

RESEARCH ARTICLE

DNA Barcoding and Genetic Characterization of *Haemaphysalis parva* (Acari: Ixodidae) from Central Anatolia, Türkiye Using Mitochondrial *cox1* Sequences

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Abstract

Ticks of the genus *Haemaphysalis* (Acari: Ixodidae) comprise a diverse group of species widely distributed across the Palearctic region and are known to harbor a variety of pathogens of medical and veterinary importance. Despite its relatively wide geographic distribution, the tick species *Haemaphysalis parva* remains relatively understudied from a molecular perspective, and reliable DNA barcode data for this species are largely lacking in global genetic databases. In the present study, we performed a mitochondrial *cox1*-based DNA barcoding and genetic characterization of *H. parva* specimens collected from active populations in Central Anatolia, Türkiye. A total of 16 adult ticks (eight females and eight males) collected from eight sampling sites were morphologically identified and subjected to molecular analyses. Sequencing of the mitochondrial *cox1* gene revealed seven distinct haplotypes among the analyzed specimens, with moderate haplotype diversity ($h = 0.6917$) and low nucleotide diversity ($\pi = 0.00162$). BLAST analyses demonstrated high sequence similarity with previously reported *H. parva* sequences from Türkiye, Iran, and Ghana. Bayesian phylogenetic reconstruction confirmed that all haplotypes identified in this study clustered within the main *H. parva* lineage and revealed two well-supported subclades within the species. The analysis also suggested possible inconsistencies among the taxonomic annotations of certain publicly available sequences in genetic databases. The generated sequences were deposited in GenBank and the Barcode of Life Data System (BOLD), establishing the first validated DNA barcode reference for *H. parva*. The findings of this study expand the currently available genetic data for this species and provide an important molecular reference for future taxonomic, ecological, and epidemiological investigations of *H. parva*.

Keywords: DNA barcoding, *cox1*, haplotype diversity, *Haemaphysalis*, phylogenetic analysis, tick genetics, Anatolia

INTRODUCTION

Ticks are among the most important arthropod vectors worldwide and represent a major threat to the health of both humans and domestic animals on a global scale [1]. Members of the genus *Haemaphysalis* (Acari: Ixodidae) have a wide geographic distribution and represent the second most species-rich genus among ticks, comprising approximately 174 recognized species worldwide [2]. Species of this genus typically exhibit a three-host life cycle and parasitize a broad range of vertebrate hosts, contributing to their wide geographic distribution and ecological diversity [3]. In the Western Palearctic region, including Türkiye, several species of this genus have been

reported, such as *Haemaphysalis parva*, *Haemaphysalis punctata*, *Haemaphysalis sulcata*, *Haemaphysalis caucasica*, *Haemaphysalis concinna*, *Haemaphysalis hispanica*, *Haemaphysalis erinacei*, and *Haemaphysalis kopetdaghica*. In addition, *Alloceraea inermis* (formerly *Haemaphysalis inermis*), which has recently been transferred to the genus *Alloceraea* but remains phylogenetically closely related to *Haemaphysalis*, is also present in this region [4-6]. Species belonging to the genus *Haemaphysalis* are also of considerable importance due to their role as vectors of a wide range of pathogens of medical and veterinary significance, including protozoan, bacterial, and viral agents [7-9]. Despite the high species diversity within the genus, the vector competence and ecological roles of



many *Haemaphysalis* species have not yet been fully characterized [6].

Haemaphysalis parva is a widely distributed tick species that exhibits a three-host life cycle and is considered an exophilic species adapted to a variety of habitats. Adult ticks parasitize medium and large wild and domestic mammals, whereas immature stages feed on a broad range of small- and medium-sized wild mammals (including hedgehogs, hares, rodents, and carnivores), ground-feeding birds, and even reptiles. Adult ticks also commonly infest domestic mammals and humans [3,10-12]. Although some populations, particularly in Anatolia, have been reported to reach relatively high abundance compared with other ixodid ticks [10], knowledge regarding the ecology, biology, and vectorial competence of this species remains limited both locally and globally. In recent years, pathogen screening studies have revealed the presence of various protozoan, bacterial, and viral agents in *H. parva* collected from different hosts, drawing increasing attention to its potential epidemiological importance [13-21]. Among these are pathogens of significant medical and veterinary importance, including Crimean-Congo hemorrhagic fever virus, *Francisella tularensis*, *Coxiella burnetii*, *Babesia ovis*, and *Babesia rossi* [22-28]. However, relatively few studies have focused on host-seeking (questing) *H. parva* ticks. A study on pathogens in questing adult *H. parva* collected in Türkiye reported the presence of *Babesia crassa*, *B. rossi*, *Rickettsia hoogstraalii*, a novel *Babesia* species (*Babesia* sp. Ucbas), and an uncharacterized *Hepatozoon* species [13]. These findings highlight the potential epidemiological significance of *H. parva* and provide additional context for future studies focusing on questing tick populations.

