INTERNATIONAL SYMPOSIUM

MAPEG-2022

PROGRAM & ABSTRACTS

MODERN ACHIEVEMENTS IN POPULATION, EVOLUTIONARY AND ECOLOGICAL CENETICS

MAPEEG ELEVENTH MEETING

VLADIVOSTOK & VOSTOK MBS

Modern Achievements in Population, Evolutionary, and Ecological Genetics: International Symposium, Vladivostok – Vostok Marine Biological Station, September 8–12, 2022: Program & Abstracts / editors: Yuri Ph. Kartavtsev, Oleg N. Katugin ; Vladivostok State University of Economics and Service. – Vladivostok, 2022. – 80 p. – Engl. ISBN 978-5-9736-0673-2.

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PROGRAM

MAPEEG-2022 Held by:

FAR EASTERN BRANCH OF RUSSIAN ACADEMY OF SCIENCES (FEB RAS), A.V. ZHIRMUNSKY NATIONAL SCIENTIFIC CENTER OF MARINE BIOLOGY, NSCMB FEB RAS; FEDERAL SCIENTIFIC CENTER OF BIODIVERSITY OF EAST-ASIA LAND BIOTA, FEB RAS; FAR EASTERN FEDERAL UNIVERSITY; VLADIVOSTOK PUBLIC FOUNDATION FOR DEVELOPMENT OF GENETICS Sponsors: HELICON COMPANY

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THURSDAY, SEPTEMBER 8

Arrival and Hotel Accommodation in Vladivostok.

FRIDAY, SEPTEMBER 9

9-30:10-15. Registration at NSCMB Conference Hall.

10-15:10-30. Opening Remarks.

Valentine I. Sergienko, President of FEB RAS (Russia).
Inessa V. Dyuizen, Acting Director of the NSCMB (Russia).
Gulab D. Khedkar, Paul Hebert Centre for DNA Barcoding and Biodiversity Studies (India).
Yuri Ph. Kartavtsev, MAPEEG-2022 Chairman (Russia).

Session 1. Evolutionary Genetics & Genomics (Oral Presentations)

(Oral Presentations)

10-30:12-10. Chair Person – Ph.D. Gulab Khedkar.

 Maria Skazina, Maria Maiorova, Irina Dolganova, Nelly Odintsova, <u>Petr Strelkov</u>. BIVALVE TRANSMISSIBLE NEOPLASIA (30'; on-line).
 <u>Petr Sibiryakov</u>, Maria Fominykh, Yuliya Davydova, Nikolay Markov, Aleksandr Sokolov, Andrei Boltunov, Maria Krokhaleva, Lidiya Yalkovskaya, Aleksandr Borodin. THE USE OF MULTIPLEX REAL- TIME PCR ASSAYS FOR SCREENING STUDIES IN POPULATION AND EVOLUTIONARY GENETICS (30').

3. Philipp E. Chetverikov, Denis S. Cheglakov, Mikhail S. Burlakovskiy,

Alexey G. Desnitskiy, Sebahat K. Ozman-Sullivan, Anna E. Romanovich, Sogdiana I. Sukhareva, Esma Kaplan. SYNHOSPITALITY IN GALL-FORMING ERIOPHYOID MITES (ACARIFORMES) ASSOCIATED WITH BROAD-LEAVED BOREAL TREES (20').

4. <u>Diana I. Kachur</u>, Sergei V. Turanov, Olga G. Shevchenko, Anna A. Ponomareva, Maria A. Shulgina. USING MOLECULAR GENETICS TECHNIQUES FOR IDENTIFICATION OF DIATOM SPECIES IN THE ORDER THALASSIOSIRALES (BACILLARIOPHYTA) IN THE SEAS OF RUSSIA (20').

12-10:13-00. Lunch.

13-00:14-10. Chair Person – Ph.D. Oleg N. Katugin.

5. <u>Elena N. Kashinskaya</u>, Evgeniy P. Simonov, Anastasia V. Shokurova, Pavel G. Vlasenko, Mikhail M. Solovyev. A METAGENOMIC ANALYSIS OF THE HOST-PARASITE SYSTEM: THE INTERACTION AMONG SYMBIOTIC MICROBES, HELMINTHS AND FISH (20').

6. <u>Ekaterina A. Nikitina</u>, Anna V. Medvedeva, Dariya M. Karovetskaya, Dariya D. Safarova, Elena V. Savvateeva-Popova. EFFECTS OF HYPOXIA ON COGNITIVE PROCESSES IN DROSOPHILA (20'; on-line).

7. Yuri Ph. Kartavtsev, <u>Alexander D. Redin</u>. ON THE MITOGENOME STRUCTURE OF RIGHTEYE FLOUNDERS OF THE FAR EASTERN SEAS WITH AN INFERENCE INTO THE MOLECULAR PHYLOGENY AND SYSTEMATICS OF THE FAMILY PLEURONECTIDAE AND THE SUBORDER PLEURONECTOIDEI (OSTEICHTIES, CARANGIFORMES) (20'). 8. **Hongtao Nie.** COMPARATIVE TRANSCRIPTOMIC ANALYSIS REVEALED DYNAMIC CHANGES OF DISTINCT CLASSES OF GENES DURING DEVELOPMENT OF THE MANILA CLAM (RUDITAPESPHILIPPINARUM) (30'; on-line).

#. Discussion on Reports (10').

Coffee Break (10').

<u>Session 2.</u> Molecular Systematics, Barcoding and Phylogenetics (Oral Presentations)

14-50:16-30. Chair Person – S.D. Alexandr V. Borodin.

9. <u>Gulab D. Khedkar</u>, Anita Tiknaik, Rahul Jamdade, Vikas Kalyankar, Rahul Surwanshi. DNA BARCODING BASED INTRA-BASIN COMPARISON OF FISH COMMUNITIES RESOLVES ISSUES RELATED TO OVERLOOKED TAXA (30').

10. <u>Anna O. Zolotova</u>, Oleg N. Katugin. THE USE OF FIVE DNA MARKERS (*COI, 16S, 12S, 28S, 18S*) IN THE STUDY OF MOLECULAR GENETIC RELATIONSHIPS AMONG SQUID OF THE FAMILY GONATIDAE (CEPHALOPODA, TEUTHIDA, OEGOPSIDA) (20').

11. <u>Kristina A. Kalinina</u>, Yulia V. Tatonova. STRUCTURAL FEATURES OF THE *ITS2* rDNA REGION FOR THE FAMILY PSILOSTOMATIDAE (TREMATODA: ECHINOSTOMATOIDAE) (20').

12. <u>Gelena V. Izotova</u>, Pavel G. Vlasenko, Elena N. Kashinskaya, Mikhail M. Solovyev. GENETIC DIVERSITY OF *DIPLOSTOMUM* SPP. (TREMATODA, DIPLOSTOMIDAE) INFESTATED FISHES FROM DIFFERENT WATER BODIES OF RUSSIA (20').

#. Discussion on Reports (10').

16-30:21-00. Welcome Party & Dinner (NCMB Lobby or Dinner Hall).

SATURDAY, SEPTEMBER 10

10-00:12-00. Chair Person – S.D. Alexander V. Rodionov (on-line).

Session 3. Molecular Systematics, Barcoding and Phylogenetics (Oral Presentations, Continued)

13. <u>Boris Levin</u>, Oleg Artaev, Alexey Bolotovskiy, Alexander Gandlin, Evgeniy Simonov, Ilya Turbanov, Ivan Pozdeev, Alexander Ruchin, Kirill Litvinov, Stepan Podolyako, Marina Levina. DNA-BARCODING OF VOLGA FISHES: UNEXPECTED DIVERSITY AND BIOGEOGRAPHICAL IMPLICATIONS (30').

14. <u>Sophia Nazarova</u>, Natalia Strelkova, Svetlana Orlova, Evgeny Genelt-Yanovskiy. PHYLOGENETIC AND BIOGEOGRAPHIC REVISION OF THE GENUS *GORGONOCEPHALUS* USING MITOCHONDRIAL DNA POLYMORPHISM (20').

15. Daria S. Pilevich, Lidia E. Yalkovskaya, Maria A. Krokhaleva, Petr A. Sibiryakov, Sergei A. Borisov, Aleksandr V. Borodin. NEW DATA OF THE *CYT B* POLYMORPHISM IN THE HARVEST MOUSE (*MICROMYS MINUTUS* P., 1771) FROM EASTERN SIBERIA, BAIKAL REGION (20').

16. <u>Irina V. Kartavtseva</u>, Irina N. Sheremetyeva, Marina V. Pavlenko. MULTIPLE CHROMOSOMAL REARRANGEMENTS IN EVORON VOLE (RODENTIA) (20'). 12-00:13-00**. Lunch.**

<u>Session 4.</u> Molecular Systematics, Barcoding and Phylogenetics (Oral Presentations, Continued)

13-00:14-20. Chair Person – Ph.D. Tatiana E. Kramina.

17. **Alexander V. Rodionov.** LIFE STORY OF PLANT GENOMES: FROM DIPLOID TO POLYPLOID AND BACK (30'; on-line).

18. <u>Sergey A. Karpov</u>, Luis Javier Galindo, David Moreira, Purificación López-García, Andrey E. Vishnyakov, Guifré Torruella, Vladimir V. Aleshin, Victoria S. Tsvetkova. SANCHYTRIOMYCOTA - A NEW PHYLUM IN THE KINGDOM FUNGI (30'; on-line).

19. <u>Boris Levin</u>, Evgeniy Simonov, Alexander Golubtsov, Marina Levina. REPEATED ADAPTIVE RADIATIONS AMONG CYPRINIDS IN ETHIOPIAN HIGHLANDS (20').

Coffee Break (10').

15-30:16-50. Chair Person – Ph.D. Evgenia I. Bondar.

20. <u>Irina A. Ekimova</u>, Ángel Valdés, Manuel A.E. Malaquias, Ekaterina Lisova, Anna L. Mikhlina, Dimitry M. Schepetov. LOOKING FOR EVIDENCE OF THE GREAT TRANS-ARCTIC INTERCHANGE: THE PHYLOGEOGRAPHY OF BOREAL AND ARCTIC NUDIBRANCH MOLLUSCS (30').

21. <u>Daria Grishina</u>, Dimitry Schepetov, Irina Ekimova. HOW TO RESOLVE TRANS-ARCTIC SPECIES COMPLEXES: THE PHYLOGEOGRAPHY OF A NUDIBRANCH SPECIES COMPLEX EUBRANCHUS RUPIUM-EXIGUUS (GASTROPODA: NUDIBRANCHIA) (20').

22. <u>Alena Yakhnenko</u>, Evgeniya Bondar, Valeria Itskovich. GENETIC DIFFERENTIATION AT THE POPULATION LEVEL IN LAKE BAIKAL ENDEMIC SPONGE *LUBOMIRSKIA BAIKALENSIS* (20').

#. Discussion on Reports (10').

16-50:17-40. **Grigory Anikin**, Field Application Scientist, Russia & CIS. OVERVIEW OF DNBSEQ TECHNOLOGY (HELICON, in Russian, 50').

SUNDAY, SEPTEMBER 11

<u>Session 5.</u> Molecular Systematics, Barcoding and Phylogenetics (Oral Presentations, Continued) 9-00:10-20. Chair Person – Ph.D. Tatiana Neretina.

23. Evgeniia I. Bondar, Alla G. Oleinik, Andrey D. Kukhlevsky,

Lubov A. Skurikhina, Natalia E. Kovpak, Natalia M. Batishcheva. POST-GLACIAL RECOLONIZATION OF THE NORTHEASTERN ASIA BY *SALVELINUS TARANETZI*: GENETIC EVIDENCE OF MULTIPLE SECOND CONTACTS AND HYBRIDIZATION BETWEEN GLACIAL CHARR LINEAGES (20').

24. <u>Sergei V. Turanov</u>, Marina A. Koltsova. NON-INVASIVE MONITORING OF POPULATION GENETIC DIVERSITY AMONG ABUNDANT SPECIES OF THE *ZOSTERA* SP. COMMUNITY IN THE NORTHERN SEA OF JAPAN (20').

25. <u>Ludmila O. Katugina</u>, Yulia V. Tatonova, Larisa A. Prozorova. TAXONOMIC STATUS OF MOLLUSCS OF THE GENUS *PARAJUGA* (SEMICULCOSPIRIDAE) (20').

26. <u>Ulyana E. Khotko</u>, Yulia V. Tatonova. AS-PCR FOR POPULATION ANALYSIS OF *METAGONIMUS SUIFUNENSIS* (20').

Coffee Break (10')

10-30:11-40. Chair Person – Ph.D. Boris Levin.

27. <u>Tatiana Neretina</u>, Margarita Ezhova, Anna Zhadan, Nikolai Neretin, Alexander Tzetlin. SEQUENCES OF *ITS* IN MARINE NVERTEBRATES METABARCODING (20').

28. Irina V. Kulikova, Sergei V. Shedko, Yuri N. Zhuravlev,

Philip Lavretsky, Jeffrey L. Peters. ASSESSING GENOMIC DIVERGENCE BETWEEN CHINESE SPOT-BILLED DUCK AND MALLARD WITH ddRAD-SEQ DATA (20').

29. Artem A. Lyubas, Alena A. Tomilova, Alexander V. Kondakov,

Ivan N. Bolotov. THE DISTINCT MtDNA LINEAGE OF THE DUCK MUSSEL (ANODONTA ANATINA) IS THE EVIDENCE FOR PLIO-PLEISTOCENE REFUGIUM IN THE AZOV SEA RIVER BASINS (20').

#. Discussion on Reports (10').

12-00:13-00**. Lunch**

Session 6. Microevolution. Population Genetic Structure of Species. Ecological Genetics (Oral Presentations)

13-00:15-00. Chair Person – S.D. Lubov V. Frisman.

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30. Maria Krokhaleva, Lidia Yalkovskaya, Aleksandr Borodin,

Petr Sibiryakov. PHYLOGEOGRAPHY OF THE SIBERIAN FLYING SQUIRREL (*PTEROMYS VOLANS* L., 1785) INFERRED FROM MITOCHONDRIAL CYTOCHROME B SEQUENCES: NEW DATA FROM CONTINENTAL AND ISLAND POPULATIONS (30').

31. <u>Alexander Ju. Dudnikov</u>, Ming Hao, Deng-Cai Liu. NUCLEAR GENE *GOT*2 DNA SEQUENCES POLYMORPHISM IN LOCAL POPULATIONS OF *AEGILOPS TAUSCHII* IN CAUCASIA HELPS TO UNDERSTAND PECULIARITIES OF THE SPECIES EVOLUTIONARY HISTORY (20').

32. Tatiana E. Kramina, Ilya G. Meschersky, Galina V. Degtjareva,

Dmitry D. Sokoloff. PHYLOGEOGRAPHY OF TWO SPECIES COMPLEXES OF THE GENUS *LOTUS* (LEGUMINOSAE): WHAT GENETIC VARIABILITY CAN TELL (20').

33. **Olga N. Antosyuk, Elena A. Sharova, <u>Anastasia K. Verbitskaya</u>. SILYBUM MARIANUM L. SEED EXTRACT AS A PROTECTOR ON THE EXAMPLE OF DROSOPHILA (20'; on-line).**

34. Mikhail M. Solovyev, <u>Elena N. Kashinskaya</u>, Anastasiya V. Shokurova, Pavel G. Vlasenko, Vadim A. Vasilenko, Evgeniy P. Simonov. GENETICAL, MORPHOLOGICAL, AND FUNCTIONAL DESCRIPTIONS OF DIGESTIVE SYSTEMS IN SYMPATRIC PAIRS OF COREGONIDS WHITEFISH: AN INTEGRATIVE APPROACH (20').

#. Discussion on Reports (10').

15-10:16-20: Chair Person – Ph.D. Alexander Yu. Dudnikov.

35. <u>Lubov V. Frisman</u>, Irina N. Sheremetyeva, Irina V. Kartavtseva, Marina V. Pavlenko, Daria V. Rodimtseva. ONE'S MORE ABOUT GENETIC DIFFERENTIATION IN EASTERN LINEAGE OF STRIPED FIELD MOUSE (*APODEMUS AGRARIUS*): STUDY OF 6 MICROSATELLITE LOCI (20').

36. <u>Sophia Nazarova</u>, Natalia Strelkova, Svetlana Orlova, Evgeny Genelt-Yanovskiy. PHYLOGENETIC AND BIOGEOGRAPHIC REVISION OF THE GENUS *GORGONOCEPHALUS* USING MITOCHONDRIAL DNA POLYMORPHISM (20').

37. <u>Ulyana V. Gorobeyko</u>, Denis V. Kazakov, Irina N. Sheremetyeva, Valentin Yu. Guskov, Anastasia A. Kadetova. PHYLOGEOGRAPHY OF MYOTIS PETAX (20').

#. Discussion on Reports (10').

Coffee Break (10')

Session 4. Poster Presentations (40')

16-30:17-10. Chair Person – S.D. Irina V. Kartavtseva.

1. Elena Yu. Andrianova, Ivan A. Vladimirov, Olga A. Pavlova,

Denis I. Bogomaz. MODELING THE ACTIVE DISTRIBUTION OF MALADAPTIVE TRAITS IN NATURAL PLANT POPULATIONS.

2. Alexandr V. Borodin, <u>Sofia V. Bulycheva</u>, Maria A. Krokhaleva, Daria S. Pilevich, Natalia A. Sokolova, Lidia E. Yalkovskaya. THE FIRST DATA ON THE GENETIC DIVERSITY OF THE EUROPEAN WATER VOLE (*ARVICOLA AMPHIBIUS* L., 1758) FROM THE YAMAL PENINSULA BASED ON *CYT B* GENE SEQUENCES.

3. <u>Natalia Batishcheva</u>, Vladimir Brykov. COMPARISON OF GENETIC DIVERSITY BETWEEN PARENTS/OFFSPRING IN *SEBASTES TACZANOWSKII* STEINDACHNER, 1880.

4. Eugenia Boulygina, Fedor Sharko, Maksim Cheprasov,

Maria Gladysheva-Azgari, Natalia Slobodova, Svetlana Tsygankova,

Sergey Rastorguev, Lena Grigorieva, Martina Kopp, Jorge M.O. Fernandes, Gavril Novgorodov, Gennady Boeskorov, Albert Protopopov, Alexei Tikhonov, <u>Artem Nedoluzhko.</u> MITOCHONDRIAL GENOME OF BROWN BEAR FOSSILS FROM THE BOLSHOY LYAKHOVSKY ISLAND (RUSSIA, REPUBLIC OF YAKUTIA) AND ITS PHYLOGENETIC IMPLICATIONS.

5. <u>Ekaterina A. Bugaeva</u>, Evgeniia I. Bondar, Alla G. Oleinik. SECONDARY CONTACT AMONG TWO GLACIAL LINEAGES OF CHARRS OF THE GENUS *SALVELINUS* IN THE RANGE OF THE SEA OF OKHOTSK COAST: ORIGIN OF THE NEIVA CHARR *SALVELINUS NEIVA*.

6. <u>Maria N. Dunaeva</u>, Mikhail Yu. Shchelkanov. PRIMER SET DESIGNING FOR THE COMPLETE GENOME SANGER - SEQUENCING OF NEWCASTLE DISEASE VIRUS.

7. <u>Irina A. Ekimova</u>, Yury V. Deart, Tatiana I. Antokhina, Anna L. Mikhlina, Dimitry M. Schepetov. LINES, TEETH AND TREES: THE FIRST ATTEMPT TO RESOLVE *CORYPHELLINA RUBROLINEATA* SPECIES COMPLEX (GASTROPODA: NUDIBRANCHIA).

8. <u>Evgeny Genelt-Yanovskiy</u>, Natalia Strelkova, Natalia Zhuravleva, Ekaterina Stratanenko, Sophia Nazarova. PHYLOGEOGRAPHY OF THE BRITTLE STAR OPHIURA SARSII LÜTKEN, 1855 (ECHINODERMATA: OPHIUROIDEA).

