



Original Research Paper

Genetic diversity of the Cameroon Western Highlands' Djallonke sheep assessed by mitochondrial d-loop

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Abstract

Indigenous livestock resources are strategic in the socio-economic of rural agricultural systems to ensure food and nutritional security in resource-poor countries. In Cameroon, small ruminant farming significantly contributes to food security and income generation. Given its great ecological variety, Cameroon hosts a large genetic diversity of sheep, but the knowledge on these is scanty. In order to contribute to a better understanding of the genetic diversity of Cameroon's Ovine genetic resources for their better use and conservation, an analysis of the Djallonke sheep genetic diversity based on the D loop of the ovine mitochondrial DNA (mtDNA) was performed on the highlands of West Cameroon Djallonke breed. To achieve this, analyses of 16 sequences of a 989bp fragment of the mtDNA D-loop region from the population were conducted in conjunction with previously published sequences from African, European, Japanese and Chinese ovine genotypes. Fourteen haplotypes were observed with a high mean haplotype diversity of 1.0000 ± 0.027 , nucleotide diversity (π) of order of 0.11082 ± 0.062 . The average nucleotide difference between sequences taken two by two was 62.945. Population specific F_{ST} indices showed a negative and significant result for the whole data set. The phylogenetic tree indicates that the mitochondrial lineages of these sheep breeds originated from a common source, the Haplogroup B. This confirms the hypothesis that the domestication of African sheep was carried out in successive waves from Asia. The results of this study contribute to the knowledge of Cameroon sheep breeds and the plan conservation programs on Djallonke sheep.

Keywords: Diversity; Haplogroup; Haplotype; Sheep; Cameroon.

Introduction

Maintaining genetic diversity in livestock is critical in order to face future challenges like climate change threats, emerging diseases, and food security for an increasing human population (Barker 2001; Groeneveld *et al.*, 2010). Indigenous African sheep genetic resources currently contribute to about 30% of agricultural gross domestic product in African countries (Muigai *et al.*, 2013). According to Anyanwu *et al.*, (2010), sheep are very important in the socio cultural and economic lives of the people of Africa and especially in Cameroon. In Cameroon, sheep are a real national asset and are raised in all regions of the country. They are made up of five major indigenous breeds which are: Djallonke, Fulani, Uda, Kirdi and Blackbelly, indicating a high biodiversity (Tchouamo *et al.*, 2005).

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These indigenous breeds have been shown to be genetically diverse and are an important storehouse of genetic materials (Pariset *et al.*, 2011; Baenyi *et al.*, 2018). This could be due to geographical isolation, natural and artificial selection for physical or productive characters, genetic drift, mutations and interpopulation gene flows that have altered gene frequencies over many generations. However, this genetic diversity has not been fully described in Africa and particularly in Cameroon where only the sheep phenotypic variability has been explored up to date. Djallonke sheep, also named broadly as West African Dwarf sheep, is known to be highly prolific, adapted to harsh humid environments and resistant to trypanosomiasis (Geerts *et al.*, 2009). Notwithstanding their potential value is not realized because of low productivity resulting from poor performance among them and the loss of genetic diversity within the same breed. In terms of biodiversity, conservation and utilization, besides the phenotypic description which has been made on this breed, there is a need to characterize these genetic resources at the molecular level for further identification and evaluation to assess their potential contribution to food and agricultural production in the future. Presently, among the various tools used for molecular genetics characterization, mtDNA has received much attention because its markers provide important phylogenetic information in relation to genetic diversities (Moradi *et al.*, 2017). Mammalian mtDNA is deemed to strictly follow maternal inheritance and is highly variable within a species, so mtDNA is an important material for phylogenetic inferences and for analysing genetic diversity (Pariset *et al.*, 2011). The climate and landscape of the western region are different from other areas of Cameroon. Traffic from other parts of Cameroon is not significant, so the region's livestock are seldom influenced by external breeds. In this study, it is only the Djallonke sheep ecotype of the western highlands of Cameroon that was selected in order to assess their variability for better use and conservation.

