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RESEARCH ARTICLE

Maternal Genetic Structure of Iraqi Faili Kurds Revealed Using Whole Mitochondrial Genome Sequencing

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ABSTRACT

Mitochondrial DNA (mtDNA) is a commonly utilized genetic marker in determining maternal ancestry because of the hypervariable regions in it, as well as haplogroups, which enable scientists to establish ancestral links among populations. This research involved the analysis of the complete mitochondrial genome of 100 Faili Kurd women in different cities in Iraq using the Illumina system. The most common haplogroups were HV14 (53%), H14a (16%), M37e2 (16%), and HV2a1*1 (15%). The pair-wise AMOVA-FST analysis in terms of population revealed that there were various levels of genetic differentiation between Faili Kurds and other populations in Iraq, Iran, and surrounding areas. The Qashqai population recorded the lowest genetic distance ($F_{ST} = 0.2203$), implying that this population is closely related in genetic terms. There was also low differentiation between Nassiriya-Iraq ($F_{ST} = 0.1826$), Assyrians ($F_{ST} = 0.1764$), Khuzestan ($F_{ST} = 0.1623$) and Tabriz ($F_{ST} = 0.1590$), which could represent shared ancestry or recent migration. Conversely, the higher F_{ST} values were found with Damascus, Syria (0.999) and Birjand, South Khorasan, Iran (0.999), whereas moderate differentiation was found with Hama, Syria (0.8381), Armenians (0.6973) and Ilam (0.6743). Such results underscore the regional affiliation and maternal genetic patterns among the Faili Kurds, supporting their long-standing presence in the Near East, and contribute to the broader body of human genetic diversity in the Middle East.

Keywords: Forensic genetics, Faili Kurds, Haplogroup diversity, Mitochondrial DNA, Next-generation sequencing, Population genetics

Introduction

The ease of detection and the haploid nature of mitochondrial DNA (mtDNA) make it an invaluable tool for studying human evolution and migration. Despite the need for caution when concluding human population history from the genealogy of a single locus, mtDNA has successfully supported or refuted numerous hypotheses regarding human evolution.¹ Because of the distinct features that set it apart from the nuclear genome, mitochondria and mtDNA are

essential to methodological research of intricate features like structure, function, and the history of the human population.² Hypermutability, copy number variation, heteroplasmy, haplogroups, lack of introns, sensitivity to environmental stimuli, mitochondrial inheritance, and epigenetic changes are some of these distinctive traits.³ Numerous aspects of mitochondrial DNA's biochemistry have been revealed by the vast research conducted since its discovery in 1963.

However, these findings often focus on specific aspects, lacking a holistic perspective that integrates

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all these features.⁴ The Kurds, an ethnic group native to West Eurasia, are recognized as the largest stateless nation globally. They communicate in an Indo-European language, though certain linguists propose that their origins in the Near East may predate the Indo-European linguistic era, suggesting an ancient, indigenous connection to their homeland.⁵ Anthropological studies further indicate that the ancestors of the Kurds were among the earliest Neolithic inhabitants of the Northern Fertile Crescent, who played a crucial role in the development of agricultural technologies in the Near East and Eurasia, subsequently spreading these innovations to Europe.⁶

The focus on archaeological excavations in the Zagros area and nearby regions have provided some of the earliest records of agriculture and domestication of animals in the Fertile Crescent. The pre-pottery Neolithic period of the Neolithic era reveals evidence of sophisticated organization of architectural planning of settlements like Cayonu in southeastern Anatolia.⁷ In the same manner, zooarchaeological and isotopic evidence of sheep and goat management was found at Neolithic sites such as Ganj Dareh, Bestansur and Jarmo (present-day western Iran and Iraqi Kurdistan) in 8000–5000 BC.⁸ The Kurdish people, indigenous to West Eurasia, represent a large and historically significant ethnic group distributed across several countries in the Middle East.⁹ Even though linguistically connected with Indo-European roots, a certain amount of evidence indicates that it is more directly connected to earlier inhabitants of the Near East.¹⁰ Archaeological estimates of the Zagros area have led to the fact that the early people helped in the evolution of agriculture in the Neolithic era, and the practices were subsequently transferred to other parts.¹¹

In spite of this historical significance, little is known about genetic studies of Kurdish populations, especially through full genome sequencing of mitochondria. Most studies that have been done previously have depended on hypervariable regions, which do not necessarily capture all the lineage diversity.¹² Faili Kurds are a relatively little-studied group that is in the form of a subpopulation and is mainly found along the Iraq-Iran border. Because of their geographic distribution and their historical background, they are of interest of particular interest in learning more about the genetic structure of the region.

