

INTERNATIONAL SYMPOSIUM

MAPEEG-2017

PROGRAM &
ABSTRACTS

MAPEEG-2015
MAPEEG-2013
**MODERN ACHIEVEMENTS IN
POPULATION, EVOLUTIONARY AND
ECOLOGICAL GENETICS**

MAPEEG-2011

MAPEEG-2009

MAPEEG-2007

MAPEEG-2005

MAPEEG-1998

MAPEEG-1995

Convener: Dr. Yuri Kartavtsev

VLADIVOSTOK & VOSTOK MBS

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PROGRAM

MAPEEG-2017 Held by:

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FEDERAL SCIENTIFIC CENTER OF BIODIVERSITY OF EAST-ASIA LAND BIOTA FEB RAS,
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Sergey Shedko, Irina Sheremetyeva, Sergey Turanov, Anna Zolotova (Members)

Program Committee:

Yuri Ph. Kartavtsev, Tadeusz Malewski

SUNDAY, SEPTEMBER 3

Arrival, Hotel accommodation in Vladivostok

MONDAY, SEPTEMBER 4

8-30: **Breakfast & Departure to the NSCMB**

9-30-10-15: **Registration**

10-15-10-30: **Opening Remarks**

Andrey V. Adrianov, NSCMB Director, RAS Academician (Russia).

Clifford W. Cunningham, Lab Head, Duke University (USA).

Wazir S. Lakra, Vice-director, ICAR-Central Institute of Fisheries
Education (India).

Tadeusz Malewski, Lab Head at Museum and Institute of Zoology (Poland).

Yuri Ph. Kartavtsev, MAPEEG-2017 Convener (Russia).

Session 1. Evolutionary Genetics & Genomics

(Oral Presentations)

10-30-12-20: **Chair Person – professor Wazir S. Lakra**

1. **Kartavtsev Yuri Ph.** Current molecular evolution data in the connection

- to Neo-Darwinism, molecular phylogenetics, and DNA barcoding (30').
2. **Shedko Sergei V.** High nucleotide substitution rate in control region of mitochondrial DNA of slowly evolving sturgeon fishes (Acipenseridae) (20').
 3. **Alekseeva Svetlana S., Vasserlauf I.A., Andreeva Yu.V.** The analysis of mosquito's metaphase chromosomes in imaginal discs of the *Aedes* genus (Diptera: Culicidae) for the interspecific identification (20').
 4. **Turanov Sergei V., Lee Y.-H., Kartavtsev Y.Ph.** Evolution and phylogenetic performance of mitochondrial control region among eelpouts (Cottoidei: Zoarcales) (20').
 5. **Semerikova Svetlana A., Semerikov V.L.** Evolution and biogeography of genus *Abies* (Pinaceae): phylogeny based on sequence data from three genomes (20').

Lunch (40')

13-00-14-20: **Chair Person – Ph.D. Tadeusz Malewski**

Session 2. Molecular Systematics, Barcoding and Phylogenetics
(Oral Presentations)

6. **Atopkin Dmitry M.** New taxonomic resolution for Hemiuridae and Lecithasteridae trematode families based on rDNA sequence data (20').
7. **Chrisanphova G., Gulyaev A., Mozharovsky L., Semyenova Seraphima.** Barcoding of Diplostomum species (Trematoda: Diplostomidae) obtained from Belorussian water ponds (20').
8. **Voronova Anastasia N., Chelomina G.N.** Molecular systematics of Foodborne Trematodes from orders Echinostomida and Plagiorchiida using secondary structures of the small subunit ribosomal RNA (SSU rRNA) (20').
9. **Oleinik A.G., Skurikhina L.A., Kukhlevsky A.D., Bondar Evgeniia I.** Genetic identification of a Lake Istihed endemic, *Salvelinus andriashevi*: discordance between morphological and genetic variation (20').

#. Discussion on Reports (10').

19-00-21-00: **Dinner & Welcome Party**

TUESDAY, SEPTEMBER 5

8-30 - 9-00: **Breakfast**

10-00-12-00: **Site Seeing Excursion, Vladivostok City**

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

Session 2. Molecular Systematics, Barcoding and Phylogenetics
(Oral Presentations, Continue)

13-00-14-30: **Chair Person – professor Clifford W. Cunningham.**

10. **Wazir S. Lakra.** Genetic conservation of fish and marine life in INDIA (30').

11. **Kartavtsev Yuri Ph., Sharina S.N., Masalkova N., Chichvarkhin Anton Y., Chichvarkhina O.V., Lutaenko K.A., Oliveira K.** Molecular evolution and phylogenetics of mussels (Mollusca, Mytilidae) (20').

12. **Chichvarkhin Anton Y., Chichvarkhina O.V., Egorova E.** Systematics and DNA barcoding of the sea stars of the genus *Henricia* (Echinasteridae) from the northwestern Pacific (20').

13. **Zhukova Alina A., Prokhorova E.E.** Polymorphisms in rDNA of *Leucochloridium vogtianum* (20').

Coffee Break (10')

14-40-16-20: Chair Person – **Ph.D. Sergei V. Shedko**

14. **Zvyagina Elena A.** Phylogeography of the genus *Suillus* s.l. (20').

15. **Kartavtseva Irina V.** Chromosomal features of the voles of the genus *Alexandromys* (RODENTIA) (20').

16. **Pavlenko Marina V.** Striped field mouse *Apodemus agrarius* (Rodentia, Muridae) as subject for genetic investigation: some results, prospect and contribution of Russian Far East (20').

17. **Roslik Galina V., Kartavtseva I.V.** Geographical differentiation of B chromosomes in *Apodemus peninsulae* (Rodentia) from East of Asia (20').

18. **Kharlamov Valery.** Sequencers of the new generation of Illumina: from amplicons to complete genomes (ALBIOGEN LLC; In Russian) (30').

#. Discussion on Reports (10').

19-00-20-00: **Dinner**

WEDNESDAY, SEPTEMBER 6

8-30-9-00: **Breakfast**

Session 2. Molecular Systematics, Barcoding and Phylogenetics
(Oral Presentations, Continue)

9-00-10-20: **Chair Person – S.D. Anatoly L. Drozdov**

19. **Turanov Sergei V., Kartavtsev Y.Ph., Shapovalov M.E.** DNA barcoding and species diversity of fishes from the Lake Khanka (20').
20. **Zolotova Anna O., Kartavtsev Y.Ph.** Analysis of sequence divergence in Pacific red fin (Cypriniformes, Cyprinidae, *Tribolodon*) based on mtDNA and nDNA markers with inferences in systematics and genetics of speciation (20').
21. **Shumenko Polina G., Tatonova Yu.V., Besprozvannykh V.V.** Genetic diversity of new species *Metagonimus suifunensis* in the Russian Southern Far East (20').
22. **Vainutis Konstantin S., Atopkin D.M., Shedko M.B.** Comparative molecular-genetic analysis of some species of parasitic flat worms from the genus *Crepidostomum* based on sequencing data of its region and 28S rDNA (20').
23. **Vasiljeva Tatjana V., Kartavtseva I.V., Sheremetyeva I.N., Moroldoev I.V., Golenishchev F.N.** Molecular genetic diversity of muya valley vole, *Alexandromys mujanensis* (20').

Coffee Break (10')

Session 3. Microevolution. Population Genetic Structure of Species. Ecological Genetics

10-50 - 12-20: **Chair Person – S.D. Galina N. Chelomina**

24. **Clifford W. Cunningham.** A Grand vision bringing together ecologists, systematists and phylogeographers from North Pacific and North Atlantic countries to study the trans-Arctic interchange (30').
25. **Shuyskaya Elena, Kolesnikov A., Rakhmankulova Z.** Genetic polymorphism of C3-C4 xero-halophyte *Sedobassia sedoides* (Chenopodiaceae) (20').
26. **Gurina A.A., Tikhodeyeva M.Y., Lebedeva M.A., Tkachenko A.A., Tvorogova V.E., Tikhodeyev Oleg N.** A unique natural population of *Trientalis europaea* (L.) with extremely high fluctuational variation in flower morphology (20').
27. **Dudnikov Alexander.** Intraspecies divergence and geographical expansion of *Aegilops tauschii* Coss. (20').

12-20 - 13-20: **Lunch**

13-20 - 15-10: **Chair Person – S.D. Tikhodeyev Oleg N.**

28. **Kalinkina Valentina A., Mikhaylova Y.V., Kislov D.E.** How many species available in the Polymorphic complex *Trifolium lupinaster*? (20').
29. **Masalkova Natalia A., Kartavtsev Y.Ph., Katolikova M.V.** Genetic and morphometric variability of two mussel species (*M. trossulus* and *M. galloprovincialis*) in the North-West Part Sea of Japan (20').
30. **Pavlova Svetlana V.** Hybrid zones between chromosomal races of the common shrew in Russia (20').
31. **Zhuikova Elena V., Kiseleva I.S.** Genetic diversity *Artemisia latifolia* Ledeb. higher in impact habitats near the Karabash copper smelter than in the background (20').
32. **Presentation by LabInstruments Co. representative** (In Russian)(30').

Session 4. Poster Presentations (30')

15-10-16-10 : Chair Person – S.D. Irina V. Kartavtseva

1. **Artemenko Elizaveta P.** Genetic diversity of dandelion populations which impacted by different levels of industrial pollution.
2. **Egoraeva Anastasiya A., Yulia V. Tatonova.** Phylogenetic relationships of *Parafossarulus* species in Primorye.
3. **Redin Alexander D., Kartavtsev Yu.Ph.** Molecular phylogeny of Russian far eastern flatfish (Pleuronectiformes, Pleuronectidae) based on sequences of mitochondrial genes.
4. **Rybnikova Irina G., Pushnikova G.M.** The use of the indicator parasite anisakis simplex in population studies of pacific herring *Clupea pallasii* (Clupeiformes: Clupeidae) in Sakhalin waters.
5. **Vu K. Than, Kartavtsev Yu.Ph., Turanov S.V.** A facility for fish rearing with experimental needs.
6. **Sheremetyeva Irina N.** The isolates of East Asia are reserves of archaic forms of some species.
7. **Vu K.Than, Kartavtsev Y.Ph.** Morphometric investigation of differences among smallmouth smelt *Hypomesus japonicus* and *H. nipponensis* (Pisces, Osmeridae) from inshore waters of north-west part of the Sea of Japan.
8. **Frolov Sergei V.** Comparison of *Hucho hucho* and *H. taimen* karyotypes.
9. **Skurikhina Lubov A., Oleinik A.G., Kovpak N.E., Kukhlevsky A.D.** Genetic diversity, phylogeography and postglacial dispersion of the pacific smelt, *Osmerus dentex*.
10. **Kartavtseva I.V., Gornikov Dmitry V., Vasiljeva T.V., Sheremetyeva I.N., Frisman. L.V.** B-chromosome of Korean field mouse *Apodemus peninsulae* from the Verkhnebureinsky depression.
11. **Frisman Liubov V., Sheremetyeva I.N., Kartavtseva I.V.,**

Pavlenko M.V. Genetic differentiation of mainland and island populations in eastern lineage of striped field mouse (*Apodemus agrarius*): study of 5 microsatellite loci.

12. **Rozhkovan Konstantin V.** Secondary structure diversity of rRNA in Monogenoidea.

13. **Petrov N.B., Drozdov Anatoliy L.** Intra- and Interspecific Divergence within Starfish and Sea Urchins.

14. **LabInstruments Co.** Posters & Exhibition.

15. **ALBIOGEN LLC.** Posters & Exhibition.

16. **KhimExpert Co.** Posters & Exhibition.

Mini Workshop/ School

16-10-17-40 : **Chair Person – S.D. Yuri Ph. Kartavtsev**

1. **Tadeusz Malewski, Marcin Kaminski.** DNA metabarcoding for Insects diet assessment: possibilities and limitations (40').

2. **Anton Yu. Chichvarkhin, Sergei V. Turanov, Yuri Ph. Kartavtsev.** Practical training on nucleotide sequences: sequencing, edition, submission, alignment, tree building and analysis of phylogeny (40').

#. General Discussion on Reports (10').

17-40-18-30: **Chair Person – Prof. Yuri Kartavtsev**

Yuri. Ph. Kartavtsev. **Concluding remarks & VFDG meeting (60').**

19-30-20-30: **Dinner**

THURSDAY, SEPTEMBER 7

8-30 - 9-00: **Breakfast**

9-00 - 19-00: **Free days, Excursions to Vostok MBS & Vladivostok City.**

19-00-23-00: **Dinner, Closing Reception & Evening party by the fire at MBS.**

FRIDAY, SEPTEMBER 8

8-30-9-00: **Breakfast**

9-00-10-00: Round Table. **International biodiversity research in the north of Pacific & Atlantic (C. Cunningham).**

10-00-10-15: **Departure to Vladivostok and Airport.**

ABSTRACTS

**THE ANALYSIS OF MOSQUITOE'S METAPHASE CHROMOSOMES IN
IMAGINAL DISCS OF THE GENUS *Aedes* (DIPTERA: CULICIDAE) FOR
SPECIES IDENTIFICATION**

¹Alekseeva S.S., ¹Vasserlauf I.A., ¹Andreeva Y.V., ¹Sibataev A.K., ¹Stegniy V.N.

