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Genetisk diversitet i mitokondrie-dna hos lokala fårpopulationer i norra Etiopien

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Abstract

This study was aimed to investigate the haplotype variations and maternal origin of four sheep populations (Highland, Abergelle, Elle and Begait) of North Ethiopia. A 1088 bp of the mtDNA control region was amplified using specific primers and the polymerase chain reaction was performed. The PCR products were purified and sequenced. Control region of the 24 mtDNA samples was sequenced. For the haplotype diversity, 1088 bp of the d-loop region of the indigenous sheep populations was analysed. Whereas, to define the maternal origins, 517 bp of mtDNA D-loop region aligned to sequences of globally defined reference sequences was used. A total of 21 haplotypes were defined from 97 segregating sites. The number of segregating sites (s) were varied among the four sheep populations. The s value of Highland, Abergelle, Begait and Elle sheep populations were (75, 19, 22 and 16 respectively). Highland sheep population had higher number of haplotypes (13) than Abergelle, Begait and Elle (4, 3 and 3 respectively). The average number of nucleotide differences (Kt) is (13.11594). Highland, Begait and Elle sheep have higher haplotype diversity values (1) than Abergelle sheep (0.9). The nucleotide diversity (π) of Abergelle sheep also reveal the same tendency (0.0082), being lower than the π value of Elle, Begait and Highland (0.0106, 0.0146 and 0.0150, respectively). The AMOVA analysis indicates 29.78% variation explain variations among the 4 populations. The phylogenetic network analysis clustered the detected haplotypes into two haplogroups, haplogroup A and B which are separated by 13 mutational sites. The mismatch analysis shows the presence of a one-time demographic expansion. Having rich matrilines in those indigenous sheep populations is essential for future conservation and breeding programmes.

Key words: Genetic diversity, Haplotype, MtDNA D-loop, North Ethiopian sheep

1. Background

In Ethiopia domestic sheep (*Ovis aries*), like other livestock species, plays vital roles in generating income to farmers, creating job opportunities, ensuring food security, providing services, contributing to asset, social, cultural and environmental values, and sustain livelihoods (Tibbo, 2006; Metaferia *et al*., 2011; Edea *et al*., 2017). The livestock subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the agricultural GDP (Metaferia *et al*., 2011). In developing countries like Ethiopia, livestock genetic resources in general have not been adequately characterised, evaluated or fully utilised through selection and in some cases local populations are threatened with extinction before their genetic value is even properly described and studied (Madalena, 1993; Gizaw *et al*., 2013).

In Ethiopia, small ruminants are mainly kept by smallholder farmers and the rural poor (Tibbo, 2006; Mourad *et al*., 2015). With 29.3 million sheep and 29.1 million goats, Ethiopia's small ruminant population is among the largest in East Africa and even within sub-Saharan Africa (FAOSTAT, 2016; http://faostat3.fao.org/browse/Q/QA/E). It is also important to notice that according to CSA (2013) 99.8% of the sheep populations in Ethiopia are local breeds.

Formulation of appropriate strategies for long-term maintenance and use of the genetic variation within livestock species requires characterisation of animal genetic resources, to identify the variation and appropriate germplasm that is optimal for each system. The domestic sheep (*Ovis aries*) has been an important farm animal species, both economically and culturally in the Near East approximately 9,000 years before present (Peters *et al*., 2004). They are raised for meat, milk and fibre production and are found in varied environments of the world (Olivieri *et al*., 2012; Pariset *et al*., 2011; Gorkhali *et at*., 2015). Characterization of sheep resources is a prerequisite for proper utilization (Solomon, 2008). Therefore, characterizing the populations in both at the level of animal phenotypes and their interaction with production systems and at the genetic level is most essential (FAO, 2011). Knowledge about genetic diversity and population structure is essential for designing effective strategies for genetic improvement, management (understanding of environmental adaptation),

utilization as well as conservation and protection against genetic erosion of farm animal genetic resources (Makina *et al*., 2014; Kawęcka *et al*., 2016; Edea *et al*., 2017). Well understanding of genetic difference embraces the key to future utilization through conservation (Agaviezor *et al*., 2012).

Research on identification, classification and description of sheep resources of Ethiopia began in the 1970s with the classification of the sheep populations into broad categories of tail and fibre types; molecular characterization has been relatively a recent development. While some Ethiopian sheep populations are now partially studied, further research may be required to fill gaps in previous projects. Sheep populations that need further characterization include: Abergelle (Tajebe *et a*l., 2011); Tigray Common highland and Afar (Mulata *et al*., 2014; Gebreyowhans and Tesfay, 2016); Begait and Nuer sheep (Tiboo, 2006; Gizaw *et al*., 2013).

Relatively few studies have looked at relevant traits in the Begait sheep. According to the study of Ashebir *et al (*2016), the reproductive performance of Begait sheep is modest and needs to improve management, nutritional and breeding practices at farm level. This study also reported that Begait sheep breed is a long thin tailed, of large size and well adapted to wide range of harsh environmental conditions in the north-western part of Tigray regional state and south-western part of Eritrea. There are also few studies on the phenotypic characterisation of sheep populations in Tigray region of Northern Ethiopia. These include Abergelle (Tajebe *et a*l., 2011), Common Highland (Mulata *et al*., 2014; Gebreyowhans and Tesfay, 2016) and Afar or Elle (Gizaw *et al*., 2007; Mulata *et al*., 2014). Among the four indigenous sheep populations, Begait has been previously identified as a promising sheep breed for meat and milk productivity improvement. As a result, Begait sheep has been introduced to different areas of Tigray for meat productivity improvement through conventional crossbreeding with other indigenous sheep breeds.

