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The complete mitochondrial genome of *Dama dama*, and their phylogenetic relationships to other Cervidae

Rebecca Barnard *, Judith Smith

University of Central Lancashire, United Kingdom of Great Britain and Northern Ireland

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ABSTRACT

This publication presents the complete mitochondrial genome of *Dama dama* along with in depth phylogenetic relationship and species divergence analysis in respect to other Cervidae. The mitochondrial genome presented here is 16,332 bp which is comprised of 13 genes, 2 rRNAs and 22 tRNAs. The mitochondrial genome for *Dama dama* is the smallest, compared to other Cervidae. Transfer RNA genes have a specific secondary structure, resembling a clover leaf, however, $tRNA^{Ser}$ (Serine 1) in *Dama dama* has been found to only have 3 arms, it is missing the dihydrouridine arm. The phylogenetic analysis conducted in this study compared the mitochondrial sequences from 25 different Cervidae species. Findings suggest that *Dama dama,* compared to other Cervidae, is most closely related to *Dama mesopotamica* and *Megaloceros giganteus*. With regards to *Dama dama*, the species divergence time from *Dama mesopotamica* and *Megaloceros giganteus* is 5.68 mya. Whereas the divergence time between *Dama mesopotamica* and *Megaloceros giganteus* is 5.35 mya. Our findings provide strong support for the distinction between *Dama dama* and *Dama mesopotamica* as a sub-species and a close evolutionary relationship between *Dama mesopotamica* and *Megaloceros giganteus*. Supporting previous reports of a sister-group relationship with a shared common ancestor. This study has provided a new perspective on the ancestral origin of the *Dama* genus, which can be further investigated using the *Dama dama* mitochondrial genome presented in this report. Understanding the evolution of *Dama dama* may help to better understand the lack of genetic diversity within the species and advance future management strategies to resolve this.

1. Introduction

Mitochondrial genomes in mammals are described as small, circular in shape and are highly conserved in both length and gene content (Simon et al., 1994; Boore, 1999; Sarvani et al., 2018). Mammalian mitochondrial genomes are approximately 16,000 bp in length which contains 37 genes that encompasses 22 transfer RNA (tRNA) genes, 13 protein-coding genes (PCGs) two ribosomal RNA (rRNA) genes and an AT rich control region (CR) (Simon et al., 1994; Boore, 1999; Sarvani et al., 2018; Huo et al., 2023). Mitochondrial genomes are highly abundant in each cell with high evolutionary rates, low levels of recombination and maternal inheritance (Curole and Kocher, 1999; Sarvani et al., 2018; Huo et al., 2023). Due to these characteristics, the mitogenome offers excellent capabilities to help understand genetic diversity within and between species, including evolutionary relationships, population genetics and phylogenetic relationships (Simon et al., 2006; Cameron, 2014; Sarvani et al., 2018; Huo et al., 2023).

Fallow deer (*Dama dama*) are wide spread across the globe, the species is considered wild and domestic whilst simultaneously being invasive and endangered. Several studies have been conducted into the European fallow deer population and comparisons between modern and archaeological samples which has revealed two clades of European *Dama dama* in the Balkans and Anatolia (Baker et al., 2024a, 2024b). These areas represent the regions where fallow deer originated from, including the middle east. Despite their large wide spread population, the species has very low genetic diversity, resulting in conservation vulnerabilities (Baker et al., 2024). It has been suggested that the bio-cultural history of the species should impact how fallow deer are managed today (Baker et al., 2024a, 2024b). The lack of genetic diversity was caused by a genetic bottle neck which effected the species population size and triggered a spike in inbreeding during the Mesolithic (8800 BCE to c. 4500 BCE) and Neolithic (4300 BCE to 2000 BCE) times (Baker et al., 2017). Due to the glacial period, the population size was reduced and squeezed into a small geographical area, they almost because extinct (Baker et al., 2017). Since this time the population of fallow has increased. Studies show that the Romans were responsible for

* Corresponding author.
E-mail address: rbarnard@uclan.ac.uk (R. Barnard).

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the translocation of fallow from mainland Europe to Britain (Baker et al., 2017; Baker et al., 2024a, 2024b). Fallow were considered a symbol of wealth and often kept in captivity on big estates. Fallow deer re-entered the wild after escaping captivity when private parks and estates were allowed to fall into disrepair during the 15th Century (Chapman and Chapman, 1997; The British Deer Society (BDS), 2016; Baker et al., 2017). This explains why their genetic diversity is still so low, despite their large populace.

This study presents the complete mitochondrial genome for Fallow deer (*Dama dama*) along with its characterisation and phylogenetic analysis. To our knowledge, the full characterisation of the complete mitochondrial genome had not been published before. Despite fallow deer being classified as least concern on the ICUN red list, the species has a profound lack of genetic diversity which is causing the species to be at risk of conservation vulnerability as well as the effect of poaching on the population. Mitochondrial sequences are commonly used to resolve species phylogenetic positions and their evolutionary relationships, hence the importance of this report in better understanding fallow deer genetic diversity, especially within the British population. This report also acts as the ground work for future studies researching the evolutionary genetics of *Dama dama*. Furthermore, phylogenetic studies involving fallow deer would help provide genetic data to support the redesigning of modern management strategies for the species, in particular, conservation breeding programs.

