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Repetitive sequences in Eurasian lynx (*Lynx lynx L.*) mitochondrial DNA control region

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Abstract
Mitochondrial DNA (mtDNA) control region (CR) of numerous species is known to include up to five different repetitive sequences (RS1–RS5) that are found at various locations, involving motifs of different length and extensive length heteroplasmy. Two repetitive sequences (RS2 and RS3) on opposite sides of mtDNA central conserved region have been described in domestic cat (*Felis catus*) and some other felid species. However, the presence of repetitive sequence RS3 has not been detected in Eurasian lynx (*Lynx lynx*) yet. We analyzed mtDNA CR of 35 Eurasian lynx (*L. lynx L.*) samples to characterize repetitive sequences and to compare them with those found in other felid species. We confirmed the presence of 80 base pairs (bp) repetitive sequence (RS2) at the 5′ end of the Eurasian lynx mtDNA CR L strand and for the first time we described RS3 repetitive sequence at its 3′ end, consisting of an array of tandem repeats five to ten bp long. We found that felid species share similar RS3 repetitive pattern and fundamental repeat motif TACAC.

Keywords: *Eurasian lynx*, Lynx lynx, *mtDNA, control region, repetitive sequences*

Introduction

Mitochondrial DNA (mtDNA) control region (CR) is a non-coding, fastest evolving sequence of the mitochondrial genome (Aquadro and Greenberg 1983; Brown et al. 1993). In mammals, it is usually located between tRNA^{Pro} and tRNA^{Phe} and it contains promoters for polycistronic RNA transcription of genes on both light and heavy strands, as well as the origin of DNA replication for the heavy strand. CR is a standard marker in population genetic studies of mammals (Nabholz et al. 2007; Galtier et al. 2009; Gomerčić et al. 2010).

The CR of most species has a central conserved region (CCR) that is surrounded by more variable A/T rich regions (Jae-Heup et al. 2001). Five different repetitive sequences (RS1–RS5) have been described within CR of numerous species (Buroker et al. 1990;

Hoelzel 1993; Hoelzel et al. 1994; Eberhard et al. 2001; Ferrando et al. 2004; Purdue et al. 2006; Wang et al. 2007). Repeats have been found at various locations, involving motifs of different length and extensive length heteroplasmy (Hoelzel et al. 1994).

The feline CR spans about 1560 base pairs (bp), from positions 16,315 to 17,009 and then from positions 1 to 865 (Lopez et al. 1996). Characteristic of domestic cat (*Felis catus*) mtDNA is the presence of two repetitive sequences (RS2 and RS3) on opposite sides of CCR (Lopez et al. 1996). These sequences are also found to be characteristic for other carnivore and especially felid species. The location of repetitive sequences in domestic cats is highly conserved compared to other mammalian CR repeats (Lopez et al. 1996). RS2 is located at the 5′ (left) end of the CR L strand and consists of three 80–82 bp monomers.

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RS3, a 294 bp-long repeat is located on the L strand 3′ end and a core unit of six to eight bp is imperfectly repeated 37 times (Lopez et al. 1996).

An 80-bp repetitive sequence (RS2) has been identified in Eurasian lynx (*Lynx lynx* L.) CR (Hellborg et al. 2002) and has been excluded from most of the population genetic studies as its high slippage rate often induces heteroplasmy (Hoelzel et al. 1994; Hellborg et al. 2002; Gugolz et al. 2008; Sindičić et al., in press). The presence of second (RS3) repetitive sequence, found in other felid species, has not been confirmed in Eurasian lynx yet. The goal of this paper was to characterize mtDNA CR repetitive sequence in Eurasian lynx. Additionally, we compared Eurasian lynx RS3 repetetive sequence to those found in other felid species. RS3 is unusual compared to other CR repetitive sequences; they differ in size, character, sequence of the repeated motif, and in the extent of variation and heteroplasmy (Hoelzel et al. 1994).

Materials and methods

We have analyzed 29 Eurasian lynx (*L. lynx* L.) samples representing a Dinaric population and six samples from Carpathian population (Slovakia). Samples were collected in the 1997–2009 period. Muscle samples (29) were taken during necropsies of animals killed by traffic, poaching, or disease, while six blood samples came from animals live-captured for radio tracking studies.

