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Mitochondrial DNA control region as a tool for species identification and distinction between wolves and dogs from Croatia

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ABSTRACT

Dogs worldwide share a single genetic origin from Asian wolves. While dog and wolf lineages are difficult to separate in terms of nuclear genes, mitochondrial lineages are clearly distinguishable for the two species, offering a good opportunity to evaluate the differences between them. Species identification from DNA is an important tool for wolf conservation in Croatia, and wolf - dog differentiation is necessary for forensic cases, wildlife management and scientific research. The goal of this paper was to provide a data set on Croatian dog and wolf mitochondrial DNA control region sequences, and to research if these sequences can be used as a reference for species identification. We analyzed 281 base pair sequences of the mitochondrial DNA (mtDNA) control region of 20 mixed breed dog blood samples, 91 grey wolf muscle samples and two muscle samples of wolf-like animals. We identified 12 dog and 4 wolf mtDNA control region haplotypes. None of the haplotypes were shared, confirming that mtDNA control region haplotypes can be used to discriminate between Croatian wolves and dogs, and to confirm the maternal ancestry of putative hybrids. The sequences of the two wolf-like animals clearly grouped into a dog cluster.

Key words: dog, *Canis lupus familiaris*, wolf, *Canis lupus*, mitochondrial DNA, control region

Introduction

The phenotypic and genetic diversity of dogs clearly indicates that their ancestors were recruited from the grey wolf population (CLUTTON-BROCK, 1995; VILÀ et al., 1997;

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WAYNE and OSTRANDER, 1999; PARKER et al., 2004). Dogs worldwide share a single genetic origin, and the domestication process took place in Asia, not more than 16,300 years ago (PANG et al., 2009). But the origin of most dog breeds probably derives from recent selective breeding practices, which are less than 200 years old (PARKER et al., 2004). Most authors describe the wolf and dog as the same species. They have identical karyotypes, can hybridize and produce fertile offspring (MENGEL, 1971; VILÀ and WAYNE, 1999). Their interaction may range from a form of predator-prey relationship to coexistence, which may lead to hybridization (VILÀ and WAYNE, 1999).

While dog and wolf lineages are difficult to separate in terms of nuclear genes (PARKER et al., 2004; VILÀ et al., 2005), mitochondrial lineages are clearly distinguishable for the two species (VILÀ et al., 1997; SAVOLAINEN et al., 2002) offering a good opportunity to evaluate the differences between them. Canine mitochondrial DNA (mtDNA) consists of around 16,728 bases (KIM et al., 1998). The control region spans positions 15,459 - 16,728 and within this there are two hypervariable regions (EICHMANN and PARSON, 2007). The canine mtDNA sequence has been used to clarify the origin of domestic dogs (SAVOLAINEN et al., 2002; VILÀ et al., 1997; VILÀ et al., 1999; PANG et al., 2009), assess hybridization with wild species (VILÀ et al., 1997; VILÀ and WAYNE, 1999; ADAMS et al., 2003a; ADAMS et al., 2003b; CIUCCI et al., 2003; VILÀ et al., 2003; VERGINELLI et al., 2005; SCHMUTZ et al., 2007) and forensic analysis (SAVOLAINEN and LUNDEBERG, 1999; WETTON et al., 2003). Several authors (OKUMURA et al., 1996; VILÀ and WAYNE, 1999; RANDI et al., 2000) have shown that wolf mtDNA control region haplotypes are not shared with any of the studied dog breeds. Only VILÀ et al. (1997) found that one wolf haplotype from Romania and western Russia is shared with dogs, suggesting recent hybridization.

The grey wolf population coexists with domestic dogs across their range and in numerous situations species differentiation based on DNA is necessary. According to the Nature Protection Law of 1994, based on the provisions of the Rule Book on the Protection of Certain Mammalian Species (*Mammalia*) (NN 31/95), the grey wolf is a protected species in Croatia, which means that any disturbance of the animal in its natural life and development, hiding, sale, purchase, stealing or any other form of acquisition, including taxidermy, is prohibited (ŠTRBENAC et al., 2005). Species identification from DNA is an important tool for wolf conservation in Croatia, and wolf - dog differentiation is necessary for forensic cases, wildlife management and scientific research. The goal of this paper was to provide a data set on control region sequences of dogs from Croatia, to research if any of them are shared with the wolf control region sequences present in Croatia and if these sequences can be used as a reference for species identification and discrimination of their hybrids.