2. Method

2.1. DNA extraction and genome sequencing

The sample used to sequence the fallow deer Mitochondrial genome was muscle collected from a male fallow, collected from Richmond Royal Park in London UK (Latitude 51.443225, longitude −0.27042112) (Barnard et al., 2023). DNA was extracted using a modified, optimised, method for Gentra Puregene (Barnard et al., 2023). The Mitochondrial genome was sequenced during WGS of the fallow deer genome using PacBio® Long-Read sequencing, on the SMRTBell™ barcoded adapter on the Sequel IIe system (Barnard et al., 2023).

The Genome data is publicly accessible on the NCBI found at the following URL, https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_033118175.1/ (Accession Number: ASM3311817v1) (Barnard et al., 2023). For the assembly of the mitochondrial genome, a previously sequenced fallow deer Mitogenome was downloaded from the NCBI and used (https://www.ncbi.nlm.nih.gov/nucleotide/NC_020700.1).

2.2. Mitochondrial genome annotation and analysis

The Mito sequence from this project was aligned with NC_020700.1 from NCBI on Bio-Edit v7.7 (Hall, 1999) and trimmed. The genome was annotated using Mitos WebServer (Bernt et al., 2013) and MitoFish (Iwasaki et al., 2013). A complete mitogenome gene map was generated using MitoAnnotator (Iwasaki et al., 2013) for *Dama dama*. tRNAscan-SE software (Lowe and Eddy, 1997; Chan et al., 2021) and Mitos2 on Galaxy.org (Bernt et al., 2013; Iwasaki et al., 2013; Afgan et al., 2022) was used to predict proteins and transfer RNA (t-RNA) along with their secondary structures. Any t-RNAs which wasn't identified using the previous software, were identified by aligning the mitogenome with the only other previously published *Dama dama* Mito data, downloaded from the NCBI (accession: NC_020700.1).

2.3. Phylogenetic analysis

For comparison to other species, mitochondrial genome sequences were downloaded from the NCBI from Artiodactyla of the same family, Cervidae, see Table 1. Sequences were aligned with ClustalW (Thompson et al., 1994) in Mega7 (Kumar et al., 2016). For the phylogenetic tree, maximum likelihood (ML) analysis was conducted using MEGA 7 (Kumar et al., 2016) on the alignment of the sequences in Table 1. Maximum likelihood was assessed using the Tamura-Nei model for substitution (Tamura and Nei, 1993; Kumar et al., 2016). Sequences from species used as an out group were African black Rhino (*Diceros bicornis*, NC_012682.1) and the Bengal tiger (*Panthera tigris tigris*, KF892541.1).

Species divergence was calculated for *Dama dama* and sub-species *Dama dama mesopotamica* on MEGA7 (Kumar et al., 2016), using the RelTime method (Tamura et al., 2018) for estimating divergence times to compute a TimeTree.

Table 1
Details of the 27 Mitochondrial Cervidae genome sequences used for comparative and phylogenetic analysis, downloaded from the NCBI.

Species	Common name	Accession number	Reference
Diceros bicornis	Black rhinoceros	NC_012682.1	(Willerslev et al., 2009)
Panthera tigris tigris	Bengal tiger	KF892541.1	(Si, 2013)
Cervus elaphus	*Cervus elaphus*	NC_007704.2	(Wada et al., 2006)
Capreolus capreolus	Roe deer	MN485773.1	(Hua, 2019)
Cervus nippon centralis	Honshū Sika Deer	AB211429.1	(Wada et al., 2006)
Cervus canadensis	Elk	NC_050863.1	(Kim et al., 2020)
Muntiacus muntjak	Southern red muntjac	NC_004563.1	(Zhang et al., 2004)
Odocoileus virginianus	White-tailed deer	NC_015247.1	(Seabury et al., 2011)
Rangifer tarandus	Reindeer	NC_007703.1	(Wada et al., 2010)
Alces alces	Moose	NC_020677.1	(Hassanin et al., 2012)
Odocoileus hemionus	Mule deer	NC_020729.1	(Hassanin et al., 2012)
Hydropotes inermis	Water deer	NC_011821.1	(Li et al., 2020)
Axis axis	Chital	NC_020680.1	(Hassanin et al., 2012)
Elaphurus davidianus	Père David's deer	NC_018358.1	(Zhang et al., 2017)
Elaphodus cephalophus	Tufted deer	NC_008749.1	(Pang et al., 2008)
Rucervus duvaucelii	Barasingha	NC_020743.1	(Hassanin et al., 2012)
Rusa timorensis	Javan rusa	NC_020745.1	(Hassanin et al., 2012)
Rucervus eldii	Eld's deer	KU133959.1	(Tabasum et al., 2017)
Axis porcinus	Indian hog deer	NC_020681.1	(Hassanin et al., 2012)
Hippocamelus antisensis	Taruca	NC_020711.1	(Hassanin et al., 2012)
Cervus albirostris	Thorold's deer	MF966595.1	(Zhao et al., 2017)
Megaloceros giganteus	Irish elk	MW802558.1	(Rey-Iglesia et al., 2021)
Antilocapra americana	Pronghorn	NC_020679.1	(Hassanin et al., 2012)
Rusa alfredi	Visayan spotted deer	NC_020744.1	(Hassanin et al., 2012)
Mazama americana	Red brocket	NC_020719.1	(Hassanin et al., 2012)
Ozotoceros bezoarticus	Pampas deer	JN632681.2	(Hassanin et al., 2012)
Dama dama mesopotamica	Persian fallow deer	JN632630.1	(Hassanin et al., 2012)

3. Results and analysis

Full annotation results from MitoFish and Mitos 2 (Bernt et al., 2013; Iwasaki et al., 2013; Afgan et al., 2022), can be found in the supplementary data file, S.7_Mitochondrial Genome Study.

