

Possible way of conservation the multipurpose Tsigai and other indigenous sheep breeds in Central-Eastern European and Balkan countries
ERFP Project

- **The project plan**
 - Organise a starting meeting to discuss the works and duties to be done – Tirana, Albania
 - Decide on details
 - Organise the final meeting to evaluate the results – Budapest, Hungary

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The first workshop in Tirana, Albania

20 – 21 November, 2004

Coordinantig countries:

ALBANIA - Prof. Dr. Kristaq Kume NC

HUNGARY - Prof. Dr. Sandor Kukovics

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Participating countries :

ALBANIA -	Dr. Lumturi Papa
BULGARIA -	Dr. Doytcho Dimov
GREECE -	Prof. Andreas Georgoudis
HUNGARY -	Tamás Szobolevszki
RUMANIA -	Prof. Condrea Draganescu
MACEDONIA -	Dr. Sreten Antonov
SLOVAKIA -	Dr. Egon Gyarmathy
SLOVENIA -	Dr. Drago Kompan

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PROJECT's OBJECTIVES:

1. To develop a common strategy for conservation, economic utilisation and improvement of the multi-purpose Tsigai and the other indigenous sheep breed populations in their traditional lowland, hill- and mountain production conditions in Central and Eastern Europe, and in the Balkan region.

2. To develop a regional cooperation among neighboring countries, in order to identify the economic and cultural values of management and conservation of an indigenous breed, at present risk of extinction.

3. To clarify relationship and genetic distances of various sheep populations in the regions.

4. To discussed about the questions: these sheep breeds are international or national breeds with some differences?

5. To organise a Regional Network of *INDIGENOUS SHEEP BREEDERS' NATIONAL ORGANISATION* with the main objective –

Implementation of the Common Strategy for conservation, economic sustainable utilisation and improvement of sheep breeds.

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Preparatory work for organizing and carried out the first workshop, Tirana, Albania

1. Preparing and fulfill a Questionnaire - *Having these data we could make a Data base and we would be able to create common programmes for conserving and management of native sheep breeds.*

2. Preparing the Country report – *having these raports like a general picture of Sheep Genetic Resources for which country, we could decided for the breeds and the topics that will be te target for our future colaboration*

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The main output of Tirana's Workshop

- 1. Elaboration of the Questionnaire and decision for the final version**
- 2. Decision regarding the local breeds that will be the target of future work.**
- 3. Discuss and decision about the methods and information that could provide for carried out the study *for clarify relationship and distances of various sheep populations in the regions.***
- 4. Identification of the main issues regarding the building up the crossborder and regional collaboration**

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**The second Workshop in Budapest, Hungary,
22-24 April, 2005.**

Coordinantig countries:

HUNGARY - Prof. Dr. Sandor Kukovics

ALBANIA - Prof. Dr. Kristaq Kume NC

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PARTICIPATING COUNTRIES :

ALBANIA

Dr. Lumturi Papa

BULGARIA

Dr. Doytcho Dimov

BOSNIA-HERZEGOVINA

Dr. Salko Muratovic

CZECH REPUBLIC

Dr. Vera Matlova

CROATIA

Dr. Ivan Vnéc

GREECE

Prof. Dr. Andreas Georgoudis

HUNGARY

Mr. Tamás Szobolevszki

MACEDONIA*

Dr. Sreten Andonov

POLAND

Dr. Elzbieta Martyniuk

ROMANIA

Prof. Dr. Condrea Draganescu

SERBIA & MONTENEGRO

Dr. Srdjan Stojanov

SLOVAKIA

Dr. Egon Gyarmathy

SLOVENIA

Dr. Drago Kompan

TURKEY

Prof. Dr. M. Ihsan Soysal

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Further participants

- **Romania** Dr. Alexandru Nagy
- **Slovakia** Dr. Jarmila Dubravská
- **Turkey** Prof. Dr. Özder Muhittin

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HUNGARY

- Prof. Dr. István Fehér
- Dr. László Zsilinszky,
- Prof. Dr. József Rátky,
- Prof. Dr. Attila Lengyel,
- Ildikó Bajúsz, PhD student,
- Tímea Németh, PhD student,
- Tímea Kupai, PhD student,
- Szilvia Kusza, PhD student
- Sándor Nagy (I), Awassi Corporation
- Sándor Nagy (II), Awassi Corporation

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Plenary papers

- **Opening of the Workshop: Sándor Kukovics and Kristaq Kume**
- **Welcome speech from:**
 - **József Rátky**, general manager, Research Institute for Animal Breeding and Nutrition, Herceghalom
 - **László Zsilinszky**, deputy general manager, Agricultural Institute for Quality Control, Budapest
 - *The practice and methods of preservation of indigenous sheep breeds in Hungary from the animal breeding authority point of view*

