

4000-year-old reindeer mitogenomes from the Volga-Kama region reveal continuity among the forest reindeer in northeastern part of European Russia

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Abstract

There are three main ecotypes of reindeer in Eurasia: tundra reindeer, boreal forest reindeer and High Arctic reindeer. Of these, especially the forest reindeer has suffered due to human over hunting and habitat fragmentation. Forest reindeer was still found in the Volga-Kama region at the beginning of the 20th century, but has since disappeared from the region. In order to investigate the genetic relationships of these historical, southernly distributed forest reindeer populations, we obtained mitogenome sequences from six individuals from Pestrechinskaya II, an archaeological site located

in Tatarstan and dated to around 4000 calibrated years before the present (cal BP). The sequences reported in this study represent the first published ancient reindeer mitogenomes. We observed genetic continuity between the historical reindeer from Tatarstan and present day wild populations from the taiga zone of northeastern part of European Russia. Interestingly, four out of the six studied individuals belong to mitochondrial control region haplogroup II, which today is a major haplogroup among the semi-domestic reindeer in Fennoscandia. Even though the haplotypes observed in Pestrechinskaya II site are not closely related to the major haplotypes observed among the Fennoscandian semi-domestic reindeer, the results suggest that this haplogroup may have its origin east of Fennoscandia. It is also interesting to note that the size of the reindeers from the Pestrechinskaya II site was one of the largest observed in the Holocene.

Key words: Reindeer, Tatarstan, ancient DNA, mitochondrial DNA

В Евразии существует три основных экотипа северного оленя: тундровый северный олень, таежный северный олень и высокоарктический северный олень. Из них лесные северные олени особенно пострадали из-за чрезмерной охоты человека и фрагментации среды обитания. Лесной северный олень еще встречался в Волго-Камском регионе в начале 20-го века, но с тех пор исчез из данного региона. Чтобы исследовать генетические связи этих исторических, южно-распределенных популяций лесных северных оленей, мы получили последовательности митогенома от шести особей из Пестречинской II стоянки, археологического памятника, расположенного в Татарстане и датированного около 4000 кал.л.н. Последовательности митохондриального ДНК, описанные в этом исследовании, представляют собой первые опубликованные древние митогеномы северного оленя. Мы выявили генетическую преемственность между историческим северным оленем из Татарстана и современными дикими популяциями из таежной зоны северо-восточной части европейской части России. Интересно, что четыре из шести исследованных особей принадлежат к гаплогруппе II, она сегодня является основной гаплогруппой среди полудомашних оленей в Фенноскандии. Несмотря на то, что гаплотипы, наблюдаемые у северных оленей из Пестречинской II стоянки,

не тесно связаны с основными гаплотипами, наблюдаемыми среди полудомашних северных оленей Фенноскандии, результаты показывают, что эта гаплогруппа может иметь свое происхождение к востоку от Фенноскандии. Интересно также отметить, что размеры северных оленей из Пестречинской II стоянки были одними из самых крупных наблюдавшихся в голоцене.

Ключевые слова: северный олень, Татарстан, древняя ДНК, митохондриальная ДНК

Introduction

Of the three main ecotypes of reindeer in Eurasia, especially the forest reindeer has suffered due to human over hunting and habitat fragmentation. At present, the Eurasian forest reindeer is found in multiple regional subpopulations in European Russia, Finland and Asia, many of which are endangered (Gunn 2016). In historical times however the range of the forest reindeer has been larger and probably more continuous. Reindeer *Rangifer tarandus* L., 1758 is known in the Middle Volga region from the Middle Pleistocene (located in Tunguz) (Alekseeva, 1990). In the second half of the Late Pleistocene (Würm) on the territory of the Middle Volga region, reindeer was a common species of periglacial forest-steppe landscapes (Turubanova, 2002; Petrova, 2009). In the early Holocene and the first half of the middle Holocene, according to the number of bone remains from archaeological sites, its number in the territory of Tatarstan was significantly lower than in Würm (Petrenko, 1984, 2007; Askeyev et al., 2009). In the second half of the middle Holocene (Subboreal period) and at the beginning of the late Holocene (SubAtlantic-1), reindeer were widely distributed throughout Tatarstan, and its populations size was the largest during the entire Holocene period (Zbrueva, 1937; Petrenko, 1984, 2007; Gasilin, 2009; Askeyev et al., 2009). According to archaeozoological data in the 4th-7th centuries and the 10th-17th centuries AD the reindeer lived throughout the territory of Tatarstan, its bones were diagnosed on 10 archaeological sites (Petrenko, 1984, 2007; Askeyev et al., 2016). In the 18th century - the first half of the 19th century, reindeer continued to be found in all large forest areas both north of the Volga and Kama rivers, and on some large woodlands south of these rivers (Eversmann, 1840; Kirikov, 1960, 1966). At the end of the 19th – beginning of the 20th

