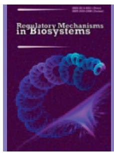

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Molecular genetic characterization of the beta-lactoglobulin gene of Bushuev dairy cattle, a local breed in Uzbekistan

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The article presents the results of a molecular genetic study of the beta-lactoglobulin (BLG) of Bushuev dairy cattle , a local breed in Uzbekistan. Primers BLG_F1 and BLG_R1 were used to amplify the complete lacto-globulin gene of a Bushuev breeding bull. Then this BLG gene was sequenced. Blast analysis of obtained nucleotide sequence of this gene against NCBI GenBank database showed that it has the highest genetic similarity (99.98%) with the Wenshangaofeng breed of Chinese zebu cattle (*Bos indicus*). As a result of multiple alignment (ClustalW) of the BLG gene nucleotide sequence of the Bushuev breed with the Chinese zebu breed "Wenshangaofeng", two novel SNP polymorphisms were identified: one 2711A>G, which is located in the 3rd intron and the second 3426G>C SNP polymorphism, which is located in the 4th intron. Phylogenetic analysis showed that the Bushuev breed is located in the Chinese cluster-1 that includes 5 Chinese zebu breeds: Wenshangaofeng, Weizhou, Guanling, Dabieshan and Jinjiang. Taking into consideration that the Bushuev dairy cattle breed was created by crossing local zebu cattle with Dutch and Swiss Brown bulls and some Simmentals and that the best crosses were bred inter se, it seems that the Bushuev breed retained the complete BLG gene from founder local zebu cattle, which possibly originated from the Chinese zebu Wenshangaofeng breed.

Keywords: local cattle breed; BLG gene; single nucleotide polymorphisms; SNP; phylogenetic analysis; evolution.

Introduction

In modern conditions, the development of livestock farming is impossible without the development of innovative methods of selection and breeding, the introduction of information technologies and the rational use of genetic resources. The experience of many countries with developed animal husbandry indicates the importance of using genetic markers that are associated with the qualitative characteristics of milk productivity in cattle (Caroli et al., 2009; Sherimbetov et al., 2020a). Currently, with the development of molecular genetics and molecular biology, it becomes possible to identify genes directly or indirectly associated with the milk productivity of farm animals. Identification of preferred variants of such genes from the point of view of selection will allow, in addition to the traditional selection of animals, selection to be carried out at the DNA level, that is, by genotype. The widespread introduction of DNA technologies in animal husbandry makes it possible to study genetic markers that control and predict important traits in farm animals. Genetic markers make it possible to test animals of any sex and age. The attention of researchers has recently been attracted by the locus of the gene of one of the main milk proteins – beta-lactoglobulin (BLG).

The protein known as beta-lactoglobulin (BLG) is considered to be a prominent component of the whey protein fraction and makes up around 10% of the total protein in cow's milk (Martorell-Aragones et al., 2015). The milk of ruminant animals contains beta-lactoglobulin, while the milk of humans, mice, and rats does not. It is thought that these proteins help transport small hydrophobic molecules. Specifically, beta-lactoglobulin has been linked to improving the absorption of vitamin A from milk (Pervaiz et al., 1985) and possibly influencing the activity of enzymes (Wodas et al., 2020).

In dairy cows, the BLG gene is located in chromosome 11, which comprises 7 exons and 6 introns and has a total length of 7877 bp (Ilie et al., 2008). The β-LG protein has a molecular weight of 36,000 Dalton, a polypeptide with a single bond of 18 kDa consisting of 162 amino acid residues. Twelve genetic variants of LGB have been found in cattle (A, B, C, D, E, F, G, W, H, I, J, and X). These variants encode different protein forms (Farrell et al., 2004). Among them, A and B variants are considered to be the most common and have been especially associated with variations in milk protein yield and composition (Lunden et al., 1997).

Evaluating genetic diversity and population structure is essential for understanding the evolutionary history of breed origins as well as for providing crucial data for local biodiversity management and conservation (Boettcher et al., 2010; Williams et al., 2016; Sherimbetov et al., 2020b; Sherimbetov et al., 2024; Xie et al., 2024). Molecular-genetic characterization of local cattle breeds offers crucial information to prevent genetic erosion, inbreeding depression, and uncontrolled cross-breeding, all of which can have detrimental effects and ultimately result in extinction (Pariset et al., 2010; Ben Jemaa et al., 2015; Ciampolini et al., 2015).

Various cattle *Bos taurus* breeds have been investigated for identification of BLG polymorphisms. Polymorphism in zebu (*Bos indicus*) and yak (*Bos grunniens*) populations has also been the subject of several researches (Bangar et al., 2022). Elevated levels of BLG protein have been associated with polymorphisms in the BLG gene (Cui et al., 2012).

The genotyping of a bull for the beta-lactoglobulin gene can serve as an additional criterion in the selection of animals. In cattle breeding, the main role in increasing the genetic potential of a breed for selection traits is played by breeding bull. The hereditary qualities of a bull determine the level of herd productivity only 4–5 years after the start of its use. Therefore, the earlier a bull is assessed, the less likely that this sire will turn out to be a deteriorator. The contribution of breeding bulls to the overall genetic improvement of a breed is from 60% to 80%.

The "Bushuev" breed is a dairy cattle local breed of Uzbekistan. It was named after the head of the "Golodnostepnaya" experimental station, Mikhail Mikhailovich Bushuev (1876–1936), who initiated its selection (1905–1918) (FAO Animal Production. Dairy Breeds Bushuev (Bushuevskaya) www.fao.org/4/ah759e/AH759E08.htm). It was created by crossing local zebu cattle with Dutch and Swiss Brown bulls and some Simmentals and the best crosses were bred inter se. Bushuev dairy cattle are well adapted to hot, dry climate conditions and are resistant to blood parasitic diseases. Their milk has a high fat content (4% and above) and protein (3.7–4.2%).

