MITOCHONDRIAL DNA PART B
2019, VOL. 4, NO. 2, 4006–4009
https://doi.org/10.1080/23802359.2019.1688695

Taylor & Francis
Taylor & Francis Group

MITO COMMUNICATION

Georgian cattle, sheep, goats: are they of Near-Eastern origins?

Nana Kunelauri, Mari Gogniashvili, Vazha Tabidze, Givi Basiladze and Tengiz Beridze

Institute of Molecular Genetics, Agricultural University of Georgia, Tbilisi, Georgia

ABSTRACT

The main aim of this research was to establish the nucleotide sequence of the highly variable region of the D loop of the mitochondrial DNA of some Georgian domestic animal species (cattle, goat, sheep) as well as their phylogenetic position among the worldwide set of domestic animals. In this study, a total of 5 haplogroups (T – 5; T3 – 7; T1 – 1; T2 – 2; T5 – 2) in 17 Georgian Mountain cattle (GMC), 4 haplogroups (A – 15; A2a1 – 3; A1a – 1; A6 – 3) in 22 Georgian goats and 3 haplogroups (A – 10; B – 16; C -15) in 41 Georgian sheeps (15 Imeretian and 26 Tushetian) were detected. This study represents the first attempt of Genetic study of native Georgian livestocks. The GMC, Georgian (Megrelian) goat, Georgian (Imeretian and Tushetian) sheep mitogenomes were grouped phylogenetically in the haplogroups indicating the closeness to the Near Eastern animals.

ARTICLE HISTORY
Received 12 August 2019
Accepted 26 October 2019

KEYWORDS
Livestock; domestication; Kartvelians; Caucasus; migration

Introduction

MtDNA sequencing

DNA extraction was carried out from blood samples by standard proteinase K/phenol-chloroform procedures (Baechtel 1989). Aliquots of blood samples were collected in EDTA vacutainer and after cell lysis with SDS was undergone enzymatic digestion by proteinase K. Further, isolation procedure was followed by Phenol/chloroform extraction and alcohol precipitation. The DNA pellet was finally dissolved in TE buffer, pH 8.0.

PCR conditions included denaturing of genomic DNA at 94 °C (1 min), 30 cycles of 94 °C denaturing (1 min), 55 °C annealing (1 min), and 72 °C extension (2 min), followed by a final extension step at 72 °C (5 min). Purified PCR products were sequenced at the Laboratory Services Division of the University of Guelph (ON, Canada) (GenBank accession; Table S1 in Supplementary material).

For detection of SNP (single-nucleotide polymorphism) and Indels (insertion/deletion) computer Mafft software was used (Katoh et al. 2002). To illustrate the evolutionary relationship among the studied animals, a phylogenetic tree was constructed based on multiple alignments using Jalview version 2 (Waterhouse et al. 2009).

Cattle

The Georgian Mountain Cattle (GMC) is a local breed from Georgia. The breed is distributed in 15 districts in the west and east part of Georgia. It is well adapted to the harsh mountain conditions of the Caucasus. The slope of grazing lands reaches 45 degree. GMC are very small: the live weight of mature cows is 220–280 kg and that of bulls is 270–370 kg; Coat color is black, black-and-white or red-and-white.

Detailed diversity analysis of a worldwide cattle (*Bos taurus*) is given in several publications (Troy et al. 2001; Di Lorenzo et al. 2016) According to recent publications, molecular diversity approach revealed that modern taurine mitochondrial genomes cluster within a number of closely related branches, termed T, T1, T2, T3, and T4, are geographically well structured: T1 predominantly found in Africa; T2 originates in the Near East and Western Asia; and T3 found in Europe and originates from the expansion of a small cattle population domesticated in the Middle East (Di Lorenzo et al. 2016).