Materials and methods

Blood samples from 20 mixed breed dogs were collected during routine veterinary examinations at the Faculty of Veterinary Medicine, University of Zagreb. Muscle samples of 91 Croatian grey wolves were sequenced for mtDNA control region by GOMERČIĆ et al. (2010). Additionally two muscle samples of wolf-like animals, submitted for necropsy examination at the Faculty of Veterinary Medicine University of Zagreb, were included in the research. Both of those animals, WCRO20 killed by a train and WCAK1 from captivity, were characterized as possible dog-wolf hybrids, based on their morphological traits.

Prior to analysis, the whole blood with EDTA, and muscle samples in 96% ethanol, were stored at -20 °C. DNA was extracted using a Promega Wizard Genomic Purification Kit. Control region amplification was carried out with primers designed by PALOMARES et al. (2002) (CR1: 5'-CCACTATCAGCACCCAAAGC-3' and CR2R: 5'-CCCGGAGCGAGAAGAGG-3'). Total reaction volume for PCR was 25 μL, containing 150 - 250 ng of genomic DNA, 1 × QIAGEN Multiplex PCR Master Mix (consisting of QIAGEN Multiplex PCR buffer with a final concentration of 3 mM MgCL2, dNTP mix, Q solution and HotStart Taq DNA polymerase) and 0.2 μM of each primer (GOMERČIĆ, 2009). The reaction was carried out on a GeneAmp PCR System 2700 (Applied Biosystems) using the following cycling parameters: 15 min at 95 °C, then 35 cycles - 40 seconds on 94 °C, 50 seconds on 55 °C, 60 seconds on 72 °C, and final 10 minutes on 72 °C. After purification with a WizardR SV Gel and PCR Clean-Up System kit, the control region was sequenced by means of a "ABI3730x1 DNA Analyzer" (Applied Biosystems). Sequence alignment was performed using Clustal W (THOMPSON et al., 1994), implemented in BioEdit software (HALL, 1999). We eventually analyzed an aligned sequence comprising 281 base pairs (bp). Haplotype frequency and the distance between haplotypes were calculated using the program Arlequin 3.1 (EXCOFFIER et al., 2005). The same program was used to estimate haplotype and nucleotide diversity (±SE), according to NEI (1987). Mega 4 (TAMURA et al., 2007) was used to construct a neighbour-joining tree, computed using a nucleotide substitution model Kimura 2-parameter distance. In total 38 dog control region sequences from all over the world (comprising 28 unique mtDNA haplotypes) from the GenBank were included in the phylogenetic analysis.

Results

We analyzed the 281 bp sequence of the mtDNA control region of 20 mixed breed dog blood samples, 91 grey wolf muscle samples taken from GOMERČIĆ et al. (2010), and two muscle samples of potential dog-wolf hybrids. We identified 12 dog and 4 wolf mtDNA control region haplotypes. The dog haplotypes had 17 polymorphic sites (6.0%) and wolves had 11 polymorphic sites (3.9%) (Table 1). The dog haplotypes showed

between 1 (0.3%) and 10 (3.6%) pairwise differences, while the four wolf haplotypes showed between 1 (0.3%) and 9 (3.2%) pairwise differences. The pairwise differences between the dog and wolf haplotypes were between 3 and 12 nucleotides, resulting in a sequence divergence of between 1.1% and 4.3% (average 2.4%).

Table 1. Haplotypes found in grey wolves (WCRO1 - WCRO6), dogs (DCRO1-DCRO12) and two wolf-like animals (WCRO20 and WCAK1) from Croatia. Polymorphic sites are identified within the 281 bp mtDNA sequence. Numbers in vertical columns refer to the aligned sites of 281 bp control region. Only variable sites are shown, dots represent identity with haplotype WCRO1 and dashes denote deletions.