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- **István Fehér**, deputy secretary, Ministry of Agriculture and Regional Development, Budapest

The importance of autochthonous animal species and the possibility of their use in the present and future animal sector of Hungary

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- **Country reports**

- Albania
- Bulgaria
- Bosnia-Herzegovina
- Czech Republic
- Croatia
- Greece
- Hungary

- **Country reports**

- Macedonia*
- Poland
- Romania
- Serbia & Mont.
- Slovakia
- Slovenia
- Turkey

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Summarising papers based on Questionnaires

- **Tímea Németh – Sándor Kukovics**

Use of blood groups and blood biochemical polymorphisms in breeding plan

- **Ildikó Bajúsz- Sándor Kukovics**

Methods of conservation (based on phenotypic and/or genotypic traits)

- **Tímea Kupai– Attila Lengyel - — Sándor Kukovics**

Use of direct gene test informations (Scrapie genotyping) and use of microsatellites in gene conservation

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Szilvia Kusza – András Jávör - Sándor Kukovics

Relationships among the various Tsigai (and Zackel) populations based on microsatellites (wool bulb DNA studies)

Sándor Nagy – Gábor Csatári – Sándor Nagy – Péter Kovács

Breeding and production possibilities of the gene reserve Gyimesi Racka sheep in the 21st century – Case study

Kristaq Kume - Sándor Kukovics

Possible way of international cooperation in conservation

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Questionnaires about breeds

- The breed groups
 - Tsigai group
 - Ruda group
 - Zackel group
 - others

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Tsigai group

**A) The autochthonous Tsigai
/black/brown/**

- Romanian Tsigai
- Hungarian
- Serbian Cokan
- Slovakian
- Czech
- Croatian
- Albanian
- Pleven Blackhead?

**B) The Milking
Tsigai**

- Sombor
/Pivnicki Tsigai
(S&M)
- Milking Tsigai
(HU)

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- **Red Tsigai**

- Romanian Rusty
- Covasna Tsigai (RO)

- **White Tsigai**

- Rodopski Tsigai (BUL)
- Staroplaninski Tsigai (BUL)
- Romanian white

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- **Tsigai relatives**

- Thrace (GR)
- Serres (GR)
- Florina (GR)
- Skopelos (GR)
- Kymi (GR)
- Kivicik (Turkey)
- Bardhoka (A)

- **Tsigai Crossbreds**

- White Maritza (BUL)
- Patch faced M. (BUL)
- Dabenska (BUL)
- Elin Pelinska (BUL)
- Lokal Karnobat (BUL)
- Spanka (RO)

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The Ruda group

- Albania
- Romania
 - Black head Ruda
 - Brown face Ruda
- Macedonia
- Bulgaria (Pleven blackhead)?
- Croatia

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Zackel Group

- Hungarian racka
- Transsylvanian Racka (HU)
- Tsurcana (RO)
- Corkscrew valashian (RO)
- Cikta (HU)
- Polish Mountain S.
- Olkuska (POL)
- Swirniarka (POL)
- Bovec (Slovenia)
- Valachian (Czech)
- Valachian (Slovakia)
- Replianska (BUL)
- Karakachan (BUL)
- Skhodrane (AL)
- Lara e Polisit (AL)
- Lara e Matit (AL)
- Baca (AL)
- Recka (AL)
- Karakachanian (MAC)

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The Pramenka subgroup

- Bardoka (S&M)
- Lipska (S&M)
- Pirotka (S&M)
- Svrljig (S&M)
- Krivovirska (S&M)
- Istrian Pr. (Slovenia)
- Bela Kraina Pramenka (Slovenia)

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Other breeds

- Ovcepolian (MAC) Z?
- Sharplanian (MAC) Z?
- Local Stara Zagora (BUL)
- Copper-red Shumenska (BUL)
- Pag Island (Cr)
- Cres Island (Cr)
- Istrian (Cr)
- Lika (CR)
- Red Karaman (Turkey)
- White Karaman (Turkey)
- Karayaka (Turkey)
- Daghe (Turkey)
- Sakis (Turkey)

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Possible use of blood biochemical polymorphisms in breeding plans, and / or preservation of breeds

(like: haemoglobine, haemopexine, esteraze, transferine, beta-lactoglobuline)

Researches on blood groups, blood biochemical polymorphisms on indigeneous sheep breeds in Hungary

Limited information about of the present use of them

- Since **1964**: only White and Black Racka --L. FÉSÜS
- **1970**: + Tsigai and Cikta (Zaupel) -- in situ
- Since **1996**: only the Milking Tsigai --. S. KUKOVICS
- Since **2000**: Σ Hungarian Tsigai varieties -- differently:
Milking and Autochthonous Tsigai --S. KUKOVICS

THE TSIGAI SHEEP

- In the comparing study of Fésüs (1978, 1991), in **Tsigai** breed no important difference was found between blood group's frequencies, but it has changed, increased or decreased, and Tfl has disappeared from this breed.