DNA was extracted using ChargeSwitch® gDNA Tissue Kit (Invitrogen, Life Technologies Corporation, Carlsbad, California, USA) and two non-overlapping fragments of CR were amplified by polymerase chain reaction (PCR). A 675-bp fragment comprising RS2 was amplified with primers L15997 (5′-CACCATTAGCACCCAAAGCT-3′) (Ward et al. 1991) and H16498 (5′-CCTGAAGTAAGAACCA-GATG-3′) (Meyer et al. 1990), while primers F20-F (5′-ACTGTGGTGTCATGCATTTGG-3′) and F20-R (5′-GACTCATCTAGGCATTTTCAG-3′) (Wu et al. 2007) were used to amplify an 838-bp fragment, which comprises RS3. The total reaction volume for the PCR was 55 μl, containing 10 μl of template DNA, 40 μl Platinum® PCR SuperMix (Invitrogen) (consisting of *Taq* DNA polymerase with Platinum® *Taq* antibody, 22 mM Tris−HCL (pH 8.4), 55 mM KCl, 1.65 mM MgCl$_2$, and 220 μM dNTP mix), and 0.2 μM of each primer. The reaction was carried out on a Veriti 96 Well Thermal Cycler (Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA) using the following cycling parameters: 2 min at 92°C, then 35 cycles of 30 s at 94°C, 30 s at 48°C for 675-bp fragment or 53°C for 838-bp fragment, and 120 s at 72°C, with a final extension for 10 min at 72°C. After purification with Wizard® SV Gel and a PCR Clean-Up System kit (Promega, Madison, Wisconsin, USA), both ampli-fied fragments of the CR were sequenced in both directions with an ABI3730x1 DNA Analyzer (Applied Biosystems). A 675 bp-long fragments were obtained from 35 samples, while 838 bp-long fragments were obtained from eight samples.

Sequence alignment was performed using Clustal W (Thompson et al. 1994) implemented in BioEdit software (Hall 1999) and alignments were manually proofed. Lynx CR sequences were aligned with the reference sequence of domestic cat (*F. catus*) complete mitochondrial genome (Lopez et al. 1996). An analyzed 675 bp-long fragment corresponds to positions 16,284–16,958; while 838 bp-long fragment corresponds to positions 76–891 of the complete domestic cat mitochondrion (Lopez et al. 1996).

Sequences were deposited in the GenBank under accession numbers JN084446, JN084447, JN0844478, JN084449, JN084450, and JN084451.

For RS3 repetitive sequence investigation, Eurasian lynx RS3 sequences from this study were manually compared with felid mtDNA sequences available in the GenBank. Analysis included two domestic cat (*F. catus*) sequences, two clouded leopard (*Neofelis nebulosa*), one snow leopard (*Panthera uncia*), two leopard (*Panthera pardus*), six tiger (*Panthera tigris*), two bobcat (*Lynx rufus*), and one cheetah (*Acinonyx jubatus*) RS3 sequence.

Results

Within the analyzed Eurasian lynx CR, we identified two repetitive sequences, which we labeled as RS2 and RS3 based on their locations and sequence characteristics following Hoelzel et al. (1994).

In Eurasian lynx, RS2 begins at position 16,532 (corresponding to complete domestic cat mitochondrion, as described by Lopez et al. 1996) and consists of 80 bp-long monomers repeated a variable number of times. We did not identify any polymorphic sites among RS2 monomers. In the 26 samples (74.3%), monomers were present 3.5 times, three full copies followed by 46 bp from the fourth copy, as identified by Hellborg et al. (2002). Eight samples (22.9%) had two 80-bp copies and 46 bp from the third copy – repetitive sequence was present 2.5 times. As PCR products were sequenced in both directions, one sample (2.8%) had 2.5 copies present in forward sequence and 3.5 RS2 copies in the reverse sequence.

RS3 repetitive sequence in Eurasian lynx starts at position 269 (corresponding to complete domestic cat mitochondrion, as described by Lopez et al. 1996) and is 305–327 bp long. We identified the fundamental repeat motif TACAC and four additional motifs consisting of the fundamental motif followed by one of the suffixes: −G, −GTA −ACGTA, or −ACACG, making TACACG, TACACGTA, TACACACGTA, and TACACACACG repeat motifs. The most frequent motif TACACG is repeated 31–33 times.

