MICROSATELLITE VARIATION WITHIN BROWN BEAR POPULATIONS: CARPATHIANS VERSUS BALKAN

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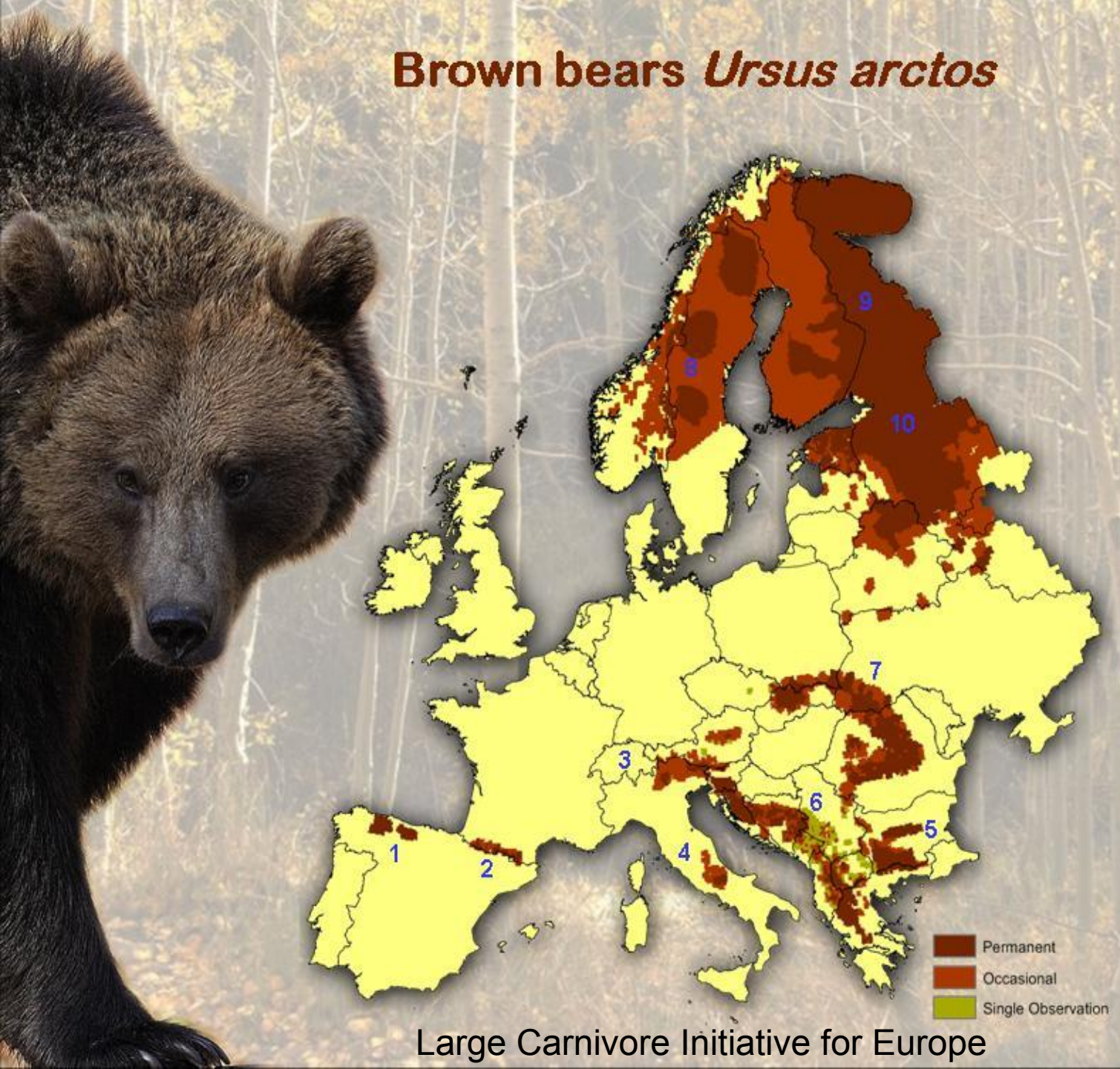
Photo Tibor Pataky

Pataky 2008

Brown bears *Ursus arctos*



Populations

1. Cantabrian
2. Pyrenees
3. Alps
4. Abruzzo
5. East Balkan
6. Dinaric-Pindos
7. Carpathian
8. Scandinavian
9. Karelian
10. Baltic

Large Carnivore Initiative for Europe

Brown bears in Europe

- formerly distributed throughout the whole continent
- extirpated from most of the distribution in Western Europe
- viable populations survived in Northeastern Europe, Scandinavia, Carpathians, Dinaric-Pindos, Rila-Rhodopes and Stara Planina Mounts.

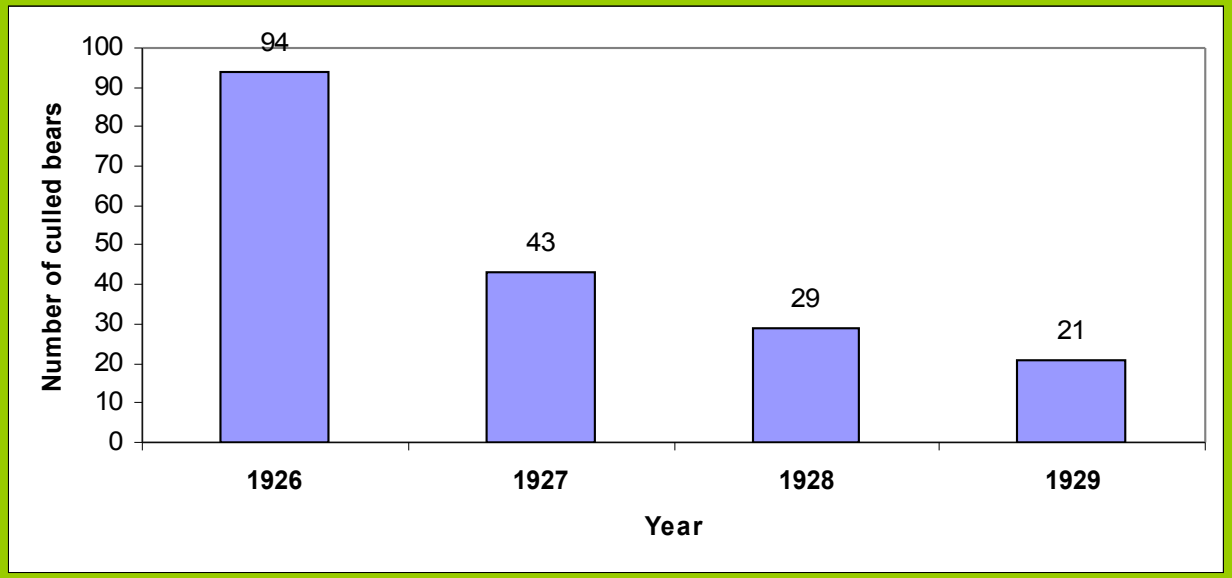
Introduction

Carpathians:

