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RESEARCH ARTICLE

Decoding the complete mitochondrial genome of *Hyalomma lusitanicum* (Parasitiformes: Ixodidae)

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ASBTRACT: The complete mitochondrial genome of *Hyalomma lusitanicum* Koch was assembled, annotated, and comprehensively characterized to investigate its genomic organization and evolutionary features. SRA reads of *Hy. lusitanicum* were retrieved from the NCBI database and assembled using the "map to reference" approach with the *Hy. rufipes* mitochondrial genome. Genome completeness was confirmed through comparative BLAST analyses with available Acari mitochondrial genomes. The circular mitochondrial genome of *Hy. lusitanicum* is 14.742 bp in length and comprises 13 protein-coding genes (PCGs), 22 transfer RNA (tRNAs), two ribosomal RNA genes (rRNAs), and two non-coding control regions. The mitogenome exhibits a pronounced AT bias and negative AT and GC skewness values. Two distinct control regions were identified. Mitogenome organization of *Hy. lusitanicum* was identical to *Hyalomma* mitotype M55. Protein-coding genes displayed strong nucleotide compositional biases, variation in start and stop codon usage, and a predominance of hydrophobic amino acids. The lengths and nucleotide compositions of tRNA genes were variable, and secondary structure predictions identified several truncated tRNAs lacking D- and/or T-arms. The presence of truncated mitochondrial tRNAs presence in *Hy. lusitanicum* represents an unexpected structural feature within Parasitiformes. The rRNA genes showed nucleotide compositions consistent with overall mitogenomic bias. Additionally, conserved and lineage-specific intergenic spacers, gene overlaps, transcriptional motifs, and tandem repeat sequences were detected throughout the mitogenome. Collectively, this study specifies a mitochondrial genome resource for *Hy. lusitanicum* and offers new insights into mitochondrial genome evolution and tRNA structural diversity in ticks.

Keywords: BLAST analysis, characterization, genomic organisation, molecular evolution, tick

Zoobank: https://zoobank.org/B44CF322-380D-4042-922C-CCC6EC2914BC

INTRODUCTION

Mitochondria produce cellular energy in the form of ATP primarily through oxidative phosphorylation (Newmeyer et al., 2003; McBride et al., 2006). Beyond this fundamental role, mitochondria play a central role in numerous vital cellular functions, including ion homeostasis, apoptosis, and the metabolism of lipids, nucleotides, carbohydrates, and amino acids (Adam-Vizi and Chinopoulos, 2006; Shah et al., 2012). To carry out these diverse functions, mitochondria contain their own genome (mtDNA), which evolves rapidly and functions in close coordination with nuclear DNA (nDNA) in the processes of replication, transcription, and translation (Burger et al., 2003).

In terms of structure and gene composition, animal mitochondrial genomes are remarkably conserved and predictable across eukaryotes (Osigus et al., 2013; Cameron, 2014). In bilaterian animals, the mitochondrial genome typically comprises 37 genes: 13 protein-coding genes (PCGs) that encode subunits of the electron transport chain, two ribosomal RNA (rRNA) genes essential for translation, and 22 transfer RNA (tRNA) genes (Desalle, 2017). In addition to coding regions, mitochondrial genomes also include non-coding regions that serve as critical binding sites for proteins involved in replication and transcription. The most prominent of these is the control region, also known as the displacement loop (D-loop) (Taanman, 1999). In addition, the "tick-box" genomic region, which has been particularly described in mites, is potentially functional in mitochondrial transcription (this region is also known as DmTTF enzyme binding site in *Drosophila*) and found between *nd1* and rrnL genes with 17 bp long (Roberti et al., 2003; Montagna et al., 2012). As well as the 7-bp long intergenic region between *nd6* and *nd4L*, which is conserved in Bilateria and is potentially considered as a transcriptional signal, is also classified as a non-coding regions (Aydemir et al., 2023).

Over the past five decades, complete mitochondrial genome sequences have been generated for numerous species, enabling extensive studies on genomic structure, gene content, organization, and expression. Comparative analyses of mitochondrial genomes reveal patterns of variation at both broad (e.g., interspecies) and fine (e.g., gene-level) scales (Aydemir and Korkmaz, 2020). Since mitochondria originated only once during evolution, their genomes serve as a singular and powerful tool for exploring evolutionary relationships and mutation dynamics (Cameron et al., 2009; Dowton et al., 2009; Nelson, et al., 2012; Wan et al., 2012; Wang et al., 2012).

Mitochondrial genome rearrangement patterns have emerged as a new resource for evolutionary and phylogenetic research (Timmermans and Vogler, 2012; Zhao et al., 2013). In a study investigating mitochondrial genome rearrangements in mites, which analyzed a total of 258 mite mitogenomes, the group was found to exhibit high levels of genomic rearrangements (Aydemir, 2024). Interestingly,

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more mobile (mostly rearranged) genes showed lower levels of sequence divergence, whereas less mobile genes exhibited higher rates of variation; and Parasitiformes mitogenomes seem to be more rearranged than Acariformes. These findings demonstrate that mitochondrial genome data are highly informative in fields such as evolutionary biology, providing strong evidence of their utility particularly in mites (Aydemir, 2024).

