIAN FAR EAST**

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A species *Apodemus peninsulae* Thomas, 1906 is widely distributed in Russia: from the Siberia to the Pacific coast in the Russian Far East (FE), as well as in northern Mongolia, China, Korea, and Japan (in Hokkaido Island) (Gromov, Erbaeva, 1995). In addition to the main set of chromosomes (A), the karyotype of *A. peninsulae* contains additional chromosomes (B-chromosomes, Bs) that vary in number, size, and morphology. We have previously studied the variability of the number and morphotypes of B-chromosomes in the FE (Kartavtseva, Roslik, 2004; Roslik, Kartavtseva, 2009; 2023). The frequencies of rare B-chromosome morphotypes have previously revealed clinal variability from the east (south and east of Primorsky Krai) to the northwest of the range (in Khabarovsk Krai, the Jewish Autonomous Region, the Amur Region), associated with a gradual loss of rare variants of B-chromosome morphotypes.

New data are presented on the variability of numerical and morphotypic parameters of the *A. peninsulae* B-chromosomes from forest-steppe and forest landscapes of the Zabaikalsky Krai. Two geographic groups have been distinguished that significantly differ from each other in the numerical parameters of B-chromosomes: spectrums of number variations, the average number of Bs per individual index ( $\bar{x}B$ ) and the conditional DNA «mass» index ( $mB$ ) and also frequency of mosaics. Numerical parameters of Bs, as well as a different number and frequencies of morphotypes and mosaics, are maintained relatively low – in the «northwestern» group I, and on the contrary, high – in the «southeastern» group II. The barrier role of the large Shilka and Onon rivers in limiting the distribution of B-chromosome numbers in two geographical groups of samples is discussed. The karyotypes of all individuals contained micro-Bs, often in combination with small and medium (in the absence of large) structures. It is possible that a decrease in the diversity of Bs morphotypes is most typical for animals from forest-steppe landscapes, probably due to the existence of individuals at the edge of the range, in the most pessimal conditions for the species.

It is suggested that the observed geographic differentiation of *A. peninsulae* populations by B-chromosome morphotypes in the Russian Far East may be associated with the adaptation of different populations to different habitat conditions, climate, spectrum of zoonotic infections in host mice, and may be of great importance for the species as a whole.

**GENETIC VARIABILITY AND POPULATION GENETIC STRUCTURE OF  
*TAKYDROMUS WOLTERI* AND *TAKYDROMUS AMURENSIS***

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Reptiles currently account for one-third of all terrestrial vertebrate diversity, with at least 21.1% of species threatened with extinction and the status of another 15% unknown. Ectothermy makes reptiles more vulnerable than other terrestrial vertebrates by virtue of their sensitivity to seasonal temperature fluctuations. However, they still remain one of the least studied groups of vertebrates.

Two species of the endemic Asian genus *Takydromus* (grass lizards or oriental racers) inhabit in the Russian Far East: the Amur Grass lizard, *Takydromus amurensis* Peters 1881, and the Mountain Grass lizard, *Takydromus wolteri* Fischer 1885. Although these species often appear in phylogenetic research concerning the genus *Takydromus* (Arnold, 1997; Lin et al., 2002; Ota et al., 2002; Lue, Lin, 2008; Chen et al., 2009; Guo et al., 2024), there are no studies on their intraspecific genetic variability. In our recent work, the use of an unconventional phylogenetic approach allowed us to divide the genus *Takydromus* members into two ecological groups: forest and meadow, the evolution of which proceeded in parallel (Sheremetyeva, Popova, 2025). To continue the research, we decided to test the hypothesis of the lower genetic diversity and a higher population subdivision in the forest group species compared to the grassland group. The work aimed to assess the mtDNA *cytb* gene variability in the two *Takydromus* species living in approximately the same macroecological conditions, but belonging to different ecological groups (Amur Grass Lizard is a forest species, Mountain Grass Lizard is a grassland species).

As a result, the first data on the genetic variability of two Far Eastern *Takydromus* species were obtained. In the Amur Grass Lizard, we identified four phylogenetic lineages with clear geographic confinement. It has been suggested that a refugium for *T. amurensis* may have existed in the past in the south of Sikhote-Alin (Sheremetyeva et al., 2025). For Mountain Grass Lizard, four phylogenetic lineages were found on the mainland with no clear geographic association and one on Jeju Island. The data obtained indicate a lower nucleotide diversity ( $\pi$ ) in Amur Grass Lizard ( $\pi=0.00905\pm0.0009$ ) compared to Mountain Grass Lizard ( $\pi=0.01423\pm0.00749$ ), which confirms the proposed hypothesis and is inconsistent with preliminary data on the *T. amurensis* variability.

