Zootaxa 4550 (4): 585–593
https://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4550.4.9
http://zoobank.org/urn:lsid:zoobank.org:pub:12E18262-0DCA-403A-B047-82CFE5E20373

Complete mitochondrial genome of the Western Capercaillie *Tetrao urogallus* (Phasianidae, Tetraoninae)

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Abstract

The Western Capercaillie (*Tetrao urogallus*) is a galliform bird of boreal climax forests from Scandinavia to eastern Siberia, with a fragmented population in southwestern Europe. We extracted the DNA of *T. urogallus aquitanicus* and obtained the complete mitochondrial genome (mitogenome) sequence by combining Illumina and Sanger sequencing sequence data. The mitochondrial genome of *T. urogallus* is 16,683 bp long and is very similar to that of *Lyrurus tetrix* (16,677 bp). The *T. urogallus* mitogenome contains the normal 13 protein-coding genes (PCGs), 22 transfer RNAs, 2 ribosomal RNAs, and the control region. The number, order, and orientation of the mitochondrial genes are the same as in *L. tetrix* and in other species of the same and other bird families. The three domains of the control region contained conserved sequences (ETAS; CSBs), boxes (F, E, D, C, B, BS box), the putative origin of replication of the H-strand (O_H) and bidirectional promoters of translation (LSP/HSP).

Key words: control region, mitogenome, Phasianidae, Tetrao urogallus

Introduction

The Order Galliformes contains about 290 species, approximately 10% of which are listed as globally Endangered or Critically Endangered (del Hoyo *et al.* 1994; Hosner *et al.* 2016; IUCN 2017). Traditionally, the species of this order are divided into seven families: Megapodiidae, Cracidae, Odontophoridae, Numididae, Phasianidae, Meleagrididae, and Tetraonidae (del Hoyo *et al.* 1994). The family Phasianidae, with more than 150 species, is distributed throughout the world (Johnsgard 1986; Fuller & Garson 2000; Fuller *et al.* 2000).

The genus *Tetrao* (subfamily Tetraoninae) includes two extant species, *T. urogallus* Linnaeus and *T. urogalloides* Middendorff. The Western Capercaillie (*Tetrao urogallus*) is the best-known species of this genus and is found typically in boreal climax forests from Scandinavia to eastern Siberia, but also in a fragmented population in southwestern Europe (Storch 2007). In this latter region, it is present in the Pyrenees (France, Andorra and Spain) and in the Cantabrian Mountains of Spain (de Juana 1994). Using morphological characteristics, up to 12 *T. urogallus* subspecies have been described (de Juana 1994), although some are not well supported by mitochondrial DNA analyses (Liukkonen-Anttila *et al.* 2004). The two capercaillie subspecies of southwestern Europe, *T. urogallus aquitanicus* Ingram of the Pyrenees and *T. urogallus cantabricus* Castroviejo of the Cantabrian Mountains, are both considered Threatened in small-scale assessments (Canut *et al.* 2004; Obeso 2004; Rodríguez-Muñoz *et al.* 2007; Charra & Sarasa 2018).

Accepted by P. Rasmussen: 12 Nov. 2018; published: 29 Jan. 2019

Although scientific information on demography over the last 20 years is scarce (Gée *et al.* 2018), the biology and ecology of *T. urogallus* is quite well-known, and information is available on reproduction, population and/or subspecific variations, and genetics (Rodríguez-Muñoz *et al.* 2007; Mollet *et al.* 2015; Fameli *et al.* 2017; Kowalczyk *et al.* 2017; Rutkowski *et al.* 2017). Genetic studies have analyzed several DNA markers, most of them mitochondrial DNA sequences [12s rRNA, 16s rRNA, cytochrome oxidase I (CoI), cytochrome B (CytB), control region (D-loop)] and several microsatellites (Segelbacher *et al.* 2008; Lucchini *et al.* 2001; Dimcheff *et al.* 2002; Kerr *et al.* 2009; Pérez *et al.* 2011).