There is also an initiative in Humera (the potential area for the breed) to develop breeding strategies for purebred Begait sheep. However, they are not genetically characterised and there is a need to further studies about their genetic diversity and population structure of those sheep populations. In fact, the potential of those sheep populations has not been exploited properly. The variable structure of mitochondrial DNA (mtDNA) D-loop region is

important to study the evolutionary history and to resolve the problem of genetic variation in domestic sheep, because of its maternal inheritance, no recombination as well as a comparatively fast evolution rate (Hiendleder *et al*., 1998b). Therefore, this project was planned to investigate the genetic diversity and population structure of the four North Ethiopian indigenous sheep populations (namely: Begait, Abergelle, Elle and Common highland) and compare them with other breeds with in the country and beyond using mtDNA D-loop genetic variation.

Aim of the study

The aim of the study was to investigate the haplotype variations and maternal origin of four sheep populations (Highland, Abergelle, Elle and Begait) in North Ethiopia.

Hypothesis

Targeted sheep populations (Abergelle, Elle, Begait and Common Highland) are genetically diverse and have unique maternal origins.

2. Literature review

2.1. Domestication of sheep

Domestication is not an event or invention rather than continuing co-evolutionary process (Gifford-Gonzalez and Hanotte, 2011). *Ovis aries* is one of the earliest farm animals to have been domesticated as well as have provided as source of food, wool and skin from the time of the Neolithic Agricultural period (Olivieri *et al.,* 2012). As indicated by archaeozoological and genetic evidences domestication of sheep was started about 11000 years ago in the Fertile Crescent region (Zeder, 2008; Pariset *et al*., 2011). Domestic sheep is one of the successful species to adapt a varied geographic range globally, because of having unique characteristics to adapt extreme climatic conditions, poor diets and their manageable size (Pariset *et al*., 2011). Molecular studies on domestication of fat-tailed sheep also confirmed that the Near East region as the main centre of origin (Rocha *et al*., 2011).

The Eastern Africa domestic sheep may have been initially spread from the Nile Valley via over land, even though they may have introduced through the Arabian Peninsula (Muigai and Hanotte, 2013). The Eastern part of African continent was critical either for the initial distribution of domestic sheep southward or for supposed subsidiary introductions from the Arabian Peninsula or Southern Asia (Resend *et al*., 2016). Given its proximity to the Arabian Peninsula, Ethiopia is believed to be one of the major genetic corridors for domestic sheep migration from Asia to Africa. Fat-tailed sheep were introduced into Africa through Horn of Africa and Northern Africa from the Middle East independently via each route (Solomon, 2008; Gizaw *et al.,* 2013; Edea *et al*., 2017). However, the study by Edea *et al*. (2017) suggested that to elucidate the evolutionary history of African sheep further genome-wide analyses of thin-tailed sheep breeds from Eastern and Western Africa as well as fat-tailed breeds from the Arabian Peninsula is important.

2.2. Mitochondrial DNA for genetic diversity and phylogenetic studies

MtDNA has been commonly used to study the origin, phylogeny and genetic diversity of sheep breeds due to its maternal inheritance, very high polymorphism within species, a lack of recombination and that it can be gained from archaeological organic materials (Hiendleder *et al*., 1998b; Koseniuk and Słota, 2016). The control region (CR) or displacement loop (D-loop), has been applied in phylogenetic studies through analysis of both the conserved

sequence of mitochondrial genes and the non-coding part of mtDNA (Koseniuk and Słota, 2016). Previous studies on CR fragment and/or the cytochrome B gene of mtDNA of domestic sheep have revealed the existence of five different haplogroups (A, B, C, D, and E) from dispersed geographical locations worldwide (Meadows *et al*., 2007; Meadows *et al*., 2011; Olivieri *et al*., 2012; Singh *et al*., 2015; Gorkhali *et at*., 2015; Koseniuk and Słota 2016; Resend *et al*., 2016). Study on the complete mitogenomes of the five mtDNA lineages of domestic sheep from diverse environments revealed to have radiation that shared a common ancestor about 920,000±190,000 years back (Meadows *et al*., 2011).

2.3. MtDNA haplogroups and their distributions in domestic sheep

The mtDNA sequence analysis is one of the very valuable method for investigating the history of modern domestic animals since the D-loop region is the main noncoding regulatory region for the transcription and replication of mtDNA (Liu *et al*., 2016). Thus, to elucidating the origins of *O. aries* and their human-mediated global migrations, analysis of mtDNA had been studied in different part of the world. For instance, from sheep breeds of the Near East (Turkey and Israel) the five (A to E) ovine mitochondrial lineages was identified (Meadows *et al*., 2007). Those five maternal inheritance that have been revealed in domestic sheep mtDNA have different origins, such as Lineage HapG A, HapG B and HapG C, D and E was originated from Asia, Europe and Near East respectively (Meadows *et al*., 2007). Gorkhali *et at*. (2015) also distinguished three haplogroups (A to C) in four indigenous sheep breeds of Nepal (Bhyanglung, Baruwal, Kage and Lampuchhre sheep) by sequencing the control region (CR) of mtDNA. Pereira *et al*. (2006) was identified two important indices (haplotype and nucleotide diversity of mtDNA) for assessing genetic diversity and differentiation. Phylogenetic study with East African and Indian sheep sequences showed that there was high frequency of haplogroup A in India sheep unlike in the East African sheep. The Kenyan sheep breeds have rich maternal inheritances, but there was no evidence that the Indian Ocean trade had been used for the spread of domestic sheep from India to East Africa (Resende *et al*., 2016).

2.4. Description of the study sheep populations

Begait sheep are long thin tailed, hairy, of large size with adult female and male body length of 62.5 ± 0.5cm and 66.1 ± 0.5 cm, respectively (Amare *et al*., 2012) and adult female and male body weight of 40 to 50 kg and 45 to 60 kg, respectively (Ashebir *et al*., 2016).

