

PCR-RFLP Authentication of Meats from Red Deer  
(*Cervus elaphus*), Fallow Deer (*Dama dama*), Roe Deer  
(*Capreolus capreolus*), Cattle (*Bos taurus*), Sheep (*Ovis aries*),  
and Goat (*Capra hircus*)

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PCR-RFLP analysis has been applied to the identification of meats from red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), cattle (*Bos taurus*), sheep (*Ovis aries*), and goat (*Capra hircus*). PCR amplification was carried out using a set of primers flanking a conserved region of ~712 base pairs from the mitochondrial 12S rRNA gene. Restriction site analysis based on sequence data from this DNA fragment permitted the selection of *MseI*, *MbolI*, *BstI*, and *ApoI* endonucleases for species identification. The restriction profiles obtained when amplicons were digested with the chosen enzymes allowed the unequivocal identification of all domestic and game meat species analyzed in the present work.

**KEYWORDS:** Game meat; species identification; 12S rRNA gene; PCR-RFLP

## INTRODUCTION

The consumption of game meat is growing in many parts of the world because of its extraordinary health and sensory characteristics: it is low in fat and cholesterol, the animals have not been treated with hormones or steroids, and the meat has a fine texture and delicate flavor when prepared properly (1, 2).

Consumers increasingly demand higher protection from falsely labeled food for a variety of economic, religious, and health reasons. However, falsification of game meat products is becoming very common due to the tremendous profit that results from selling less costly meat as meat from higher priced species in great demand. Thus, the prevention of such fraudulent practices, nowadays, constitutes an important part of food regulatory control and quality assurance systems. In this context, development of reliable and simple tools for the authentication of meat from game species is necessary for wildlife law enforcement and to assess their proper use in processed game products (3, 4).

Conventional methodology used for the determination of the species origin in meat products has been predominantly based on the immunochemical and electrophoretic analysis of proteins (5, 6). Alternatively, DNA analysis constitutes an attractive strategy for meat species identification. In comparison with proteins, DNA is stable against technological treatments and independent of the considered tissue. For these reasons, nucleic acid based techniques are now the preferred approaches for species identification in food products (7–9).

In recent years, an extensive number of works have been developed that use Polymerase Chain Reaction (PCR) coupled with techniques such as nucleotide-sequencing, single-strand conformation polymorphism (SSCP), random amplified polymorphic DNA (RAPD), or restriction fragment length polymorphism (RFLP) for the identification of domestic meat species such as cattle, sheep, goat, pig, turkey, or chicken (10–14). However, fewer studies have been published so far reporting the application of DNA-based techniques for game meat authentication (1, 2, 4, 15, 16).

Meat from venison is becoming increasingly popular in European markets. The three main species consumed in Europe are the red deer (*Cervus elaphus*), the fallow deer (*Dama dama*), and the roe deer (*Capreolus capreolus*). These animals are reported to produce a leaner, more flavorful, and also more expensive meat than their domestic counterparts. In the present work we describe a method for the specific identification of game meats from red deer, fallow deer, and roe deer based on PCR-RFLP analysis of a conserved fragment from the mitochondrial 12S rRNA gene. The assay is also intended to enable the differentiation between these cervid meats and those from cattle, sheep, and goat domestic species.

## MATERIALS AND METHODS

**Sample Selection and DNA Extraction.** Authentic muscle samples of red deer, fallow deer, and roe deer were obtained from the Department of Animal Pathology (Facultad de Veterinaria, Universidad Autónoma de Barcelona, Spain) and from several meat-cutting installations of the Comunidad de Madrid (Parque Natural “El Pardo”), Andalucía (Parque Natural Sierra de Cazorla, Segura y Las Villas),

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and Castilla-La Mancha (Ciudad Real and Toledo). Cattle, sheep, and goat meat samples were obtained from local abattoirs (Madrid, Spain). All specimens were morphologically identified before the samples were obtained.

Several processed game meat products were also obtained from different retail markets. These included dried salt-cured and heat-treated meat samples from red deer and roe deer species.

Genomic DNA was extracted from meat using a Wizard DNA Clean-up System kit (Promega Corp., Madison, WI). The extraction was performed as follows: 0.2 g of meat was homogenized with 860  $\mu\text{L}$  of extraction buffer (10 mM Tris, pH 8.0; 150 mM NaCl, 2 mM EDTA, and 1% SDS), 100  $\mu\text{L}$  of 5 M guanidine-hydrochloride, and 40  $\mu\text{L}$  of 20 mg/mL proteinase K (Boehringer Mannheim GmbH, Mannheim, Germany). The samples were incubated overnight at 55 °C with shaking at 60 rpm (C24KC, New Brunswick Scientific Co., Edison, NJ). After incubation, they were left to cool at room temperature. Five hundred microliters of chloroform (Sigma-Aldrich, Steinheim, Germany) was added to the lysate before centrifugation at 13000 rpm for 10 min. The aqueous phase (500  $\mu\text{L}$ ) was carefully transferred to a fresh tube to purify the DNA using the Wizard DNA Clean-up System kit (Promega) with a vacuum manifold, according to the manufacturer's instructions. Finally, the DNA was eluted in 50–100  $\mu\text{L}$  of sterile deionized water, and its concentration was determined by spectrophotometry at 260 nm.

**PCR Amplification of a Conserved 12S rRNA Gene Fragment from Red Deer, Fallow Deer, Roe Deer, Cattle, Sheep, and Goat Meats.** The set of primers used for amplification consisted of 12S-FW and 12S-REV oligonucleotides:

12S-FW: 5'-GGTAAATCTCGTGCCAGCCA-3'

12S-REV: 5'-TCCAGTATGCTTACCTTGTTACGAC-3'

They were designed for the amplification of a conserved fragment of the 12S rRNA gene, based on sequences available in the Genbank/EMBL database for various mammal species. This set of primers was expected to produce amplicons of the same length (~712 bp) in the six meat species analyzed in this work.