The complete structure of the mitochondrial genome for *Dama dama*

is presented in Fig. 1. The total mitochondrial genome for *Dama dama* is 16,322 bp in length, consisting of 13 protein coding genes, 2 rRNAs and 22 tRNAs, see Table 2. This is similar length as the previously published *Dama dama* Mito data (16, 330 bp) (accession: NC_020700.1). The notable difference with the mitogenome for *Dama dama* is that it is the smallest compared to all other mitochondrial genomes analysed in this study.

All genes have been mapped to the mitochondrial genome and their arrangement is detailed in Table 2. There is marginal discrepancies between the arrangement of the genes in fallow deer and that of other deer species. Between species base pair location of genes differs by only a few bases and there is minor distinctions with the tRNA genes. Overall, the arrangement is almost identical.

As part of mapping the genes on the mitochondrial genome, the protein coding regions have been represented in a graphical format, see Fig. 2, based upon their location and the quality of the base-calling. Protein-coding genes present are identical to that of other Cervidae species (Frank et al., 2016). The 13 protein-coding genes amounts to 11,193 bp in length, which is slightly shorter than other deer species (Wada et al., 2010; Ju et al., 2015; Frank et al., 2016; Tabasum et al., 2017; Sarvani et al., 2018), which is to be expected as the whole genome is the smallest compared to other Cervidae species analysed in this study. The longest gene is ND5, at 1821 bp, and the shortest is the ATP8 gene, at 201 bp.

Along with the 13 protein-coding genes, 22 tRNA genes have been identified, with a total length of 1517 bp. The size of the tRNA genes range from 61 to 75 bp, the smallest being $tRNA^{Ser}$ and the largest being $tRNA^{Leu}$. Transfer RNA genes have a specific secondary structure, see Fig. 3 apart from $tRNA^{Ser}$ (Serine 1) which lacks the dihydrouridine arm, the rest resemble a clover leaf secondary structure (Okimoto and Wolstenholme, 1990; Frank et al., 2016). As with other Mammalia, there are 2 rRNA genes present on the mitochondrial genome, located between $tRNA^{Phe}$ and $tRNA^{Val}$ is the 12S rRNA and located between $tRNA^{Val}$ and $tRNA^{Leu}$ is the 16S rRNA. 12S rRNA in the *Dama dama* mitochondrial genome is 12S rRNA bp's and the 16S rRNA is 1570 bp's in length, making it the larger of the two rRNA genes.