**MORPHOTYPICAL DIFFERENCES IN THE STRUCTURE OF THE THIRD UPPER MOLARS FOUND FOR THE FIRST TIME IN TWO CHROMOSOMAL RACES OF *ALEXANDROMYS EVORONENSIS* (ARVICOLINAE, RODENTIA)**

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The Evoron vole *Alexandromys evoronensis* is an endemic species of northeastern Asia and the southern Russian Far East. It has three isolated populations and two-chromosome races - "evoron" (2n = 38-41) and "argi" (2n = 34-37) (Kartavtseva et al., 2021). Karyotype polymorphism is associated with multiple chromosomal structural rearrangements of eleven chromosomes - two Robertsonian fusions and five tandem fusions. There are no studies of the morphotypic variability of the molars in chromosomal races with multiple tandem fusions.

It is known that molar morphotypes are controlled by a number of genes. It has been suggested that multiple chromosomal rearrangements, including tandem chromosome rearrangements, may affect the morphotypic variability of the third upper molars.

We have shown for the first time that animals of the chromosomal race "evoron" have both M3 morphotypes with fused first two prisms and lower morphotypic variability compared to the chromosomal race "argi". It has also been shown that chromosomal races have differences in the number of morphotypes, their combinations, asymmetry index and frequency of morphotype combinations. (Kartavtseva et al., 2025). It has been concluded that chromosomal rearrangements could affect the genes responsible for molar formation.

The work was carried out within the framework of the state assignment of the Ministry of Science and Higher Education of the Russian Federation (No. 124012200182-1).

**GENETIC LINEAGES AS A STRATEGY FOR ECOLOGICAL SUCCESS: THE CASE  
OF THE PARTHENOGENETIC COSMOPOLITAN SPRINGTAIL  
*PARISOTOMA NOTABILIS* S.L.**

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Molecular-genetic methods are being widely implemented in modern soil zoology. Their application has revealed intraspecific genetically distant forms in many widespread soil invertebrates. Genetic distances between such forms (genetic lineages) often reach interspecific levels. Morphological differences between genetic lineages are frequently absent. Based on their extensive range, high abundance, and strong intraspecific genetic diversity, such species are in a state of biological progress. A striking example is the cosmopolitan parthenogenetic springtail *Parisotoma notabilis*. This most abundant species in Europe occurs across all natural zones, demonstrating ecological plasticity and tolerance to anthropogenic disturbances. Previous genetic studies in the Nearctic and western Palearctic regions have revealed 12 genetic lineages within *P. notabilis* s.l. Ecological divergence has been demonstrated for several lineages. In particular, on the East European Plain, three genetic lineages widespread in the Nearctic and western Palearctic occupy habitats with varying degrees of disturbance. In disturbed soils, the ruderal lineage L1 dominates, exhibiting an r-strategy as demonstrated in our laboratory experiments. Another example of ecological adaptation is the Mediterranean lineage L-Crimea, found in dry subtropical forests at Cape Martyan, Crimea, suggesting its tolerance to high temperatures and aridity. Equally remarkable is the discovery of lineage L-Cheget in the nival zone of the Greater Caucasus Mountains, indicating potential frost tolerance. Notably, the Caucasus region - a known of species diversity hotspot - also revealed the highest intraspecific diversity of *P. notabilis* s.l., harboring 10 of the 12 known lineages. While these findings suggest a possible Caucasian origin for the species, conclusions remain premature given the limited genetic data from eastern Palearctic regions. Our study provides the first genetic data from the Urals and Far East (Yakutia and Kamchatka), where we identified a novel lineage designated L-East.

The complex of genetic lineages within *P. notabilis* s.l. can be considered a polytypic species undergoing both ecological (primarily in lowlands) and geographic (mainly in mountainous regions) differentiation. To date, no morphological differences between genetic lineages have been detected, which hinders their recognition as distinct species.