9. <u>**Pavel V. Grebenkin</u>, Olesya A. Rutenko.** A BRIEF OVERVIEW OF MODERN METHODS OF RESEARCH OF CIS-REGULATORY ELEMENTS OF GENOMES.</u>

10. <u>Jana V. Indriksone</u>, Yury V. Deart, Tatiana I. Antokhina, Dimitry M. Shepetov, Irina A. Ekimova. MORPHOLOGICALLY CONSERVATIVE BUT

GENETICALLY DIVERGENT: MOLECULAR SYSTEMATICS OF THE PHYLLIDIID NUDIBRANCH MOLLUSCS IN SOUTHERN VIETNAM.

11. <u>**Diana R. Iunusova</u>, Maria A. Polezhaeva.** THE PRELIMINARY DATA ABOUT INTERSPECIFIC GENETIC VARIABILITY IN SAKHALIN *LEDUM* SPECIES BASED ON cpDNA.</u>

12. <u>Yana I. Ivashko</u>, Dmitry M. Atopkin, Alexander A. Semenchenko. CHARACTERIZATION OF MITOCHONDRIAL GENOME OF *SWIFTOPECTEN SWIFTII* (BERNARDI, 1858) AND THE PHYLOGENETIC RELATIONSHIPS WITHIN FAMILY PECTINIDAE.

13. <u>Anna V. Izrailskaia</u>, Yulia V. Tatonova. NEW DATA FOR THE FAMILY NOTOCOTYLIDAE LUHE, 1909.

14. Daria N. Kamenskaya, Vladimir A. Brykov. EXON AND INTRON DIVERGENCE IN SALMONIDS GROWTH HORMONE GENES.

15. <u>Masalkova N.A.</u>, Kartavtsev Yu. Ph., Katolikova M.V. GENETIC AND MORPHOMETRIC VARIABILITY IN SETTLEMENTS OF TWO MUSSEL SPECIES (MYTILUS EX. GR. EDULIS), *MYTILUS TROSSULUS* AND *MYTILUS GALLOPROVINCIALIS*, IN THE NORTHWESTERN SEA OF JAPAN.

16. Artem Nedoluzhko, Fedor Sharko, Svetlana Tsygankova, Eugenia Boulygina, Natalia Slobodova, Anton Teslyuk, Jorge Galindo-Villegas,

<u>Sergey Rastorguev</u>. INTERGENERIC HYBRIDIZATION OF TWO STICKLEBACK SPECIES LEADS TO INTROGRESSION OF MEMBRANE-ASSOCIATED GENES AND INVASIVE EXPANSION.

17. Nhi D.D. Nguen, <u>Artur Yu. Nikulin</u>, Vecheslav Yu. Nikulin. DIVERSITY IN *OROSTACHYS SPINOSA* (CRASSULACEAE) CHLOROPLAST DNA MARKERS IN THE ALTAI MOUNTAINS.

18. <u>Alla G. Oleinik</u>, Andrey D. Kukhlevsky, Lubov A. Skurikhina. PHYLOGENY AND DIVERGENCE IN ARCTIC LINEAGE OF CHARRS (*SALVELINUS*, SALMONIDAE) IN THE NORTHEASTERN ASIA AND NORTH AMERICA.

19. <u>Maria A. Polezhaeva</u>, Makar V. Modorov, Elena A. Marchuk. GENETIC STRUCTURE AND VARIABILITY OF SOME SPECIES OF RHODODENDRONS OF THE RUSSIAN FAR EAST.

20. <u>Irina N. Sheremetyeva</u>, Irina V. Kartavtseva, Alexander S. Lapin,

Igor V. Moroldoev. THE VARIABILITY OF MITOCHONDRIAL DNA CONTROL REGION IN THREE INVASIVE POPULATIONS OF THE EAST EUROPEAN VOLE (*MICROTUS ROSSIAEMERIDIONALIS*) IN THE FAR EAST OF RUSSIA.

21. <u>Oksana A. Shumenko</u>, Yulia V. Tatonova. USING *CYTB* mtDNA GENE FOR ANALYSISOF POPULATION STRUCTURE OF *CLONORCHIS SINENSIS*.
22. <u>Polina G. Shumenko</u>, Yulia V. Tatonova. POPULATION STRUCTURE ANALYSIS OF *METAGONIMUS SUIFUNENSIS* BASED ON A HIGHLY VARIABLE MT-MARKER, THE *NAD1* GENE. **23.** Evgeniya S. Soboleva, Gleb N. Artemov. CHARACTERISTICS OF INVERSION POLYMORPHISM IN THE SIBERIAN NATURAL POPULATIONS OF MALARIA MOSQUITOES *ANOPHELES BEKLEMISHEVI* USING FLUORESCENT *IN SITU* HYBRIDIZATION.

24. Daria A. Solodovnik. NEW MORPHOLOGICAL AND GENETIC DATA FOR *METORCHIS* SP. (TREMATODA: OPISTHORCHIIDAE) IN THE RUSSIAN FAR EAST.

25. <u>Natalia Sukhikh</u>, Victor Alekseev. DESCRIBING OF THE *EURYTEMORA* (CRUSTACEA: COPEPODA) SPECIES USING MORPHOLOGICAL AND MOLECULAR-GENETIC METHODS.

26. <u>Viktor A. Sviderskiv</u>, Sergei V. Turanov. EXPERIMENTAL EVALUATION OF PCR PRIMERS FOR IDENTIFICATION OF SAKHALIN STURGEON *ACIPENSER MIKADOI* HILGENDORF, 1892.

27. <u>Pavel G. Vlasenko</u>, Sergei G. Sokolov, Evgeniy P. Ieshko, Evgeniy V. Frolov, Alexander P. Kalmykov, Aleksey A. Parshukov, Yulia K. Chugunova, Elena N. Kashinskaya, Anastasiya V. Shokurova, Nikolai A. Bochkarev, Karl B. Andree, Mikhail M. Solovyev. GENETIC DIVERSITY AND PHYLOGENY OF *TRIAENOPHORUS* SPP. (CESTODA, BOTHRIOCEPHALIDEA, TRIAENOPHORIDAE) PARASITIZING FRESHWATER FISHES IN EURASIA.

#. Discussion of Young Scientists Reports and Announce of the Concurs' Presentations (Referee Committee Report 10').

Mini Workshop/School (Presentations in Russian)

17-20:18-50. Chair Person – S.D. Yuri Ph. Kartavtsev.

1. Yuri Ph. Kartavtsev. MOLECULAR MARKERS AND SOCIETY NEED: FROM BIODIVERSITY ASSESSMENT AND MODERN GENERAL BIOLOGY PARADIGM VALIDATION TO SEAFOOD MISLABELING DETECTION (30'). 2. Sergei V. Turanov. USING METABARCODING OF DNA FROM AQUATIC ENVIROMNENT FOR MONITORING SPECIES AND GENETIC DIVERSITY (30').

3. **Mikhail B. Konashev.** ON SOVIET GENETICS AND GENETICISTS IN THE DIARY OF TH. DOBZHANSKY (30').

Coffee Break (10')

19-00:19-20. Chair Person – Prof. Yuri Kartavtsev

Yuri. Ph. Kartavtsev. Concluding remarks & VFDG meeting (20').

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MONDAY, SEPTEMBER 12

- 9-30:12-30. Trip to Vostok MBS
- 12-30:13-30. **Dinner**
- 13-30:14-30. Accommodation
- 14-30:15-30. Excursions to Vostok MBS
- 15-30:18-30. Excursions to Vostok Bay Vicinities
- 18-30:21-30. Banquet & Party by the Fire

TUESDAY, SEPTEMBER 13

- 9-30: 9-00. Breakfast
- 9-00:19-00. Back trip to Vladivostok. Free day at Vladivostok City
- 13-00:23-00. **Departures**

MAPEEG-2022: Program & Abstracts

ABSTRACTS

MODELING THE ACTIVE DISTRIBUTION OF MALADAPTIVE TRAITS IN NATURAL PLANT POPULATIONS

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This work devoted to the consideration of the mechanisms of fixing non-adaptive traits in the plant population, for example, as an agrobacterial insertion. It is known that in the evolution of some plant genera (*Nicotiana, Linaria, Ipomoae*) the insertion of T-DNA (transferred DNA) was fixed after agrobacterial transformation. It should be noted that the T-DNA insert carries sequences unfavorable for plants. Subsequently, such an insertion propagates throughout the populations of plants in the same species, but if it occurred without an insertion, it is eliminated. There is a phenomenon of repeated fixation in populations of different types of "harmful" sequence and its subsequent distribution. A hypothesis stated in this work tries to describe the process of trait distribution in the population. A software has been created to simulate the corresponding processes and confirm the hypothesis. The simulation results suggest that the corresponding fixation of non-adaptive features is not random. and associated with the breakdown of the mechanism of self-incompatibility and the transition to self-pollination.

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SILYBUM MARIANUM L. SEED EXTRACT AS A PROTECTOR ON THE EXAMPLE OF DROSOPHILA

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The ethanol extract of two types of seeds of *Sílybum mariánum* L. in 5% concentration relative to the volume of the nutrient medium (with dark and light color of the peel) was used in the work. The protective properties were evaluated when combined with the antitumor drugs methotrexate and etoposide (concentration of 800 μ g / kg of medium). The study was carried out on a model object of *Drosophila melanogaster*.

The total mortality of individuals is less in the case of using dark seeds. Since when using the extract of light seeds, the mortality of individuals increases relative to the control group, and when used together with etoposide exceeds 50%.

The presence and changes in the genetic activity of antitumor drugs in the tested concentration in the presence of dark seed extract were analyzed. It was found that etoposide has genetic activity, whereas methotrexate $800 \mu g/kg$ does not have pronounced genetic activity. When combined with the extract of *Sílybum mariánum* L., the genetic activity of methotrexate increases, which is manifested in an increased number of cases of mosaicism. Whereas, in the group where etoposide and 5% seed extract were used together, the genetic activity decreased to the indicators of the control group.

COMPARISON OF GENETIC DIVERSITY BETWEEN PARENTS/OFFSPRING IN SEBASTES TACZANOWSKII STEINDACHNER, 1880

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A detailed pedigree of offspring provides clues to addressing a variety of problems: monitoring the levels of genetic variability, inbreeding, effective population size, mechanism and level of mutation processes, identification of genetic mating systems and reproductive tactics.

Earlier, we, for the first time, investigated the family polymorphism and identified allelic diversity of five microsatellite DNA markers in white-edged rockfish (*Sebastes taczanowskii* Steindachner, 1880) by multilocus intermicrosatellite DNA analysis. After analyzing the variation of loci, we found mutant DNA alleles in the first (F1) generation at four of the five loci with different mutation levels and could determine what kind of the genetic mating system of this species has.

In this study, we used microsatellites in combination with DNA-based kinship analysis to assess the genetic diversity variation in 177 individuals of F1 offspring in 46 family-groups of *S. taczanowskii*, a species representing the genus *Sebastes*. This species is characterized by internal fertilization and viviparity, which makes it a valuable model for family analysis.

High variation of microsatellite loci was observed both in the maternal sample and in the offspring population. Compared to the maternal sample, the F1 offspring showed greater genetic variation at three loci (SR 7-2, SR 7-7, and SR 7-25), which was manifested as an increase in the number of alleles. Locus SR 7-25 turned out to be the most polymorphic one, with 24 allelic diversity recorded. The average allele richness was 13 in the parental population and 14 in the offspring samples. One of the loci (SR 7-2) showed an extremely high instability. The level of mutations at this locus was higher than in others, 4.8×10^{-2} . One locus (Sma 3) with null alleles showed a decrease in variation and the lack of mutations *de-novo*.

In further study, we are going to estimate the amount of the variation and genotypic diversity in *S. taczanowskii* at family-groups associated with polygamy of both sexes (promiscuity), and also, the part that can be explained by mutations of microsatellite repeats.

POST-GLACIAL RECOLONIZATION OF THE NORTHEASTERN ASIA BY SALVELINUS TARANETZI: GENETIC EVIDENCE OF MULTIPLE SECOND CONTACTS AND HYBRIDIZATION BETWEEN GLACIAL CHARR LINEAGES

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For the high latitude biota of the Northern Hemisphere, the Pleistocene glaciations had a dominant and well-documented role in shaping patterns of genetic diversity. Habitats were disturbed during the rotation of glacial and interglacial periods and these changes of the environment gave great opportunities for the secondary contact between glacial lineages. Consequences of such contacts can vary from complete reproductive isolation and absence of gene flow to complete genomic introgression.

Charrs of the genus Salvelinus have multiple secondary contact zones between glacial lineages, which arose from allopatry during the Pleistocene. We investigated post-glacial recolonization of Northeast Asia by charrs and examined potential hybridization between different glacial lineages upon secondary contact. New data obtained allowed us to clarify the distribution of three phylogenetic lineages of charrs genus Salvelinus in Northeast Asia. Analysis of the genetic variability using mitochondrial DNA (mtDNA) and microsatellite loci of the nuclear DNA (msDNA) confirmed wide historical dispersal of the Arctic group Salvelinus taranetzi sensu Oleinik et al. (2015) along the Sea of Okhotsk coast. Based on the combined approach, hypotheses about belonging of the lake charrs to (1) Arctic group Salvelinus taranetzi; (2) Bering group of northern Dolly Varden Salvelinus malma malma; (3) Atlantic group Salvelinus alpinus were tested. We have demonstrated secondary contact and the historic hybridization between the ancestral Bering and Arctic charr lineages including a unique case of the mitochondrial capture. It is important to emphasize that introgressive hybridization does not lead to changes in the morphological, ecological, and ethological characteristics of the corresponding charr species. Results suggest one of the most interesting consequences of the secondary contact emerged during fixation of the historical introgressive hybridization in the regions, where none of the participating taxa currently presented. We proposed to consider the founder effect or genetic drift after hybridization as the possible reason of the fixation of the alien mitochondrial genome.

Our study sought to better understand the recolonization of Northeast Asia by charrs following the last glaciation. According to our results, the boundary of the Arctic lineage in Asia shifts south from the Kamchatka Peninsula tip and, therefore, merges with the southern border of the Bering lineage and the border of the Late Wisconsin glaciation. Charr's survival in the water bodies during the Last Glacial Maximum (LGM) is unlikely as the conditions for colonization of rivers of the Sea of Okhotsk coast and western territory of Kamchatka Peninsula emerged only after the end of the Ice Age. Genetic similarity (with the presence of homing and the absence of the anadromous stage of development in the lake charrs) was observed between isolated populations of these geographical regions, possibly, connected to the colonization from the common sources and not to the modern gene flow.

The work was supported by the Russian Foundation for Basic Research under grant number № 20-04-00205.

SECONDARY CONTACT AMONG TWO GLACIAL LINEAGES OF CHARRS OF THE GENUS SALVELINUS IN THE RANGE OF THE SEA OF OKHOTSK COAST: ORIGIN OF THE NEIVA CHARR SALVELINUS NEIVA

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Glaciation events are a significant driver of evolution, physically isolating species into separate glacial refugia, which can undergo allopatric divergence for thousands of years. During allopatry, populations may experience differential selection and gene drift resulting in the formation of genetically distinct glacial lineages. Retreating glaciers allowed access to new environments, sometimes facilitating secondary contact. Upon secondary contact, glacial lineages may demonstrate: (a) extensive gene flow, leading to complete genomic introgression; (b) complete reproductive isolation and an absence of gene flow; and (c) some intermediate level of gene flow. The amount of genetic divergence accumulated between glacial lineages and the degree of erosion of this divergence in secondary contact zones can significantly influence the contemporary genetic structure of a species. The Pleistocene glaciation event prompted the allopatric divergence of multiple glacial lineages of charrs of the genus *Salvelinus*, some of which have come into secondary contact upon their recolonization of the Holarctic. Several main lineages of charrs have been described based on mitochondrial (mt) DNA: Atlantic, Acadia, Arctic, Siberia, Bering (Central), Western Pacific, and Eastern Pacific.

Divergence of the phenotypically different and often geographically isolated forms is a substantial problem for the taxonomy and phylogeny of the charrs genus *Salvelinus*. Origin, relationships, and taxonomic status of the lake charrs from the Arctic regions of Northeastern Asia, most of which are described as separate species, are actively discussed. Based on the analysis of nine microsatellite loci (msDNA) and nucleotide sequences of the mitochondrial DNA control region (*CR* mtDNA), the relationships of neiva *Salvelinus neiva*, narrow-ranged species from the lakes of the Okhota river basin were estimated. The hypothesis that *S. neiva* belongs to the Arctic lineage of *Salvelinus taranetzi*; Bering lineage of the northern Dolly Varden *Salvelinus malma malma*; Atlantic lineage of *Salvelinus alpinus* were tested. According to the analysis of the *CR* mtDNA, *S. neiva* belongs to the Bering lineage, however, msDNA confirms the phylogenetic proximity of *S. neiva* to the Arctic lineage. Incongruence between mtDNA and msDNA testify historic introgression of mtDNA from *S. malma malma* to *S. taranetzi*. The present study provides an example of the historical hybridization and introgression on the southernmost borders of *S. taranetzi* Arctic lineage distribution.

Our results clearly demonstrate the secondary contact of the Arctic and Bering glacial lineages of charrs of the range of the Sea of Okhotsk coast, Russia. These two glacial lineages have likely introgressed extensively in this region. We demonstrate that charrs are an ideal model species for future investigation of secondary contact zones and the influence of historical allopatry on contemporary genetic structure and niche divergence.

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THE FIRST DATA ON THE GENETIC DIVERSITY OF THE EUROPEAN WATER VOLE (*ARVICOLA AMPHIBIUS* L., 1758) FROM THE YAMAL PENINSULA BASED ON *CYT B* GENE SEQUENCES

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The phylogeographic position of the European water vole (*Arvicola amphibious*) populations from the most northern known localities in Western Siberia was estimated. For 18 individuals from three localities (Yamal Peninsula: Erkuta, Salekhard, Labytnangi) six haplotypes of *cyt b* (1143 bp) were described, all of which were new to the species.

Phylogeographic reconstructions were carried out using *cyt b* fragments (999 bp), which made it possible to use the GenBank data across the species range. There were four haplogroups on the phylogenetic tree. The first haplogroup (I) was formed by populations of Great Britain, Northern and Central Europe, territories of Siberia and Altai. The second (II) included populations of Turkey. The third (III) consisted of populations of Great Britain, France, Spain. Populations from Italy formed the fourth group (IV). Within the group I a division of haplotypes into two subgroups was revealed. One subgroup was formed by *A. amphibius* from Belgium, France, Austria, Germany, Hungary, Romania, Slovenia, Serbia, Bosnia; the other was formed by *A. amphibious* from Finland, Denmark, Great Britain, Germany, Western Siberia (including haplotypes which were sequenced by us) and Altai. Within the second subgroup haplotypes from Western Siberia and Altai were relatively isolated from European populations. The results of phylogeographic reconstructions were confirmed by the analysis of median-joining network.

Our results showed that the phylogeography structure of the European water vole is more complex than it was observed in previous studies (Kryštufek et al., 2015; Mahmoudi et al., 2019).

The phylogenetic links of the European water vole from the north of Western Siberia with the northern European (Great Britain, Denmark, Germany, and Finland) and Altai populations have been established. The most probable way of species expansion into the high latitudes of the central part of northern Eurasia was dispersal from the northern European part of the range. To verify this assumption, it is necessary to obtain data from the territory of the East European Plain, the western and eastern slopes of the Urals.

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MAPEEG-2022: Program & Abstracts

MITOCHONDRIAL GENOME OF BROWN BEAR FOSSILS FROM THE BOLSHOY LYAKHOVSKY ISLAND (RUSSIA, REPUBLIC OF YAKUTIA) AND ITS PHYLOGENETIC IMPLICATIONS

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In the present study, we describe the mitochondrial genome of the brown bear (*Ursus arctos* L., 1758), which inhabited Northeast Asia in the Middle Holocene (3460 ± 40). The carcass of a brown bear was excavated in 2020 from permafrost on Bolshoy Lyakhovsky Island (Novosibirsk Islands) and was used for paleogenomics analysis. The modern distribution range of the *U. arctos* does not include this region.

