

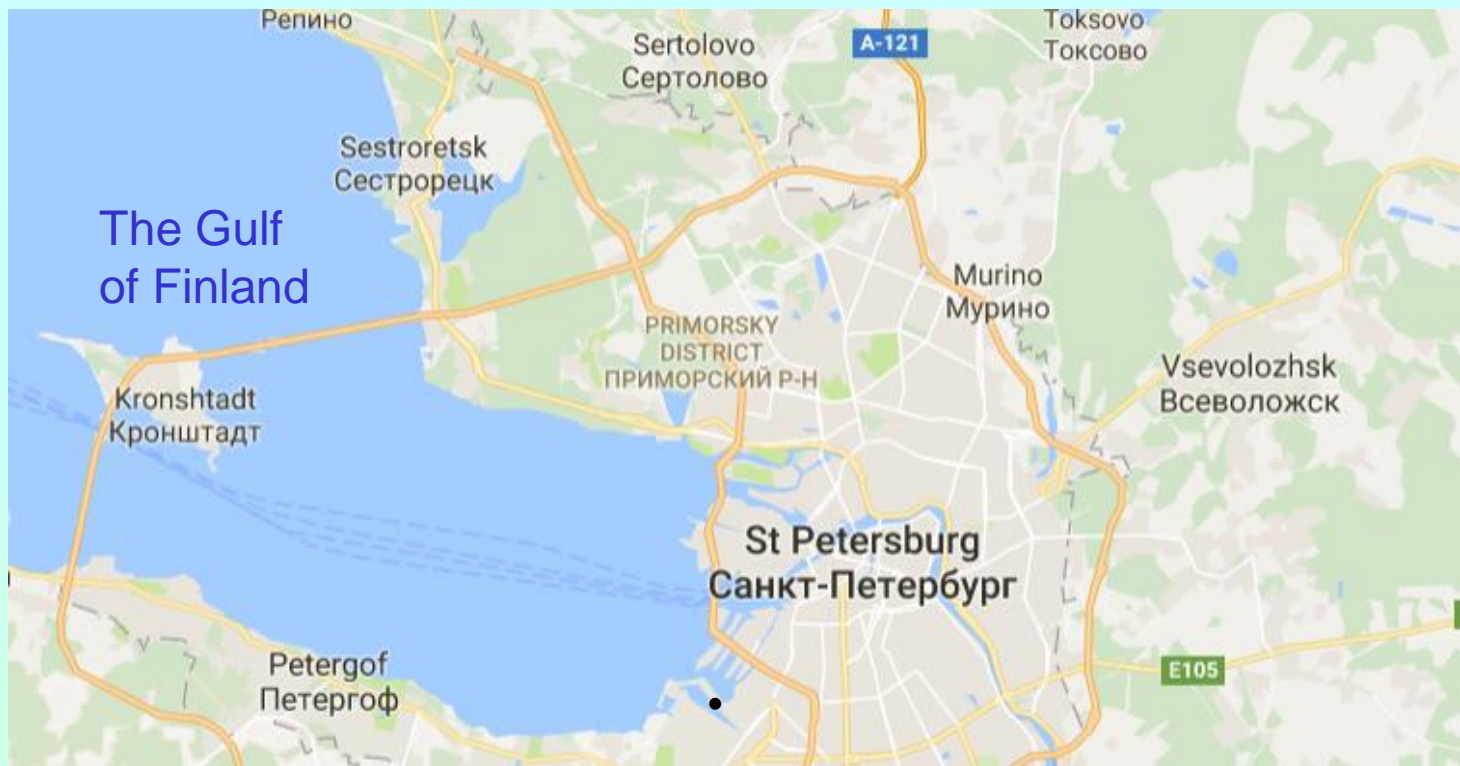


The amphipod *Gmelinoides fasciatus*  
as an example species  
used to investigate  
the Gulf of Finland biological systems  
by means of molecular genetic methods.

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Anthropogenic pressure has a profound impact on the biological system of the Gulf of Finland.

- Ship industry is the major factor.
- Industrial and economic activities carried out to maintain sustainability of the coastal areas are having an impact on the aquatic state of the littoral zone.



**Various genetic approaches are the source for a long-term forecast, therefore they are indispensable for complex assessment of water system state including investigations on water organisms.**

Genetic methods make it possible:

- to estimate genotoxic effects of water pollution,
- to give a prognosis on the possible structural changes of the analysed biosystem .

**The monitoring is based on genetic test-systems, which include indicator species and criteria for measuring deleterious effect on genetic material.**



## indicator species

The amphipod  
*Gmelinoides fasciatus*  
is one of the most  
numerous species  
among  
alien organisms.



Due to its broad ecological plasticity this hydrobiont got widespread in the water bodies of the North-West regions of Russia as the result of deliberate introduction.



North-  
West  
of  
RUSSIA



Deliberate  
introduction



The ecological niche for autochthonous populations of the sideswimmer *G.fasciatus* is the Lake Baikal.