Hyalomma lusitanicum Koch, 1844 is a metastriate tick species belonging to the family Ixodidae, which comprises the largest and most medically important group of ticks worldwide. *Hy. lusitanicum* is primarily distributed in the western Mediterranean region, including the Iberian Peninsula and parts of North Africa, where it is commonly associated with warm and dry environments (Espírito-Santo et al., 2017; Valcárcel et al., 2020, 2023). The species parasitizes a wide range of vertebrate hosts, with immature stages frequently feeding on small mammals and birds, while adults preferentially infest large mammals such as ungulates. Similar to other *Hyalomma* species, *Hy. lusitanicum* has been associated with several tick-borne pathogens of veterinary and potential public health relevance, underscoring its epidemiological importance (Santos-Silva et al., 2011; Johnson, 2023). Owing to its ecological adaptability and vector potential, *Hy. lusitanicum* represents a relevant model for studies on tick evolution, population genetics, and mitochondrial genome organization. In the NCBI database (https://www.ncbi.nlm.nih.gov/nuccore), 793 mitogenome sequences, from 12000 to 35000 bp long, were found for 171 distinct Ixodidae species (January, 2026). 137 of the sequences were verified and deposited as a RefSeq data of which of three were *Hyalomma* species. This study aims to contribute to the understanding of the mitogenome patterns of this complex group, Ixodidae by including *Hyalomma lusitanicum* mitogenome data.

MATERIALS AND METHODS

Mitochondrial genome assembly

Oxford_Nanopore raw reads of *Hyalomma lusitanicum* were downloaded from NCBI with ERR13381542 accession number. Mitochondrial genome sequences were assembled using "map to reference" option with medium-low sensitivity parameters in Geneious R9 with a closely related *Hyalomma rufipes* mitogenome data in NCBI (Accession number: MW884229) as a reference (Kearse et al., 2012). Only reads that mapped concordantly to the reference mitochondrial genome were used for generating contig sequences. Regions with low coverage were manually inspected to avoid potential misassemblies or reference-induced bias. The resulting contig was validated, and mitochondrial genome completeness was confirmed by comparing against mitogenomes of Acari with BLAST search.

Mitogenome annotation

The assembled mitochondrial genome was subsequently annotated to identify protein-coding genes (PCGs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), and non-coding regions.

Organizations of PCGs were identified using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder) and MITOS2 (http://mitos2.bioinf.uni-leipzig.de/index.py) with the invertebrate mitochondrial genetic code as the prior (Bernt et al., 2013). Annotations were manually evaluated using BLAST searches and compared with verified RefSeq mitogenome data from NCBI.

The precise ends of rRNA genes were determined based on the locations of adjacent genes. tRNA sequences were identified and extracted from these data through internal bioinformatics processing. The resulting tRNA sequences were then subjected to secondary structure prediction and visualization using the R2DT (RNA 2D Templates) tool available on the RNAcentral platform (https://rnacentral.org/) (McCann et al., 2025).

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 settings under basic option (Benson, 1999).

Nucleotide compositions (length, A%, T%, C% and G%), dinucleotide percentages (AC%, AT% and GC%) and skewness values (AT- and GC- skew) were calculated for each gene and whole mitogenome using MEGA 12.1 (Kumar et al., 2024).

For protein-coding genes, the relative synonymous codon usage (RSCU) values of the mitogenome and amino acid compositions of each gene were calculated.

RESULTS AND DISCUSSION

Mitogenome architecture of *Hyalomma lusitanicum*

The *Hyalomma lusitanicum* complete circular mitogenome was annotated as 14.742 bp-long and have been deposited in GenBank under the provisional submission number ID3048678. The mitogenome of *Hy. lusitanicum* is visualized in Figure 1, and the corresponding feature table is presented in Table 1. Nucleotide compositions of *Hy. lusitanicum* mitogenome were as follows: 39.7%= T%; 38.5%= A%; 12.5%= C% and 9.3%= G% with negative AT- (-0.0152) and GC- (-0.1453) skewness values.

In the mitogenome of *Hy. lusitanicum*, overall lengths of PCGs, tRNAs, rRNAs and Control-Regions (CRs) are 10850, 1375, 1895 and 611 bp, respectively. A CR1 non-coding control region was determined between rrnS and trnI, 304 bp long. Another non-coding region, CR2, is between trnL1 and trnC with 307 bp long.

Hyalomma mitotype M55 was determined in *Hy. lusitanicum* mitogenome, differentiated from M1 with some TDRL and transpositions events (Arribas et al., 2020; Aydemir, 2024).

The intergenic regions were 77 bp in total length and determined between 10 adjacent genes, with minimum 2- (*nd4L*-trnT) and maximum 20- (trnQ-trnF) bp long (Table 1).