- The observed 8 Hungarian Tsigai flocks can be separated by blood biochemical polymorphisms (Kukovics, 2004). The lowest difference is in **Hpx** and **Blg** genotypes

- The lambing rate depends on:

-- **Blg** and

-- **Hb** genotype, but its affects differently by flocks and

-- **Tf** 's effect is the strongest.

HpxA is dominant in every flock, but ... **HpxAB** affects as stronger as HpxA. The lambing rate of **Es-** 's ewes is higher than **Es+** ewes (Kukovics, 2004, 2005).



In the case of Cikta („Zaupelschaf”) and Racka („Zackelschaf”) only the frequencies of blood groups alleles were analysed and compared by L. Fésüs.



The frequency distributions of blood group factors in **Cikta** breed are different, but the tendency is not consistent. The present of Tfl isn't obvious; it has been introduced into this breed from Merino or other Zaupel.

In the case of **Black-** and **White Racka**, there isn't found difference in the frequencies of transferrin between Black and White Racka, except TfB allele (Fésüs, 1978).



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**The use of phenotypic and genotypic, or both
traits in the breeding programmes and / or
preservations of breeds**

- ☺ Some countries and some breeds these traits are used,**
- ☹ But in some others no information received**

COMPARATIVE TABLE 1.

<i>Name of native breed</i>	<i>Country</i>	<i>Genetical improvement based on</i>	<i>wool</i>	<i>milk</i>	<i>meat</i>	<i>reproduction</i>
Tsigai	Romania	phenotypic				
		genetic traits				
		both	X	X	X	
	Croatia	phenotypic				
		genetic traits				
		both			X	X
	Slovak Republic	phenotypic				
		genetic traits				
		both		X	X	
	Czech Republic	phenotypic				
		genetic traits				
		both		X	X	X
	Hungary	phenotypic				
		genetic traits				
		both	X			X

COMPARATIVE TABLE 2.

<i>Name of native breed</i>	<i>Country</i>	<i>Genetical improvement based on</i>	<i>wool</i>	<i>milk</i>	<i>meat</i>	<i>reproduction</i>
Ruda	Romania	phenotypic				
		genetic traits				
		both	X	X	X	X
	Croatia	phenotypic			X	
		genetic traits				
		both		X		X
	Albania	phenotypic		X	X	
		genetic traits				
		both				

COMPARATIVE TABLE 3.

<i>Name of native breed</i>	<i>Country</i>	<i>Genetical improvement based on</i>	<i>wool</i>	<i>milk</i>	<i>meat</i>	<i>reproduction</i>
Valachian	Romania	phenotypic			x	x
		genetic traits				
		both	x	x		
	Czech Republic	phenotypic			x	
		genetic traits				
		both				x
	Slovak Republic	Phenotypic			x	x
		genetic traits				
		both		x		

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Use of direct gene test information (Scrapie genotyping) and use of microsatellites in gene conservation

The questionnaire contains items intended to ask about using of:

- - microsatellites for marker assisted selection (QTL)
- - other DNA / molecular genetic studies
- - other examination methods

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In Hungary the following researches are in progress:

- Measuring the frequency of Scrapie genotype
 - Milk protein polymorphism measures
 - Effect of Booroola mutation
 - QTL mapping (especially for weight gain)
- Characterisation of indigenous breeds by microsatellites
 - Identification of breeds by microsatellites

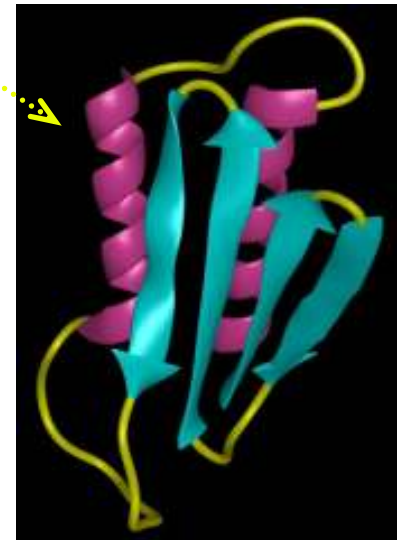
Scrapie

TSE (Transmissible Spongiform Encephalopathy)

- connection with BSE (bovin) and CJD (human)
- non-heritable
- needs infective agent (prion protein) and inherent susceptibility

EU regulations: 999/2001/EC;
1003/2002/EC;
100/2003/EC;
260/2003/EC;
848/2003/EC;
1494/2003/EC

In Hungary: 69/2003 (VI.25.) and 22/2004 (II.27.)



