THE SYSTEMATIC RELATIONSHIPS OF CERVIDS WITH SPECIAL REFERENCE TO THE SOUTH AMERICAN RADIATION

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THE SYSTEMATIC RELATIONSHIPS OF CERVIDS WITH SPECIAL REFERENCE TO THE SOUTH AMERICAN RADIATION

by

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ABSTRACT

Mitochondrial DNA (mtDNA) sequences were used to investigate systematic relationships of 21 species of deer (family Cervidae), with special attention directed towards the poorly understood South American taxa. The nucleotide region examined was a 410 base pair (bp) region of the 12S ribosomal RNA (rRNA) in combination with 401-bp cytochrome *b* data set. In addition to the cervids, nine other ungulate genera were also sequenced.

Among the 811-bp of sequence data available, 365 nucleotide positions were variable, of which 296 were phylogenetically informative. The data suggest that cervids consist of two monophyletic clades or subfamilies, corresponding to a previously recognized alternative conditions of the metacarpals of the lateral digits. The plesiometacarpalian state or the loss of the distal portion of the second and fifth metacarpals, is characteristic of the cervines (subfamily Cervinae), whereas the telemetacarpalian state or the loss of the proximal metacarpal portions in the lateral digits, is characteristic of the odocoileines and *Hydropotes* (subfamily Odocoileinae). Within Cervinae, three taxa were identified: *Cervus* (including *Elaphurus*), *Axis*, and *Muntiacus*. The Odocoileinae includes three monophyletic tribes: Capreolini (*Capreolus* and *Hydropotes*), Alcini (*Alces* only), and Odocoileini (endemic New World deer and holarctic *Rangifer*). The *Odocoileus* species were consistently the sister group to *Mazama* (*M. americana*, *M. nana*, and *M. bororo)* in at least 52% (NJ) of the bootstrap replicates from all three methods of phylogenetic analysis [maximum-parsimony (MP)

bootstrap value 77%; maximum-likelihood (ML) bootstrap value 69%; and, neighbour-joining (NJ)]. *Hydropotes* was identified as a sister species of *Capreolus* in at least 80% (MP) of the bootstrap replicates and thus is not representative of the plesiomorphic ancestral state for cervids. Relationships among *Alces* and the remaining odocoileine genera were not well resolved.

The data challenge conventional assumptions about New World cervid evolution and taxonomy. *Odocoileus* is distributed throughout North and Central America, and the occurrence of *O. virginianus* in South America north of the Amazon basin has been taken to suggest that all South American deer evolved from *O. virginianus*. The molecular data instead show that the endemic South American genera (*Pudu*, *Ozotoceros*, *Blastocerus*, and *Hippocamelus*) as well as one South American species of *Mazama* (*M. gouazoupira*) form a monophyletic lineage whereas *Odocoileus* is more closely related to the remaining species of Central and South American *Mazama* (*M. americana*, *M. nana*, and *M. bororo*). In all three analyses, *H. bisulcus*, *B. dichotomus*, *P. puda*, and *O. bezoarticus* clustered together; with *M. gouazoupira* being the sister group to these other four genera (NJ bootstrap value 76%), with *M. gouazoupira* and *H. bisulcus* being the sister group to the latter three genera (MP bootstrap value 53%), and with *P. puda* being the sister taxa to these other three genera along with *M. gouazoupira* (ML bootstrap value 88%). All analyses placed the four *Mazama* species in at least three different clades and the *M. americana* individuals were often split between two clusters, suggesting a large degree of genetic variability within this genus and species, respectively.

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INTRODUCTION

The Cervidae or deer family is in the order Artiodactyla, the even-toed ungulates or hoofed mammals. Cervids (superfamily Cervoidea) are included in the infraorder Pecora of the suborder Ruminantia (Nowak, 1991). The ruminant Artiodactyla is a diverse and complex group, and the phylogenetic position of the Cervidae within this suborder has been widely debated (Scott and Janis, 1987; Gentry and Hooker, 1988). Central to the controversy is the argument that horn-like organs probably originated in deer independently from giraffoids and bovoids (Hamilton, 1978). The classic arrangement of the four major families within the infraorder Pecora pairs Cervidae and Giraffidae together, and Bovidae with Antilocapridae, based on morphological characteristics. However, it is currently agreed that this traditional arrangement is not well-supported (Janis, 1988) although no new consensus has emerged concerning the correct cladistic arrangement of ruminants (Scott and Janis, 1987; Hassanin and Douzery, 2003). Similarly, the phylogenetic placement of deer genera within subfamilies and the number of subfamilies within Cervidae vary greatly according to different authors. That is, considerable differences exist among various classifications of this family and few authors are in complete agreement (Simpson, 1984).

Conventional deer taxonomy is based on the presence or absence of antlers, defined as bony outgrowths of the frontal bone (Geist, 1966; Bubenik, 1983; Lister, 1987; Bubenik, 1990). However, attempts to define species and genera based on antler morphology have historically been complicated by intraspecific variation (Smith *et al.*,

1983; Ullrey, 1983; Scott and Janis, 1987). Antlers are characteristically seen only in male deer, except for *Rangifer* where both sexes are antlered. *Hydropotes,* the Chinese water deer, is antlerless and is most often placed as the sister species to the antlered deer. *Hydropotes* also possesses enlarged canines, setting this genus apart from the antlered deer. One explanation is that with the continued evolution of antlers the canine declined in importance and that the antlerless state represents the ancestral plesiomorphic condition for deer. This is the basis of the conventional taxonomy of the Cervidae. However, Carr (1996) inferred from molecular data that the Chinese water deer does not represent the plesiomorphic state, and that this genus is more closely related to the roe deer (*Capreolus*), a species with simple branched multi-tined antlers. Carr (1996) hypothesized that Old World deer with large palmate antlers such as moose (*Alces*), and caribou (*Rangifer*) represent the ancestral condition, whereas multi-tined antler patterns typical of New World deer (such as the *Odocoileus*) represent a modification of this ancestral condition. It is important to point out that there is no fossil record of antlered forms in North America until the early Pliocene (4.5 MYBP) when it is assumed that they must have entered from Eurasia (Eisenberg, 1987; Carr and Hughes, 1993; Carr, 1996; Geist, 1998).

Historical classification of the antlered cervids using morphological and paleontological information has also taken into account foot structure. The anterior surface of the metatarsals in cervids is highly fused with a closed gully, resulting in a reduction of the lateral digits (Scott and Janis, 1987). "Plesiometacarpalian" refers to the loss of the distal portion of the second and fifth metacarpals, whereas "telemetacarpalian"

refers to a loss of the proximal metacarpal portions in the lateral digits. These two patterns have formed the basis of the historical classification of antlered cervids into the two subfamilies Cervinae and Odocoileinae (Brook, 1878; Ellerman and Morrision-Scott, 1966; Simpson, 1983; Wilson and Reeder, 1993). The ancestral plesiometacarpalian condition is characteristic of the Old World deer or Cervinae, and includes over 20 species allocated among four to nine genera (*Muntiacus* or barking deer, *Dama* or fallow deer, *Axis* or chital deer, *Cervus*, *Elaphurus* or Pere David's deer, *Panolia* or Eld's deer, *Rucervus* or swamp deer or barasingha, *Rusa* or sambar deer, and *Sika*). The latter five genera have been described as distinct from *Cervus*, which has often been used as a "catch-all" taxon for any Old World cervid (personnel communication, S. Carr, 2003).

The telemetacarpalian condition is characteristic of the "New World" deer or Odocoileinae, as well as *Hydropotes*, altogether about fourteen species allocated to ten genera (Groves and Grubb, 1987). Among those that are limited to the New World, or neocervines, *Odocoileus hemionus* subspecies including mule deer and black-tailed deer are Nearctic, whereas white-tailed deer (*Odocoileus virginianus)* extend from the Nearctic into the Neotropics. An additional five genera are exclusively neotropical: *Ozotoceros*, *Blastocerus*, *Hippocamelus*, *Mazama* and *Puda*. Additional species identified as telemetacarpalian are New World caribou and Old World reindeer (*Rangifer tarandus*), moose (*Alces alces)* and *Capreolus* species, with moose and *Capreolus* spp. being holarctic and are paleoarctic, respectively. The presence of tarsal glands on the hocks of the neocervines and holarctic species distinguishes these groups from

Capreolus. Likewise, the absence of a vomerine septum in *Alces* distinguishes this genus from the neocervines and *Rangifer* (Groves and Grubb, 1987).

A survey of contemporary literature revealed that most commonly, the phylogeny of the family Cervidae is described to include the subfamilies Muntiacinae (muntjacs), Hydropotinae (Chinese water deer), Cervinae (most Old World cervids like the axis, fallow, red deer and elk), and the Odocoileinae (most New World cervids like the white-tailed deer, caribou, moose, and all South American taxa, along with the European roe deer) (Groves and Grubb, 1987; Scott and Janis, 1987; Miyamoto *et al.*, 1993). This traditional view suggests that subfamilies Cervinae, Muntiacinae, and Odocoileinae form a monophyletic group within the family Cervidae and that Hydropotinae is the sister to these antlered deer (Groves and Grubb, 1987). Mitochondrial DNA (mtDNA) investigations by Miyamoto *et al.* (1993) suggested that the subfamilies Cervinae and Muntiacinae are sister taxa, with odocoileines being more distantly derived. Miyamoto *et al.* (1993) furthermore suggested that Odocoileinae most likely originated in the Old World during the Late Miocene. Simpson (1984) added a fifth subfamily, Moschinae, to include the antlerless musk "deer", *Moschus*. Scott and Janis (1987) however suggest that *Moschus* and a number of fossil genera should be placed separately in the family Moschidae, rather than in the Cervidae. A more recent molecular analyses of seven mitochondrial and nuclear markers by Hassanin and Douzery (2003), concluded that Bovidae, Cervidae and Moschidae were closely related, with the musk deer as the sister group of bovids rather than cervids. Similarly, the mouse deer (Tragulidae) have sometimes been included as cervids, but there is now agreement this family should be

excluded. It is interesting to note that the canines of *Hydropotes* resemble *Tragulus*. (Webb and Taylor, 1980; Groves and Grubb, 1987).

