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Mitochondrial genetic variation in two Podolian cattle breeds in Croatia

¹ Ivanković, A., ¹ Ramljak, J., ² Dovč, P.

¹ *Department of animal production and technology, Faculty of Agriculture, Zagreb, Croatia*

² *Department of Animal Science, Biotechnical Faculty, Ljubljana, Slovenia*

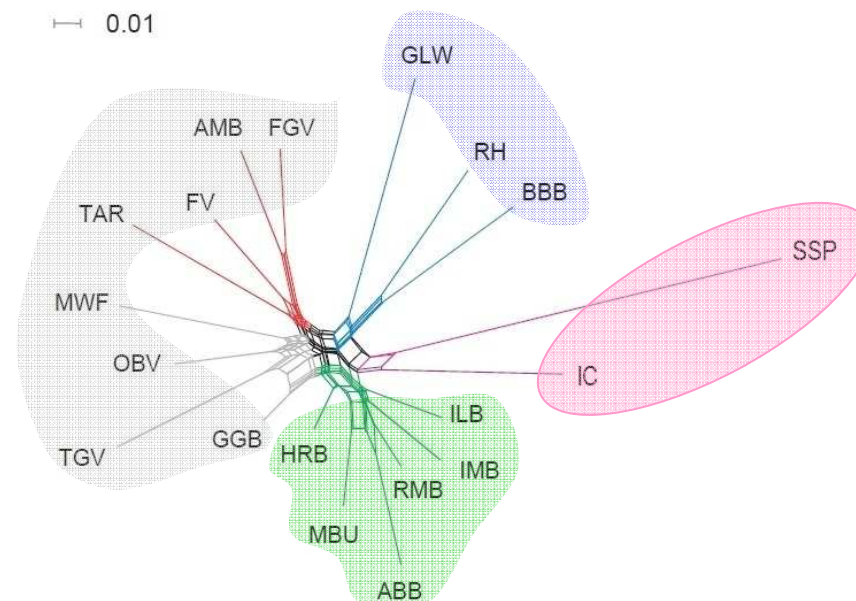


Introduction

- In the framework of breed conservation, genetic characterisation is an important step allowing preservation of the breed integrity and is a prerequisite for efficient management of genetic resources (Bjørnstad and Røed, 2002).
- Molecular analysis provides a reliable tool which can be used together with the quantitative approach and traditional breeding strategies for an efficient design of preservation strategy (*Dovč et al., 2006*).
- The control region of the mitochondrial DNA (mtDNA) is, due to its high mutation rate, lack of recombinations and maternal inheritance, a very useful marker system for population and evolutionary biology.

Introduction

- two of three autochthonous cattle breeds in Croatia belongs to the Podolian type of cattle:
 - Slavonian – Sarmian Podolian cattle
 - Istrian cattle
- performed studies of genetic structure based on microsatellite loci indicate their considerable genetic diversity

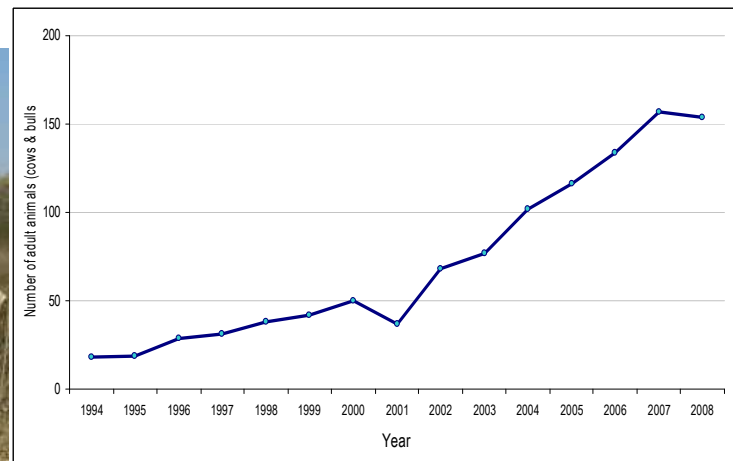


(Ramljak et al., 2011)