Genotype	Group of risk
ARR/ARR	R1
AHQ/AHQ	
ARR/AHQ	R2
AHQ/ARH	
AHQ/ARQ	
ARR/ARH	R3
ARR/ARQ	
ARH/ARH	
ARH/ARQ	
ARQ/ARQ	R4
ARR/VRQ	
AHQ/VRQ	
ARH/VRQ	R5
ARQ/VRQ	
VRQ/VRQ	

The aim of the EU regulations:
to improve the frequency of
ARR prion (PrP) allele, which is
joined to resistance of Scrapie

obligatory:
from 1st of April 2005.

R1 = low risk of Scrapie disease

R5 = high risk ...

PrP genotypes in indigenous breeds (%)

Genotype	Improved Valachian¹ (106)	Slovakian Tsigai¹ (117)	Hortobágyi racka² (140)	Transylvanian Racka² (57)	Hungarian Tsigai² (64)	Cikta² (69)
ARR/ARR	9.4	15.4	5	5.26	3.13	1.45
AHQ/AHQ	0.9	-	4.29	-	-	-
ARR/AHQ	10.4	-	6.43	-	6.25	4.35
AHQ/ARH			7.14	-	-	-
AHQ/ARQ	14.2	-	12.14	1.75	-	14.49
ARR/ARH	-	-	4.29	-	-	-
ARR/ARQ	19.8	42.7	25	42.11	45.31	33.33
ARH/ARH			0.71	-	-	-
ARQ/ARQ	16	20.5	17.86	47.37	40.63	46.38
ARH/ARQ	-	-	10	-	3.13	-
ARR/VRQ	15.1	12.8	2.14	1.75	-	-
AHQ/VRQ			1.43	-	-	-
ARH/VRQ			1.43	1.75	-	-
ARQ/VRQ	13.2	6.8	2.14	-	1.56	-
VRQ/VRQ	0.9	1.7	-	-	-	-

Group of risk	R1	R2	R3	R4	R5	ARR	AHQ	ARH	ARQ	VRQ
Jezerško-Solcava¹ (78)	-	-	-	-	-	-	-	-	-	12.82
Bovec¹ (49)	-	-	-	-	-	-	-	-	-	5.1
Bela Krajina Pramenka¹ (69)	-	-	-	-	-	-	-	-	-	1.45
Istrian Pramenka¹ (66)	-	-	-	-	-	-	-	-	-	7.85
Hortobágyi racka² (140)	5	10.71	48.57	32.14	3.57	23.93	17.86	12.14	42.5	3.57
Transylvanian racka² (57)	5.26	-	43.86	49.12	1.75	27.19	0.88	-	70.18	1.75
Hungarian Tsigai² (64)	3.13	6.25	45.31	43.75	1.56	28.91	3.13	1.56	65.63	0.78
Hungarian Cíkta² (69)	1.45	4.35	47.83	46.38	-	20.29	9.42	-	70.29	-
Bergschaf³ (6)	-	50	16.7	33.4	-	-	58.3	8.32	33.3	-
Tirolk Steinschaf³ (4)	25	-	25	50	-	25	12.5	-	62.5	-
Tirol Steinschaf⁴ (27)	-	-	51.8	48.1	-	14.8	11.1	3.7	70.3	-
Tirol Bergschaf⁴ (35)	2.9	5.7	51.4	40.4	-	25.8	8.6	-	65.7	-
Waldschaf⁴ (26)	11.5	-	26.9	61.5	-	19.2	5.8	3.8	71.2	-
Charintian sheep⁴ (24)	4.2	-	45.9	45.8	4.2	23	4.2	4.2	64.6	4.2

Characterisation and determine the distance of indigenous breeds

Macedonia:

- Ovchepolian, Sharplanian and Karakachanian.
- microsatellites : BM8125, ETH225, ILSTS11, ILSTS28, SRCSP1, SRCSP3, SRCSP7, SRCSP8, SRCSP9, OARFCB11, OARFCB48, OARFCB193, OARJMP5, MAF214 and McM527.