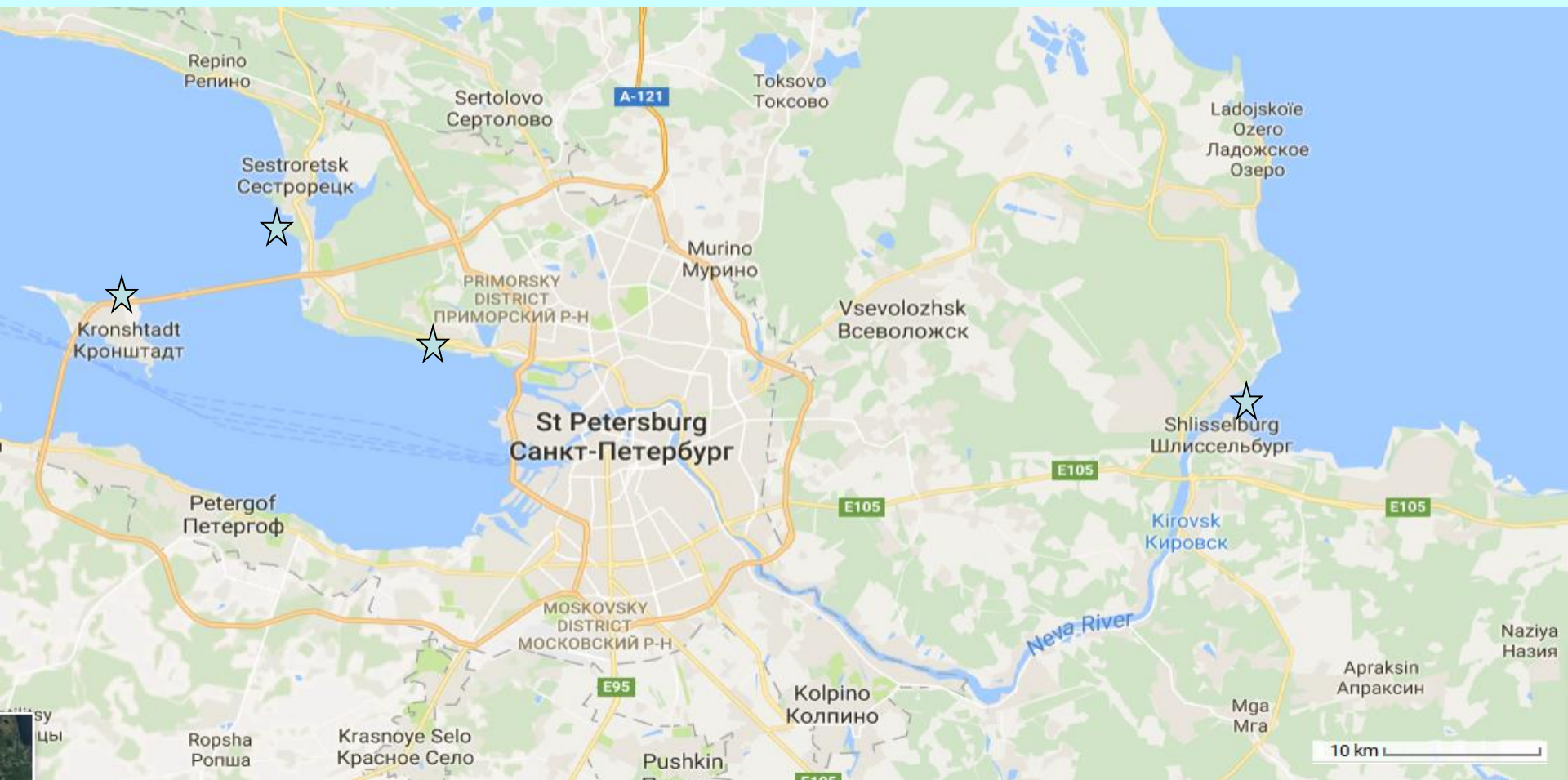
*Gmelinoides fasciatus* Stebbing, 1899 (Amphipoda, Crustacea)  
from the Lake Baikal was introduced into a number of water  
bodies of Northwest Russia  
to expand food reserves of commercial fish.



The interest in this crustacean has been attributed to its successful adaptation and expanding habitat area in the region.

**Therefore, it would be an ideal experimental set up because the same species could be used to examine the influence of differing environments.**





- ☆ - sample collection sites in the vicinity of St.Petersburg:  
in the Gulf of Finland (Sestroretsk - Park “Dubki”,  
Kronshtadt, Olgino) and in the Lake Ladoga.

