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An Assessment of Applicability of Illumina GoatSNP50 BeadChip for Genetic Studies of Caucasian tur (*Capra caucasica*) †

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Abstract: Caucasian tur (*Capra caucasica*) is native to Greater Caucasus Mountain Chain from Azerbaijan and Georgia in the East to Krasnodar region of Russia in the West. This species is divided into two subspecies—East-Caucasian tur and West-Caucasian tur and an admixed subpopulation referred to as Mid-Caucasian tur. Up to date most of genetic studies of Caucasian tur were based on mtDNA sequences and comprehensive investigation of nuclear DNA is required for clarification of its genetic diversity and population structure. In our work, we assessed applicability of Illumina GoatSNP50 BeadChip for genetic studies of Caucasian tur. Fifteen specimens of *Capra caucasica* including East Caucasian tur from Dagestan (E_TUR, $n = 5$), West Caucasian tur from Karachay-Cherkessia (W_TUR, $n = 5$) and Mid-Caucasian tur from Kabardino-Balkaria (M_TUR, $n = 5$) were genotyped. After quality control, 4758 polymorphic loci, which were distributed all over 29 autosomes, were detected. The lowest number of SNPs was found on the 25th chromosome—68, and the highest on the 1st chromosome—348. It was shown that all the three groups of Caucasian tur clustered separately. A total of 2061 SNPs were common for all the subpopulations, 594 were found only in W_TUR, 689 in E_TUR and 530 in M_TUR. Individual heterozygosity ranged from 0.309 to 0.319 in W_TUR, from 0.244 to 0.278 in E_TUR and from 0.288 to 0.323 in M_TUR. Thus in our study we demonstrated that the Illumina GoatSNP50 BeadChip designed for domestic goats can be used as useful tool for genetic studies of Caucasian tur.

Keywords: wild goats; single nucleotide polymorphisms; genetic diversity; population structure

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1. Introduction

Caucasian tur (*Capra caucasica*) habitat is limited to Greater Caucasus Mountain Chain from Azerbaijan and Georgia in the East to Krasnodar region of Russia in the West (Figure 1). Its range is one of the smallest habitats of all ungulates—around 770 km in length and 80 km in width [1]. Taxonomically, the species is divided into two subspecies: West-Caucasian tur (*C. c. severtzovi*) and East-Caucasian tur (*C. c. cylindricornis*). Sometimes these populatins are even considered as different species [2]. The areas of these subspecies are united and in the territory between them a hybrid form, which is referred to as a Mid-Caucasian tur, is distinguished [3]. To clarify the taxonomy of Caucasian tur

molecular genetic studies are needed. Currently, one of the most effective technologies for molecular genetic research is the genome-wide analysis of single-nucleotide polymorphisms (SNPs). Recent advances in the development of high-throughput genotyping platforms (SNP chips) have turned SNPs into a powerful tool for genetic studies of domestic animals [4,5]. Despite the fact that such chips are not available for most of wild animals, the use of the SNP chips designed for their domestic relatives were found to be suitable. Thus, Tokarska et al. [6] used the BovineSNP50 BeadChip created for cattle (*Bos taurus*) to infer the population structure of European bison (*Bos bonasus*). Kharzinova et al. [7,8] have shown the applicability of the BovineSNP50 BeadChip and BovineHD BeadChip for whole-genome studies of reindeer (*Rangifer tarandus*). The OvineSNP50 BeadChip, created for domestic sheep, was successfully used to study wild Ovis species: bighorn (*Ovis canadensis*), thinhorn (*Ovis dalli*) [9,10] and snow sheep (*Ovis nivicola*) [11].

Figure 1. Map with sampling sites of Caucasian tur used in this study and the area of the species habitat. W_TUR = West-Caucasian tur, E_TUR = East-Caucasian tur, M_TUR = Mid-Caucasian tur.

The aim of our study was to assess the applicability of Illumina GoatSNP50 BeadChip, created for domestic goats, for genetic studies of Caucasian tur (*Capra caucasica*).

2. Materials and Methods

A total of 15 specimens of *Capra caucasica,* including East Caucasian tur from Dagestan (E_TUR, n = 5), West Caucasian tur from Karachay-Cherkessia (W_TUR, n = 5) and Mid-Caucasian tur from Kabardino-Balkaria (M_TUR, n = 5) were selected for this study. The sampling sites are presented in Figure 1. All the samples were taken from trophy hunters of the Mountain Hunters Club (www.kgo-club.ru), who were licensed to hunt Caucasian tur.

