Complete mitochondrial genome sequence of *Lepus yarkandensis* Günther, 1875 (Lagomorpha, Leporidae): characterization and phylogenetic analysis

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Abstract

*Lepus yarkandensis* is a national second-class protected animal endemic to China and distributed only in the hot and arid Tarim Basin in Xinjiang. We sequenced and described the complete mitogenome of *L. yarkandensis* to analyze its characteristics and phylogeny. The species’ DNA is a 17,047 bp circular molecule that includes 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes, and one control region. The overall base composition was as follows: A, 31.50%; T, 29.40%; G, 13.30% and C, 25.80%, with a high A+T bias of 60.9%. In the PCGs, ND6 had deviation ranges for AT skew (–0.303) and GC skew (0.636). The Ka/Ks values of ND1 (1.067) and ND6 (1.352) genes were >1, indicating positive selection, which might play an important role in the adaptation of *L. yarkandensis* to arid and hot environments. The conserved sequence block, the central conserved domain, and the extended termination-associated sequences of the control region and their features were identified and described. The phylogenetic tree based on the complete mitogenome showed that *L. yarkandensis* was closely related to the sympatric *Lepus tibetanus pamirensis*. These novel datasets of *L. yarkandensis* can supply basic data for phylogenetic studies of *Lepus* spp., apart from providing essential and important resource for further genetic research and the protection of this species.

Keywords

mitogenome, molecular phylogeny, synonymous/non-synonymous substitution, Yarkand hare
Introduction

The Yarkand hare (Lepus yarkandensis) is endemic to China and is restricted to scattered oases around the Taklamakan Desert in the Tarim Basin of Xinjiang (Luo 1988; Smith et al. 2008, 2018). These hares live in hot, arid environments with scarce food and open terrain. Thus, this species is highly morphologically specialized, with smaller bodies, longer ears, and larger tympanic bullae than other Lepus species in China (Shan et al. 2011; Wu et al. 2011). This species is also listed as a second-class protected animal (Wang 1998). Several studies have been published on L. yarkandensis, including its morphology, skull morphometrics, genetic diversity, and genetic structures based on partial mitochondrial DNA (mtDNA) markers, microsatellites, and several nuclear genes (Li et al. 2005; Li et al. 2006; Aerziguli et al. 2010; Shan et al. 2011). The complete mtDNA sequence of L. yarkandensis has been reported (Huang et al. 2019), but without the details given of its characteristics, particularly those adapting to such extremely arid environments.

Characterized by small size, stable gene content, high evolutionary rate, relatively conserved gene arrangement, high information content, and maternal inheritance, animal mitogenomes are powerful tools used to investigate molecular evolution, phylogenetic relationships, and protective biology for many animals (Yu et al. 2017; Zhang et al. 2018; Song et al. 2019; Hu et al. 2020; Wu et al. 2020).

In the present study, we successfully sequenced and characterized the complete mtDNA of L. yarkandensis, including its base composition, gene structure, and arrangement of protein-coding genes (PCGs) and a control region. We also constructed a phylogenetic tree based on complete mitogenome sequences to elucidate the relationship of L. yarkandensis with other Lepus spp. Therefore, this study provides essential scientific data and contributes to population genetics, adaptation, and phylogenetic studies of L. yarkandensis.

Materials and methods

A male adult L. yarkandensis was collected from Alar, Xinjiang, China (40°34'00"N, 81°19'33"E) on 24 December 2016. Complete mtDNA was extracted from muscle tissue using standard phenol-chloroform (Psifidi et al. 2010). The complete mitogenome of the species was sequenced by next-generation sequencing using an Illumina HiSeq platform by Hengchuang Gene Technology Co., Ltd (Shenzhen, China) and assembled using SOAPdenovo 12.04 (Luo et al. 2012). The genome structure was mapped using the CGView software (Stothard et al. 2005). The complete mitogenome sequences of 25 other lagomorph species were downloaded from GeneBank (Table 1). The base composition, Ka and Ks (Ka, Ks, Ka/Ks) values, and composition skew were analyzed using MEGA7, together with the following formulas: AT skew = [A − T]/[A + T] and GC skew = [G − C]/[G + C] (Perna et al. 1995). A conserved sequence block (CSB) in the control region was identified based on previously published se-
Table 1. Lagomorph mitogenomes used in the phylogenetic analysis of the present study.