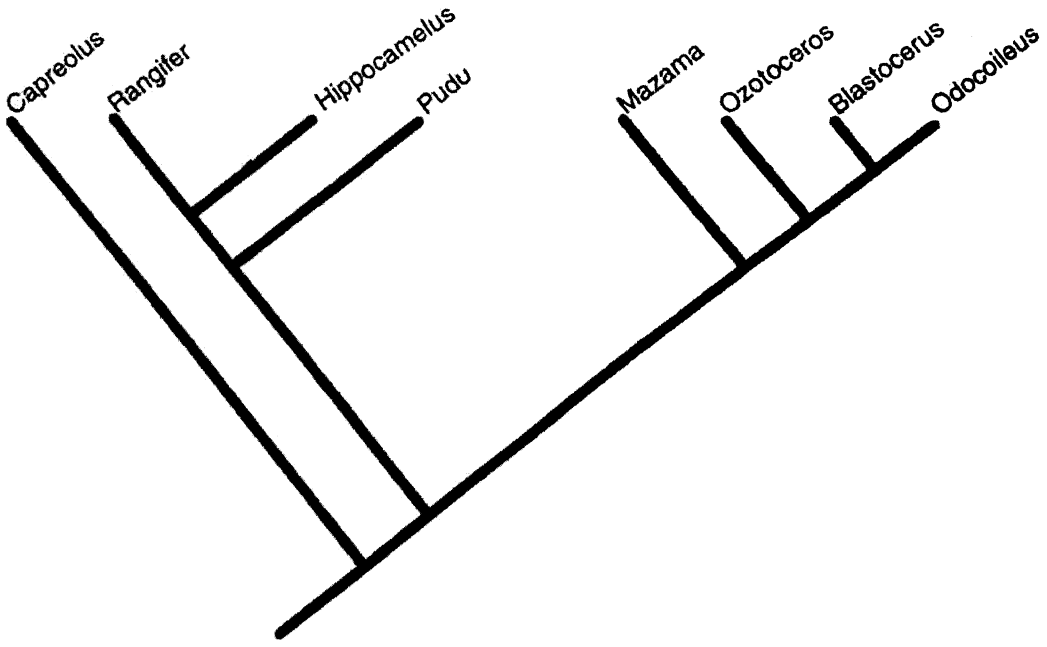
Alternatively, Groves and Grubb (1987) and Carr (1996) have suggested very similar provisional classifications of cervids, based on a composite of morphological characters (e.g. foot structure and antlers), karyotype data, geographical information, and mtDNA evidence. Based on morphological characters and karyotypic evidence, Groves and Grubb (1987) concluded that there are three deer subfamilies, with Hydropotinae the sister to the antlered deer, and telemetacarpalian Odocoileinae (including tribes Odocoileini, Capreolini, and Alcini) the sister to the monophyletic plesiometacarpal Cervinae (including tribes Cervini and Muntiacini). Odocoileini and Alcini constitute a clade, with Capreolini being the sister group, thus the telemetacarpalian Odocoileinae are not monophyletic. However, the authors suggest that this classification would require a better knowledge of fossil cervids before any absolute conclusions can be confidently stated. A very similar classification is put forth by Carr (1996) based on geographical, morphological, karotypic, and mtDNA evidence, in which he describes a division of deer into two monophyletic subfamiles. Telemetacarpalian New World deer, or Odocoileinae, are divided into three monophyletic tribes: Capreolini (*Capreolus* and *Hydropotes*), Alcini (*Alces* only), and Odocoileini (endemic New World deer, including *Odocoileus* and Neotropical species as well as *Rangifer*). Plesiometacarpalian Old World deer or Cervinae (including *Cervus* and *Elaphurus*) form a clade and each Cervinae genus is a monophyletic subgenus of *Cervus*. In this phylogeny, *Axis* is the sister to *Cervus* and *Muntiacus* is the sister to the other cervines. Available karotypic work, summarized by

Neitzel (1987), further points to a six subfamily classification scheme, in which Alcinae and Rangiferinae are elevated to the subfamilial level, otherwise the conventional cervid taxonomy is maintained. According to a compilation of literature through 1970, Whitehead (1993) divided the deer of the world into two families: Moschidae (non-antlered artiodactyls with one subfamily of Moschinae) and Cervidae (antlered artiodactyls with six subfamilies).

Most recently, McKenna and Bell (1997) presented a similar classification to Groves and Grubb (1987), with the exception of listing Rangiferini as a monotypic tribe. Webb (2000), further expanded the Groves and Grubb (1987) study of morphological comparisons, to present an arrangement in which *Hippocamelus* and *Pudu* were united with *Rangifer* within the tribe Rangiferini. This phylogenetic hypothesis proposes that the telemetacarpalian Odocoileinae, should be split into two separate tribes, namely Odocoileini (*Odocoileus*, *Blastocerus*, *Ozotoceros*, and *Mazama*) and Rangiferini (*Hippocamelus*, *Pudu,* and *Rangifer*) (Figure 1). According to Webb, these two tribes diversified in parallel, beginning possibly in Asia, then in North America, and extending together into South America.

Evidently, many outstanding questions at different taxonomic levels remain unresolved about the phylogeny and evolution of the cervids. In particular, subfamilial relationships among antlered deer have not been convincingly resolved. Equally puzzling is the evolutionary relationships of South American deer, which are very poorly understood. Conventional assumptions about cervid evolution and taxonomy suggest that all South American deer evolved very recently from North American deer. Eisenberg

Figure 1: A recent phylogenetic hypothesis of relationships among genera of the subfamily Odocoileinae. Cladogram is modified from Webb (2000) to exclude the extinct genera: *Navahoceros* and *Eocoileus*.


Capreolus
Rangifer
Hippocamelus
Pudu
Mazama
Ozotoceros
Blastocerus
Odocoileus

(1987) theorizes that at the completion of the land bridge between North America and South America (during the late Pliocene), deer entered the southern continent and began an adaptive radiation, filling niches that would have normally been occupied by bovids. This traditional interpretation classifies all South American deer as a monophyletic group derived from North American white-tailed deer, *Odocoileus virginianus* (Eisenberg, 1987; Hershkovitz, 1982; Geist, 1998 regarding *Pudu*). Sometimes the Neotropical genera are included in *Odocoileus* (Haltenorth, 1963, cited in Wilson and Reeder, 1993). The South American deer include eleven species grouped into six genera: *Odocoileus*, *Blastocerus*, *Ozotoceros*, *Hippocamelus*, *Mazama*, and *Pudu*. All six genera are investigated in this thesis (Table 1), and apart from *Odocoileus*, the genera noted above are exclusively Neotropical. Currently, almost all of the South American cervid genera are listed by CITES as being either endangered or vulnerable.

Odocoileus is the typical deer of North America and has been widely studied, especially with regards to inter- and intraspecific hybridization (Carr *et al.*, 1986; Gavin and May, 1988; Cronin *et al.*, 1988; Cronin, 1991; Cronin 1992, Ballinger *et al.* 1992; Hughes and Carr, 1993; Carr and Hughes, 1993; Greenslade, 1998). There are two species in this genus, *O. hemionus* (mule deer and black-tailed deer) and *O. virginianus* (white-tailed deer); only the latter species is found in South America (Figure 2a), where it is sympatric with several of the Neotropical genera (Eisenberg, 1987). This genus differs morphologically from *Cervus* by an absence of the upper canine teeth. Like *Cervus*, *Odocoileus* species also have multi-tined antlers (Figure 3d). *Odocoileus* is distinguished from the related South American genera *Blastocerus* and *Ozotoceros* by the presence of

Table 1. List of scientific and common names of Cervid species investigated.

Scientific Name	Common Name(s)
Alces alces	moose
Axis axis	chital or spotted deer
Blastocerus dichotomus	marsh deer
Capreolus capreolus	roe deer
Cervus duvauceli	swamp deer or barashinga
Cervus elaphus canadensis	American elk or wapiti
Cervus (Rusa) unicolor	sambar
Elaphurus davidianus	Pere David's deer or milu
Hippocamelus bisulcus	huemul
Hydropotes inermis	Chinese water deer
Mazama americana	brocket deer
Mazama bororo	brocket deer
Mazama gouazoubira	brocket deer
Mazama nana	brocket deer
Muntiacus muntjac	muntjac or barking deer
Odocoileus hemionus	mule deer
Odocoileus hemionus columbianus	black-tailed deer
Odocoileus virginianus	white-tailed deer
Ozotoceros bezoarticus	pampas deer
Pudu puda	Andean pudu
Rangifer tarandus	caribou (New World) or reindeer (Old World)

Figure 2. Distribution of South American deer: (a). *Odocoileus virginianus*; (b) *Blastocerus dichotomus*; (c) *Ozotoceros bezoarticus*; (d) *Hippocamelus bisulcus*; (e) *Mazama americana*; and (f) *Pudu puda*. Modified from Eisenberg, 1987.

