UNIVERSITY OF ZAGREB

Faculty of Agriculture

DNA Sequence Variation in the Mitochondrial Control Region of Oryctolagus cuniculus from Croatia

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Background

Oryctolagus cuniculus

↓

key species in biology

↓

animal model

↓

human diseases – biotechnology applications

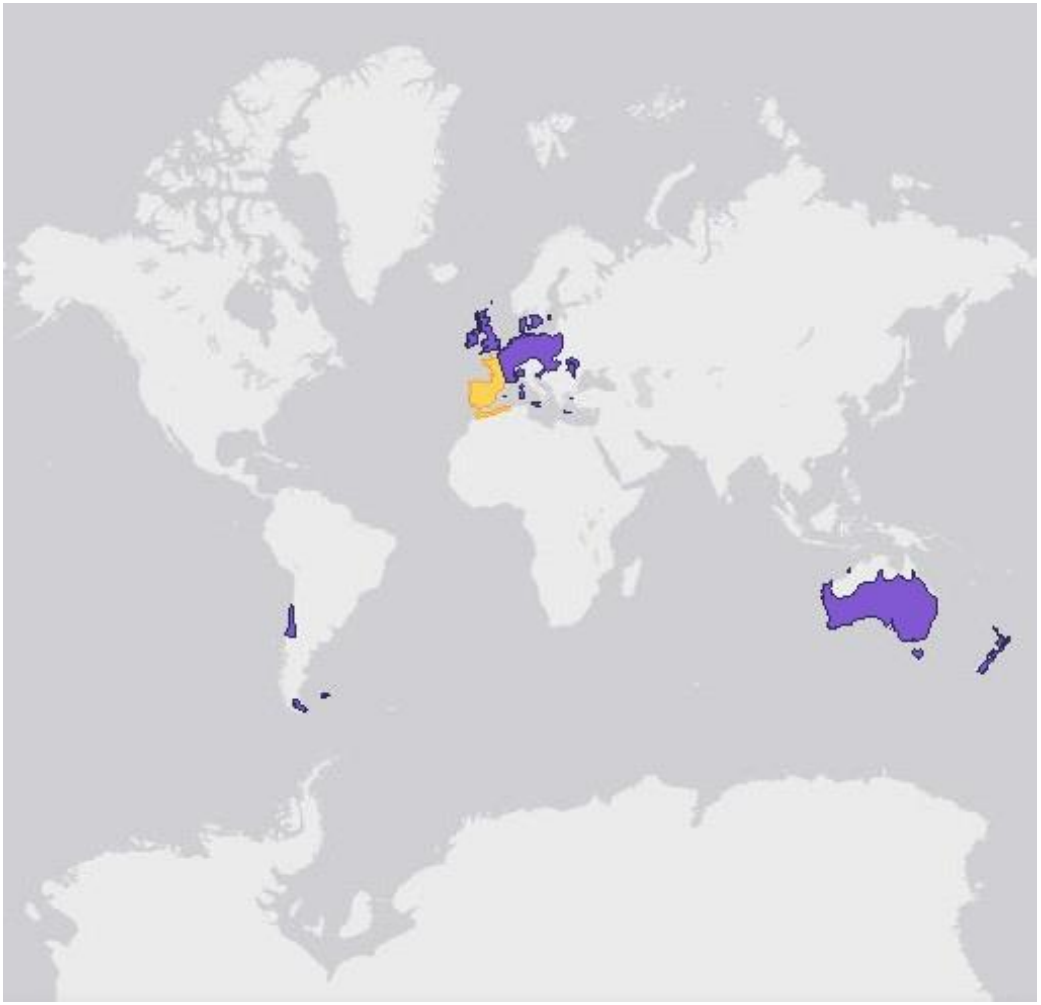
↓

wild type

→

domesticated type

Background



native introduced

Kingdom:	*Animalia*
Phylum:	*Chordata*
Class:	*Mammalia*
Order:	*Lagomorpha*
Family:	*Leporidae*
Genus:	*Oryctolagus*
Species:	*O. cuniculus*

Common name:

European Rabbit

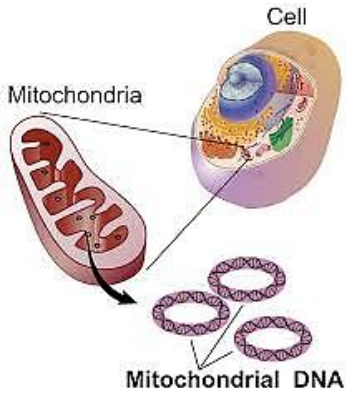
Population trend: ↓ decreasing

Upper elevation limit (metres): 1500

Background


Maribor
Varaždin
Pécs
Baja
Udine
Slovenia
Treviso
E70
Trieste
Venice
Osijek
Padua
Poreč
Opatija
Rijeka
E71
Rovinj
Slavonski Brod
E70
Brijuni
L. europaeus
Bosnia and Herzegovina
Bijeljina
Tuzla
Ravenna
5
Pag
Zadar
Zenica
Cesena
Rimini
San Marino
6
7
9
E65
Sarajevo
Šibenik
Ancona
Levrnaka
O. cuniculus
Split
Arezzo
Mostar
Makarska
Međugorje
E35
Italy
San Benedetto del Tronto
Adriatic Sea
Ascoli Piceno
Terni
Montene
Podgorica

