Genetic analysis on common carps (*Cyprinus carpio* L.) in Croatia

Tea Tomljanović

University of Zagreb
Faculty of Agriculture
Department for Fisheries, Beekeeping and Special Zoology



- common carp (*Cyprinus carpio* L. 1758) has a long history of breeding and introduction worldwide, resulting in a large number of locally adapted wild and cultured populations
- the culture of common carp (*Cyprinus carpio* f. *domestica*) in Croatia developed considerably at the turn of the nineteenth to twentieth century
- the first carp stocks were introduced primarily form Germany and other parts of the Austro-Hungarian empire

- during a period before and after the Second World War common carp was a major fish species in monoculture farming
- Yugoslav type of carp, which originated from the Galician and Aischgrundsy carp type from Germany, was primarily produced

- in some ponds carps were characterized by special morphological traits
- after noticing the differences in economic traits of carp, within various localities in Croatia starts the selection of important economic parameters:
  - growth rate
  - survival
  - utilization of food
  - resistance to adverse environmental factors and diseases
  - late maturity
  - meat quality

- decades of the efforts on selection evolved into well-konown carp's strains such as: Grudnjak, Koncanica and Draganic
- on fish farm "Nasice" long-term selection program on height-length (high proportion of height to length of fish) developed a very quality line of Nasice carp

- fry of Nasice carp was sent to Israel 1970th year
- the hybrids between Nasice and Israeli Dor-70 strains happen to be most successful crossbreeds in Israel
- they had the best growth within the numerous investigated carp strains
- today, the strain crosses of Dor 70 and Nasice carp produce as a standard material for commercial fish ponds in Israel

Israel‘s common carp line Dor-70

- Common carp lines from fish pond "Poljana" were also very successful in selection programs, especially in Hungary

- development of the modern technology increased production of consume fish
- in this production chain an important role has had a hatcheries
- beside the introduction of broadstocks from other fish ponds, spawn distribution is the main reason for population mix or disappearance of the difference between the strains of common carp in Croatia
- although artificial propagation helped the uncontrolled mixing of strains the fish farmers tried to keep their own stocks

- for the protection of original carp strains collected from different parts of Europe and Asia a living gene bank HAKI (1962) was formed in Szarvas (Hungary)
- 32 fish ponds, 1 to 4 ha each were used for genetic research, including broodfish ponds, nursery, rearing and testing ponds

Strains of common carp maintained at the gene bank of HAKI

Bikal mirror carp
Dinnyés mirror carp
Felsősomogy mirror carp
Göd mirror carp
Hortobágy mirror carp
Nagyatád mirror carp
Palkonya mirror carp
Sumony mirror carp
Szarvas mirror carp
Szarvas red mirror carp
Szeged mirror carp
Tata scaly carp
Tisza wild carp

Szarvas 22 mirror carp
Szarvas 15 mirror carp
Szarvas P33 scaly carp
Szarvas P31 scaly carp
Szarvas P34 scaly carp
Szarvas 215 mirror carp

Amur wild carp
Czech scaly carp
Czech mirror carp
Fresinet scaly carp
German mirror carp
Nasice mirror carp
Polish linear carp
Polish mirror carp
Poljana scaly carp
Poljana mirror carp
Ropsha scaly carp
Thai scaly carp
Ukrainian scaly carp
Vietnam scaly carp

- Three high quality hybrids have been produced in HAKI using the strains of the live common carp gene bank

Strains intended for food production

Strain intended for fishing

- Nasice mirror carp (1976. year)

- Poljana scaly carp (1988. year)

- Poljana mirror carp (1988. year)

Objectives of the „study”

- During 2005. a research project of the Department of Fisheries, Faculty of Agriculture in Zagreb and Institute for Fisheries, Aquaculture and Irrigation (HAKI) started
- The project aim was to compare the genetic characteristics of the Croatian and Hungarian carps populations using microsatellite DNA markers
- describe the genetic distance between the strains
- searching for signs of inbreeding
- propagate and repatriate the Croatian strains