centuries, this species was very rarely found in the northern and northeastern regions of Tatarstan (Bogdanov, 1871; Kirikov, 1960, 1966). The last reliable data on the findings of reindeer in Tatarstan fall on the twenties of the 20th century (Bashkirov, Grigoriev, 1931; Kirikov, 1960, 1966). In order to study the faith of these southernly distributed reindeer from the boreal forest regions of the Volga-Kama region, we obtained genetic data from 4000-year-old reindeer samples from Tatarstan and compared it with data from modern Eurasian populations. We also compared the body size estimates of the reindeer with estimates obtained from other ancient sites in Russia (see Appendix 2).

Material and methods

Samples and DNA extraction

We subjected six samples from the Pestrechinskaya II site to DNA analysis (Table 1). The samples consisted of post-cranial skeletal parts and teeth. All genetic work prior the sequencing library amplifications was conducted in ancient DNA laboratory located at the Swedish Museum of Natural History. Around 50 mg of bone powder was obtained from each sample by drilling inside the bone. DNA was then extracted using the protocol outlined in Ersmark et al. (2015). This protocol is a modified version of the protocol C in Yang et al. (1998).

Library preparation, mitochondrial genome capture and sequencing

Uracil-DNA-glycosylase (UDG) treated sequencing libraries were built according to step (g) Library preparation: full uracil–DNA–glycosylase treatment (III) as in Rohland et al. (2015), which is based on the methods described in Meyer and Kircher (2010) and Kircher et al. (2012). The six amplified libraries of the reindeer from the Pestrechinskaya II site were pooled together with five other ancient reindeer libraries in equimolar ratios. Each library had a unique barcode combination. The pool was then subjected to mitogenome capture as described in Maricic et al. (2010) using deer-specific baits. After the capture, the pool was turned into a complete sequencing library by PCR, using indexing primers as in Meyer and Kircher (2010). The quality and concentration of the purified

DNA sample code	Sample no	Bone	Archaeological lable (in English and Russian)
P3	3	humerus	Pestrechinskaya II site 2013, Digging 1, plot G/5, layer 9, sector B, 12.08.13, p. 57 (Пестречинская II стоянка 2013, Р.1, уч.Г/5, пласт 9, сектор Б, 12.08.13, стр. 57)
P5	5	metatarsus	Pestrechinskaya II site 2013, Digging 1, plot B/9, layer 11, without location (Пестречинская II стоянка 2013, Р.1, уч.Б/9, пласт 11, б/м)
P10	10	humerus	Pestrechinskaya II site 2013, Digging 1, plot G/5, layer 8, out clusters of bones, 11.08.13 (Пестречинская II стоянка 2013, Р.1, уч.Г/5, пласт 8, вне скопления костей, 11.08.13)
P13	13	metatarsus	Pestrechinskaya II site 2013, Digging 1, plot V 4,5,6, abreast layers 8 – 9, bones from a landslide outcrop and scree (Пестречинская II стоянка 2013, Р.1, уч. В 4, 5, 6, уровень пласта 8-9, кости из обнажения оползня и осыпи)
P17	17	teeth	Pestrechinskaya II site 2013, Digging 1, plot B/10, layer 10 (Пестречинская II стоянка 2013, Р.1, уч.Б/10, пласт 10)
P20	20	phalanx1	Pestrechinskaya II site 2013, Digging 1, plot G/5, layers 9, depth 162,5 cm, 12.08.13 (Пестречинская II стоянка 2013, Р.1, уч.Г/5, пласт 9, гл. - 162,5 см, 12.08.2013)

Table 1. Reindeer samples analyses in the study.

library pool was quantified on a 2100 Bioanalyzer (Agilent), and the pool was combined with other capture pools that had different barcode combinations and indexes into a single pool in equimolar concentrations. The final pool was sequenced on one Illumina MiSeq lane with a 2x151bp setup and on one HiSeq lane with 2x126bp setup.