From a genetic perspective, the available data on *H. parva* are derived from studies based on different genetic markers and specimen sets [29-33]. In addition, several cases of misidentification have been reported in previous molecular studies [34,35]. Validated DNA barcode records for *H. parva* in the Barcode of Life Data System (BOLD) remain underrepresented, underscoring the importance of expanding reference datasets for molecular identification. Therefore, the aim of the present study was to provide a validated genetic characterization and DNA barcode reference for *H. parva* based on mitochondrial *cox1* sequences obtained from active populations in Central Anatolia, Türkiye, to assess intraspecific haplotype diversity, and to investigate the phylogenetic relationships of this species within the genus *Haemaphysalis*.

MATERIAL AND METHODS

Ethical Statement

This study did not involve the use of live animals or human participants. All tick specimens were collected as

host-seeking individuals from the environment without direct interaction with vertebrate hosts. Therefore, ethical approval was not required.

Study Area and Tick Collection

A total of 16 adult tick specimens (eight females and eight males) used in this study were collected from several localities within Ankara Province, Türkiye, including the districts of Kızılcahamam, Çubuk, Çamlıdere, Akyurt, Kalecik, and Sincan, during 2021-2022 (Fig. 1). The specimens consisted of host-seeking (questing) adults collected from vegetation using the flagging method. After collection, the ticks were transported to the Ticks and Tick-Borne Diseases Research Laboratory (TTBDRL), Department of Parasitology, Faculty of Veterinary Medicine, Ankara University. The specimens were preserved in 70% ethanol and stored at +4°C under appropriate laboratory conditions until further analyses were performed. Detailed information on the collected specimens, including sampling localities and specimen codes, is provided in Table 1.

Morphological Identification

All collected tick specimens (eight females and eight males) were morphologically identified to species level using standard taxonomic keys [4,36]. Based on the diagnostic morphological characters, all specimens were confirmed as adult *H. parva*. Following identification, the specimens were examined and photographed under a Zeiss Stemi 2000-C stereomicroscope (Zeiss, Germany) equipped with an AxioCam digital camera and the ZEN imaging software. Dorsal and ventral images were obtained for each specimen, and representative dorsoventral microscopic images of all examined individuals are presented according to sex in Fig. 2 (females) and Fig. 3 (males).

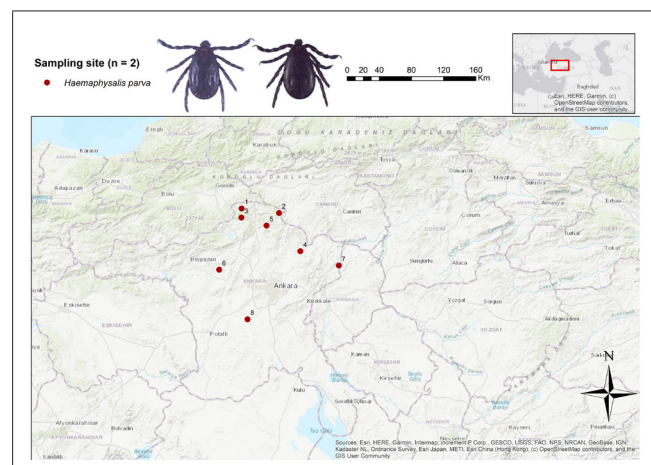


Fig 1. Geographic distribution of the sampling sites of *Haemaphysalis parva* in Ankara Province, Türkiye. Sixteen specimens (one female and one male from each locality) were collected from eight sampling sites. The inset map indicates the location of the study area within Türkiye