Haplotypes	19	43	44	62	71	90	123	132	148	149	157	158	162	164	169	173	176	180	187	189	202	247
WCRO1	C	T	C	T	-	A	A	T	T	T	T	T	C	A	C	T	A	A	T	A	T	C
WCRO2	·	·	·	·	-	G	·	C	·	·	·	C	T	·	·	·	·	G	·	·	·	·
WCRO3	·	·	·	C	C	·	·	C	C	·	·	C	T	·	·	C	·	·	·	G	·	·
WCRO6	·	C	·	C	C	·	·	C	C	·	·	C	T	·	·	C	·	·	·	G	·	·
DCRO1	·	·	·	·	-	·	·	·	·	C	·	C	T	·	T	·	G	G	·	·	·	·
DCRO2	·	·	·	C	-	·	·	C	·	·	C	C	T	G	·	·	·	·	·	G	·	·
DCRO3	·	·	·	C	-	·	·	C	·	·	·	C	T	·	·	·	·	·	·	G	·	·
DCRO4	·	·	T	·	-	·	·	C	C	·	·	C	T	·	·	·	G	·	C	G	·	T
DCRO5	·	·	·	C	-	·	·	C	·	·	·	C	T	·	·	·	·	·	·	G	C	·
DCRO6	·	·	·	C	-	·	·	C	·	·	·	C	T	·	·	·	T	·	·	G	·	·
DCRO7	·	·	·	C	-	·	·	C	·	·	·	C	T	G	T	·	·	·	·	G	·	·
DCRO8	·	·	·	C	-	·	T	C	·	·	·	C	T	·	·	·	T	·	·	G	·	·
DCRO9	·	·	·	·	-	·	·	C	·	C	·	C	T	·	T	·	G	G	·	·	·	·
DCRO10	·	·	·	·	-	G	·	·	·	C	·	C	T	·	T	·	G	G	·	·	·	·
DCRO11	·	·	T	·	-	·	·	C	C	·	·	C	T	·	·	·	G	·	C	G	·	·
DCRO12	T	·	·	C	-	·	·	C	·	·	·	C	T	G	·	·	·	·	·	G	·	·
WCAK1	·	·	·	·	-	·	·	·	·	C	·	C	T	·	T	·	G	A	·	·	·	·
WCRO20	·	·	·	·	-	·	·	·	·	C	·	C	T	·	T	·	G	G	·	·	·	·

The neighbour-joining tree containing dog haplotype sequences from this research, wolf haplotypes from Croatia (GOMERČIĆ et al., 2010), two wolf-like animals from this research and worldwide dog haplotypes from the Genbank, clearly separated dogs and wolves into different clusters (Fig. 1). The sequences of the two wolf-like animals (WCRO20 and WCAK1) that were characterized as possible dog-wolf hybrids based on their morphological traits, clearly grouped into the dog cluster. Sequence analyses revealed that sample WCRO20 was identical to the dog haplotype DCRO1, while WCAK1 haplotype was not identical to any of the Croatian dog haplotypes analyzed, but

it differed only in one base pair (transition) from the haplotype DCRO1. A dog haplotype identical to that of WCAK1 was registered in the GenBank.