- in Middle ages large continuous population
- Czech Republic – last brown bear shot in 1850's
- at the end of 19th century bears were extirpated from western Slovakia
- in central Slovakia – before WW1 about 120 individuals
- continuous hunting pressure and persecution led to split of population into Western and Eastern Carpathians

Introduction

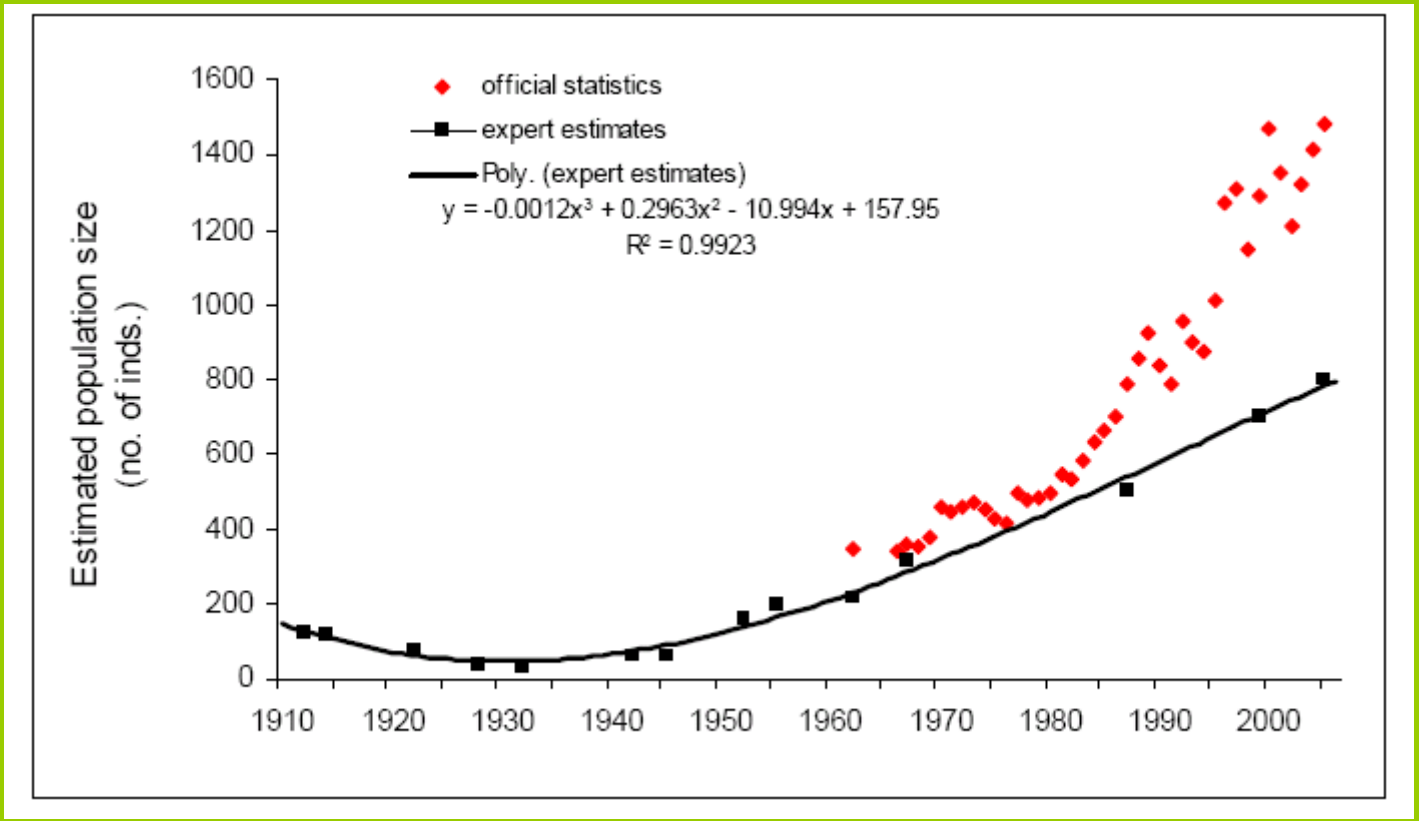
- high cull rates in 1920's
- according to Žuffa (1932) only 15–25 animals survived in Slovakia
- Tobiáš (1933) gave tree-times higher estimates



Annual cull rates at the end of 20's (Žuffa 1932)