Table 1. Sampling details of <i>Haemaphysalis parva</i> specimens collected from eight localities in Ankara Province, Türkiye						
Study Site	Sample Code	Species	Gender	Location (Village/District)	Coordinates (Lat./Long.)	Collection Date
1	Hpa-1785	<i>Haemaphysalis parva</i>	Female	Gebeler/Kızılcahamam	40.628955/32.49913833	24.09.2021
1	Hpa-1786	<i>Haemaphysalis parva</i>	Male	Gebeler/Kızılcahamam	40.628955/32.49913833	24.09.2021
2	Hpa-1787	<i>Haemaphysalis parva</i>	Female	Semer/Kızılcahamam	40.59123167/32.91630667	13.10.2022
2	Hpa-1788	<i>Haemaphysalis parva</i>	Male	Semer/Kızılcahamam	40.59123167/32.91630667	13.10.2022
3	Hpa-1789	<i>Haemaphysalis parva</i>	Female	Kuşçu/Çamlıdere	40.552806/32.497469	12.10.2021
3	Hpa-1790	<i>Haemaphysalis parva</i>	Male	Kuşçu/Çamlıdere	40.552806/32.497469	12.10.2021
4	Hpa-1791	<i>Haemaphysalis parva</i>	Female	Karadana/Çubuk	40.26625667/33.15500333	06.04.2022
4	Hpa-1792	<i>Haemaphysalis parva</i>	Male	Karadana/Çubuk	40.26625667/33.15500333	06.04.2022
5	Hpa-1793	<i>Haemaphysalis parva</i>	Female	Yıldırımören/Çubuk	40.48433333/32.77777667	18.04.2022
5	Hpa-1794	<i>Haemaphysalis parva</i>	Male	Yıldırımören/Çubuk	40.48433333/32.77777667	18.04.2022
6	Hpa-1795	<i>Haemaphysalis parva</i>	Female	Ahmetadil/Akyurt	40.10857167/33.24836333	15.04.2022
6	Hpa-1796	<i>Haemaphysalis parva</i>	Male	Ahmetadil/Akyurt	40.10857167/33.24836333	15.04.2022
7	Hpa-1797	<i>Haemaphysalis parva</i>	Female	Buğra/Kalecik	40.14484833/33.58530667	01.03.2022
7	Hpa-1798	<i>Haemaphysalis parva</i>	Male	Buğra/Kalecik	40.14484833/33.58530667	01.03.2022
8	Hpa-1799	<i>Haemaphysalis parva</i>	Female	Ücret/Sincan	39.68348/32.56499	24.09.2022
8	Hpa-1800	<i>Haemaphysalis parva</i>	Male	Ücret/Sincan	39.68348/32.56499	24.09.2022

DNA Extraction, PCR Amplification and Sequencing

Prior to molecular analyses, the tick specimens were rinsed again with 70% ethanol and air-dried on sterile filter paper. Each adult *H. parva* specimen was processed individually for nucleic acid extraction. Ticks were mechanically homogenized in bead-containing tubes using a SpeedMill PLUS cooling homogenizer (Analytik Jena, Jena, Germany) to ensure efficient tissue disruption. Genomic DNA was subsequently isolated from the homogenates using the BlackPREP Tick DNA/RNA Kit

(IST Innuscreen GmbH, Berlin, Germany) according to the manufacturer's instructions. The extracted DNA samples were stored at -20°C until further analysis.

PCR amplification targeted a 710 bp fragment of the mitochondrial cytochrome C oxidase subunit I (*cox1*) gene using the universal primers LCO1490 and HCO2198 [37]. The thermal cycling conditions consisted of an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, with a final extension



Fig 2. Dorsal and ventral views of female *Haemaphysalis parva* specimens examined in this study. (a-b) Hpa-1785; (c-d) Hpa-1787; (e-f) Hpa-1789; (g-h) Hpa-1791; (i-j) Hpa-1793; (k-l) Hpa-1795; (m-n) Hpa-1797; (o-p) Hpa-1799. For each specimen, the left image shows the dorsal view and the right image shows the ventral view

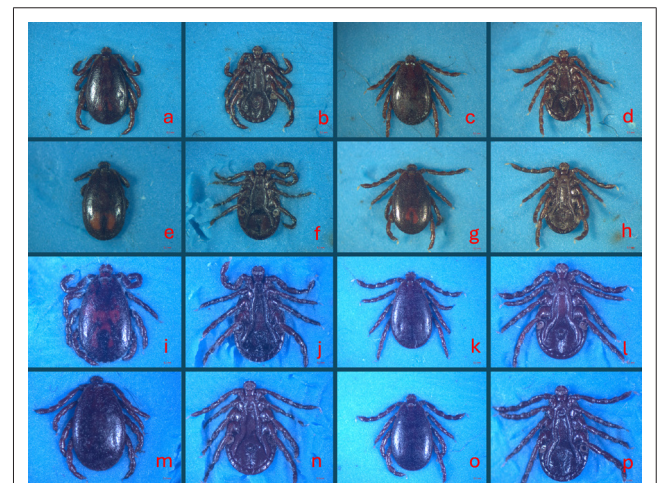


Fig 3. Dorsal and ventral views of male *Haemaphysalis parva* specimens examined in this study. (a-b) Hpa-1786; (c-d) Hpa-1788; (e-f) Hpa-1790; (g-h) Hpa-1792; (i-j) Hpa-1794; (k-l) Hpa-1796; (m-n) Hpa-1798; (o-p) Hpa-1800. For each specimen, the left image shows the dorsal view and the right image shows the ventral view

step at 72°C for 7 min. Positive amplicons were stored at -20°C until sequencing analyses. PCR products were purified using the PureLink™ Quick Gel Extraction Kit (Invitrogen, Löhne, Germany) following the manufacturer's protocol. The purified DNA samples were sequenced bidirectionally using the Sanger sequencing method on an Applied Biosystems™ 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions.