At present, a complete mitogenome sequence is not available for either of the two *Tetrao* species. However, the mitogenome of the Black Grouse, *Lyrurus tetrix* Linnaeus, a closely related species previously included in genus *Tetrao*, has been described (Li *et al.* 2016). In this study, we sequenced and described the complete mitochondrial genome of *T. urogallus aquitanicus*, which we then compared with that of *L. tetrix*.

Materials and methods

DNA extraction, sequencing, and mitogenome assemblage. Genomic DNA was extracted from liver tissue of an individual of *T. urogallus aquitanicus* using the DNeasy Blood & Tissue Kit (Qiagen). For genome sequencing, 3 µg of genomic DNA were used for the construction of a library with 750 bp fragments. This library was used in Illumina® Hiseq™ 2000 paired-end sequencing with 100 bp reads. Two Gbp of sequences were obtained (coverage about 1.5X). We assembled the sequence for the *T. urogallus* mitogenome using the MITObim v1.8 program (Hahn *et al.* 2013) with the "--quick" option and with the default mismatch of 15%. For this purpose, we randomly selected one million read pairs with seqTK (https://github.com/lh3/seqtk) and used as a reference the mitogenome of *Lyrurus tetrix* (accession number KF955638.1; Li *et al.* 2016). A region containing the D-loop and the gene Nd6 of the mitochondrial genome was not recovered completely and so was amplified by PCR using the same *T. urogallus* sample and sequenced. For PCR amplification the primer pair Pro+ (5'-ACCATCAGCACCCAAAGCTG-3') and Phe- (5'-AAGCATTTTCAGTGCTTTGCTT-3') were used (Haring *et al.* 2000). Sequences were analyzed with Bioedit (version 7.0.9.0) (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The annotation of the *T. urogallus* mitogenome was fulfilled using web-based services MITOS (http://mitos.bioinf.uni-leipzig.de/help.py) (Bernt *et al.* 2013) and tRNA scan-SE (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe & Eddy 1997). The annotations of protein coding genes (PCGs), transfer RNAs (tRNAs) and rRNA genes were refined by comparing manually with the *L. tetrix* mitogenome (Li *et al.* 2016). The circular drawing of the mitogenome was carried out using the OrganellarGenomeDRAW tools (http://ogdraw.mpimp-golm.mpg.de/) (Lohse *et al.* 2013).

Results and discussion

Genome organization and gene arrangement. The mitochondrial genome of *T. urogallus* was assembled and submitted to GenBank (accession number MG583885). It is composed of 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and the non-coding region (D-loop) (Table 1, Fig. 1). The mitogenome of *T. urogallus* is 16,683 bp long, similar to the *L. tetrix* mitogenome of 16,677 bp (Li *et al.* 2016); both have a percentage of identity of 94.70%. The total base composition of the *T. urogallus* mitochondrial genome is 30.15% A, 30.55% C, 13.62% G, and 25.67% T; the A-T content is 55.82%, which is also very similar to the *L. tetrix* base composition and A-T content (30.37% A, 30.42% C, 13.38% G, 25.83% T; A-T content 56.20%) (Li *et al.* 2016).

Protein coding genes (PCGs). The total length of the 13 PCGs of *T. urogallus* was 11,392 bp (68.23% of the total length of the mitogenome); the similarities with the 13 PCGs of *L. tetrix* is very high (varying between 96.10% of the Nd1 and 92.66% of the Nd6) (Table 2). The main differences affect the Nd6 gene of *L. tetrix*, which has an 18-bp nucleotide deletion and gives rise to a smaller protein; this deletion is absent from the same gene in *T. urogallus* and other Phasianidae species (Nishibori *et al.* 2001; Guan *et al.* 2010; Kan *et al.* 2010; Shen *et al.* 2010; Li *et al.* 2016). In addition, as in other bird species including *L. tetrix*, the Nd3 gene of *T. urogallus* has an extra nucleotide in position 174 (mitogenome nt 10,855, C) that is not translated (Mindell *et al.* 1998; Li *et al.* 2016).

