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GENETIC DIFFERENTIATION OF THE WILD GOATS (GENUS *Capra*) BASED ON THE ANALYSIS OF MITOCHONDRIAL GENE CYTOCHROME *B* AND FRAGMENT OF NUCLEAR GENE SRY

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Abstract

The phylogenetic analysis of sequences of the mitochondrial (cytochrome *b*, 1128 b.p.) and nuclear DNA (intron of gene SRY, 1832 b.p.) of *Capra* species was carried out. Our study includes both original data (46 samples) and data published by other authors (Takada 1997, Hassanin *et al.* 1998, Manceau *et al.* 1999, Kahila & Greenblatt 1999 *et al.*). It is shown that two geographical forms of *C. sibirica* exist, and the level of their differentiation is close to the species level. *C. falconeri,* which has a peculiar morphology, genetically is not remote from other species. Genetic distance between *C. caucasica* and *C. cylindricornis* is appreciable. Our data confirm the assumption (Pidancier *et al.* 2006) that ancient interspecific hybridization might have had a great significance for formation of the modern genetic diversity of *Capra* species. Furthermore, there is some genetic evidence of recent and contemporary hybridization in wild goats. According to our data an intensive hybridization takes place between *C. caucasica* and *C. cylindricornis.* Also there are actual proofs of the crossing events between Caucasian goats and *C. aegagrus* in the Caucasus, and between two distinct forms of *C. sibirica* in Siberia. Moreover, some of the Siberian goats are thought to have foreign mtDNA haplotypes as a result of hybridization with either *C. falconeri,* or *C. aegagrus* or *C. hircus*. According to mtDNA sequences *C. aegagrus* is represented by 4 haplogroups, two of them are similar to those of the Caucasian goats, another to *C. falconeri* and the last to *C. hircus.* So it is questionable, if *C. aegagrus* has its own mtDNA haplotypes. On the whole the variability of the investigated mitochondrial DNA fragment as well as distances between *Capra* species is quite large and comparable with that of other species of ungulates. Although the variability of nuclear gene SRY is very low it gives the ability to estimate the order of the divergence of many species of *Capra*.

Key words: *Capra*, phylogeny, cytochrome *b*, SRY, hybridization.

Resumen

Diferenciación genética de la cabra silvestre (género Capra*) basada en un análisis del gen mitocondrial citocromo b y el fragmento del gen nuclear SRY*

Hicimos un análisis filogenético de las secuencias del ADN mitocondrial (citocromo b, 1128 pb) y nuclear (intrón del gen SRY, 1832 pb) de la especie *Capra*. Nuestro estudio incluye datos

originales (46 muestras), además de datos publicados por otros autores (Takada 1997, Hassanin *et al.*, 1998, Manceau *et al.*, 1999, Kahila and Greenblatt 1999). Se demuestra que existen dos formas geográficas de *C. sibirica*, con un nivel de diferenciación que se acerca al que se encontraría entre especies. La especie *C. falconeri* tiene una morfología particular pero genéticamente no se diferencia demasiado de otras especies. La diferencia genética entre *C. caucasica* y *C. cylindricornis* es notable. Nuestros datos confirman la hipótesis (Pidancier *et al.*, 2006) de que una hibridación antigua interespecífica pudiera haber tenido un impacto importante en la formación de la diversidad genética actual de la especie *Capra*. Existe, además, evidencia genética de hibridación reciente y contemporánea en las cabras silvestres. Según nuestros datos, ocurre una hibridación intensiva entre *C. caucasica* y *C. cylindricornis* e incluso hay pruebas reales de cruzamiento entre el tur del Cáucaso y *C. aegagrus* en el Cáucaso, así como entre dos formas distintas de *C. sibirica* en Siberia. Además, se piensa que algunos íbices siberianos tienen haplotipos foráneos en el ADNmt como consecuencia de la hibridación con *C. falconeri*, *C. aegagrus* o *C. hircus*. Según las secuencias de ADNmt, C. aegagrus está representada por 4 haplogrupos, dos de los cuales son similares al tur del Cáucaso occidental, otro a *C. falconeri* y el cuarto a *C. hircus*. Por lo tanto, se pregunta si *C. aegagrus* tiene sus propios haplotipos de ADNmt. En conjunto, la variabilidad del fragmento de ADN mitocondrial estudiado, así como las diferencias entre las especies de *Capra*, son grandes y comparables con las que se observan en otras especies de ungulados. Aunque la variabilidad del gen nuclear SRY es muy baja, nos permite estimar el grado de divergencia de muchas especies de *Capra*.

Palabras claves: *Capra*, citocromo b, filogenia, hibridación, SRY.

Introduction

A considerable ammount of classical zoological papers are dedicated to the problem of phylogeny and taxonomy of the wild goats (Schwarz 1935, Tsalkin 1950, Ellerman & Morrison-Scott 1951, Vereschagin 1959, Sokolov 1959, Bannikov 1954, Heptner *et al.* 1961, Haltenorth 1963, Sokolov 1979, Veinberg 1993, Sokolov & Tembotov 1993, Pavlinov 2003, Danilkin *et al.* 2005). Lately this group has been investigated by many authors using the molecular-genetic analysis (Takada 1997, Hassanin *et al.* 1998, Manceau *et al.* 1999, Luikart *et al.* 2001, Mannen et *al.* 2001, Sultana *et al.* 2003, Pidancier *et al.* 2006). The amount of characteristics used for the estimation of variability of *Capra* species is increasing continuously. Consequently, the number of phylogenetic and taxonomic hypotheses is rising too.