Material and methods

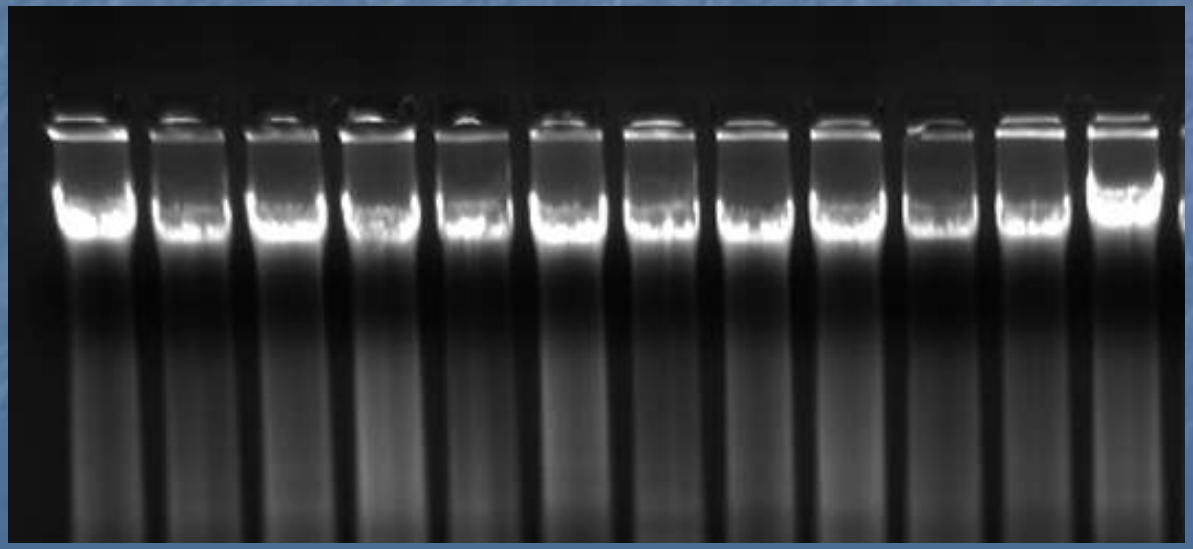
4 common carp populationes;

- Fishfarm "Nasice" Croatia (30)
- Fishfarm "Poljana" Croatia(30)
- Nasice strain live genebank Szarvas (30)
- Poljana strain live genebank Szarvas (30)

- Total DNA was isolated from fin clips, preserved in 96% ethanol

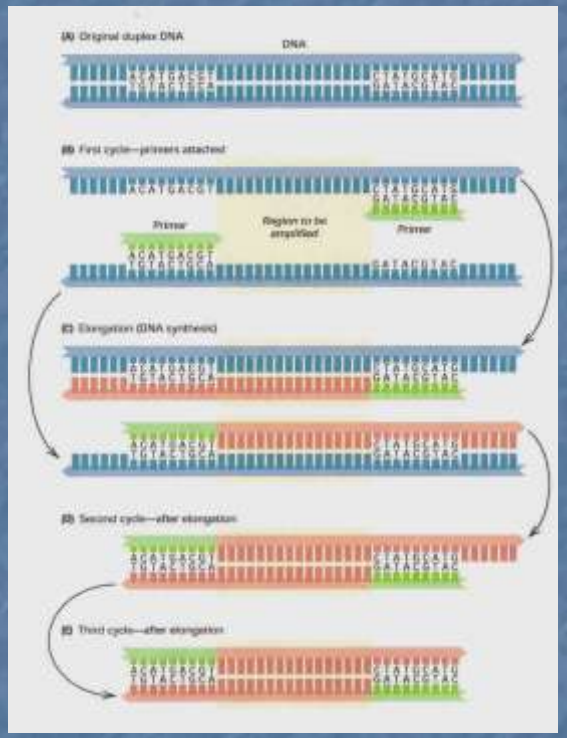
Material and Methods

- extraction of total genomic DNA:
  - digestion of proteins with Proteine-Kinase enzyme
  - using high salt concentration, phenol, chlorophorm, isopropanol (Miller et al., 1988)
- checking DNA quality and quantity using agarose gel-electrophoresis and spectophotometry



Material and methods

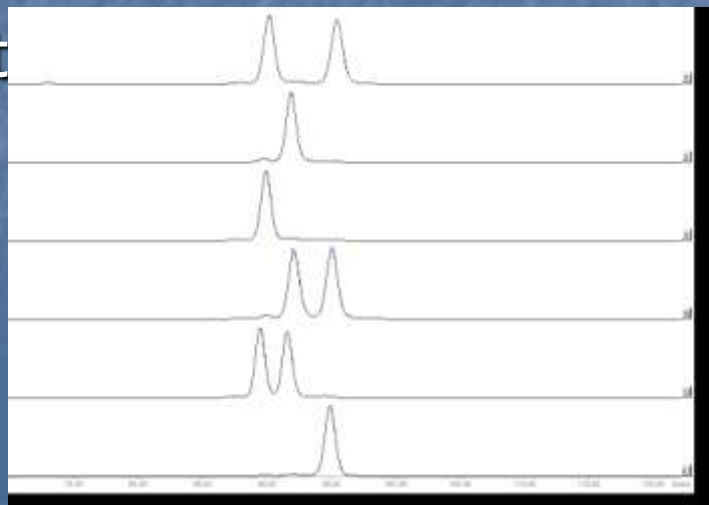
- 6 microsatellite DNA markers: MFW1, MFW4, MFW6, MFW7, MFW16, MFW28 (**Crooijmans et al. 1997.**)