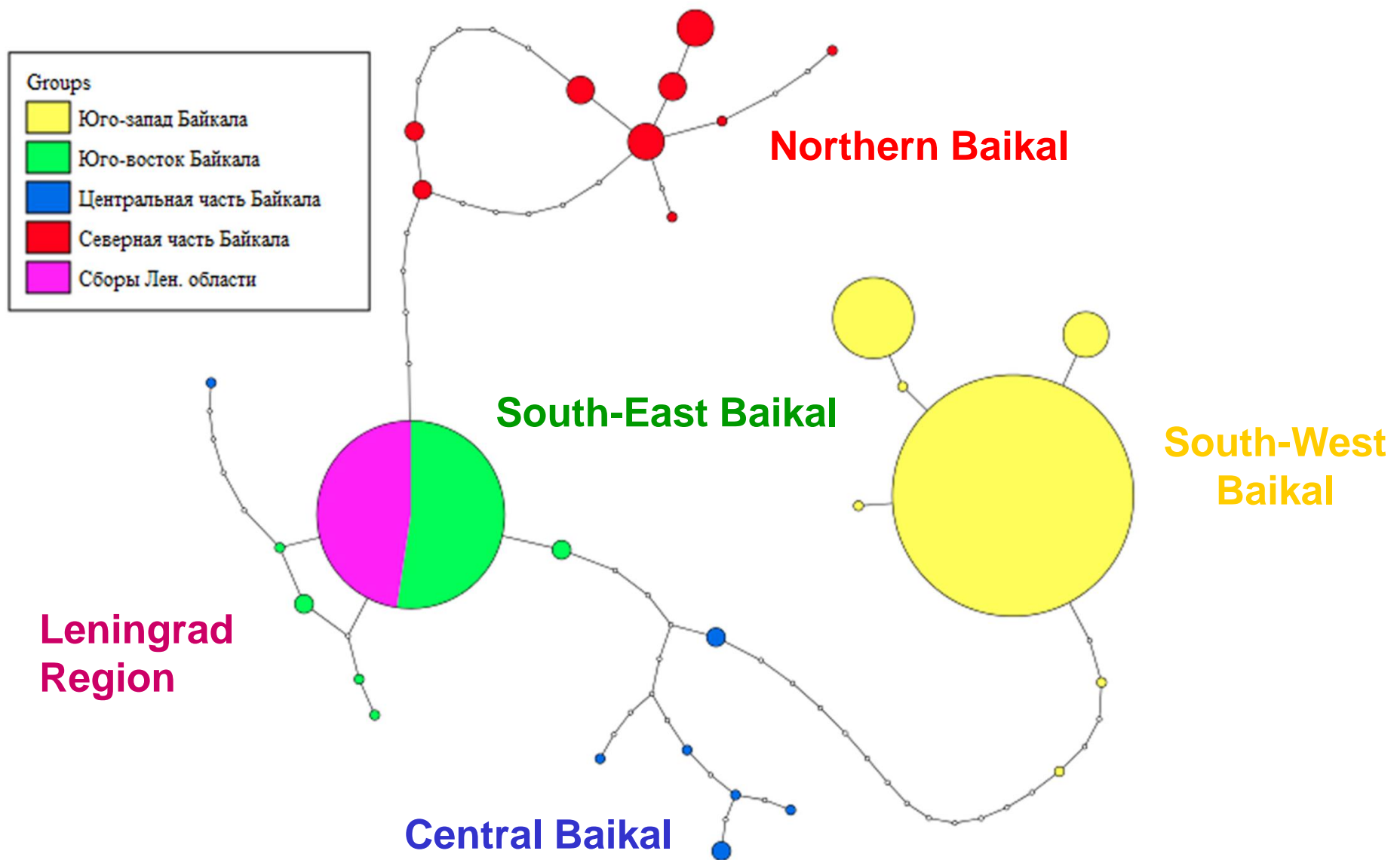


In order to assign the analyzed specimens to *G. fasciatus* we used the standard phylogeographic marker mtDNA cytochrome *c* oxidase subunit I (COI) gene.

It was also necessary to determine the origin of introduced population of this species as well as to assess its variability at different points in the Leningrad Region.







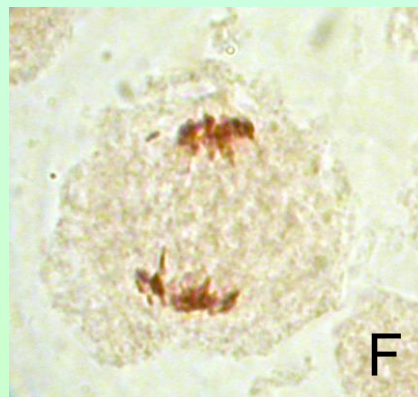
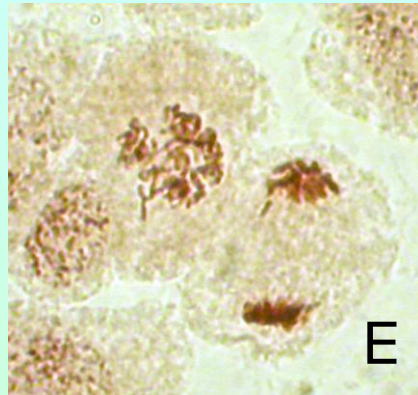
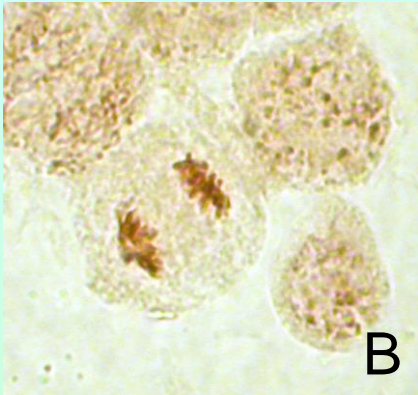
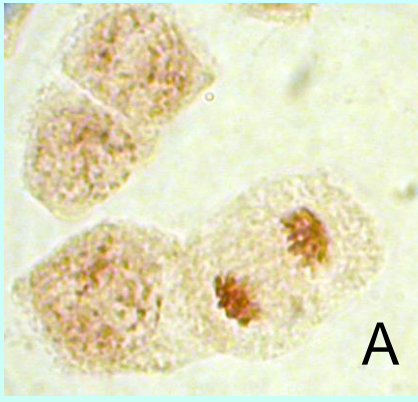
The network of haplotypes of the CO1 gene of *G. fasciatus* with samples taken from the Lake Baikal and the Gulf of Finland in the Leningrad Region, constructed by the method of median binding (Abramson et al. 2017 unpublished)

The sideswimmer *G. fasciatus* has not yet been subjected to substantial genetic studies although

it has already been proposed as an indicator species to be used in long-term complex monitoring of the state of environmental conditions in various water bodies.

In order to assess **the role of genetic mechanisms in adaptation to new conditions** the frequency of chromosomal aberrations (ChA) at Anaphase and Telophase stages of mitosis in *G. fasciatus* embryos were studied in two sequential years.