PCR amplification reactions were performed in a total volume of 50  $\mu\text{L}$ . Each reaction mixture contained 100–200 ng of template DNA, 2 mM  $\text{MgCl}_2$ , 50 mM KCl, 200  $\mu\text{M}$  of each dNTP, 5 pmol of each primer, and 2 units of *Tth* DNA polymerase (Biotools, Madrid, Spain) in a reaction buffer containing 75 mM Tris-HCl, pH 9.0, 50 mM KCl, 20 mM  $(\text{NH}_4)_2\text{SO}_4$ , and 0.001% BSA.

PCR amplification was carried out in a Progene thermal cycler (Techne Ltd., Cambridge, U.K.). Thirty-five cycles of amplification with the following step cycle profile were programmed: strand denaturation at 93 °C for 30 s, primer annealing at 65 °C for 30 s, and primer extension at 72 °C for 45 s. An initial denaturation at 93 °C for 2 min and a final extension at 72 °C for 5 min improved the product yield. PCR products (10  $\mu\text{L}$ ) were mixed with 2  $\mu\text{L}$  of gel loading solution (Sigma) and loaded in a 1.5% D1 (Hispanlab S.A., Torrejón, Spain) agarose gel containing 1  $\mu\text{g}/\text{mL}$  ethidium bromide in Tris-acetate buffer (0.04 M Tris-acetate and 0.001 M EDTA, pH 8.0). Electrophoretic separation was performed at 110 V for 30 min. The resulting DNA fragments were visualized by UV transillumination and analyzed using a Geldoc 1000 UV Fluorescent Gel Documentation System-PC (Bio-Rad Laboratories, Hercules, CA).

**Sequencing of the PCR Products.** PCR products (90  $\mu\text{L}$ ) amplified with 12S-FW and 12S-REV oligonucleotides from red deer, fallow deer, roe deer, cattle, sheep, and goat were loaded in a 2% LM2 (Hispanlab) agarose gel containing 1  $\mu\text{g}/\text{mL}$  ethidium bromide in Tris-acetate buffer and electrophoresed at 90 V for 70 min. Each DNA fragment was excised from the agarose gel under UV light using a sterile scalpel. The gel slice was purified with the QIAquick Gel Extraction kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The concentration of the PCR products was estimated by agarose gel electrophoresis using a standard as reference marker (REAL, Durviz S.L., Valencia, Spain). A Geldoc 1000 System-PC (Bio-Rad) was used for that purpose. Purified PCR products were sequenced at Sistemas Genómicos S.L. (Parque Tecnológico de Valencia, Spain). DNA

sequencing was accomplished in an ABI Prism model 377 DNA sequencer (Perkin-Elmer/Applied Biosystems Division, Foster City, CA) using 12S-FW and 12S-REV primers with the dRhodamine Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer).

**Restriction Site Mapping and Enzymatic Digestion of the Amplified DNA Fragments.** Alignment and restriction site mapping of 12S rRNA gene sequences obtained from red deer (AJ 885204, AJ 885205, AJ 885206), fallow deer (AJ 885203), roe deer (AJ 885202, AJ 972679, AJ 972680, AJ 972681, AJ 972682, AJ 972683), cattle (AJ 885201), sheep (AJ 885200), and goat (AJ 885199) were performed using the EMBOSS software package, version 2.2.0. From the detailed comparison of the sequence maps, *MseI*, *MboII*, *BslI*, and *ApoI* endonucleases (New England BioLabs, Beverly, MA) were selected for meat species identification.

Digestions were performed in a total volume of 20 L containing 100–200 ng of amplified DNA, 5–10 units of enzyme, and 10 L of 10 $\times$  digestion buffer recommended by the manufacturer and were incubated at the appropriate temperature of each endonuclease during 16 h. The resulting fragments were separated by electrophoresis in a 3.5% MS8 (Hispanlab) agarose gel at 70 V for 90 min. The sizes of the resulting DNA fragments were estimated by comparison with a commercial standard (Biomarker Low, BioVentures Inc., Murfreesboro, TN).

## RESULTS AND DISCUSSION

The food analyst is confronted with providing proof of fraudulent substitution of more expensive meats with cheaper ones (8). To detect such frauds, analysis of RFLP of PCR products has gained acceptance among meat species identification methods (3, 4, 12). The present study aimed to develop a PCR-RFLP technique for the specific identification of meats from the cervid species red deer (*C. elaphus*), fallow deer (*D. dama*), and roe deer (*C. capreolus*), targeting sequences of the 12S rRNA gene. The technique was also applied to the differentiation of these game meats from those of bovine, caprine, and ovine species.

PCR has the potential sensitivity and specificity required to achieve detection of a target sequence from template DNA (8, 17). The mitochondrial 12S rRNA gene has been selected in this study as template for DNA amplification because it has an acceptable length and an adequate grade of mutation and there are numerous sequences available in the databases (10, 18, 19). The mitochondrial primers 12S-FW and 12S-REV used in the PCR technique developed in this work successfully amplified a conserved 712 bp region from the 12S rRNA gene of all red deer, fallow deer, roe deer, cattle, sheep, and goat individuals analyzed (results not shown).