According to different authors, the genus *Capra* includes from one to thirteen species. In the present study, I hold the view of the genus composition

(Heptner *et al.* 1961, Sokolov 1959) that distinguishes eight species of wild goats: bezoar (*C. aegagrus*), Alpine ibex (*C. ibex*), Siberian ibex (*C. sibirica*), Nubian ibex (*C. nubiana*), Spanish ibex (*C. pyrenaica*), markhor (*C. falconeri*), Kuban tur (*C. caucasica*), and Dagestan tur (*C. cylindricornis*). According to the classification of Wilson & Reeder (2005) there are also 8 species, but they have other compositions: Caucasian goats are combined into one species, bezoar is considered a part of *C. hircu* species, and *C. walie* is separated from *C. nubiana*. Many other scientists classify Caucasian wild goats (*C. caucasica* and *C. cylindricornis*) as one species (Sokolov 1959, Tembotov 1961, 1974, Gromov *et al.* 1963, Abdurahmanov 1977, Baryshnikov *et al.* 1981, Sokolov & Tembotov 1993). Ellerman and Morison-Scott (1951) group them in five species, *C. ibex*, *C. sibirica*, *C. nubiana*, *C. caucasica*, and *C. pyrenaica,* into the *C. ibex* group. Haltenorth (1963) also places *C. cylindricornis* in the *C. ibex* group. In recent years, the theory on the isolation of complex ibex group has become increasingly popular. On the other hand, in contrast to extreme "grouping," Lydekker (1913) distinguishes Nubian (*C. nubiana*) and Abyssinian (*C. walie*) goats, while Sokolov distinguishes *C. nubiana*, *C. sinaitica*, and *C. walie* (Sokolov 1959). The group of Caucasian goats is subjected to even stronger subdivision with the isolation of about five species, *C. caucasica*, *C. falconeri*, *C. severtzowi*, *C. raddei*, and *C. dinniki* (Razevig 1904, Dinnik 1910). Pointing to morphological specificity of *C. falconeri*, some authors (Heptner *et al.* 1961, Sokolov 1959, Pavlinov 2003) consider it a separate subgenus, *Orthaegoceros* (Trouessart, 1905), while Schwarz (1935) adopts the status of a genus for this species and attributes only one species, *C. ibex*, to the genus *Capra*.

The additional difficulties in estimating taxonomic status are connected with the absence of reproductive barrier. A lot of data prove the possibility of hybridization in captive *Capra* populations.

The main goal of our investigation was to find the correspondence between molecular-genetic *Capra* populations' divergence and their position in the taxonomic system, *Capra* phylogeny revision and search of the genetic evidence of *Capra* species hybridization in nature.

Materials and methods

Phylogenetic analysis of the mitochondrial gene cytochrome *b* and the nuclear gene SRY, which is located on the Y-chromosome, was carried out in eight *Capra* species. DNA was isolated from the ethanol-fixed muscle samples. DNA extraction was performed using the standard phenol–chloroform method (Sambrook *et al.* 1989) or a special kit «Diatom DNA Prep 200» («Izogen», Moscow).

46 samples of *Capra* were analyzed (Table 1). Cytochrome b fragments (1128 b.p.) were amplified for part of the samples (28 items) using primers GLU (L14724) 5'–CGA AGC TTG ATA TGA AAA ACC ATC GTT G–3' (Ozawa *et al.* 1997) and R (H15915) 5'–GGA ATT CAT CTC TCC GGT TTA CAA GAC–3' (Kocher *et al.* 1989). Polymerase chain reaction was conducted in the following conditions: 94ºC for 3 min (1 cycle); then 94ºC for 30 s, 50ºC for 15 s, 72ºC for 2 min (35 cycles); and 72ºC for 3 min (1 cycle).

Amplification of two fragments of the SRY gene intron was carried out using originally designed primers specific to horned ungulates: SRYL1 forward (5`–GCA TGT AGC TCC AGA ATA TTT CAC T–3`), SRYH1 reverse (5`–ATA AAT C(T/C)(G/A) T(G/A)A GGC AAA CTT GAA A–3`) –for the first fragment, SRYL2 forward (5`–TGC TTC TGC TAT GTT CAG AGT ATT G –3`), SRYH3 reverse (5`–GCA ATT TAC AAA GAG GTG GAA AGT A–3`) –for the second fragment. Design of the primers was made using online program "primer-3". Fragment amplification was conducted in the following conditions: 94ºC for 3 min (1 cycle); 94ºC for 30 s, 62ºC for 24 s, and 72ºC for 1 min (30 cycles); 72ºC for 6 min (1 cycle). PCR was run in a Tertsik autonomous thermal cycler (DNA-Technology, Russia).

Amplification products sequencing was performed using the ABI PRISM BigDye Terminator v. 3.1 kit. The sequencing reaction products were visualized on an ABI 3130 PRISM™ DNA sequencer (Applied Biosystems). The numbers of obtained sequences in GenBank are DQ246768-DQ246781, DQ246788, DQ246792, DQ246799-DQ246801. Additionally 1 SRY sequence and 26 cytochrome *b* sequences from GenBank were included in the alignment.

TABLE 1
Sample list.