| Name                        | Accession number | Collection places       | Size  |
|------------------------------|------------------|-------------------------|-------|
| Lepus americanus1            | NC024043         | Montana, USA            | 17042 |
| Lepus americanus2            | KJ397613         | Montana, USA            | 17042 |
| Lepus capensis               | GU937113         | Yancheng, Jiangsu       | 17722 |
| Lepus coreanu                | KF040450         | Incheon, Korea          | 17472 |
| Lepus europaeus1             | AJ421471         | Skane, Sweden           | 17734 |
| Lepus europaeus2             | KY211025         | North-east Greece       | 16680 |
| Lepus gomontensis1           | NC024042         | León, Spain             | 16916 |
| Lepus gomontensis2           | KJ397610         | León, Spain             | 16916 |
| Lepus hainanus               | JQ219662         | Hainan, China           | 16646 |
| Lepus timidus                | KM362831         | Hefei Anhui             | 17438 |
| Lepus timidus1               | KR019013         | Harbin, Heilongjiang    | 17762 |
| Lepus timidus2               | KJ397605         | Finland                 | 17755 |
| Lepus timidus3               | KR030070         | Harbin, Heilongjiang    | 17748 |
| Lepus timidus4               | KR030072         | Harbin, Heilongjiang    | 17749 |
| Lepus timidus5               | KR030069         | Harbin, Heilongjiang    | 17744 |
| Lepus timidus6               | KR013248         | Harbin, Heilongjiang    | 17759 |
| Lepus tolai                  | KM609214         | Hefei Anhui             | 17472 |
| Lepus townsendii1            | NC024041         | Wyoming, USA            | 17732 |
| Lepus townsendii2            | KJ397609         | Wyoming, USA            | 17732 |
| Lepus yarkandensis1          | MG279351         | Alar, Xinjiang          | 17047 |
| Ochotona carozaniae          | EF535828         | Qinghai, China          | 17313 |
| Ochotona collaris            | AF348080         | Not mentioned           | 16968 |
| Ochotona princeps            | AJ537415         | Not mentioned           | 16481 |
| Oryctolagus cuniculus        | AJ001588         | Not mentioned           | 17245 |

Results and discussion
Mitochondrial genome organization

The mitogenome of *L. yarkandensis* was a circular, double-stranded DNA molecule 17047 bp in size (GenBank accession number: MG279351) which is slightly longer than reported *L. yarkandensis* (MN450151) with 17011 bp (Huang et al. 2019). It contained all 37 typical vertebrate mitogenomes—13 PCGs, two rRNA genes, 22 tRNA genes, and one control region—among which 28 genes were encoded on the heavy strand (H strand), except for eight tRNA genes and the ND6 gene (Fig. 1; Table 2). Eleven
overlapping nucleotides with lengths ranging from 1 bp to 47 bp were present in the *L. yarkandensis* mitogenome, comprising a total length of 140 bp, with the longest nucleotide located between ND4 and tRNA-His. Moreover, 70 bp of intergenic spacer sequences spread over 12 regions in the hare mitogenome, ranging from 1 bp to 32 bp in size, with the longest was located between tRNA-Asn and tRNA-Cys (Table 2).

**Genome composition and skewness**

AT skew, GC skew, and A + T content were selected as parameters for investigating the pattern of the mitogenome nucleotide composition (Wei et al. 2010; Hassanim et al. 2005). The *L. yarkandensis* mitogenome had a base nucleotide composition of 31.50% for A,
Table 2. Mitochondrial genome organization of *Lepus yarkandensis*.