Ancient DNA (aDNA) was isolated from brown bear tissues and multiplexed DNA libraries were constructed in the specially equipped aDNA facilities of the National Research Center "Kurchatov Institute". In total, 535,049,211 DNA reads were generated using the Illumina platform. These genomic data were used for *de novo* assembly of the brown bear mitochondrial genome using the SPAdes tool after filtering by quality. The resulting sequence was annotated using the MITOS. The obtained annotation was then used to define partitions in the subsequent phylogenetic analysis.

As a result, the mitogenome of brown bear (U. *arctos*) consists of 16,654 base pairs and includes 13 protein-coding genes, 2 rRNA genes and 22 tRNA genes. The order of genes and the direction of their transcription in the brown bear mitogenome are identical to the mitochondrial genomes of other mammals.

The primary comparative phylogenetic analysis by Neighbor-Joining as well as Maximum-Likelihood methods show a significant genetic similarity of the studied specimen with modern brown bears inhabiting Northeast Asia.

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SYNHOSPITALITY IN GALL-FORMING ERIOPHYOID MITES (ACARIFORMES) ASSOCIATED WITH BROAD-LEAVED BOREAL TREES

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Eriophyoidea (gall mites) is an ancient group of phytoparasitic chelicerates closely related to the soil mites of the family Nematalycidae. In the course of the colonization of plants, synhospital complexes of eriophyoid species have been formed on numerous host plant species. The eriophyoid complexes associated with different host plants often share similar spectra of gall types and the mechanisms underlying this phenomenon remain unclear.

We aimed to investigate the mechanisms leading to synhospitality in gall-forming eriophyoids using the complexes of mite species that induce galls on broad-leaved, woody, Palearctic dicotyledons in two families (Betulaceae, Malvaceae) and three genera, *Betula*, *Alnus* and *Tilia*, as a research model. These host plants are inhabited by numerous eriophyoid species that are morphologically difficult to distinguish and cause various types of galls, including pouch galls, leaf margin rolling, erineum, vein angle galls and bud galls, often on the same shoot.

In this study, we focused on the mites from genera *Aceria* Keifer, 1944, *Acalitus* Keifer, 1965, and *Eriophyes* von Siebold, 1851 collected in 2020-2021 and tested the following three hypotheses. (1) Species of gall-forming mites are grouped on a host plant according to the type of gall they form, regardless of the phylogenetic relatedness of their hosts. (2) Species of gall-forming mites are grouped by host, regardless of the type of gall they cause. (3) The formation of different synhospital complexes is determined by different mechanisms: (i) in some cases "star-like" speciation on one host is the main mechanism, (ii) in other cases, the mites switch to a new host, meaning that the resistance to phylogenetically non-related mite species by a given host plant is suppressed, or (iii) a combination of these mechanisms occurs.

We sequenced fragments of two marker genes, mitochondrial *Cox1* (1158 bp, 386 amino acids) and segments D1-D5 of nuclear 28S rDNA (1750 bp), and reconstructed phylogenetic relationships between the selected mites species. Our data suggest that scenario (1) is the main one explaining the evolution of gall-forming eriophyoids on the studied model tree genera. Scenario (2) was inferred as most plausible only for a group of *Eriophyes* spp. causing erinea between and along veins of leaves of *Tilia cordata*. Overall, we conclude that "gall type" is the main factor grouping the mite species belonging to investigated synhospital complexes. We also hypothesize that the "star-like" speciation is not typical for gall-forming eriophyoids possibly because of the conservatism of the mechanisms of the formation of a certain gall type and constraints, explained by the morphogenetic characteristics of the host-plant epiderma. We also found several examples of superficially similar but histologically very different galls caused by phylogenetically non-related mite species on the leaves of the studied model plants. Finally, we show that similarity of the galls induced on the same or on closely related hosts does not always mean phylogenetic relatedness of the gall-inducers.

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NUCLEAR GENE GOT2 DNA SEQUENCES POLYMORPHISM IN LOCAL POPULATIONS OF AEGILOPS TAUSCHII IN CAUCASIA HELPS TO UNDERSTAND PECULIARITIES OF THE SPECIES EVOLUTIONARY HISTORY

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Aegilops tauschii Coss. is a diploid goat-grass (genome DD), a wild relative of common wheat, Triticum aestivum L. (genome AABBDD). Its range is from Turkey to Kirgizstan, plus Yellow River basin which is very far to the east from the main area. The study of Ae. tauschii in Dagestan (Russia), revealed that some local habitats at the very edge of Ae. tauschii range were able to accumulate very high level of genetic variability. We investigated what phylogenetic lineages made impact to this variability in Dagestan local habitats "4" and "6". It was found that in addition to major lineage of Ae. tauschii subsp. tauschii, one of the rare relict lineages of this subspecies was pointed out there, the lineage previously found in places very distant from Dagestan, - in Iran, Turkmenistan and Afghanistan. In Ae. tauschii subsp. strangulata one of relict lineages and two major lineages, all those that were known to inhabit Dagestan region, were found in Dagestan local habitats "4" and "6". It was a surprise that despite of very high migration capacity of Ae. tauschii, we have not found in these habitats neither of the other three major lineages of Ae. *tauschii* subsp. *strangulata*, the two of which are common in the regions just near Dagestan - in Azerbaijan and Armenia. These data obtained revealed that each of several relict lineages of subsp. tauschii previously for some time span occupied the most part or the whole subspecies range, as the single one major lineage of subsp. tauschii do now. In contrast, in subsp. strangulata the subspecies range is being shared between several major lineages neither of which ever occupied the whole range.

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PRIMER SET DESIGNING FOR THE COMPLETE GENOME SANGER -SEQUENCING OF NEWCASTLE DISEASE VIRUS

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Newcastle disease virus (NDV), or Avian paramyxovirus type 1 (Paramyxoviridae, Avulavirinae: *Orthoavulavirus*, Newcastle antigenic complex), is the etiological agent of the disease of the same name, causing epizootics with a high mortality rate in bird populations. The NDV genome is represented by single-stranded RNA (15 kb) of negative polarity and is divided into 18 genotypes and a number of subgenotypes (Ia-c, II, III, IV, Va-d, VIa-h, VIIa-i, VIII, IX, X, XI, XII, XIIIa-c, XIVa-b, XV, XVI, XVIIa-b, XVIIIa-b), which differ in nucleotide sequences in the genome, virulence level and geographical distribution. The natural reservoir of NDV are wild birds of both aquatic and terrestrial ecological complex. From wild populations, NDV penetrates into poultry populations. The virus is transmitted by droplet-air and oral-fecal route.

Genome sequencing is the most reliable method for identifying, determining some biological properties and forming hypotheses about the origin of pathogenic microorganisms. At the same time, Sanger sequencing, which requires a system of primers that flank overlapping nucleotide sequences, remains the most technologically and economically accessible approach to viral sequencing. Several sequencing schemes are known for NDV, but it should be taken into account that the high degree of genetic variability of this virus requires the development of region-specific primer systems. This paper describes such a system verified for NDV strains isolated from agricultural and wild birds in Primorsky Krai.

DIFFERENT EVOLUTIONARY HISTORIES OF MHC I AND II REFLECT DISTINCT FUNCTION AND ECOLOGY

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The molecular characteristics of the major histocompatibility complex (MHC) have been a central topic of a plethora of studies in all major vertebrate taxa since the introduction of modern sequencing methods. There are two major reasons for this: First, the MHC plays a major role in initiating the adaptive immune response in all vertebrates, both humoral and cell-mediated, and second, the MHC is the most genetically variable coding region across vertebrate taxa. Thus, the MHC is among few proteins in which selection is often detected at the molecular level within and between extant populations. Particularly noteworthy are studies in which novel or emerging pathogens have been shown to exert selective pressure on MHC peptide (antigen) binding regions within the gene complex. Despite the large number of MHC studies over the last few decades, however, how the most important proteins in the complex, the MHC I and MHC II, have evolved and interact with each other and with pathogens remains unresolved, particularly in non-model organisms. Here, we explore the different evolutionary trajectories of the MHC I and MHC II in two species of closely related passerine birds. We argue that confusion abounds regarding the different functions of these two proteins, and that genetic analysis reveals how these functions have evolved over time. The focal taxa are two sister species of sparrows that were introduced to the USA in the 1800's, one (European house sparrows) that has become ubiquitous across North and South America, while the other (the Eurasian tree sparrow) remains confined to a relatively small geographic area. We extend our argument to include general patterns of MHC evolution across all vertebrates.

LINES, TEETH AND TREES: THE FIRST ATTEMPT TO RESOLVE CORYPHELLINA RUBROLINEATA SPECIES COMPLEX (GASTROPODA: NUDIBRANCHIA)

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Nudibranch species *Coryphellina rubrolineata* O'Donoghue, 1929 is a well-known, brightly colored cladobranch sea slug, which shows an abnormally wide range from the Mediterranean and the Red seas to Philippines and Australia. The specific morphological identification traits are perfoliate rhinophores and three red, purple or violet pigmental lines on the dorsal and lateral sides, which normally continues from a head to a tail. However, different varieties may have discontinuous stripes, or even none. The molecular study has been never conducted to confirm the species identity of molluscs with those color varieties in the Indo-West Pacific.

We have studied several specimens collected from coastal waters of Central and Southern Vietnam. Our methods included traditional anatomical dissections, SEM studies of jaws and the radula and the molecular phylogenetic analysis of four genes, accompanying by modern species delimitations methods.

We have shown that the species *C. rubrolineata* is restricted to areas, which are close to the type locality (the Mediterranean Sea, the Red Sea). In Southern Vietnam *Coryphellina* species represent a species complex, with at least six distinct species found. Four of these species are new for science, while one species is identical to recently described *Coryphellina lotos* Korshunova et al., 2017 from Japan. Species differ from each other in coloration pattern and arrangements of red bands and pigmental lines. Also, we have found some differences in radular characters, while the morphology of the reproductive system was similar in all species, including the nominative species *C. rubrolineata*. Further revisions are needed for deeper understanding of *Coryphellina* diversity in the Indo-West Pacific region and to uncover processes underlying cryptic and pseudocryptic speciation of these mollusks.

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LOOKING FOR EVIDENCE OF THE GREAT TRANS-ARCTIC INTERCHANGE: THE PHYLOGEOGRAPHY OF BOREAL AND ARCTIC NUDIBRANCH MOLLUSCS

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The species delineation is particularly acute in boreal seas, which are often inhabited by species with broad geographic ranges and high degrees of intraspecific morphological and molecular diversity. Environmental conditions in boreal and Arctic regions significantly changed multiply times in recent past, promoting the formation of geographic barriers and leading to allopatric speciation events. Unlike with sympatric species, no separation in ecology happens, and truly cryptic species can be formed with overlapping morphological variation, but significantly distant genetically.

In this study we tested the species identity and observe morphological variation across amphiboreal species of three common nudibranch families: Onchidorididae, Dendronotidae and Coryphellidae. For this purpose, we used a set of 5 standard mitochondrial and nuclear markers: *COI, H3, 16S-, 28S-* and *18S* rDNA and a large variety of species delimitation (ABGD, GMYC, bPTP) and phylogeographic methods (population analysis; ancestral area reconstruction). The morphological analysis included standard morpho-anatomical examination using the light microscopy and scanning electron microscopy.

Based on our integrative results we identified cases of true amphiboreal species, but also cases of cryptic species being formed allopatrically following Pliocene-Pleistocene Climate Change. Ancestral area reconstruction (AAR) provides evidence for a Pacific origin of both families. Different lineages of Onchidorididae, *Dendronotus* and *Coryphella* demonstrate different level of genetic differentiation and, according to our molecular clocks calibration, different divergence times. This indicates that the invasion of the Arctic and Atlantic regions occurred multiply times starting from the first opening of the Bering Strait in late Miocene.

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ONE'S MORE ABOUT GENETIC DIFFERENTIATION IN EASTERN LINEAGE OF STRIPED FIELD MOUSE (APODEMUS AGRARIUS): STUDY OF 6 MICROSATELLITE LOCI

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The striped field mouse inhabits a wide area from central Europe to the Pacific coast of Asia including nearest islands. The species range is subdivided into two allopatric parts with disjunction in Transbaikalia. Due to the investigation of 5 microsatellite loci it was found higher affinity of continental populations within each of the lineages and somewhat greater genetic differentiation between these lineages (Frisman et al., 2019). The aim of a current work was to investigate the polymorphism and differentiation of islands and mainland populations in eastern lineage of the species. Animals were caught on 4 islands of the Peter the Great Bay in Sea of Japan as well as in 6 continental localities in Russian Far East. Mainland localities were distributed from the coast of the Sea of Japan in the southern Primorye to the northwestern end of the area of the lineage in the Amur region. Local samples contained between 21 and 30 specimens, and total sample size comprised 278 individuals. Six microsatellite loci were selected for the analysis, CAA2A, GSADT7, GTTDS8, GATAE10A, GTTF9A that developed after Makova et al. (1998) and DSFM2 that developed according Wu et al. (2008).

A higher allelic diversity was found in the mainland part of the range than on the island part of the range. Opposite to that, a significantly higher level of genetic differentiation was found between islands' part of the range. On the mainland part of the range there was a decrease in allelic diversity in populations in the direction from south to north. The greatest differences for overall dataset were observed for the population of Big Pelis island. This island, characterized among those considered (Russkii, Popov's, Putyatin's), by the smallest area, the greatest distance and longest time of separation from the continent (9.5 thousand years) (Velizhanin, 1976). Populations of other islands different in size of area, but separated from the mainland by a distance of 0.6-1.6 km showed a level of differentiation comparable to the differentiation of the western and eastern continental lineages. The latter can be a confirmation of no more than the Holocene time of the division of the area of the striped field mouse in Transbaikalia.

PHYLOGEOGRAPHY OF THE BRITTLE STAR *OPHIURA SARSII* LÜTKEN, 1855 (ECHINODERMATA: OPHIUROIDEA)

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The brittle star *Ophiura sarsii* Lütken, 1855 is a circumpolar cold-water species widely distributed throughout the North Pacific, the North Atlantic, and the Arctic ocean, where it can reach maximum depths of ca. 3000 m. The subspecies *Ophiura sarsii vadicola* Djakonov, 1954 was described from the Sea of Japan, where it occurs along with *O. sarsii* f. *typica*. Interestingly, in the Yellow Sea only the *O. sarsii vadicola* subspecies was found. The genetic structure of *O. sarsii* was broadly evaluated across the Pacific (Li et al., 2020). We analyzed the mitochondrial cytochrome c oxidase subunit I (COI) gene to examine the genetic diversity of *O. sarsii* across the range.

Sampling was conducted in the Barents Sea during the 17th joint Barents Sea Ecosystem Survey cruise of the Institute of Marine Research (Norway) and the Polar branch of VNIRO (PINRO) in September 2019, 114th cruise of RV "Vilnyus" (PINRO), and in the Laptev and the East-Siberian Seas during TRANSARCTIC-2019 expedition of the Arctic and Antarctic Research Institute. Barcode fragment of the COI (Folmer et al., 1994; Layton et al., 2016) was used for the molecular analysis. The total set consists of 64 new sequences from the Russian Arctic Seas and 104 sequences published in NCBI GenBank from the Atlantic and the Pacific.

Four haplotype groups were identified. Three of them included *O. sarsii* f. *typica* specimens only, while all *O. sarsii* f. *vadicola* specimens from the Yellow Sea formed a single clade that was 61 mutation steps away from the nearest *O. sarsii* f. *typica* clade.

Two *O. sarsii* f. *typica* haplotype clades were found in the Atlantic section of the Arctic. The first clade comprised specimens from the Barents Sea, Iceland, Baffin Bay and St.Lawrence Bay. Brittle stars from the North Sea, the Barents Sea and Iceland formed the second clade. In the Barents Sea, both clades were present, and both comprised specimens from the northern part of the sea (near Svalbard) as well as from the southern one. Star-shape of the first Atlantic clade with the unimodal mismatch distribution and significantly negative values of Fu's Fs test for both Atlantic clades indicates a recent expansion. Bayesian skyline plots confirm demographic expansion for both Atlantic clades of *O. sarsii*, yielding median estimates of time since the last population expansion as ca. 18,000 years for the clade 1 and ca. 58,000 years for the clade 2.

Siberian-Pacific clade consisted of the *O. sarsii* f. *typica* specimens from the Laptev, the East Siberian, the Chukchi, the Bering Seas, the Hudson Bay, and the British Columbia. A significant genetic distance between the Siberian-Pacific and the Arctic clades corresponds to the presence of a geographical gap: according to the collection of the Zoological Institute RAS, *O. sarsii* is almost absent in the Kara Sea. Bayesian skyline plots show an older time of expansion (ca. 110,000 years) compared to Atlantic clades, which is in good agreement with the observed phenomenon of dispersal of the echinoderm fauna in the Arctic (Mironov, Dilman, 2010).

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RECOMBINATION-INDEPENDENT RECOGNITION OF DNA HOMOLOGY FOR MEIOTIC SILENCING IN NEUROSPORA CRASSA

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Homologous chromosome pairing represents a critical aspect of meiosis in nearly all sexually reproducing species. While meiotic pairing relies on the formation of double-strand DNA breaks in some organisms, in many others it can proceed in the apparent absence of DNA breakage and recombination. The mechanistic nature of such recombination-independent pairing represents a fundamental question in molecular biology. Using "meiotic silencing by unpaired DNA" (MSUD) in the fungus Neurospora crassa as a model system, we demonstrate the existence of a principally new solution to the problem of inter-chromosomal homology recognition during meiosis. Here we take advantage of the unique ability of MSUD to efficiently detect and silence (by RNA interference) any relatively short DNA fragment lacking a homologous allelic partner. We show that MSUD does not require the function of eukaryotic RecA proteins and the type II topoisomerase-like protein Spo11. We further show that MSUD recognizes weak interspersed homology in which units of sequence identity as short as 3 base-pairs (bp) are spaced apart with a periodicity of 11 bp, approximating double-helical DNA pitch and corresponding to an overall sequence identity of only 27%. Taken together, these results reveal the role of a recombinationindependent homology-directed process in guiding the expression of small interfering RNAs and suggest that meiotic chromosomes can be evaluated for sequence homology at base-pair resolution by a mechanism that operates on intact DNA molecules.

PHYLOGEOGRAPHY OF MYOTIS PETAX

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The intraspecific variability of *Myotis petax* control region was studied for 147 specimens from all species range. We found three highly divergent genetic lineages named I, II and III. The II and III lineages are distributed only on South Korea and Kunashir Island, respectively. The distribution of the most widespread lineage I include mainland part of the range and Sakhalin Island.

The lineage I is subdivided in three separated groups: IA, IB, IC. The IA group is found throughout the west part of the range, including West and East Siberia and the Altai Mountains. Besides that this group prevails in the south part of Primorsky Krai. The most of the specimens from Primorsky Velican cave and the Khasan District are identified as IA group. Also the single individuals of this group were detected in Khabarovsk Krai.

The distribution of the IB group includes Khabarovsk Krai, the Republic of Yakutia and Sakhalin Island. The single specimens were found in the Primorsky Krai and China (Jilin).

The IC group is differing from other groups in the presence of additional tandem repeats in control region. The range of this group is restricted the Upper and Middle Amur and the Transbaikalia, but the single individuals are occurred in Primorsky Krai.

Thus, we revealed all three groups of lineage I in the Primorsky Krai. The other regions when different groups found together are Transbaikalia (IA and IC) and Khabarovsk Krai (IA and IB).

Summarizing, we can conclude that the genetic structure of *Myotis petax* is explored only in outline and partly correlated with the previously described morphological forms. A detailed study of the formation of the observed intraspecific structure should be the purpose of further research.