TABLE 1. Gene organization of the *Tetrao urogallus* mitogenome.

Gene	Strand	Nucleotide positions	Size (bp)	Anticodon	Intergenic nucleotide
D-loop	H	1–1141	1141		
$tRNA^{Phe}$	H	1142–1208	67	TTC	
12S rRNA	H	1209–2171	963		
$tRNA^{Val}$	H	2172–2244	73	GTA	
16S rRNA	H	2245–3858	1614		
$tRNA^{Leu\ (uur)}$	H	3859–3932	74	TTA	
Nd1	H	3944–4918	975		11
$tRNA^{Ile}$	H	4919–4992	74	ATC	
$tRNA^{Gln}$	L	4999–5069	71	CAA	6
$tRNA^{Met}$	H	5069–5137	69	ATG	-1
Nd2	H	5138–6176	1039		
$tRNA^{Trp}$	H	6177–6253	77	TGA	
$tRNA^{Ala}$	L	6260–6328	69	GCA	6
$tRNA^{Asn}$	L	6333–6405	73	AAC	4
$tRNA^{Cys}$	L	6408–6473	66	TGC	2
$tRNA^{Tyr}$	L	6473–6543	71	TAC	-1
COI	H	6545–8095	1551		1
$tRNA^{Ser\ (ucn)}$	L	8087–8161	75	TCA	-9
$tRNA^{Asp}$	H	8164–8232	69	GAC	2
COII	H	8234–8917	684		1
$tRNA^{Lys}$	H	8919–8989	71	AAA	1
Atp8	H	8991–9155	165		1
Atp6	H	9146–9829	684		-10
COIII	H	9829–10,612	784		-1
$tRNA^{Gly}$	H	10,614–10,681	68	GGA	1
Nd3	H	10,682–11,033	352		
$tRNA^{Arg}$	H	11,035–11,103	69	CGA	1
Nd4L	H	11,104–11,400	297		
Nd4	H	11,394–12,771	1378		-7
$tRNA^{His}$	H	12,772–12,840	69	CAC	
$tRNA^{Ser}$	H	12,842–12,906	65	AGC	1
$tRNA^{Leu}$	H	12,908–12,978	71	CTA	1
Nd5	H	12,979–14,796	1818		
CytB	H	14,801–15,943	1143		4
$tRNA^{Thr}$	H	15,946–16,014	69	ACA	2
$tRNA^{Pro}$	L	16,017–16,086	70	CCA	2
Nd6	L	16,093- 16,614	522		6
$tRNA^{Glu}$	L	16,616–16,683	68	GAA	1

Intergenic nucleotide: denotes the number of overlapping nucleotides (negative values) or the number of spacer nucleotides (positive values) between two consecutive genes.

Most of the 13 PCGs of *T. urogallus* started with the ATG start codon, the exception being CoI, which has GTG as the start codon. In *L. tetrix*, both the CoI and Nd5 genes also start with GTG. In both these species, the genes Nd1, CoII, Atp8, Atp6, Nd3, Nd4L, Nd5, and CytB all have TAA stop codons, while gene Nd6 has a TAG

stop codon, CoI has AGG and Nd2, CoIII, and Nd4 have incomplete T-- stop codons (Table 2). Incomplete stop codons (T--), which occur in three PCGs, are common in mitogenomes and may be completed by the polyadenylation of the 3'-end of the mRNA after transcription (Ojala 1981; Mouchaty *et al.* 2000). There were 3795 codons for the 13 PCGs, excluding incomplete termination codons; the most frequently occuring amino acid was Leu (13.6%).