Materials and methods

Samples collection

A total of 100 samples were obtained from Faili Kurdish women above 18 years of age, recruited from

Baghdad, Wasit, Basra, Missan, Sulaymaniyah, and Diyala governorates along the Iraq–Iran border. Ethical approval for this study was first obtained from the Universiti Putra Malaysia Institutional Review Board (vide letter no. UPM/TNCPI./RMC/.1.4.18.2. (JKEUPM), and additional approval for sample collection in Iraq was granted by the University of Baghdad – College of Pharmacy Research Ethics Committee (vide letter no. 1–24/2022). Written informed consent was obtained from all participants prior to sample collection. Buccal swabs were collected using sterile PureGene collection tubes (Qiagen, Mansfield, MA) and served as the primary source for DNA extraction. Peripheral venous blood (3–5 mL) was collected into EDTA tubes only when buccal samples were unavailable or insufficient. Biospecimens were anonymized and stored under the accession number SUB14835651 in the NCBI Bio-Samples database.

Data accessibility

Data will be accessible under BioProject ID PRJNA1182475.

Whole genome sequencing

DNA extraction for each sample was carried out using the ThermoFisher Genomic DNA purification (Cat#K0512), following the manufacturer's protocol. Whole genome sequencing was performed on all samples using the Illumina NextGen platform with paired-end sequencing.

For downstream analysis, we utilized the mtDNA Server2 tools from Mitoverse, a publicly accessible online resource for haplogroup assignment.¹³ This tool integrates previously established haplocheck and haplogrep3¹⁴ tools to analyze user-uploaded BAM files. This haplogroup analysis starts by validating input files and produces a MultiQC report of the FASTQ data. The analysis either uses mutserve or mutect2, depending on the user's preference. We applied the fusion mode, a combination of mutserve and Mutect2, to enhance variation discovery in our work. Once we identified variants, we annotated them, confirmed they were not contaminated and assigned haplogroups to them. A summary of the results is presented in an interactive HTML report.

The tool is versatile, whereby the parameters can be modified by the user to increase prediction effectiveness. Sensitivity can be controlled with detection limits, depth and uniformity can be evaluated with coverage estimates, and mean coverage, base quality and mapping quality thresholds can be specified. These environments assist in making sure that only quality data is being analyzed.

In our study, we maintained the default settings to study 100 in-house samples. These consisted of a detection limit of 0.02, subsampling of coverage to 2000x, and both minimum base and mapping quality of 20. Such an arrangement was adopted to obtain a haplogroup assignment of high quality and accuracy.

Sample collection from published data

The data on mitochondrial DNA were synthesized out of a variety of earlier published work on many populations. Sahakyan et al.¹⁵ provided data on people of Afghanistan, Iran, Iraq, Jordan, Kuwait, Pakistan, Palestine, Saudi Arabia, Syria, Tajikistan, Turkey, and Yemen. Equally, Pala et al.¹⁶ provided information on Iran, Iraq, Jordan, Kuwait, Romania, Saudi Arabia, Syria, Turkey, the United Arab Emirates and Yemen.

Further genetic data for populations in Iran, Romania, Saudi Arabia, and Turkey were obtained from Kushniarevich et al.,¹⁷ while additional Iranian sequences were sourced from De Fanti et al.¹⁸

In addition, 352 complete Iranian mtDNA published by Derenko et al.¹⁹ were incorporated to strengthen the comparative framework. Table 1 summarizes all mitochondrial sequence data included in this study.

Table 1. Details of individuals with mitochondrial genome sequencing data from different populations included in this study.