- PCR:
- 2 minute - 94°C (denatuing step)
- 30 cycles
  - 40 sekundi - 94°C (denaturing),
  - 50 sekundi - 55°C (primer-annealing)
  - 90 sekundi - 72°C (elongation)
- 10 minuta - 72°C (for T*aq* polymerase terminal transpherase activity)

Material and methods

- separation of PCR products using 7% polyacrylamid gel (32% formamide, 5% urea), ALF Express II (Amersham Biosciences) fragment analyser
- sizing of PCR products : size standards
  - 50,100,150,200,250,300,350,400,450,500 bp standards
  - standard samples with known lenght
  - Fragment Analyser 1.03



Material and methods: data analysis

:

- Genepop (**Raymond and Rousset, 1995**): allele-frequences mean number of alleles, H_e, H_o, deviation from Hardy-Weinberg equilibrium (p values)
- Convert 1.31 (**Glaubitz, 2004**): private alleles
- F-Stat (**Goudet, 1995**): pairwise fixation index (F_{st} -value), allelic richness
- Populations (**Langella, 1999**): Nei's genetic distance between populations (**Nei, 1972**; **Nei, 1978**; **Nei et al., 1983**)
- Populations (**Langella, 1999**): dendrogram (Neighbour Joining method- NJ)
- Treeview (**Page, 1996**): drawing dendrogram
- GeneClass (**Piry és mtsai., 1999**):

  assignment test, self-classification, Bayesian method (**Ranala és Mountain, 1997)** checking individual genotypes in order to assign them to a population

Results:

Genetic variabiliy within the strains

- Allele numbers by strain and locus, (number of privete alleles)

	Population			
Locus	Nasice	Genbank Nasice	Poljana	Genbanka Poljana
MFW1	10	11 (3)	10 (4)	14 (4)
MFW4	10 (2)	7	8 (1)	13 (2)
MFW6	6	7	10 (3)	10 (3)
MFW7	10 (2)	9 (1)	11 (1)	13 (3)
MFW16	6 (1)	11 (5)	6 (1)	14 (4)
MFW28	9	11 (1)	13 (1)	10 (1)
∑	51 (5)	56 (10)	58 (11)	74 (17)
Mean	8,5	9,33	9,6	12,33

Expected (He) and observed (Ho) heterozygosity

Poljana	He	Ho
MFW1	84,02	25
MFW4	86,40	54,83
MFW6	87,17	76,66
MFW7	79,67	75,86
MFW16	63,84	6,66
MFW28	89,09	40
Mean	81,69	46,50