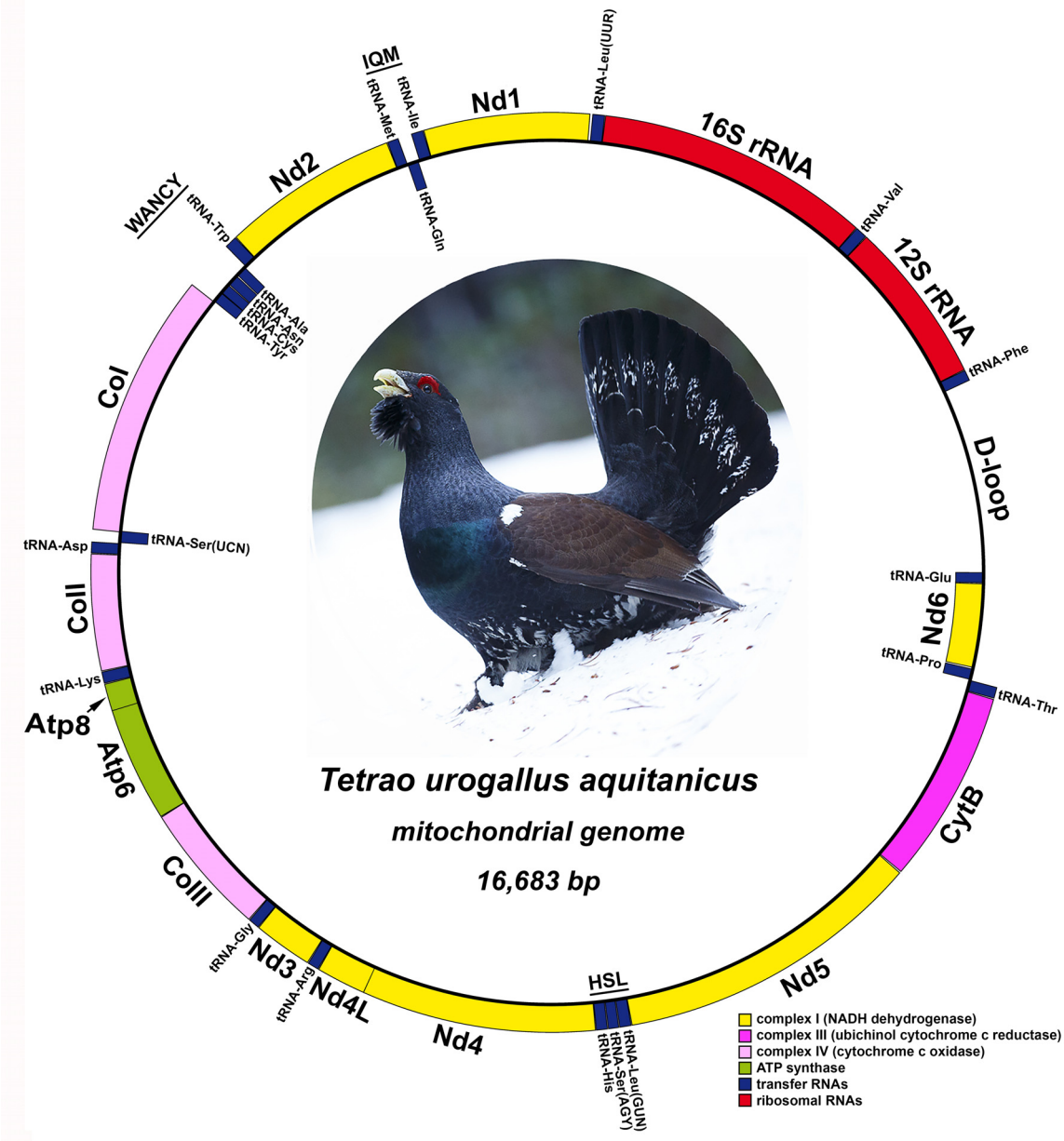

FIGURE 1. Map of the mitochondrial genome of *Tetrao urogallus*. Genes encoded by the heavy strand are shown outside the circle, while those encoded by the light strand are shown inside the circle.