Sequence Processing and Population Genetic Analyses

Chromatograms were visually inspected, edited, and assembled into consensus sequences using AliView v1.26 [38]. The obtained sequences were subsequently compared against the GenBank (National Center for Biotechnology Information, <https://www.ncbi.nlm.nih.gov/genbank>) and BOLD (<http://www.boldsystems.org>) databases using nucleotide similarity searches (BLAST) to confirm species identification. All sequences generated in this study were compiled into a single dataset for haplotype-based population genetic analyses.

Prior to downstream analyses, the sequences were translated into amino acid sequences in AliView v1.26 to check for potential stop codons and NUMTs (nuclear mitochondrial DNA segments). Because *cox1* is a protein-coding gene, sequence alignment was performed using the MUSCLE algorithm [39] while considering the translated amino acid sequences. The aligned dataset was then used for population genetic analyses, including calculation of nucleotide diversity (π), number of haplotypes (k), haplotype diversity (h), relationships among haplotypes, and the distribution of haplotypes among sampling sites using DnaSP v6.12.03 [40].

Phylogenetic Analyses

For phylogenetic analyses, the haplotypes identified in this study were combined with available *cox1* sequences of *H. parva* and other *Haemaphysalis* species (also *Alloceraea* spp.) retrieved from GenBank. In addition, two *Bothriocroton* species (*B. undatum* and *B. concolor*) were included as outgroup taxa. Prior to alignment, the reliability of the dataset was evaluated using GUIDANCE2 [41], and sequences or columns with low confidence scores were excluded if necessary. The remaining sequences were aligned again using the MUSCLE algorithm based on the translated amino acid sequences. The reliability of the final alignment was further assessed by calculating p-distances (mean genetic distances) in MEGA v11.0.13 [42].

The most appropriate nucleotide substitution model for phylogenetic reconstruction was determined using jModelTest v2.1.10 [43]. Bayesian phylogenetic inference

was performed using the Markov chain Monte Carlo (MCMC) method implemented in the BEAST2 software package (BEAUti v2.7.6, BEAST v2.7.6, and TreeAnnotator v2.7.4) [44]. The MCMC chain was run for 100 million generations, and convergence as well as effective sample size (ESS) values were evaluated in Tracer v1.7.2 [45]. After discarding the first 20% of trees as burn-in, a maximum clade credibility tree was generated using TreeAnnotator v2.7.4. The resulting phylogenetic tree was visualized and edited using FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree>).

Data Availability

The genetic and barcode data generated in this study were deposited in the NCBI and BOLD databases. For this purpose, the *cox1* sequences of *H. parva* specimens together with detailed barcode metadata—including dorsoventral photographs of all examined specimens and their corresponding geographic coordinates—were submitted to BOLD, where the specimens were assigned to the barcode index number BIN:ADA9786. The same *cox1* sequence data were also deposited in the GenBank database of the NCBI and assigned accession numbers PQ725627-PQ725642. These records ensure public availability and traceability of the barcode data generated in this study.

RESULTS

A total of 16 adult ticks were collected from eight different localities in Ankara Province, Türkiye, with one female and one male specimen obtained from each site (Table 1). The geographic distribution of the sampling sites is shown in Fig. 1. All specimens were morphologically identified as *H. parva* based on established diagnostic characters, and no morphological anomalies were observed. However, descriptive morphological variation was observed among the examined individuals. The most prominent differences included variation in the density and depth of punctations on the scutum and conscutum. In addition, some male specimens exhibited distinct light and dark areas on the scutum, and the pattern and extent of these color variations differed among individuals. No quantitative assessment of these traits was performed, and these observations were considered to represent normal intraspecific variation within the species. Representative dorsal and ventral views of the examined females and males are presented in Fig. 2 and Fig. 3, respectively. Consequently, all specimens were included in subsequent genetic analyses.

Genomic DNA was successfully extracted from all 16 *H. parva* specimens, and PCR amplification of the mitochondrial *cox1* gene yielded amplicons of the expected size in all individuals. The PCR products were sequenced bidirectionally, and the resulting sequences

were validated through BLAST homology searches against the GenBank and BOLD databases. The characterized sequences were deposited in GenBank under accession numbers PQ725627-PQ725642 and in BOLD under the BIN:ADA9786 (Table 2).