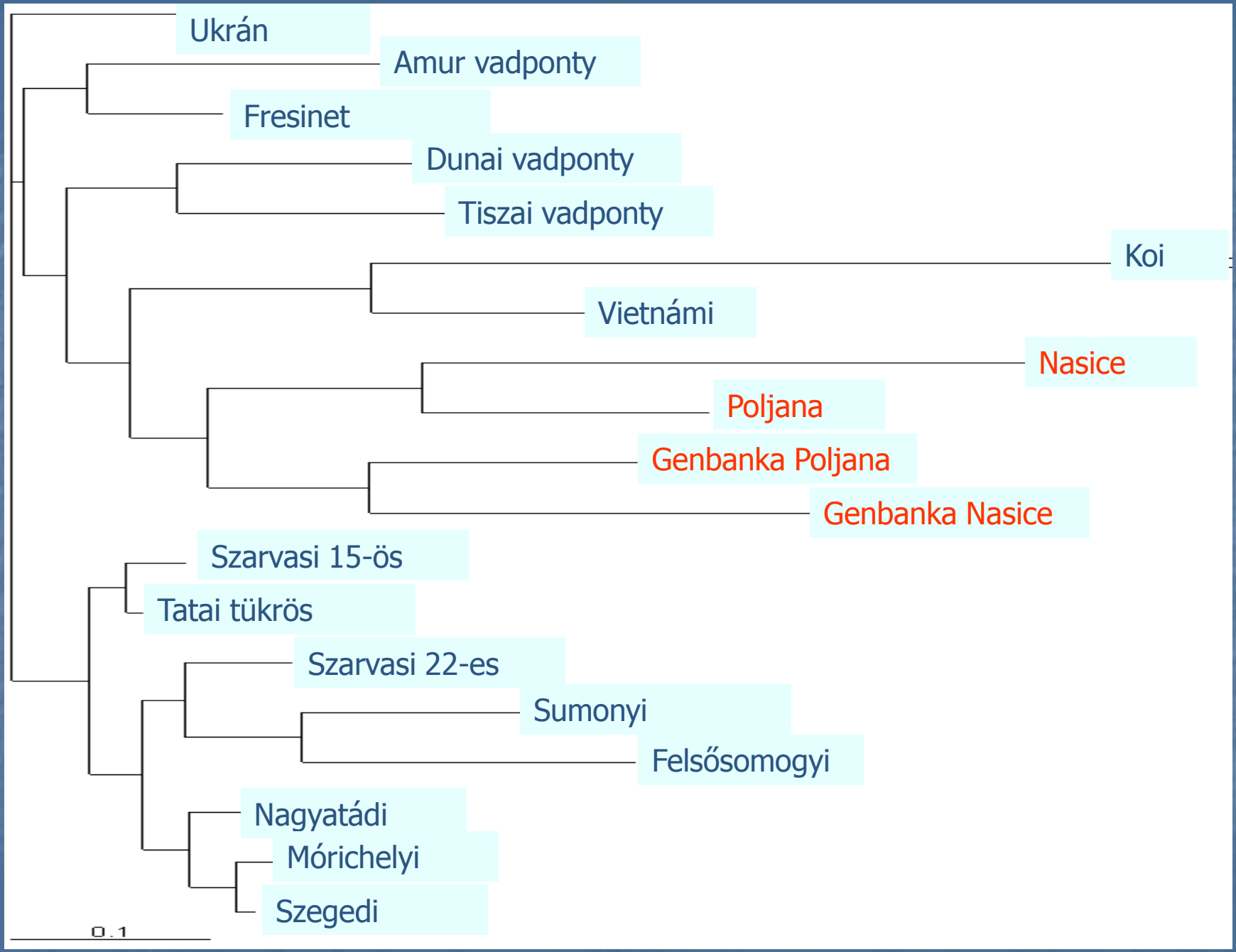
Genbank Poljana	He	Ho
MFW1	88,64	63,33
MFW4	88,58	93,33
MFW6	80,15	20,68
MFW7	88,53	86,66
MFW16	86,27	26,66
MFW28	82,66	72,72
Mean	85,80	60,56

-in most cases the strains are significantly deviate from Hardy-Weinberg equilibrium

-the mean observed heterozygosity is lower than the mean expected heterozygosity in case of all strains (sign of inbreeding!!!)

Genetic Distance between the strains (Da distance by Nei)

	Nasice	Poljana	Genbank Poljana	Genbank Nasice
Nasice	0			
Poljana	0,44	0		
Genbank Poljana	0,69	0,46	0	
Genbank Nasice	0,67	0,57	0,38	0


Ukrán
Amur vadponty
Fresinet
Dunai vadponty
Tiszai vadponty
Koi
Vietnámi
Nasice
Poljana
Genbanka Poljana
Genbanka Nasice
Szarvasi 15-ös
Tatai tükrös
Szarvasi 22-es
Sumonyi
Felsősomogyi
Nagyatádi
Mórichelyi
Szegedi
0.1

Conclusions

- in most cases the strains are significantly deviate from Hardy-Weinberg equilibrium
- the mean observed heterozygosity is lower than the mean expected heterozygosity in case of all strains
- all strains are inbred
- strains from the genebank are more variable
- genebank strains are good source of repatriating projects

- in the spring of 2006. from the HAKI Institute three carp strains were brought to Croatia
- 100.000 larvae/strain was brought the farm of origin

- the Croatian carp strains have settled succesfully at their original farm environment

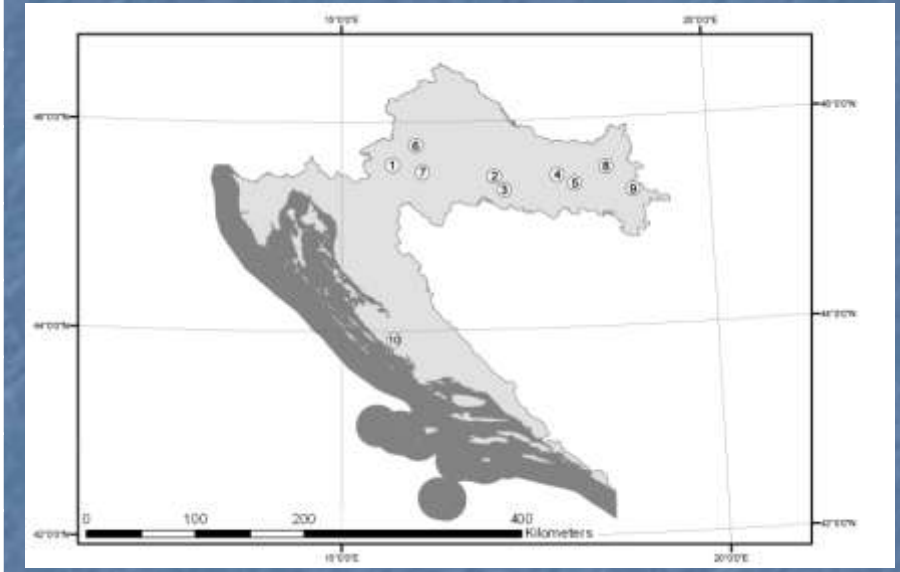
Morphological and genetic differences among common carp populationes in Croatia