Common highland sheep is a small size, short fat-tailed, coarse-wool sheep with average live weight of 28 and 23 kg for mature male and female, respectively (Mulata *et al*., 2014; Gebreyowhans and Tesfay, 2016). Abergelle sheep is short fat tailed with average adult male and female body weight of 24.42 and 23.13 kg respectively (Tajebe *et al*., 2011) and Elle sheep belong to the hairy with long fat-rumped (Gizaw *et al*., 2007; Mulata *et al*., 2014). The overall mean body weight and body length of Elle ewe is 25.95kg and 54.03cm, respectively. The corresponding values for male Afar sheep is 22.7±1.42 kg and 51.2±1.76 cm, respectively. Under pastoral management the age at sexual maturity for Afar ram lambs is 7.10 months (Gizaw *et al*., 2013). Hayelom *et al*. (2014) also reported that on-farm reproductive performance of Elle, Abergelle and Common highland sheep in six selected districts of Tigray regional state have acceptable age range for breeding though it is late compared to temperate breeds under traditional management system. As shown in (Appendix Figure 1) the four studied sheep populations have different phenotypic appearance both between and within populations.

3. Materials and methods

3.1. Description of sampling sites

The samples were collected from Tigray and Afar regional state of North Ethiopia. It is important to take into account that Ethiopia is characterized by highly contrasting ecological zones modified mainly by altitude ranging from 126 metres below sea level to 4620 metres above sea level (masl). The ecology of Tigray and Afar regional state is also within this range. For example, the attitude ranges from 560 – 1849 masl in Setit and Kafta humera district of Western Tigray (Amare *et al*., 2012), from 1200 - 1500 masl in Abergelle district of Central Tigray (Tajebe *et al*., 2011); from less than 1000 - 1900 masl in Afar regional state and bordering of Tigray (Solomon, 2008). The various agro-ecological zones in the country might have led the country to have highly differentiated indigenous sheep populations which include Abergelle, Elle (Afar), Begait and Common highland sheep populations (Solomon, 2008).

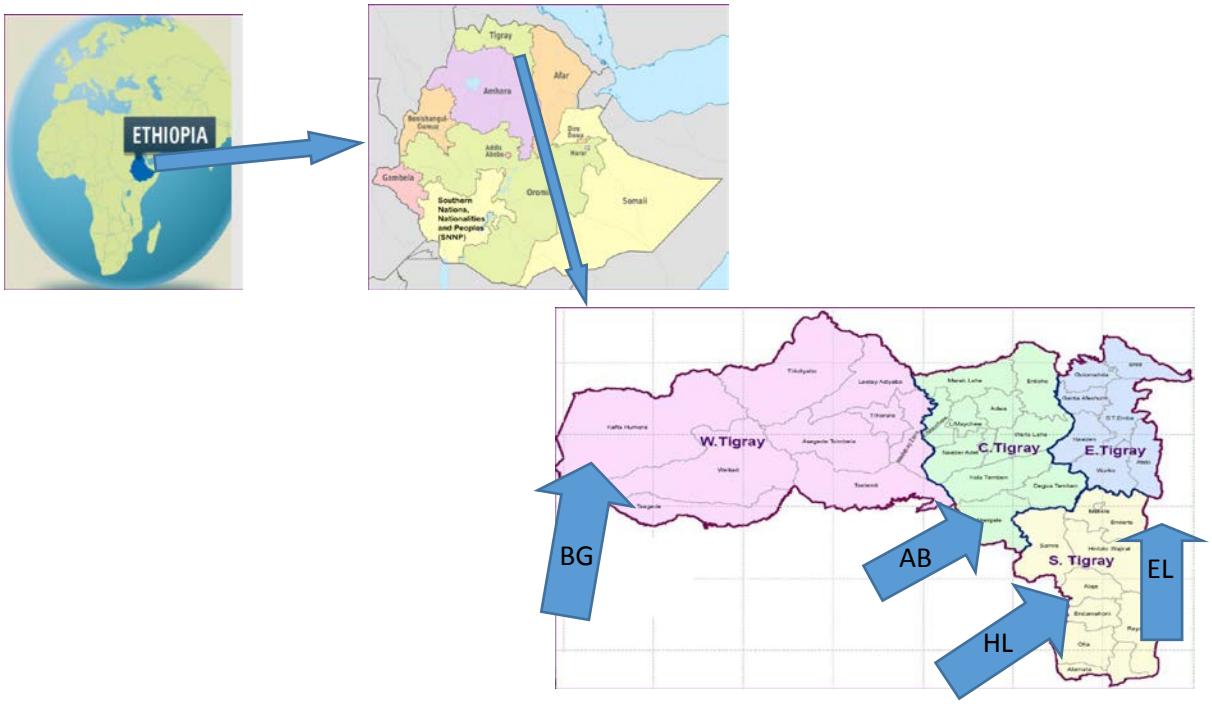

Figure 1. An outline map of Tigray regional state illustrating sampling districts (Setit humera for Begait sheep (BG)); Abergelle District for Abregelle sheep (AB)); Alagie, Ofla and Maichew districts for highland sheep (HL)), and Abala district for Elle sheep (EL)) of Northern Ethiopia domestic sheep populations used in this study. The blue arrow indicates the location of Ethiopia and the sampled districts within Tigray and Afar regional state (ethiovisit.com, 2018).

3.2. Sampling strategy and DNA extraction

Nasal swab samples were collected by using Performagene LIVESTOCK's nasal swab DNA collection kit (DNA Genotek, Kanata, ON, Canada) from a total of 154 animals from different geographic origins within the regional state of Tigray and Afar of Northern Ethiopia, representing the above mentioned four sheep populations. Both female and male animals were sampled randomly from flocks of small holder farmers. From 35 to 41 animals were sampled from each sheep population (41 Abergelle, 40 common Highland, 38 Elle and 35 Begait). From those we had found 42 samples that have good sequence results. Ten reverse and six forward sequences could not have aligned properly. However, 24 samples were successfully aligned. This could be because of the purity problems in nasal swab samples of extensive farms, any systematic errors during lab work (such as light, mixing of working solutions), small amount of initial material (1 µL genomic DNA). Genomic DNA of nasal samples collected from those four populations were extracted at Holeta National Biotechnology Research Centre (Animal biotechnology, molecular laboratory) in Ethiopia with laboratory protocol for manual purification of DNA from 0.5 mL of Performagene^{TM} sample. The mtDNA d-loop region was sequenced at the Department of Animal Breeding and Genetics laboratory (SLU, Uppsala, Sweden).

