

**Original Article****The Complete Mitogenome of the Iraqi Awassi Sheep Breed and the Maternal Lineage Utilizing High Throughput Sequencing Raw Reads**

Sarbast Ihsan Mustafa✉

Department of Animal Production, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region-Iraq

ARTICLE INFO	ABSTRACT
<p>Article history Received: 21 Sep 2021 Accepted: 17 Nov 2021 Published: 31 Dec 2021</p> <p>Keywords Genetic diversity, Mitochondrial genome, Maternal phylogeny, Next Generation Sequencing data</p> <p>Correspondence Sarbast Ihsan Mustafa ✉: sarbast.ihsan@uod.ac</p>	<p>Background: The Awassi sheep are the dominant indigenous fat-tailed sheep breed found in Iraq, in the Fertile Crescent, within the center of the domestication and diversity of the animal species. Their productive traits and morphology are well defined. However, the genetic landscape regarding the assembly of their complete mitogenome and maternal phylogeny is not characterized yet. Materials and Methods: High throughput genomic sequencing data and bioinformatics analysis were performed to assemble the complete mitogenome, identify maternal lineage and determine patterns of genetic diversity of the Iraqi Awassi sheep. Results: Phylogenetic analysis of the complete mitogenome (16617bp) positioned the maternal lineage of the Iraqi Awassi sheep into the most predominant European maternal haplogroup B. Furthermore, analysis of nucleotide diversity showed high level of mitogenomic similarity of the Iraqi Awassi sheep to Turkish Karakas, African Djallonke, Iraqi Karadi, Israeli Assaf and Jordanian Awassi sheep breeds. Conclusion: The present findings revealed the maternal phylogeny and the genetic biodiversity of the Iraqi Awassi sheep. This study contributes to the better understanding of genetic relatedness of the Iraqi native sheep with other domestic and wild sheep worldwide in the context of origin, breeding and conservation.</p>
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Introduction

Domestic sheep (*Ovis aries*) belong to the Caprini tribe, Caprinae subfamily, to the Bovidae family within the order Artiodactyla (Bibi, 2013). Archaeological and genetic studies demonstrated that sheep were one of the first clusters of livestock species that domesticated around 8000-11000 years ago in the Fertile Crescent (Zeder, 2008). Sheep are one of the most important farm animals that produce valuable and diverse products (Chessa et al., 2009). The Awassi sheep are the most abundant and widespread fat-tailed sheep breed in many countries that are well adapted to a variety of harsh environmental conditions in the southwestern regions of Asia (Galal et al., 2008). In Iraq, the Awassi breed is one of the indigenous sheep breeds which encompasses nearly two-thirds of the existing Iraqi sheep population distributed between the northern and central regions of the country and is mainly raised for lamb production and carpet-wool production (Alkass and Juma, 2005). Genetic diversity is the raw material

for adaptation, conservation and evolution of animals and therefore, the preservation of natural levels of genetic integrity in animal species is the fundamental target for breeding and management of animal genetic resources (Toro et al., 2009; Groeneveld et al., 2010). Polymorphism within mitochondrial DNA (mtDNA) sequences has widely been used as one of the vital markers to study genetic diversity. The biodiversity of maternal phylogeny has been explored in different animal species, which can be associated with their geographical zones to resolve ancestry (Kimura et al., 2011 (donkey); Yang et al., 2017 (dogs); Al-Jumaili et al., 2020 (chickens)). Correspondingly, several studies have been used PCR-based methods and whole genome sequencing data to examine the mitogenomic sequences and ascertain the phylogenetic relationship between domestic sheep breeds and as a results five major maternal haplogroups known as HPG-A, HPG-B, HPG-C, HPG-D and HPG-E have been classified (Meadows et al., 2007; Lv et al., 2015). Phenotypic and productive characteristics of the Iraqi Awassi sheep

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have been well studied, but their complete mitogenome and maternal identity have not been considered yet. Hence, we aimed to assemble the complete mitogenome, reveal maternal lineage and patterns of genetic diversity of the Iraqi Awassi sheep using high throughput sequencing (HTS) genomic data.

Materials and Methods

High throughput sequencing (HTS) data

About 412186220 high throughput sequencing (2x150bp) raw reads of the Iraqi Awassi sheep breed were retrieved from the European Nucleotide Archive database, sample accession SAMN14590377, run SRR11657624 of the study (Li et al., 2020). HiSeq X Ten platform from Illumina was used in their study to sequence the whole genome of the domestic Awassi sheep breed from Iraq (Sample code: AWA-21) (Table 1).