**SPECIES COMPOSITION OF THE GENUS *METAGONIMUS* (TREMATODA: HETEROPHYIDAE) IN THE RUSSIAN FAR EAST**

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Two species of trematodes of the genus *Metagonimus*, *M. suifunensis* and *M. pusillus*, circulate in the south of the Russian Far East, which was confirmed by both morphological and molecular data (Shumenko et al., 2017; Tatonova et al., 2018). At the same time, 10 species from this genus were described in Japan (Nakao et al., 2022).

For the first time, metacercariae from the Odyr River (the Khabarovsk Territory), Bira River (the Jewish Autonomous Oblast), and Ilistaya River (the Primorsky Region) were studied, which expanded the known localities for Russian parasites. *M. pusillus*, previously recorded only in the Bolshaya Ussurka River, was also found in the Ilistaya River and Bira River. Molecular genetic analysis revealed new data for *M. suifunensis* and *M. pusillus*. In addition, two sequences were identified as new species: *Metagonimus* sp. 1 in the Ilistaya River and *Metagonimus* sp. 2 in the Odyr River. Phylogenetic reconstruction using the 28S rRNA gene showed that the first species is phylogenetically close to *M. suifunensis*, while the second one forms a separate branch, which is combined with *Metagonimus yokogawai* from Japan. The nuclear marker results were confirmed using a mitochondrial gene.

Nevertheless, the description of the cryptic species requires a morphological description of the adult worms and clarification of the life cycle of these trematodes. These data are difficult to obtain, foremost because they are rare in natural habitats and also because there are difficulties in finding adequate morphological criteria for differentiation at the species level. However, the data obtained suggest that this group of parasites in southern Russian Far East should be studied more thoroughly, as the genus includes epidemiologically significant trematodes.

**COMPARATIVE ANALYSIS OF *ALECTRIAS CIRRATUS* (LINDBERG, 1938) (PERCIFORMES: STICHAETIDAE) BASED ON THE MITOCHONDRIAL *COXI* GENE FROM PETER THE GREAT GULF (RUSSIA) AND THE SOUTHEASTERN COAST OF HOKKAIDO ISLAND (JAPAN)**

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*Alectrias cirratus*, a small stichaeid (prickleback) fish, inhabits the coastal waters of Primorye (Peter the Great and Vladimir Gulfs) and the northern Japanese archipelago, including Hokkaido and the eastern coast of Honshu. The species is poorly studied; published data are scarce and its morphology remains incompletely described. We compared populations from Peter the Great Gulf (Russia) and Uchiura Gulf (Japan) using sequences of the mitochondrial cytochrome-*c* oxidase subunit I (*COXI*) gene.

Our dataset comprised 11 *COXI* sequences (628 bp) obtained from sexually mature *A. cirratus* collected in Paris Bay, Peter the Great Gulf. Specimens were identified morphologically. For comparison, we retrieved 12 larval *COXI* sequences of *A. cirratus*, four of *Anoplarchus purpureus*, one of *A. insignis* and one of *Pseudalectrias tarasovi* from the NCBI database.

Neighbour-joining analysis and haplotype-network reconstruction revealed pronounced interspecific divergence between *A. cirratus* and *A. purpureus*. Intraspecific variability within *A. cirratus* was low, and haplotypes lacked clear geographic structuring. Considering spring water exchange mediated by the Schrenk (Limannoye) Current, we suggest that the minimal genetic differentiation between the two regions results from regular recruitment of the Primorye population by pelagic larvae originating from Hokkaido waters.

## GENETIC DIVERSITY OF *CARASSIUS GIBELIO* IN NORTHERN ASIA

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*Carassius gibelio* is among the most widespread and successful invasive fish species in Eurasia. Despite its importance in aquaculture and active ongoing range expansion, its phylogeography and phylogeny remain insufficiently studied. In this work, we studied the genetic diversity of *C. gibelio* in a vast territory of Northern Asia in the Amur, Lena and Ob river's basins. Analysis of the mtDNA control region (CR or D-loop) revealed a low level of genetic diversity across the vast area of northern Eastern Siberia. All individuals sampled in this region belonged to a single, unusual mitochondrial lineage, C7, which is considered more basal compared to other East Asian mitochondrial lineages of *C. gibelio*. We suggest that the low genetic diversity is due to a combination of historical evolutionary processes and strong selection pressures associated with harsh environmental conditions, such as a short growing season, low temperatures, and variable oxygen levels. Additionally, populations from the Ob and Amur basins exhibited similar mitochondrial lineage compositions, with no detectable genetic differentiation. These results provide indirect evidence supporting the hypothesis that indigenous haplotypes in Western Siberia were replaced by Amur-origin haplotypes following the introduction of *C. gibelio* from the Amur river populations in the 20th century.