| Gene name          | Position | Size (bp) | Location | Codon | Intergenic nucleotide bp |
|--------------------|----------|-----------|----------|-------|-------------------------|
|                    | From     | To        | H/L strand | Start | Stop        |
| tRNA-Phe           | 1        | 67        | H         | 0     |
| t2S rRNA           | 68       | 1022      | H         | 0     |
| tRNA-Val           | 1023     | 1088      | H         | 0     |
| t1S rRNA           | 1087     | 2668      | H         | –2    |
| tRNA-Leu (UUR)     | 2669     | 2743      | H         | 0     |
| ND1                | 2746     | 3702      | H         | 0     |
| tRNA-Ile           | 3767     | 3838      | L         | 0     |
| tRNA-Met           | 3848     | 3916      | H         | 0     |
| ND2                | 3917     | 4960      | H         | 0     |
| tRNA-Arg           | 4966     | 5032      | H         | 0     |
| tRNA-Ala           | 5035     | 5101      | L         | 0     |
| tRNA-Asn           | 5102     | 5174      | L         | 0     |
| tRNA-Cys           | 5207     | 5273      | L         | 0     |
| tRNA-Tyr           | 5274     | 5339      | L         | 0     |
| COI                | 5347     | 6888      | H         | 0     |
| tRNA-Ser (UCN)     | 6891     | 6959      | L         | 0     |
| tRNA-Asp           | 6963     | 7031      | H         | 0     |
| COII               | 7032     | 7715      | H         | 0     |
| RNA-Lys            | 7719     | 7789      | H         | 0     |
| ATP8               | 7791     | 7994      | H         | 0     |
| ATP6               | 7952     | 8632      | H         | 0     |
| COIII              | 8632     | 9435      | H         | 0     |
| tRNA-Gly           | 9416     | 9485      | H         | 0     |
| ND3                | 9486     | 9842      | H         | 0     |
| tRNA-Arg           | 9833     | 9899      | H         | 0     |
| ND4                | 9901     | 10197     | H         | 0     |
| tRNA-His           | 11569    | 11637     | H         | 0     |
| tRNA-Ser (AGY)     | 11638    | 11696     | H         | 0     |
| tRNA-Leu (CUN)     | 11697    | 11766     | H         | 0     |
| ND5                | 11767    | 13578     | H         | 0     |
| ND6                | 13575    | 14099     | L         | 0     |
| tRNA-Glu           | 14100    | 14167     | L         | 0     |
| Cytb               | 14171    | 15310     | H         | 0     |
| tRNA-Thr           | 15310    | 15377     | H         | 0     |
| tRNA-Pro           | 15378    | 15443     | L         | 0     |
| D-Loop             | 15444    | 17047     | H         | 0     |

(Overlap is denoted as “−”. Spacer regions are denoted as “+”. No overlap or interval is denoted as “0”.)

29.40% for T, 13.30% for G, and 25.80% for C, with an A+T bias of 60.90%. Moreover, A and C were more popular than T and G with overall AT skew = 0.034 and GC skew = –0.320 in the entire *L. yarkandensis* mitogenome (Table 3). These overall genome composition and skewness are highly similar to those of other *Lepus* spp., such as *L. yarkandensis* (MN450151), *Lepus coreanus* and *Lepus tolai* (Yu et al. 2015; Huang et al. 2019; Shan et al. 2020). However, in species such as *Caenorhabditis elegans*, *Ascaris suum*, and *Mytilus edulis*, different AT and GC skew values were determined-negative AT skew and positive GC skew (Perna et al. 1995). In *Arbacia lixula* and *Anopheles cracens*, both AT and GC skews were negative (Perna et al. 1995; Mao et al. 2019). Moreover, an AT-rich region
is typically observed in vertebrates (Quinn et al. 1993; Zhao et al. 2016; Sarvani et al. 2018). Thus, this variation in AT and GC skews shows a degree of similarity within the same genus but not in different classes, which can also be used as an auxiliary reference for evaluating phylogenetic relationships.

### Protein-coding genes

The total length of PCGs in the *L. yarkandensis* mitogenome was 11,417 bp, and its base composition was 30.50% for A, 30.90% for T, 12.00% for G, and 26.50% for C with an A+T bias of 61.40%. Among the 13 PCGs, 12 were located on the heavy strand (H strand), whereas ND6 was located on the light strand (Tables 2, 3), as observed in other *Lepus* species (Ding et al. 2014; Shan et al. 2020).

The skewness of the entire PCGs in *L. yarkandensis* (Table 3) indicated a higher occurrence of T than A, with a negative AT skew (–0.007), and C than G with a negative GC skew (–0.337) (Table 3). The negative AT skew value was inconsistent with that for most mammals, which had positive AT skew values (Sarvani et al. 2018; Priyono et al. 2020). However, the result of the current study is highly similar to the result obtained for *Camelus dromedarius* (both AT and GC skews were negative), a heat-tolerant mammal (Sarvani et al. 2018; Manee et al. 2019).