Table 1. Maternal haplogroup, GenBank ID, mitogenome size, total HTS data and assembled mitochondrial reads of Awassi sheep

Sheep breed	Maternal haplogroup ^a	GenBank accession	Mitogenome size (bp)	Total HTS raw reads ^b	Assembled reads (n) ^c	Coverage ^d
Awassi	HPG-B	MZ664406	16617	412186220	104745	945X

^aHaplogroup was indicated based on the phylogenetic analysis see Figure 2 and Table 2.

^bHTS reads= total high throughput sequencing raw reads collected from the [European Nucleotide Archive](#) database.

^cn= numbers of HTS raw reads assembled to reference to produce complete mitogenome

^dCoverage= number of assembled reads ×150bp [average read length]/16617bp (mitogenome size)

Assembly of complete mitogenome

The complete mitogenome of the Iraqi Awassi sheep breed was assembled after alignment 412186220 raw reads with the complete mitogenome of the domestic Karadi sheep breed (GenBank Accession No.: MF004245, Mustafa et al., 2018) as a reference. Then, the consensus of assembled reads was extracted and annotated in order to identify the lengths and order of the 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and D-loop region. The Geneious Prime software (Kearse et al., 2012; <http://www.geneious.com>) was used for the bioinformatics analysis including setting paired end raw reads, reference mapping and mitogenome annotation.

Phylogenetic analysis

The phylogenetic relationship was built based on the multiple alignment of the complete mitogenome of the Iraqi Awassi sheep with published mitogenomes from domestic and wild sheep representing the main five maternal haplogroups (HPG-A, HPG-B, HPG-C, HPG-D, and HPG-E) (Figure 2 and Table 2). The phylogenetic status was identified by constructing a Bayesian tree using MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001) within the Geneious Prime software. The parameters for the Bayesian tree were set on General Time Reversible (GTR) as a substitution model, gamma with four gamma categories as a rate variation, 10,000 as a burn-in length and 100,000 as a chain length. The goat mitogenome was used as the out-group.

Assessment of genetic diversity

The complete mitogenome was used to estimate the genetic distance based on the nucleotide diversity and the polymorphic sites (variable sites) between the Iraqi Awassi sheep breed and other sheep breeds

representing the main maternal haplogroups using DnaSP v.5.1 software (Librado and Rozas, 2009).

Results and Discussion

Assembly and structure of complete mitogenome of Awassi sheep

Previous studies used 17-22 pairs of primer to amplify and assemble the complete mitogenome of sheep (Dong et al., 2019; Wang et al., 2020). In contrast, we used the high throughput sequencing (HTS) data and bioinformatics software to assemble the complete mitogenome. As a result of mapping of the 412186220 HTS raw reads to a reference mitogenome from the domestic Karadi sheep breed, in total 104745 HTS reads with 945X coverage were assembled and consensus (16617bp) representing the complete mitogenome of the Iraqi Awassi sheep was formed (Table 1 and Figure 1). Similarly, Mustafa et al. (2018) assembled the complete mitogenomes of the Iraqi Karadi and Hamdani sheep breeds using next generation sequencing data. As reported by previous studies regarding the structure and composition of mitogenome of other domestic sheep breeds (Lv et al., 2015), the mitogenome structure of the Awassi sheep is a circular molecule with size 16617bp included 13 protein coding genes named ND1-6, ND4L, COX1-3, CYTB, ATP6, and ATP8; 22 transfer RNA (tRNAs) genes; 2 ribosomal RNA (rRNAs) genes and one control (D-loop) region (Figure 1). In agreement with the results of the study by Hu and Gao (2016), the nitrogenous bases of the complete Awassi mitogenome is 33.67% (A: Adenine), 27.38% (T: Thymine), 25.84% (C: Cytosine), and 13.11% (G: Guanine). This means that the overall base composition is substantially rich in A+T nucleotides (61.05%), but is low in a G+C content (38.95%). Our results are compatible with the characterization of complete

mitogenomes of other vertebrates such as goats, chicken, cattle, and fish (Colli et al., 2015; Peng et al., 2018; De et al., 2019; Xu et al., 2019). The annotation features including the start and stop codons, position, sizes and strand distribution of all 37 genes and the

control region of the Awassi sheep mitogenome are available under the accession number MZ664406 of the NCBI database.