Haplotype-based population genetic analyses revealed that the 16 sequences were grouped into seven distinct haplotypes ($k=7$). The calculated haplotype diversity (h) was 0.6917, while the nucleotide diversity (π) was 0.00162. Among the detected haplotypes, Haplotype 6 represented the most common variant and included nine specimens (Hpa-1800, Hpa-1795, Hpa-1786, Hpa-1789, Hpa-1790, Hpa-1792, Hpa-1793, Hpa-1796, and Hpa-1798). Haplotype 7 comprised two specimens (Hpa-1785 and Hpa-1788), whereas the remaining haplotypes were each represented by a single specimen (Table 2). Sequence polymorphism analysis indicated the presence of eight segregating sites ($S=8$), including seven singleton variable sites and one parsimony-informative site. Detailed haplotype information and sequence similarity values are presented in Table 2, while the geographical distribution of haplotypes across the sampling sites is illustrated in Fig. 4.

BLAST homology searches of the detected haplotypes revealed high similarity with previously reported *H. parva* sequences available in public databases. When considering matches with $\geq 90\%$ query coverage, the highest similarity was observed with a sequence obtained from *H. parva* in Türkiye (GenBank accession PX122095),

showing 99.42-99.71% identity at 100% coverage. In addition, the haplotypes showed 99.37-100% identity with 92-93% coverage to a sequence of *H. parva* reported from Ghana (PV125849). BLAST similarity values and corresponding reference sequences are summarized in Table 2. Furthermore, the haplotypes obtained in this study displayed 99.81-100% identity with approximately 75% coverage to two *H. parva* sequences previously reported from Iran (MH532296 and MH532297). BLAST searches also revealed high similarity with four sequences deposited under the name "*Hyalomma impeltatum*" from Israel (KT989628-KT989631), showing 98% coverage and 98.96-99.40% identity. In contrast, no barcode records of *H. parva* were available in the BOLD database, and therefore a direct sequence similarity comparison within the BOLD system could not be performed.

To infer the phylogenetic relationships of the detected haplotypes, a comprehensive dataset was constructed based on all available *H. parva* *cox1* sequences deposited in GenBank. A total of six *H. parva* records were available (accessed 11 March 2026) and included in the dataset, comprising three sequences from Türkiye, two from Iran, and one from Ghana. In addition, four sequences from Israel deposited under the name "*Hyalomma impeltatum*" and one sequence from Türkiye recorded as "*Haemaphysalis inermis*" were also incorporated into the dataset because BLAST analyses indicated close sequence similarity with *H. parva*. The final dataset consisted of

Table 2. Mitochondrial *cox1* haplotypes of *Haemaphysalis parva* identified in this study and their corresponding GenBank and BOLD accession numbers with BLAST similarity values

Study Site	Sample Code	Haplotype Name	GenBank Accession No.	BOLD ID.*	BLAST Homology**
1	Hpa-1785	Haplotype7	PQ725627	HAPA001-24	99.56 ^a , 99.53 ^b
1	Hpa-1786	Haplotype6	PQ725628	HAPA002-24	99.71 ^a , 99.69 ^b
2	Hpa-1787	Haplotype3	PQ725629	HAPA003-24	99.56 ^a , 99.53 ^b
2	Hpa-1788	Haplotype7	PQ725630	HAPA004-24	99.53 ^b , 99.42 ^a
3	Hpa-1789	Haplotype6	PQ725631	HAPA005-24	99.69 ^b , 99.56 ^a
3	Hpa-1790	Haplotype6	PQ725632	HAPA006-24	99.71 ^a , 99.69 ^b
4	Hpa-1791	Haplotype1	PQ725633	HAPA007-24	99.42 ^a , 99.37 ^b
4	Hpa-1792	Haplotype6	PQ725634	HAPA008-24	99.71 ^a , 99.69 ^b
5	Hpa-1793	Haplotype6	PQ725635	HAPA009-24	99.71 ^a , 99.69 ^b
5	Hpa-1794	Haplotype4	PQ725636	HAPA010-24	99.56 ^a , 99.53 ^b
6	Hpa-1795	Haplotype6	PQ725637	HAPA011-24	99.71 ^a , 99.69 ^b
6	Hpa-1796	Haplotype6	PQ725638	HAPA012-24	99.69 ^b , 99.57 ^a
7	Hpa-1797	Haplotype5	PQ725639	HAPA013-24	99.69 ^b , 99.56 ^a
7	Hpa-1798	Haplotype6	PQ725640	HAPA014-24	99.71 ^a , 99.69 ^b
8	Hpa-1799	Haplotype2	PQ725641	HAPA015-24	100 ^b
8	Hpa-1800	Haplotype6	PQ725642	HAPA016-24	99.71 ^a , 99.69 ^b