Table I. Encoded repetitive array sequence RS3 found in Eurasian lynx.

GenBank accession number	Encoded RS3 sequence	Repetitive sequence length	No of sequences (%)
JN084448	$(X_3Y)_5 \ X_9Z_2 \ X_7Z_6 \ Q$	305	6 (66.67)
JN084449	$(X_3Y)_6 \ X_7Z_2 \ X_7Z_6 \ Q$	321	1 (11.11)
JN084450	$(X_3Y)_6 \ X_8Z_2 \ X_7Z_6 \ Q$	327	1 (11.11)
JN084451	$(X_3Y)_5 \ X_3W \ X_7 \ Z_2 \ X_7Z_6 \ Q$	319	1 (11.11)

Notes: X = TACACG; Y = TACACACGTA; Z = TACACGTA; W = TACACACACG; Q = TACAC.

Furthermore, we identified four different patterns of RS3 repeat motifs (Table I). One sample (12.5%) expressed length variation when sequenced in both directions. One sequence was 305 bp long and other 319 bp long.

We compared Eurasian lynx RS3 sequences from this study with mtDNA sequences of five felid species for which RS3 presence was previously confirmed – domestic cat (*F. catus*), clouded leopard (*N. nebulosa*), snow leopard (*P. uncia*), leopard (*P. pardus*), and tiger (*P. tigris*). We also identified RS3 repetitive sequence among cheetah (*A. jubatus*) and bobcat (*L. rufus*) sequences, for which RS3 has not been previously described. When aligned with complete domestic cat mitochondrion (Lopez et al. 1996) RS3 repetitive sequences in all analyzed species start at position 269 (corresponding to complete domestic cat mitochondrion, as described by Lopez et al. 1996) and have various lengths (Figure 1). The shortest repeat sequence is 179 bp long in snow leopard (*P. uncia*), while leopard (*P. pardus*) has the longest RS3 sequence (395 bp). Fundamental repeat motif shared among all analyzed species is TACAC. In tiger (*P. tigris*), leopard (*P. pardus*), clouded leopard (*N. nebulosa*), and bobcat (*L. rufus*) the slightly changed motif –TACGC appears in addition to TACAC. We identified a total of 10 longer motifs, consisting of fundamental repeats TACAC or TACGC with 10 different suffix patterns –G, –GCG, –GCA, –GTA, –ACG, –ACGTA, –ACGTG, –ACACG, –ACGC-ACACG, and –ACACACACGCGCACACACGGA-CTCG (Figure 1, Table II). Several repeat motifs are species specific. Two longest motifs (TACACACAC-ACACGCGCACACACGGACTCG and TACACA-CGCACACG) were found only in clouded leopard (*N. nebulosa*). One motif (TACACACGTG) is specific for the domestic cat (*F. catus*), while motif TACAC-ACACG was found in only one Eurasian lynx (*L. lynx*) sequence. Motif TACACGCA was found only in snow leopard (*P. uncia*). Most frequent motifs are TACACACG and TACACG (Table II).

Discussion and conclusions

Felid CR has been divided into three segments – left domain (containing RS2), CCR, and right domain (containing RS3) (Jae-Heup et al. 2001). The presence of both repetitive sequences in CR of a particular species is rare (Jae-Heup et al. 2001) and among felids until now it has been confirmed in domestic cat (Lopez et al. 1996), five species from *Panthera* genus (Jae-Heup et al. 2001) and Eurasian lynx (this study). Ketmaier and Bernardini (2005) compared CR repetitive sequences among nine carnivore taxa and besides tiger, spotted hyena (*Crocuta crocuta*) was the only species with both RS2 and RS3.