Table 2. GenBank ID, mitogenome size, maternal haplogroups and geographical locations of domestic and wild sheep used for constructing the phylogenetic tree as shown in Figure 2

Common name (Country)	GenBank ID	Mitogenome size (bp)	Maternal Haplogroup	References
<i>Merino (Australia)</i>	HM236174	16617	HPG-A	Meadows et al. (2011)
<i>Romney (Australia)</i>	HM236175	16617	HPG-A	
<i>Hamdani (Iraq)</i>	MF004242	16619	HPG-A	Mustafa et al. (2018)
<i>Hamdani (Iraq)</i>	MF004243	16618	HPG-A	
<i>Hamdani (Iraq)</i>	MF004244	16618	HPG-A	
<i>Karadi (Iraq)</i>	MF004245	16617	HPG-B	
<i>Karadi (Iraq)</i>	MF004246	16617	HPG-B	
<i>O. musimon (Germany)</i>	HM236184	16616	HPG-B	Meadows et al. (2011)
<i>Karakas (Turkey)</i>	HM236176	16616	HPG-B	
<i>Awassi (Jordan)</i>	MW260509	16617	HPG-B	Brake et al. (2021)
<i>Assaf (Israel)</i>	HE577848	16617	HPG-B	Reicher et al. (2012)
<i>Oxford Down (UK)</i>	KF938359	16617	HPG-B	Lv et al. (2015)
<i>Lanzhou Large-tailed (China)</i>	KF938335	16617	HPG-B	
<i>Altay (China)</i>	KF938320	16621	HPG-C	
<i>Karakas (Turkey)</i>	HM236178	16620	HPG-C	Meadows et al. (2011)
<i>Morkaraman (Turkey)</i>	HM236180	16616	HPG-D	
<i>Morkaraman (Turkey)</i>	HM236181	16616	HPG-D	
<i>Awassi (Israel)</i>	HM236182	16620	HPG-E	
<i>Tuj (Turkey)</i>	HM236183	16620	HPG-E	
Awassi (Iraq)	MZ664406	16617	HPG-B	Present study
<i>O. ammon Argali (Kazakhstan)</i>	HM236188	16613	wild sheep	Meadows et al. (2011)
<i>O. vignei Urial (Kazakhstan)</i>	HM236186	16694	wild sheep	
<i>O. canadensis (Canada)</i>	JN181255	16463	bighorn sheep	Miller et al. (2012)
<i>Ovis ammon hodgsoni (China)</i>	JX101654	16,688	wild sheep	Jiang et al. (2013)
<i>Vietnamese (Capra hircus)</i>	GU295658	16,643	goat	Hassanin et al. (2010)

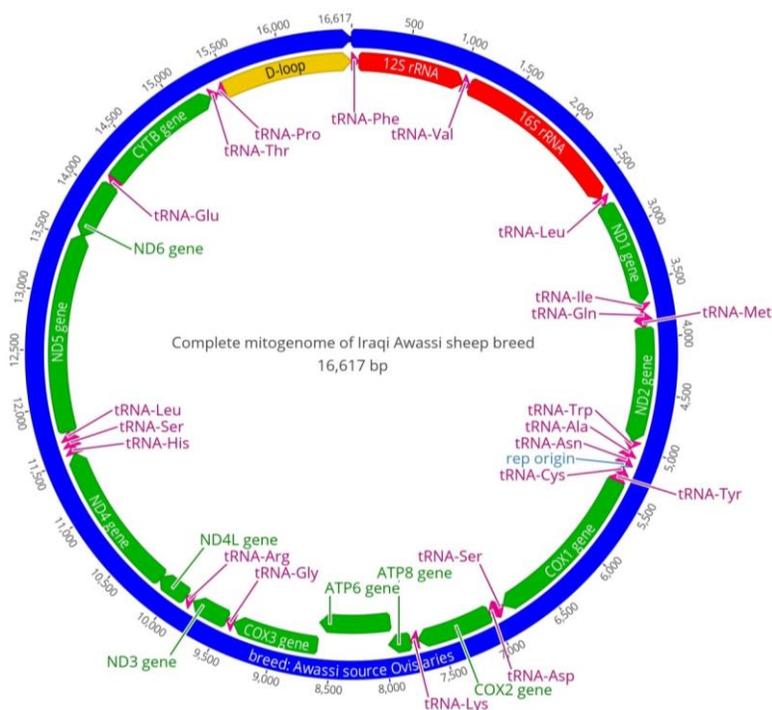


Figure 1. Mitogenome structure of Awassi sheep. The assembled complete mitogenome *Ovis aries* Awassi source (blue colour) (16617bp) (GenBank accession number MZ664406) with major features: 13 protein-coding genes (green bars, with the arrow pointing in the transcription directions), 22 transfer RNA (tRNA) genes (pink triangles), the 12S and 16S rRNA genes (red colour) and the control region (orange colour). Table 1 shows details of assembled data