To further estimate and understand the level of base bias between all PCGs, we calculated the AT and GC skew ratios for each PCG in the mtDNA genome of *L. yarkandensis* (Fig. 2). All values for the skewness of GC (except for ND6) in PCGs were negative, with C being more prevalent that G in the nucleotide composition. The ATP6, ATP8, ND2, and ND3 genes had positive AT skews, whereas the remaining genes (9 of 13) had negative values. Notably, ND6 had deviation ranges for AT skew (–0.303) and GC skew (0.636) when compared with the other 12 PCGs in the *L. yarkandensis* mtDNA sequence, and the deviation range is highly similar to some mammals, such as *Moschiola indica*, *Camelus dromedarius*, and *Bubalus quarlesi* (Sarvani et al. 2018; Manee et al. 2019; Priyono et al. 2020).

As with the vertebrate mtDNA genome, the majority of PCGs in the *L. yarkandensis* mitogenome used ATG as the start codon, although ND2, ND3, and ND5 used ATT as the start codon. Most PCGs used typical stop codons (TAA for ND2, COI, COII, ATP8, ATP6, ND4L, and ND5; TAG for ND6 and COII), whereas a small

| Table 3. Nucleotide composition and skewness of the *Lepus yarkandensis* mitogenome. |
|-----------------------------------------------|
| **A%** | **T%** | **G%** | **C%** | **Size** | **A+T%** | **ATskew** | **GCskew** |
| Total PCGs | 30.50 | 30.90 | 12.00 | 26.50 | 11417 | 61.40 | –0.007 | –0.377 |
| Overall | 31.50 | 29.40 | 13.30 | 25.80 | 17047 | 60.90 | 0.034 | –0.320 |
| rRNAs | 36.10 | 24.70 | 17.80 | 21.40 | 2535 | 60.80 | 0.188 | –0.092 |
| tRNAs | 31.20 | 29.90 | 12.30 | 26.70 | 8295 | 61.10 | 0.021 | –0.369 |
| D-Loop | 28.70 | 27.40 | 13.00 | 30.90 | 1604 | 56.10 | 0.023 | –0.408 |
| CDs | 21.80 | 27.10 | 21.10 | 30.0 | 317 | 48.90 | –0.108 | –0.174 |
| CSB | 30.00 | 26.2 | 11.4 | 32.4 | 920 | 56.2 | 0.068 | –0.480 |
| ETAS | 31.60 | 30.80 | 9.80 | 27.80 | 367 | 62.40 | 0.013 | –0.479 |

is typically observed in vertebrates (Quinn et al. 1993; Zhao et al. 2016; Sarvani et al. 2018). Thus, this variation in AT and GC skews shows a degree of similarity within the same genus but not in different classes, which can also be used as an auxiliary reference for evaluating phylogenetic relationships.
number of abnormal stop codons were observed, including AGG (Cytb), T (ND1, COII, ND4), and TA (ND3). Moreover, nine of 13 PCGs had complete stop codons, and four genes had incomplete stop codons (Table 2), which could be completed via posttranscriptional polyadenylation (Anderson et al. 1981; Ojala et al. 1981). Both PCGs of our *L. yarkandensis* (MG279351) and reported Yarkand hare (MN450151) have identical start and end codons, but different skewness.

The Ka, Ks, and Ka/Ks values of PCGs were estimated using substitution rates (Fig. 3). If Ka/Ks > 1, a positive selection effect was considered; if Ka/Ks = 1, a neutral effect was assumed; and if Ka/Ks < 1, purification selection was considered (Hurst et al. 2002). Except for ND1 and ND6, all PCGs in *L. yarkandensis* had average Ka/Ks values < 1, indicating purification selection. Meanwhile, for ND1 and ND6, Ka/Ks > 1 indicated positive selection. The function of the mitochondrial genome is crucial because it mainly undergoes evolutionary neutral or purifying selection. Other studies have reported that mitochondrial genes are also influenced by positive selection, particularly in animals adapting to harsh environments (Luo et al. 2008; Hichem et al. 2017; Jin et al. 2018). In the present study, positive selection in ND1 and ND6 might be beneficial to organisms and may confer to *L. yarkandensis* the ability to adapt to harsh and arid environments.

