

Evolutionary Medicine

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Isotopic reconstruction and
multielemental analysis of
ancient diet
and health: implications for
evolutionary medicine
My Recherche

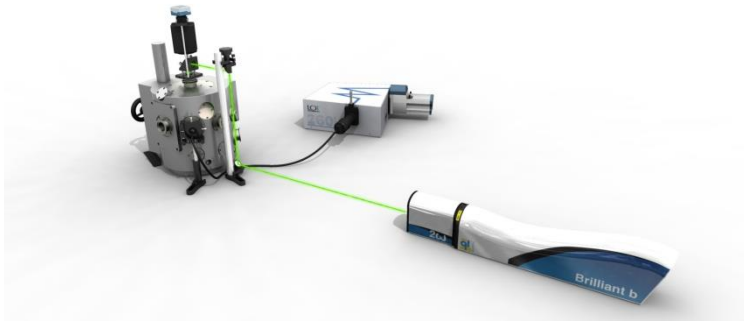


Analytical chemie

Analysis of fossil samples using laser ablation

Methods

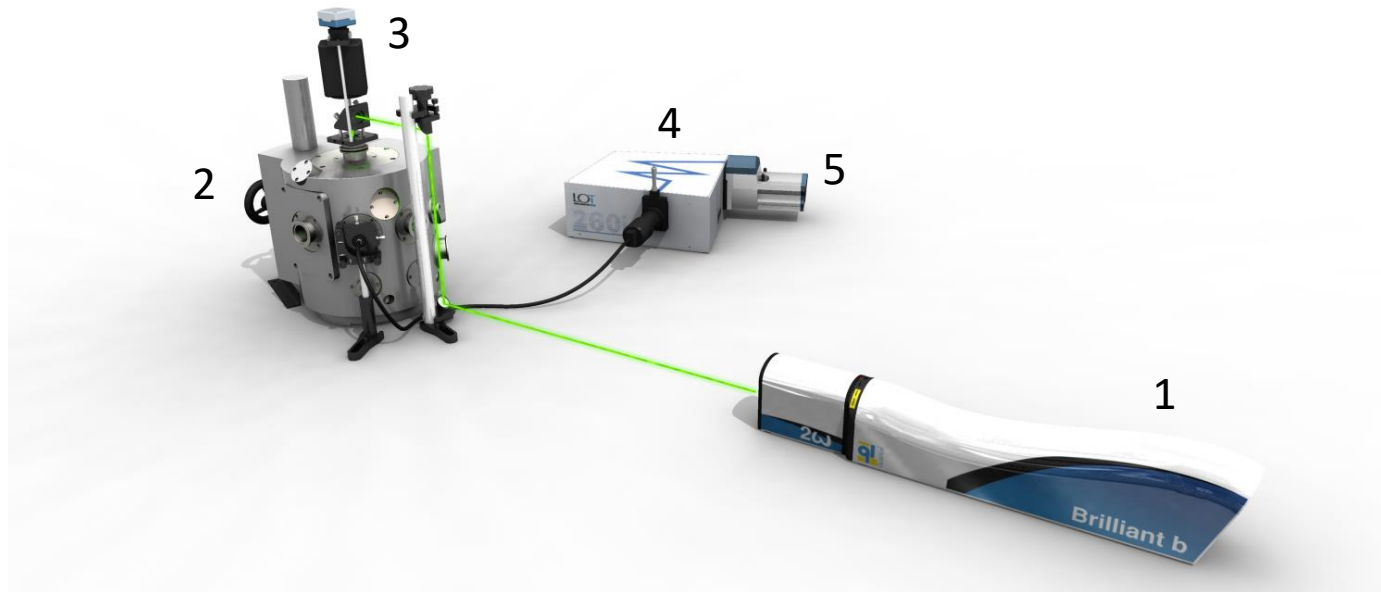
SP and DP LIBS



LA-ICP-MS



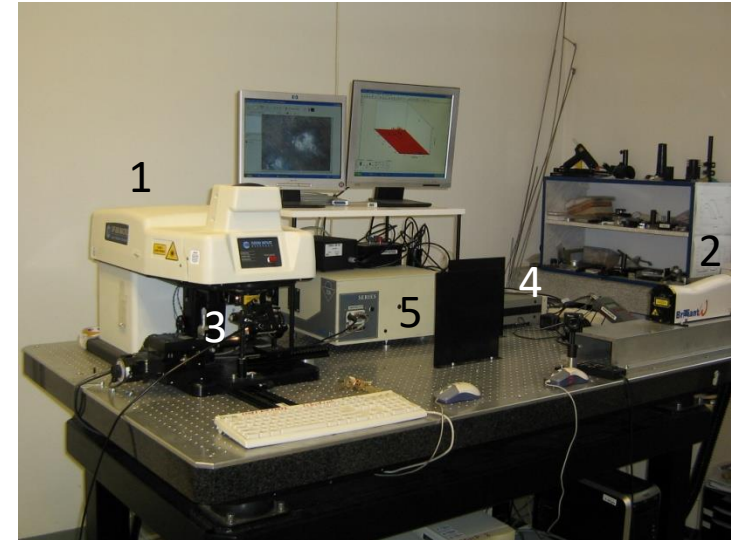
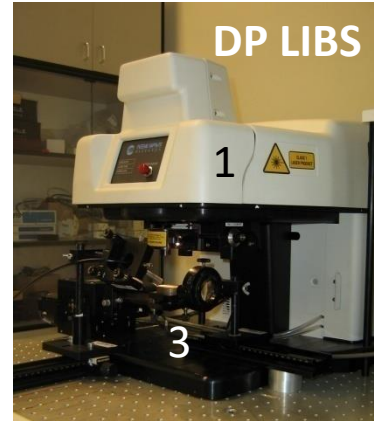
SP LIBS



1 – ablation laser (Quantel Brilliant B), 2 – ablation chamber with sample holder and fine slides (Tescan s.r.o.), 3 – CCD camera for sample observation/laser pulse focusing, 4 – spectrometer (LOT Oriel 260i), 5 – ICCD camera (Andor Istar DH734i)

DP LIBS a LA-ICP-MS

1 – ablation laser (New Wave, MACRO 266 nm), 2 – re-excitation laser (Quintel Brilliant, 1064 nm), 3 – sample holder and displacements, 4 – delay generators (Stanford RS) 5 – spectrometer and ICCD camera (Jobin Yvon, Triax).



LA-ICP-MS

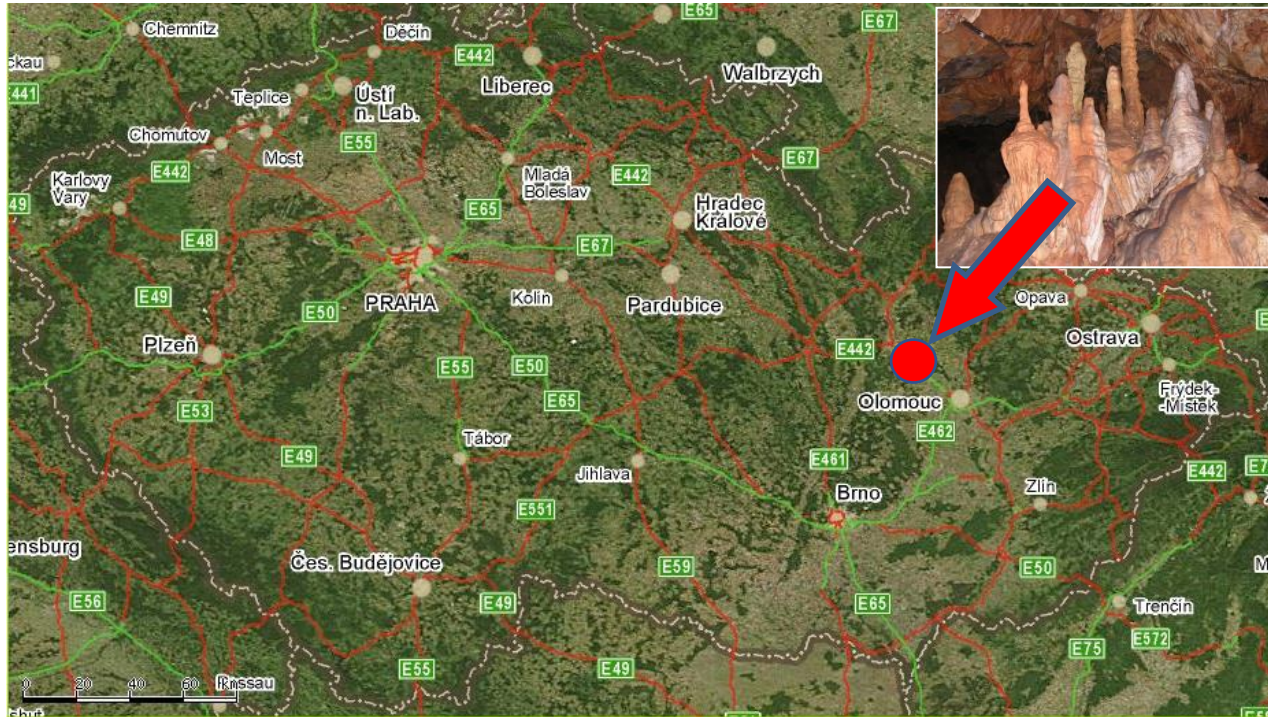


- ☞ Ablation system – UP 213 (New Wave, USA)
- ☞ Ablation chamber – SuperCell (New Wave, USA)
- ☞ ICP-MS spectrometer - Agilent 7500 CE (Agilent, Japan)

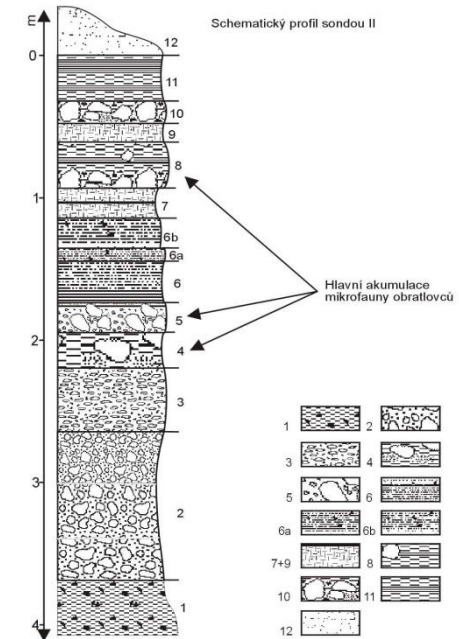
The story of the snake






Natrix natrix



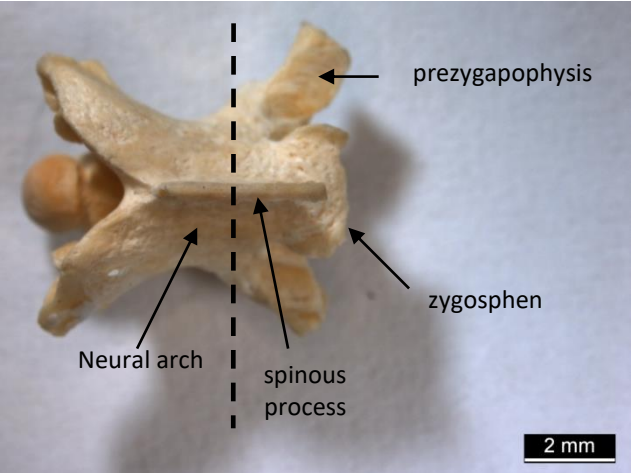
Mladečské jeskyně waal



-  The fossil material comes from the locality of Mladeč cave, which lies about 4 km WNW from the village of Litovel in Olomouc. The location is located on the Třesín hill (345 m above sea level). It is a hill stretched in the E-W direction, located approximately 1.5 km WNW from the village of Mladeč.
-  All of the fossil snake material comes from the cinder cone sediments in the "House of the Dead", the II probe, dug in 1958-1959.
-  Fauna of Probe II sediments documents a Lower Pleistocene age (Lower Bihar). Undoubtedly, this is the fauna of one of the interglacials of the Lower Pleistocene, it could probably be the Waal interglacial, or the end of the Waalian interglacial (Ivanov 1993, 2007).

Matrix matrix

The main component is carbonized hydroxyapatite
 $Ca_{10}(CO_3,PO_4)_6(OH)_2$
Ca/P ratio 2:1 in healthy bone.

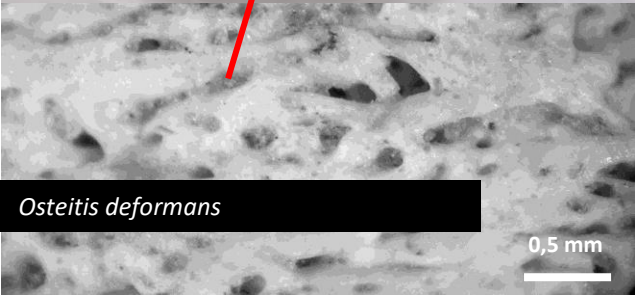
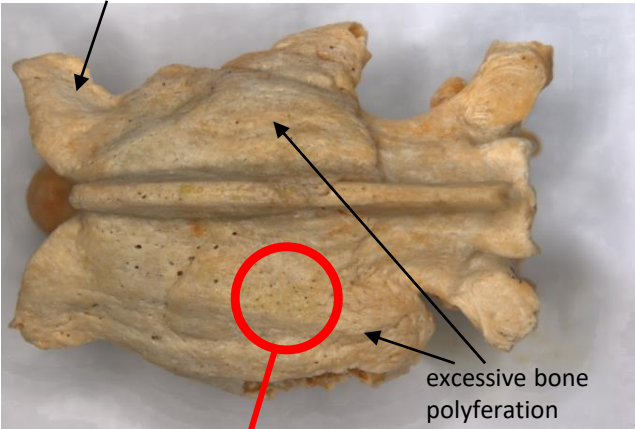
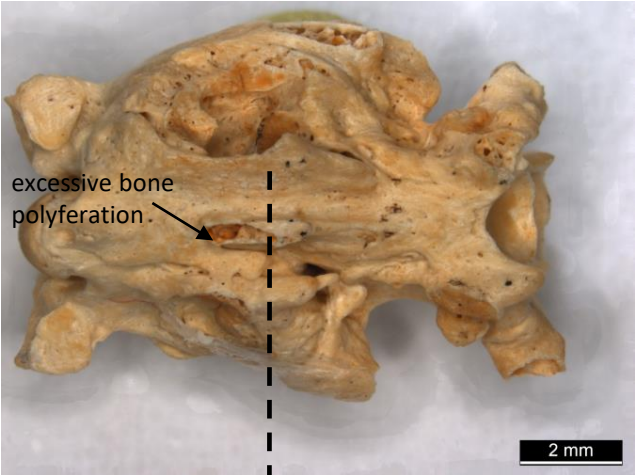


A healthy vertebra:

A vertebra from the middle part of the precaudal section is characterized by a strongly elongated vertebral center, a distinctly arched neural arch with a high spinous process.

Pathological vertebra:

Two fused precaudal vertebrae. Bearing signs of an advanced stage of osteitis deformans. Spinal segmentation disorder is accompanied by signs of severe pathological involvement of the periosteal part of the bone. This is characterized by deformations associated with excessive pathological growth of highly porous bone tissue (so-called felt bone).

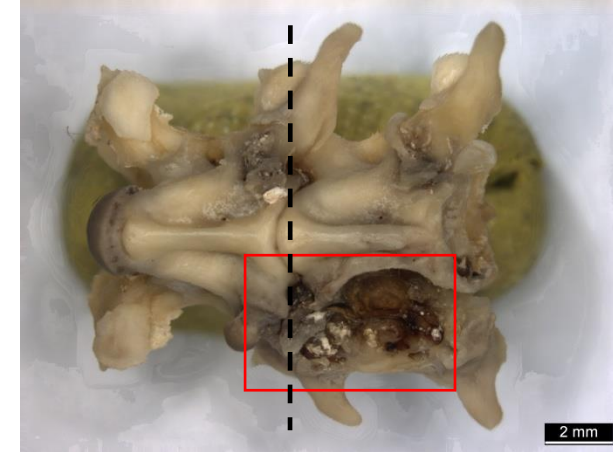


Osteitis deformans

Elaphe guttata



Red snake (*Elaphe guttata*) – captive



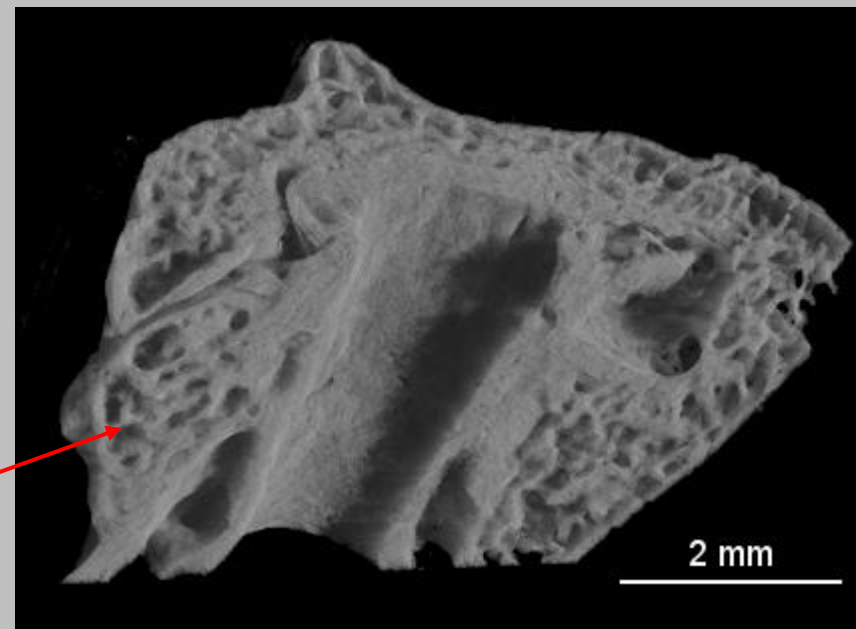
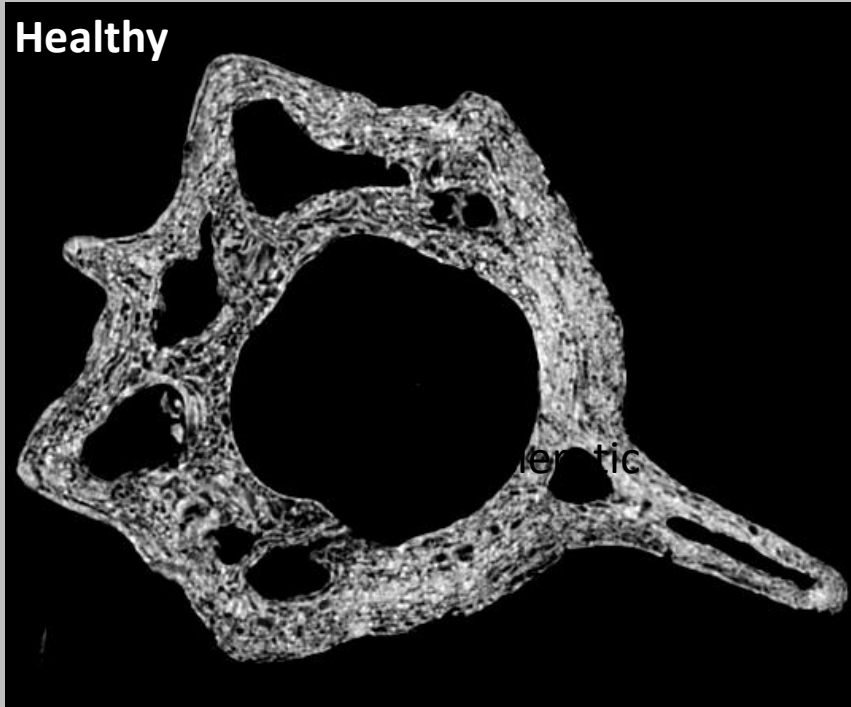
Healthy vertebra

1 vertebra from the middle part of the trunk compartment. The transverse section is made through the center of the prezygapophyses and zygosphenes. The ventro-lateral section is guided by the region of the synapophyses.

A pathological vertebra

2 fused vertebrae from the back of the trunk compartment. A transverse incision is made near the caudal edge of the vertebra at the postzygapophyses. Laterally, the pathologically affected prezygapophysis of the following vertebra is captured in the section.

Matrix Matrix



Different stages of osteitis deformans (Paget's disease): 1 – resorption phase (osteolytic phase); 3 - mix of osteolytic and osteoblastic phase;

3 – osteosclerotic phase.

Solution analysis by ICP-MS



ICP-MS spectrometer - Agilent 7500 CE (Agilent, Japan)



Contents [mg/kg]	Fossil vertebrae		Recent vertebrae	
	Pathological	Healthy	Pathological	Healthy
Mg	1226.71	1025.45	3377.94	3539.08
Al	3399.84	1798.90	33.12	64.20
Na	2466.61	2113.27	5533.81	628.10
Si	5287.27	1652.69	137.05	159.68
P	204503.11	209780.44	171565.84	173914.39
Ca	331288.82	338822.36	266298.93	269602.98
Fe	2812.89	1495.51	~	136.29
Zn	1177.80	1212.57	970.82	632.75
Sr	661.72	597.80	139.61	151.67
Ba	319.10	281.74	65.87	44.08

Measured elements and experimental conditions

Ca (II) 396,85 nm, Ca (I) 452,69 nm, P (I) 253,56 nm

Mg (II) 279,55 nm, 280,27 nm, Mg (I) 285,21 nm

Sr (I) 460,73 nm, Sr (II) 407,77 nm

Fe (I) 302,40 nm, Ba (II) 455,40 nm, Al (I) 396,15 nm, Zn (I) 334,50 nm,

Si (II) 288,75 nm



laser wavelength 266 nm + 1064 nm



energy 10 mJ/pulse and 90 mJ/pulse



pulse length 5 ns



grid 2400 vr./mm



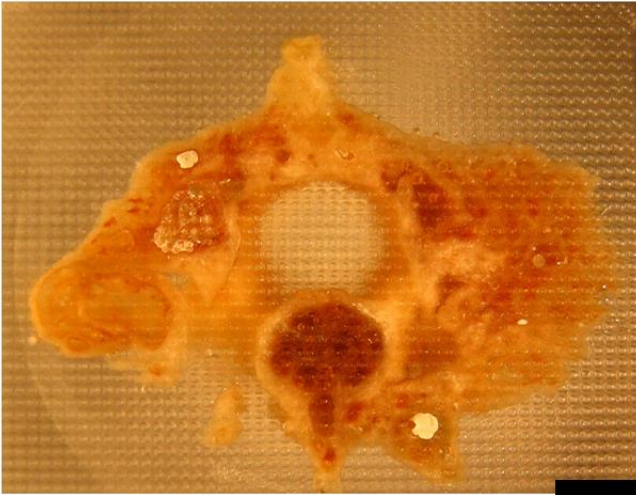
delay 1 μ s, integration time 10 μ s, time between pulses 500 ns, entrance slit 50 μ m



distance of ablation craters 150 μ m (x, y)

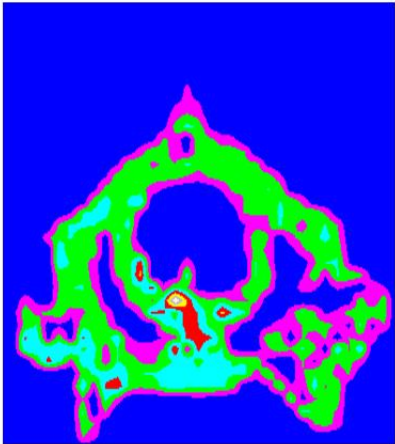
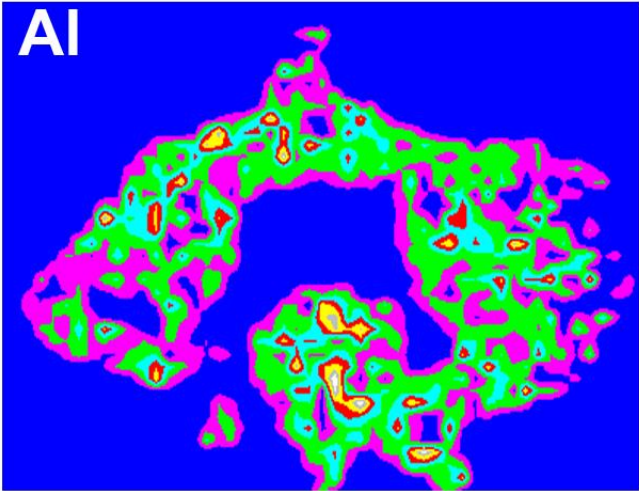
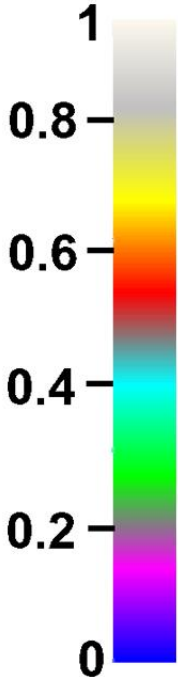
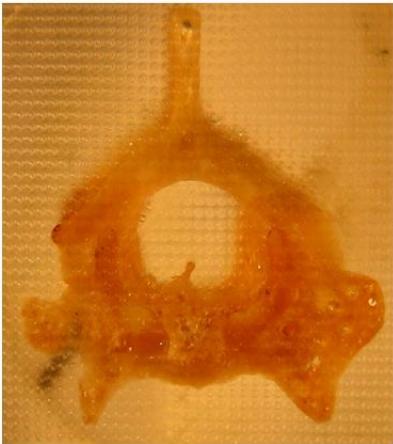
Matrix matrix – distribution of AI

Pathological



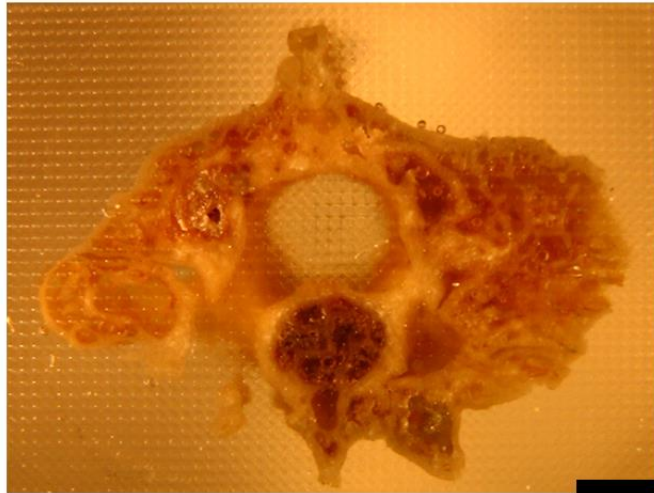
1 mm

Healthy



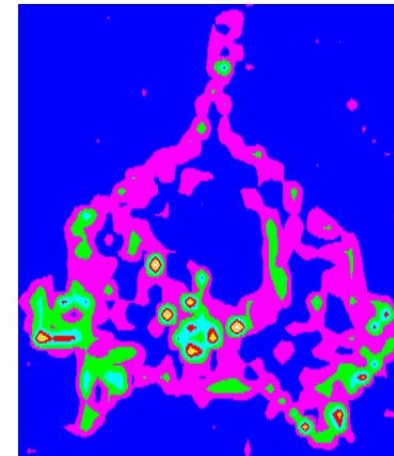
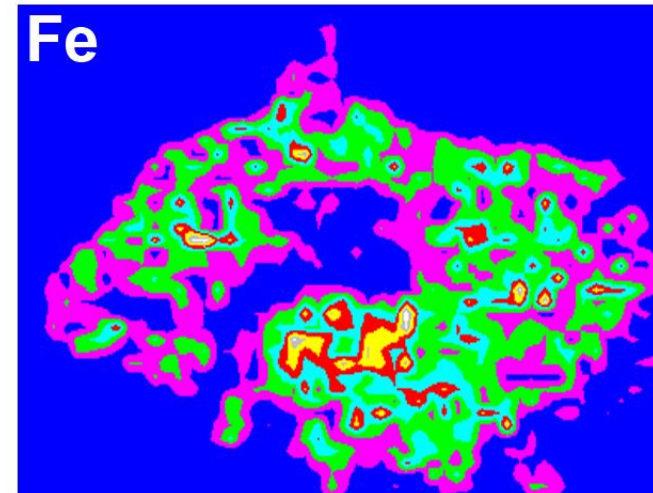
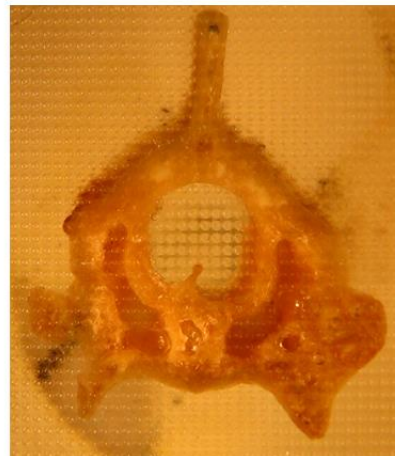
Matrix matrix – distribution of Fe

