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SEQUENCE ANALYSIS OF THE 5' COI GENE REGION FROM *DAMA DAMA* (LINNAEUS, 1758) (MAMMALIA: CERVIDAE)

LUIS OVIDIU POPA, OANA PAULA POPA,
PETRE GĂRGĂREA, DUMITRU MURARIU

Abstract. The DNA sequence of the mitochondrial 5'COI gene region was analysed in the follow deer *Dama dama* (Linnaeus, 1758) (Mammalia: Cervidae). The sequence was submitted to GenBank under the accession number EF675699 and will allow the identification of the species through the DNA barcoding technique.

Résumé. Dans ce travail on présente l'analyse de la séquence de la région 5' de la gène COI (cytochrome oxydase) du niveau de l'ADN mitochondrial pour l'espèce *Dama dama* (Mammalia: Cervidae). La région génique respective est utilisée pour l'identification des espèces animales, dans le cadre de la technique nommée «DNA barcoding». La séquence analysée a été publiée dans la base de données Gen Bank avec le numéro d'accès EF675699.

Key words: *Dama dama*, COI gene, barcode.

INTRODUCTION

Comparative studies of mitochondrial DNA among different organisms have revealed both a general conserved organization across metazoa, and the existence of significant differences between groups (Morlais & Severson, 2002). These differences allowed the use of the DNA sequence of some specific mitochondrial DNA regions as a molecular biology tool for species identification, technique called DNA barcoding (Moritz & Cicero, 2004; Rubinoff, 2006). This approach led to the appearance of the Consortium for the Barcoding of Life (CBOL), which proposed as a standard for species identification, a 648bp mtDNA sequence, from the 5' end of the cytochrome oxidase gene (Hebert et al., 2003; Stoeckle, 2003). For those groups where the COI gene proved unable to distinguish between species, CBOL proposed alternative mtDNA regions to be used as barcode (Stoeckle, 2003). The COI gene was chosen as a barcode for the following reasons: i) the DNA sequence is easily amplified with the same set of primers across different groups (Folmer et al., 1994); ii) the third position of the codons shows a high incidence of nucleotide substitutions, as compared to other protein genes (McClellan, 2000, Perna & Kocher, 1995); iii) the overall mutation rate of the COI gene is lower than that of other mitochondrial genes (Yi et al., 2002).

We report here for the first time the DNA sequence of the 5' end of the COI gene of the follow deer (*Dama dama*). The sequence was submitted to GenBank with the accession number EF675699.

MATERIALS AND METHODS

Sample collection

One specimen of follow deer (*Dama dama*) was hunted in Spring 2007, in the Șarlota hunting area, Timiș county, Romania (45°.95' N, 21° .51' E). A fragment of

muscle tissue has been taken and preserved in 96% ethanol. The sample was deposited as a voucher specimen (inventory number TIS1) at the National Museum of Natural History “Grigore Antipa”, Bucharest.

DNA extraction

Total genomic DNA was extracted from a small amount of muscle tissue with the NucleoSpin® Tissue DNA extraction kit (Macherey-Nagel, Düren, Germany), following the manufacturer specifications. The DNA concentration and purity were assessed by spectrophotometry using the UV-VIS Nanodrop ND-1000 equipment (NanoDrop Technologies, Wilmington, USA). Complete spectra 220-350nm was recorded for every sample. DNA purity was determined by calculating the absorbance ratio A260/280. Organic contamination was assessed by calculating the absorbance ratio A260/230.

Amplification of 5'COI gene region

The amplification of the 5'COI gene region from the mitochondrial genome was performed with the Folmer universal primers (Folmer et al., 1994), modified by the addition of M13 tails (see table 1).

