Acta Parasitologica (2026) 71:52
https://doi.org/10.1007/s11686-026-01243-y

RESEARCH

The Complete Mitogenome of *Haemaphysalis parva* (Arachnida: Ixodidae) and Comparative Mitogenomics of *Haemaphysalis* Species

Habeş Bilal Aydemir[1] · **Adem Keskin**[2]

Received: 4 November 2025 / Accepted: 6 February 2026 / Published online: 27 February 2026
© The Author(s) 2026

Abstract

Introduction

Ticks are globally recognised as the second most important vectors of infectious diseases, posing significant threats to human and animal health. *Haemaphysalis parva* (Acari: Ixodidae) is frequently reported infesting humans and domestic animals and has been experimentally demonstrated to transmit *Babesia ovis*, with field associations to ovine babesiosis during the colder months. It has also been reported to harbour several zoonotic pathogens, including *Coxiella burnetii*, *Francisella tularensis*, and various *Rickettsia* species. Here, we aim to report the complete mitochondrial genome of *Haemaphysalis parva* (Ixodida: Ixodidae), a zoonotic tick species with significant public health relevance in Türkiye.

Methods

For this purpose, we isolated total genomic DNA from *H. parva* and sequenced using Illumina HiSeq 2000 platform, raw reads were processed, and then the mitogenome was assembled using the Geneious R9 program with "map to reference" and verified via "de novo assembly" options.

Results and Discussion

The mitogenome of *H. parva* is a circular DNA molecule of 14,843 bp, comprising the canonical 37 genes (13 PCGs, 22 tRNAs, and 2 rRNAs) and two major non-coding regions (312 bp and 304 bp). Strand-specific compositional bias revealed a strong A+T enrichment (77.8%) and pervasive negative AT- and GC-skew values, diverging from the typical skew profiles observed in most arthropods and possibly reflecting lineage-specific replication asymmetries. All PCGs exhibited AT-biased codon usage, preferentially encoding hydrophobic amino acids. Several genes (*cox1*, *cytB*, *nd2*, *nd6*) showed dN/dS ratios >1, suggesting positive adaptive evolution. Comparative mitogenomic analysis of 27 *Haemaphysalis* species confirmed overall structural conservation but identified a rearranged *nd1*–rrnS gene block relative to the *Ixodes* reference genome. Collinearity and synteny analyses revealed multiple conserved sequence blocks, including a putative humanin-like ORF within the rrnL gene region, indicating potential dual-coding or regulatory elements within non-PCG regions.

Keywords Mitochondrial genome · *Haemaphysalis* · Ticks · Comparative mitogenomics

Introduction

In terms of genome structure and gene content, animal mitogenomes are stable and predictable in eukaryotic groups [7, 37]. In bilateral animals, the mitogenome is generally fixed with 37 genes: 13 protein-coding genes (PCGs) that code subunits of the electron transport chain, two ribosomal RNA (rRNA) genes involved in the translation of these PCGs, and 22 transfer RNA (tRNA) genes [26]. PCGs constitute the subunits of four of the five mitochondrial complexes embedded in the mitochondrial inner membrane that are involved in oxidative phosphorylation. Of these genes,

✉ Habeş Bilal Aydemir
hbilalaydemir@hotmail.com

[1] Department of Biotechnology, Department of Molecular Biology and Genetics, Faculty of Science and Lecture, Tokat Gaziosmanpaşa University, Tokat, Turkey

[2] Department of Zoology, Department of Biology, Faculty of Science and Lecture, Tokat Gaziosmanpaşa University, Tokat, Turkey

NADH dehydrogenase subunit 1 (*nd1*), *nd2*, *nd3*, *nd4*, *nd4L*, *nd5* and *nd6* encode the proteins of complex 1 (NADH-ubiquinol oxidoreductase); *cytochrome b* (*cytb*) complex 3 (ubiquinone-cytochrome-c-oxidoreductase); *cytochrome-c-oxidase subunit 1* (*cox1*), *cox2* and *cox3* complex 4 (cytochrome-c-oxidase); and finally *ATP synthase Fo subunit 6* (*atp6*) and *atp8* complex 5 (ATP synthase). Two rRNA genes encode the large (16S) and small (12S) subunit genes participating in ribosome structure; 22 tRNA genes encode tRNA molecules with one copy for each amino acid, except for serine and leucine, which have two copies [12]. In addition to the coding gene content, the mitogenome contains one or more non-coding regions. These regions function as binding sites for proteins involved in genome replication or transcription. The major non-coding region is called the major control region or displacement loop (D-loop) [32].

Over the last 50 years, numerous total mitochondrial genome sequences have been obtained, and many studies have evaluated genome structure, gene content, organization, and expression from different perspectives. Comparative mitochondrial genome studies allow assessment of mitochondrial genome variations from both general (supra-species level) and specific (intra-species and gene level) perspectives [2]. The fact that the mitogenome has emerged only once in the evolutionary process makes mitogenome data a unique resource for evolution and mutation studies. Phylogenetic trees constructed with genes encoded from nDNA and mtDNA show strikingly similar constructions [31].

Therefore, studies about small, circular, and simple mtDNA are easier than studies with large, chromosome-packaged, and highly complex nDNA. Mitogenome-level rearrangement patterns are also being used as a new source for both phylogenetic and evolutionary biology studies [8]. Nuclear mtDNA remnants (NUMTs) are informative in mutational and evolutionary processes. Horizontal and lateral gene transfers, genetic code differences, asymmetric replication and transcription processes, and transcriptome studies are also used mtDNA as a template for many studies [12].

Ticks are worldwide arthropod parasites that feed on blood from terrestrial vertebrates and are vectors of various diseases. Ticks, particularly those parasitizing humans, play a significant role in the transmission of viral, rickettsial, bacterial, and protozoan diseases to humans [9, 19, 20]. Mitochondrial genomes have provided substantial insights into the evolutionary history, population genetics, and phylogeography of mites and particularly strong contributions to studies on ticks. Currently, more than 300 complete mitochondrial genomes for 125 tick species are available in the NCBI database, and mitogenomic studies on ticks are expanding rapidly as new species are sequenced each year [3, 11, 34, 36].

Haemaphysalis parva is a hard tick species distributed in the Palearctic region, with a particular prevalence in the Mediterranean Basin and Middle East. This species is especially active during the autumn months and primarily infests livestock. It also parasitizes a range of wild animals, including wild boars, rabbits, foxes, *Eurasian lynx*, as well as both domestic and wild horses [35]. *H. parva* can occasionally infest humans and is recognized as a vector of several zoonotic agents, including *Coxiella* sp., *Francisella tularensis* subsp. *holarctica*, *Babesia* spp., and *Hepatozoon* spp., with a particular association with *Rickettsia* species such as *R. massiliae* and '*Candidatus* Rickettsia goldwasserii' [41]. In this study, the total mitochondrial genome of *Haemaphysalis parva* (Ixodida: Ixodidae), a species that frequently infects humans in Türkiye, was sequenced and compared with other *Haemaphysalis* species, whose mitogenome data are available in the NCBI database.