Material and Methods

Breed description

The Djallonke breed is found in all agro-ecological zones of Cameroon. It dwells in all agroecological regions, notably in humid and sub-humid forest. The Djallonke breed is very hardy and trypanotolerant. The main value of these small ruminants is the production of meat which is of excellent quality, not fatty.

The coat colour can be monochromic (white or black), dichromic (black and white) or trichomic (black, white and brown) and varies from white, brown to black and spotted white. The Djallonke sheep is a hypo-metric, rectilinear and median-line animal. The horns are usually present in males, often absent in females. The ears are thin and narrow, dropping or semi-pendant (Geerts *et al.*, 2009).

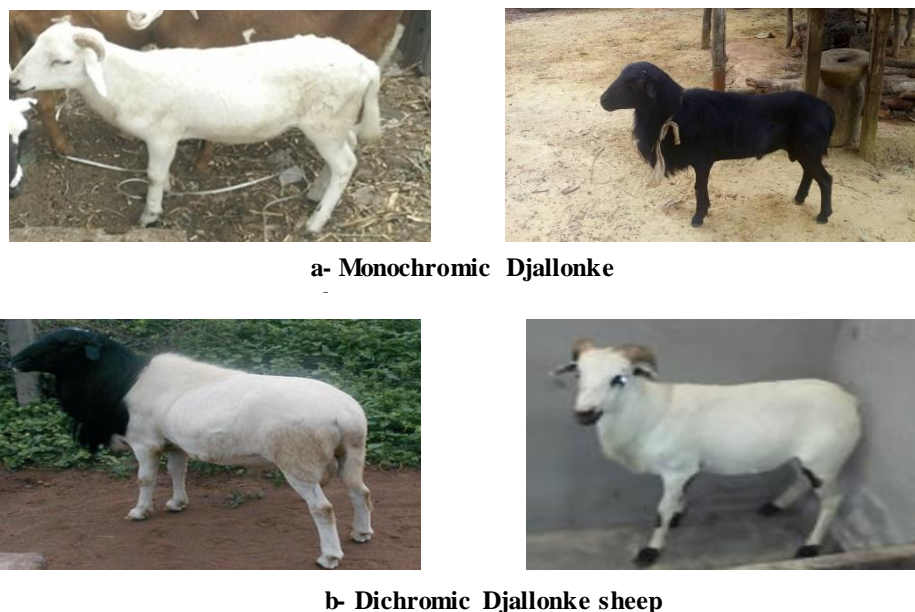


Figure 1. Coat color polymorphism in Djallonke

Sample collection and DNA extraction

A total of 16 Ear blood samples were collected on FTA® cards from Djallonke sheep breed in 8 localities of the western highlands of Cameroon. According to the simple random sampling method, individual animals were chosen based on the knowledge of local herdsman, to ensure that they were not closely related. DNA was extracted from these specimens using phenol/chloroform as described by Sambrock *et al.*, (2001). Twenty reference sheep sequences were retrieved from Genebank, AY829388, DQ097443, DQ097445, DQ097447, DQ097449, DQ097451, DQ097452, DQ852286, DQ852287, AF010407, DQ852282, DQ852285, DQ097460, DQ097462, DQ852283, DQ852284, DQ852288, DQ852289, DQ852280, DQ852281 (Mariotti *et al.*, 2013).

Table 1: Accession numbers summary

Accessions	Haplogroups
AY829388, DQ097443, DQ097445, DQ097447, DQ097449, DQ097451, DQ097452, DQ852286, DQ852287, AF010407	Haplogroup A
DQ852282, DQ852285	Haplogroup B
DQ097460, DQ097462, DQ852283, DQ852284	Haplogroup C
DQ852288, DQ852289	Haplogroup D
DQ852280, DQ852281	Haplogroup E

Source: (Mariotti *et al.*, 2013)