Pathological

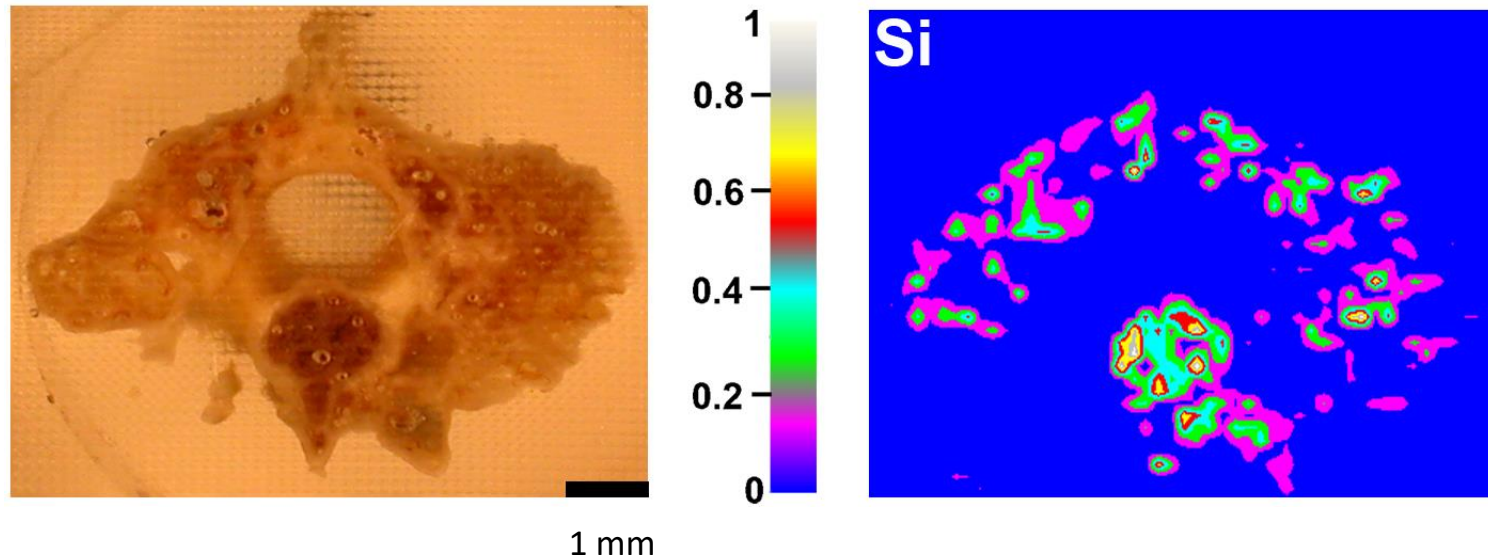


1 mm

Healthy



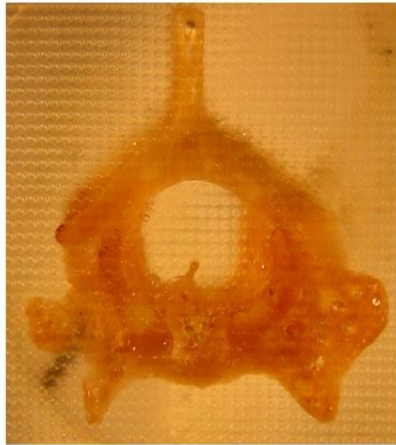
Pathological



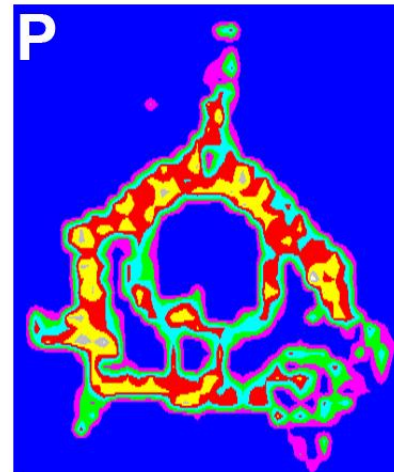
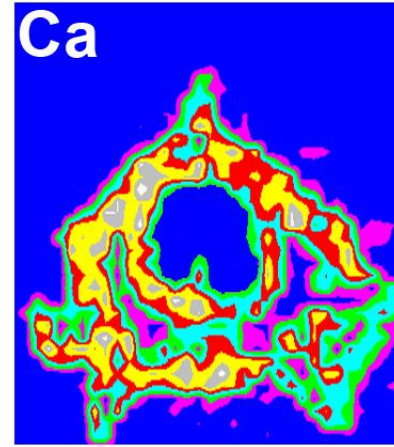
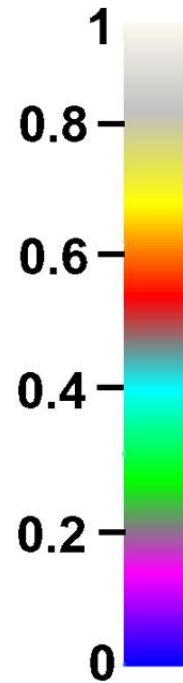
- High content of trace elements (Al, Fe, Si) observed in free places of bone tissue. Their presence does not contribute to the structure of the pathological vertebra.
- Increased contents caused due to migration of elements during **diagenesis**.

Matrix matrix – distribution of matrix elements(Ca, P)

Healthy

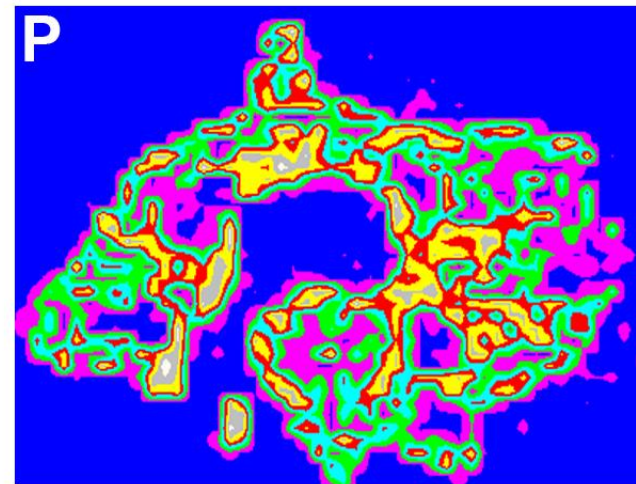
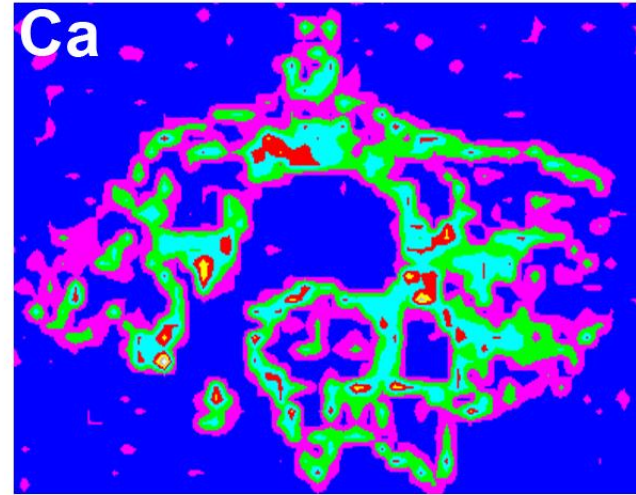
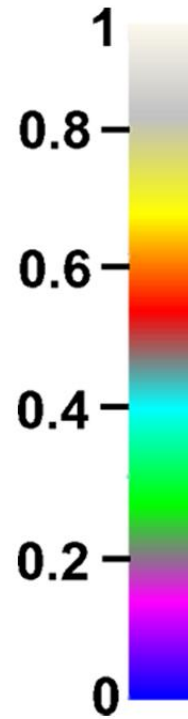
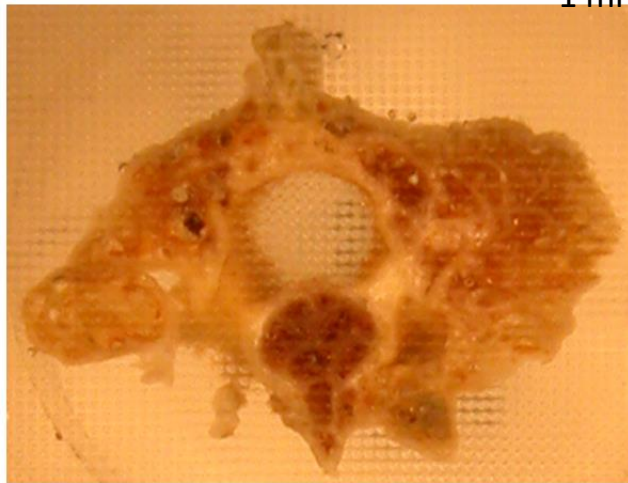
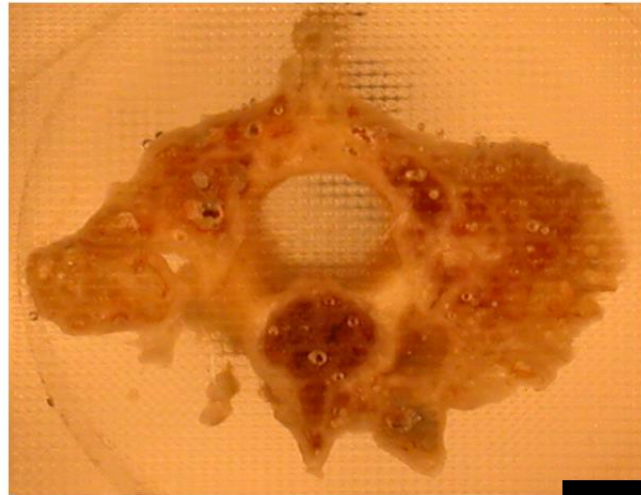


1 mm



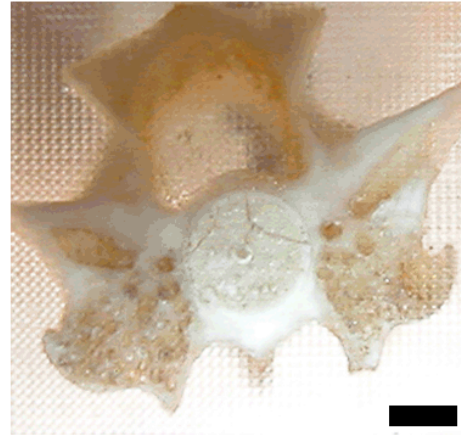
Matrix matrix – distribution of matrix elements(Ca, P)

Pathological

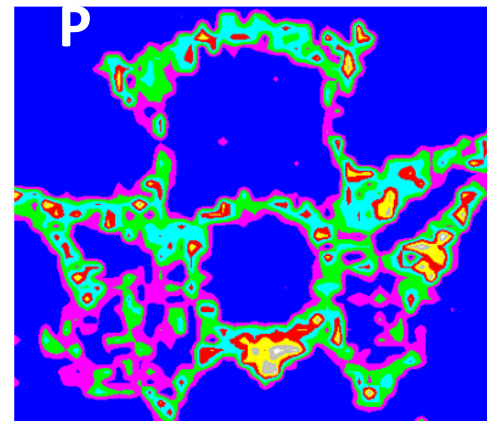
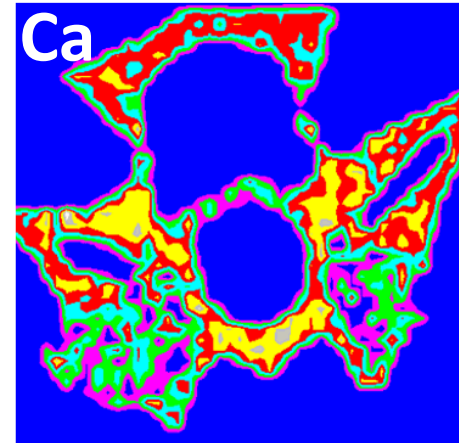
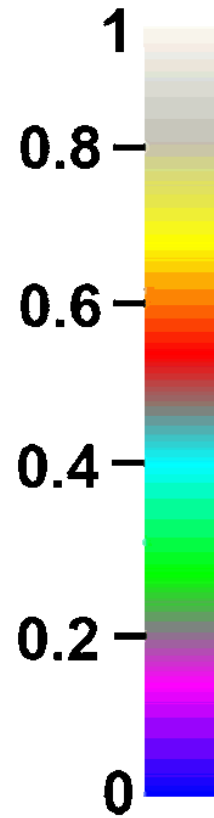
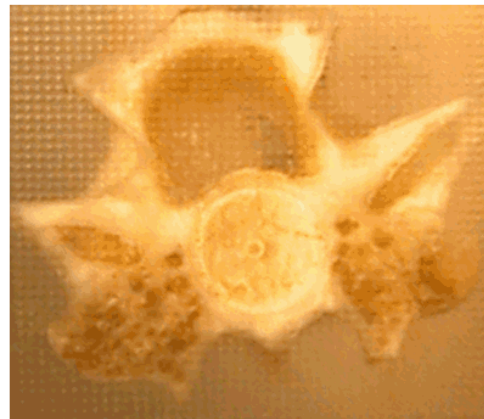


Elaphe guttata – distribution of matrix elements (Ca, P)

Healthy

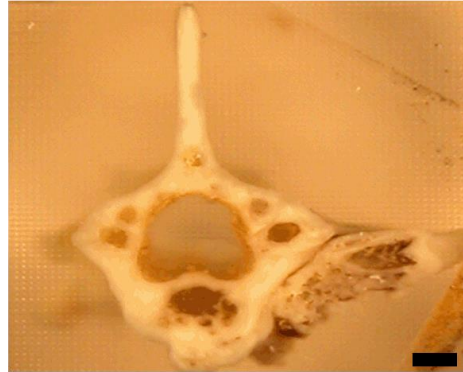


1 mm

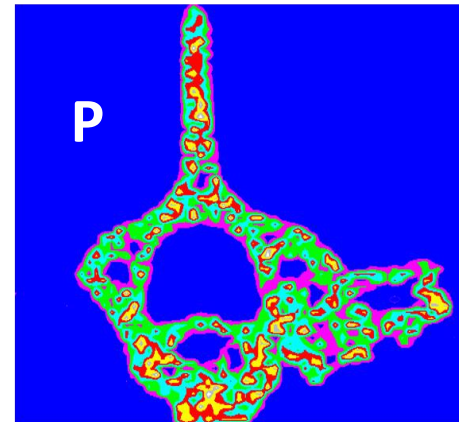
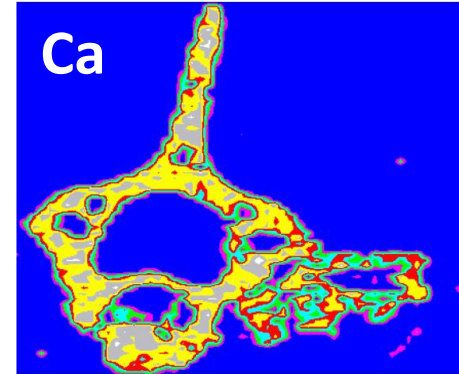
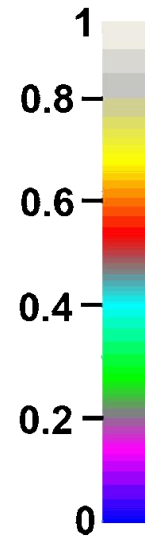
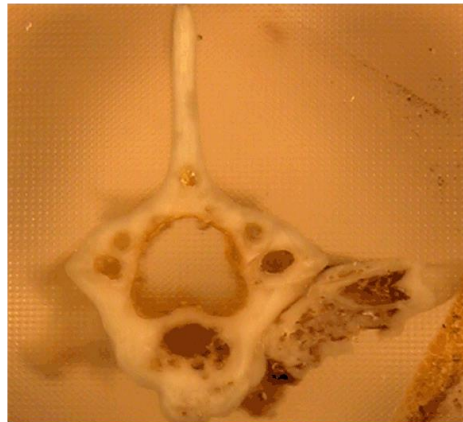


Elaphe guttata – distribution of matrix elements (Ca, P)

Pathological



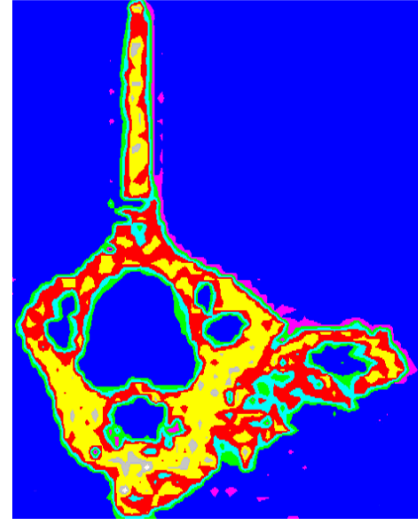
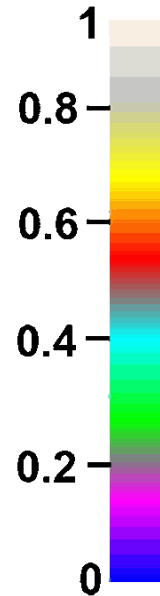
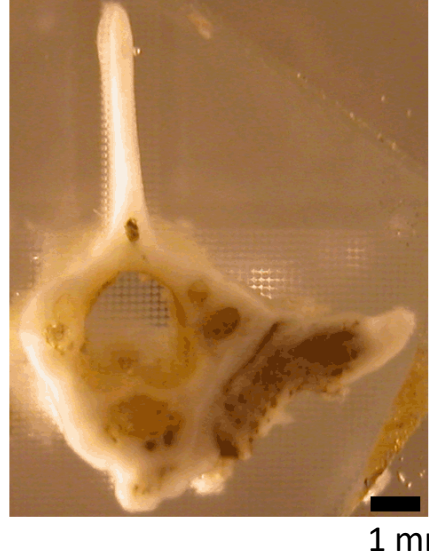
1 mm



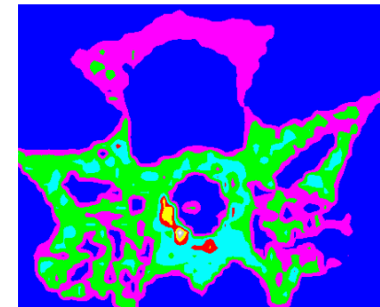
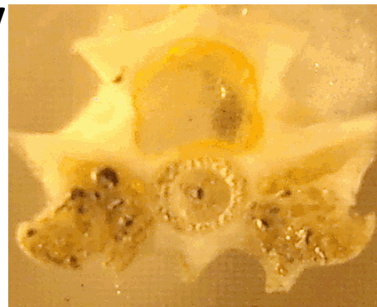
A high Ca/P ratio in both healthy and diseased vertebra indicates the initial phase (resorption) of the disease.

Elaphe guttata – distribution of Na

Pathological



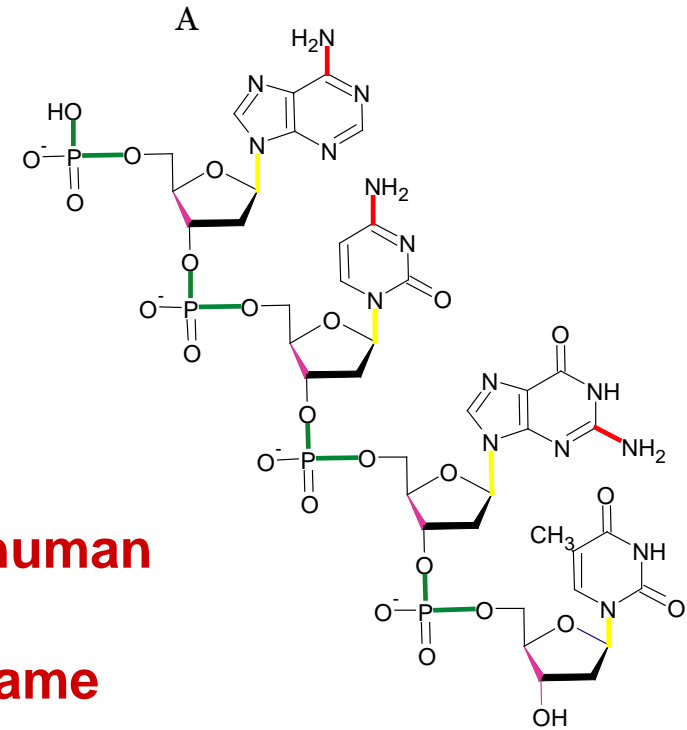
Healthy



The increased Na content in the pathological vertebra is due to increased K-Na channel activation as a result of inflammation.

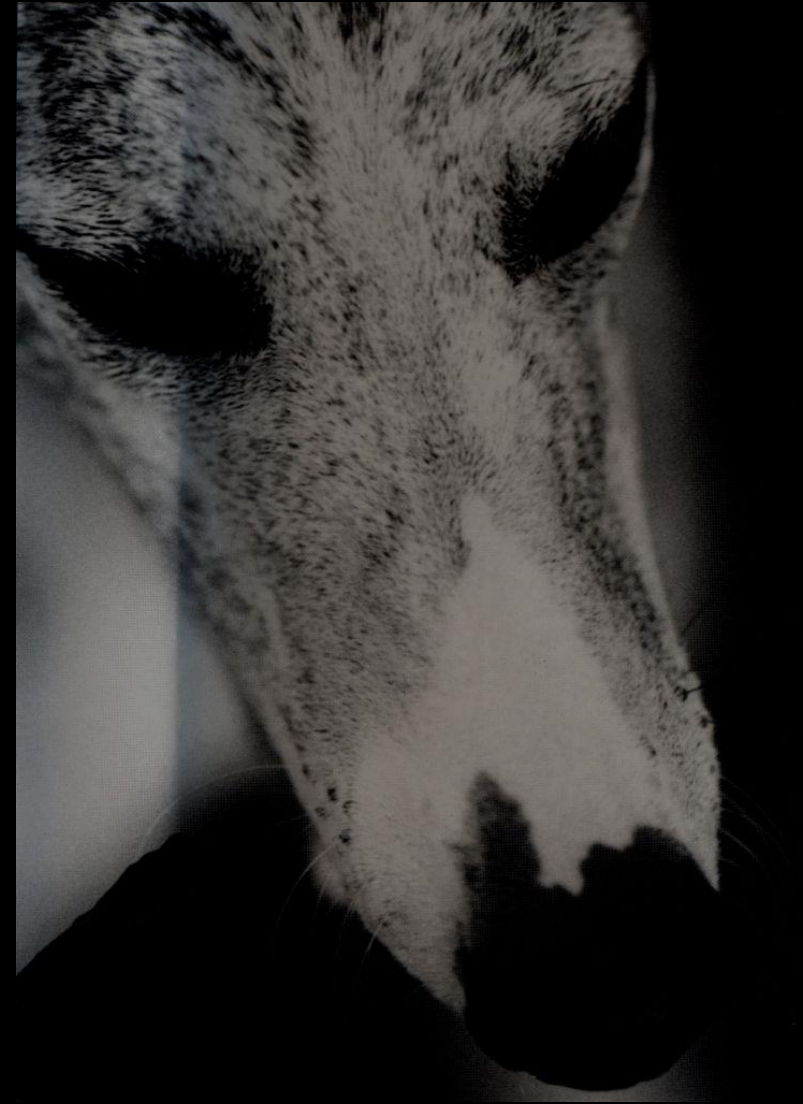
Archaeogenetics

- it emerged as a new field in 1990 first she focused on human DNA,
- it first focused on human DNA, in recent years she became interested in animal and plant archaeological material,
- breeds, sexes, crosses with wild species,
- the movement of domestic and wild animals can be determined from population genetics,
- from DNA analysis it is possible to determine the migrations of individual pieces of animals and thereby determine the trade and migration routes of animals and people



DNA The story of the greyhound from Chotěbuz-Podobora

- It transmits hereditary information
- Unchangeable throughout life
- Relatively stable
- In all nucleated cells

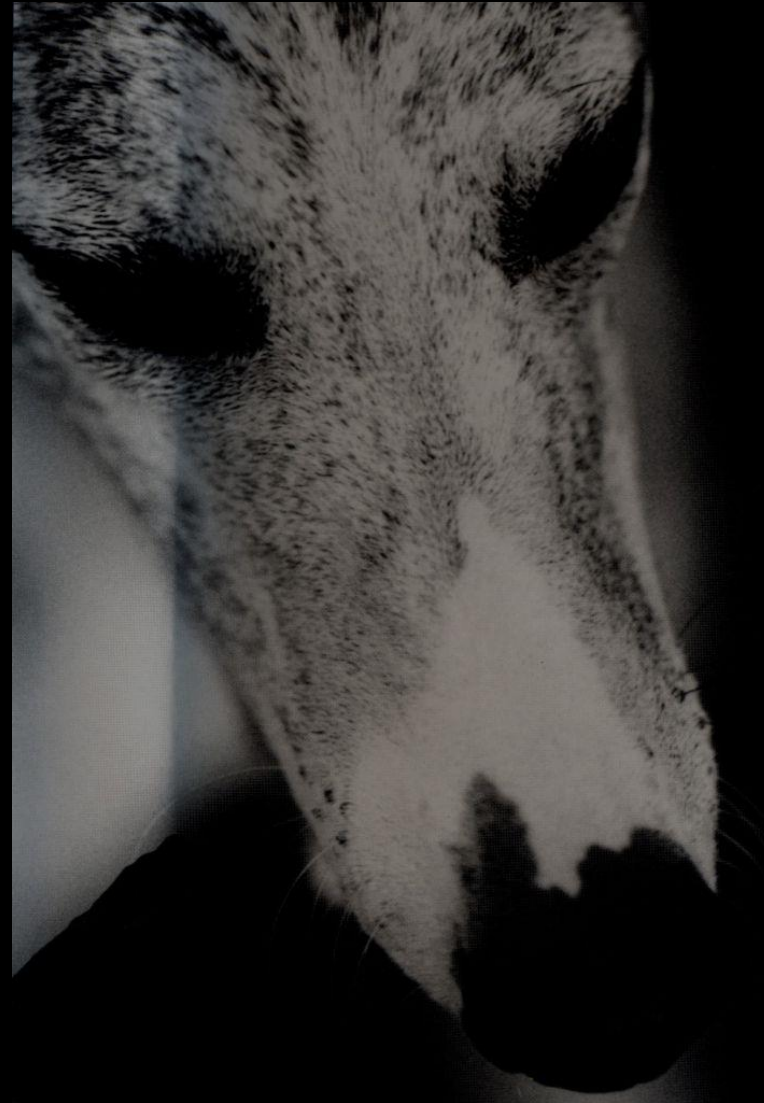


DNA SOURCE

- Nuclear DNA (nDNA)
- double chain (ds)linear
- Chromosome
- 2 copies in a cell from father and mother
- protected by nuclear membrane (? sufficiency)
- Mitochondrial DNA (mt DNA)
- double-strandedcircular
- in the mitochondria organelles inherited through the maternal line
- many copies
- smaller and more stable structure