A BRIEF OVERVIEW OF MODERN METHODS OF RESEARCH OF CIS-REGULATORY ELEMENTS OF GENOMES

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Cis-regulatory elements (CREs) are non-coding DNA sequences that regulate transcription of nearby genes (Wittkopp, Kalay, 2012). CREs are characterized by open unmethylated chromatin, acetylated histones, and interaction with transcription factors (Schmitz et al., 2022). Mutational changes in CREs, especially in enhancers, are important for the divergence of phenotypic evolution and speciation. These changes affect the patterns of gene expression, which is expressed in the divergence of phenotypes. At the same time, CREs mutations are often less harmful than mutations in structural genes or trans-regulatory elements that affect many genes at once. This circumstance suggests a large accumulation of mutations in CREs, which has a significant impact on evolution (Wittkopp, Kalay, 2012). The study of CRE is possible both based on *in silico* predictions and by molecular methods to identify and validate potential sites. In this paper, current molecular methods of searching and determining CRE will be considered.

A group of methods for determining chromatin availability is based on the location of active CRE in the euchromatin regions. Methods such as BS-seq, EM-seq (Feng et al., 2020), FAIRE-seq (Bianco et al., 2015), ATAC-seq (Buenrostro et al., 2013), MN-seq (Cui, Zhao, 2012), DNase-seq (Sabo et al., 2006) can differentiate active and inactive chromatin, and thereby predict the location of CREs within the entire genome (Klein, Hainer, 2020; Schmitz et al., 2022; Shlyueva et al., 2014).

A group of methods for profiling protein-chromatin interactions relies on specific interactions between antibodies and DNA-interacting proteins. It is usually the modified histones that characterize CREs. A family of ChIP-based methods (Perez-Romero, Imperiale, 2007; Zhao et al., 2019) and a younger group of CUT&RUN methods (Skene, Henikoff, 2017) make it possible to identify binding sites with predefined proteins. These are highly accurate methods, but their common weak point is a relatively narrow sample of stable, high-quality monoclonal antibodies suitable for research (Klein, Hainer, 2020).

A special group of methods for studying CRE are methods for capturing chromatin conformation (3C, 4C, 5C, Hi-C). These methods are effective for mapping CRE connections and their controlled genes (Peng et al., 2019; Hughes et al., 2014; Shlyueva et al., 2014).

The study of CRE expands the understanding of gene regulation, allows the construction of gene regulatory maps, complements evolutionary models based on divergence and development of regulatory areas, opens new aspects of the study of phenotypic evolution. However, in addition to fundamental biological issues, research in this area makes a significant contribution to applied areas – bioengineering, gene therapy, medicine, and many others.

HOW TO RESOLVE TRANS-ARCTIC SPECIES COMPLEXES: THE PHYLOGEOGRAPHY OF A NUDIBRANCH SPECIES COMPLEX EUBRANCHUS RUPIUM-EXIGUUS (GASTROPODA: NUDIBRANCHIA)

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Nudibranch mollusks of the *Eubranchus rupium* - exiguous species complex have similar external morphology. Based on the published data, it is impossible to conclude whether they are separate species or should be reduced to synonyms. The representatives of this complex show an amphiboreal distribution. Species with this type of distribution often represent complexes of cryptic or pseudo-cryptic species. It makes them an appropriate model to study modes of speciation in boreal and Arctic regions.

A total of 199 samples, collected from the White, Barents and Japanese Seas, and Norway. Integrative taxonomy methods were used to investigate species identity of studied groups and phylogenetic relationships between them, including molecular genetic methods (phylogenetic analysis using sequence *COI*, *16S*- and *18S* rDNA as markers), haplotype networks and morphological analysis (specimen dissection, light and scanning electron microscopy).

Our analysis recovered *Eubranchus rupium* and *Eubranchus exiguus* as separate distinct species. They can be distinguished by coloration, radular and reproductive system morphology. Both are recovered as supported monophyletic clades in our phylogeny reconstruction. Additionally, we found two pseudocryptic species - one is sister similar to *Eubranchus rupium* and another - to *Eubranchus exiguus*.

The *Eubranchus rupium* has a wide distribution and a fragmented range. In the waters of the Sea of Japan a similar species was found. This new species is 4.7% different in sequence divergence from *E. rupium* in *COI* marker and also shows a specific coloration.

The second pseudocryptic species, similar to *Eubranchus exiguus*, is found sympatrically with true *Eubranchus exiguus*. This new species is 11% different from *E. exiguus* in *COI* marker. Morphological differences are found in the coloration of individuals and the structure of the digestive gland enclosed in cerates. Eubranchus taxonomy should be revised to accommodate these new findings, and two new species should be described.

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MORPHOLOGICALLY CONSERVATIVE BUT GENETICALLY DIVERGENT: MOLECULAR SYSTEMATICS OF THE PHYLLIDIID NUDIBRANCH MOLLUSCS IN SOUTHERN VIETNAM

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Nudibranchia is a group of shell-less and strikingly colored marine molluscs, encountering more than 4,000 species. They are distributed worldwide with the highest diversity in the Indo-West Pacific region. Family Phyllidiidae is the less typical of all families included in Nudibranchia. Their unique traits are the lack of radula and partly external digestion. Phyllidiidae has always been a complicated group for taxonomical studies. Due to lack of the radula the species identification is much more difficult than in other nudibranch families. Despite some several recent works using molecular methods that have been published, the taxonomy of the family is a challenge due to a great number of true cryptic species. In the present study, we observed diversity of Phyllidiidae in Southern Vietnam using an integrative approach included traditional morphological (anatomical research, SEM) and modern molecular (phylogenetic analysis and species delimitation) methods. The material was collected in 2016-2021 from Nha Trang Bay and Spratly Islands in Vietnam waters using SCUBA diving and snorkeling. A cryptic diversity was shown within several genera, e.g., the species *Phyllidiella pustulosa* (Cuvier, 1804) is represented by nine highly divergent clades.

THE PRELIMINARY DATA ABOUT INTERSPECIFIC GENETIC VARIABILITY IN SAKHALIN LEDUM SPECIES BASED ON cpDNA

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For a long time *Ledum palustre* L. was considered as the single highly polymorphic species of *Ledum* throughout the entire Eurasian part of its range. V. L. Komarov was first to divide *L. palustre* s.l. into four distinct species: *L. decumbens* (Ait.) Lodd., *L. palustre* s. str. L., *L. hypoleucum* Kom. and *L. dilatatum* (Wahlb.) Kom. (= *L. macrophyllum* Tolm) (Komarov, 1932). However there is still no single generally accepted taxonomy for *Ledum* species.

L. decumbens is an arctic-alpine Asian-American species and L. palustre is a circumboreal species, both are widely distributed in the Northern Hemisphere. On the contrary, L. hypoleucum and L. macrophyllum have a rather narrow range in the Far East. L. decumbens can grow in subarctic tundra as well as in high mountains, L. palustre is abundant in swampy habitats, L. hypoleucum and L. macrophyllum mainly occur in damp forests. All these species are distributed in Sakhalin Island, which represents the biogeographic boundary between the boreal and temperate biota. The existing problem of distinguishing the species is due to extremely similar floral characters of them. Their vegetative characters vary continually across the geographic range. Although morphological features have traditionally been considered as a key to the unraveling of taxonomic relationships within Ledum group, in this study we apply molecular methods to consider species boundaries.

This study investigated genetic structure of *Ledum* complex in Sakhalin. The study aimed to find and optimize plastid molecular markers for the analysis of genetic variability of four *Ledum* species throughout the island.

The polymorphism of non-coding regions of chloroplast DNA (cpDNA) was found in *trnV-ndh*C (Shaw J. et al., 2017) and *petB-petD* (Löhne C., Borsch T., 2005) fragments using DNA sequence data. In total 188 *Ledum* samples (all four species throughout Sakhalin) were included in the analysis of chloroplast genetic variability using Arlequin and Network programs. The chloroplast data indicated eight chloroplast haplotypes in *Ledum* populations, but none of them were species-specific. Our current level of observed variability cannot clarify the interspecies genetic structure.

This study lays the groundwork for future phylogenetic studies within *Ledum*. It illustrates the needs to involve alternative genetic markers (ITS, EST, AFLP) and include more regions of the range in order to capture a complex genetic and biogeographic history.

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CHARACTERIZATION OF MITOCHONDRIAL GENOME OF SWIFTOPECTEN SWIFTII (BERNARDI, 1858) AND THE PHYLOGENETIC RELATIONSHIPS WITHIN FAMILY PECTINIDAE

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Family Pectinidae is purely studied group of marine bivalves in regard to systematic position, phylogenetic relationships and evolution. Species of this family are important objects of active trading and aquaculture. One of the most common mollusks in Primorye region, *Swiftopecten swiftii*, is one of major objects of aquaculture at this territory. However, there are extremely few data on biodiversity and genetic variation of this species in the Far East of Russia.

The first data on a mitochondrial genome (mitogenome) of *S. swiftii* (Bivalvia: Pectinidae) was generated using the next-generation sequencing (NGS) approach. We obtained partial sequence of the mitochondrial genome (mitogenome) of this species, including two fragments, 11484 b.p. and 5618 b.p. in length, and working on establishment of the complete sequence of the mtDNA of *S. swiftii*. Currently we defined 11 protein-coding genes, two ribosomal genes, 12 tRNA genes, as well as a unidentified region between *trnM* and *trnA* of tRNA genes. We have compared our results with the previously obtained data on the scallop *Mizuhopecten yessoensis* (Jay, 1857) (FJ595959, Wu et al., 2009), which considered as closely related species to *S. swiftii* (Matsumoto, Hayami, 2000). However, at a first glance there is a lot of gene rearrangements in the mitogenome of *S. swiftii* in comparison with *M. yessoensis*.

The phylogenetic relationships of *S. swiftii* within of Pectinidae have been reconstructed on the basis of protein-coding genes from available in GenBank mitogenomes of the Pectinid species. In the current report the results of the phylogenetic analysis are discussed.

GENETIC DIVERSITY OF *DIPLOSTOMUM* SPP. (TREMATODA, DIPLOSTOMIDAE) INFESTATED FISHES FROM DIFFERENT WATER BODIES OF RUSSIA

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Trematodes of the genus *Diplostomum* are worldwide distributed and at the stage of metacercariae cause various forms of diplostomosis of fishes. These trematodes are, in most cases, localized in the lens and the inner media of the fish eyes. The taxonomic identification of these trematodes on metacercariae stage is a difficult task due to the low variability of their morphological features and limited number of them. In the present study, the diversity of metacercariae of *Diplostomum* spp. was evaluated based on the variability of the cytochrome c oxidase (cox1) gene of mtDNA. Since species identification of *Diplostomum* in fish eyes from Siberian lakes was based only on the morphological features, the previous studies had a number of biases and contradictions in taxonomical diversity in this region.

The parasite samples were collected from fishes inhabiting three large lakes: Chany, Teletskoye, and Baunt. Chany is a shallow eutrophic lake inhabited by 14 species of fishes, whereas lakes Teletskoe and Baunt are oligotrophic mountain lakes inhabited by 13 and more than 12 species of fishes, respectively. The present study was performed during 2019-2021 years.

Total DNA was isolated from metacercariae using a 5% aqueous solution of Chelex ion exchange resin (Bio Rad). Proteinase K was used to completely dissolve soft tissues. A fragment of the first subunit of the cox1 gene with a length of 595 nucleotides was used as a DNA marker. PCR uses primers and conditions that developed by Steenkiste et al. (2014). Amplicon purification and further sequencing were performed at the «Genomics» Research Center of the SB RAS. All operations with the obtained sequences are performed in the MEGA 11 program. For comparison with the homologues available in the GenBank database, the BLAST NCBI service was used.

In total, nucleotide sequences of 77 metacercariae from ten species of fish were obtained from Chany Lake (common carp, Prussian and crucian carps, roach, dace, ide, bream, perch, pike perch, and northern pike), 32 metacercariae from seven species of fishes (whitefish, bream, perch, siberian bullhead, burbot, dace and common minnow) from Teletskoye Lake and 42 metacercariae from eight species of fish (roach, dace, Prussian carp, ide ruff, burbot, perch and whitefish) from Baunt Lake.

As a result of phylogenetic analysis, sequences from the studied lakes formed 10 clades of the species level. Reference sequences with a high percent of similarity (>99%) were found for 6 clades and the following types of *Diplostomum* spp were determined: *D. pseudospathaceum*, *D. spathaceum*, *D. baeri* complex sp. 2, *D. mergi* and *D. mergi* complex sp. 2. Four undetermined species of trematodes were also found. Therefore, 3 species of *Diplostomum* were found in fishes from Chany Lake, whereas for fishes from Teletskoye Lake and Baunt lakes - 7 and 8 species were identified, respectively.

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NEW DATA FOR THE FAMILY NOTOCOTYLIDAE LUHE, 1909

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In Izrailskaia et al. (2019), as a result of studying the morphology and obtaining molecular data for representatives of the family Notocotylidae Luhe, 1909, it was found that the main criteria used for the differentiation of genera (the number and location of tegumental formations) were not effective in resolving taxonomy issues. On the phylogenetic reconstruction in the study, species with three rows of papillae were combined into one cluster; species with two rows of papillae and a crest were grouped into another one, while some worms with both variants of these formations were placed in a separated cluster.

In this work, the above research is continued. Namely, new morphological and molecular data are obtained for several species of the genus *Notocotylus* Diesing, 1839. The reconstruction, based on new data for partial sequences of the 28S rRNA gene, confirms results that previously obtained.

In addition to molecular studies, the specific features of the circulation are studied for parasites from the family Notocotylidae, namely, the list of the first intermediate hosts participating in the life cycle is analyzed for these worms. Three ecological groups of intermediate hosts are identified: prosobranch freshwater, marine and pulmonate snails. Unfortunately, data on the first intermediate hosts are available not for all trematodes with nucleotide sequences in GenBank. However, the analysis of the phylogenetic reconstruction obtained by our team shows that the worms are combined into separate clades corresponding with snails involved in their life cycles. And remarkably, the most numerous group on the phylogenetic tree includes trematodes developing with the participation of pulmonate and prosobranch freshwater snails. In our opinion, the separation of notocotylids into different clades, regardless of the structure of their tegumental formations, is the result of the formation of their life cycles and, in particular, highly specific relationships with one of the ecological groups of snails playing a role of the first intermediate hosts.

USING MOLECULAR GENETICS TECHNIQUES FOR IDENTIFICATION OF DIATOM SPECIES IN THE ORDER THALASSIOSIRALES (BACILLARIOPHYTA) IN THE SEAS OF RUSSIA

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Members of the order Thalassiosirales (Bacillariophyta) are among the most common marine planktonic diatoms of the temperate zone of the World Ocean. In the summer-autumn period they represent a major part of the population and phytoplankton biomass and cause "blooming" of the coastal waters. In the seas of Russia all currently recognized families of the order Thalassiosirales are occurred and the species of the genus *Skeletonema* and *Thalassiosira* are most abundant among them. Due to the difficulty in the identification of many representatives of the order Thalassiosirales, data on the number of species in the Russian Federation is scattered.

In this study, we identified several species of the genera *Skeletonema* and *Thalassiosira* using molecular genetic methods. For this we used clone cultures of these genera taken from plankton and benthic samples in the Russian seas (Japan sea and Black sea) during the period from 2009 to 2019. Identification was based on the direct sequencing and phylogenetic analysis of 28S rDNA and 18S rDNA sequences. Using these methods, it was possible to distinguish in Japan sea three species from the genus *Skeletonema* (*S. marinoi*, *S. dorhnii* and *S. japonicum*) and point out the presence at least two species from the genus *Thalassiosira* (*T. antarctica* and *T. punctigera*).

STRUCTURAL FEATURES OF THE *ITS2* rDNA REGION FOR THE FAMILY PSILOSTOMATIDAE (TREMATODA: ECHINOSTOMATOIDAE)

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The family Psilostomatidae Odhner, 1913 is a cosmopolitan group of trematodes that parasitizes in birds, fish and amphibians. This family is studying the entire world but most of the works are devoted to morphological description and there're relatively few works with genetic data. There aren't studies on the analysis of spacer regions (*ITS1* and *ITS2*) of rDNA for the family Psilostomatidae. Transcribed spacers are important for rRNA processing and biogenesis of active ribosomal subunits therefore in this study we analyzed the secondary structures of the *ITS2* region for four trematode species from the Russian Far East: *Sphaeridiotrema ussuriensis*, *S. aziaticus*, *S. pyriforme* and *Psilotrema limosum*. In addition, the data presented in the GenBank were also included in the analysis

The secondary structure of the *ITS2* rDNA region for most trematodes has four domains (Morgan, Blair, 1998; Ataev, 2016) but the number of domains may vary in some cases (Prasad, 2008). Within the genus *Sphaeridiotrema* two species, *Sphaeridiotrema ussuriensis* and *S. aziaticus*, have a classical structure with conservative A and B domains. In contrast, variable secondary structures with 3–4 domains were found for *Sphaeridiotrema pyriforme* and *S.pseudoglobulus*. Despite that usually the domain A has a very conservative topology, it varies on all secondary structures of both species, and is absent in two of the three models for *S.pseudoglobulus*. The length of the *ITS2* rDNA region of *Psilotrema limosum* is two or more times that spacer length of other members of the family resulting different secondary structures of *ITS2* for this species. These features of psilostomids could appear as a result of adaptation to host types and individual tissues in which the parasite lives.

EXON AND INTRON DIVERGENCE OF GROWTH HORMONE GENES IN SALMONIDS

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Growth hormone is the most studied polypeptide hormone. It performs a number of vital functions, being responsible for the regulation of somatic growth and involved in numerous physiological processes, including ion balance, lipid and protein metabolism, immune response, reproduction, and various aspects of behavior. Genes encoding growth hormone have been described in many taxonomic groups and usually include 5 exons and 4 introns. In most vertebrates, including some fish species, there is a single copy of the growth hormone gene. In salmonid fishes, there are two copies of the growth hormone gene: gh1 and gh2, which indicate a gene duplication in the ancestral form. Genes gh1 and gh2 have the same structure. Both genes consist of 6 exons and 5 introns. Differences in the size of this gene, both in different species and between the two copies of a gene of the same species, arise due to the different lengths of introns. Despite the fact that, one of the copies of duplicated genes may be under reduced selection pressure and accumulate changes at a higher frequency, growth hormone genes remain quite conservative. The absence of additional stop codons in the nucleotide sequence, open reading frame with a total length of 630 bp, high conservation of the predicted amino acid sequence, including the positions of cysteine residues, demonstrate the saving of functional potential of both genes. However, different parts of the genome can accumulate mutations at varying rates. It is known that proteincoding sequences and regulatory elements are under the pressure of strong negative selection. In non-coding sequences, mutations occur much more frequently. Comparison of the nucleotide diversity in each exon and intron of the paralogous salmon gh1 and gh2 genes makes it possible to evaluate the patterns of divergence in the case of the origin of duplicated genes and further potential pathways of their evolution.

SANCHYTRIOMYCOTA - A NEW PHYLUM IN THE KINGDOM FUNGI

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Compared with filamentous fungi and unicellular yeasts, fungi with spreading flagellar stages (zoospores) remain poorly understood, and their phylogenetic position is often very uncertain. The seemingly parasitic chytridiomycetes that we have studied over the past 5 years have amoeboid zoospores with highly reduced flagella - pseudocilia. Ultrastructural studies of zoospores of two such species showed their extremely unusual structure: kinetosomes are reduced to single microtubules, but, at the same time, reach a length of up to 2 µm. According to morphological features, they are sharply distinguished from chytridiomycetes, and the first molecular phylogenetic analysis allowed them be assigned to the class Monoblepharidomycetes, where these new species Amoeboradix gromovi and Sanchytrium tribonematis were assigned to two new genera and composed the new order Sanchytriales. A more thorough phylogenetic analysis of the rRNA genes showed that they form a monophyletic group not closely related to any known fungal clade. To establish their phylogenetic position and possible evolutionary transformations, the genomes of both species were sequenced. Phylogenomic analysis using various sets of proteins, as well as a wide and carefully designed sample of taxa, made it possible to resolve almost completely the tree of the kingdom Fungi, especially zoosporic fungi. It turned out that chytridiomycetes, which are the sister branch to all other fungi, are the first to branch off in the evolution of fungi. Sanchytrids form a well-supported, rapidly evolving branch, sister to the phylum Blastocladiomycota. Comparative genomic analysis of Sanchytriales with other representatives of Holomycota reveals a significantly reduced metabolic repertoire in sanchytrids, which indicates their deep adaptation to a parasitic lifestyle. Based on the phylogenetic position of sanchytrids in combination with their unique morphological features, it was proposed to separate this group of zoosporic fungi into a new phylum Sanchytriomycota, within which an independent loss of the flagellar apparatus occurred.