Introduction

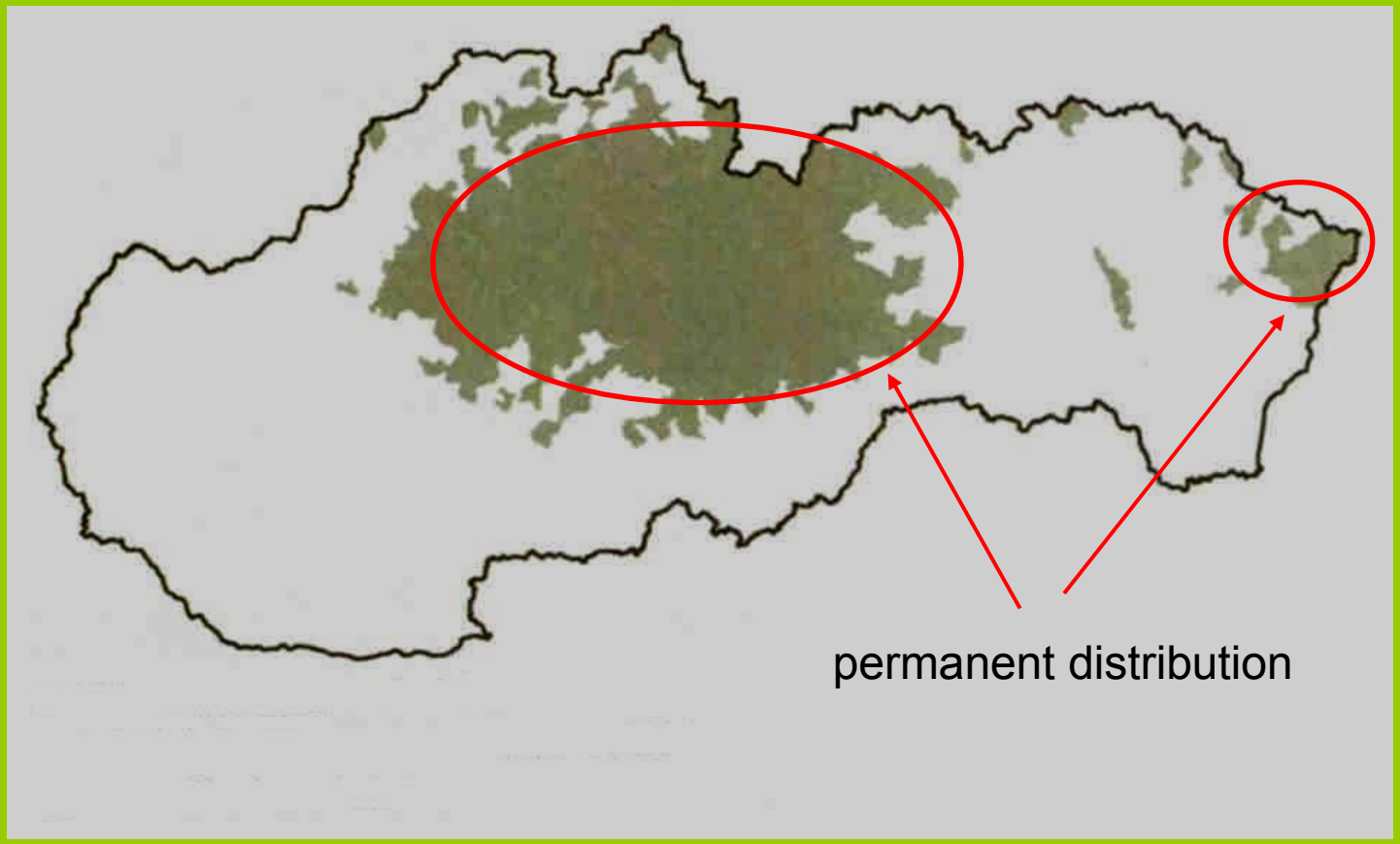
- protection since 1932 – population recovery



Rigg & Adamec (2007)