Country	Subpopulation(s)	N
Afghanistan	—	9
Iran	General, Arab, Azari, Balouchi, Iranian, Khorasan, Khuzestan, Kordestan, Lur, Mazandaran, Yazd	91
Iraq	—	28
Jordan	—	5
Kuwait	—	23
Pakistan	—	9
Palestine	—	3
Romania	—	10
Saudi Arabia	—	6
Syria	—	5
Tajikistan	—	6
Turkey	—	19
United Arab Emirates	—	3
Yemen	—	4

Calculate a pairwise population distance matrix using FST

We have used Arlequin (ver 3.5.2.2) to obtain AMOVA and a pairwise population distance matrix based on pair fixation indices (FST). Arlequin is a highly reputed package in the realm of population genetics. It can provide a thorough anal-

ysis of population subdivision under the AMOVA framework.²⁰

PCoA and MDS

The principal coordinate analysis (PCoA) and MDS plot were plotted based on the population pairwise fst distance matrix obtained from Arlequin.²¹

Results and discussion

All the samples passed the basic QC implemented by mtDNA Server2. No contamination was observed in any of the samples. Sequencing of all samples covered 16,569 bases with a coverage percentage of 100%, a mean mapping quality of 37, a mean base quality score of ~34, and a mean depth ranging from 1986.55 to 2004.27. An example of the resulting QC check can be seen in Fig. 1. In total, four major haplogroups were identified among the Faili Kurdish samples: HV14 (53%), H14a (16%), M37e2 (16%), and HV2a1*1 (15%).

In the present study, approximately 15% of the Faili Kurdish mitochondrial DNA samples were classified under the HV2a1*1 haplogroup, supported by a confidence level of 98%. According to the phylogenetic reconstruction using the mtDNA Server2 platform, HV2a1*1 descends from haplogroup HV Fig. 2. Haplogroup HV is a key ancestral clade from which both H and V haplogroups emerged.²² Earlier studies of the mitochondria revealed a significant proportion of HV lineages in places where Faili Kurds had lived and continue to live, especially western Iran, Mesopotamia, and the South Caucasus.²³

Haplogroup H14a was detected in 16% of the analysed samples, with an assignment confidence of 92%. This lineage is characterized by 10 defining mutations and originates from two HVS-I variants. Phylogenetic analysis as shown in Fig. 2 a performed using mtDNA Server2, which places H14a within haplogroup H. In earlier research, the root H14 has been associated primarily with populations of northwestern Europe, and specifically with the Irish, although its subclade H14a is found to be more widely distributed among the Armenians and Sardinians.²⁴

Similarly, the haplogroup HV2a1*1 was found in 15% of the samples and was high with a 98 level of confidence. Phylogenetic reconstructions based on the mitochondrial genome (mtDNA Server2) place this lineage in the haplogroup HV, the ancestral branch of haplogroup H and V Fig. 3. Haplogroup HV has been reported to be predominant in parts of western Iran, Mesopotamia and South Caucasus.²⁵