**Control region**

The control region 1604 bp in length was organized between *trnP* and *trnF* genes in the *L. yarkandensis* mitogenome (Table 2; Fig. 4). In vertebrate mitogenomes, the control region is a noncoding segment and consists of several control elements. These elements regulate genome replication and transcription (Boore 1999). In the current study, we successfully identified several highly conserved domains within the control region of the *L. yarkandensis* mitogenome-conserved sequence blocks (CSB) I–III, con-
served domain (CD), and extended termination associated sequence (ETAS) I–II—on the basis of their homology with other members of Lagomorpha and mammals (Elisabetta et al. 1997) (Table 4; Fig. 4). Characteristic motifs were used to detect the CSB domains: CSBI (GACATA), CSBII (CAAACCCCCC), and CSBIII (TGCCAAACCCCAAAAAC) (Gemmell et al. 1996; Elisabetta et al. 1997). We found from the sequence alignment results among hares and other mammals (Elisabetta et al. 1997) that more variations existed in Yarkand hare, including base insertions and deletions in the whole control region. CD was conservative with a narrow length range. The ETAS and

**Figure 3.** Evolutionary rates of the *Lepus yarkandensis* mitogenome by Ka/Ks.

**Figure 4.** A schematic of the structural organization of the mitochondrial control region in *Lepus yarkandensis*. Control region flanking genes tRNA-Phe and tRNA-Pro presented in red. Conserved elements in the control region denoted by gray boxes: TAS, termination associated sequence; CD, central conserved domain; CSB, conserved sequence block. SR, short repeat; LR, long repeat.
CSB regions widely varied in the length of the control region, which is also the main reason for variations in mitogenome size in different species (Xu et al. 2012). In CSB regions, CSB1 and CSB3 were relatively conservative, and CSB2 widely varied in L. yarkandensis. This finding contradicted the results for Felis catus and Mustelidae species (Elisabetta et al. 1997l; Zhang et al. 2009). In the present study, an ACCCCC motif in the ETAS I sequence of L. yarkandensis was found, similar to that of the horseshoe bat (Sun et al. 2009). In some taxa such as species of Mustelidae, cattle, and Cervidae, the sequences were GCCCCC (Zhang et al. 2009; Douzary et al. 1997). Between CSB I and CSB II, a number of short tandem repeat motifs, which commonly characterize mitogenomes, were observed in the L. yarkandensis mitogenome (Ren et al. 2009). The short repeat CGTCTACGCGACGTACACCCA was 22 bp with 14 repetitions (Table 4), whereas the long repeat ACAATACGACATAGCAGCCTTCTCTCTTCTCCAACAGGCTAAACACACATTAGATTACACCTTCTTCTTCCTAATATTATTTCACAAAA occurred twice. Similarly, the short repeats CSB3 CGTCTACGCGACGTACACCCA in L. yarkandensis (Fig. 4) occurred twice, which was also found in other Lepus species in this study. Notably, tandem repeats have been described in the control region of metazoans (Lunt et al. 1998; Rand et al. 1993; Yokobori et al. 2004) and the family Veneridae.

Transfer RNAs and ribosomal RNAs

Except for tRNA-ser (AGY), which lacked a D stem, the other 21 tRNAs formed complete secondary structures (Suppl. material 1). Aberrant loops have been found in some tRNA genes. These mismatches could be rectified by the post-transcriptional RNA-editing mechanism to maintain tRNA functions (Tomita et al. 2002).

Phylogenetic analysis

We constructed NJ and Bayesian trees based on the complete mtDNA genome of L. yarkandensis in this study and 25 other lagomorphs published on NCBI (Fig. 5). The topological structures of both trees were consistent and supported by high bootstrap values. The phylogenetic tree confirmed the existence of three distinct lineages—hares, rabbits, and pikas—which is consistent with Smith et al. (2018). In the present study, L. yarkandensis was not closely related to neither Lepus europaeus nor Lepus americanus.
but was closely related to *Lepus tibetanus* in Xinjiang, China. The latter was misnamed as *Lepus capensis pamirs* in our previous study (Shan et al. 2015) and was renamed by Smith et al. (2018). Our *L. yarkandensis* and *L. yarkandensis* (MN450151) were clustered on the same branch. One reason for this close relationship could be the relatively close habitat. *Lepus t. pamirensis* are mainly distributed in the Pamir plateau of southeastern Kashgar, Xinjiang, China, bordering the Tarim Basin. The *L. yarkandensis*
sample used in the current study was from Alar City in western Tarim Basin, which is near the *L. t. pamirensis* distribution. Another reason could be similarly extreme environments. Both habitats are dry with scarce rainfall and a lack of food (Shan et al. 2011). However, the phylogenetic relationship between *L. yarkandensis* and *L. t. pamirensis* remains uncertain, as hybridization has occurred between them (Wu et al. 2011). Further analysis with more samples and more extensive markers is required.