The purpose of the research is molecular genetic characterization of beta-lactoglobulin (BLG) gene of a Bushuev breeding bull in the State Breeding Enterprise "Uznaschilik".

Materials and methods

Ethical approval was granted by the Ethics Committee of the Institute of Genetics and plant experimental biology for the use of the cattle genotypes in this study. Blood sampling were carried out by official veterinarian under animal welfare standards and in strict accordance following the guidelines (https://arriveguidelines.org, www.ncbi.nlm.nih.gov/books/NBK54050).

Written informed consent was obtained from the calf/farm owner before enrolment to the study. The farm owner was informed about the objectives of the study and his rights to withdraw from the study at any time.

The object of the research was a breeding bull of the Bushuev breed nicknamed "Sherkhan", used as semen donor at the State Breeding Enterprise "Uznaslichilik". Blood obtained from the breeding bull was added to test tubes with 100 mM EDTA to a final concentration of 10 mM.

DNA extraction was performed using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific). The bovine lactoglobulingene was amplified using primers BLG_F1 (5'-GTCCCAG CCAAGGTCTGTAG-3') and BLG_R1 (5'-GCTCACCCTATCTT TTCCCTCT-3'). These primers were designed using Primer3Plus at www.bioinformatics.nl/primer3plus (Untergasser et al., 2007). Measurement of quantity and quality of genomic DNA was performed using a spectrophotometer NanoDrop Eight (Thermo Fisher Scientific, USA), then the DNA samples were stored at –20 °C until used for PCR (Table 1).

For PCR, the Platinum™ SuperFi™ DNA Polymerase amplification reagent mix (Thermo Fisher Scientific, USA) was used. The PCR mix (25 µL) contained DNA (4 µL), 13.35 µL dd H_2O, 5 µL 5X SuperFi™ Buffer, 0.4 µL 10 mM dNTP mix, 1 µL 10 mM forward primer, 1 µL 10 mM reverse primer, 0.25 µL Platinum™ SuperFi™ DNA polymerase. PCR was performed on a MiniAmp Plus amplifier (Thermo Fisher Scientific, USA). The following thermocycling program was used for PCR: initial denaturation (98 °C, 30 s), denaturation (98 °C, 10 s), annealing (60 °C, 10 s) , elongation (72 °C, 4 min) – 35 cycles, final elongation (72 °C, 5 min).

The resulting PCR product was analysed by gel electrophoresis with 1xTBE buffer (pH 8.3) in 2% agarose gel, followed by gel staining with 0.5 µg/mL ethidium bromide (EtBr) solution.

Electrophoresis was performed using the horizontal electrophoresis system SE-1 (Helicon, Russia) at 100 V for 100 minutes. PCR products were visualized by UV light and photodocumented by using a gel document imaging system BK-AG100 (Biobase Kings Co., Ltd, China).

Table 1
Primers used in this study

Primer name	Primer nucleotide sequence
BLG_F1	GTCCCAGCCAAGGTCTGTAG
BLG_R1	GCTCACCCTATCTTTTCCCTCT

Sequencing was performed using Oxford Nanopore technology on the MinION sequencer equipped with an R10.4.1 flow cell. The sequencing process was controlled by the MinKNOW program (v.19.12.5). Base-calling was performed using Guppy (version 3.2.10).

The similarity analysis of the nucleotide sequence of the lactoglobulingene was performed using the GenBank database using the BLAST program of the US National Center for Biotechnology Information (NCBI) (Altschul et al., 1990; https://blast.ncbi.nlm.nih.gov/Blast.cgi) and closely related nucleotide sequences of this gene were taken from the NCBI GeneBank database for subsequent phylogenetic analysis. Multiple alignment of nucleotide sequences was performed using the ClustalW algorithm and phylogenetic trees were constructed by the neighbor-joining method using the Kimura 2-parameter model (1000 boostrap repeats) using the MEGA 11 program (Molecular Evolutionary Genetics Analysis version 11 (Boettcher et al., 2010).