Introduction

- present distribution of bears in Slovakia
- about 600–800 individuals



Finďo *et al.* 2007

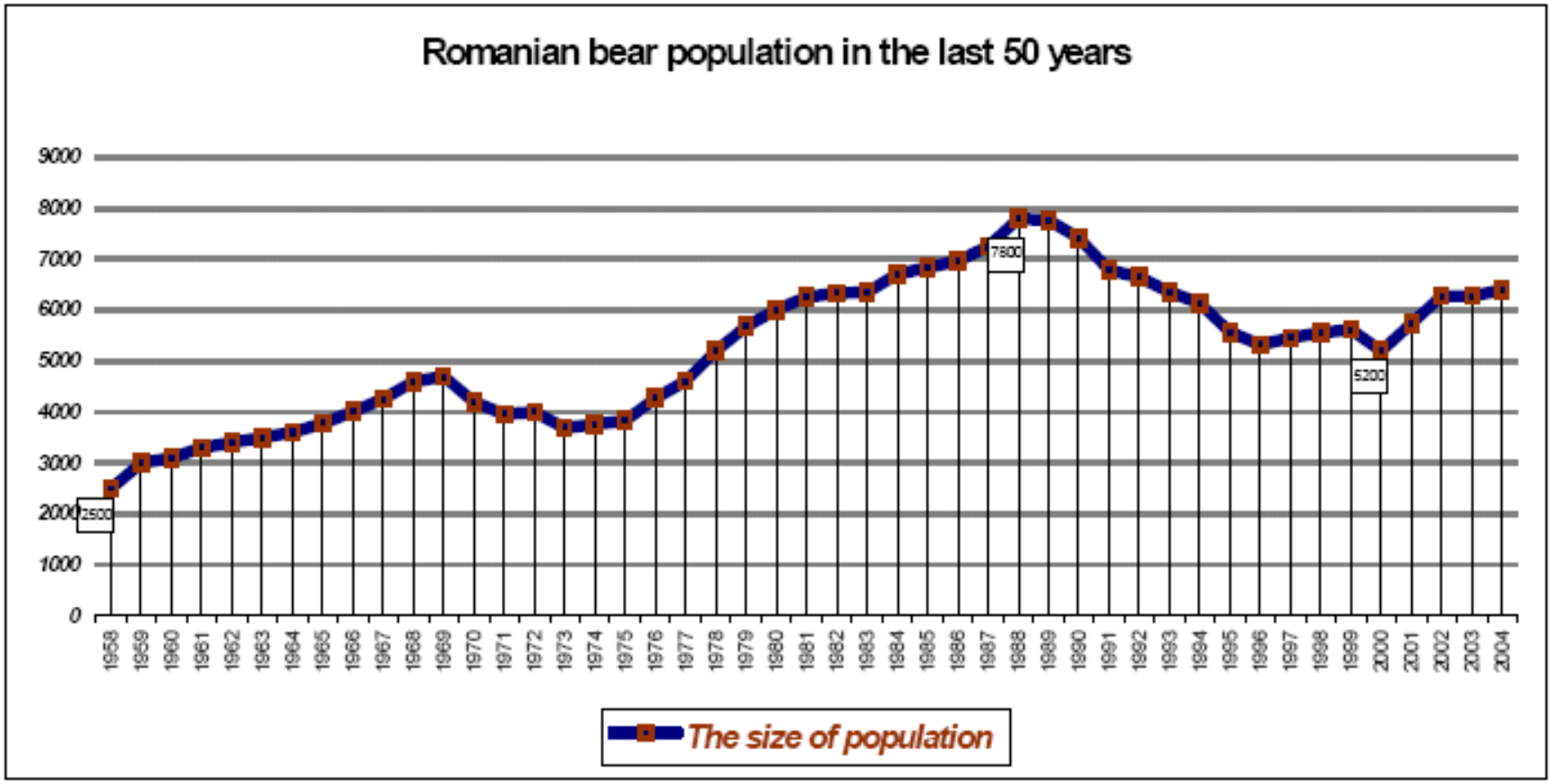
Introduction

- few literature sources from Romania
- minimum population size (about 900 individuals) at the beginning of 1950's
- since 1953 – protection, led to increase of bear numbers
- Ceausescu's era – measures for population growth
- at the end of 1980's – population maximum reached about 8,000 individuals

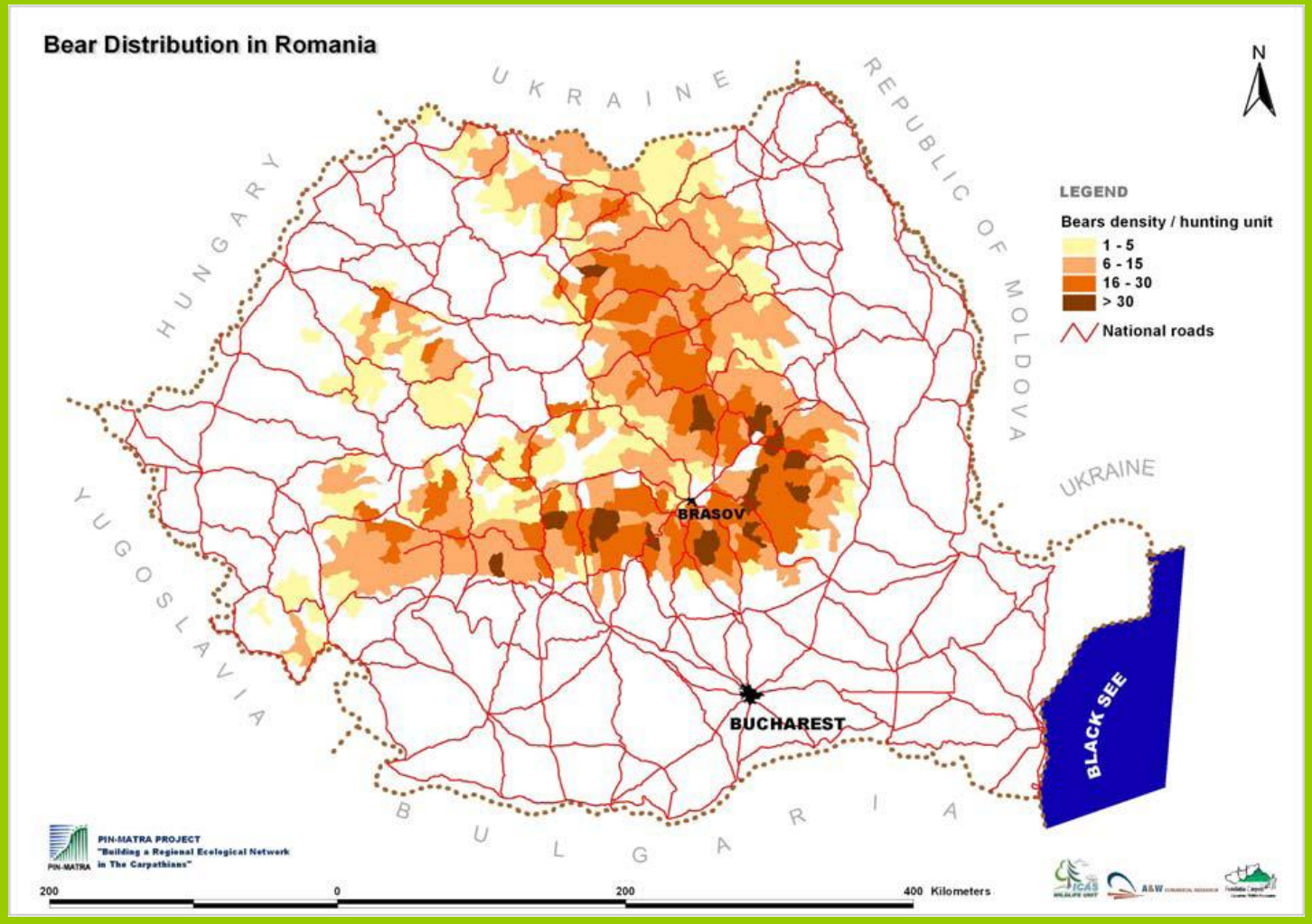
Introduction

- frequent bear-human conflicts
- during 1990–1999: 119 attacks on human, 18 mortal
- at present approximately 6,000 individuals

Introduction



Introduction



Management and action plan for the bear population in Romania

Introduction

Greece

- similar population history to Slovakia
- bears formerly occupied whole country
- during last two centuries bears survived only in northwestern and northeastern mountain ranges
- today population is recovering and the population size is estimated for 200 individuals

Introduction

Brown bear distribution in Greece

Introduction

- sampling in Greece – part of the project called “Southwestern Balkan Bear Register” and assessing the impact of Egnatia highway building (Arcturos)
- laboratory analyses performed in Zvolen, Slovakia

Main goals

- assessing the population structure of bears in Carpathians
- evaluation the amount of genetic differentiation between Carpathian and Greek populations
- measuring the level of genetic diversity within studied population (affected by changes of population size in the recent history?)