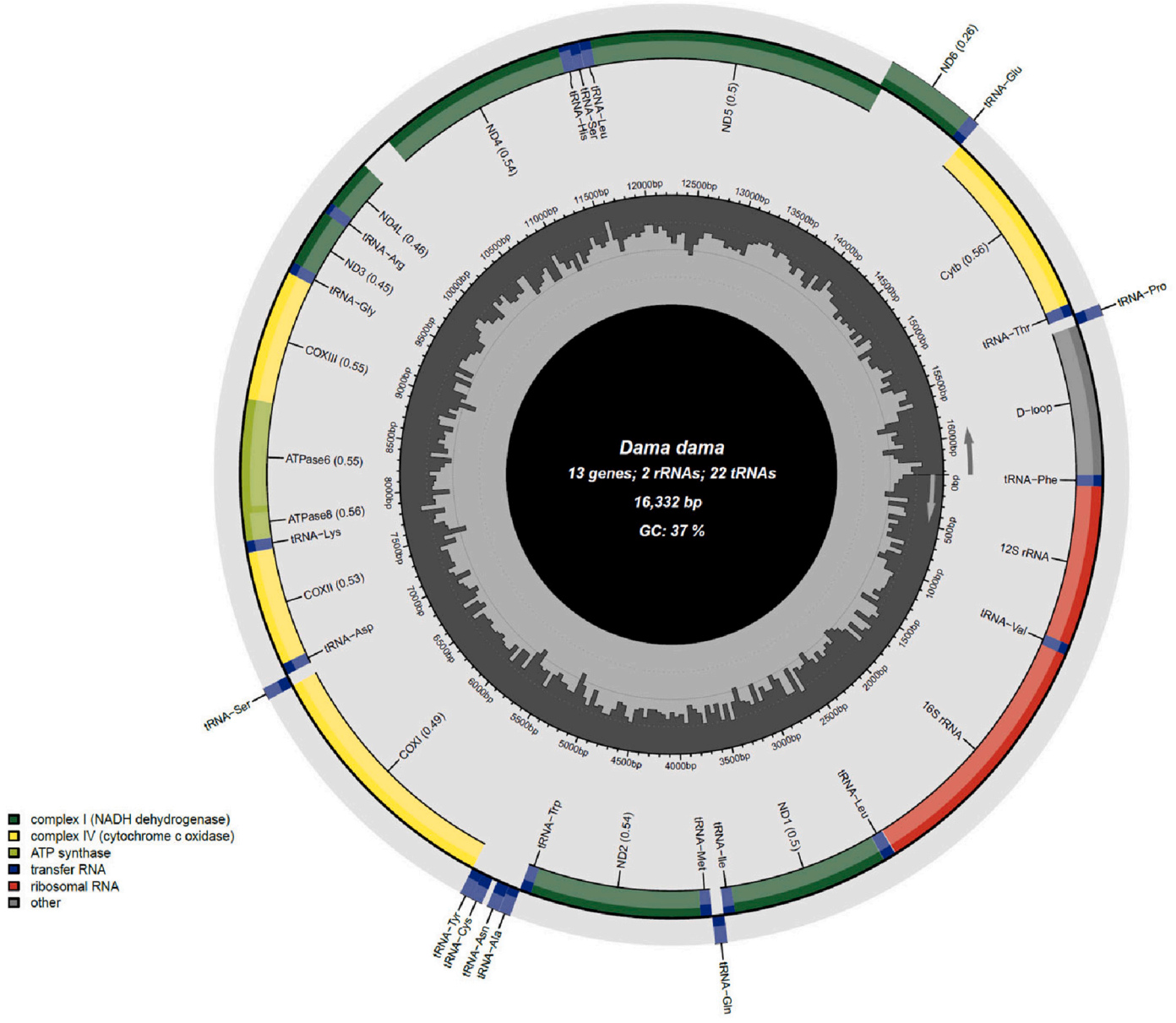

Fig. 1. Diagram depicting the circular structure of the mitochondrial genome, including the arrangement of genes for *Dama dama*. The figure was developed using the MitoAnnotator software (Iwasaki et al., 2013).

Table 2
Genes and their arrangement, for the Fallow deer (*Dama dama*) mitochondrial genome.

Name	Site	Length (bp)	Intergenic nucleotide*	Strand	Start codon	Stop codon	Anti-codon
$tRNA^{Phe}$	1–69	69	1	H			GAA
12S rRNA	70–1024	955	0	H			
$tRNA^{Val}$	1025–1091	67	0	H			UAC
16S rRNA	1091–2660	1570	−1	H			
$tRNA^{Leu}$	2666–2740	75	5	H			UAA
ND1	2743–3698	956	2	H	ATG	ATA	
$tRNA^{Ile}$	3699–3767	71	0	H			GAU
$tRNA^{Gln}$	3765–3836	72	−1	L			UUG
$tRNA^{Met}$	3839–3907	69	2	H			CAU
ND2	3908–4949	1042	0	H	ATA	AGT	
$tRNA^{Trp}$	4950–5017	68	0	H			UCA
$tRNA^{Ala}$	5019–5087	69	1	L			UGC
$tRNA^{Asn}$	5089–5161	73	1	L			GUU
$tRNA^{Cys}$	5194–5260	67	31	L			GCA
$tRNA^{Tyr}$	5261–5329	69	0	L			GUA
COX1	5331–6875	1545	1	H	ATG	TAA	
$tRNA^{Ser}$	6873–6941	69	−1	L			UGA
$tRNA^{Asp}$	6949–7016	68	7	H			GUC
COX2	7018–7701	683	1	H	ATG	TAA	
$tRNA^{Lys}$	7705–7772	68	3	H			UUU
ATP8	7774–7974	201	1	H	ATG	TAA	
ATP6	7935–8614	678	−37	H	ATG	TAA	
COX3	8615–9398	784	0	H	ATG	AT	
$tRNA^{Gly}$	9399–9467	69	0	H			UCC
ND3	9468–9814	347	0	H	ATA	AAT	
$tRNA^{Arg}$	9815–9883	69	0	H			UCG
ND4L	9884–10,180	297	0	H	ATG	TAA	
ND4	10,383–11,553	1171	2	H	ATG	ATT	
$tRNA^{His}$	11,554–11,622	69	0	H			GUG
$tRNA^{Ser}$	11,623–11,683	61	0	L			GCU
$tRNA^{Leu}$	11,685–11,754	70	1	H			UAG
ND5	11,755–13,575	1821	0	H	ATA	TAA	
ND6	13,559–14,086	528	−14	L	TTA	AT	
$tRNA^{Glu}$	14,087–14,155	69	0	L			UUC
CYTB	14,160–15,299	1140	4	H	ATG	AGA	
$tRNA^{Thr}$	15,302–15,371	70	2	H			UGU
$tRNA^{Pro}$	15,371–15,436	66	−1	L			UGG
Control Region	15,437–16,331	895	0	H			

* Overlapping adjacent genes are indicated by negative numbers.