The project was supported by the Russian Science Foundation, grant No. 21-74-20089. Cultivation of sanchytrids as part of the Fond Collection of the Zoological Institute of the Russian Academy of Sciences, was supported by a grant of the Ministry of Science and Higher Education of the Russian Federation (no. 075-15-2021-1069).

ON THE MITOGENOME STRUCTURE OF RIGHTEYE FLOUNDERS OF THE FAR EASTERN SEAS WITH AN INFERENCE INTO THE MOLECULAR PHYLOGENY AND SYSTEMATICS OF THE FAMILY PLEURONECTIDAE AND THE SUBORDER PLEURONECTOIDEI (OSTEICHTIES, CARANGIFORMES)

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In this paper, we for the first time report the complete sequence of the mitochondrial genome (mitogenome) of the yellow-striped flounder Pseudopleuronectes herzensteini (Pleuronectoidei: Pleuronectidae). The total length of the genome is 16,845 bp, including 13 protein-coding genes, 22 tRNA genes, 12S and 16S rRNA genes, and a control region. 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 deviated significantly from expected equilibrium proportion. The submission to the global database (GenBank) of two new mitogenomes along with 106 analyzed GenBank sequences could contribute to phylogenetics and systematics studies of flounders at the family and suborder levels. Based on 26 and 108 nucleotide sequences of protein coding genes (PCGs), the molecular phylogeny of flounders is investigated and the analysis performed for two sets of sequences, including those of members of the family Pleuronectidae and the suborder Pleuronectoidei. Their significance to the taxonomy at these two levels have estimated. Data that obtained by 4-6 techniques of multi-gene phylogenetic reconstructions support the monophyly of the family Pleuronectidae with high statistical confidence while the conclusion on the suborder that also supports monophyly needs further investigation. They also reviled paraphyletic and weakly supported branches that are especially numerous in the suborder which property suppose the necessity for taxonomic revisions firstly for the suborder level and with less intense to the family level.

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MULTIPLE CHROMOSOMAL REARRANGEMENTS IN EVORON VOLE (RODENTIA)

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The Evoron vole *A. evoronensis* is the endemic vole species found in the intermountain landscape of the southern Russian Far East. It inhabits the Evoron-Chukchagir lowland, the Upper Zeya Plain, and the Upper Bureya Depression. The Evoron voles of the Evoron-Chukchagir lowland, with the maximum number of chromosomes for the species (2n = 38-41, NF = 54-59), belong to the "Evoron" chromosomal race, as they were the first to be found on the shores of Lake Evoron. Voles with the minimum number of chromosomes for the species (2n = 34, 36, 37, NF = 51-56) were assigned to the "Argi" chromosomal race.

Using GTG-, GTC-, NOR staining methods we described multiple structural chromosomal rearrangements in the Evoron vole (Kartavtseva et al., 2021 a, b).

Two isolated populations of the "Argi" chromosomal race from Upper Zeya Plain, and the Upper Bureya Depression have identical polymorphism. We revealed the tandem fusions (Mev11/19, Mev13/15, Mev17/18, Mev6/7/14) and the Robertsonian translocations (Mev13.15 and Mev17.18) that led to eight new variants of the karyotype described. We observed the tandem fusion (Mev6/7/14) of chromosomes in heterozygous states in both populations. In the "Evoron" chromosomal race from Evoron-Chukchagir lowland the 4 chromosomes (Mev1, Mev4, Mev17, and Mev18) took part in tandem fusion.

Tandem and Robertsonian rearrangements (Mev17/18 and Mev17.18) were revealed in both "Evoron" and "Argi" chromosomal races. The combination of all the variations of chromosomes for the species made it possible to describe 20 variants of the *A. evoronensis* karyotype, with 11 chromosomes which being involved in multiple structural rearrangements.

Prolific offspring from the individuals with tandem fusion in the karyotype and a high percentage of this rearrangement in individuals from natural sample sets indicates the absence of a harmful effect of current aberration on the viability of voles.

MOLECULAR MARKERS AND SOCIETY NEED: FROM BIODIVERSITY ASSESSMENT AND MODERN GENERAL BIOLOGY PARADIGM VALIDATION TO SEAFOOD MISLABELING DETECTION

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Application of molecular genetic markers is very fruitful in many directions. One is validation of modern paradigms, the Synthetic Theory of Evolution (STE) plus Biological Species Concept (BSC) and another is a Biodiversity estimation for the variety of social needs including false labelling of marine species as food sources. Vast sequence data on nDNA and mtDNA are gathered and analyzed by a variety of software tools.

The main issues of the report are focused on 5 items. (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 obvious the introgression among many taxa, 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 marine and terrestrial realms are introgressed, as it is still evident for many cases, then we should recognize that the BSC, in terms of complete lack of gene flow among species, is inadequate due to the fact, that many zoological species are not biological species yet. However, vast modern molecular data proved that sooner or later they will definitely become biological species (Kartavtsev, 2013, 2018; Hedges et al., 2015). (4) The recent investigation of fish taxa divergence (Kartavtsev, 2017, 2018) using BOLD database shows that most gene trees are basically monophyletic and interspecies reticulations are rare. (5) A variety of evidence are available globally that proved a fraud of seafood; thus, scientists must to develop a molecular control for this and similar industry spheres.

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A METAGENOMIC ANALYSIS OF THE HOST-PARASITE SYSTEM: THE INTERACTION AMONG SYMBIOTIC MICROBES, HELMINTHS AND FISH

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The impact of parasites on gut microbiota of the host is well documented, but the role of the relationship between the parasite and the host in the formation of the microbiota is poorly understood. Using 16S rDNA amplicon sequencing we characterize the gut microbiota of the sympatric pair of whitefish Coregonus lavaretus complex and pike Esox lucius and the associated microbiota of cestodes parasitizing their intestine. The essence of the proposed approaches is, firstly, to use the method of successive washes of the microbiota from the cestode's surfaces to analyze the degree of bacterial association to the tegument of the parasite. Secondly, to use a method combining the sampling of intestinal content and mucosa with the wash-out procedure from the mucosa to understand the real structure of the fish gut microbiota. Our results demonstrate that the type of sample analyzed (mucosa, content), segment of gastrointestinal tract (anterior, posterior), infection status (infected, uninfected) were significant factors that affected on the associated microbiota of fish. Additional environmental niches for settlement of bacteria in the intestine are formed by the parasitic helminths that caused the restructuring of the bacterial community in infected fish compared to those uninfected. Using the desorption method in Ringer's solution, we have demonstrated that cestodes possess their own microbial community which is put together from "surface" bacteria received from the host, bacteria which are weakly and strongly associated with the tegument, and microbiota obtained after removal of the tegument from the cestodes.

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TAXONOMIC STATUS OF MOLLUSCS OF THE GENUS PARAJUGA (SEMICULCOSPIRIDAE)

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Freshwater molluscs are an important part of benthos, which prevails in most rivers and lakes in terms of abundance and biomass (Prozorova, 2013). They play a role of aquatic biofilters, take part in the decomposition of organic matter, and are also source of food for many birds and mammals. At the same time, molluscs are the first intermediate hosts for various parasites such as *Nanophyetus salmincola* and *Metagonimus suifunensis*.

In 2009, based on molecular analysis, Strong and Köhler (2009) indicated that *Parajuga* is a paraphyletic and an invalid taxon. Later, Köhler (2016), based on molecular data for one specimen of *Parajuga amurensis*, suggested that it belongs to *Koreoleptoxis amurensis*. However, the systematic position of other members of the genus *Parajuga* is not resolved due to the lack of molecular, anatomical, and morphological criteria.

In this study, the nucleotide sequences of the *16S* rRNA gene of mitochondrial DNA are analyzed to clarify the phylogenetic position of representatives of the genus *Parajuga* inhabiting the territory of the Russian southern Far East. Partial sequences of the gene are identical for *Koreoleptoxis amurensis*, *P. nodosa* and *Parajuga* sp., and the *p*-distances between them and *P. heukelomiana* and *P. amurensis* have intraspecific level. Therefore, it is not excluded that all specimens in this analysis are belong to the single species *Koreoleptoxis amurensis*.

DNA BARCODING BASED INTRA-BASIN COMPARISON OF FISH COMMUNITIES RESOLVES ISSUES RELATED TO OVERLOOKED TAXA

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Five major river basins were sampled over a period of ten years for their entire fish fauna. The rivers selected are the Godavari, Narmada, Krishna, Mahanadi and Tapi. These rivers basins were sampled by selecting all ecological regions. Sampling was undertaken over three seasons for multiple time. The collected fish specimens were taxonomically scrutinized followed by preparation of DNA barcode library. The barcode data is deposited to BOLD system under individual project for respective rivers. The data obtained were analyzed individually for every individual river basin which includes the congener, conspecific distances were analyzed followed by barcode gap discovery. Similarly, various datasets were created by combining the data of two basins, three basins, four basins and five basins.

The individual data sets were taxonomically following 3% rule for the species boundary and not conflicting with taxa in the dataset. Interestingly, when inter-basin datasets were combined and data was analyzed, in over 10% species records representing conflicting taxa. To resolve such conflicting taxa several records were reanalyzed and partitioned for cryptic taxa. This approach has helped in discovering cryptic diversity and probed for careful use of 3% rule for species boundary.

AS-PCR FOR POPULATION ANALYSIS OF METAGONIMUS SUIFUNENSIS

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The trematode *Metagonimus suifunensis* was first described as a separate species few years ago (Shumenko et al., 2017). This species inhabits the south of the Russian Far East and was previously recorded in this area as *Metagonimus yokogawai* by Shatrov (1974). According to morphological data, *M. suifunensis* and *M. yokogawai* have no visual distinctions, and the difference in nuclear ribosomal DNA sequences formed the basis for identifying a new species. Sequence analysis for population of *M. suifunensis* revealed a single nonsynonymous polymorphism at position 419 bp in the conserved region of the cytochrome b redox center (Tatonova, Shumenko, 2021). The authors suggested that close location of the replacement to the functional center and the presence of both variants of amino acids in equal proportions in the population may be associated with the adaptive ability of specimens for surviving in different environmental conditions. Therefore, the study of this variability type for the sample with a larger size can be useful for assessing the prevalence of allelic variants in different area parts of *M. suifunensis*.

One of the most accessible methods for the analysis of single nucleotide polymorphisms is the allele-specific polymerase chain reaction (as-PCR). It can be used to analyze a large number of samples in a short period of time. In this study, allele-specific primers are developed for *M. suifunensis*, which can help in studying the population structure of a new to the Russian Far East species. In addition, these primers can be used for medical purposes. Diagnostic kits based on the designed primers can help quickly identify the parasite infestation from various parts of the studied region.

ON SOVIET GENETICS AND GENETICISTS IN THE DIARY OF TH. DOBZHANSKY

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Feodosiy Grigoryevich Dobrzhansky or Th. Dobzhansky (1900-1975), Russian (Soviet) and American geneticist, evolutionist and humanist. Dobzhansky was born in Nemirov, Podolsk province of the Russian Empire (now the Vinnytsia region of Ukraine), graduated from Kiev University. In the 1920s, at the invitation of Yu. A. Filipchenko, head of the country's first Department of Genetics at Leningrad State University, he worked at this department. In December 1927, as a fellow of the Rockefeller Foundation, he went on an internship at the laboratory of T.H. Morgan in the USA. In 1931 after long and painful thoughts, he decided to stay there, and in 1936 he became an American citizen.

Throughout his life, from a young age, Dobzhansky kept a diary, which is a unique source on the history of evolutionary biology of the XX century, especially evolutionary genetics. The diary contains a number of entries about Soviet genetics and Soviet geneticists, with whom Dobzhansky met at international genetic congresses and corresponded first from 1928 to 1934, and then from 1958 to 1975. His main correspondents were Yu.A. Filipchenko, N.I. Vavilov, G.A. Levitsky, G.D. Karpechenko, B.M. Zavadovsky, Yu.Ya. Kerkis, N.N. Medvedev, A.S. Serebrovsky, N.P. Dubinin, B.L. Astaurov, D.K. Belyaev, N.N. Vorontsov, J.A. Medvedev, V.N. Soyfer and other scientists.

The diary is kept in the Library of the American Philosophical Society (APSL).

О СОВЕТСКОЙ ГЕНЕТИКЕ И ГЕНЕТИКАХ В ДНЕВНИКЕ Ф.Г. ДОБРЖАНСКОГО

М.Б. Конашев

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Феодосий Григорьевич Добржанский или Th. Dobzhansky (1900-1975), русский (советский) и американский генетик, эволюционист и гуманист. Добржанский родился в г. Немирове Подольской губернии Российской империи (в настоящее время Винницкая область Украины), закончил Киевский университет. В 1920-е годы по приглашению Ю. А. Филипченко, заведующего первой в стране кафедры генетики Ленинградского государственного университета, работал на этой кафедре. В декабре 1927 г. в качестве стипендиата фонда Рокфеллера отправился на стажировку в лабораторию Т. Х. Моргана в США. В 1931 г. после долгих и мучительных раздумий принял решение остаться там, в 1936 г. стал американским гражданином.

Всю свою жизнь, с юношеского возраста, Добржанский вёл дневник, который представляет собой уникальный источник по истории эволюционной биологии XX в., в особенности эволюционной генетике. В дневнике имеется ряд записей о советской генетике и советских генетиках, с которыми Добржанский встречался на международных генетических конгрессах и переписывался сначала с 1928 по 1934 г, а затем с 1958 по 1975 г. Основными его корреспондентами были Ю.А. Филипченко, Н. И. Вавилов, Г. А. Левитский, Г. Д. Карпеченко, Б. М. Завадовский, Ю. Я. Керкис, Н. Н. Медведев, А. С. Серебровским, Н. П. Дубинин, Б. Л. Астауровым, Д. К. Беляев, Н. Н. Воронцов, Ж. А. Медведев, В. Н. Сойфер и другие ученые.

Дневник хранится в Библиотеке Американского философского общества (APSL).

PHYLOGEOGRAPHY OF TWO SPECIES COMPLEXES OF THE GENUS *LOTUS* (LEGUMINOSAE): WHAT GENETIC VARIABILITY CAN TELL

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Genus *Lotus* includes ca. 130 species of annual and perennial herbs, semishrubs and small shrubs of the tribe Loteae (Leguminosae). The genus is widely distributed in the Old World and has the main diversity center in the Mediterranean, from where the genus presumably originated. *Lotus* includes several species complexes, i. e. groups of species with complicated structure and unclear interspecific boundaries. Our study was focused on the genetic structure in two species complexes of the northern evolutionary branch of the genus *Lotus*.

Lotus dorycnium s. l. is a complex of taxa traditionally regarded as members of Dorycnium, however molecular phylogenetic data support placement of the complex in the genus Lotus. The complex has a wide Mediterranean range, extending in the north to Central and Eastern Europe, and in the east to the Crimea, the Caucasus, and the Western Caspian region. The results of the morphological analyses demonstrated sertain degree of differentiation, with some taxa (treated as subspecies) more or less well defined, whereas the others hardly distinguished from each other. Analyses of the L. dorycnium complex based on nrITS revealed a tendency towards a geographic differentiation into Western, Eastern, and South-Eastern (=Turkish) groups. Phylogenetic and phylogeographic analyses of the same set of specimens using concatenated plastid markers trnL-F, rps16, and psbA-trnH demonstrated a low resolution between the L. dorycnium complex and L. hirsutus, as well as among the taxa within the L. dorycnium complex, which can be interpreted as evidence of an incomplete lineage sorting or hybridization. The haplotype network of the L. dorycnium complex is branched, with many missing/hypothetical haplotypes and a predominance of singletons. The evolutionary processes responsible for incongruence in phylogenetic signals between plastid and nuclear sequences of the morphologically well-defined species L. dorycnium and L. hirsutus were most likely localized in the Eastern Mediterranean. A possibility of rare gene exchange between the L. dorycnium complex and the group of L. graecus is also revealed.

Another set of species that studied from the genus *Lotus* is the *L. corniculatus* complex. The geographical range of the complex includes the Mediterranean basin, the high mountains of eastern sub-Saharan Africa, main part of Europe (except the northernmost Arctic regions) and southwestern, central and eastern Asia. A haplotype network for this complex was constructed using cpDNA trnL-F region. Several important differences can be outlined between cpDNA haplotype networks of L. dorycnium and L. corniculatus species complexes, associated with their different evolutionary histories. It is assumed that the origin of both complexes is associated with the Mediterranean, and then the members of the complexes spread to more northern and eastern regions. Some representatives of the L. corniculatus complex have migrated much further to the north and east (up to Southern Siberia and North-Western China) and have undergone a recent expansion there, as evidenced by the presence of widespread haplotypes and a low number of derived haplotypes. In contrast, most subspecies of L. dorycnium apparently existed for a long time in the Mediterranean region, undergoing fluctuations in abundance, as evidenced by the presence of many missing haplotypes and the multimodal distribution of pairwise substitutions. We hypothesize that L. dorycnium ssp. herbaceus may have undergone a relatively recent expansion to the east as evidenced by the unimodal mismatch distribution.

PHYLOGEOGRAPHY OF THE SIBERIAN FLYING SQUIRREL (*PTEROMYS VOLANS* L., 1785) INFERRED FROM MITOCHONDRIAL CYTOCHROME B SEQUENCES: NEW DATA FROM CONTINENTAL AND ISLAND POPULATIONS

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The biological specificity (wood dependent species) and wide spread (boreal forests from Finland to Eastern Siberia, Korean Peninsula, Sakhalin and Hokkaido Islands) of siberian flying squirrel (*Pteromys volans*) assume that the current genetic diversity and the history of the species formation are associated with the dynamics of the Eurasian boreal forest zone in the Quaternary. Although the previous phylogeographic studies of the species cover almost the entire species distribution area and propose the hypotheses to explain modern genetic structure of the species (Oshida et al., 2005; Yalkovskaya et al., 2015), a number of regions both from the continental and islands parts of the range, which are probably important for verifying the proposed evolutionary scenarios, remain unexplored.

In this study we present the data on 9 complete *cyt b* gene sequences (1140 bp) of *P. volans* from previously unstudied area: two localities in the north-west of Western Siberia (a region located on the way of the species' expansion from the eastern to the western Palearctic); and three localities in Sakhalin Island, assumed as migrants on a way to Hokkaido (Oshida et al., 2005). Four haplotypes are identified, and all of them are new. The results of phylogenetic reconstructions (BI, MN, NJ, MJ-network) do not contradict in general the previously reconstructed phylogenetic structure of the species. Three phylogroups "Hokkaido", "Far East", and "Northern Eurasia" with the subgroup "Northwestern Eurasia" within the latter are distinguished. The Western Siberian haplotypes are included in the "Northwestern Eurasia" subgroup together with haplotypes from the European part of the species range, the Urals and southeastern part of Western Siberia. The haplotypes from Sakhalin belong to the "Far East" phylogroup, and form a highly supported group within it.

The obtained results demonstrate that the northern West Siberian populations of *P. volans* are included to the "Northwestern Eurasia" subgroup. This subgroup distributions from Europe to Eastern Siberia. Significant genetic distance between the Sakhalin and Hokkaido haplotypes does not confirm the hypothesis of the colonization of Hokkaido through Sakhalin.