Seventeen cattle of GMC breed were selected in the Dusheti District (Georgia). A 350-bp fragment located in the most highly variable region of the D-loop of mtDNA (including hypervariable region I, nucleotide positions 16,023–16,262) was amplified using the primers AN4 (5′-GGTAATGTACATAACATTAATG-3′) and AN3(5′-CGAGATGTCT TATTTAAGAGG-3′), as described by Cymbron et al. (1999) to positions 15,959–16,334. The highly variable regions of the D loop of the mtDNA of 17 cattle of GMC were sequenced (Table 1). Sequence analysis of PCR products revealed that 17 animals belong to 5 haplogroups: T – 5; T3 – 7; T1 – 1; T2 – 2; T5 – 2. Four novel haplotypes were observed which have not been found in any other cattle breed so far. The GMC mitogenomes are grouped phylogenetically in the T-haplogroup indicating the closeness to the Near Eastern Cattle as well as to the South European Red Mountain Cattle

CONTACT Mari Gogniashvili m.gogniashvili@agruni.edu.ge Institute of Molecular Genetics, Agricultural University of Georgia, #240 D.Agmashenebeli Alley, Tbilisi 0159, Georgia

Supplemental data for this article can be accessed here.

Table 1. Mitochondrial control region sequence variations observed in 17 Georgian mountain cattle.

	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
	6	6	6	6	6	6	6	6	6	6	6	6	6	6		
	0	0	0	0	1	1	1	1	2	2	2	2	2	3		
	5	5	5	8	1	8	9	9	0	0	4	4	5	0		
No. of sample	0	7	8	5	3	5	2	5	0	8	7	8	5	1	Haplogroup	New haplotype (NH)
	C	G	C	A	T	G	C	A	G	T	C	C	T	C	T3 .BRS.Ref(V00654)	
1,6,10,14										C	T		C		T	NH
2,12															T3	
3		C				A							C	T	T2	
13		C				A							C		T2	
4,5,7,8				C											T3	
9		C	T					G	A					T	T3	NH
11	T		T		C								C		T1	NH
15													C		T5	
16							A					T	C		T5	NH
17											T		C		T	