Among felids, RS2 has been identified in domestic cat (*F. catus*) (Lopez et al. 1996), ocelot (*Leopardus pardalis*) and margay (*Leopardus wiedii*) (Eizirik et al. 1998), *Panthera* genus (Jae-Heup et al. 2001), African cheetahs (*A. jubatus*) (Freeman et al. 2001), wildcat (*Felis silvestris*) (Hertwig et al. 2009), snow leopard (*P. uncia*) (Wei et al. 2009), and Eurasian lynx (Hellborg et al. 2002; this study). In those species, RS2 had an 80-bp motif, with variable number of repeats (1–4) among species and clones. Phylogenetic relationship and comparison of RS2 repetitive sequence among felids have been presented by Jae-Heup et al. 2001. Contrary to other felid species, Eurasian lynx RS2 sequence analyzed in this study has no polymorphic sites. This might be a characteristic of reintroduced and inbred Dinaric population, as polymorphic sites were also not found in rest of the CR (Sindičić et al., in press). Alternatively, this could be a species level phenomenon. Only seven mtDNA CR haplotypes of Eurasian lynx were described until now and low CR variability seems to be characteristic to all lynxes in Europe, resulting from their survival in single glacial refugium (Hellborg et al. 2002; Gugolz et al. 2008).

RS3 was located in domestic cat (*F. catus*) (Lopez et al. 1996), *Panthera* genus (Jae-Heup et al. 2001), clouded leopard (*N. nebulosa*) (Wu et al. 2007), snow leopard (*P. uncia*) (Wei et al. 2009), cheetah (*A. jubatus*), bobcat (*L. rufus*), and Eurasian lynx (this study). We found that all analyzed felid species share the similar repetitive pattern and that fundamental repeat TACAC is characteristic for felids. Similarly, Jae-Heup et al. (2001) propose TACACG as common structural motif among great cats, while ACGT was identified as basic motif in carnivore RS3 (Hoelzel et al. 1994; Ketmaier and Bernardini 2005; Pérez-Haro et al. 2005). Duplication, deletion, and substitution of the CA element generate CG and TA, basic nucleotide pairs that combine to form RS3 fundamental repeats (Hoelzel et al. 1994; Jae-Heup et al. 2001). RS3 sequence variations
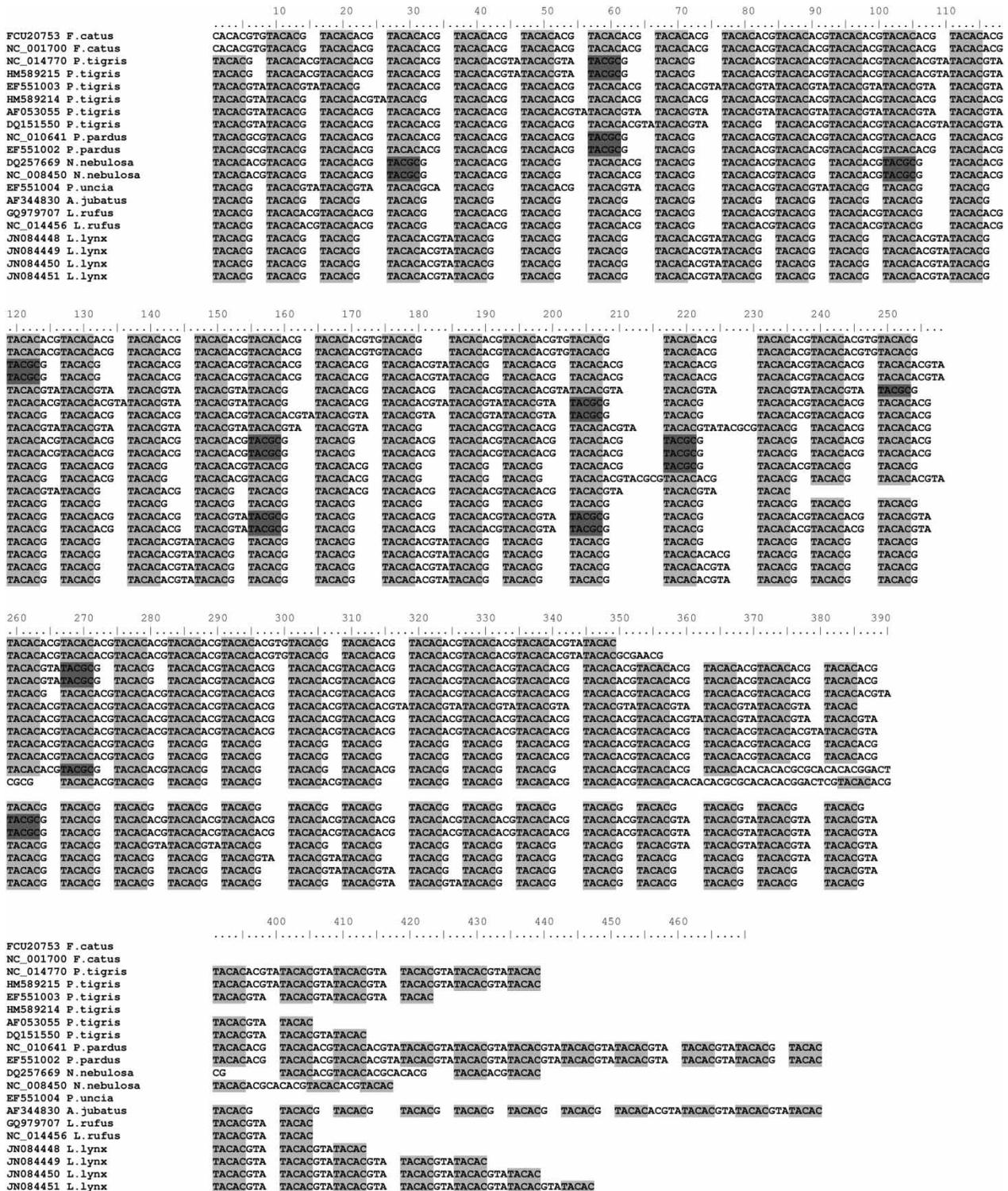
Figure 1.   Aligned RS3 sequences of analyzed felid species. Fundamental repeat motif is marked light grey (TACAC) and dark grey (TACGC).