## EFFECTS OF DIFFERENT LIGHT SPECTRA ON THE DEVELOPMENT OF WHITEFISH IN AQUACULTURE

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Light is an extremely important environmental factor that synchronizes the different stages of fish development and has a significant impact on physiological processes in the organism. The aim of this study was to investigate the factor of artificial lighting and to identify the patterns of influence of different light spectra on the development of embryos, larvae and juveniles of Baikal whitefish and its hybrids with the Yenisei hump-snout whitefish. Telomere length, telomerase enzyme activity, *hsp-90* (heat shock protein 90) and *ck* (creatinine kinase) genes expression and blood profile analysis, including the ratio of neutrophils to lymphocytes, were selected as biomarkers of fitness.

The eggs were incubated until hatching at the temperature of 3°C in specially designed boxes. Differences in key stress biomarkers under the influence of different light spectra were observed at VII, IX, XIII and XIV stages of early development, according to Chernyaev (2017) with a full light spectrum (1.8 and 20  $\mu\text{mol/s}\cdot\text{m}^2$ , LDF), in complete darkness, in the red spectrum (LDR, 631 nm, 2.2  $\mu\text{mol/s}\cdot\text{m}^2$ ), in the blue spectrum (LDB, peak at 459 nm, 1.7  $\mu\text{mol/s}\cdot\text{m}^2$ ), in the green spectrum (LDG, peak at 507 nm, 2.1  $\mu\text{mol/s}\cdot\text{m}^2$ ). Full spectrum light of moderate intensity (LDF, 1.8  $\mu\text{mol/s}\cdot\text{m}^2$ ), close to natural environmental conditions, was used as a control.

The results obtained confirm the significant influence of lighting on the early developmental stages of whitefish. Baikal whitefish and their hybrids are able to intensify the restoration of telomere length at early developmental stages in response to stress, probably due to the telomerase enzyme activity. An optimal technology for incubating Baikal whitefish eggs has been proposed, taking into account lighting conditions: the most optimal light spectrum is LDG, as well as the LDF spectrum at 1.8  $\mu\text{mol/s}\cdot\text{m}^2$ . Baikal whitefish and their hybrids are more sensitive to darkness and the LDR spectrum during embryogenesis when they are directly exposed to this factor. The hybrids also show a delayed cumulative effect of the negative influence of the LDB spectrum and darkness in the postnatal period. We conclude that the optimization of light conditions for embryonic development under artificial conditions can lead to an increase in the survival rate and quality of juvenile fish and thus to an increase in the economic efficiency of aquaculture.

This study was conducted at the Large-Scale Research Facilities "Experimental Freshwater Aquarium Complex for Baikal Hydrobionts" and supported by the Russian Science Foundation grant no. RSF 25-24-00634.

## POPULATION GENETIC STUDIES OF BAIKAL ENDEMIC SPONGES

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In recent years, the ecosystem of Lake Baikal has been subjected to intensified anthropogenic pressures and is responding to global climate change. Since 2011, widespread cyanobacterial blooms, proliferation of filamentous algae, and disease outbreaks leading to mortality among endemic sponges have been observed. In light of these challenges, alongside routine monitoring, it is critical to investigate the population-genetic structures of Baikal's aquatic organisms, particularly those experiencing significant population fluctuations.

The study of genetic diversity in Lake Baikal's endemic sponges is fundamentally important for assessing their viability, especially amid the mass mortality events documented in the lake. This research marks the first application of developed microsatellite markers to analyze the population-genetic structure of Baikal's endemic sponges, using *Lubomirskia baikalensis* as a model. A clear spatial-genetic subdivision was identified, with the species divided into three distinct genetic clusters corresponding to the lake's major basins. Reconstruction of historical effective population size trends revealed a sharp increase in the recent past.

According to the data, all eight populations adhere to Hardy-Weinberg equilibrium and exhibit no signs of past genetic bottlenecks. This indicates that population sizes remain sufficient to maintain genetic diversity, even in the context of mass mortality and disease events observed over the past decade.