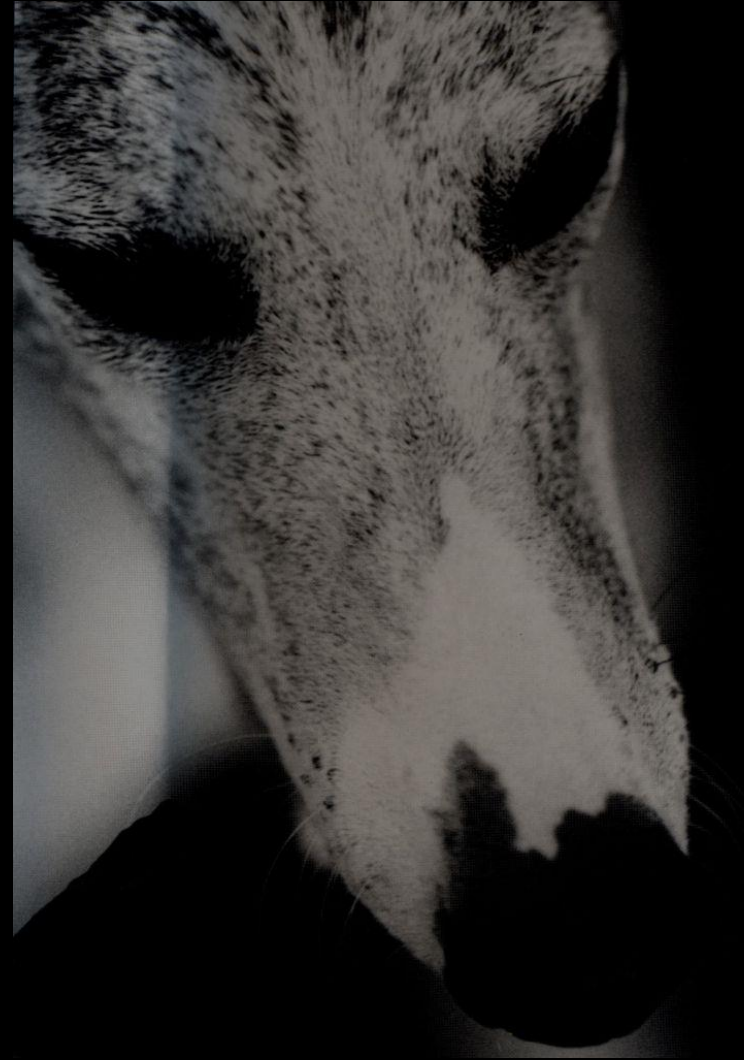
mt DNA

- passed from mother to offspring
- 2 strings: light (L) and heavy (H) hypervariable region, the so-called D-loop
- single point mutations (single nucleotide polymorphisms – SNP)
- specific for individual breeds of dogs
- length: 16727 bp (NC_002008) in the range 15458..16727 D-loop



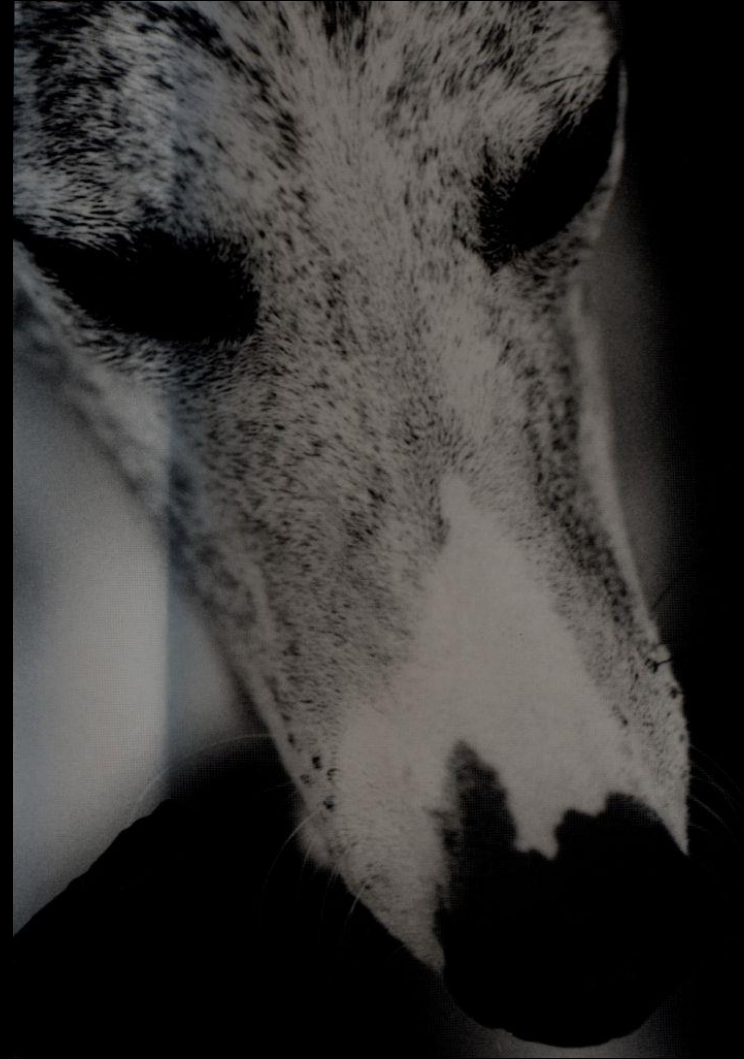
PROCEDURE

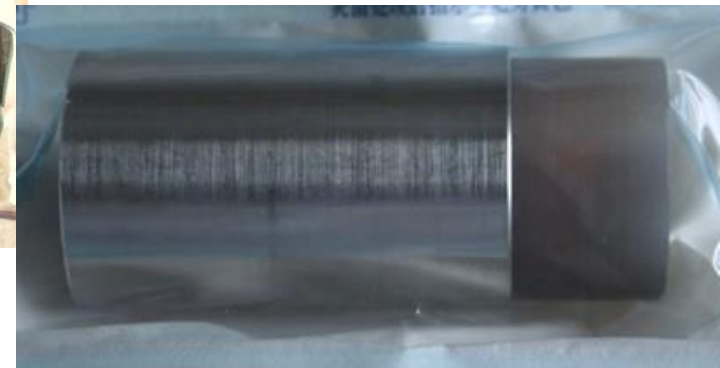
- MATERIAL EXAMINED
- part of the bone
- COMPARATIVE MATERIAL
- recent greyhound imported from Great Britain racing greyhound
- verifiable ancestor from 1840
- Buccal (mouth) swab taken



Bone

- mechanical and chemical cleaning
- Finesandpaper
- ethanolcutting
- bone oscillating
- sawcrushing the cut slices
- hydraulic press





Bone

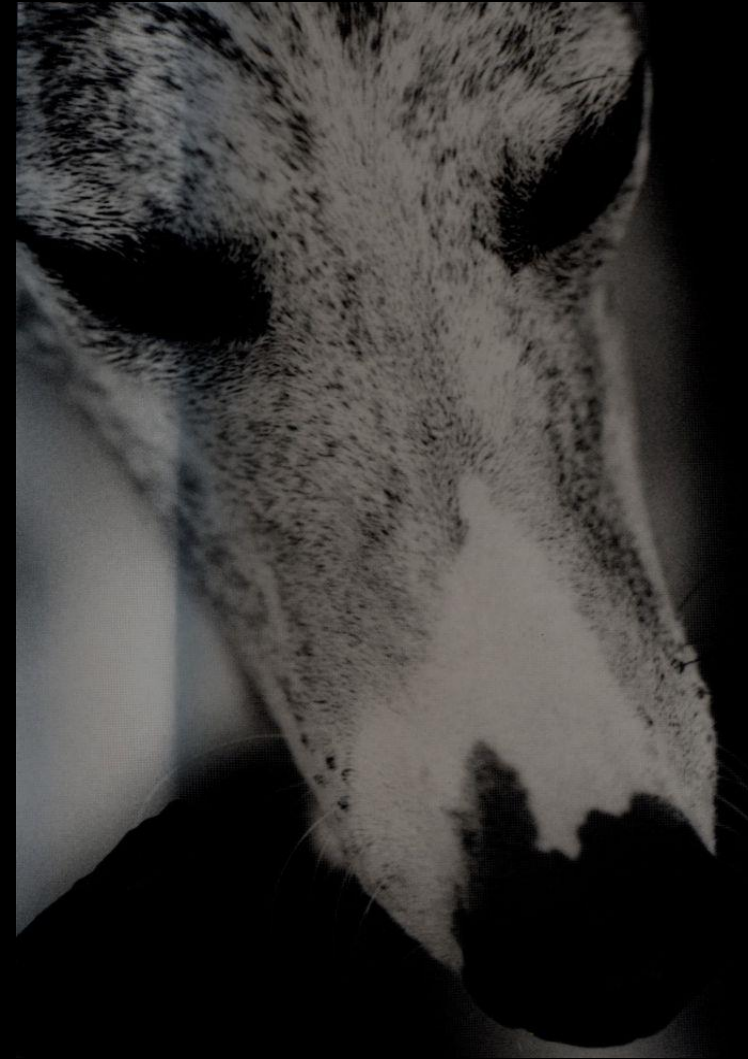
- Grind
- Retsch grinder and agate balls in a roller
- Final weight of bone meal
2.47 g





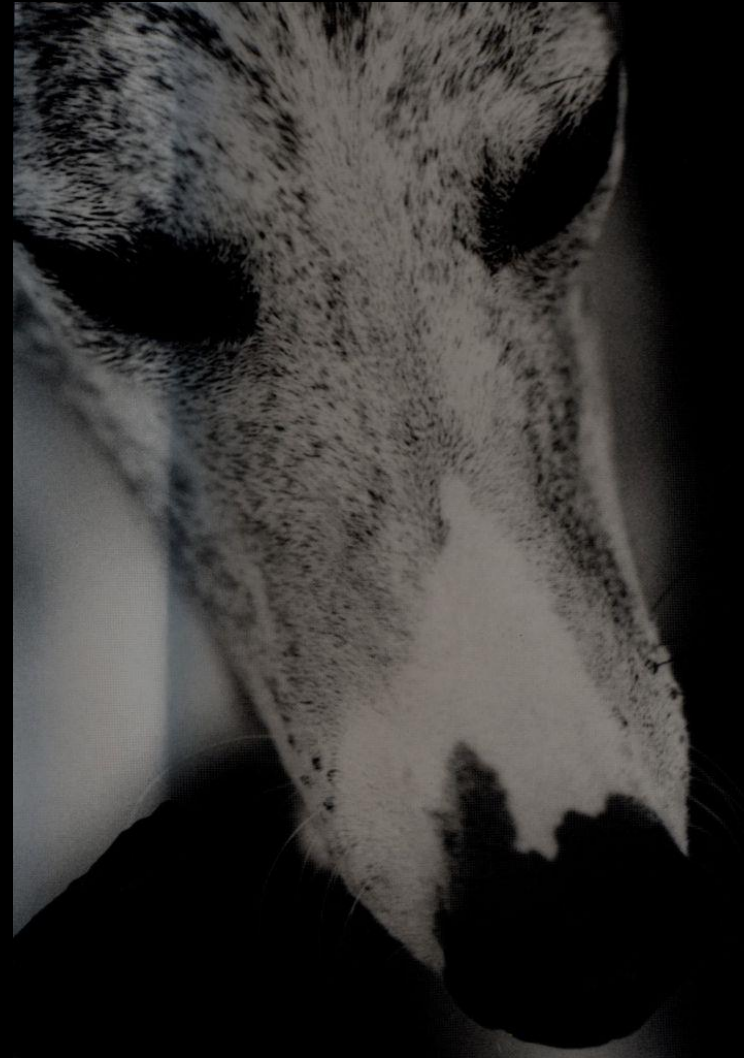
Bone

- Removal of excess calcium
- EDTA solution (pH 8) with a volume of 40 ml
- Incubate at 4 °C for at least 24 hours
- After the specified time, the Exchange centrifugation
- EDTA (ethylenediaminetetraacetic acid)+SARCO (pH 8)
- Final pellet at the bottom of the 50 ml test tube



COMPARATIVE SAMPLE

- buccal smear of oral cavity cells
- insulation with a 5% Chelex solution
- separation of two phases:
- aqueous with DNA chelex with cellular debris
- final volume of isolate 200 μ l



PCR (Polymerase Chain Reaction)

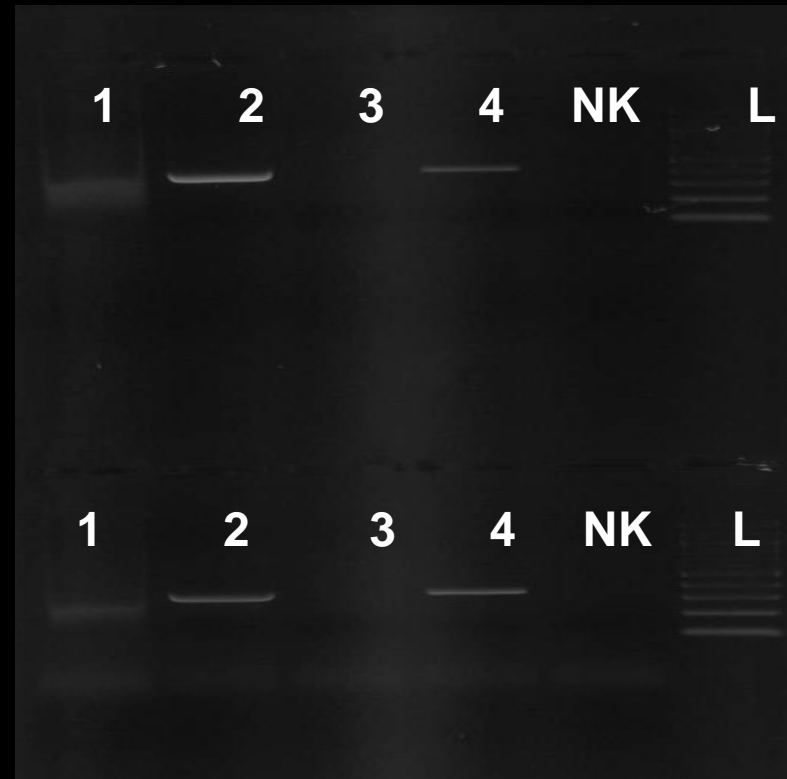
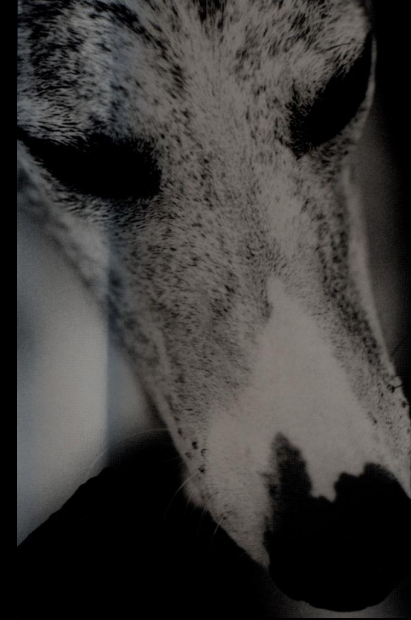
- used to multiply DNA
- Primers – definition of the multiplied section
- reaction optimization – gradient

- 2 primer pairs for the region in the D-loop:
 - F15719 + R16114
 - F16431 + R42
- Publication: Rebekah L. Gundry et al., Mitochondrial DNA Analysis of the Domestic Dog: Control Region Variation Within and Among Breeds, J Forensic Sci, May 2007, Vol. 52, No. 3



PCR products

- Reaction success verified using electrophoresis (agarose gel)



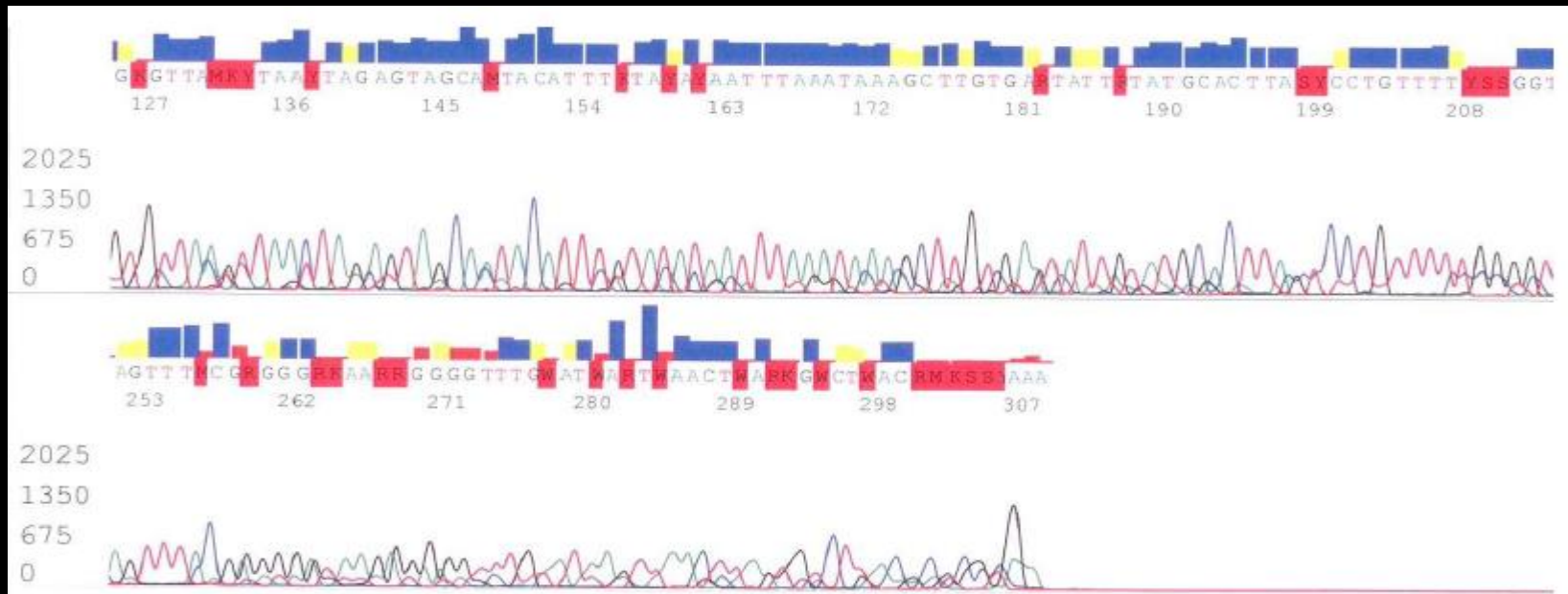
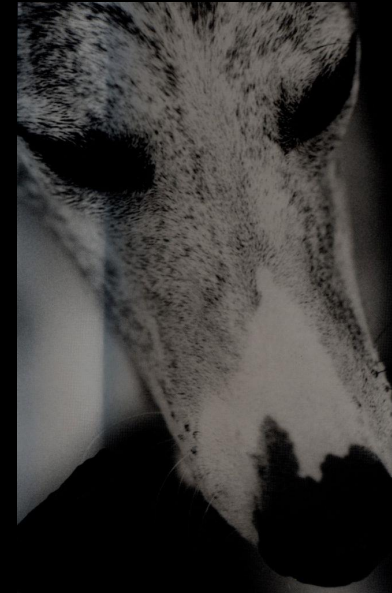
pair of primer pair A

primer pair B

1, 3 = undiluted DNA samples
2, 4 = 10x diluted DNA samples
NK = negative contro
IL = 100bp ladder

SEQUENCE

- reading the amplified section written letter by letter (nucleotide by nucleotide)
- Registration:



FINÁLNÍ SEKVENCE

- F 15719 + R16114

```
CCTGGCATCT GGTTCCTACT TCAGGGGCAT AACTTTATTT ACTCCAATCC TACTAATTCT
CGCAAATGGG ACATCTCGAT GGACTAATGA CTAATCAGCC CATGATCACA CATAACTGTG
GTGTCATGCA TCTGGTATCT TTTAATTTT AGGGGGGAA TCTGCTATCA CTCATCTACG
ACCGCAACGG CACTAACTCT AACTTATCTT CTGCTCTCAG GGATATGCC CGTCGCGGGCC
CTAATGCAGT CAATAACTT GTAGCTGGAC TTATTCATTA TCATTTATCA ACTCACGCAT
AAATCAAGG TG
```



```
Query 1 CTTTATTTACTCCAATCCTACTAATTCTC GCAAATGGGACATCTCGATGGACTAATGACT 60
|||||
Sbict 1 CTTTATTTACTCCAATCCTACTAATTCTC GCAAATGGGACATCTCGATGGACTAATGACT 60

Query 61 AATCAGCCC ATGATCACAC ATAAC TGTGGTGT CATGCATCTGGTATCTTTAATTTTAA 120
|||||
Sbict 61 AATCAGCCC ATGATCACAC ATAAC TGTGGTGT CATGCATCTGGTATCTTTAATTTTAA 120

Query 121 ggggggAATCTGCTATCACTCATCTACGACCGCAACGGC ACTAACTCTAACTTATCTTCT 180
|||||
Sbict 121 GGGGGGAATCTGCTATCAC TCATCTACGACCGCAACGGC ACTAACTCTAACTTATCTTCT 180

Query 181 GCTCTCAGGGAATATGCCC GTCGC GGCCCTAATGCAGTC AAATAACTTGTAGCTGGACTT 240
|||||
Sbict 181 GCTCTCAGGGAATATGCCC GTCGC GGCCCTAATGCAGTC AAATAACTTGTAGCTGGACTT 240

Query 241 ATTCATTATCATTTATCAACTCAC GCATAAAATCAAGG 278
|||||
Sbict 241 ATTCATTATCATTTATCAACTCAC GCATAAAATCAAGG 278
```

FINÁLNÍ SEKVENCE

- F16431 + R42

```
TAAACTCATG TCATCTATTA
TACACTTATT TATGTCCCGC CAAACCCCAA AAACAGGACT AAGTGCATAC AATACTCACA
AGCTTTATTT AAATTATATA CAAATGTATT GCTACTCTAG TTAACCTAAC ACAACAGTCT
TACACGCATT TGGTCTCGTA GTCTATCTAT AGATAGCATT CCcttttttt Tccctctcat
|
ATTTACTATG TATTTTATTT ATTACGCACA CTCATTTC AGTATAA
```



Query	1	AACTCATGTCATCTATTATACACTTATTTATGTC CCGCCAAACC CCAAAAACAGGACTAA	60
Sbjct	3	AACTCATGTCATCTATTATACACTTATTTATGTC CCGCCAAACC CCAAAAACAGGACTAA	62
Query	61	GTGCATACAATACTCACAAGCTTTATTTAAATTATATACAAATGTATTGCTACTCTAGTT	120
Sbjct	63	GTGCATACAATACTCACAAGCTTTATTTAAATTATATACAAATGTATTGCTACTCTAGTT	122
Query	121	AACTTAACACAACAGTCTTACACGCATTTGGTCTCGTAGTCTATCTATAGATAGCATTCC	180
Sbjct	123	AACTTAACACAACAGTCTTACACGCATTTGGTCTCGTAGTCTATCTATAGATAGCATTCC	182
Query	181	CCCtttttttttCCTCTCAT-TT-ACTATGTATTTTATTTATTACGCACACTACAATTTTC	238
Sbjct	183	C--TTTTTTTCCCTCTCATATTTACTATGTATTTTATTTATTACGCACACTACAATTTTC	240
Query	239	AGTATAA 244	
Sbjct	241	AGTATA 246	