are not informative for phylogenetic analysis (Jae-Heup et al. 2001; Ketmaier and Bernardini 2005).

The study of repetitive sequences is important as it may provide insight into genome function and evolution of nuclear and mitochondrial genomes (Ray and Densmore 2003). The diverse functional roles of the CR have likely led to contrasting patterns of selective pressures among its different segments and as repetitive sequences may have important functional roles, number of repeats is possibly under both positive and negative selective pressures (Jae-Heup et al. 2001). Some reports have indicated

Table II. Description and number of motifs identified among felid RS3 repetitive sequences.

GenBank no.	Species	Sequence length (bp)	Number of each repeat (%)												Total no. of repeats
			TACAC	TAC ACG	TAC GCG	TACA CGCG	TACA CGCA	TACA CGTA	TACA CACG	TACAC ACGTA	TACAC ACGTG	TACAC ACACG	TACAC ACGCA CACG	TACACACACA CACGGCGCACA CACGGACTCG	
FCU20753	*F. catus*	301	0	5 (13.16)	0	1 (2.63)	0	0	26 (68.42)	2 (5.26)	4 (10.53)	0	0	0	38
NC_001700	*F. catus*	302	1 (2.63)	5 (13.16)	0	0	0	0	26 (68.42)	2 (5.26)	4 (10.53)	0	0	0	38
NC_014770	*P. tigris*	375	1 (2.08)	5 (10.42)	3 (6.25)	0	0	7 (14.58)	27 (56.25)	5 (10.42)	0	0	0	0	48
HM589215	*P. tigris*	375	1 (2.08)	5 (10.42)	3 (6.25)	0	0	7 (14.58)	27 (56.25)	5 (10.42)	0	0	0	0	48
EF551003	*P. tigris*	363	1 (2.17)	3 (6.52)	1 (2.17)	0	0	18 (39.13)	20 (43.48)	3 (6.52)	0	0	0	0	46
HM589214	*P. tigris*	331	1 (2.33)	4 (9.30)	1 (2.33)	0	0	12 (27.91)	21 (48.84)	4 (9.30)	0	0	0	0	43
AF053055	*P. tigris*	347	1 (2.27)	3 (6.82)	1 (2.27)	0	0	16 (36.36)	20 (45.45)	3 (6.82)	0	0	0	0	44
DQ151550	*P. tigris*	363	1 (2.22)	4 (8.89)	1 (2.22)	0	0	12 (26.67)	23 (51.11)	4 (8.89)	0	0	0	0	45
NC_010641	*P. pardus*	395	1 (1.92)	11 (21.15)	3 (5.77)	1 (1.92)	0	7 (13.46)	28 (53.85)	1 (1.91)	0	0	0	0	52
EF551002	*P. pardus*	395	1 (1.92)	11 (21.15)	3 (5.77)	1 (1.92)	0	7 (13.46)	28 (53.85)	1 (1.91)	0	0	0	0	52
DQ257669	*N. nebulosa*	335	1 (2.13)	18 (38.30)	4 (8.51)	0	0	0	19 (40.43)	1 (2,13)	0	0	2 (4.26)	2 (4.26)	47
NC_008450	*N. nebulosa*	335	1 (2.13)	18 (38.30)	4 (8.51)	0	0	0	19 (40.43)	1 (2,13)	0	0	2 (4.26)	2 (4.26)	47
EF551004	*P. uncia*	179	1 (4.00)	9 (36.00)	0	0	1 (4.0)	7 (28.00)	7 (28.00)	0	0	0	0	0	25
AF344830	*A. jubatus*	325	1 (1.89)	49 (92.45)	0	0	0	2 (3.77)	0	1 (1.89)	0	0	0	0	53
GQ979707	*L. rufus*	323	1 (2.27)	10 (22.73)	3 (6.82)	0	0	8 (18.18)	22(50.00)	0	0	0	0	0	44
NC_014456	*L. rufus*	323	1 (2.27)	10 (22.73)	3 (6.82)	0	0	8 (18.18)	22 (50.00)	0	0	0	0	0	44
JN084448	*L. lynx*	305	1 (2.04)	34 (69.39)	0	0	0	8 (16.33)	0	5 (11.11)	0	0	0	0	49
JN084449	*L. lynx*	321	1 (2.08)	32 (68.09)	0	0	0	8 (16.67)	0	6(12.77)	0	0	0	0	48
JN084450	*L. lynx*	327	1 (2.13)	33 (67.35)	0	0	0	8 (17.02)	0	6 (12.24)	0	1 (2.13)	0	0	47
JN084451	*L. lynx*	319	1 (2.27)	30 (68.18)	0	0	0	8 (18.8)	0	5 (11.36)	0	0	0	0	44