Following amplification, the use of PCR-RFLP analysis to identify the origin of an unknown sample may be possible thanks to the use of the appropriate restriction endonucleases (13, 20). However, previous sequence data from authentic specimens are needed to provide species-specific reference restriction patterns. For that purpose, 12S PCR products from at least 11 individuals of each selected meat species were purified from the gel and sequenced. Restriction map analysis of the 12S rRNA gene sequences obtained from red deer (AJ 885204, AJ 885205, AJ 885206), fallow deer (AJ 885203), roe deer (AJ 885202, AJ 972679, AJ 972680, AJ 972681, AJ 972682, AJ 972683), cattle (AJ 885201), sheep (AJ 885200), and goat (AJ 885199) allowed the selection of *MseI* endonuclease, as well as a combination of *MboII*, *BslI*, and *ApoI* enzymes, as potential tools for the suitable identification of meats from the six analyzed species (Figure 1). As shown in the figure, the presence of a few nucleotide polymorphisms within some individuals of red deer and roe deer generated more than one 12S rRNA gene sequence type for these two species. The cleavage patterns predicted from sequence map analysis are indicated in Table 1.

1 12S-FW→ MseI MseI 70

C. elaphus1 (AJ 885204) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. elaphus2 (AJ 885205) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. elaphus3 (AJ 885206) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 D. dama (AJ 885203) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. capreol1 (AJ 885202) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. capreol2 (AJ 972679) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. capreol3 (AJ 972680) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. capreol4 (AJ 972681) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. capreol5 (AJ 972682) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. capreol6 (AJ 972683) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 C. hircus (AJ 885199) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 B. taurus (AJ 885201) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG  
 O. aries (AJ 885200) GGTAATCTC GTGCCAGCCA CCGCGGTCAT ACGATTAAACC CAAGTTAATA GGCATACGGC GTAAAGTGTG

71 MseI MseI ApoI MseI MseI 140

C. elaphus1 (AJ 885204) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. elaphus2 (AJ 885205) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. elaphus3 (AJ 885206) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 D. dama (AJ 885203) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. capreol1 (AJ 885202) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. capreol2 (AJ 972679) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. capreol3 (AJ 972680) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. capreol4 (AJ 972681) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. capreol5 (AJ 972682) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. capreol6 (AJ 972683) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 C. hircus (AJ 885199) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 B. taurus (AJ 885201) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT  
 O. aries (AJ 885200) TTAAGCACT ATACTAAATA AAGTTAAATT CCAATTAAGC TGTA AAAAGC CATAATCGTA ACAA AATAT

141 MseI BslI 210

C. elaphus1 (AJ 885204) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. elaphus2 (AJ 885205) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. elaphus3 (AJ 885206) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 D. dama (AJ 885203) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. capreol1 (AJ 885202) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. capreol2 (AJ 972679) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. capreol3 (AJ 972680) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. capreol4 (AJ 972681) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. capreol5 (AJ 972682) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. capreol6 (AJ 972683) ATAACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 C. hircus (AJ 885199) ATGACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 B. taurus (AJ 885201) ATGACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT  
 O. aries (AJ 885200) ATGACGAAAG TAACCTTACA ACCGCTGAAA CACGATAGCT AGGACCCAAA CTGGGATTAG ATACCCCCT

211 MseI MseI MseI280

C. elaphus1 (AJ 885204) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. elaphus2 (AJ 885205) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. elaphus3 (AJ 885206) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 D. dama (AJ 885203) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. capreol1 (AJ 885202) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. capreol2 (AJ 972679) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. capreol3 (AJ 972680) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. capreol4 (AJ 972681) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. capreol5 (AJ 972682) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. capreol6 (AJ 972683) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 C. hircus (AJ 885199) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 B. taurus (AJ 885201) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA  
 O. aries (AJ 885200) ATGCCTAGCC TTAACACAAA ATAGTTATGC AAACAAAAC ATTGCGCCAGA GTACTACCGG CAATAGCTTA

281 BslI 350

C. elaphus1 (AJ 885204) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. elaphus2 (AJ 885205) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. elaphus3 (AJ 885206) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 D. dama (AJ 885203) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. capreol1 (AJ 885202) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. capreol2 (AJ 972679) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. capreol3 (AJ 972680) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. capreol4 (AJ 972681) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. capreol5 (AJ 972682) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. capreol6 (AJ 972683) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 C. hircus (AJ 885199) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 B. taurus (AJ 885201) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT  
 O. aries (AJ 885200) AAACCTCAAG GACTTGGCGG TGCTTTATAC CCTTCTAGAG GAGCCTGTTC TATAATCGAT AAACCCCGAT

351 MboII BslI 420

C. elaphus1 (AJ 885204) AAACCTCACC ATTCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. elaphus2 (AJ 885205) AAACCTCACC ATTCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. elaphus3 (AJ 885206) AAACCTCACC ATTCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 D. dama (AJ 885203) AAACCTCACC ATTCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. capreol1 (AJ 885202) AAACCTCACC ACCCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. capreol2 (AJ 972679) AAACCTCACC ACCCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. capreol3 (AJ 972680) AAACCTCACC ACCCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. capreol4 (AJ 972681) AAACCTCACC ACCCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. capreol5 (AJ 972682) AAACCTCACC ACCCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. capreol6 (AJ 972683) AAACCTCACC ACCCTTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 C. hircus (AJ 885199) AAACCTCACC AATCCTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 B. taurus (AJ 885201) AAACCTCACC AATCCTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA  
 O. aries (AJ 885200) AAACCTCACC AATCCTGTCT AATACAGTCT ATATACCGCC ATCTTCAGCG AACCTTAAAA A-GGTACAAA