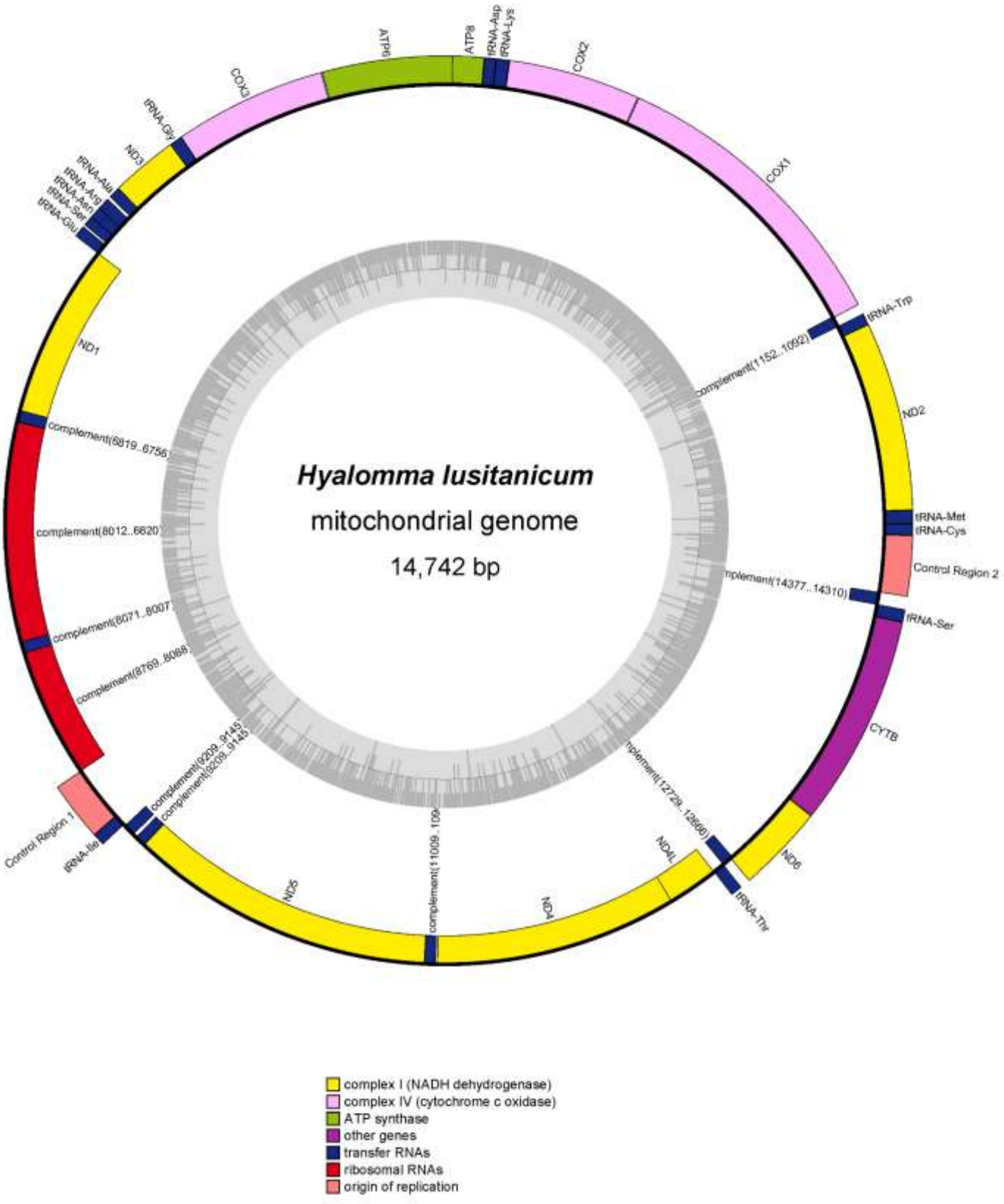

Figure 1. Schematic representation of *Hyalomma lusitanicum* mitogenome.

Tick-box motif (TTGYRTCHWWWTWWGDA) was found only between trnQ-trnF as TTGCATCATTTTTTGTA sequence, distinct from other *Hyalomma* species (Montagna et al., 2012; Liu et al., 2018). Conserved Bilaterian transcription motif (WHWGHTW) was found between *nd4L* and trnT, upstream of *nd4L* gene as TAAGATT sequence (Aydemir et al., 2023).

On the other hand, the overlapping regions were dispersed among different gene junctions, 65 bp in total length.

Table 1. Annotation, characterization and organisation of *Hyalomma lusitanicum* mitogenome.