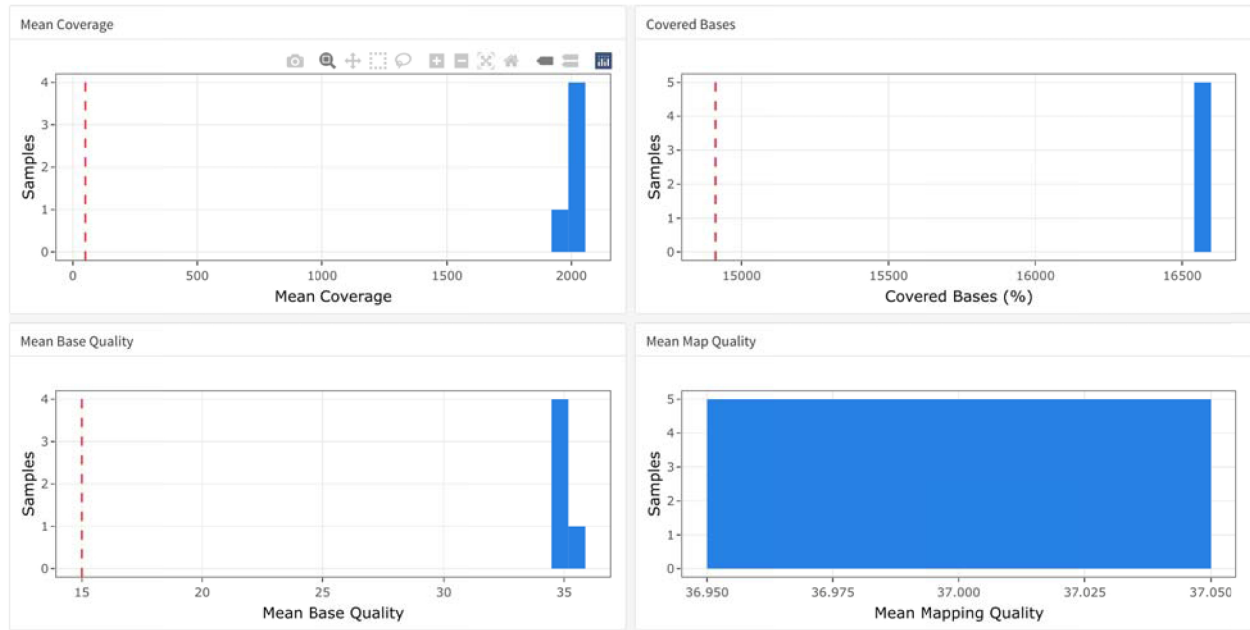


Fig. 1. The outcomes of mitochondrial DNA-sequencing quality control, as a representation of read depth, coverage distribution and base quality in all samples. These findings show that the data could be used in the further haplogroup assignment and phylogenetic analysis.

H14a and HV2a1*1 presence in the analyzed population demonstrates the heterogeneity of the maternal lineages among the Faili Kurds. These haplogroups probably indicate a complicated history of interactions and gene flow throughout the Middle East and surrounding areas.

Haplogroup HV14 was detected in (53%) of analyzed samples, with a probability of assignment between 92 and 95. Previous studies have reported this haplogroup in Iran, India, Sri Lanka, and Yemen, as well as in ancient remains from Turkmenistan dated to about 4.5 kya,²⁶ indicating the presence of HV14 in West Eurasia.

Current indications show that HV14 is likely to have been present in southern Iran, with an estimated date of emergence of 12.1–13.4 kya or 8.4–12.6 kya.²⁵ The high proportion of HV14 in the current study indicates a close maternal relationship between the Faili Kurds and the ancient Iranian plateau and adjacent populations [Fig. 4](#).

Approximately 16% of the analysed samples were assigned to haplogroup M37e2 with a prediction probability of 97%. The phylogenetic reconstruction performed using the mtDNA Server2 platform placed this lineage within haplogroup M, a clade widely distributed across multiple continents.

Haplogroup H14a

Phylogenetic Tree

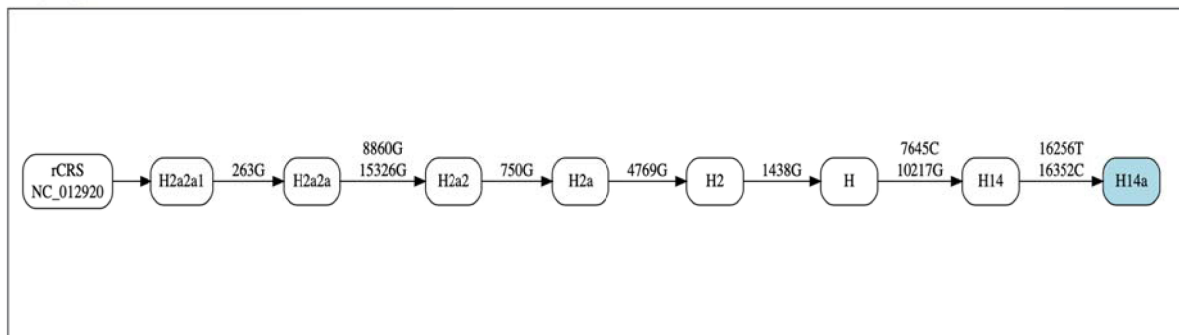


Fig. 2. MtDNA Server2 phylogenetic tree of haplogroup H14a, including its location in haplogroup H and the mutational paths that characterized this lineage.