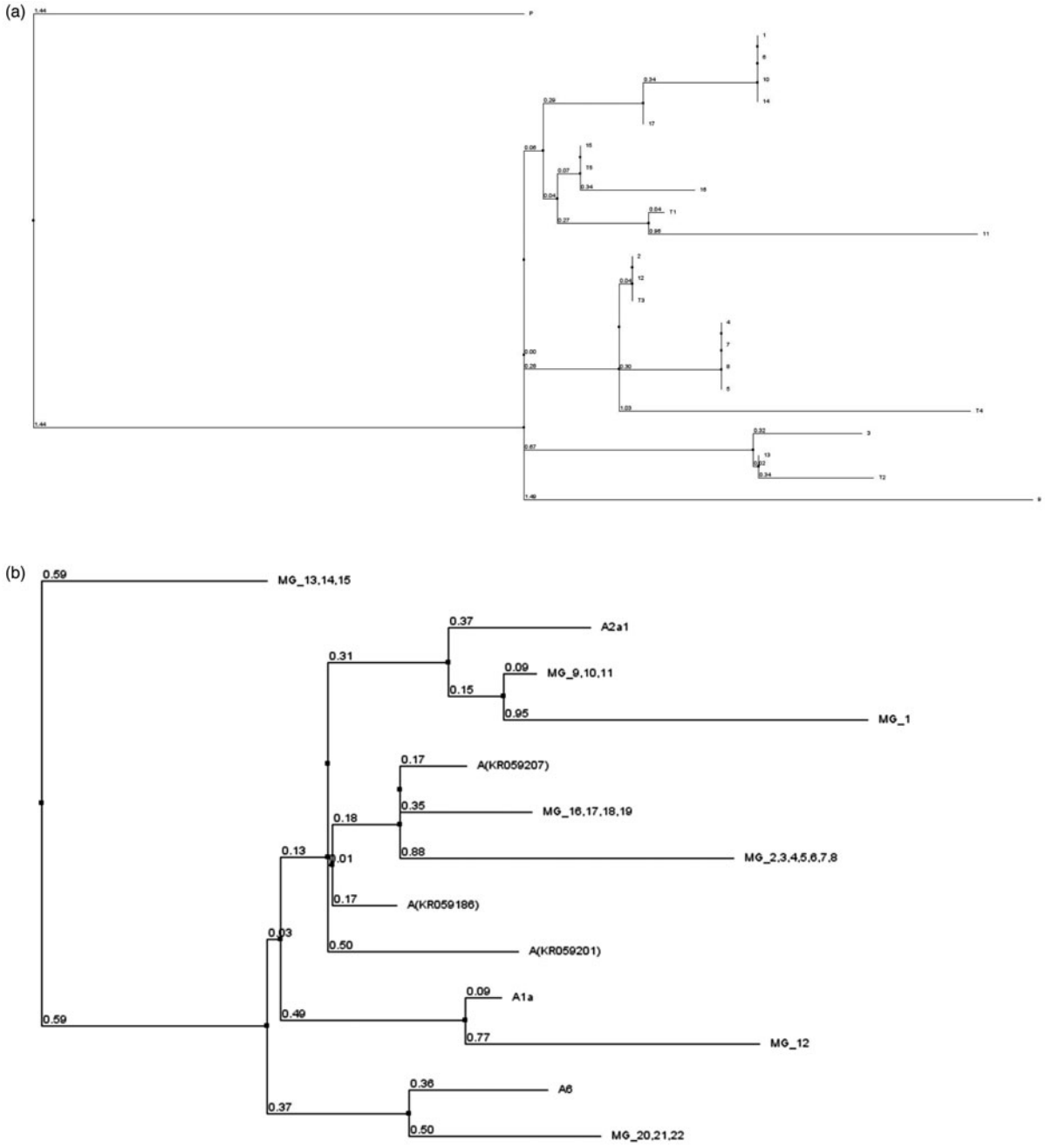

Figure 1. MtDNA phylogeny of GMC (a), Georgian (Megrelian) goat (b) and Georgian (Imeretian and tushetian) sheep (c). Neighbor joining tree using PID

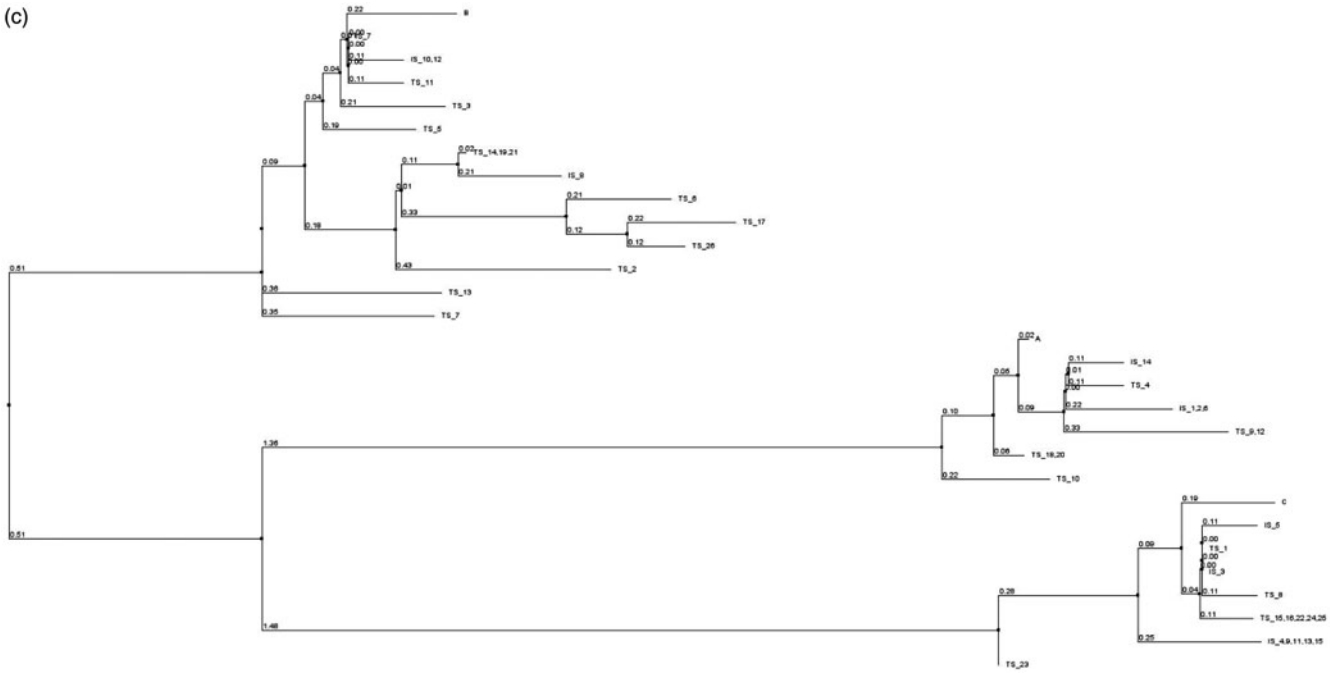

Figure 1. Continued.