* BOLD BIN:ADA9786; ** BLAST homology with over 90% coverage has been considered

^a PX122095 *Haemaphysalis parva*; ^b PV125849 *Haemaphysalis parva* voucher T5

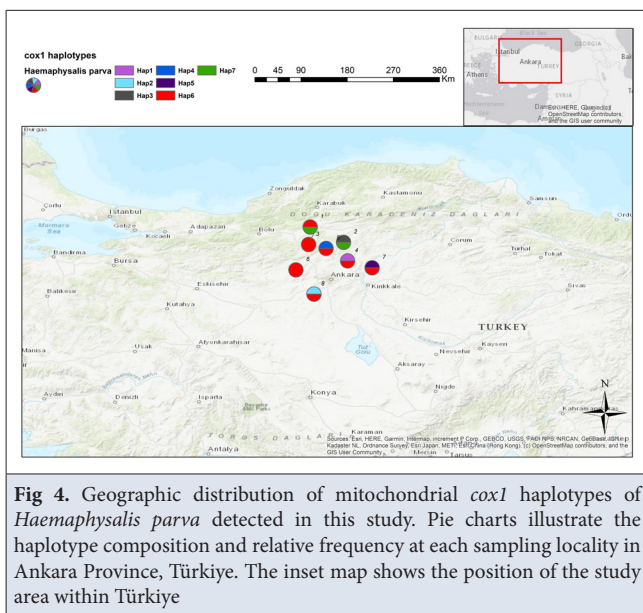


Fig 4. Geographic distribution of mitochondrial *cox1* haplotypes of *Haemaphysalis parva* detected in this study. Pie charts illustrate the haplotype composition and relative frequency at each sampling locality in Ankara Province, Türkiye. The inset map shows the position of the study area within Türkiye

66 sequences, including the seven haplotypes identified in this study, while the remaining sequences represented various species of the genus *Haemaphysalis* retrieved from GenBank. Four sequences belonging to *Alloceraea* spp. were included as sister taxa, whereas *B. undatum* and *B. concolor* were used as outgroup taxa.

The Bayesian phylogenetic tree showed that all haplotypes identified in this study clustered within the main *H. parva* clade together with the previously reported sequences from Ghana and Iran. The *H. parva* lineage was strongly supported and split into two well-defined subclades with maximum posterior probability (posterior=1). One subclade contained the haplotypes identified in this study together with the sequences from Ghana and Iran, whereas the second subclade comprised three sequences from Türkiye together with one sequence recorded as "*Haemaphysalis inermis*" from Türkiye and four sequences from Israel deposited under the name "*Hyalomma impeltatum*". Furthermore, *H. parva* formed a monophyletic lineage with *H. sulcata* and *Haemaphysalis kashmirensis*, supported by a high posterior probability value (posterior=0.89) (Fig. 5). Overall, the phylogenetic reconstruction confirmed the taxonomic placement of the studied specimens within the *H. parva* lineage.

DISCUSSION

The present study provides the first validated DNA barcode reference for *H. parva* based on mitochondrial *cox1* sequences obtained from active populations in Central Anatolia, Türkiye. Despite the relatively wide geographic distribution of this species across the Palearctic region [10,11,36,46], genetic information available for *H. parva* remains relatively limited and is based on a small number of studies and specimens [29-33]. In the present study, all

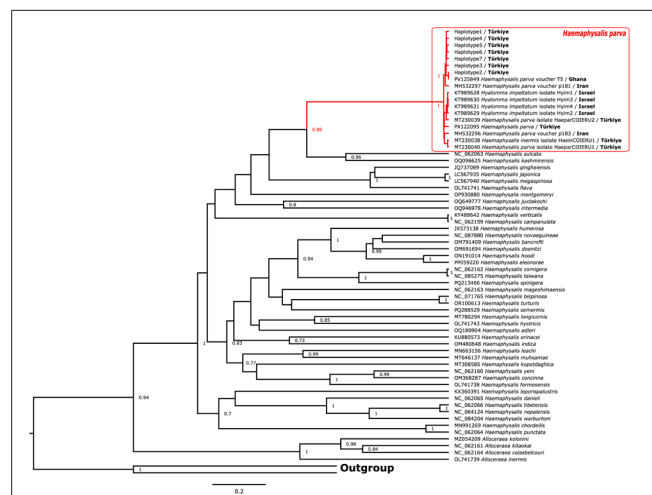


Fig 5. Bayesian phylogenetic tree inferred from mitochondrial *cox1* sequences of *Haemaphysalis* ticks. The analysis was performed using a dataset of 66 sequences and 686 aligned nucleotide positions under the TN93+I+G4 substitution model. *Bothriocroton undatum* (NC_017757) and *Bothriocroton concolor* (NC_017756) were used as outgroup taxa to root the tree. Node labels represent posterior probability values, with values < 0.7 omitted for clarity. Haplotypes identified in this study are highlighted in red, and the *Haemaphysalis parva* clade is indicated by a boxed frame. GenBank accession numbers are provided before species names, and the country of origin of sequences within the *Haemaphysalis parva* clade is indicated after the sequence names. The scale bar represents the number of nucleotide substitutions per site