Table 1

Primer name	Primer sequence (5'-3')
M13f_LCO1490	cac gac gtt gta aaa cga cgg tca aca aat cat aaa gat att gg
M13r_HCO2198	gga taa caa ttt cac aca ggt aaa ctt cag ggt gac caaa aaa tca

The reaction was performed in a final volume of 50µl, containing 40ng of genomic DNA, 67mM Tris-HCl, 16.6mM (NH₄)₂SO₄, 0.45% Triton X-100, 0.2mg/ml Gelatin, 2.5mM MgCl₂, 50µM₂ dNTPS, 0.125µM of each primer and 1U of Taq Pol (Jena Bioscience GmbH, Germany). The samples were initially denaturated at 94°C for 1 minute, followed by 5 cycles of amplification at lower stringency (denaturation at 94°C for 30 sec, annealing at 45°C for 90 sec and extension at 72°C for 60 sec), 30 cycles of higher stringency amplification (denaturation at 94°C for 30 sec, annealing at 51°C for 90 sec and extension at 72°C for 60 sec), and a final extension step at 72°C for 5 minutes. The reaction was performed on a MJ Research PTC-100 thermal cycler.

Electrophoresis conditions and gel extraction

After the completion of the PCR reaction, the samples were mixed with 10 µl of loading buffer (Glycerol 40%, Orange G 0.025%) and electrophoresed through an 1.5% agarose gel in SB conductive medium (10mM NaOH brought to pH 8.5 with boric acid), with 0.5µg/ml ethidium bromide incorporated in the gel. The gel was run for 30 minutes at 7V/cm constant voltage, then it was placed on a sterile piece of plastic wrap, visualized under UV on a standard transilluminator, and photographed with an Olympus C770 Ultrazoom digital camera equipped with an orange filter.

The PCR band representing the 5'COI gene region was excised from the gel with a sterile razor blade, then the DNA was extracted from the gel with the Agarose Gel Extraction Kit (Jena Bioscience GmbH, Germany), according to the manufacturer specifications. The DNA concentration was measured by

spectrophotometry using the UV-VIS Nanodrop ND-1000 equipment (NanoDrop Technologies, Wilmington, USA).

DNA sequencing

The purified PCR product was bi-directionally sequenced with M13 5' labeled primers (see table 2).

Table 2

Primer name	5' label	Primer sequence (5'-3')
M13fwd	IRD800	cacgacggtgtaaacgac
M13rev	IRD700	ggataacaattcacacagg

The sequencing reaction was performed with the DNA Cycle Sequencing Kit (Jena Bioscience GmbH, Germany), according to the manufacturer specifications, with 200fmol of purified PCR product as a template. The reaction was performed on a MJ Research PTC-100 thermal cycler. At the end of the sequencing reaction, the samples were mixed with LI-COR stop solution, denatured at 94°C for 3 minutes, then placed on ice. The sequencing electrophoresis was performed on the LI-COR4300L instrument, according to the manufacturer specifications. Proofreading and editing of each sequence were performed using eSeq software (LI-COR Biosciences, Lincoln, USA).

Data analysis

The published sequences of the cytochrome oxidase I gene of different Cervidae species and subspecies (*Muntiacus muntjak* AY225986, *Muntiacus reevesi micrurus* EF035447, *Muntiacus crinifrons* AY239042, *Muntiacus reevesi* AF527537, *Rangifer tarandus* AB245426, *Muntiacus muntjak* AY225986, *Elaphodus cephalophus* DQ873526, *Cervus elaphus* AB245427, *Cervus nippon taiouanus* DQ985076, *Cervus unicolor swinhoei* EF035448, *Cervus nippon yesoensis* AB210267, *Cervus nippon yakushimae* AB218689, *Cervus nippon centralis* AB211429) and one Bovidae species used as outgroup (*Bos taurus* AB074968) were downloaded from GenBank and aligned to the new *Dama dama* COI sequence using the ClustalX software package (Thompson et al., 1997).

The follow deer COI sequence was submitted to the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) for homology searches. The COI sequence analysis was performed with the DAMBE software package (Xia & Xie, 2001). The protein sequence corresponding to the obtained DNA sequence was analysed with the TMPred software (Hofmann & Stoffel, 1993) available online at http://www.ch.embnet.org/software/TMPRED_form.html.

RESULTS AND DISCUSSION

A 690bp fragment of the mitochondrial COI gene was obtained for the analysed sample of *Dama dama* (Fig. 3). The base composition (Tab. 3) is similar to the other members of the Cervidae family (data not shown). The obtained sequence was submitted to GenBank under accession no. EF675699.