Sample Nº	Species	Origin	Sex
4	*C. sibirica*	Kirgizia	♂
5-6	*C. caucasica*	Karachaevo–Cherkessia	♂
7	*C. cylindricornis*	Kabardino–Balkaria	♂
8	*C. caucasica*	Karachaevo–Cherkessia	♂
9-11	*C. cylindricornis*	Kabardino–Balkaria	♂
12-13	*C. cylindricornis*	North Ossetia	♂
14	*C. sibirica*	Kirghizia	♂
15-16	*C. sibirica*	Altai	♂
17-18	*C. aegagrus*	Dagestan	♂
19	*C. cylindricornis*	Azerbaijan	-
20-21	*C. sibirica*	Tajikistan	♂
25	*C. caucasica*	Karachaevo–Cherkessia	♂
27	*C. sibirica*	Kirghizia	♂
28	*C. sibirica*	Kirghizia	-
29	*C. sibirica*	Kirghizia	♂
30	*C. falconeri*	Tajikistan	♂
41	*C. sibirica*	Mongolia, Gobi	-
42	*C. cylindricornis*	Kabardino–Balkaria	♂
43	*C. sibirica*	Irkutsk region	♂
44	*C. sibirica*	Kirghizia	♂
53-55	*C. caucasica*	Karachaevo–Cherkessia	♂
77	*C. sibirica*	Krasnoyarsk region	♂
90-94	*C. falconeri*	Hindu Kush mountain range	♂
115	*C. cylindricornis*	Azerbaijan	♂
116	*C. sibirica*	Mongolia, South Goby	♂
117	*C. sibirica*	Altai	♂
118	*C. sibirica*	-	♂
125	*C. aegagrus*	Dagestan	-
126	*C. aegagrus*	Dagestan	♂
127	*C. aegagrus*	Dagestan	-
129	*C. aegagrus*	Dagestan	-
130	*C. aegagrus*	Dagestan	-
6/Nº	*Pseudois nayaur*	-	-
6/Nº	*Ovis ammon*	Turkmenia	♂

Alignment was performed manually using the BioEdit software program (Hall 1999). Phylogenetic trees were constructed with *Pseudois nayaur* and *Nemorhaedus goral* mtDNA sequences from GenBank as the outgroups. Sequence analysis, estimation of the nucleotide diversity, Net Distances and construction of the trees, with the help of Neighbor-Joining method were conducted using the Kimura-2-Parameter model in the MEGA3 software program (Kimura 1980, Saitou & Nei 1987, Kumar *et al.* 2004). Construction of phylogenetic trees by the Maximum Parsimony (MP) method was performed using the PAUP 4.10 software package (Swofford 2000), Maximum Likelihood method – METAPIGA (Lemmon & Milinkovitch 2002). Net distance between groups was calculated using the following algorithm: D= dXY–(dX - dY)/2, where dXY is the mean genetic distance between haplotypes of the groups X and Y, and dX, dY is the mean genetic distance between haplotypes within those groups. Statistical significance of the obtained reconstructions was tested by bootstrap method (1000 replicates) (Felsenstein, 1985). Median-joining network of the haplotypes was constructed in the Network program (Bandelt *et al.* 1999).

Results

Cytochrome *b*. Within the cytochrome b fragment (1128 b.p.) there are 227 (20,1%) variable sites and 179 among these are parsimony informative. The mean number of substitutions per one sequence is 53 transitions (7, 5, and 41 substitutions at the first, second, and third positions, respectively), and 4 (1, 0, and 3) transversions.

Phylogenetic trees constructed using different methods have the similar topology (Figure 1). There are minimum 7 separated clusters. Species *C .sibirica, C. nubiana* and *C. falconeri* have their own clusters. *C. ibex* and *C. pyrenaica* are combined in one cluster. Caucasian goats are divided into two independent groups. The position of *C. aegagrus* is not stable on the cladogram. Sequences of this species are included into different clusters.

All the samples were divided into 9 groups in accordance with their specific names. Sequences *C. caucasica* AF034738 from GenBank were included into the "*C. cylindricornis*" group and sequences N12, 13 - in the "*C. caucasica*" group according