Amplification, purification, and DNA sequencing

For amplification, the primers used according to the procedure of Luo *et al.*, (2005) were: forward: 5'-GCCCCACTATCAACACCCAAAG-3' and reverse: 5'-AATGGGCGATTTTGA TGAGATG GC-3'. The PCR amplification reaction system consisted of genomic DNA 3µl, dNTPs 0.5 µl, primers 1.5 µl, MgCl₂ 2.5 µl, Taq DNA polymerase (5U/µl) 0.2 µl. The reaction profiles included an initial denaturation at 95°C for 3 min, followed by 35 cycles, each consisting of 1 min denaturation at 94°C, 1 min primer annealing at 64°C, 1 min extension at 72°C, and then a final 10 min extension at 72°C. The PCR products were electrophoresed through 2.0% (wt/vol) agarose gel which was stained with ethidium bromide solution. The amplified products were purified with a DNA purification kit according to the manufacturer's instructions (TW-Biotech. CO., LTD.). The PCR products were purified and sequenced using Big Dye Terminator v3.1 cyclic sequencing chemistry and the 3130XL automatic capillary sequencer (Applied Biosystems, USA).

Data analysis

All chromatograms were generated, visualized and edited with the BioEdit 7.0 software (Hall, 1999). Multiple sequence alignments were performed using the ClustalW algorithm (Thompson *et al.*, 1994) and manually edited in MEGA 6 (Tamura *et al.*, 2011). Variations in the D-loop region were defined by direct comparison with the reference *O. orientalis* mtDNA sequence (Accession No. AF039579; Hiendleder *et al.*, 1998). Gaps in the aligned sequences were excluded from the analysis. The number of polymorphic sites, haplotype diversity, nucleotide diversity and average number of nucleotide differences were also estimated using Dnasp v5.10.01 software (Librado and Rozas, 2009) and Arlequin 3.0 (Excoffier *et al.*, 2005). For the analysis of the structure and genetic relationships between individuals, the appropriate substitution model for the data set was determined using hierarchical likelihood ratio tests implemented in Mega 6 (Tajima and Nei, 1984). The phylogenetic tree was constructed using the NJ (Neighbor-Joining) algorithm implemented in MEGA 6. To analyze the level of confidence that can be associated with each bifurcation, the NJ tree was reconstructed after 1000 bootstrap replications. The best substitution model for the analysis of phylogenetic relationships was the "T92 :Tamura 3-parameter".

Results

Sequence polymorphism

From the analysis of the sequences of the mtDNA D-loop, a total of 354 variable sites were observed across a 989 bp length fragment that generated 14 haplotypes. Mitochondrial DNA has been very widely used by Geneticists to analyze the phylogenetic relationships at inter- or intra- species level in cattle, swine, goats, and other species. It has also been used to investigate the genetic variation of species (Tanaka *et al.*, 1996; Giuffra *et al.*, 2000; Sultana *et al.*, 2003; Sasazaki *et al.*, 2006).