Data processing

Fastq-data from both runs was merged and demultiplexed based on the unique sample barcodes (custom python script), removing reads with an incorrect barcode pairing (~1% of reads). We then removed sequencing adapters using Trimmomatic (Bolger et al. 2014) and subsequently merged the reads with AdapterRemovalV2 (Schubert et al. 2016). The first and the last 7 base pairs of each read were removed as these represent the barcodes. Merged reads were then mapped to the reindeer mitogenome reference (GenBank accession number KM506758, Ju et al. 2016) using bwa aln, (Li and Durbin 2009) excluding reads below 15 basepairs. During the mapping, the human mitogenome (hg19 and PhiX genome (NC_001422) reference sequences were used as decoys. We then removed duplicates (samtools rmdup, Li et al. 2009). Mitogenomes were constructed by calling the major allele at each site covered by at least three independent reads and above 90% of reads agreeing on the major allele.

Mitogenome sequence phylogeny

The consensus sequences with at least 3x coverage were used in the following analyses. We included a published mitogenome of an *Aoluguya* reindeer (GenBank accession number KM506758, Ju et al. 2016), and aligned the sequences using MAFFT online version 7 (<https://mafft.cbrc.jp/alignment/server/>, Katoh et al. 2002; Katoh and Standley 2013; Katoh et al. 2017). In order to infer phylogenetic relationships among the study samples, we then built a Bayesian phylogenetic tree using MrBayes version 3.2 (Ronquist et al. 2012), running the analysis for 2,500,000 generations and saving every 1000th sample. HKY+I substitution model was used in the run, as this was inferred as the most optimal according to jModelTest version 2.1.4 (Guindon and Gascuel 2003; Darriba et al. 2012) that could be used in MrBayes. The first 250,000 samples were discarded as burn-in, and a 50 percent majority rule tree was visualized using FigTree version 1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

MtDNA control region haplotype sharing

Due to the limited number of complete mitogenomic sequences for comparative purposes, we made further analyses using only the control region, from which there is more reference data available. First

we studied possible haplotype sharing between the historical reindeer from Tatarstan and present day populations. We included a large number of sequences representing both wild and domestic Eurasian reindeer diversity (Røed et al. 2008; Kholodova et al. 2011; Baranova et al. 2012; Kvie et al. 2016a; Kvie et al. 2016b; Korolev et al. 2017), aligned these together with our sequences, and truncated the dataset to 179 base pairs in order to accommodate all the sequences. We then identified shared haplotypes within the dataset using PopART version 1.7 (<http://popart.otago.ac.nz>).

MtDNA control region haplogroup affiliations

In order to identify to which mtDNA control region haplogroup each sample belonged to, we made a phylogenetic tree with representative haplotypes of each haplogroup from Kvie et al. (2016b). This was done with BEAST version 1.10.4 (Suchard et al. 2018) using tip dates (Drummond et al. 2002), HKY+gamma+invariant sites as a substitution model with 4 gamma categories, strict clock and GMRF Bayesian Skyride (Minin et al. 2008) as a tree prior. The analysis was run for 100000000 iterations logging parameters every 10000 iterations. Maximum clade credibility tree was built after discarding the first 10000000 states as burnin. The tree was visualized with FigTree version 1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results and discussion

Mitogenome sequence phylogeny

98-99% of the sequence was resolved at 3X coverage, for all samples except P13, which was resolved to 87%. These sequences have been submitted in GenBank under the accession numbers MK608014-MK608019. Each sample has a unique haplotype. The general relationships of the mitogenomes are shown in Figure 1. All groupings have a high support. Samples P20 and P17 group together and further form a group with a modern Aoluguya reindeer from China. Samples P3 and P10 are closely related to each other, and together group with P13. Sample P5 is basal to this latter group.