(Figure 1(a)) Four mitogenomes of one of the maternal founder lineages of GMC coincide with the mitogenome of the Red Mountain Cattle (Samples #4,5,7,8) (Ludwig et al. 2014).

Goat

More than 1,200 breeds are described in the world domestic goat (*Capra hircus*) (Colli et al. 2015). In the domestic goat six distinctive domestic haplogroups of mtDNA were identified – A, B (B1, B2), C, D, F, and G (Naderi et al. 2007). Globally, haplogroup A has the largest geographic distribution, more than 90% of goats examined were in haplogroup A (Colli et al. 2015). Haplogroup B occurs in eastern and southern Asia and at low frequencies in South Africa and Namibia. Haplogroup C occurs at low frequencies in Mongolia, Switzerland, Slovenia, Pakistan, and India, while haplogroup D occurs only in Pakistan and India. Haplogroup F is exclusive to 'Sicily (Italy)' while haplogroup G has been observed in Turkey, Iran, Iraq, Saudi Arabia, Kenya and Egypt (Colli et al. 2015; Tarekegn et al. 2018)

The Georgian 22 (Megrelian) goat is an ancient breed from West Georgia. The blood samples of Georgian (Megrelian) goats have been taken in West Georgia, villages Maghlaki (Tskaltubo District), Nakiani and Akhuti (Chkorotsku District). The variable fragment (HVI segment) of the control region of the goat mtDNA was amplified by PCR analysis using the primers CAP-F (5′-CGTGTATGCAAGTACATTAC-3′) and CAP-R (5′-CTGATTAGTCATTAGTCCATC-3′). The 638 bp fragment, which correspond the 15,635–16,273 positions of the reference (LS992661_1) DNA (Naderi et al. 2007) was amplified. 15,679–16,254 region of HVI segment was sequenced. Twenty-two animals belong to four haplogroups: A – 15; A2a1 – 3; A1a – 1; A6 – 3. Seven novel haplotypes were observed which have not been found in any other goat breed so far (Table S2 in Supplementary material). The goat mitogenomes are grouped phylogenetically in the A-haplogroup indicating the closeness to the Near Eastern goats (Figure 1(b)).

Sheep

More than 1,400 breeds are nowadays recognized in the world domestic sheep (*Ovis aries*) diversity (Scherf 2000). Four haplogroups A, B, C and D have been found in the domestic sheep. Group B has been found in European domestic sheep, although archeological evidence supports sheep domestication in the central part of the Fertile Crescent (Zeder 2008). Four haplogroups A, B, C, and D were observed in Caucasus, three A, B and C in Central Asia, and two A and B in the Eastern Europe. Only one example of haplogroup D was detected in a single Caucasian sheep (Tapio et al. 2006). Culture of sheep farming has been known in Georgia for centuries predominately in mountainous regions (Rcheulishvili 1957). Coverage of the Tushetian sheep is located in east part of Georgia. Specifically, it is very popular for its high resistance against severe environmental conditions. The Imeretian sheep was obtained by the selection of folk cultures in VI-VII century. The 15 samples of Imeretian sheep have been taken in West Georgia, from Imereti Province (village Maghlaki, Tskaltubo District), 26 samples from Alvani village in East Georgia (Akhmeta District). The mtDNA control region (positions 1–33, 15451–16616 of the reference was analyzed (Hiendleder et al. 1998). PCR analysis was used for amplification of two overlapping fragments (for fragment 1, SIF: 5′-CCCCACTATCAACACCCAAA-3′ and SIR: 5′-CATGGT GAACAAGCTCGTGA-3′, and for fragment 2, SIIF: 5′-TGCTTGACCGTACATAGTACAT-3′ and SIIR: 5′-CATCTAGGC ATTTTCAGTGCC-3′) (Pereira et al. 2006). The highly variable

regions of the D-loop of mtDNA of 41 Georgian (15 Imeretian and 26 Tushetian) sheep were sequenced. They belong to 3 haplogroups: A – 10; B – 16; C – 15. Twenty-six novel haplotypes were observed that have not been found in any other sheep breed so far (Table S3 in Supplementary material). No specific difference between Imeretian and Tushetian sheep were observed. The sheep mitogenomes are grouped phylogenetically in the A, B and C haplogroups indicating the closeness to the Near Eastern sheep's as well as to the European type of sheep's (haplogroup B) (Figure 1(c)).