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C. elaphus1 (AJ 885204) AGTAAGCACA ATCATAATAC ATAAAGACGT TAGGTCAAGG TGTAACCTAT GGAACGGAAA GAAATGGGCT
C. elaphus2 (AJ 885205) AGTAAGCACA ATCATAATAC ATAAAGACGT TAGGTCAAGG TGTAACCTAT GGAACGGAAA GAAATGGGCT
C. elaphus3 (AJ 885206) AGTAAGCACA ATCATAATAC ATAAAGACGT TAGGTCAAGG TGTAACCTAT GGAACGGAAA GAAATGGGCT
D. dama (AJ 885203) AGTAAGCACA ATCATAATAC ATAAAGACGT TAGGTCAAGG TGTAACCTAT GGAATGGGAA GAAATGGGCT
C. capreol1 (AJ 885202) AGTAAGCACA ACCATCATAC ATAAAAACGT TAGGTCAAGG TGTAACCTAT GAGGTGGGAA GAAATGGGCT
C. capreol2 (AJ 972679) AGTAAGCACA ACCATCATAC ATAAAAACGT TAGGTCAAGG TGTAACCTAT GAGGTGGGAA GAAATGGGCT
C. capreol3 (AJ 972680) AGTAAGCACA ACCATCATAC ATAAAAACGT TAGGTCAAGG TGTAACCTAT GAGGTGGGAA GAAATGGGCT
C. capreol4 (AJ 972681) AGTAAGCACA ACCATCATAC ATAAAAACGT TAGGTCAAGG TGTAACCTAT GAGGTGGGAA GAAATGGGCT
C. capreol5 (AJ 972682) AGTAAGCACA ACCATCATAC ATAAAAACGT TAGGTCAAGG TGTAACCTAT GAGGTGGGAA GAAATGGGCT
C. capreol6 (AJ 972683) AGTAAGCACA ACCATCATAC ATAAAAACGT TAGGTCAAGG TGTAACCTAT GAGGTGGGAA GAAATGGGCT
C. hircus (AJ 885199) AGTAAGTCA ATCACAACAC ATAAAGACGT TAGGTCAAGG TGTAACCTAT GGAATGGGAA GAAATGGGCT
B. taurus (AJ 885201) AGTAAGCGTA ATTATGATAC ATAAAAACGT TAGGTCAAGG TGTAACCTAT GGAATGGGAA GAAATGGGCT
O. aries (AJ 885200) AGTAAGCTCA ATAATAACAC ATAAAGACGT TAGGTCAAGG TGTAACCTAT GAGGTGGGAA GAAATGGGCT

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C. elaphus1 (AJ 885204) ACATTTTCTA ATCTAAGAAA ATCCAACACG AAAGTTATTA TGAAATTAAT AACCAAAGGA GGATTTAGCA
C. elaphus2 (AJ 885205) ACATTTTCTA ATCTAAGAAA ATCCAACACG AAAGTTATTA TGAAACTAAT AACCAAAGGA GGATTTAGCA
C. elaphus3 (AJ 885206) ACATTTTCTA ATCTAAGAAA ATCCAACACG AAAGTTATTA TGAAATTAAT AACCAAAGGA GGATTTAGCA
D. dama (AJ 885203) ACATTTTCTA ACCTAAGAAA ATCTAATACG AAAGTTATTA TGAAACTAGT AACCAAAGGA GGATTTAGCA
C. capreol1 (AJ 885202) ACATTTTCTA ATTTAAGAAA ACTTAAACAG AAAGTTATTA TGAAATTAAT AACTAAAGGA GGATTTAGCA
C. capreol2 (AJ 972679) ACATTTTCTA ATTTAAGAAA ACTTAAACAG AAAGTTATTA TGAAATTAAT AACTAAAGGA GGATTTAGCA
C. capreol3 (AJ 972680) ACATTTTCTA ATTTAAGAAA ACTTAAACAG AAAGTTATTA TGAAATTAAT AACTAAAGGA GGATTTAGCA
C. capreol4 (AJ 972681) ACATTTTCTG ATTTAAGAAA ACTTAAACAG AAAGTTATTA TGAAATTAAT AACTAAAGGA GGATTTAGCA
C. capreol5 (AJ 972682) ACATTTTCTG ATTTAAGAAA ACTTAAACAG AAAGTTATTA TGAAATTAAT AACTAAAGGA GGATTTAGCA
C. capreol6 (AJ 972683) ACATTTTCTG ATTTAAGAAA ACTTAAACAG AAAGTTATTA TGAAATTAAT AACTAAAGGA GGATTTAGCA
C. hircus (AJ 885199) ACATTTTCTA CCTTAAGAAA ATTTAA-TACG AAAGCCATTA TGAAATTAAT GACCAAGGA GGATTTAGCA
B. taurus (AJ 885201) ACATTTCTA CACCAAGGA ATCAAGCAGG AAAGTTATTA TGAAACCAAT AACCAAAGGA GGATTTAGCA
O. aries (AJ 885200) ACATTTTCTA CCCAAGAAA TTTAA-TACG AAAGCCATTA TGAAATTAAT AGCCAAGGA GGATTTAGCA

561
C. elaphus1 (AJ 885204) GTAAACTAAG AATAGAGTGC TTAGTTGAAC TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. elaphus2 (AJ 885205) GTAAACTAAG AATAGAGTGC TTAGTTGAAC TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. elaphus3 (AJ 885206) GTAAACTAAG AATAGAGTGC TTAGTTGAAC TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
D. dama (AJ 885203) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. capreol1 (AJ 885202) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. capreol2 (AJ 972679) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. capreol3 (AJ 972680) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. capreol4 (AJ 972681) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. capreol5 (AJ 972682) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. capreol6 (AJ 972683) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
C. hircus (AJ 885199) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
B. taurus (AJ 885201) GTAAACTAAG AATAGAGTGC TTAGTTGAAT TAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC
O. aries (AJ 885200) GTAAACTAAG AATAGAGTGC TTAGTTGAAT CAGGCCATGA AGCACGCACA CACCGCCCGT CACCCTCCTC