„Stories“

**Seasonality, paleoenvironment
and animal migration in the
Gravettian period**

Season of death

Summer increment

Winter increment



Principle of dental cementum increment analysis



Lubná I

Mamutowa cave

Krakow-Spadzista

Deszczowa cave

Ostrava-Petřkovice

Přerov-Předmostí

Spythněv

Jarošov II

Boršice

Dolní Věstonice-Pavlov

Milovice

Trenčianské Bohuslavice

Stiefried-Grub/Kranawetberg

Krems-Wachtberg

Moravany II-Lopata

Krems-Hundssteig

Willendorf II

Seasonality of Gravettian locality



Mammoth migration

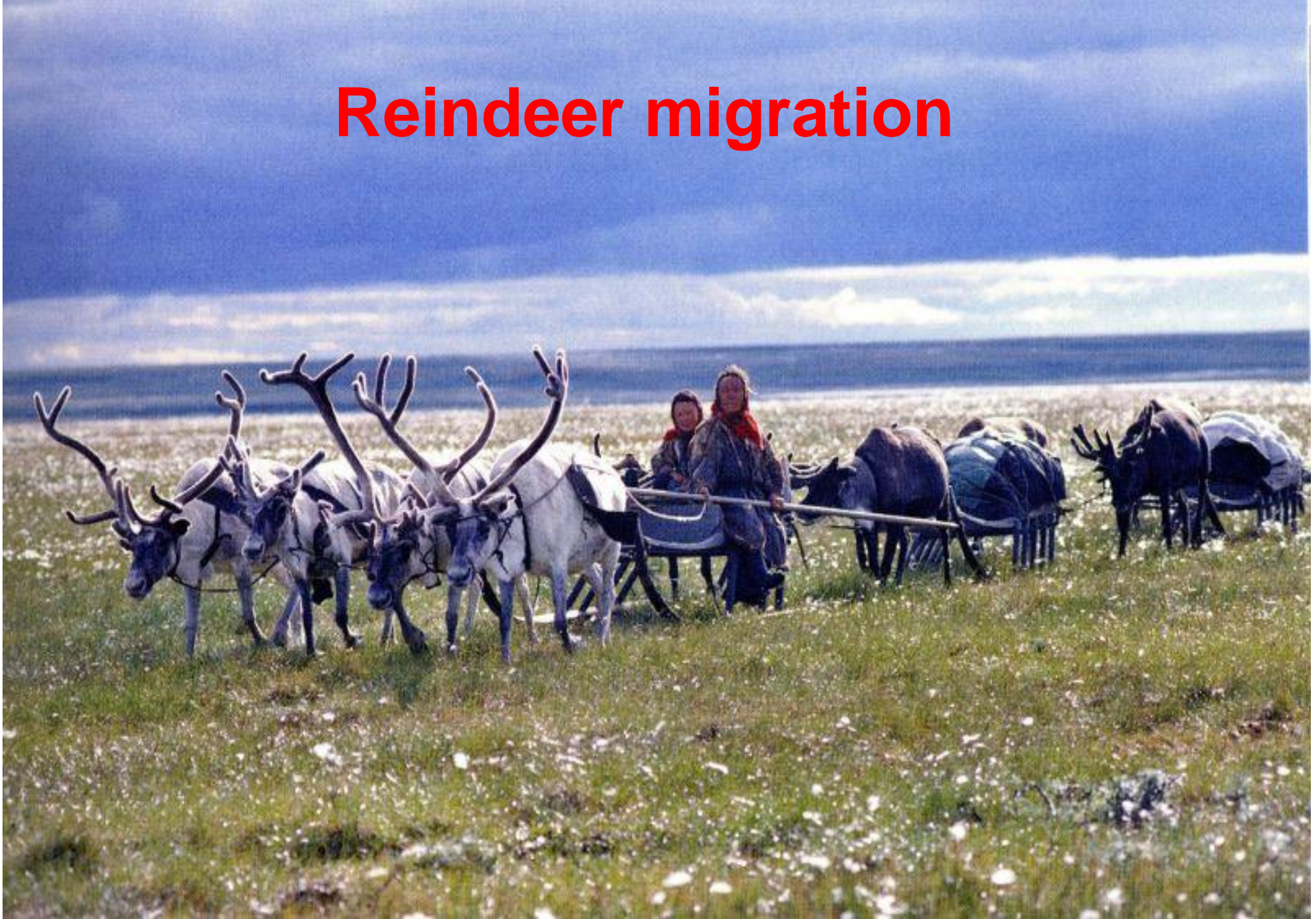




Mammoth migration

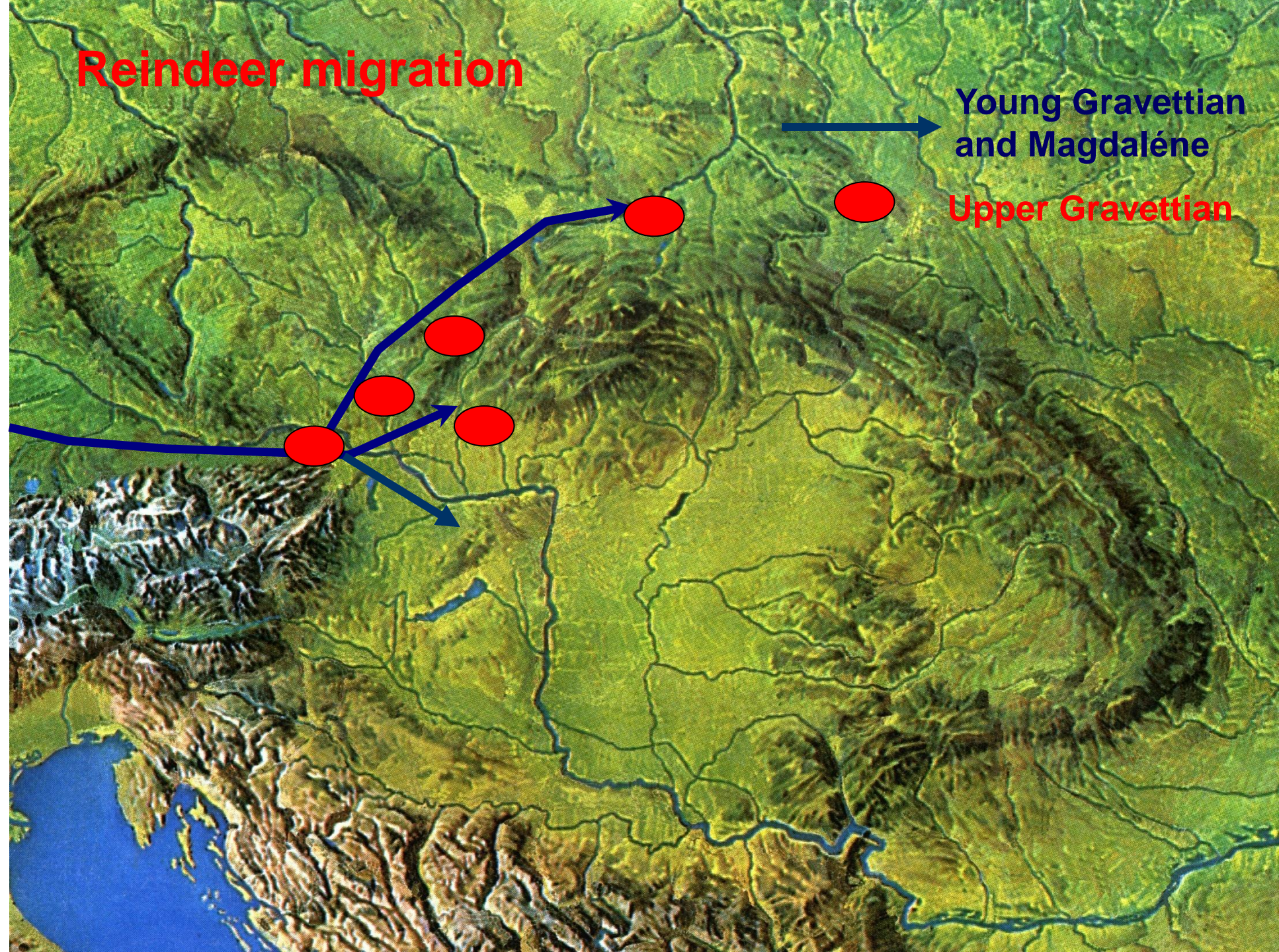


Reindeer migration





Reindeer migration

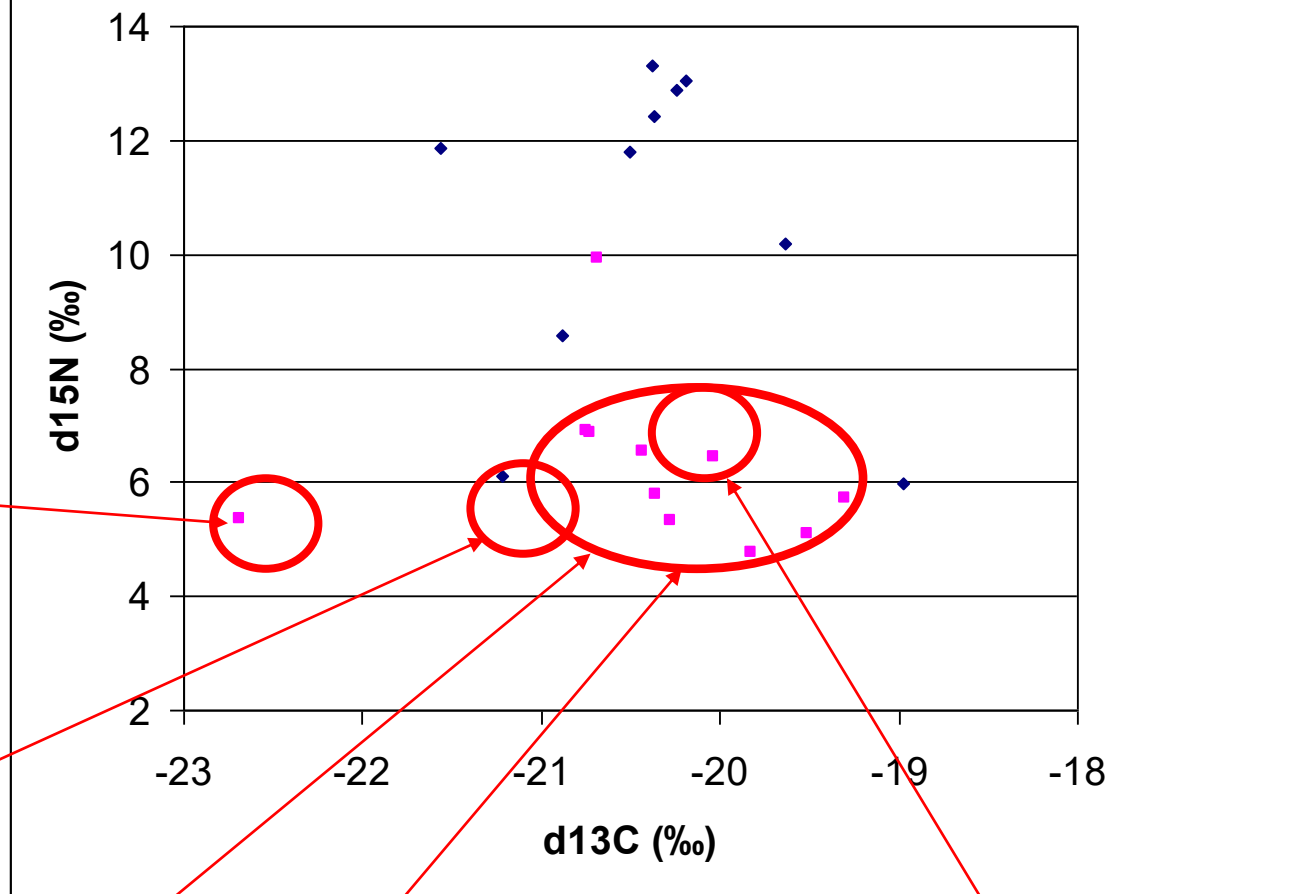
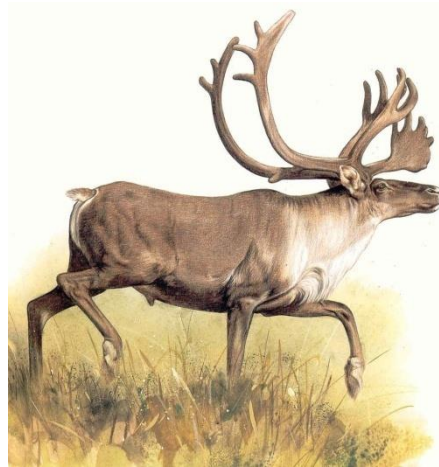
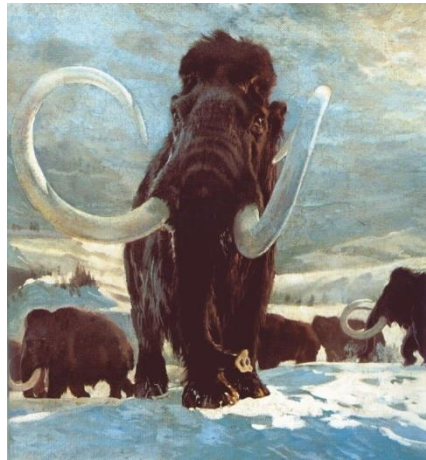


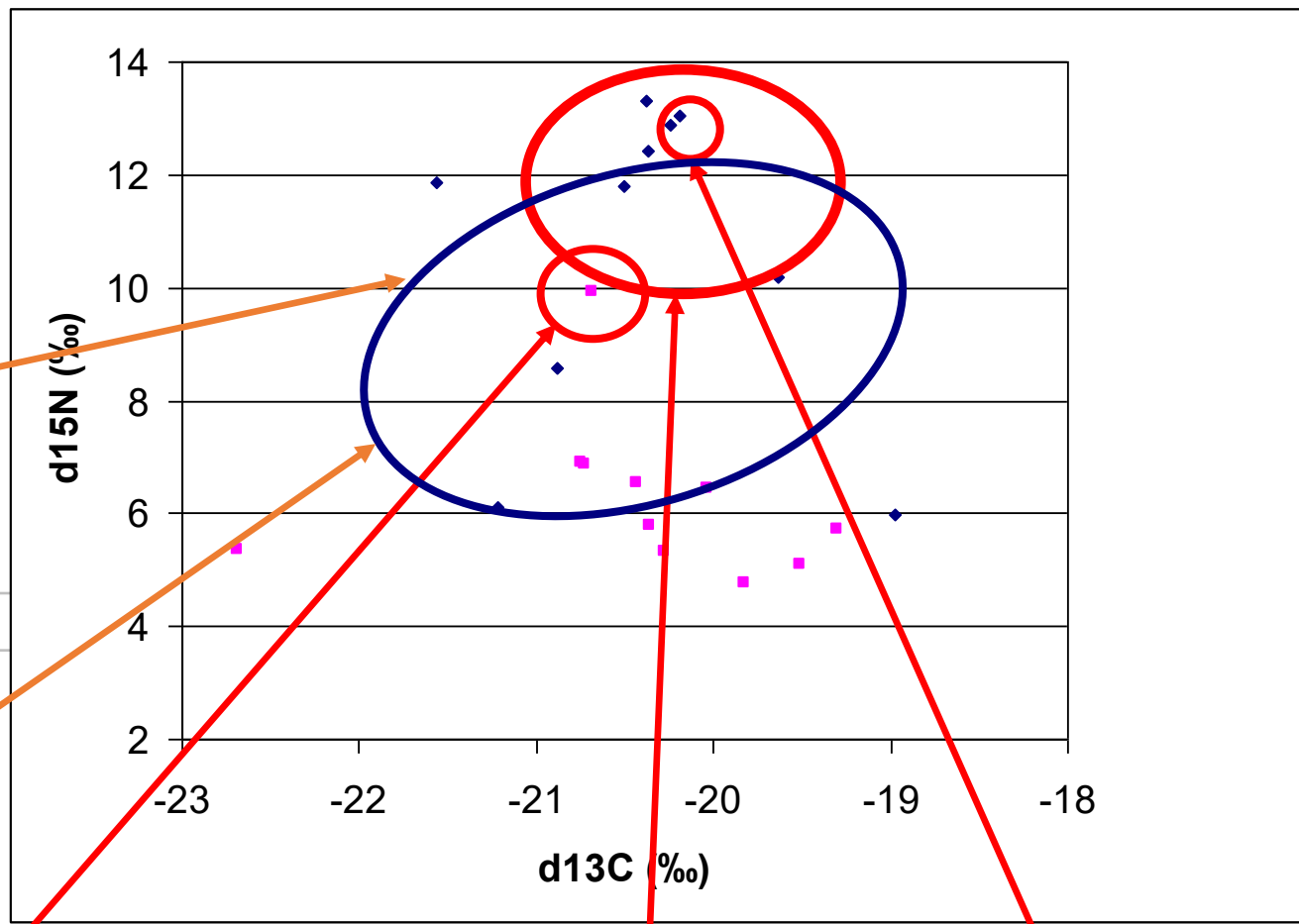
Young Gravettian
and Magdaléne

Upper Gravettian

Animal nutrition







Palaeocology



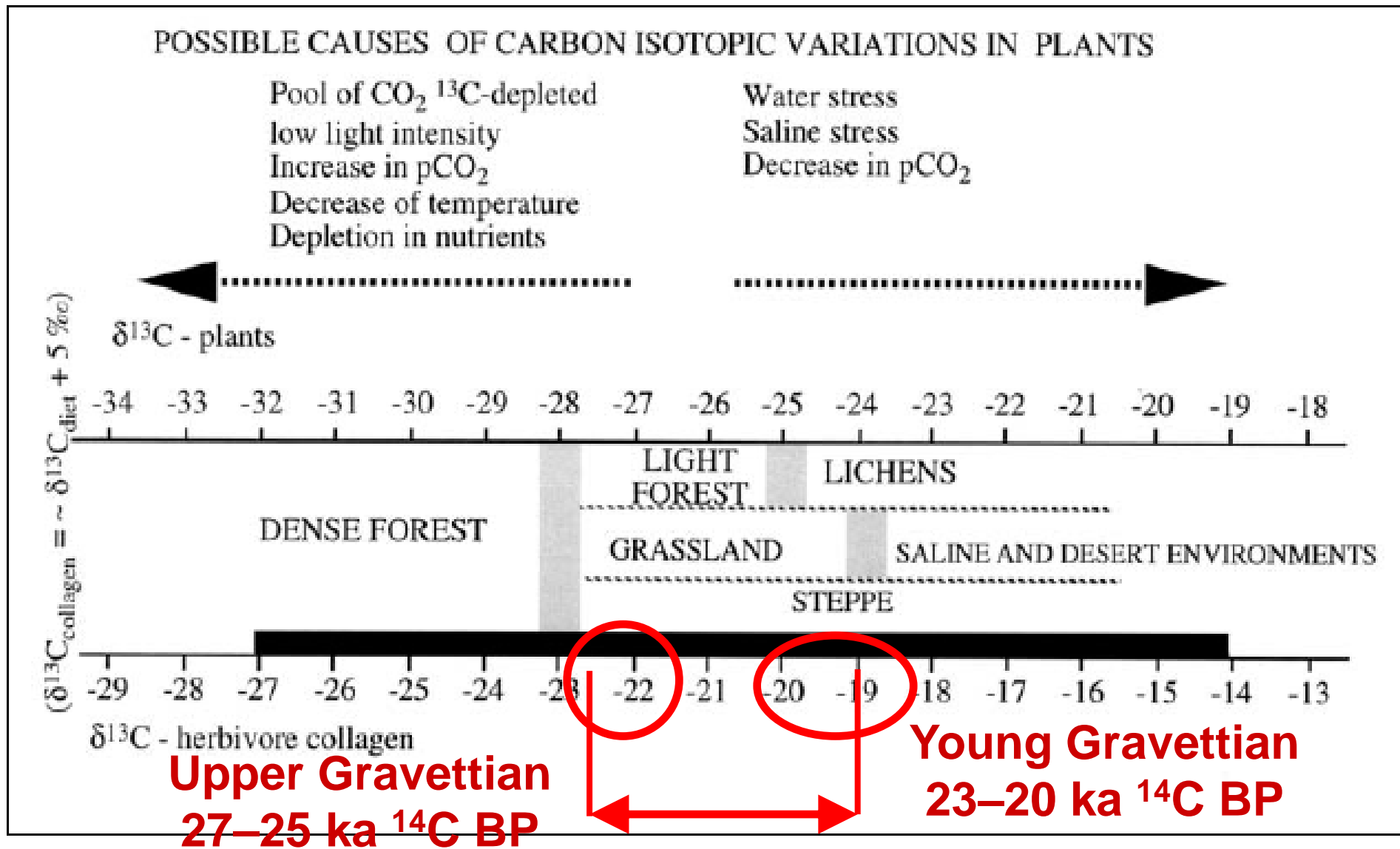


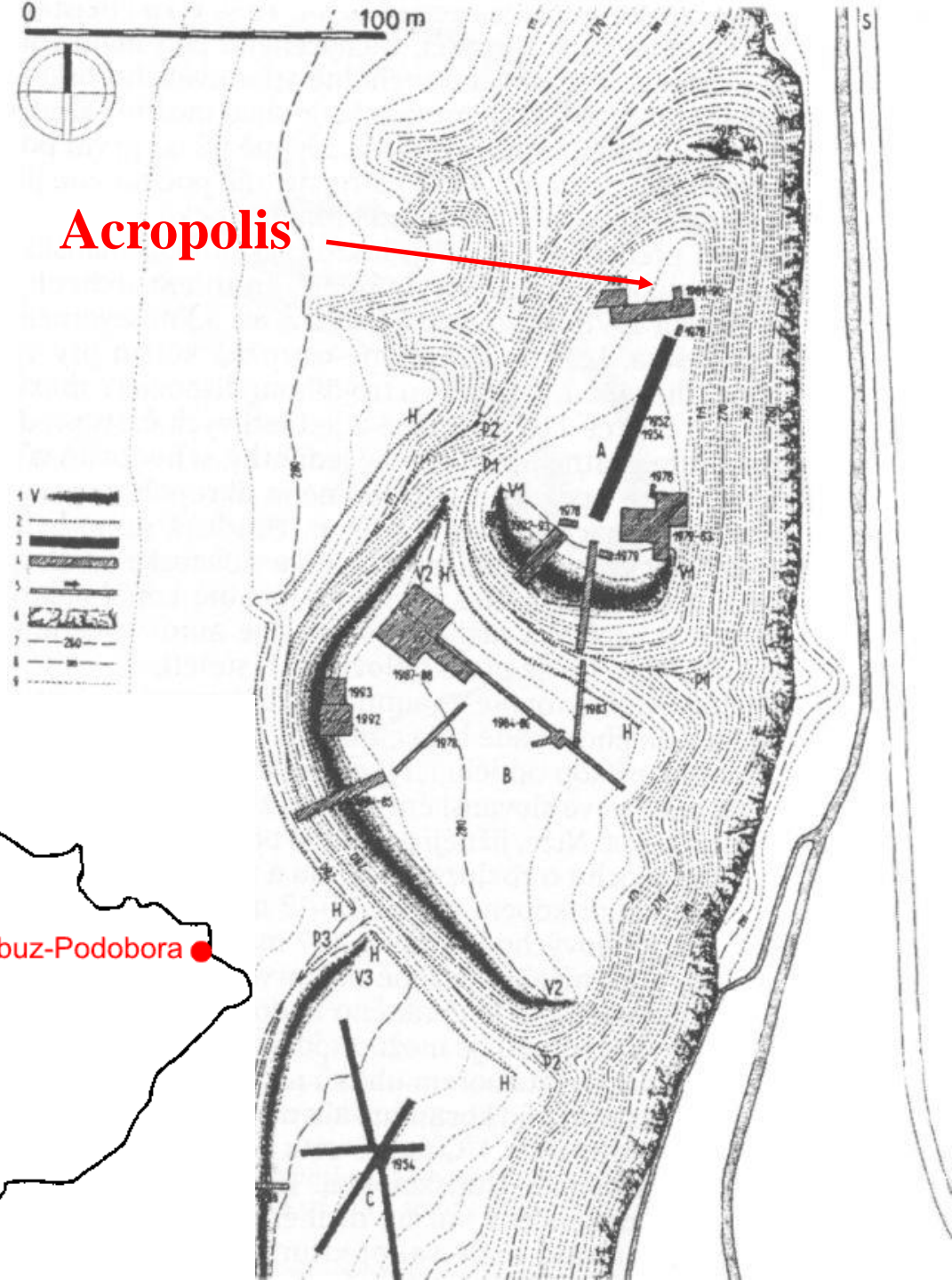
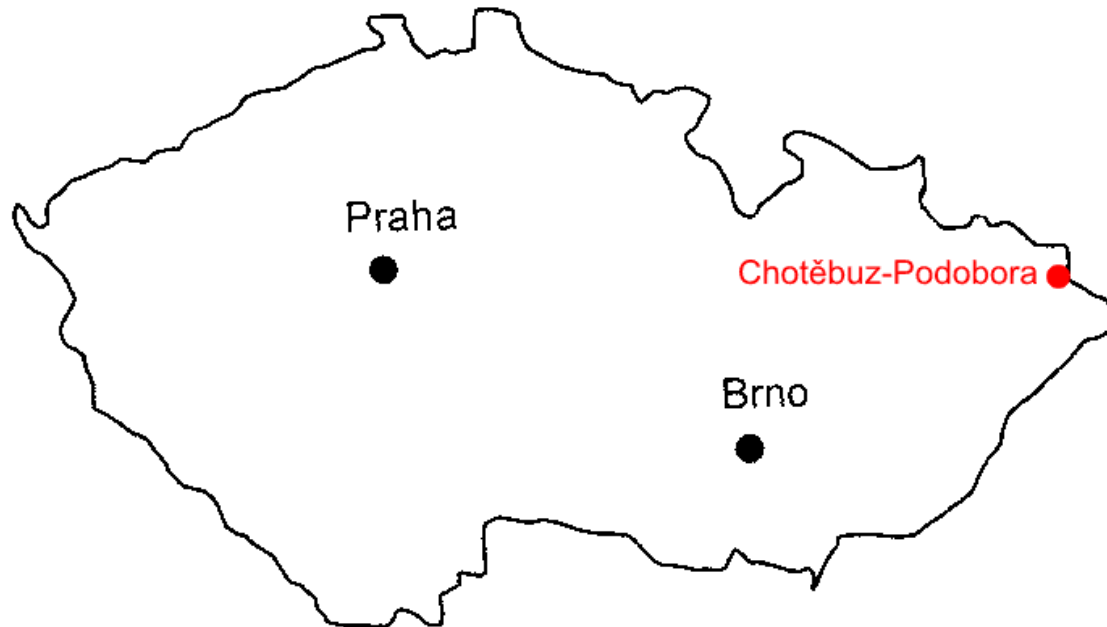
Figure 1 Range of herbivore collagen $\delta^{13}\text{C}$ values measured in modern arctic, temperate and steppic areas, with possible causes of carbon isotopic variations in plants and herbivores. Values are compiled from Nelson et al. (1986), Bocherens et al. (1994, 1996, 2000), Rodière et al. (1996).

**Nutrition, migration and human paleoekology from Chotěbuz locality
(Hallstatt)**

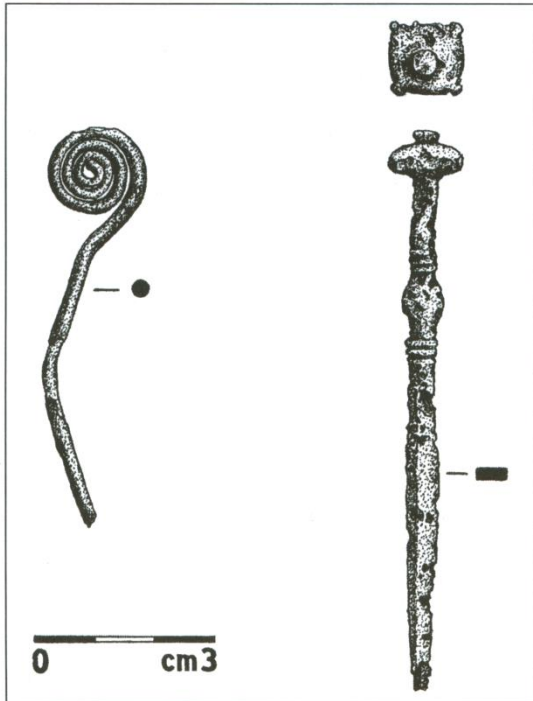
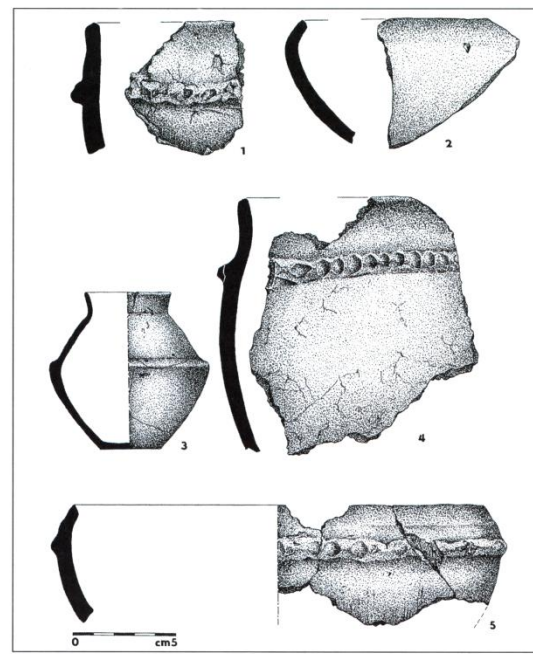
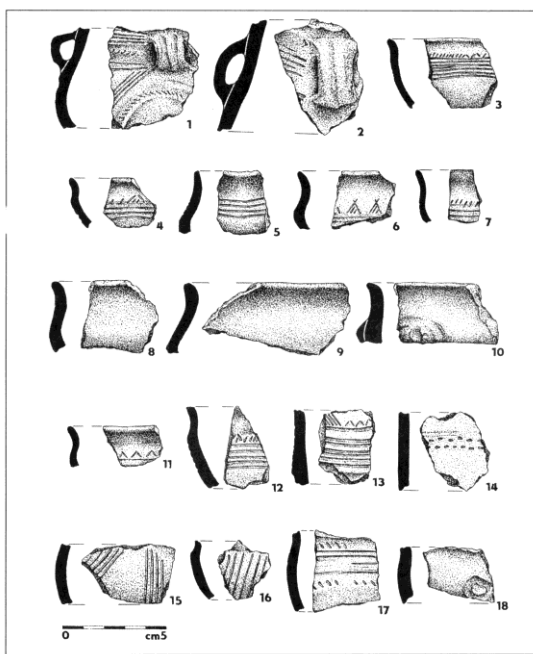


Localities Chotebuz-Podobora

Chotěbuz – Podobora is located near the border with Poland near Český Těšín.



