

The conservative region of the 16s rRNA gene differentiating selected species of the Bovidae family

K. R. Lipka, W. Olech, Z. Nowak

Warsaw University of Life Sciences, Faculty of Animal Sciences, Department of Genetics and Animal Breeding, ul. Ciszewskiego 8, 02-786 Warsaw

Introduction



Taxonomic determination of an ancient material derived from European bison (*Bison bonasus*), buffalo (*Bison bison*), aurochs (*Bos primigenius*), and from domestic cattle (*Bos taurus*) is problematic due to a poor condition of the remains. Therefore the explicit identification of this species belonging to one family and having common origin is not always possible among others as a result of introgression, interspecies hybrids – natural or derived intentionally – pose additional problem. Therefore, when biometric species identification of the remains does not give a clear answer, it is necessary to use more explicit methods like mitochondrial DNA (mtDNA) analysis.

Material



- Bone samples of wisent, hybrids of a wisent and a bison and samples described as wisent subspecies hybrids from the State Darwin Museum in Moscow collection, Cherga city in Altai Republic (Russia) and from Białowieża Primeval Forest in Poland – ancient material
- A peripheral blood derived from wisents living in Białowieża Primeval Forest and from the American bisons bred in Kurozwęki (Poland) – contemporary material
- Beef meat samples (*Bos taurus*) from the supermarket
- Wisent, bison, aurochs and cattle 16s rRNA gene sequences obtained from National Center for Biotechnology Information (NCBI)

Methods



- Primers design (Primer3web 4.0.0)
- Genomic DNA extraction
- Amplification of mtDNA fragments in PCR. The product was a 489bp part of 16s rRNA gene sequence.
- Sequencing (Genetic Analyzer ABI 3500)
- Taxonomic similarity determination of the obtained samples (nucleotide BLAST)
- Sequences alignment and trimming to 358bp; identification of polymorphic sites (GeneDoc 2.7.0.00)
- Minimal evolution method (ME) based phylogenetic tree construction (Mega6)

F: 5' GTGACAACCTGTTTAAACGGC3'
R: 5' CAAACGAACCTTTGATAGCG3'

Fig. 1. Primers sequences

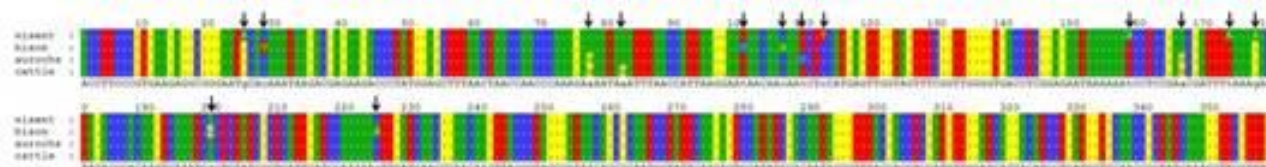


Fig. 2. Polymorphic sites differentiating examined species in 358bp fragment of 16s rRNA gene sequence

Results & Conclusions



- As a result of comparison of the 358bp sequences of the 16s rRNA gene derived from species *Bison bison*, *Bison bonasus*, *Bos taurus* and *Bos primigenius*, overall, 17 polymorphic sites were uncovered, among them 14 differentiating examined species.
- Dendrogram shows evolutionary similarity of cattle and aurochs. It is consistent with the studies of Achilli et al. (2008), which suggested that aurochs mitochondrial genome was transmitted to cattle more than once after domestication.
- Homogenous cluster of the individuals described as *Bison bonasus bonasus*, *Bison bonasus caucasicus* and their hybrids, as well as high probability (99%) with which they collocate indicates low genetic diversity of this species. At this point it is necessary to highlight that examined samples were derived from different periods.
- High genetic similarity of wisent and aurochs confirms theory about introgression of the aurochs genome in to the wisent which happened after distinction of the *Bos* and *Bison* genus (Nowak, Olech, 2008).
- Bison clearly diverges from wisent. Introgression of the yak genome during bisons migration to North America through Asia about 700.000 years ago (Zeyland i wsp., 2012), would explain this much high genetic diversity between this species from the *Bison* family.
- Every hybrid individual was assigned to the cluster of their mother species. Only *Bison-Bos* hybrids established slightly distinct cluster as a result of a single mutation typical only of this hybrids.

Bibliography



- Achilli A., Olivieri A., Pellecchia M., Iliopoulou C., Coll L., Al-Jahery N., Accetturo M., Pala M., Hooshiar Kashani S. H., Perego U. A., Battaglia V., Fornari S., Kalamati J., Houshmand M., Negri R., Semino O., Richards M., MacAulay V., Ferretti L., Bonardi H. J. R., Ajmone-Marsan P., Torroni A. (2008). Mitochondrial genomes of extinct aurochs survive in domestic cattle. *Current Biology*, 18(4), R157-R160.
- Nowak Z., Olech W. (2008). Verification of phylogenetic hypothesis concerning the evolution of genus *Bos*. *Annals of Warsaw University of Life Sciences, Animal Science*, 45, 65-72.
- Zeyland J., Wolk L., Lipiński D., Wasznick A., Nowak A., Szatalska M., Bocianowski J., Storski K. (2012). Tracking of wisent-bison-yak mitochondrial evolution. *Journal of applied genetics*, 53(3), 317-322.

Acknowledgements



We thank the State Darwin Museum in Moscow and all the people involved in the samples collection.

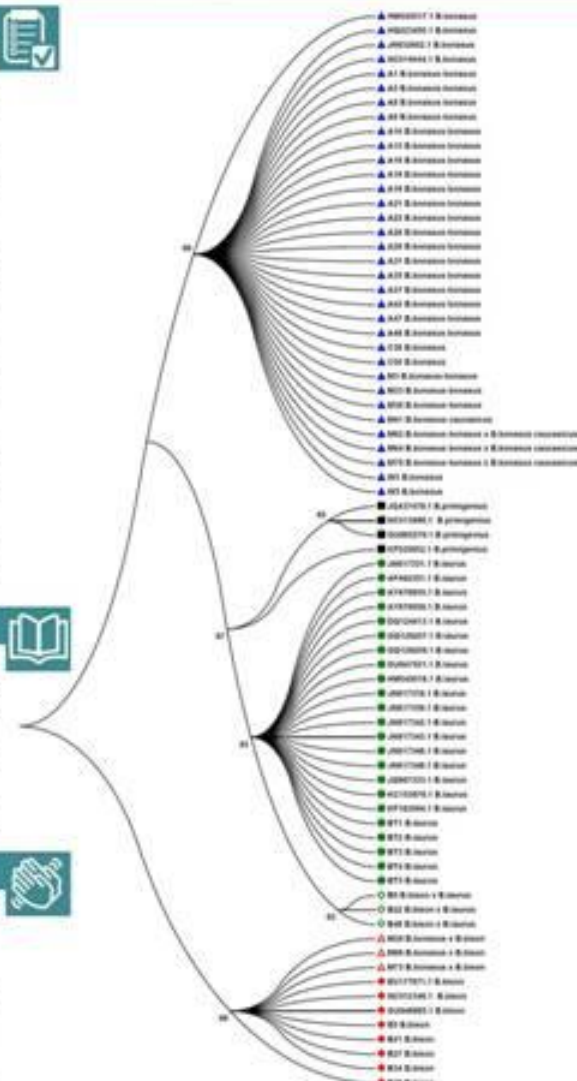


Fig. 3. Phylogenetic tree presenting evolutionary relationship between examined species based on 358bp fragment of 16s rRNA gene sequence