Results

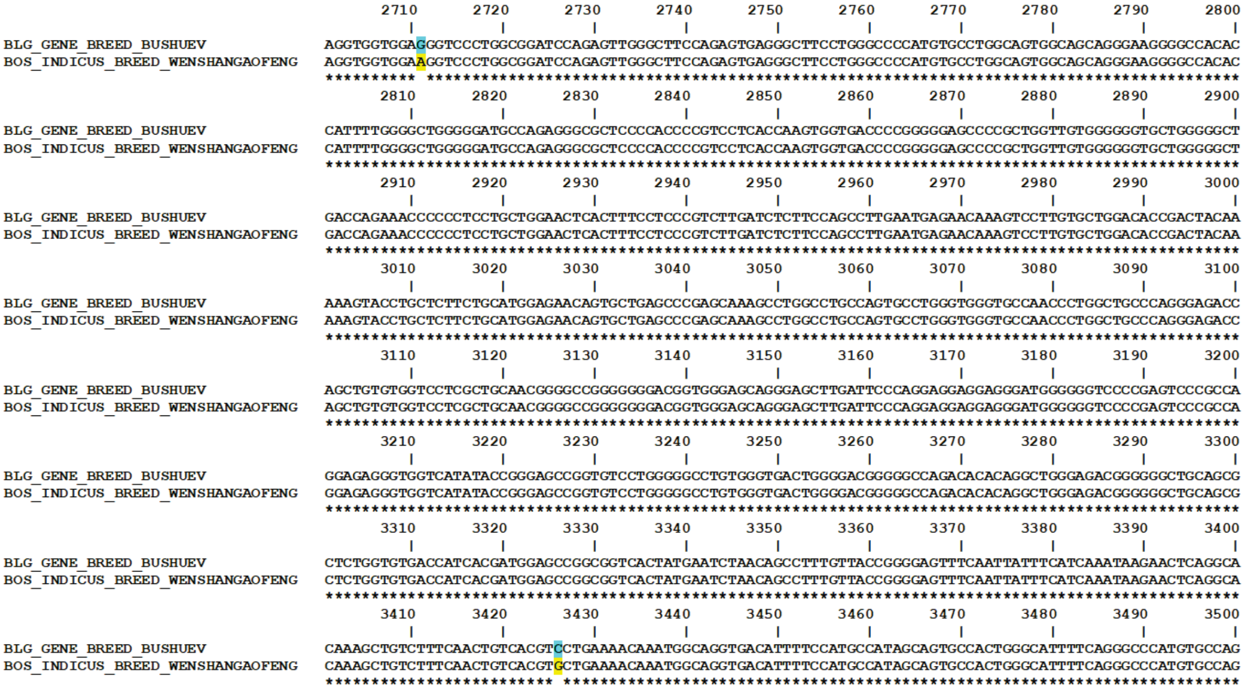
As a result of amplification of the lacto-globulingene region, a PCR product of the expected size (~8500 bp) was obtained. The resulting amplicon was purified and sequenced. The BLG gene nucleotide sequence of the Bushuev breed has been deposited in GenBank NCBI database with accession number PV038021. The results of the Blast analysis of the sequenced lacto-globulingene of the investigated bull show that it has the highest genetic similarity (99.98%) with the Wenshangaofeng breed of Chinese zebu cattle (*Bos indicus*) (Genbank accession number JAKQXP010000003) (Table 1).

As a result of multiple alighnment (Clustal W) of the BLG gene nucleotide sequence of the Bushuev breed with the Chinese zebu (*Bos indicus*) breed "Wenshangaofeng", two SNP polymorphisms were identified: one 2711A>G and the second 3426G>C. 2711A>G SNP polymorphism was located in the 3rd intron and 3426G>C SNP polymorphism waslocated in the 4th intron of BLG gene. Multiple alighnment of the BLG gene with ten other breeds (Weizhou, Guanling, Dabieshan, Jinjiang, Lincanggaofeng, Weining, Leiqiong, Yiling, Xiangxi, Gir) revealed additional 57 SNP and 2 deletions. In total, 59 SNPs and 2 deletions were identified (Table 2).

A phylogenetic tree was constructed that included the sequence of the BLG gene of the investigated Bushuev breeding bull isolate as well as the closest sequences of the BLG gene taken from the NCBI GeneBank database (Fig. 6).

Table 2
Summary of BLASTn results of BLG sequence obtained in this study against the NCBI database

Blastn match	GenBank accession #	Pairwise similarity, %	Query cover, %	E-value
Bos indicus breed Wenshangaofeng	JAKQXP010000003	99.98	100	0.0
Bos indicus breed Dabieshan	JAKQXV010000003	99.94	100	0.0
Bos indicus breed Jinjiang	JAJUAF010000003	99.94	100	0.0
Bos indicus breed Weizhou	JAJUAN010000003	99.93	100	0.0
Bos indicus breed Guanling	JAJUAI010000003	99.93	100	0.0
Bos indicus breed Lincanggaofeng	JAKQXR010000003	99.77	100	0.0
Bos indicus breed Weining	JAJUAU010000003	99.76	100	0.0
Bos indicus breed Leiqiong	JAJUAJ010000003	99.76	100	0.0
Bos indicus breed Gir	PRDE01000003	99.62	100	0.0
Bos indicus breed Yiling	JAKQXO010000003	99.42	100	0.0
Bos indicus breed Xiangxi	JAKQXN010000003	99.36	100	0.0