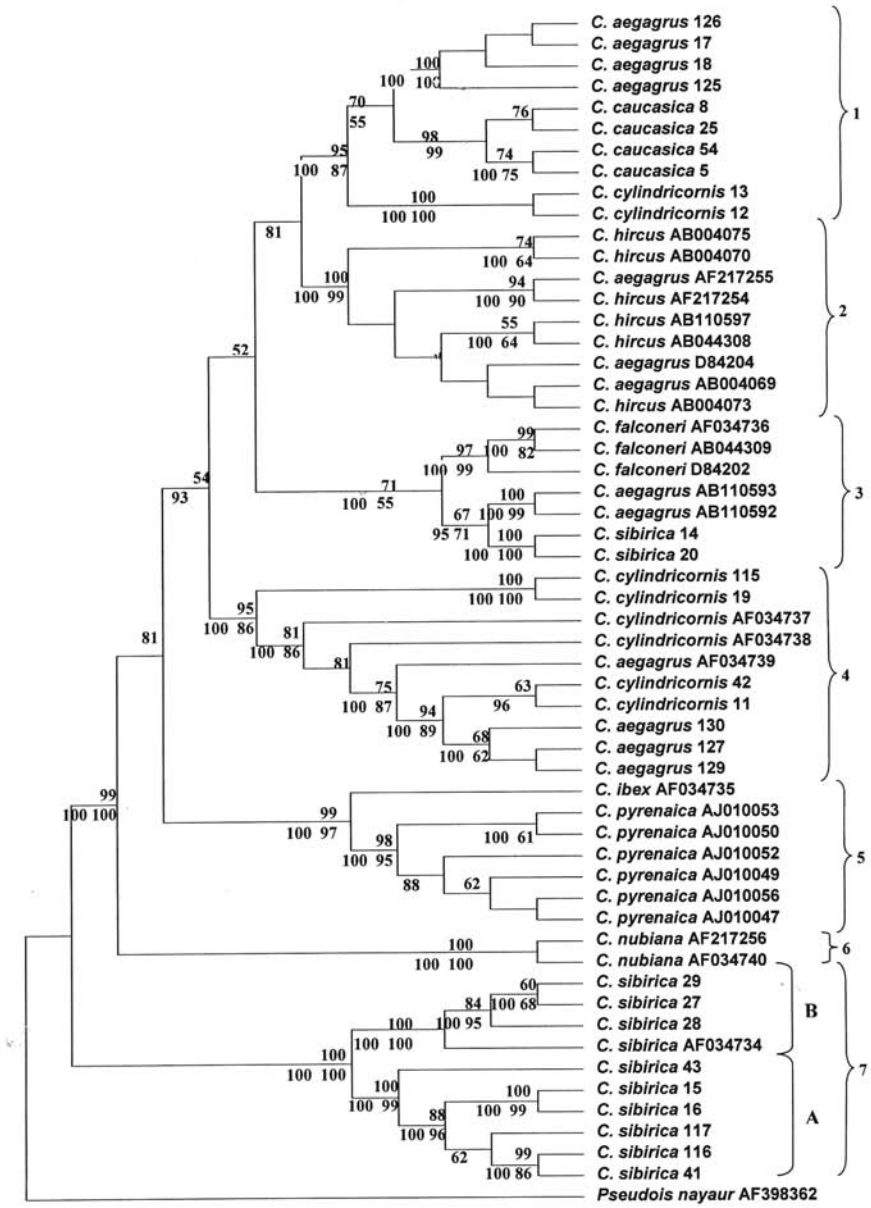

Figure 1. Phylogenetic tree based on cytochrome b gene sequences (1128 b.p.) obtained using NJ method and Kimura-2 parameter. The number above the branching node is the result of bootstrap analysis. Bootstrap index for appropriate node in a tree obtained with Maximum Parsimony model is in the right under the node. Supporting by Maximum Likelyhood method is in the left under the node. 1-7 are the main phylogenetic branches of wild goats.

to their position on the phylogenetic tree. 2 samples of *C. sibirica* (number 14, 20) which obviously have foreign mDNA haplotypes were excluded. For all these 9 groups within- and interspecific distances were calculated (Tables 2, 3).

TABLE 2
Mean intraspecific distances (nucleotide diversity), based on cytochrome b sequences (1128 b.p.), calculated using Kimura-2 parameter.

Species	Number of sequences	π	S.E.
C. caucasica	6	0.008	0.002
C. aegagrus	13	0.026	0.003
C. falconeri	3	0.016	0.003
C. cylindricorns	6	0.012	0.002
C. hircus	6	0.005	0.001
C. nubiana	2	0.016	0.004
C. ibex	1	-	-
C. pyrenaica	6	0.002	0.001
C. sibirica	10	0.031	0.004

TABLE 3
Interspecific distances (Net distances) based on cytochrome b sequences (1128 b.p.), calculated using Kimura-2 parameter.

	S.E. / net distance	1	2	3	4	5	6	7	8	9
1	*C. sibirica*	–	0.0085	0.0078	0.0084	0.0082	0.0080	0.0075	0.0086	0.0087
2	*C. falconeri*	0.0659	–	0.0053	0.0068	0.0073	0.0062	0.0037	0.0053	0.0055
3	*C. cylindricornis*	0.0610	0.0264	–	0.0056	0.0062	0.0053	0.0014	0.004	0.005
4	*C. pyrenaica*	0.0680	0.0414	0.0314	–	0.0043	0.0065	0.0047	0.0061	0.0065
5	*C. ibex*	0.0668	0.0447	0.0366	0.0181	–	0.0070	0.0052	0.0063	0.0067
6	*C. nubiana*	0.0582	0.0339	0.0270	0.0393	0.0451	–	0.0047	0.0054	0.0065
7	*C. aegagrus*	0.0568	0.0171	0.0066	0.0268	0.0304	0.0256	–	0.0015	0.0022
8	*C. caucasica*	0.0662	0.0280	0.0195	0.0364	0.0387	0.0322	0.0077	–	0.0044
9	*C. hircus*	0.0669	0.0289	0.0271	0.0385	0.0421	0.0413	0.0115	0.0231	–

The most significant distance separates *C. sibirica* from other species. The small distances are between *C. aegagrus* and *C. caucasica, C. falconeri* and *C. aegagrus*, *C. aegagrus* and *C. cylindricornis, C. aegagrus* and *C. hircus, C. ibex* and *C. pyrenaica*. The mean within-group distance (nucleotide diversity) is maximal in *C. sibirica* and *C. aegagrus* (Table 2). It can be explained by existence of several cytochrome *b* haplogroups within each of these species (Figure 2). Nucleotide diversity is even higher in *C. nubiana*, *C. falconeri*, and *C. cylindricornis*.