DNA extraction was carried out using Nexttec columns (Nexttec Biotechnology GmbH, Leverkusen, Germany) in accordance with the manufacturer's recommendations. SNP genotyping was performed using the Illumina GoatSNP50 BeadChip containing 53,347 SNPs.

SNP quality filtering was performed in PLINK v1.9 [12]. SNPs that were genotyped in less than 90% of individuals (–geno 0.1) with a minor allele frequency (MAF) < 5% (–maf 0.05) and in linkage disequilibrium (–indep-pairwise 50 5 0.5) were pruned. The observed (Ho) and unbiased expected (He) heterozygosity [13], inbreeding coefficient (Fis), and rarified allelic richness (Ar) were calculated in the R package "diveRsity" [14]. Pairwise F_{ST} genetic distances [15] were calculated in the R package "StAMPP" [16]. Principle component analysis was performed with PLINK 1.9 (–pca 4) and visualized in the R package "ggplot2" [17]. An individual tree based on the pairwise identity-by-state (IBS) distance matrix (–distance 1-ibs) was constructed using the Neighbor-Net algorithm implemented in SplitsTree 4.14.6 [18]. To estimate and visualize the distribution of heterozygosity at the individual level, multilocus heterozygosity (MLH) was calculated in the R package "inbreedR" [19]. Venn diagram was constructed in the R package "VennDiagram" [20].

The map with sampling sites was created using R packages "maps" [21] and "ggplot2" [17].

3. Results and Discussion

In our study we assessed the applicability of Illumina GoatSNP50 BeadChip, created for domestic goats (*Capra hircus*), for genetic studies of Caucasian tur (*Capra caucasica*). 15 individuals belonging to three groups of Caucasian tur (West-Caucasian tur, East-Caucasian tur and Mid-Caucasian tur) were genotyped. From the initial set of 53,347 SNPs available in the Illumina GoatSNP50 BeadChip, after quality control, 4758 polymorphic loci (8.92%), distributed all over 29 autosomes, were selected. The lowest number of SNPs was found on the 25th chromosome—68, and the highest on the 1st chromosome—348 (Figure 2).

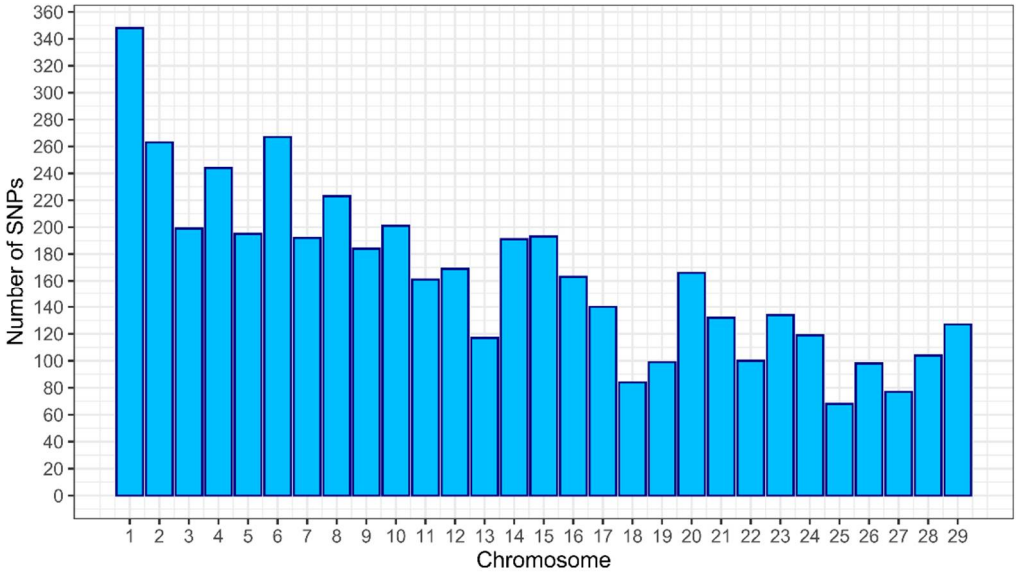

Figure 2. Number of polymorhic SNPs per chromosome in Caucasisan tur (*Capra caucasica*).