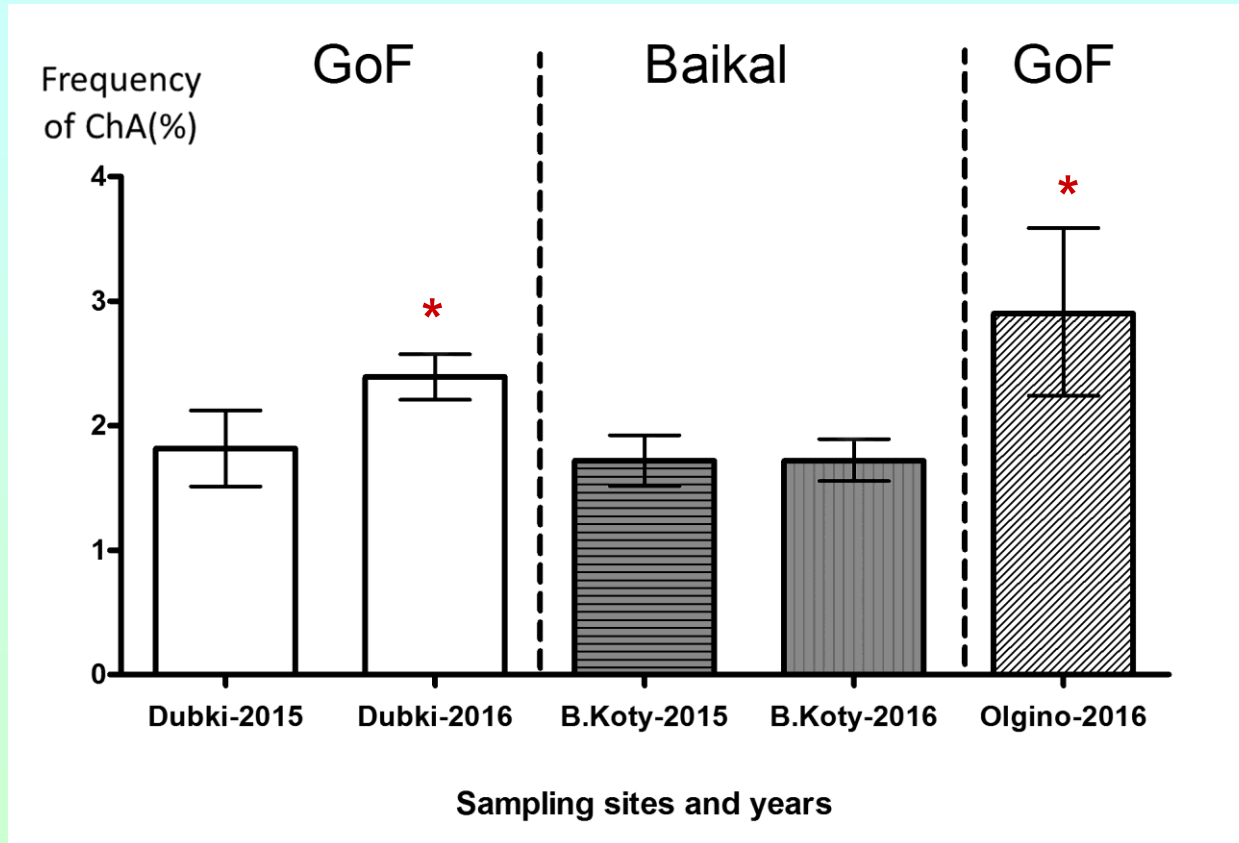


Cytogenetic analysis is being performed using early embryos of *G.fasciatus* as the source of cells, nuclei and chromosomes.

The number of dividing cells in developing embryos is sufficient to find all stages of mitosis, and the sufficiency of cells at Anaphase/Telophase stages of mitosis allowed us to assess the frequency of various chromosomal aberrations.

A,B,C - Ana-Telophase stages of mitosis without irregularities, (D) - with a “bridge”, (E) - lagging chromosome, (F) - broken “bridge” and acentric fragment.

The frequency of chromosomal aberrations (ChA) at Anaphase and Telophase stages of mitosis in *G. fasciatus* embryos from one local population of Lake Baikal and 2 invasive populations of the Gulf of Finland.



The average level of ChA varied between 1.8% in the samples collected in Lake Baikal and 3,0% in those from the Gulf of Finland.

A significant increase in the frequency of ChA was found at two locations in the Gulf of Finland in 2016 (\*).



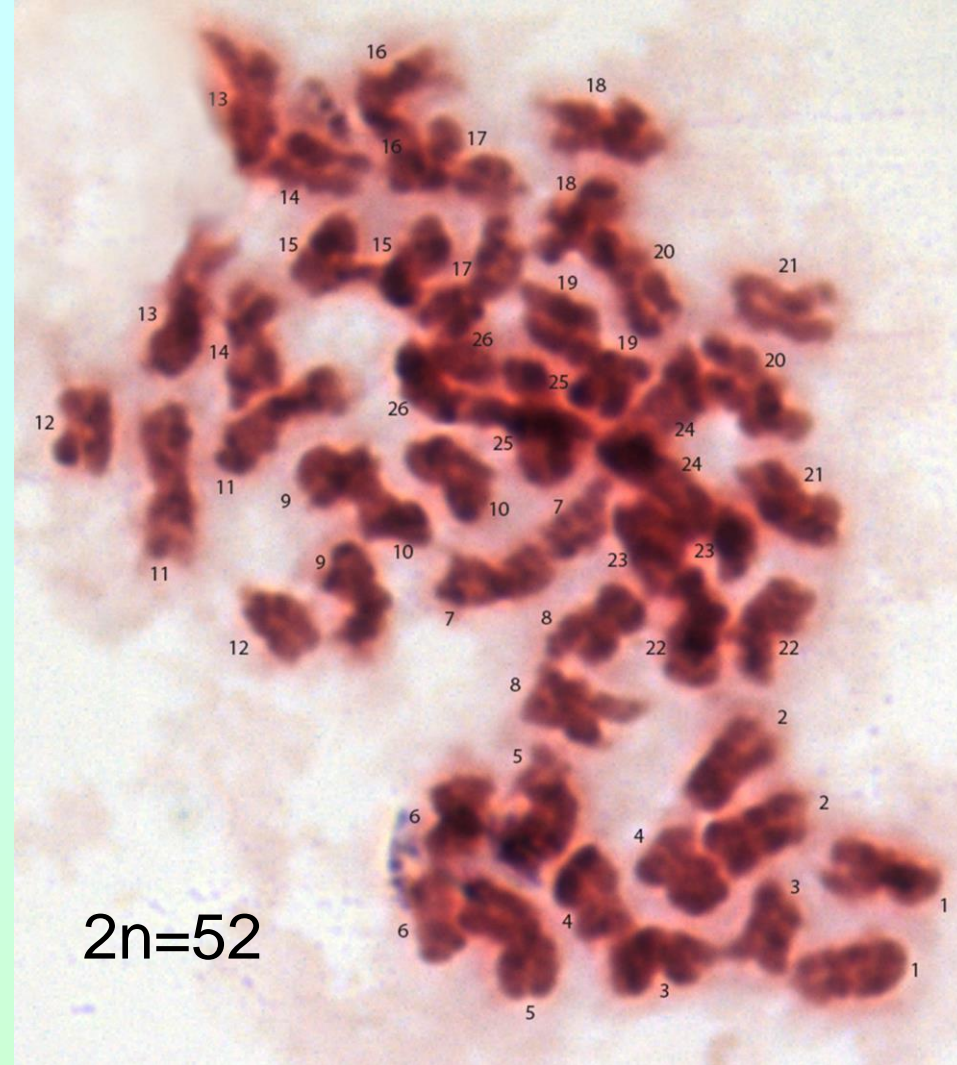
Cytogenetic characteristics of an individual species make it possible to expand data on biodiversity of biosystems.