Materials and Methods

Sampling of *Haemaphysalis parva* and DNA extraction

Haemaphysalis parva species were collected from infected humans in Tokat province (40° 19' North and 36° 43' East) and stored in Tokat Gaziosmanpaşa University, Department of Biology, Parasitology research laboratory in Tokat (Türkiye) between 2009 and 2011. Species were preserved in −20 °C in 100% ethanol and was identified at the species level using morphological characteristics via various species identification keys [13, 14] under a stereomicroscope (Leica MZ16 and Olympus SZ61). All specimens were preserved in 100% ethanol at −20 °C before molecular analyses. In addition to the specimens used in this study, tick specimens collected from humans in the same localities are preserved in the Tokat Gaziosmanpaşa University, Department of Biology, Parasitology Research Laboratory in Tokat (Türkiye), providing a reference material for future taxonomic verification. The universal *cox1* barcode region was amplified for verification of morphological characterization and identification molecularly via BOLD system after DNA extraction (as detailed in the following paragraph).

Total DNA was extracted from the leg set of selected organisms using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions with minor changes. The extracted DNA were measured qualitatively and quantitatively via agarose gel electrophoresis and MicroDrop (Invitrogen), respectively.

The universal *cox1* barcode region was amplified for verification of morphological characterization and identification molecularly via BOLD system after DNA extraction and PCR. For amplification of *cox1* barcode region *cox1*-F 5′-AATGTAATTGTAACTGCTCATGC-3′ and *cox1*-R 5′-CTATTCCTACTGTAAATATRTGATG-3′ primers were designed, synthesized, and used with 45 °C annealing temperature in Biorad T-100 thermal cycler. A 729 bp long amplicon were sequenced using Standart Sanger sequencing, conducted at Macrogen Inc. (Netherlands). Reads were initially proceeded with end-trimming and assembling. After the consensus *cox1* sequence was generated and searched in BOLD System v3 [27], the species has been confirmed to be *Haemaphysalis parva*.

Sequencing and Data Analysis

The total of 100 ng of the extracted DNA was sequenced as 150 bp paired end reads using Illumina HiSeq 2000 platform (Macrogen Inc. (Netherlands)) to generate ~66 million high quality reads, 10 GB raw data. Raw NGS reads were filtered qualitatively using FastQC v0.11.4 (http://www.bioinformatics.babraham. ac.uk/projects/fastqc). The filtered high-quality reads were assembled into contigs with "map to reference" option in Geneious R9 using other *Haemaphysalis* RefSeq data in NCBI (NC_085275, NC_037246, NC_039765, NC_037493) under the parameters of 'medium–low sensitivity' and 'iterate up to five times' [18]. After consensus sequences were generated, BLASTn search was conducted. The assemblies with a high score that matched *Haemaphysalis* mitogenomes were fully annotated to provide a final assembly.

Mitogenome Characterization of *Haemaphysalis parva*

For the standard characterization of mitogenomes, organizations of the PCGs were identified using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder) and MITOS (http://mitos2.bioinf.uni-leipzig.de/index. py) with the invertebrate m itochondrial genetic code prior [5]. Amino acid sequences of the putative ORFs were verified with BLASTp. Conserved domains of protein coding genes were determined via BLAST CD-search [24]. tRNA genes were annotated with tRNA-Scan SE and ARWEN servers under the mito/chloroplast genetic code and the default search options [22, 23]. The precise ends of rRNA genes were determined based on the locations of adjacent genes and their putative secondary structures were constructed according to the commonly accepted comparative approach and algorithm-based methods. XRNA 1.2.0b (http://rna.ucsc.edu/rnacenter/xrna/xrna.html) was used to draw the folding structure with the results of the CRW site as reference [10]. Intergenic spacers and overlapping regions were determined manually. The boundary of the A+T-rich region was determined by considering the adjacent genes. The number, position and length of the repeated sequence motifs were searched by Tandem Repeat Finder v4.07b using default setting under basic option [4]. Finally, the whole mitogenome sequence of *Haemaphysalis parva* was deposited in the GenBank under accession number PX122095.

Nucleotide and amino acid compositions of genes and mitogenomes were calculated and strand asymmetry values of each genomic compartment and each gene were counted using the following formulae: AT-skew = (A − T) / (A + T); GC skew = (G − C) / (G + C) [15].

Comparative Analysis of *Haemaphysalis* Mitogenomes

Complete mitogenome sequence of *Haemaphysalis parva* obtained from this study, and 26 different *Haemaphysalis* and mitogenome sequences from NCBI were used for comparative analyses (Table 1). *Ixodes columnae* (Arachnida: Ixodidae) were included in comparative analysis for determination of rearrangement events. For comparison of PCGs, relative synonymous codon usage (RSCU) value of genes was calculated with MEGA 11 software [33]. The ratio of the nonsynonymous substitution rate (dN) to the synonymous substitution rate (dS) was calculated with DNAsp v6 [28]. To assess the selective pressures acting on the analyzed protein-coding genes, the ratio of nonsynonymous to synonymous substitution rates (dN/dS, ω) was calculated. The dN/dS ratio provides a widely used measure for distinguishing between purifying, neutral, and positive selection acting at the protein level by comparing the rates of amino acid–altering and silent substitutions [38].

Gene rearrangement events in *Haemaphysalis* were heuristically evaluated via CREx and qMGR with an Ixodidae reference, *Ixodes columnae* (NC_067861) [6, 40].

Collinearity analysis were conducted to compare *Haemaphysalis* species with riparian plot using pyGenomeViz v1.6.1 (https://pygenomeviz.streamlit.app/).