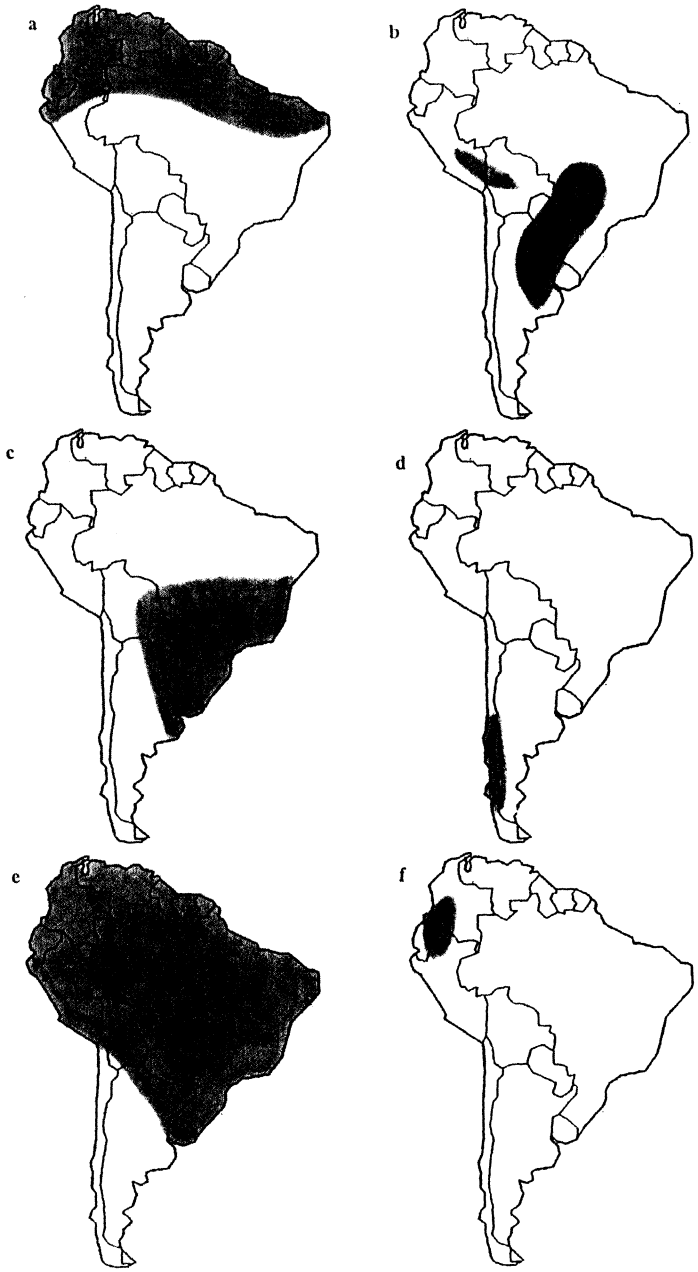
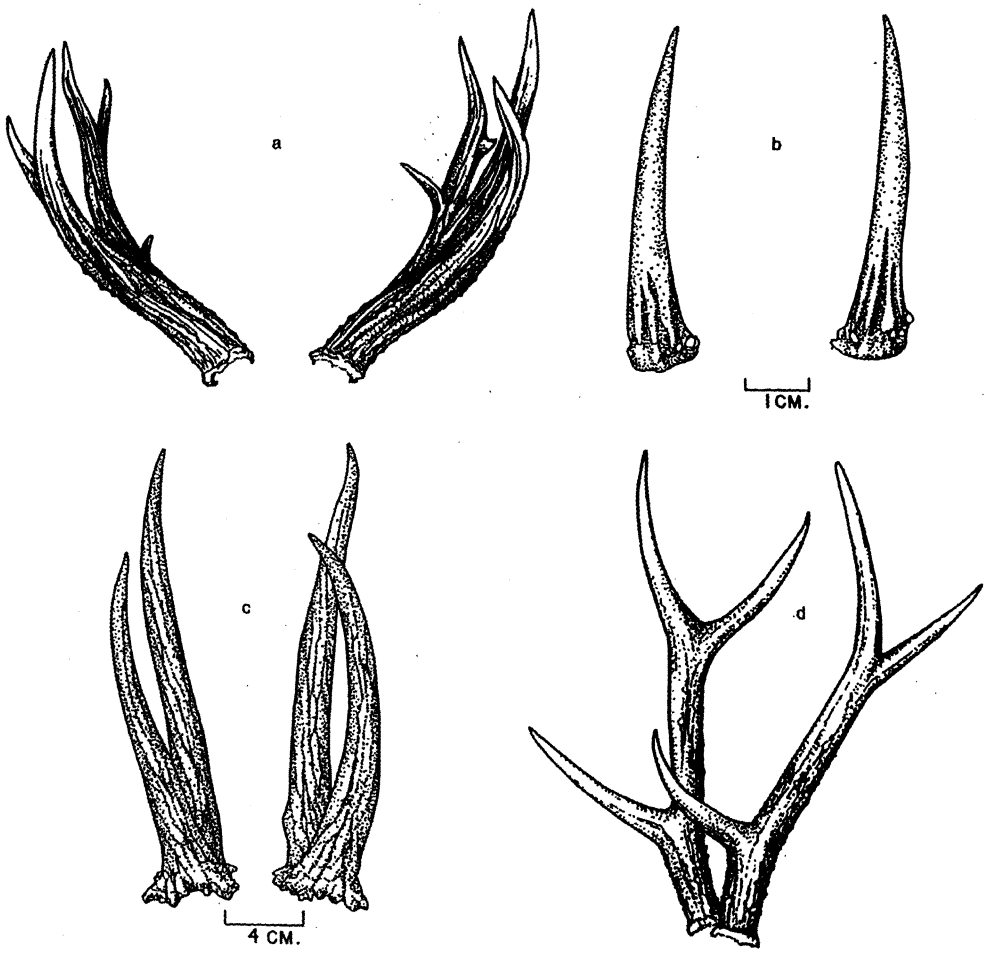

a
b
c
d
e
f

Figure 3. Antler form displayed by the six genera of South American cervids: (a) *Blastocerus*; (b) *Pudu* and *Mazama*; (c) *Hippocamelus*; (d) *Odocoileus* and *Ozotocerus*. Figure taken from Redford and Eisenberg, 1992.


a
b
1 CM.
c
4 CM.
d

metatarsal glands. Mitochondrial studies show that the subspecies of *Odocoileus hemionus* (mule deer and black-tailed deer) are paraphylectic, perhaps as a result of sorting and/or introgressive hybridization between species and subspecies (Cronin, 1991; Carr and Hughes, 1993; Cathy *et al.*, 1998). Allozyme investigations suggests that black tailed deer and mule deer are conspecific and distinct from white-tailed deer (Gavin and May, 1988). The suggested earlier fossil appearance of white-tailed deer and wide distribution of this species has been argued to explain that North American mule deer and black-tailed deer are derived from white-tailed deer (Kurten and Anderson, 1980). According to Mendez (1984), *Odocoileus* continues to decline in Mexico and Central America due to habitat destruction, illegal hunting and expanding agricultural activity. In particular, in South America the number of animals killed illegally is greater than that taken legally (Nowak, 1991).

In the southern portion of South America, *Odocoileus virginianus* is replaced by two species, the marsh deer, *Blastocerus dichotomus*, and the pampas deer, *Ozotoceros bezoarticus* (Figure 2b and 2c). *B. dichotomus* is the largest of the Neotropical deer and it's antlers are double-forked, usually having four points (Figure 3a). The antlers of *O. bezoarticus* usually have three tines (3d) and this genus closely resembles the white-tailed deer (*Odocoileus*) in its social behavior (Nowak, 1991). *Blastocerus* and *Ozotoceros* each have only a single species. Both species are listed by CITES as endangered: their numbers and distributions of both species have declined considerably through the loss of habitat to agriculture and marsh drainage, uncontrolled hunting, and possibly the transmission of disease from domestic livestock (Duarte, 1997; Nowak, 1991).

The genus *Hippocamelus*, of which only a single species, *H. bisulcus* or huemul (Figure 2d), is investigated in this study, is reported to have adaptively radiated to fill the sheep niche with regards to the areas they inhabit (Eisenberg, 1987). The only other species within this genus is *H. antisensis* or taruca. The antlers of both species in this genus are bifurcated (Figure 3c). Figure 3c actually depicts a sketch of the antler of *H. antisensis,* where the antler bifurcates at the base of the tine (proximal end) immediately above the pedicel, whereas in contrast, the antler of the *H. bisulcus* bifurcates several centimeters further up the tine from the pedicle (Díax and Smith-Flueck, 2000). The steep decline of the huemul is widely discussed (Miller *et al.*, 1983; Smith-Flueck and Flueck, 1997; Smith-Flueck, 2000) and it has been suggested that it has almost been exterminated as a result of hunting, predation by dogs and other animals, disease, and competition with cattle and other deer (Redford and Eisenberg, 1992); however, to date there is no empirical evidence or antidotal reports to support these claims. Investigations show that competition could possibly occur between huemul populations and introductions of domestic cattle (Frid, 2001) and European red deer (*Cervus elaphus*) (Smith-Flueck, 2000). A study by Smith-Flueck and Flueck (1997) in the Province of Rio Negro, Argentina, confirmed that the Mountain lion (*Felis concolor*) was a predator of the huemul in the area surveyed. Puma predation is also a limiting factor affecting huemul populations (Smith-Flueck and Flueck, 2001).

The smaller endemic deer of South America are the *Mazama* (Figure 2e) and the *Pudu* (Figure 2f). Both have simple spikes for antlers (Figure 3b). Eisenberg (1987) hypothesizes that spikes may be the result of reduced selection for large antlers

accompanying an overall reduction in body size. *Pudu* or pudus is the smallest of the South American deer and there are two species: *P. puda and P. mephistophiles*; they are listed as threatened and indeterminate, respectively. Only the former species is investigated in this study. *P. puda and P. mephistophiles* are also the two smallest deer in the world (Geist, 1998; Smith-Flueck, 2000). Tusks do not occur in the upper jaw of pudus and an external tail is practically lacking. Hershkovitz (1982) has summarized the existing data concerning life history and morphology of the genus *Pudu*. The geographic distribution of the two species of *Pudu* are probably separated (Eisenberg, 1987). Conventional taxonomy outlines four species of brocket deer or *Mazama*; however, at least six species and many more subspecies have been suggested (Duarte, 1997). This study looks at only four species: the red brocket or *M. americana*, *M. gouazoupira*, *M. nana*, and *M. bororo*. The upper canines in *Mazama* may be present or absent and there is no metatarsal gland. Brocket deer are intensively hunted for use as food, and because they frequently damage bean and corn crops.