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In the present study we have analyzed the mitotic chromosomes in imaginal discs of three mosquito's species of the genus *Aedes* (Diptera: Culicidae). The purpose of our study was to conduct a karyotype analysis of three mosquito's species such as *Aedes behningi*, *A. excrucians* and *A. euedes*. The analysis has been conducted on mitotic cells on mosquito's imaginal discs. Diploid chromosome's set of the genus *Aedes* is 6 ($2n=6$). Mosquitos of *Aedes* species have three pairs of metacentric chromosomes. It was shown by calculating the centromeric index which was determined according to the formula $p/(p+q)$, where "p" is short arm of a chromosome and "q" is long arm of a chromosome. Slides with chromosomes were processed with acetic-orcein (Stegniy, 1979) and DAPI (Sayfitdinova, 2008). The analysis of the results was carried out using light and fluorescent microscopes. Chromosomes were numbered according to the classification of chromosomes (McDonald et al, 1970).

Based on chromosome's banding and its length we created ideograms which show us clear differences among three mosquitos species: *A. behningi*, *A. euedes* and *A. excrucians*. Acetic-orcein staining showed clear difference among the species in chromosome one. DAPI staining showed the difference in all three pairs of chromosomes. Measurement of the chromosomes was made with ImageJ program. The lengths of the 1st, 2nd and 3rd chromosomes of *A. behningi* are 2,432 mkm, 3,821 mkm and 3,790 mkm, respectively; *A. excrucians* - 2,760 mkm, 7,722 mkm, 7,001 mkm; and *A. euedes* - 3,937 mkm, 6,092 mkm and 5,961 mkm, respectively. Therefore, we were able to reveal differences between *A. euedes*, *A. excrucians* and *A. behningi* in chromosome banding patterns and length.

**NEW TAXONOMIC RESOLUTION FOR HEMIURIDAE AND
LECITHASTERIDAE TREMATODE FAMILIES BASED ON RIBOSOMAL DNA
SEQUENCE DATA**

Atopkin D.M.

*Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of
the Russian Academy of Sciences, Vladivostok 690022, Russia*

Present study compiled first molecular data for *A. mugilis* *Aphanurus mugilis* Tang, 1981 (Hemiuridae: Aphanurinae) and several *Lecithaster* trematode species along with taxonomical and phylogenetic analyses of Hemiuridae and Lecithasteridae. *Aphanurus mugilis* was in the same clade as most hemiurid subfamilies (lineage H1) and was closely related to Dinurinae, Elytrophallinae and Hemiurinae ($d = 2.8 \pm 0.57\% - 3.0 \pm 0.64\%$ by 28S rRNA gene sequence data). These results appear to support previous findings on the systematic position of Aphanurinae within Hemiuridae. However, genetic *p*-distances between Aphanurinae, Dinurinae, Elytrophallinae and Hemiurinae were considerably lower in comparison with values calculated between each of these four subfamilies and Plerurinae and Lecithochiriinae. These results indicate that lineage H1 represents the true Hemiuridae family. On the other hand, our data show that the taxonomic status of Aphanurinae, Dinurinae, Elytrophallinae and Hemiurinae should be reconsidered in light of the molecular differentiation found in the present study, with the inclusion of these trematodes into the same subfamily, Hemiurinae.

The major diagnostic feature of different hemiurid subfamilies is the ecsoma, a protrusible, posterior region of the body that has been entirely lost or is vestigial in four subfamilies, including Aphanurinae. The results of our molecular phylogenetic study indicate that the presence or absence of an ecsoma was not associated with molecular data for hemiurid subfamilies differentiation. The basal position of Bunocotylineae on the molecular-based phylogenetic tree indicated a primordial nature of ecsoma of hemiurid trematodes. Considerable molecular differentiation of Bunocotylineae from other hemiurids indicated the possibility of the recognition of the family Bunocotylidae Dollfus, 1950.

Bayesian phylogenetic analysis revealed paraphyly for the family Lecithasteridae. This same analysis revealed polyphyly for the family Lecithasteridae. The genus *Lecithaster* was located separately from other Lecithasterinae representatives: *Aponurus* and *Lecithophyllum* genera. There is considerable molecular differentiation between these three genera; *p*-distance values ranged from 8.5% (*Lecithophyllum/Aponurus*) to 17.5% (*Lecithaster/Aponurus*). The genus *Lecithaster* was considered as a member of Hemiuridae (subfamily Lecithasterinae) for a long time, until Skrjabin and Gushanskaya (1954) erected the family Lecithasteridae. Paraphyly of the Lecithasterinae has been previously shown using the V4 region of 18S rRNA gene sequence data by Blair et al., 1998. Our results support this point of view, assuming that *Machidatrema chilostoma* as a taxon within Bunocotylineae by Leon-Regagnon et al. (1998), which in turn is contrary to the concept of Bray and Cribb (2000) that *Machidatrema* shares a lineage with hysterolecithine lecithasterids.

COPY NUMBER VARIATION OF mtDNA AND rDNA REPEATS IN PATIENTS WITH SCHIZOPHRENIA

¹Chestkov I.V., ²Jestkova E.M., ¹Ershova E.S., ³Golimbet V.G., ³Lezheiko T.V., Kolesina N.Yu., R.V. ¹Veiko, ¹Izevskaya V.L., ¹Kutsev S.I., ¹Veiko N.N., ¹Kostyuk S.V.

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OBJECTIVE: Approximately 1% of the world's population suffer from schizophrenia (SZ). SZ is a highly heritable neuropsychiatric disorder of complex genetic etiology. Mitochondria (mt), the cell energy source, have a crucial role in intracellular Ca²⁺ homeostasis, producing ROS and activating the apoptotic pathway. Several studies have evaluated the influence of SZ on mtDNA copy number (CN), but the results are controversial to each other. The ribosome is a critical component of translation machinery. The key components of ribosomes is ribosomal RNA. Dysregulation of rRNA biogenesis has been implicated in some human diseases. One of the factors affecting rRNA biogenesis is the rRNA genes (rDNA) copy number in the genome. Studies of the number of rDNA copies in the genomes of SZ patients have not previously been conducted. The aim of this study was to examine the leukocyte mtDNA and rDNA copy number of the unmedicated and medicated patients with paranoid schizophrenia (SZ) in comparison with the healthy controls (HC).

METHODS: We evaluated leukocyte mtDNA and rDNA CN of 179 subjects with SZ (108 male/71 female) in comparison with 122 HC (60 male /62 female) by the qPCR (ratio (mtDNA or rDNA)/nDNA (gene B2M) was detected). SZ patients were further divided into two subgroups. The patients of the subgroups SZ (m+) (N=121) were treated with standard antipsychotic medications in the hospital. The patients of the subgroup SZ (m-) (N =58) were not treated with antipsychotic medications before venous blood was sampled.

RESULTS: The mtDNA CN in the unmedicated SZ subgroup was significantly higher than that in the medicated subgroup or in HC group ($p < 0.0001$). The leukocyte mtDNA CN showed no significant difference in medicated SZ patients and HC. The leukocyte mtDNA CN of the male HC was significantly lower than that of the female HC (median 138 vs 238, $p < 0.001$). This is not true for SZ. rDNA CN was much higher in SZ patients than in controls (median 542 vs 384, $p = 10^{-25}$). The leukocyte rDNA CN showed no significant difference in female and male SZ patients or HC.

CONCLUSION: The leukocytes of the unmedicated SZ patients with acute psychosis contain more mtDNA than the leukocytes of the SZ patients treated with antipsychotic medications or the healthy controls. An increase in mtDNA content may precede mitochondrial dysfunction as an adaptive response to oxidative stress and could therefore be a predictive biochemical marker. Genomes of SZ patients contain more ribosomal genes than those of the healthy controls. The elevated ribosomal genes copy number in human genome can be one of the genetic factors of schizophrenia development and could therefore be a predictive genetic biomarker.

Keywords: schizophrenia, leukocytes, mtDNA copy number, rDNA copy number

**SYSTEMATICS AND DNA BARCODING OF SEA STARS OF THE GENUS
HENRICIA (ECHINASTERIDAE) FROM THE NORTHWESTERN PACIFIC**

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Sea stars of the genus *Henricia* are abundant in all world seas estimating nearly 80 extant species, about 45 of them occur in the northern Pacific. Despite their wide distribution and abundance, their systematics is severely confused and poorly developed due to their extremely variable morphology. Also, poor attention was paid to this group during last five–six decades, although these animals are a popular object in drug discovery, biomedical and cytological research. Recently, we have confirmed distribution of several species in the waters of the northwestern Pacific, where three new species were described.

We have sequenced and mined from GenBank the 63 partial sequences of mitochondrial DNA for 16S rRNA that formed 598 b.p. alignment. These sequences belong to presumed 16 identified and five unidentified *Henricia* species, 12 of them inhabit Russian waters of the Pacific. The Automated Barcode Gap Discovery (ABGD), using Jukes-Cantor and *p*-distances, revealed 19 presumed species at *p*-distance threshold of 0.077. Thus, most species were successfully delimited. But this method failed to distinguish *H. hayashii* vs. *H. obesa* and *H. compacta*. Also, three species belonging to the fine-spined group of the subgenus *SetiHenricia*, i.e. *H. lineata*, *H. uluudax*, and *H. olga* were not delimited. *H. obesa* and *H. compacta* occur in the Southern Hemisphere off Australian shore, while the others are distributed in the northern Pacific; recent studies confirmed their significant level of morphological divergence. Therefore, we suppose that ABGD method failed to distinguish these young but unequivocally distinct species using the 16S rRNA marker. The Neighbor-Joining tree based on *p*-distance and rather high bootstrap support values suggests delimitation of all presumed species including those, that were not distinguished by ABGD.

Kimura-2-Parameter NJ tree supports the monophyly of the subgenera *Henricia* and *SetiHenricia*, while the interrelationships between presumed members of the subgenus *AleutiHenricia* and *H. pachyderma* remained unresolved due to low bootstrap support. Basal position of Australian *H. tahia* suggests that this species may not belong to *Henricia*. This tree also confirms the hypotheses of three newly described taxa, *H. djakonovi*, *H. alexeyi*, and *H. olga*, as well as supported recently described *H. lineata* and *H. uluudax* known from the Aleutians. *H. aspera robusta* sampled from the northern Pacific is identical with Atlantic *H. oculata*, hence the name *robusta* is synonymized with the latter. *H. pachyderma* is reported from Russian waters for the first time.

We found that life coloration as a very important species-specific character, which was often ignored by earlier scholars. The morphology of aboral spines distinguishes the species rather good within the subgenus *SetiHenricia*, while the species of the subgenera *Henricia* and *AleutiHenricia* possess rather similar spines.

**A RESEARCH NETWORK TO STUDY COMMUNITY ASSEMBLY AND
EVOLUTIONARY HISTORIES OF FOUR COASTS FIRST JOINED BY
THE TRANS-ARCTIC INVASION**

Cunningham C.W.

Department of Biology Duke University Durham, NC., 27708-0338, USA

The trans-Arctic interchange followed the opening of the Bering Strait ≈ 3.5 ma. This opening joined the temperate biotas of the North Atlantic and North Pacific – which had evolved in isolation for over 50 million years. As with ALL meetings of great biotas (Suez Canal, the Great American Interchange) this invasion was almost entirely in one direction – from the North Pacific to the North Atlantic. One consequence of this massive invasion (80% of New England USA rocky intertidal species have a Pacific origin) was to place closely related species on 4 coasts in the Northern Pacific and North Atlantic (See cover of the volume I edited for Ecology for a map of the invasion). I led an NSF Research Coordination Network from 2002-2008 – CoOrdinating Research on the North Atlantic (CORONA) – dedicated to foster international and interdisciplinary of the North Atlantic as A SINGLE BASIN, instead of disconnected research on both sides of that Ocean each country. Our focus is not the Arctic biota but the boreal, temperate zones. Now it is time to add Japanese, Korean, and mainly Russian scientists to this enterprise, expanding to the North Pacific.