631
C. elaphus1 (AJ 885204) AAGTAGGCAC AGTACACTCA AATTTATTTC CACGTATTAA TCATATGAGA GGAGACAAGT CGTAACAAGG
C. elaphus2 (AJ 885205) AAGTAGGCAC AGTACACTCA AATTTATTTC CACGTATTAA TCATATGAGA GGAGACAAGT CGTAACAAGG
C. elaphus3 (AJ 885206) AAGTAGGCAC AGTACACTCA AATTTATTTC CACGTATTAA TCATATGAGA GGAGACAAGT CGTAACAAGG
D. dama (AJ 885203) AAGTAGGCAC AATACACTCA AATTTATTCA TACGTATTAA TCATATGAGA GGAGACAAGT CGTAACAAGG
C. capreol1 (AJ 885202) AAATAAGCAA AATACATTAA AATTTATTTC TACGTATAAA CCATATGAGA GGAGACAAGT CGTAACAAGG
C. capreol2 (AJ 972679) AAATAAGCAA AATACATTAA AATTTATTTC TACGTATAAA CCATATGAGA GGAGACAAGT CGTAACAAGG
C. capreol3 (AJ 972680) AAATAAGCAA AATACATTAA AATTTATTTC TACGTATAAA CCATATGAGA GGAGACAAGT CGTAACAAGG
C. capreol4 (AJ 972681) AAATAAGCAA AATACATTAA AATTTATTTC TACGTATAAA CCATATGAGA GGAGACAAGT CGTAACAAGG
C. capreol5 (AJ 972682) AAATAAGCAA AATACATTAA AATTTATTTC TACGTATAAA CCATATGAGA GGAGACAAGT CGTAACAAGG
C. capreol6 (AJ 972683) AAATAAGCAA AATACATTAA AATTTATTTC TACGTATAAA CCATATGAGA GGAGACAAGT CGTAACAAGG
C. hircus (AJ 885199) AAGTAATATC AATGCACTCA AGCCTATTAA CACGCATCAA CTACATGAGA GGAGATAAGT CGTAACAAGG
B. taurus (AJ 885201) AAATAGATTC AGTGCATCTA ACCCTATTAA AACGCACTAG CTACATGAGA GGAGACAAGT CGTAACAAGG
O. aries (AJ 885200) AAGTAATATGAT GATATACTTA AACCTATTAA CATATATCAA CCACACGAGA GGAGACAAGT CGTAACAAGG

701 ←12S-REV713
C. elaphus1 (AJ 885204) TAAGCATACT GGA
C. elaphus2 (AJ 885205) TAAGCATACT GGA
C. elaphus3 (AJ 885206) TAAGCATACT GGA
D. dama (AJ 885203) TAAGCATACT GGA
C. capreol1 (AJ 885202) TAAGCATACT GGA
C. capreol2 (AJ 972679) TAAGCATACT GGA
C. capreol3 (AJ 972680) TAAGCATACT GGA
C. capreol4 (AJ 972681) TAAGCATACT GGA
C. capreol5 (AJ 972682) TAAGCATACT GGA
C. capreol6 (AJ 972683) TAAGCATACT GGA
C. hircus (AJ 885199) TAAGCATACT GGA
B. taurus (AJ 885201) TAAGCATACT GGA
O. aries (AJ 885200) TAAGCATACT GGA

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**Figure 1.** DNA sequences from part of the 12S rRNA gene of red deer (*C. elaphus*), fallow deer (*D. dama*), roe deer (*C. capreolus*), goat (*C. hircus*), cattle (*B. taurus*), and sheep (*O. aries*). Restriction sites for *MseI* (T/TAA), *MbolI* (GAAGA(N)<sub>8</sub>), *BslI* (CCNNNNN/NGG), and *ApoI* (R/AATY) are shaded (N = A, T, C, or G; Y = T or C; R = A or G). Boldfaced nucleotides indicate the position of primers 12S-FW and 12S-REV used for PCR amplification.

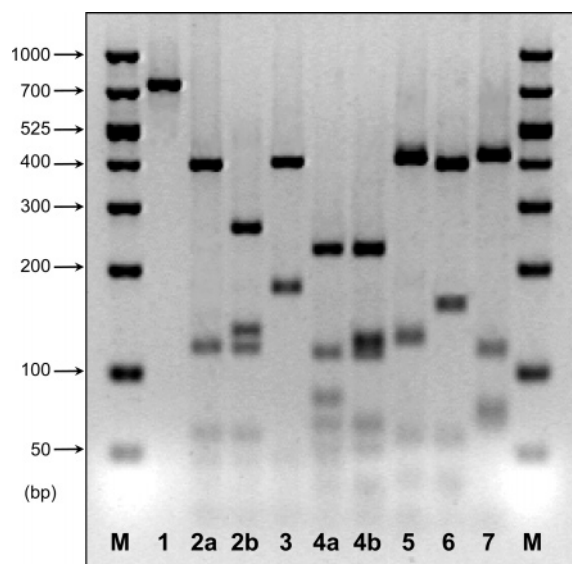
**Figure 2** shows the results obtained following restriction analysis of 12S rRNA gene from red deer, fallow deer, roe deer, cattle, sheep, and goat, after incubation with *MseI* endonuclease. As can be seen, different DNA banding profiles were obtained for each of the six meat species analyzed with this enzyme. However, the few existing 12S rRNA sequence point mutations

within individuals from red deer and roe deer originated two different *MseI* electrophoretic patterns on each of these two species. In this way, *MseI* digestions of red deer samples caused either 9 (line 2a, pattern A) or 10 (line 2b, pattern B) DNA fragments of 388 to 10 bp or 257 to 10 bp, respectively. Similarly, roe deer 12S rRNA gene digestions originated either