Materials and methods

- different types of biological material
- samples from culled bears, samples taken from immobilized animals, hunting trophies, museum specimens
- special group of samples – non-invasive samples (hair, feces)

Materials and methods

Country	Soft tissues	Bones	Hair	Feces	Blood	DNA aliquots	Σ
Slovakia	146	4	5	50	12	–	217
Romania	102	3	4	–	–	–	109
Greece	7	–	67	–	–	49	123

Materials and methods

DNA extraction:

- phenol – chloroform deproteinisation, ethanol precipitation
- kits for isolation (Qiagen, Macherey-Nagel)
- Chelex

Materials and methods

PCR amplification:

- amplification of 13 microsatellite loci
- sex specific SRY marker
- primers amplified in two multiplexes

Fragment analysis:

- ABI 3130 automated genetic analyzer (Applied Biosystems)

Materials and methods

Microsatellite markers used in this study

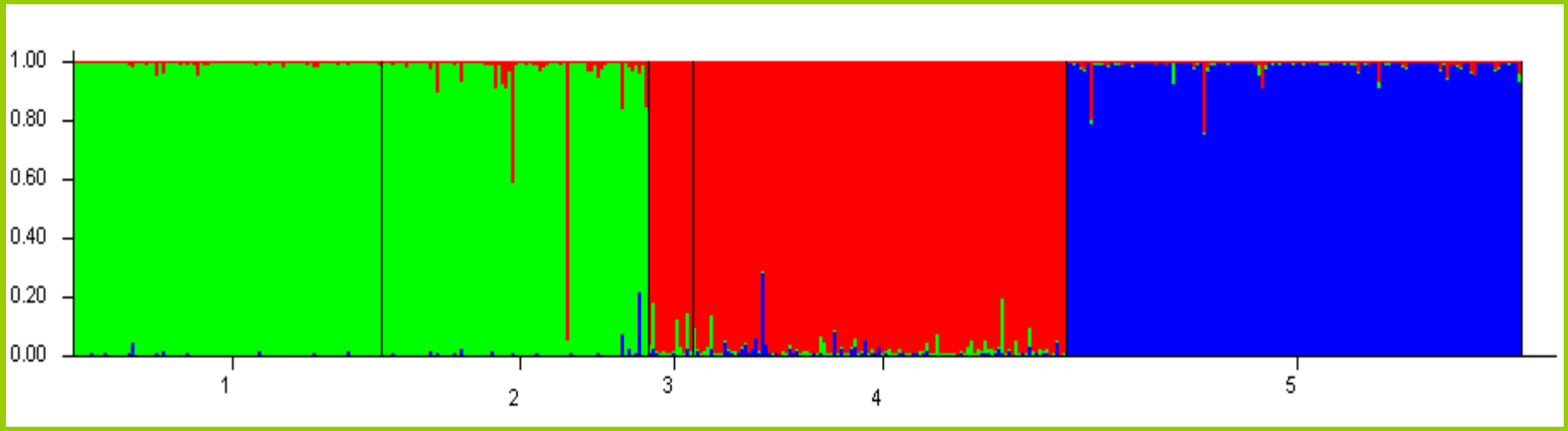
	Sequence	Annealing temp. (°C)	Dye	Conc. (ul)	Range (bp)	Reference
Mu10	F: ATTCAGATTTCATCAGTTTGACA R: TCAGCATAGTTACACAAATCTCC	60	FAM	0,50	108-132	Bellemain *et al.* 2004
Mu23	F: GCCTGTGTGCTATTTTATCC R: TAGACCACCAAGGCATCAG	60	NED	0,60	136-156	Bellemain *et al.* 2004
Mu50	F: GTCTCTGTCATTTCCCCATC R: AACCTGGAACAAAAATTAACAC	60	FAM	0,40	76-102	Bellemain *et al.* 2004
Mu51	F: AGCCAGAATCCTAAGAGACCT R: AAAGAGAAGGGACAGGAGGTA	60	HEX	0,60	105-129	Bellemain *et al.* 2004
Mu59	F: GCTCCTTTGGGACATTGTAA R: TGACTGTCACCAGCAGGAG	60	NED	0,50	90-122	Bellemain *et al.* 2004
G10L	F: ACTGATTTTATTCACATTTCCC R: GATACAGAAACCTACCCATGCG	60	HEX	0,50	141-161	Bellemain *et al.* 2004
SRY	F: GAACGCATTCTTGGTGTGGTC R: TGATCTCTGAGTTTTGCATTTG	60	HEX	0,50	75	Taberlet *et al.* 1997
G10B	F: GCCTTTTAATGTTCTGTTGAATTTG R: GACAAATCACAGAAACCTCCATCC	58	FAM	0,22	130-152	Paetkau *et al*., 1995
G10C	F: AAAGCAGAAGGCCTTGATTTCCTG R: GGGACATAAACACCGAGACAGC	58	FAM	0,13	87-109	Paetkau *et al*., 1995
G10D	F: GATCTGTGGGTTTATAGGTTACA R: CTACTCTTCCTACTCTTTAAGAG	58	FAM	0,25	167-181	Paetkau *et al*., 1995
G10J	F: GATCAGATATTTTCAGCTTT R: AACCCCTCACACTCCACTTC	52	HEX	0,18	73-103	Paetkau & Strobeck, 1994
G10M	F: TTCCCCTCATCGTAGGTTGTA R: GATCATGTGTTTCCAAATAAT	52	FAM	0,35	202-218	Paetkau *et al*., 1995
G10P	F: AGTTTTACATAGGAGGAAGAA R: TCATGTGGGGAAATACTCTGAA	58	HEX	0,15	141-173	Paetkau *et al*., 1995
G10X	F: CCCTGGTAACCACAAATCTCT R: TCAGTTATCTGTGAAATCAAAA	58	NED	0,12	132-156	Paetkau *et al*., 1995

Materials and methods

- Bayesian clustering method (Structure)
- Factorial correspondence analysis (Genetix)
- Pairwise F_{st} (Arleqiun)
- Genetic diversity measures A, H_o, H_e (Arleqiun)