tRNA and rRNA genes. All 22 tRNAs sequences have similar lengths, which is 1,547 bp and 1,546 bp for *T. urogallus* and *L. tetrix*, respectively, and an identity percentage of 96.82% when comparing the mitogenomes of both species. The size of the tRNAs varied between 65 bp for $tRNA^{Ser}$ and $tRNA^{Cys}$, and 77 bp for the $tRNA^{Trp}$ (Table 1). As in other mitogenome studies of species from the same family, most of the tRNA genes are located on the heavy strand, the exceptions being eight ($tRNA^{Gln}$ / $tRNA^{Ala}$ / $tRNA^{Asn}$ / $tRNA^{Cys}$ / $tRNA^{Tyr}$ / $tRNA^{Ser}$ / $tRNA^{Pro}$ / $tRNA^{Glu}$) that are located on the light strand (Table 1) (Li *et al.* 2016; Chen *et al.* 2017). Three clusters of tRNAs—IQM, WANCY, and HSL—are present in the mitogenome of the two species, as has previously been described in other avian species (Chen *et al.* 2017) (Fig. 1).

TABLE 2. Comparison between the sequences 13 PCGs of *T. urogallus* and *L. tetrix* mitogenomes.

Gene	Length (bp)		AT Content		% Identity	Start/Stop codons		Protein length	
	T. uro	*L. tet*	*T. uro*	*L. tet*		*T. uro*	*L. tet*	*T. uro*	*L. tet*
Nd1	975	975	54.15	54.05	96.10	ATG/TAA	ATG/TAA	324	324
Nd2	1039	1039	58.52	58.81	92.88	ATG/T--	ATG/T--	346	346
COI	1551	1551	54.16	54.93	94.62	GTG/AGG	GTG/AGG	516	516
COII	684	684	56.29	55.26	96.05	ATG/TAA	ATG/TAA	227	227
Atp8	165	165	60.61	59.39	92.12	ATG/TAA	ATG/TAA	54	54
Atp6	684	684	55.41	55.26	94.44	ATG/TAA	ATG/TAA	227	227
COIII	784	784	54.59	54.85	95.54	ATG/T--	ATG/T--	261	261
Nd3*	352	352	52.27	54.26	93.73	ATG/TAA	ATG/TAA	116	116
Nd4L	297	297	55.89	55.56	93.60	ATG/TAA	ATG/TAA	98	98
Nd4	1378	1378	56.31	57.33	93.69	ATG/T--	ATG/T--	459	459
Nd5	1818	1818	55.83	56.88	93.67	ATG/TAA	GTG/TAA	605	605
CytB	1143	1143	53.37	54.24	93.61	ATG/TAA	ATG/TAA	380	380
Nd6	522	504	53.64	52.78	92.66	ATG/TAG	ATG/TAG	173	167
Total	11,392	11,374	55.26	55.71	94.18				

*Nd3 has one additional C that is not translated

In *T. urogallus*, the 12s rRNA and 16s rRNA genes are situated between the $tRNA^{Phe}$ and the $tRNA^{Leu}$. They are separated by the $tRNA^{Val}$ and have a length of 963 bp and 1614 bp, respectively. This situation is the same in *L. tetrix* but with respective lengths of 971 bp and 1611 bp (Li *et al.* 2016). The 12s rRNA and 16s rRNA of both species have identity percentages of 97.51% and 94.97%, respectively. In addition, the 12s rRNA and 16s rRNA in the two species are slightly A-T rich (54.83% in *T. urogallus* and 54.38% in *L. tetrix* 12s rRNA; 56.13% in *T. urogallus*; and 56.36% in *L. tetrix* 16s rRNA), as in other Phasianidae (e.g., *Bonasa sewerzowi* Przevalski) (Li *et al.* 2014) and other avian species (Chen *et al.* 2017; Shi *et al.* 2017).