**Table 1.** Lengths of Fragments Generated by Digestion of PCR Products from 12S rRNA Gene with the Indicated Restriction Enzymes<sup>a</sup>

n = 25	red deer		fallow deer	roe deer		goat	cattle	sheep
<i>MseI</i>	A (n' = 6)	B (n' = 19)		A (n' = 12)	B (n' = 13)			
	<b>388</b>	<b>257</b>	<b>388</b>	<b>224</b>	<b>224</b>	<b>397</b>	<b>379</b>	<b>407</b>
	<b>116</b>	<b>131</b>	<b>173</b>	<b>112</b>	<b>122</b>	<b>121</b>	<b>152</b>	<b>112</b>
	<b>57</b>	<b>116</b>	<b>46</b>	<b>79</b>	<b>112</b>	<b>57</b>	<b>55</b>	<b>71</b>
	46	<b>57</b>	35	<b>65</b>	<b>65</b>	36	36	<b>65</b>
	35	46	26	<b>51</b>	<b>51</b>	35	35	23 (2)
	26	35	23	43	35	23 (2)	32	7
	23	26	11	35	34	11	23	4
	11	23	10	34	26	9		
	10	11		26	23			
		10		23	10 (2)			
			10(2)					
<i>MboII</i>			<b>384</b>			<b>384</b>	<b>384</b>	<b>384</b>
			<b>328</b>	<b>489</b>	<b>384</b>	<b>223</b>	<b>223</b>	<b>223</b>
				<b>223</b>	<b>223</b>	<b>105</b>	<b>105</b>	<b>105</b>
<i>BsII</i>			<b>223</b>	<b>223</b>	<b>303</b>	<b>240</b>	<b>303</b>	<b>303</b>
			<b>191</b>	<b>191</b>	<b>191</b>	<b>191</b>	<b>191</b>	<b>191</b>
			<b>126</b>	<b>126</b>	<b>126</b>	<b>126</b>	<b>126</b>	<b>126</b>
			<b>92</b>	<b>92</b>	<b>92</b>	<b>92</b>	<b>92</b>	<b>92</b>
			<b>80</b>	<b>80</b>	<b>92</b>	<b>63</b>	<b>92</b>	<b>92</b>
<i>ApoI</i>			<b>553</b>	<b>553</b>	<b>649</b>	<b>616</b>	<b>616</b>	<b>412</b>
			<b>96</b>	<b>96</b>	<b>63</b>	<b>96</b>	<b>96</b>	<b>204</b>
			<b>63</b>	<b>63</b>				<b>96</b>

<sup>a</sup> n = number of individuals analyzed from each species; n' = number of individuals yielding profile A or B. Boldface type indicates visible DNA fragments in gel images.



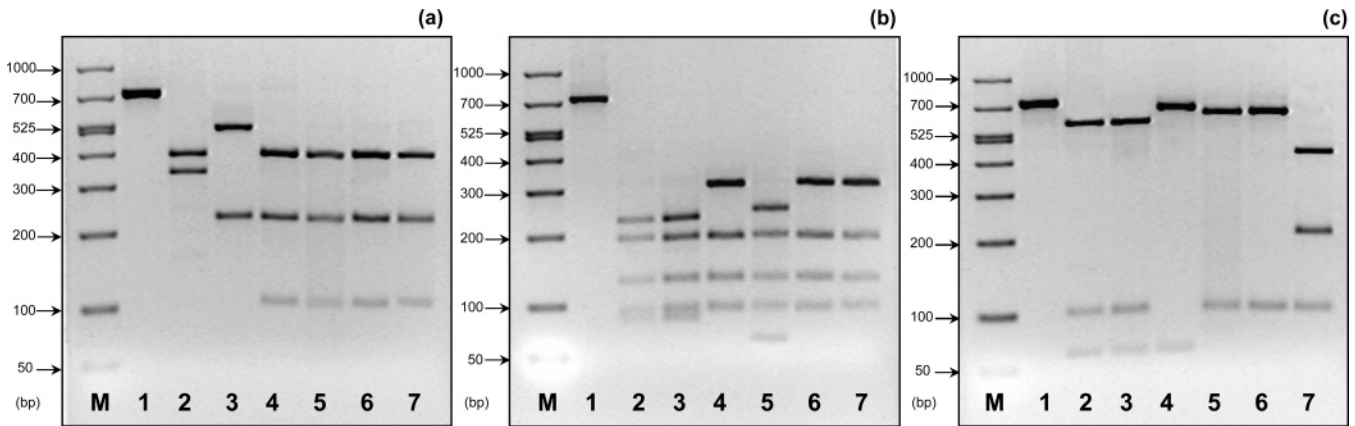
**Figure 2.** Electrophoretic analysis (3.5% MS8 agarose) of the restriction fragments obtained from 12S rRNA gene PCR products digested with *MseI* endonuclease. Samples: (1) undigested PCR product; (2a) red deer RFLP pattern A; (2b) red deer RFLP pattern B; (3) fallow deer; (4a) roe deer RFLP pattern A; (4b) roe deer RFLP pattern B; (5) goat; (6) cattle; (7) sheep. M, molecular weight marker 50–1000 bp ladder (Biomarker Low).

12 (line 4a, pattern A) or 11 (line 4b, pattern B) DNA fragments of 224 to 10 bp. On the other hand, digestions performed with *MseI* endonuclease resulted in eight DNA fragments (388 to 10 bp) in fallow deer and another eight fragments (407 to 4 bp) in sheep. Six restriction sites for this enzyme found in cattle PCR products yielded seven DNA fragments of 379 to 23 bp. Finally, goat samples originated nine fragments of 397 to 9 bp, as expected from the presence of eight recognition sites for *MseI* endonuclease. It should be noted that a number of small DNA fragments resulting from 12S rRNA gene digestions could not be detected after electrophoresis of the samples. However, the

*MseI* cleavage bands visualized in the gel were enough and suitable for the discrimination of all game and domestic species analyzed.