3.3. Primer design and polymerase chain reaction (PCR)

A pair of PCR and sequencing primers which were used by Lui *et al* (2016) was employed for this study. The nucleotide sequences of forward primer CsumF and reverse primer CsumR were 5'-GGCTGGGAC CAAACCTAT-3' and 5'-GAACAACC AACCTCCCTAAG-3', respectively. PCR was performed in a 9700-thermal cycler with a total reaction volume of approximately 10 µL, containing 1 µL genomic DNA (4 ng/µL), 1.5 µL M13-tailed PCR Primer mix (0.8µM each primer), 5 µL BigDye Direct PCR Master Mix, and 2.5 µL ddH2O. The PCR conditions were as follows: initial denaturation for 5 min at 96°C, 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 45 s, and extension at 72°C for 2 min. The PCR amplification products were subsequently stored at 4°C until use.

3.4. Cycle sequencing and purification of sequencing products

For cycle sequencing, PCR was also performed by 9700-thermal cycler with a total reaction volume of 13 µL mix of 2 µL of BigDye® Direct Sequencing Master Mi; 1 µL of BigDye® Direct M13 Fwd primer or BigDye® Direct M13 Rev primer, and 10 µL of mix from PCR1

(1 µL genomic DNA (4 ng/µL), 1.5 µL M13-tailed PCR Primer mix (0.8µM each primer), 5 µL BigDye® Direct PCR Master Mix, and 2.5 µL ddH2O). The PCR conditions were as follows: hold for 37°C 15 min, for 82°C 2min and for 96°C 1min followed by three steps of 25 cycles at 96°C for 10 s, 50°C for 5 s and final step of 60°C for 4 min. Sequenced products were purified using a BigDye XTerminator® Purification Kit (Applied Biosystems, Foster City, CA, USA). After getting the purified products Sanger sequencing was made using Applied Biosystems 3500 Series Genetic Analyzers Machine for DNA Sequencing and Fragment Sizing.

3.5. Data Analysis

Population genetic diversity: Consensus sequences generated by the forward and reverse primers were generated using CodonCode aligner software (Richterich peter, 2017). Multiple alignment including the reference sequences was carried out with MEGA6 (Tamura *et al*., 2013). Reference sequences from globally identified haplogroups of *Ovis aries* (Haplogroup A to E) were included in the multiple alignment as shown in Table 1 and used to define maternal origins of Ethiopian indigenous sheep populations.

The level of genetic diversity, determined as the number of haplotypes (h), haplotype diversity (hd), nucleotide diversity (π), and average number of nucleotide differences (k) between haplotypes and their standard deviations, were computed for each population and across all populations using Arlequin 3.5 (Excoffier & Lischer, 2010). The same statistical package was also employed to evaluate analysis of molecular variance (AMOVA) and to calculate F_{ST} (Weir and Cockerham, 1984) pair-wise genetic distances among populations.

Phylogenetic and population structure analysis: To investigate the genetic relationships between populations, median-joining (MJ) network and neighbour joining (NJ) phylogenetic trees were constructed (Bandelt et al., 1999) and MEGA6 software (Tamura et al., 2013), respectively. The population expansion was investigated with mismatch distribution using Arlequin ver 3.5.1.2 (Excoffier and Lischer, 2010). Arlequin ver 3.5.1.2 (Excoffier and Lischer, 2010). To complement the mismatch distributions, the same package was also used to calculate the neutrality tests, Fu's FS (Fu, 1997) and Tajima's D (Tajima,1989) statistics.

Table 1. Reference sequences for different haplogroups of domestic sheep mtDNA.

Haplogroup	GenBank accession no.	Breed name	Country of origin	Sources
A	AY829388.1,	Kazakh Fat-Rumped	China	Guo *et al*., 2005
	DQ097443.1,	Akkaraman	Turkey	Pedrosa *et al*., 2005
	DQ097445.1,	Karayaka	Turkey	Pedrosa *et al*., 2005
	DQ097447.1,	Karayaka	Turkey	Pedrosa *et al*., 2005
	DQ097449.1,	Tuji	Turkey	Pedrosa *et al*., 2005
	DQ097451.1,	Tuj	Turkey	Pedrosa *et al*., 2005
	DQ097452.1,	Hemsin	Turkey	Pedrosa *et al*., 2005
	DQ852286.1,	Karakas	Turkey	Meadows *et al*., 2007
	DQ852287.1	Karakas	Turkey	Meadows *et al*., 2007
B	DQ852282.1,	Karakas	Turkey	Meadows *et al*., 2007
	DQ852285.1	Karakas	Turkey	Meadows *et al*., 2007
C	DQ097460.1,	Hemsin	Turkey	Pedrosa *et al*., 2005
	DQ097462.1,	Akkaraman	Turkey	Pedrosa *et al*., 2005
	DQ852283.1,	Karakas	Turkey	Meadows *et al*., 2007
	DQ852284.1	Karakas	Turkey	Meadows *et al*., 2007
D	DQ852288.1,	Karakas	Turkey	Meadows *et al*., 2007
	DQ852289.1	Karakas	Turkey	Meadows *et al*., 2007
E	DQ852280.1,	Awassi	Israel	Meadows *et al*., 2007
	DQ852281.1	Awassi	Israel	Meadows *et al*., 2007

4. Result and discussion

4.1. Mitochondrial DNA sequence variation and genetic diversity

Haplotype diversity (Hd) and nucleotide diversity (π) was used to measured sequence based genetic diversity. The former only measures the probability of occurrences of different haplotypes of two sequences when they were selected randomly in a population, whereas the latter (π) is the average number of nucleotide differences per site between two sequences. The value of Hd is sample size dependent unlike the π value. The mtDNA genetic diversity of the four North Ethiopian sheep populations is presented in Table 2. The number of segregating sites (s) in the Highland, Abergelle, Begait and Elle sheep populations were (75, 19, 22 and 16 respectively). There are total of 21 haplotypes (h) investigated from all 24 sequences of the four sheep populations. Highland sheep population has higher haplotype diversity (13) than Abergelle, Begait and Elle (4, 3 and 3 respectively) (Table 2).