Aim



MtDNA dloop anaylsis

↓

Oryctolagus cuniculus

↓

very distinct maternal lineages

A and **B**

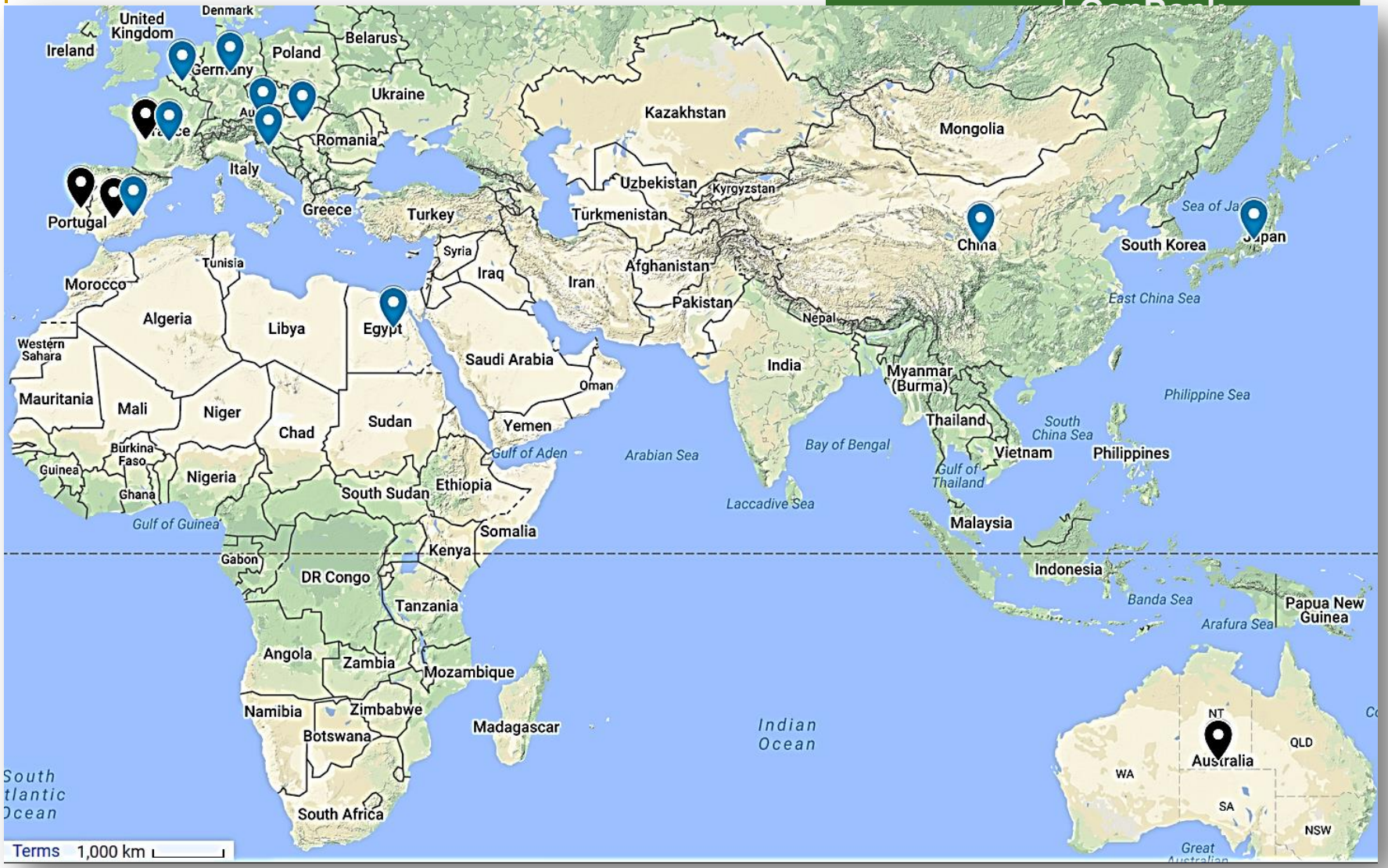
south west of the
Iberian Peninsula

rest of Europe,
including Northern Spain

- **Aim** of the present study→ characterize **maternal genetic diversity** and origin of **wild rabbits** in **Croatia**

Material and methods

Localities | **Sequences from**



Reference sequence = NC_001913
(Gissi et al., 1998)

[1]wild rabbit = 67
[2]domestic rabbit = 140

Material and methods

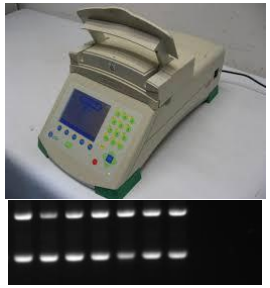
- **DNA extraction** - EZ-10 spin column genomic DNA miniprep kit (BioBasic, Canada)

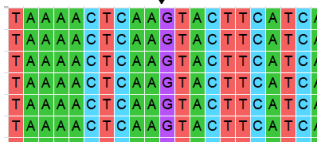
- A 509 bp **D-loop** region (15460 -15968) was amplified by iCycler (Biorad, Germany) **forward** (5′-CACCATCAGCACCCAAAG-3′) (Melo-Ferreira et al., 2007) and **reverse** (5′-ATTTAAGAGGAACGTGTGGG-3′) (Pierpaoli et al., 1999) **primers**

- Wizard SV Gel and **PCR** Clean-Up Kit (Promega, USA)

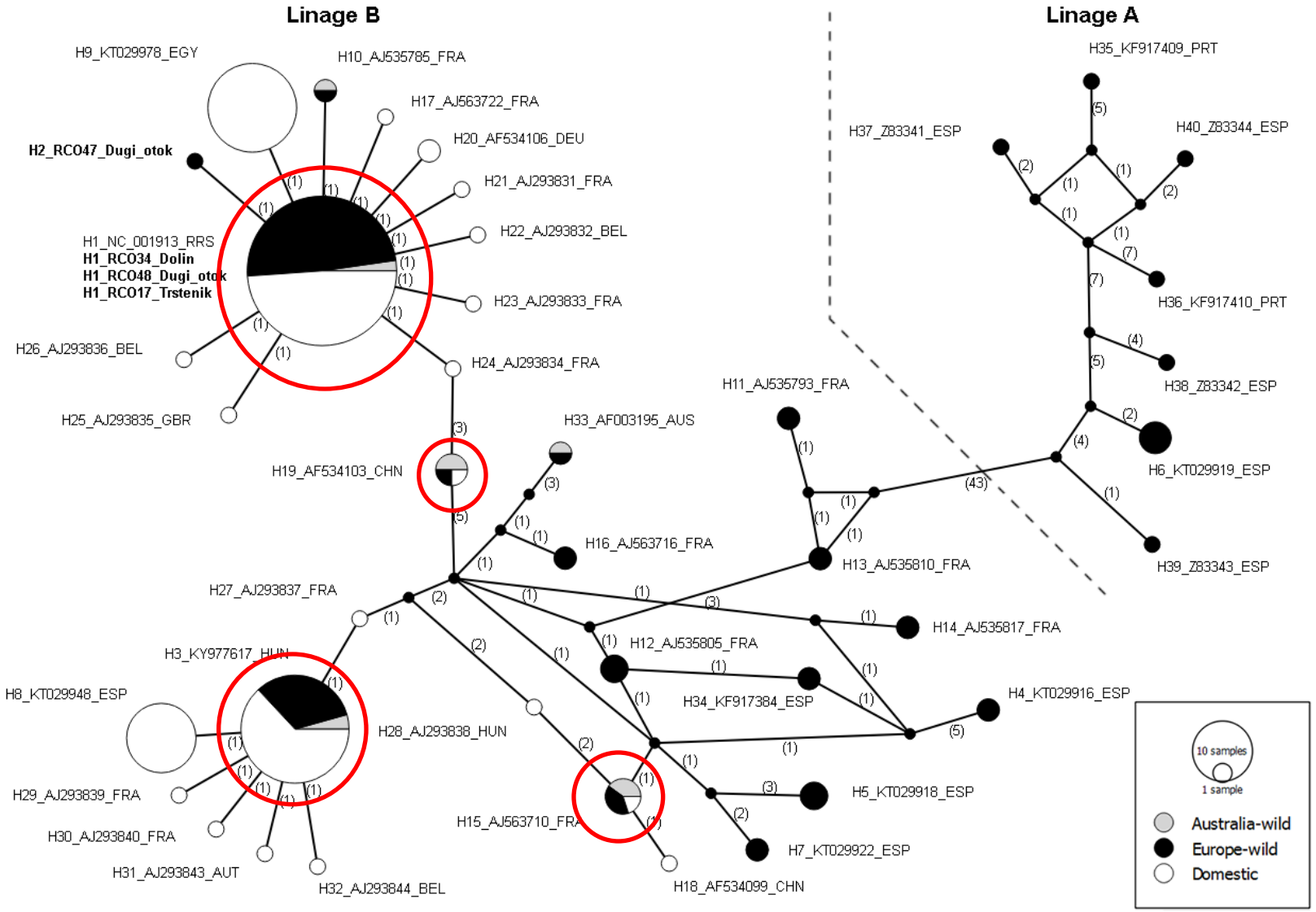
- ABI 3130 DNA automated **sequencer** (Applied Biosystems, USA)