J. Anim. Breed. Genet. 128 (2011) 73–84

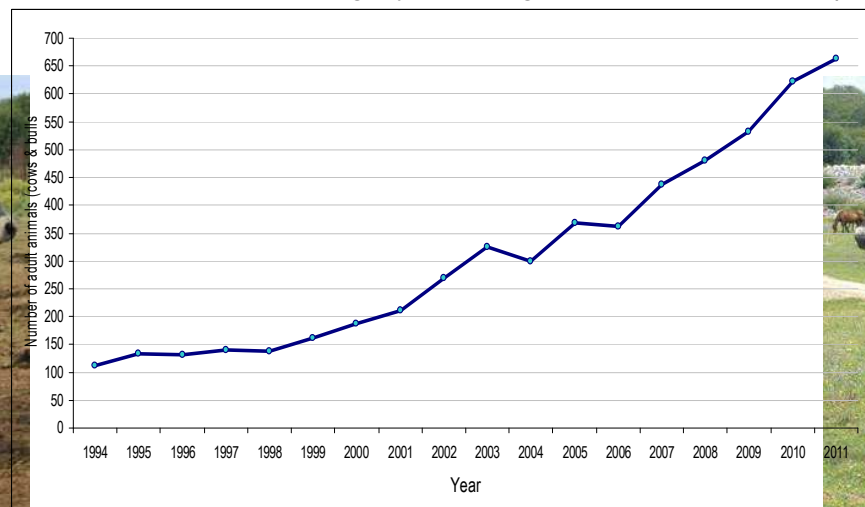
Introduction – Slavonian – Strymian Podolian cattle

- **History of breed:** has populated the area of Croatian planes two thousand years ago, during the offensive of Roman legions to the planes of the Danube confluence. Half millennium later, the podolian cattle reached the planes of Croatia again, together with the arrival of Avarian tribes from the East.
- **Phenotype and production characteristics:** late maturing breed of moderate frame (withers height 125 to 140 cm). Colour is grey-white to dark grey. Milk production is around 800 to 1000 litres. Needs very little food, and is kept in pasture the major part of the year.
- **Breeding area:** area of Posavina and Slavonia.
- **Present state of the breed** from 1997 systematic protection program has been implemented in order to protect the remaining population. Although the protection programme was started a decade ago, preservation of the population primarily at one limited locality has limited its growth. The breed is included in the national cattle breeding programme (2007)
- **Level of endangerment:** FAO/EAAP - critically endangered; $N_e = 33,9$ (9 bulls + 145 cows)



Introduction - Istrian cattle

- **History of breed:** is phenotype similar to podolian cattle whose ancient homeland is the area of Podolia and Volhinia from where it spread to the larger part of Panonia, and along the Adriatic Coast to South Europe and North Africa. At the end of the 18th century a targeted introduction of podolian primigen Italian breeds, Podolian, Romagnola and Maremana.
- **Phenotype and production characteristics** is late mature, longevity breed of moderate size of frame (withers height 135 to 150 cm). Milk production of Istrian cattle is 800 to 1500 kg. Most common are uni coloured light grey. Bulls are commonly darker then cows and heifers. Calves are mostly red upon birth, but can also be white to grey.
- **Breeding area:** Istrian peninsula and Kvarner islands.
- **Present state of the breed:** The Association of Breeders of Istrian Cattle was founded in 1990. From 1994 a program of systematic monitoring, establishment and breeding was started. In 2008 the Breeding program for the Istrian cattle was adopted.
- **Level of endangerment:** FAO/EAAP - highly endangered; Ne = 136.18 (36 bulls + 627 cows)