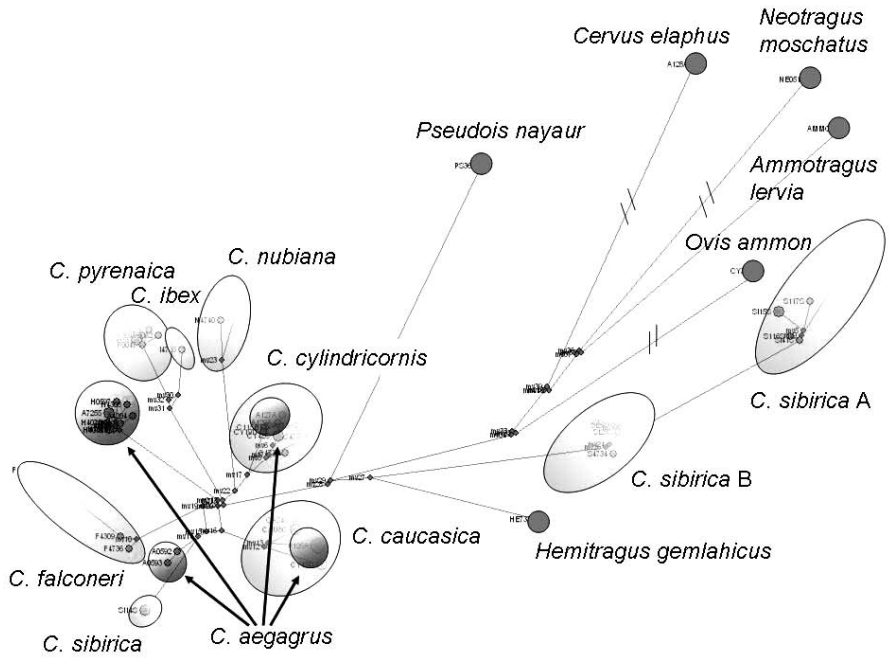

Figure 2. Median-joining network based on cytochrome b gene sequences (1128 н.п). The branch length is proportional to the number of the mutations.

The species *C. sibirica* forms two groups (A, B) and the distances between them are comparable with the interspecific distances (Table 4). Also there are two *C. sibirica* cytochrome b haplotypes which are close to the haplotypes of

the other species: *C falconeri* and *C. aegagrus*. *C. aegagrus* is represented by 4 haplogroups. Two of them are similar to those of the Caucasian goats, another to *C. falconeri* and the last to *C. hircus.*

Table 4
Intergroup genetic distances (Net distances) for some *Capra* populations, based on cytochrome b sequences, calculated using Kimura-2 parameter.

Phylogenetic lines	*C. sibirica* A	*C. aegagrus* (*C. cylindricornis*)	*C. aegagrus* (*C. caucasica*)	*C. aegagrus*	*C. hircus*
C. sibirica B	0.037	-	-	-	-
C. aegagrus (*C. caucasica*)	-	0.034	-	-	-
C. aegagrus	-	0.024	0.025	-	-
C. aegagrus (*C. hircus*)	-	0.038	0.030	0.031	0.000
C. caucasica Karachaevo-Cherkessia	-	-	0.012	-	-
C. caucasica North Osetia	-	-	0.020	-	-
C. cylindricornis Kabardino-Balkaria	-	0.001	-	-	-
C. cylindricornis Azerbaidjan	-	0.015	-	-	-

According to mitochondrial DNA data analysis, *C. sibirica* is the most ancient species but it is difficult to estimate the order of further divergences. These results confirm the data of other authors (Manceau *et al.* 1999, Pydancier *et al.* 2006 and others). Moreover, according to the figure 3, genus *Capra* is not a strong monophyletic group and two separated groups can be distinguished. One of them includes haplotypes of *C. sibirica*, second – the rest of the species.

SRY gene. In the SRY gene (1932 b.p.), 26 positions are variable (1,4%). From these, 25 positions are parsimoniously-informative. The mean number of substitutions per one sequence is 5 transitions and 3 transversions. The percentages of T, C, A, and G are 32.7, 20.3, 30.3, and 16.7% respectively.