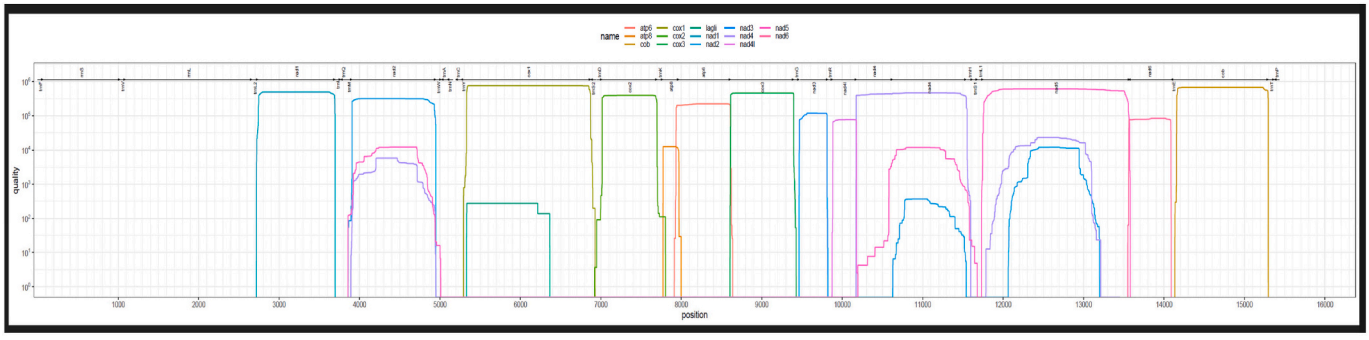

Fig. 2. Graphical depiction of the protein coding regions and their location along the mitochondrial genome (Bernt et al., 2013; Iwasaki et al., 2013; Afgan et al., 2022).

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (−73,461.31) is shown in Fig. 4. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The analysis involved 28 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 7358 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

A TimeTree, see Fig. 5, was inferred using the RelTime method (Tamura et al., 2012) and estimates of branch lengths inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The analysis involved 28 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 7358 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

4. Discussion

Over the years the genetic variation of fallow deer has been of

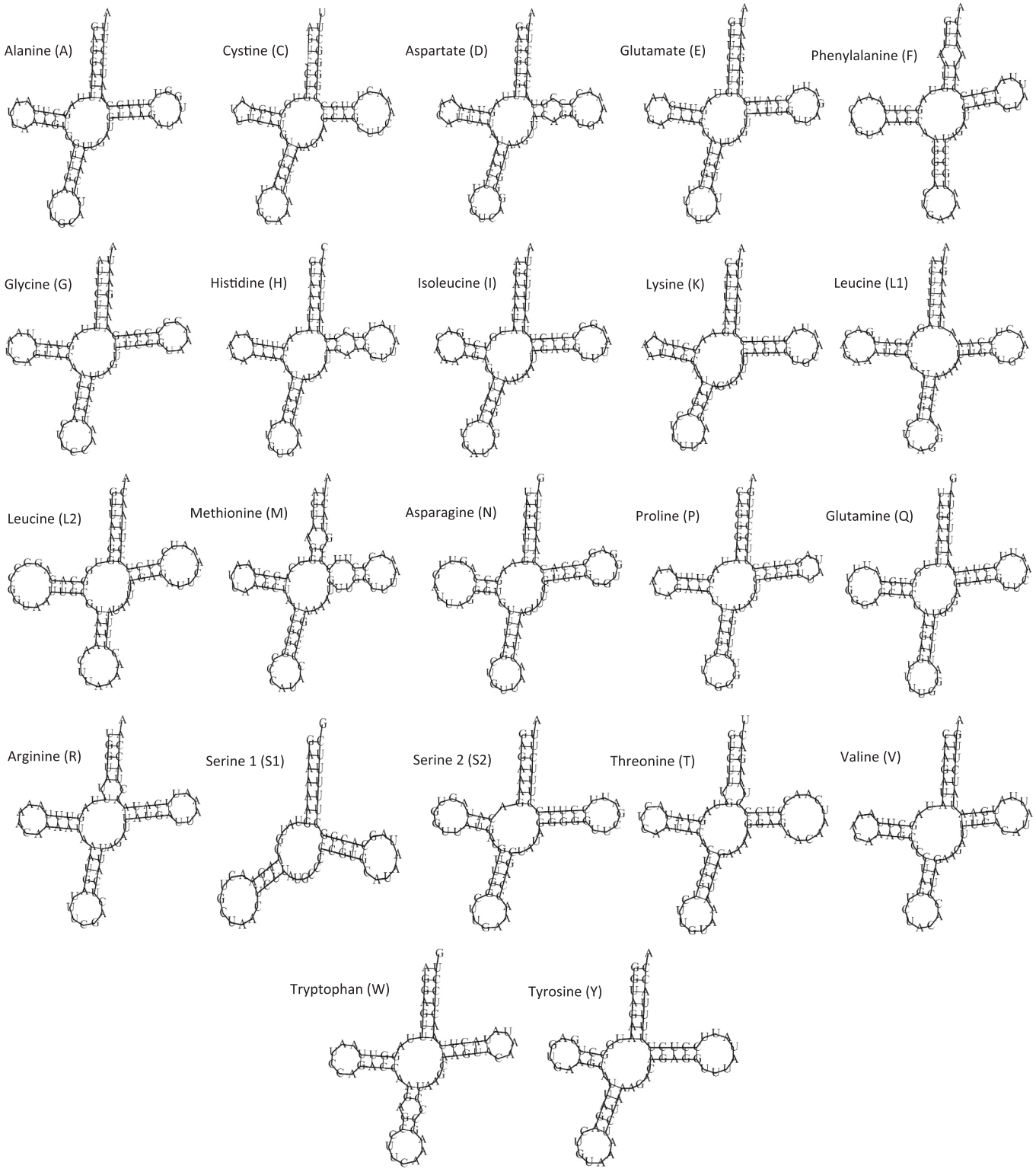

Fig. 3. The 22 tRNA genes of the *Dama dama* mitochondrial genome, and their secondary structures.