		Start	End	Strand	Length	Intergenic-overlapping	T	C	A	G	%AT	AT-skew	GC-skew	Anticodon	Start	Stop
Hy. lusitanicum	Whole mitogenome	1	14742		14742		39.7	12.5	38.5	9.3	78.2	-0.0152	-0.1453			
trnM	tRNA	4	70	J	67	0	32.8	13.4	41.8	11.9	74.6	0.1200	-0.0588	CAT		
nad2	PCG	71	1033	J	963	-1	47.8	11.3	36.1	4.8	83.9	-0.1386	-0.4065		ATT	TAA
trnW	tRNA	1033	1093	J	61	-2	36.1	8.2	50.8	4.9	86.9	0.1698	-0.2500	TCA		
trnY	tRNA	1092	1152	N	61	-8	32.8	11.5	31.1	24.6	63.9	-0.0256	0.3636	GTA		
cox1	PCG	1145	2683	J	1539	3	38.7	16.5	31.1	13.7	69.8	-0.1099	-0.0925		ATT	TAA
cox2	PCG	2687	3359	J	673	0	38.2	15.6	37.3	8.9	75.5	-0.0118	-0.2727		ATG	T--
trnK	tRNA	3360	3426	J	67	0	37.3	11.9	34.3	16.4	71.6	-0.0417	0.1579	CTT		
trnD	tRNA	3427	3486	J	60	0	33.3	10.0	43.3	13.3	76.7	0.1304	0.1429	GTC		
atp8	PCG	3487	3648	J	162	-7	39.5	13.0	45.7	1.9	85.2	0.0725	-0.7500		ATC	TAA
atp6	PCG	3642	4307	J	666	4	47.7	10.8	32.1	9.3	79.9	-0.1955	-0.0746		ATG	TAA
cox3	PCG	4312	5089	J	778	0	45.5	12.9	29.6	12.1	75.1	-0.2123	-0.0309		ATG	T--
trnG	tRNA	5090	5154	J	66	-4	36.4	4.5	54.5	4.5	90.9	0.2000	0.0000	TCC		
nad3	PCG	5151	5495	J	345	-2	50.7	7.0	32.8	9.6	83.5	-0.2153	0.1579		ATA	TAG
trnA	tRNA	5494	5554	J	61	16	42.6	8.2	39.3	9.8	82.0	-0.0400	0.0909	TGC		
trnR	tRNA	5571	5634	J	64	-2	43.8	10.9	31.3	14.1	75.0	-0.1667	0.1250	TCG		
trnN	tRNA	5633	5693	J	61	-2	34.4	11.5	37.7	16.4	72.1	0.0455	0.1765	GTT		
trnS1	tRNA	5692	5744	J	53	11	32.1	15.1	43.4	9.4	75.5	0.1500	-0.2308	TCT		
trnE	tRNA	5756	5815	J	60	-2	45.0	11.7	30.0	13.3	75.0	-0.2000	0.0667	TTC		
nad1	PCG	5814	6758	N	945	-3	44.2	9.9	34.8	11.0	79.0	-0.1191	0.0505		ATA	TAA
trnL2	tRNA	6756	6819	N	64	0	32.8	12.5	40.6	14.1	73.4	0.1064	0.0588	TAA		
rrnL	rRNA	6820	8012	N	1193	-6	39.1	7.2	42.6	11.1	81.6	0.0431	0.2146			
trnV	tRNA	8007	8071	N	65	-4	44.6	7.7	40.0	7.7	84.6	-0.0545	0.0000	TAC		
rrnS	rRNA	8068	8769	N	702	0	38.5	8.4	40.3	12.8	78.8	0.0235	0.2081			
CR1	CR	8770	9073		304	0	35.2	16.8	33.6	14.5	68.8	-0.0239	-0.0737			
trnI	tRNA	9074	9136	J	63	8	38.1	7.9	36.5	17.5	74.6	-0.0213	0.3750	GAT		
trnQ	tRNA	9145	9209	N	65	20	43.1	4.6	44.6	7.7	87.7	0.0175	0.2500	TTG		
trnF	tRNA	9230	9289	N	60	0	40.0	6.7	43.3	10.0	83.3	0.0400	0.2000	GAA		
nad5	PCG	9290	10948	N	1659	0	47.6	8.4	34.1	9.9	81.7	-0.1646	0.0855		ATT	TAA
trnH	tRNA	10949	11009	N	61	7	42.6	3.3	42.6	11.5	85.2	0.0000	0.5556	GTG		
nad4	PCG	11017	12333	N	1317	-7	46.6	9.0	32.0	12.4	78.7	-0.1853	0.1601		ATG	TAA
nad4l	PCG	12327	12602	N	276	2	51.1	4.7	33.7	10.5	84.8	-0.2051	0.3810		ATG	TAA
trnT	tRNA	12605	12665	J	61	0	42.6	9.8	36.1	11.5	78.7	-0.0833	0.0769	TGT		
trnP	tRNA	12666	12729	N	64	-13	37.5	4.7	39.1	18.8	76.6	0.0204	0.6000	TGG		
nd6	PCG	12717	13163	J	447	3	48.5	10.1	36.2	5.1	84.8	-0.1451	-0.3235		ATA	TAA
cytB	PCG	13167	14246	J	1080	0	41.6	15.7	32.2	10.5	73.8	-0.1267	-0.2014		ATG	TAG
trnS2	tRNA	14247	14311	J	66	-2	39.4	6.1	45.5	9.1	84.8	0.0714	0.2000	TGA		
trnL1	tRNA	14310	14377	N	68	0	38.2	5.9	44.1	11.8	82.4	0.0714	0.3333	TAG		
CR2	CR	14378	14685		307	0	36.2	16.0	34.2	13.7	70.4	-0.0278	-0.0769			
trnC	tRNA	14686	14742	J	57	3	43.9	7.0	43.9	5.3	87.7	0.0000	-0.1429	GCA		

Table 2. Amino acid composition of *Hyalomma lusitanicum* mitogenome.

