Journal of Mammalogy, 97(1):234–245, 2016
DOI:10.1093/jmammal/gyv172
Published online November 18, 2015

The complete mitochondrial genomes of nine white-tailed deer subspecies and their genomic differences

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The white-tailed deer (*Odocoileus virginianus*) is an important, sustainable-use species in Mexico; 14 subspecies are widely distributed throughout the Mexican territory. The criteria for classifying subspecies is based on morphological features throughout their geographical range; however, the complete genetic characterization of Mexican subspecies has not been established. The objective of the present work is to report the mitogenomes of 9 of the 14 white-tailed deer subspecies from Mexico and identify their unique variations. Typical vertebrate mitogenomes structures (i.e., 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes) were observed in the studied subspecies. The greatest numbers of polymorphisms were identified in the D-loop, ND4, ND5, CYTB/COI, ATP6, and COIII genes. Phylogenetic analyses showed that the southern and southeastern subspecies were distinct from the central and northern subspecies; the greatest genetic distances were also observed between these 2 groups. These subspecies-specific variations could be useful for designing a strategy to genetically characterize the studied subspecies.

El venado cola blanca es una de las especies de mayor importancia dentro del aprovechamiento de la fauna silvestre de México, donde se distribuyen de manera natural 14 subespecies. Actualmente, estas subespecies se han clasificado de acuerdo a sus variaciones fenotípicas que presentan a lo largo de su rango de distribución, sin embargo no se ha establecido la caracterización genética completa de las mismas. Es por esto que el objetivo del presente estudio es reportar los mitogenomas de 9 de las 14 subespecies de venado cola blanca, así como identificar las variaciones únicas de cada subespecie. En las 9 subespecies se observó la estructura típica de los mitogenomas de vertebrados (13 genes que codifican para proteínas, 22 ARNt, 2 ARNr). Los genes con mayor polimorfismo fueron D-loop, ND4, ND5, CYTB/COI, ATP6 y COIII. El análisis filogenético mostró la separación de las subespecies del sur y sureste de las subespecies del centro y norte del país, a su vez las distancias genéticas entre estos dos grupos fueron las más altas. Estas variaciones subespecie-específicas podrían ser útiles para diseñar una estrategia para caracterizar genéticamente las subespecies estudiadas.

Key words: central and northern subspecies, mitogenomes, southern and southeastern subspecies, white-tailed deer

The white-tailed deer (*Odocoileus virginianus*) is a species of the Cervidae family and is found exclusively on the American continents, ranging from southern Canada to South America (i.e., northern Brazil and northern Peru). Based on their morphological traits, 38 subspecies have been identified (Smith 1991), and 37% of these subspecies are distributed throughout the Mexican territory (Halls 1984; Smith 1991). Due to their widespread distribution in the country, the white-tailed deer represents an important sustainable food source for native communities (Galindo and Weber 2005). Additionally, the northern subspecies have specific aesthetic features, such as their large body size and their big, branched antlers that make them popular for sport hunting (Galindo and Weber 1998), which provides significant economic benefit to the states in which they are distributed (Villarreal 2012).

Due to demands for larger-sized subspecies, the northern subspecies are commonly expanded beyond their original

habitat toward central and southern Mexico (Logan et al. 2007). However, these relocations could result in the loss of genetic identity in natural, local white-tailed deer (Villarreal 1999; Logan et al. 2007; Hernández et al. 2014). The southern subspecies are genetically differentiated from the central and northern subspecies, as was determined using short sequences from mitochondrial DNA and nuclear microsatellites (De La Rosa et al. 2012; Logan et al. 2012).

Despite the significant differentiation between the southeastern subspecies and those from central and northern Mexico, there have been no reports of specific genetic differences between the subspecies that would allow us to discriminate them. Studies of the complete mitochondrial genome increase the chances of identifying subspecies-specific variability and could generate information to design a strategy to identify the genetic differences between subspecies (Kitpipit et al. 2009, 2011). Additionally, the analysis of whole mitochondrial genomes increases the resolution of a high level of polytomy and decreases the number of branches with low support (Morin et al. 2010).

Therefore, this work focuses on analyzing the structure of 9 mitogenomes of the white-tailed deer subspecies from Mexico and identifies subspecies-specific nucleotide variations.

Materials and Methods

Sample collection and DNA extraction.—Previously collected samples were selected from a sample bank from the Animal Biotechnology Laboratory of Centro de Biotecnología Genómica, Instituto Politécnico Nacional Reynosa Tamaulipas Mexico. The samples were representative of the geographical distribution of each subspecies; their collection sites were paired with the corresponding subspecies. The *O. v. texanus*, *O. v. couesi*, *O. v. veraecrucis*, *O. v. sinaloae*, and *O. v. mexicanus* samples were obtained from white-tailed deer natural to central and northern Mexico. Similarly, the *O. v. acapulcensis*, *O. v. oaxacensis*, *O. v. toltecus,* and *O. v. yucatanensis* samples were obtained from southern Mexico (Fig. 1). For all but *acapulcensis*, 1 animal per subspecies was selected. Equimolar DNA concentrations of 7 *acapulcensis* animals were combined to determine intraspecific variability (Appendix I). DNA was isolated from hair or skin samples using the GenElute Mammalian Genomic DNA Kit (Sigma, St. Louis, Missouri) and from the antlers using the Puregene Tissue Core Kit B (Gentra Systems, Minneapolis, Minnesota).

PCR and sequencing.—Thirty-seven primer pairs were designed based on the complete mitochondrial genome of *O. virginianus* (Seabury et al. 2011) and were used in combination to generate overlapping 1,500–3,000 bp PCR fragments. Subsequently, internal primers were used to sequence smaller overlapping 750 bp fragments within these fragments (Appendix II). All PCR reactions were achieved in a total volume of 25 µl using 2.5 mM $MgCl_2$, 0.2 mM DNTPs, 0.2 µM of each primer, 1.2 U Taq polymerase, and 50 ng DNA. The PCR cycle was as follows: 95°C for 5 min, 5 cycles of 95°C for 45 s, 62°C for 45 s, and 72°C for 45 s; the aligning temperature was sequentially decreased by 2°C each cycle; 30 cycles of 95°C for 45 s, 55°C for 45 s, and 72°C for 45 s, and one final step of 72°C for 10 min.

The sequencing was performed by primer walking using the internal primers in an Applied Biosystems 3130 Genetic Analyzer; the fragments were sequenced in both directions.

Sequence assembly and comparative analysis.—SeqMan software from DNASTAR LASERGENE, Inc. (Madison, Wisconsin) was used to assemble genomes. The reference sequence HQ332445 was used to identify all mitogenome structures and for annotating coding and noncoding regions.