increasing interest (Chapman and Chapman, 1997; Pemberton and Smith, 1985; Hartl et al., 2009; Randi and Apollonio, 1988; Arslan-gündoğdu et al., 2010, Ludwig et al., 2011; Baker et al., 2017; Baker et al., 2024a). With the profound lack of genetic diversity with the species, many researchers have turned to mitochondrial DNA to help answer questions of phylogeny and heredity (Baker et al., 2017; Baker et al., 2024a, 2024b). Most current genetics research into *Dama dama* focusses on a known section of the Control region (Baker et al., 2017; Baker et al., 2024a), however, whole genome data provides the opportunity for a more detailed and robust analysis of phylogenetic relationships. This study provides an insight to the full length mtDNA genome for *Dama dama* and delivers analysis on their phylogenetic status and divergence time in relation to other Cervidae. As this project is the first to do this, the findings from this study help to better

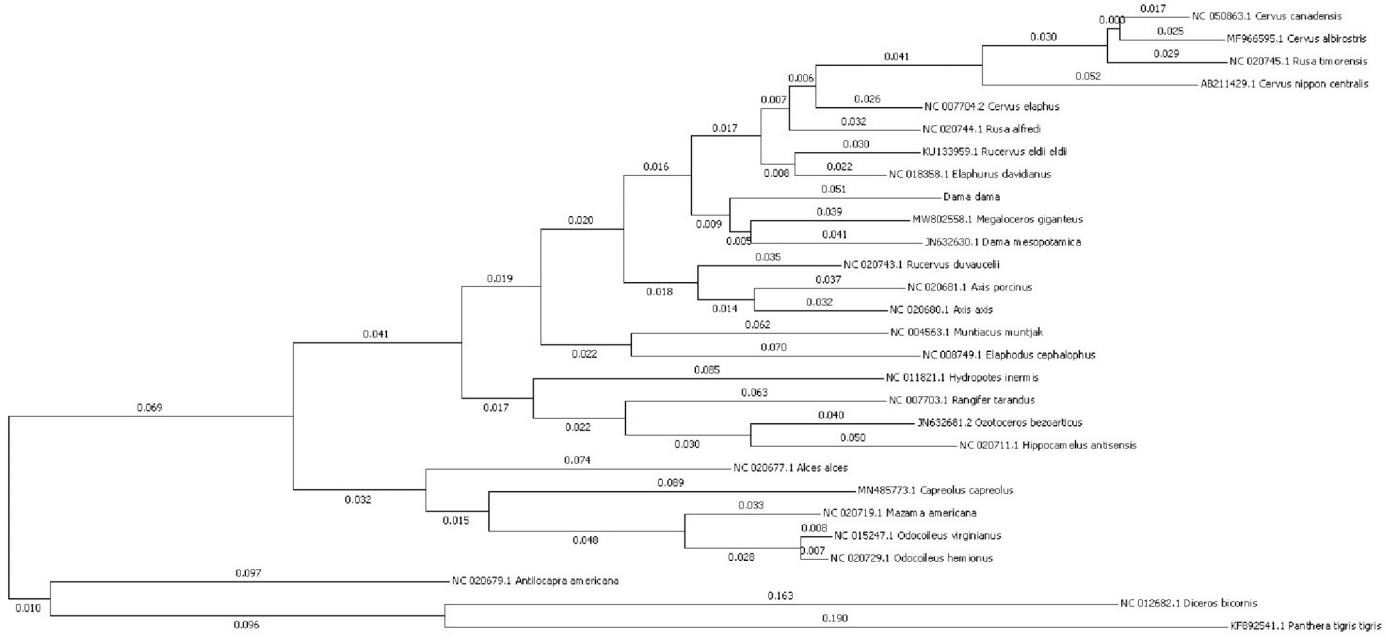

Fig. 4. Molecular phylogenetic analysis by Maximum Likelihood method.

understand the species evolutionary status and relationship with other Cervidae to help unpiece why this species has such low genetic variation.