The analyses of chromosome structural features will allow:

- to establish phylogenetic links within the systematic category,
- to disclose genetic mechanisms underlying high degree of adaptation of this species to varying environmental conditions.



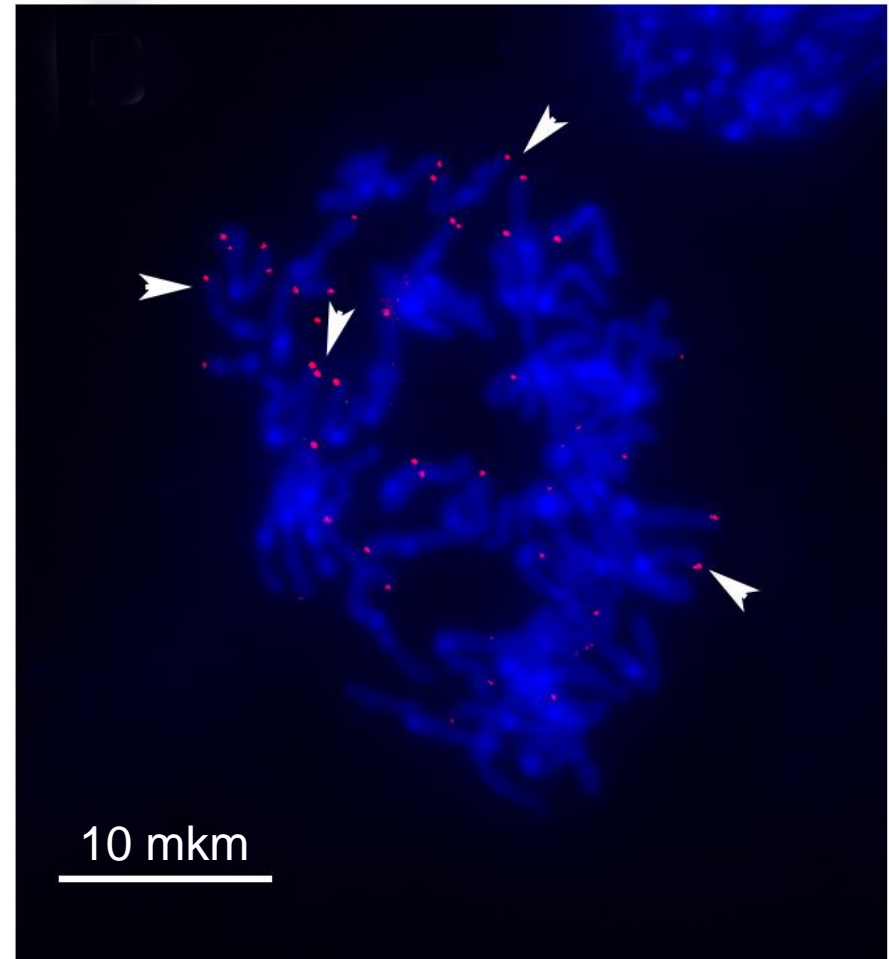
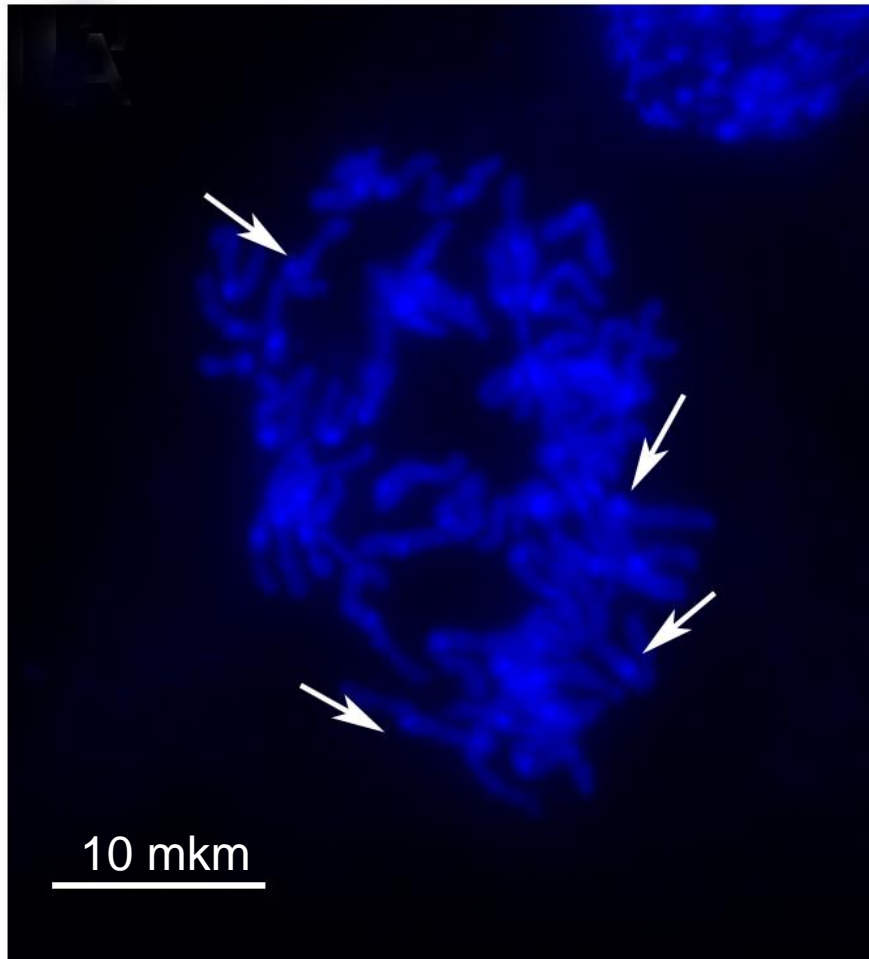
# Karyotype analyses