Table 2. Haplotype (Hd) and nucleotide (π) diversities of the four North Ethiopian sheep populations.

Population	**N**	**S**	**h**	**Hd**	**π**	**k**
Highland	13	75	13	1	0.0150	15.0256
Abergelle	5	19	4	0.9000	0.0082	8.2000
Begait	3	22	3	1	0.0146	14.6666
Elle (Afar)	3	16	3	1	0.0106	10.6666
Total	24	97	21	0.9891	0.0131	13.1159

N, sample size; S, number of polymorphic sites; h, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity; k, average number of nucleotide differences.

Highland, Begait and Elle sheep populations have higher haplotype diversity (1) than Abergelle sheep (0.9). As shown in Table 2, the nucleotide diversity of Abergelle sheep also reveal the same tendency (0.0082), being lower than the nucleotide diversity of Elle, Begait and Highland (0.0106, 0.0146 and 0.0150), respectively. The π and Hd value of this study are similar with the study of Lui *et al*. (2016), also found that the nucleotide diversity and haplotype diversity of fifteen Tibetan sheep based on mtDNAD-Loop sequences were between 0.013 to 0.027, and 0.90 to 1 respectively.

4.2. Genetic differentiation

AMOVA analysis incorporating the 4 populations assuming no hierarchical clusters showed that 99.082% of the total genetic variation present in North Ethiopian indigenous sheep occurred within populations, 4.85% of the variation was due to genetic differences among populations (Table 3). The Molecular diversity indexes also showed that there is difference between the four studied populations in relation to the mean and standard deviations of the number of transition sites, transversion sites and substitution sites (see: Appendix Table1).

Table 3. Results of AMOVA based on the analysis of the 1088 pb of the mtDNA *D*-loop in 4 North Ethiopian sheep populations including the reference populations.

Source of variations	df	Sum of squares	Variance components	Percentage of variation
Among populations	1	14.292	0.54570 Va	4.850
Among populations within groups	2	18.243	-0.44248 Vb	-3.930
Within populations	19	211.812	11.14801 Vc	99.080
Total	22	244.348	11.25123	

To examine the genetic differentiation between the four indigenous North Ethiopian sheep populations, we analyzed the average pairwise differences between populations and within populations (Table 4) using the estimated pairwise F_{ST} values. As shown in Table 4 the results show that the F_{ST} values between Ethiopian sheep in decreasing order were 0.35697 (Highland and Elle sheep), 0.31289 (Abergelle and Elle sheep), 0.29201 (Begait and Elle sheep), 0.04773 (Begait and Abergelle sheep), 0.10131 (Abergelle and Highland sheep) and -0.01716 (Begait and Highland sheep). Although the FST values were smaller than 0.25, indicating that significant genetic differentiation has not occurred among the highland, Abergelle and Begait sheep populations the nucleotide and haplotype diversity and AMOVA showed that there is genetic vartion among the populations.

Table 4: Population pairwise F_{ST} differences

	Highland	Abergelle	Begait
Abergelle	0.10131	0.0000	
Begait	-0.01716	0.04773	0.0000
Elle (Afar)	0.35697**	0.31289**	0.29201**

F_{ST} =Wright's F-statistics of a subpopulation within total population; The F*ST* values were each calculated with 110 permutations. **Significant at P-value = 0.0001.

4.3. Population phylogenetic relationship

Neighbor-joining tree based on 517 bp of control region mtDNA of 24 sheep samples from four selected districts of Northern Ethiopia and rooted with previously studied sheep sequences showed the presence of two distinct haplogroups, namely; A and B, out of the five lineages reported in *Ovis aries* (Figure 3). As shown in Figure 2 and Figure 3 all the haplotypes except haplotype12 were grouped under haplogroup B. Haplotype12 was aligned to haplogroup A. in the current study, haplogroup A and B are separated by 13 mutational sites between haplogroup A and haplogroup B (Figure 2).

This result agreed with literatures which reported that haplogroup B is dominant in Eastern and Northern Africa countries. For instance, the study by Resende *et al.* (2016) reported that haplogroup B was commonly spread in the maternal diversity of Kenyan sheep, whereas haplogroup A was detected only from a single individual of Kenyan sheep. Othman (*et al*., 2014) also reported that haplogroup B and A were found in five Egyptian sheep breeds (Barki, Ossimi, Rahmani, Saidi and Sohagi). According to Othman (*et al*., 2014), majority (76 out of 90) of the tested animals was clustered with haplogroup B whereas nine tested animals cluster with haplogroup A. Unlike in the Egyptian sheep breeds, haplogroup C and haplogroup E are not found in the North Ethiopian sheep populations.