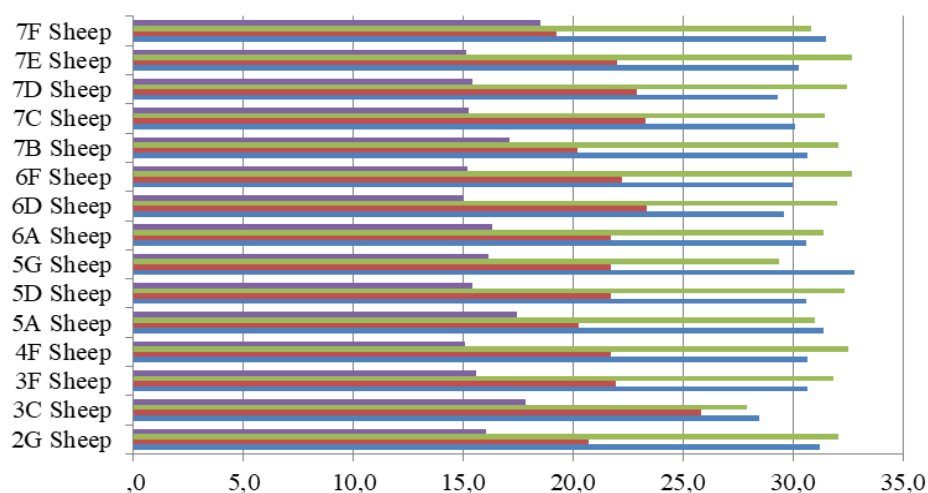


Figure 2. Nucleotide composition of Djallonké sheep sequences

The aligned sequences had stable regions in most of their sections. However, some regions appeared, with relatively high frequencies of deletion and nucleotide substitution. The haplotype diversity was 1.0000 ± 0.027 and the average number of nucleotide differences was 62.945 (Table 2) This finding is consistent with archeological data and other genetic diversity studies (Luo *et al.*, 2005; Lei *et al.*, 2006; Wang *et al.*, 2007).

The nucleotide composition (Figure 2) of all the haplotypes was 31.48% A; 30.52% T; 21.92% C and 16.08% G with a high dominance of nucleotides A and T. The nucleotide diversity was 0.11082 ± 0.062 . All the Djallonke sheep sequences in this study showed high haplotype diversity and nucleotide diversity meaning that there is a good genetic variability among them. The composition of the nucleotides in this study was similar to that of Meadows *et al.*, (2007). Such genetic diversity may be caused by a rapid population growth (Meka *et al.*, 2019), generations' overlapping, mixing of populations from different geographical locations, natural selection favoring heterozygosis or

subdivision accompanied by genetic drift (Zhao *et al.*, 2011; Djoufack *et al.*, 2020) and accumulation of mutations from a population with a low effective population size (Meadows *et al.*, 2007).

The mean pair-wise genetic distance of all the haplotypes calculated using the Kimura 2-parameter model was 0.034. The values of the Tajima D: -2.23540 $p < 0.01$ and Fu FS statistics: -1.053 $p = 0.00$ were negative and significant for all data. According to the negative Fu's test result, the samples of the Djallonke sheep ecotype of the western highlands of Cameroon had been subject to a recent population expansion. It was similar to the study of Mariotti *et al.*, (2013).

Table 2: Genetic diversity parameters of Djallonke mtDNA sequences

Parameters	Values
N	15
h	14
Hd \pm e.t.	1.0000 \pm 0.027
K	62.945
S	354
Indels	40
$\pi \pm$ e.t.	0.11082 \pm 0.062
D	-2.23540 $p < 0.01$
Fu's test	-1.053 $p = 0.00$

N: number of individuals sampled; **h:** the number of haplotypes; **Hd:** the haplotype diversity; **K:** the average number of differences; **S:** the number of segregating sites (excluding indels); **D:** Tajima's D statistic; **π :** the nucleotide diversity.

Phylogenetic analysis

In this study, 24 widely-used models were investigated for phylogenetic analyses. Models with least Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC) and Maximum Likelihood (ML) information indices were used for description of the best substitution pattern.

At the end, Tamura 3 (T92) model was selected as the best model for phylogenetic studies. In order to assess the phylogenetic relationships, the neighbor-joining (NJ) and median-joining (MJ) dendrograms were generated based on the sequences obtained in this study together with the sequences from GenBank.

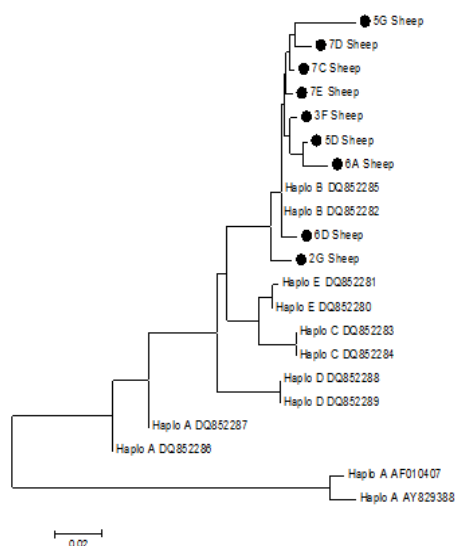


Figure 3. Neighbor-Joining tree constructed from mtDNA haplotypes belonging to Cameroon Djallonke sheep, European, Japanese and Chinese sheep.