Control region. The mtDNA CR is mainly involved in the replication, termination, and transcription of the mitochondrial genome. The control region (D-loop) is the most variable region between the two mitogenomes that have a sequence identity in *T. urogallus* and *L. tetrix* of 93.60%, a length of 1,141 bp and 1,147 bp, and a A-T content of 58.98% and 59.98%, respectively. Huang & Ke (2014) found that the length of the control region (about 1150 bp) is relatively well-conserved in Phasianidae. We aligned the control region of both mitogenomes and identified the three domains —domain I, the central conserved domain II, and domain III (positions 1–315, 316–782, and 783–1148 respectively; Fig. 2) —and several other conserved regions, as in other Phasianidae species (Randi & Lucchini 1998; Huang & Ke 2014).

Domain I contained two conserved extended termination-associated sequences, ETAS1 (62–121) and ETAS2 (108–167), as well as the shorter TAS (TATAT or TACAT motifs) that can form stable secondary structures (Fig. 2). At the beginning of this domain a C-stretch interrupted by three Ts (goose hairpin) conserved in Galliformes and Anseriformes is able to form a hairpin structure along with a string of guanines, located a short distance downstream (Ruokonen & Kvist 2002; Buehler & Baker 2003). Two TCCC motifs are found in both *T. urogallus* and *L. tetrix*; in other species three such motifs have been described and are linked to the termination of H-strands in mammalian and bird D-loops (Douzery & Randi 1997; Randi & Lucchini 1998). Finally, two CSB-1-like sequences exist in both these grouse species and also in other avian species, including other Phasianidae (Fig. 2) (Randi & Lucchini 1998; Yang *et al.* 2015).

In Domain II, five highly conserved sequence boxes were localized and identified as boxes F, E, D, C, and B (Fig. 2). The four first boxes (but not the B-box) were also identified in Phasianidae by Randi & Lucchini (1998) and Huang & Ke (2014). We identified the B-box by comparison with the sequences described by Ruokonen & Kvist (2002) in their study of the avian mitochondrial control region. Furthermore, we identified a very well-conserved bird similarity box (BS Box) (Fig. 2), which has identical sequences in several analyzed avian groups

(Ruokonen & Kvist 2002; Buehler & Baker 2003; Li *et al.* 2014). The function of these six boxes is not yet clear, although they could be related to D-loop formation and H-strand replication (Yang *et al.* 2015).

In Domain III, we identified the conserved sequence blocks 1 and 3 (CSB-1 and CSB-3) (Fig. 2) but were not able to identify conserved sequence block 2 (CSB-2). In previous studies in the Domain III of Phasianidae and other avian groups CSB-1 was identified, although with considerable sequence variation; however, the conserved sequences CSB-2 and CSB-3 were not found (Baker & Marshall 1997; Huang & Ke 2014). The characteristic motif of CSB-1 is GACATA, identical to several vertebrate mtDNA CSB-1sequences (Sbisà *et al.* 1997), while that of CBS-3 is a poly(C) track (Zhang *et al.* 2009). These sequences (CSB) are involved in potential secondary structures and have been proposed as regulatory signals for the processing of the RNA primers for H?strand replication (Sbisà *et al.* 1997).

Domain III also included a poly(C) sequence, which was similar to a replication initiation of a mammalian heavy chain (O_H), and the bidirectional promoters of translation (LSP/HSP) also identified in other birds (L'Abbé *et al.* 1991) including Phasianidae species (Randi & Lucchini 1998; Li *et al.* 2014), as well as a long track of poly(T) between CSB-1 and LSP and HSP bidirectional promoters (Fig. 2).