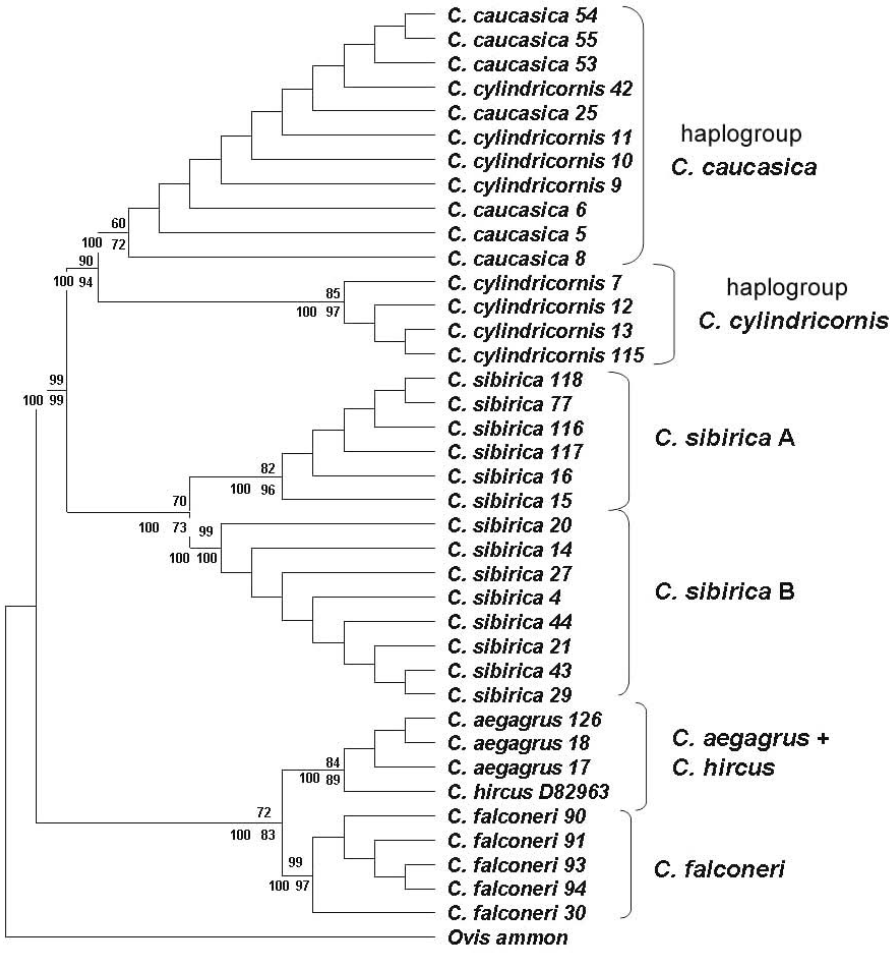

Figure 3. Phylogenetic tree based on SRY gene sequences (1832 b.p.) obtained using NJ method and Kimura-2 parameter.

Our phylogenetic analysis shows that there are two separated evolutional lines: *C. falconery* + *C. aegagrus* + *C. hircus* and *C. sibirica* + *C. caucasica* + *C. cylindricornis* (Figures 3, 4). *C. sibirica* has lost its basal position. All *C. sibirica* samples (including NN 14 and 20) form a common monofiletic group, which is divided into two well separated haplogroups. All sequences of the *C. aegagrus* are

separated from those of the other species. Caucasian goats are represented by two groups, but there is no distinct geographical boundary between them. According to our data the sequence of the divergence of species is the same as it was shown in the previous research made by N. Pydancier (Pydancier *et al.* 2006).

In accordance with clustering, I formed 7 groups and calculated interspecific distances (Table 5) and within group distance. Within group distance (nucleotide diversity) tends to zero. As nuclear data shows the remotest species are *C. falconeri* and *C. aegagrus*, and the greatest distances are between makhur and Siberian goat, makhur and Caucasian goats. The level of divergence between two groups of *C. sibirica* is very high and is comparable with that of other species.

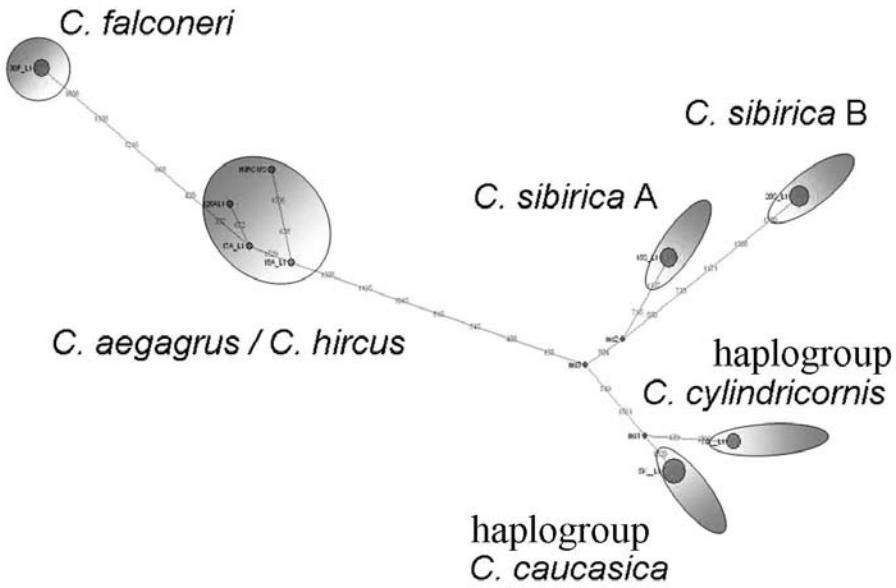

Figure 4. Median-joining network based on SRY gene sequences (1832 н.п).

TABLE 5
Interspecific distances (Net distances) for *Capra* species, based on SRY sequences (1832 b.p.), calculated using Kimura-2 parameter.

net dsitance \ S.E.		1	2	3	4	5	6	7
1	*C. falconeri*	–	0.0015	0.0020	0.0023	0.0020	0.0022	0.0014
2	*C. aegagrus*	0.0033	–	0.0017	0.0019	0.0017	0.0019	0.0005
3	*C. sibirica* A	0.0077	0.0055	–	0.0014	0.0013	0.0014	0.0018
4	*C. sibirica* B	0.0088	0.0066	0.0033	–	0.0016	0.0017	0.0020
5	*C. caucasica*	0.0078	0.0056	0.0033	0.0040	–	0.0010	0.0018
6	*C. cylindricornis*	0.0083	0.0061	0.0039	0.0050	0.0019	–	0.0019
7	*C. hircus*	0.0039	0.0005	0.0061	0.0072	0.0062	0.0066	–

Capra evolution history reconstraction

Basal phylogeny of the genus. Explanation for mtDNA and Y-chromosome data discordance was given by Pydancier *et al.* (2006). Our data do not contradict this construction. So I developed a scenario of *Capra* evolution but presented it in another form making some additions (Figure 5).

As the scenario shows there could have been two centers of *Capra* evolution (the "Ibex type" in Central Asia and the "Bezoar type" near the Mediterranean Sea), and then a possible hybridization and introgression of mtDNA toward the "Ibex type" took place. Ancient genus divergence in two groups is recorded in the differentiation of Y-chromosome haplotypes and sharp distinction of *C. sibirica* mtDNA. Introgression of the "Bezoar type" mtDNA results in similarity of the majority of mtDNA haplotypes with those of *C. aegagrus*. This scheme is in accordance with morphological data. Under the hypothesis *C. ibex, C. nubiana and C. caucasica,* whose appearances are very similar to those of *C. sibirica*, are direct offsprings of the "Ibex type" and they only inherited mtDNA from the "Bezoar type".

