ABSTRACT
Shorthorn Rhodopean cattle breed is one of two endemic for Bulgaria cow breeds. The breed is characterized by high vitality and fertility, calving ease, resistance to diseases, and low requirements for feed. Milk yield is low, but fat and protein content of milk are high. Therefore it is of high significance to reveal the genotype profile of that cattle population which represents the aim of present study. 38 animals of that breed were genotyped for two lactoprotein genes: kappa-casein and alfaS1-casein through PCR-RFLP analysis. The results for kappa-casein locus show slight superiority of heterozygous AB genotype and prevalence of uncommon B allele. Analysis of polymorphism of alfaS1-casein gene displays that about 53% of the animals are with homozygous BB genotype, about 34% - with heterozygous BC genotype and only ~13% (5 cows) – with homozygous CC genotype. It may be concluded that Shorthorn rhodopean cow breed is with specific genotype profile similar to other cattle population in South-Eastern Europe.

Keywords: Shorthorn Rhodopean cattle breed, milk protein genes, genotyping, biodiversity

Introduction
The bovine casein locus, mapped on BTA6q31-33 (14), contains four milk protein genes which are closely linked, and in the order αs1-casein (CSN1S1), β-casein (CSN2), αs2-casein (CSN1S2), and κ-casein (CSN3). The genes are organised in a cluster of approximately 250 kb (13). The locus is considered to influence on economically important traits in dairy cattle (6, 16).

Genetic polymorphism of milk proteins provokes a significant interest because some of genetic variants are of economic importance as they can be used as a DNA marker for milk traits (8). The marker assisted selection (MAS) acts as an initial step to identification and increase the frequency of favourable alleles and decrease the frequency of unfavourable alleles within a cattle population with regards to milk production, fat and protein content. Classical selection methods used for milk quantitative and quantitative traits improvement is restricted only to mature lactating females. Usage of MAS allows determining the potential of each animal even before being expressed phenotypically. That could be applicable for definition of the genetic variants of any gene, independently of age and sex, and thus contribute to genetic improvement of dairy cattle populations.

One of the two native cow breeds in Bulgaria is a Shorthorn Rhodopean cattle breed. It is considered as one of the last forms of the prehistoric European cattle breeds together with Albanian, Illyric Dwarf and Montenegro cattle. Today, less than 300 animals are kept by farmers in Rhodopa mountains and according to Food and Agriculture Organization (FAO) data that breed become extinct. Due to the fact that the gene fund of highly productive dairy cows is getting narrower in the course of years of selection, this breed is a genetic resource for the enrichment of other breeds used in the region. On the other hand, it is of great importance to preserve its own population structure as a native cattle breed for Bulgaria.

Our study aimed to analyze the genetic diversity of CSN1S1 and CSN3 milk protein genes in Shorthorn Rhodopean cattle breed.

Materials and Methods
Sample collection and DNA extraction
A total of 38 blood samples were collected from Shorthorn Rhodopean cows. DNA was extracted from whole blood by commercial GeneJet™ Genomic DNA Purification Kit (Fermentas).

PCR-RFLP analysis
For amplification of the polymorphic region of the CSN1S1 gene, primers described by Koczan et al. (7) were used. They covered parts of the 5'-flanking region and exon 1 (in total 310 bp fragment). Polymorphic region of the CSN3 gene located between exon 4 and intron 4 (in total 350 bp fragment) was amplified using the primers described by Medrano and Cordova (9). Reaction conditions for both investigated gene regions were as followed: initial denaturation 95°C for 5 min.; 35 cycles (denaturation 95°C for 30 sec.; primer annealing 50°C for 30 sec.; extension 72°C for 1 min.) and final extension 72°C for 10 min. PCR products were visualized on 1% agarose gel with ethidium bromide under UV light. Fragments size

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was determined using 1 kb Ladder Plus (Fermentas).
Amplified fragments of CSN1S1 and CSN3 genes were restricted with Tsp45I and HinfI specific endonucleases (Fermentas), respectively for 1 hour on 65°C and 1 hour on 37°C according to manufacturer’s instructions. Restriction products were visualized on 2% agarose gel with ethidium bromide under UV light. Fragments size was determined using GeneRuler™ 100 bp Ladder Plus (Fermentas).

**Statistical analysis**
Analysis of genotype and allele frequencies was calculated independently for both genes. Validity of Hardy-Weinberg equilibrium for the population was evaluated using χ² test (12).

**Results and Discussion**

**CSN1S1 gene polymorphism**
A total of 36 animals were analyzed for CSN1S1 gene polymorphism. Alleles B and C of the CSN1S1 gene differ from each other in one amino acid substitution 192Glu(B)/Gly(C) due to single nucleotide polymorphism (SNPs) at position 26181 bp (A/G). About 53% (20 cows) were homozygous BB animals, while CC animals were presented with the lowest frequency - 13% (5 cows). The heterozygous BC genotype was presented by frequency of 34% (13 cows). The frequency of B allele (0.697%) of the gene was more than twice higher than C allele (0.303%). A recent review of the milk protein nomenclature (5) has indicated nine genetic variants of the CSN1S1 gene (A, B, C, D, E, F, G, H and I) in the genus Bos. For this gene, the most common allele is B followed by C. These allele forms can be found in all cattle breeds.