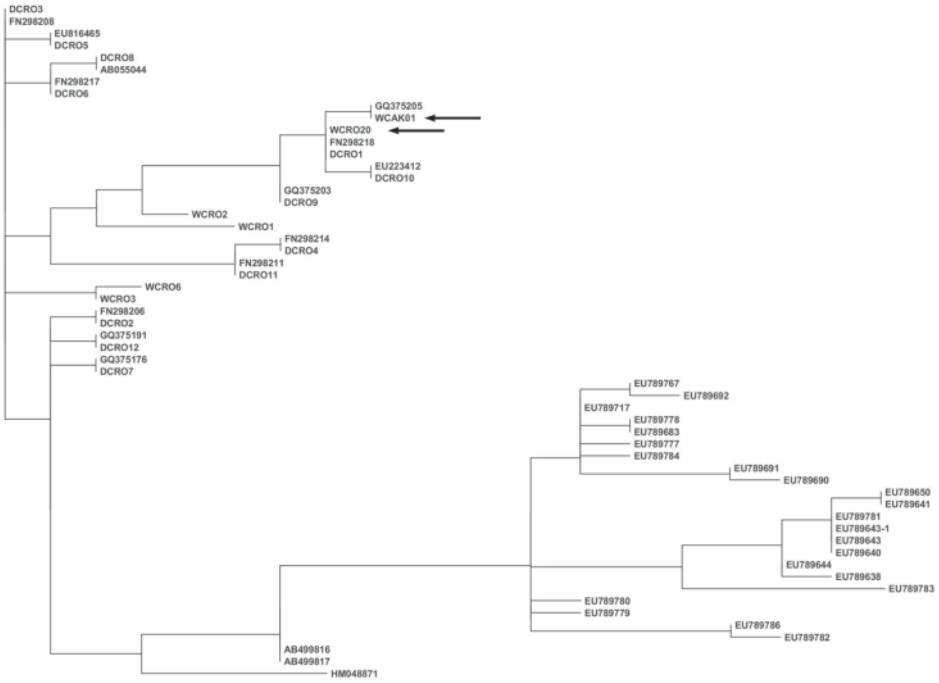

Fig. 1. Neighbor-joining tree, computed using nucleotide substitution model Kimura 2-parameter distance, showing the phylogenetic relationship among dog (DCRO) and wolf (WCRO) haplotypes found in Croatia, two wolf - like animals (WCRO20 and WCAK1, marked with an arrow) and worldwide dog mtDNA haplotypes (indicated under their GenBank accession number).

Discussion

Differentiation between wolves and dogs deserves serious attention because of its ecological and management consequences. By analyzing the 281 bp part of the mtDNA control region of 20 dog blood samples and 91 wolf muscle samples, we identified 12 dog and 4 wolf haplotypes. None of the haplotypes were shared, confirming that mtDNA control region haplotypes can be used to discriminate between Croatian wolves, dogs and their hybrids. The dog haplotypes from this study were registered in the GenBank, under accession numbers: GU324475, GU324476, GU324477, GU324478, GU324479, GU324480, GU324481, GU324482, GU324483, GU324484, GU324485, GU324486. All

these sequences match previously described dog haplotypes, registered in the GenBank. Based on the analysis of the control region sequences, two wolf-like animals, WCRO20 and WCAK1, were identified as dogs.

As the wolf is a strictly protected species in Croatia, poaching control is a prerequisite for wolf management and conservation. Poachers sometimes try to justify their act by the fact that they confused a stray dog with a wolf due to their morphological similarity. In these cases scientific species identification is necessary for the lawsuit.

Wolf predation of livestock is a major management problem in Croatia and limited hunting quotas have been issued to help reduce the damage since 2005, when the wolf management plan for Croatia was adopted. In Croatia, compensation for damage caused by a strictly protected species is paid from the state budget, while no compensation is paid for damage done by dogs. Although evidence left at the site of an attack usually differs between wolves and dogs, because wolves are more skilful hunters, the identification of the culprit is not always clear (SUNDQVIST et al., 2008). Predator identification at the damage site is sometimes possible only from blood, saliva, hair or scant samples found at the site (SUNDQVIST et al., 2008). Our results can be applied for a more reliable method to distinguish if a livestock attack was conducted by a wolf or a dog. This is important because if wolves are blamed for attacks they are not responsible for, wolf conservation and management may be hampered (SUNDQVIST et al., 2008). Also, correct identification of the predator is of economic interest, both for the livestock owner and for the state.