While 2061 polymorphic SNPs were common for all the Caucasian tur groups, the number of unique SNPs for W_TUR, E_TUR and M_TUR was 594, 689 and 530, respectively. The most shared SNPs among two groups was found in W_TUR—M_TUR pair—869, and the least number of shared loci were observed for W_TUR—E_TUR pair—420 (Figure 3).

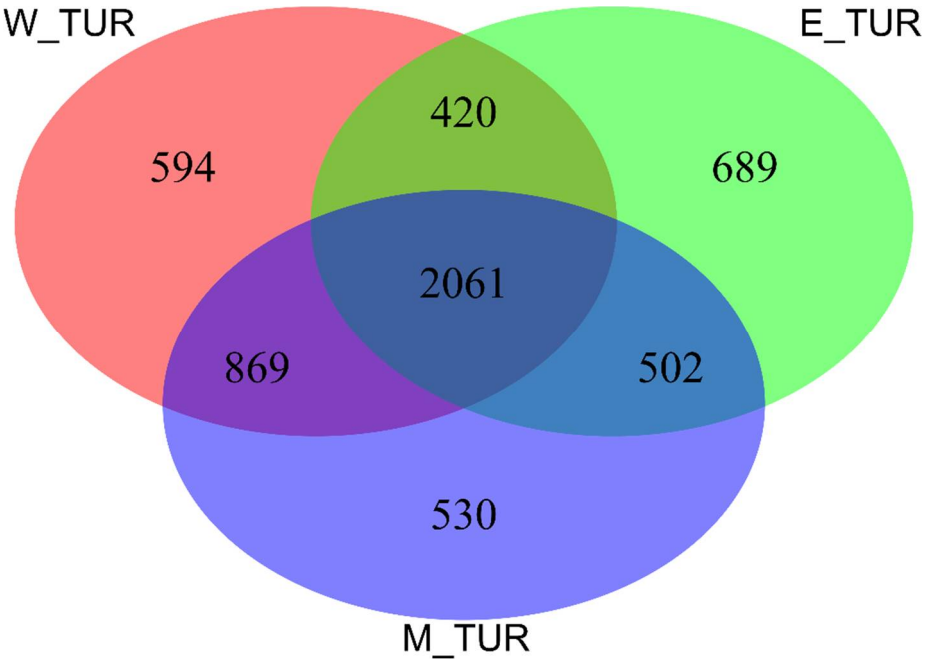

Figure 3. Venn diagram representing the number of unique and shared polymorphic SNPs in the three groups of Caucasian tur (*Capra caucasica*). W_TUR = West-Caucasian tur, E_TUR = East-Caucasian tur, M_TUR = Mid-Caucasian tur.

The results of principal component analysis (PCA) revealed that all the studied groups of Caucasian tur clustered separately (Figure 4). The first component (PC1), which explained 2.57% of genetic variability, divided Caucasian tur into two groups. The first group consisted of E_TUR (PC1 < 0) and the second one included W_TUR and M_TUR (PC1 > 0).

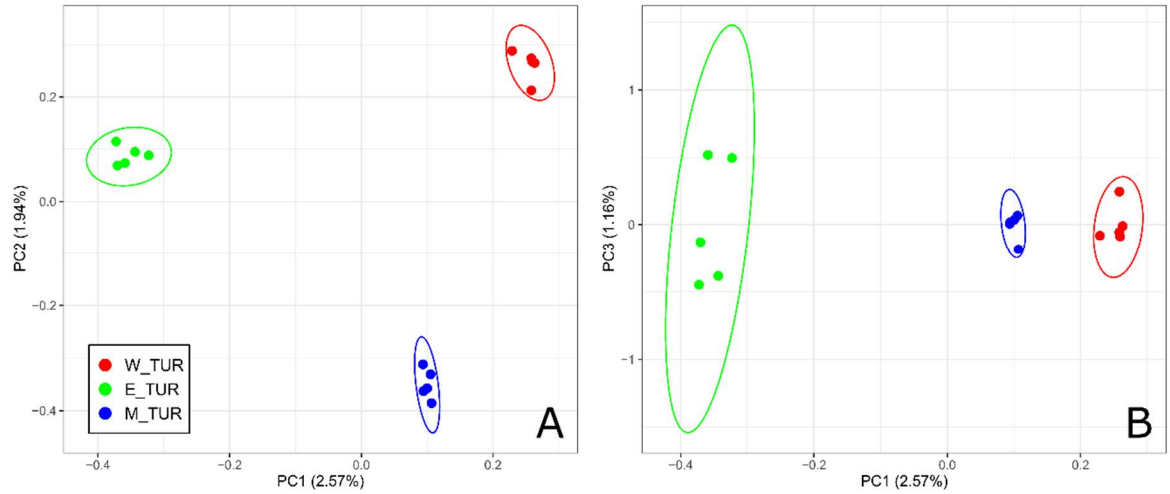

Figure 4. Principal component analysis (PCA) of the groups of Caucasian tur (*Capra caucasica*): (**A**) the first two components (PC1 and PC2), (**B**) the first and third components (PC1 and PC3). W_TUR = West-Caucasian tur, E_TUR = East-Caucasian tur, M_TUR = Mid-Caucasian tur.