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                                        2710      2720      2730      2740      2750      2760      2770      2780      2790      2800
                                           |         |         |         |         |         |         |         |         |         |
BLG_GENE_BREED_BUSHUEV            AGGTGGTGGAGGGTCCCTGGCGGATCCAGAGTTGGGCTTCCAGAGTGAGGGCTTCCTGGGCCCCATGTGCCTGGCAGTGGCAGCAGGGAAGGGGCCACAC
BOS_INDICUS_BREED_WENSHANGAOFENG  AGGTGGTGGAAGGTCCCTGGCGGATCCAGAGTTGGGCTTCCAGAGTGAGGGCTTCCTGGGCCCCATGTGCCTGGCAGTGGCAGCAGGGAAGGGGCCACAC
                                  ********** *****************************************************************************************
                                        2810      2820      2830      2840      2850      2860      2870      2880      2890      2900
                                           |         |         |         |         |         |         |         |         |         |
BLG_GENE_BREED_BUSHUEV            CATTTTGGGGCTGGGGATGCCAGAGGGCGCTCCCCACCCCGTCCTCACCAAGTGGTGACCCCGGGGGAGCCCCGCTGGTTGTGGGGGGTGCTGGGGGCT
BOS_INDICUS_BREED_WENSHANGAOFENG  CATTTTGGGGCTGGGGATGCCAGAGGGCGCTCCCCACCCCGTCCTCACCAAGTGGTGACCCCGGGGGAGCCCCGCTGGTTGTGGGGGGTGCTGGGGGCT
                                  ****************************************************************************************************
                                        2910      2920      2930      2940      2950      2960      2970      2980      2990      3000
                                           |         |         |         |         |         |         |         |         |         |
BLG_GENE_BREED_BUSHUEV            GACCAGAAACCCCCCTCCTGCTGGAACTCACTTTCCTCCCGTCTTGATCTCTTCCAGCCTTGAATGAGAACAAAGTCCTTGTGCTGGACACCGACTACAA
BOS_INDICUS_BREED_WENSHANGAOFENG  GACCAGAAACCCCCCTCCTGCTGGAACTCACTTTCCTCCCGTCTTGATCTCTTCCAGCCTTGAATGAGAACAAAGTCCTTGTGCTGGACACCGACTACAA
                                  ****************************************************************************************************
                                        3010      3020      3030      3040      3050      3060      3070      3080      3090      3100
                                           |         |         |         |         |         |         |         |         |         |
BLG_GENE_BREED_BUSHUEV            AAAGTACCTGCTCTTCTGCATGGAGAACAGTGCTGAGCCCGAGCAAAGCCTGGCCTGCCAGTGCCTGGGTGGGTGCCAACCCTGGCTGCCCAGGGAGACC
BOS_INDICUS_BREED_WENSHANGAOFENG  AAAGTACCTGCTCTTCTGCATGGAGAACAGTGCTGAGCCCGAGCAAAGCCTGGCCTGCCAGTGCCTGGGTGGGTGCCAACCCTGGCTGCCCAGGGAGACC
                                  ****************************************************************************************************
                                        3110      3120      3130      3140      3150      3160      3170      3180      3190      3200
                                           |         |         |         |         |         |         |         |         |         |
BLG_GENE_BREED_BUSHUEV            AGCTGTGTGGTCCTCGCTGCAACGGGGCCGGGGGGGACGGTGGAGCAGGGAGCTTGATTCCCAGGAGGAGGAGGGATGGGGGGTCCCCGAGTCCCGCCA
BOS_INDICUS_BREED_WENSHANGAOFENG  AGCTGTGTGGTCCTCGCTGCAACGGGGCCGGGGGGGACGGTGGAGCAGGGAGCTTGATTCCCAGGAGGAGGAGGGATGGGGGGTCCCCGAGTCCCGCCA
                                  ****************************************************************************************************
                                        3210      3220      3230      3240      3250      3260      3270      3280      3290      3300
                                           |         |         |         |         |         |         |         |         |         |
BLG_GENE_BREED_BUSHUEV            GGAGAGGGTGGTCATATACCGGGAGCCGGTGTCCTGGGGGCCTGTGGGTGACTGGGGACGGGGGCCAGACACACAGGCTGGGAGACGGGGGGCTGCAGCG
BOS_INDICUS_BREED_WENSHANGAOFENG  GGAGAGGGTGGTCATATACCGGGAGCCGGTGTCCTGGGGGCCTGTGGGTGACTGGGGACGGGGGCCAGACACACAGGCTGGGAGACGGGGGGCTGCAGCG
                                  ****************************************************************************************************
                                        3310      3320      3330      3340      3350      3360      3370      3380      3390      3400
                                           |         |         |         |         |         |         |         |         |         |
BLG_GENE_BREED_BUSHUEV            CTCTGGTGTGACCATCACGATGGAGCCGGCGGTCACTATGAATCTAACAGCCTTTGTTACCGGGGAGTTTCAATTATTTCATCAAATAAGAACTCAGGCA
BOS_INDICUS_BREED_WENSHANGAOFENG  CTCTGGTGTGACCATCACGATGGAGCCGGCGGTCACTATGAATCTAACAGCCTTTGTTACCGGGGAGTTTCAATTATTTCATCAAATAAGAACTCAGGCA
                                  ****************************************************************************************************
                                        3410      3420      3430      3440      3450      3460      3470      3480      3490      3500
                                           |         |         |         |         |         |         |         |         |         |
BLG_GENE_BREED_BUSHUEV            CAAAGCTGTCTTTCAACTGTCACGTCCTGAAAACAAATGGCAGGTGACATTTTCCATGCCATAGCAGTGCCACTGGGCATTTTCAGGGCCCATGTGCCAG
BOS_INDICUS_BREED_WENSHANGAOFENG  CAAAGCTGTCTTTCAACTGTCACGTGCTGAAAACAAATGGCAGGTGACATTTTCCATGCCATAGCAGTGCCACTGGGCATTTTCAGGGCCCATGTGCCAG
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Fig. 1. Multiple sequence alignment of the BLG gene of the investigated Bushuev breed (Uzbekistan) and *Bos indicus* breed Wenshangaofeng: nucleotide positions are shown in relation to the start codon reference point