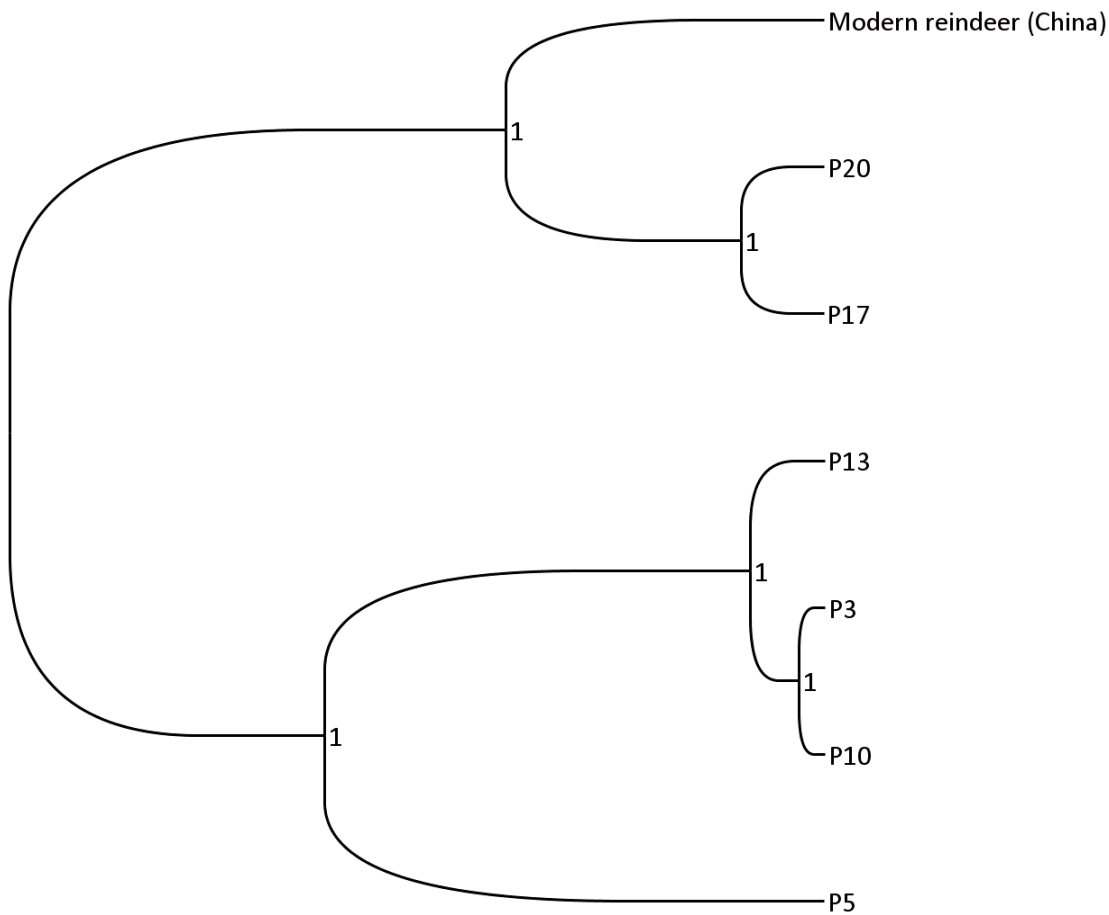


Figure 1. Bayesian phylogenetic tree depicting the relationships of the study samples and a modern Aoluguya reindeer from China.

MtDNA control region haplotype sharing

Because this part of the analysis is based on very short sequences, the results should be interpreted with some caution. We, however, observed mtDNA continuity between the historical reindeer of the Volga-Kama region and present day wild populations of the northeastern part of the European Russia: Sample P13 had the same haplotype as some wild reindeer from Cispolur Urals and Taimyr. Samples P3 and P10 shared a haplotype with wild reindeer from Mezen and Peza–Kosminsk regions. Sample P5 shared a haplotype with wild reindeer from Cispolur Urals as did the sample P17. Sample P20 had a unique haplotype. All in all, the historical reindeer from Tatarstan shared haplotypes especially with modern reindeer from the taiga zone of the northeastern part of European Russia, implying genetic relatedness between these populations. It is also worth noting that we didn't observe any haplotype sharing with Eurasian domestic reindeer nor the Finnish forest reindeer.