Searching for unique polymorphisms for each subspecies.—CodonCode Aligner v.4.2.1 software (CodonCode Corporation 2013) was used to search for unique polymorphisms for each subspecies. The 9 complete mitochondrial genomes and the reference sequence of *O. virginianus* HQ332445 were aligned and compared to visually identify the polymorphisms specific for each subspecies and the synonymous and nonsynonymous substitutions by gene.

Genetic distances between subspecies.—A Kimura 2 parameters model was used to determine the genetic distances between the complete mitochondrial genomes of the 9 subspecies, the reference sequence of *O. virginianus* HQ332445, *O. virginianus* from French Guiana (OvirgF.G) JN632671, *Odocoileus hemionus* (*hemionus*) JN632670, and *Mazama nemorivaga* (*Maz nem*) JN632660.

Phylogenetic relationship.—All sequences were aligned using the L-INS-i algorithm implemented in the online version of MAFFT (Katoh and Standley 2013). The phylogeny of the genus *Odocoileus* was determined, including the 9 *O. virginianus* mitochondrial genomes sequenced in this study and 18 sequences reported by Hassanin et al. (2012; Appendix III). Afterward, the sequence alignment was edited using Mesquite (Maddison and Maddison 2014), and the D-loop region was excluded because of the number of missing values and gaps observed between species. A total of 15,748 characters were analyzed using 2 methods: the maximum likelihood (ML) method in RAxML 7.2.6 (Stamatakis 2006) and the Bayesian method in MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003). RAxML was run with 1,000 rapid bootstrap inferences and with an ML search using the substitution model GTR + G + I, which was chosen from the JModel test using the Akaike information criteria (AIC), AIC_c, and Bayesian information criteria (Darriba et al. 2012). The MrBayes program was run with 4 independent Markov chain Monte Carlo chains, 5,000,000 generations, a sample frequency of 5,000 and a burn criterion of 0.25. The convergence between the chains was confirmed in Tracer (Rambaut et al. 2014). A 50% majority rule consensus tree was obtained in Sumtrees (Sukumaran and Holder 2010).

Results

Genome organization and comparative analysis.—The 9 mitogenomes used herein are available at GenBank (Benson et al. 2013): *veraecrucis* (KM612271), *yucatanensis* (KM612272), *texanus* (KM612273), *sinaloae* (KM612274),

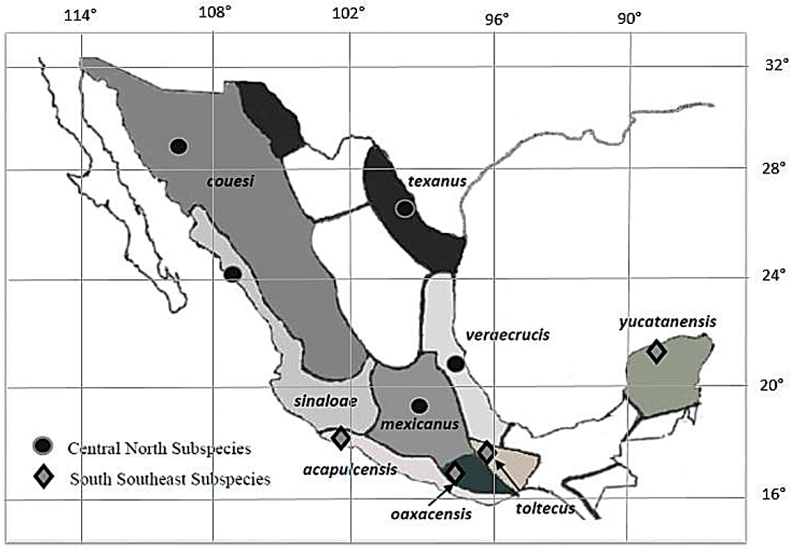

Fig. 1.—Geographic distribution of 9 *Odocoileus virginianus* subspecies in Mexico according to Villarreal 1999; the circles and diamonds represent the collection sites from central north and south southeast subspecies. The shaded areas in map represent the area of distribution of each subspecies.

couesi (KM612275), *toltecus* (KM612276), *oaxacensis* (KM612277) *mexicanus* (KM612278), and *acapulcensis* (KM612279). A typical vertebrate organization was observed in the 9 mitogenomes: 13 protein-coding genes, 1 D-loop region, 12S and 16S ribosomal RNA genes, and 22 transfer RNA genes. The heavy strand contained 12 protein-coding genes, the genes for 12S and 16S ribosomal RNA and 14 transfer RNA genes; the light strand contained the gene for ND6 protein and 8 transfer RNA genes.

Compared with the previously reported sequence of *O. virginianus*, 7 of the 9 mitogenomes showed variations in their genomic length. In most cases, the lengths were shorter than the previously reported sequence (Table 1). In the reported mitogenomes, the D-loop region was 1,044 bp. The *texanus*, *mexicanus*, and *toltecus* subspecies had the same 1,044 bp length, whereas the *veraecrucis* and *sinaloae* subspecies had 1,045 bp region sizes, the *couesi* subspecies had a 1,046 bp D-loop region, and the *yucatanensis*, *oaxacensis*, and *acapulcensis* subspecies had 1,037 bp sizes. In the latter 3 subspecies, deletions of 10 bp in the D-loop region belonging to the left peripheral domain between positions 127 and 136 were observed (Fig. 2). The same deletion was found in 1 individual of *O. virginianus* French Guiana (JN632671).

This deletion was confirmed by PCR–RFLP using the Rsa I enzyme in 10 animals each from *veraecrucis*, *texanus*, and *yucatanensis*. In *acapulcensis*, this deletion was confirmed by sequencing 6 animals.

In the coding regions, no changes were observed in the start codons for the 9 mitogenomes. The ATP8 and ATP6 genes are coded in the same transcript and overlap by 40 bp. However, in *yucatanensis*, 2 nonsynonymous variations were found. The first was in the 95 G/A position, which changed the AAT Asn codon to AGT Ser codon. This variation was verified in 11 *yucatanensis* subspecies animals, which all presented the variation. The second was in the 199 C/T position, which changed the TAA stop codon to the CAA Gln codon. A TCC Phe codon and an additional TAG stop codon followed this 2nd variation. This variation was also evaluated in 11 *yucatanensis* subspecies animals, of which 7 presented the variation. Thus, these 7 animals would have 2 additional amino acids in the ATP8 gene.

Similarly, the *texanus* and *veraecrucis* subspecies presented the A/G variation in the CYTB gene that generated the AAA Lys codon instead of the AGA stop codon. After this codon, another stop codon sequentially followed between the CYTB gene and the gene for the tRNA for Thr was present. Therefore, animals with this variation would have one additional amino acid in their CYTB protein compared with animals without this variation. This variation was evaluated in 10 *texanus* animals and 10 *veraecrucis* animals and was found at frequencies of 0.7 and 0.3 for A and G, respectively, for both subspecies. Therefore, this variation was not fixed in these populations, and individuals with the A variation would have one additional lysine amino acid.