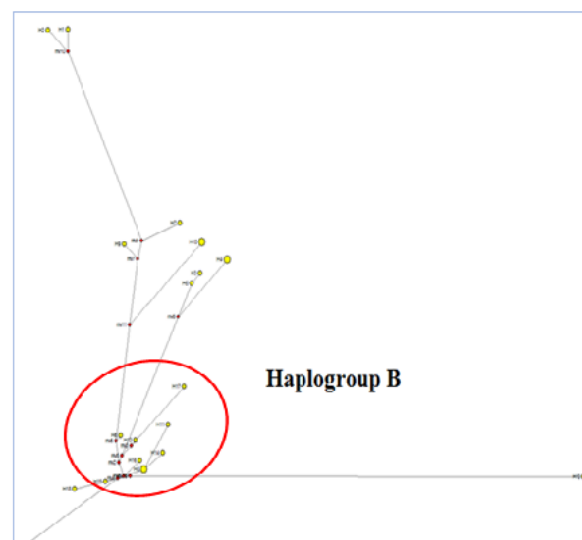


Figure 4. Phylogenetic origins of Djallonke sheep

The major feature showed by the neighbor-joining tree (Fig.3) was the presence of different clades in the tree, which shows a high variability of Djallonke sheep sequences. In the Neighbour-Joining tree, mtDNA sample sequences were distributed throughout the tree with generally short branches and scarcely robust structure, indicating a recent origin of the diversity and suggesting a pattern of a relatively recent population expansion. These findings suggest a common origin and maternal evolutionary history due to human-mediated breeding (Lavery *et al.*, 1996).

All sample sequences belonged to the clade corresponding to haplogroup B. The fact that the sample sequences belonged to a classic haplogroup places the maternal origin of Cameroonian Djallonke sheep in Central Asia in haplogroup B. The results obtained in this study are in perfect agreement with those of Mariotti *et al.*, (2013), where the affiliation of certain Italian sheep breeds to the maternal lineage of haplogroup B was established on the basis of the analysis of mtDNA variations as in the case of the Djallonke breed in Cameroon.

The Median Joining network of Djallonke sheep haplotypes and haplogroup confirms that Djallonke sheep belong to haplogroup B, whose ancestral base is located in Central Asia. This observation is consistent with the results of Luo *et al.*, (2004) and Hiendleder *et al.*, (1998), in other sheep populations. The fact that Djallonke sheep belong to haplogroup B would confirm the hypothesis of Roger *et al.*, (1999); Naderi *et al.*, (2007) and (2008) and Pereira *et al.*, (2009), according to which the domestication of African sheep was carried out in successive waves from Asia.

Conclusion

This study based on mtDNA D-loop assessment found that the Djallonke sheep of the western highlands of Cameroon had high genetic diversity and all belong to haplogroup B. This finding suggests a probable origin from the very first domestic sheep ancestors in Asia. They could have reached the country in several waves. Successive migration and relative selection could explain the current mitochondrial DNA variability. These results could enhance further and deep exploration using other molecular tools targeting some peculiar traits, and then imperative for better usage of Cameroon sheep genetic resources. The results of this study could have implications for managing improvement and long-term conservation of Cameroon Djallonke sheep.

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Authors' contribution

Meutchieye designed, collected and processed mtDNA; **Ntsoli and Meutchieye** made analysed the data; Ntsoli wrote manuscript, **Meka Zibi II and Ayagirwe** revised and improved the paper contents.

Ethics

Author declare that there are no ethical issues that may arise after the publication of this manuscript.