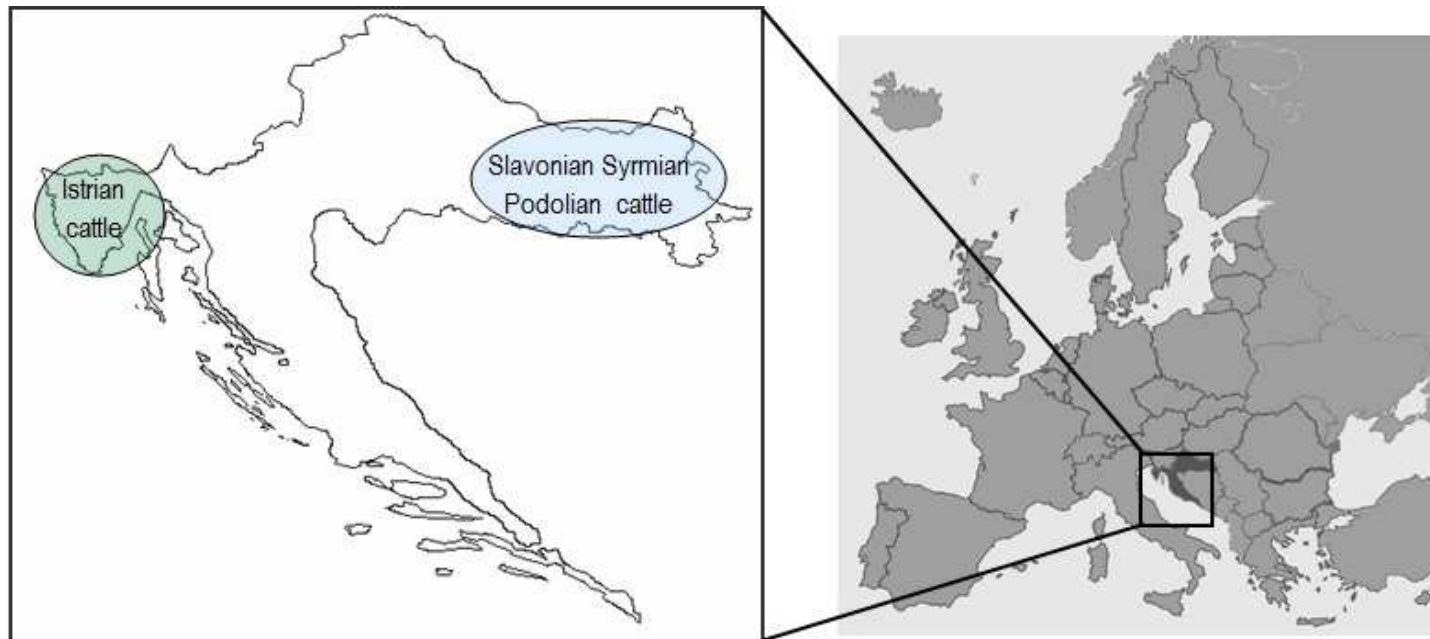


Aim of research

- was estimation of genetic variability in two local Podolian cattle breeds based on analysis of the mtDNA D-loop region:
 - Slavonian – Sarmian Podolian cattle
 - Istrian cattle
- reasons:
 - evaluation of genetic variability
 - improving existing *in situ* conservation programs (*management of genetic variability*)
 - help in the decision for storing the genetic material in the Gene Bank

Material and methods

- blood samples were collected from
 - 25 Istrian cattle (IC)
 - 25 Slavonian–syrmian podolian cattle (SSP)



- sampling included unrelated individuals from several locations per population (10 herds / breeds; 2-3 animals / herd)



Material and methods

- mtDNA was isolated with procedure by White and Densmore (1992)
- the proximal part of the D-loop region (780-bp fragment of mtDNA D-loop region; nt 15 923–16 342; 1-365) was PCR amplified using primers
 - P28 (5'-GTAAAACGACGGCCAGTCTCACCATCAACCC CCAAAGC-3')
 - HF (5'- GCCCCATGCATATAAGCAAG-3')
- the PCR reaction was performed on MJ Research PTC-100 thermal cycler under next conditions:
 - 95°C /5 min; followed by 32 cycles of 94°C/60 s, **52°C**/30 s, 72°C/60 s; 72°C/5 min
- used analytical software:
 - Clustal-W (Thompson et al., 1994)
 - Arlequin ver 3.5 (Excoffier et al., 2005)
 - MEGA 5.05 (Tamura et al., 2011)