In contrast to the monophyletic derivation of South American deer from North American white-tailed deer previously discussed, recent studies of mtDNA sequences of South American species by Carr (1996) indicate that deer have invaded Latin America at least twice. This study suggested that Central American *Mazama* are more closely related to North American *Odocoileus* species whereas endemic South American genera (*Pudu*, *Ozotoceros*, and *Blastocerus*) represent a separate, more ancient clade. That is, these data indicate that Neotropical deer taxa have a paraphyletic or maybe even a polyphyletic origin. Available karyotypic work by J. M. B. Duarte at UNESP Jaboticabal in Brazil has

documented unsuspected karyotypic diversity among South American deer, indicating that there are many more species of South American *Mazama* than previously suspected (Duarte and Giannoni, 1995a and 1995b). Neitzel (1987) also reports a very high degree of karyotypic evolution within the family Cervidae, including the South American genera.

The phylogenetic analyses of South American deer presented by Carr (1996) are based on a single, relatively short portion of maternally-inherited mtDNA molecule, the cytochrome *b* gene. The cytochrome *b* gene is extensively used to determine relationships between closely related genera but may poorly resolve deeper branches, such as those between the plesiometacarpalian and telemetacarpalian taxa. In order to determine accurately the evolutionary history of South American cervids, sufficient mtDNA sequence data are required to generate a gene tree that would be more strongly robust than the cytochrome *b* mtDNA phylogeny. The 12S rRNA gene is a more slowly evolving molecule and complements the resolution of the more rapidly evolving cytochrome *b* gene. The primary goal of this study was to obtain sufficient additional mtDNA sequence data to allow for the unequivocal construction of the South American deer phylogeny by augmenting the existing mtDNA cytochrome *b* evidence. Such information can help to clarify the systematic relationships of especially the South American cervids and perhaps more broadly, aid in the phylogenetic subfamilial resolution of the family Cervidae.

MATERIALS AND METHODS

A portion of the mitochondrial 12S ribosomal RNA (rRNA) was sequenced for 38 Artiodactyla samples, representing eight taxonomic families. Thirty-one samples were from the Cervidae family and one sample was from each of the following families: Hippopotamidae, Camelidae, Suidae, Tayassuidae, Giraffidae, Bovidae, and Antiocapidae. Two Perissodactyla samples belonging to Equidae and Rhinocerotidae were successfully sequenced.

SAMPLE COLLECTION

The 40 samples used in this study came from a variety of sources.

The individual roe deer [*Capreolus capreolus*], red brocket [*Mazama americana*], muntjac or barking deer [*Muntiacus muntjac*], Pere David's deer or milu [*Elaphurus davidianus*], sambar [*Cervus (Rusa) unicolor*], American elk or wapiti [*Cervus elaphus canadensis*], and swamp deer or barashinga [*Cervus duvauceli*] samples analyzed are the same as those analyzed by Cronin (1991) and Carr (1996). Genomic DNA extracts of these Cervid samples were provided by Matt Cronin. The Andean pudu [*Pudu puda*], chital or spotted deer [*Axis axis*] and Chinese water deer [*Hydropotes inermis*] samples were obtained from the frozen collection at the London Zoo, courtesy of Rob Wayne (Carr, 1996). The collection of the pampas deer [*Ozotoceros bezoarticus*] and marsh deer [*Blastocerus dichotomus*] samples, and the brocket deer or Mazama samples [*M. nana*, *M. americana*, *M. gouazoubira*, and *M. bororo*] were provided by the Brazilian

researchers: Prof. José Mauricio Barbanti Duarte, FCAV-UNESP Campus de Jaboticalbal and José Eduardo Garcia, IB/UNESP Campus de Botucatu, respectively. An additional sample of the pampas deer [*O. bezoarticus*] was obtained from the San Diego Zoo, courtesy of Leona Chemnick and Oliver Ryder (Carr, 1996). Joanne Smith-Flueck, National University of Comahue, Bariloche, Argentina provided the huemul [*Hippocamelus bisulcus*] tissue sample. The New World caribou [*Rangifer tarandus*] and moose [*Alces alces*] samples were compliments of the Provincial Department of Forest Resources and Agrifoods from the island of Newfoundland, Newfoundland and Labrador, Canada. The Old World reindeer [*Rangifer tarandus*] was provided by Magrath and Waterton Lakes National Park, Alberta, Canada (Greenslade, 1998). Samples of mule deer [*Odocoileus hemionus*] and black-tailed deer [*Odocoileus hemionus columbianus*] were provided by Steven Carr and are the same as those analyzed by Hughes and Carr (1993). White-tailed deer [*Odocoileus virginianus*] samples used in this study were provided by David M. Irwin and Steven Carr (Hughes and Carr, 1993).

David M. Irwin provided domestic cow [*Bos taurus*], pronghorn antelope [*Antilocapra americana californica*], giraffe [*Giraffa camelopardalis*], domestic pig [*Sus scrofa*], collared peccary [*Tayassu tajacu*], Grevy's zebra [*Equus grevyi*] and black rhinoceros [*Diceros bicornis*] tissue samples (Irwin *et al.*, 1991). David M. Irwin also supplied samples of llama [*Llama glama*] and hippopotamus [*Hippopotamus amphibius*] samples (Irwin *et al.*, 1991).

Hereinafter, the genus or subgenus names used above will be used for ease and specificity of reference, with no intention to prejudge the systematic conclusions.

DNA EXTRACTION

Samples obtained from Matt Cronin and David M. Irwin were provided in the form of extracted DNA. Samples analyzed by Carr and Hughes (1993), Carr (1996), and Greenslade (1998) were extracted from tissue samples (liver, heart, muscle or blood) by previous students in the Genetics, Evolution, and Molecular Systematics (GEMS) Laboratory, Department of Biology, Memorial University of Newfoundland, using the AGPC extraction procedure described below. Genomic DNA extracts were obtained from preserved muscle samples provided by José Mauricio Barbanti Duarte and José Eduardo Garcia using the AGPC method. Using this same protocol DNA was isolated from frozen muscle specimens obtained from New World *R. tarandus* and *A. alces*.

DNA was isolated from frozen, ethanol- or DMSO-preserved specimens by the acid guanidium thiocyanate-phenol-chloroform (AGPC) extraction procedure of Chomezynski and Sacchi (1987) as modified by Bartlett and Davidson (1991). The majority of the samples were extracted using this AGPC protocol. Approximately 100 – 200 mg of tissue was homogenized using a sterile plastic pestle in 450 µL of a guanidium extraction buffer (stored at 0°C) in 150 µL and 300 µL aliquots, respectively. This buffer solution contained 4 M guanidium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% Sarkosyl^{R}, and 0.1 M 2-mercaptoethanol. To the resulting homogenate, 50 µL of sodium acetate (2 M, pH 4.1) was added, followed by 300 µL of Tris-saturated phenol and 150 µL of chloroform/isoamyl alcohol (24:1, v/v). The solution was vortexed and incubated on ice or in the freezer for 15 minutes. During this step the DNA visibly precipitates. The sample was then centrifuged at 10,000 to 12,000 x g for 15 minutes at 4°C, after

which the top aqueous phase was transferred into a new microfuge tube, containing 450 μL of chloroform/isoamyl alcohol (24:1, v/v). The sample was then gently mixed by inverting the tube several times in succession. The sample was centrifuged at 10,000 x g for 15 minutes at 4°C, following which the upper aqueous phase was transferred to a new microfuge tube containing 400 μL of cold (0°C) isopropanol. The sample was then mixed by inverting the tube several times and incubated overnight at –20°C to precipitate the nucleic acids. The sample was then centrifuged at 10,000 to 12,000 x g for 15 minutes at 4°C. The supernatant was then removed and discarded, being careful not to disturb the resulting nucleic acid pellet in the tube. The pellet was then washed with ice-cold (-20°C) 70 – 75% ethanol and subsequently centrifuged at 10,000 to 12,000 x g for 15 minutes at 4°C. The supernatant ethanol was then removed, again being careful not to disturb the pellet. Lastly, the pellet was dried under vacuum and resuspended in 20 μL of sterile distilled water.

The *H. bisulcus* sample was provided in the form of dry tissue (skin and hair). DNA was extracted from this specimen using a commercial kit, the Qiagen QIAMP Tissue Kit™ (QIAGEN Inc., Chatsworth, CA), in accordance with manufacturer's instructions.

Extracted DNA was stored at –20°C. If severe evaporation occurred an additional 20 μL of sterile distilled water was added prior to depletion of the sample.

AMPLIFICATION OF DNA

PCR (Polymerase chain reaction) (Kessing *et al.*, 1989) was used to amplify 410-base pair sequences of the mitochondrial 12S ribosomal RNA gene. The primers used were 12Sb (5'– AGGGTGACGGGCGGTGTGT–3') (modified from L1091 in Kocher *et al.*, 1989) and 12Sa (5'– CAAACTGGGATTAGATACCCCACTAT-3') (modified from H1478 in Kocher *et al.*, 1989). Primers were synthesized by the Oligonucleotide Synthesis Laboratory, Queen's University, Kingston, ON.

Amplifications were performed in 100 μL reaction volume containing: 67 mM of Tris-HCL (pH 9.0 at 25°C), 2 mM $MgCl_2$, 10mM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO), 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Pharmacia), 10 pmol each of the oligonucleotide primer, 0.3 – 1 units of Amplitaq® DNA polymerase (Perkin-Elmer, Mississauga, Ontario) and 2 μL of isolated DNA. One drop of light white mineral oil was placed in each tube to prevent evaporation. Amplification was carried out in a Perkin Elmer Cetus DNA Thermal Cycler with an initial denaturation at 95°C for 5 minutes. This was followed by 40 cycles consisting of 93°C for 1 minute, 50°C for 1 minute, 55°C for 30 seconds, followed by 72°C for 3 minutes. A final elongation step of 72°C for 10 minutes was then performed.