that repetitive segments act to regulate function in the course of replication and transcription of mtDNA (Delarbre et al. 2001; Delport et al. 2002); however, some authors find that repetitive segments have no important biological function and are just trifle sequences (Casane et al. 1997; Wu et al. 2007). All five repetitive sequences (RS1–RS5) are situated in regions where the H-strand replication is regulated (Savolainen et al. 2000). Repeat units have a high degree of internal complementarity and the capability to fold into complex secondary structures (Buroker et al. 1990; Wilkinson and Chapman 1991; Stewart and Baker 1994; Petri and Von Haeseler 1996; Lopez et al. 1996; Savolainen et al. 2000; Freeman et al. 2001). It was hypothesized that secondary structures may be associated with replication control sites (Brown et al. 1986; Hoelzel et al. 1991; Sbisá et al. 1997) and different nuclear-coded proteins have been identified that bind to repetitive elements in the mtDNA D-loop (Madsen et al. 1993; Kumar et al. 1995; Wilkinson et al. 1997). These proteins may cause DNA polymerase to stall, causing instability and increasing the likelihood of reiterative strand slippage (Weitzmann et al. 1997). The RS2 cloverleaf structures become more energetically stable as more repeat units are added and may be further stabilized by a protein interaction (Hoelzel et al. 1991; Fumagalli et al. 1996; Lopez et al. 1996; Sbisá et al. 1997). The carnivore RS3 can form stable stem structures, which can stabilize loops and help account for the slippage of the long motifs (Hoelzel et al. 1994). Another function of repetitive elements could be compensation for deleterious mutations as they may provide a redundant signal if a mutation in one repeat alerts the binding ability of a regulatory protein (Wilkinson et al. 1997).

Further investigation on RS3 sequence pattern should provide better insight into evolutionary processes that shape their structure and possible function.

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References

Aquadro CF, Greenberg BD. 1983. Human mitochondrial DNA variation and evolution: Analysis of nucleotide sequences from seven individuals. Genetics 103:287–312.

Brown GG, Gadaleta G, Pepe G, Saccone C, Sbisá E. 1986. Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. J Mol Biol 192: 503–511.

Brown JR, Beckenbach AT, Smith MJ. 1993. Intraspecific DNA sequence variation of the mitochondrial control region of white sturgeon (*Acipenser transmontanus*). Mol Biol Evol 10:326–341.