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ASSESSING GENOMIC DIVERGENCE BETWEEN CHINESE SPOT-BILLED DUCK AND MALLARD WITH ddRAD-SEQ DATA

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Mallard *Anas platyrhynchos* and Chinese Spot-billed Duck *A. zonorhyncha* are closely related and partly sympatric species with their breeding ranges overlapping in East Asia. Hybridization between them is well known but does not appear to be frequent. Several phenotypic hybrids are found each year in the south of Russian Far East. Mitochondrial and nuclear DNA markers were unable to differentiate these species due to their very recent divergence, incomplete lineage sorting, and gene flow caused by episodic hybridization. We used double-digest restriction-associated DNA sequencing (ddRAD-seq) to examine genomic divergence between Mallard and Chinese Spot-billed Duck. In addition to the overall ddRAD-seq allele frequency differences between the species, several diagnostic SNPs located on the Z-chromosome that clearly discriminate Mallard and Chinese Spot-billed Duck were discovered. These SNPs are the first species-specific molecular markers revealed among mallard-group species.

REPEATED ADAPTIVE RADIATIONS AMONG CYPRINIDS IN ETHIOPIAN HIGHLANDS

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Multiple repeated patterns of adaptive radiation were revealed in cyprinid fishes of the genus *Labeobarbus* inhabiting the rivers of Ethiopian Highlands. We detected at least four independently evolved radiations in the evolutionary lineage of hexaploids (2n = 150) in *Labeobarbus* genus based on matrilineal relationships of more than 800 individuals. Each radiation manifested similar patterns of mouth phenotype diversity, and included ecomorphs/species of the generalized, lipped, scraping (one or two), and large-mouthed (one to three) types. All radiations were detected in geographically isolated rivers, and originated from different ancestral populations. This is the first documented case in which multiple parallel radiations of fishes occurred in a riverine environment. Some radiations are very recent and monophyletic, while others are older and include ecomorphs that originated in separate mini-flocks and further combined into one. The diversification bursts among Ethiopian *Labeobarbus* were detected in the middle-to-upper reaches of rivers (1050-1550 m above the sea level), which likely offer ecological opportunities (diverse habitats) and relaxed selection (depauperated fauna and subsequently lower competition).

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DNA-BARCODING OF VOLGA FISHES: UNEXPECTED DIVERSITY AND BIOGEOGRAPHICAL IMPLICATIONS

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Volga is the longest European river (3690 km) with a catchment area of about 1360000 square kilometers. Volga basin is known to experience increased anthropogenic impact because of its location in the area that is densely populated by humans. Almost half of the population in Russia (ca. 70 million people) lives around Volga basin. Fish fauna of the Volga River includes ca. 90 species. This study represents the first comprehensive molecular assessment of freshwater fishes and lampreys from the Volga River basin. In total, 1070 DNA barcodes belonging to 74 species of 24 families and 54 genera from 175 localities were produced. Our study revealed highly underestimated species diversity within some indigenous species, local endemism for several species inhabited Kama subbasin and highly complex history of Volga colonization. Biogeographically, Volga was colonized from almost all adjacent basins and may represent one of the largest melting pots for widely distributed species that experienced secondary contact.

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THE DISTINCT MtDNA LINEAGE OF THE DUCK MUSSEL (ANODONTA ANATINA) IS THE EVIDENCE FOR PLIO-PLEISTOCENE REFUGIUM IN THE AZOV SEA RIVER BASINS

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Freshwater mussels (Bivalvia: Unionoida) have a significant importance in freshwater habitats as ecosystem engineers of the water environment. The duck mussel (Anodonta anatina) is widely distributed throughout Europe, Siberia, Western and Central Asia, which makes it a suitable model object for biogeographic studies. We have analyzed the divergence of A. anatina populations, and discovered an existence of the separate genetic lineage that distributed in rivers of the Azov Sea basin. This finding was confirmed by high genetic distances between this group and previously defined populations, and by the position of this clade in the Bayesian phylogeny that built and time calibrated. Biogeographic scenarios of A. anatina dispersal in Europe, Northern, Western, and Central Asia over the Neogene–Quaternary were simulated using the approximate Bayesian computation (ABC) analysis. The isolation of this mitochondrial DNA haplogroup in rivers of the Azov Sea basin most likely occurred in the Late Pliocene that was probably facilitated by rearrangement of freshwater basins boundaries in the Ponto-Caspian region. Population genetic indices show, that this group exists in river basins of the region for a long time. The studying of a long-term refugium in rivers of the Azov Sea helps to a better understanding of freshwater fauna evolution in the Neogene–Quaternary and highlighted the importance of conservation of these freshwater animals in the region as a source of unique genetic diversity.

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GENETIC AND MORPHOMETRIC VARIABILITY IN SETTLEMENTS OF TWO MUSSEL SPECIES (MYTILUS EX. GR. EDULIS), M. TROSSULUS AND M. GALLOPROVINCIALIS, IN THE NORTHWESTERN SEA OF JAPAN

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Genetic and morphometric variability in mussels are important issues that attract attention of numerous researchers with a special emphasis on hybrid occurrence and genetic introgression in recent years. Genetic and multidimensional discriminant function analysis (DFA) that included morphometric traits (MT) were performed on mussels, *Mytilus trossulus* and *Mytilus galloprovincialis*, taken from the northwestern Sea of Japan (NWSJ).

The analysis, based on eight polymorphic enzyme loci and two nuclear DNA markers, used samples from eight settlements in 2011 and samples from six settlements in 2012 to 2013 for a smaller set of loci but jointly with MT analysis. If the average generation length is taken as three years, the number of immigrants (Nm) per generation was estimated approximately as Nm = 5. Assuming that interspecific gene flow is from offspring of F2, F3, and Fb generations rather than F1, the fraction of interspecific migrants estimated as Fb + F2 etc. are equal to $0.9\% \pm 0.7\%$. The data suggest a continuing invasion of *M. galloprovincialis* into NWSJ. Judging from the occurrence of hybrids of all types, the rate of genetic introgression between the two taxa is low. During 14 years in the Vostok Bay area, it varied from 0% in 2012-2013 to $8.95\% \pm 1.68\%$ back in 1999. Our data support the concept of an existing bimodal hybrid zone, with the contact zone showing a limited degree of hybrids differ was determined by DFA using GLU-5 genotypes as a diagnostic grouping variable. *M. trossulus* and F1 offspring collected in 2012 and 2013 were detected correctly with a high accuracy, of about 94%. Despite that, DFA showed generally weak differentiation among settlements even with the use of a combined set of traits and indices.

PHYLOGENETIC AND BIOGEOGRAPHIC REVISION OF THE GENUS GORGONOCEPHALUS USING MITOCHONDRIAL DNA POLYMORPHISM

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Basket stars of the genus *Gorgonocephalus* live in coldwater environments including the Arctic, Antarctic, and deep-sea habitats. In the Russian Arctic seas, four species were found. *G. lamarckii* (Müller et Troschel, 1842) lives only in the western Barents Sea. *G. caryi* (Lyman, 1860) was described from the North Pacific and observed also in the southern Chukchi Sea. Currently, *G. caryi* is included in the *G. eucnemis* species (WoRMS, 2022). *G. eucnemis* (Müller et Troschel, 1842) and *G. arcticus* Leach, 1819 are widespread circum-Arctic species that often form mixed and abundant settlements.

In the Barents and the Kara Seas, species identification of *G. arcticus and G. eucnemis* is clear because diagnostic features are discrete and there are no mixed features. In the Laptev, East Siberian and Chukchi seas, basket stars with intermediate features could be found along with the typical forms of both species. The presence of continuous series of transitional forms served as the basis for merging these species into one, so that *G. arcticus* became a senior synonym and *G. eucnemis* a junior synonim (Smirnov, Smirnov, 1990, 1994, 2006, 2009; List of species... 2001). At the same time, in the World register of marine species, both species are listed (WoRMS, 2022). Therefore, the taxonomic status of G. *arcticus* and *G. eucnemis* is uncertain and requires clarification.

Thirty-eight *Gorgonocephalus* specimens from the Russian Arctic seas were collected in 2019-2020 in VNIRO expeditions. There were 14 specimens of typical *G. arcticus*, 17 typical *G. eucnemis* and 7 specimens with mixed diagnostic features. Barcode fragment of the cytochrome c oxidase gene, *COI* (Folmer et al., 1994) was used for the molecular analysis. Also, 27 *G. arcticus* and 17 *G. eucnemis* sequences were mined from the NCBI GenBank.

The total aligned *COI* data set consisted of 449 nucleotide positions for 72 sequences. Among 22 identified haplotypes, 7 were common for the *G. arcticus* and *G. eucnemis*. Specimens with mixed morphological features had 3 different haplotypes: one of these haplotypes was found also in *G. arcticus*, and two others were common for both species. The number of species defined using the Automatic Barcode Gap Discovery method (Puillandre et al., 2012) varied with the different prior thresholds ranging from 0.0028 to 0.02 prior intraspecific divergences. The lowest threshold value clustered haplotypes into 5 groups, but the highest threshold combined all haplotypes together. There are no clear geographical patterns in haplotype distribution. One common haplotype for the Atlantic (Fundy bay), Pacific (British Columbia) and the Kara Sea was found. The existence of common haplotype in the basket stars from the Barents, Kara, Laptev and Chukchi seas shows that there is no gap between eastern and western arctic populations, in contrast, e.g., to the other circum-Arctic brittle star species *Ophiura sarsii* (Genelt-Yanovskiy et al., 2021). Therefore, mitochondrial DNA data support combining *G. arcticus* and *G. eucnemis* into one species, but investigation of nuclear markers is also necessary to clarify this issue.

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INTERGENERIC HYBRIDIZATION OF TWO STICKLEBACK SPECIES LEADS TO INTROGRESSION OF MEMBRANE-ASSOCIATED GENES AND INVASIVE EXPANSION

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Interspecific hybridization has occurred relatively frequently during the evolution of vertebrates. This process usually eliminates reproductive isolation between the parental species. Moreover, it results in the exchange of genetic material and can lead to hybridogenic speciation. Hybridization between species has predominately been observed at the interspecific level, whereas intergeneric hybridization is rarer. In this study, using whole-genome sequencing analysis, we describe clear and reliable signals of intergeneric introgression between the three-spined stickleback (*Gasterosteus aculeatus*) and its distant freshwater relative the nine-spined stickleback (*Pungitius pungitius*) that inhabit northwestern Russia. Through comparative analysis, we demonstrated that such an introgression phenomenon take place in the low-salinity White Sea basin, although it is not detected in Japanese sea stickleback populations. Bioinformatical analysis of the sites influenced by introgression showed that they are located near transposable elements, whereas those in protein-coding sequences are mostly found in membrane-associated and alternative splicing-related genes.

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SEQUENCES OF ITS IN MARINE INVERTEBRATES METABARCODING

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Metabarcoding studies of marine invertebrates have used various regions of nuclear small-(18S) and large-subunit (28S) rRNA genes, which allow accurate classification of novel sequences and reliable amplification with conservative primers. However, this may underestimate species diversity in a community under investigation due to their low nucleotide distances (if any) in closely related species. To assess faunistic diversity at the species level, more variable genes are needed. Widely used Folmer DNA barcoding region COI is not often suitable because of common pseudogene amplification or pure primer match. We propose to use the *ITS* (internal transcribe spacer of rRNA) region for metabarcoding of invertebrates in the Arctic seas. For some taxa the ITS has been used quite often as a nuclear marker in recent years, in particular, for the representatives of Cnidaria. The use of this marker for the representatives of this group is rather successful, but in many cases, its use is limited due to intragenomic polymorphism that associated with multicopy of this marker (and as a result it cannot be used in the Sanger sequencing). Amplicon sequencing by NGS with relatively long reads (such as offered by MiSeq chemistry) makes the application of this marker highly effective. We obtained sequences of ITS2 region for more than 300 species from different taxa using the Illumina Miseq technology. Thus, we have now a representative Database and hope that we can use metabarcoding analysis for monitoring of Arctic marine ecosystems.

DIVERSITY IN OROSTACHYS SPINOSA (CRASSULACEAE) CHLOROPLAST DNA MARKERS IN THE ALTAI MOUNTAINS

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Mostly biennial plant *Orostachys spinosa* (L.) Sweet is a small, slow-growing succulent belonging to the genus *Orostachys* Fisch., family Crassulaceae J.St.-Hil. It grows in steppe and forest-steppe zones on sandy soils, on dry slopes of ravines from the sea level up to 3000 m (Goncharova, 2006; Nikulin et. al., 2020). Representatives of the genus are distributed in the temperate mountainous zone of Asia from Kazakhstan and Mongolia to the Far East of Russia and Japan. It is interesting species not only from the point view of plant physiology (it lives in different altitudes and climate zones), but also due to its valuable medical properties (it is a source of some bioactive compounds), and also because of genetic features. Despite the fact that this species is of great theoretical and practical interest, at the present moment, there are only a few studies of its genetic characteristics, and natural populations coverage in some parts of its geographic range is insufficient.

In this research, we analyzed a total of 96 plant samples from 16 different populations located in the Altai Mountains using DNA sequence analyses for 3 regions of intergenic spacers of chloroplast DNA (cpDNA): *trnH–psbA*, *rpL32–trnL*, *trnQ–rpS16*.

Analysis of the data sets showed that the length variation in all markers was due to indels (insertions/deletions). In addition, inversions were found in the *trnH–psbA* and *rpl32–trnL* spacers.

The total length of the combined sequences of the three regions was 2342 bp; 108 variable sites were found, including 84 sites informative according to the maximum parsimony method. Analysis of 96 sequences revealed 49 haplotypes, of which 21 (43%) were unique.

On the haplotype network, we defined 2 groups and one cluster of haplotypes (A, B and C, respectively). Divergence of haplotypes within the group A was less (from 1 to 3 mutational steps) than that in the group B (6–8 steps) and cluster C (1–16 steps). Haplotypes found in different populations were included in groups irregularly. Only two populations clearly belonged to one group – A. Furthermore, analysis of the dataset in the Arlequin program allowed us to characterize the studied populations based on the parameters of genetic diversity. The number of polymorphic sites (pS) and the index of haplotype diversity (h) were high in almost all populations, except for the populations 7 (MAR) and 1 (SEM).

Our data confirm the hypothesis proposed by Nikulin et al. (2020) that *O. spinosa* could have originated in the Altai mountains, which is not only the center of ancestral genetic diversity, but also a refugium.

COMPARATIVE TRANSCRIPTOMIC ANALYSIS REVEALED DYNAMIC CHANGES OF DISTINCT CLASSES OF GENES DURING DEVELOPMENT OF THE MANILA CLAM (RUDITAPESPHILIPPINARUM)

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The Manila clam *Ruditapesphilippinarum* is one of the most economically important marine shellfish. However, the molecular mechanisms of early development in Manila clams are largely unknown. In this study, we collected samples from 13 stages of early development in Manila clam and compared the mRNA expression pattern between samples by RNA-seq techniques.

We applied RNA-seq technology to 13 embryonic and larval stages of the Manila clam to identify critical genes and pathways involved in their development and biological characteristics. Important genes associated with different morphologies during the early fertilized egg, cell division, cell differentiation, hatching, and metamorphosis stages were identified. We detected the highest number of differentially expressed genes in the comparison of the pediveliger and single pipe juvenile stages, which is a time when biological characteristics greatly change during metamorphosis. Gene Ontology (GO) enrichment analysis showed that expression levels of microtubule protein-related molecules and Rho genes were upregulated and that GO terms such as ribosome, translation, and organelle were enriched in the early development stages of the Manila clam. Kyoto Encyclopedia of Genes and Genomes pathway analysis showed that the foxo, wnt, and transforming growth factor-beta pathways were significantly enriched during early development. These results provide insights into the molecular mechanisms at work during different periods of early development of Manila clams. These transcriptomic data provide clues to the molecular mechanisms underlying the development of Manila clam larvae. These results will help to improve Manila clam reproduction and development.

EFFECTS OF HYPOXIA ON COGNITIVE PROCESSES IN DROSOPHILA

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The existence of common mechanisms underlying the formation of stress response and learning has now been experimentally proven. This allows us to think about the existence of a common mechanism that triggers adaptive reactions, and, possibly, environmental factors, on the impact of which these reactions are formed. One of the important, but insufficiently studied external factors is changes in the geomagnetic field, inextricably linked with daily rhythms. The primary magnetic field acceptor of the cell are the cryptochrome family proteins (CRY), which function as blue light receptors and are known as repressors of the main circadian transcription complex CLOCK/BMAL1. We have previously shown the effect of a weakened static magnetic field on cognitive processes in *Drosophila*. Another important aspect of the influence of magnetic field is its protective effect in the development of oxidative stress and hypoxia. This indicates the commonality of the mechanisms underlying these processes, which allows through the arsenal of Drosophila neurological mutations and the use of another stressful agent, hypoxia, to expand ideas about the formation of adaptive reactions. Severe forms of hypoxia suppress the processes of neuroplasticity, cause disturbances of learning and memory. According to our data, training flies in hypoxia increases learning ability. From the point of view of finding targets of hypoxic exposure, Drosophila mutant cardinal (cd) with a disturbance of the kynurenine tryptophan exchange pathway, which causes the accumulation of 3-hydroxykinurenine, an oxidative stress generator, is extremely interesting. Differences in the frequency of double-strand DNA breaks, after exposure to hypoxia, were identified in *cd* mutant and wild-type *CS* strain. Double-strand breaks are essential in the implementation of cognitive processes, being an indicator of physiological activity in neurons. Double-strand breaks in chromatin remodeling are hypothesized to be necessary for expression of genes involved in memory formation and learning processes. The CRY regulation in transcription of genes controlling circadian rhythms through the CLOCK/BMAL1 heterodimer extends magnetic field effects to the expression of a key transcriptional regulator of oxygen starvation adaptation, the HIF1, whose promoter contains a regulatory motif for daily rhythm control genes. Due to the bidirectional interaction of these gene systems, a common mechanism of influence of the magnetic field and hypoxia on the chromosomal apparatus is also possible. The CRY/CLOCK/BMAL1 system controls circadian rhythms and promotes adaptation of living organisms to changing environmental conditions by linking together magnetoreception, hypoxia, circadian rhythm regulation, cognitive function, and double-strand DNA breaks.

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PHYLOGENY AND DIVERGENCE IN ARCTIC LINEAGE OF CHARRS (SALVELINUS, SALMONIDAE) IN THE NORTHEASTERN ASIA AND NORTH AMERICA

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Charrs of the genus *Salvelinus* are ecologically important components in diverse fish communities in the northeastern Asian and North American freshwater ecosystems. In spite of a rich history of systematic investigations of charrs, there is extensive conflict among previous hypotheses that may be due to restricted taxa or characters sampling. Current views on the phylogenetic groups have been identified that unite the closely related species of charrs (Arctic, Atlantic, Siberia, Acadia, Bering, West Pacific, and East Pacific). Previously, we also proposed to distinguish an independent Arctic phylogenetic group of Taranetz charr *Salvelinus taranetzi* (Oleinik et al. 2015). Further studies of charr mtDNA considerably extended the knowledge about their genetic diversity and phylogenetic relationships in the Pacific basin in East Siberia, and in North American Arctic. A revision of the genus systematics was suggested, which implied consideration of monophyletic groups, revealed by mtDNA analysis, as separate species.

Here we present the phylogenetic analysis of *Salvelinus* that combines DNA sequence data from both the mitochondrial and nuclear genomes and includes Asian and North American charrs with Arctic group haplotypes. Gene sequence data were collected from the three mtDNA fragments (*Cytb, COI* genes, and *CR*), and a complete nuclear DNA growth hormone gene (*GH2*).

We confirmed earlier proposed subdivision of Taranetz charr *S. taranetzi* and Arctic charr *S. alpinus* into separate phylogenetic groups (Oleinik et al. 2015). The Arctic phylogenetic group of *S. taranetzi* and a phylogenetic group of *S. alpinus* (*S. alpinus alpinus* and *S. alpinus oquassa*) have reached the state of reciprocal monophyly without maintaining ancestral polymorphism. There is no doubt that charrs *S. a. erythrinus* from the North American Arctic phylogroup (syns. *S. a. stagnalis* and *S. a. taranetzi*) and Northeast Asia belong to a single species which was first described as the Taranetz charr *S. taranetzi* Kaganovsky, 1955 from Lake Achchen, Chukotka. This conclusion is supported both by the genus phylogeny based on mitochondrial genomes and by the phylogeny based on sequences of the nuclear growth hormone gene *GH2*. Phylogenetic networks (MJ and Neighbor-Net) also confirm the closeness of the taxa that we combined into the Arctic group (*Salvelinus* sp. 4, *S. alpinus erythrinus* (NWT), *S. elgyticus*, *S. boganidae*, *S. andriashevi*).