Successful amplification was confirmed by the electrophoresis of 5 μL of the amplification product through a 2% agarose gel containing ethidium bromide (1 μg/mL). DNA was visualized by exposure to 312 nm ultraviolet light on a ultraviolet light transilluminator (Ultra-Violet Products Inc., San Gabriel, CA). To estimate the size of the product and ensure amplification of the appropriate fragment, a molecular weight

standard, *HaeIII* digest of Φ X phage DNA (Amersham Biosciences, Montreal, PQ) was also run on the gel.

PURIFICATION AND QUANTIFICATION OF PCR PRODUCT

To remove excess primer and other reactants prior to sequencing, amplification products were purified with the Wizard™ PCR Preps DNA Purification System (Promega Corp., Madison, WI), according to manufacturer's instructions. Concentration of the purified DNA products was then ascertained using a DNA Fluorometer, model TKO 100 (Hoefer Scientific Instruments, San Francisco, CA), and a 250 μg/mL calf thymus DNA (Clontech, BD Biosciences, Mississauga, ON) concentration standard as a reference.

DNA SEQUENCING

Sequencing of both strands of each fragment was carried out using the PE Applied Biosystems ABI Prism™ Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit following the manufacturer's instructions. Each DNA sample was resuspended in a mixture containing 12.8 μL of distilled sterile water, 8 μL of reaction premix (PE Applied Biosystems ABI Prism™ Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit), and 0.325 μL of 1 μM primer. Primers 12Sa and 12Sb were used in separate reactions and the amount of double-stranded DNA template added to each sequencing reaction varied between 200 and 500 ng depending on the length and purity of the PCR product. Sequencing reactions were carried out in a Perkin Elmer Cetus DNA

Thermal Cycler (TC-1) using 50 cycles of 96°C for 30 seconds, 50°C for 15 seconds and 60°C for 4 minutes.

Excess primers and unincorporated dye were removed from the samples by running the reaction through a Sephadex® G-50 (fine) spin purification column (Amersham Biosciences, Montreal, PQ). The eluted DNA was completely dried under vacuum, and then resuspended in 4 μL of a 5:1 mixture of deionized formamide and 50 mM disodium EDTA (Sigma Chemical Co., St. Louis, MO).

All samples were sequenced using the Applied Biosystems model 373A automated DNA sequencer (Applied Biosystems Inc., Foster City, CA), except for the *H. bisulcus* sample, which was sequenced using the long-read, 96-lane Applied Biosystems 377-XL instrument. Prior to being loaded on a 6% polyacrylamide denaturing gel samples were denatured by heating to 90°C for 3 minutes and then held at 5°C until loaded. Standard electrophoresis conditions were used (Hillis *et al.*, 1996).

DATA ANALYSIS

DNA sequence data were collected using the ABI collection analysis software package. Sequences were edited with the SeqEd™ 675 DNA Sequence Editor (Applied Biosystems Inc., Foster City, CA) and the Eyeball Sequence Editor (ESEE) program (Cabot and Beckenbach, 1989). Maximum-likelihood (ML), neighbour-joining (NJ), and maximum-parsimony (MP) analyses were performed with the Phylogenetic Analysis Using Parsimony (PAUP) program (version 4, release d63) (Swofford, 1998). To complement the 12S rRNA data analysis, a 401-bp segment of the cytochrome *b* gene

sequenced (Carr, 1996) was added to the 12S rRNA data to produce a combined data set of 811-bp. Cytochrome *b* sequences were available for all 30 species of cervids in the 12S rRNA database. For all three methods of phylogenetic analysis, the cervines (*M. muntjac*, *A. axis*, *C. duvauceli*, *E. davidianus*, *C. elaphus canadensis* and *C. (Rusa) unicolor)* were used as the outgroup.

ML analyses (Felsenstein, 1981) were completed with estimates of the transition (Ts) to transversion (Tv) ratio as 7.4 and the gamma parameter (γ) as 0.30, a heuristic search with ten random taxon additions, and the nearest-neighbour branch-swapping option. Bootstrap analyses (Felsenstein, 1985) were performed by a heuristic search with a taxon addition order determined by NJ and a heuristic search with a single nearest-neighbour-interchange branch swapping for each of the 1000 replicates. NJ analysis (Saitou and Nei, 1987) was done on ML and Tamura-Nei distances (Tamura and Nei, 1993) using the same Ts/Tv ratio and γ as indicated above, and bootstrap analysis performed with 1000 replicates. MP trees were obtained using the heuristic search algorithm, with 10 random taxon additions and the tree-bisection and reconnection branch-swapping option. Ts/Tv ratios of 3:1, 10:1, and transversions only were used for the combined data sets. Bootstrap analysis of parsimony trees were completed with 1000 replicates using 10 random taxon additions and the nearest-neighbour interchange branch-swapping option.

RESULTS

DNA sequences for twenty-one species (thirty-one individuals) of cervids, seven Artiodactyls, and two Perissodactyls in a 410-bp region of the 12S rRNA gene are given in Appendix I (Figure 9). Due to the only one nucleotide variant between the two *Axis* sequences and the lack of a cytochrome *b* 401-bp complement for this individual, the *A. axis* D33 sequence was subsequently removed from phylogenetic analyses, which were thus done on 30 individuals. For all three methods of phylogenetic analyses, the cervines (*M. muntjac*, *A. axis*, *C. duvauceli*, *E. davidianus*, *C. elaphus canadensis* and *C. (Rusa) unicolor)* were used as the outgroup for the rest of the taxa. Preliminary results (not shown) indicated that the cervines were the appropriate outgroup.

Among the 811-bp of sequence data available, 365 nucleotide positions were variable, 296 of which were phylogenetically informative (Nei, 1987). Neighbour-Joining analysis from Maximum Likelihood distances (ML) (Figure 4), Neighbour-Joining (NJ) analysis from Tamura-Nei distances (Figure 5), and Maximum Parsimony (MP) (Figure 6) methods all produced trees with similar topographies, although some distinct differences could be noted.

With regards to the cervines, *C. capreolus* and *H. inermis* were consistently sister taxa, in at least 80% (MP) of the bootstrap replicates. *A. alces* repeatedly came out as a sister to these taxa, but with poor bootstrap support. In all three analyses, *E. davidianus*, *A. axis,* and all species of *Cervus* cluster together, with *M. muntjac* appearing as a sister

Figure 4. Phylogenetic tree produced by Neighbour-Joining analysis from Maximum Likelihood distances for 811-bp of mtDNA (401-bp of the cytochrome *b* and 410-bp of the 12S rRNA genes) from 21 species of cervids. The numbers above each branch indicates the percent occurrence of that branch among 1000 bootstrap replicates. Ts/Tv = 7.4 and gamma parameter = 0.30.