- Common carp is economically very important species for Croatian aquaculture
- In central and eastern Europe there are many different varieties of carp, but their origin and relationships have not yet been well studied
- populationes of wild carp in Croatia are very rare and seriously endangered
- the change of genetic composition in populations coming by; putting of genetically inappropriate strains in river systems and their cross with members of indigenous populations
- molecular markers allow the identification and description of the hybrid populations

Objectives of the „study”

- to determine the relationship between common carp populations in Croatia
- to determine the percentage of crossing wild carp with those cultivated in our fishponds

Population		Genetic analysis
Fishfarm Draganici		37
Fishfarm Koncanica		37
Fishfarm Nasice		37
Fishfarm Grudnjak		37
Fishfarm Poljana		37
River Sava		4
River Dunav		22
River Drava		16
River Kupa		13
Vranjsko lake		11



Genetic analysis

- DNA isolation
  - Qiagen DNeasy Blood and & Tissue Kit
- 15 microsatellite DNA markers (Crooijmans et al. 1997) :
  - MFW1, MFW9, MFW31, MFW12, MFW4, MFW7
  - MFW20, MFW23, MFW29, MFW16
  - MFW13 MFW17, MFW26, MFW28, MFW3
- PCR amplification

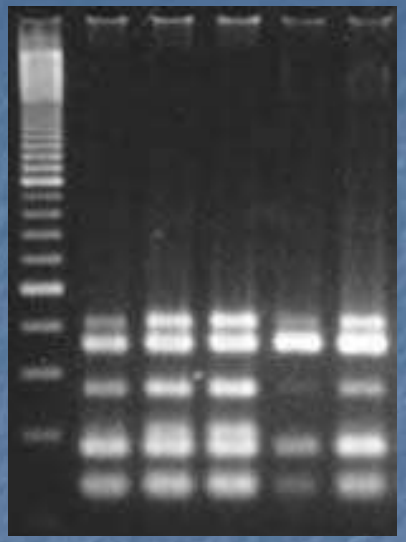
Analiza mikrosatelitske DNK

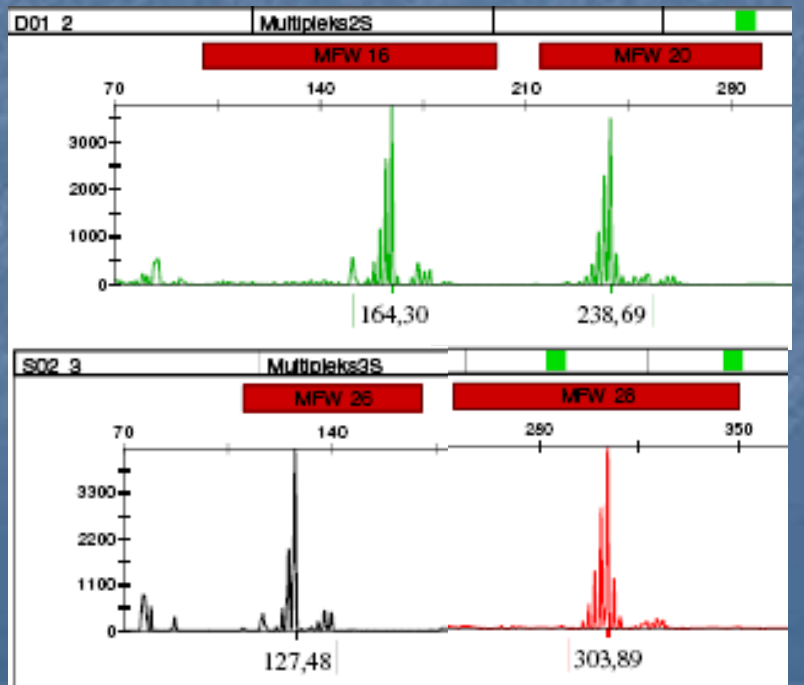
- 15 polimorfnih mikrosatelita (Crooijmans i sur., 1997)