An advantage of using the endonuclease *MseI* is that only one enzyme allows one-step differentiation of all the species selected in this study. Nevertheless, *MseI* restriction analysis presents two important disadvantages: (i) this enzyme has many recognition sites, thus generating electrophoretic profiles with a high number of DNA fragments that may be difficult to visualize in the gel; (ii) because DNA point mutations may affect *MseI* restriction sites, more than one banding pattern is likely to be generated for a species, as occurs in the present study with red deer and roe deer.

In this work, another approach was used to select a combination of more than one enzyme that could enable PCR-RFLP differentiation of meats from the six game and domestic species, avoiding the limitations of the *MseI* endonuclease. **Figure 3** shows the results obtained following restriction analysis of 12S PCR products from red deer, fallow deer, roe deer, cattle, sheep, and goat, after incubation with *MboII*, *BsII*, and *ApoI* endonucleases. As can be deduced from this figure, the combined use of the three mentioned enzymes allowed the specific identification of the six species analyzed in this study: *MboII* endonuclease cleaved the 12S rRNA gene products of both red deer and fallow deer into two DNA fragments of 384/328 and 489/223 bp, respectively, as expected from the presence of a single recognition site in different positions of their sequences. However, two identical *MboII* restriction sites for roe deer, goat, cattle, and sheep PCR products yielded three conserved DNA fragments of 384, 223, and 105 bp (**Figure 3a**). Similarly, the presence of four restriction sites for *BsII* endonuclease in both red deer and fallow deer 12S rRNA gene sequences caused five DNA fragments of 223, 191, 126, 92, and 80 bp. The three restriction sites for this enzyme found in roe deer, cattle, and sheep PCR products yielded four DNA fragments of 303, 191, 126, and 92 bp, and goat amplicons were cleaved into five DNA fragments of 240, 191, 126, 92, and 63 bp (**Figure 3b**). Finally, digestions performed with *ApoI* endonuclease resulted in three DNA fragments of 553, 96, and 63 bp in red deer and fallow



**Figure 3.** Electrophoretic analysis (3.5% MS8 agarose) of the restriction fragments obtained from 12S rRNA gene PCR products digested with (a) *MboII*, (b) *BsiI*, and (c) *ApoI* endonucleases. Samples: (1) undigested PCR product; (2) red deer; (3) fallow deer; (4) roe deer; (5) goat; (6) cattle; (6) sheep. M, molecular weight marker 50–1000 bp ladder (Biomarker Low).

deer, whereas a single restriction site present in roe deer sequence generated two DNA fragments of 649 and 63 bp. Two restriction sites for this enzyme found in sheep samples yielded three DNA fragments of 412, 204, and 96 bp, and cattle and goat samples, with one *ApoI* restriction site, caused two DNA fragments of 616 and 96 bp (Figure 3c). In this case, it should be also noted that resolution of the agarose gel did not allow visualization of the smallest fragments that resulted from digestions.

The results obtained suggest that, compared with *MseI* endonuclease profiles, the DNA restriction patterns obtained after digestion of the amplicons with *MboII*, *BsiI*, and *ApoI* enzymes consisted of a more discrete number of bands, which facilitated the interpretation of the results. Besides, it is worth mentioning that the band sizes obtained by agarose gel electrophoresis after cleavage of PCR products with all selected endonucleases were in agreement with the expected sizes for the restriction fragments inferred from sequence analysis.

Many of the studies published to date about game species identification by the PCR-RFLP technique rely on the use of the cytochrome *b* gene included in the mitochondrial DNA (3, 4, 16). These works have been shown to provide a valuable means for rapidly testing species. However, mutations among individuals make the selection of endonucleases in conserved restriction sites difficult, and several enzymes are often needed to discriminate between phylogenetically related species (4). In the present work, the intraspecific nucleotide substitutions within red deer and roe deer 12S rRNA gene were shown to interfere with some of the recognition sites for *MseI*, giving rise to two different restriction profiles on these species. However, conserved restriction sites found for *MboII*, *BsiI*, and *ApoI* endonucleases among all red deer, roe deer, fallow deer, cattle, sheep, and goat 12S rRNA gene sequences facilitated consistent and unequivocal species-specific identification. Twenty-five specimens from each species obtained from different origins were analyzed, suggesting reproducibility of PCR-RFLP patterns with the use of all selected endonucleases.

To check the influence of processing treatments on the suitability of the PCR-RFLP method developed in this work, 12 commercial game products, including 3 dried salt-cured and 3 heat-treated meats from each red deer and roe deer species, were also assayed. The first samples (dried–cured) were correctly analyzed by the PCR-RFLP technique developed, because amplification of the 712 bp DNA fragment was successfully achieved with the conserved primers, and subsequent restriction of the amplicons with the selected endonu-

cleases was, thus, possible. Results obtained after PCR-RFLP analysis indicated that the species origin of the dried salt-cured products was in accordance with their label statements. On the contrary, PCR amplification of the 712 bp fragment could not be accomplished in commercial samples subjected to more destructive processing, such as the case of the thermally treated meat (results not shown). This is ascribed to the fact that thermal treatment strongly accelerates DNA degradation from the samples and, consequently, amplification of particularly large DNA fragments such as the 712 bp one is impeded. Thus, PCR-RFLP based on the mitochondrial 12S rRNA fragment targeted in our study cannot be applied for species identification in thermally treated meats. In the analysis of food matrices in which thermal action or other processing effects may degrade the DNA present in the food tissues, PCR using specific primers targeting short DNA fragments may be used as an alternative technique for species identification (21).