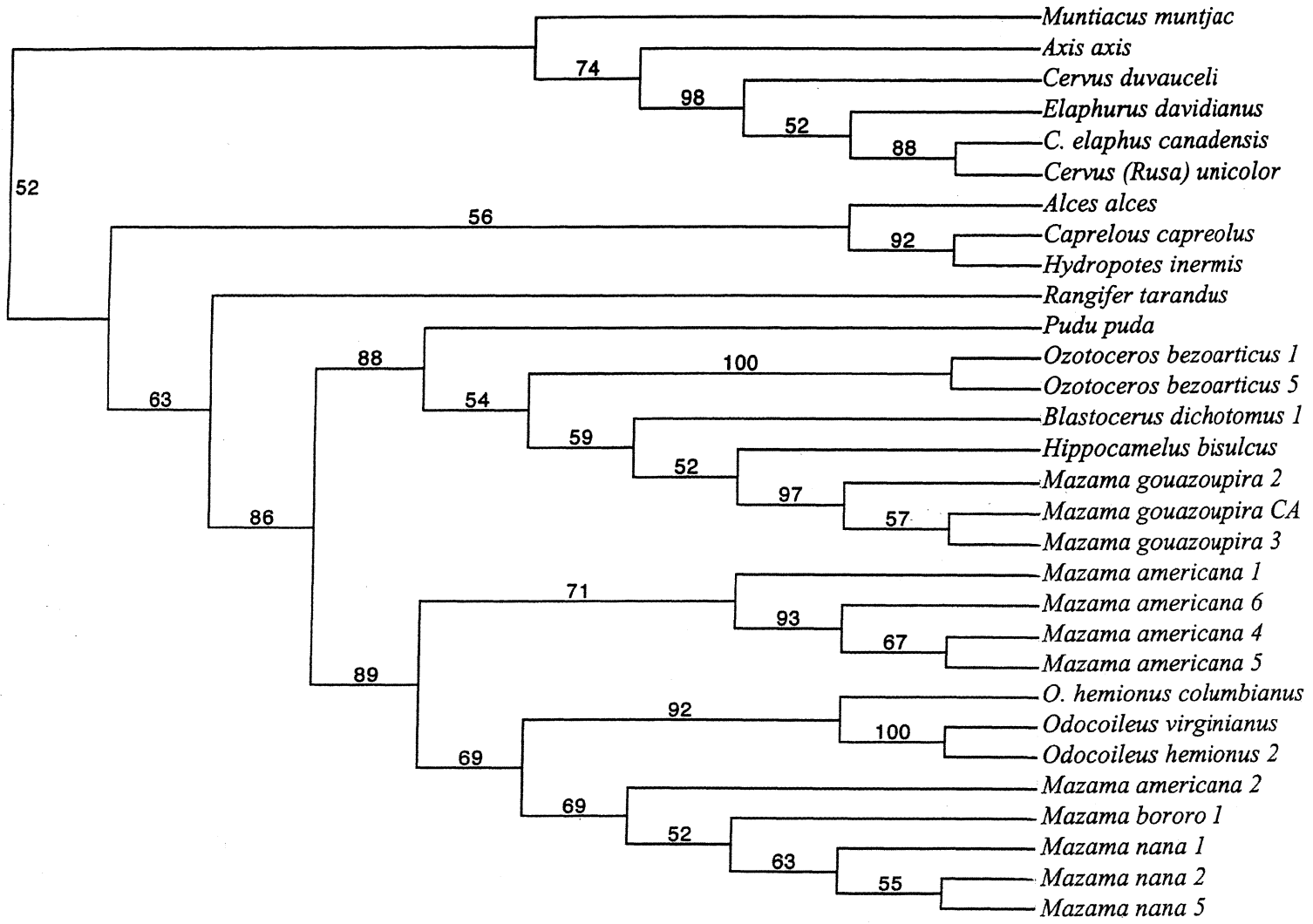

Muntiacus muntjac
Axis axis
Cervus duvauceli
Elaphurus davidianus
C. elaphus canadensis
Cervus (Rusa) unicolor
Alces alces
Caprelous capreolus
Hydropotes inermis
Rangifer tarandus
Pudu puda
Ozotoceros bezoarticus 1
Ozotoceros bezoarticus 5
Blastocerus dichotomus 1
Hippocamelus bisulcus
Mazama gouazoupira 2
Mazama gouazoupira CA
Mazama gouazoupira 3
Mazama americana 1
Mazama americana 6
Mazama americana 4
Mazama americana 5
O. hemionus columbianus
Odocoileus virginianus
Odocoileus hemionus 2
Mazama americana 2
Mazama bororo 1
Mazama nana 1
Mazama nana 2
Mazama nana 5
74
98
52
88
52
56
92
63
88
100
54
59
52
97
57
86
71
93
67
89
92
100
69
69
52
63
55

Figure 5. Phylogenetic tree produced by Neighbour-Joining analysis from Tamura-Nei distances for 811-bp of mtDNA (401-bp of the cytochrome *b* and 410-bp of the 12S rRNA genes) from 21 species of cervids. The numbers above each branch indicates the percent occurrence of that branch among 1000 bootstrap replicates. Ts/Tv = 7.4 and gamma parameter = 0.30.

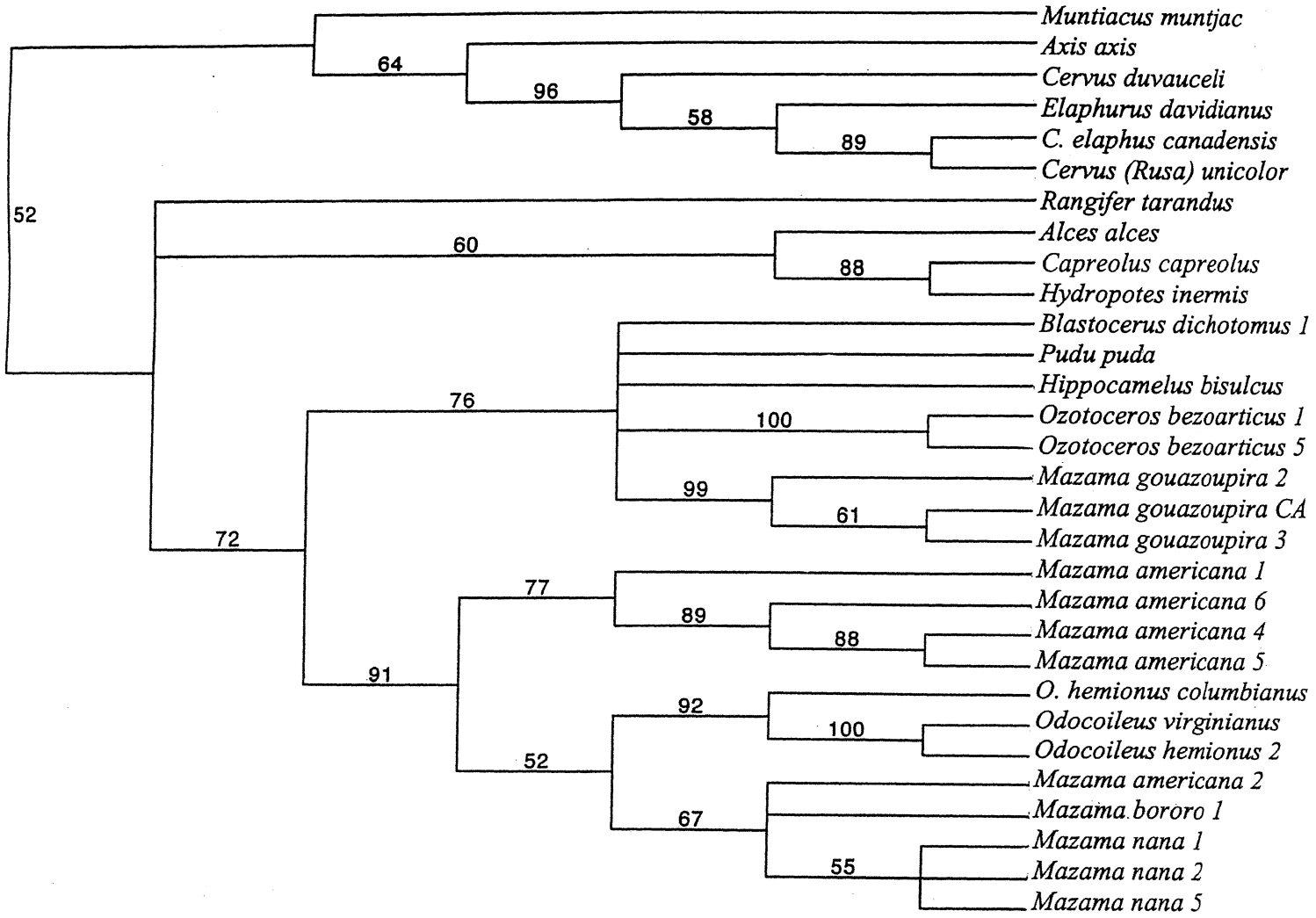
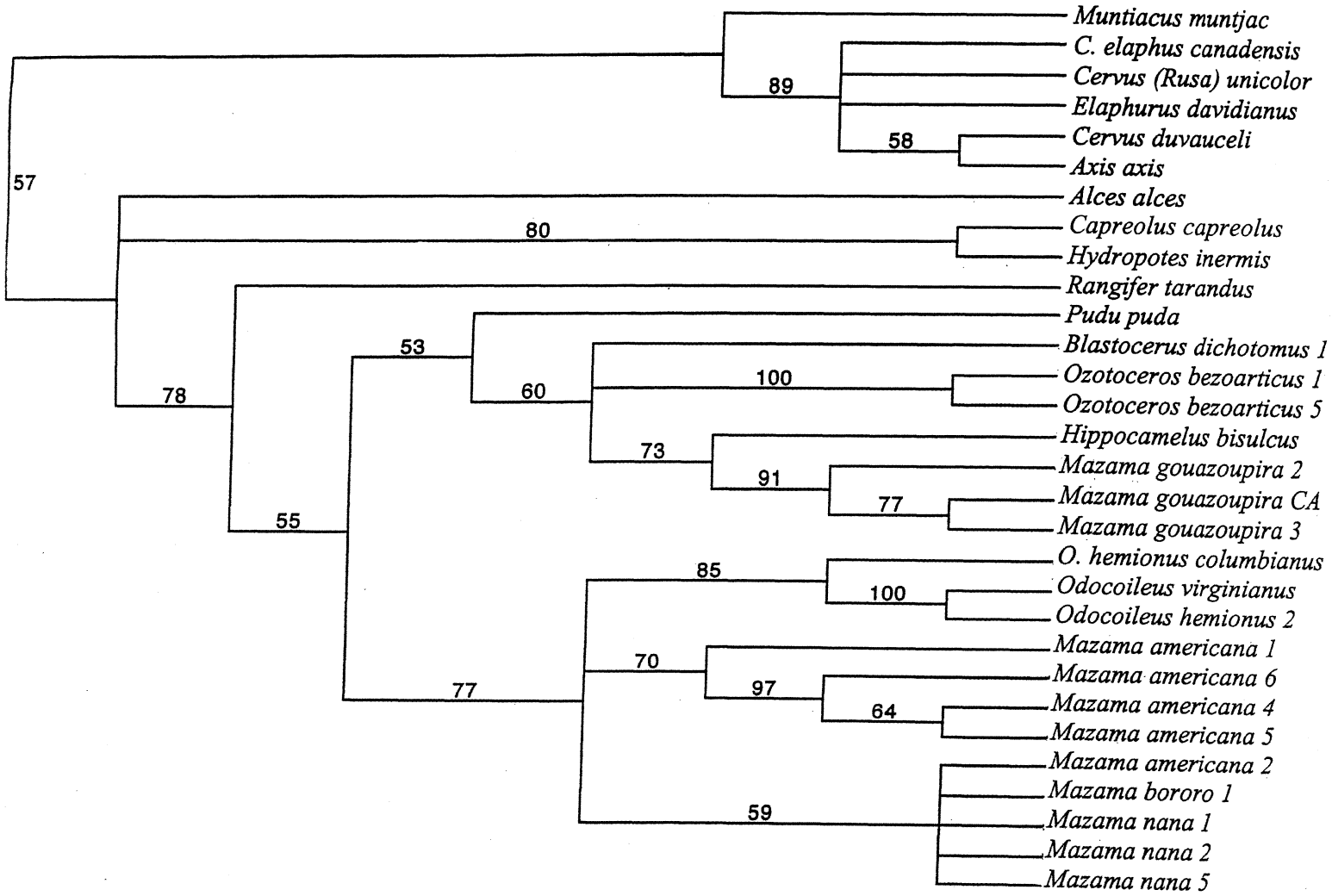

Muntiacus muntjac
Axis axis
Cervus duvauceli
Elaphurus davidianus
C. elaphus canadensis
Cervus (Rusa) unicolor
Rangifer tarandus
Alces alces
Capreolus capreolus
Hydropotes inermis
Blastocerus dichotomus 1
Pudu puda
Hippocamelus bisulcus
Ozotoceros bezoarticus 1
Ozotoceros bezoarticus 5
Mazama gouazoupira 2
Mazama gouazoupira CA
Mazama gouazoupira 3
Mazama americana 1
Mazama americana 6
Mazama americana 4
Mazama americana 5
O. hemionus columbianus
Odocoileus virginianus
Odocoileus hemionus 2
Mazama americana 2
Mazama bororo 1
Mazama nana 1
Mazama nana 2
Mazama nana 5
64
96
58
89
52
60
88
76
100
99
61
72
77
89
88
91
92
100
52
67
55

Figure 6. Phylogenetic tree produced by Maximum Parsimony analysis for 811-bp of mtDNA (401-bp of the cytochrome *b* and 410-bp of the 12S rRNA genes) from 21 species of cervids. The numbers above each branch indicates the percent occurrence of that branch among 1000 bootstrap replicates. Ts/Tv = 7.4 and gamma parameter = 0.30.