This comparative analysis allowed us to identify subspecies-specific variations. *Yucatanensis* was the most variable subspecies, with 124 specific variations, followed by *toltecus* with 68, *oaxacensis* with 55, *acapulcensis* with 36, and *mexicanus* with 29. The subspecies with the least amount of specific variations were *sinaloae* with 17, *veraecrucis* with 8 *couesi* with 7, and *texanus* with 5 (Supporting Information S1). The most variable genes were also identified and were the D-loop, ND4, ND5, CYTB/COI, ATP6, and COIII genes (Fig. 3).

The GC content (G 13.33%, C 23.6%) was less than the AT content (A 33.6%, T 29%; Supporting Information S2), which is characteristic of mitochondrial genomes. Synonymous and nonsynonymous variations were identified in the coding genes, synonymous variations were greater than nonsynonymous variations

Table 1.—Comparative analysis of the mitogenomes from 9 white-tailed deer subspecies.

Region	*texanus*		*veraecrucis*		*mexicanus*		*sinaloae*		Start codon	End codon
	Position	Size bp	Position	Size bp	Position	Size bp	Position	Size bp		
tRNA-Phe	1–69	69	1–69	69	1–69	69	1–69	69		
12S	70–1023	954	70–1023	954	70–1023	954	70–1023	954		
tRNA-Val	1024–1091	67	1024–1091	67	1024–1091	67	1024–1091	67		
16S	1092–2659	1,568	1092–2659	1,568	1092–2659	1,568	1092–2659	1,568		
tRNA-Leu	2660–2734	474	2660–2734	474	2660–2734	474	2660–2734	474		
ND1	2737–3692	956	2737–3692	956	2737–3692	956	2737–3692	956	ATG	TA-
tRNA-Ile	3693–3761	68	3693–3761	68	3693–3761	68	3693–3761	68		
tRNA-Gln	3759–3830	71	3759–3830	71	3759–3830	71	3759–3830	71		
tRNA-Met	3833–3901	68	3833–3901	68	3833–3901	68	3833–3901	68		
ND2	3902–4943	1,042	3902–4943	1,042	3902–4943	1,042	3902–4943	1,042	ATA	T--
tRNA-Trp	4944–5012	68	4944–5012	68	4944–5012	68	4944–5012	68		
tRNA-Ala	5013–5081	68	5013–5081	68	5013–5081	68	5013–5081	68		
tRNA-Asn	5083–5155	72	5083–5155	72	5083–5155	72	5083–5155	72		
tRNA-Cys	5188–5254	36	5188–5254	36	5188–5254	36	5188–5254	36		
tRNA-Tyr	5255–5323	68	5255–5323	68	5255–5323	68	5255–5323	68		
COI	5325–6869	1,545	5325–6869	1,545	5325–6869	1,545	5325–6869	1,545	ATG	TAA
tRNA-Ser	6867–8935	2,068	6867–8935	2,068	6867–8935	2,068	6867–8935	2,068		
tRNA-Asp	6943–7010	67	6943–7010	67	6943–7010	67	6943–7010	67		
COII	7072–7695	684	7072–7695	684	7072–7695	684	7072–7695	684	ATG	TAA
tRNA-Lys	7699–7767	68	7699–7767	68	7699–7767	68	7699–7767	68		
ATP8	7769–7969	201	7769–7969	201	7769–7969	201	7769–7969	201	ATG	TAA
ATP6	7930–8610	681	7930–8610	681	7930–8610	681	7930–8610	681	ATG	TAA
COIII	8610–9393	784	8610–9393	784	8610–9393	784	8610–9393	784	ATG	T--
tRNA-Gly	9394–9462	68	9394–9462	68	9394–9462	68	9394–9462	68		
ND3	9463–9809	347	9463–9809	347	9463–9809	347	9463–9809	347	ATA	TA-
tRNA-Arg	9810–9878	68	9810–9878	68	9810–9878	68	9810–9878	68		
ND4L	9879–10175	297	9879–10175	297	9879–10175	297	9879–10175	297	GTG	TAA
ND4	10169–11546	1,378	10169–11546	1,378	10169–11546	1,378	10169–11546	1,378	ATG	T--
tRNA-His	11547–11615	68	11547–11615	68	11547–11615	68	11547–11615	68		
tRNA-Ser	11616–11676	60	11616–11676	60	11616–11676	60	11616–11676	60		
tRNA-Leu	11676–11746	70	11676–11746	70	11676–11746	70	11676–11746	70		
ND5	11747–13567	1,821	11747–13567	1,821	11747–13567	1,821	11747–13567	1,821	ATA	TAA
ND6	13551–14078	528	13551–14078	528	13551–14078	528	13551–14078	528	ATG	TAA
tRNA-Glu	14079–14147	68	14079–14147	68	14079–14147	68	14079–14147	68		
CYT B	14152–15294	1,143	14152–15294	1,143	14152–15294	1,140	14152–15294	1,140	ATG	TAA
tRNA-Thr	15295–15364	69	15295–15364	69	15295–15364	69	15295–15364	69		
tRNA-Pro	15364–15429	65	15364–15429	65	15364–15429	65	15364–15429	65		
D-loop	15434–16477	1,044	15434–16478	1,045	15434–16477	1,044	15434–16477	1,045		
Genome		16,477		16,478		16, 477		16,478		

Region	*couesi*		*toltecus*		*oaxacensis*		*yucatanensis*		*acapulcensis*		Start codon	Stop codon
	Position	Size bp	Position	Size bp	Position	Size bp	Position	Size bp	Position	Size bp		
tRNA-Phe	1–69	69	1–69	69	1–69	69	1–69	69	1–69	69		
12S	70–1023	954	70–1023	954	70–1023	954	70–1023	954	70–1023	954		
tRNA-Val	1024–1091	67	1024–1091	67	1024–1091	67	1024–1091	67	1024–1091	67		
16S	1092–2659	1,568	1092–2659	1,568	1092–2659	1,568	1092–2659	1,568	1092–2659	1,568		
tRNA-Leu	2660–2734	474	2660–2734	474	2660–2734	474	2660–2734	474	2660–2734	474		
ND1	2737–3692	956	2737–3692	956	2737–3692	956	2737–3692	956	2737–3692	956	ATG	TA-
tRNA-Ile	3693–3761	68	3693–3761	68	3693–3761	68	3693–3761	68	3693–3761	68		
tRNA-Gln	3759–3830	71	3759–3830	71	3759–3830	71	3759–3830	71	3759–3830	71		
tRNA-Met	3833–3901	68	3833–3901	68	3833–3901	68	3833–3901	68	3833–3901	68		
ND2	3902–4943	1,042	3902–4943	1,042	3902–4943	1,042	3902–4943	1,042	3902–4943	1,042	ATA	T--
tRNA-Trp	4944–5012	68	4944–5012	68	4944–5012	68	4944–5012	68	4944–5012	68		
tRNA-Ala	5013–5081	68	5013–5081	68	5013–5081	68	5013–5081	68	5013–5081	68		
tRNA-Asn	5083–5155	72	5083–5155	72	5083–5155	72	5083–5155	72	5083–5155	72		
tRNA-Cys	5188–5254	36	5188–5254	36	5188–5254	36	5188–5254	36	5188–5254	36		
tRNA-Tyr	5255–5323	68	5255–5323	68	5255–5323	68	5255–5323	68	5255–5323	68		
COI	5325–6869	1,545	5325–6869	1,545	5325–6869	1,545	5325–6869	1,545	5325–6869	1,545	ATG	TAA