**INTRASPECIFIC DIVERGENCE AND GEOGRAPHIC EXPANSION OF
AEGILOPS TAUSCHII COSS**

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Aegilops tauschii is a goat-grass, which donated its genome D to a common wheat. It is the only one of all diploid *Aegilops* species, which was able to occupy vast area. The aim of the study was to understand how the geographic expansion of *A. tauschii* has happened, and what are the peculiarities of *A. tauschii* biology, which provided evolutionary success of the species. Allozyme variation at 18 genes was studied in a set of 320 *A. tauschii* accessions representing the entire species distribution range. In 60 of these accessions DNA of one of the genes, *Got2*, about 3000 b.p., was sequenced. In 111 *A. tauschii* accessions, polymorphism of the sequence of about 3000 b.p. of non-coding chloroplast DNA was studied. Each of essentially polymorphic enzyme-encoding genes has its own specific geographic pattern of allozyme polymorphism; and all these patterns correspond well with climatic variation through the species range. It was revealed that, in *A. tauschii*, allozymes are reliable as genetic markers of different phylogenetic lineages. Geography of allozyme variation displays adaptive nature of *A. tauschii* intraspecific divergence. *A. tauschii* is presented in nature by many local populations, which could have an opportunity of rather independent long-term evolution. When a new effective adaptation originates in a local population, *A. tauschii* plants having such advantageous genotype rapidly occupy large area forcing out some of the "older" phylogenetic lineages from their habitats and/or expanding the species range. Phylogeographic study reveals that there were many such "migration waves" through the vast species range. The level of cross-pollination in *A. tauschii* is very low. When different lineages "meet" in some habitat they do not form a "hybrid" population. As a rule, each lineage retains its identity; and the lineage which is adapted better to the habitat's environmental conditions forces out the other lineages. At the same time, existing low level of cross-pollination seems to be sufficient enough for the evolution of local populations to go not only by mutations, but by "hybridization" as well. Since migration capacity is high in *A. tauschii*, the high level of genetic differentiation of *A. tauschii* populations, with G_{ST} being about 0.65, seems to be mostly due to natural selection: in hilly regions, which the species mostly inhabit, the habitats that are located not far from each other could considerably differ ecologically. Nowadays existing phylogeographic patterns in *A. tauschii* is a result of a long and complex history of competition between different phylogenetic lineages. And since "younger", better adapted, lineages force out "older" lineages rapidly and effectively, it seems very likely that these phylogenetic patterns are in equilibrium state, i.e., each phylogenetic lineage occupies part of the area for which it is relatively better adapted. And no essential changes will take place until in some of many local populations of *A. tauschii* a new lineage having new effective adaptation originates.

**GENETIC DIFFERENTIATION OF MAINLAND AND ISLAND POPULATIONS
IN EASTERN LINEAGE OF THE STRIPED FIELD MOUSE (*Apodemus agrarius*):
A STUDY OF 5 MICROSATELLITE LOCI**

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The striped field mouse inhabits a wide geographical area from the central Europe to the Pacific coast of Asia including nearest islands. The species range is subdivided into two allopatric parts (European-Siberian- Kazakh versus Russian Far Eastern-Chinese-Korean) with disjunction in Transbaikalia. Using a fragment analysis of 5 microsatellite loci (GTTDS8, GATAE10A, CAA2A, GTTF9A and GSADT7), it was shown that allelic diversity in the western lineage is lower than in the eastern one (Frisman et al. 2016). Perhaps, for *A. agrarius*, this was due to much longer period of living in the Eastern Palearctic than in Siberia and Europe. It was found that the affinity of continental populations within each lineage is higher, and genetic differentiation between these lineages is larger (Frisman et al., 2016).

The aim of this study was to compare differentiation of mainland and island populations within the eastern lineage. A total of 205 animals were caught in five continental localities as well as on two islands in the Peter the Great Bay (Sea of Japan). To perform the fragment analysis we used the same microsatellite markers as before. GTTDS8 locus was monomorphic in all the samples except for one population in the southern Primorye (Khasan district) where the second allele was found. The number of alleles in continental populations were higher than in populations on the islands. The smallest number of alleles was found in population on the Bolshoi Pelis Island.

The highest genetic similarity was revealed within both groups of populations "Middle Priamurye" and "Primorye" ($D_{Nei1978} < 0.06$). There was somewhat lower similarity between populations in these groups ($D_{Nei1978} 0.074-0.092$). Island populations presented higher differentiation both among themselves and when compared to continental ones ($D_{Nei1978} 0.109-0.382$). It suggests the importance of genetic drift in formation of their genetic structure.

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COMPARISON OF *HUCHO HUCHO* AND *H. TAIMEN* KARYOTYPES

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Karyotypes of Danube salmon *Hucho hucho* and Siberian taimen *H. taimen* were studied earlier. Karyotype of *H. hucho* was determined as $2n = 82$, $NF = 106$ (Sofradzija, 1979), and $2n = 82$, $NF = 126$ or $NF = 124$ in different European population (Rab, Liehman, 1982). However, *H. taimen* has $2n = 82, 83, 84$, $NF = 112 - 116$ in Amur River basin (Viktorovsky et al., 1985; Frolov et al., 1999).

Further analysis of karyotypes of European and Siberian taimens has shown, that both species really have $2n = 82$ in their karyotypes. However, determination of the numbers of chromosome arms in their karyotypes called doubts. From permission of the authors, who described the karyotypes of European and Siberian taimens (Dr. Rab, and Dr. Viktorovsky), the known karyotypes were updated under the uniform scheme with separation of meta-, submeta-, submeta-subtelo-, subtelo- and acrocentric chromosomes.

It appeared that the karyotypes of European and Siberian taimens ($2n = 82$) are practically identical and very similar in their morphology: 11 pairs of metacentric, 2 pairs of submetacentric, 1 pair of submeta-subtelocentric, 7 pairs of subtelocentric and 20 pairs of acrocentric chromosomes. Therefore, chromosomes in karyotypes of both species at $2n = 82$ have, first of all, identical number of chromosome arms: $NF = 108+2$.

However, there are differences in certain karyotypes parts of European and Siberian taimens. In European taimen - in pericentric inversion which resulted in different number of chromosome arms in its karyotype in different populations in Europe (Rab, Liehman, 1982), in Siberian taimen - in Robertsonian translocation, which resulted in polymorphism of chromosome number in Amur River population (Viktorovsky et al., 1985; Frolov et al., 1999).

**A UNIQUE NATURAL POPULATION OF *TRIENTALIS EUROPAEA* (L.)
WITH EXTREMELY HIGH FLUCTUATIONAL VARIATION IN FLOWER
MORPHOLOGY**

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The phenotype of a certain organism is traditionally believed to depend on three factors: the genotype, environment, and developmental stage. However, there are multiple phenomena (e.g. incomplete penetrance, variable expressivity, and fluctuational asymmetry) where remarkable phenotypic variation occurs even under strict control of all three abovementioned factors. This is due to intrinsic molecular stochasticity, which can also significantly affect the phenotype establishment. The resulting variation is called random or fluctuational; it is still poorly studied, especially in nature. One of the useful models to investigate the role of fluctuational variation in nature is *Trientalis europaea*, a small pseudo-annual herb plant very common in the boreal zone of Eurasia. In this plant, dramatic variation of flower morphology have described. The typical *T. europaea* flower possesses 7 sepals, 7 petals, 7 stamens, and 1 pistil (such flowers are designated as regular heptamerous; R₇), but the number of sepals, petals, and stamens can significantly vary even on the same plant. Statistical analysis of such variation revealed two types of the underlying events. First, three outer whorls of a floral meristem undergo equal alteration of their merosity to 5, 6, 8 or 9; as a result, a regular pentamerous (R₅), hexamerous (R₆), octamerous (R₈), or nanomerous (R₉) flower is produced. Second, a single whorl in the developing meristem is affected by a local deviation leading to either 1 lacking (-1) or 1 extra (+1) organ; the corresponding flowers are designated as irregular (Ir). We analyzed flower morphology in 7 natural populations of *T. europaea* on Konevitsa Island (Ladoga Lake, North-West Russia). In 5 populations, the rate of the Ir flowers was low (about 5-10%), that is typical for this species. One population displayed high rate of Ir (about 40%), and another one was even more atypical (about 60% of Ir). Although the latter population was rather small (about 150 plants), it produced more than 40 different types of flowers, that is unique for *T. europaea*. Despite significant differences in the weather, the rate of Ir in each population was stably reproduced at least for 4 years, while the ratio between the flowers with different merosity dramatically varied. Thus, natural variation in the rate of Ir was not environmental. Moreover, in two-flower plants, the rate of Ir did not depend on the order of flower opening; so, variation in this rate was not ontogenetic. To study the role of the genotype in flower morphology variation we compared the DNA polymorphism in 3 studied populations (typical, unique, and intermediate) by RAPD analysis. The typical and intermediate populations were highly polymorphic, while the unique one appeared to be mostly clonal. So, extremely high variation of flower morphology in this population was neither genotypic, nor environmental, nor ontogenetic. In our previous studies we demonstrated that each type of the -1 or +1 local deviations fit the Poisson distribution, and therefore was induced by some random events. All these data suggest that flower morphology variation in *T. europaea* is predominantly fluctuational, and the key origin of this variation is intrinsic molecular stochasticity.

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**HOW MANY SPECIES AVAILABLE IN THE POLYMORPHIC COMPLEX
TRIFOLIUM LUPINASTER?**

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The Leguminosa is the largest family of flowering plants, and genus of *Trifolium* L., is one of the largest genera in the family. The species of *Trifolium* was found in the old and new world, as well in Africa and South America. The systematics of the genus is very complicated because the genus has high degree of polymorphism in some species. Currently, the taxonomy of the section *Lupinaster* (Fabr.) Ser. from the *Trifolium* Moench subgenus is still controversial. Main disputes of botanists are focused on systematic position of *T. lupinaster*, because this species is characterized a disjunctive range, wide environmental amplitude and high morphological variability.

Different contribution of plant traits (morphometric features of lamina, morphological variability of underground part, biochemical variability), polyploidy and wide distribution area lead to division of polymorphic species *T. lupinaster* to a number of distinct species. Today, different scientists treated the *T. lupinaster* as an aggregation of species (*T. lupinaster* L.) or variety of names (*T. baicalense*, *T. litwinowii*, *T. tundricum*, *T. romanicum*, *T. criswogense*, *T. spryginii*, *T. pacificum* *Lupinaster popovii*, *Lupinaster albus*).

Molecular phylogenetic analysis showed that the variability of ITS and trnL-trnF sequences in *Lupinaster* section is very low. Sequences of the ITS region of the *T. lupinaster* complex, including highlighted as a separate genus *Lupinaster*, are identical and represented by a single haplotype.

Chloroplast haplotype pattern of *T. pacificum* collected from Japan is slightly differed. Statistical analysis of the leaf sheet features of *T. pacificum*, showed no significant differences in the leaf sheet traits, but find that they are significantly different in leaf shape, and they have a quite narrow habitat, and specific development of specimens. This may be caused by isolated island state for this population, which, in turn, leads to the accumulation of variability.

Against the background of significant morphological plasticity of species in *Lupinaster* section, and in the complex *T. lupinaster* particularly, obtained evidence show it is surprisingly uniform genetically.

After complex morphology and molecular study we consider *T. lupinaster*, *T. pacificum*, *T. gordejevii*, *T. eximium* as separate species of section *Lupinaster*. Dedicated earlier species *T. baicalense*, *T. litwinowii*, *T. criswogense*, *T. spryginii*, and *L. albus* are not the independent taxa, but represent different variants of *T. lupinaster* life forms.

**CURRENT MOLECULAR EVOLUTION DATA IN THE CONNECTION TO
NEO-DARWINISM, MOLECULAR PHYLOGENETICS, AND DNA
BARCODING**

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The evidences of possible impact of gene introgression on species evolution, evolutionary fate of taxa, including reticulations in phylogenetic trees, and consistency of the latest molecular genetic data with the main modern paradigm, Neo-Darwinism, are considered in many publications (Barton, Hewitt, 1985; Campton, 1987; Avise, 2000; Gerber, 2001; Arnold, 2009; Arnold, Fogarty, 2009; Kartavtsev, 2013). In this assignment, the author will focus on animals, although many ideas suit to other phyla too.

The main issues of the report are as follows: (1) What methods are most appropriate for the hybrid detection and estimation of genetic introgression (gene flow)? (2) What facts, obtained on genetic introgression by nDNA and mtDNA markers, are evidencing for? (3) Is there in the literature any data on correspondence of molecular diversity in lineages or in taxa with Biological Species Concept (BSC). (4) How frequently reticulations in gene trees are observed, and what is a major signal from the tree topology?

(1) A combination of nDNA and mtDNA markers best suits for the hybrid identification and estimating the genetic introgression or gene flow. (2) The available facts for both nDNA and mtDNA diversity seemingly make the introgression among many taxa of animals and plants obvious, although even in wide hybrid zones of *Mytilus* ex. group *edulis*, for example, 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 orthodox 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, sooner or later they definitely become biological species. This conclusion is supported by the genetic distance increasing with taxa rank and by the lowest diversity at intraspecies level as for single mtDNA genes, for complete mitogenome, and for nDNA data (Kartavtsev, 2013; Kartavtsev et al., 2016; Hedges et al., 2015). (4) The recent investigation of fish taxa divergence (Kartavtsev, 2017) using vast BOLD (www.boldsystem.org) data shows that gene trees for taxa up to the family level are basically monophyletic and interspecies reticulations are rare for most of gene trees.