(Source: Questionnaire)

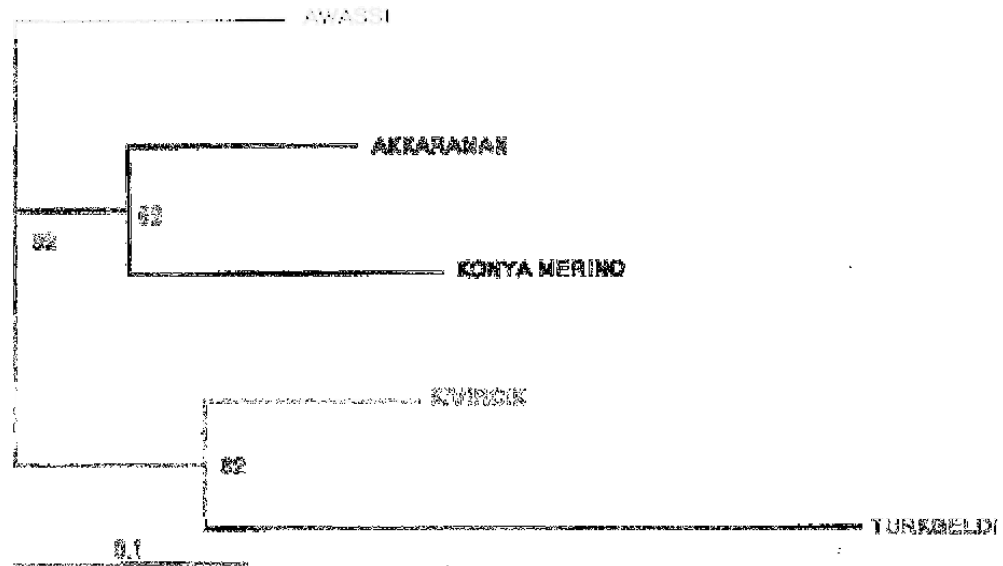
Serbia-Montenegro:

- the within-population diversity were studied in Tsigai sheep populations, especially Cokan Tsigai comparing to Pivnicki var.
- microsatellites : BM0757, BM1314, BM1818, BM4621, BM6506, BM6526, BM8125, CSSM31, MAF214, MAF36, MAF48, MAF65, McM527, OarCP20, OarCP34, OarCP38, OarFCBII, OarFCB128, OarFCB304, OarFCB48, OarHH47, OarHH64 and OarVH72.

Turkey:

- Awassi, White Karaman, Western Thrace (Kivircik) and crossbreeds: Türkgeldi, Konya Merino were studied for polymorphism of the microsatellites.
- The following microsatellites were genotyped:
 - OarFCB304,
 - OarFCB20,
 - MAF65.

Determine the distance of breeds



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**GENETIC DIVERSITY OF EASTERN AND
MIDDLE EUROPEAN TSIGAIA SHEEP
BREED VARIATION**

Based on microsatellites

THE MOST FREQUENT USED METHODS FOR DIVERSITY EXAMINATION

LabelName

AFLP	Amplified Fragment Length Polymorphism
EST	Expressed Sequence Tag
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat (Microsatellite)
STS	Sequence Tagged Site

EXAMINED SAMPLES

<i>Country</i>	<i>Variates</i>	<i>Number</i>	<i>Label</i>
Hungary	Indigenous	217	HU-SMA-AC HU-KMKK-AC HU-KMNP-AC HU-SZICS-AC HU-MRDJ-TAC
	Csokai (Cokan) Tsigai	125	HU-DE-CSC
	Milking Tsigai	159	HU-PG-ZC HU-LB-TCZ HU-OJ-TC
	Rusty Tsigai (Zuku)	50	HU-PG-TRC
Rumania	Rumanian Ruda	40	RO-RUDA
	Romanian Rusty	40	RO-RUST-TS
Albania	Albanian Tsigai	39	AL-TS
	Albanian Ruda	37	AL-RUDA
	Bardhoke sheep	31	AL-BARDH
Bulgaria	Patch Faced Maritza sheep	39	BU-PFMAR
	Pleveny Blackhead sheep	35	BU-PLBH
	Rodopski Tsigai	30	BU-ROD-TS
	Staroplaninski Tsigai	42	BU-STAR-TS
	White Maritza sheep	41	BU-WFMAR
Croatia	Croatian Tsigai	50	CR-TS