Haplogroup HV2a1*1

Phylogenetic Tree

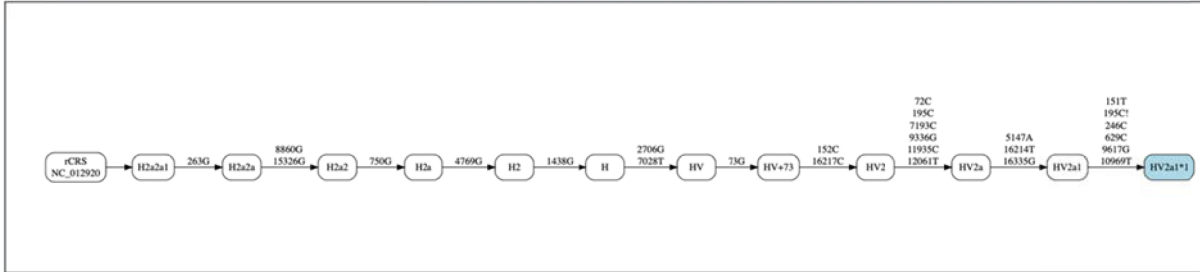


Fig. 3. Phylogenetic tree of haplogroup HV2a1*1 generated using the mtDNA Server2 platform, showing its position within haplogroup HV and its ancestral relationship to haplogroups H and V based on complete mitochondrial genome data.

Haplogroup HV14

Phylogenetic Tree

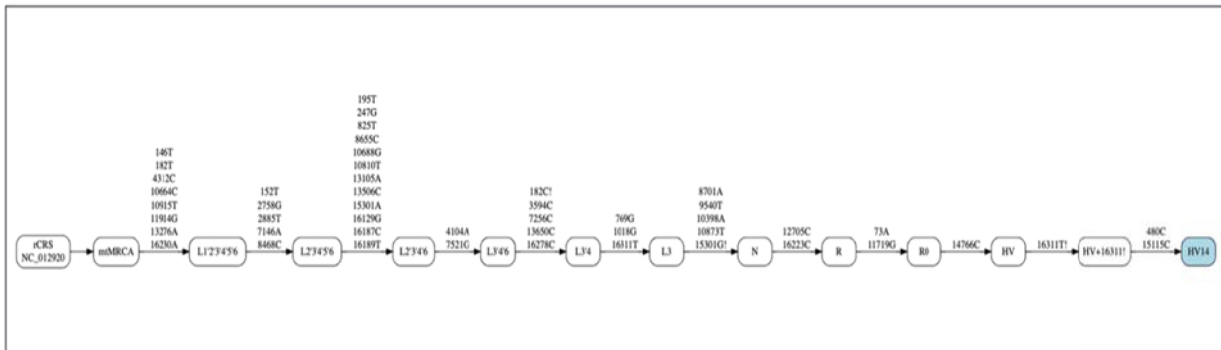


Fig. 4. Phylogenetic tree of haplogroup HV14 obtained from mtDNA Server2, showing its branching pattern within haplogroup HV and the mutations that define this maternal lineage.

Haplogroup M37e2

Phylogenetic Tree

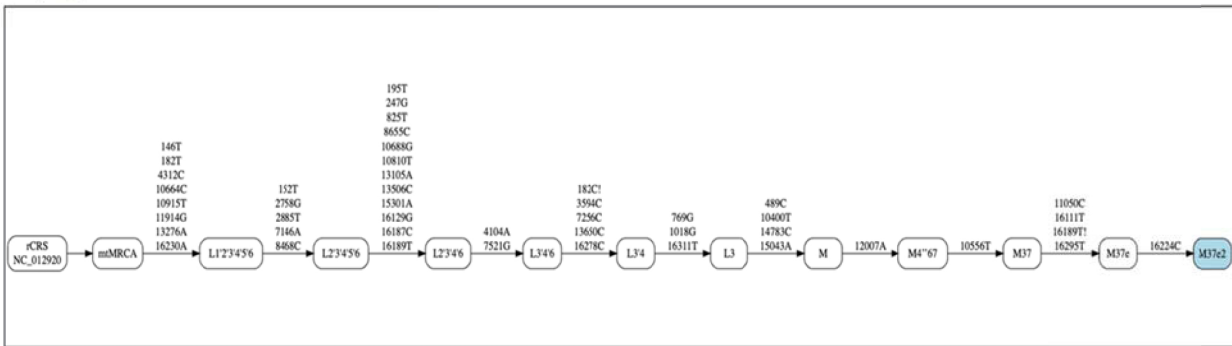


Fig. 5. Phylogenetic tree of haplogroup M37e2 showing its evolutionary relationship within the macrohaplogroup M. The tree was reconstructed based on complete mitochondrial genome sequences, highlighting the defining mutations that characterize each node and the specific lineage identified in the Faili Kurdish population.