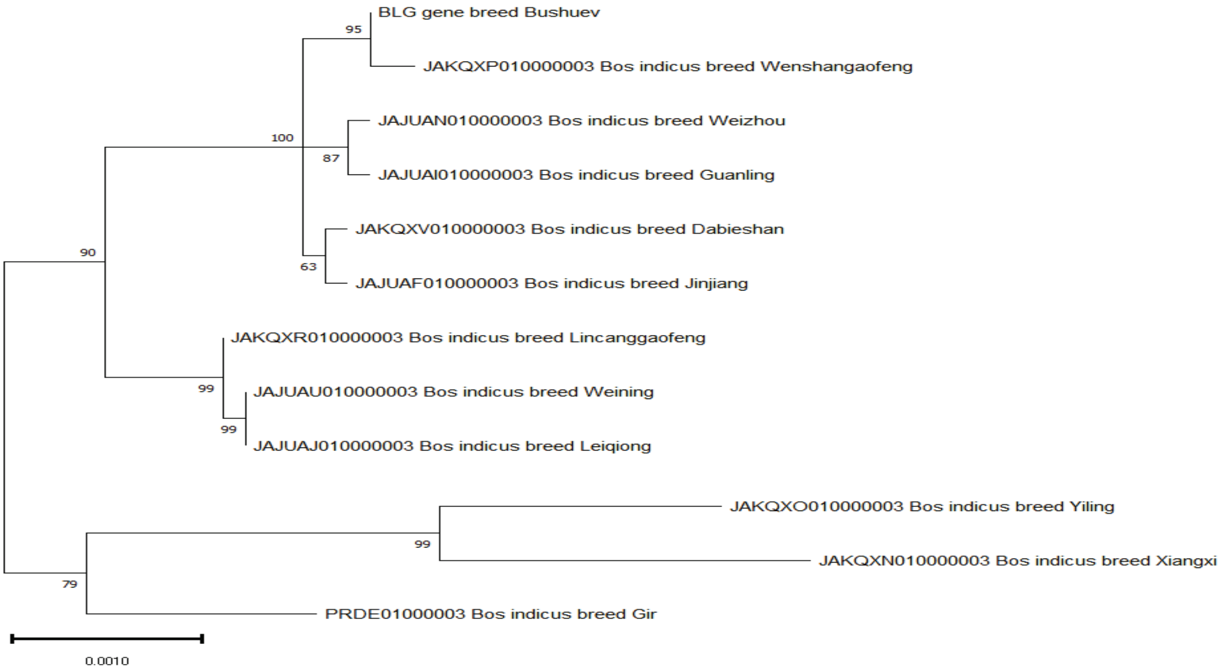

Fig. 2. Neighbor-joining phylogenetic tree of zebu (*Bos indicus*) breeds

Phylogenetic analysis revealed that breeds grouped in three clusters. The phylogenetic tree showed that the Bushuev breeding bull isolate is located in the Chinese cluster-1 that includes 5 Chinese zebu breeds: Wenshangaofeng, Weizhou, Guanling, Dabieshan and Jinjiang. The second cluster consists of 3 Chinese zebu breeds: Lincanggaofeng, Weining, Leiqiong. and the third cluster includes 2 Chinese zebu breeds (Yiling, Xiangxi) and one Indian zebu breed Gir.

Analysis of encoded protein of the BLG gene of the investigated Bushuev breeding bull isolate reveals that this isolate has a homozygous genotype BB (Fig. 4).

Discussion

The results from this study are consistent with several prior studies suggesting that zebu cattle were domesticated 8,000–9,000 years ago (B.P.) in India and dispersed throughout northwestern South Asia through China, Afghanistan, Southern Central Asia and modern day Iran by 6,000 (Fig. 3) (Pérez-Pardal et al., 2018; Semenenko et al., 2019; Ajmone-Marsan et al., 2010; Chen et al., 2010). The results of our phylogenic analysis regarding the close relatedness of the native breed in Uzbekistan with Chinese zebu breeds reveal profound influence of the Silk Road (an ancient network of trade routes that connected the East and West) on genetic diversity of cattle in Uzbekistan. Our data confirm the existence of a so called "The biological silk road" that involved the exchange of crops, livestock, pathogens, and genes along the Silk Road. It is worth noting the positive consequences associated with the zebu genetic founder effect during breeding of the Bushuev breed. There are several reports emphasizing that milk of zebu cattle offers numerous health benefits. It has higher nutritional quality (Siddiqui et al., 2024), enhances the immune system (Sharma et al., 2019) and improves cognitive function and memory in people (Ogata et al., 2016). Analysis of encoded protein of the BLG gene of the investigated Bushuev breeding bull isolate reveal sthat this isolate has a homozygous genotype BB. Understanding of the BLG genotype of cattle is very important since it is linked to a number of factors that are significant to the dairy industry. The BLG B variant is linked to greater cheese yields (Meza-Nieto et al., 2013). It also has been shown that the β-lactoglobulin BB genotype resulted in the higher fat content (Cendron et al., 2021). Since human milk contains a comparatively high concentration of α-LA and lacks BLG (de Wit, 1998), whey composition linked to the BLG variant B is advantageous when utilized as a component for infant nutrition. Knowledge on BLG genotypes might be useful for Uzbekistan farmers who manufacture their milk into cheese.

Table 3
SNPs and deletions identified in this study

Breed name	SNPs according to the nucleotide position (in relation to the start codon reference point)																														
	32	54	102	169	279	375	426	569	577	583	648	726	808	915	1063	1077	1372	1539	1624	1640	1680	1772	1916	1917	1926	1997	1998	2084	2089	2137	2164
BUSHUEV	T	T	C	C	G	T	A	T	C	C	C	C	C	A	C	C	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
WENSHANGAOFENG	T	T	C	C	G	T	A	T	C	C	C	C	C	A	C	C	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
WEIZHOU	T	T	C	T	G	T	A	T	C	C	C	C	C	A	C	C	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
GUANLING	T	T	C	C	G	T	A	T	C	C	C	C	C	A	C	C	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
DABIESHAN	T	T	C	C	G	T	A	T	C	C	C	C	C	A	C	C	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
JINJIANG	T	T	C	C	G	T	A	T	C	C	C	C	C	A	C	C	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
LINCANGGAOFENG	T	C	C	C	G	T	A	T	C	T	C	C	C	A	C	T	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
WEINING	T	C	C	C	G	T	A	T	C	T	C	C	C	A	C	T	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
LEIQIONG	T	C	C	C	G	T	A	T	C	T	C	C	C	A	C	T	C	A	A	C	T	G	G	A	G	T	G	G	C	A	G
YILING	C	C	G	C	A	C	G	C	C	C	T	G	C	C	T	T	C	G	G	T	T	G	A	G	G	C	G	A	T	A	G
XIANGXI	C	T	G	C	A	T	G	C	T	C	T	G	delC	A	T	C	C	G	G	T	G	A	A	G	A	C	A	G	T	delA	A
GIR	T	C	G	C	A	T	A	T	C	C	C	G	delC	A	C	T	T	A	A	C	T	G	A	G	A	C	A	G	T	A	G