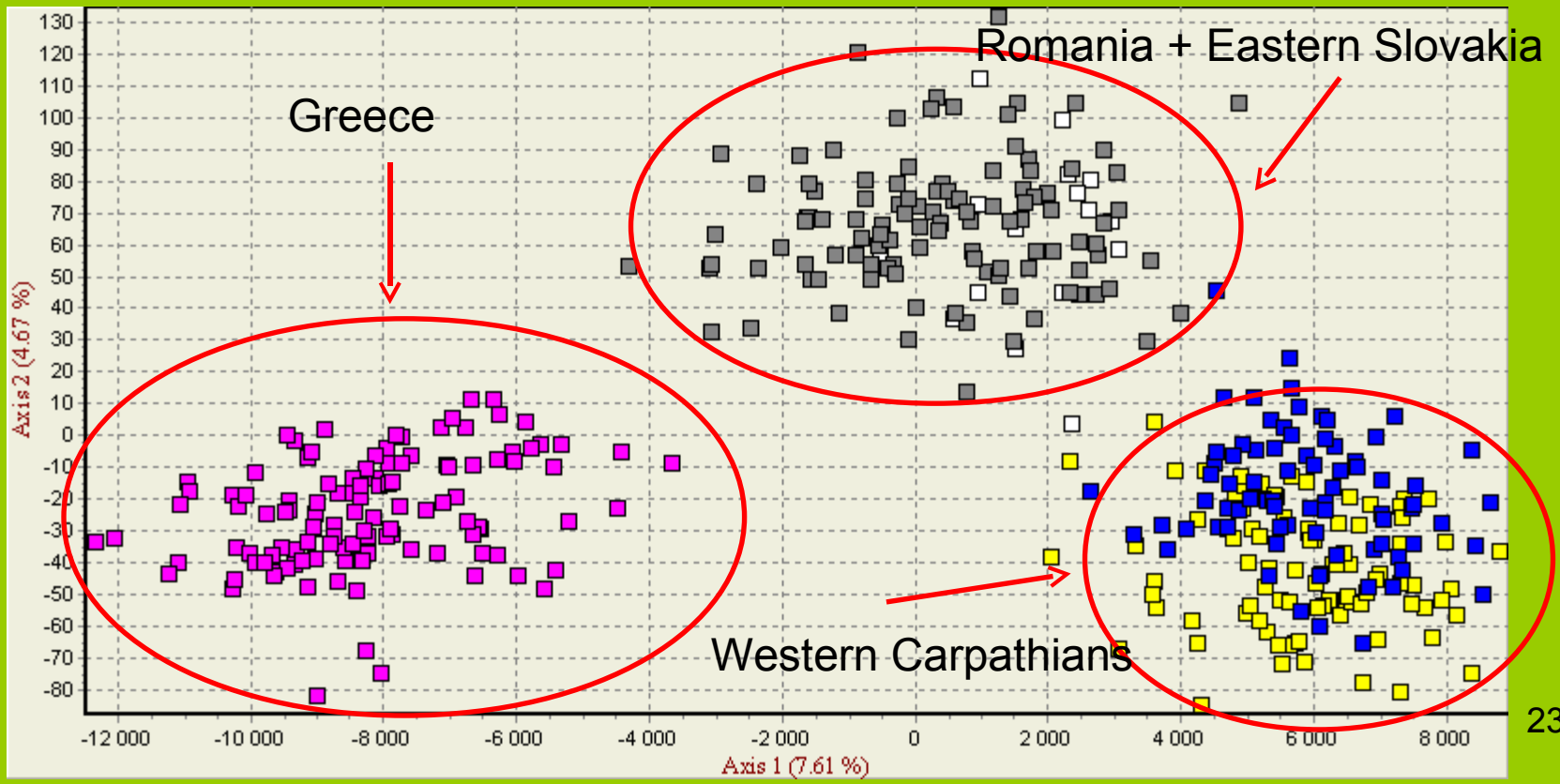
Results – genetic structure

1 – Northern Slovakia
2 – Central Slovakia
3 – Eastern Slovakia
4 – Romania
5 – Greece