of conventionally stained metaphases of mitosis allowed us to determine the diploid chromosome number in *G. fasciatus* as  $2n=52$  or 26 pairs of chromosomes and to build the karyogram to characterize the karyotype of this amphipod.

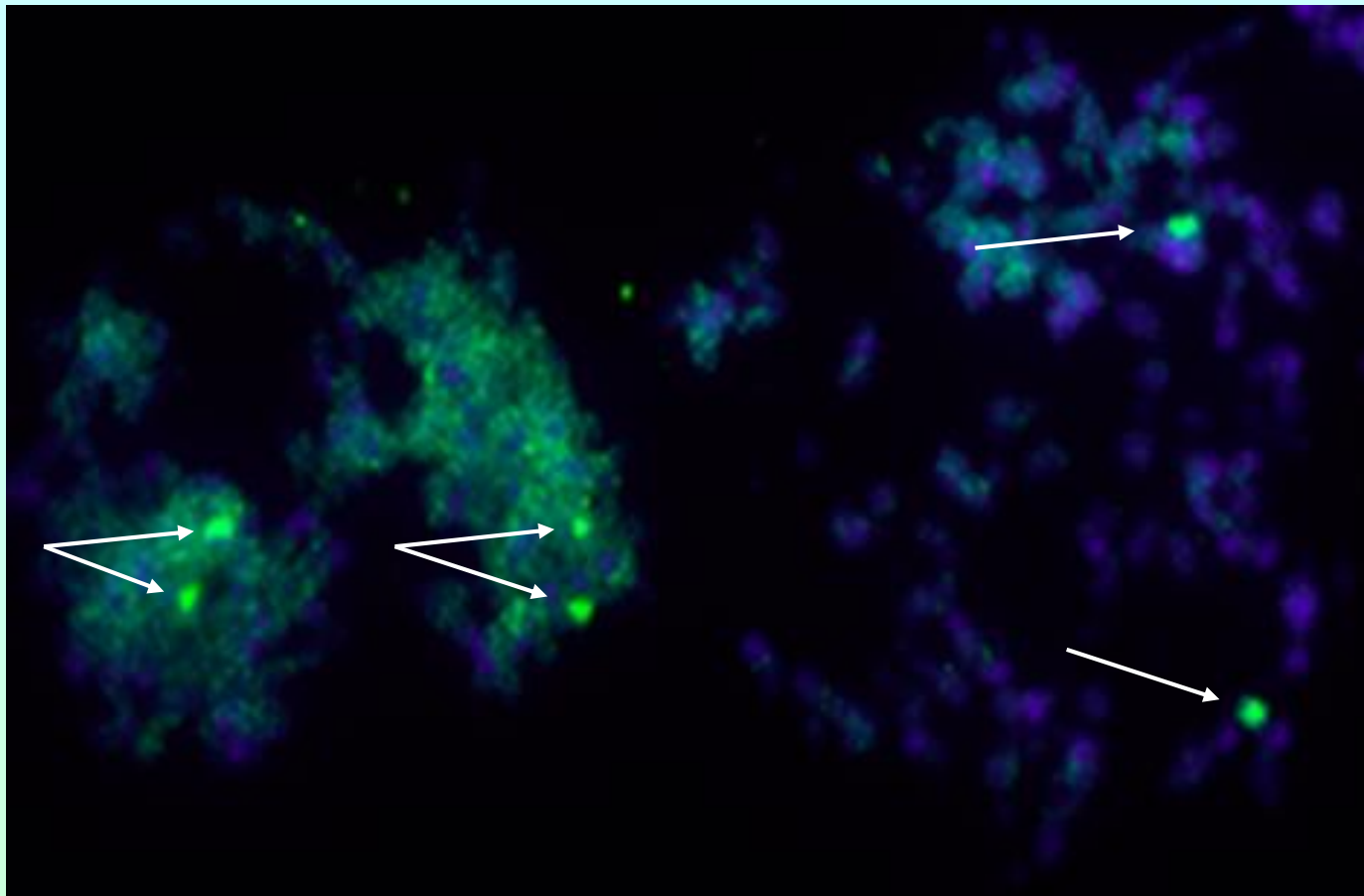


# Fluorescent DNA-DNA hybridization *in situ*



**FISH experiments revealed that (TTAGG)<sub>n</sub> repeats hybridize to chromosome ends. A – DAPI-bright fluorescing Adenine-Thymine base-pair (A-T) rich pericentromeric regions (arrows); B – red telomeres (arrowheads).**