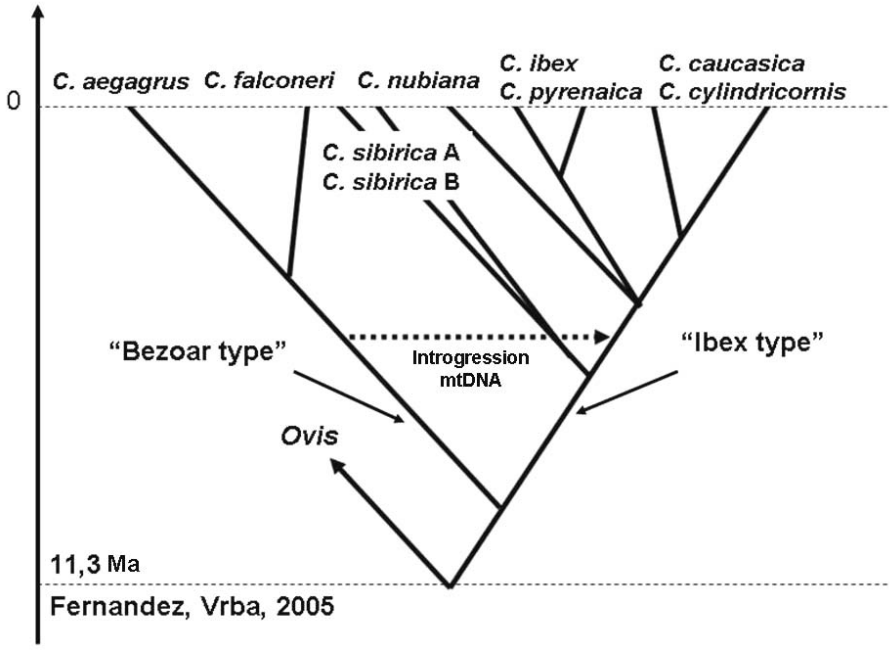

Figure 5. Supposed evolutional scenario of the genus *Capra*

***C. falconeri* phylogeny**. *C. falconeri* is the brightest species in the genus. But genetically this species is not remote from the other goats. MtDNA and Y-chromosome distances between *C. falconeri* and other *Capra* species are not very high. According to our data, *C. falconeri* could not have appeared much earlier than other species. Probably makhur was formed as an independent species approximately in the same time as the other species of wild goats.

***C. sibirica* phylogeny**. The internal structure of *C. sibirica* is also worth discussing. There were a lot of different races of Siberian goat by the beginning of the XX century. Most of these races were described by the single samples. In the work of Flerov (1935) only two forms of *C. sibirica* were kept: *C. sibirica sibirica* (South Siberia, Mongolia) and *C. s. sakeen* (the rest of the range). Fifteen years later Tzalkin (1950) determined another composition of this species, and distinguished five differents subspecies: *C. s. sibirica* (Altai, Sayany mountains,

Zaaltaiskaya Goby desert), *C. s. alaiana* (Eastern and Central Tian Shan, Pamir), *C. s. formosovi* (Western Tian Shan), *C. s. dementievi* (Western Kunlun to the Keriyskiy mountain range). This point of view was also supported by the famous Russian zoologist Sokolov (1959). Later Heptner (1961) returned to the standpoint of Flerov (1935) and described two forms of Siberian goat: *C. s. sibirica* from Altai and *C. s. alaiana* from Central Asia.

Our data support the constructions of Flerov (1935) and Heptner (1961) and reveal the high level of genetic differentiation and geographical isolation of the two *C. sibirica* forms. Also we confirmed morphological differentiation of these two forms using multivariate analysis of cranial characteristics in our previous study (Zvychaynaya & Puzachenko 2009). Evidently these forms have a long evolution history and they arose at the same time as other *Capra* species. One sample haplotype (N43) from the Altai has "B type" Y-chromosome, so there might be hybridization between these two forms.

Two samples of *C. sibirica* (N14, 20) stand out against the background: they have Y-chromosome typical for *C. sibirica* and different mtDNA sequence. These mtDNA might be obtained from other species such as: *C. falconeri, C. aegagus,* or *C. hircus*. This fact might be evidence of hybridization with domestic goats in nature, at the same time it could be indicate the vulnerability of wild *Capra* gene pool and must be investigated in following essays.

***C. ibex* and *C. pyrenaica* phylogeny**. In this study I had only cytochrome b sequences from GeneBank for *C. ibex* and *C. pyrenaica*. I compared genetic distances between them with those from other *Capra* species. It was shown that genetic net distance is smaller than those between two groups of *C. sibirica,* and than between *C. ibex* (or *C. pyrenaica*) and any species of the "Ibex" group (such as *C. nubiana, C. sibirica, C. caucasica*), and even smaller than distance which separates Caucasian goats. It was concluded, that the divergence of *C. ibex* and *C. pyrenaica* took place much later, when other *Capra* species had already been formed.

***C. aegagrus* phylogeny.** MtDNA sequences of *C. aegagrus* form 4 different haplogroups. Two of them are similar to those of the Caucasian goats, another (from Genbank) to *C. falconeri* and the last to *C. hircus.* At the same time, Y-

chromosome sequences of *C. aegagrus* Caucasian samples are close to the *C. hircus* and are combined in one cluster. I examined two hypotheses: this mtDNA variability is the heritage of ancestral polymorphism (1) or it is a result of hybridization (2).