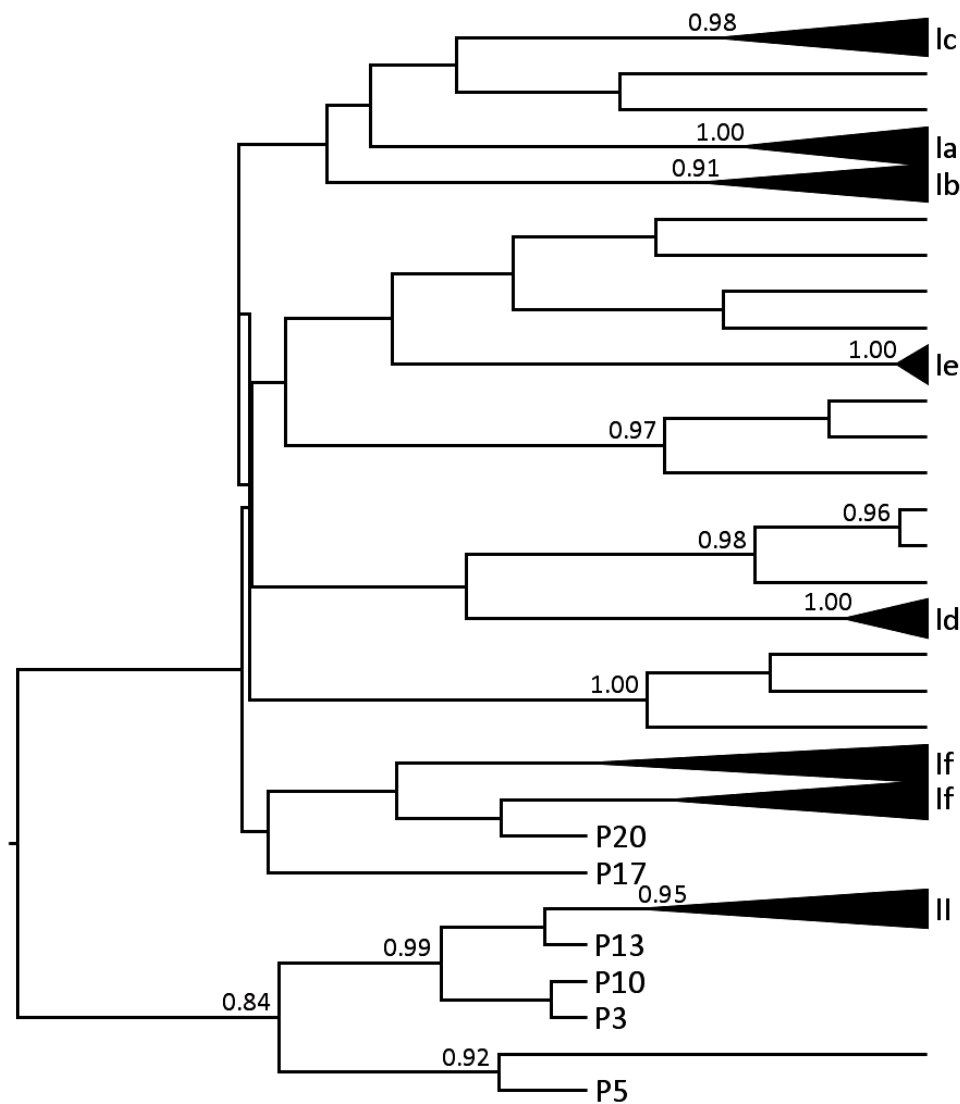


Figure 2. Bayesian phylogenetic tree showing the mitochondrial control region haplogroup affiliations of the study samples. Posterior probabilities above 0.80 are shown above the nodes. The branches that are unlabelled, represent undefined mtDNA control region haplogroups.