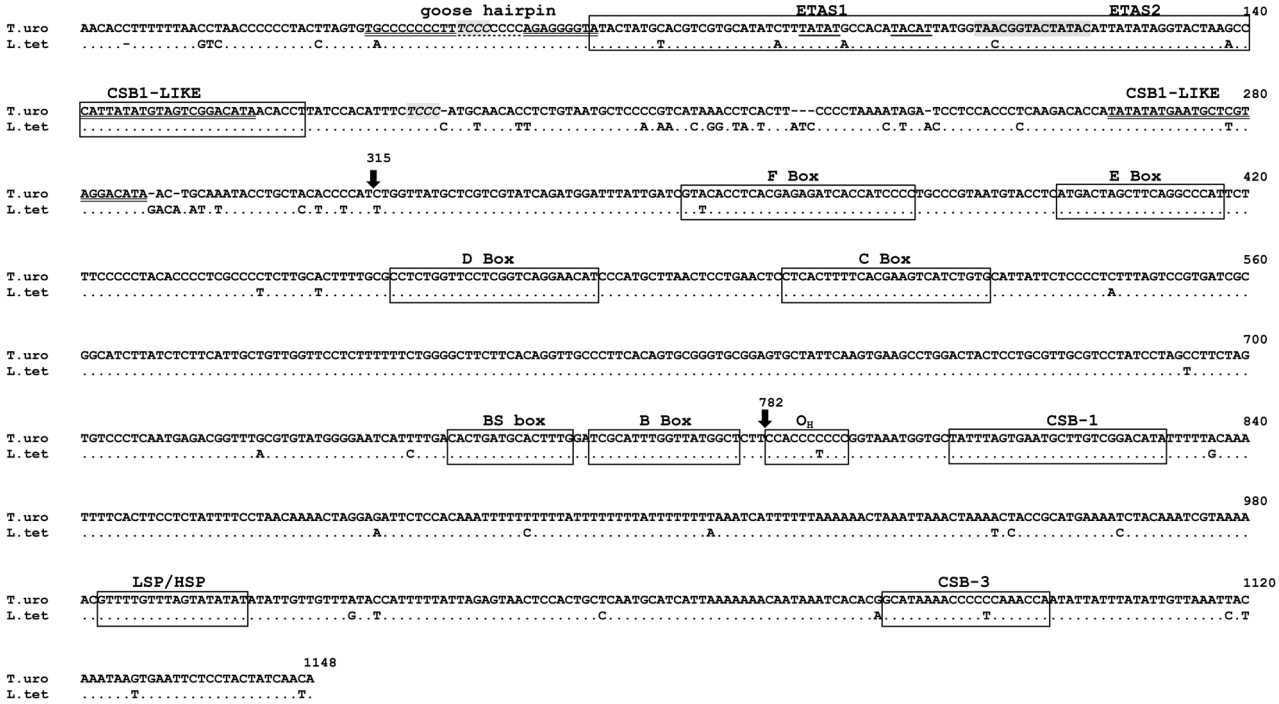

FIGURE 2. Aligned nucleotide sequences of the control region of *Tetrao urogallus* (T.uro) and *Lyrurus tetrix* (L.tet). Dots indicate the identity of the nucleotides and dashes the indels. The limits between Domains I to III are indicated by arrows. Domain I: The putative ends of H-strand DNA synthesis at TCCC motifs are shown in italics and highlighted in gray. The stem and loop of the goose hairpin are underlined with double lines and dots, respectively. Sequences ETAS1 (62–121) and ETAS2 (108–167) sequences are both included in a single large box, with the overlap nucleotides in gray and TATAT and TACAT sequences underlined. The CSB-1-like motifs are double-underlined. Domain II: The six conserved blocks identified F, E, D, C, B, and Bird Similarity (BS) box are in boxes. Domain III: The putative origin of the replication of the H-strand (O_H), CSB1, CSB-3, and the sequence corresponding to the avian bidirectional LSP/HSP promoters are also shown in boxes.

Acknowledgements

This work and the research activities of GAM are funded by the Fédération Nationale des Chasseurs (France), Fédération Départementale des Chasseurs de l'Ariège (France), Fédération Départementale des Chasseurs des Hautes Pyrénées (France) (project FNC-PSN-PR16-2014), the Fundación para la Conservación del Quebrantahuesos (Spain) (project FCQ-UJ-2017), and the Government of Andorra (project GA-UJ-2017). The research activities of authors are partially funded by the PAIDI, Junta de Andalucía (RNM-118 group). The authors express their gratitude to Edgar Madrenys for the picture of the *T. urogallus* in Figure 1.

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