Above-listed outcomes are highly important for understanding of species notion. Their impact is also obvious to the paradigms of General Biology, Evolutionary Genetics, and iBOL (www.ibol.org), and to the practice of species delimitation in particular. Available common successful delimiting of species by barcoding technique is achieved due to the geographic speciation mode that prevailing nature and allows random accumulation of numerous mutations/substitutions during isolation history of sister taxa, which nowadays are detectable by means of molecular markers (barcodes). It seems that evidences on the invalidity of the modern BSC paradigm (Arnold, Fogarty, 2009) due to

the large-scale gene introgression and phylogeny reticulations are too contradictive. Contrary to that, data available in the literature and that listed in this assignment show that molecular genetic evidences are basically concordant with the BSC and Neo-Darwinism.

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**CURRENT MOLECULAR PHYLOGENETICS EVIDENCE ON
RELATIONSHIPS AND SYSTEMATICS OF MUSSELS (MOLLUSCA,
MYTILIDAE) USING SEQUENCES
ON 28S rRNA, 18S rRNA AND H3 MARKERS**

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Based on partial nucleotide sequences of three nDNA genes 28S rRNA, 18S rRNA and histon *H3* and using complex approaches of molecular phylogenetics and evolutionary genetics, the phylogeny and systematics of mussels, one of largest taxon of bivalve mollusks, the family Mytilidae is studied. The phylogeny for the family Mytilidae and nearest relatives of the order Mytilida, which has no consensus currently under orthodox approach, is reconstructed on the base of three single gene trees build and combined tree constructed on the concatenated super-matrix for 28S rRNA and *H3*. Obviously, using the nucleotide sequences of three nDNA genes 28S rRNA, 18S rRNA and *H3* such a consensus for Mytilidae could be established. Some concerns of mussel systematics resolved, in particular the monophyly of the family Mytilidae Rafinesque, 1815 was established; most strongly was supported the branch of the subfamily Mytilinae Rafinesque, 1815. Our data did not support Distel's (1999) conclusion on a polyphyly of the subfamily Mytilinae Rafinesque, 1815 (Fig. 1). The topological signal from gene trees have also proved two subfamilies, Modiolinae G. Termier & H. Termier, 1950 and Bathymodiolinae Kenk & Wilson, 1985 within Mytilidae, as well as family Septiferidae Scarlato et Starobogatov, 1979 b. The rank of last taxon is not widely recognized and this info will take an extra examination.

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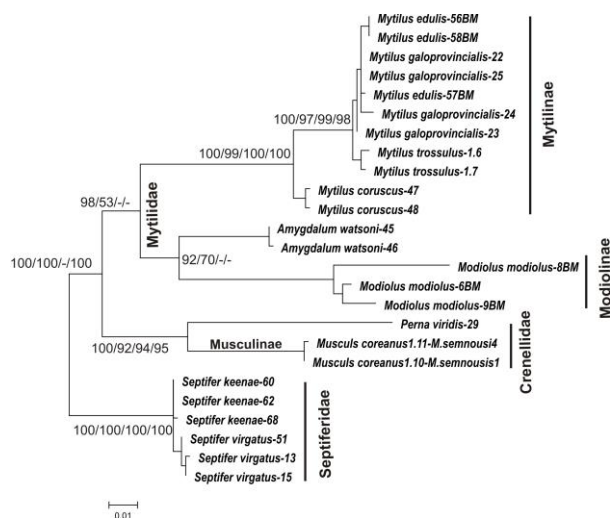


Fig. 1. Rooted consensus Bayesian tree that show phylogenetic relations for 25 concatenated sequences for 28S rRNA and *H3* of mussels (Mollusca, Mytilidae).

**B-CHROMOSOME OF KOREAN FIELD MOUSE *APODEMUS PENINSULAE*
FROM THE VERKHNEBUREINSKY DEPRESSION**

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Korean field mouse, *Apodemus peninsulae* (Thomas, 1906) is widely distributed throughout the mixed woods of Asian continent and on some East Asia islands (Sakhalin, Hokkaido, Russky and Stenina). The species karyotype contains both the 48 acrocentric A chromosomes of the basic set and additional B chromosomes, late are varying in number and morphology. Animals have either stable karyotype (2n is constant within the cells of one individual) or mosaic, with several cellular clones (the number of chromosomes varies in different cells in the same individual). Among animals with stable karyotypes there are both individuals with B-chromosomes, and without B-chromosomes (Kartavtseva 2002). As a rule, samples with more than 5 individuals do not have 100 percent of a stable karyotype. The phenomenon of mosaicism or karyotype instability by number and morphology in chromosomes is still not explained.

A study of karyotypes from 367 sampled animals in the Russian Far East populations: Primorskii krai (n = 280), Khabarovsk krai (Lower Amur Territory) (n = 67), Jewish Autonomous Oblast (n = 1), Amur Oblast (n = 4), Magadan Oblast (n = 1) (Roslik, Kartavtseva 2012) allowed to assume the leading role of natural selection in the formation of the critical mass of B chromosomes and its weakening in individuals with stable karyotype. According to morphology, these chromosomes were recently divided into two groups: (i) ordinary - small and medium metacentric and (ii) rare - large meta-, submetacentric, subtelocentric and mini B chromosomes (Roslik et al. 2016).

We investigated karyotypes of *A. peninsulae* (5 males and 2 females) from the Bureya River depression, east coast of the Bureya river bank, near the village of Chegdomyn, Khabarovsk krai (Middle Amur Territory). All individuals had stable karyotype with bi-armed B chromosomes of small sizes. Three individuals had one B chromosome, two individuals - two B chromosomes and two individuals - four B chromosomes. According to morphology of B chromosomes, all mice had B chromosomes of ordinary morphology. Such a variant of the number and morphology of chromosomes had been described earlier for four populations of mice (n=19) from the Amur-Sungari plain (Middle Amur Territory) (Roslik et al. 2016). The C- banding of chromosomes showed that the arms of B chromosomes have a C-staining of a weaker color than the centromere of the autosomes and the Y chromosome. The centromere of the B chromosomes had no staining. Such staining of B chromosome is typical for *A. peninsulae* from Primorskii krai (Kartavtseva et al. 2000).

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**CHROMOSOMAL FEATURES OF THE VOLES OF THE GENUS
ALEXANDROMYS (RODENTIA)**

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Data on morphology, hybridization (Meyer et al., 1996), chromosome composition (Meyer et al., 1996; Mekada et al., 2001; Borodin et al., 2010), along with genetic analysis of nuclear and mtDNA (Conroy, Cook, 2000; Jaarola et al., 2004; Galewsky et al., 2006; Bannikova et al., 2010) showed independence of the vole from Asian phylogenetic line of *Microtus* from the rest lines and allowed to raise taxonomic level of East Asian voles *Alexandromys* Ognev, 1914 from subgenus up to genus (Abramson, Lisovsky, 2012). According to the genetic data separation of *Alexandromys* have happened approximately 1.2 Mya (Bannikova et al., 2010) and chromosomal rearrangements played an important role in the speciation (Frisman et al., 2016). The chromosomal rearrangements (Robertsonian, tandem fusions, para- and pericentric inversions, centric reposition and changes of heterochromatin material) have played an important role in speciation of the taxon (Modi, 1987; Mazurok et al., 2001; Lemskaya et al., 2010). In species of *Microtus* a polymorphism of chromosome numbers is a rare phenomenon, and polymorphism on the tandem fusion is absent completely (Modi, 1987, Zima, 1993, 2000). The East Asian voles of the genus *Alexandromys* is characterized by a high frequency of intra species polymorphism. We divided the species group into four types: (I) The species, which have stable karyotype in diploid number and number of arms of chromosomes: *A. montebelli*, *A. kikuchii*, *A. clarkei*, *A. sachalinensis* ($2n = 50$ NFa=60), *A. gromovi* ($2n = 44$), (II) Species with changeable karyotype in number chromosomes: *A. oeconomus* ($2n=30-32$) and *A. mongolicus* ($2n = 49-50$), (III) Species with changeable karyotype in number of chromosome arms: *A. fortis* ($2n = 52$, NFa=62-64) *A. middendorffii* ($2n = 50$, NFa = 54-56), *A. limnophilus* Büchner, 1889 ($2n=38$, NFa=56-58) and *A. mujanensis* ($2n = 38$, NFa = 46-50), (IV) Species with changeable karyotype in number of chromosomes and chromosome arms: *A. evoronensis* ($2n = 38-40$, NFa=51-54) and *A. maximowiczii* ($2n = 36-44$, NFa=50-60).

Recently, the genus *Alexandromys* was isolated from genus *Microtus*. According to the genetic and molecular data separation *Alexandromys* happened between approximately 1.2 Mya (Bannikova et al. 2010).

**GENTIC CONSERVATION OF FISH AND MARINE LIFE IN INDIA
WAZIR SINGH LAKRA**

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The paper highlights the potential application of genetic information for conservation challenges of both marine and freshwater taxa. India is endowed with vast inland and marine habitats which harbour nearly 1,500 marine and 765 freshwater fish species. These aquatic genetic resources need to be authenticated using powerful molecular markers for biodiversity utilization and conservation. The recent conservation efforts include genetic stock identification using molecular markers, development of brood banks and ex-situ gene banking of endangered and commercially important species. A recent spurt in the development of cell lines for various fish species provides new dimensions to germline conservation. A concept of a State Fish has been an innovative approach introduced recently for fisheries conservation involving State Governments as stakeholders. The technological and innovative applications of DNA barcoding in taxonomic identification and improved fisheries management are discussed.

**DNA METABARCODING FOR INSECTS DIET ASSESSMENT:
POSSIBILITIES AND LIMITATIONS**

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Characterization of biodiversity has been extensively used to confidently monitor and assess environmental status. A major part in this struggle for existence is played by predator–prey, host–parasite and herbivore–plant interactions, between which multidimensional webs of interactions have arisen. An important part of this process is analysis of precisely what has been eaten in the field, information that is difficult to obtain for generalist predators and herbivores. Metabarcoding, the combination of DNA taxonomy and high-throughput sequencing, is a promising tool for the rapid assessment and monitoring of biodiversity in mixed, bulk samples. Metabarcoding has been successfully applied to taxa that are difficult to assess with traditional methods. Although arthropods constitute the most abundant and diverse non-microbial organisms on Earth, comprehensive information on large-scale patterns of richness, endemism and biogeography are lacking.

The COI gene is by far the most commonly used marker for metazoan metabarcoding, for which thousands of reference sequences (Barcode of Life Database - BOLD, over 1,000,000) and several hundreds of amplification primers (over 400 COI) are available. It has also been found to be an effective tool for assessing the diversity of insects collected from traps and characterize the diet of predators and herbivores. One limitation of metabarcoding is the efficiency of assigning taxonomy to molecular operational taxonomic units (MOTUs). Though the percentage of MOTUs assigned to order level is usually high, this is not the case for assignments at a lower taxonomic level. This problem is not due to the metabarcoding pipeline used, but rather to the lack of comprehensive and taxonomically reliable barcode databases for most taxa. To enhance the utility of metabarcoding a new primer combination targeting a 400 bp fragment of the COI gene and Illumina high-throughput sequencing is used.

PCR amplification of targeted genes is employed as the sole approach to acquiring sufficient barcode sequences that are used for species identification. An inherent drawback to this approach is that primers amplify of undesirable species. The amplification of DNA from the predator can be particularly problematic because it is often more abundant than the prey templates and can prevent their detection. Several solutions have been devised to deal with this difficulty. One set of approaches is based on the targeted removal of undesired dominant DNA templates. A promising alternative, termed ‘suicide polymerase endonuclease restriction’, involves the design of a target-specific PCR primer and identification of a restriction site within the amplicon. During a joint PCR and endonuclease digest, only double-stranded target DNA is cut, leaving single stranded rare DNA templates intact. Another strategy is to block the amplification of predator DNA. This can be accomplished using an artificially synthesized DNA analogue, such as peptide nucleic acid or locked nucleic acid designed to attach somewhere along the target predator DNA fragment and prevent DNA polymerase from extending the broad coverage PCR primers along the entire length of the fragment. The use of PNA in such ‘PCR clamping’ has been applied successfully to dietary analysis.