Turkey	Sakiz	49	TR-SAKIZ
	Gokceada	42	TR-GOKCE
	Kivircik (Marmara region)	46	TR-KIV-MAR
	Kivircik (Trakya region)	53	TR-KIV-TRA
Slovak Republic	Handel	25	SL-HAN-TS
	Jugat	22	SL-JUG-TS
	Kamo	19	SL-KAO-TS
	Sirig	22	SL-SIR-TS
	Vojin	5	SL-VOJN-TS
	Jurbis	24	SL-JUR-TS
	Kamendin	16	SL-KAM-TS
	Olymp	5	SL-OLYM-TS
	Ondrej	16	SL-OND-TS
	Rybar	16	SL-RYB-TS
	Vancouver	15	SL-VAN-TS
	Brend	10	SL-BREN-TS
Serbia & Montenegro	Pivnicki (Milking) Tsigai	41	SM-ZP-TS
	Cokan Tsigai	12	SM-CS-TS
	Svrgig Zackel Pramenka	48	SM-SVR-PR
	Krivovirska Zackel Pramenka	32	SM-KRI-PR

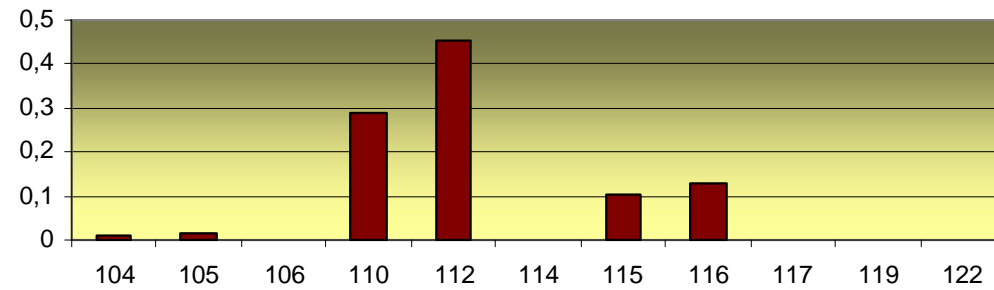
METHODS

- DNA EXTRACTION (from blood, hair)
- MICROSATELLITE ANALYSIS
- Start with 21, but only the followings could be evaluated :
(MAF35, CSSM43, MCM527, TGLA53, MCMA7, OarFCB 20, TGLA357, INRA127, MAF70, MAF65, ILSTS11, OarCP20, OarCP49, BM1314, BM6506 and OarAE 119)
- STATISTICAL ANALYSIS