Results and Discussion

Mitogenome of *Haemaphysalis parva*

The *Haemaphysalis parva* complete mitogenome was annotated as 14,843 bp-long and can be accessed from NCBI under accession number PX122095. The mitogenome was visualized and given in Fig. 1, and the mitogenome feature table was given in Table 2. In the mitogenome, overall

Table 1 Mitogenome sequences and nucleotide characters which used in comparative analysis

Species	AT%	AT-skew	Accession number	Genome lenght	T	C	A	G	GC%	GC-skew	Purine_ Pyrimi-dine Bias	Amino_ Keto Bias	AT_ GC Bias	NB
Haemaphysalis parva	77,8	−0,010879972	**PX122095**	14,843	39,3	12,8	38,5	9,5	22,2	−0,147031963	−4,1	2,4	55,5	2,09283
Haemaphysalis flava	76,9	−0,018149624	NC_005292	14,686	39,2	12,9	37,8	10,2	23,1	−0,115,895,016	−4,1	1,3	53,8	1,984,753
Haemaphysalis formosensis	78,3	−0,013577023	NC_020334	14,676	39,7	12,4	38,6	9,3	21,7	−0,140,615,191	−4,1	2,0	56,6	2,19,572
Haemaphysalis inermis	78,8	−0,007690993	NC_020335	14,846	39,7	12,1	39,1	9,0	21,2	−0,146,310,433	−3,7	2,5	57,6	2,251,718
Haemaphysalis concinna	78,0	−0,009875033	NC_034785	14,675	39,4	12,8	38,6	9,2	22,0	−0,16,382,781	−4,4	2,8	56,0	2,154,286
Haemaphysalis japonica	77,6	−0,016062495	NC_037246	14,685	39,4	12,6	38,2	9,8	22,4	−0,125	−4,0	1,5	55,3	2,09461
Haemaphysalis longicornis	77,2	−0,010567101	NC_037493	14,718	39,0	13,1	38,2	9,8	22,8	−0,143,367,043	−4,1	2,5	54,3	2,019866
Haemaphysalis hystricis	77,2	−0,006335797	NC_039765	14,716	38,9	12,9	38,4	9,8	22,8	−0,136,038,186	−3,6	2,6	54,4	2,027621
Haemaphysalis bancrofti	78,4	−0,010698443	NC_041076	14,673	39,6	12,2	38,8	9,4	21,6	−0,130,352,645	−3,7	2,0	56,7	2,203,575
Haemaphysalis montgomeryi	78,5	0,004861955	NC_058312	14,681	39,0	12,6	39,4	9,0	21,5	−0,169,143,218	−3,3	4,0	56,9	2,224,394
Haemaphysalis sulcata	76,4	−0,011145787	NC_062063	14,679	38,6	13,7	37,8	9,9	23,6	−0,160,508,083	−4,6	2,9	52,8	1,919,978
Haemaphysalis punctata	78,0	−0,005320541	NC_062064	14,697	39,2	12,7	38,8	9,2	22,0	−0,158,872,716	−3,9	3,1	56,1	2,154,409
Haemaphysalis danieli	81,1	−0,014060931	NC_062065	14,739	41,1	10,7	40,0	8,2	18,9	−0,131,494,088	−3,6	1,4	62,1	2,628,976
Haemaphysalis tibetensis	77,7	−0,011801731	NC_062066	14,715	39,3	12,7	38,4	9,6	22,3	−0,13,980,464	−4,0	2,2	55,5	2,105,623
Haemaphysalis qinghaiensis	77,5	−0,015725204	NC_062067	14,683	39,4	12,7	38,2	9,8	22,5	−0,129,090,909	−4,1	1,7	55,1	2,077448
Haemaphysalis doenitzi	77,4	−0,009595915	NC_062158	14,671	39,1	13,0	38,3	9,6	22,6	−0,153,381,643	−4,2	2,7	54,8	2,067735
Haemaphysalis campanulata	78,2	−0,003483714	NC_062159	14,691	39,2	12,3	38,9	9,5	21,8	−0,127,454,036	−3,1	2,5	56,3	2,169,252
Haemaphysalis yeni	78,0	−0,012820513	NC_062160	14,690	39,5	12,6	38,5	9,4	22,0	−0,145,454,545	−4,2	2,2	56,0	2,150,088
Haemaphysalis kitaokai	77,3	0,000865951	NC_062161	14,936	38,6	13,1	38,7	9,5	22,7	−0,158,205,431	−3,5	3,7	54,6	2,015628
Haemaphysalis cornigera	78,1	−0,013172817	NC_062162	14,681	39,6	12,3	38,5	9,6	21,9	−0,124,922,312	−3,8	1,7	56,2	2,160,052
Haemaphysalis mageshimaensis	78,1	−0,012356422	NC_062163	14,721	39,5	12,5	38,6	9,4	21,9	−0,139,671,725	−4,0	2,1	56,1	2,154,261
Haemaphysalis colasbelcouri	78,0	−0,008532276	NC_062164	14,885	39,3	12,7	38,6	9,3	22,0	−0,156,002,438	−4,1	2,8	55,9	2,115,937
Haemaphysalis nepalensis	77,8	−0,010572302	NC_064124	14,720	39,3	12,7	38,5	9,5	22,2	−0,145,038,168	−4,0	2,4	55,5	2,107,832
Haemaphysalis bispinosa	78,7	−0,013965517	NC_071765	14,732	39,9	12,0	38,8	9,2	21,3	−0,130,906,769	−3,9	1,7	57,5	2,254,885
Haemaphysalis warburtoni	77,8	−0,015650957	NC_084204	14,695	39,5	12,6	38,3	9,6	22,2	−0,136,279,926	−4,2	1,8	55,7	2,122,546
Haemaphysalis taiwana	78,2	−0,012805994	NC_085275	14,685	39,6	12,3	38,6	9,5	21,8	−0,128,509,046	−3,8	1,8	56,3	2,173,331
Haemaphysalis novaeguineae	78,0	−0,017369294	NC_087880	14,681	39,7	12,3	38,3	9,6	22,0	−0,122,828,784	−4,1	1,3	56,1	2,154,562

The bold value indicates the GenBank accession number of *Haemaphysalis parva* mitogenome data generated in this study and deposited in NCBI