Table 1.—*Continued*

Region	*couesi*		*toltecus*		*oaxacensis*		*yucatanensis*		*acapulcensis*		Start codon	Stop codon
	Position	Size bp	Position	Size bp	Position	Size bp	Position	Size bp	Position	Size bp		
tRNA-Ser	6867–8935	2,068	6867–8935	2,068	6867–8935	2,068	6867–8935	2,068	6867–8935	2,068		
tRNA-Asp	6943–7010	67	6943–7010	67	6943–7010	67	6943–7010	67	6943–7010	67		
COII	7072–7695	684	7072–7695	684	7072–7695	684	7072–7695	684	7072–7695	684	ATG	TAA
tRNA-Lys	7699–7767	68	7699–7767	68	7699–7767	68	7699–7767	68	7699–7767	68		
ATP8	7769–7969	201	7769–7969	201	7769–7969	201	7769–7975	207	7769–7975	207	ATG	TAA
ATP6	7930–8610	681	7930–8610	681	7930–8610	681	7930–8610	681	7930–8610	681	ATG	TAA
COIII	8610–9393	784	8610–9393	784	8610–9393	784	8610–9393	784	8610–9393	784	ATG	T--
tRNA-Gly	9394–9462	68	9394–9462	68	9394–9462	68	9394–9462	68	9394–9462	68		
ND3	9463–9809	347	9463–9809	347	9463–9809	347	9463–9809	347	9463–9809	347	ATA	TA-
tRNA-Arg	9810–9878	68	9810–9878	68	9810–9878	68	9810–9878	68	9810–9878	68		
ND4L	9879–10175	297	9879–10175	297	9879–10175	297	9879–10175	297	9879–10175	297	GTG	TAA
ND4	10169–11546	1,378	10169–11546	1,378	10169–11546	1,378	10169–11546	1,378	10169–11546	1,378	ATG	T--
tRNA-His	11547–11615	68	11547–11615	68	11547–11615	68	11547–11615	68	11547–11615	68		
tRNA-Ser	11616–11676	60	11616–11676	60	11616–11676	60	11616–11676	60	11616–11676	60		
tRNA-Leu	11676–11746	70	11676–11746	70	11676–11746	70	11676–11746	70	11676–11746	70		
ND5	11747–13567	1,821	11747–13567	1,821	11747–13567	1,821	11747–13567	1,821	11747–13567	1,821	ATA	TAA
ND6	13551–14078	528	13551–14078	528	13551–14078	528	13551–14078	528	13551–14078	528	ATG	TAA
tRNA-Glu	14079–14147	68	14079–14147	68	14079–14147	68	14079–14147	68	14079–14147	68		
CYT B	14152–15291	1,140	14152–15291	1,140	14152–15291	1,140	14152–15291	1,140	14152–15291	1,140	ATG	TAA
tRNA-Thr	15295–15364	69	15295–15364	69	15295–15364	69	15295–15364	69	15295–15364	69		
tRNA-Pro	15364–15429	65	15364–15429	65	15364–15429	65	15364–15429	65	15364–15429	65		
D-loop	15434–16479	1,046	15434–16477	1,044	15433–16470	1,037	15432–16469	1,037	15432–16469	1,037		
Genome		16,479		16,477		16,470		16,469		16,470		

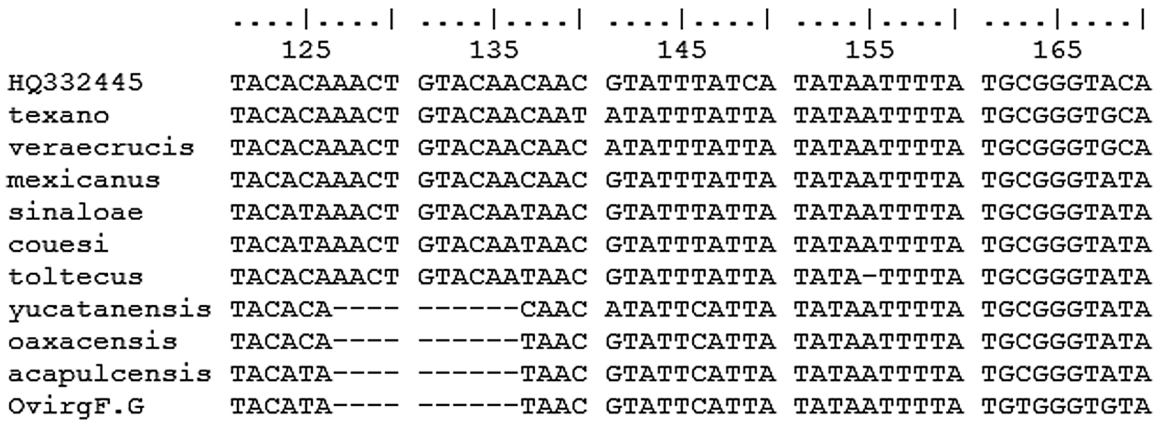

Fig. 2.—Ten base pair deletion in D-loop region in 3 subspecies from south and southeast and 1 animal of *Odocoileus virginianus* from French Guiana represented by OvirgF.G; HQ332445 correspond to the sequence of *O. virginianus* reported by Seabury et al. (2011).

with 88% and 12%, respectively, the ATP6 and ND5 genes presented a high number of nonsynonymous variations with 14 and 12 variations, respectively (Supporting Information S3).

The strategy implemented in the *acapulcensis* subspecies wherein equimolar DNA concentrations from 7 animals were mixed, could not detect intraspecific variability because more than one allele was unidentified from the same peaks resolved on electropherograms.