LOCUS	DD070606 690 bp DNA 13-iun-2007					
BASE COUNT	188	a	166	c	116	g 220 t
ORIGIN						
1	aagatattgg	taccctgtat	ctactatttg	gtgcctgagc	ggcatagta	
51	ggaacagctt	taagcctatt	gattcgtgct	gaactgggcc	aacctgggtac	
101	cctacttgga	gatgaccaa	tttataatgt	tattgtaacc	gcacatgcat	
151	tcgtaataat	tttctttata	gttataccaa	ttataatcgg	aggatttggg	
201	aactgactag	ttcccttaat	aattgggtgcc	ccagatatag	cattccctcg	
251	aataaacaat	atgagctttt	gactccttcc	tcctctttc	ttactacttc	
301	tagcatcatc	tatagttgaa	gctggcgcag	gaacaggctg	aactgtgtac	
351	ccccctctag	ctggtaactt	agctcacgca	ggagcctcag	tggacctaac	
401	tatcttttct	ctacacctgg	caggtgtctc	ttcaattcta	ggggccatta	
451	actttattac	aacaattatc	aatataaaac	cccctgctat	gtcacataac	
501	caaactcccc	tatttgtgtg	atccgtacta	gtcaactgctg	tattactact	
551	tctctcactc	ccagtactag	cagctggaat	tacaatatta	ttaacagacc	
601	gaaatttaaa	tacaaccttt	tttgatccag	caggaggcgg	agatcccatt	
651	ctatatcaac	acttattctg	atTTTTTggt	caccctgaag		

Fig. 1 – Nucleotide sequence of the *Dama dama* 5' cytochrome oxydase subunit 1 gene region (GenBank accession number EF675699).

Table 3

DNA molecule: DD070606 [organism= <i>Dama dama</i>]		
G+C content = 40.87%		
A+T content = 59.13%		
Nucleotide	Number	Mol%
A	188	27.25
C	166	24.06
G	116	16.81
T	220	31.88

The homology searches performed with the BLAST program for the *Dama dama* COI sequence issued a 93% sequence similarity with *Cervus nippon*, 92% with *Cervus unicolor* and *Cervus elaphus*, 89% with *Elaphodus cephalophus* and 88% with *Muntiacus muntjak* and *Rangifer tarandus*. The same species, plus *Bos taurus* as outgroup were used to infer the UPGMA dendrogram based on the TN83 genetic distance (Nei & Tajima, 1983). The resulting tree is presented in figure 2. In this tree, all *Cervus* sp. sequences clustered together with the *Dama dama* sequence. The results are in agreement with previous studies (Gilbert et al., 2006).

The amino acid sequence of the corresponding 5'COI gene region comprises five transmembranar helices joined by two external and two internal lops (Fig. 3), in accordance with the topographical model of the COI protein (Saraste, 1990).

In this paper we report the 5'COI gene region DNA sequence from the cervid species *Dama dama*. The sequence was submitted to GenBank and will allow the identification of the follow deer using the barcode technique.