	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
nd2	1.875	0.3125	0.3125	0.9375	13.75	1.25	1.25	18.438	7.1875	15.313	9.0625	6.5625	1.875	0.9375	0.3125	9.6875	4.375	0.9375	1.875	3.75
cox1	5.8594	0.1953	2.5391	1.3672	8.9844	8.5938	2.9297	12.109	2.1484	10.938	7.0313	5.2734	4.8828	1.3672	1.7578	8.2031	5.8594	4.4922	2.7344	2.7344
cox2	2.6786	1.3393	4.0179	4.9107	6.6964	3.125	2.2321	16.518	3.5714	7.5893	9.375	6.6964	4.4643	3.125	1.7857	10.268	5.3571	0.8929	2.2321	3.125
atp8	1.8868	0	0	0	15.094	0	0	18.868	15.094	11.321	9.434	11.321	3.7736	1.8868	0	3.7736	1.8868	0	3.7736	1.8868
atp6	3.1674	1.3575	0.4525	1.81	12.67	1.81	1.81	14.027	3.6199	14.932	7.2398	7.2398	3.6199	0.905	0.905	10.86	4.5249	4.0724	2.7149	2.2624
cox3	3.4749	0.7722	1.1583	2.7027	14.672	5.0193	4.6332	11.197	3.0888	10.039	5.0193	3.0888	3.0888	2.3166	1.1583	11.969	5.4054	3.4749	4.2471	3.4749
nd3	0	0.8772	1.7544	5.2632	13.158	2.6316	0	21.053	7.0175	14.912	7.0175	4.386	4.386	0	0.8772	8.7719	0	3.5088	0.8772	3.5088
nd1	3.1847	1.2739	0.9554	4.1401	10.828	4.4586	0.6369	15.605	3.8217	11.783	8.5987	5.414	2.8662	1.9108	2.2293	10.51	1.5924	2.8662	1.5924	5.7325
nd5	2.8986	1.087	2.1739	1.2681	13.406	3.8043	0.9058	11.051	4.3478	17.754	8.8768	7.4275	1.9928	1.087	0.7246	9.058	3.0797	3.442	1.8116	3.8043
nd4	2.7397	1.8265	0.6849	1.8265	12.785	4.7945	1.3699	10.731	3.4247	15.525	10.959	4.5662	2.2831	0.9132	1.1416	9.8174	2.7397	4.3379	2.2831	5.2511
nd4L	1.0989	2.1978	0	3.2967	13.187	3.2967	1.0989	13.187	1.0989	25.275	8.7912	6.5934	0	0	0	10.989	2.1978	3.2967	0	4.3956
nd6	2.027	1.3514	0	2.027	12.838	1.3514	0.6757	15.541	6.0811	17.568	14.865	7.4324	2.7027	0.6757	0	6.7568	1.3514	2.7027	0.6757	3.3784
cytB	4.4568	0.5571	1.9499	1.1142	11.978	5.571	2.2284	13.092	3.6212	10.585	8.6351	5.8496	5.2925	1.1142	1.3928	7.2423	5.571	2.7855	2.507	4.4568