Genetic distances between subspecies.—The subspecies from the central and northern regions (*texanus*, *veraecrucis*, *couesi*, *mexicanus*, and *sinaloae*) and the *toltecus* subspecies were more closely related than the subspecies from the southern and southeastern regions (*oaxacensis*, *acapulcensis*, and *yucatanensis*; Table 2). The smallest genetic distance between 2 subspecies was observed for the *texanus* and *veraecrucis* subspecies, with genetic variations of only 0.1%, followed by the *couesi* and *sinaloae* subspecies, with genetic variations of 0.2%. The variations among the *texanus* from the United States, *texanus* from Mexico, and *veraecrucis* subspecies were approximately 0.3%. The *mexicanus* subspecies presented similar variations (0.4–0.5%) when compared with the *texanus* from Mexico, *texanus* from the United States, and *veraecrucis* subspecies. The most distant subspecies were the *yucatanensis* and *toltecus*, with variations of 2.4%, followed by the *toltecus* and *oaxacensis*, with variations of 2.3%. The *O. virginianus* from French Guiana (OvirgF.G) presented similar genetic distances in the same rank of the *yucatanensis*. The *Mazama nemorivaga* presented similar genetic variations

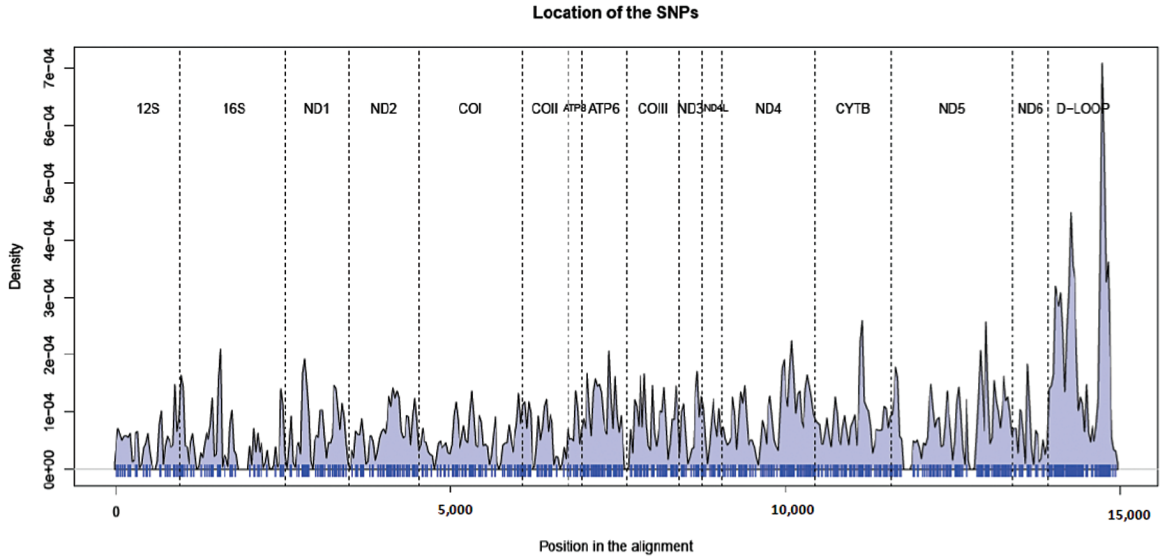

Fig. 3.—SNPs densities from an alignment of 15,000 bp corresponding to 13 protein-coding genes, 2 ribosomal genes, and the D-loop region excluding all transference RNAs. SNP = single nucleotide polymorphism.

Table 2.—Genetic distances between complete mitochondrial genomes of 9 white-tailed deer subspecies, *Odocoileus virginianus* reference sequence HQ332445.1, *O. virginianus* French Guiana (OvirgF.G), *Odocoileus hemionus*, and *Mazama nemorivaga* (*Maz nem*), using the K2P model with a final alignment of 16,490 bp.

Subspecies	*HQ332445*	*texanus*	*veraecrucis*	*mexicanus*	*sinaloae*	*couesi*	*toltecus*	*oaxacensis*	*acapulcensis*	*yucatanensis*	OvirgF.G	*hemionus*
HQ332445												
texanus	0.003											
veraecrucis	0.003	0.001										
mexicanus	0.004	0.004	0.005									
sinaloae	0.009	0.009	0.010	0.010								
couesi	0.009	0.009	0.009	0.009	0.002							
toltecus	0.009	0.009	0.010	0.010	0.010	0.010						
oaxacensis	0.020	0.020	0.020	0.021	0.021	0.020	0.023					
acapulcensis	0.019	0.019	0.019	0.020	0.020	0.020	0.021	0.007				
yucatanensis	0.022	0.021	0.022	0.022	0.023	0.022	0.024	0.018	0.018			
Ovirgf.G	0.021	0.021	0.021	0.022	0.023	0.022	0.023	0.015	0.015	0.021		
hemionus	0.009	0.009	0.009	0.010	0.011	0.010	0.007	0.022	0.021	0.023	0.023	
maz nem	0.104	0.104	0.1104	0.104	0.105	0.105	0.106	0.107	0.106	0.105	0.108	0.106

(from 10.4% to 11%) compared with all studied *Odocoileus* subspecies (Table 2).

Phylogenetic analysis.—The 9 subspecies were grouped into one clade in the Odocoileini tribe. Two *O. virginianus* animals from South America and 1 *O. hemionus* animal were included in this clade and therefore, the Mexican subspecies are categorized as a paraphyletic group. There were 2 principal clades inside this clade. Clade 1 comprised subspecies from the central and northern regions of the country and was further subdivided into 3 groups. The 1st group was composed of the *texanus*, *veraecrucis*, *texanus* from the United States (HQ332445), and *mexicanus*; the 2nd group was formed by 1 animal each from *O. hemionus* and *toltecus*; and the 3rd group was formed by the *couesi* and *sinaloae* subspecies.

Clade 2 comprised subspecies from the southern and southeastern regions of the country (*oaxacensis*, *acapulcensis*, and *yucatanensis*); within this group, there was also 1 *O. virginianus* animal, which was from French Guiana (Fig. 4).

Similar to the study of Hassanin et al. (2012), in this work, the genus of the South American deer did not form monophyletic groups. The *Odocoileus* genus was polyphyletic because 1 animal of the *O. virginianus* species was grouped with 2 animals from the *Mazama americana* species (Fig. 4).