References

- Anyanwu J. Ohaeri PC. Iheshiulor OM. and Etela 2010.** Preliminary investigations into the socio economic and production characteristics of sheep and goat farming in the Owerri zone of Stata Nigeria. *Glo. J. Agriculture and Biology Research*. 1(1):6-9.
- Baenyi P. Meutchieye F. Ayagirwe B.R. Bwihangane B. Karume K. Mushagalusa NG. Ngoula F 2018.** Biodiversity of indigenous Djallonke sheep (*Ovis aries*) in Sudano Guinean region in Cameroon. *Genetics and Biodiversity Journal*. J. 2 (2): 1-10
- Barker T. Moore M. Matheson and Selvaraj 2001.** Genetic variation and relationships among populations of Asian goats (*Capra hircus*). *Journal of Animal Breeding and Genetics*, 118: 213233. doi:10.1046/j.14390388.2001.00296.x

- Djoufack YT. Meutchieye F. Manjeli Y 2020.** Phenotypic and genetic differentiation of qualitative traits in sheep ecotype from Cameroon Western Highlands. *Genetics and Biodiversity Journal*. J: 4 (1):2329.
- Excoffier LG. Laval and Schneider S 2005.** Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47 50.
- Geerts S. Osaer S. Goossens B. & Faye D 2009.** Trypanotolerance in small ruminants of sub-Saharan Africa. *Trends Parasitol*, 25, 132-138.
- Giuffra E. Kijas JMH. Amarger V. Carlborg O. Jeon JT. and Andersson L 2000.** The origin of the domestic pig: Independent domestication and subsequent introgression. *Genetics*. 154:17851791.
- Groeneveld LF. Lenstra JA. Eding H. Toro MA. Scherf B. Pilling D. Negrini R. Finlay EK. Jianlin H. Groeneveld E. Weigend S 2010.** Genetic diversity in farm animals a review. *Animal Genetics*, 41: 6-31. Doi :10.1111/j.1365-2052.2010.02038.x.
- Hall TA 1999.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series* (Vol. 41, No. 41, pp.95-98). [London]: Information Retrieval Ltd., c1979-c2000.
- Hiendleder S. Mainz K. Plante Y. and 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. *Journal of Heredity*. 89 (2), 113-120.
- Lavery S. Moritz C. Fielder DR 1996.** Genetic patterns suggest exponential population growth in a declining species. *Molecular Biology and Evolution* 13:1106–1113
- Lei XQ. Xu TS. Chen YL. Chen H. Yuan ZF 2006.** Microsatellite markers on genetic relationships of six Chinese indigenous sheep breeds. *Chinese Journal of Biochemistry and Molecular Biology* 22:81–85.
- Librado P. and Rozas J 2009.** DnaSP v5: software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451-1452.
- Luo YZ. Cheng SR. Lkhagva B. Badamdorj D. Hanotte O. and Han JL 2005.** Origin and genetic diversity of Mongolian and Chinese sheep using mitochondrial DNA D-loop sequences. *Acta Genetica Sinica* 32:12561265.
- Mariotti M. Valentini A. Ajmone MP. and Pariset L 2013.** Mitochondrial DNA of seven Italian sheep breeds shows faint signatures of domestication and suggests recent breed formation. *Mitochondrial DNA*. 24(5): 577-583.
- Meadows JRS. Cemal I. Orhan K. Gootwine E. and James WK 2007.** Five Ovine Mitochondrial Lineages Identified from Sheep Breeds of the Near East. *The Genetics Society of America* DOI: 10.1534/ 106.068353.
- Meka Zibi II M.A. Meutchieye F. Ntsoli J. Tadakeng Y. Fonteh F 2019.** Factors affecting the global diffusion of an African animal genetic resource: the case study of the Cameroon Blackbelly. *PKFokam Journal of Applied Science and Technology*, Inaugural issue June 2019: 21-29.
- Moradi M. Phua S. Hedayat N. Khodaei M. and Razmkabir M 2017.** Haplotype and Genetic Diversity of mtDNA in Indigenous Iranian Sheep and an Insight into the History of Sheep Domestication. *Journal of Agricultural Science and Technology*. 19. 591-601.
- Muigai. Anne and Hanotte O. 2013.** The Origin of African Sheep: Archaeological and Genetic Perspectives. *African Archaeological Review*. 30. 10.1007/s10437-013-9129-0.
- Naderi S. Rezaei HR. Pompanon F. Blum MGB. Negrini R. Naghash HR. Balkız O. Mashkour M. Gaggiotti OE. Ajmone-Marsan P. Kence A. Vigne JD. And Taberlet P 2008.** The goat domestication process inferred from large-scale mitochondrial DNA analysis of wild and domestic individuals. *Proceedings of the National Academy of Sciences of the United States of America*. Vol. 105 (46) 17659-17664; doi.org/10.1073/pnas.0804782105.
- Naderi S. Rezaei HR. Taberlet P. Zundel S., Rafat SA. Naghash HR. and Econogen Consortium 2007.** Large scale mitochondrial DNA analysis of the domestic goat reveals six haplogroups with high diversity. *PLoS One*, 2(10), e1012.
- Pariset L. Mariotti M. Gargani M. Joost S. Negrini R. Perez T. Valentini A 2011.** Genetic Diversity of Sheep Breeds from Albania, Greece, and Italy Assessed by Mitochondrial DNA and Nuclear Polymorphisms (SNPs). *The Scientific World Journal*, 11, 1641–1659. doi:10.1100/2011/186342.