Hallstatt



^{14}C dating
 $929 \pm 68 \text{ BC}$
 $568 \pm 155 \text{ BC}$
 $583 \pm 166 \text{ BC}$

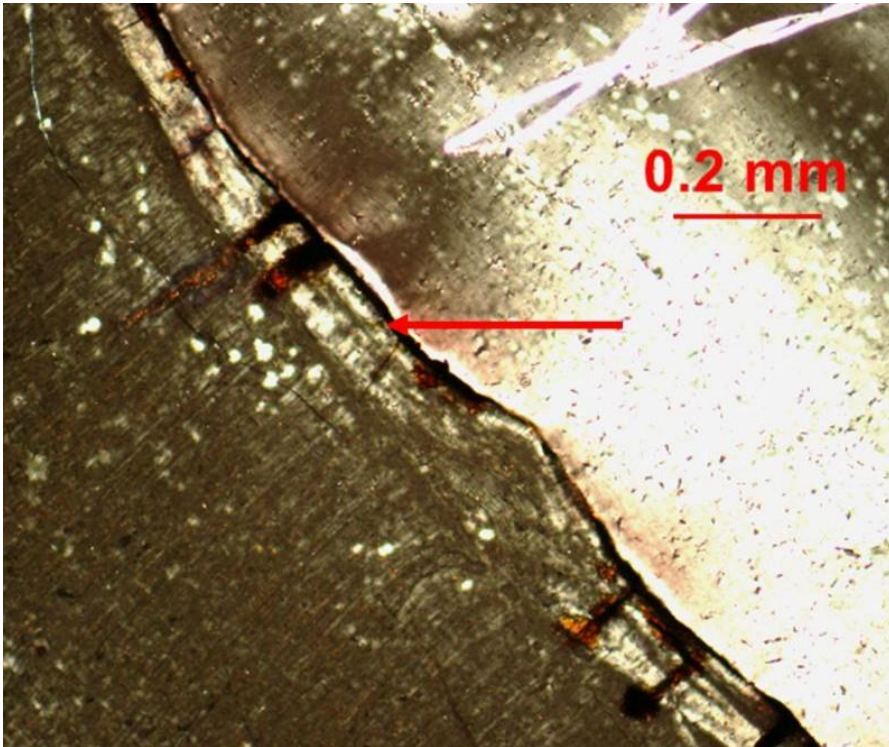
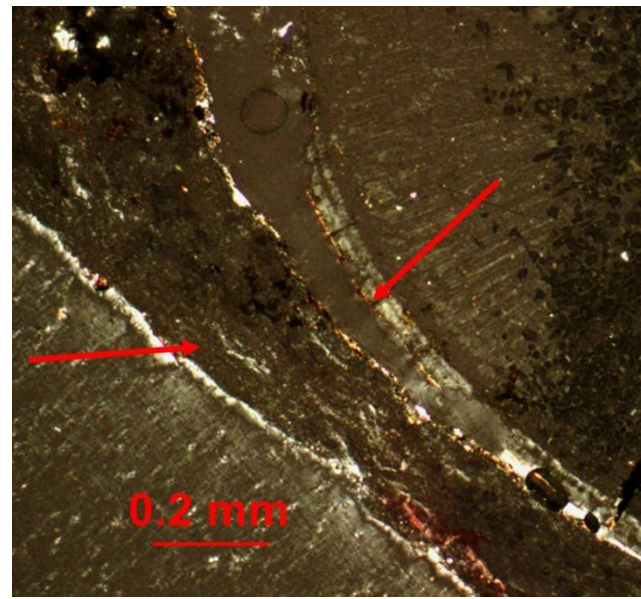
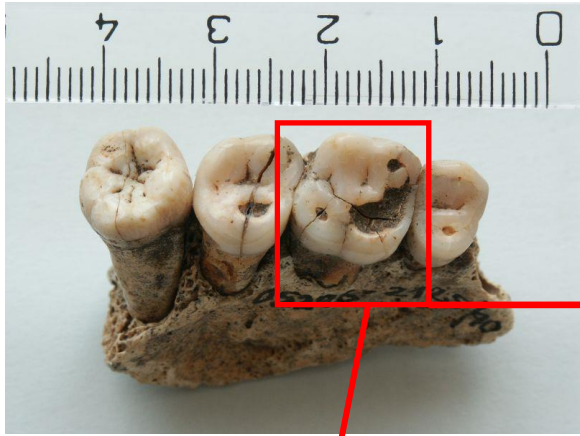


The discovery of human remains that were not properly stored



?man - age 30- 40 years





a person died in early November



Geochemical Analysis

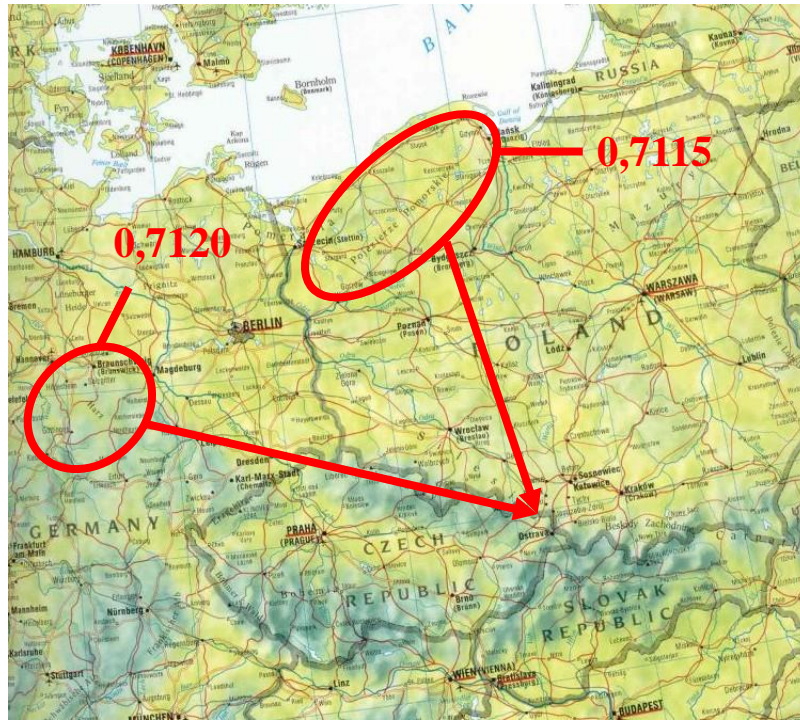
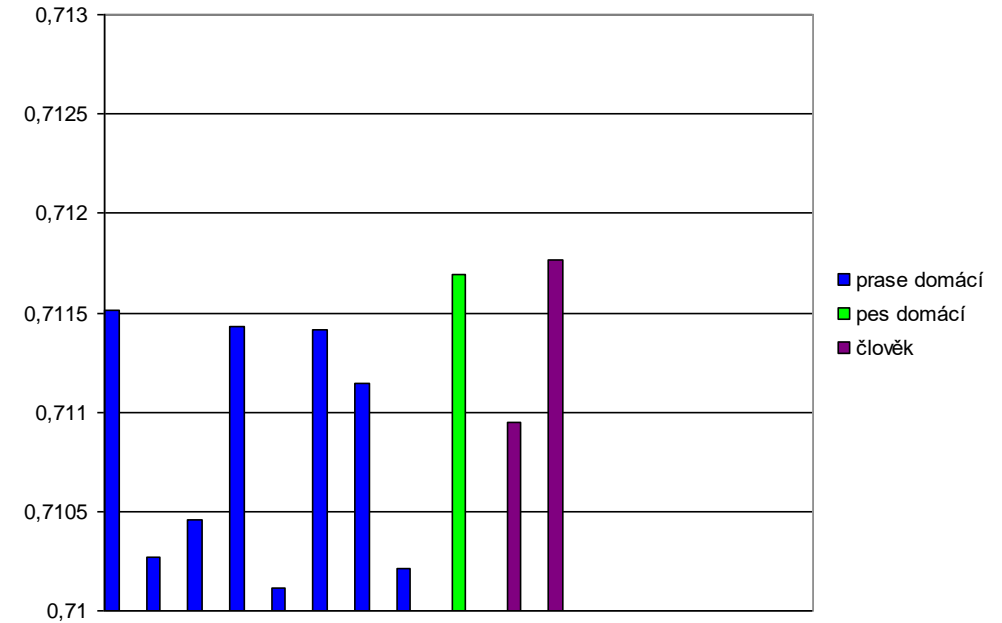
Strontium analyses

$^{87}\text{Sr}/^{86}\text{Sr}$

Human jaw: 0.7116 (2σ - 0.000013)

Human molar: 0.710947 (2σ - 0.000011)

Domestic pig: 0.7102 (2σ - 0.000017)



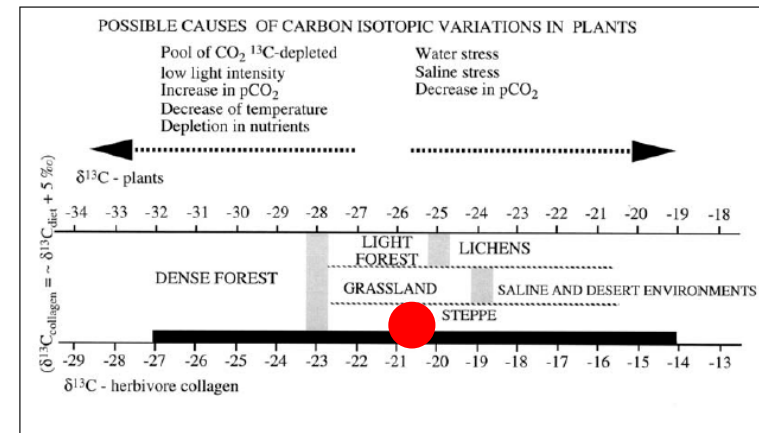
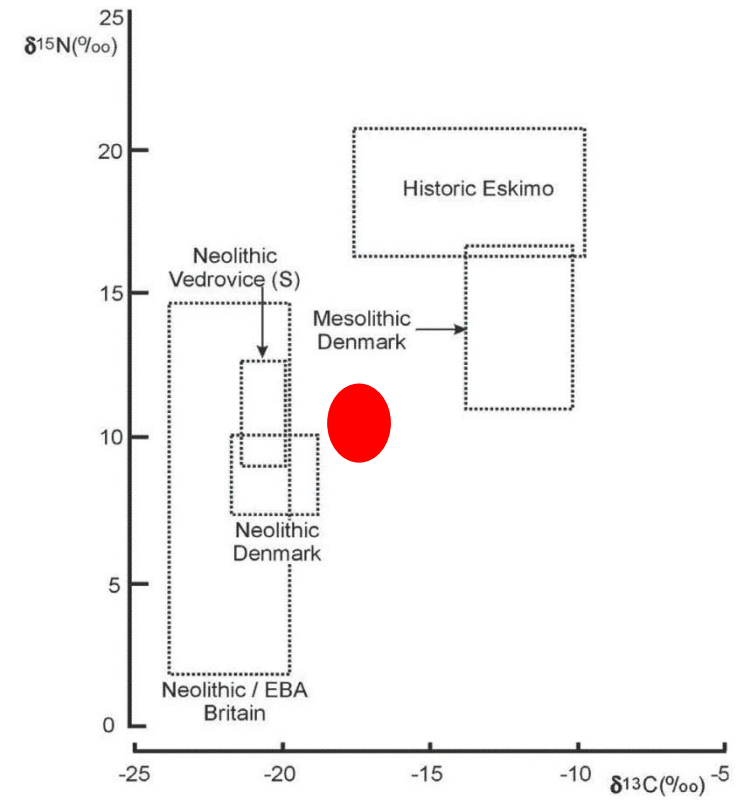
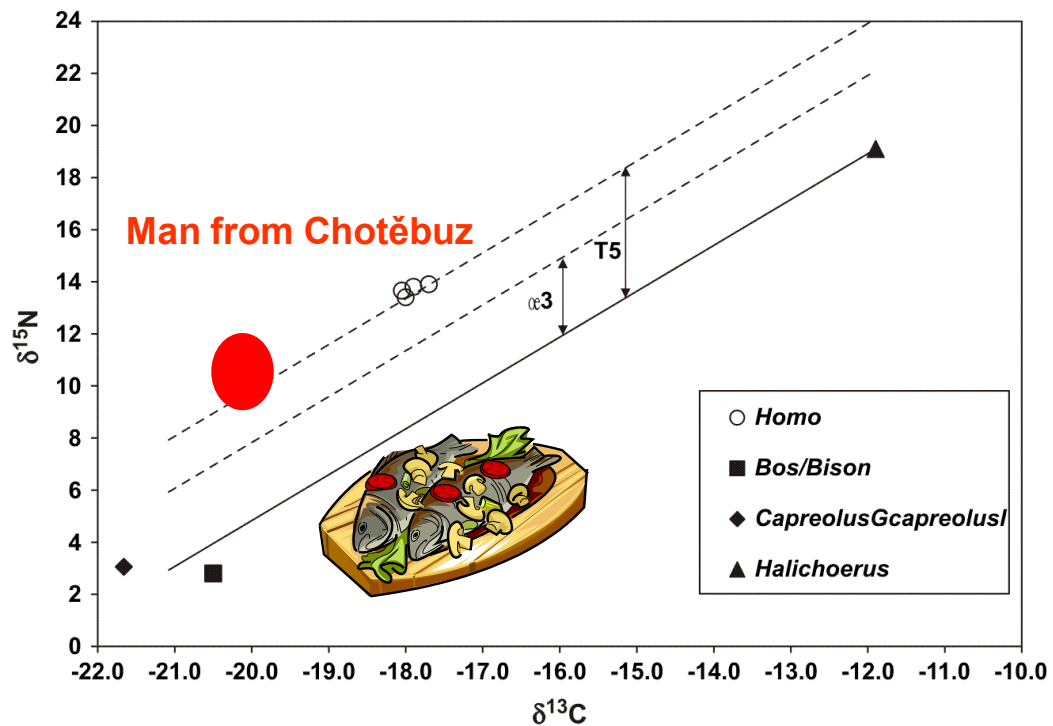
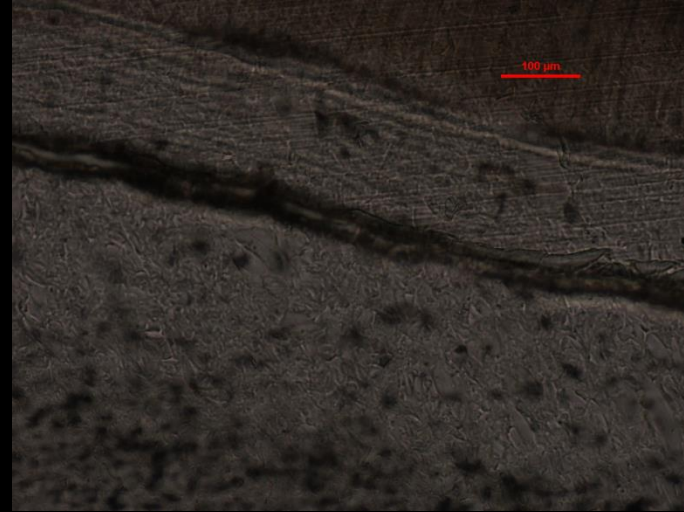


Figure 1 Range of herbivore collagen $\delta^{13}\text{C}$ values measured in modern arctic, temperate and steppe areas, with possible causes of carbon isotopic variations in plants and herbivores. Values are compiled from Nelson *et al.* (1986), Bocherens *et al.* (1994, 1996, 2000), Rodière *et al.* (1996).

Women from Krumlovský les

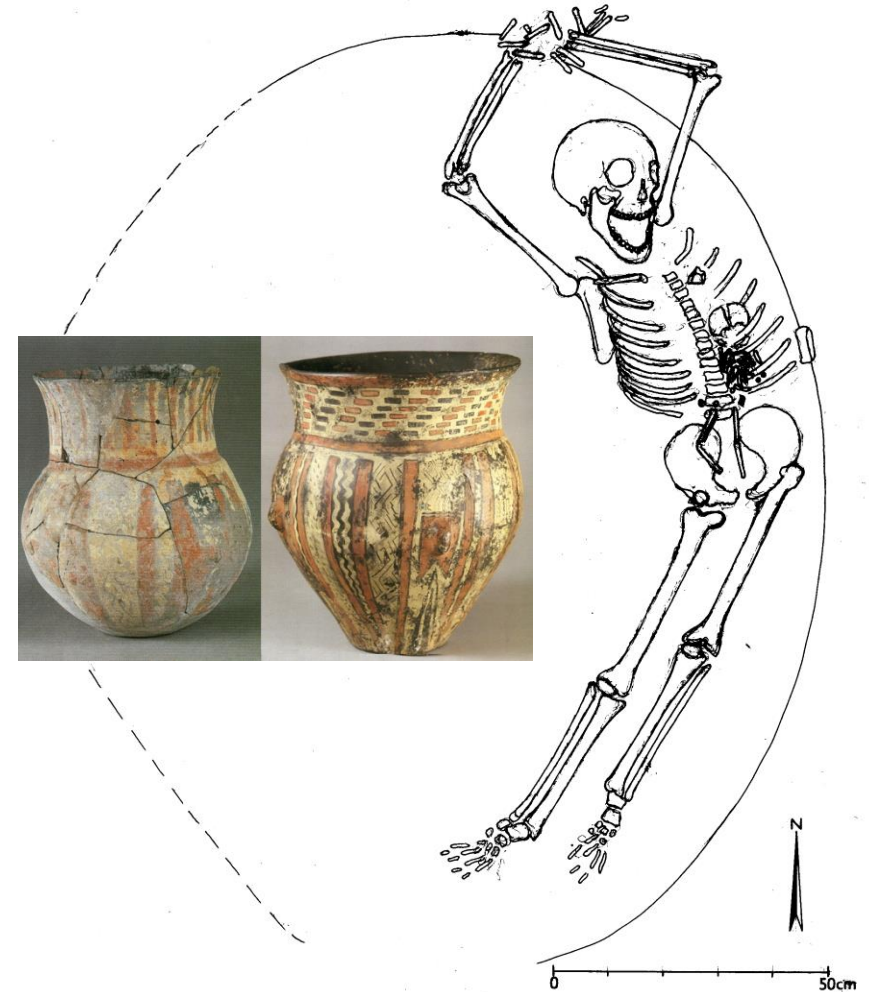


Season of death



Archeology

One of Europe's largest prehistoric mining areas is located on the southeastern slopes of the Krumlovský les (Oliva 2005). The skeleton of a woman aged 25-30 was discovered in shaft No. 4 at a depth of 6 m. The skeleton was lying in a horizontal, strictly crouched position, either on the right side or on the back with the legs curled under the pelvis. The human bone yielded a GrA-22839 date: 5380 ± 50 BP uncalibrated. Another skeleton of a woman in an anatomical position was discovered about 60 cm deeper. The body of a woman (age 35-40 years) was lying on her back with her hands clasped behind her head, her head looking slightly to the left. On her chest lay the skeleton of a newborn. Above the first skeleton in the backfill lay a Lengyel bowl on a foot with a missing rim. Charcoal in the vicinity gave a date of 5490 ± 50 BP uncalib. The rearranged bones of a small dog were placed above the head of the second woman, and above her palms were bones from the leg of a frog, probably a frog (Oliva 2008).



Nutrition of women from the Krumlov Forest

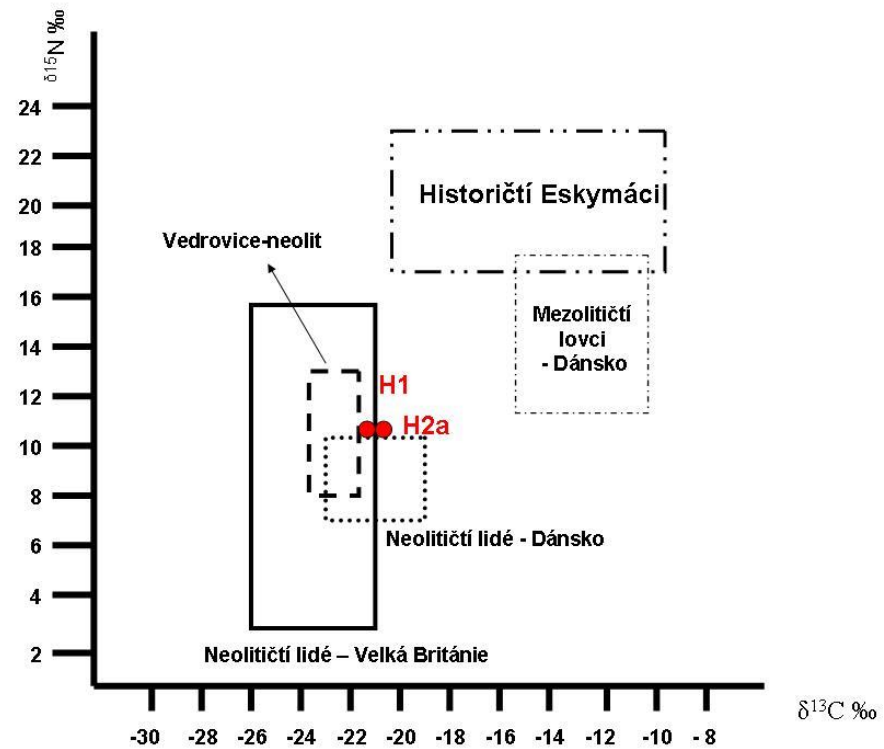


H2a

^{14}C 5630 \pm 40

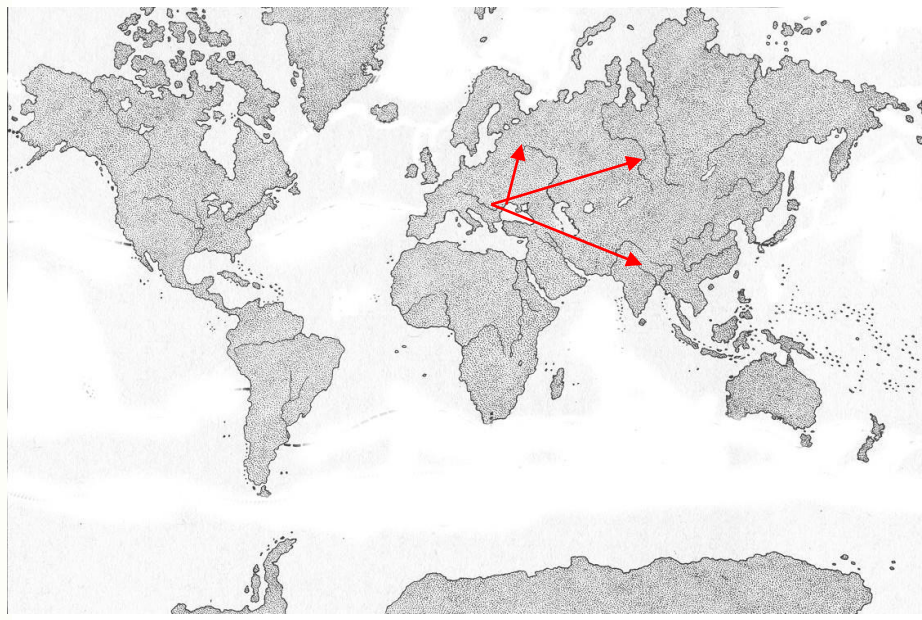


H1



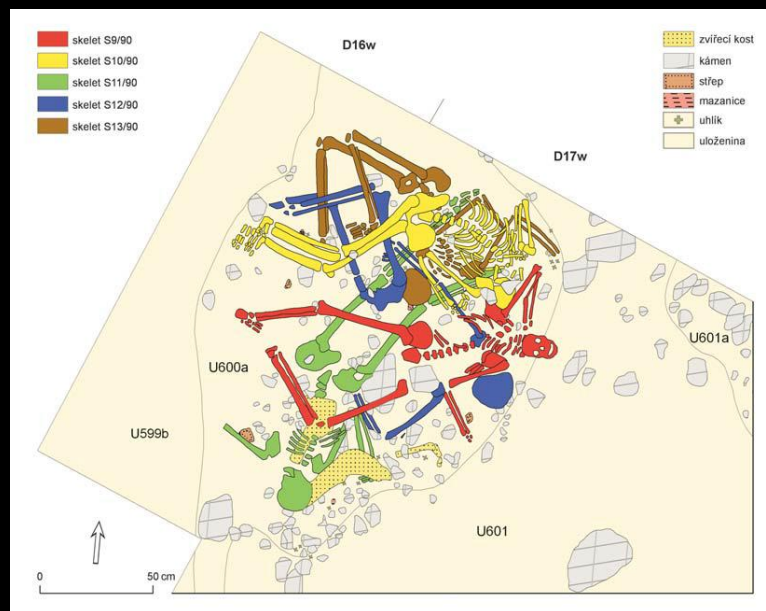


	H1	H2
D3S1358		11
TH01		
D21S11		
D18S51		
Penta_E		
D5S818	11	
D13S317	14	14
D7S820		7
D16S539	8	14
CSF1PO	8	14
Penta_D	376.8	9
AMEL	X	X
vWA	12	12
D8S1179		11
TPOX		
FGA		26

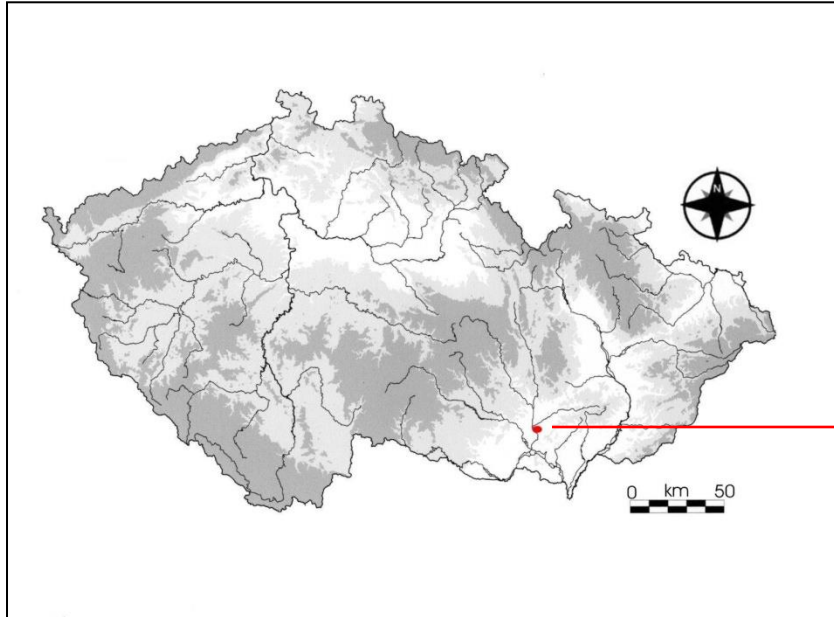


Genetics

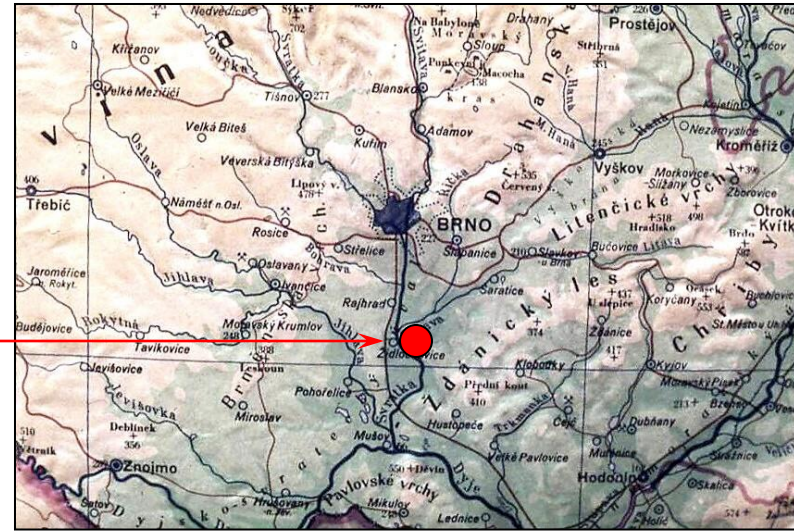
Paleodiet and paleoenvironment of people from Blučina locality



Geographical and topographical location



Blučina (district Brno-venkov), 20 km south of Brno

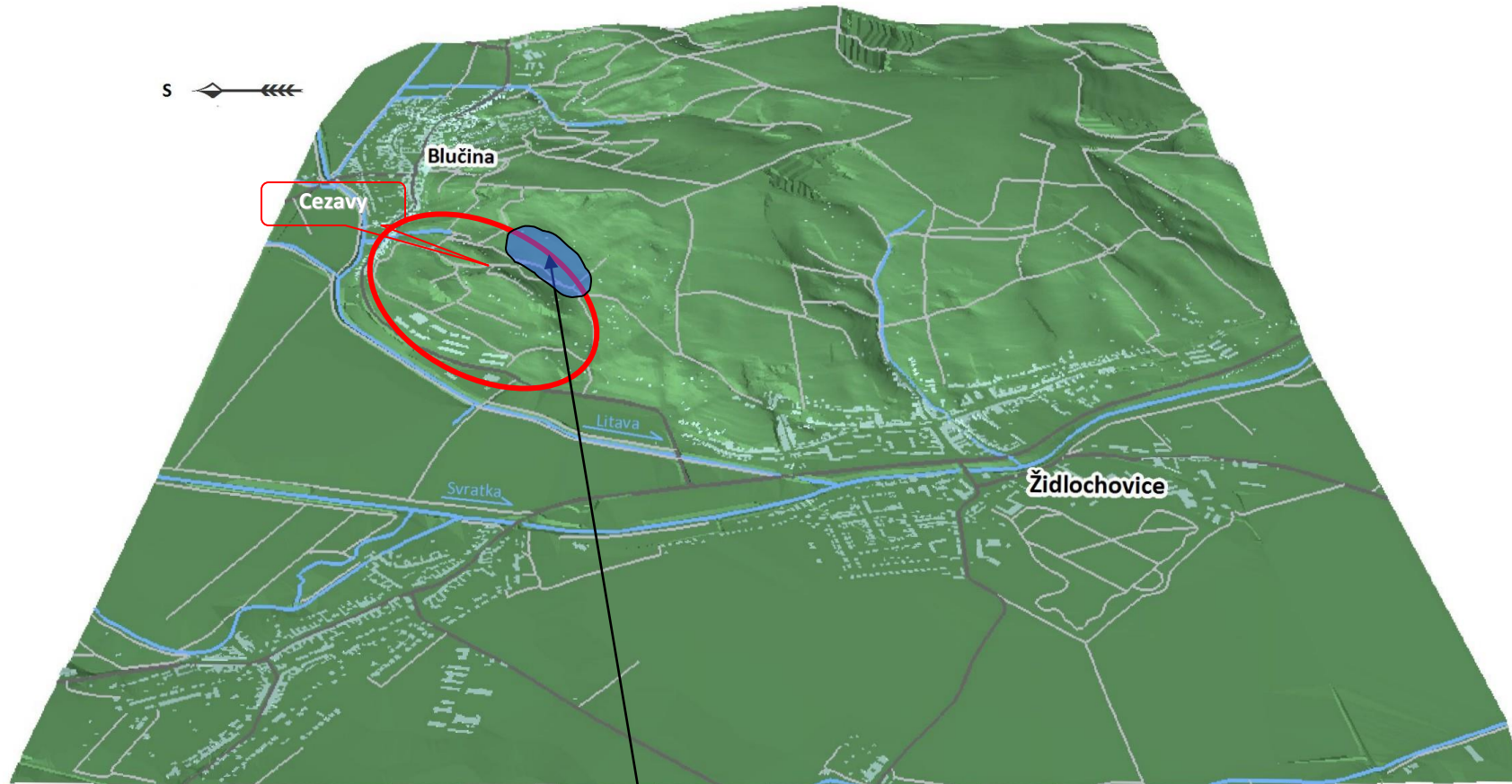


Výchon: geomorphological district, isolated elevation between Blučina and Židlochovice, 354 m above sea level - the highest point of the Dyjsko-Svratecký valley geomorphological unit