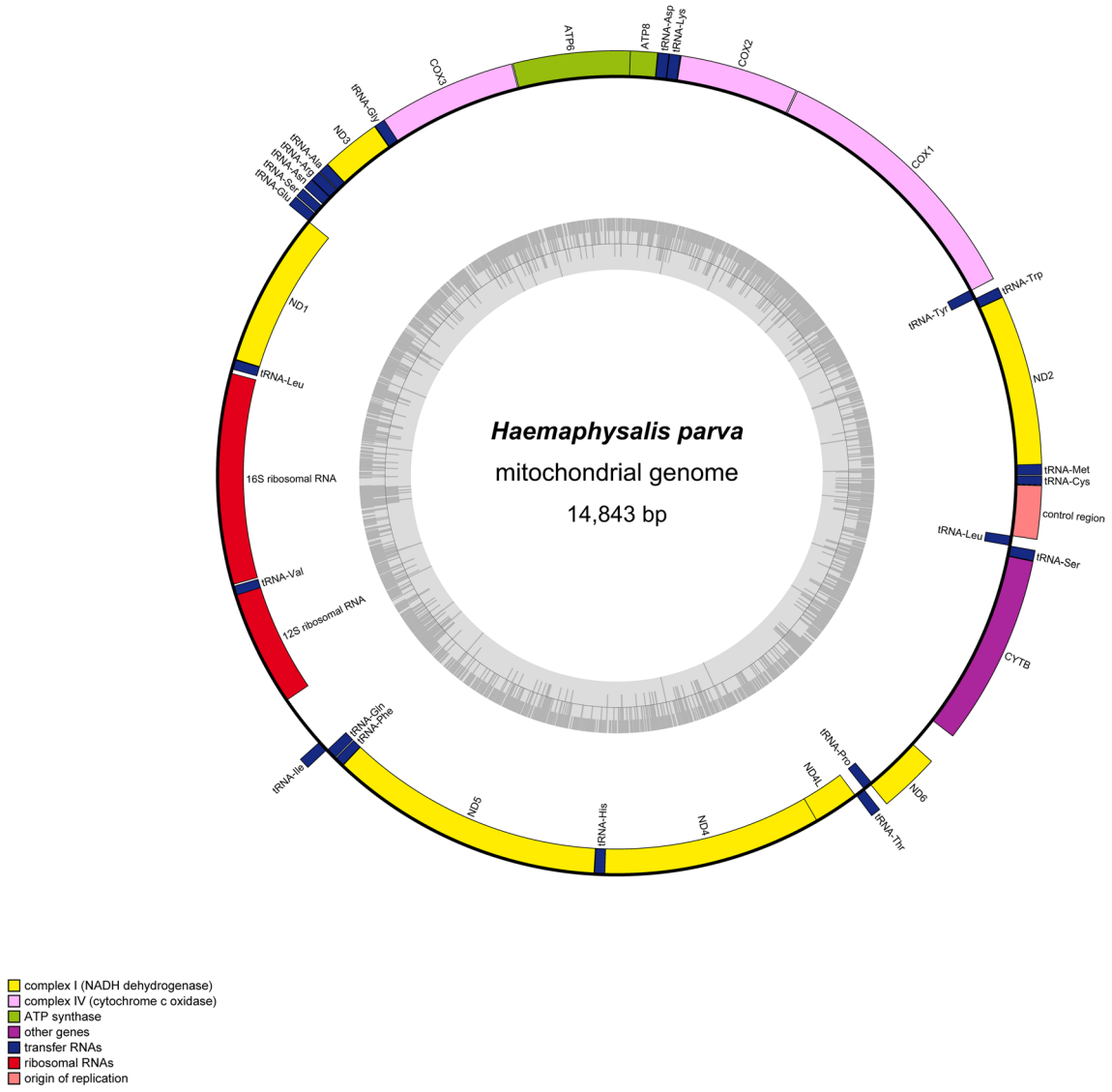

Fig. 1 Mitochondrial genome visualization of *Haemaphysalis parva* (Ixodida: Ixodidae)

lengths of PCGs, tRNAs, and rRNAs are 10,749, 1354 and 1969 bp, respectively. Two major non-coding regions were determined between rrnS-trnI and trnL1-trnC genes with 312 and 304 bp long, respectively. The intergenic regions were 238 bp in total length and determined between 12 adjacent genes, except for major non-coding regions. On the other hand, the overlapping regions were dispersed among different gene junctions 101 bp in total length (Table 2). 15 neighbouring genes overlapped with from 1 to 23 nucleotides (Table 2). Conserved *atp6-atp8* and *nd4-nd4L* overlaps were determined as 7 bp conserved length. The conserved and newly determined transcriptional sequence "WHWGHTW" identified in Ecdysozoa was detected in *H. parva* mitogenome immediately upstream (positions 12,647–12,653) of the *nd4L* gene [1].

Haemaphysalis H parva mitogenome consists of T%=39,3, A%=38,5, C%=12,8 and G%=9,5 of nucleotides; indicating the negative AT- and negative GC-skewness values. Like other invertebrates, the mitogenome of *H. parva* was also biased to A+T nucleotides (%77,8). All PCGs, except *atp8*, show negative AT-skew, and; except L-strand encoded *nd1*, *nd4*, *nd4L, nd5,* and H-strand encoded *nd3* genes, show negative GC-skew.

Both rrnL and rrnS genes have positive AT- and positive GC-skewness values. Most of the tRNA genes show positive AT- and positive GC-skew values, except trnK, trnY, trnE, trnR, trnI, trnQ, trnH and trnS2 which have negative AT-skewness value, and except for trnW, trnG, trnA, trnS1, trnV and trnC which shown negative GC-skewness.

Each protein-coding gene was biased towards having a high AT content, with hydrophobic amino acids, including