Discussion

Genome organization and comparative analysis.—Compared with the reference genome (Seabury et al. 2011), the 9 genomes presented the same number and order of genes. This feature has been reported in other vertebrate species and even in invertebrates, with the exception of a few arthropods (Chen et al. 2011). Another important feature of the mitochondrial genome is that it is highly variable among species of the same genus and even between subspecies (Wada et al. 2007), which was also observed in this study. Of the 13 mitochondrial protein-coding genes, 7 (12S, ND2, COII, ATP6, COIII, ND4, and CYTB) presented unique polymorphisms in 7 of the 9 subspecies. Moreover, the COI gene and the D-loop region were polymorphic for 8 of the 9 subspecies. These variations could

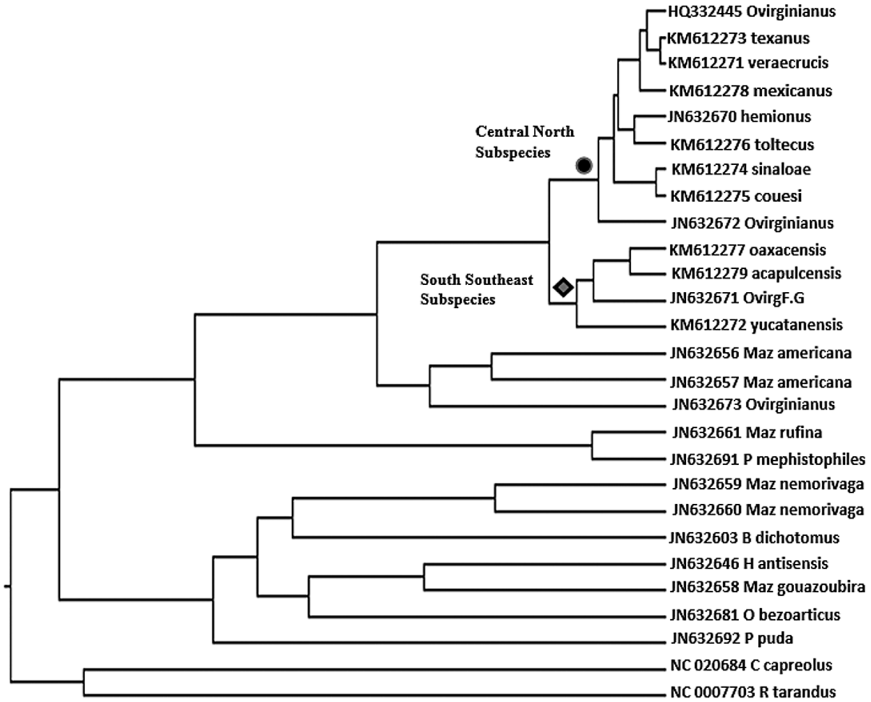

Fig. 4.—Consensus phylogenetic tree obtained by maximum likelihood and Bayesian analysis, in both methods all the branch nodes were supported by bootstrap values of 100%, the central north clade is represented by circle and south southeast clade by diamond.

be useful for developing a strategy to uniquely identify each of these subspecies.

The nonsynonymous variations found in the ATP8 genes of *yucatanensis* and the CYTB genes of the *texanus* and *veraecrucis* subspecies would increase the number of amino acids for each of these proteins. Therefore, it is important to study the impact of these additional amino acids in the function of these proteins.

In the CYTB gene, nonsynonymous nucleotide variation has been reported. This variation generates important amino acidic variations in the functional sites of the proteins associated with specialized metabolic requirements in various species adapted to extreme and contrasting environments (da Fonseca et al. 2008).

Because *veraecrucis* and *texanus* are the biggest subspecies in the country and, along with the *carminis*, *miquihuanensis*, and *couesi* subspecies, inhabit the most xeric environment (Mandujano et al. 2010), we hypothesized that the nonsynonymous A/G variation found in the CYTB gene in 70% of the individuals from *veraecrucis* and *texanus* would promote the adaptation of these subspecies to their environment. However, this hypothesis should be carefully validated because the resultant additional amino acid does not form part of the functional sites of the protein (Hunte et al. 2000). This additional lysine amino acid should be further investigated to determine whether it would significantly affect the folding of the protein. It is also important to analyze the *carminis*, *miquihuanensis*, and more *couesi* animals to determine whether this variation is a synapomorphy exclusive of the subspecies of xeric environments. Because both amino acids involved in the G/A variation in *yucatanensis*, i.e., Ser (AGT) and Asn (AAT), are polar, this variation may not affect the protein structure. However, because this variation was verified in 11 animals from this subspecies, the variation could be used to discriminate this subspecies from the other subspecies.

In addition to the nonsynonymous variations reported in CYTB, some studies have reported adaptive variations in the ATPase complex. Hassanin et al. (2009) observed adaptive variations in the ATPase complex in sheep from different altitudes. Peng et al. (2012) found, in the same complex, that the rate of nonsynonymous substitutions with respect to synonymous substitutions (dN/dS) was greater in larger sheep compared with smaller sheep, and although the values were less than 1, they suggested that these variations were involved in the adaptability of these species to their environment.

In the case of the 2 additional amino acids found in the ATP8 gene of some animals of the *yucatanensis* subspecies, these amino acids could promote the adaptation to this subspecies to their environment because this subspecies is one of the smallest subspecies in the country (Mandujano et al. 2010). The *yucatanensis* subspecies is found exclusively in the Yucatan Peninsula, which could be considered a discrete province because it presents an environment (Rzedowski 1978; Morrone 2005) in which the flora and fauna are more related to Cuba and the Antilles than to the continent (Espinosa 2008). However, investigations of the effect of these additional amino acids on protein folding and changes to energy requirements for this subspecies are important.

The detection of intraspecific variability was limited in this study because only one animal per subspecies was used for sequencing the complete mitochondrial genomes. Additionally, the strategy implemented in *acapulcensis* was not useful to detect variability. More than one mitochondrial genome was not detected in the electropherograms. However, we were able to detect intraspecific variability by validating

the nonsynonymous variations in the D-loop region in *acapulcensis* ATP8 and CYTB genes from *yucatanensis*, *texanus*, and *veraecrucis*, respectively. These variations were not fixed within the studied subspecies population and thereby reflected intraspecific variability.

Phylogenetic analysis and genetic distances.—The differences in the genetic structure observed in the genetic distances and the phylogenetic analysis of the subspecies from the southern and southeastern regions, with respect to the subspecies from the central and northern regions, could be explained by the historic physiography of the Mexican territory. Two biogeographic regions, the Nearctic and Neotropic, converge in central Mexico. These regions give rise to a great diversity of environments and endemisms (Espinosa 2008). The transition zone between the 2 regions comprises the biogeographic provinces of Sierra Madre Occidental, Sierra Transvolcanica Mexicana, and Sierra Madre del Sur (Escalante et al. 2004).

The Sierra Transvolcanica Mexicana is a volcanic belt at altitudes ranging from 1,000 to 5,000 m (Escalante et al. 2004) that recently formed in the tertiaries (Gesundheit and Macías 2005). It extends approximately 920 km from the Pacific Ocean to the Gulf of Mexico through the states of Jalisco, Guanajuato, Michoacán, Guerrero, Edo. de México, Morelos, Distrito Federal, Tlaxcala, Puebla, Oaxaca, and Veracruz (Morrone 2005).