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**GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIPS OF THE  
DAGESTAN PINE VOLE *MICROTUS (TERRICOLA) DAGHESTANICUS* FROM  
REPUBLIC OF DAGESTAN, THE NORTH-EASTERN CAUCASUS  
BASED ON *CYT B* DATA**

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Dagestan pine vole, a representative of the subgenus *Terricola*, inhabits alpine meadows in the Caucasus Mountains and the adjacent Eastern Black Sea Mountains of northeast Turkey. Significant genetic polymorphism has been observed in *M. (T.) daghestanicus* at the chromosomal and molecular levels (Akhverdyan et al., 1992; Bogdanov et al., 2021) suggesting that the data from poorly studied parts of the species range are necessary to reveal the patterns of genetic differentiation and the ways of modern genetic structure formation. Genetic variability of the Dagestan vole in the Republic of Dagestan (southwestern Russian Federation) has been studied at the chromosome level and the Robertsonian chromosome fan has been revealed (Akhverdyan et al., 1992). However, the molecular data on the Dagestan pine vole from this region has been lacking.

The study presents the results of the genetic diversity and phylogenetic relationship analysis of *M. (T.) daghestanicus* from the Republic of Dagestan based on complete *cyt b* sequences (1143 bp). *Cyt b* sequences were obtained for eight specimens of *M. (T.) daghestanicus* from two localities: the vicinity of Chirag village (41.818N 47.434E) and the Shalbuzdug Mountain (41.327N 47.799E). Five haplotypes were described, all of which were new for the species.

Phylogenetic reconstructions showed the division of *M. (T.) daghestanicus* into four main clades, that in general corresponded to the results of previous studies (Bogdanov et al., 2021) and four haplotypes from the southern slope of the Main Caucasus (Krasnaya Polyana, Krasnodar Region) were poorly differentiated. The first clade was formed by haplotypes from the Kabardino-Balkar and Karachay-Cherkess Republics of Russia, the second - by haplotypes from Turkey, the third clade - by haplotypes from the North Ossetia-Alania Republic. All haplotypes of *M. (T.) daghestanicus* from Dagestan together with a single haplotype from Georgia were included in the fourth clade. Taking into account the significant distances between the localities of Georgian and Dagestanian populations included in a single clade, and the uncertain position of four haplotypes from Krasnodar Region, the future genetic studies with inclusion of data from both slopes of the Caucasus are needed to clarify the species' genetic structure and evolutionary history of its formation.

The study was supported by the Russian Science Foundation (grant no. 22-14-00332-II).

## PHYLOGENETIC STATUS OF *ECHINOCHASMUS* SPP. IN THE SOUTH OF THE RUSSIAN FAR EAST

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Representatives of the family Echinochasmidae Odhner, 1910 are parasitic flatworms with a wide host range. Intermediate hosts are molluscs, amphibians, and freshwater fish. The definitive hosts are birds and mammals, including humans. Species of the genus *Echinochasmus*, such as *E. milvi*, *E. japonicus* and others, have been repeatedly recorded in humans. Although these parasites have high epidemiological significance, the species composition of the genus has not yet been studied due to the presence of the cryptic species. Therefore, the nuclear marker, the 28S rRNA gene (28S), and the mitochondrial marker, the *cox1* gene (*cox1*), were used to identify the species composition of representatives of the genus *Echinochasmus* in the south of the Russian Far East.

Based on partial sequences of *cox1*, three new species of the genus *Echinochasmus* with long-tailed cercariae morphologically similar to the species *E. milvi* were identified. The genetic distances (*p*-distances) between all species are at an interspecific level. In addition, two nucleotide substitutions were detected for 28S, separating one of the obtained cryptic species and *Echinochasmus milvi*. No other nucleotide substitutions were detected.

The identified cryptic species have different geographical distributions in the territory of the southern Far East. The habitats of these trematodes are associated with the Amur River basin. One of the identified cryptic species was also recorded on the islands of Japan. This wide range may be due to the involvement of birds in the movement of worms.

These results indicate the need for additional studies of representatives of the genus *Echinochasmus* to establish the full species composition of this genus in the south of the Russian Far East using morphological and molecular genetic methods.