examined specimens were morphologically confirmed as *H. parva* and subsequently characterized using *cox1*-based molecular analyses, revealing the presence of seven haplotypes among 16 morphologically confirmed specimens. In addition, the generated sequences were deposited in both GenBank and BOLD, thereby establishing the first barcode reference dataset for this species within the BOLD framework. The availability of such validated barcode records represents an important step toward improving the molecular identification and taxonomic reliability of *H. parva*, particularly given the previously reported cases of misidentification [34,35] and the limited representation of this species in global genetic databases.

The molecular characterization of *H. parva* has historically been based on relatively few specimens and genetic markers. The first partial genetic data for this species were generated from two specimens collected from sheep in Romania, in which the nuclear ITS1 and ITS2 regions were sequenced and reported to be identical between the samples [29]. Subsequently, Burger et al. [34] sequenced the mitochondrial genome together with partial nuclear 18S and 28S rRNA genes from a specimen preserved in the Queensland Museum and suggested that *H. parva*, together with *A. inermis*, was highly divergent from the remaining species of the genus *Haemaphysalis*. However, this interpretation was later questioned by Orkun [30], who performed a molecular

characterization of ticks collected in Türkiye based on mitochondrial 16S rRNA sequences. In that study, a questing *H. parva* specimen collected in Ankara showed the highest nucleotide similarity (92.2%) to *H. sulcata*, and the phylogenetic reconstruction did not support the placement of the previously published *H. parva* sequence reported by Burger et al.^[34]. These findings suggested that the Romanian specimen might actually represent *A. inermis* rather than *H. parva*. This issue was subsequently clarified when the authors of the original study re-evaluated the specimen and acknowledged a morphological misidentification, correcting the record as *A. inermis* ^[35]. More recently, additional molecular data have been generated from a small number of specimens. For instance, Faghihi et al.^[31] analyzed *H. parva* ticks collected from sheep and goats in Iran and sequenced the partial mitochondrial *cox1* gene from two specimens as well as the ITS2 region from four specimens. Very recently, a partial *cox1* sequence from a specimen collected from a dog in Ghana was reported ^[32], and the complete mitochondrial genome of a specimen collected from a human in Türkiye was also characterized ^[33]. Despite these contributions, the number of publicly available sequences for *H. parva* remains limited, and most studies rely on one or a few specimens. In comparison, the present study provides a substantially expanded molecular dataset based on 16 morphologically validated specimens, revealing seven distinct *cox1* haplotypes within a single regional population and establishing the first DNA barcode reference for this species within the BOLD system. These findings expand the currently available molecular data for *H. parva* and provide a valuable genetic reference for future taxonomic, ecological, and epidemiological studies.

Molecular genetic approaches have become increasingly important for the genetic characterization, DNA barcoding, population structure analysis, and phylogenetic reconstruction of vector ticks, thereby contributing to our understanding of tick evolution and tick-borne diseases ^[47,48]. Among the available genetic markers, the mitochondrial genome occupies a central role in arthropod molecular studies and has been widely used to investigate the phylogeny and genetic diversity of ticks ^[6,49,50]. Within the mitochondrial genome, the *cox1* gene is one of the most widely used evolutionary markers, representing a relatively conserved yet sufficiently informative region for investigating genetic variation among closely related taxa ^[47]. Consequently, *cox1* has been extensively applied in genetic characterization and population genetic studies of ticks ^[51-56]. In particular, Lv et al.^[53] emphasized that the *cox1* region should be considered a primary marker for standard DNA barcoding studies of ticks. For these

reasons, the mitochondrial *cox1* gene was selected as the barcode marker in the present study.