Breed name	SNPs according to the nucleotide position (in relation to the start codon reference point)																													
	2297	2319	2349	2391	2494	2532	2711	2765	2806	2807	2890	2910	2940	2953	2965	3054	3130	3228	3239	3289	3300	3426	3475	3527	3572	3893	3943	3957	4013	4041
BUSHUEV	C	G	C	G	A	C	G	A	T	G	T	C	C	T	T	C	G	G	G	G	G	C	G	G	C	C	C	C	C	T
WENSHANGAOFENG	C	G	C	G	A	C	A	A	T	G	T	C	C	T	T	C	G	G	G	G	G	G	G	G	C	C	C	C	C	T
WEIZHOU	C	G	C	G	A	C	G	A	T	G	T	C	C	T	T	C	G	G	G	G	G	C	G	G	C	C	C	C	C	T
GUANLING	C	G	C	G	A	C	G	A	T	G	T	C	C	T	T	C	G	G	G	G	G	C	G	G	C	C	C	C	C	T
DABIESHAN	C	G	T	G	A	C	G	A	T	G	T	C	C	T	T	C	G	G	G	G	G	C	G	G	C	C	C	C	C	T
JINJIANG	C	G	T	G	A	C	G	A	T	G	T	C	C	T	T	C	G	G	G	G	G	C	G	G	C	C	C	C	C	T
LINCANGGAOFENG	C	G	C	G	A	C	G	A	T	G	T	C	C	T	C	C	G	G	G	G	G	C	G	T	C	C	T	C	C	C
WEINING	C	G	C	G	A	C	G	A	T	G	T	T	C	T	C	C	G	G	G	G	G	C	G	T	C	C	T	C	C	C
LEIQIONG	C	G	C	G	A	C	G	A	T	G	T	T	C	T	C	C	G	G	G	G	G	C	G	T	C	C	T	C	C	C
YILING	T	G	C	A	G	T	G	G	C	A	C	C	C	A	C	C	G	G	G	G	A	C	G	T	G	C	C	T	C	C
XIANGXI	C	A	C	A	G	T	G	G	C	A	C	C	T	A	C	T	G	A	A	G	G	C	T	T	C	C	C	C	C	C
GIR	C	G	C	A	A	C	G	A	T	G	T	C	C	T	C	C	A	G	G	C	G	C	T	T	C	T	C	C	T	C