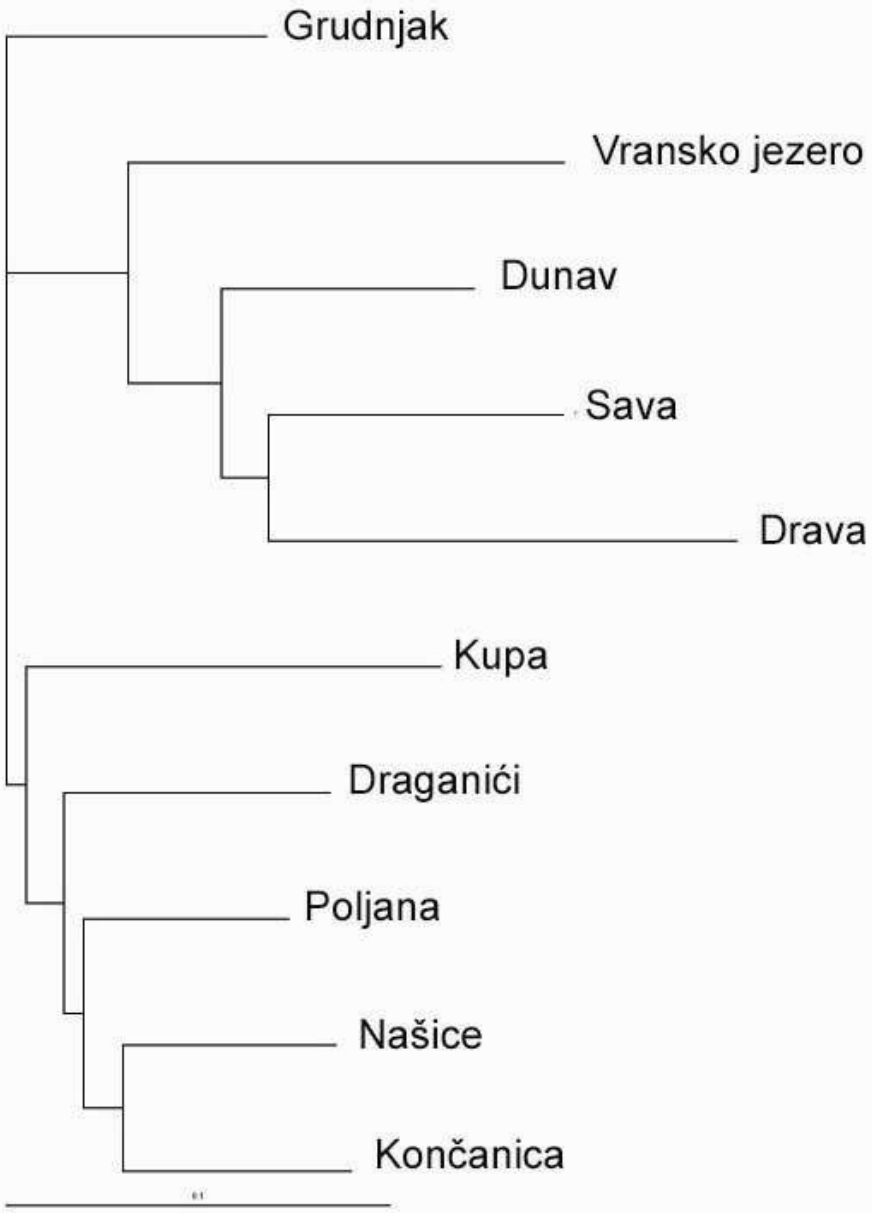
Ime lokusa	Flourescentna oznaka	Ponavljajući motiv	Broj alela	Raspon alela	Nukleotidni slijed početnice
MULTIPLEX 1					
MFW1	6-Fam	(CA)n	5	163-224	5'GTCCAGACTGTCATCAGGAGGA GGTGTACACTGAGTCACGC 3'
MFW4	Ned	(CA)n	5	132-152	5'TCCAAGTCAGTTTAATCACCGGG GAAGCGTTGACAACAAGC 3'
MFW7	Pet	(CA)n	7	120-276	5'TACTTTGCTCAGGACGGATGCAT CACCTGCACATGGCCACTC 3'
MFW9	6-Fam	(CA)n	6	84-139	5'GATCTGCAAGCATATCTGTCGAT CTGAACCTGCAGCTCCTC 3'
MFW12	Vic	(CA)n	5	116	5'TTTATTAGAATAATTAATTAGCA GATAGAAGTCGATGGAAAGTCC 3'
MFW31	6-Fam	(CA)n	3	284-308	5'CCTTCCTCTGGCCATTCTCACTA CATCGCAGAGAATTCGTAAG 3'
MULTIPLEX 2					
MFW16	Vic	$(CA)_n$	7	115-181	5'GTCCATTCTGTCAAGATAGAGTC TTCATTTCAGGCTGCAAAG 3'
MFW20	Vic	(CA)n	6	249	5'CAGTGAGACGATTACCTTGG GTGAGCAGCCCACATTGAAC 3'
MFW23	Ned	(CA)n	6	145	5'GTATAATTGGGAGTTTTAGGG CAGGTTTATCTCCCTTCTAG 3'
MFW29	Pet	(CA)n	4	158	5'GTTGACCAAGAAACCAACATGC GAAGCTTTGCTCTAATCCACG 3'
MULTIPLEX 3					
MFW3	Vic	$(CA)_n$	5	131	5'GATCAGAAGGTACAGAGAAG CCTTACAGAAAACCTGTTTGC 3'
MFW13	6-Fam	(CA)n	7	178-204	5'ATGATGAGAACATTGTTTACAG TGAGAGAACAATGTGGATGAC 3'
MFW17	Vic	(CA)n	6	234-315	5'CTCAACTACAGAGAAATTTCATC GAAATGGTACATGACCTCAAG 3'
MFW26	Ned	(CA)n	7	122-150	5'CCCTGAGATAGAAACCACTG CACCATGCTTGGATGCAAAAG 3'
MFW28	Pet	(CA)n	6	291	5'GATCCCTTTTGAATTTTTCTAGAC AGTGAGGTCCAGAAGTCG 3'