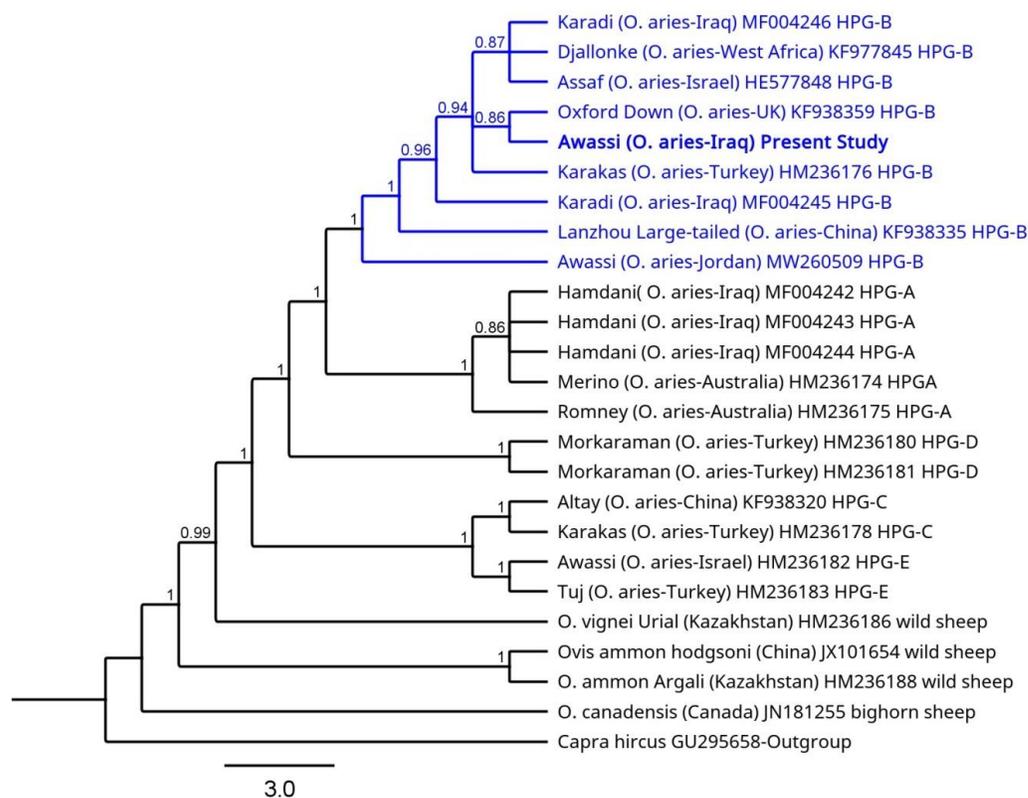


Figure 2. Phylogenetic relationships of Iraqi Awassi sheep breed to the main maternal haplogroups of domestic and wild sheep. Nodes are characterized with posterior probabilities. Table 2 refers to the accession numbers, geographical locations, maternal haplogroups of sheep breeds and their references used for phylogenetic tree.

Phylogenetic status

Phylogenetic relationships in domestic sheep breeds have been extensively studied. Some researchers investigated partial fragments of mtDNA, while others studied the complete genome of mitochondria. As a result, five major maternal haplogroups known as HPG-A, HPG-B, HPG-C, HPG-D and HPG-E have been classified in which the most abundant haplogroups in Europe and Asia are HPG-B and HPG-A respectively, while the other three haplogroups HPG-C, HPG-D and HPG-E are less frequent (Hiendleder et al., 1998; Tapio et al., 2006; Meadows et al., 2007; Lv et al., 2015; Mustafa et al., 2018). The results of the phylogenetic analysis clustered the Iraqi Awassi mitogenome within the most abundant European maternal haplogroup B (HPG-B) in which the Iraqi Karadi, African Djallonke, Jordanian Awassi, Israeli Assaf, Turkish Karakas, British Oxford down and Chinese Lanzhou sheep breeds are included (Figure 2 and Table 2). Our results are supported by Pedrosa et al. (2007), when they found that the majority of the analyzed Awassi sheep sampled from Israel belonged to maternal haplogroup B. Similarly, and most recently, Brake et al. (2021) demonstrated that the complete mitogenome of the Awassi sheep from Jordan is also fitted into the maternal haplogroup B. Nevertheless, the Iraqi Awassi sheep (HPG-B) are phylogenetically distant from the

Israeli Awassi sheep (HPG-E) that were sampled by Meadows et al. (2011) (Figure 2). Compatibly, different maternal haplogroups (HPG-A, HPG-C and HPG-E) have been found and this is perceptibly due to the existence of high genetic diversity in Awassi breed worldwide (Meadows et al., 2007; Pedrosa et al., 2007). Overall, Awassi sheep from the Fertile Crescent countries are the most diverse sheep breed by having various maternal haplogroups HPG-A, HPG-B, HPG-C and HPG-E. On the other hand, in comparison to the Iraqi Hamdani sheep (HPG-A), the Iraqi Awassi shared the same common ancestor (Figure 2). Our findings infer that the presence of high variability in the mitogenomic compositions of the Awassi sheep from the Middle East countries could be due to their geographical separation.