Our results allow us to conclude that PCR-RFLP of the mitochondrial 12S rRNA gene is a suitable alternative that can be applied to the detection of fraudulent or unintentional mislabeling of domestic and game meat species present in commercialized products such as raw and dried salt-cured meats. Compared to alternative methods, such as DNA-sequencing and nucleotide sequence analysis (1, 19), PCR-RFLP combines simplicity, speed, resolving power, and low cost. Consequently, the reported technique can be routinely applied by meat industries as a tool to warrant the quality and authenticity of the products offered for sale (4, 13).

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#### LITERATURE CITED

- (1) Brodmann, P. D.; Nicholas, G.; Schaltenbrand, P.; Ilg, E. C. Identifying unknown game species: experience with nucleotide sequencing of the mitochondrial cytochrome *b* gene and a subsequent basic local alignment search tool search. *Eur. Food Res. Technol.* **2001**, *212*, 491–496.

- (2) Matsunaga, T.; Chikuni, K.; Tanabe, R.; Muroya, S.; Nakai, H.; Shibata, K.; Yamada, J.; Shinmura, Y. Determination of mitochondrial cytochrome b gene sequence for red deer (*Cervus elaphus*) and the differentiation of closely related deer meats. *Meat Sci.* **1998**, *49*, 379–385.
- (3) Pfeiffer, I.; Burger, J.; Brenig, B. Diagnostic polymorphisms in the mitochondrial cytochrome b gene allow discrimination between cattle, sheep, goat, roe buck and red deer by PCR-RFLP. *Genetics* **2004**, *5*, 30.
- (4) Wolf, C.; Rentsch, J.; Hübner, P. PCR-RFLP analysis of mitochondrial DNA: a reliable method for species identification. *J. Agric. Food Chem.* **1999**, *47*, 1350–1355.
- (5) Chen, F. C.; Hsieh, Y. H. Detection of pork in heat-processed meat products by monoclonal antibody-based ELISA. *J. AOAC Int.* **2000**, *83*, 79–85.
- (6) McCormick, R. J.; Collins, D. A.; Field, R. A.; Moore, T. D. Identification of meat from game and domestic species. *J. Food Sci.* **1992**, *57*, 516–517.
- (7) Buntjer, J. B.; Lenstra, J. A. Mammalian species identification by interspersed repeat PCR fingerprinting. *J. Ind. Microbiol. Biotechnol.* **1998**, *21*, 121–127.
- (8) Herman, B. L. Determination of the animal origin of raw food by species-specific PCR. *J. Dairy Res.* **2001**, *68*, 429–436.
- (9) Sáez, R.; Sanz, Y.; Toldrá, F. PCR-based fingerprinting techniques for rapid detection of animal species in meat products. *Meat Sci.* **2004**, *66*, 659–665.
- (10) Girish, P. S.; Anjaneyulu, A. S. R.; Viswas, K. N.; Anand, M.; Rajkumar, N.; Shivakumar, B. M.; Bhaskar, S. Sequence analysis of mitochondrial 12S rRNA gene can identify meat species. *Meat Sci.* **2004**, *66*, 551–556.
- (11) Koh, M. C.; Lim, C. H.; Chua, S. B.; Chew, S. T.; Phang, S. T. W. Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. *Meat Sci.* **1998**, *48*, 275–285.
- (12) Sun, Y. L.; Lin, C. S. Establishment and application of a fluorescent polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for identifying porcine, caprine, and bovine meats. *J. Agric. Food Chem.* **2003**, *51*, 1771–1776.
- (13) Meyer, R.; Höfelein, C.; Lüthy, L.; Candrian, U. Polymerase chain reaction–restriction fragment length polymorphism analysis: a simple method for species identification in food. *J. AOAC Int.* **1995**, *78*, 1542–1551.
- (14) Rea, S.; Chikuni, K.; Avellini, P. Possibility of using single strand conformation polymorphism (SSCP) analysis for discriminating european pig and wild boar meat samples. *J. Food Sci.* **1996**, *3*, 211–220.
- (15) Colombo, F.; Cardia, A.; Renon, P.; Cantóni, C. A. Note on the identification of *Rupicapra rupicapra* species by polymerase chain reaction product sequencing. *Meat Sci.* **2004**, *66*, 753–755.
- (16) Verkaar, E. L. C.; Nijman, I. J.; Boutaga, K.; Lenstra, J. A. Differentiation of cattle species in beef by PCR-RFLP of mitochondrial and satellite DNA. *Meat Sci.* **2002**, *60*, 365–369.
- (17) Comincini, S.; Sironi, M.; Bandi, C.; Giunta, C.; Rubini, M.; Fontana, F. RAPD analysis of systematic relationships among the *Cervidae*. *Heredity* **1996**, *76*, 215–221.
- (18) Avise, J. C.; Arnold, J.; Ball, R. M.; Bermingham, E.; Lamb, T.; Neigel, J. E.; Reeb, C. A.; Saunders, N. C. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu. Rev. Ecol. Syst.* **1987**, *18*, 489–522.
- (19) Kocher, T. D.; Thomas, W. K.; Meyer, A.; Edwards, S. V.; Pääbo, S.; Villablanca, F. X.; Wilson, A. C. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6196–6200.
- (20) Cronin, M. A. Analysis of total mitochondrial DNA (mtDNA) with restriction enzymes to identify mtDNA genotypes among North American cervids: moose, caribou, elk, white-tailed deer and mule deer. *J. Mammal.* **1992**, *73*, 70–82.
- (21) Matsunaga, T.; Chikuni, K.; Tanabe, R.; Muroya, S.; Nakai, H.; Shibata, K.; Yamada, J.; Shinmura, Y. A quick and simple method for the identification of meat species and meat products by PCR assay. *Meat Sci.* **1999**, *51*, 143–148.

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