Table 2 Summary of mitochondrial genome of *Haemaphysalis parva*

Name	Minimum	Maximum	Length	Direction	intergenic/overlapping	start codon	stop codon	anticodon	T%	C%	A%	G%	AT-skew	GC-skew
trnM(cat)	2	64	63	forward	−12			CAT	29,7	14,1	42,2	14,1	0,173,913	0
nad2	53	1024	972	forward	−2	ATT	TAA		45,1	9,6	40,2	5,1	−0,05797	−0,3007
trnW(tca)	1023	1083	61	forward	−2			TCA	37,7	8,2	49,2	4,9	0,132,075	−0,25
trnY(gta)	1082	1143	62	reverse	−8			GTA	40,3	8,1	33,9	17,7	−0,08696	0,375
cox1	1136	2674	1539	forward	4	ATT	TAA		38,6	15,5	32,5	13,4	−0,08592	−0,07416
cox2	2679	3353	675	forward	0	ATG	TAG		37,5	15,6	37,3	9,6	−0,00198	−0,23,529
trnK(ctt)	3354	3418	65	forward	−1			CTT	36,9	13,8	35,4	13,8	−0,02128	0
trnD(gtc)	3418	3481	64	forward	0			GTC	35,9	7,8	48,4	7,8	0,148,148	0
atp8	3482	3637	156	forward	−7	ATC	TAA		42,3	12,2	42,9	2,6	0,007519	−0,65,217
atp6	3631	4296	666	forward	3	ATG	TAA		48,0	12,2	32,3	7,5	−0,19,626	−0,23,664
cox3	4300	5086	786	forward	−9	ATG	T—		44,7	13,1	30,2	11,9	−0,19,322	−0,04569
trnG(tcc)	5078	5141	64	forward	0			TCC	37,5	7,8	48,4	6,3	0,127,273	−0,11,111
nad3	5142	5480	339	forward	−2	ATT	TAG		50,1	9,1	31,3	9,4	−0,23,188	0,015873
trnA(tgc)	5479	5540	62	forward	1			TGC	38,7	9,7	43,5	8,1	0,058824	−0,09091
trnR(tcg)	5542	5601	60	forward	−1			TCG	40,0	11,7	35,0	13,3	−0,06667	0,066667
trnN(gtt)	5601	5661	61	forward	9			GTT	32,8	13,1	37,7	16,4	0,069767	0,111,111
trnS1(tct)	5671	5726	56	forward	4			TCT	37,5	10,7	44,6	7,1	0,086957	−0,2
trnE(ttc)	5731	5791	61	forward	0			TTC	44,3	9,8	34,4	11,5	−0,125	0,076923
nad1	5792	6724	918	reverse	0	ATT	T—		43,7	9,5	34,8	12,0	−0,11,354	0,114,428
trnL2(taa)	6725	6785	61	reverse	22			TAA	34,4	9,8	39,3	16,4	0,066667	0,25
rrnL	6808	8075	1268	reverse	8				35,9	10,1	40,0	14,0	0,055255	0,16,041
trnV(tac)	8084	8142	59	reverse	−9			TAC	39,0	11,9	39,0	10,2	0	−0,07692
rrnS	8134	8834	701	reverse	312				38,1	8,9	39,6	13,4	0,018382	0,205,128
trnI(gat)	9147	9211	65	forward	1			GAT	41,5	7,7	36,9	13,8	−0,05882	0,285,714
trnQ(ttg)	9213	9279	67	reverse	3			TTG	43,3	4,5	40,3	11,9	−0,03571	0,454,545
trnF(gaa)	9283	9343	61	reverse	−1			GAA	39,3	4,9	47,5	8,2	0,09434	0,25
nad5	9343	10,998	1656	reverse	0	ATT	TAA		45,8	7,6	36,2	10,4	−0,11,717	0,157,191
trnH(gtg)	10,999	11,063	65	reverse	−23			GTG	44,6	1,5	43,1	10,8	−0,01754	0,75
nad4	11,041	12,378	1338	reverse	−7	ATG	TAA		48,8	8,5	30,0	12,7	−0,23,909	0,197,183
nad4l	12,372	12,647	276	reverse	2	ATG	TAA		49,3	6,9	34,4	9,4	−0,17,749	0,155,556
trnT(tgt)	12,650	12,709	60	forward	0			TGT	40,0	8,3	41,7	10,0	0,020408	0,090909
trnP(tgg)	12,710	12,770	61	reverse	−15			TGG	39,3	4,9	41,0	14,8	0,020408	0,5
nad6	12,756	13,103	348	forward	180	ATA	TAA		45,6	12,3	31,9	10,2	−0,17,736	−0,09091
cytB	13,284	14,363	1080	forward	−1	ATG	TAG		44,1	13,8	31,8	10,4	−0,16,239	−0,14,176
trnS2(tga)	14,363	14,425	63	forward	−1			TGA	42,9	7,9	39,7	9,5	−0,03846	0,090909
trnL1(tag)	14,425	14,484	60	reverse	304			TAG	36,7	8,3	40,0	15,0	0,043478	0,285,714
trnC(gca)	14,789	14,841	53	forward	1			GCA	35,8	7,5	50,9	5,7	0,173,913	−0,14,286

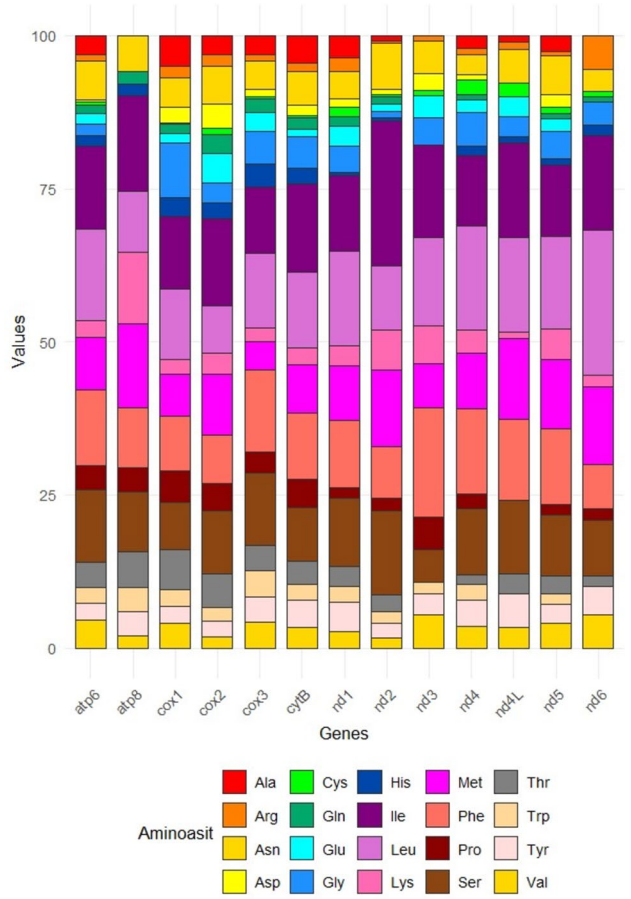

Fig. 2 Aminoacid compositions of *Haemaphysalis parva* mitochondrial PCGs

isoleucine (13.69%), leucine (13.64%), phenylalanine (11.23%), and serine (10.22%). On the other hand, the existences of cysteine (0.92%), arginine (1.34%), and glutamine (1.42%) amino acid levels were the lowest (Table S1, Fig. 2).

Initiation codons of *cox2*, *cox3*, *cytB*, *atp6*, *nd4,* and *nd4L* genes were standard ATG- methionine codon; *cox1, nd1*, nd2, *nd3* and *nd5* genes were started with ATT- isoleucine codon; *nd6* and *atp8* genes were found to initiate with ATA-methionine and ATC- isoleucine codons, respectively.

While *atp6*, *atp8*, *cox1*, *nd2*, *nd4*, *nd4L*, *nd5,* and *nd6* genes have complete TAA stop codons; *cox2*, *cytB,* and *nd3* genes terminate with TAG codon; and *cox3* and *nd1* genes have incomplete T—stop codons supposed to be completed with polyadenylation.

Comparative Mitogenomics of *Haemaphysalis* Species

Total length of *Haemaphysalis* mitogenomes varies from 14,671 (*H. doenitzi*) to 14,936 (*H. kitaokai*); with an average of 14,722 bp. There is no significant length variation within *Haemaphysalis* species ($p > 0.001$). Total nucleotide compositions of the *Haemaphysalis* mitogenomes are A and T-rich, with an 78% A + T content on average (Table S2). The arthropod mitogenomes commonly display positive AT and negative GC skew values, because the asymmetric nature of replication and transcription processes induces deamination mutations [29]. However, all analysed *Haemaphysalis* species show negative AT and GC-skewness values (Table S2). The existence of multiple replication origins may be interpreted as a skewness bias. Although rearrangement events are not detected between *Haemaphysalis* mitogenomes, *nd1*-rrnS gene block were rearranged in *Haemaphysalis* species when comparing the reference *Ixodes* mitogenome (Fig. 3).