Results and discussion

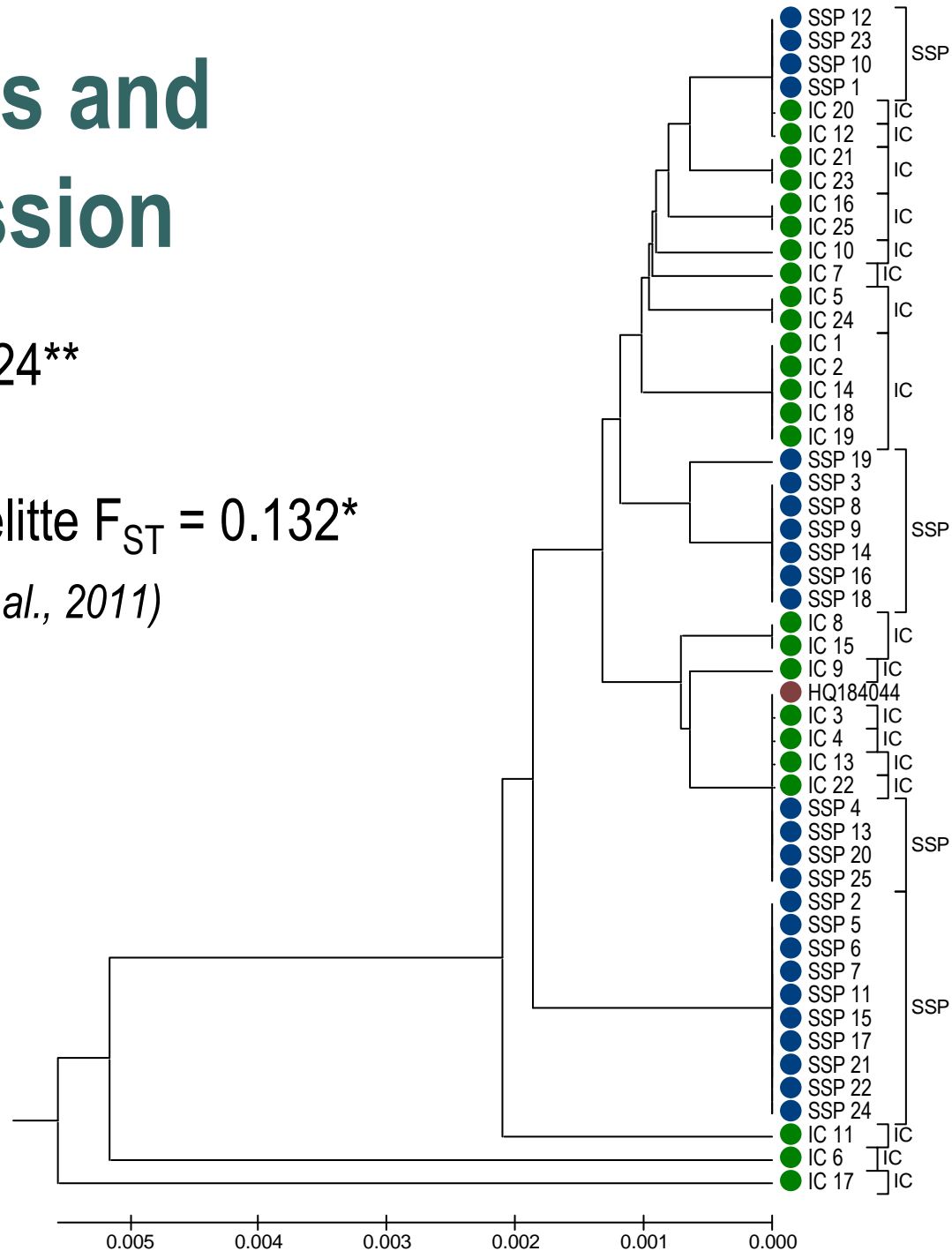
Analysis of cattle population at intrapopulation level

| | Istrian cattle | Slavonian-syrmian podolian cattle |
|--|---------------------|--------------------------------------|
| No. of sequences | 13 | 5 |
| No. of polymorphic sites | 22 | 5 |
| Number of observed transitions | 20 | 5 |
| Number of observed transversions | 2 | 0 |
| Gene diversity | 0.9300 ± 0.0294 | 0.7600 ± 0.0514 |
| Mean number of pairwise differences | 3.1972 ± 1.7089 | 2.1458 ± 1.2332 |
| Nucleotide diversity (average over loci) | 0.0041 ± 0.0024 | 0.0028 ± 0.0018 |
| Theta(k) | 10.2059 | 1.5904 |
| Theta(H) | 11.8064 | 2.4663 |
| Theta(S) | 5.5615 | 1.3242 |
| Theta(Pi) | 3.1833 | 2.5917 |



Results and discussion

- $F_{ST} = 0.124^{**}$
- microsatellite $F_{ST} = 0.132^*$
(Ramljak et al., 2011)