Cezavy: oval hill (20 ha) on the NW edge of Výchon



Geographical and topographical location



Cezavy:

- on the W side from Výhon separated by a saddle, originally with a periodic lake
- on the N and W sides of the Litava (confluence of the Svratka and Litava) with the Svratka valley floodplain (asl. 260 m, elevation 70 m)

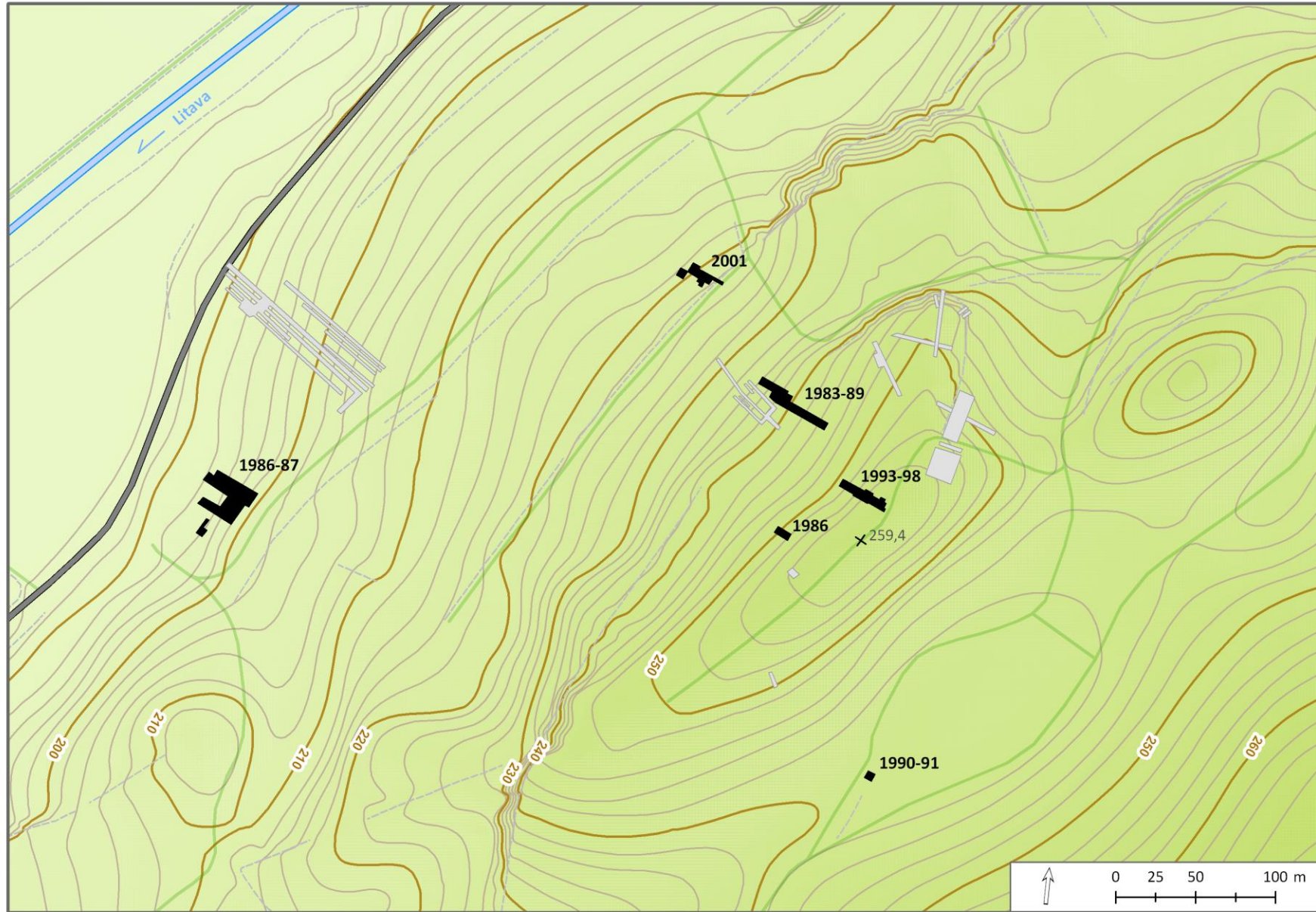
Settlement of Cezav in the Younger Bronze Age

- older (velatic) phase of KSPP - early velatic stage - Blučina horizon (approx. 1300-1250 BC)

Specifics of the early Velatic horizon in Cezava:

- absence of fortifications
- absence of settlement pits
- specific finding situations - area accumulations: including human skeletal remains, bronze depots; documents of metallurgical production

Blučina - Cezavy: site plan



part of the areas investigated in 1948-1974

areas investigated in 1983-2001

Findings

ARTIFACTS:

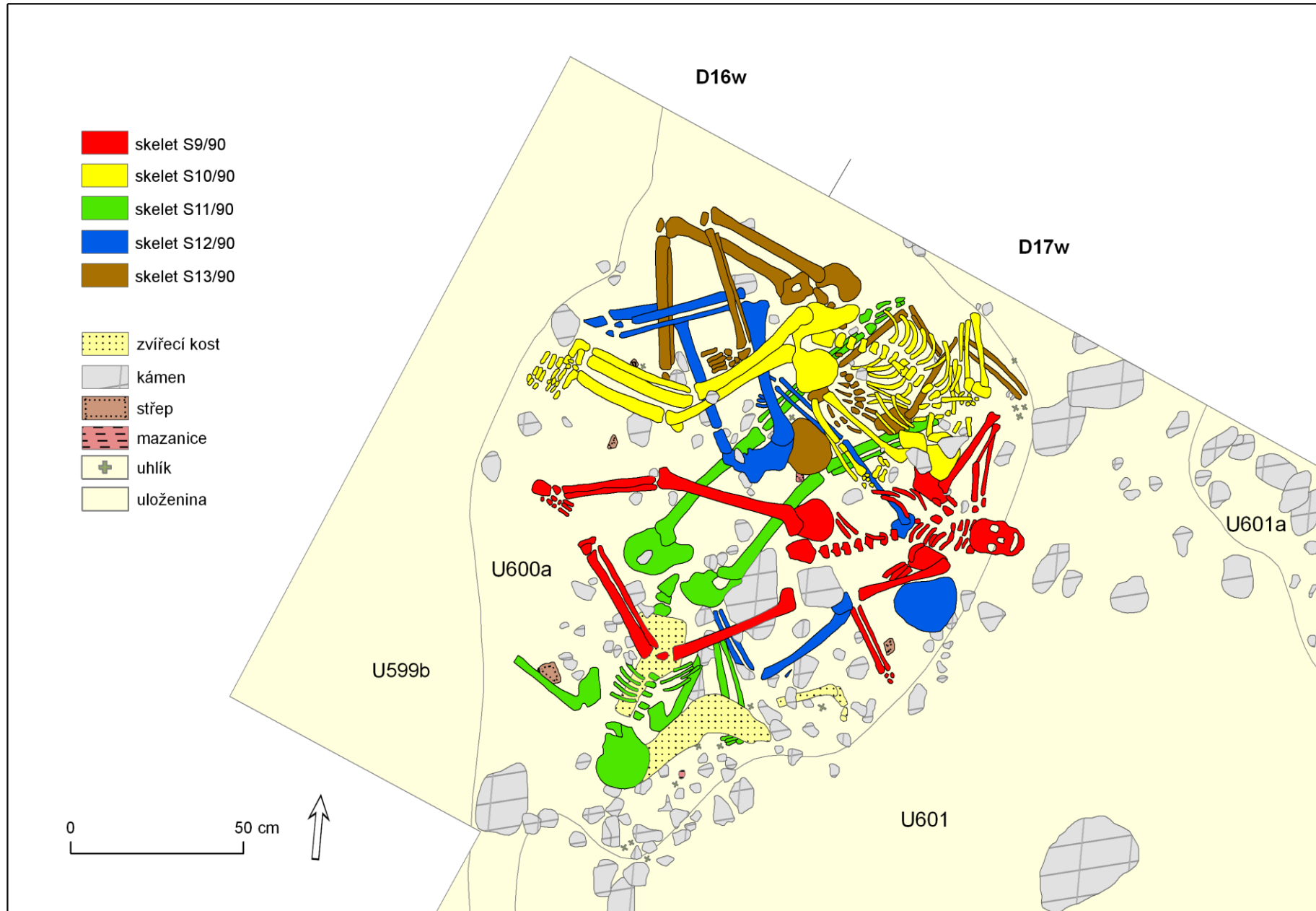
- ceramics (ceramic vessels)
- other ceramic artifacts (weights, whorls, wheels, casting molds)
- metal (bronze, copper) industry,
- bronze depots bone and antler industry
- stone chipped industry
- other stone industry, including stone hulls glass and amber beads grease gun

NATURAL FACTS:

- animal bones
- Malacofauna
- archaeobotanical material (carbons, plant macroremains)
- anthropological material (human skeletal remains in non-standard - mostly "non-ritual", i.e. non-funeral storage: whole skeletons, their parts and individual bones)
- rough stones

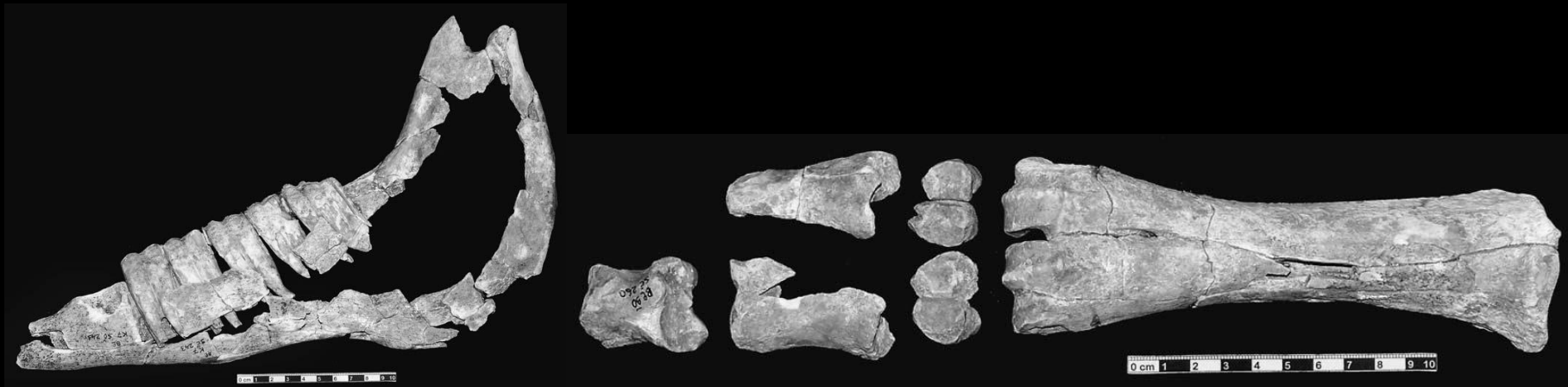
Find accumulation K7 (area 2.85 m²)

D17w, D16w SV/230 cm



Zooarchaeological analysis

The remains of only two types of domestic animals were found in the building, namely the domestic deer and the domestic horse. bones of the autopodium and scapula were preserved from the tur the horse's lower jaw was preserved



Zooarchaeological analysis

- the most abundant animal in the Younger Bronze Age was the sheep and the goat,
- followed by the domestic pig and less is the turn of the domestic pig,
- domestic dog and horse are represented at least,
- wild animals as well, from which we can judge a small share of hunting,

Anthropology (Dr. M. Dočkalová a dr. I. Jarošová)

A total of 5 human skeletons were found in the object

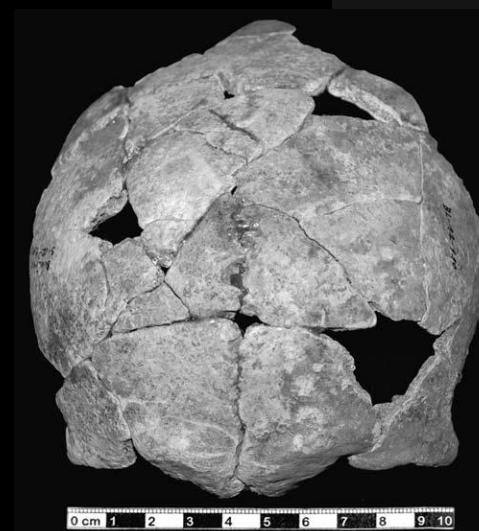
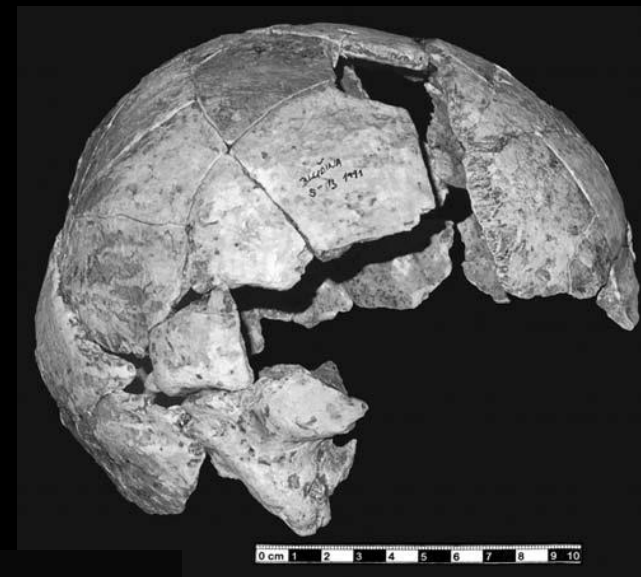
Skeleton S9/90 – male, age 40 - 55 years

Skeleton S10/90 – male, age 35 - 45 years

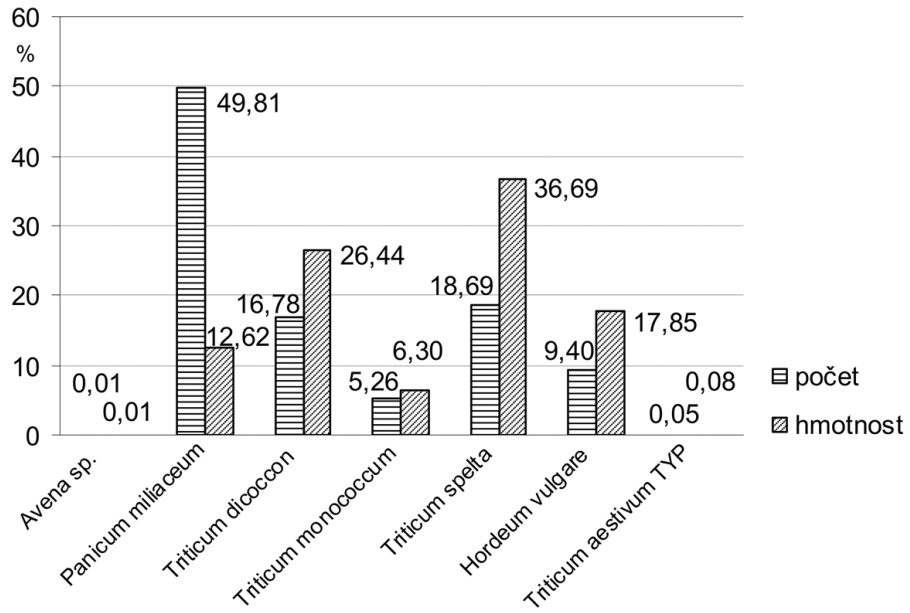
Skeleton S11/90 – male 19 - 22 years

Skeleton S12/90 – male 20-30 years

Skeleton S 13/90 – male 26-29 years



Paleobotany (Kočár)

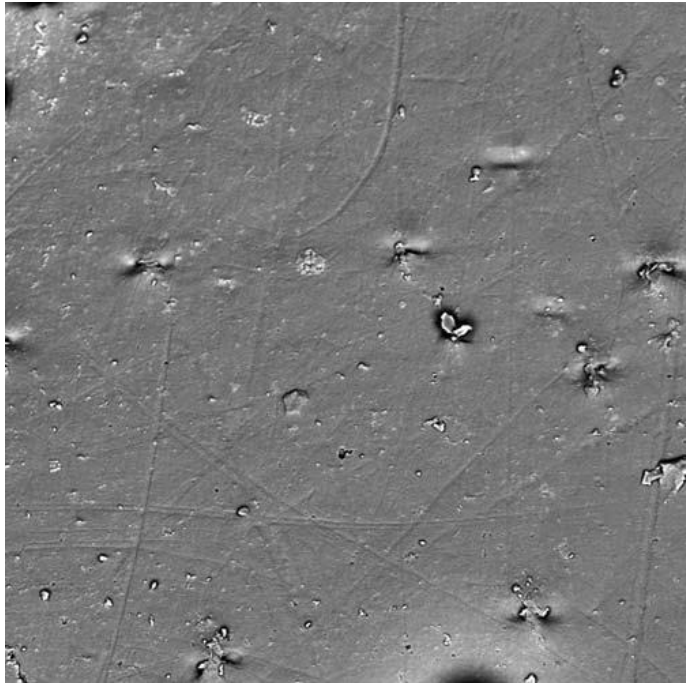


Cereals

- From the legumes, the remains of lentils and peas were discovered
- Acorns were found from other plants.

Reconstruction of men's diet based on tooth microabsorption I. Jarošová)

Using this method, food can be found during the last six months of life



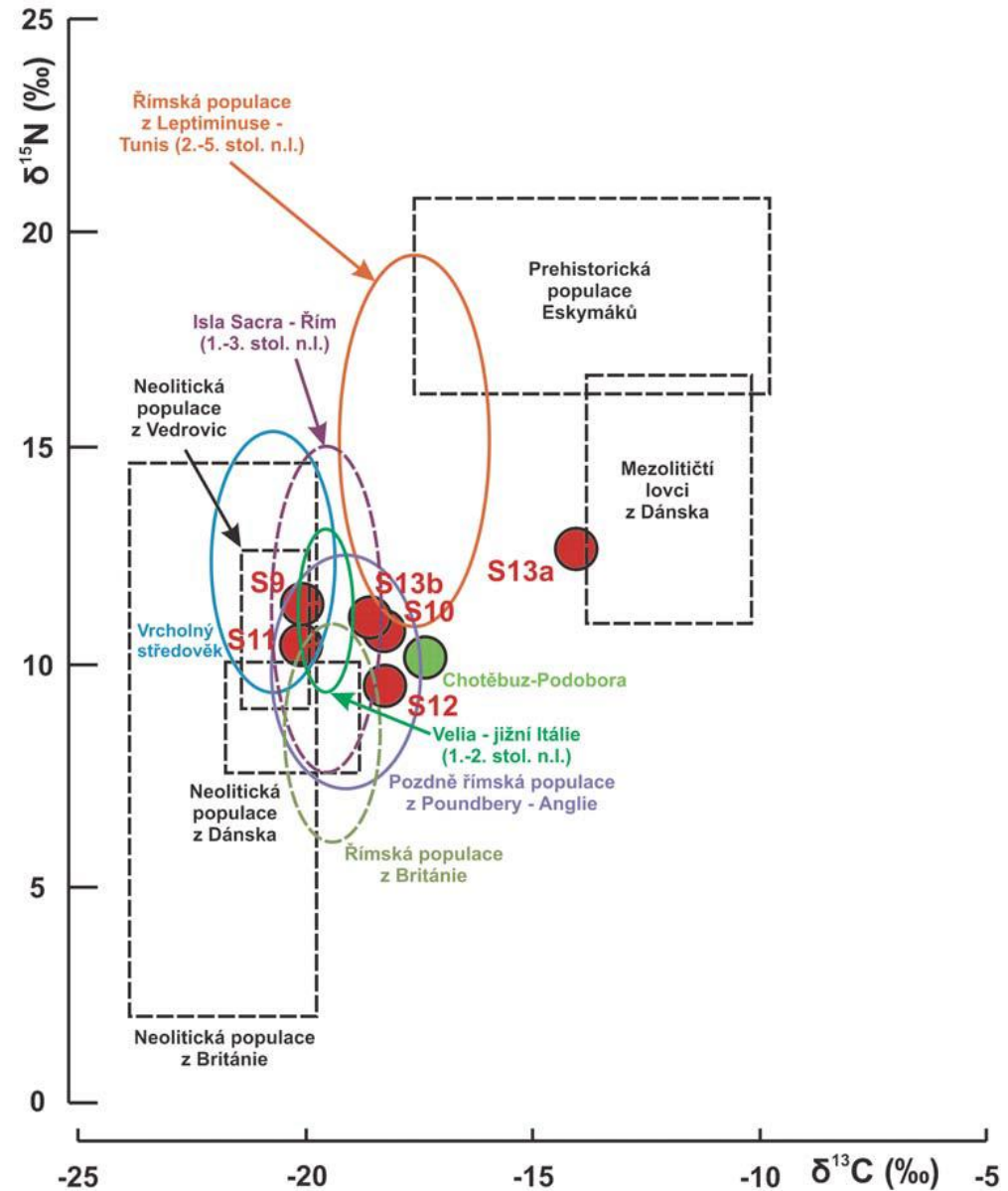
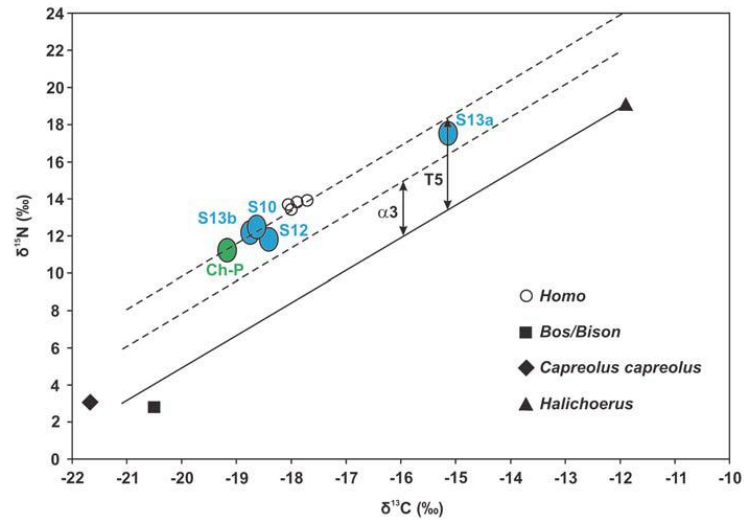
Males S12 and S13 ate mostly meaty food