Buroker NE, Brown JR, Gilbert TA, Ohara PJ, Beckenbach AT, Thomas WK, Smith MJ. 1990. Length heteroplasmy of sturgeon mitochrondrial-DNA: An illegitimate elongation model. Genetics 124:157–163.

Casane D, Dennebouy N, de Rochambeau H, Mounolou JC, Monnerot M. 1997. Nonneutral evolution of tandem repeats in the mitochondrial DNA control region of lagomorphs. Mol Biol Evol 14:779–789.

Delarbre C, Rasmussen A, Arnason U, Gachelin G. 2001. The complete mitochondrial DNA genome of the hagfish *Myxine glutinosa*: Unique features of the control region. J Mol Evol 53: 634–641.

Delport W, Ferguson JWH, Bloomer P. 2002. Characterization and evolution of the mitochondrial DNA control region in Hornbills (Bucerotiformes). J Mol Evol 54:794–806.

Eberhard JR, Wright TF, Bermingham E. 2001. Duplication and concerted evolution of the mitochondrial control region in the parrot genus Amazona. Mol Biol Evol 18:1330–1342.

Eizirik E, Bonatto SL, Johnson WE, Crawshaw PG, Jr, Vie J, Brousset DM, O'Brien SJ, Salzano FM. 1998. Phylogeographic patterns and evolution of the mitochondrial DNA control region in two Neotropical cats (Mammalia, Felidae). J Mol Evol 47: 613–624.

Ferrando A, Ponsà M, Marmi J, Domingo-Roura X. 2004. Eurasian Otters, *Lutra lutra*, have a dominant mtDNA haplotype from the Iberian Peninsula to Scandinavia. J Hered 95:430–435.

Freeman AR, Machugh DE, Mckeown SN, Walzer C, Mcconnell DJ, Bradley DG. 2001. Sequence variation in the mitochondrial DNA control region of wild African cheetahs (*Acinonyx jubatus*). Heredity 86:355–362.

Fumagalli L, Taberlet P, Favre L, Hausser J. 1996. Origin and evolution of homologous repeated sequences in the mitochondrial DNA control region of shrews. Mol Biol Evol 13:31–46.

Galtier N, Nabholz B, Glemin S, Hurst GDD. 2009. Mitochondrial DNA as a marker of molecular diversity: A reappraisal. Mol Ecol 18:4541–4550.

Gomerčić T, Sindičić M, Galov A, Arbanasić H, Kusak J, Kocijan I, Đuras Gomerčić M, Huber Đ. 2010. High genetic variability of grey wolf (*Canis lupus* L.) population from Croatia as revealed by mitochondrial DNA control region sequences. Zool Stud 49: 816–823.

Hellborg L, Walker CW, Rueness EK, Stacy JE, Kojola I, Valdmann H, Vila C, Zimmermann B, Jakobsen KS, Ellegren H. 2002. Differentiation and levels of genetic variation in northern European lynx (*Lynx lynx*) populations revealed by microsatellites and mitochondrial DNA analysis. Conserv Genet 3: 97–111.

Gugolz D, Bernasconi MV, Breitenmoser-Würsten C, Wandeler P. 2008. Historical DNA reveals the phylogenetic position of the extinct Alpine lynx. J Zool (Lond) 275:201–208.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/97/NT. Nucleic Acids Symp Ser 41:95–98.

Hertwig ST, Schweizer M, Stepanow S, Jungnickel A, Böhle UR, Fischer MS. 2009. Regionally high rates of hybridization and introgression in German wildcat populations (*Felis silvestris*, Carnivora, Felidae). J Zool Sys Evol Res 47:283–297.

Hoelzel AR, Hancock JM, Dover GA. 1991. Evolution of the cetacean mitochondrial D-loop region. Mol Biol Evol 8: 475–493.

Hoelzel AR. 1993. Evolution by DNA turnover in the control region of vertebrate mitochondrial DNA. Curr Opin Genetics Dev 3: 891–895.

Hoelzel AR, Lopez JV, Dover GA, O'Brien SJ. 1994. Rapid evolution of a heteroplasmic repetitive sequence in the mitochondrial DNA control region of carnivores. J Mol Evol 39:191–199.

Ketmaier V, Bernardini C. 2005. Structure of the mitochondrial control region of the Eurasian Otter (*Lutra lutra*; Carnivora, Mustelidae): Patterns of genetic heterogeneity and implications for conservation of the species in Italy. J Hered 96:318–328.