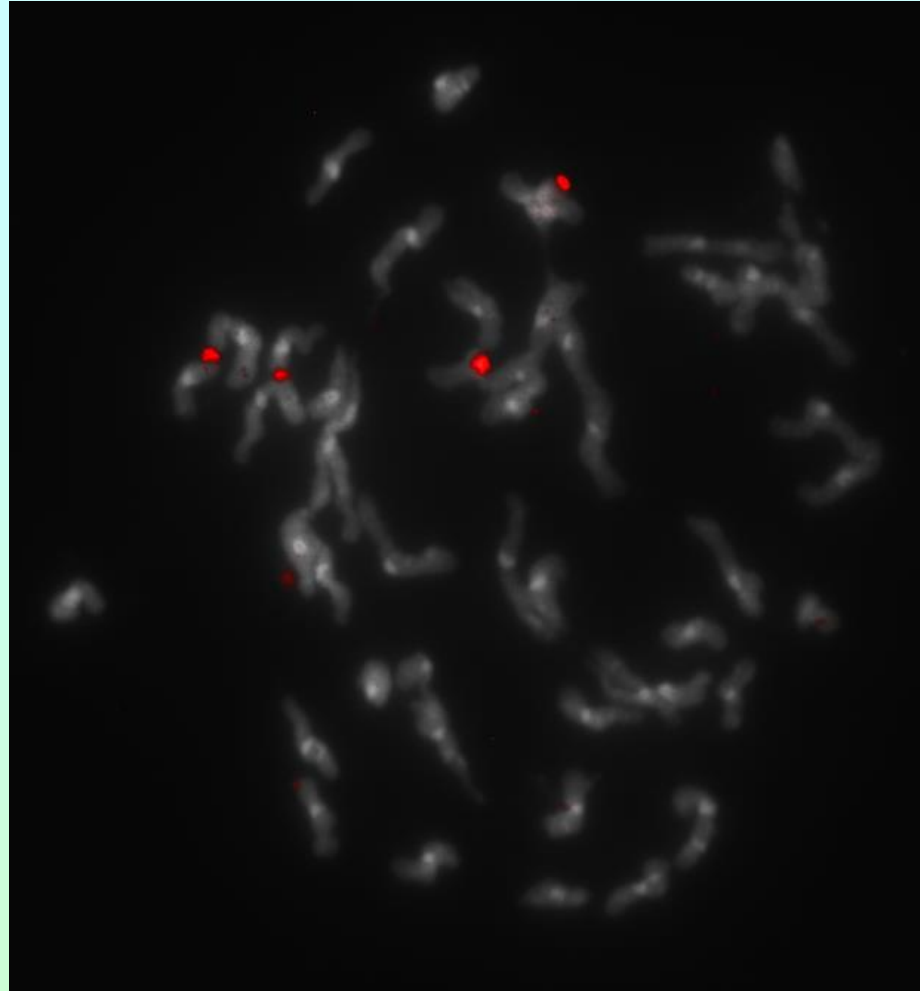




The 18S rDNA probe, amplified using a pair of primers specific for *G. fasciatus*, revealed two hybridization sites at different stages of the cell cycle (white arrows) in the Gulf of Finland population. Pairs of hybridization sites are visible in one chromosome set at the right hand side, and in two prophase nuclei (at the left).

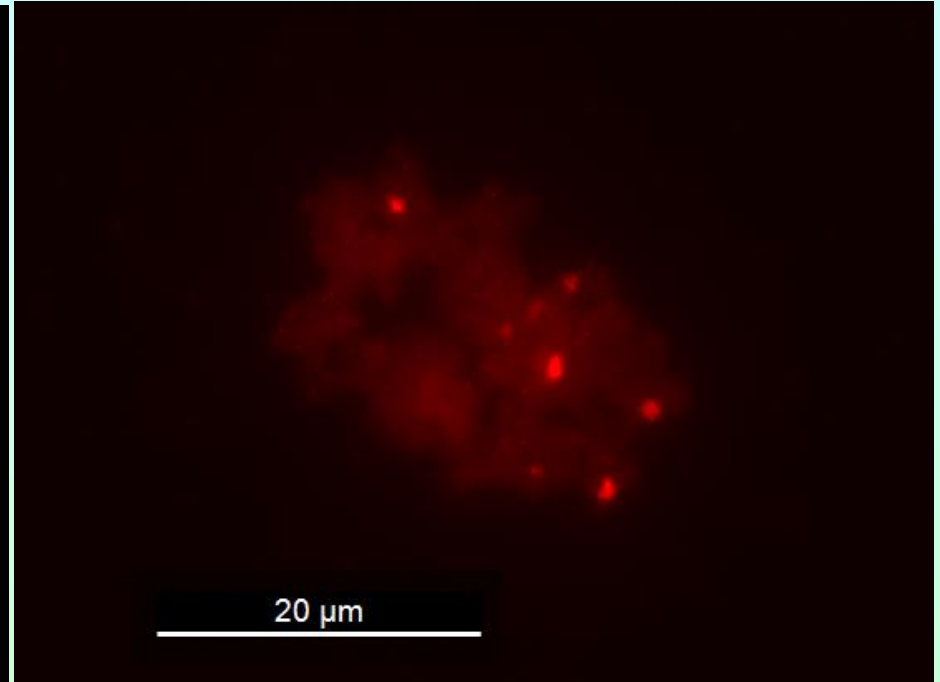
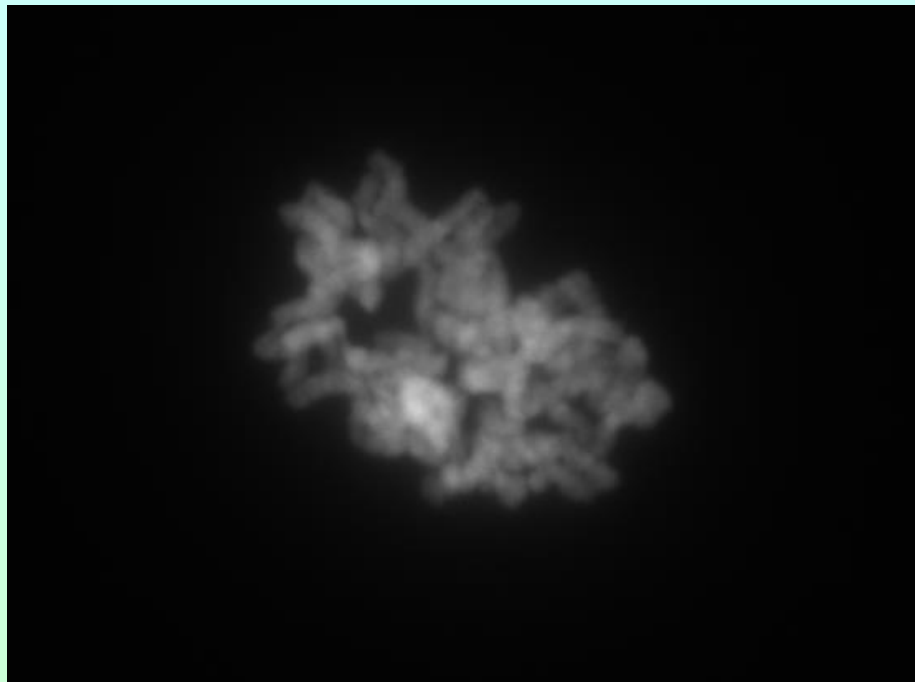