Comparisons of the RSCU values of degenerate codons revealed a significant correlation between codon preference and nucleotide composition. The most frequently used codons were counted as AUU (I), UUU (F), UUA (L2), AUA (M), AAU (N) and AAA (K) codons, which all of them have only A and U nucleotides. As expected for determinants in nucleotide compositions, both two-fold and four-fold degenerated codons are biased specifically to A and U nucleotides (Fig. 4).

The ratios of nonsynonymous to synonymous substitution rates (ω: dN/dS) between *Haemaphysalis* species in

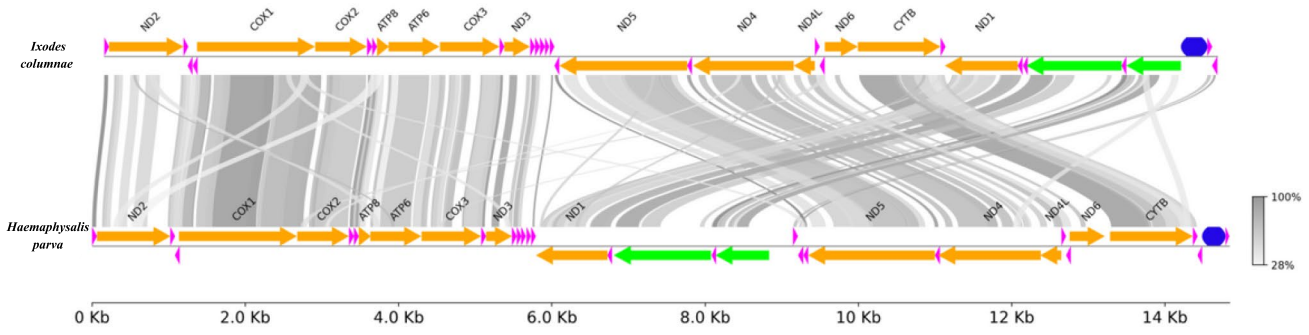

Fig. 3 Riparian plot representation of mitogenome sequences of *Haemaphysalis parva* and *Ixodes columnae* using BlastProtein genome comparison method

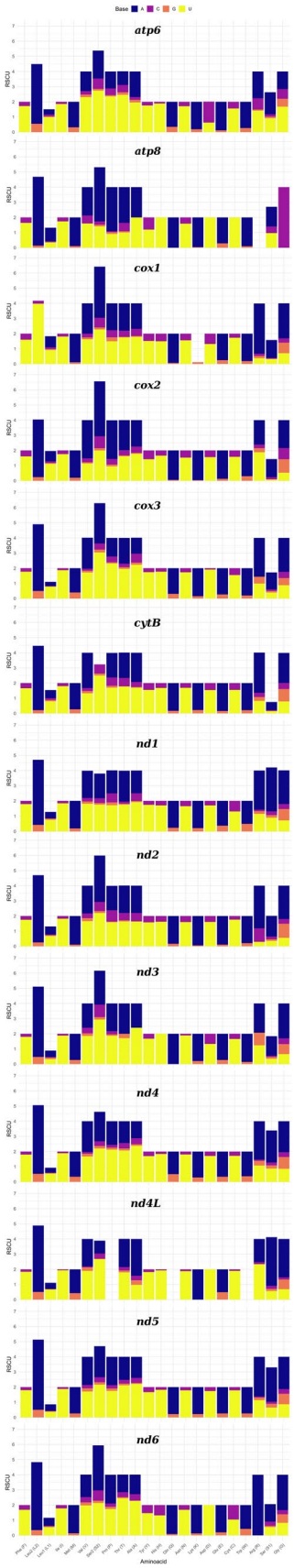

Fig. 4 Relative synonymous codon usage (RSCU) of the *Haemaphysalis* mitogenomes. Amino acids are provided on the x axis with alternative 3rd codon positions with different colors

the mitochondrial PCGs are just about 1. Although dN/dS is generally less than 1, for some genes (*cox1*, *cytB*, *nd2* and *nd6*) the average dN/dS ratio is higher than 1 ($p < 2.2e\text{-}16$) (Fig. 5). ω values support that mitogenomes of *Haemaphysalis* species are still evolved and not saturated yet. The overgeneralized guess about mitogenome evolutions have some handicaps.

Collinearity analysis was conducted by comparing the mitogenome of *H. parva* (represented identical organized *Haemaphysalis* species) with reference *Ixodes columnae* mitogenomes. Three locally collinear blocks (LCBs) and numerous collinear blocks were identified, as illustrated in Fig. 3. In this study, using the MUMmer protein comparison method, the coding capacity of rrnL gene (probably the *humanin*-like gene) was detected as conserved sequence block (Fig. 3). Some sequence blocks represented in non-PCGs are conserved and may indicate conserved functional regions like replication- transcription origins or nuclear peptides like humanin and/or MOTS-c.

Conclusion

The complete mitochondrial genome of *Haemaphysalis parva* shows typical features of tick mitogenomes in terms of size, gene content, and high A+T bias. However, the consistent negative AT- and GC-skew values across all analyzed *Haemaphysalis* species deviate from the common arthropod pattern, suggesting alternative replication or transcription dynamics, possibly due to multiple replication origins [16, 30].

Codon usage was strongly influenced by nucleotide composition, favoring A+T-rich codons and hydrophobic amino acids, consistent with selective pressures on mitochondrial translation efficiency [25]. Overlapping gene regions and highly biased codon usage patterns may reflect genome compaction and regulatory complexity in mitochondrial gene expression.

Notably, some protein-coding genes (e.g., *cox1*, *cytB*, *nd2*, *nd6*) exhibited dN/dS ratios above 1, indicating potential positive selection and ongoing adaptive evolution in certain lineages. Additionally, the identification of conserved non-coding motifs and a putative humanin-like sequence within the rrnL gene points to functional elements beyond canonical OXPHOS-related genes [17, 21, 39].