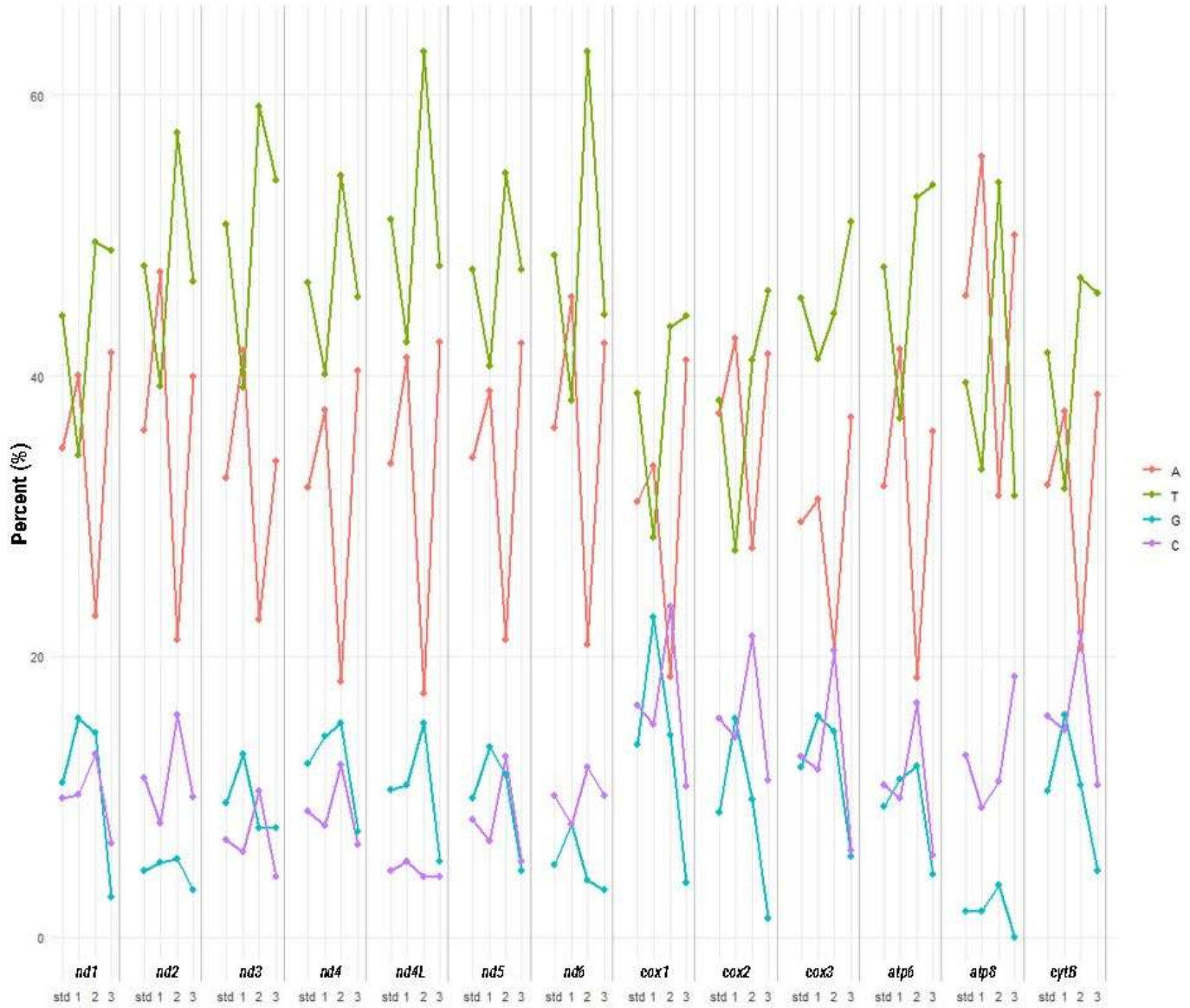

Figure 2. Nucleotide composition of codon positions of each protein-coding gene.