Because this province separates the northern areas with Nearctic affinity and the southern areas with Neotropical affinity, the Sierra Transvolcanica Mexicana is responsible for various important vicariance events, as reported, for example, in the following studies: Escalante et al. (2004), with mammals; Devitt (2006), with snakes; Castañeda et al. (2014), with rodents; Marshall and Liebher et al. (2000), with insects, fishes, reptiles, and plants; and Miguez et al. (2013), with beetles, gymnosperms, snakes, and lizards.

Various studies suggest that the glaciations of the Pleistocene era affected the distribution of species (Moscarella et al. 2003; Gilbert et al. 2006; Duarte et al. 2008). However, in this specific situation, the grouping of subspecies did not coincide with the Mexican Pleistocene biogeographic corridors reported by Ceballos et al. (2010).

The ancestors of the Odocoileini tribe have been considered to originate from Asia approximately 4.2–5.7 Ma in the late Miocene era by way of the Bering land bridge to the American continent. They are hypothesized to have colonized South America in the late Pliocene era and the beginning of the Pleistocene era, once the Panamanian land bridge was uplifted (Gilbert et al. 2006; Duarte et al. 2008).

Thus, it is possible that once the animals of the Odocoileini tribe arrived in the current region and adapted to the local environments, the presence of the physiographic barrier of the Sierra Transvolcanica Mexicana prevented genetic flow, which could account for the genetic differences between the north central and southern subspecies.

Moscarella et al. (2003) found that *O. virginianus* from South America were clearly separated from North American *O. virginianus*. Similarly, in the present study, 1 *O. virginianus* animal from Colombia was grouped closer to the *Mazama americana* animals (isolate 1 from French Guiana, isolate 2 from Peru) instead of with its conspecific *O. virginianus* from the central and northern regions of America. This reflected the marked differences between Nearctic and Neotropic deer. However, further studies are necessary to include more samples of deer from the Nearctic and Neotropic areas.

Logan et al. (2012) reported the genetic separation of subspecies by comparing 480 bp sequences from the D-loop regions of 9 subspecies of white-tailed deer. They found phylogenetic discontinuity between the southeastern subspecies and the remaining Mexican subspecies. Similarly, using 12 nuclear microsatellite loci, De La Rosa et al. (2012) found strong differentiation in the *yucatanensis* subspecies compared with the remaining subspecies. This result was verified in the present study because we found the greatest number of single nucleotide polymorphisms specific to *yucatanensis*, which indicated that this subspecies was genetically different from the rest.

These results are consistent with the study by Mandujano et al. (2010), in which they found that 13 of the 14 subspecies of white-tailed deer can be separated into 2 groups based on their morphologic features: the northern subspecies (*texanus*, *carminis*, *miquihuanensis*, *veraecrucis*, *mexicanus*, and *couesi*), which grow to larger sizes, and the southern subspecies (*sinaloae*, *thomasi*, *yucatanensis*, *truei*, *oaxacensis*, *acapulcensis*, and *nelsoni*) whose sizes are smaller.

Based on the geographic location, the *toltecus* subspecies would be expected to group with the southern and southeastern subspecies. However, it was found in the clade of the central and northern subspecies closely related to *O. hemionus*. An analysis of the geographically closest southeastern subspecies (*O. v. thomasi*, *O. v. nelsoni*, and *O. v. truei*) could confirm this observed topological difference.

On the one hand, *O. v. yucatanensis* and *O. virginianus* from French Guiana could be descendants from the same ancestor that lived during the radiation of deer to South America in the Pleistocene era. On the other hand, the paraphyly of *O. virginianus* with respect to *O. hemionus* found in the phylogenetic analysis is consistent with the observations of other studies (Moscarella et al. 2003; Duarte et al. 2008; Hassanin et al. 2012). The paraphyly has been suggested to be produced by hybridization (Hassanin et al. 2009) because these 2 species share a broad distribution range. In this study, the sample of *O. hemionus* was sourced from Arizona, which is a location where these 2 species converge. The findings in the present study support the hybridization theory because *O. hemionus* presented genetic distances within the same range of the subspecies of *O. virginianus*. However, it is necessary to include more samples from overlapping and nonoverlapping regions to clarify this issue.

By sequencing the complete mitogenomes of 9 subspecies of white-tailed deer from Mexico, we identified nucleotide variations specific to each subspecies, which enables the development of a differential diagnostic strategy for each subspecies. Thus, the current study provides the basis for the genetic identification of the subspecies of white-tailed deer in Mexico. Due to the great differentiation between the subspecies of central-northern and southern-southeastern regions of

Mexico, conservation and sustainability management programs should be focused on taking special care to preserve the genetic integrity of the southern and southeastern subspecies by avoiding the introduction of subspecies from central and northern Mexico (specially *O. v. texanus* subspecies, which is the most economically important subspecies in Mexico and therefore its introductions are very common around the country). The preservation of this subspecies would contribute to the conservation of the diversity of genetic resources in the country.

Future work should focus on obtaining complete mitogenomes for the 5 remaining subspecies and finding nucleotide variation specific to each subspecies.

Acknowledgments

Authors acknowledge financial and technical support from Asociación Nacional de Criadores de Cérvidos de México. Projects SIP-IPN 20120885 and CONACYT 89749 Grants supported this research and the authors acknowledge the Texas A&M University Brazos HPC cluster that contributed to the research reported here: brazos.tamu.edu.

Supporting Information

The Supporting Information documents are linked to this manuscript and are available at Journal of Mammalogy online (jmammal.oxfordjournals.org). The materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.—Specific variations by subspecies by gene in 9 subspecies of Mexican white-tailed deer.

Supporting Information S2.—Percentage of nucleotides by subspecies using complete mitochondrial genomes of 9 subspecies of Mexican white-tailed deer.

Supporting Information S3.—Synonymous and non-synonymous variations by gene.

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Submitted 13 July 2015. Accepted 10 October 2015.

Associate Editor was Jorge Ortega.

APPENDIX I

Sampling sites and geographic coordinates for subspecies of *Odocoileus virginianus*. UMA = Unidad para la Conservacion, Manejo y Aprovechamiento Sustentable de la Vida Silvestre, in Spanish.