Results – genetic structure

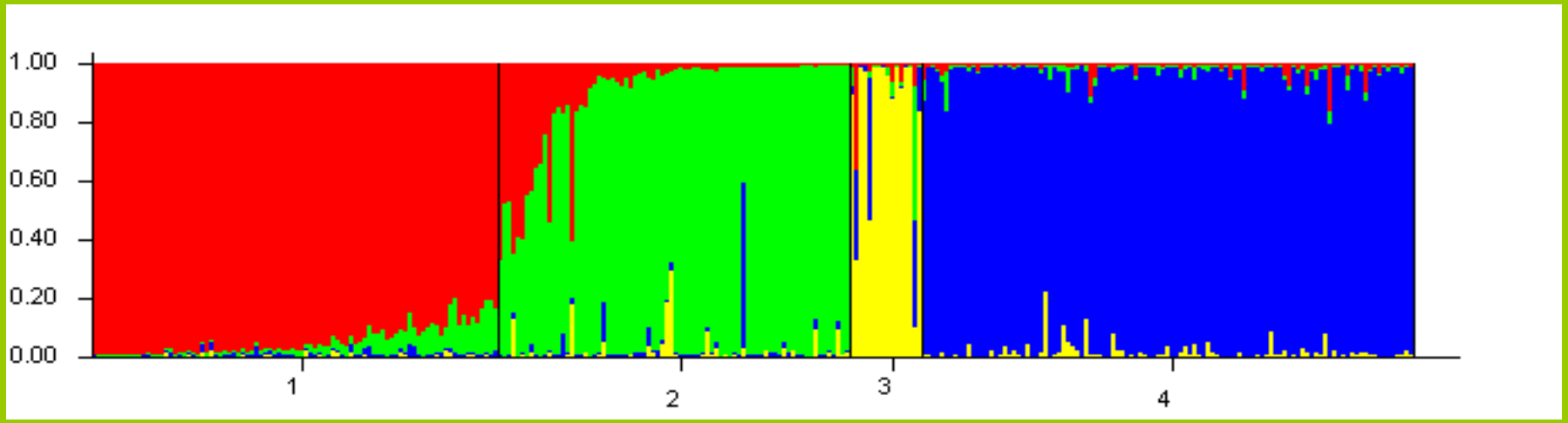
Factorial correspondence analysis



Results – genetic structure

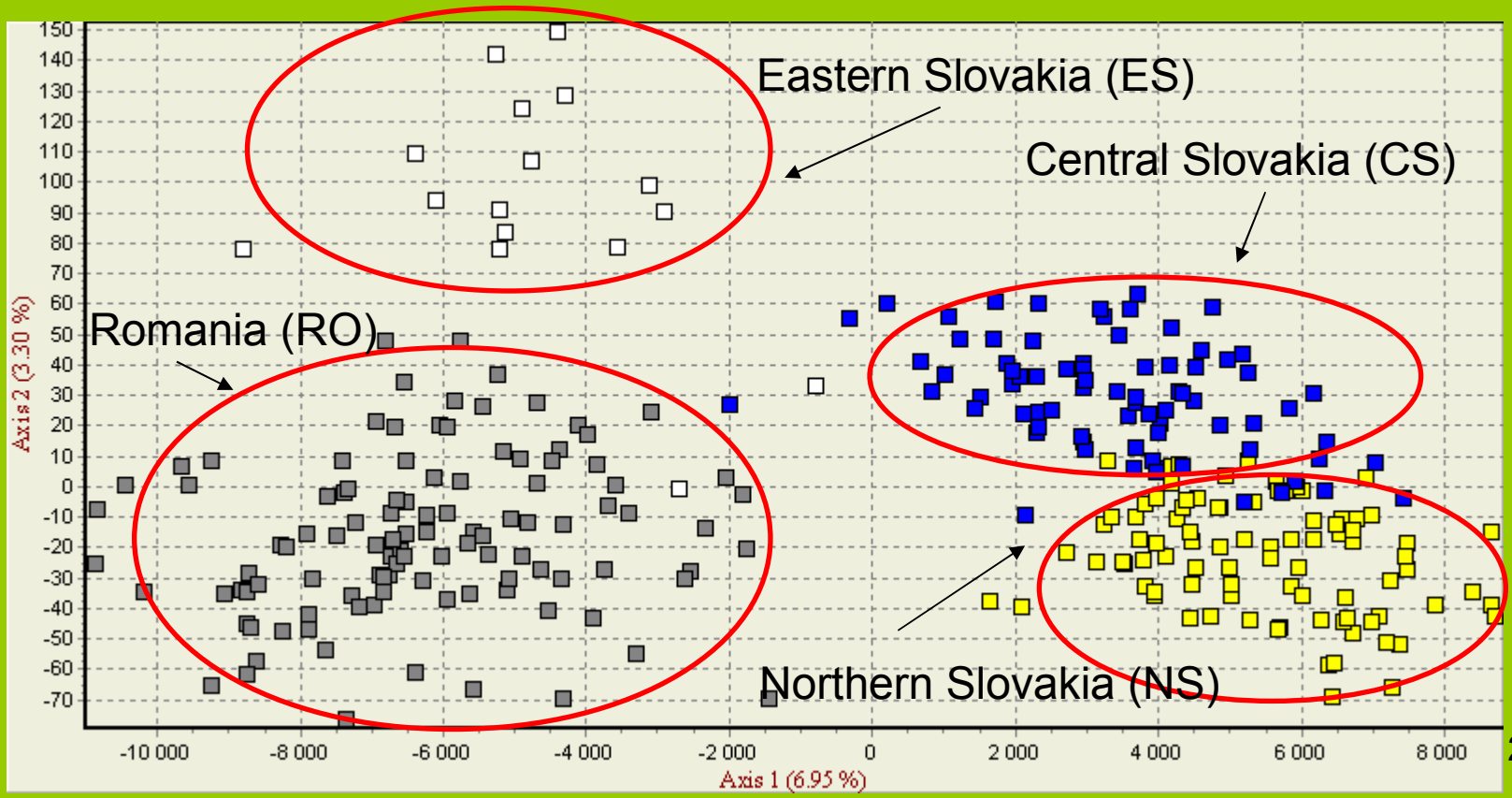
Populations from Carpathians

1 – Northern Slovakia
2 – Central Slovakia
3 – Eastern Slovakia
4 – Romania

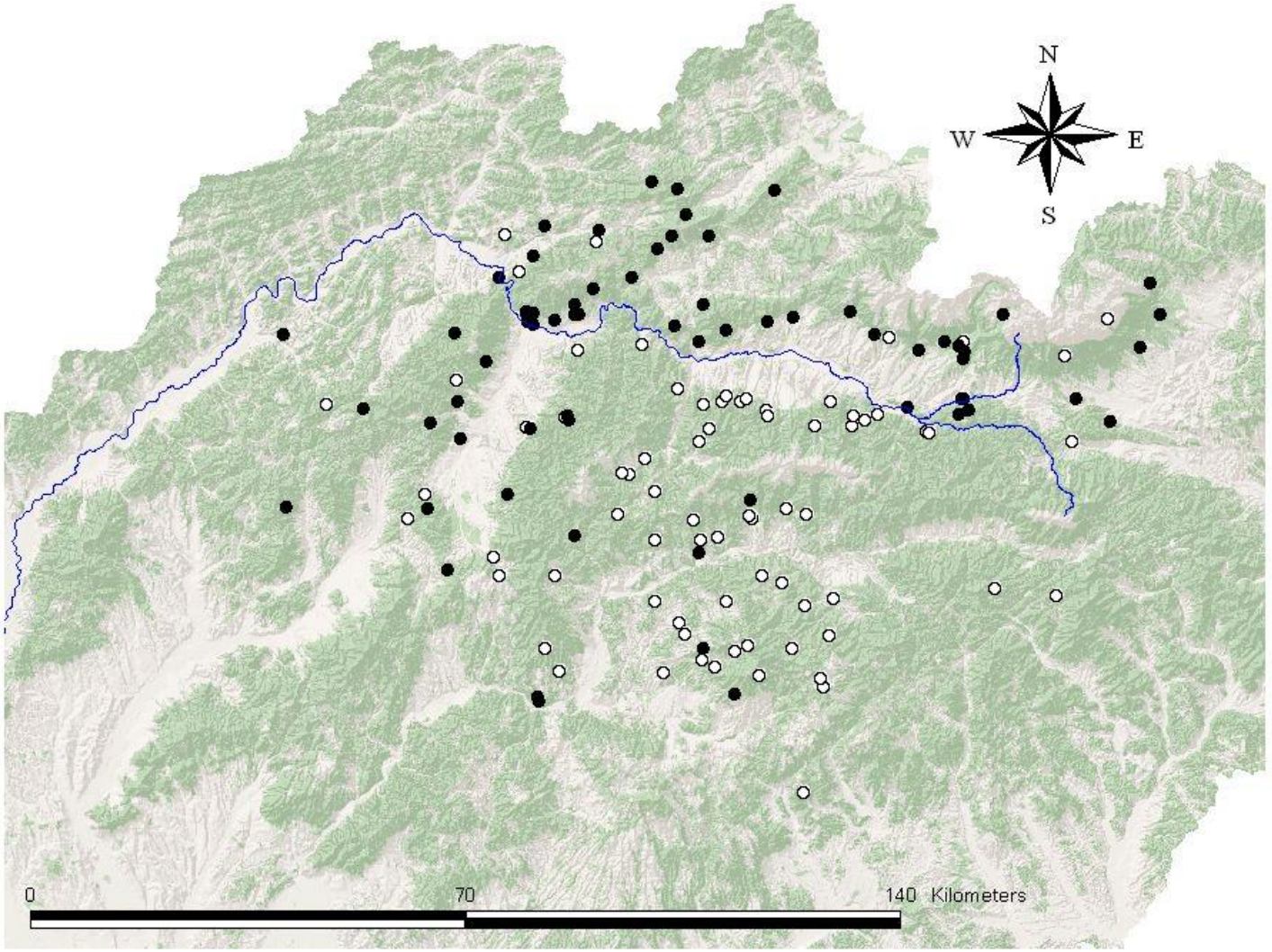


Results – genetic structure

Factorial correspondence analysis – Carpathian populations



Distribution of clusters Northern Slovakia (yellow) and Central Slovakia (blue) in Western Carpathians



•main barrier valley of river Váh

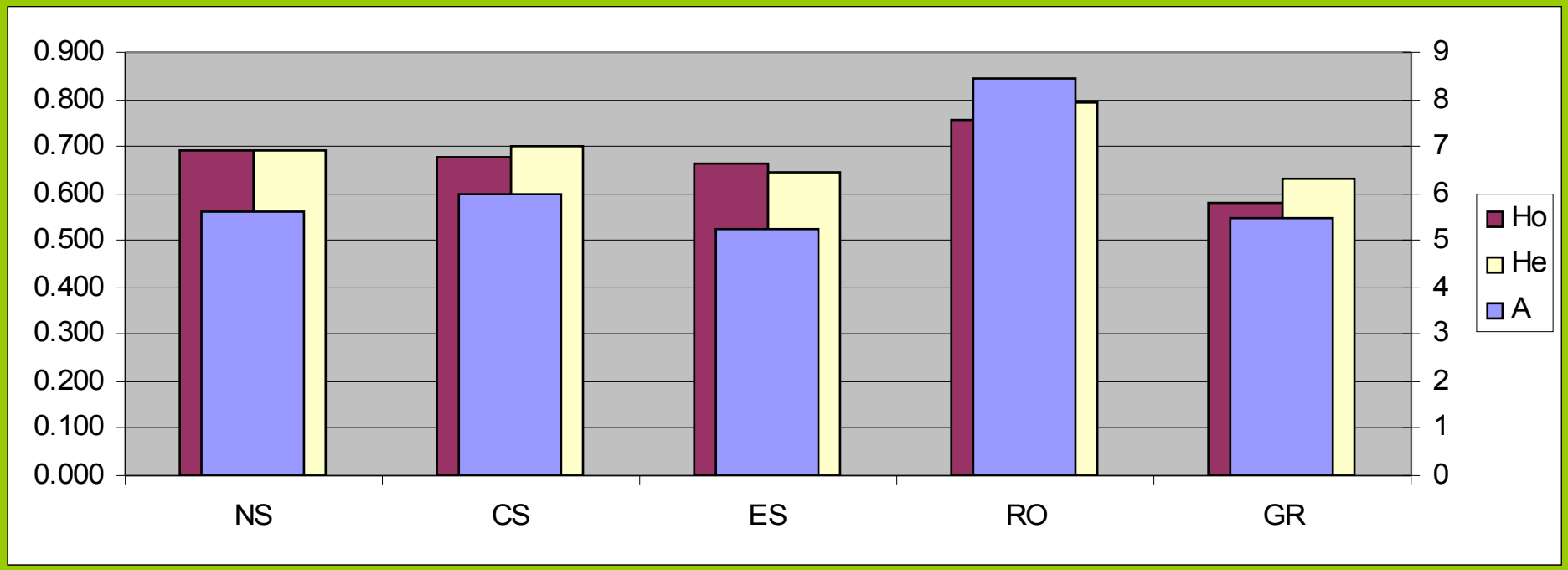
Results – pairwise *Fst*

	Northern Slovakia	Central Slovakia	Eastern Slovakia*	Romania	Greece
Northern Slovakia	0				
Central Slovakia	0.06203	0			
Eastern Slovakia*	0.18361	0.12716	0		
Romania	0.10828	0.09253	0.1006	0	
Greece	0.19942	0.21283	0.24553	0.13875	0

*) results could be biased due to low sample size of Eastern Slovakia

Results – genetic diversity

- the highest number of alelles and observed and expected heterozygosity found in Romanian population
- slightly lower heterozygosity in Western Carpathians, the lowest in Greece



Comparison of *He* with different populations (based on 7 loci)

Population	n	He
Romania	109	0.77
Kluane, Yukon	50	0.77
Scandinavia NS	108	0.70
Kuskokwim Range, Alaska	55	0.70
Scandinavia NN	29	0.69
Scandinavia S	156	0.68
Scandinavia M	88	0.66
Slovakia Central	78	0.65
Slovakia North	90	0.65
Slovakia East	16	0.63
Greece	123	0.57
Yellowstone	57	0.54
Kodiak Island	34	0.22

data from Paetkau *et al.* 1998, Waits *et al.* 2000

Conclusions

- populations from Western, Eastern Carpathians and Greece form 3 different clusters
- Carpathian bears are further subdivided into 4 clusters: Northern Slovakia, Central Slovakia, Eastern Slovakia, Romania
- Greek population the most differentiated
- population in Eastern Slovakia supported by gene flow from Ukraine and Romania

Conclusions

- population in Western Carpathians is formed by 2 different subpopulations – despite geographic proximity
- hypothesis – they may have founded by two small isolated stocks which survived the radical decline of populations numbers at the beginning of 20th century
- genetic diversity in Carpathians relatively high, in Greece slightly lower


Thank you for attention!