Assessment of mtDNA variability and phylogenetic relationships of Siberian local horse breeds

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Abstract. Local horse breeds of Siberia have universal economic characteristics and good adaptive qualities. The most valuable gene pool of these breeds has been formed over the centuries, and is of considerable interest for study at the molecular genetic level. The aim of our research was to study the sequence of the hypervariable region of the D-loop of mtDNA in 88 horses of the Buraytskaya, Zabaikalskaya, Tuvinckaya, and Yakutskaya breeds. Analysis of the sequenced D-loop sequence of 530 bp was performed using the Neighbor-Joining method in combination with bootstrap analysis in the MEGA7 program. When studying the polymorphism of the hypervariable region of the D-loop of mtDNA in local horses of Siberia, we identified 16 haplogroups: A, B, C, D, E, G, I, L, M, N, O, P, Q, and R, according to the modern classification. Three additional mtDNA haplogroups with a high level of bootstrap support (100%) were found in Buryatskaya horses. Sequenced fragments of the D-loop from 15471 to 16000 nucleotide positions included up to 113 polymorphic sites, mainly represented by transitions. The studied horse breeds differed among themselves in the structure of the mitochondrial genome and demonstrated a high level of diversity of haplotypes and haplogroups typical of the Mongolian population and other horses of Eurasia.

1. Introduction
Purposeful preservation of the genetic diversity of animal breeds is of decisive importance for agriculture, food production, the development of many regions, ensuring the employment of the population and the independence of the country's economy as a whole. The global trend of increasing demand for livestock products puts forward a number of urgent tasks, aimed at increasing the production of meat for all types of farm animals, including horse meat and products from it.

The Russian Federation possesses a significant variety of horse breeds for sporting, productive and universal purposes. The State Pedigree Register of Breeding Achievements includes 44 breeds and 5 pedigree types of horses, half of which are represented by local breeds. The greatest variety of factory and local horse breeds is presented in the Siberian Federal District, where 419.3 thousand horses are...
concentrated, which is 32.7% of the total horse population in the country. Today Siberia is a leading region for meat herd horse breeding with positive dynamics of development over the past years [1].

The most valuable gene pool of horse breeding in the Russian Federation is the unique local horse breeds of Siberia, including the Altaiskaya, Buryatskaya, Zabaikalskaya, Priobskaya, Tuvinckaya and Yakutskaya horse breeds, which were formed for centuries under the influence of folk selection and natural selection. All of them are well adapted to harsh climatic conditions, year-round herd and pasture maintenance and do not require significant costs for the production of high-quality meat [2].

Assessment of biological diversity of populations and breeds at the genetic level is part of the monitoring of animal genetic resources [3]. Studies of the molecular genetic characteristics of horses of domestic breeds using DNA microsatellites have shown the uniqueness of the allele pool of many local breeds [4, 5, 6, 7]. In horses of the native breeds of Siberia, including the Altaiskaya, Priobskaya, Tuvinckaya and Yakutskaya, private alleles were identified, indicating a high level of biological diversity [6, 8]. It was found that local domestic horse breeds form one common subcluster, confirming the commonality of their origin, and differ markedly from cultivated breeds in the spectrum of alleles [9]. The high variability of the mtDNA D-loop sequence and maternal inheritance make it a unique object for studying the origin and phylogenetic relationships of breeds. Fundamental studies of mitochondrial DNA polymorphism have shown the presence of several wild ancestors in horses and the existence of several regions of domestication [10]. The first information about the matrilineal structure of horse breeds in Russia appeared somewhat later; previously unknown mtDNA sequences were found in horses of the Akhal-Teke, Vyatskaya, Mezenskaya, Orlov Trotter and Yakutskaya breeds [11]. Further studies confirmed the high level of mtDNA polymorphism in domestic horse breeds [12, 13].