GENETIC AND MORPHOMETRIC VARIABILITY OF TWO MUSSEL SPECIES (*M. TROSSULUS* AND *M. GALLOPROVINCIALIS*) IN THE NORTH-WEST PART SEA OF JAPAN

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Genetic variability of Pacific mussel, *Mytilus trossulus* and Mediterranean *M. galloprovincialis*, was studied in the North West part Sea of Japan, Peter the Great Bay. The genotyping of individuals made from 8 settlements at 8 polymorphic enzyme loci and 2 nuclear DNA markers, *Me-5* and *ITS-1,2* in 2011 and 2012-2013 years jointly with the analysis of occurrence of parent species and their hybrids in samples. Enzyme and nuclear markers exhibited concordant genetic variability in data obtained. In the settlements the prevalence of local species *M. trossulus* was obtained. The fraction of introduced species *M. galloprovincialis* in the whole material is relatively low. Main outcome is made on the continuation of the invasion of *M. galloprovincialis* in west-pacific part of Japan Sea; it is stated that in the Possjeta Bay even permanent settlements of this mussel are already available which never recorded here before. Second conclusion is that genetic introgression still present between two taxa of *Mytilus* ex. gr. *edulis* investigated from the area in Peter the Great Bay and its vicinities. Although it is keeps at a low level. Hybrid occurrence for instance vary across years judging on obvious differences in amount of all types of hybrids in sampled 2011 vs. 2012-2013 and in other records of the Vostok Bay area for 14 years interval: 2012-2013: 0%, 2011: 0%, 2003: 1.60±0.90%, and 1999: 8.95±1.68%. The number of immigrants (Nm) per generation was estimated approximately as Nm = 5. Assuming that not F1 hybrids but mostly offspring of the next generation like F2, F3 and Fb take part in gene flow, the fraction of interspecific migrants estimated as Fb+F2 etc. was equal to 0.9±0.7%.

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**SHIELDING OF GEOMAGNETIC FIELD AS A NEW TYPE OF STRESS: FROM
NUCLEAR ORGANIZATION TO COMPLEX BEHAVIOR**

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Neurodegenerative diseases (NDD) are caused by a complex interaction of unfavorable external factors and specific genome features that predispose to the development of a disease. *Drosophila* constitutes a convenient model for studying the link between genome organization, chromosome architecture and cognitive disturbances observed in neurodegenerative disorders. Despite existence of genetic risk factors, the "start" of many NDD is spontaneously under the action of external stress factors. One of the new insufficiently studied stress factor is a weakened magnetic field of low intensity, which is capable to make an impact on live organisms including human. Shielding of live objects from the natural geomagnetic field renders harmful and today still misunderstood impact on a nervous system. We analyzed the influence of shielding geomagnetic field on transcriptional activity of the genome, learning ability and medium-term memory formation in *Drosophila melanogaster*. Under stress we observed the dependence of transcriptional activity modification on the structure of a gene LIMK1, the key enzyme of actin remodeling cascade. Increased attention of neurobiologists to the signal cascade of actin remodeling, integration of different neurodegenerative disorders under the name «cophilinopathies» pointed to a wide spectrum of inner adaptive processes related to this cascade. The signal cascade of actin remodeling: receptors of neurotransmitters – small Rho GTPases (RhoA, Cdc42 and Rac1) – LIM kinase 1 (LIMK1) - cofilin – actin – is believed to play the main role in dendrite- and synaptogenesis. LIMK1 – is the key enzyme of actin remodeling which controls dendritic spine morphology necessary for synaptic plasticity during learning and memory formation. The analysis of histone methylation has revealed high level of transcription in both stocks, *Berlin* (control) and *agn^{ts3}* (defect of *limk1* gene), in normal conditions. However, we have found significant suppression of transcription after impact of the weak static magnetic field in both stocks, especially in *agn^{ts3}* mutant. Therefore, the change of transcriptional activity in the conditions of weakening a geomagnetic field can be caused by disturbances of actin remodeling cascade. Disturbances of medium-term memory were observed under the impact of a weak static magnetic field in a wild type stock *Canton-S*. On the contrary, in mutant *agn^{ts3}*, this stress action results in restoration of learning ability and memory formation. Obviously, stress influences are necessary for this mutant and sufficient for restoration of training ability and formation of memory.

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GENETIC IDENTIFICATION OF A LAKE ISTIHED ENDEMIC *SALVELINUS ANDRIASHEVI*: DISCORDANCE BETWEEN MORPHOLOGICAL AND GENETIC VARIATION

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The divergence of phenotypically different and often geographically isolated forms is a significant problem for taxonomy and phylogeny of charr of the genus *Salvelinus*. Of special interest are isolated lacustrine charr populations known in many water bodies of the Northeast Asia. Among such charrs is the Chukchi charr, an endemic narrow-range species represented by a single population in land-locked Lake Istihed (64°29' N/173°33' W) in the Chukotka Peninsula (Chereshnev et al. 2002). Originally described as a separate species, *Salvelinus andriashevi* Berg (1948), it was subsequently synonymized with *Salvelinus alpinus malma* var. *andriashevi* (Barsukov 1958) or regarded as a subspecies *Salvelinus alpinus* (Glubokovsky et al. 1979). Relying on the identity of karyotypes (Frolov and Frolova 2001) and 22 protein nuclear loci (Omel'chenko et al. 1998) from Istihed and Achchen lakes, it was concluded that the Chukchi charr represents an isolated local population of the Taranetz charr. However, the species status of Chukchi charr was later confirmed based on morphological characters (Chereshnev et al. 2002).

The present study attempts to resolve the existing taxonomic problem using molecular genetic markers. We have sequenced the three mitochondrial DNA (mtDNA) regions, among them those in *S. andriashevi* for the first time, for comparison with other lake charrs and for more precise phylogenetic analysis: the entire cytochrome *b* gene (*Cytb*; 1141 bp), segments of the 5' end of the cytochrome *c* oxidase I gene (*COI*; 1200 bp) and control region (*CR*; 1021 bp). The sequences are very similar in size and gene arrangement and composition to the charr genomes published previously. The total length of mtDNA nucleotide sequences was 3362 bp. The overall base composition was 26.5% A, 29.8% T, 16.6% G and 27.2 % C. The combined sequence (1-1021 bp *CR*, 1022-2221 bp *COI*, and 2222-3362 bp *Cytb*) had 124 variable sites (92 parsimony-informative) and 37 different haplotypes. The analyzed mtDNA region in two specimens of *S. andriashevi* had one haplotype.

Our results suggest that the specimens of Chukchi charr belong to the arctic group of Taranetz charr according to Oleinik et al. (2015). The genealogy of mtDNA haplotypes supports the phylogenetic closeness of *S. andriashevi* with *S. taranetzi* and their recent divergence and/or origin from a common ancestor. *S. andriashevi* is the least diverged in the arctic group and can be regarded as an isolated population of *S. taranetzi*. The level of divergence between *S. andriashevi* and other taxa within the phylogenetic group was relatively low ($D_{xy} = 0.001 \pm 0.000 - 0.003 \pm 0.001$). At the same time, the level of divergence of allopatric *S. andriashevi* and *S. malma malma* ($D_{xy} = 0.014 \pm 0.002$), *S. andriashevi* and *S. alpinus alpinus* ($D_{xy} = 0.015 \pm 0.002$) matched the estimates for allopatric and sympatric populations of *S. taranetzi* and *S. malma malma* ($D_{xy} = 0.014 \pm 0.002$), *Salvelinus* sp. 4 and *S. malma malma* ($D_{xy} = 0.013 \pm 0.002$), *S. krogiusae* and *S. malma malma* ($D_{xy} = 0.013 \pm 0.002$). Therefore, the ratio of within- to between-population divergence of the mtDNA nucleotide sequences was analogous to that previously reported for *S. taranetzi* and *S. m. malma* populations (Oleinik et al. 2015).

We encountered the problem of non-conformity of taxonomic differentiation of charrs based on morphological and genetic analyses. The reason for the observed discrepancy between morphological and genetic differentiation is likely to be recent divergence of the populations, and/or uneven evolutionary dynamics of qualitatively different characters against the background of exceptional ecological plasticity of charrs. Another reason may be limitations imposed by some adaptive morphological characters as phylogenetic markers. Our study also indicates the need for more thorough analysis of the morphological and genetic diversity of charrs from this region.

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**STRIPED FIELD MOUSE *APODEMUS AGRARIUS* (RODENTIA MURIDAE) AS
A SUBJECT FOR GENETIC INVESTIGATION: SOME RESULTS, PROSPECT
AND CONTRUBUTION OF THE RUSSIAN FAR EAST**

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Striped field mouse *Apodemus agrarius* Pallas, 1771 – is the widespread ecologically plastic species of the Palearctic rodents. The extensive area of this species, divided by disjunction in Transbaikalia and Mongolia, extend from central Europe to the Pacific coast of Asia, including China, Korea, south of the Russian Far East. In the Northeast Palearctic expansion of the boundary of the species area was observed in recent decades: the boundaries are dynamic and formed under the influence of human activities. As a subject of genetic research, the striped field mouse is involved in phylogeographic and phylogenetic analysis insufficiently. Populations from east part of species range demonstrated higher level of genetic diversity in comparison with west ones. Ambiguously interpreted data (Suzuki et al., 2008; Sakka et al., 2010), reflecting the expansion of the species from East Asia and the probable existence of several Pleistocene refuges were obtained. Populations the Korean Peninsula and the adjacent shelf islands were studied based on analysis of the mitochondrial genome variability. The level of genetic diversity was assessed, and the essential differentiation for the island isolates and relatively poorly expressed between continental subspecies of the Korean Peninsula and Northeast China was shown (Yoon et al., 2004; Koh et al., 2011; 2014; Oh et al., 2013; Kim, Park, 2015). The study of both nuclear and mitochondrial genomes revealed a high level of genetic variation in this species in the south of the Russian Far East, including in Primorskii krai (Pavlenko, 1997; Atopkin et al., 2007; Zasytkin et al., 2007; Dokuchaev et al., 2008; Sakka et al., 2010; Frisman et al., 2015, 2016). The hypothesis that the continental population of the southern Russian Far East can be considered as one of the centers of diversification of this species is discussed (Sakka et al., 2010). The genetic variability of molecular markers was estimated based on a small number of limited local samples, which sometimes included single specimens (Suzuki et al., 2008; Sakka et al., 2010) in spite of a giant species area and population abundance. Recently the variability of the cytochrome b gene of the striped field mouse in the South of Far East of Russia (Pereverzeva, Pavlenko, 2014) was investigated in more detail for representative local samples. High indices of molecular diversity were obtained. The topology of the phylogenetic tree and the median networks suggest that the population of striped field mice in the south of Primorskii krai originated from three maternal lines. It was noted that clustering of haplotypes on a territorial basis is absent, and population of *Apodemus agrarius* in southern Primorskii krai can be considered as one of the key points in maintaining a high genetic diversity of the species. Also genetic features and the putative sources of formation for invasive isolated populations of the striped field mouse in Magadan oblast have been studied. Magadan's enclave include of several isolated local settlements originated from South of Russian Far East and Central China (Pereverzeva, Primak, Pavlenko et al., 2017). And an analysis of genetics diversity and differentiation of mainland and islands population in the Far East of Russia on the base of microsatellites loci was started (Frisman et al., 2015, 2016). Morphological basis for genetic study also was developed (Sheremetyeva et al., 2017).

INTRA- AND INTERSPECIFIC DIVERGENCE WITHIN STARFISH AND SEA URCHINS

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A fragment of the mitochondrial *COI* gene from isolates of several echinoderm species was sequenced. The isolates were from three species of starfish from the Asteroidea family (*Asterias amurensis* and *Aphelasterias japonica* collected in the Sea of Japan and *Asterias rubens* collected in the White Sea) and from the sea urchin *Echinocardium cordatum* (family Loveniidae) collected in the Sea of Japan. Additionally, regions including internal transcribed spacers and 5.8S rRNA (*ITS1* – 5.8S rDNA – *ITS2*) were sequenced for the three studied starfish species.

Phylogenetic analysis using *COI* sequences together with earlier determined homologous *COI* sequences from *Ast. forbesii*, *Ast. rubens*, and *E. laevigaster* from the North Atlantic and *E. cordatum* from the Yellow and North Seas (GenBank) placed them into strictly conspecific clusters with high bootstrap support (99% in all cases). Only two exceptions – *Ast. rubens* DQ077915 sequence placed with the *Ast. forbesii* cluster and *Aph. japonica* DQ992560 sequence placed with the *Ast. amurensis* cluster – were likely results of species misidentification. The intraspecific polymorphism for the *COI* gene within the Asteroidea family varied within a range of 0.2–0.9% as estimated from the genetic distances. The corresponding intrageneric and intergeneric values were 10.4–12.1 and 21.8–29.8%, respectively (Fig. 1).

Analysis of relations at a higher taxonomic level showed that *Ast. amurensis* (North Pacific) and *Ast. rubens* (North Atlantic) are the closest species within the *Asterias* genus despite significant geographical remoteness of their areas. The proximity of these two species is emphasized by the number of common specific features: out of 36 phylogenetically informative sites, 13 supported clustering (vs. 7 sites for clustering of *Ast. rubens* and *Ast. forbesii*). At the same time, the North Atlantic species *Ast. forbesii* is equidistant from the other two species and localizes to the base of a group combining the three species of this genus.