It is well known that the widespread fossil species was *C. prisca*, which is thought to be close to the modern bezoar *C. aegagrus* according to morphological signs (Heptner *et al.* 1961). So I had to pay attention to the first hypothesis. But eventually it was not supported by SRY data and I chose the second suggestion. It is essential that the distances between four haplogroups are very high and quite the contrary these haplotypes are utterly close to other species haplotypes. In the historical times *C. aegagrus* range and *C. falconeri* range were very close to each other and nowadays *C. aegagrus* and *C. cylindricornis* inhabit the mountains of the east Caucasus together. So all prerequisites existed to make hybridization possible. Eventually, if *C. aegagrus* had been polytypical species from the beginning, the introgression mtDNA towards the "Ibex type" (Figure 5) would have been impossible. In this case the hybridization and introgression mtDNA must have taken place at least three times independently, but the probability of that tends to zero. So the fist hypothesis also is not supported in the context of the "basal phylogeny" (Figure 5).

Contemporary range of *C. aegagrus* in the Caucasus is microfocal. This species only inhabits Dagestan, Azerbaijan, Chechen Republic, Ingushetia, Armenia and Georgia. But before, it was widely spread on the Greater Caucasus mountain range and also in the central and eastern part of the Lesser Caucasus (Vereschagin 1949, 1959, Heptner *et al.* 1961, Baryshnikov 1978). The Pleistocene age fossil remains of *C. aegagrus* in the Caucasus have not been found yet, although *C. caucasica* and *C. cylindricornis* remains have been discovered in great numbers. It might be the evidence of recent *C. aegagrus* appearance in the Caucasus. Invasion of *C. aegagrus* must have gone from Asia through the central Caucasus. At that moment hybridization with *C. caucasca* and the mtDNA introgression might have occurred. Our data confirm this suggestion because *C. aegagrus* cytochrome b nucleotide sequences are very similar to the *C. caucasica* and *C. cylindricornis* from Karachaevo-Cherkessia and Kabardino-Balkaria republics respectively. The

genetic distance between *C. aegagrus* and *C. cylindricornis* is smaller, and it might signify their late hybridization.

***C. caucasica* and *C. cylindricornis* phylogeny**. Molecular-genetic data gave evidence of deep divergence of this group. Also some mtDNA and Y-chromosome haplotype mixture is observed in the Central Caucasus which is the evidence of *C. caucasica* and *C. cylindricornis* hybridization.

Caucasian goats are thought to arise in the Caucasus. But it is unclear why *C. cylindricornis* has specialized very quickly but morphological similarity between *C. caucasica* and *C. ibex*, *C. nubiana* and *C. sibirica* was saved.

Paleontological data show that Pleistocene large "ibex" (*Ibex cebennarum, I. priscus, I. cenomanus, Capra prisca*) were widespread in Europe including the Crimea and the Caucasus (Gromova 1935, 1948, Gromov 1948, Vereschagin 1959). At that time the Crimea and the Caucasus had a strong resemblance in mammal species compositions, which apparently had the same origin. It might be explained by the "bridge" that existed between these territories on the Azov sea at the beginning of Pleistocene (Vereschagin 1959). The nearest fossil findings of "Ibex" were discovered nearby Odessa (about 350 km from the Crimean site) and on the banks of the Dnestr and the Prut rivers (about 600 km). Animals could have probably migrated from the Caucasus to central Europe and backwards. Also there is some evidence that Pleistocene goats from Europe show the most resemblance to contemporary *C. caucasica* (Rivals & Testu 2006) and it might be possible that an ancestor or a close relation of *C. caucasica* was widespread in Europe.

So there might be three scenarios of evolution in Caucasian goats. First of them: the common ancestor appeared in the Caucasus, and the population was divided into eastern and western parts. Then each of them evolved separately. Later the "obstacle" disappeared and the hybridization began. But in this case the cause of their long isolation remains mysterious as well as the *C. caucasica* and Pleistocene "ibex" resemblance. Second scenario: *C. cylindricornis* was formed in the Caucasus and *C. caucasica* appeared in Central Europe and later spread to the west (and became the ancestor of *C. ibex*) and in the Caucasus. Third scenario:

C. caucasica and *C. cylindricornis* both have the Caucasian origin, but after their appearance *C. caucasica* could have spread in Europe, where it evolved separately from *C. cylindricornis*.

Whether *C. caucasica* has close relations with *C. ibex* and *C. pyrenaica* is still unknown. I have not found any genetic evidence of the close relation between *C. caucasica* and *C.ibex* or *C. pyrenaica*. Evidently *C. caucasica* evolved separately from *C. cylindricornis,* and it is possible that the forming of *C. caucasica* took place out of the Caucasus. Which scenario is close to the truth it is unclear. Nowadays we have two morphologicaly and genetically different species in the small territory, which are hybridizing. Now there is a lot of genetic evidence pointing to hybridization. Most animals from Kabardino-Balkaria republic have mtDNA from *C. cylindricornis* and Y-chromosome from *C. caucasica*. Evidently, the permanent hybridization results in the clinal morphological variability and the gradual obliteration in the considerable difference in their appearance.

***Capra* taxonomy**. Two contemporary forms *C. sibirica sibirica* and *C. s. sakeen* (Flerov 1935) are differentiated geographically, genetically and morphologically. The level of their differentiation is very high and comparable with that of other *Capra* species. In my opinion, within the limits of the present taxonomy (Wilson & Reeder 2005, Pavlinov 2003, Sokolov 1979) they could be considered as the good species.

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