It is well documented that different molecular markers tend to have different evolutionary rates across taxa (Huang, 2008; Frank et al., 2016). Mutation rates are important when reconstructing phylogenies using single genes or short sequences of genes as it may cause errors or inaccuracies when producing phylogenetic trees (Huang, 2008; Frank et al., 2016). Using the complete mitochondrial genome for analysis, the results are more robust and a greater degree of support is provided for the study of evolutionary relationships between individuals and between species. The protein coding genes identified within the *Dama dama* mitochondrial genome, have the similar positions and lengths to that of other Cervidae species (Wada et al., 2010; Ju et al., 2015; Frank et al., 2016; Tabasum et al., 2017; Sarvani et al., 2018). The start and stop codon details can be found in Table 2. For the most part, the start codons are either ATG or ATA which are methionine start codons, apart from ND6 which has a TTA start codon. Furthermore, the stop codons are mainly all complete with only two truncated to AT (COX3 and ND6), truncation of stop codons is common in animal mitochondrial genomes (Frank et al., 2016). The secondary structures of the 22 tRNA genes located within the *Dama dama* mitochondrial genome can be found in Fig. 3. Looking at the structure of $tRNA^{ser}$ (Serine S1) closely it can be seen that there is a missing DHU arm, this has also been reported in other deer species (Frank et al., 2016). The sequencing of the mitochondrial genome of the fallow deer using NGS has provided highly reliable genome data which includes the full control region. This non-coding region is important for in-depth population studies due to the high mutation rate and conserved regions (Bronstein et al., 2018).

A Phylogenetic trees were drawn using the maximum likelihood method, see Fig. 4. Several species of Cervidae have grouped together within a clade, therefore all those arranged on the same node share a common ancestor (Baum, 2008). For example, all deer from the *Cervus* genus are all grouped together on the same node of the tree. On the same outer node as the *Cervus* genus is *Dama dama*. *Dama dama* shares a node with two other species which have formed a separate clade, *Dama mesopotamica* and *Megaloceros giganteus*. It is documented that *Dama mesopotamica*, a critically endangered species, is a sub-species of *Dama dama* (Werner et al., 2015; Baker et al., 2017). The findings from the phylogenetic tree presented in this study support this claim. With *Dama dama* being on the same node but on its own branch, not part of the *Dama mesopotamica* clade. From this, it can be concluded that there is enough genetic variation between these two mitochondrial genomes that they are no longer the same species and that genetic divergence has occurred. This has most likely been caused by the founder effect, where a population of *Dama dama* became separated and formed a new gene pool, forming *Dama mesopotamica* (Baker et al., 2024b). Furthermore, as *Dama mesopotamica* is now critically endangered and is only found naturally in the middle-eastern areas, Natural selection could also have a part to play. Genetic drift and natural selection often both occur simultaneously to cause genetic divergence and result in the occurrence of a new sub-species. It is also interesting to note the close presence of the *Megaloceros giganteus* species with *Dama dama* and in particular *Dama mesopotamica*. *Megaloceros giganteus* shares a clade with *Dama mesopotamica* therefore are closely related, even more so than to *Dama dama*. *Megaloceros giganteus* is a much older species, dating back approximately 450,000 years ago, it is known as Irish elk or Giant deer, however it has also been called, the Giant Fallow deer, and with the findings from this study to support, it is likely that the *Megaloceros giganteus* is the common ancestor of *Dama dama* and *Dama mesopotamica* and the origin species of these two sub-species.

Estimation of divergence times was inferred using the RelTime method (Tamura et al., 2012) and the results can be viewed in Fig. 5. According to the time tree, the origin of the Cervidae sample group used in this study is estimated to be a mean value of 17.02 mya. This indicates that the Cervidae family dates back to later than previous evidence has indicated. The *Cervus* genus node, diverges from the *Dama* family node at 6.24 mya. The largest divergence time is 17.02 mya for *Antilocapra americana* and the smallest is 0.92 mya for the *Odocoileus* clade. Another more recent divergence time is 1.13 mya for the *Rusa/Cervus* clade. This suggests that *Rusa timorensis*, formerly known as *Cervus timorensis* (Pangau-Adam et al., 2022), is still a closely related species of the *Cervus* genus and may not belong to two separate species as the name suggests. With regards to *Dama dama*, the species divergence time from *Dama mesopotamica* and *Megaloceros giganteus* is 5.68 mya. Whereas the divergence time between *Dama mesopotamica* and *Megaloceros giganteus* is 5.35 mya. This suggests that there has been a divergence between *Dama dama*, which happened prior to the divergence of *Dama mesopotamica* and *Megaloceros giganteus*. This provides support for the distinction between *Dama mesopotamica* and *Dama dama* as sub-species