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**References**

Aerziguli S, Mairepati P, Dilixiati A, Mahemut H (2010) Comparison of skull morphological characters of Yarkand Hare (*Lepus yarkandensis*) of different geographical populations. Xinjiang Agricultural Sciences 47: 1627–1631.

Anderson S, Bankier AT, Barell BG, De-Bruijn MH, Coulson AR, Drouin J (1981) Sequence and organization of the human mitochondrial genome. Nature 290: 457–465. https://doi.org/10.1038/290457a0

Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Research 27: 1767–1780. https://doi.org/10.1093/nar/27.8.1767

Ding L, Chen CM, Wang H, Zhang BW (2014) Complete mitochondrial DNA sequence of *Lepus tolai* (*Leporidae: Lepus*). Mitochondrial DNA 27: 1711–1712. https://doi.org/10.3109/19401736.2014.982568

Douzery E, Randi E (1997) The mitochondrial control region of Cervidae: evolutionary patterns and phylogenetic content. Molecular Biology and Evolution 14: 1154–1166. https://doi.org/10.1093/oxfordjournals.molbev.a025725

Elisabetta S, Tanzariello F, Reyes A, Pesole G, Saccone C (1997) Mammalian mitochondrial d-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. Gene 205: 125–140. https://doi.org/10.1016/S0378-1119(97)00404-6

Gemmell NJ, Western PS, Watson JM (1996) Evolution of the mammalian mitochondrial control region-comparisons of control region sequences between monotreme and therian mammals. Molecular Biology and Evolution 13: 798–808. https://doi.org/10.1093/oxfordjournals.molbev.a025640

Hassain, A, Léger N, Deutsch J (2005) Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of Metazoa, and consequences for phylogenetic inferences. Systematic Biology 54: 277–298. https://doi.org/10.1080/10635150590947843
Hichem BS, Helmut S, Felix K, Franz S (2017) Selection on the mitochondrial ATP synthase6 and the NADH dehydrogenase 2 genes in hares (Lepus capensis L., 1758) from a steep ecological gradient in North Africa. BMC Evolutionary Biology 17: e46. https://doi.org/10.1186/s12862-017-0896-0

Hu CY, Wang SB, Huang BS, Liu HG, Xu L, Hu ZG, Liu YF (2020) The complete mitochondrial genome sequence of Scolopendra mutilans L. Koch, 1878 (Scolopendromorpha, Scolopendridae), with a comparative analysis of other centipede genomes. ZooKeys 925: 73–88. https://doi.org/10.3897/zookeys.925.47820

Huang YL, Chen YX, Guo HT, Xu YH, Liu HY, Liu DW (2019) The complete mitochondrial genome sequence of Yarkand hare (Lepus yarkandensis). Mitochondrial DNA Part B 4: 3727–3728. https://doi.org/10.1080/23802359.2019.1681321

Hurst LD (2002) The Ka/Ks ratio: Diagnosing the form of sequence evolution. Trends in Genetics 18: 486–487. https://doi.org/10.1016/S0168-9525(02)02722-1

Jin YT, Wo YB, Tong HJ, Song S, Zhang LX, Brown RP (2018) Evolutionary analysis of mitochondrially encoded proteins of toad-headed lizards, Phrynocephalus, along an altitudinal gradient. BMC Genomics 19: e185. https://doi.org/10.1186/s12864-018-4569-1

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Li Z, Xia L, Li Y, Yang Q, Liang M (2006) Mitochondrial DNA variation and population structure of the yarkand hare Lepus yarkandensis. Acta Theriologica 51: 243–253. https://doi.org/10.1007/BF03192676

Li ZC, Xia L, Yang QS, Liang MY (2005) Population genetic structure of the Yarkand hare (lepus yarkandensis). Acta Theriologica Sinica 25: 224–228.

Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic acids research 25: 955–964. https://doi.org/10.1093/nar/25.5.955

Lunt DH, Whipple LE, Hyman BC (1998) Mitochondrial DNA variable number tandem repeats (VNTRs): utility and problems in molecular ecology. Molecular Ecology 7: 1441–1455. https://doi.org/10.1046/j.1365-294x.1998.00495.x

Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He GZ, Chen YX, Pan Q, Liu YJ, Tang JB, Wu GX, Zhang H, Shi YJ, Liu Y, Yu C, Wang B, Lu Y, Han CL, Cheung DW, Yiu SM, Peng SL, Zhu XQ, Liu GM, Liao XK, Li YR, Yang HM, Wang J, Lam TW, Wang J (2012) Soapdenovo2: an empirically improved memory-efficient short-read de novo assembler. Giga Science 1: 1–18. https://doi.org/10.1186/2047-217X-1-18

Luo YJ, Gao WX, Gao YQ, Tang S, Huang QY, Tan XL, Chen J, Huang TS (2008) Mitochondrial genome analysis of Ochotona curzoniae and implication of cytochrome c oxidase in hypoxic adaptation. Mitochondrion 8: 352–357. https://doi.org/10.1016/j.mito.2008.07.005

Luo ZX (1988) The Chinese Hare. China Forestry Publishing House, Beijing.