The aim of our research was to study the variability of the mitochondrial genome in local horses of Siberia, including the Buraytskaya, Zabaikalskaya, Tuvinckaya, and Yakutskaya breeds. The objectives of our research included:

- a comparative study of the variability of the mtDNA D-loop region in horses of the Buraytskaya, Zabaikalskaya, Tuvinckaya, and Yakutskaya breeds;
- assessment of the level of genetic divergence of the matrilineal structure of local breeds of horses in Siberia;
- determination of the phylogenetic relationship of modern populations of local horses.

2. Materials and methods

Hair samples from 88 local horses, including 15 Buryatskaya, 31 Zabaikalskaya, 12 Tuvinckaya and 30 Yakutskaya horses were used as material for the research. DNA was isolated from hair follicles using the ExtraGene DNA Prep 200 kit manufactured by the Isogen laboratory (Moscow) according to the manufacturer’s instructions. The design of primers for amplification of a given region of the D-loop of mtDNA (nucleotide positions 15471-1600) was developed [12] taking into account the reference sequence of the fossil Swedish horse X79547 [15].

Separation of PCR fragments in agarose gel was performed at OOO Genetics (Moscow) using a Bigdye Terminator Cycle Sequencing Kit (PE Applied Biosystems, USA) on an ABI 3130xl genetic analyzer (PE Applied Biosystems, USA) in accordance with the manufacturer’s recommendations.

All obtained 530 bp mtDNA sequences were aligned using BioEdit 7.2.1 software. To identify mtDNA sequences, GenBank data on 18 known haplogroups (A-R), placed under accession numbers JN398377-JN398457, as well as information on haplotypes of the Yakut horse (20: DQ32280-DQ328057) were used as a standard. For comparison, data from the D-loop sequences of 64 Mongolian horses (GenBank JQ936335-JQ936399) were also taken there. For phylogenetic analysis of the mtDNA D-loop region, the maximum composite likelihood (MCL) model was used in combination with bootstrap analysis using the MEGA7 program. The Neighbor-Joining method was used to construct a phylogenetic tree. Comparative analysis of horse mtDNA D-loop sequences, taking into account haplotypes and nucleotide substitutions, was performed using the DnaSP program [17].
3. Results and discussion

In the horse genotypes of four local breeds of Siberia, a wide variability of mtDNA haplotypes (88 to 88 individuals) was revealed, belonging to 16 out of 18 known haplogroups according to the classification of Achilli et al [16]. The tested horses of local breeds lacked the rare haplogroup K, as well as the haplogroup F, typical for *E. ferus Przewalski* [11]. In addition, 3 new mtDNA haplogroups not included in the standard list, A-R, were found in Buryatskaya horses (figure 1).

Local horse breeds differed markedly among themselves in their matrilineal structure (table 1). For Buryat horses, the most typical haplogroups were B, L, O-P Q (13.33%). The proportion of horses belonging to three additional haplogroups was 20%. In the studied region of the mtDNA D-loop in these horses, transitions (36) and transversions (67) were quite common; the ratio of nucleotide substitutions Rsi/sv was 0.5.

![Figure 1. Phylogenetic tree of mtDNA D-loop sequences from haplotypes of native horse breeds constructed by the Neighbor-Joining method in combination with bootstrap analysis. For identification of haplogroups were used GenBank data (JN398377- JN398457) and their classification according to Achilli et al. [16].](image)