DISTRIBUTION OF ALLELE FREQUENCIES

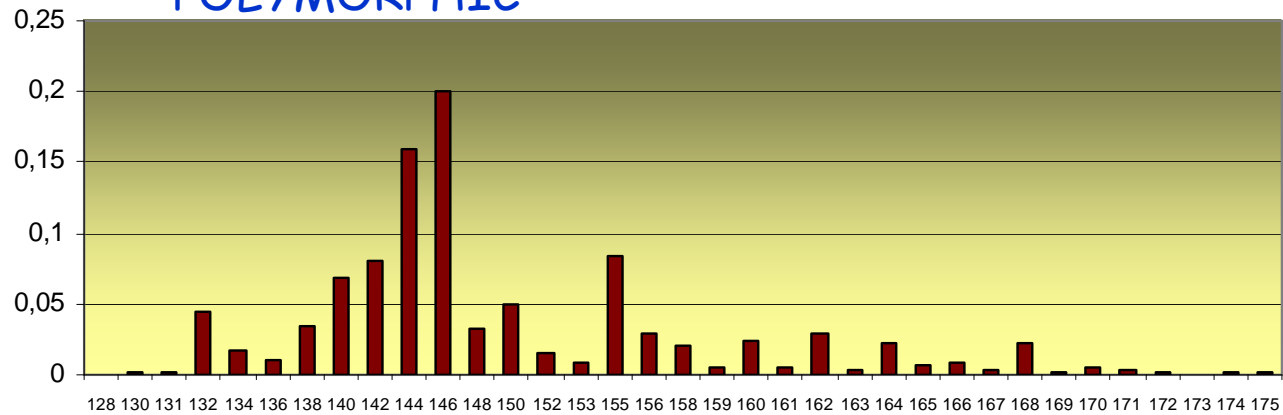
• THE LESS
POLYMORPHIC

MAF35

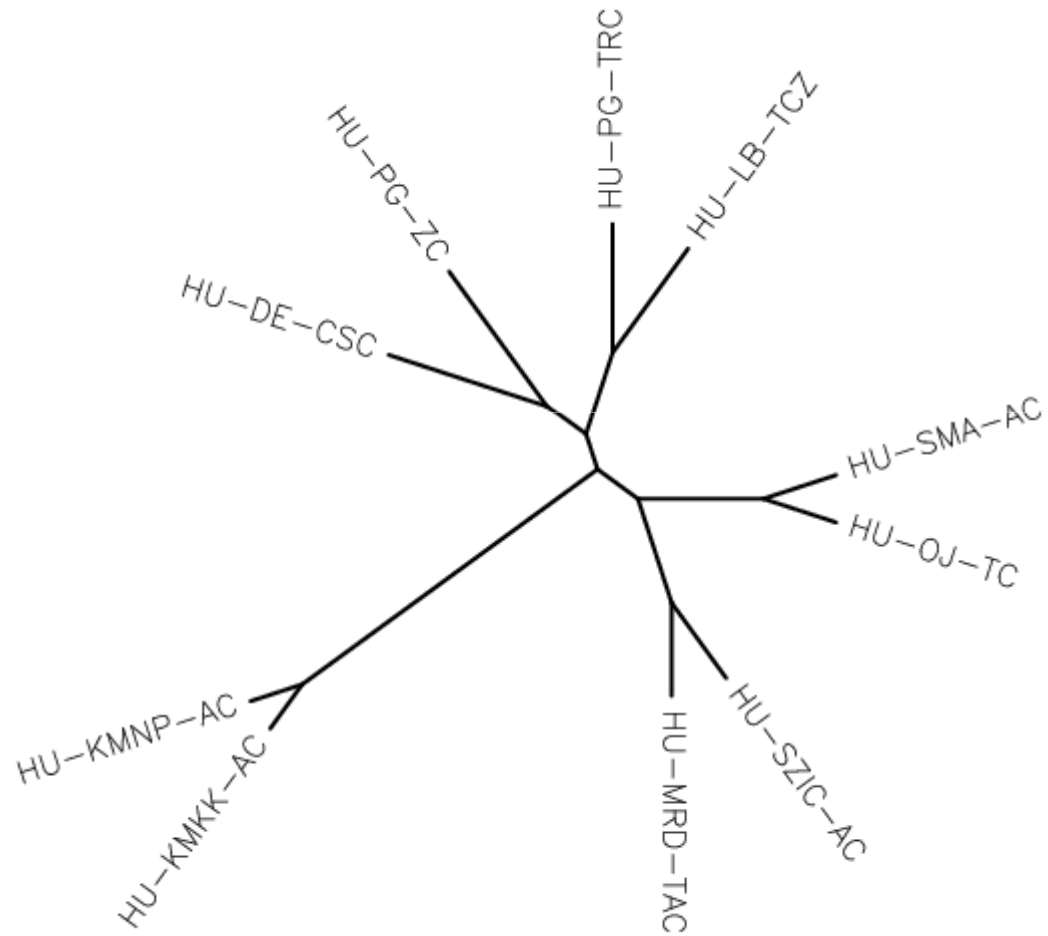


• THE MOST
POLYMORPHIC

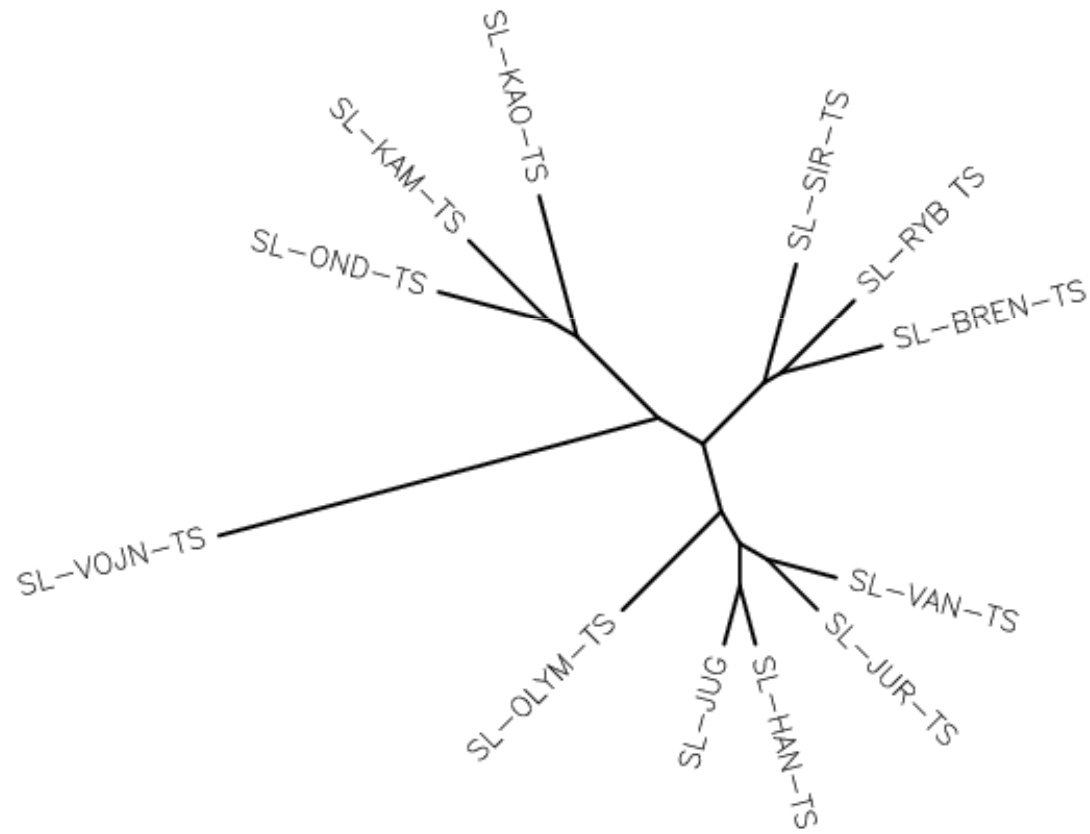
MAF70



UNROOTED NEIGHBOUR-JOINING TREE ABOUT HUNGARIAN VARIATION



UNROOTED NEIGHBOUR-JOINING TREE ABOUT SLOVAKIAN VARIATION



Possible way of conservation the multipurpose Tsigai and other indigenous sheep breeds in Central-Eastern European and Balkan countries
ERFP Project

Cooperation possibilities in preservation of studied sheep breeds

- Cross-border co-operations concerning the breeds in questions,
- Genetic co-operations regarding the studied breeds

Cooperation possibilities in preservation

- Conditions
 - Relationships within Tsigai group
 - Relationships within Ruda group
 - Relationships within Zackel group
 - Similarity between Tsigai and Ruda group
 - Geographical distance
 - Genetical distance

Possible cooperation I

- **Racka group** → Hungary – Romania
- **Valachian group** → Czech – Slovakia – Hungary – Romania
- **Tsigai group** → Czech R. – Slovakia – Hungary – Romania – Croatia – Serbia & Montenegro – Albania – Turkey - Greece
- **Ruda group** → Romania – Albania – Macedonia – Croatia – Bulgaria
- **Zackel group** → All of the countries

Possible cooperation II.

Cross border cooperation

- Ruda sheep → Albania – Macedonia – Kosovo
- Ruda sheep → Romania – Bulgaria – Croatia -
Albania
- Tsigai sheep → Czech R. – Slovakia – Hungary
– Romania – Croatia – Serbia & M. – Albania –
Greece – Turkey
- Valachian sheep → Czech R. – Slovakia –
Romania - Poland

Possible cooperation III.

Cross border cooperation

- **Karakachanian** → Macedonia – Bulgaria
- **Patch faced Maritza** → Bulgaria – Greece (Florian, Thrace)
- **Stara Zagore (BUL)** → Zakintos (GR) – Bergamasca (IT)