MtDNA control region haplogroup affiliations

As seen from the Figure 2, samples P5, P13, P3 and P10 take basal positions in haplogroup II. Based on mitochondrial control region data, this haplogroup is at present mostly found in western parts of the reindeer distribution in Eurasia, and is especially common among the semi-domestic reindeer of Fennoscandia, where together with haplogroup Ib, it is the dominant haplogroup (Flagstad and Røed 2003; Røed et al. 2008; Kvie et al. 2016b). The fact that a lot of basal diversity regarding this haplogroup is observed among the ancient reindeer from the Pestrechinskaya II site, might suggest that this haplogroup has its origin east of Fennoscandia. The haplogroup II haplotypes observed

among the ancient reindeer from the Pestrechinskaya II site are not however particularly closely related to the haplotypes observed among the Fennoscandian semi-domestic reindeer, which together with the absence of haplogroup Ib in Pestrechinskaya II site may suggest that the Fennoscandian domestic reindeer lineages have probably not directly originated from the population presented by the Pestrechinskaya II site. Samples P17 and P20 are placed on the base of haplogroup If, but without statistical support due to the low resolution on the deeper nodes in haplogroup I.

Conclusions

Our results suggest that there is genetic continuity between the historical reindeer from the Volga-Kama region and present day wild reindeer from northeastern part of the European Russia, especially from the taiga zone. Even though our sample size was rather small, we further observed surprisingly lot of basal diversity within mitochondrial haplogroup II, and this finding may have significance regarding the deep history of this haplogroup.

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TABLE AND FIGURE LEGENDS

Table 1. Reindeer samples analysed in the study.

Figure 1. Bayesian phylogenetic tree depicting the relationships of the study samples and a modern Aoluguya reindeer from China.

Figure 2. Bayesian phylogenetic tree showing the mitochondrial control region haplogroup affiliations of the study samples. Posterior probabilities above 0.80 are shown above the nodes. The branches that are unlabelled, represent undefined mtDNA control region haplogroups.

APPENDIX 1.

SELECTION, ETIQUETTE AND EXAMPLE OF PREPARATION OF SAMPLES OF ANCIENT BONES OF THE REINDEER OF TATARSTAN REPUBLIC (LABORATORY BIOMONITORING OF THE INSTITUTE OF PROBLEMS IN ECOLOGY AND MINERAL WEALTH, TATARSTAN ACADEMY OF SCIENCES, KAZAN, RUSSIA)



APPENDIX 2.

THE SIZE OF THE REINDEER FROM HOLOCENE TIME IN THE RUSSIA

As for the dimensional variability of reindeers in the Holocene, very little research is done mainly on the measurement of bones from individual archaeological sites without significant comparative aspects with similar osteological collections. I. Tsalkin's scientific publications (1961, 1962) provide data on the measurement of the bones of reindeer from a number of archaeological sites in the forest zone of the Upper Volga region dating back to the period of the beginning of the first millennium AD. A fairly extensive database on the size of the bones of the reindeer from the Holocene of the Urals and Western Siberia is given in the articles and PhD thesis of P. Kosintsev (1991, 1992, 1997a, b, 2009), (Razhev, Kosintsev, Ulitko, 2005) and the thesis PhD of O. Bachura (2006). They conclude that in the Holocene the reindeers of the Urals had large bones of the skeleton compared to the late Pleistocene and apparently belonged to the forest form. At the same time, in the late Holocene of Western Siberia P. Kosintsev (1997a, b) and at the end of the Middle Holocene of the Polar Urals (Kosintsev, 2009), based on comparatively large osteological material, concludes that reindeers in the forest-tundra and the northern taiga belt were very similar to the representatives modern tundra subspecies *Rangifer tarandus tarandus*. Interesting results with the use of statistical methods of research on reindeer osteology and osteometry of the early Holocene frozen site in the Siberian High Arctic (study on the Zhokhov site faunal remains, De Long Islands LE-3534: LE-3529 Reindeer bone fragments 8050 ± 70 , LE-3536 Reindeer antler 8610 ± 220 , Reindeer bone fragments 7810 ± 180 , GIN-6400 Reindeer humerus 7930 ± 40) were received (Pitulko, Kasparov, 1998; Pitulko et al., 2015). Comparison of the size of the reindeer bones from the island of Zhokhov with the similar sizes of the reindeer of the late Pleistocene of the Urals and Transbaikalia and with modern tundra populations was made: the reindeer of the early Holocene of the island of Zhokhov were somewhat larger than the late Pleistocene Ural reindeer and much larger than the Transbaikalian ones, while they were almost identical to the representatives modern tundra reindeer (*Rangifer tarandus tarandus*), however, the reindeer of the island of Zhokhov were much more graceful (Pitulko, Kasparov, 1998).