Male S9 - fleshy food

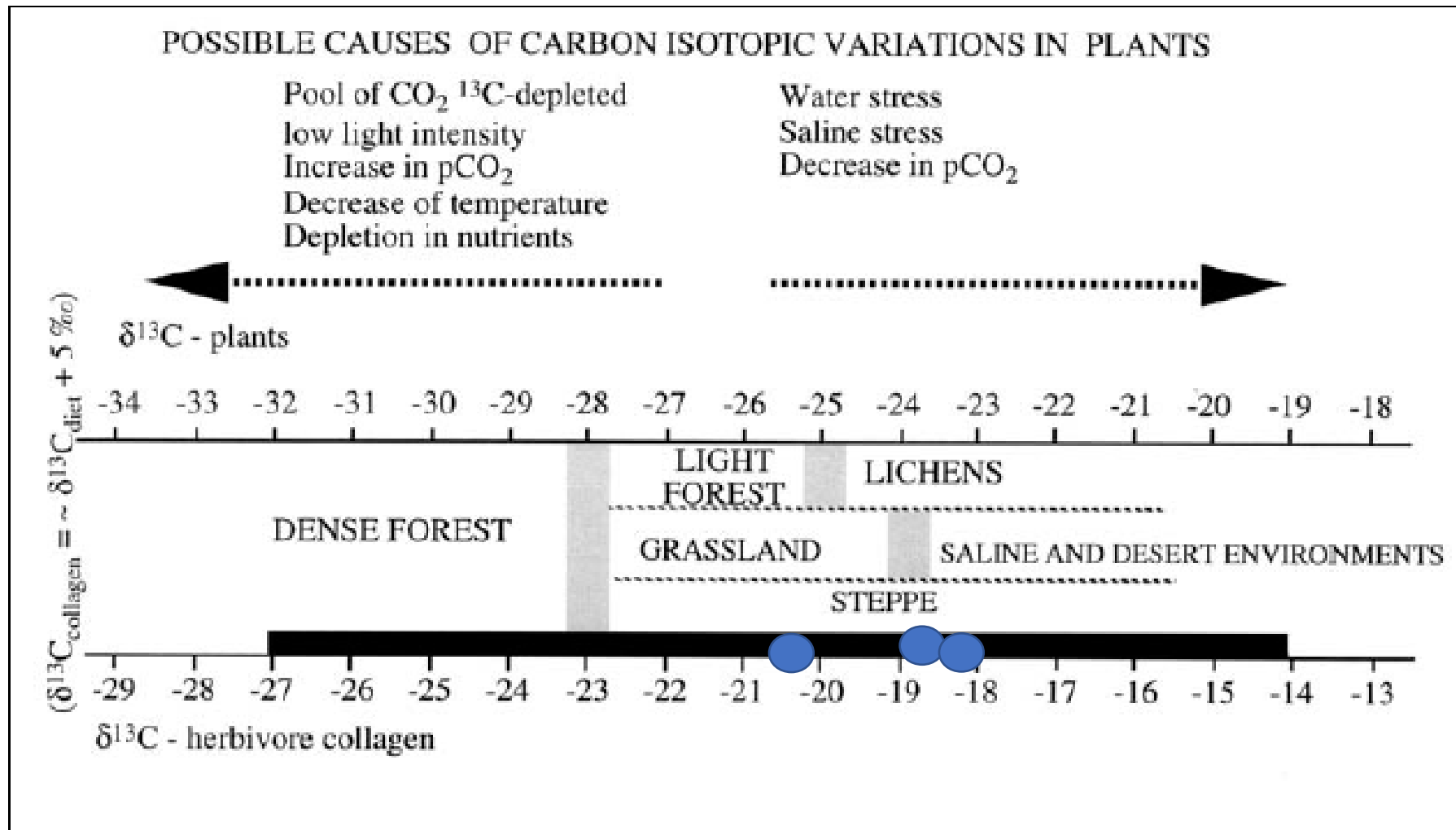
S10 and S11 – mainly plant food

Isotope analyzes of C, N

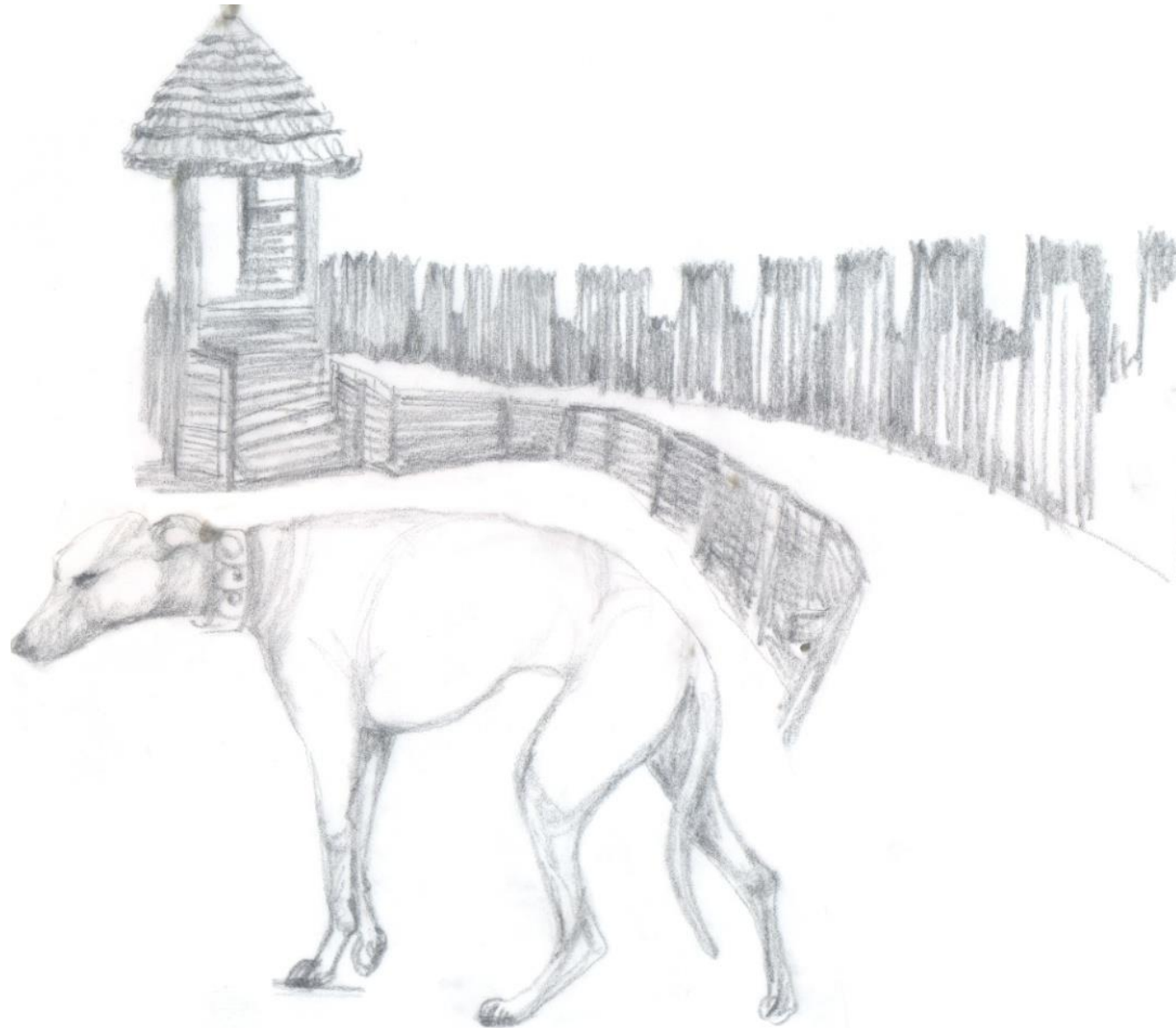
Food



Paleocology

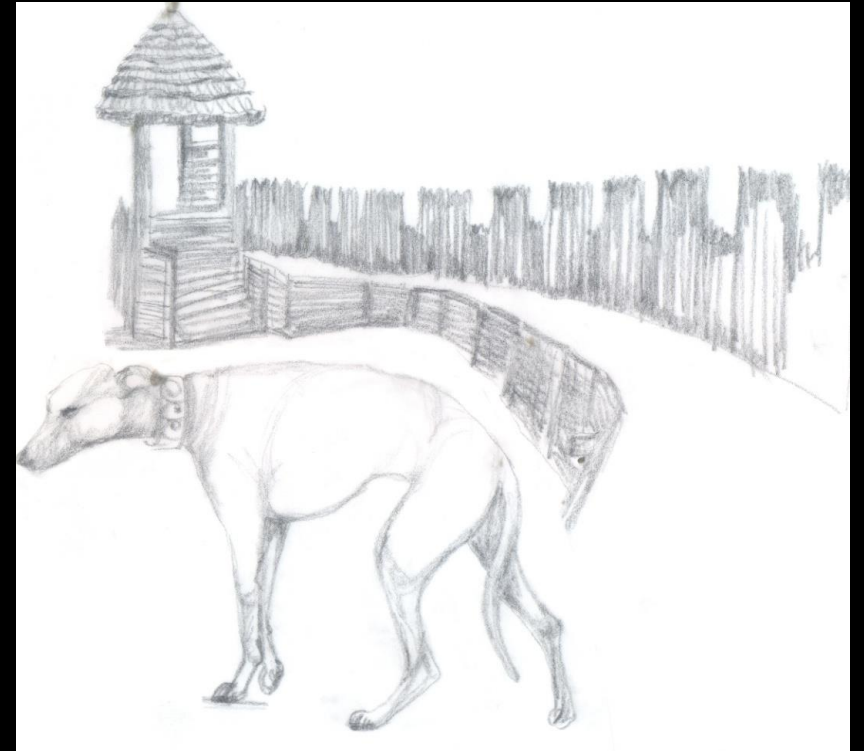
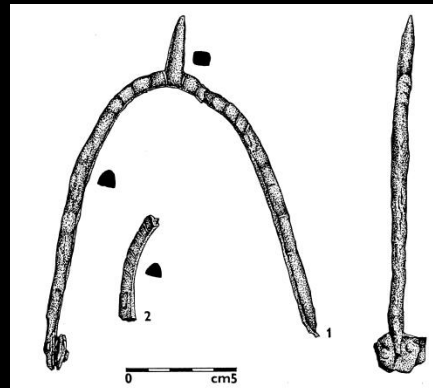
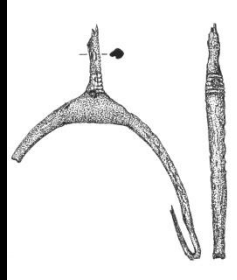
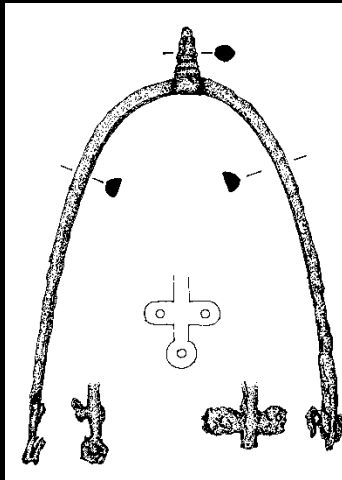


Greyhound from Chotěbuz

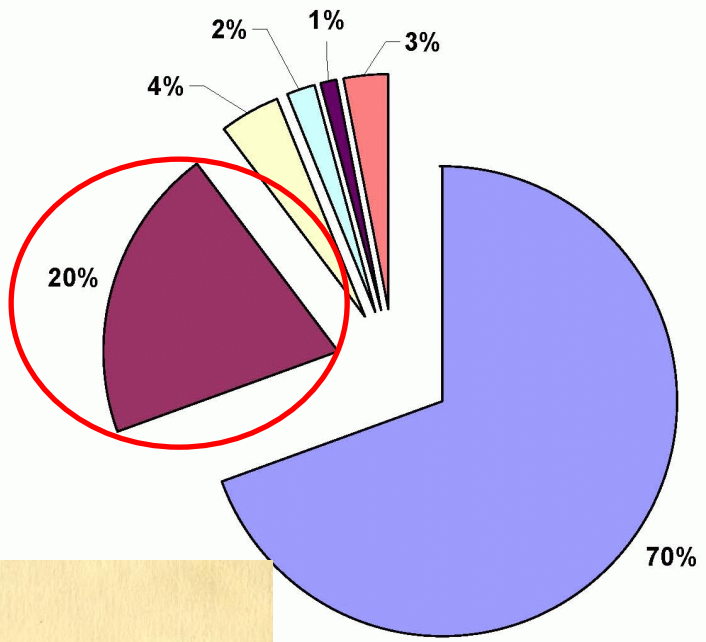
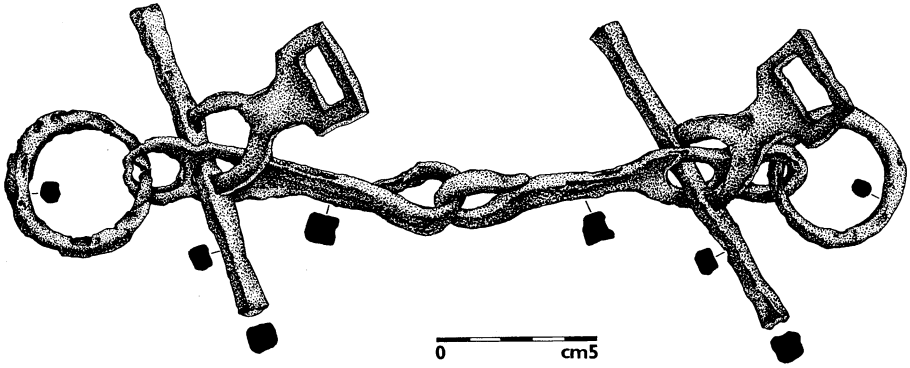


Local Elite

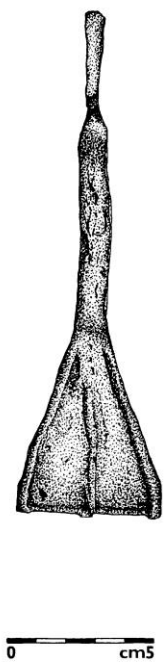
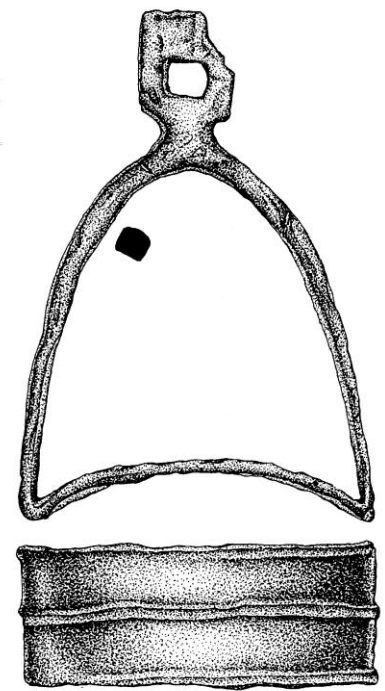
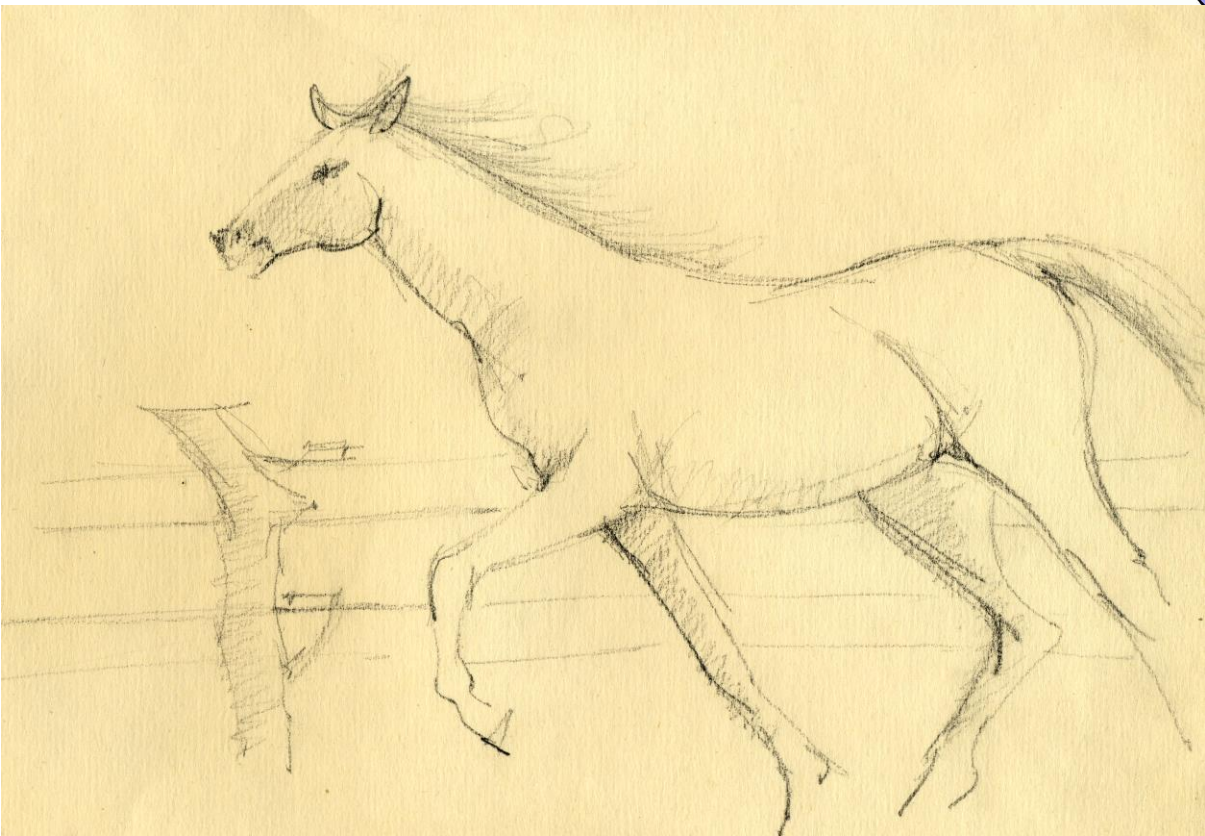
According to Kouřil (2007), the local elite lived in the fort in the second half of the 8th century. to the fourth quarter of the 9th century AD The people at the fort controlled the northern exit from the Moravian Gate and mining at the local iron ore deposits. The elite was mainly concentrated on the acropolis, where they lived in log structures with heating devices. A large number of artifacts (weapons, spurs, stirrups, bits and jewelry) have been found on the acropolis, which indicate the presence of a local ruling elite and free men and warriors gathered around the local ruler. Some of the found artefacts indicate relations to the north and north-west, i.e. to today's Polish territory.



Local Elite

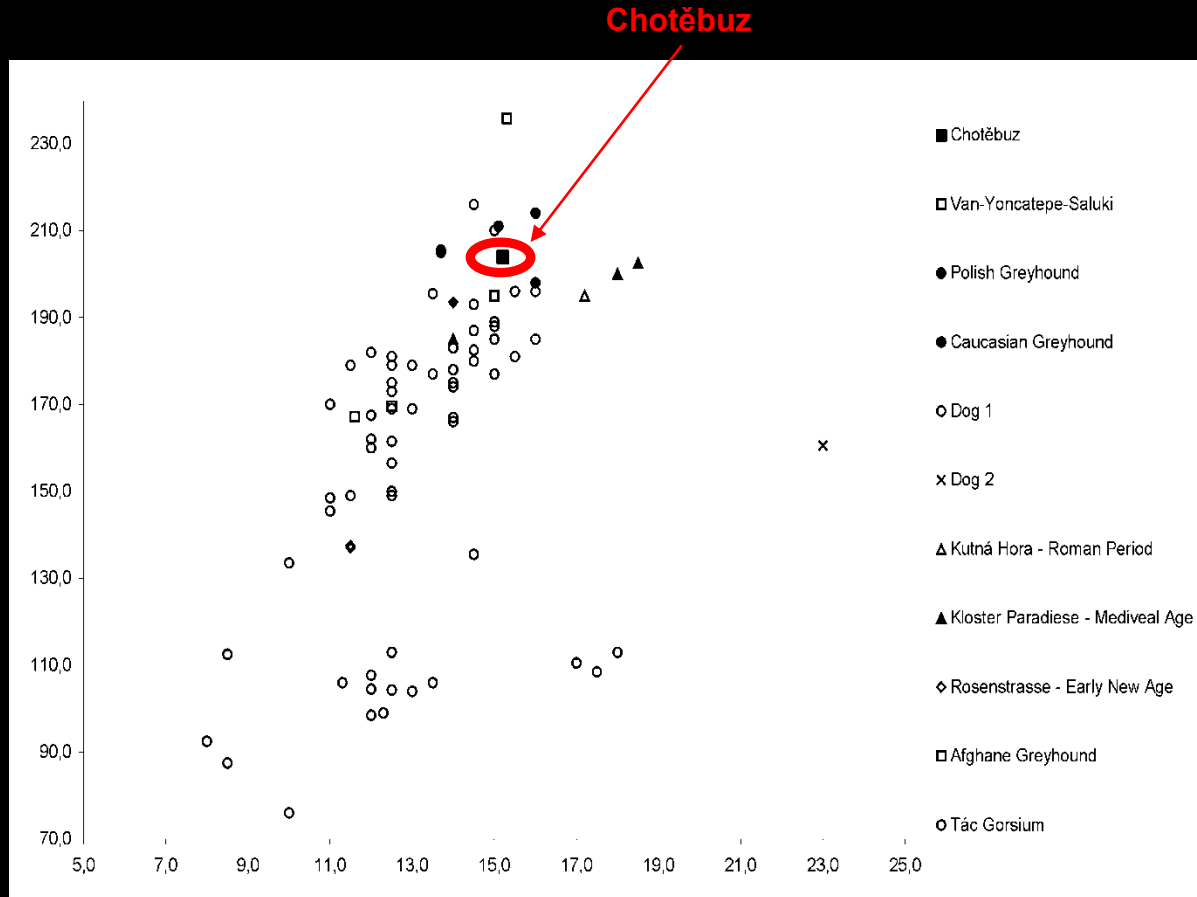
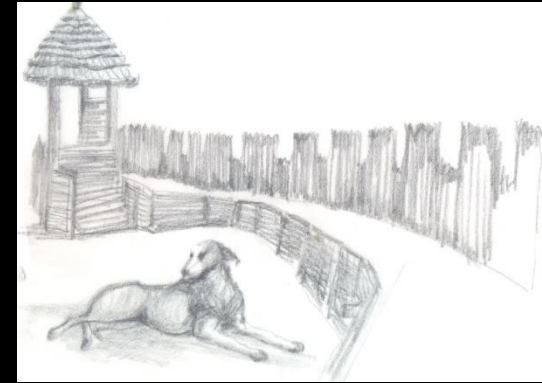


- cows
- horses
- dogs
- pigs
- sheep/goat
- wild animals



Greyhound find

- two spindle bones from a greyhound were found in object S 44 on the acropolis,
- this is the first finding of such a dog in the Slavic period in our country,
- the greyhound could come from the Normans (who had greyhounds), who traded in the Baltic region at this time.



$^{14}\text{C} - 800 \pm 60 \text{ AD (calibrated)}$

Height of faucet: 70 cm



Geochemical analysis

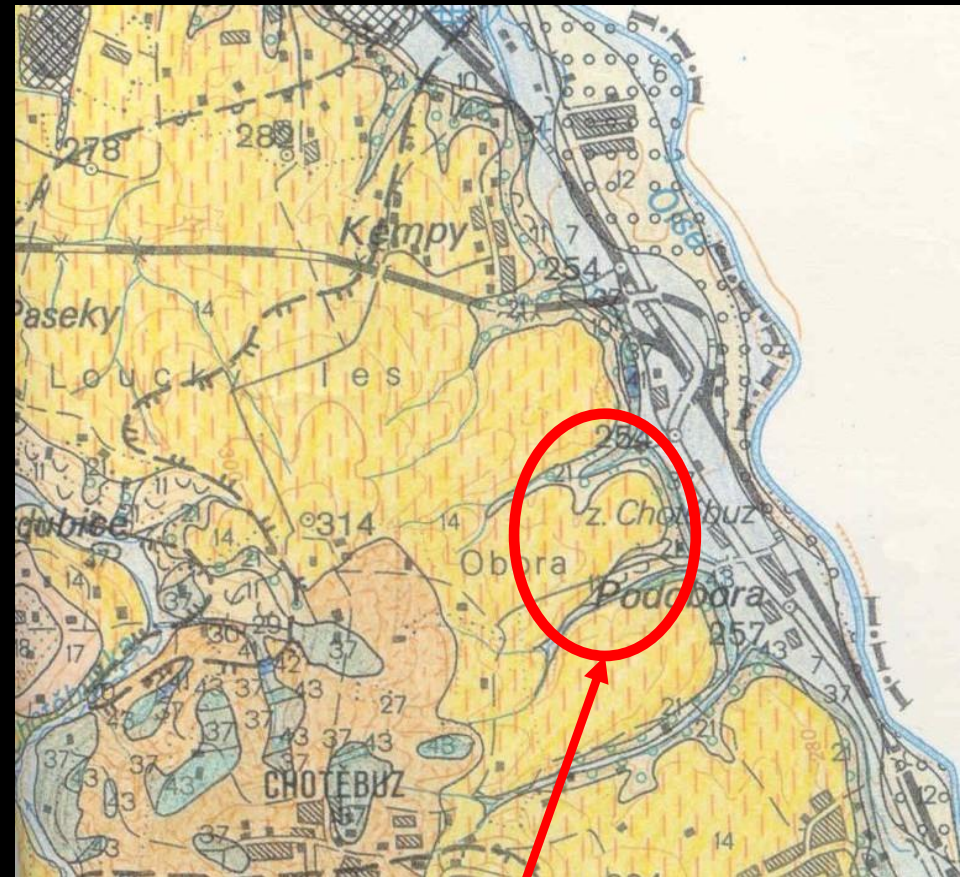
- an analysis of the $^{86}\text{Sr}/^{87}\text{Sr}$ ratio was performed
- dog bone samples were taken to determine local or foreign origin and domestic pig (domestic pig is assumed to be of local origin),
- sediment samples were taken to determine the subsoil signal

$^{87}\text{Sr}/^{86}\text{Sr}$

Dog – 0.7116 (2σ - 0.000009)

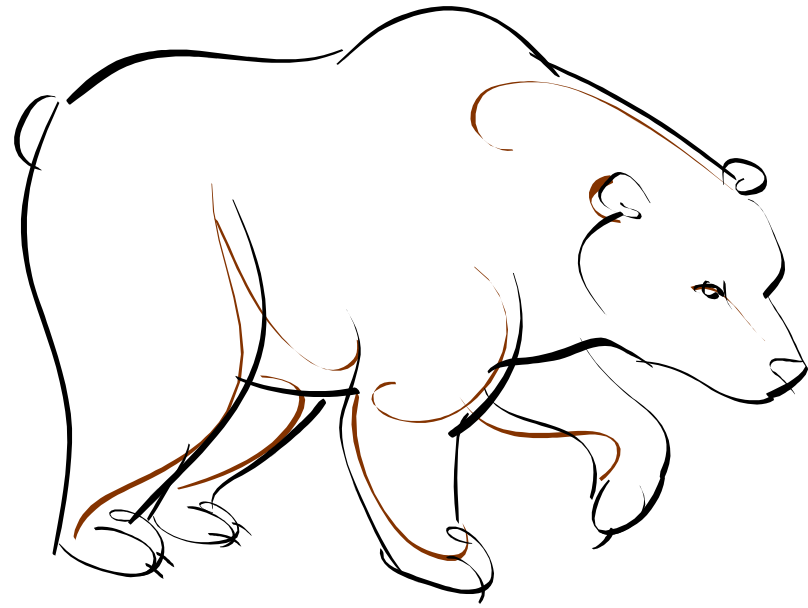
Domestic pig – 0.7102 (2σ - 0.000017)

loess clays – 0,7298 - 0,7354
(2σ - 0,000009)

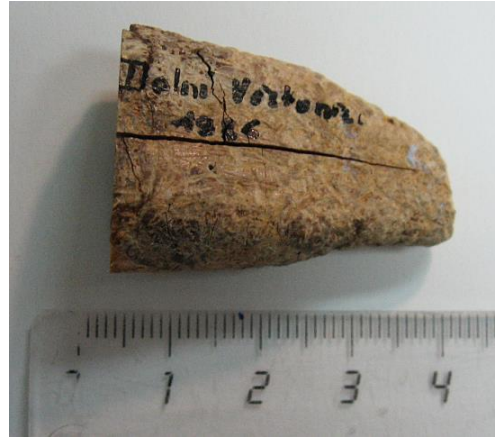


The subsoil consists of loessclays and glacial fluvial clays and gravels

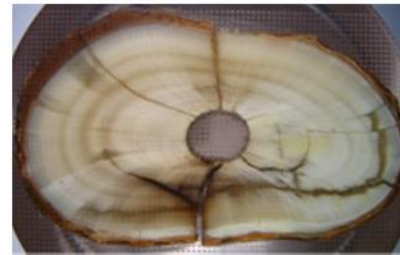
The story of the bear



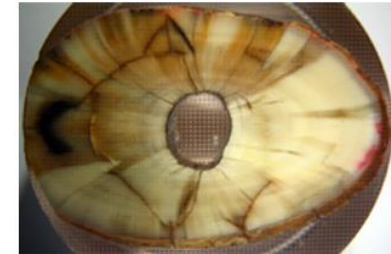
Ursus arctos



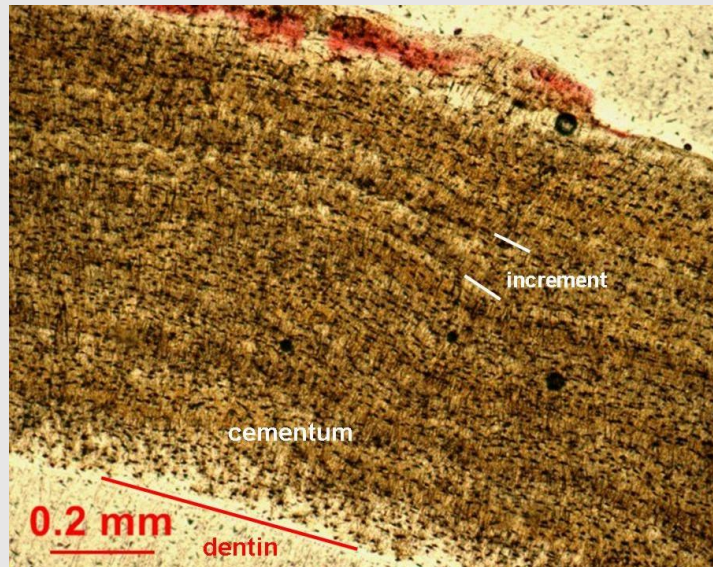
root



crown



- 🐾 Bear (*Ursus arctos*) canine found in Dolní Věstonice
- 🐾 age of the site $26,640 \pm 110$ years uncalibrated.



- 🐾 Age and seasonality determined on the basis of the study of microstructures of dental cement deposits on the root part of the tooth.
- 🐾 The bear died at the age of 14. On the basis of the unfinished summer increment and the missing winter increment, the period between summer and autumn (August - October) was determined.