Despite overall structural conservation, collinearity analysis revealed rearrangements (e.g., *nd1*–rrnS block) compared to *Ixodes*, supporting the utility of mitochondrial architecture in phylogenetic studies. These findings

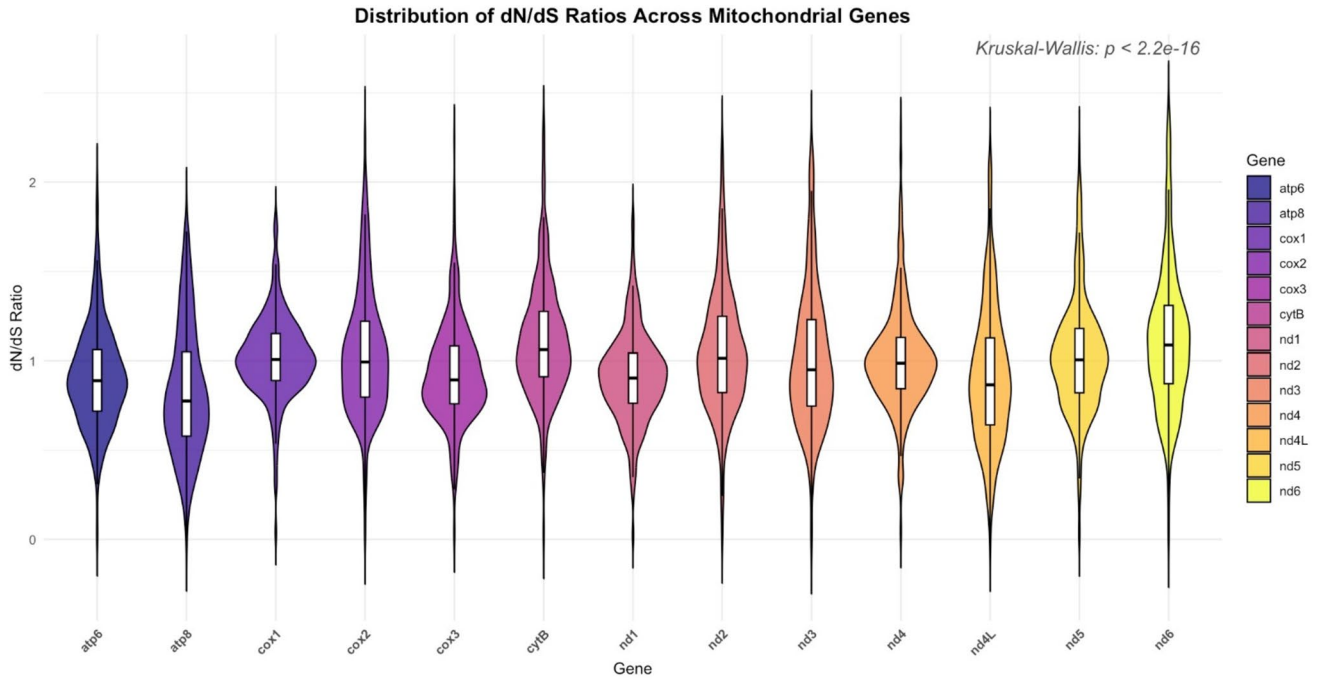

Fig. 5 Schematic representations of dN/dS (ω) values of mitochondrial PCGs of *Haemaphysalis*. For each gene, the bottom and top of the line indicates the minimum and maximum values, respectively

contribute valuable insight into the evolution, structure, and functional potential of tick mitogenomes and highlight the importance of further comparative and functional analyses across broader taxa.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11686-026-01243-y.

Acknowledgements This study was financially supported by Tokat Gaziosmanpaşa University BAP with grant number 2021-71. We thank Dr. Merve Nur AYDEMİR for her valuable contribution of evaluating results.

Author Contribution H.B.A. conceived and designed the study, performed data analysis, and drafted the manuscript. A.K. contributed to data interpretation, manuscript editing, and critical review. Both authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This study was financially supported by Tokat Gaziosmanpaşa University BAP with grant number 2021–71.

Data Availability H. parva mitogenome data were deposited into the NCBI database under accession number PX122095 and are available at the following URL: https://www.ncbi.nlm.nih.gov/.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Aydemir MN, Aydemir HB, Budak M, Kızıltepe B, Çelebi MŞ, Korkmaz EM, Başıbüyük HH (2023) A novel, conserved and possibly functional motif "WHWGHTW" in mitochondrial transcription across Bilateria. Mitochondrion 68:72–80. https://doi.org/10.1016/j.mito.2022.11.004
2. Aydemir MN, Korkmaz EM (2020) Comparative mitogenomics of Hymenoptera reveals evolutionary differences in structure and composition. Int J Biol Macromol 144:460–472. https://doi.org/10.1016/j.ijbiomac.2019.12.135
3. Barker SC, Kelava S (2022) What have we learned from the first 600 mitochondrial genomes of Acari? Zoosymposia. https://doi.org/10.11646/zoosymposia.22.1.8
4. Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580. https://doi.org/10.1093/nar/27.2.573
5. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF (2013) Mitos: improved de novo metazoan mitochondrial genome annotation. Mol