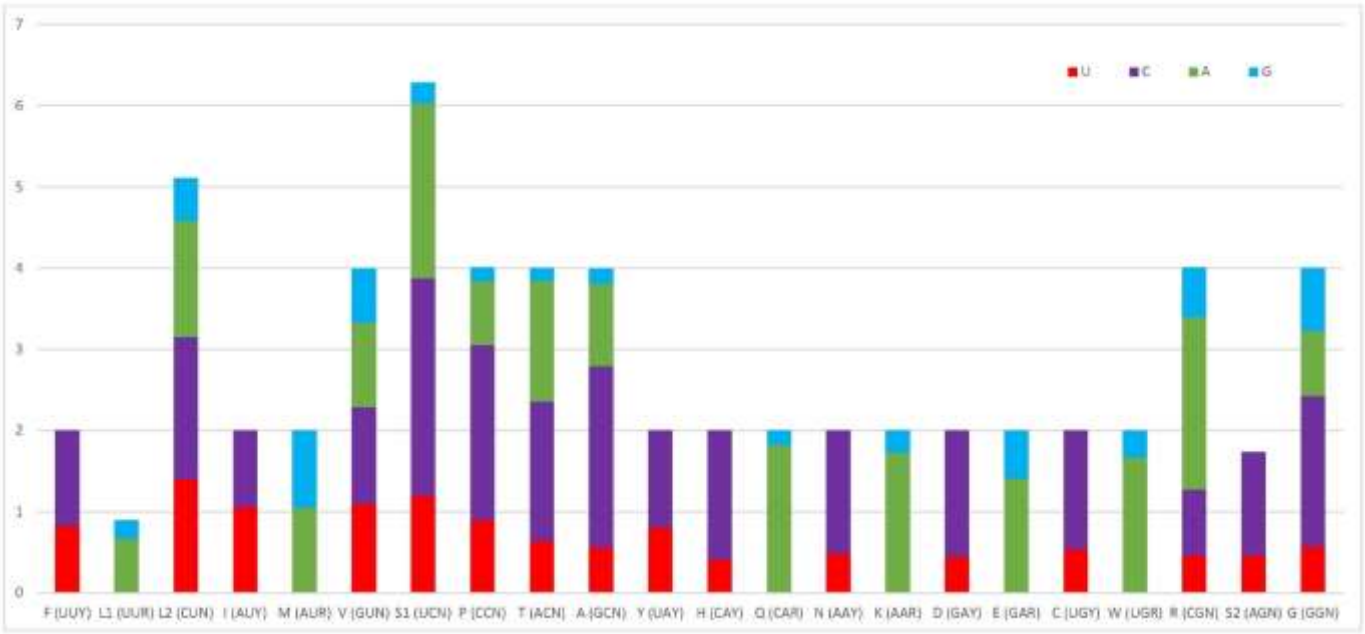

Figure 3. Relative synonymous codon usage (RSCU) values of *Hyalomma lusitanicum* mitogenome.

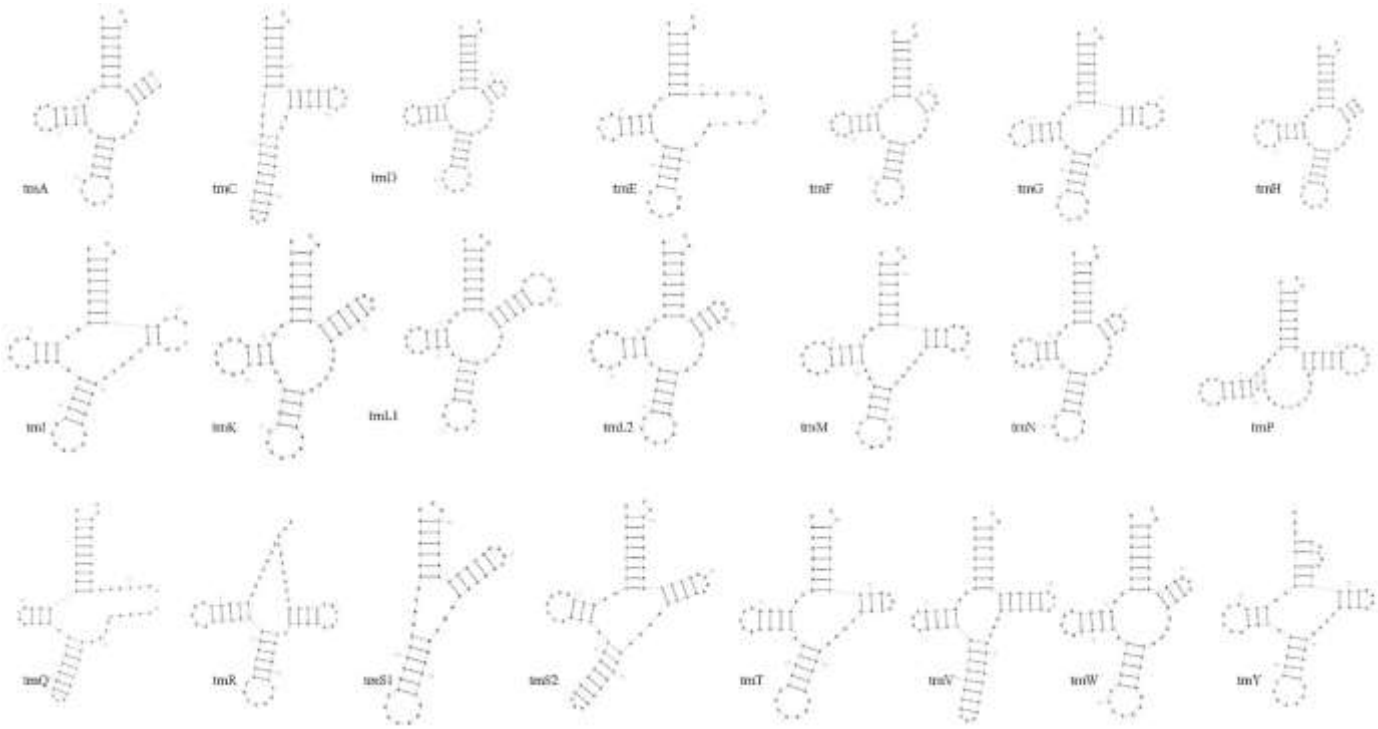

Figure 4. Putative secondary structures of *Hyalomma lusitanicum* mito-tRNAs.

Table 3. Potential tandem repeats determined in *Hyalomma lusitanicum* mitogenome via TRF.

Repeat	position	Size (bp)	Gene	Copy number	Percent matches	Score	A	C	G	T	Entropy (0-2)
1	4556-4611	28	*cox3*	2	82	69	14	8	7	69	1.35
2	5152-5201	15	*nd3*	3.3	82	64	32	0	2	66	1.03
3	9439-9485	21	*nd5*	2.2	84	58	34	6	0	59	1.23

15 neighbouring genes overlapped with from 1 to 13 nucleotides (Table 1). Conserved *atp8-atp6* and *nd4L-nd4* overlaps were determined as 8 and 7 bp in length, respectively. Interestingly, 13-bp-long overlap was identified between the trnP and *nd6* genes. Conserved *cox1-cox2* overlap in *Hyalomma asiaticum asiaticum* and *Hy. kozlovi* seems to be shifted between trnY and *cox1* genes in *Hy. lusitanicum* like *Hy. anatolicum* (Liu et al., 2018; Che et al., 2025).