Rezultati analize mikrosatelitske DNA

- PCR
- elektroforeza na agaroznom gelu
- veličina alela mikrosatelitskih lokusa automatskim sekvencerom 3730 *GeneticAnalyzer*






Grudnjak
Vransko jezero
Dunav
Sava
Drava
Kupa
Draganići
Poljana
Našice
Končanica

Genetic divergence between Asian (*Cyprinus carpio haematopterus)* and European subspecies of carp (*C. carpio carpio)*

- Common carp has been cultivated in fish ponds of Europe for few hundred years
- His orgin is still not clear
  - Vooren: ancestor of European carp was introduced from Asia during Greek and Roman period
  - Kirpichnikov: as the result of domestification of wild carp from Danube river, German domestic carp line to be the progenitor in Europe

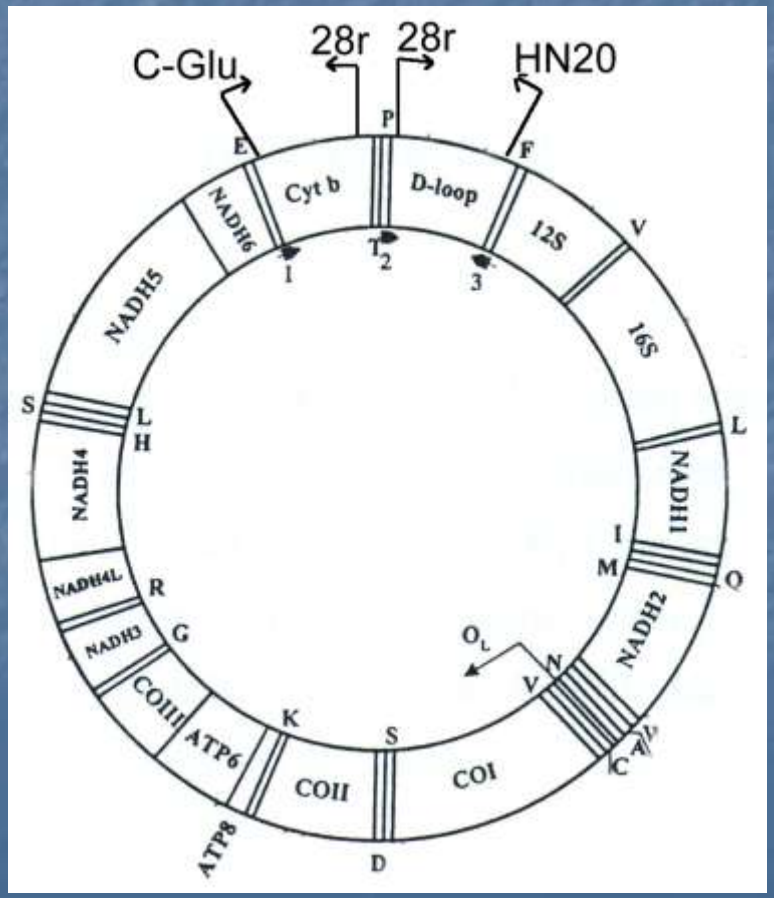
C. c. carpio (19 specimens)