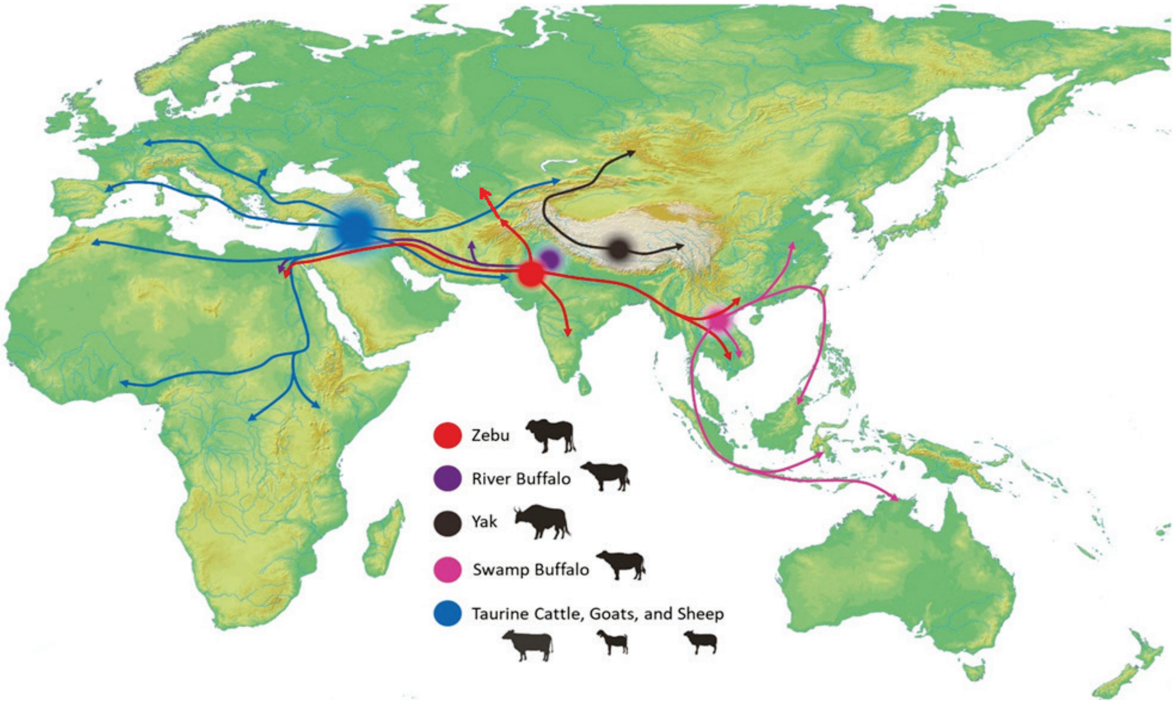

Fig. 3. Migration and evolution ways of livestock animals

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                         10        20        30        40        50        60        70        80        90       100
                          |         |         |         |         |         |         |         |         |         |
Bushuev_BLG      MKCLLLALALTCGAQALIVTQTMKGLDIQKVAGTWYSLAMAASDISLLDAQSAPLRVYVEELKPTPEGDLEILLQKWENGECAQKKIIAEKTKIPAVFKI
BLG_B_allele     MKCLLLALALTCGAQALIVTQTMKGLDIQKVAGTWYSLAMAASDISLLDAQSAPLRVYVEELKPTPEGDLEILLQKWENGECAQKKIIAEKTKIPAVFKI
                 ****************************************************************************************************
                        110       120       130       140       150       160       170
                          |         |         |         |         |         |         |
Bushuev_BLG      DALNENKVLVLDTDYKKYLLFCMENSAEPEQSLACQCLVRTPEVDDEALEKFDKALKALPMHIRLSFNPTQLEEQCHI
BLG_B_allele     DALNENKVLVLDTDYKKYLLFCMENSAEPEQSLACQCLVRTPEVDDEALEKFDKALKALPMHIRLSFNPTQLEEQCHI
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Fig. 4. Amino acid sequence alignment of BLG encoded protein of Bushuev breed with BLG B reference allele

Conclusion

The Bushuev dairy cattle breed has the greatest genetic similarity to the Wenshangaofeng *Bos indicus* breed in terms of the beta-lactoglobulin (BLG) gene. Taking into consideration that the Bushuev dairy cattle breed was created by crossing local zebu cattle with Dutch and Swiss Brown bulls and some Simmentals and that the best crosses were bred inter se, it seems that the Bushuev breed retained the complete BLG gene from founder local zebu cattle, which possibly originated from Chinese zebu of the Wenshangaofeng breed.

All authors declare that they have no conflicts of interest.

References

Ajmone-Marsan, P., Garcia, J. F., & Lenstra, J. A. (2010). On the origin of cattle: How aurochs became cattle and colonized the world. Evolutionary Anthropology: Issues, News, and Reviews, 19(4), 148–157.

Bangar, Y. C., Patil, C. S., Magotra, A., & Yadav, A. S. (2021). Meta-analysis of gene polymorphism of beta-lactoglobulin gene in Indian dairy cows. Biochemical Genetics, 60(3), 1039–1048.

Ben Jemaa, S., Boussaha, M., Ben Mehdi, M., Lee, J. H., & Lee, S.-H. (2015). Genome-wide insights into population structure and genetic history of tunisian local cattle using the illumina bovinesnp50 beadchip. BMC Genomics, 16(1), 677.

Boettcher, P. J., Tixier-Boichard, M., Toro, M. A., Simianer, H., Eding, H., Gandini, G., Joost, S., Garcia, D., Colli, L., & Ajmone-Marsan, P. (2010). Objectives, criteria and methods for using molecular genetic data in priority setting for conservation of animal genetic resources. Animal Genetics, 41(s1), 64–77.

Caroli, A. M., Chessa, S., & Erhardt, G. J. (2009). Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. Journal of Dairy Science, 92(11), 5335–5352.

Cendron, F., Franzoi, M., Penasa, M., De Marchi, M., & Cassandro, M. (2021). Effects of β- and κ-casein, and β-lactoglobulin single and composite genotypes on milk composition and milk coagulation properties of Italian Holsteins assessed by FT-MIR. Italian Journal of Animal Science, 20(1), 2243–2253.

Chen, S., Lin, B.-Z., Baig, M., Mitra, B., Lopes, R. J., Santos, A. M., Magee, D. A., Azevedo, M., Tarroso, P., Sasazaki, S., Ostrowski, S., Mahgoub, O., Chaudhuri, T. K., Zhang, Y.-P., Costa, V., Royo, L. J., Goyache, F., Luikart, G., Boivin, N., … Beja-Pereira, A. (2009). Zebu cattle are an exclusive legacy of the South Asia neolithic. Molecular Biology and Evolution, 27(1), 1–6.

Ciampolini, R., Casu, S., Mastrangelo, S., Flori, L., Moazami, G. K., Sechi, T. (2015). Genetic diversity of Mediterranean cattle breeds related to geography and climate. In: Proceedings of the 21st animal science and production association congress, 14, 65–66.

de Wit, J. N. (1998). Nutritional and functional characteristics of whey proteins in food products. Journal of Dairy Science, 81(3), 597–608.

Farrell, H. M., Jimenez-Flores, R., Bleck, G. T., Brown, E. M., Butler, J. E., Creamer, L. K., Hicks, C. L., Hollar, C. M., Ng-Kwai-Hang, K. F., & Swaisgood, H. E. (2004). Nomenclature of the proteins of cows' milk – sixth revision. Journal of Dairy Science, 87(6), 1641–1674.

Ilie, D. E., Gavojdian, D., Kusza, S., Neamţ, R. I., Mizeranschi, A. E., Mihali, C. V., & Cziszter, L. T. (2023). Kompetitive allele specific PCR genotyping of 89 SNPs in Romanian Spotted and Romanian Brown cattle breeds and their association with clinical mastitis. Animals, 13(9), 1484.