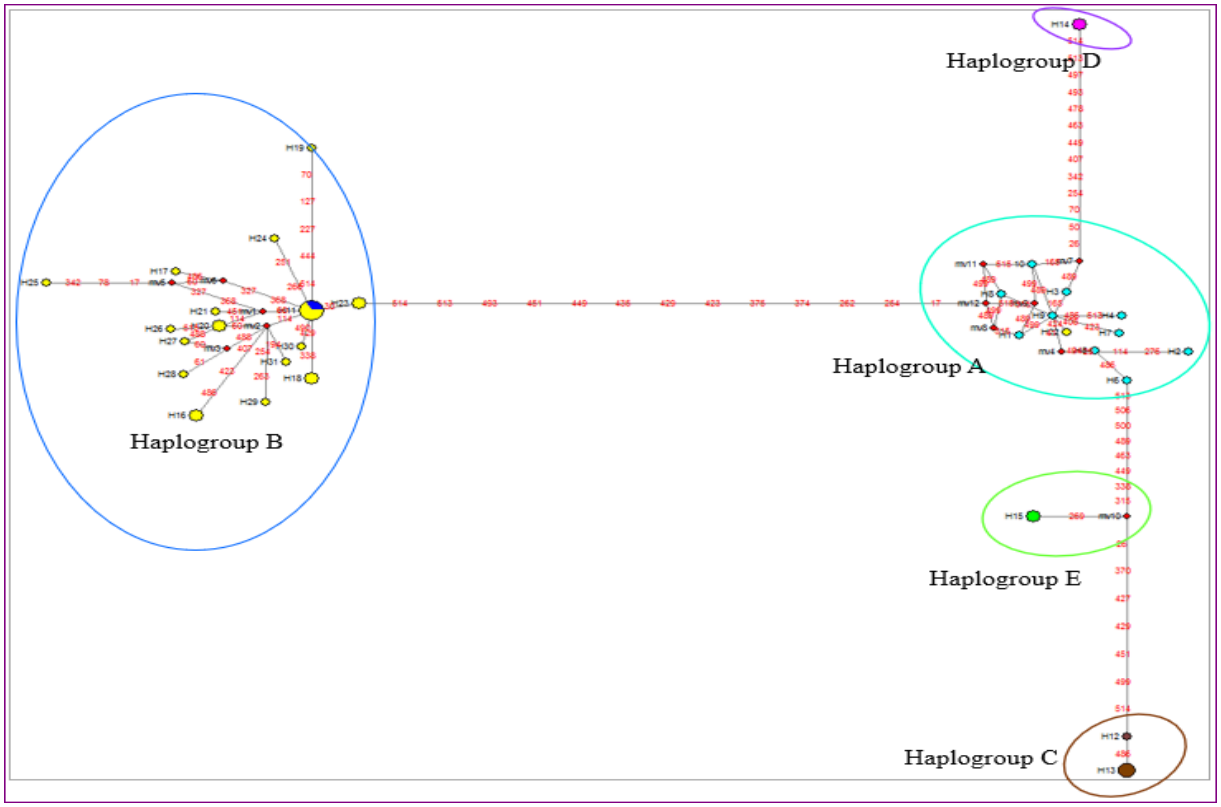

Figure 2. Median-joining network had been constructed based on the analysis of 517 bp of the *mtDNA* D-loop involving the 21 haplotypes observed in 4 North Ethiopian sheep populations (yellow circles) including the reference haplogroups of the five *mtDNA* haplogroups in *Ovis arise* (Table 1). Branch lengths are proportional to number of mutations.