Our findings are in agreement with observations of many authors, who reported that the domination of BB over the BC genotype is characteristic for all European cattle breeds (10, 17). Distribution of the casein variants shows plain dependence on the geographic origin of the European breeds (11). The authors estimated higher frequency of C allele in south-eastern European breeds in comparison with breeds with north-western and central European origin. For example, in south-eastern European countries like Turkey and Spain (Anatolian Black, Turkish Grey Steppe and Asturian Mountain breed) the frequency of C allele is over 40%. In contrast, cattle populations from the north-western and central European regions like UK, France and Germany (British Friesian, Charolais and German Yellow breed) have frequency of C allele lower than 10%. There is no data for Bulgarian cattle population. Our results clearly show that the frequency of C allele of CSN1S1 gene of Shorthorn Rhodopean cows is identical with that in cattle breeds from south-eastern European countries like Aurochs (Bos primigenius) started ~11 000 years ago from Eurasian ancestor with common name aurochsen (Bos primigenius) (2). They suggested that prevalence of B allele was predominantly found in southern European countries like Turkey, Italy and Spain with frequency lower than 50% (11). Mentioned above genetic variants are the most typical among all cattle breeds but there are others that also show independent geographic distribution (e.g. E allele (UK) and H allele (Near East)). H allele is typical for indicine cattle breeds (Bos indicus) and it is possible to be introduces in Bos taurus population in Near East with frequency ~30% (11). In our study we did not analyze H allele but if it presents in Bulgarian population set it will be indentified like A allele.

**TABLE 1**
Genotype and allele frequencies for the CSN1S1 and CSN3 genes of Shorthorn rhodopean cattle population. NS, non-significant differences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Genotype frequencies</th>
<th>Allele frequencies</th>
<th>χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSN1S1</td>
<td>BB</td>
<td>0.526 0.484</td>
<td>0.697 0.303</td>
<td>0.04</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>0.132 0.868</td>
<td>1.000 0.000</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>0.342 0.658</td>
<td>0.422 0.578</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>CSN3</td>
<td>AA</td>
<td>0.257 0.743</td>
<td>0.213 0.787</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>0.316 0.684</td>
<td>0.291 0.709</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>0.447 0.553</td>
<td>0.497 0.503</td>
<td>0.01</td>
<td>0.99</td>
</tr>
</tbody>
</table>

**Origin, evolution and biogeography of domestic cattle**
According to recent studies cattle domestication started 11 000 years ago from Eurasian ancestor with common name aurochsen (Bos primigenius) (2). Now there are two basic domesticated cattle species – Bos taurus with geographic distribution in
Europe, Near East and North Africa, and Bos indicus (zebu) distributed in South Asia and Africa (4). These species are provisionally differentiated by morphologically and geographical location but some authors define them as subspecies. That is due to their ability of crossbreeding (15) which leads to hybrid cattle breeds’ creation. Cattle origin is not fully clarified but still there are three hypotheses. One of them concerns monophyletic domestication from Near East during the Neolith and secondary worldwide spreading (2). Another hypothesis is based on multiple independent domestications of wild aurochs from different geographical regions (Near East, Indus Valley and North Africa) and time periods (2). Although cattle are one of the most popular domestic species their origin is difficult to clarify because the population of wild animals has disappeared. Only recent researches were focused on present-day cow breeds genetic diversity explanation.

Basic studies utilized mitochondrial D-loop region (1) and hypervariable T region (3). According to the obtained sequence data several haplotype groups were defined for both mtDNA regions characteristic for particular geographic areas (North Africa, Near East, Central and North Europe and South Europe). Published data clearly show that wild cattle in Europe have different haplotypes than present-day domestic cows (1, 2, 3). It is more obviously visible when compare with data from British Isles. These facts show possible introgression of domestic cattle in Europe. Another fact is that haplogroups of domestic cattle in Europe are geographically distinguishable between Mediterranean region, Central and North Europe. Domestic cattle in Mediterranean countries have mixed profiles comprise North African, Near East and common European haplotypes. Cattle in Central and North Europe have predominantly common European haplotype (2).

Genotyping by somatic genes allelic forms also showed a similar geographic relationship. Basically investigations were performed for casein genes – CSN1S1, CSN1S2, CSN2 and CSN3 (11). These investigations are concentrated on identification of allelic variants and their distribution among domestic cattle breeds and geographic regions. For example, B and C alleles of CSN1S1 gene and A and B alleles of CSN3 gene are the most common for European cow breeds (11). The typical for Bos indicus H allele of CSN3 gene also presents in cattle population from Near East and the Balkans (11). Allelic forms frequencies of both genes show different geographic distribution in Europe (Fig. 1).

There were no data for Bulgarian cattle population with respect to genetic polymorphism. This geographic region is boundary area of Europe with Near East and obtained data from genotyping is possible to explain the introgression of Bos indicus haplogroups in southern and eastern European cattle breeds. Our results for Shorthorn Rhodopean, this endemic for Rhodopa mountains cow breed showed genetic identity with southern European cattle breeds (Fig. 1).

Conclusions
In this study endemic for Bulgaria cow breed (Shorthorn Rhodopean cattle) is genotyped by CSN1S1 and CN3 genes. Our results show that B allele of CSN1S1 is predominantly found in its population with frequency of 69%. B allele of CSN3 gene was identified to be 53%. These results show that this ancient cow population may be defined as genetically similar to other cattle population in South Europe.

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REFERENCES