Subspecies	Sampling site	UMA	Number of samples	Geographic location
Odocoileus virginianus texanus	Anáhuac, Nuevo León	Cuevas	1	27°13′58″N 100°08′04″W
Odocoileus virginianus veraecrucis	Panuco, Veracruz	El Indio	1	22°03′09″N 98°10′58″W
Odocoileus virginianus mexicanus	México D.F.	Desierto de los Leones	1	19°18.491″N 98°18.751″W
Odocoileus virginianus couesi	Yecora, Sonora	Rancho San Salvador	1	28°22′17.67″N 108°55′36.84″W
Odocoileus virginianus sinaloae	Mazatlán, Sinaloa	Acuario	1	23°14′01″N 106°25′45″W
Odocoileus virginianus toltecus	Santa María Yahuiche Ixtlan de Juárez, Oaxaca	Wildlife	1	17°19′50.00″N 96°29′14.00″W
Odocoileus virginianus oaxacensis	San Miguel Ahuehuetitlan, Oaxaca	Wildlife	1	17°40′21″N 98°19′14″W
Odocoileus virginianus yucatanensis	Mérida, Yucatán	Wildlife	1	20°58′47″N 89°36′57″W
Odocoileus virginianus acapulcensis	Aquila y Arteaga, Michoacán	Wildlife	7	18°16′27.01″N 103°20′46.99″W 18°18'19.40''N 103°16'1.82''W

Appendix II

Nucleotide sequence of primers used for sequencing the 9 Mexican white-tailed deer subspecies, each pair of primer amplify fragments of approximately 750 bp.

Forward primer	5′-3′ sequence	Reverse primer	5′-3′ sequence
MT1	AACAATAAAGCAAGGCACTG	MT1′	CCATTTCTTTCCACTCCATAG
MT2	CAAAACTATTCGCCAGAGTA	MT2′	GACAACTCATTATGCAAAAGG
MT3	TACTGGAAAGTGTGCTTGG	MT3′	TGACTTAAACTCGTGCGG
MT4	TGGTGATAGCTGGTTGTC	MT4′	TATGGTATTCCCGCCTCT
MT5	CCAAAAACATCACCTCCAG	MT5′	GTTTTACGCAATTACCGGG
MT6	ATCGACAATAGGGTTTACG	MT6′	AATTGTTTGTGCTACTGCTC
MT7	GGTCCCTATGGCTTACTC	MT7′	TAACGGAATCGAGGGTATG
MT8	TAGAATATGCAGCAGGGC	MT8′	TCTGTAGCTCGTGGGTTG
MT9	GCTCCCAATTACACCAAAC	MT9′	GTGTGAATATGGTGGAGGT
MT10	CAGAAGTAACACAAGGCATC	MT10′	GAAATATTGTTAGTGCTGTGG
MT11	GCTATCCTGATTCTCGTAACC	MT11′	AGTTGAAAATAATCAGCGGT
MT12	CTTGGTAAAAAGAGGAGTATGG	MT12′	CCGCCAAGTGTAGAGAAAA
MT13	CCCCGAATAAATAACATAAGC	MT13′	TGTTGCCTCCATGAAGTG
MT14	CCTGTTCTGATTTTTCGGA	MT14′	GGATGGAAATGCTTCTCAGAT
MT15	CCATTATAGGTGGGTTTGTC	MT15′	CTGTTTCTACTTCTTGGGC
MT16	CGAACCCCCTATAGCTGG	MT16′	GTTGTTTGGTTTAAGCGTCC
MT17	CATCAGAATTAAAGCCAGGAG	MT17′	GTCTGTCCTTTGGTATTGTG
MT18	CTTAGTGATATGCCGCAAC	MT18′	GCGGTGATATTGGCTGTTA
MT19	CTCATTCACACCAACCAC	MT19′	GGATGGATGCCTGTTGGA
MT20	CATATAGTAAACCCAAGCCC	MT20′	CCACATCCACGAAGTGTC
MT21	TCTGACGGAGTATATGGC	MT21′	CTAATCCTTTTTGGGTTCATTC
MT22	CTAGCCCTCCTAACCAACT	MT22′	TGATAATCAGGTCAGAGGCA
MT23	TACGGCACCGATTATGTTC	MT23′	GGCTTCTACATGGGCCTT
MT24	ATCAAAAGAGAGCTTAACTCG	MT24′	TAGATGAGGTAAGGCCGT
MT25	CCCCATTGCAGGATCTAT	MT25′	GCTCCTATTATCAGATTCACA
MT26	GTAATTACCGCCTTATATTCCC	MT26′	GACAAATAATGCTACTGGAAC
MT27	CCCCATTATAACTACAAGCTC	MT27′	GTTGAAAGTCTCAGGCGT
MT28	GTTCCAGTAGCATTATTTGTC	MT28′	GATAATAGAGCCGGAGCA
MT29	AAGGCCCTACTCCTGTCT	MT29′	GCTAAGGCTGTTATTTTTAGGT
MT30	TAGGACAACCCCGATTTC	MT30′	TCTTTATTGATTGGTGTGGTAG
MT31	AATCATACACCGCCTGAC	MT31′	GTGCTTGGTTTTGTAGGA
MT32	TATCTCGGGATACTGCTCT	MT32′	TACCCTACGAATGCTGTG
MT33	CATCCGACACAATAACAGC	MT33′	ATGGTGTAGTAGGGGTGAAT
MT34	CCTCTCAGCAATCCCATA	MT34′	ATTGGTTGCTCTCCTTTTC
MT35	CATCTAAACAACGCAGCATAA	MT35′	ACGGGATACGCATGTTGA
MT36	ACTATATACCCCATGCTTACA	MT36′	ATAGCTGAGTCCAAGCATC
MT37	TTCTTCAGGGCCATCTCA	MT37′	GGCGCTTATATACTTACCTC

Appendix III

Complete genomes of the tribe Odocoileini reported by Hassanin et al. (2012).

Species	Isolated	Origin	Accession no.
Pudu puda	JP/M92144	Unknown	JN632692
Pudu mephistophiles		Colombia	JN632691
Rangifer tarandus		Unknown	AB245426
Blastocerus dichotomus		Bolivia	JN632603
Hippocamelus antisensis	MRGHa9	Argentina	JN632646
Ozotoceros bezoarticus	MRGOb2	Bolivia	JN632681
Mazama americana	MAZ9472	French Guiana	JN632656
Mazama americana	MRGMa40	Peru	JN632657
Mazama gouazoubira	MRGMsp2	Colombia	JN632658
Mazama nemorivaga	MRGMa36	Peru	JN632659
Mazama nemorivaga	T1627	French Guiana	JN632660
Mazama rufina	MRGMr4	Colombia	JN632661
Odocoileus hemionus	T1766	Arizona	JN632670
Odocoileus virginianus	T4887	French Guiana	JN632671
Odocoileus virginianus	CYTO 02.133	Unknown	JN632672
Odocoileus virginianus	MRGOv14	Colombia	JN632673
Odocoileus virginianus		Texas	HQ332445
Rangifer tarandus		Unknown	NC_020684
Capreolus capreolus		Unknown	NC_007703