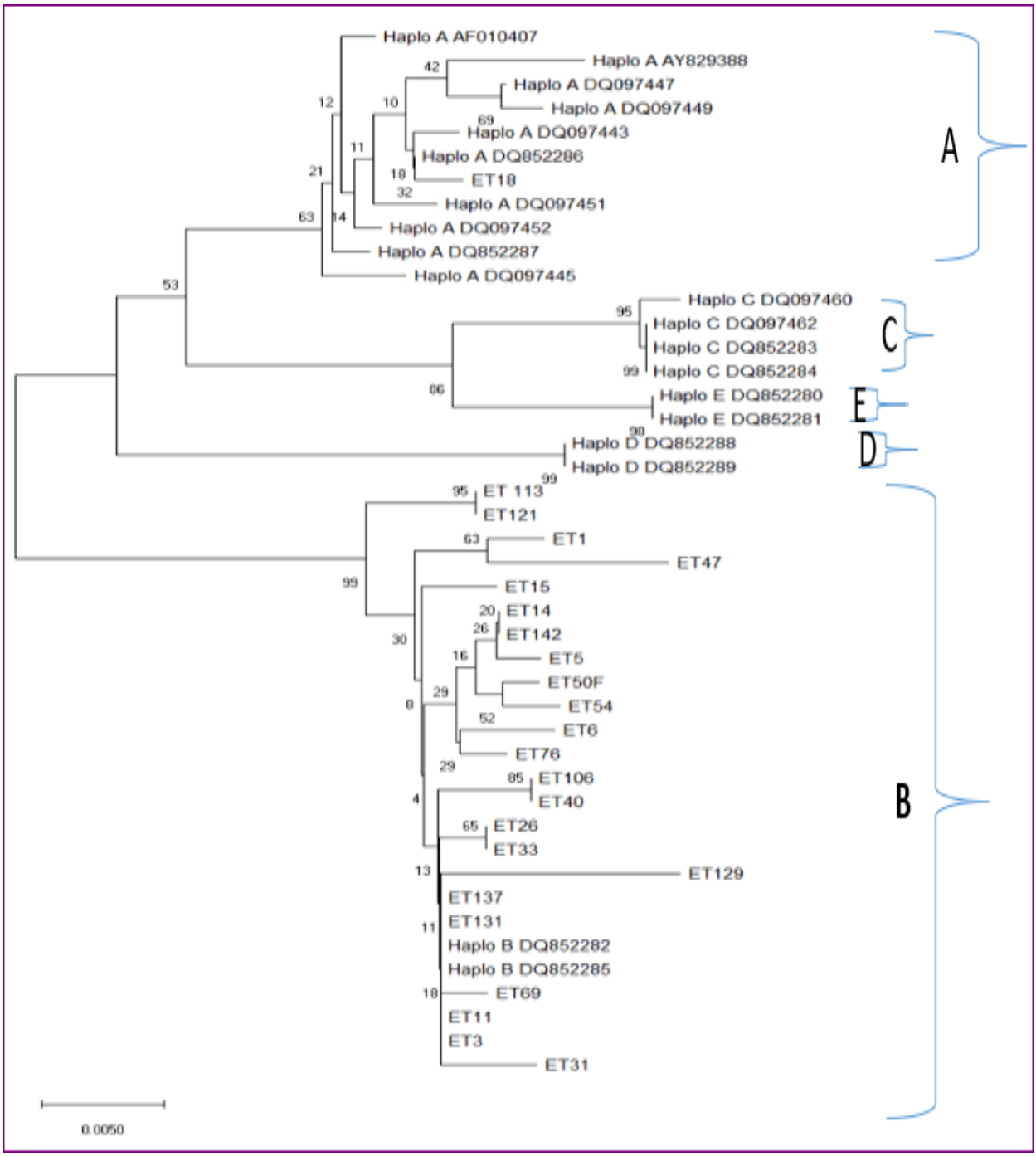

Figure 3. Neighbor-joining tree of the study population sequences (ET1 to ET 142) and reference sequences of the five haplogroups of *ovis aries* (haploG A to E) based on 517bp of mtDNA D-loop.

4.4. Population expansions

Mismatch distribution is the distribution of the number of pairwise differences between sequences (Harpending *et al.*, 1998). From the aspect of both population diversity and population demographic history this distribution is an informative method. Information about

population expansion is provided from the shape of mismatch distribution (Jobling *et al*., 2004). The mismatch distribution analysis of the four indigenous Ethiopian sheep populations of 517 bp mtDNA D-loop is shown in Figure 4 and the result indicated a one-time population major expansion and followed by considerable slow population expansion. This is because a smooth bell-shaped distribution designates a rapid population expansion while the multimodal, ragged distribution indicates a constant population size. This was confirmed by Neutrality tests such as; Tajima's D test (1989) and Fu's F_S test (Fu, 1997)) as showed in (Table 5). Neutrality tests (tests for selection) such as were used to reveal the population history of haplogroups by compared the observed diversity of a population to the expected under neutral evolution.

Tajima's D neutrality test of the Highland, Elle, Abergelle and Begait sheep was not significant (with mean Tajima´s D value of 0.29902, p = 0.68200) (Table 5). Fu's FS mean value was 0.14396 for the four indigenous sheep populations were also not significant ($p<0.01$, $p<0.05$ or $p<0.001$) that could be because of small sample size used in those statistical tests.

Table 5. Demographic expansion of the North Ethiopian sheep populations

Statistics	Highland	Elle	Abergelle	Begait	Mean	S.D.
SSD	0.0346	0.4059	0.0000	0.0787	0.1089	0.1684
	(0.1800)	(0.0200)	(0.0000)	(0.3700)	(0.1220)	(0.1556)
Raggedness	0.0711	1.0000	0.0000	00.1600	0.2564	0.4196
index	(0.3400)	0.3300)	(0.0000)	(0.6200)	(0.2640)	(0.2556)
Tajima's D test	-1.3330	2.1562	0.0000	-0.3817	0.2990	1.3449
	(0.0970)	(0.9880)	(1.0000)	(0.4420)	(0.6820)	(0.3984)
Fu´s FS test	-1.2614	3.5263	0.0000	-1.3451	0.1439	1.9857
	(0.2030)	(0.9350)	(N.A.)	(0.1170)	(N.A.)	(N.A.)

The charts of the mismatch distribution for the total samples of the four North Ethiopian sheep populations were bimodal (Figure 4). As showed from the mismatch distribution analysis pattern in (figure 4) this finding suggests the occurrence of two expansion events in the demographic history of the four North Ethiopian sheep populations. As reviewed by Muigai and Hanotte (2013) between 7500 and 7000 BP the first sheep (likely of the thin-

tailed type) entered Africa, via the Isthmus of Suez and/or the southern Sinai Peninsula. On the other hand, the fat-tailed sheep entered Africa through its north eastern part and the Horn of Africa (Muigai and Hanotte, 2013), suggesting that Northern Ethiopia could be one of the main entry points of domestic sheep in to the continent and particularly in to Ethiopia.

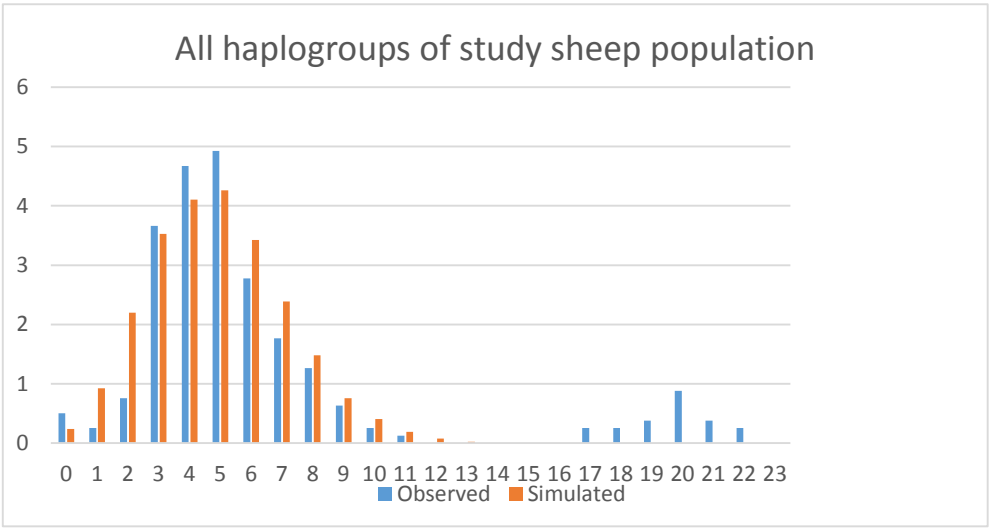

Figure 4. Mismatch distribution pattern of all haplotypes revealed by the MJ network analysis.