Manee MM, Alshehri MA, Binghadir SA, Aldhafer SH, Alswailem RM, Algarni AT, Al-Shomrani BM, Al-Fageeh MB (2019) Comparative analysis of camelid mitochondrial genomes. Journal of Genetics 98: e88. https://doi.org/10.1007/s12041-019-1134-x
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Mao QM, Li TJ, Fu WB, Yan ZT, Chen B (2019) Sequencing of the complete mitochondrial genome of *Anopheles lindesayi* and a phylogenetic analysis of the genus *Anopheles* (Diptera: Culicidae) based on mitochondrial genomes. ACTA Entomologica Sinica 62: 101–116.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature 290: 470–474. https://doi.org/10.1038/290470a0

Perna NT, Kocher TD (1995) Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. Journal of Molecular Evolution 41: 353–358. https://doi.org/10.1007/BF01215182

Priyono DS, Solihin DD, Farajallah A, Purwantara B (2020) The first complete mitochondrial genome sequence of the endangered mountain anoa (*Bubalus quarlesi*) (Artiodactyla: Bovidae) and phylogenetic analysis. Journal of Asia-Pacific Biodiversity 13: 123–133. https://doi.org/10.1016/j.japb.2020.01.006

Psifdi A, Dovas CI, Banos G (2010) A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. Mol Cell Probes 24: 93–98. https://doi.org/10.1016/j.mcp.2009.11.001

Quinn TW, Wilson AC (1993) Sequence evolution in and around the mitochondrial control region in birds. Journal of Molecular Evolution 37: 417–425. https://doi.org/10.1007/BF00178871

Rand DM (1993) Endotherms, ectotherms, and mitochondrial genome-size variation. Journal of Molecular Evolution 37: 281–295. https://doi.org/10.1007/BF00175505

Ren J, Shen X, Sun M, Jiang F, Yu Y, Chi Z (2009) The complete mitochondrial genome of the clam *Meretrix petechialis* (Mollusca: Bivalvia: Veneridae). Mitochondrial DNA 20: 78–87. https://doi.org/10.1080/19401730902964425

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Song R, Zhang D, Gao JW, Cheng XF, Xie M, Li H, Wu YA (2019) Characterization of the complete mitochondrial genome of *Brentisentis yangtzensis* Yu & Wu, 1989 (Acanthocephala, Illiosentitidae). ZooKeys 861: 1–14. https://doi.org/10.3897/zookeys.861.34809

Sun KP, Feng J, Jin LR, Liu Y, Shi LM, Jiang TL (2009) Structure, DNA sequence variation and phylogenetic implications of the mitochondrial control region in horseshoe bats. Mammalian Biology 74: 130–144. https://doi.org/10.1016/j.mambio.2008.09.002

Sarvani R K, Parmar D R, Tabasum W, Thota N, Sreevivas A, Gaur A (2018) Characterization of the complete mitogenome of Indian Mouse Deer, *Moschiola indica* (Artiodactyla: Tragulidae) and its evolutionary significance. Scientific reports 8: e2697. https://doi.org/10.1038/s41598-018-20946-5

Sbisà E, Tanzariello F, Reyes A, Pesole G, Saccone C (1997) Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. Gene 205: 125–140. https://doi.org/10.1016/S0378-1119(97)00404-6
Shan WJ, Liu J, Yu L, Robert WM, Mahmut H, Zhang YP (2011) Genetic consequences of postglacial colonization by the endemic Yarkand hare (*Lepus yarkandensis*) of the arid Tarim Basin. Chinese Science Bulletin 56: 1370–1382. https://doi.org/10.1007/s11434-011-4460-9

Shan WJ, Liu YG (2015) The complete mitochondrial DNA sequence of the cape hare *Lepus capensis pamirensis*. Mitochondrial DNA 27: 4572–4573. https://doi.org/10.3109/19401736.2015.1101569

Shan WJ, Tursun M, Zhou SY, Zhang YC, Dai HY (2020) The complete mitochondrial genome sequence of *Lepus tolai* in Xinjiang. Mitochondrial DNA Part B 5: 1336–1337. https://doi.org/10.1080/23802359.2020.1735267

Smith AT, Johnston CH, Alves PC, Hacklander K (2018) Lagomorphs: Pikas, Rabbits, and Hares of the World. Johns Hopkins University Press, Baltimore.