The level of genetic differentiation was estimated with pairwise F_{ST} distances (Table 1). We observed a high F_{ST} value among W_TUR and E_TUR—0.161. M_TUR was genetically more close to W_TUR than to E_TUR, but in both cases the differtiation was moderate—0.100 and 0.134, respectively.

Table 1. Pairwise F_{ST} genetic distances between the groups of Caucasian tur (*Capra caucasica*).

	W_TUR	E_TUR	M_TUR
W_TUR	0.000		
E_TUR	0.161	0.000	
M_TUR	0.100	0.134	0.000

Notes: W_TUR = West-Caucasian tur, E_TUR = East-Caucasian tur, M_TUR = Mid-Caucasian tur. F_{ST} values in the range 0–0.05 indicate low genetic differentiation; value between 0.05 and 0.15, moderate differentiation; values between 0.15 and 0.25, high differentiation; and values above 0.25, very high genetic differentiation [22,23].

The above-mentioned results were also supported with Neighbor-Net dendrogram (Figure 5). All the studeid individuals were placed in clades according to their geographical origin. This analysis also revealed that there were no close relatives among the 15 genotyped animals.

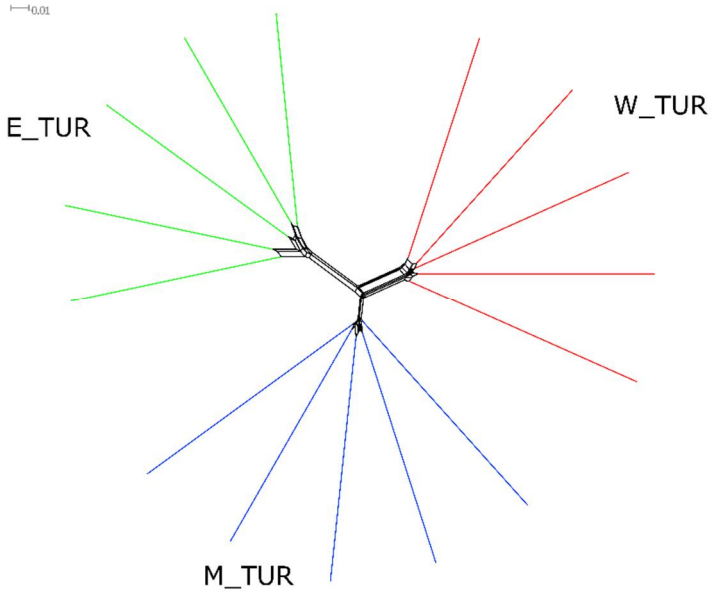

Figure 5. Neighbor-Net dendrogram of fifteen Caucasian tur (*Capra caucasica*) individuals, based on IBS distances.

Expected heterozygosity (He) and allelic richness (Ar) in W_TUR were significantly higher than in E_TUR (Table 2). But the highest genetic diversity parameters were observed in M_TUR. The within population inbreeding coefficient (Fis) was close to zero in W_TUR and M_TUR which showed that these groups were in a fairly stable state, and slightly higher than zero in E_TUR, which indicated weak heterozygote deficit.

Table 2. Genetic diversity in the groups of Caucasian tur (*Capra caucasica*).

Group	*n*	Ho (±SE)	He (±SE)	Fis [95% CI]	Ar (±SE)
W_TUR	5	0.334 ± 0.004	0.339 ± 0.003	0.016 [0.003; 0.029]	1.825 ± 0.006
E_TUR	5	0.279 ± 0.004	0.308 ± 0.003	0.08 [0.066; 0.094]	1.772 ± 0.007
M_TUR	5	0.333 ± 0.004	0.345 ± 0.003	0.03 [0.017; 0.043]	1.844 ± 0.006

Notes: *n*—number of samples, Ho—observed heterozygosity, He—expected heterozygosity, Fis—inbreeding coefficient, Ar—allelic richness, SE—standard error, CI—confidence interval, W_TUR = West-Caucasian tur, E_TUR = East-Caucasian tur, M_TUR = Mid-Caucasian tur.