Conclusion

This study represents the first attempt of genetic study of some native Georgian livestocks.

The GMC, Georgian (Megrelian) goat, Georgian (Imeretian and Tushetian) sheep mitogenomes are grouped phylogenetically in the haplogroups indicating the closeness to the Near Eastern animals (Figure 1(a–c)).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Knowledge Fund, a funding organization of the Free University of Tbilisi and Agricultural University of Georgia.

References

Baechtel FS. 1989. The extraction, purification and quantification of DNA. In: Proceedings of the International Symposium on the Forensic Aspects of DNA Analysis. Washington, DC: U.S. Government Printing Office; p. 25–28.

Colli L, Cardinali H, Olivieri I, Capodiferro A, Pellecchia MR, Rzepus M, Zamani M, Naderi W, Gandini S, Vahidi F, et al. 2015. Whole mitochondrial genomes unveil the impact of domestication on goat matrilineal variability. BMC Genomics. 16:1115–1127.

Cymbron T, Loftus RT, Malheiro MI, Bradley DG. 1999. Mitochondrial sequence variation suggests an African influence in Portuguese cattle. Proc R Soc Lond B. 266(1419):597–603.

Di Lorenzo P, Lancioni H, Ceccobelli S, Curcio L, Panella F, Lasagna E. 2016. Uniparental genetic systems: a male and a female perspective in the domestic cattle origin and evolution. Electron J Biotechnol. 23: 69–78.

Hiendleder S, Mainz K, Plante Y, Lewalski H. 1998. Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: no evidence for contributions from urial and argali sheep. J Heredity. 89(2):113–120.

Katoh K, Misawa K, Kuma K-I, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acids Res. 30(14):3059–3066.

Ludwig A, Lieckfeldt D, Hesse UGW, Froelich K. 2014. Tracing the maternal roots of the domestic Red Mountain Cattle. Mitochondrial DNA A DNA Mapp Seq Anal. 2016. 27(2):1080–1083.

Naderi S, Rezaei H-R, Taberlet P, Zundel S, Rafat S-A, Naghash H-R, Mohamed AA, El-Barody Ertugrul O, Pompanon F. 2007. Large-scale mitochondrial DNA analysis of the domestic goat reveals six maternal lineages with high haplotype diversity. PLoS One. 10:e1012.

Pereira F, Davis SJM, Pereira L, McEvoy B, Bradley DG, Amorim A. 2006. Genetic signatures of a mediterranean influence in iberian peninsula sheep husbandry. Mol Biol Evol. 23(7):1420–1426.

Rcheulishvili MD. 1957. To the history of Georgian sheep. Tbilisi: Academy of Science Publishing House; p. 219.

Scherf BD, editor. 2000. World Watch List for domestic animal diversity. 3rd ed. Rome: Food and Agriculture Organization of the United Nations; p. 58.

Tapio M, Marzanov N, Ozerov M, Cinkulov M, Gonzarenko G, Kiselyova T, Murawski M, Viinalass H, Kantanen J. 2006. Sheep mitochondrial DNA variation in European, Caucasian, and Central Asian Areas. Mol Biol Evol. 23(9):1776–1783.

Tarekegn G, Tesfaye K, Mwai O, Djikeng A, Dessie T, Birungi J, Osama S, Zergaw N, Alemu A, Achieng G, et al. 2018. Mitochondrial DNA variation reveals maternal origins and demographic dynamics of Ethiopian indigenous goats. Ecol Evol. 8(3):1543–1553.

Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT, Sykes BC, Bradley DG. 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature. 410(6832):1088–1091.

Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. 2009. Jalview version 2: a multiple sequence alignment and analysis workbench. Bioinformatics. 25(9):1189–1191.

Zeder M. 2008. Domestication and early agriculture in the Mediterranean Basin: origins, diffusion, and impact. PNAS. 105(33):11597–11604.