The interspecific divergence for the *COI* gene in the sea urchin of *Echinocardium* genus (family Loveniidae) was significantly higher (17.1–17.7%) than in the starfish, while intergeneric divergence (14.6–25.7%) was similar to that in asteroids. The interspecific genetic distances for the nuclear transcribed sequences (*ITS1* – 5.8S rDNA – *ITS2*) within the Asteroidea family were lower (3.1–4.5%), and the intergeneric distances were significantly higher (32.8–35.0%), compared to the corresponding distances for the *COI* gene. The genetic distance between *COI* sequences of *E. cordatum* specimens from the Northern Sea and the Sea of Japan (6.8%) raises some doubt if these animals belong to the same species, although we cannot neglect the fact that their populations are very geographically distant.

Our results suggest that the investigated molecular-genetic markers could be used for segregation and identification of echinoderm species.

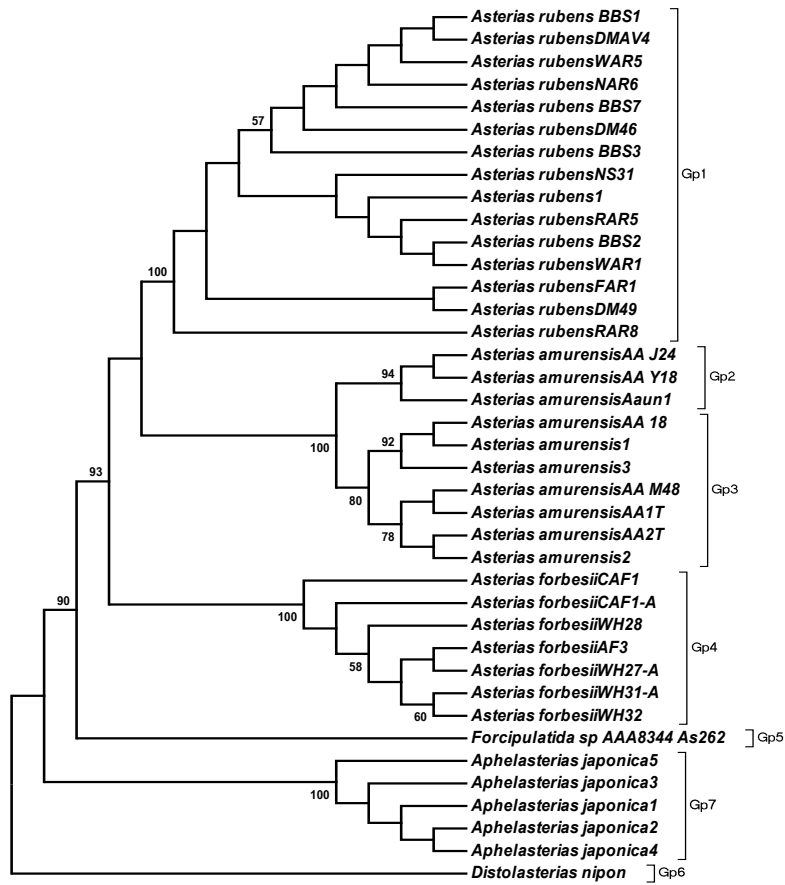


Fig. 1. Phylogenetic ML tree for the 5'-region of the mitochondrial *COI* gene from starfish of the Asteroiidae family.

**MOLECULAR PHYLOGENY OF RUSSIAN FAR EASTERN
FLOUNDERS (PLEURONECTIFORMES, PLEURONECTIDAE) BASED ON
MITOCHONDRIAL SEQUENCES FOR GENES, *CO-1* AND *CYT-B*, AND
COMPLETE MITOGENOME**

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To increase knowledge about the systematics of Pleuronectidae the primary sequence of nucleotides at subunit 1 cytochrome oxidase *c* (*Co-1*) and cytochrome *b* (*Cyt-b*) genes were determined. In total 17 newly collected species and some species from GenBank were analyzed in this research. Phylogenetic relationships among representatives of flounders were based on four types of trees: neighbor joining, maximum parsimony, Bayesian and maximum likelihood. These trees showed similar topology. Two separate clusters on the trees support subfamily Hippoglossoidinae and Hippoglossinae subdivision and monophyletic status of these taxa. The subfamily Pleuronectinae also can be considered monophyletic, if the tribe Microstomini is excluded from it and genus *Lepidopsetta* is moved in the tribe Pleuronectini. The phylogenetic status of *Hippoglossoides elassodon* and *H. robustus* is uncertain and need to be resolved in further investigation. Mitogenome of 38-45 complete sequences from NCBI GenBank were analyzed. After alignment two sets of nucleotide sequences were formed and investigated independently, one set included only structural genes (15,068 bp) while the second set comprised by the whole mitogenome (15,120 bp). Both data sets gave congruent phylogenetic signal basically agreed with conventional views on the taxonomic system for the order Pleuronectiformes. In particular, the node which includes the representatives of suborder Pleuronectoidei and superfamily Pleuronectoidea is highly supported. The incongruities between morphological and molecular issues that also were obtained suggest the need for reassessing the systematic value of some morphological characters and phylogeny both at family and order levels.

ANOVA was carried out in the software package Statistica 10. This analysis showed the relationship between the two variables, "comparison group" and "*p*-distance". The statistically highly significant relationship proves that with an increase in the rank of the comparison group (taxon), the *p*-distance is increases (Figure).

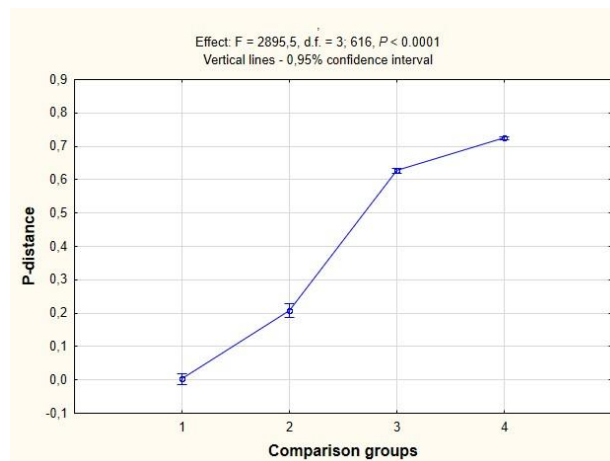


Figure. Variability of the average values of p -distances by 4 comparison groups: 1, p -distances within the species, between individuals of the same species; 2, p -distances within the genus, between individuals of different species of the same genus; 3, p -distances within the family, between species of different genera of the same family; 4, p -distances within the order, between the species of different families of the same order.

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GEOGRAPHIC DIFFERENTIATION OF B CHROMOSOMES IN *APODEMUS PENINSULAE* (RODENTIA) FROM THE EAST ASIA

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A species *Apodemus peninsulae* Thomas, 1906 has a wide distribution range on the territory of Russia - from the Ob' river in Siberia to the Pacific coast in the Far East, as well as in the north of Mongolia, China, Korea, Hokkaido Island of Japan (Gromov, Yerbaeva, 1995). Karyotypes of this species have supernumerary (B) chromosomes in addition to chromosomes of the basic set.

We have previously studied karyotypes of *A. peninsulae* in the East Asia on a large material and described polymorphism in terms of the number and frequency of B chromosome morphotypes (Roslik, Kartavtseva, 2009; 2012; Roslik et al., 2016).

We performed a comparative analysis of different geographic populations of *A. peninsulae* in the East Asia, using a new parameter: the variability of rare B chromosome morphotypes. Both frequently occurred B chromosome morphotypes and rare ones were identified. Small and medium metacentric B chromosomes were prevalent. All other morphotypes, large meta-, submeta-, subtelocentric; medium and small submeta-, subtelocentric and mini B chromosomes were classified as rare (Roslik et al., 2016; Roslik, Kartavtseva, 2017). Moreover, clinal variability in frequency of rare B chromosome morphotypes was revealed from the East to the Northwest of the area. According to the revealed pattern in variability, from the East (South and East Primorskii Krai), with a maximum diversity of B chromosome morphotypes, to the Northwest (→ center → West Primorskii Krai → Khabarovsk Krai → Jewish Autonomous Oblast → Amur Oblast), there is a gradual loss of some rare B chromosome morphotypes. Only large morphotypes and/or very small mini B chromosomes remain in populations of *A. peninsulae* in Jewish Autonomous and Amur Oblasts. According to another characteristic: presence of individuals without B chromosomes, differences were also found between populations. Such animals have been recorded in the mainland populations in Primorskii Krai and Khabarovsk Krai; however, findings of specimens without B chromosomes are extremely rare in Jewish Autonomous and Amur Oblasts and further in Siberia (Kartavtseva, Roslik, 2004, etc.).

Therefore, we detected two parameters: frequency of rare B chromosome morphotypes and frequency of individuals without B chromosomes, which to a greater or lesser extent indicate differentiation between populations of *A. peninsulae* in the East Asia.

**THE USE OF THE INDICATOR PARASITE *ANISAKIS SIMPLEX* IN
POPULATION STUDIES OF PACIFIC HERRING *CLUPEA PALLASII*
(CLUPEIFORMES: CLUPEIDAE) IN SAKHALIN WATERS**

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Mass parasites registered in all the Far Eastern seas are larvae of the nematode *Anisakis simplex* found in 42 species of fish, including the Pacific herring (56.6% infestation rate). Quantitative indicators of infestation with the larvae of *A. simplex* were used in differentiation of populations of pink salmon in Sakhalin waters. Indicator parasites allowed to reveal differentiation among local populations of sockeye salmon. Information about infestation of the Pacific herring with larvae of this parasite is vital and of great practical interest. As a mass kind of parasite, sufficiently large and easily recognizable larvae of *A. simplex* can be used as indicator parasites of the exploited Pacific herring populations. Previous studies conducted with the use of the methods of biochemical genetics showed the existence of differentiation between local population of Pacific herring in the Japan and Okhotsk seas. The use of parasites as biological indicators confirmed the earlier revealed differentiation pattern. Quantitative indicators of infestation of herring in Sakhalin waters reveal high infection rate of the fish in the Sea of Okhotsk, off eastern coast of Sakhalin. Moreover, in some years, almost 100% of herring were infested. In Sea of Japan, off western coast of Sakhalin, the indicator values of herring infestation are much lower. The analysis of herring infestation rate in these marine areas allows to confirm our earlier conclusions about population structure of herring inhabiting waters around Sakhalin Island.

**EVOLUTION AND BIOGEOGRAPHY OF THE GENUS *ABIES* (PINACEAE):
PHYLOGENY BASED ON SEQUENCE DATA FROM THREE GENOMES**

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Phylogenetic methods, based on multiple independent loci, take into account the stochasticity of individual locus genealogy; therefore, these methods have an advantage over the methods, which employ cytoplasmic and single locus nuclear markers. At the same time, the use of genetic markers with different inheritance mode for phylogenetic reconstructions makes it possible to obtain additional information shedding light on past migrations, hybrid contacts and captures of cytoplasmic genomes.

In order to study the phylogeny, evolutionary history and molecular systematics of the genus *Abies* (ca. 50 fir tree) we performed a phylogenetic reconstruction of the genus using nucleotide sequences of ten single-copy nuclear genes and complemented those with chloroplast and mitochondrial DNA phylogenies. The phylogenetic tree, obtained from five regions of paternally inherited cpDNA and nuclear “species tree” largely corresponded to each other. The presence of several clades of firs, inhabiting both North America and Eurasia, points to repeated intercontinental migrations. MtDNA haplotype tree, however, has shown a strong contradiction to the phylogeny of nuclear and chloroplast DNA. It consisted of two clusters: one cluster included mainly American haplotypes, while the other one was composed by solely Eurasian haplotypes. Presumably, this conflict is due to the intercontinental migrations and introgressive hybridization, accompanied by the mitotype transfer among species. Based on these data we suggest the hypothesis of American origin of extant firs including several migrations from North America to Asia. Calibrations of the divergence times were based on paleobotanical data and on estimation of mutation rate. The occurrence of the genus *Abies*, as well as many other conifers, dates back to the Cretaceous; however, the diversification of modern lines coincided with the Neogene cooling, and expanding the territory, suitable for temperate and boreal vegetation.