Results and discussion

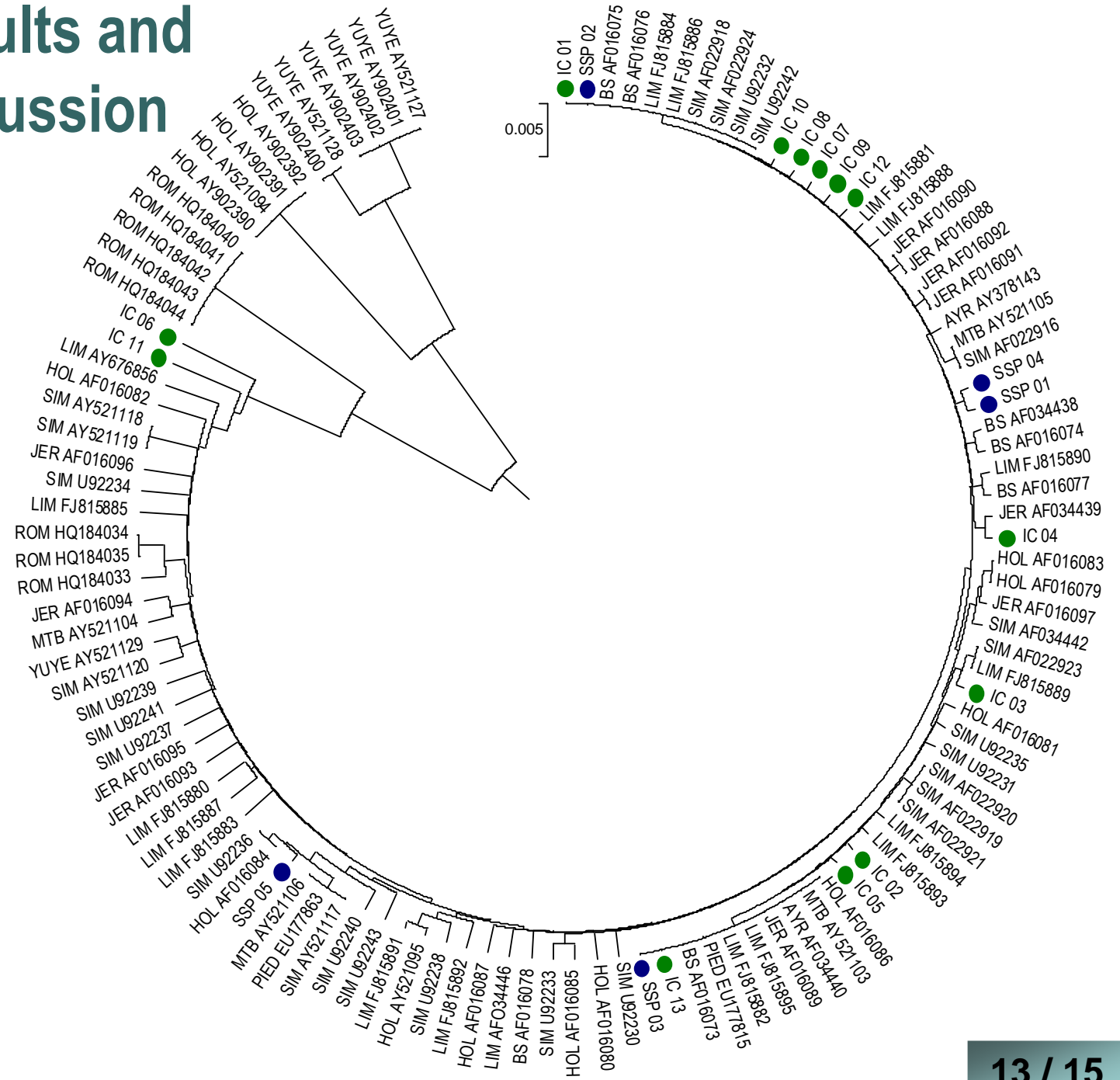
Results from Tajima's D and Fu's F_S Neutrality Test

| <i>Population</i> | <i>Tajima's D</i> | <i>Fu's F_S</i> |
|--|-------------------|---------------------------|
| Istrian Cattle (IC) | -1.553* | -4.839* |
| Slavonian – Sarmian Podolian cattle (SSP) | 1.765 | 1.943 |

* $P < 0.05$

- Istrian cattle – signature of rapid population demographic expansion
- Slavonian – Sarmian Podolian cattle – has not passed thorough population expansion in the past

Results and discussion





Conclusions

- our results show a relatively moderate genotypic diversity within and between the two analyzed cattle breeds
- the high numbers of identified haplotypes in population of Istrian cattle indicate a high genetic variability of the maternal component, while in population of the Slavonian – Syrmian Podolian cattle lower genetic variability was observed
- the genetic diversity information based on mtDNA will serve to select mothers for future breeding conservation strategies



Thank you for your attention!