| Haplogroup | Buraytskaya N=15 | Tuvinskaya N=12 | Yakutskaya N=30 | Zabaikalskaya N=31 | Mongolian N=64 |
|------------|------------------|-----------------|-----------------|-------------------|---------------|
| A          | 0.00             | 41.67           | 16.67           | 0.00              | 6.20          |
| B          | 13.33            | 0.00            | 3.33            | 19.35             | 11.0          |
| C          | 0.00             | 8.33            | 20.00           | 6.45              | 6.20          |
| D          | 6.67             | 0.00            | 3.33            | 0.00              | 3.10          |
| E          | 0.00             | 0.00            | 16.67           | 0.00              | 6.20          |
| F          | 0.00             | 0.00            | 0.00            | 0.00              | 0.00          |
| G          | 6.67             | 8.33            | 0.00            | 16.13             | 3.10          |
| H          | 0.00             | 0.00            | 0.00            | 12.90             | 1.60          |
| I          | 6.67             | 0.00            | 3.33            | 0.00              | 0.00          |
| J          | 0.00             | 25.00           | 6.67            | 0.00              | 0.00          |
| K          | 0.00             | 0.00            | 0.00            | 0.00              | 0.00          |
| L          | 13.33            | 0.00            | 16.60           | 3.23              | 17.20         |
| M          | 0.00             | 0.00            | 0.00            | 9.68              | 11.00         |
| N          | 6.67             | 0.00            | 0.00            | 0.00              | 0.00          |
| O-P        | 13.33            | 16.67           | 10.00           | 0.00              | 12.50         |
| Q          | 13.33            | 0.00            | 3.33            | 25.81             | 18.80         |
| R          | 0.00             | 0.00            | 0.00            | 6.45              | 3.10          |
The horses of the Zaikal'skaya breed were also characterized by a high individual variability in the haplotypes of the mitochondrial genome. Among the 8 defined mtDNA haplogroups, haplogroups Q (25.81%), G (16.3%), H (12.90%) and M (9.68%) dominated. The sequenced region of the mitochondrial genome of these horses included 17 nucleotide substitutions, represented mainly by transitions. The average number of substitutions per site was 0.03 ± 0.022, which indicates a relatively high level of individual diversity in this breed.

The genetic structure of the Tuvinskaya horse breed, represented by individuals from 3 farms, was dominated by the “ancient” haplogroup A (41.67%), typical for fossil horses of Northern Europe [11]. A rare haplogroup J was found in 25% of Tuvinskaya horses, indicating that ancestors of eastern origin participated in the creation of this breed [18]. Horses with haplogroups C (8.33%), G (8.33%) and O-P (16.67%) were found much less frequently in the breed. According to GenBank, the matrilineal structure of the Tuvinskaya horse breed is also represented by haplogroups D, E, H, K, L, Q, and R [13], which indicates its relationship with the Mongolian horse. In the analyzed region of the mtDNA D-loop of 12 Tuvinskaya horses, 7 nucleotide substitutions, represented by transitions, were found.

The Yakutskaya horse, the northernmost horse population in Siberia, was characterized by a wide variety of mitochondrial genome sequences. In 30 Yakut horses, belonging to 11 out of 18 known mtDNA haplogroups was determined, among which A (16.67%), C (20.00%), E (16.67%), and L (16.67%) were most common. A characteristic feature of Yakutskaya horses is the high frequency of occurrence of the rare haplogroup E, found in the Mongolian population. The sequenced region of the mtDNA D-loop of the tested Yakut horses included 18 nucleotide substitutions represented by transitions.

The clustering of haplogroups of local horse breeds shown in the figure indicates a high individual variability of mtDNA haplotypes that support biodiversity of populations, and a certain commonality of the matrilineal structure of these breeds, formed under the influence of horse breeding of Asian nomadic tribes.

4. Conclusions
Until recently, information on the genetic structure of native breeds of horses in Russia was practically absent, which did not allow obtaining a complete picture of horse domestication. Comparative analysis of the mtDNA D-loop sequence of four local horse breeds in Siberia revealed a high level of variability of haplotypes and haplogroups (A-R, n = 16) in horses of all breeds studied, as well as their relationship with ancient horses of Northern Europe and East Asia. In Buryatskaya horses, three new mtDNA haplogroups and the maximum number of nucleotide substitutions in the sequenced sequence were additionally found. This allows us to consider the territory of Siberia as one of the centers of domestication and centuries-old migration of horses, which had a significant impact on the formation of horse breeds in Europe.

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