- Pereira F. Queiros F. Gusmao L. Nijman IJ. Cuppen E. Lenstra JA. Davis SJM. Nejmeddine F. and Amorin A 2009.** Tracing the history of goat pastoralism: new clues from mitochondrial and Y chromosome DNA in the North Africa Molecular Biology and Evolution. 26 (12):2765-2773. Doi:10.1093/molbev/msp 200. Publishing.
- Sasazaki SS. Odahara C. Hiura F. Mukai and Mannen 2006.** Mitochondrial DNA variation and genetic relationships in Japanese and Korean cattle. Asian-Australasian Journal of Animal Sciences. 19(10):1394-1398.
- Sultana SH. Mannen and Tsuji S 2003.** Mitochondrial DNA diversity of Pakistani goats. Animal Genetic. 34:417-421.
- Tajima F. and Nei M. 1984.** Estimation of evolutionary distance between nucleotide sequences. Molecular Biology and Evolution 1:269-285.
- Tamura K. Peterson D. Peterson N. Stecher G. Nei M. and Kumar S 2011.** MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution, 28(10): 2731-2739
- Tanaka KC. Solis DJ. Masangkay S. Maeda K. Kawamoto Y. and Namikawa T 1996.** Phylogenetic relationship among all living species of the genus *Bubalus* based on DNA sequences of the cytochrome b gene. Biochemical Genetics. 34:443-452.
- Tchouamo IR. Tchoumboué J. and Thibault L 2005.** Caractéristiques socioéconomique et techniques de l'élevage de petits ruminants dans la province de l'ouest Cameroun. Tropicultura 23(4): 201-211.
- Thompson JD. Higgins DG. And Gibson TJ 1994.** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research, 22, 4673–4680.
- Wang X. Ma YH. Chen H. and Guan WJ 2007.** Genetic and phylogenetic studies of Chinese native sheep breeds (*Ovis aries*) based on mtDNA D-loop sequences. Small Ruminant Research 72:232–236. doi: 10.1016/j.smallrumres.2006.10.016
- Zhao Y. Zhang J. Zhao E. Zhang X. Liu X. Zhang N 2011.** Mitochondrial DNA diversity and origins of domestic goats in Southwest China (excluding Tibet). Small Ruminant Research 95:40-47 doi:10.1016/j.small rumres.2010.09.004.
- Brown TA. 2016.** Gene cloning and DNA analysis: an introduction. Hoboken: John Wiley & Sons. 165-183.
- Roger B. and MacDonald KC 1999.** The Origins and Development of African Livestock, Routledge, 1re éd.
- Sambrook J. Fritsch EF. And Maniatis T 2001.** Molecular cloning: a laboratory manual (third edition). Cold Spring Harbor, New York: Cold spring Harbor Laboratory Press.