The haplotype-based analyses performed in the present study revealed the presence of seven distinct *cox1* haplotypes among 16 *H. parva* specimens, indicating a relatively moderate haplotype diversity within the examined population. Although the number of haplotypes was relatively high compared with the sample size, the overall nucleotide diversity was low, suggesting limited sequence divergence among haplotypes. Such a pattern, characterized by multiple closely related haplotypes with low nucleotide divergence, has been reported in geographically restricted populations of various organisms ^[57,58]. However, the underlying demographic processes cannot be inferred from the present dataset alone. The dominance of a single haplotype (Haplotype 6), which was represented by the majority of specimens, further supports the presence of a central haplotype surrounded by several less frequent variants. Similar patterns have been reported in mitochondrial studies of other ixodid ticks, where closely related haplotypes often differ by only a small number of mutational steps due to the relatively conserved nature of mitochondrial genes such as *cox1* ^[56,59,60]. In this context, the haplotype structure observed in the present study is consistent with intraspecific genetic variation within the local *H. parva* population rather than the presence of deeply divergent lineages. Nevertheless, it should be noted that the present analysis was based on a relatively limited number of specimens collected from a geographically restricted area. Therefore, broader sampling across the distribution range of *H. parva* would be necessary to better understand the overall genetic diversity and population structure of this species.

The BLAST similarity analyses and phylogenetic reconstruction conducted in the present study collectively support the taxonomic placement of the analyzed specimens within the *H. parva* lineage. The obtained *cox1* haplotypes showed the highest nucleotide similarity with previously reported *H. parva* sequences from Türkiye ^[33], Iran ^[31], and Ghana ^[32], indicating a clear genetic affinity among geographically distant populations of this species. Such high levels of sequence similarity across different regions may reflect the relatively conserved nature of the mitochondrial *cox1* gene and the wide geographic distribution of *H. parva* throughout the Palearctic and adjacent regions. The Bayesian phylogenetic tree further confirmed this relationship, as all haplotypes identified in the present study clustered within the main *H. parva* clade together with sequences originating from Ghana and Iran ^[31,32]. The phylogenetic reconstruction also revealed two well-supported subclades within the *H. parva* lineage. One of these subclades included the haplotypes identified in the present study together with

sequences from Ghana and Iran, whereas the second subclade contained sequences reported from Türkiye as well as several sequences originally deposited under the name "*Hyalomma impeltatum*" (KT989628-KT989631) from Israel and one sequence recorded as "*Haemaphysalis inermis*" from Türkiye (MT230038). The placement of these sequences within the *H. parva* clade may indicate possible inconsistencies in taxonomic annotation in public databases. However, additional morphological and multilocus molecular data would be required to clarify their taxonomic status. These findings also highlight potential inconsistencies in publicly available sequence annotations and emphasize the importance of generating well-validated reference datasets for reliable molecular identification of tick species. In this context, the present study also provides morphologically validated reference sequences that may facilitate future assessments of taxonomic annotation in public databases.

Although the present study provides the first validated DNA barcode reference for *H. parva*, several limitations should be acknowledged. First, the analyses were based on a relatively small number of specimens collected from a geographically restricted region of Central Anatolia, Türkiye, which may not fully represent the genetic diversity of the species across its distribution range. Second, the present study relied on a single mitochondrial marker (*cox1*), and additional nuclear or genomic markers may provide further insights into population structure and phylogenetic relationships. Therefore, future studies incorporating broader geographic sampling and multilocus approaches will be important for obtaining a more comprehensive understanding of the genetic diversity and evolutionary history of *H. parva*.

Overall, the present study expands the currently available molecular data for *H. parva* by providing a validated *cox1*-based DNA barcode reference from active populations in Central Anatolia, Türkiye. These findings contribute to improving the molecular identification of *H. parva* and provide a valuable genetic resource for future taxonomic studies of this species. Considering the increasing evidence that *H. parva* may harbor a variety of pathogens of medical and veterinary importance^[11,13], a better understanding of its genetic diversity and phylogenetic relationships is also essential for future ecological and epidemiological investigations. The barcode data generated in this study therefore provide a valuable molecular resource that may facilitate future taxonomic, ecological, and epidemiological investigations of *H. parva*.

DECLARATIONS

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author (ÖÖ) on reasonable request.

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Competing interests: The authors declare no competing interests.

Ethical Statement: This study did not involve the use of live animals or human participants. All tick specimens were collected as host-seeking individuals from the environment without direct interaction with vertebrate hosts. Therefore, ethical approval was not required.

Declaration of Generative Artificial Intelligence (AI): Authors declare that the article, tables, and figures were not written or created by AI and AI-assisted technologies.

Authors' contributions: Conceptualization: ÖÖ, Data curation: ÖÖ, Formal analysis: MNG and Ö.O, Investigation: MNG and Ö.O, Methodology: ÖÖ, Project administration: MNG and ÖÖ, Resources: ÖÖ, Software: ÖÖ, Supervision: ÖÖ, Validation: ÖÖ, Writing - original draft: ÖÖ, Writing-review and editing: MNG and ÖÖ. All authors have read and agreed to the published version of the manuscript.

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