Lundén, A., Nilsson, M., & Janson, L. (1997). Marked effect of β-lactoglobulin polymorphism on the ratio of casein to total protein in milk. Journal of Dairy Science, 80(11), 2996–3005.

Martorell-Aragonés, A., Echeverría-Zudaire, L., Alonso-Lebrero, E., Boné-Calvo, J., Martín-Muñoz, M. F., Nevot-Falcó, S., Piquer-Gibert, M., & Valdesoiro-Navarrete, L. (2015). Position document: IgE-mediated cow's milk allergy. Allergologia et Immunopathologia, 43(5), 507–526.

Meza-Nieto, M. A., González-Córdova, A. F., Piloni-Martini, J., & Vallejo-Cordoba, B. (2013). Effect of β-lactoglobulin A and B whey protein variants on cheese yield potential of a model milk system. Journal of Dairy Science, 96(11), 6777–6781.

Ogata, S., Tanaka, H., Omura, K., Honda, C., & Hayakawa, K. (2016). Association between intake of dairy products and short-term memory with and without adjustment for genetic and family environmental factors: A twin study. Clinical Nutrition, 35(2), 507–513.

Pariset, L., Mariotti, M., Nardone, A., Soysal, M. I., Ozkan, E., Williams, J. L., Dunner, S., Leveziel, H., Maróti-Agóts, A., Bodò, I., & Valentini, A. (2010). Relationships between Podolic cattle breeds assessed by single nucleotide polymorphisms (SNPs) genotyping. Journal of Animal Breeding and Genetics, 127(6), 481–488.

Pérez-Pardal, L., Sánchez-Gracia, A., Álvarez, I., Traoré, A., Ferraz, J. B. S., Fernández, I., Costa, V., Chen, S., Tapio, M., Cantet, R. J. C., Patel, A., Meadow, R. H., Marshall, F. B., Beja-Pereira, A., & Goyache, F. (2018). Legacies of domestication, trade and herder mobility shape extant male zebu cattle diversity in South Asia and Africa. Scientific Reports, 8(1), 18027.

Pervaiz, S., & Brew, K. (1985). Homology of β-lactoglobulin, serum retinol-binding protein, and protein HC. Science, 228(4697), 335–337.

Semenenko, A. A. (2019). The spread of zebu cattle from South Asia to the East Mediterranean region as a marker of Indo-European population dispersal. Bulletin Social-Economic and Humanitarian Research, 2(4), 3–27.

Sharma, A., Shandilya, U. K., Sodhi, M., Mohanty, A. K., Jain, P., & Mukesh, M. (2019). Evaluation of milk colostrum derived lactoferrin of Sahiwal (*Bos indicus*) and Karan Fries (cross-bred) cows for its anti-cancerous potential. International Journal of Molecular Sciences, 20(24), 6318.

Sherimbetov A. G. Namazov, S. E., Adilov, B. S., Ruzmetov, D. R., Sadiqov, K. R., Matyoqubov, S. K., & Karimov, E. Y. (2020a). Investigation and identification of phytopathogenic and saprophytic *Fusarium* species in the agricultural fields soil layers of the Republic of Uzbekistan. Plant Cell Biotechnology and Molecular Biology, 21, 101–108.

Sherimbetov, A. G., Adilov, B. S., Kadirova, Z. N., Makhmudov, T. X., Mambetnazarov, A. B., Ruzmetov, D. R., Yuldashov, U. X., & Karimov, E. Y. (2020b). Molecular verification of species identity of some isolates of the genus *Fusarium* deposited in the phytopathogen collection in Uzbekistan. Plant Cell Biotechnology and Molecular Biology, 21, 94–98.

Sherimbetov, A. G., Zaynitdinova, L. I., Adilov, B. S., & Ruzmetov, D. R. (2024). *Trichoderma afroharzianum* species associated with the anthropogenically polluted soils in Uzbekistan. SABRAO Journal of Breeding and Genetics, 56(5), 1946–1957.

Sherimbetov, A., Sherimbetov, S., Adilov, A., Ruzmetov, D., & Shavkiev, J. (2024) Rapid detection of *Alternaria* spp. by PCR in the newly created forest plantations on the drained bottom of the Aral Sea. Regulatory Mechanisms in Biosystems, 15(2), 361–366.

Siddiqui, S. A., Schulte, H., Golik, A. B., Pandiselvam, R., Venkidasamy, B., Homayouni, A., & Maqsood, S. (2024). Traditional and commercial dairy products from yak, camel, zebu-brahma, mithun, reindeer and sow: A review on current research status. International Dairy Journal, 152, 105879.

Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., & Leunissen, J. A. (2007). Primer3Plus, an enhanced web interface to Primer3. Nucleic acids research, 35, W71–W74.

Williams, J. L., Hall, S. J., Del Corvo, M., Ballingall, K. T., Colli, L., Ajmone Marsan, P., & Biscarini, F. (2016). Inbreeding and purging at the genomic level: the Chillingham cattle reveal extensive, non-random SNP heterozygosity. Animal genetics, 47(1), 19–27.

Wodas, L., Mackowski, M., Borowska, A., Puppel, K., Kuczynska, B., & Cieslak, J. (2020). Genes encoding equine β-lactoglobulin (LGB1 and LGB2): Polymorphism, expression, and impact on milk composition. PloS One, 15(4), e0232066.

Xie, X., Shi, L., Zhong, Z., Wang, Z., Pan, D., Hou, G., & Xiao, Q. (2024). Danzhou chicken: A unique genetic resource revealed by genome-wide resequencing data. Poultry Science, 103(8), 103960.