Results from phylogenetic analysis supported the common origin of *Salvelinus* sp. 4 and *S. alpinus erythrinus* from North America (NWT). New evidences that *Salvelinus* sp. 4 population from Nachikinskoye Lake is an isolated population of *S. taranetzi* were obtained. *Salvelinus* sp. 4 position on the phylogram is strictly determined and statistically confirmed as there are sister relationships between *Salvelinus* sp. 4 – *S. a. erythrinus* and *S. taranetzi*. Assuming the common origin of the *Salvelinus* sp. 4 and *S. a. erythrinus* from the geographically isolated populations, we evaluated the divergence of mitochondrial genomes, which resulted from the common ancestor range fragmentation. Genetic similarity among charrs observed among and within geographical regions probably originated from postglacial colonizations of common ancestors rather than from extensive gene flow. All these findings improve our understanding of the *Salvelinus* systematics and could affect respective evolutionary studies, as well as highlight the importance of revisiting phylogenies using integrative research.

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NEW DATA OF THE CYT B POLYMORPHISM IN THE HARVEST MOUSE (MICROMYS MINUTUS P., 1771) FROM EASTERN SIBERIA, BAIKAL REGION

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The study on phylogeography in wide-range species occupies a key sector in evolutionary ecology research, in particular, in solving issues related to reconstruction of fauna history, understanding the ways and mechanisms of development of taxa and ecosystems in general. One of these species is the harvest mouse *Micromys minutus*, which is widely distributed in the forest and forest-steppe zones in the Palearctic: from northern Spain and Great Britain to Vietnam, China, Japan, and Taiwan. Previously, in the course of genetic analysis using mtDNA, the division of the species into four large clades was shown, and a hypothesis was suggested that, during the course of *M. minutus* modern genetic structure formation, the species passing through few cycles of geographic range contraction and expansion under the influence of Quaternary glacial cycles (Yasuda et al., 2005). It was also shown that the chinese and vietnamese populations form another species, *M. erythrotis* (Abramov et al., 2009). However, at present, a large part of the range of the harvest mouse still remains unexplored, and therefore, the analysis of phylogeny and phylogeography of this species was carried out without taking into account data on the populations from a number of major regions, in particular, eastern Siberia and the Russian Far East.

This paper presents the results of phylogeographic analysis of the harvest mouse based on complete sequences of the *cyt b* gene (1140 bp) mtDNA with the inclusion of new data from eastern Siberia (five individuals from Irkutsk), as well as western Siberia (10 individuals), Ural region (15 individuals) and northeast of the East European Plain (one individual from the Komi Republic), which also remain "blank spots" in the genetic studies of the species.

A total of 13 haplotypes have been described, 12 of which were new, including three haplotypes from eastern Siberia. Phylogenetic reconstructions showed the division of the species into five clades: four, in general, correspond to those identified earlier, and the fifth East-Siberian clade is formed by haplotypes from Irkutsk, sequenced by us. Haplotypes obtained from the Urals, western Siberia and northeast of the East European Plain joined the Central-Eurasian clade, previously called the Russian clade (Yasuda et al., 2005). Also, significant differentiation of the harvest mouse populations from the Russian Far East was revealed; the phylogenetic status of them is still unclear.

Significant differentiation of *M. minutus* from eastern Siberia and the Russian Far East indicates the important role of these regions in the origin of harvest mouse modern genetic diversity; however, further studies using extensive database are needed to substantiate any hypotheses.

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GENETIC STRUCTURE AND VARIABILITY OF SOME SPECIES OF RHODODENDRONS OF THE RUSSIAN FAR EAST

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The vast territory of East Asia, including southwestern Beringia, is considered to have been almost ice free during the Pleistocene. Cold-resistant flora may have persisted in this region expanding or contracting its range during the climate cooling. Only a few plant genera have been studied with a sampling area across their entire geographic range in East Asia; therefore, the understanding of the biogeographic history of alpine flora in this region remains limited. In the present study, genetic variation and population structure of two cold resistant shrubs, alpine shrub Rhododendron aureum and subalpine R. brachycarpum across its range in East Asia were studied. We use nuclear microsatellites as markers (18 loci in 21 populations of *R. aureum* and 14 loci in 7 populations of *R. brachycarpum*) to estimate the level of genetic variation within and between populations. High diversity in all populations of both species was revealed, except remote marginal populations of R. brachycarpum in Sikhote-Aline and Kuril Islands. Strong population differentiation and clear distinction among the geographical groups also were revealed for both species. Phylogenetic analyses for R. aureum revealed three main genetic groups: Siberia, Northeast, and North Pacific. According to the geographical pattern of genetic diversity, the North Pacific group includes populations from Kamchatka, south of Russian Far East, and territories close to central Japan. This group is the most diverse and likely diverged (ABC modeling data) earlier than the Siberia and Northeast groups and predated the Last Glacial Maximum. The pattern of genetic diversity of *R. aureum* supports the survival of the species at high latitudes during the Pleistocene with limited contribution of the southern populations to expansion of the species range to the Northeast region and Siberia. For R. brachycarpum bayesian clustering and principal coordinate analyses indicated that two Russian populations, Sikhote-Alin and Iturup Island, represent extremes of two different migration routes, with one derived from the mainland and one from Japan.

This study was supported by Russian Science Foundation, project № 20-04-00417. This work (partial collection of samples) was carried out within the framework of the state assignment of the Institute of Plant and Animal Ecology, UB RAS; molecular genetic analysis was supported by the Russian Foundation for Basic Research, grant no. 20-04-00417.

LIFE STORY OF PLANT GENOMES: FROM DIPLOID TO POLYPLOID AND BACK

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Repeated cyclic events have occurred through the evolutionary history of recent flowering plants taxa: interspecific hybridization – polyploidization – genome shock (loss of some genes, reorganization of the karyotype, variability of genome, repeatome and transcriptome) stabilization of polyploid genome/karyotype - secondary diploidization - interspecific hybridization. In each coil of this dialectical spiral, from the initial uniformity, through maximum possible diversity to the final uniformity, a genome can become stable at the polyploid stage, which is often accompanied by a transition to apomixis and vegetative reproduction. Such eupolyploids account for about 15–30% of modern flowering plants species; they are especially numerous among plants with a low basic number of chromosomes in the genome (x=2-7), as well as among species of the Arctic and high mountains flora (Wood et al., 2009). But in other plants, a polyploid state of the genome/karyotype exists only as an intermediate and transitional stage. We believe this ephemeral state primarily is an effective way to both destabilizing of a genome and a source of many new combinations of alleles that pass through a rigid filter of selection and are realized later at a secondary diploid stage. Among mechanisms of diploidization of a neopolyploid karyotype are chromosomal rearrangements that lead to a change in the chromosome number in genomes, the so-called dysploidy. In parallel with the processes of karyotype diploidization, contributing to it and supplementing it, there exist processes of structural and epigenetic diploidization of the genome, the loss of some of the duplicated genes. The transition from a polyploid to a diploid state is justified by the fact that neopolyploids cannot provide a high percentage of gametes carrying balanced chromosome sets due to problems with chromosome pairing in meiosis I. Mechanisms of transition to strict pairwise chromosome pairing automatically lead to diploidization of both genome and karyotype. It is also important that the diploid karyotype provides more stringent, faster, more efficient selection of adaptively important new combinations of alleles, thereby contributing to the accumulation of taxonomically significant traits, speciation and, as a result, to progressive evolution.

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THE VARIABILITY OF MITOCHONDRIAL DNA CONTROL REGION IN THREE INVASIVE POPULATIONS OF THE EAST EUROPEAN VOLE (MICROTUS ROSSIAEMERIDIONALIS) IN THE FAR EAST OF RUSSIA

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The East European vole (*Microtus rossiaemeridionalis*) is one of the gray vole species of the "arvalis" group. The native range of this species covers vast territory from southern Finland and Baltic region eastward to the western Siberia and southward to the southern Caucasus and northern Iran, Turkey and Greece. The East European vole is characterized by a rapid expansion of its range and its occurrence outside the known native range. In the last years, this vole migrated with transport into certain areas of western and eastern Siberia and the Russian Far East. The aim of this work was to evaluate the genetic diversity in three invasive populations of the East European vole recently discovered in the Russian Far East.

The obtained results demonstrated lower genetic diversity in the population of the East European vole from the city of Sovetskaya Gavan (Sheremetyeva et al., 2021) compared to the populations from the cities of Khabarovsk and Ulan-Ude. At the same time, the H1 haplotype was common in individuals of all three populations, and the H3 haplotype in the population of the cities of Khabarovsk and Sovetskaya Gavan. The remaining haplotypes had a small number of substitutions in the H1 haplotype, with the exception of two highly differentiated haplotypes, one from the population of the city of Khabarovsk (H21) and the other from the city of Ulan-Ude (H20). It can be assumed that the donor of the invasive voles in the three cities was one population, which is within the range of the European lineage (EU subclade). Low genetic diversity in the Sovetskaya Gavan populations (genetic drift). The presence of highly differentiated haplotypes in the populations of Khabarovsk and Ulan-Ude may indicate, on the one hand, that the invasion of the East European vole occurred repeatedly from different regions of the native range. On the other hand, it is possible that there are also highly differentiated haplotypes in the donor population.

USING CYTB mtDNA GENE FOR ANALYSIS OF POPULATION STRUCTURE OF CLONORCHIS SINENSIS

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Previously, using the nucleotide sequences of the *cytb* mtDNA gene, it was revealed that the trematode *Metagonimus suifunensis* (Heterophyidae) is subdivided into northern and southern populations in the southern Russian Far East. This isolation of parasite individuals could be associated with the possible drying up of the Sungach River, which led to the impossibility of movement of intermediate hosts (mollusks and fish) (Tatonova and Shumenko, 2021). Another trematode from a sister family, *Clonorchis sinensis* (Opisthorchiidae), inhabits the same area, and it has not been analyzed using the *cytb* mtDNA gene. It is possible that this marker will also find a subdivision within a population of *C. sinensis*. Therefore, the aim of this work is to analyze the population structure of *C. sinensis* using the *cytb* mtDNA gene.

For amplification of the full-length sequence of the *cytb* mtDNA gene, several pairs of primers were designed in OligoAnalyzer. As result of the analysis of PCR products of one of the primer pairs using gel electrophoresis, it was detected that the haplotypes of *C. sinensis* individuals from Vietnam differ from those of this parasite from Russia. However, confirmation of the ability of the marker to reveal the population structure requires increasing the sample size for *C. sinensis* from different regions.

POPULATION STRUCTURE ANALYSIS OF *METAGONIMUS SUIFUNENSIS* BASED ON A HIGHLY VARIABLE MT-MARKER, THE *NAD1* GENE

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Metagonimus suifunensis (Trematoda: Heterophyidae), distributed in the Russian southern Far East, was described in 2017 based on the nuclear ribosomal DNA sequences of the *ITS2* region and 28S gene of rDNA. In addition to clarifying the taxonomic status, it is also important to study the variability within parasite population. Previously, we obtained data on the population structure of *M. suifunensis* based on the *cox1* and *cytb* genes of mitochondrial DNA (mtDNA) (Tatonova et al., 2019; Tatonova, Shumenko, 2021). Analysis of the *cox1* sequences revealed low level of nucleotide diversity. The level of variability obtained using the *cytb* sequences was also low; however, the MST showed a clear subdivision of the parasite population into two haplogroups with a similar size that are associated with the geographic distribution of the species. Based on the data obtained for the *cox1* gene, we assumed the spread of the parasite from north to south, from the Khabarovsk Region to the Primorsky Region. It was confirmed by population analysis of *M. suifunensis* using data on the *cytb* gene.

In this work, we use sequences of the *nad1* mtDNA gene to estimate intraspecific variability in *M. suifunensis*. This marker is usually more variable than the *cox1* and *cytb* genes and has been successfully used to study population structure in various trematode species. The level of genetic diversity in the studied populations using this marker is higher than using the *cox1* gene, but is similar to the *cytb* gene. However, the MST based on the *nad1* gene has a simple starlike structure, similar to the *cox1* gene, with one ancestral haplotype. Most haplotypes on this network slightly differ from the main one (1–3 mutational steps), and two of them are more differentiated and have 4 and 7 mutations. Therefore, despite higher level of variability, the *nad1* gene is not an optimal marker for assessing population structure in *Metagonimus suifunensis*. It shows that the level of variability in individual genes is not always a determining factor in selection of suitable nucleotide sequences for population analysis.

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THE USE OF MULTIPLEX REAL-TIME PCR ASSAYS FOR SCREENING STUDIES IN POPULATION AND EVOLUTIONARY GENETICS

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Real-time PCR is widely used in biology and medicine for quantitative analysis of RNA expression. Another application of this method is the detection of specific DNA. These DNA could be both endogenous and exogenous in relation to the biological material under consideration. Real-time PCR with fluorescently labeled probes allows creating multiplex assays for detection of several (up to 6 in general) specific DNA or RNA markers within a single tube. The use of quantitative detection in population and evolutionary studies is sporadic and most often applied in the studies of species composition of microbiomes or assessment of mRNA expression in various groups of organisms.

The aim of this study was to demonstrate the broad potential of this method in population and evolutionary studies in mammals, as an example. Here we present four ongoing studies, where the real-time PCR is one of the main methods of laboratory analysis:

(1) species identification in sibling-species *Microtus arvalis* and *Microtus rossiaemeridionalis*. We use modification of the previously described method of species identification. Modified method allows species identification particularly from the museum samples with a high level of contaminating DNA from the second species;

(2) sex identification in walrus (*Odobenus rosmarus*). We use the sites located on the X and Y chromosomes. We developed a new method applicable both for high-quality (fresh and well preserved) tissue samples and those with degraded DNA (the minimum length of detected DNA fragments in a sample is 100 base pairs);

(3) genotyping of wild boar (*Sus scrofa*) males using several weakly variable markers located on the Y chromosome. A new laboratory method was developed to assign animals to the previously described phylogenetic lineages. Taking into account low variability of the markers under study, the method allows to successfully replace expensive Sanger sequencing;

(4) genotyping of Northern red-backed (*Clethrionomys rutilus*) and European bank (*Clethrionomys glareolus*) voles by markers of mitochondrial and nuclear DNA. We developed a method to express assessment of mtDNA introgression and identification of hybrids of Redbacked voles. This method is currently used for screening the occurrence of interspecific hybrids and mtDNA introgression both in space and time.

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Study 2 was carried out within the framework of the project "Comprehensive study of walruses in the territory of the Yamalo-Nenets Autonomous District" supported by the Government of the Yamalo-Nenets Autonomous District and Gazprom PJSC.

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MAPEEG-2022: Program & Abstracts

BIVALVE TRANSMISSIBLE NEOPLASIA

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Clonally transmissible cancer (CTC) is a neoplastic disease passed from individual to individual by physical transfer of cancer cells. The first inkling of a transmissible cancer came from a study of canine transmissible venereal tumor, CTVT, dating back to 1876. Since then, CTC has been confirmed for CTVT and the facial tumor of Tasmanian devil Sarcophilus harrisii and, more recently, for eight lineages of bivalve transmissible neoplasia (BTN). Etiologically, BTN is disseminated neoplasia – a cancer of hemolymph. The disease is transmitted between individuals through water by free cancer cells. The list of species affected by BTN includes Mya arenaria, Cerastoderma edule, Polititapes aureus, Venus verruscosa, Macoma balthica and four species of blue mussels *Mytilus*. In some cases, BTN lineages are parasitized in species other than those in which they originated. In some cases, two independent lineages are parasitized in the same species. For example, the Pacific mussel *M. trosuulus* gave rise to two lineages, MtrBTN1 and MtrNTN2, of which the latter spread throughout the ocean with mussels fouling the bottoms of ships, and affected other species of the genus *Mytilus*. Obviously, 8 lineages in 9 host species are the tip of the iceberg, and we have yet to learn about the true diversity of BTN. A straightforward method of CTC diagnostics is DNA genotyping. The genotype of CTC cancer cells is different from that of the host cells. The result is genetic chimerism, when an individual possesses cells with different genotypes. At the same time, cancer cells of the same lineage have the same genotype in different infected individuals. Such lineage-specific genotypes are thought to derive from the "patient zero", the host individual in which the cancer originated. In the report, we demonstrate approaches to finding and describing BTNs using original *Mytilus* data as an example.

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CHARACTERISTICS OF INVERSION POLYMORPHISM IN THE SIBERIAN NATURAL POPULATIONS OF MALARIA MOSQUITOES ANOPHELES BEKLEMISHEVI USING FLUORESCENT IN SITU HYBRIDIZATION

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Anopheles beklemishevi Stegnii et Kabanova is a malaria mosquito and member of the *Maculipennis* group inhabiting the Palearctic. Its distribution area is the northernmost among malaria mosquitoes of Eurasia and covers the harshest climatic zones in western and eastern Siberia as well as northern Europe. The genetic mechanisms of cold resistance in *Anopheles* mosquitoes may be related to adaptive inversion polymorphism. *A. beklemishevi* is a rare species of malaria mosquitoes, in which inversions in X chromosome are widespread in natural populations. Until now polymorphic inversions X1 and X2 have been found, according to the literature and our own observations, only in heterozygous state. This could be seen as a Hardy-Weinberg disequilibrium. Because of peculiarities in localization of inversions, the analysis of the order of chromosomal banding in polytene chromosomes does not allow reliably detect inverted homozygous.

The aim of this study was to determine homozygote frequencies for inversions in the X chromosome in natural populations of *A. beklemishevi* using the MD-FISH method. Using the microdissection and PCR we developed a fluorescence DNA-probe marking 1a-3a region of standard X chromosome (X0) of *A. beklemishevi*. This region includes only one, distal, breakpoint of polymorphic inversions X1 and X2. After FISH with a standard X chromosome the signal of DNA-probe appears to be solid; however, in inverted variants of chromosome X1 and X2 the labeled region is divided into two parts in a specific way for each inversion which makes it easy to detect the genotype.

Materials for the study were samples of *Anopheles* mosquitoes larvae that had been caught in natural reservoirs in Siberia from 2019 to 2021. The geography of samples includes specimens from Yamalo-Nenets Autonomous Okrug (YaNAO), Khanty-Mansi Autonomous Okrug (KhMAO), Krasnoyarsk Krai (KK), Tomsk Oblast (TO) and Altay Republic.

A total of 685 larvae of malaria mosquitoes were identified from 19 natural populations. The distance between the northernmost (village Shuryshkary, KhMAO) and southernmost populations (village Ozernoye, Altay Rep.) was almost 2000 kilometers along the straight line. Larvae of *A. beklemishevi* usually occur together with *A. messeae* and *A. daciae*; however, in the north and in the highlands, as well as during the early summer period, mainly only individuals of the former species can be found. The standard homozygous cytotype was found in all populations with a sharp predominance in frequency of occurrence over the inverted homozygotes. X1 inversion in homozygous state was found with the frequencies from 3.6 % to 3.9 % in only two populations (villages Choya, Altay Rep., and town Strezhevoi, TO), while homozygous individuals with X2 cytotype were found in seven populations with the frequencies from 7.2% to 33.33% in foothills of Altay, middle (TO and KK) and northern (KhMAO) latitudes of Siberia. Inversions were also found in heterozygous state in the same populations as homozygotes. This research showed the effectiveness of using MD-FISH method to detect homozygotes for inversions in the X chromosome *A. beklemishevi* which can occur with high frequency.