Protein-coding genes of *Hy. lusitanicum* mitogenome

Average nucleotide content of protein-coding genes of *Hy. lusitanicum* was as follows: T: 45.2%, C: 11.1%, A: 34.4%, G: 9.2%. AT content of each protein-coding gene was varied from 69.8 (*cox1*) to 85.2 (*atp8*) percent. When the first codon position was evaluated in terms of nucleotide composition, A was the most frequently preferred nucleotide. At the second codon position, more than half of the sites showed a bias toward thymine T. As expected, the third codon position exhibited the lowest frequency of guanine G. Dramatic nucleotide biases among codon positions were remarkable (Fig. 2).

Protein-coding genes showed variation in start codon usage. *cox2*, *atp6*, *cox3*, *nd4*, *nd4L*, and *cytB* genes initiate translation with the standard ATG (methionine) start codon, whereas *nd3*, *nd1*, and *nd6* use the alternative ATA codon, which also encodes methionine. In contrast, *nd2*, *cox1*, and *nd5* genes start with the ATT codon, and the *atp8* gene begins with the ATC start codon. Most protein-coding genes terminate with the TAA stop codon; however, *cytB* and *nd3* genes end with the TAG codon. *cox3* gene possesses an incomplete stop codon (T– –), which is presumed to be completed by post-transcriptional polyadenylation (Table 1).

Amino acid preferences of protein-coding genes were biased to hydrophobic amino acids with an average of 60.81%, from minimum 50.44% in *cox2* to maximum 68.92% in *nd6* (Table 2). Amino acid composition and RSCU values of PCGs were given in Table 2 and Figure 3.

tRNA and rRNA genes of *Hy. lusitanicum* mitogenome

The lengths of tRNA genes of *Hy. lusitanicum* mitogenome were varied from 53 (trnS1) to 68 (trnL1) bp long, with average 63 bp-long. Nucleotide compositions of tRNA genes were T: 38.6%, C: 8.8%, A: 40.6%, G: 12.0%. The anticodon sequences of each tRNA genes were given in Table 1. Secondary structure of tRNA molecules were predicted and visualised (Fig. 4). Truncated tRNAs, particularly those lacking D- and T-arms, were identified in the *Hy. lusitanicum* mitochondrial genome, specifically in the trnC, trnE, and trnS1 genes. Truncated tRNA structures have been widely reported in the mitochondrial genomes of Acariform mites; however, this pattern is generally unexpected in Parasitiformes, with only a few reported exceptions (Xue et al., 2016). In a more recent study, similar truncated tRNA patterns were also identified in Trombidiformes mitochondrial genomes (Zhang et al., 2025). Therefore, the detection of truncated tRNAs in *Hy. lusitanicum* mitogenome represents an unexpected structural pattern within Parasitiformes and suggests a greater level of mitochondrial tRNA structural diversity than previously recognized.

The lengths of rrnS and rrnL genes were determined as 702 and 1193 bp long, respectively. rRNA genes consist 38.8% of T; 7.8% of C; 41.4% of A and 12.0% of G nucleotides (Table 1).

As a result of Tandem Repeat Finder analysis, three different tandem repeat were found (Table 3). Repeats were dispersed in *cox3*, *nd3* and *nd5* genes in with two or three repeat numbers and 15- 28 bp period size. The high T-content of repetitive sequences is remarkable. Sequences with a minimum T-ratio of 59% and a maximum of 68% have a closest content A, ranging from 14% to 34%.

CONCLUSION

In this study, the complete mitochondrial genome of *Hyalomma lusitanicum* was successfully characterized. The mitogenome exhibits a compact organization with an AT bias, two non-coding control regions, and a genome organisation corresponding to *Hyalomma* mitotype M55, shaped by transposition and TDRL events. Detailed analyses of nucleotide composition, codon usage, and amino acid preferences revealed strong compositional biases, consistent with patterns commonly observed in tick mitochondrial genomes.

Notably, the identification of truncated tRNA structures lacking D- and/or T-arms in trnC, trnE, and trnS1 represents an unexpected finding for Parasitiformes and suggests a higher level of mitochondrial tRNA structural diversity than previously recognized in this group. The presence of conserved transcriptional motifs, lineage-specific overlaps, and tandem repeats further highlights the dynamic nature of the *Hy. lusitanicum* mitogenome.

Taken together, these findings expand current knowledge of *Hyalomma* mitochondrial genome architecture and evolution, providing a valuable genomic resource for future studies on tick phylogeny, comparative mitogenomics, and molecular systematics within Ixodidae.

Statement of ethics approval

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Conflict of interest

There is no conflict of interest.

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