On the basis of the algorithm proposed by Weinstock (1997a, b, 2000, 2006), we made a preliminary calculations of the Variability Size Index (VSI). The VSI is calculated according to this formula: $VSI = (x - m / 2 \times s) \times 50$; where x represents the actual measurement for which the index is being calculated, m is the arithmetical mean of the standard population for the dimension in question and s is the standard deviation of the standard population for that dimension. For all the VSI's of a bone fragment, the mean is calculated and used further. Combining all the individual 'mean VSI's' from a site ensures that the sites can be compared to each other. VSI calculated based on the data of osteometric studies of Holocene reindeer from Russia (see Figure) : 1. Early Holocene - island of Zhokhov, The Novosibirsk Islands (76°08'N 152°43'E (Pitulko, Kasparov, 1998; Pitulko et al., 2015); 2. Yanganape 2 (Layer 3. 3320 ± 50 BP, CO AN - 3930; (67°42'N 67 ° 51'E)), the Polar Urals (Kosintsev, 2009); 3. As a standard population, of the reindeers data from Ust-Poluy site (Salekhard, 66°56'N 66°56'E) were used (the tundra-forest zone, the end of the first millennium BC - the beginning of the first millennium AD) (Kosintsev, 1997a); 4. Vermulegan 1 (15th-16th centuries AD) (65°47'N 64°04'E) (Kosintsev, 1997b); 5. The settlements of the Upper Volga region (the beginning of the first millennium AD) (Tsalkin, 1961, 1962); 6. Pestrechinskaya II site (end of the Middle Holocene 3700 BP), Republic of Tatarstan (55°72'N. 49 ° 63'E) (Askeyev I. V. personal data); 7. Grotto Bobylek, Middle Ural (56°23'N. 57 ° 37'E) (1743±110 BP – IEPA – 139a, 1713±110 BP – IEPA – 140a), (Razhev, Kosintsev, Ulitko, 2005).

Based on the results of the calculation of the Variability Size Index (VSI), it can be concluded that the reindeer of the forest belt of Eastern Europe in the Holocene were very large (Pestrechinskaya II site – VSI = 69,6 and settlements of the Upper Volga region - VSI = 52,64) and should refer to a large forest reindeer form similar to the *Rangifer tarandus fennicus* Lonnberg, 1908 (modern forest reindeer is VIS = 46,65. Calculations are performed according to osteometric data from Sokolov, Chernyavsky, 1962) and middle size (Grotto Bobylek – VSI = 14,45). Eduard Friedrich von Eversmann also drew attention to the very large sizes of taiga reindeer from Kazan province compared to semi-domestic reindeer from Siberia (Eversmann, 1840). Professor E. F. von Eversmann was the

first zoologist who not only saw, but also carried out measurements (8 specimens) of reindeer from the taiga forests of the Volga – Ural region. The reindeer of the Holocene tundra and forest-tundra, as well as the northern part of the taiga zone of Western Siberia, were approximately of the similar size (VSI = 1,95; -0,94; 0,2 (standart population) and in their size should be referred to the tundra form (The modern tundra reindeer is VIS = 4,93. Calculations are performed according to osteometric data from Kuzmina, 1971). The reindeer of the early Holocene from the Island of Zhokhov according to the results of VSI (-5,49) were not large - High Arctic ecotype, that's probably consistent with the fact that they lived on the northern edge of its range, and the population had an insular character. Thus, the VSI -method applied to reindeer showed the existence of three main ecotypes of reindeer on the territory of Russia in the Holocene: tundra reindeer, boreal forest reindeer and High Arctic reindeer. In addition, this method is very effective for determining the assessment of the climatic parameters of the existence of different populations in ecotypes of reindeer. Reindeer body size variability could be used as a reliable proxy for environmental conditions during Holocene.



Figure. Representation of reindeer body size and mean VSIs from Holocene sites of the Russia. Standard population (3) from Ust-Poluy site (Kosintsev, 1997a).

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