Muntiacus muntjac
C. elaphus canadensis
Cervus (Rusa) unicolor
Elaphurus davidianus
Cervus duvauceli
Axis axis
Alces alces
Capreolus capreolus
Hydropotes inermis
Rangifer tarandus
Pudu puda
Blastocerus dichotomus 1
Ozotoceros bezoarticus 1
Ozotoceros bezoarticus 5
Hippocamelus bisulcus
Mazama gouazoupira 2
Mazama gouazoupira CA
Mazama gouazoupira 3
O. hemionus columbianus
Odocoileus virginianus
Odocoileus hemionus 2
Mazama americana 1
Mazama americana 6
Mazama americana 4
Mazama americana 5
Mazama americana 2
Mazama bororo 1
Mazama nana 1
Mazama nana 2
Mazama nana 5
89
58
57
80
78
53
60
100
73
91
77
55
85
100
70
97
64
77
59

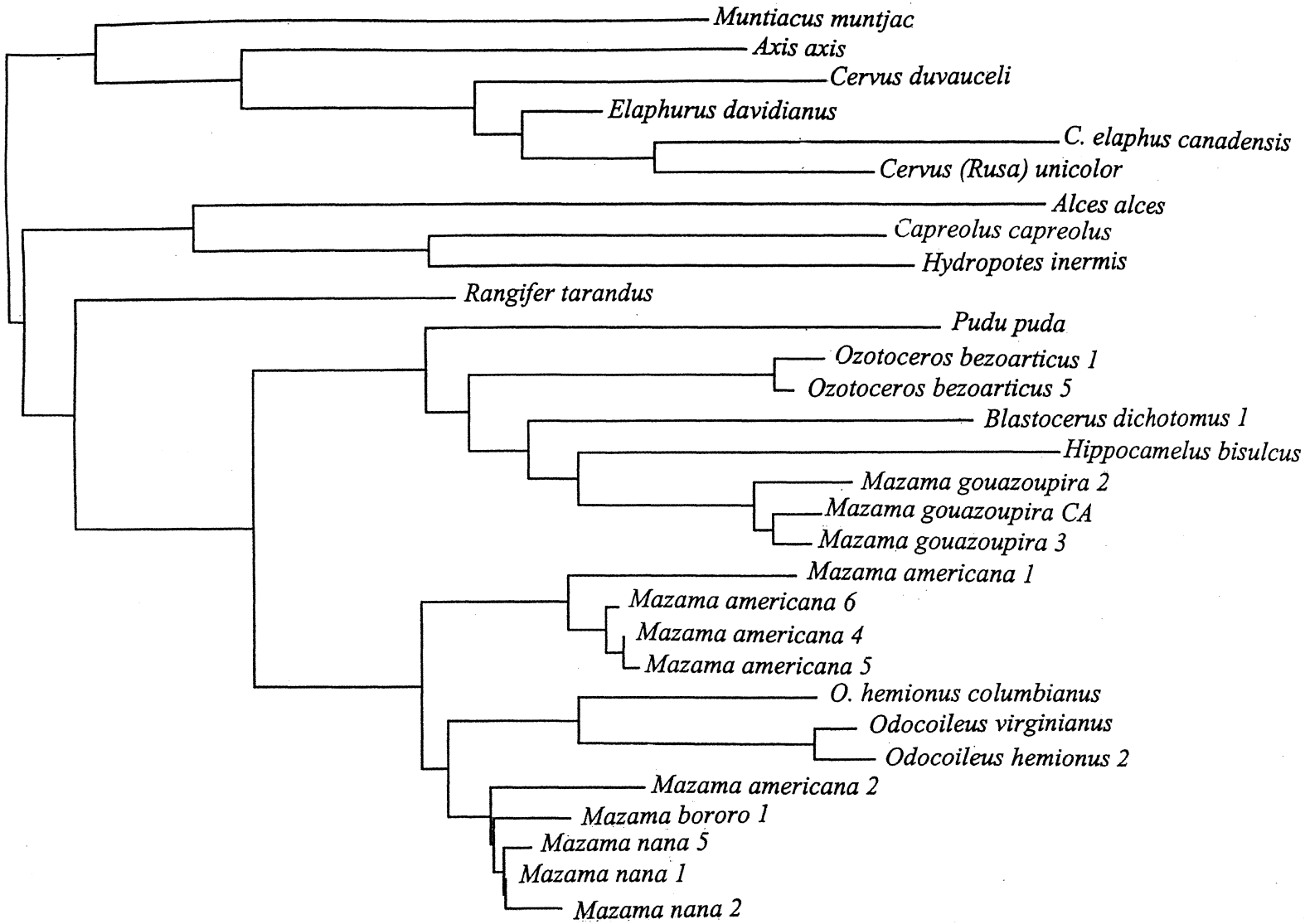
to this group. As *A. alces* falls outside this clade, its exact relationship was indeterminate. *R. tarandus* often occurred as the sister taxa to the remaining odocoileines.

In all three analyses, *H. bisulcus*, *B. dichotomus*, *P. puda*, and *O. bezoarticus* clustered together; with *M. gouazoupira* being the sister group to these other four genera (NJ), with *M. gouazoupira* and *H. bisulcus* being the sister group to the latter three genera (MP), and with *P. puda* being the sister taxa to these other three genera along with *M. gouazoupira* (ML). In all three analyses, the *Mazama* species (*M. gouazoupira*, *M. americana*, *M. bororo*, and *M. nana*) were identified in at least three different clades. ML and NJ identified *M. americana* in two different clusters as one of the *M. americana* individuals were found grouped with *M. Bororo* and *M. nana* in 69% and 67% of the bootstrap replicates, respectively. The three *Odocoileus* species were consistently the sister group to *Mazama* (including *M. nana*, *M. bororo*, and at least one *M. americana* individual). The bootstrap support for this arrangement was 52% (NJ), 69% (ML) and 77% (MP); the higher percentage being representative of the configuration where all *Mazama* taxa (excluding *M. gouazoupira*) cluster together. However, even in the MP analysis, *M. americana* was split among two sister taxa. In this study, the influence of intraspecific variation within this genus (Cronin, 1991; Cronin, 1992; Cronin 1993) was considered by sequencing several different specimens of *Mazama*.

Separate analyses of the 12S rRNA sequences identified the same groups described above; however, bootstrap supports were considerably weaker than those in the combined analysis causing some of the deeper branches to collapse (results not shown). A hypothetical reconstruction of the origins and distributions of the 21 species of cervids

was produced, based on the MP analysis of the combined cytochrome *b* and 12S rRNA data sets (Figure 7).

Figure 7. Hypothetical reconstruction of the biogeographic origins and distributions of 21 species of cervids as inferred from mtDNA sequence data. The cladogram is based on a MP analysis for 811-bp of mtDNA (401-bp of the cytochrome *b* and 410-bp of the 12S rRNA genes).