- MEGA 7, DNA Sp 5.10 , PopART





Results



The median-joining (MJ) network for the 317 bp D-loop region data set showing phylogenetic positions found in Croatian wild rabbits

Results

- **low diversity** of Croatian wild rabbits **belonging to linage B**

- Hap **H2** is one (**T→C at 15803**) transversion remote from **H1** where is **RRS (NC_001913)**

- **19** unique **hap** in **wild** rabbit

- **17** unique **hap** in **domestic** rabbit

- **4** hap shared between **wild** and **domestic**

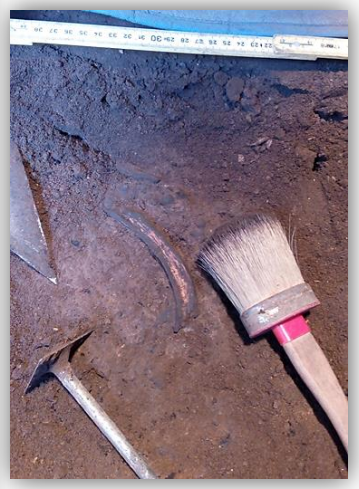
Localities	Hap	Seq	Hap/Seq
[1]Australia	6	10	0.6
[1]Croatia	2	36	0.06
[1]France	7	9	0.77
[1]Portugal	2	2	1
[1]Spain	17	46	0.37
[2]Austria	1	1	1
[2]Belgium	3	3	1
[2]China	3	11	0.27
[2]Egypt	3	68	0.04
[2]France	8	8	1
[2]Germany	3	8	0.36
[2]Great Britain	1	1	1
[2]Hungary	3	6	0.5
[2]Japan	1	2	0.5
[2]Spain	2	32	0.06

[1]wild rabbit = 103
[2]domestic rabbit = 140

Conclusion

- **Low diversity** of Croatian wild rabbits
- 2 haplotypes detected → **H1** and **H2**
- All Croatian samples belong to the **lineage B**
- Indication of **feral origin** of Croatian wild rabbits

Archeogenetic analysis of Lagomorphs



- **COST** “A collaborative European Network on Rabbit Genome Biology – RGB-Net“ - FEM - dr. Cristiano Vernesi
- **NGS → Mitogenom**: ***Oryctolagus cuniculus***, ***Lepus europaeus*** and ***Lepus timidus***
- Enlarged number of mitogenomes from Balkans and Mediterranean basin
- Preparation for publication

Acknowledgment

http://mitotauromics.agr.hr

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http://mendthegap.agr.hr

Provided partial travel support & know-how building.

Thank you!