Kumar S, Suzuki H, Onoue S, Suzuki S, Hattori N, Ozawa T. 1995. Rat mitochondrial mtDNA-binding proteins to inter-specifically conserved sequences in the displacement loop region of vertebrate mtDNAs. Biochem Mol Biol Int 36:973–981.

Lopez JV, Cevario S, O'Brien SJ. 1996. Complete nucleotide sequences of the domestic cat (*Felis catus*) mitochondrial genome and a transposed mtDNA tandem repeat (*Numt*) in the nuclear genome. Genomics 33:229–246.

Madsen CS, Ghivizzani SC, Hauswirth WW. 1993. Protein binding to a single termination-associated sequence in the mitochondrial DNA d-loop region. Mol Cell Biol 13:2162–2171.

Meyer A, Kocher TD, Basasibwaki P, Wilson AC. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature 347:550–553.

Jae-Heup K, Eizirik E, O'Brien SJ, Johnson WE. 2001. Structure and patterns of sequence variation in the mitochondrial DNA control region of the great cats. Mitochondrion 14:279–292.

Nabholz B, Mauffrey J, Bazin E, Galtier N, Glemin S. 2007. Determination of mitochondrial genetic diversity in mammals. Genetics 178:351–361.

Petri B, Von Haeseler A. 1996. Extreme sequence heteroplasmy in bat mitochondrial DNA. Biol Chem 661–667.

Pérez-Haro M, Viñas J, Mañas F, Batet A, Ruiz-Olmo J, Pla C. 2005. Genetic variability in the complete mitochondrial control region of the Eurasian otter (*Lutra lutra*) in the Iberian Peninsula. Biol J Linn Soc 86:397–403.

Purdue JR, Oleksyk TK, Smith MH. 2006. Independent occurrences of multiple repeats in the control region of mitochondrial DNA of white-tailed deer. J Hered 97:235–243.

Ray DA, Densmore LD. 2003. Repetitive sequences in the Crocodilian mitochondrial control region: Poly-A sequences and heteroplasmic tandem repeats. Mol Biol Evol 20: 1006–1013.

Savolainen P, Arvestad L, Lundeberg J. 2000. mtDNA tandem repeats in domestic dogs and wolves: Mutation mechanism studied by analysis of the sequence of imperfect repeats. Mol Biol Evol 17(4):474–488.

Sbisá E, Tanzariello F, Reyes A, Pesole G, Saccone C. 1997. Mammalian mitochondrial D-loop region structural analysis: Identication of new conserved sequences and their functional and evolutionary implications. Gene 205:125–140.

Sindičić M, Gomerčić T, Skrbinšek T, Polanc P, Jelenčič M, Huber Đ, Trontelj P. In press. Low genetic diversity of Dinaric lynx population.

Stewart DT, Baker AJ. 1994. Patterns of sequence variation in the mitochondrial D-loop region of shrews. Mol Biol Evol 11:9–21.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680.

Wang T, Tzeng C, Teng H, Chang T. 2007. Phylogeography and identification of a 187-bp-long duplication within the mitochondrial control region of *Formosania lacustre* (Teleostei: Balitoridae). Zool Stud 46:569–582.

Ward RH, Frazier BL, Dew-Jager K, Paabo S. 1991. Extensive mitochondrial diversity within a single Amerindian tribe. P Natl Acad Sci USA 88:8720–8724.

Wei L, Wu X, Jiang Z. 2009. The complete mitochondrial genome structure of snow leopard *Panthera uncia*. Mol Biol Rep 36: 871–878.

Weitzmann MN, Woodford KJ, Usidin K. 1997. DNA secondary structures and the evolution of hypervariable tandem arrays. J Biol Chem 272:9517–9523.

Wilkinson GS, Chapman AM. 1991. Length and sequence variation in evening bat d-loop mtDNA. Genetics 128:607–617.

Wilkinson GS, Mayer F, Kerth G, Petri B. 1997. Evolution of repeated sequence arrays in the D-loop region of bat mitochondrial DNA. Genetics 146:1035–1048.

Wu X, Zheng T, Jiang Z, Wei L. 2007. The mitochondrial genome structure of the clouded leopard (*Neofelis nebulosa*). Genome 50: 252–257.