

# ARCHAEOGENETIC ANALYSIS OF LATE PLEISTOCENE AND HOLOCENE BISON FOSSILS FROM SLOVENIA AND HUNGARY

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During the last 130,000 years, two species of Bison were traditionally acknowledged to be present in Europe: the steppe bison (*Bison priscus*) and the European bison or wisent (*Bison bonasus*) [1]. The steppe bison appeared already during the Middle Pleistocene and occupied an area ranging from Western Europe to North America [1,2]. It became extinct in Europe at the end of the Late Pleistocene [3], while in some areas, it survived into the Early [4] or Middle Holocene [5]. The morphologically distinct osteological remains of the extant European bison first appear in the fossil record around the start of the Holocene [6]. Phylogenetic inferences based on mitochondrial DNA prove the existence of three clades in the last 50,000 years: Bp, which includes the steppe bison, and Bb1 and Bb2, which are related to the European bison [1,7-9]. Although several dozen Late Pleistocene and Holocene bison remains have been discovered in Slovenia and Hungary, genetic analysis has been mostly performed on (sub)fossils from Western Europe, Poland, the Caucasus, and Siberia. Therefore, our study aimed to analyse the ancient DNA (aDNA) from teeth/bones found in these two regions in order to assign the specimens to the existing clades. For this purpose, we extracted the DNA from 8 samples from the second half of the Late Pleistocene from Slovenia, and 2 Holocene samples from Hungary, morphologically attributed to *Bison sp.*, in a specialised aDNA laboratory. After using an amplicon-based approach with a maximum amplicon length of 198 base pairs, targeting the 12S, 16S, ND2, COI, ND4, and cytochrome b regions of the mitochondrial DNA, we sequenced the amplicons on the Ion Torrent S5 next-generation sequencing system. We processed the sequencing results using an in-house developed bioinformatics pipeline and constructed phylogenetic trees using the BEAST software. We successfully amplified and sequenced aDNA from the samples, followed by the assembly of 2,195 bp long concatenated consensus sequences of the mitochondrial DNA. Our results show that most of the Late Pleistocene *Bison* from the studied areas belong to the Bp clade, while the one close to the Pleistocene/Holocene transition and the Holocene specimens belong to the Bb2 clade. We did not detect the presence of the extinct Bb1 clade. Our research can, therefore contribute new insights into the paleogeography of

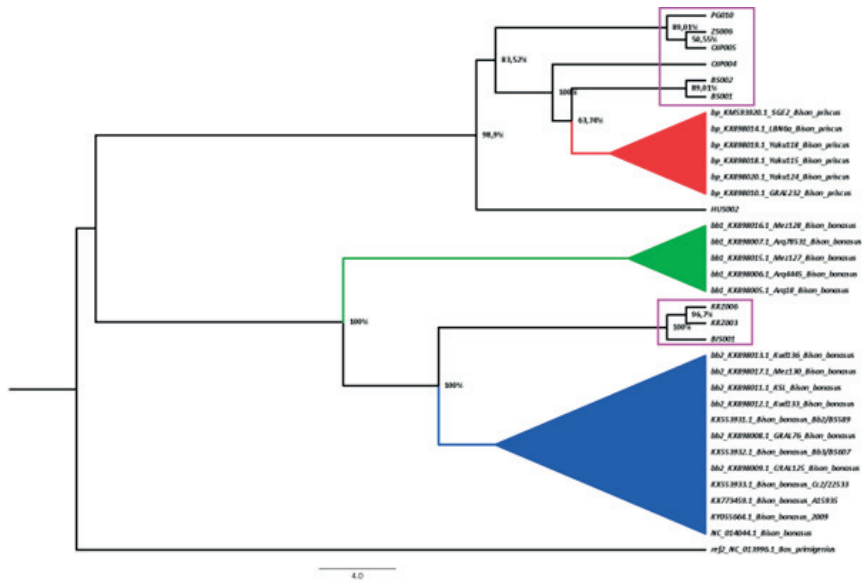


Fig. 1: Phylogenetic tree, constructed with BEAST. The tree shows the relationship between the clades: Bb1 (green) and Bb2 (blue) are closely related to each other, and the Bp clade (red) represents the steppe bison. Our samples are marked with a purple square.

**References:**

1. Massilani D, Guimaraes S, Brugal J-P, Bennett EA, Tokarska M, Arbogast R-M, et al. Past climate changes, population dynamics and the origin of Bison in Europe. *BMC Biol* 2016;14(1):93.
2. Markova AK, Puzachenko AY, van Kolfschoten T, Kosintsev PA, Kuznetsova T V., Tikhonov AN, et al. Changes in the Eurasian distribution of the musk ox (*Ovibos moschatus*) and the extinct bison (*Bison priscus*) during the last 50 ka BP. *Quat Int* 2015;378:99–110.
3. Kurtén B. Pleistocene mammals of Europe. *AldineTransaction*; 1968. 317 p.
4. Kirillova I V., Zanina OG, Kosintsev PA, Kul’kova MA, Lapteva EG, Trofimova SS, et al. The first finding of a frozen Holocene bison (*Bison priscus* Bojanus, 1827) carcass in Chukotka. *Dokl Biol Sci* 2013; 452:296-99.
5. Froese D, Stiller M, Heintzman PD, Reyes A V., Zazula GD, Soares AER, et al. Fossil and genomic evidence constrains the timing of bison arrival in North America. *Proc Natl Acad Sci* 2017;114(13):3457–62.
6. Verkaar ELC, Nijman IJ, Beeke M, Hanekamp E, Lenstra JA. Maternal and paternal lineages in cross-breeding bovine species. Has wisent a hybrid origin? *Mol Biol Evol.* 2004;21(7):1165–70.
7. Soubrier J, Gower G, Chen K, Richards SM, Llamas B, Mitchell KJ, et al. Early cave art and ancient DNA record the origin of European bison. *Nat Commun* 2016;7:1–7.
8. Grange T, Brugal JP, Flori L, Gautier M, Uzunidis A, Geigl EM. The evolution and population diversity of bison in Pleistocene and Holocene Eurasia: Sex matters. *Diversity* 2018;10(3):1–25.
9. Wang K, Lenstra JA, Liu L, Hu Q, Ma T, Qiu Q, et al. Incomplete lineage sorting rather than hybridization explains the inconsistent phylogeny of the wisent. *Commun Biol* 2018; 1:169.