The 18S rDNA probe revealed **four hybridization sites at Pro-Metaphase** (red colour) in the Lake Ladoga population of *G.fasciatus*.



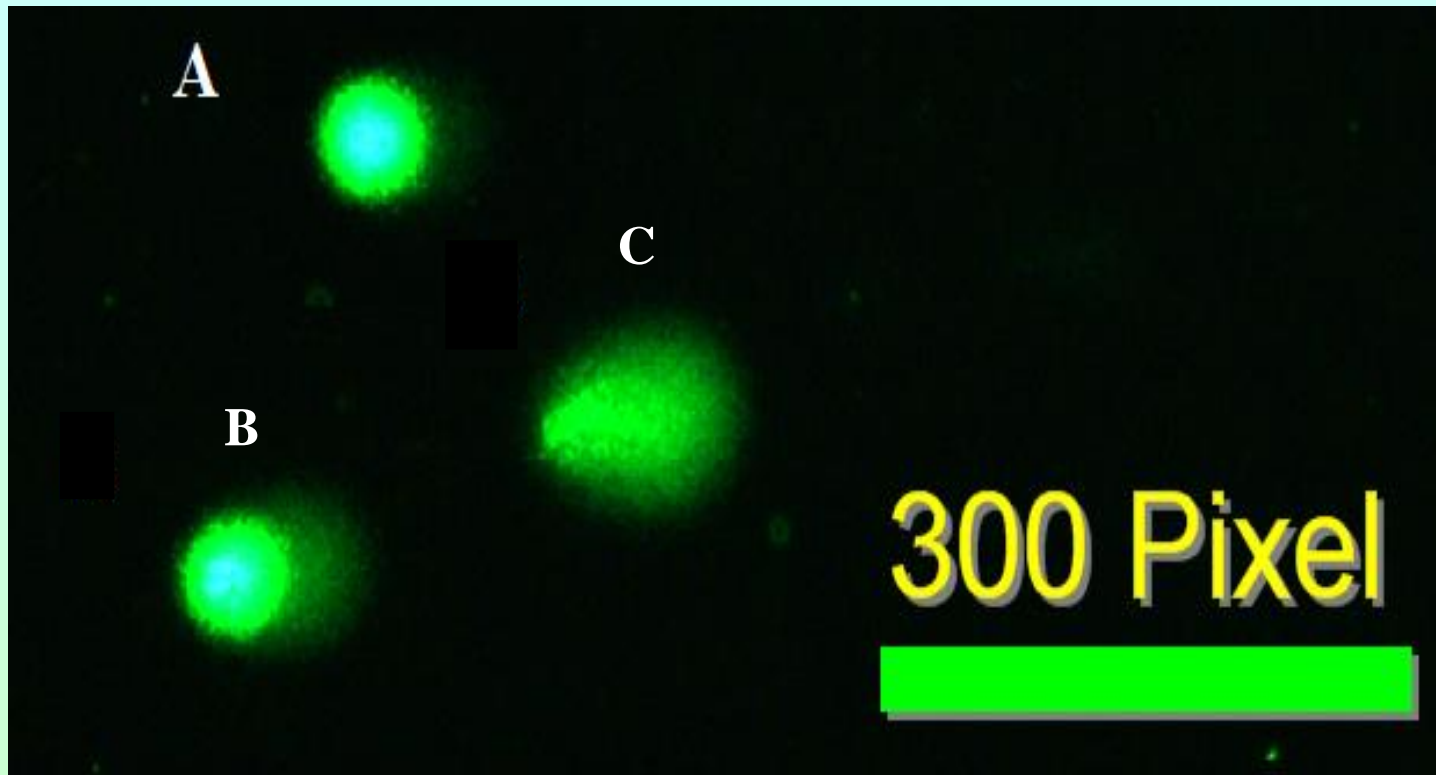


**Eight hybridization sites** (four major and 4 minor)  
at Pro-Metaphase in the Lake Ladoga population.



# Comet alkaline electrophoresis

allows to assess frequencies of primary disturbances at DNA level, which might be prerequisites of chromosome aberrations.



A – comets of embryonic nuclei with intact DNA, B – partial destruction of DNA, C – high level of destruction of DNA in the nucleus



## Molecular biology approach

The traces of adaptation process can remain as diverse micro-changes in the DNA (single nucleotide substitutions, micro-deletions, inversions, transpositions, *etc.*).

Our sequencing project is underway and we intend to compare the genomes of *G. fasciatus* from initial Baikal population and that of the non-native species from the Gulf of Finland, using rDNA and mtDNA cytochrome *c* oxidase subunit I (COI) gene as the first targets.

Comparative genome analyses could give an idea of whether ecological specialization took place along with the invasion process.



The results of our investigations enable us to expand the range of models to study possible mechanisms of biological adaptation, and at the same time to suggest *Gmelinoides fasciatus* as a natural bioindicator of the environmental state.





## The Team

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