Hybridization can threaten the integrity of the gene pool of wild canids and has the potential to produce morphological, physiological, behavioral and ecological changes in captive and wild-living canids (MENGEL, 1971; BOITANI, 1984; THURBER and PETERSON, 1991; LARIVIERE and CRETE, 1993; GOTTELLI et al., 1994; ANDERSONE et al., 2002). Hybridization between wolves and dogs has been observed in the wild in several studies (ANDERSONE et al., 2002; RANDI and LUCCHINI, 2002; SUNDQVIST, 2008). In total 3.4% F1 hybrids have been found in the Spanish wolf population, all of them being a result of a female wolf mating with a male dog (SUNDQVIST, 2008). The author proposes that this biased direction of hybridization could be the result of the physiological differences between the mating seasons of dogs and wolves. While wolves have a well defined mating season, dogs can often reproduce twice in the same year, and male dogs show high testosterone levels during the entire year. This makes male dogs able to fertilize all female wolves, while male wolves are unlikely to be sexually active at the time most female dogs are receptive (SUNDQVIST, 2008). As mtDNA is inherited from the mother, only the maternal parent of the hybrid can be detected by analyzing mtDNA. Unfortunately, analyses of the mtDNA cannot detect if the paternal parent was a dog. Paternal ancestry can only be investigated using nuclear markers, such as microsatellite loci. Our analysis showed that the wolf-like animals, WCRO20 and WCAK1, were the offspring of a female

dog, with the slight possibility of their father being a wolf, due to the reason previously described.

References

ADAMS, J. R., J. A. LEONARD, L. P. WAITS (2003a): Widespread occurrence of a domestic dog mitochondrial DNA haplotype in south eastern US coyotes. Mol. Ecol. 12, 541-546.

ADAMS, J. R., B. T. KELLY, L. P. WAITS (2003b): Using faecal DNA sampling and GIS to monitor hybridization between red wolves (*Canis rufus*) and coyotes (*Canis latrans*). Mol. Ecol. 12, 2175-2186.

ANDERSONE, Z., V. LUCCHINI, E. RANDI, J. OZOLINS (2002): Hybridisation between wolves and dogs in Latvia as documented using mitochondrial and microsatellite DNA markers. Mamm. Biol. 67, 79-90.

BOITANI, L. (1984): Genetic considerations on wolf conservation in Italy. Boll. Zool. 51, 367-373.

CIUCCI, P., V. LUCCHINI, L. BOITANI, E. RANDI (2003): Dewclaws in wolves as evidence of admixed ancestry with dogs. Can. J. Zool. 81, 2077-2081.

CLUTTON-BROCK, J. (1995): Origins of the dog: domestication and early history. In: The Domestic Dog: its Evolution, Behavior and Interactions with People (Serpell, J., Ed.). Cambridge University Press. Cambridge. pp. 8-20.

EICHMANN, C., W. PARSON (2007): Molecular characterization of the canine mitochondrial DNA control region for forensic applications. Int. J. Leg. Med. 121, 411-416.

EXCOFFIER, L., G. LAVAL, S. SCHNEIDER (2005): Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinf. Online 1, 47-50.

GOMERČIĆ, T., M. SINDIČIĆ, A. GALOV, H. ARBANASIĆ, J. KUSAK, I. KOCIJAN, M. ĐURAS GOMERČIĆ, Đ. HUBER (2010): High genetic variability of Croatian grey wolf (*Canis lupus L.*) population as revealed by mitochondrial DNA control region sequences. Zool. Stud. 49, 816-823.

GOMERČIĆ, T. (2009): Genetic diversity of gray wolf (*Canis lupus*) in Croatia. Dissertation. Faculty of Veterinary Medicine University of Zagreb. Zagreb, Croatia.

GOTTELLI, D., C. SILLERO-ZUBIRI, G. D. APPLEBAUM, M. S. ROY, D. J. GIRMAN, J. GARCIA-MORENO (1994): Molecular genetics of the most endangered canid - the Ethiopian wolf *Canis simensis*. Mol. Ecol. 3, 301-312.

HALL, T. A. (1999): BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids. Symp. Ser. 41, 95-98.