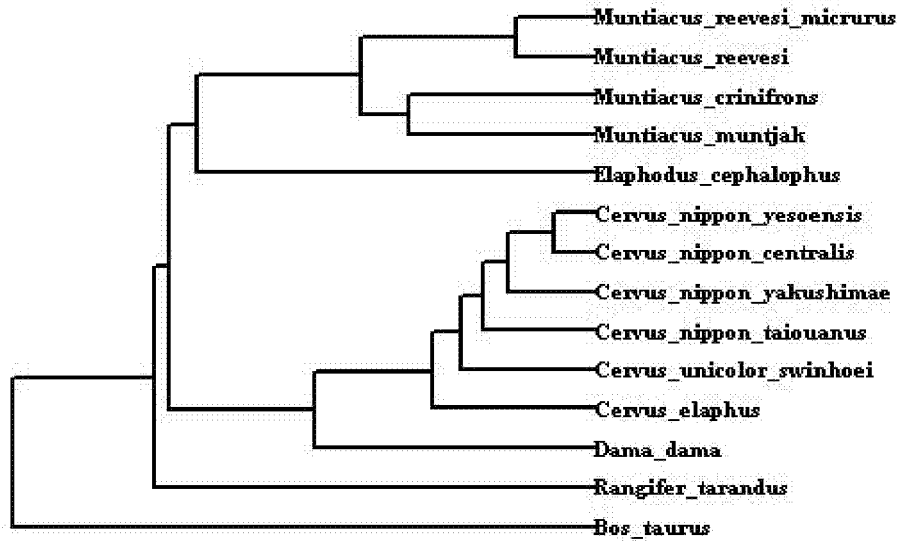


Fig. 2 – Dendrogram based on the alignment of the 5'COI sequences of different cervids species, constructed using DAMBE. The sequence from *Bos taurus* was used as the outgroup.

Tmpred output for *Dama dama*

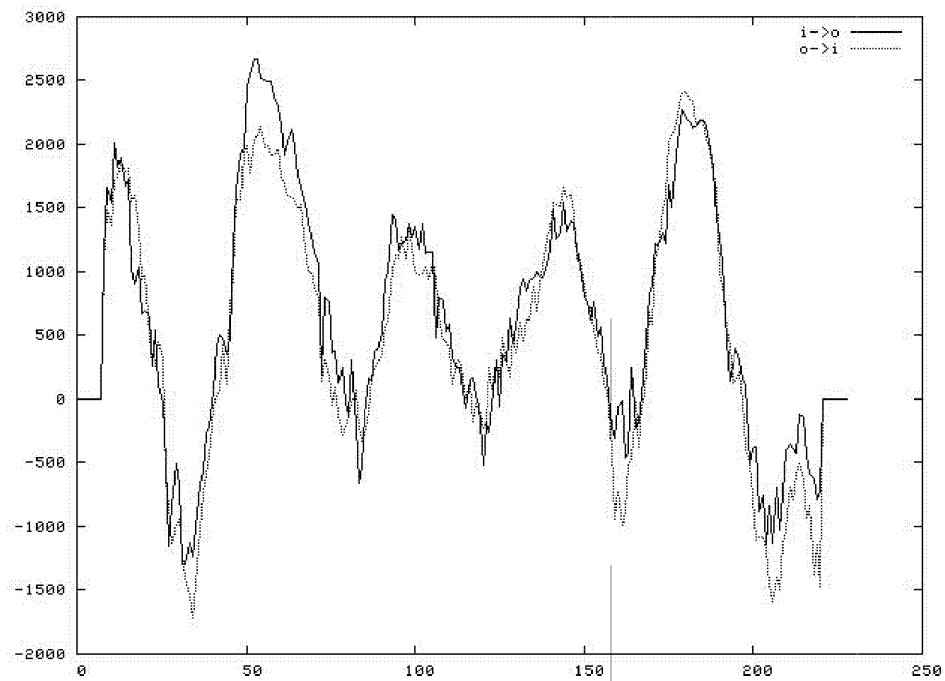


Fig. 3 – Hydropathy plot of *Dama dama* 5'COI gene region. The plot was generated with TMPred.

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ANALIZA SECVENȚEI ADN A REGIUNII GENICE 5'COI LA SPECIA *DAMA DAMA*
(LINNAEUS, 1758) (MAMMALIA: CERVIDAE)

REZUMAT

În această lucrare prezentăm analiza secvenței regiunii 5' a genei COI (cytochrome oxidase subunit I) de la nivelul ADN mitocondrial pentru specia *Dama dama* (Mammalia: Cervidae). Regiunea genică respectivă este utilizată pentru identificarea speciilor animale, în cadrul tehnicii denumite „DNA barcoding”. Secvența analizată a fost publicată în baza de date Gen Bank cu numărul de acces EF675699.

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Luis Ovidiu Popa, Oana Paula Popa, Dumitru Murariu
Muzeul Național de Istorie Naturală „Grigore Antipa”
Șos. Kiseleff nr. 1, 011341 București 2, România
e-mail: popaluis@antipa.ro
oppopa@antipa.ro
dmurariu@antipa.ro

Petre Gărgărea
Regia Națională a Pădurilor ROMSILVA
Bd. Magheru nr. 31, 010325 București 1, România
e-mail: s.vanat@rosilva.ro