## SYSTEMATICAL MORPHOLOGY AND GENETIC COMPARISON DEFINED THE TWO SUBSPECIES OF *AEGILOPS TAUSCHII*

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*Aegilops tauschii* (DD,  $2n = 2x = 14$ ) is a well-studied wild species since it is the D genome donor of bread wheat. The mismatching between the botanically- and genetics-based subspecies structure is a long-term puzzle for the species. Based on [the](#) variation in spike morphology, the species has been conventionally subdivided into subspecies *tauschii* and ssp. *strangulata*. The classification remains controversial due to the existence of morphologically intermediate types; subspecies *tauschii* and *strangulata* is not well matched with lineages L1 and L2 demarcated by molecular phylogeny. The present study addressed the puzzle by using spikelet index (SI, the ratio between the width of the glume and the rachis segment) as an objective criterion to score spike morphology. The analysis revealed the presence of two distinct clusters, named SI-C1 (low SI) and SI-C2 (high SI). Then, fluorescence *in situ* hybridization (FISH) karyotyping was used in the analysis of the subspecies structure at chromosome level, which also defined two distinct clusters (FISH-C1 and FISH-C2). FISH markers 2D-CTT-4 and 2D-535 can be used in the accurately distinguishing of the two clusters. Interestingly, SI-C1 and SI-C2 were matched perfectly with FISH-C1 and FISH-C2, which also corresponded with linkage L1 and L2 that was a previously assembled phylogeny, respectively. This study presents the conclusion that *Ae. tauschii* can be divided into two categories, i. e. SI-C1/FISH-C1/L1 and SI-C2/FISH-C2/L2, matching each other at the morphological, cytological, and molecular levels. The former category was populated exclusively by ssp. *tauschii* accessions, while the latter harbored both ssp. *strangulata* and morphologically intermediate accessions. As the consequence, the intermediate accessions were placed into ssp. *strangulata*. The treatment achieved a match between the botanically- and genetics-based categorizations, i.e. that ssp. *tauschii* becomes synonymous with SI-C1 (FISH-C1, L1), and ssp. *strangulata* with SI-C2 (FISH-C2, L2).

## IT'S NOT WHAT IT SEEMS: BARCODING OF A COMMON TINDER FUNGUS AS A REASON FOR RECONSIDERATION

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The tinder fungus (*Fomes fomentarius* sensu lato; Fungi, Basidiomycota, Agaricomycetes, Polyporales) is a widespread wood-decay fungus in the northern hemisphere that causes white rot mainly of deciduous trees. It has a long ethnomycological history and is still widely used in traditional medicine in various countries and regions. Because of this, it now attracts much attention as a promising source of biologically active and medicinal compounds. The species *Fomes fomentarius* (L.) Fr. is widespread and widely known not only among specialists. For decades, researchers have noted its morphological and later biochemical variability, as well as ecological plasticity, but the identified intraspecific groups and additionally described species have not received general distribution and recognition.

The revolution was made by molecular geneticists, who sequenced the internal transcribed spacers (*ITS*) 1 and 2 of ribosomal DNA and the 5.8S rRNA gene and then confirmed the results by sequencing partial translation elongation factor 1- $\alpha$  (*efa*) region and 25S large subunit ribosomal RNA gene. Due to this approach, in a decade we have moved from a single species to two pairs of phylogenetic lineages with geographical and ecological individuality, some of which are described as sympatric cryptic species.

The study of the true polypore helps to shed light on the structure of widespread xylotrophic fungi species. Such studies raise some universal, fundamental questions: can species boundaries be defined by a single standard value of sequence similarity, can the concept of a type specimen be transferred to a sequence, is sufficient attention paid to the study of geographic variability of various features, how to reduce the limitations of data and herbarium collections of common species, and others. Some ideas on these issues will be highlighted in the report.

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STUDY OF NUCLEOTIDE SEQUENCES OF MITOCHONDRIAL (*CO-1*, *16S* rRNA) AND NUCLEAR (*28S* rRNA, HISTONE *H3*) GENE MARKERS OF CHAETOGNATHS (CHAETOGNATHA, SAGITTOIDEA) USING THE EXAMPLES OF *LEPTOSAGITTA ALBA* AND *PARASAGITTA ELEGANS*

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Chaetognaths (phylum Chaetognatha), commonly known as arrow worms, are abundant zooplanktonic organisms inhabiting all seas and oceans with salinities exceeding 8‰. They occupy a wide range of depths, from shallow waters to the abyssal zone. To date, over 150 species of chaetognaths have been described.