Smith AT, Xie Y (2008) Mammal of China. Princeton university press, Princeton, New Jersey

Stothard P, Wishart DS (2005) Circular genome visualization and exploration using CGView. *Bioinformatics* 21: 537–539. https://doi.org/10.1093/bioinformatics/bti054

Tomita K, Yokobori S, Oshima T, Ueda T, Watanabe K (2002) The cephalopod *Loligo bleekeri* mitochondrial genome: multiplied noncoding regions and transposition of tRNA Genes. Journal of Molecular Evolution 54: 486–500. https://doi.org/10.1007/s00239-001-0039-4

Wang S (1998) China Red Data Book of Endangered Animals. Science Press, Beijing, 247 pp.

Wei SJ, Shi M, Chen XX, Sharkey MJ, van Achterberg C, Ye GY, He JH (2010) New Views on Strand Asymmetry in Insect Mitochondrial Genomes. *PloS ONE* 5: e12708. https://doi.org/10.1371/journal.pone.0012708

Wu Y, Xia L, Zhang Q, Yang Q, Meng X (2011) Bidirectional introgressive hybridization between *Lepus capensis* and *Lepus yarkandensis*. Molecular Phylogenetics & Evolution 59: 545–555. https://doi.org/10.1016/j.ympev.2011.03.027

Wu YA, Gao JW, Cheng XF, Xie M, Yuan XP, Liu D, Song R (2020) Characterization and comparative analysis of the complete mitochondrial genome of *Azygia huangtsiyui* Tsin, 1933 (Digenea), the first for a member of the family Azygiiidae. *ZooKeys* 945: 1–16. https://doi.org/10.3897/zookeys.945.49681

Xu XD, Wu XY, Yu ZN (2012) Comparative studies of the complete mitochondrial genomes of four *Paphia clams* and reconsideration of subgenus *Neotapes* (Bivalvia: Veneridae). *Gene* 494: 17–23. https://doi.org/10.1016/j.gene.2011.12.002

Yokobori S, Fukuda N, Nakamura M, Aoyama T, Oshima T (2004) Long-Term Conservation of Six Duplicated Structural Genes in Cephalopod Mitochondrial Genomes. Molecular Biology and Evolution 21: 2034–2046. https://doi.org/10.1093/molbev/msh227

Yu J, Nam B, Yoon J, Kim E B, Park J Y, Kim H, Yon SH (2017) Tracing the spatio-temporal dynamics of endangered fin whales (*Balaenoptera physalus*) within baleen whale (Mysticeti) lineages: a mitogenomic perspective. *Genetica* 145: 603–612. https://doi.org/10.1007/s10709-017-9988-4

Yu JN, Chung CU, Kwak M (2015) The complete mitochondrial genome sequence of the Korean hare (*Lepus coreanus*). Mitochondrial DNA 26: 129–130. https://doi.org/10.3109/19401736.2013.815170
Complete mitochondrial genome of *Lepus yarkandensis*

Zhao C, Zhang HH, Liu GS, Yang XF, Zhang J (2016) The complete mitochondrial genome of the *tibetan fox* (*vulpes ferrilata*) and implications for the phylogeny of Canidae. Comptes Rendus Biologies 339: 68–77. https://doi.org/10.1016/j.crvi.2015.11.005

Zhang H, Xu C, Ma J (2009) Structure of the mtDNA control region and phylogeny of the Mustelidae species. Journal of Ecology 29: 3585–3592.

Zhang QH, Huang P, Chen B, Li TJ (2018) The complete mitochondrial genome of *Orancistrocerus aterrimus aterrimus* and comparative analysis in the family Vespidae (Hymenoptera, Vespidae, Eumeninae). ZooKeys 790: 127–144. https://doi.org/10.3897/zookeys.790.25356

**Supplementary material I**

**Figure S1a, S1b**
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Data type: multimedia

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