Phylogenet Evol 69:313–319. https://doi.org/10.1016/j.ympev.2012.08.023
6. Bernt M, Merkle D, Ramsch K, Fritzsch G, Perseke M, Bernhard D, Schlegel M, Stadler PF, Middendorf M (2007) Crex: inferring genomic rearrangements based on common intervals. Bioinformatics 23:2957–2958. https://doi.org/10.1093/bioinformatics/btm468
7. Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Res 27(8):1767–1780. https://doi.org/10.1093/nar/27.8.1767
8. Boore JL, Brown WM (1998) Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. Curr Opin Genet Dev 8(6):668–674. https://doi.org/10.1016/S0959-437X(98)80035-X
9. Bursali A, Tekin S, Keskin A, Ekici M, Dundar E (2011) Species diversity of Ixodid ticks feeding on humans in Amasya, Turkey: seasonal abundance and presence of Crimean-Congo hemorrhagic fever virus. J Med Entomol 48:85–93. https://doi.org/10.1603/me10034
10. Cannone JJ, Subramanian S, Schnare MN, Collett JR, Souza LMD, Du Y, Feng B, Lin N, Madabusi LV, Müller KM, Pande N, Shang Z, Yu N, Gutell RR, Souza LMD', Du Y, Feng B, Lin N, Madabusi LV, Müller KM, Pande N, Shang Z, Yu N, Gutell RR (2002) The Comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinformatics. https://doi.org/10.1186/1471-2105-3-2
11. Che J, Chai Y, Li W, Zhao S, Luo J, Ren Q, Niu Q, Guan G, Wang J, Yin H (2025) The complete mitogenome of *Hyalomma anatolicum*: novel insights into the phylogenetics of *Hyalomma*. Parasitol Res 124:67. https://doi.org/10.1007/s00436-025-08497-x
12. Desalle R (2017) MtDNA: the small workhorse of evolutionary studies. Front Biosci 22(5):873–887. https://doi.org/10.2741/4522
13. Estrada-Peña A, Mihalca AD, Petney TN (2017) Ticks of Europe and North Africa: a Guide to Species Identification. Springer, Cham, Switzerland
14. Filippova N (1997) Fauna of Russia and neighbouring countries. Ixodid ticks of subfamily Amblyomminae. Nauka Publishing House, St. Petersburg
15. Frank AC, Lobry JR (1999) Asymmetric substitution patterns: a review of possible underlying mutational or selective mechanisms. Gene 238:65–77. https://doi.org/10.1016/S0378-1119(99)00297-8
16. Gissi C, Iannelli F, Pesole G (2008) Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity (Edinb) 101:301–320. https://doi.org/10.1038/hdy.2008.62
17. Ju YS, Tubio JMC, Mifsud W, Fu B, Davies HR, Ramakrishna M, Li Y, Yates L, Gundem G, Tarpey PS, Behjati S, Papaemmanuil E, Martin S, Fullam A, Gerstung M, Nangalia J, Green AR, Caldas C, Borg Å, Tutt A, Lee MTM, Van'T Veer LJ, Tan BKT, Aparicio S, Span PN, Martens JWM, Knappskog S, Vincent-Salomon A, Børresen-Dale AL, Eyfjörd JE, Flanagan AM, Foster C, Neal DE, Cooper C, Eeles R, Lakhani SR, Desmedt C, Thomas G, Richardson AL, Purdie CA, Thompson AM, McDermott U, Yang F, Nik-Zainal S, Campbell PJ, Stratton MR (2015) Frequent somatic transfer of mitochondrial DNA into the nuclear genome of human cancer cells. Genome Res 25(6):814–824. https://doi.org/10.1101/gr.190470.115
18. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647–1649. https://doi.org/10.1093/bioinformatics/bts199
19. Keskin A, Bursali A, Keskin A, Tekin S (2016) Molecular detection of spotted fever group rickettsiae in ticks removed from humans in Turkey. Ticks Tick Borne Dis 7(5):951–953. https://doi.org/10.1016/j.ttbdis.2016.04.015
20. Keskin A, Keskin A, Bursali A, Tekin S (2015) Ticks (Acari: Ixodida) parasitizing humans in Corum and Yozgat provinces, Turkey. Exp Appl Acarol 67(4):607–616. https://doi.org/10.1007/s10493-015-9966-4
21. Kim SK, Tran LT, NamKoong C, Choi HJ, Chun HJ, Lee Y, Cheon MH, Chung CH, Hwang J, Lim HH, Shin DM, Choi YH, Kim KW (2023) Mitochondria-derived peptide SHLP2 regulates energy homeostasis through the activation of hypothalamic neurons. Nat Commun 14:4321. https://doi.org/10.1038/s41467-023-40082-7
22. Laslett D, Canbäck B (2008) ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics 24:172–175. https://doi.org/10.1093/bioinformatics/btm573
23. Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25(5):955–964. https://doi.org/10.1093/nar/25.5.955
41. Mumcuoglu K, Keskin A, Mans BJ, Dantas-Torres F (2025) Ticks of the Middle East Taxonomy, Biology, Ecology, Medical, and Veterinary Significance. Academic Press, Elsevier. https://doi.org/10.1016/C2024-0-01611-6
24. Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY, Bryant SH (2017) CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. Nucleic Acids Res 45:D200–D203. https://doi.org/10.1093/nar/gkw1129
25. Nakashima H, Nishikawa K, Ooi T (1990) Distinct character in hydrophobicity of amino acid compositions of mitochondrial proteins. Proteins Struct Funct Bioinform 8(2):173–178. https://doi.org/10.1002/prot.340080207
26. Osigus HJ, Eitel M, Bernt M, Donath A, Schierwater B (2013) Mitogenomics at the base of Metazoa. Mol Phylogenet Evol 69:339–351. https://doi.org/10.1016/j.ympev.2013.07.016
27. Ratnasingham S, Hebert PDN (2007) BOLD: the barcode of life data system: barcoding. Mol Ecol Notes 7:355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x
28. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 34:3299–3302. https://doi.org/10.1093/molbev/msx248
29. Saccone C, De Giorgi C, Gissi C, Pesole G, Reyes A (1999) Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. Gene 238:195–209. https://doi.org/10.1016/s0378-1119(99)00270-x
30. Schultheis aS, Weigt La, Hendricks aC (2002) Arrangement and structural conservation of the mitochondrial control region of two species of Plecoptera: utility of tandem repeat-containing regions in studies of population genetics and evolutionary history. Insect Mol Biol 11:605–610. https://doi.org/10.1046/j.1365-2583.2002.00371.x
31. Springer MS, DeBry RW, Douady C, Amrine HM, Madsen O, de Jong WW, Stanhope MJ (2001) Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. Mol Biol Evol 18:132–143. https://doi.org/10.1093/oxfordjournals.molbev.a003787
32. Taanman J-WW (1999) The mitochondrial genome: structure, transcription, translation and replication. Biochim Biophys

Acta-Bioenerg 1410:103–123. https://doi.org/10.1016/s0005-2728(98)00161-3
33. Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120
34. Uribe JE, Kelava S, Nava S, Cotes-Perdomo AP, Castro LR, Rivera-Paéz FA, Perea S, Mans BJ, Gofton A, Teo EJM, Zardoya R, Barker SC (2024) New insights into the molecular phylogeny, biogeographical history, and diversification of *Amblyomma* ticks (Acari: Ixodidae) based on mitogenomes and nuclear sequences. Parasit Vectors 17:139. https://doi.org/10.1186/s13071-024-06131-w
35. Vatansever Z (2017) *Haemaphysalis parva* (Neumann 1897) (Figs 100–102). In: Ticks of Europe and North Africa. Springer International Publishing, Editors: Agustín Estrada-Peña, Andrei Daniel Mihalca, Trevor N. Petney, Cham, pp. 259–263
36. Wang T, Zhang S, Pei T, Yu Z, Liu J (2019) Tick mitochondrial genomes: structural characteristics and phylogenetic implications. Parasit Vectors 12:451. https://doi.org/10.1186/s13071-019-3705-3
37. Wolstenholme DR (1992) Animal mitochondrial DNA: structure and evolution. Int Rev Cytol 141:173–216. https://doi.org/10.1016/s0074-7696(08)62066-5
38. Yang Z, Bielawski JR (2000) Statistical methods for detecting molecular adaptation. Trends ecol evol 15(12):496–503. https://doi.org/10.1016/s0169-5347(00)01994-7
39. Zárate SC, Traetta ME, Codagnone MG, Seilicovich A, Reinés AG (2019) Humanin, a mitochondrial-derived peptide released by astrocytes, prevents synapse loss in hippocampal neurons. Front Aging Neurosci 11:123. https://doi.org/10.3389/fnagi.2019.00123
40. Zhang J, Kan X, Miao G, Hu S, Sun Q, Tian W (2020) qMGR: A new approach for quantifying mitochondrial genome rearrangement. Mitochondrion 52:20–23. https://doi.org/10.1016/j.mito.2020.02.004

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.