Molecular genetics analysis of several species of the phylum Chaetognatha was performed on the set of four markers of the *Co-1*, *16S* rRNA, *28S* rRNA genes, and histone *H3*. It was appeared that the studied sequences of *Leptosagitta alba* and *Aidanosagitta crassa* in fact belong to *Leptosagitta alba* individuals that, contrary to *Aidanosagitta crassa*, were lacked the gut outgrowths (diverticula).

Also, we discovered a number of morphological features distinguishing *Parasagitta elegans* that inhabits the Sea of Japan from *Parasagitta elegans* that occur in other regions.

1. **Fins:** The presence of a single pair of fins (on the trunk and caudal segments), whereas typical *Parasagitta elegans* possess two pairs of fins.

2. **Midgut Diverticula:** The midgut lacks diverticula and begins posteriorly to the septum, while in typical *Parasagitta elegans*, diverticula are always present.

3. **Corona Ciliata:** The corona ciliata is located posteriorly to the eyes, on the neck, whereas in other *Parasagitta elegans*, it is positioned anteriorly to the eyes and primarily on the trunk.

4. **Fin Rays:** The potentially new species lacks rays in the fins, while typical *Parasagitta elegans* always possess fin rays.

5. **Musculature:** The potentially new species exhibits transverse musculature in both the trunk and caudal segments.

These, along with the estimated *p*-distances for *Co-1*, *16S* rRNA, and histone *H3*, were suggested the existence of more than one species in *Parasagitta elegans*.

The analysis has shown that the *28S* rRNA marker failed to identify and discriminate between the Sagittoidea species considered in the present study. The revealed differences between *Aidanosagitta regularis* and *Ferosagitta robusta* were at intraspecific level for the *Co-1* and *16S* rRNA genes, which indicates a recent divergence of these species from each other. There was an issue of species identification of a number of *Eukrohnia hamata* individuals combined into the cluster *Eukrohnia hamata* + *Eukrohnia bathypelagica*. The necessity of a new study that would confirm either the existence of the species *Eukrohnia bathypelagica* or the presence of lineages of the species *Eukrohnia hamata* is shown.

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## ОТ НАШИХ СПОНСОРОВ

ПАО ЦГРМ «Генетико» — один из крупнейших лабораторно-производственных комплексов на территории России, построенный по стандартам GLP. В состав ПАО ЦГРМ «Генетико» входит собственная медико-генетическая и биоинформатическая службы. Специалисты Центра — члены Российского общества медицинских генетиков и Европейского общества генетики человека, авторы публикаций в российских и зарубежных научных журналах. В 2017 году в составе Центра была открыта лаборатория высокопроизводительного секвенирования, на базе которой проводится секвенирование нового поколения (next generation sequencing, NGS), которое позволяет в больших масштабах определить последовательность как нескольких генов, так и всего генома с высокой точностью и скоростью. Проводимые в Центре исследования по секвенированию являются самыми быстрыми и доступными по стоимости. ПАО ЦГРМ «Генетико» сотрудничает с ведущими медицинскими центрами, научными лабораториями, образовательными учреждениями и научно-исследовательскими институтами.

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- Уникальный опыт исследований в области генетики — более 10 лет на рынке. Используем только высокотехнологичное оборудование и оригинальные реагенты.
- Лучшие сроки выполнения исследования. Наша лаборатория и специалисты находятся в Москве и не зависят от международных логистических цепочек.
- Высокий уровень безопасности полученных данных. Все данные проведенных исследований хранятся на защищённых серверах в России и не передаются в сторонние организации.

Компания ПАО ЦГРМ «Генетико» выполняет следующие услуги:

- Выделение ДНК, РНК
- Подготовка библиотек для высокопроизводительного секвенирования
- Секвенирование генома, экзона и транскриптомов
- Секвенирование единичных клеток
- Секвенирование готовых библиотек
- Секвенирование 16s рРНК
- Секвенирование по Сэнгеру
- Биоинформатический анализ и составление технических отчетов
- Сборка генома
- Оценка уровня метилирования
- Секвенирование метагенома

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Международный симпозиум

Владивосток: Морская биологическая станция "Восток"

2-5 сентября 2025

Тезисы докладов

Ответственный за выпуск Ю.Ф. Картавец

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