THE ISOLATES OF THE EAST ASIA ARE RESERVES FOR ARCHAIC FORMS OF SOME SPECIES

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The problem of biodiversity conservation is related to the reserves of intrapopulation variability of a species. On the one hand, the genetic diversity of natural populations is the basis for adaptive and evolutionary changes. On the other hand, this is the main mechanism of species stability. Isolation is a key factor that results in barriers, which prevent crossbreeding. This leads to an increase and consolidation of interpopulation differences due to the weakened flow of migrants. Islands (including mountain isolates) are natural laboratories of isolated populations for studying the processes of microevolution. The features of these processes in island populations lead to a lower level of genetic diversity and panmixia. Nevertheless, isolation, gene drift, and low population size allow each isolated population to change independently and lead to genetic differentiation and formation of a unique gene pool. In addition, isolates are also able to be reserves of ancient haplotypes or archaic forms. Archaic forms are relic populations of the species. Being isolated they are the remains of an ancient species, which was earlier distributed in this area. Often the definition of relic populations is difficult.

This study includes the analysis of genetic diversity in some rodent species with isolated populations with the purpose to detect archaic forms. As a result, archaic forms were discovered in isolated populations in the reed vole and greater long-tailed hamster.

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GENETIC DIVERSITY OF NEW SPECIES *METAGONIMUS SUIFUNENSIS* IN THE RUSSIAN SOUTHERN FAR EAST

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Representatives of the genus *Metagonimus* (Trematoda: Heterophyidae) have an important epidemiological significance in the Far Eastern region, where cases of human infection by *M. yokogawai* have been repeatedly registered.

However, using both the ITS2 region and the 28S gene of nuclear ribosomal DNA as the markers, we have proved that species, inhabiting the Russian southern Far East, is not *M. yokogawai*, as it was supposed until recently. This species with no significant morphometric differences from *M. yokogawai* was described as a new species, *M. suifunensis* Shumenko, Tatonova & Besprozvannykh, by molecular data (Shumenko et al., 2017). In addition, in the analysis of 64 *M. suifunensis* samples from 6 localities in the Russian southern Far East using ITS1 sequences, no variability was found in the whole investigated area.

In this study, using the *cox1* gene of mitochondrial DNA (513 bp), we confirmed the status of this species: the genetic distance between *M. suifunensis* and *M. yokogawai* is in a range of distances between species of sister family Opisthorchiidae. Besides, using this sensitive marker, the genetic diversity of *M. suifunensis* in the Russian southern Far East was estimated. We revealed that nucleotide diversity in this species is 10 times lower, than in *Clonorchis sinensis* (Trematoda: Opisthorchiidae) in the same territory of Russia (Chelomina et al., 2014). The haplotype diversity level is also 3.5 times lower in *M. suifunensis*, than in *C. sinensis*. Moreover, the mismatch distribution suggests recent population “bottleneck” in *M. suifunensis*.

**GENETIC POLYMORPHISM OF C₃-C₄ XERO-HALOPHYTE *Sedobassia sedoides*
(CHENOPODIACEAE)**

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The polymorphism of 8 enzyme systems in 21 populations of annual xero-halophyte *Sedobassia sedoides* was studied in two geographic regions with different climatic (index aridity) and edaphic (soil type, K⁺ and Na⁺ contents) conditions (the South Urals and the Caspian Lowland). 24% of the populations were monomorphic across 14 loci of these enzyme systems. Most polymorphic *S. sedoides* populations are characterized by low heterozygosity and significant deviations from the Hardy-Weinberg equilibrium. A higher level of intra- and interpopulation genetic diversity, and differentiation of populations is characteristic of *S. sedoides* in the Southern Urals, which indicates considerable isolation of the populations, possibly caused by mosaic edaphic conditions. In the arid climate of the Caspian Lowland, on soils with lower potassium and sodium contents, significant genes flow was revealed between *S. sedoides* populations. On average, the level of genetic variability in the polymorphic populations of *S. sedoides* is 1.5-2-fold lower under these conditions as compared with the Southern Urals.

The work was supported by the Russian Foundation for Basic Research (project no. 17-04-00853-a).

**GENETIC DIVERSITY, PHYLOGEOGRAPHY AND POSTGLACIAL
DISPERSION OF THE PACIFIC SMELT *Osmerus dentex***

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The Pacific or Arctic rainbow smelt *Osmerus dentex* Steindachner et Kner, 1870 (Nellbring 1989) is a member of the polytypic genus *Osmerus* within the family Osmeridae. *O. dentex* is widespread in the North Pacific and Arctic seas and might shed light on evolutionary history of arctic marine and estuarine fauna. We assessed the impact of global climatic and geological changes on the formation of genetic structure of *O. dentex* in the Eurasian parts of the species range using a variety of phylogenetic methods and molecular dating interpreted in conjunction with paleoclimatic evidence. The phylogeographic patterns were analyzed based on *cytb* and *coI* sequences in 87 individuals, and RFLP-analysis mtDNA in 462 individuals from 25 localities in the Sea of Japan, Sea of Okhotsk, as well as in the Bering, Kara, Barents and White seas. For comparison, we used thirteen sequences from GenBank, ten of which belong to *O. dentex* from the Bering Sea and the Chukchi Sea, North American coast and Hokkaido (Japan).

Nucleotide diversity was low (0.005 ± 0.000 and 0.004 ± 0.002 , respectively) and not significant. Haplotype diversity was high (0.844 ± 0.016 from PCR-RFLP analysis and 0.864 ± 0.036 from sequencing of the *cytb/coI*); however, for populations from the Bering Sea and the Arctic seas was not statistically supported ($p > 0.05$). The BA phylogenetic tree for *cytb* data, including all our haplotypes and the haplotype downloaded from GenBank, revealed no supported geographical clustering, probably, because of the short length of the combined sequences (196 bp). However, in the BA tree for *coI* (596 bp) the Sea of Japan haplotypes were monophyletic. Hierarchical analysis of molecular diversity (AMOVA) has shown that the bulk of the variability of the total molecular dispersion (from 87 to 98%) falls on the intrapopulation component.

The most significant differentiation among geographical populations of *O. dentex* by their grouping associated with marine basins was observed for the Sea of Japan. The phylogeographic survey of Pacific smelt throughout its distribution on the opposite coasts of Sea of Japan and the Sea of Okhotsk revealed the existence of two phylogroups (or clades), with the boundary between them probably lying in the Nevelskoy Strait. Modern genetic structure of the taxon reflects historical isolation in ancestral refugia with subsequent colonization along the eastern and Arctic coasts of Eurasia during global ocean transgressions. Our results suggest that geographic distribution observed in mtDNA haplotypes resulted from influences of historical range expansions, episodes of long-distance colonization and restricted dispersal. We supposed that the main refugium of *O. dentex* was on the western Pacific coast (the Sea of Japan and the southern Sea of Okhotsk). The results of analyses suggest probable historical fragmentation of the *O. dentex* range associated also in the White Sea. The isolation could have occurred if any population in the White Sea survived in an ice-free refugium and then expanded along its shores. However, the contribution of the supposed refugium on the Arctic coast to recolonization throughout the range of *O. dentex* can be considered as negligible. The loss of mtDNA diversity in the smelt from the Arctic region can be associated with both historical and modern severe ecological conditions. In this connection, Arctic populations could repeatedly pass through a bottleneck.

EVOLUTION AND PHYLOGENETIC PERFORMANCE OF MITOCHONDRIAL CONTROL REGION AMONG EELPOUTS (COTTOIDEI: ZOARCALES)

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Control region (*CR*) is a major non-coding domain of mitochondrial DNA. It contains promoters for replication and transcription of mitochondrial genome as well as binding sites for metabolic machinery thus being a vital element for the integrity of mitochondrial genome as a replicator. The origin and diversity of structural elements within control region have been intensively studied in the recent years with involvement of new diverse taxa. Here we report new data on nucleotide and structural patterns of *CR* evolution and its phylogenetic performance among eelpouts (Cottoidei: Zoarcales). We carried out the phylogenetic and structural analysis of 29 *CR* sequences belonging to long shanny *S. grigorjewi* and 9 sequences of other eelpouts representing 4 families and compared them with evolutionary patterns of mitochondrial protein-coding fragments. The *CR* organization within *S. grigorjewi* as well as all other eelpouts is consistent with common three-domain scheme known for most vertebrates. We reveal a hidden *CR* variation constrains observed on the landscape level together with a lack of nucleotide saturation. These findings demonstrate an advantage of *CR* in phylogenetic reconstructions among eelpouts.

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**DNA BARCODING AND SPECIES DIVERSITY OF FISHES
FROM THE LAKE KHANKA**

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Khanka is the largest lake in the Far East of Russia, and contains valuable resources for freshwater fishery. The fauna of Lake Khanka has a low level of endemism due to the recent origin and connection with large river systems of Amur and Ussuri. Currently the lake is experiencing an increasing impact from human activity, e.g., through intentional introduction of non-indigenous fish species with a purpose to stabilize relationships among communities and increase natural productivity. Therefore, it is challenging to use modern powerful techniques to describe species diversity of the Lake Khanka.

Previous attempts to describe fish species diversity of the Lake Khanka have been made based on the classic methods. Here, for the first time, we present the complex approach to document fish species diversity.

We collected 65 fish specimens of 17 species representing 4 families within 3 orders of ray-finned fishes and analyzed their taxonomy and species diversity using both classic methods and DNA barcoding techniques and protocols. Specimens have been genotyped based of *Co-1* mitochondrial gene marker. Mean values of *K2P*-corrected intraspecific genetic distances were 0.1% (0-0.7), distances between different species within the same genera were 4.07% (0.15-6.6) and distances within families between different genera varied from 3.8 to 23.4 with 14.1% as a mean value. Phylogenetic analysis revealed monophyletic origin of all species clusters with large support (98-100% of bootstrap values), thus supporting reciprocal complement of morphological and genetic species boundaries and confirming high performance of the *Co-1* DNA barcoding in the field of documentation of fish species diversity in the Lake Khanka.

This study was supported by the Russian Foundation for Basic Research (project no. 15-29-02456 and 15-29-02456-ofi) as well as by the Russian Science Foundation grant no. 14-50-00034.

COMPARATIVE MOLECULAR-GENETIC ANALYSIS OF SOME SPECIES OF PARASITIC FLAT WORMS FROM THE GENUS *CREPIDOSTOMUM* BASED ON SEQUENCING DATA OF ITS REGION AND 28S rDNA

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The present study raises the question of the taxonomic state of some species of parasitic flat worms from genus *Crepidostomum* (Digenea: Allocreadidae), collected in Europe and in the Far East. We also included in the research some representatives from genus *Bunodera*. Moreover, several species of *Crepidostomum*, kindly provided by prof. Takeshi Shimazu, were also used. In previous molecular-genetic studies on these species, inadequate number of taxa was used for more complete comparison and further analysis, namely: several species from the genus *Crepidostomum*: *C. farionis*, *C. metoecus*, *C. auriculatum*, *C. chaenogobii* and *C. nemachilus*; one species from *Bunodera*: *B. acerinae*; and also the gene bank data were used.

Due to difficult definition of taxonomic status of some *Crepidostomum* species, we decided to increase the sample and added the same species of the genus from Kamchatka.

In view of the use of only one site in early studies, 28S (Atopkin, Shedko, 2014), it was necessary to include one more rDNA marker, ITS, in the analysis. For the taxonomic revision, we applied two genetic markers of ribosomal DNA, ITS and 28S rDNA, and a morphological description of some of the species studied, with the exception of the Far Eastern forms, whose morphological description had not been previously reported. We sequenced 188 individuals from the genera *Bunodera* and *Crepidostomum*.

According to the data obtained, we aligned all sequences with the total length of 880 bp (base pairs) and reconstructed phylogenetic tree using ML method (Maximum Likelihood) in Mega 6.0 and MrBayes 3.1.2. In addition, genetic distances between distinct species, each combined in individual groups, were calculated.

Phylogenetic analysis revealed differences between species of the genus *Crepidostomum* at intrageneric level. One of the species, *C. auriculatum*, was distinguished by genetic isolation with other members of this genus, as previously mentioned by many authors. And one subspecies, earlier identified as *Bunodera luciopercae acerinae*, was identical with the *B.l. luciopercae* forming one common well-supported clade. Previously validity of both subspecies was confirmed (Petkeviciute, 2010; Sokolov et al., 2013).

Therefore, an increase in number of studied individuals from different geographic localities helps to determine the pattern of their variability, and make assumptions about their genetic divergence, which will bring us closer to resolving the issue of species affiliation of some Far Eastern forms.