Genetic diversity

The results of the nucleotide diversity and the polymorphic sites between mitogenomic sequences of the Iraqi Awassi sheep and other ovine breeds within the same maternal haplogroup B were ranged from 0.00090 to 0.00284 and 15 to 47 respectively (Table 3). Remarkably, these findings indicated a high level of mitogenomic similarity between Iraqi Awassi sheep and Turkish Karakas, West African Djallonke, Iraqi Karadi, Israeli Assaf, Jordanian Awassi, and Chinese Lanzhou

(Table 3). However, a higher value of nucleotide diversity (0.00698 to 0.01011) and polymorphic sites (116 to 168) was resulted between the mitogenome of the Iraqi Awassi sheep and sheep breeds from other maternal haplogroups HPG-A, HPG-C, HPG-D and HPG-E (Table 3) indicating higher genetic variation between them. Consequently, the highest genetic diversity (0.01011 nucleotide diversity; 168 polymorphic sites)

was found between the Iraqi Awassi (HPG-B) and the Awassi from Israel (HPG-E). Our results are in agreement with the findings of Pedrosa et al. (2007), when they investigated the fragment of the mitochondrial control region of 24 samples of the Awassi sheep of Israel and the highest nucleotide diversity (0.0243) was recorded in comparison to 19 Iberian sheep and 5 foreign sheep breeds.

Table 3. Nucleotide diversity and polymorphic sites between complete mitogenome of Iraqi Awassi sheep with other domestic sheep breeds representing the main maternal haplogroups

Sheep breeds (Representatives of five maternal haplogroups)	Awassi (<i>O. aries</i> -Iraq)	
	(Present study-MZ664406)	
	Nucleotide diversity	Polymorphic sites
<i>Karakas (O. aries-Turkey) HM236176 HPG-B</i>	0.00090	15
<i>Djallonke (O. aries-West Africa) KF977845 HPG-B</i>	0.00096	16
<i>Karadi (O. aries-Iraq) MF004245 HPG-B</i>	0.00120	20
<i>Assaf (O. aries-Israel) HE577848 HPG-B</i>	0.00120	20
<i>Karadi (O. aries-Iraq) MF004246 HPG-B</i>	0.00126	21
<i>Oxford Down (O. aries-UK) KF938359 HPG-B</i>	0.00144	24
<i>Awassi (O. aries-Jordan) MW260509 HPG-B</i>	0.00199	33
<i>Lanzhou (O. aries-China) KF938335 HPG-B</i>	0.00284	47
<i>Hamdani (O. aries-Iraq) MF004242 HPG-A</i>	0.00698	116
<i>Merino (O. aries-Australia) HM236174 HPGA</i>	0.00698	116
<i>Hamdani (O. aries-Iraq) MF004244 HPG-A</i>	0.00704	117
<i>Hamdani (O. aries-Iraq) MF004243 HPG-A</i>	0.00722	120
<i>Altay (O. aries-China) KF938320 HPG-C</i>	0.00849	141
<i>Morkaraman (O. aries-Turkey) HM236181 HPG-D</i>	0.00897	149
<i>Awassi (O. aries-Israel) HM236182 HPG-E</i>	0.01011	168

Conclusion

The maternal phylogeny of the Iraqi Awassi sheep breed indicated that their lineage belongs to the most abundant European maternal haplogroup B (HPG-B). Additionally, nucleotide diversity unveiled that Awassi sheep from Iraq are mitogenomically in close relationship with other sheep breeds worldwide within the same maternal lineage (HPG-B). Nonetheless, the Iraqi Awassi sheep share a common ancestor with the Iraqi Hamdani sheep from the maternal haplogroup A (HPG-A).

Data availability statement

The annotated complete mitogenome of the Awassi sheep breed is available in GenBank of the NCBI database under the accession number MZ664406.

Author contribution

The retrieval of the high throughput sequencing raw reads from databases, assembly of mitogenome, submission of mitogenome to NCBI, genomics and phylogenetic analysis, writing and reviewing of the manuscript were all performed by the corresponding author.

Competing interests

The author has declared that no competing interests exist.

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