Individual heterozygosity values, which were represented by multilocus heterozygosity (MLH) showed that all the E_TUR individuals had lower heterozygosity than both

W_TUR and M_TUR (Figure 6). Although mean values of observed heterozygosity were almost equal in W_TUR and in M_TUR, the range of values was wider in M_TUR than in E_TUR.

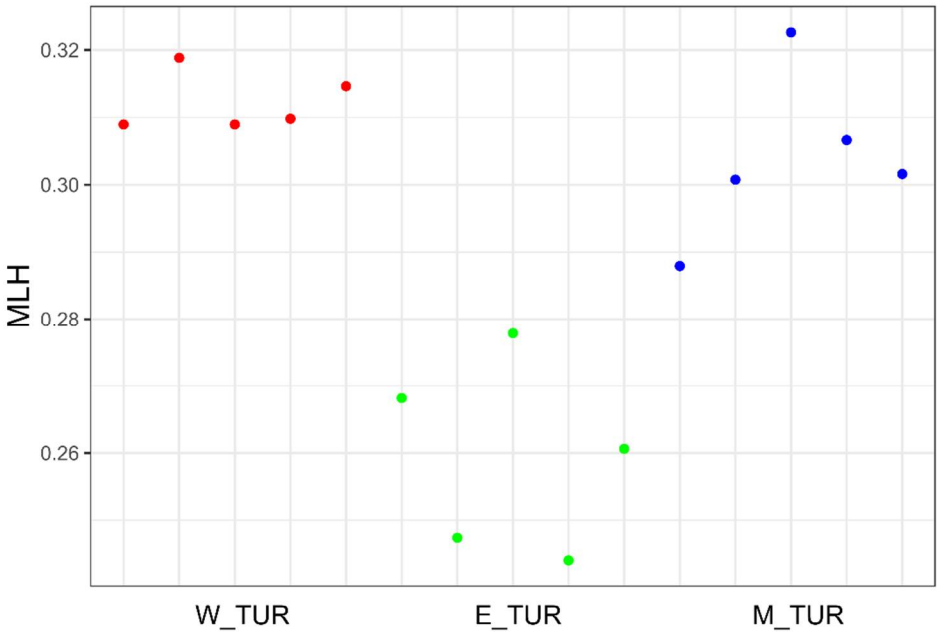

Figure 6. Individual multilocus heterozygosity (MLH) in the groups of Caucsian tur (*Capra caucasica*).

To date molecular genetic studies of Caucasian tur were based on the investigation of mitochondrial DNA [24–26]. Manceau et al. [24] examined concatenated fregments of cytochrome b and control region (500 bp in length) of 3 samples of East-Caucasian tur and 9 samples of West-Caucasian tur. Kazanskaya et al. [25] examined fragments of cytochrome b and control region of 8 samples of East-Caucasian tur and 9 samples of West-Caucasian tur. Kashinina and Kholodova [26] examined a 715 bp fragment of cytochrome b gene in 6 samples of East-Caucasian tur, 11 samples of West-Caucasian tur and 8 samples of Mid-Caucasian tur. In all these studies clear separation of East-Caucasian tur from West-Caucasian tur was determined. The authors emphasized that further studies on nuclear markers should be performed. Thus, the results obtained in our research will make it possible to clarify the taxonomic status of the Caucasian tur, reconstruct the natural history of the species formation and assess the genetic diversity.

4. Conclusions

In our study we demonstrated that the Illumina GoatSNP50 BeadChip designed for domestic goats can be used as useful tool for genetic studies of Caucasian tur. It was shown that West-Caucasian tur, East-Caucasian tur and Mid-Caucasian tur clustered separately from each other. Genetic diversity parameters in East-Caucasian tur were lower than in the other groups. To obtain more accurate information about population structure and genetic diversity of Caucasian tur, more studies based on Illumina GoatSNP50 BeadChip with larger number of samples are needed.

Author Contributions: A.V.D., K.W. and N.A.Z. conceived and designed the experiments; A.N.R. and H.R. performed the experiments; A.V.D. analyzed the data; A.V.D., D.G.M., K.W., N.A.Z. contributed reagents/materials/analysis tools; A.V.D. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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