**MOLECULAR GENETIC DIVERSITY OF THE MUYA VALLEY VOLE
*ALEXANDROMYS MUJANENSIS***

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The Muya Valley vole *Alexandromys* (= *Microtus*) *mujanensis* Orlov et Kovalskaya, 1978 was first described based on chromosomal analysis and hybridization in Muya River valley (in the environs of Muya vill.) in Buryatia. This is the sibling species to Maximowicz's vole *A. maximowiczii* Schrenck, 1858, who is polymorphic in diploid number of chromosomes ($2n = 36-44$) while chromosome number for Muya Valley vole is constant ($2n = 38$). Until recently, *A. mujanensis* was known from Muya Valley only on the basis of chromosomal studies (Orlov and Kovalskaya, 1978; Meyer et al., 1996; Lemskaya et al., 2015). According to data published in 2015 (Golenishchev et al., 2015), Muya Valley vole was found also in Dzherginsky Nature Reserve and in the environs of Baunt Lake (Buryatia) by the study of karyotype and cytochrome b gene sequence (mitochondrial DNA). We analyzed control region sequence of mtDNA, which is studied for a number of species within genus *Alexandromys* (Haring et al., 2010) and is characterized by higher level of variability compared to cyt b gene. Small genetic distances between groups of individuals within one species could be detectable by using control region. We analyzed 55 specimens belonging to three mentioned localities. When DNA analysis was performed it was confirmed that all specimens belong to Muya Valley vole. Phylogenetic tree was inferred and it showed that all investigated specimens split into three clusters corresponding to three geographic populations. At the same time, genetic distances were lower between geographically close populations of Baunt Lake region and Dzherginsky Nature Reserve, while population near Taksimo vill. (vicinity of the type locality) is characterized by higher distances in relation to the other two clusters. Moreover, this clade (Taksimo) differs at a higher intrapopulation diversity level. Therefore, our study showed that the three geographical populations of Muya Valley vole differ in mtDNA control region. Possible colonization paths are discussed.

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SECONDARY STRUCTURE PREDICTION FOR THE 18S RRNA ACROSS TREMATODA FAMILIES

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Parasitic flatworms, known as flukes are internal parasites of molluscs and vertebrates, including humans. Elucidation of the phylogenetic relationships of trematodes is important for species identification, diagnosis and management of parasitic diseases. Small subunit (SSU) 18S rRNA gene is one of the most frequently used genes for these purposes, but very often phylogenetic signal of individual nucleotide substitutions can't be entirely estimated, since any particular gene mutations is not necessarily followed by changes in its secondary structure. The main variability of 18S rRNA is concentrated in expansion segments, ES, alternating with the conserved gene sequences. Studies on secondary structures modeling of the 18S rRNA from trematodes previously has not been performed. RNA secondary structure modeling is a challenging problem, and recent successes have raised the standards for accuracy, consistency, and tractability. For the first time, we predict minimal free energy secondary structures for the variable regions (helices) and 5 rRNA expansion segments (ES3^S, ES6^S, ES7^S, ES9^S, and ES12^S) of 19 animal and human pathogenic trematodes of the three orders: Echinostomida, Plagiorchiida, and Strigeida, based on comparative sequence analyses with other taxa and reference to recently published crystal structure of the ribosome. This can clarify whether the parasites differ from other animals. ES6^S is an extensive region that occupies central position in the entire 80S models. For this ES, a total of 65 secondary structures of studied trematodes were modeled, 42 models were obtained for worms of the orders Echinostomida and Plagiorchiida, and 23 models for schistosomes of the order Strigeida. Yet, a number of significant differences between the models for trematoda were present. Percentage of difference within families varies from 0.3% in the Echinostomatidae to 23% in the Shistosomatidae. ES6^S provide an effective framework, thus the specificity of the structure for each family solves some controversial systematic issues. Noteworthy, the degree of uniqueness of secondary structures of both ES and helices also depends on the length of their primary sequences. In short: one length - one structure. Comparison of phylogenetic trees with secondary structures suggests the direction of their evolution. Phylogenetic trees based on 18S rRNA gene using ML and BI methods showed similar topology of all taxa with a high level of support. The program BEAST (1.8.2) was used to estimate the rates. The order Echinostomida is older (87 Ma) than the Plagiorchiida (73 Ma) and presumably its representatives have quickly occupied all possible ecological niches in parasitic communities. Using ES6^S we tried to find out if there any connections of structures with patterns of life cycles and evolution in the Trematoda. However, the secondary structures of ES6^S and ES9^S during divergence time did not change their overall architecture. But additional elements present in the Trematoda 18S models may reflect an increased complexity of translation regulation in eukaryotic cells, as it is evident for assembly, translation initiation, and development. All these observations suggest that the evolution of 18S secondary structures continues, and may be related to new acquisition strategies and adaptive mechanisms.

A FACILITY FOR REARING OF FISH IN THE AQARIUM CONDITIONS FOR THE EXPERIMENTAL NEEDS

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Description of an original experimental aquarium facility for scientific purposes is given. This facility comprises a system of small, flowing boxes (aquariums, poly-propen made) with water removal at a wet table. Water supply arranged via main aquarium (tank) equipped with cooler, rough and fine water filters and aquarium skimmer; a system of tubes with taps allows water flow and temperature regulation. The facility construction enables make factor experiments for fish rearing by applying a diallelic genetic scheme of parent cross and offspring analysis in repetitions under controlled environment. The facility designed for experiments in the fields of genetics, biochemistry, toxicology, and other branches of biology and aquaculture. Rearing of eggs, larvae and fry is under an automated control of environment within the facility, at least for three parameters of water: temperature, pH, and oxygen concentration. Preliminary results obtained for smelt, *Hypomesus japonicus* are discussed.

This study was supported by the Russian Foundation for Basic Research (project no. 15-29-02456 and 15-29-02456-ofi) as well as by the Russian Science Foundation grant no. 14-50-00034.

**MORPHOMETRIC INVESTIGATION OF DIFFERENCES AMONG
SMALLMOUTH SMELT *HYPOMESUS JAPONICUS* AND *H. NIPPONENSIS*
(PISCES, OSMERIDAE) FROM INSHORE WATERS OF NORTH-WEST PART
OF THE SEA OF JAPAN**

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To specify taxonomic value of morphometric traits in smallmouth smelt *Hypomesus japonicus* and *H. nipponensis* (Pisces: Osmeridae) the research is performed that extends on the area of their habitat in the north-west part Sea of Japan. By means of the multidimensional analysis of traits and indices the differences among smelt individuals in the studied area is revealed. Data of multidimensional analysis support findings that *H. japonicus* and *H. nipponensis* differed by known traits, such as smaller diameter of the eyes and position of the dorsal fin ahead the ventral fin in the former species, as well as by traits that were newly obtained in the investigation like longer dorsal and adipose fins in the former species. Among *H. japonicus* representatives individuals from Olga Bay and that of locality of the shore near Ternay Village are the most similar, while representatives of this species fished near Russkiy Island and that from Olga Bay are extremely different. Discrimination accuracy for two species identification in the set of indices is 98.4% (Figure).

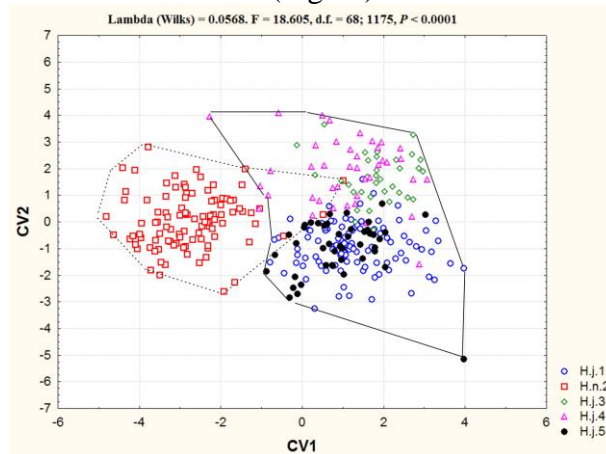


Figure. Plot of canonical variables (CV1 and CV2) distribution, obtained at DFA analysis for the individuals of two species samples of smallmouth smelt, *Hypomesus japonicus* (H.j.) and *H. nipponensis* (H.n.).

Analysis includes 5 localities: H.j.1, H.n.2, H.j.3, H.j.4 and H.j.5. The individuals from them are measured at 16 basic indices and an index that measured relative distance of dorsal fin ahead of position of ventral fin, correspondingly for H.j. as compared to H.n. (*DIF-DV*). Broken lines depict area of dots for CV1 and CV2 scores for two species. Data shows that two species are differ basically by the scores of CV1, while the north (H.j.3, H.j.4) and south localities (H.j.1, H.j.5) of H.j. by the scores of CV2 (Figure).

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**ANALYSIS OF SEQUENCE DIVERGENCE IN PACIFIC REDFIN
(CYPRINIFORMES, CYPRINIDAE, *TRIBOLODON*) BASED ON mtDNA AND
nDNA MARKERS WITH INFERENCES IN SYSTEMATICS AND GENETICS
OF SPECIATION**

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The genus *Tribolodon* (Sauvage, 1883) is a member of the most common fish family, Cyprinidae that possibly is one of the largest families of vertebrates. To clarify relationship of species of the genus *Tribolodon* in the Russian part of their distribution ranges, 2 mitochondrial markers (*Co-1* and *Cyt-b*), nuclear markers of rhodopsin gene (*Rho*) and internal transcribed spacer of rRNA (*ITS-1,2*) were used. Depending on the marker, the different number of the species groups were detected by the gap finder software, ABGD. These results were compared with the analysis of phylograms and data showed generally the support for known species clusters and regional intraspecies groups. A complex analysis of sequences from three redfin species within the area of the study, based on four marker genes and methods of molecular phylogenetics, ordination of genetic distances (ABGD software), recombinant analysis (RDP software), and population genetic approaches (MEGA-6 and DNA-SP5 software) is performed. Presented data revealed clusters of three commonly recognized species, regional intraspecific groups or individuals of local populations, and few hybrid individuals. The capture of hybrids in Kievka and Vostok bays supports the theoretical possibility of existence of a hybrid zone for *Tribolodon* in southern Primorsky Krai. However, the frequency of hybrid occurrence remains poorly studied. The findings of the present study support the existence of three formerly established species of the genus *Tribolodon* in southern Primorsky Krai and Sakhalin Island: *T. hakonensis*, *T. brandtii*, and *T. sachalinensis*. DNA barcoding technique proved to be efficient with the use of two mtDNA markers: *Co-1* and *Cyt-b*. Some insertions and substitutions within the *ITS-1,2* have been found. This nuclear rRNA gene marker has a relatively high variability that allowed us to identify each of three the redfin species of the genus *Tribolodon*. *ITS-1,2* marker has been sequenced for *Tribolodon* species for the first time, and it can be used in further studies for species identification and phylogenetic reconstructions of redfins, which is a challenging task. The results obtained on inter- and intraspecies differentiation did not confirm a sufficient level of divergence for defining any new taxa of a species rank in the genus *Tribolodon*.

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GenSeq™ DNA Library kit

таргетное секвенирование
на платформах Illumina и Ion Torrent
стало доступней



GenSeq™ DNA Library kit - кроссплатформенный набор для создания библиотек ампликонов на основе панелей AmpliSeq™ для последующего анализа методом высокопроизводительного секвенирования. Позволяет проводить секвенирование полученной библиотеки на двух платформах: Illumina и Ion Torrent. Впервые панели AmpliSeq™ стали доступны пользователям Illumina.

GenSeq™ DNA Library Kit производится в России компанией АНПРО. Выгодная цена набора позволяет секвенировать больше в рамках прежнего бюджета. А удобный формат наборов на 48/96/384 реакции подходит для широкого круга задач.

Высокие показатели однородности и специфичности делают набор незаменимым при проведении таргетного секвенирования. Для приготовления библиотеки достаточно всего 1-10 нг геномной ДНК на 1 пул праймеров.

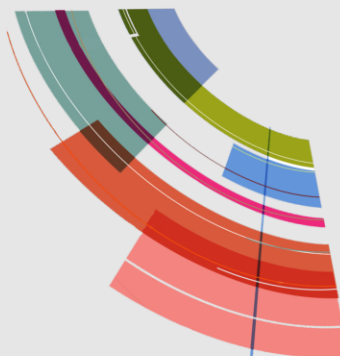
Панели праймеров AmpliSeq™ решают широкий круг задач методом таргетного секвенирования: имеются как готовые панели для исследования заболеваний человека, так и возможно создавать собственные, пользовательские панели.

Пользовательскую панель можно применять для изучения любых видов живых организмов - достаточно лишь загрузить геном исследуемого вида и выбрать мишени, программа сама проведет дизайн панели с учетом указанного пользователем качества входящей ДНК.

On-demand панели праймеров применимы только для изучения заболеваний человека - они позволяют составить панель из всего множества представленных в он-лайн приложении AmpliSeq™ Designer генов, ассоциированных с определенными заболеваниями. Все панели on-demand прошли проверку в "мокрой" лаборатории, что является дополнительным гарантом качества их работы.

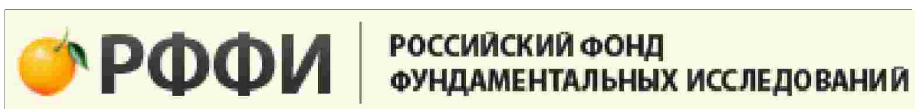
AmpliSeq™ panels


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