Ursus arctos – measured elements and experimental conditions

SP LIBS

- 👉 Ca (I) (452,69 nm), Sr (I) (460,73 nm), Ba (II) (455,40 nm)
- 👉 laser wavelength 532 nm
- 👉 grid 2400 vr./mm
- 👉 energy 30 mJ/pulse
- 👉 detection parameters: delay 1 μ s, integration time 10 μ s
- 👉 entrance slit 3 μ m

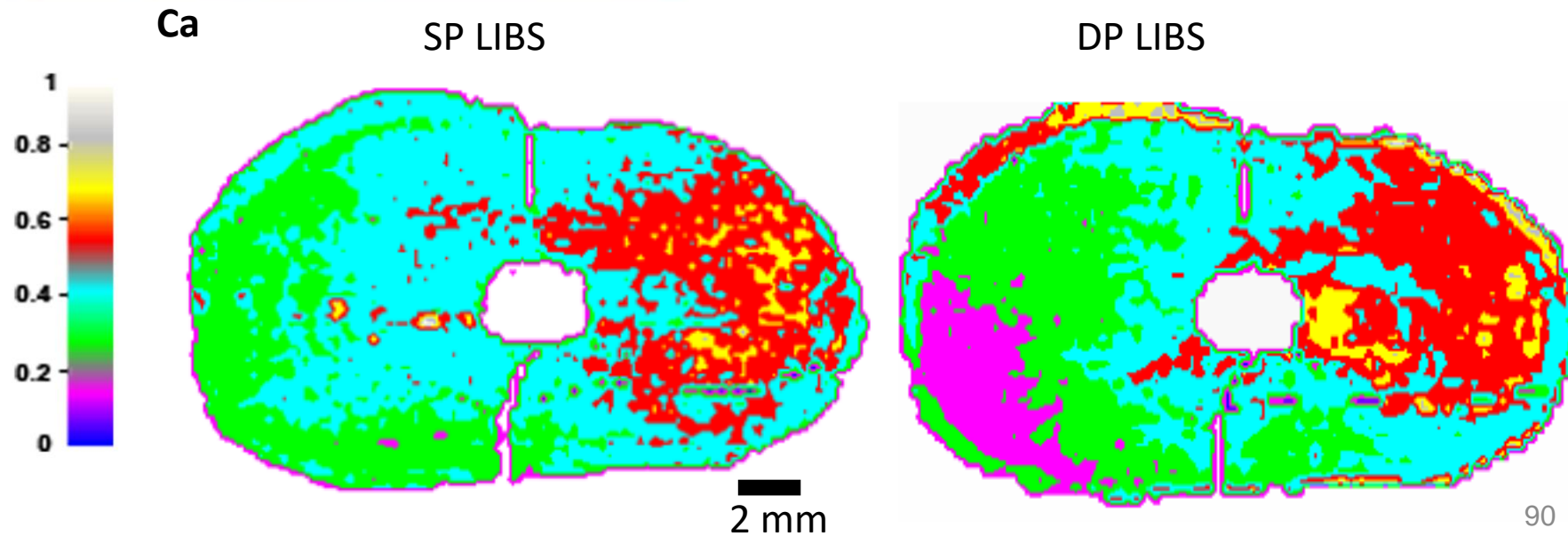
DP LIBS

- 👉 Ca (I) (452,69 nm), Sr (I) (460,73 nm), Ba (II) (455,40 nm)
- 👉 laser wavelengths 266 nm + 1064 nm
- 👉 grid 2400 vr./mm
- 👉 time between pulses 500 ns
- 👉 energy 10 mJ/pulse + 90 mJ/pulse
- 👉 detection parameters: delay 1 μ s, integration time 10 μ s
- 👉 entrance slit 50 μ m

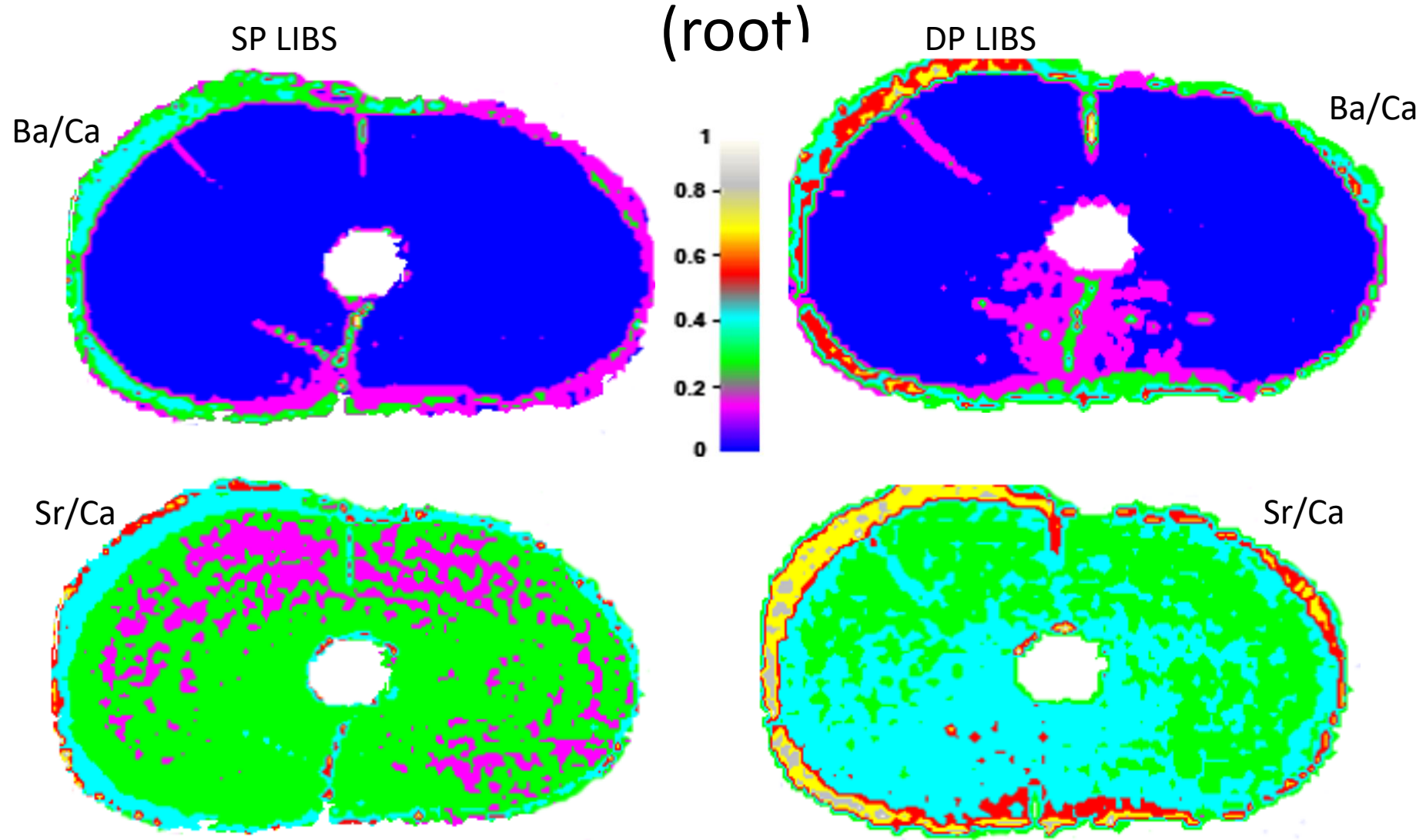
Ursus arctos – mapping Ca distribution using SP and DP LIBS (root)



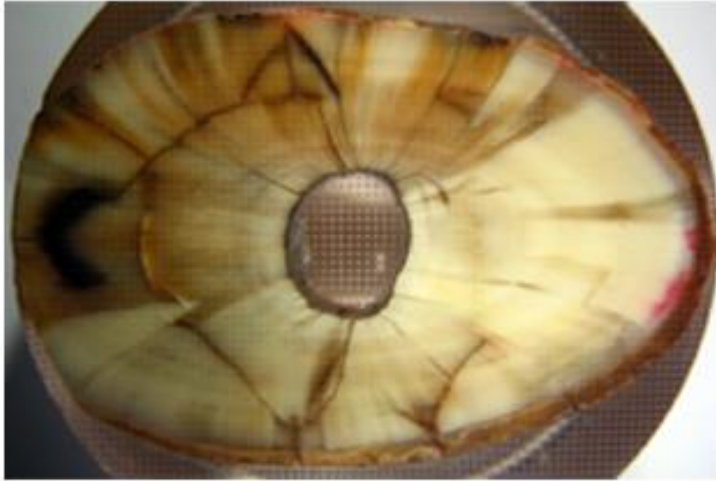
Comparison of Ca distribution in a section of the root part of a bear tooth.



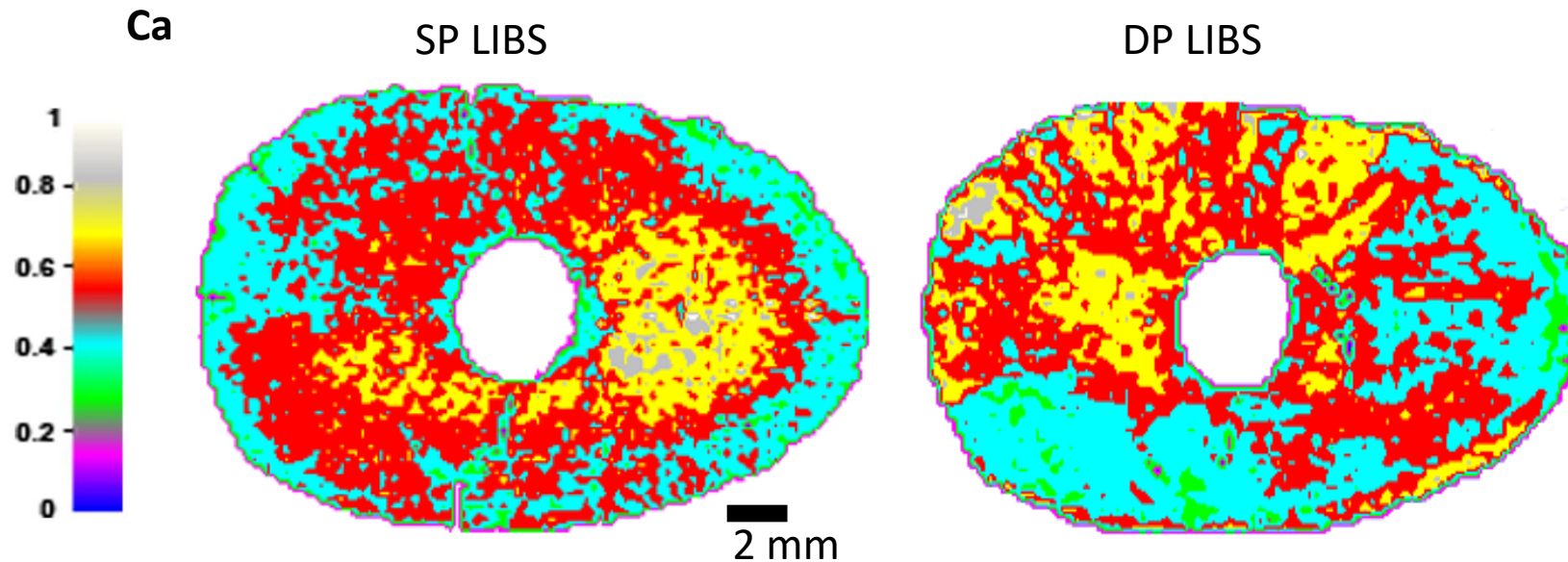
Ursus arctos – mapping of Ba, Sr distribution using SP and DP LIBS (root)



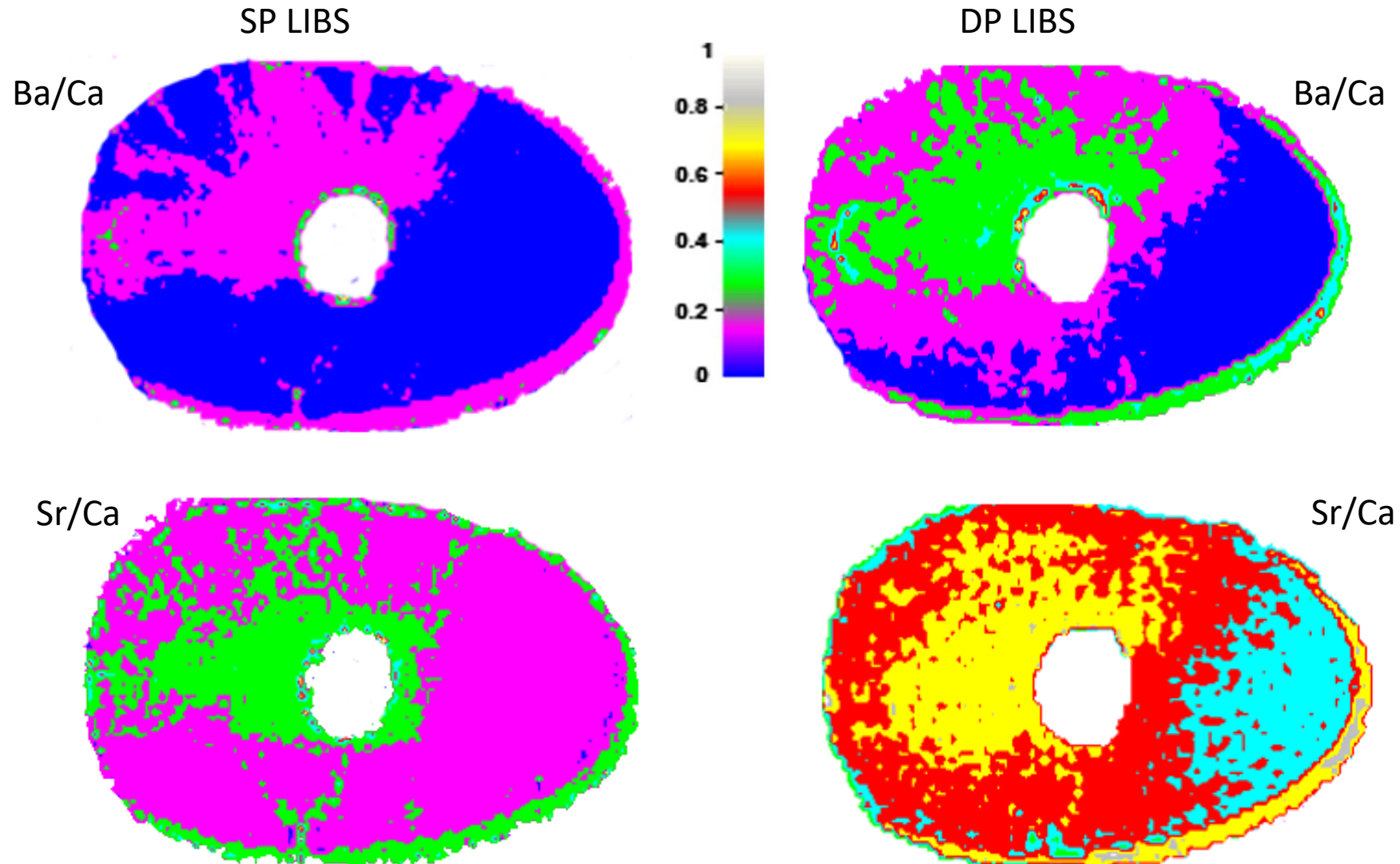
Ursus arctos – Ca distribution mapping using SP and DP LIBS (crown)



Comparison of Ca distribution in a cross section of a bear's tooth crown.



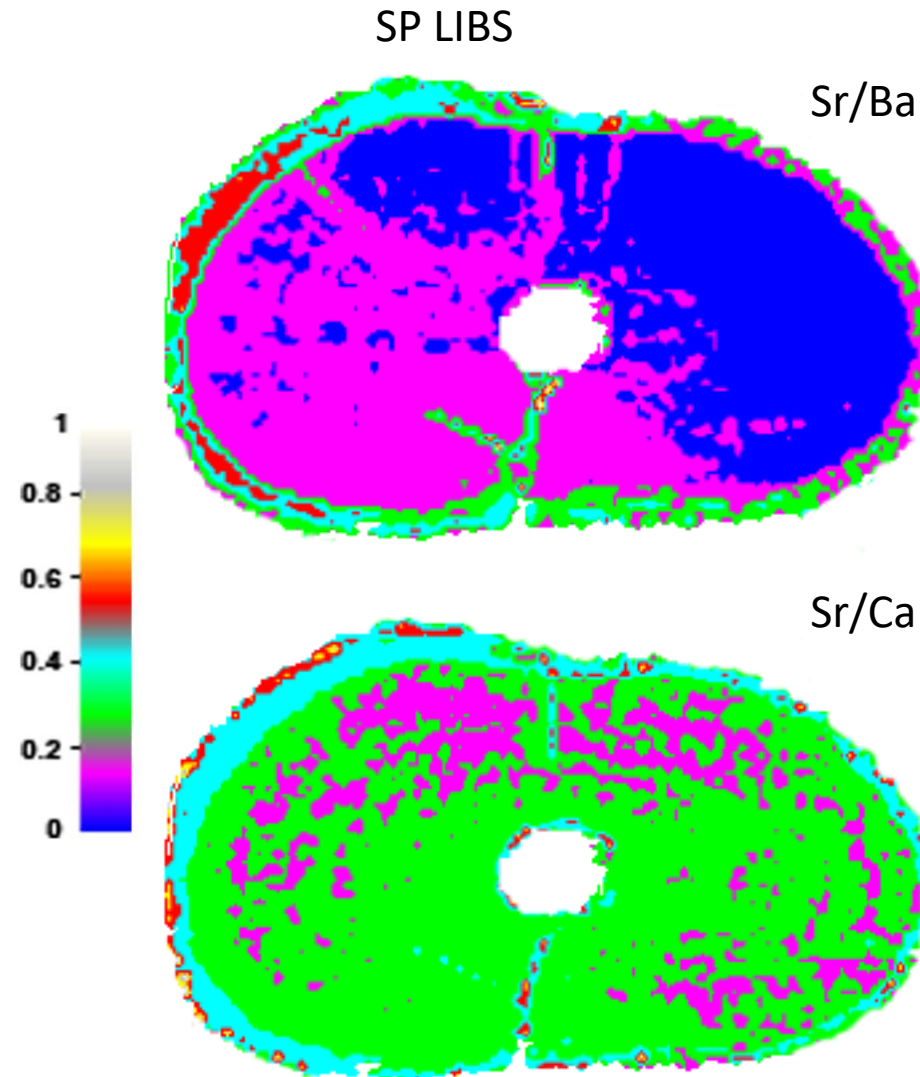
Ursus arctos – Ba, Sr distribution mapping using SP and DP LIBS (crown)



Ursus arctos – etology (root)



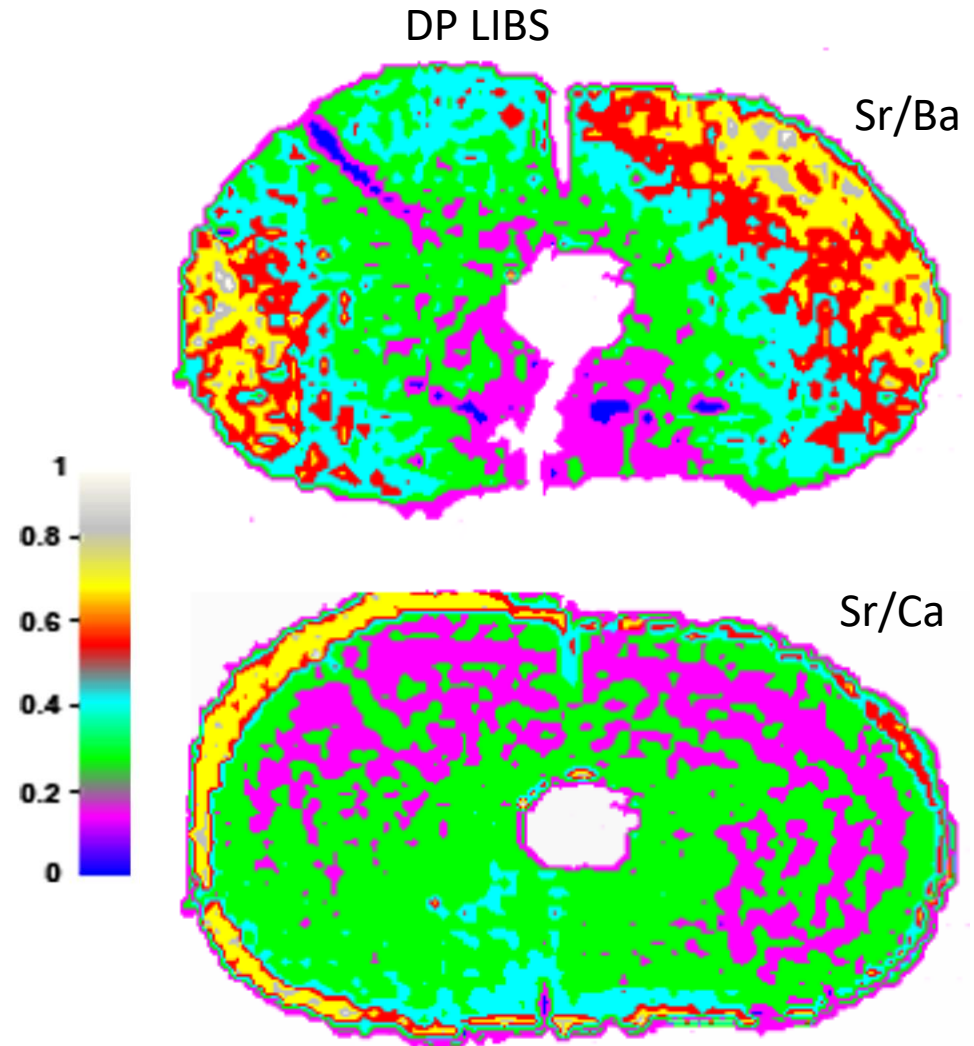
Using DP LIBS and LA-ICP-MS techniques, seasonal fluctuations of Sr/Ca and Sr/Ba were detected, indicating the migration of the animal between the place where the sample was found and the place of hibernation.



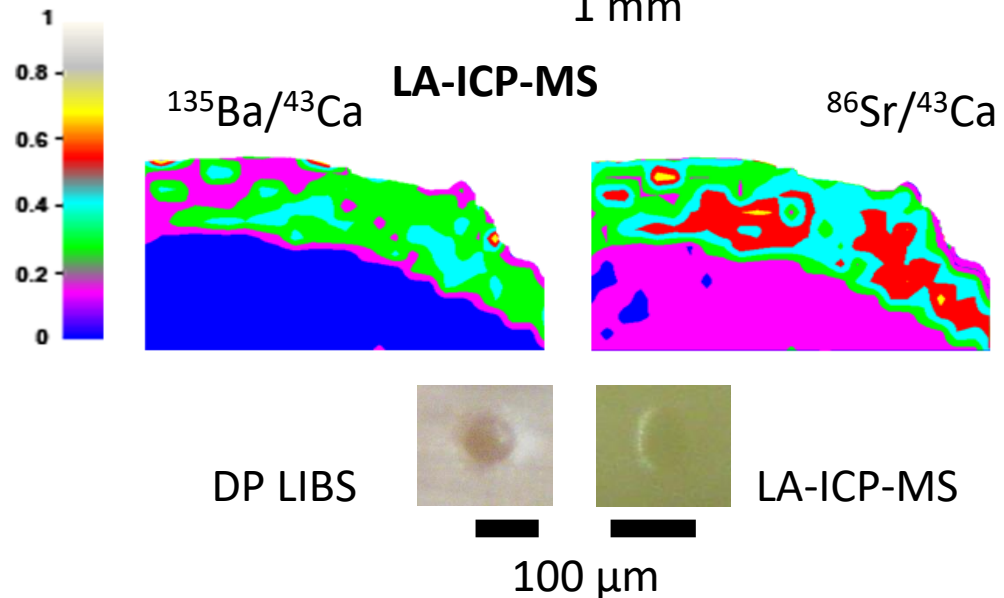
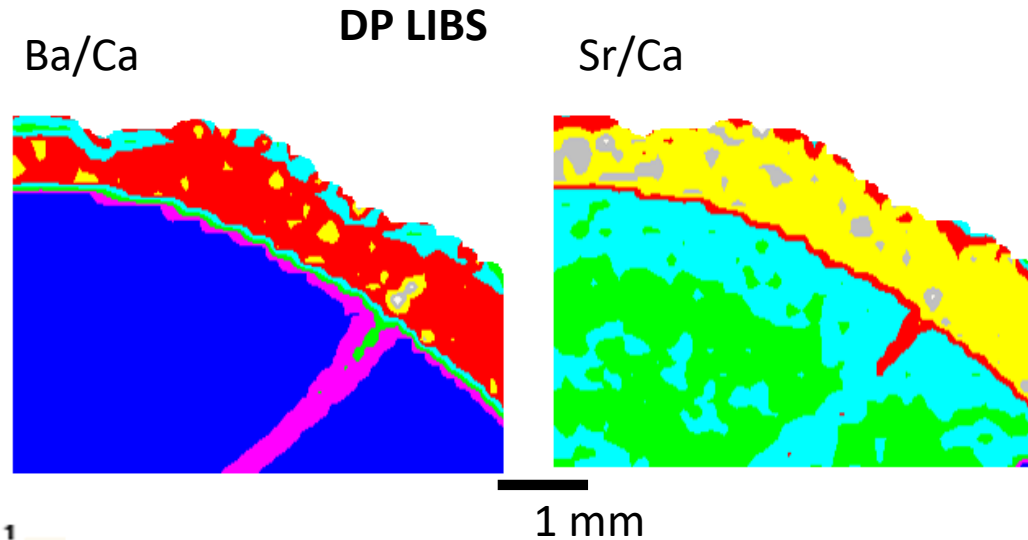
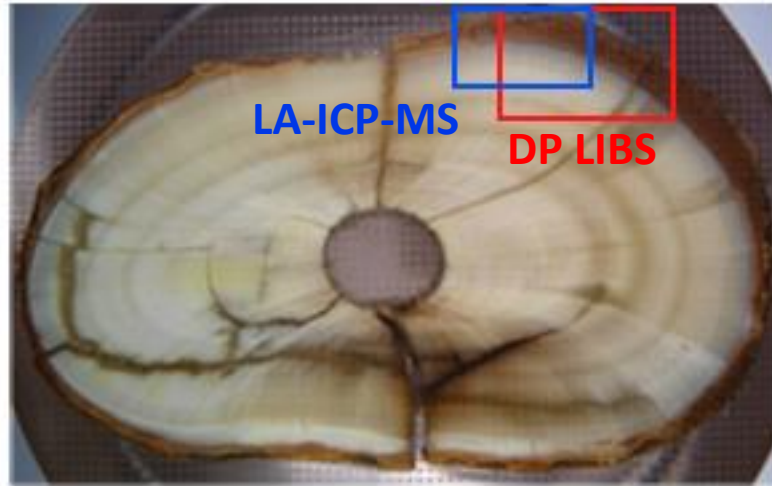
Ursus arctos – etology (root)



Dark areas on the bear canine root sample correspond to areas with lower intensity of Sr/Ca and Sr/Ba ratios in the maps shown. These areas represent narrow winter increments lines.

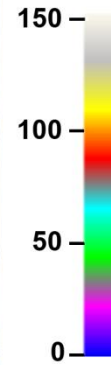


Ursus arctos – comparison DP LIBS a LA-ICP-MS

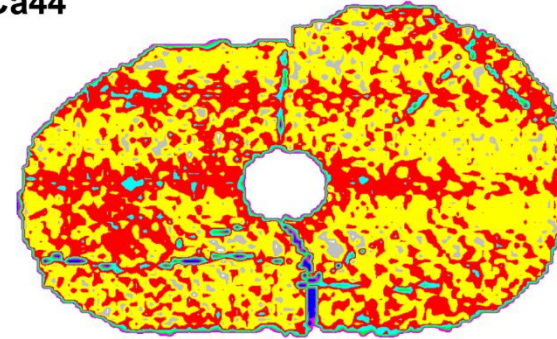


- DP LIBS and LA-ICP-MS ablation grids placed close to each other.
- Comparable ablation crater diameters and similar areal resolution achieved.

Ursus arctos – LA-ICP-MS

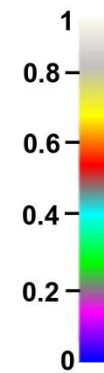
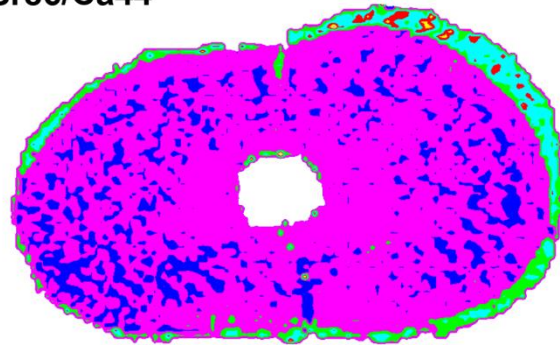


Ca44

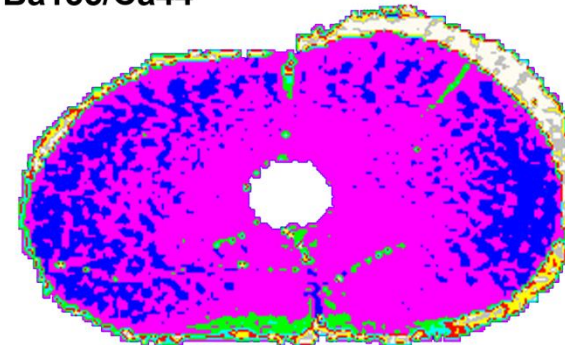


500 μ m

Sr86/Ca44



Ba135/Ca44



**Thank you for
your attencion!**