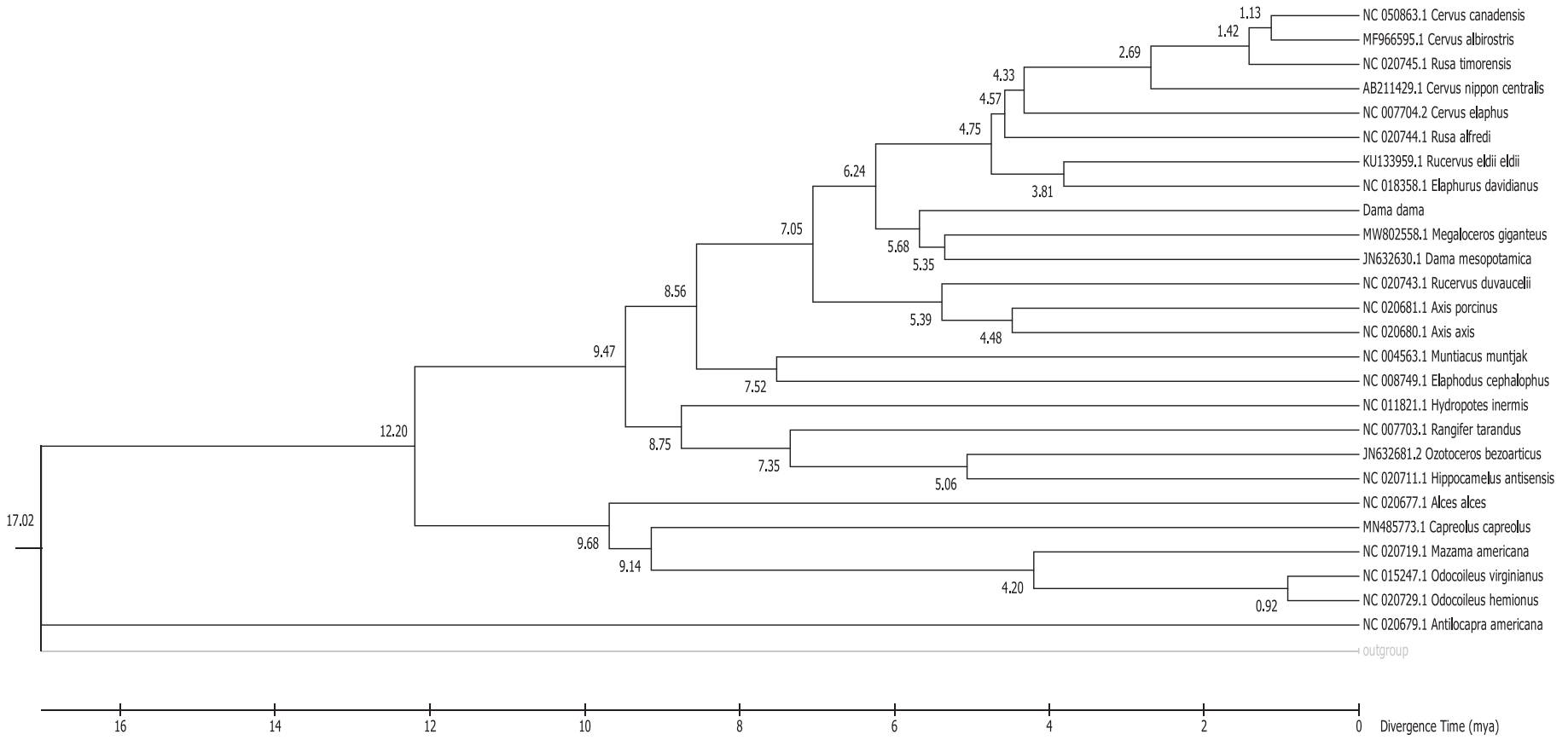

Fig. 5. A TimeTree inferred using the RelTime method to determine the evolutionary relationships of taxa (Tamura et al., 2012).

(Baker et al., 2017). The divergence time between *Dama mesopotamica* and *Megaloceros giganteus* is small, compared to that of the Cervidae family, this provides evidence for the theory that they are less of two separate species but more of the same species, much more closely related than some previously documented and providing support for a sister-group relationship with a shared common ancestor (Lister et al., 2005). It suggests that as *Megaloceros giganteus* is the older of the *Dama* species, *Dama mesopotamica* has held onto more of the ancestral genetic sequence than *Dama dama*, and that it is *Dama dama* that diverged first and has mutated away from the ancestral lineage.

Overall, the phylogenetic and divergence time analysis has provided support for previously published theories but has also given a new perspective on the divergence of the *Dama dama* species and its sub-species *Dama mesopotamica* which has been a popular topic over the years due to the rapid decline of the *Dama mesopotamica* species. The overall divergence times of the Cervidae family studies in this report are concordant with that reported by Frank et al. (2016). However, Frank et al. (2016) notes the complexities around divergence estimation for the Cervidae family, as the figures reported here, as within their paper, are higher than previously reported and gathered from fossil data. Fossil records for *Dama dama* date back to the Mesolithic/neolithic times, which was 9000–4000 years ago (Breda and Lister, 2013; Baker et al., 2017). The divergence times for *Dama dama* from other Cervidae species supports this, at 6.24 mya. This study has also provided a new perspective on the ancestral origin of the *Dama* genus, which can be further investigated using the *Dama dama* mitochondrial genome presented in this report.

Ethics statement

Appropriate Ethical clearance was obtained from The Animal Welfare and Ethics Review Board at the University of Central Lancashire, for the collection of the male sample used in this study, from the *Dama dama* species and the undertaking of the project. The experiment was undertaken in accordance with the EU Directive 2010/63/EU for animal experiments involving wildlife. No Animals were killed or harmed for the sole purpose of this study; samples were taken as a bi-product of the annual deer cull season.

CRediT authorship contribution statement

Rebecca Barnard: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Writing – original draft, Writing – review & editing. **Judith Smith:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The new mitochondrial genome for *Dama dama* has been deposited in GenBank, GenBank: JASJUW010000034.2 (https://www.ncbi.nlm.nih.gov/nuccore/JASJUW010000034.2?report=fasta). This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JASJUW000000000. The version described in this paper is version JASJUW020000000. The other datasets analysed during the current study are available in GenBank, as referenced in Table 1.

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