The findings suggest that the Fails Kurds are genetically related to the neighbouring populations but also feature very evident distinctions. This trend is probably indicative of a complicated demographic history based on historical trends, interactions, and relative isolation. This suggests past gene flow into populations around Iran, Syria and surrounding areas due to observed affiliations with them, whereas the observed differences may reflect unique evolutionary processes within this group.

As helpful as these observations are, a number of limitations must be taken into account. Although the present sample size is sufficient in terms of the preliminary observations, it might not be sufficient to provide a complete view of the larger genetic diversity of the Faili Kurdish population. Moreover, the study using the mtDNA alone can only analyse the aspect of maternal inheritance and thus cannot give the entire genomic data.

More extensive sample sets, including Y-chromosome and genome-wide data, should be used in future work, and larger and more heterogeneous sample sets are needed. These methods would give a more detailed picture of the genetic background of the Faili Kurds and how they relate to other peoples within the region and wider.

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Authors' declaration

- Conflict of interest: None.
- We hereby confirm All the Figures and Tables in the manuscript are ours. Any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript
- Authors signed on ethical consideration's approval.
- Ethical approval: The project was approved by the Ethics Committee for Research involving Human Subjects (JKEUPM) from Universiti Putra Malaysia (JKEUPM-2022-987), and the Research Ethics Committee of Al-Nahrain University (24/2022).

Authors' contribution statement

M.K.I.: Conceptualization, data curation, formal analysis, investigation, methodology, visualization, and original draft writing. S.A.B.: Supervision, validation, resources, critical review, and manuscript editing. C.Y.K. and M.A.S.: Co-supervision, validation, critical review, and manuscript editing.

Data availability

The datasets generated and analyzed during the current study are available in the NCBI BioProject repository under accession number PRJNA1182475.

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السلالة الأمومية للأكراد الفيليين في العراق المجموعات الفردانية للحمض النووي المتقدري باستخدام التسلسل الجيني

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الخلاصة

يُعد الحمض النووي للميتوكوندريا (mtDNA) علامة وراثية شائعة الاستخدام في تحديد الأصول الأمومية بسبب وجود المناطق شديدة التباين فيه، بالإضافة إلى مجموعات الهابلوغروب (Haplogroups)، والتي تمكّن العلماء من تحديد الروابط السلالية بين المجموعات السكانية. شملت هذه الدراسة تحليل الجينوم الكامل للميتوكوندريا لـ 100 امرأة من الكرد الفيليين في مدن مختلفة في العراق باستخدام نظام الإليومينا (Illumina). وكانت أكثر المجموعات الهبلوغروبية شيوعاً هي (HV14) 53%، و(H14a) 16%، و(M37e2) 16%، و(HV2a1*1) 15%. أظهر تحليل التباين الجزيئي (AMOVA-FST) على مستوى المجموعات السكانية وجود درجات مختلفة من التمايز الوراثي بين الكرد الفيليين وسكان آخرين في العراق وإيران والمناطق المجاورة. وقد سجلت مجموعة القشفاي أقل مسافة وراثية (FST = 0.2203)، مما يشير إلى أن هذه المجموعة قريبة وراثياً. كما وُجد تمايز منخفض بين ناصرية العراق (FST = 0.1826)، والأشوريين (FST = 0.1764)، وخوزستان (FST = 0.1623)، وتبريز (FST = 0.1590)، مما قد يعكس أصلاً مشتركاً أو هجرة حديثة. في المقابل، سُجلت قيم مرتفعة لـ FST مع دمشق في سوريا (0.999) وبيرجند في خراسان الجنوبية بإيران (0.999)، بينما وُجد تمايز متوسط مع حماة في سوريا (0.8381)، والأرمن (0.6973)، وإيلام (0.6743). تؤكد هذه النتائج الانتماء الإقليمي والأنماط الوراثية الأمومية لدى الكرد الفيليين، وتدعم وجودهم التاريخي الطويل في منطقة الشرق الأدنى، كما تسهم في توسيع قاعدة التنوع الوراثي البشري في الشرق الأوسط.

الكلمات المفتاحية: علم الوراثة الجنائي، الكرد الفيليين، تنوع الهبلوغروبس، الحمض النووي للميتوكوندريا، التسلسل الجيني من الجيل التالي، علم الوراثة السكانية.