The study was supported by RSF grant №20-74-10040 and by the Tomsk State University Development Programme (Priority 2030).
NEW MORPHOLOGICAL AND GENETIC DATA FOR *METORCHIS* SP. (TREMATODA: OPISTHORCHIIDAE) IN THE RUSSIAN FAR EAST

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Flukes of the genus *Metorchis* Looss, 1899 are cosmopolitan worms that parasitize the gallbladder of birds and mammals, including humans (Bray et al., 2008). According to Ai with coauthors (2010) the genus includes 26 species, of which eight species are parasites of mammals, and 16 species infect birds. Over the past 10 years, some species have been reduced to synonyms, for example, Sitko (2016) showed that the species *Metorchis albidus* (Braun, 1893), *Metorchis crassiusculus* (Rudolphi, 1809) and *Metorchis bilis* (Braun, 1790) are one species, where *M. albidus* and *M. crassiusculus* are currently synonyms of *M. bilis*. In addition, a new species was described in the Far East of Russia, e.g., *Metorchis ussuriensis* Besprozvannykh (Tatonova, Shumenko, 2019). It is difficult to determine the actual composition of the genus due to limited research, despite the fact that these trematodes are epidemiologically important for public health throughout the world.

At the moment, six species of the genus *Metorchis* have been recorded in the East Asian region: *Metorchis orientalis* Tanabe, 1920, *Metorchis taiwanensis* Morishita, 1929, *Metorchis elegans* Belogurov & Leonov, 1963, *Metorchis butoridi* Oschmarin, 1963, *Metorchis kimbangensis* Ha, 2005, and *M. ussuriensis* (Besprozvannykh et al., 2019).

During field research in an unnamed storage reservoir near Bezymyannoe village (Amur Oblast, Russia) and in Magdykovoe Lake (Primorsky Krai, Russia) cyprinids infected with opisthorchiid metacercariae were found. In the result of life cycle experiment in the laboratory, it was shown that worms develop in the gallbladders of ducklings. Adult worms were assigned to the genus *Metorchis* according to morphology data. Genetic studies based on nuclear and mitochondrial genes have shown that the obtained worms cluster with representatives of the genus *Metorchis*, but differ at the species level from all members of the genus for which nucleotide sequences at the moment are presented in the GenBank.

GENETICAL, MORPHOLOGICAL, AND FUNCTIONAL DESCRIPTIONS OF DIGESTIVE SYSTEMS IN SYMPATRIC PAIRS OF COREGONIDS WHITEFISH: AN INTEGRATIVE APPROACH

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Coregonids are widely spread group of whitefishes in the Northern Hemisphere that may form different sympatric pairs in lakes. Two of such pairs inhabit lakes Teletskoye (Altai Reuplic) and Baunt (Republic of Buryatia). In Teletskoye Lake, the sympatric pair is formed by *Coregonus lavaretus pidshian* (zoobenthivorous) and *C. pravdinellus* (zooplanktivorous). In Baunt Lake the sympatric pair is represented by also zoobenthivorous and zooplanktivorous forms/species: *C. l. pidshian* and *C. baunti sp. nova*, respectively. These fish are very interesting models to study the evolution process in closely related fish. The main aim of the study was to compare different biochemical parameters of digestive enzymes and microbial communities in gut of whitefish.

All whitefish were caught by nets with mesh size 18-40 mm. After their capture, fish were dissected and their guts extracted and frozen in liquid nitrogen for further analysis. The activities of pepsin (stomach) and α -amylase, lipase, non-specific esterase, total alkaline proteases, trypsin, chymotrypsin, carboxypeptidases, aminopeptidase, alkaline phosphatase, and maltase (intestine) were assayed. The number of alkaline protease bands on SDS-PAGE electrophoresis with casein as a substrate was assayed. For structural identification of proteins the anterior part of intestine was chosen. In order to identify the spectrum of digestive enzymes the transcriptome and proteome analyses were applied.

In the digestive tract of whitefish, such enzymes and their isoforms as alkaline phosphatase, maltase-glucoamylase, carboxypeptidases aminopeptidase A1,A2,B,M,O,D,Y,Z, N. phospholipase A2, B, B1, trypsin 1, 2, 3, chymotrypsin A, B, alpha-amylase, BSD-lipase, and several chitinases were identified by transcriptome and proteome analyses. The level of activity of studied digestive enzymes was significantly higher in intestinal content than in intestinal mucosa. For the majority of digestive hydrolases, significantly higher activity was found in the anterior intestine compared to the posterior one. The activity of key digestive enzymes in whitefish from Baunt Lake was similar to the same enzymes in whitefish from Teletskoye Lake. The optima of pH level were similar for almost all studied enzymes in whitefish from lakes Baunt and Teletskoye. The temperature optima of digestive enzymes of whitefish from lakes Baunt and Teletskoye varied significantly. It depended more on the type of enzyme, than on the form/species of studied whitefish. Pepsin and lipase retained up to 40% and more of their maximum activity, which indicated the importance of these enzymes for whitefish during digestion in the low temperature zone. All other enzymes were characterized by significantly higher temperatures (30-80°C) required to achieve maximum activity.

The gut microbial community in whitefish from Teletskoye Lake was dominated by phyla Proteobacteria, Firmicutes, and Tenericutes. The most dominant phyla in mucosa and content of stomach were Proteobacteria in both whitefish. Throughout the intestine in *C. l. pidschian* the dominant phyla in mucosa were Proteobacteria and Firmicutes, whereas in *C. l. pravdinellus* - Tenericutes and Proteobacteria. The microbiota of whitefish gut from Baunt Lake was dominated by the phylum Proteobacteria. Moreover, bacteria of the phyla Chlamydiae, Firmicutes, and Planctomycetes dominated the microbiota of benthivorous whitefishes, while Bacteroidetes, Firmicutes, Planctomycetes, and Tenericutes dominated the planktivorous whitefishes.

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DESCRIBING OF THE EURYTEMORA (CRUSTACEA: COPEPODA) SPECIES USING MORPHOLOGICAL AND MOLECULAR-GENETIC METHODS

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Our understanding of the systematics and species richness in *Eurytemora affinis* complex has evolved at a fast pace over the last decades. This happened in many ways thanks to the methods of molecular genetics. Formerly considered as a complex of cryptic species, it includes now three valid species: *Eurytemora affinis* (Poppe), *Eurytemora carolleeae* Alekseev et Souissi and *Eurytemora caspica* Sukhikh et Alekseev. Morphological species delimitation of these new species was supported by the analysis of fragments of the mtDNA *CO1*, as well as *nITS* and *18S* rRNA genes. High nucleotide divergence level was observed among the species: 9.4-11.8% in the *CO1* and 3.9-5.0% in the *nITS*. Morphological differences among these *Eurytemora* species were based on measurements and features of mandibles, caudal rami, genital somite and some structures in P4 and P5 in both sexes.

Holarctic distribution has been divided into 3 different areas occupied by each species. The main area of *E. affinis* is European estuaries. The native area of *E. carolleeae* is North American Atlantic coast. *E. caspica* inhabits the northern Caspian Sea along with the Volga River basin. It is interesting that *E. cf. affinis* from the Russian Far East and Japan is closer morphologically and genetically to *E. caspica* from the Caspian area and, in the near future, a new subspecies of *E. caspica* will be described. Possibly common origin of the Caspian and Far Eastern-Japanese *Eurytemora* populations is a result of the Tethys Sea evolution.

For this work, the Federal Collection of Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) was used. It was conducted in accordance with the national initiative 122031100274-7 and supported by grant from the Russian Foundation for Basic Research (RFBR 20-04-00035).

EXPERIMENTAL EVALUATION OF PCR PRIMERS FOR IDENTIFICATION OF SAKHALIN STURGEON ACIPENSER MIKADOI HILGENDORF, 1892

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Sturgeons (family Acipenseridae) are valuable commercial fish and aquaculture resources. The *Acipenser* genus includes about 20 species, 12 of which are recorded in Russia. The Sakhalin sturgeon *A. mikadoi* Hilgendorf, 1892 is one of the rarest species. Its population has been significantly decreased and the species is now close to extinction. Natural populations of the species have survived in the Tumnin River in Khabarovsk Region and, possibly, in the Viakhtu River in Sakhalin Region. Due to scarce nature of this rare and endangered fish species, noninvasive approaches, including the use of environmental DNA appear to be the most suitable for monitoring of species populations and genetic diversity among them. The method is well established and has been successfully tested for monitoring of species of sturgeons. Here we present the results of evaluation of previously developed primers specific to the mitochondrial DNA of the species *A. mikadoi*. Primers were designed on the basis of the *D*-loop and *COI* fragments.

The range of the Sakhalin sturgeon may overlap with the range of the Amur sturgeon *A. schrenckii* and Kaluga *A. dauricus*. This had to be taken into account during preparation for the experiment. Experimental verification of primers [*D-loop* (114 bp), *COI* (219 bp) and *D-loop* (119 bp)] was carried out by amplification of corresponding fragments based on the total DNA of the Amur sturgeon, Kaluga and Sakhalin sturgeon, and showed the strict specificity of designed primers to *A. mikadoi*. The applied combinations of primer pairs based on the *D-loop* and *COI* fragments made it possible to specifically identify the Sakhalin sturgeon using PCR.

NON-INVASIVE MONITORING OF POPULATION GENETIC DIVERSITY AMONG ABUNDANT SPECIES OF THE ZOSTERA SP. COMMUNITY IN THE NORTHERN SEA OF JAPAN

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Intraspecific genetic variation is widely used to assess the resistance of living organisms to changes in environmental conditions and anthropogenic pressure. Measures of genetic diversity in natural populations obtained during monitoring can be effective for preliminary estimates of natural population structure and preparation for selective multilocus analyses. Nowadays, in addition to non-invasive high throughput species monitoring, as well as aiming at estimating of approximate biomass, there have been attempts to use eDNA to assess intraspecific genetic variation. The limitations of this approach are related to sequencing artifacts that may be a source of false haplotypes, as well as the lack of preliminary data on variation of the standardized region of the COI gene (~313 bp) at the population-genetic level. In this study, we aimed to address these challenges: to assess intraspecific variation in different taxa based on the universal fragment sequences, available in the GenBank, as well as experimentally evaluate the possibility to identify sequenced amplicon variants in abundant species of the Zostera sp. community in the northern Sea of Japan: Hexagrammos octogrammus, Pholidapus dybowskii, and Pandalus latirostris. A total of 100 published sequence data sets were collected in some common groups of multicellular organisms (Mollusca, Echinodermata, Crustacea, Polychaeta and Actinopterygii) through the search on assignment to the mitochondrial COI gene in the popset database of the NCBI. A total of 20 datasets with at least 15 sequences in each were retrieved for every group. Searching for the region (313 bp of COI) and adjusting the sequences to the target length was done by alignment on reference datasets with a correct open reading frame from each group. The sequence set from each species was translated into amino acids and aligned. Next, separate sets of sequences of target length were generated based on the sets in which a 313 bp region was found. Then, the values of haplotypic variability, as well as the number of haplogroups of the same dataset were calculated for the region of original length (~650 bp) and 313 bp region. The results produced for the both regions reflect the possibilities and limitations of using 313 bp region for high-performance monitoring of genetic diversity. We found that the length of the Leray fragment can vary in the Echinodermata and Polychaeta groups due to indels. Three abovementioned species (10 individuals from each taxon) were collected at two distant locations in the Peter the Great Bay in the Japan Sea and placed into separate 150-liter aquaria. The eDNA (900 ml) was collected with syringe filters both from the aquaria representing two locations and individually, when exposing in small boxes. For this, each individual was placed into a separate aquarium of 1.2 liters and 30 minutes after eDNA was collected in the same way. Then all individuals were placed in a container with a 10% urethane solution for sedation, measured, weighed, and genotyped individually for 650 bp and 313 bp COI gene fragments. eDNA was isolated from the syringe filters, and COI region of 313 bp length was amplified using a pair of primers with individual 7-nucleotide tag for each sample. The amplicons were purified, normalized, pooled together, and sequenced on Illunima 250 bp pair-end platform. Along with the taxonomy we were able to retrieve individual haplotypes from the artificial communities of the studied marine organisms. In addition, we have gathered and analyzed natural water samples from the location of Zostera sp. community with a little (standard) sequence coverage and failed to retrieve any information about OTUs of taxa in mock communities, which may indicate much higher biomass of non-target organisms in the studied community which imposes certain restrictions on obtaining population genetic data for important commercial taxa.

GENETIC DIVERSITY AND PHYLOGENY OF *TRIAENOPHORUS* SPP. (CESTODA, BOTHRIOCEPHALIDEA, TRIAENOPHORIDAE) PARASITIZING FRESHWATER FISHES IN EURASIA

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Tapeworms of the genus *Triaenophorus* are widely distributed parasites of *Esocidae*, *Percidae*, *Salmonidae*, *Thimallidae*, *Cobitidae*, *Osmeridae*, *Cyprinidae*, *Cottidae*, *Gadidae* and several others fish families in the Holarctic. Their taxonomic arrangements produced by different authors, based on morphological and ecological-biogeographic characters, suggest the presence of two to five species of this genus in Eurasia. But the lack of information on genetic diversity in *Triaenophorus* sp. did not allow to substantiate any of the taxonomic models. The aim of this study is to characterize genetic diversity of *Triaenophorus* in Eurasia using partial sequences of the *cox1* gene obtained from the regional fish hosts.

Samples of cestodes were collected in 15 localities across Russia from Karelia to Sakhalin Island. Total DNA was extracted from plerocercoids using the DNA-sorb B kit manufacturers' protocols (kit for DNA extraction, Central Research Institute of Epidemiology, Russia). Amplification by PCR was conducted using the primers and conditions described by Steenkiste et al. (2015). Analysis of genetic distances was conducted in MEGA 7 (Kumar et al., 2016). The number of haplotypes and levels of DNA polymorphism were calculated using the program DNAsp 6 (Rozas et al., 2017). Phylogenetic reconstructions within the genus *Triaenophorus* were performed using the Maximum Likelihood (ML) and the Bayesian inference (BI) approaches. Popart 1.7 software (https://popart.otago.ac.nz) was used to calculate and visualize the median-joining network of phylogenetic relationships among haplotypes (Bandelt et al. 1999).

A total of 63 specimens of *Triaenophorus* spp. from different fish species and waterbodies of Eurasia were examined. The studied cestode specimens are distributed among five species-level clades: *T. amurensis*, *T. crassus*, *T. meridionalis*, *T. orientalis* and *T. nodulosus* (Fig. 3). Tree topologies constructed using ML and BI approaches were identical, excluding clustering of the samples within the species-level clades. Within the species-level clades, mean *p*-distance values for *T. crassus*, *T. orientalis*, *T. meridionalis*, *T. nodulosus* and *T. amurensis* were 0.54±0.13%, 0.17±0.12%, 0.57±0.22%, 1.25±0.24%, and 0.63±0.20% respectively. Mean *p*-distances between these clades varied in range from $10.9\pm1.3\%$ (*T. crassus* by *T. orientalis*) to 18.0 ± 1.6 (*T. meridionalis* by *T. amurensis*). *T. nodulosus* was characterized by the highest levels of haplotype (0.99±0.02) and nucleotide (0.012) diversity. The species-level haplogroups are distinctly separated in the haplotype network. Geographically specific haplogroups were found only in one widespread *species*, *T crassus*. Results from phylogenetic analyses confirmed the hypothesis of Kuperman (1968, 1973); there are five species within the genus *Triaenophorus* parasitizing fishes in Eurasia.

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GENETIC DIFFERENTIATION AT THE POPULATION LEVEL IN LAKE BAIKAL ENDEMIC SPONGE LUBOMIRSKIA BAIKALENSIS

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Lake Baikal is a unique natural ecosystem with a high level of endemism. Since the lake is close to marine ecosystems in some hydrological characteristics, some species of organisms have acquired features inherent to marine related groups. For example, due to stable living conditions, representatives of the endemic sponge family Lubomirskiidae have lost the ability to form gemmules and have acquired a long-life cycle, which is typical for representatives of marine sponge species. Despite great progress in the study of genetic characteristics of Baikal endemic sponges, genetic structure of populations remain completely unexplored. In the last decade, mass disease and mortality of sponges have been observed in Lake Baikal. In the light of these events, population structure studies look even more relevant. Microsatellites are the most suitable markers for conducting population genetic studies in sponges on a short time scale. Since there is currently no published set of microsatellite markers for endemic Baikal sponges, it was necessary to develop them. Based on the previously published analysis of the Lubomirskia baikalensis draft genome, we developed a set of 10 polymorphic microsatellite markers. This set was used to analyze sponge samples from the southern, middle, and northern basins, ranging from 60 to 120 specimens from each basin. Based on the results of the analysis, the presence of genetic differentiation between groups of individuals from different basins was revealed. These data were obtained for the Baikal endemic sponges for the first time and require further more thorough study.

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THE USE OF FIVE DNA MARKERS (*COI, 16S, 12S, 28S, 18S*) IN THE STUDY OF MOLECULAR GENETIC RELATIONSHIPS AMONG SQUID OF THE FAMILY GONATIDAE (CEPHALOPODA, TEUTHIDA, OEGOPSIDA)

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Squids of the family Gonatidae (Oegopsida, Teuthida, Cephalopoda) inhabit subpolar seas and oceans in the Northern and Southern Hemispheres. The gonatid squid are highly abundant in boreal waters of the North Pacific Ocean, where they are key components in marine pelagic communities. Currently, there remain controversial issues in taxonomy of this family, which could be resolved using molecular genetic approach. We used sequences from five mitochondrial and nuclear gene markers, *COI, 16S rDNA, 12S rDNA* (mtDNA), *28S rDNA* and *18S rDNA* (nDNA), to analyze phylogenetic relationships among the Gonatidae.

Squid for subsequent analysis of DNA sequences were taken from two major sources. First, most specimens were collected during research surveys in the Okhotsk, Bering, and Japan seas, and in the Northwest Pacific Ocean using midwater and bottom trawl nets. Second, we used sequences deposited in the GenBank NCBI for a number of the Gonatidae species. In total, 13 new sequences of *COI* (\approx 580 bp), 52 new sequences of *16S* (\approx 550 bp), 56 new sequences of *12S* (\approx 260 bp), 32 new sequences of *28S* (\approx 1635 bp), and 54 new sequences of *18S* (\approx 400 bp) markers were deposited to the GenBank for the gonatid squid. We used ABGD-analysis (Automatic Barcoding Gap Discovery) of species-groups for each marker to understand relationships among the Gonatidae. In the result, 14 species-groups were revealed using *COI*; 10 species-groups using *16S*; 8 species-groups using *12S* (JC, K2P, pairwise distance); 9 species-groups using *28S* (K2P method), 8 (JC method) and 1 (pairwise distance); and 6 species-groups using *18S* (K2P and JC) and 1 (pairwise distance).

Phylogenetic reconstructions, based on *COI*, *16S*, and *12S* markers, revealed 14 clusters, corresponding to 12 nominal species, previously identified using morphological characters. There were 13 clusters for 12 nominal species on the *28S* tree. The obtained phylogenetic trees (Maximum Likelihood, Neighbor–joining, MrBayes) and ABGD analysis suggested that the *18S* marker is not suitable for establishing genetic relations among the Gonatidae.

The applied array of partially sequenced genes revealed certain non-conformity of the observed genetic relationships to the existing systematics in the Gonatidae. It appeared that individuals, identified using morphology as *Gonatus berryi*, consisted of two genetically distinct taxonomic units, or sister species, which we provisionally named *Gonatus* cf. *berryi* 1 and *Gonatus* cf. *berryi* 2. According to phylogenetic and ABGD analyses based on the four gene markers, two species clusters were also identified in the polymorphic species *Boreoteuthis borealis*, for which large- and small-sized forms, or cohorts are known. These two size cohorts definitely represent different species. It was also shown that, despite clear morphological differences between *Gonatus* (species with five rows of teeth in the radula and tentacles in adults), representatives of these two genera cluster together on the genetic trees. Revealed genetic differentiation among the Gonatidae clearly indicates the need for further systematic revision of this squid family.

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