

Phylogenetic reconstructions performed for ancient and modern individuals belonging to Canidae family from the territory of Siberia and Central Asia

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Motivation and Aim: Our work included ancient and recent animals of three extant species from different sites of the Siberia region and an ancient sample of *Cuon alpinus*, extinct species of Siberia. Except for the wolf (*Canis lupus*) and the dog (*Canis lupus familiaris*) species, the data concerning mtDNA sequences of Canidae species in open databases is poor and often restricted by Eastern Asia or Europe regions. Our goal is to add some data about mtDNA sequences of Canidae species for future comprehensive phylogenetic analyses. Existing phylogenetic analyses for *Vulpes vulpes*, *Vulpes corsac* and *Cuon alpinus* are based on some short regions of mtDNA sequence and on the restricted part of an area. Moreover, full ancient mitogenomes are received only for *Canis lupus*, the history of other species' evolution is to be revealed in future. Hence, the aim of the study is to define the phylogenetic connections among ancient and modern representatives of canids from the regions of Siberia and Central Asia.

Methods and Algorithms: In our work ancient samples were collected by paleontologists from different regions of Siberia, such as the Omsk Oblast, the Republic of Khakassia, the Altai Republic, the Irkutsk Oblast, the Krasnoyarsk Krai. One sample was collected from the location in Tajikistan. Modern samples were collected from the Novosibirsk Oblast, the Russian Far East and the Sakha Republic. Ancient DNA was isolated according to Yang's protocol [1] with modifications [2], the libraries for high-throughput sequencing were constructed using TruSeq® Nano DNA Sample Preparation Kit with index sequences from Truseq DNA HT Library Preparation Kit or Truseq Nano DNA LT Sample Preparation Kit (Illumina). According to the protocol of Maricic et al. [3] with modifications [4] enrichment with target fragments in two rounds was carried out. Modern libraries were enriched in one round. Consensus sequences were obtained by alignments against reference sequences. Then models of evolution for every subset in the genome were determined, models of damage were constructed by MapDamage v2.2.0 package [5]. The phylogenetic tree for Canidae was built using the MrBayes program

[6]; the haplogroup median-joining network for ancient and modern fox species was constructed in the Network program (Fluxus Technology Ltd).

Results: We have constructed 34 DNA libraries from ancient and modern isolated DNA samples. Only few ancient samples demonstrated enough mitogenome coverage to use in the phylogenetic reconstruction. However, all 9 modern samples achieved enough coverage (more than 94 %). The haplogroup network was obtained showing some new Siberian haplotypes and some known from available literature haplotypes of the cytochrome B gene. For *Vulpes corsac* there was only one available mitogenome in GenBank database, so we obtained another three haplotypes for this species. An ancient sample took the special position in both reconstructions which was relevant to its geographical location. We gained 68 % of mitogenome coverage for *Cuon alpinus* sample and confirmed its species affiliation. We look forward to increase this value to get the first complete or nearly complete mitogenome of ancient *Cuon alpinus*.

Conclusion: The phylogenetic position for a number of ancient and modern individuals of Canidae family was determined. Furthermore, the group obtained the decent mitogenome coverage value for *Cuon alpinus* sample, which is the first result of that quality for the Siberian individual.

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References

1. Yang D.Y. et al. Technical note: Improved DNA extraction from ancient bones using silica- based spin columns. *Am J Phys Anthropol.* 1998;105(4):539-543. doi 10.1002/(SICI)1096-8644(199804)105:4<539::AID-AJPA10>3.0.CO;2-1
2. Vorobieva N.V., Makunin A.I. et al. High genetic diversity of ancient horses from the Ukok Plateau. *PLoS One.* 2020;15(11):e0241997. doi 10.1371/journal.pone.0241997
3. Maricic T., Whitten M., Pääbo S. Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. *PLoS One.* 2010;5(11):e14004. doi 10.1371/journal.pone.0014004
4. Sanderson C., Radley K., Mayton L. Ethylenediaminetetraacetic acid in ammonium hydroxide for reducing decalcification time. *Biotech Histochem.* 1995;70(1):12-18. doi 10.3109/10520299509108310
5. Jónsson H., Ginolhac A., Schubert M., Johnson P.L.F., Orlando L. MapDamage2.0: Fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics.* 2013;29(13):1682-1684. doi 10.1093/bioinformatics/btt193
6. Ronquist F.R., Huelsenbeck J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics.* 2003;19(12):1572-1574. doi 10.1093/bioinformatics/btg180