KIM, K. S., S. E. LEE, H. W. JEONG, J. H. HA (1998): The complete nucleotide sequence of the domestic dog (*Canis familiaris*) mitochondrial genome. Mol. Phylogenet. Evol. 10, 210-220.

LARIVIERE, S., M. CRETE (1993): The size of eastern coyotes (*Canis latrans*): A comment. J. Mammal. 74, 1072-1074.

MENGEL, R. M. (1971): A study of dog-coyote hybrids and implications concerning hybridization in Canis. J. Mammal. 52, 316-336.

NEI, M. (1987): Molecular evolutionary genetics, Columbia University Press. New York.

OKUMURA, N., N. ISHIGURO, M. NAKANO, A. MATSUI, M. SAHARA (1996): Intra- and interbreed genetic variations of mitochondrial DNA major non-coding regions in Japanese native dog breeds (*Canis familiaris*). Anim. Genet. 27, 397-405.

PALLOMARES, F., J. A. GODOY, A. PIRIZ, S. J. O'BRIEN, W. E. JOHNSON (2002): Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. Mol. Ecol. 11, 2171-2182.

PANG, J. F., C. KLUETSCH, X. J. ZOU, A. B. ZHANG, L. Y. LUO, H. ANGLEBY, A. ARDALAN, C. EKSTRÖM, A. SKÖLLERMO, J. LUNDEBERG, S. MATSUMURA, T. LEITNER, Y. P. ZHANG, P. SAVOLAINEN (2009): mtDNA data indicate a single origin for dogs south of Yangtze River, less than 16,300 years ago, from numerous wolves. Mol. Biol. Evol. 26, 2849-2864.

PARKER, H. G., L. V. KIM, N. B. SUTTER, S. CARLSON, T. D. LORENTZEN, T. B. MALEK, G. S. JOHNSON, H. B. DEFRANCE, E. A. OSTRANDER, L. KRUGLYAK (2004): Genetic structure of the purebred domestic dog. Science 304, 1160-1164.

RANDI, E., V. LUCCHINI, M. F. CHRISTENSEN, N. MUCCI, S. M. FUNK, G. DOLF, V. LOESCHKE (2000): Mitochondrial DNA variability in Italian and east European wolves: Detecting the consequences of small population size and hybridization. Conserv. Biol. 14, 464-473.

RANDI, E., V. LUCCHINI (2002): Detecting rare introgression of domestic dog genes into wild wolf (*Canis lupus*) population by Bayesian admixture analyses of microsatellite variation. Conserv. Genet. 3, 31-45.

SAVOLAINEN, P., J. LUNDEBERG (1999): Forensic evidence based on mtDNA from dog and wolf hairs. J. Forensic Sci. 44, 77-81.

SAVOLAINEN, P., Y. P. ZHANG, J. LUO, J. LUNDEBERG, T. LEITNER (2002): Genetic evidence for an East Asian origin of domestic dogs. Science 298, 1610-1613.

SCHMUTZ, S. M., T. G. BERRYERE, J. L. BARTA, K. D. REDDICK, J. K. SCHMUTZ (2007): Agouti sequence polymorphisms in coyote, wolves and dogs suggest hybridization. J. Hered. 98, 351-355.

SUNDQVIST, A. K. (2008): Conservation Genetics of Wolves and their Relationship with Dogs. Dissertation. Faculty of Science and Technology, Uppsala University. Uppsala, Sweden.

SUNDQVIST, A. K., H. ELLEGREN, C. VILÀ (2008): Wolf or dog? Genetic identification of predators from saliva collected around bite wounds on prey. Conserv. Genet. 9, 1275-1279.

ŠTRBENAC, A., Đ. HUBER, J. KUSAK, A. MAJIĆ-SKRBINŠEK, A. FRKOVIĆ, Ž. ŠTAHAN, J. JEREMIĆ-MARTINKO, S. DESNICA, P. ŠTRBENAC (2005): Wolf Management Plan for the Republic Croatia, State Institute for Nature Protection. Zagreb. pp. 77-83.

TAMURA, K., J. DUDLEY, M. NEI, S. KUMAR (2007): MEGA 4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596-1599.