5. Conclusions

This study concluded that the North Ethiopian sheep populations revealed two major haplotypes of *Ovis aries* and this showed that there is high mtDNA haplotype diversity. The two haplogroups, haplogroup A and B were detected in the study population of which the latter is the dominant haplogroup. The observed haplogroups were reported in the Horn of Africa and Middle East suggesting the gene flow of Ethiopian sheep gene pool is from the Middle East through Egypt crossing the Red Sea. Based on the mismatch distribution analysis, there was a one time major and a considerable but slow sheep population expansion in Ethiopia. Presence of multiple maternal origins in the North Ethiopian indigenous sheep populations is very essential for future conservation and breeding programmes. Therefore, unravelling the whole genome landscape of the indigenous resources will be to fully understand their genetic potentials.

6. Possible consequences

6.1. Societal Values

To guide decision-making in livestock development and breeding programmes a good understanding of breed characteristics is required as recognized by the Global Plan of Action for Animal Genetic Resources (FAO, 2011). Conservation-based breeding programs considering breeding objectives of communities, adaptive merits of breeds and full involvement of the community in the design and implementation of breeding programs are required (Solomon, 2011). Since Ethiopia has different agro-climatic zones, the availability of diverse sheep genetic resources is essential for the conservation of breeds for future generation and improves the production and productivity of sheep populations within the given environment. Accordingly, knowledge on the genetic background will further assist to further improve the indigenous sheep breeds of Northern Ethiopia. Therefore, this study will contribute to develop conservation-based breeding programmes of those study sheep breeds to sustain the livelihood of the rural populations by increasing the income from their live animals and animal products especially meat from those potentially adapted breeds.

6.2. Ethical and other consideration

We declare that there is no professional or other personal interest of any nature and we have no any personal and financial relationships with other people or organizations that can inappropriately influence our work. We have been observed the local regulations for import and export of DNA samples. Accordingly, the Material Transfer Agreement (MTA) for export genetic material for genotyping was done with the Ethiopian institute of biodiversity conservation (IBC) based on the guideline of FAO (2011) for molecular genetic characterization. This research was using Nasal swab DNA collection kits, which does not require injury to the animal nor impose pain. The project contains no special gender aspects that need to be addressed.

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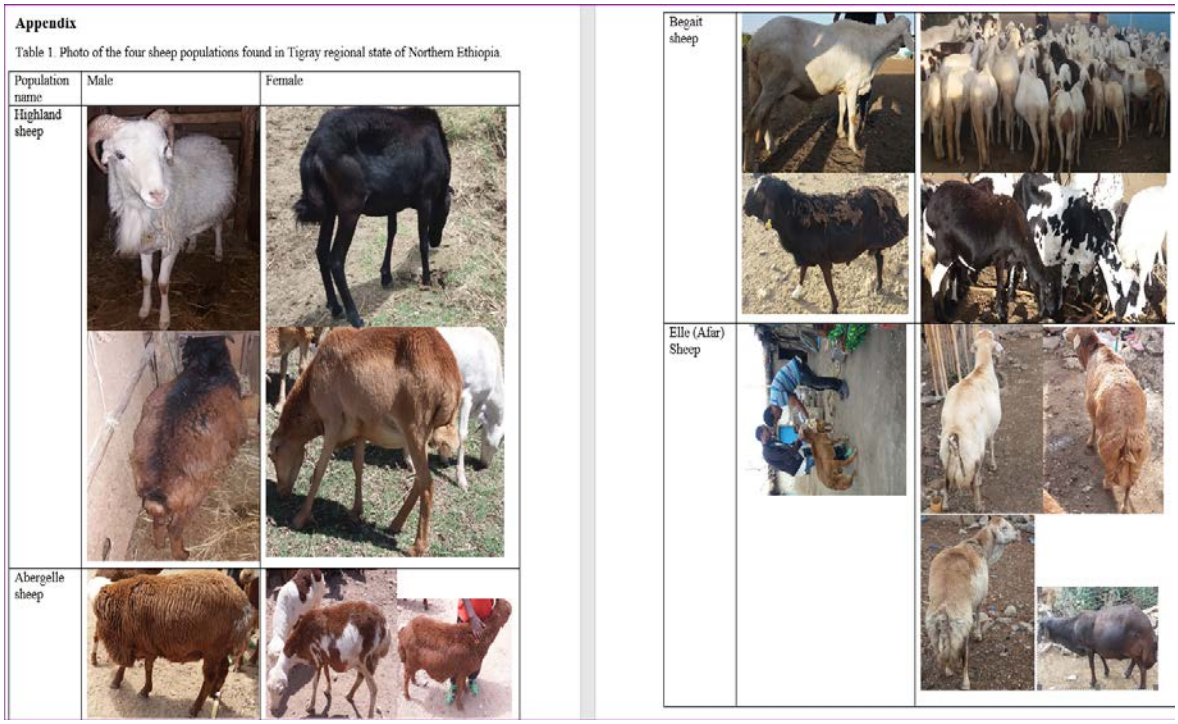
8. Appendices:

Figure 1. Photo of the four studied sheep populations in Northern Ethiopia.



Appendix Table 1. Molecular diversity indexes

Statistics	Highland	Elle	Abergelle	Begait	Mean	s.d.
No. of transitions	24	6	0	10	15.800	15.691
No. of transversions	3	0	0	1	1.000	1.225
No. of substitutions	27	6	0	11	16.800	16.392
No. of indels	0	0	0	0	0.000	0.000
No. of ts. sites	24	6	0	10	15.800	15.691
No. of tv. sites	3	0	0	1	1.000	1.225
No. of subst. sites	26	6	0	11	16.600	16.242
No. private subst. sites	4	0	0	5	5.400	7.403
No. of indel sites	0	0	0	0	0	0
Pi	6.622	4.000	0.000	5.000	5.87155	5.02849