- **Pramenka group** → Slovenia – Croatia – Serbia & M.
- **Swiniarska (POL)** → Poland – Lithuania – Belarus
- **Polish heath** → Poland – Lithuania – Belarus
- **Olkuska** → Poland - Slovakia

Proposed future

- Working out plans for future activities
- Determined programmes might be started
- Suggested programmes
 - Based on DNA (microsatellite) studies
 - Based on blood protein characteristics
 - Based on phenotypic characteristics

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The need of publishing the collected information

- Good and up to data of the present situation of the sheep sectors in 14 countries of the region
- Inventory of some 62 autochthonous sheep breeds with characteristics and traits, along with pictures and maps where are they bred
- Relationships among the breeds to be used for future works: preservation of breeds
- Determined methods for suggested crossborder and genetic cooperations in the future

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Based on the results of the Project

- There are possibilities to prevent and preserve the autochthonous sheep breeds of the region as common activities
- Working out cooperative projects for international awards

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Most important outputs of the project

- strengthening of our capacities in field of collaboration,
- carry out good experiences in field of experience exchange between us, and
- identification of the most important issues targeted of our future collaboration in field of conservation , development and, sustainable use of sheep genetic resources.

THANK YOU FOR YOUR
ATTENTATION









**Possible way of conservation the multipurpose Tsigai and other indigenous
sheep breeds in Central-Eastern and South Eastern European countries -
ERFP Project**

SEE YOU NEXT TIME