THOMPSON, J. D., D. G. HIGGINS, T. J. GIBSON (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acids. Res. 22, 4673-4680.

THURBER, J. M., R. O. PETERSON (1991): Changes in body size associated with range expansion in the coyote (*Canis latrans*). J. Mamm. 72, 750-755.

VERGINELLI, F., C. CAPELLI, V. COIA, M. MUSIANI, M. FALCHETTI, L. OTTINI, R. PALMIROTTA, A. TAGLIACOZZO, I. D. G. MAZZORIN, R. MARIANI-COSTANTINI (2005): Mitochondrial DNA from prehistoric canids highlights relationships between dogs and south-east European wolves. Mol. Biol. Evol. 22, 2541-2551.

VILÀ, C., P. SAVOLAINEN, J. E. MANDONALO, I. R. ARMORIN, J. E. RICE, R. L. HONEYCUTT, K. A. CRANDALL, J. LUNDEBERG, R. K. WAYNE (1997): Multiple and ancient origins of the domestic dog. Science 276, 1687-1689.

VILÀ, C., R. K. WAYNE (1999): Hybridization between wolves and dogs. Conserv. Biol. 13, 195-198.

VILÀ, C., J. E. MALDONADO, R. K. WAYNE (1999): Phylogenetic relationships, evolution, and genetic diversity of the domestic dog. J. Hered. 90, 71-77.

VILÀ, C., C. WALKER, A. K. SUNDQVIST, O. FLAGSTAD, Z. ANDERSONE, A. CASULLI, I. KOJOLA, H. VALDMANN, J. HALVERSON, H. ELLEGREN (2003): Combined use of maternal, paternal and bi-parental genetic markers for the identification of wolf-dog hybrids. Heredity 90, 17-24.

VILÀ, C., J. SEDDON, H. ELLEGREN (2005): Genes of domestic mammals augmented by backcrossing with wild ancestors. Trends Genet. 21, 214-218.

WAYNE, R. K., E. A. OSTRANDER (1999): Origin, genetic diversity, and genome structure of the domestic dog. Bioassays 21, 247-257.

WETTON, J. H., J. E. HIGGS, A. C. SPRIGGS, C. A. RONEY, C. S. TSANG, A. P. FOSTER (2003): Mitochondrial profiling of dog hairs. Forensic Sci. Int. 133, 235-241.

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SAŽETAK

Psi diljem svijeta imaju jedinstveno genetsko podrijetlo od azijskih vukova. Dok je vukove i pse teško razlikovati na temelju jezgrenih gena, mitohondrijska DNA jasno se razlikuje što daje dobru mogućnost za istraživanje razlika između tih dviju vrsta. Identifikacija vrste na temelju DNA važan je alat za zaštitu vuka u Hrvatskoj, a razlikovanje između vuka i psa prijeko je potrebno u forenzičkim slučajevima, upravljanju divljim životinjama i u znanstvenim istraživanjima. Cilj ovoga rada bio je dobiti podatke o sekvencijama mitohodrijske DNA vuka i psa iz Hrvatske te istražiti da li se te sekvencije mogu upotrijebiti kao referencija za razlikovanje vrsta. Analizirali smo sekvenciju

kontrolne regije mitohondrijske DNA dugu 281 parova baza izdvojenu iz 20 uzoraka krvi pasa mješanaca, 91 uzorka mišića sivoga vuka i dva uzorka mišića životinja sličnih vuku. Pronašli smo 12 psećih i 4 vučja haplotipa kontrolnoga područja. Nijedan od haplotipova nije bio zajednički psima i vukovima, potvrđujući da se kontrolno područje mitohondrijske DNA može rabiti za razlikovanje pasa i vukova, te za određivanje majčinskih predaka mogućih hibrida. Sekvencije dviju životinja sličnih vuku jasno su pripadale psima.

Ključne riječi: pas, *Canis lupus familiaris*, vuk, *Canis lupus*, mitohondrijska DNA, kontrolno područje