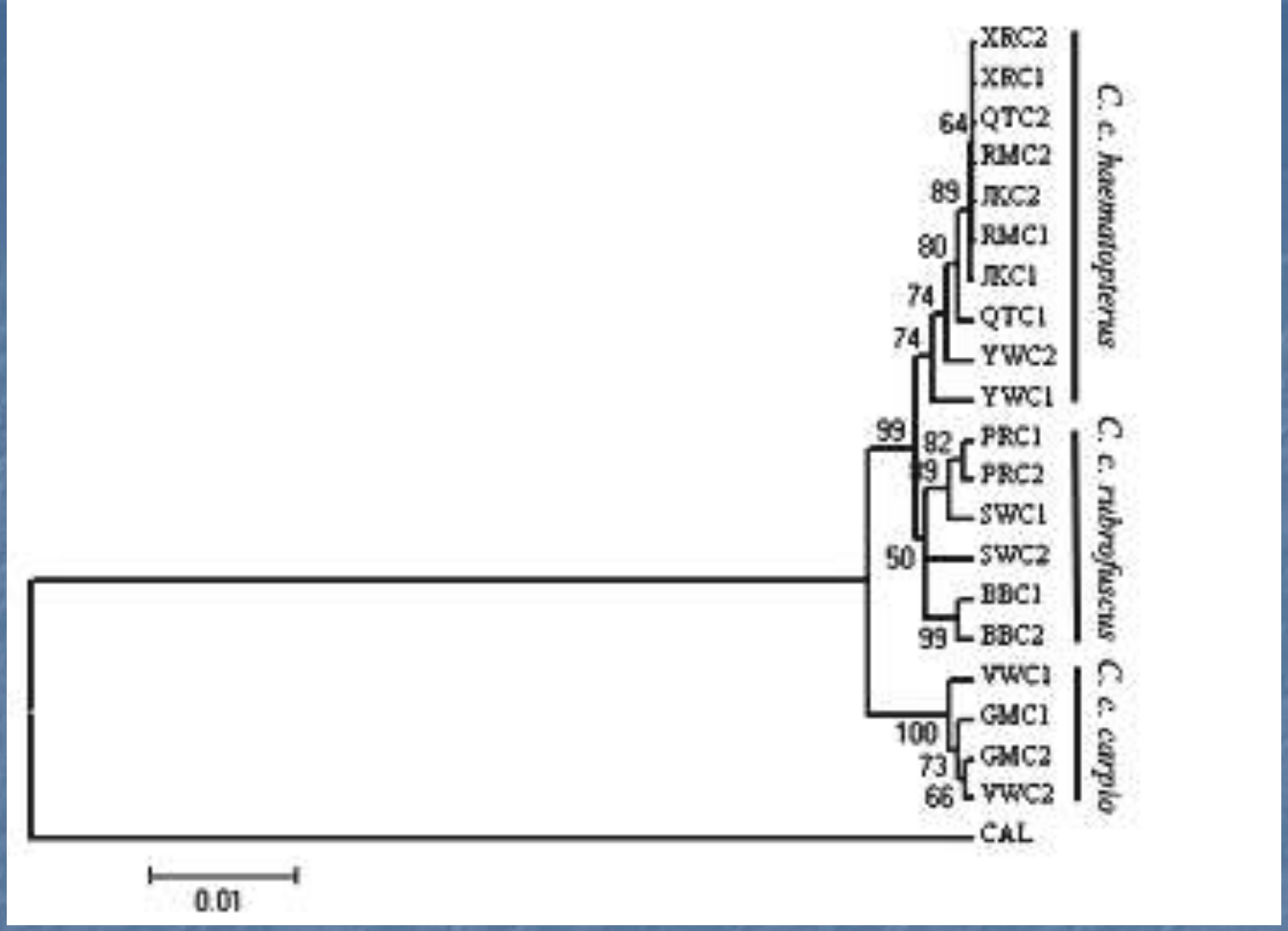
GENETIC DIVERGENCE ?

C. c. haematopterus (17 specimens)

DNA amplification and sequencing

- Comparative nucleotide analyses included the regions recognized as informative
  - mtDNA control region





- European and Asian carps belong to separate branches of phylogenetic tree
- Analysed sequence of D-loop region showes that common carps from Croatian fish ponds belong to already known hapolotype of European wild carp from Volga river (VWC)
- They are in the same branch with German wild carp (GMC)
- Yangtze river carp (YW) belong to the other branch of phylogentic tree

Thank you for your attention!