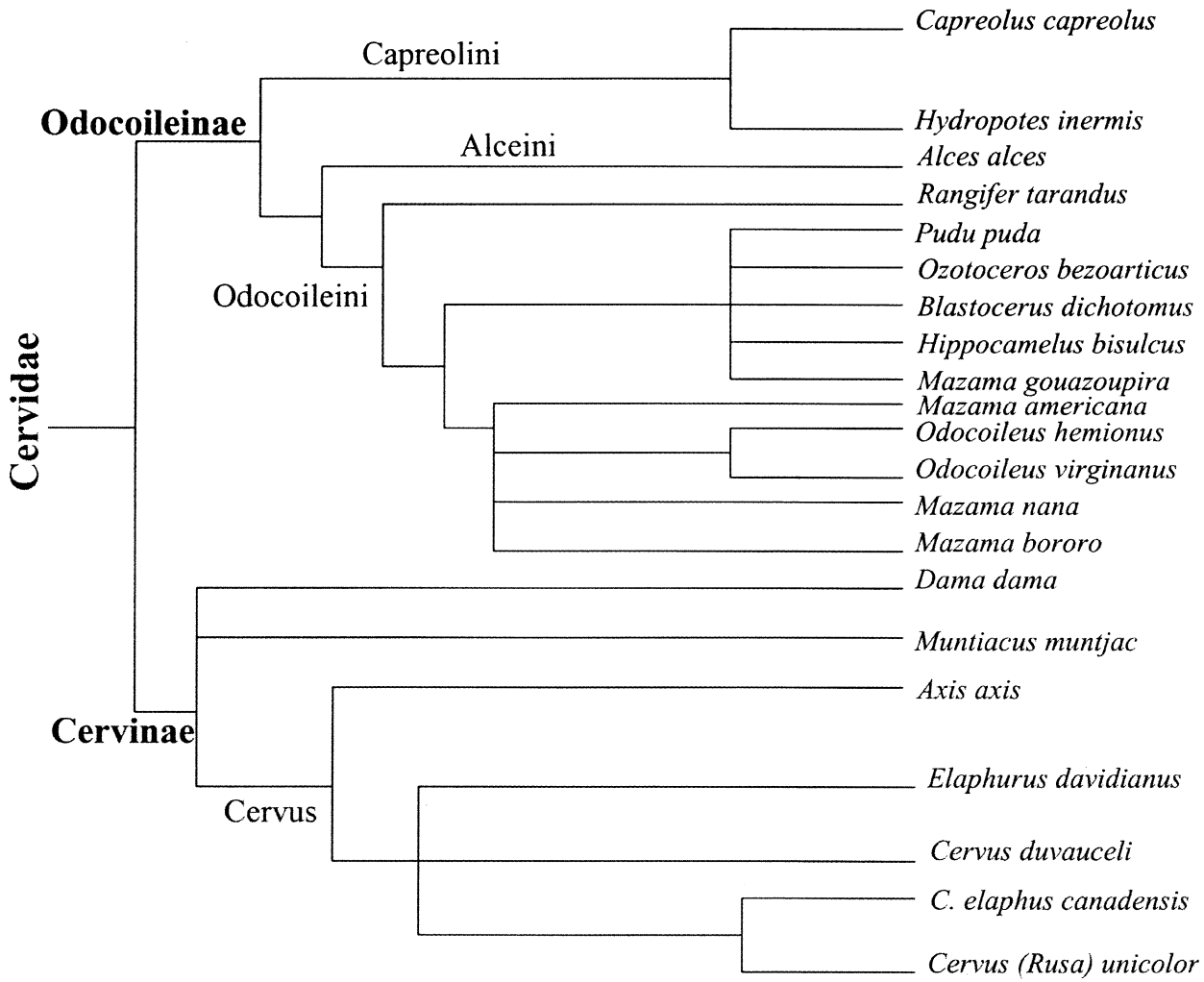

Muntiacus muntjac
Axis axis
Cervus duvauceli
Elaphurus davidianus
C. elaphus canadensis
Cervus (Rusa) unicolor
Alces alces
Capreolus capreolus
Hydropotes inermis
Rangifer tarandus
Pudu puda
Ozotoceros bezoarticus 1
Ozotoceros bezoarticus 5
Blastocerus dichotomus 1
Hippocamelus bisulcus
Mazama gouazoupira 2
Mazama gouazoupira CA
Mazama gouazoupira 3
Mazama americana 1
Mazama americana 6
Mazama americana 4
Mazama americana 5
O. hemionus columbianus
Odocoileus virginianus
Odocoileus hemionus 2
Mazama americana 2
Mazama bororo 1
Mazama nana 5
Mazama nana 1
Mazama nana 2

DISCUSSION

Phylogenetic studies of mitochondrial DNA (mtDNA) gene sequences are yielding an enhanced resolution of evolutionary relationships and molecular evolution within the family Cervidae. These investigations of mtDNA, when combined with morphological, cytogenetic, and other information have considerably clarified systematic relationships and evolutionary patterns within and among deer species. The molecular data obtained in this thesis are compared with previous morphological and other relevant data because all views are informative and should not be considered in isolation of one another (Hillis *et al.*, 1996). The results obtained in this thesis support the effectiveness of molecular systematics and population genetics in studying the evolutionary relationships of artiodactyls.

The conventional division of Cervidae into four subfamilies (Muntiacinae, Hydropotinae, Cervinae, and Odocoileinae), or more contemporary division into three subfamilies (where in the muntjacs are combined with the odocoileines), is not supported by the phylogenetic relationships among cervid taxa as indicated by cytochrome *b* sequences analyzed by Car (1996) or by the results of this study when combining rRNA and cytochrome *b* sequences (Figure 7). Instead, two monophyletic clades or subfamilies are recognized (Figure 8). Under this new phylogeny, *Hydropotes* is no longer identified as a cladistically distinct lineage, but as a sister species of *Capreolus*. This resolution corresponds to the classical division of cervids into two groups based on alternative conditions of the metacarpals of the lateral digits: namely, plesiometacarpalian and

Figure 8: A hypothesis of the relationships of subfamilies within Cervidae. This consensus phylogeny is based on a collection of information including: geographical, morphological, cytogenetic, and molecular evidence (Hershkovitz, 1982; Eisenberg, 1987; Groves and Grubb, 1987; Scott and Janis, 1987; Neitzel, 1987; Gavin and May, 1988; Bubenik, 1990; Cronin, 1991; Miyamoto *et al.*, 1993; Whitehead, 1993; Duarte and Giannoni, 1995a and 1995b; Carr, 1996; McKenna and Bell, 1997; Díax and Smith-Flueck, 2000).


Cervidae
Odocoileinae
Capreolini
Alceini
Odocoileini
Cervinae
Cervus
Capreolus capreolus
Hydropotes inermis
Alces alces
Rangifer tarandus
Pudu puda
Ozotoceros bezoarticus
Blastocerus dichotomus
Hippocamelus bisulcus
Mazama gouazoupira
Mazama americana
Odocoileus hemionus
Odocoileus virginanus
Mazama nana
Mazama bororo
Dama dama
Muntiacus muntjac
Axis axis
Elaphurus davidianus
Cervus duvauceli
C. elaphus canadensis
Cervus (Rusa) unicolor

telemetacarpalian. The plesiometacarpalian state or a reduction of the distal portions of the metacarpals in the lateral digits is characteristic of the cervines (subfamily Cervinae); whereas, the telemetacarpalian state exhibiting a reduction in the proximal portions, is characteristic of the odocoileines and *Hydropotes* (subfamily Odocoileinae). Specifically, within Cervinae there are three sister taxa identified: *Cervus* (*Cervus*, *Elaphurus,* and *Axis*), *Dama*, and *Muntiacus*; whereas, Odocoileinae can be divided into three monophyletic tribes including Caproeolini (*Capreolus* and *Hydropotes*), Alcini, and Odocoileini (endemic New World deer and holarctic *Rangifer*) (Figure 8). With the exclusion of Hydropotinae, which is the sister group of the antlered deer, this topology is supported by Groves and Grubb (1987). *Rangifer* is included in the Odocoileini, which is consistent with karyotypic data and the unique condition of the vomerine septum. Carr and Hughes (1993) report that New and Old *Rangifer* are no more distinct than conspecific *Odocoileus* species. *Alces* also sometimes occur as sister to the Odcoileini. However, it should be acknowledged that neither the independent cytochrome *b* nor the combined rRNA and cytochrome *b* analyses unambiguously resolved the relationships among the three Odocoileine clades. In particular, relationships among *Alces* and *Rangifer* and the remaining Odocoileine genera were not resolved. Also, while most of the cervine genera including *Muntiacus*, *Cervus*, *Elaphurus*, and *Axis* constituted a monophyletic clade, the latter three genera formed a subclade (Figure 7). Most analyses placed *Muntiacus* as the outgroup to the other cervines (Figures 4 - 7). Nonetheless, it can be suggested that the alternative conditions of the contemporary foot structure, may

have originated independently from an ancestral holometacarpalian state, in which the metacarpals were complete (e.g. *Cerocervus*) (Groves and Grubb, 1987).

The endemic South American cervid genera, *Pudu*, *Blastocerus*, *Ozotoceros*, *Hippocamelus*, and *Mazama* were cladistically distinct from the genus *Odocoileus* in all analyses (Figures 4 - 7). Thus, contrary to common suggestion (Haltenorth, 1963, cited in Wilson and Reeder, 1993), these South American genera cannot be considered as species monophyletically derived from the genus *Odocoileus*; but, alternatively having at least a paraphyletic, if not a polyphyletic origin. Recent studies of mtDNA sequences of South American species by Carr (1996) indicated that deer have invaded Latin American at least twice. Carr (1996) suggests that Central American *Mazama* are more closely related to North American *Odocoileus* species, whereas endemic South American genera (*Pudu*, and *Ozotoceros*) represents a separate, more ancient clade. i.e. these data indicate that Neotropical deer taxa have a paraphyletic origin. The present study was expanded to also investigate the neotropical *Hippocamelus, Blastocerus*, and three additional species of *Mazama*. Only *M. americana* was investigated by Carr (1996). A paraphyletic origin for South American cervids was also obtained using this expanded data set (Figures 4 - 8). *Mazama* genera including *M. bororo*, *M. nana*, and *M. americana* were more closely related to *Odocoileus* species; whereas, *B. dichotomus*, *O. bezoarticus*, *H. bisulcus*, and *M. gouazoupira* represented a separate more ancient clade. All analyses placed the four *Mazama* species (12 individuals) in at least three different clades and the *M. americana* individuals were often split among two clusters, suggesting a large degree of genetic variability among this genus and species, respectively.

It is important to note that while the 12S rRNA data on their own did not give strong bootstrap support, it was nonetheless found to be useful in resolving lineages when combined into a larger data set. By using the combined data set to produce longer sequences (the 12S rRNA data complemented by the cytochrome b data) the resolution of the deeper branches, such as those between the plesiometacarpalian and telemetacarpalian taxa were convincingly resolved. Although, Carr (1996) obtained good resolution of the relationships between closely related genera, his investigation was unable to adequately resolve the deeper branches. Miyomoto *et al.* (1990) similarly sequenced the 12S and 16S rRNA genes from several cervids and combined the data sets to produce a finer resolution to ascertain a number of phylogenetic questions.

Inter- and intraspecific phylogenetic relationships among some of the clades described above may be explained by a review of available cytogenetic evidence (Groves and Grubb, 1987; Neitzel, 1987). The family Cervidae has a very high degree of karyotypic variability. Within the Cervinae lineage as described in Figure 8 (excluding *Muntiacus*), only Robertsonian translocations contributes to differentiation of the karyotypes; however, chromosomal divergence is extreme within the genus *Muntiacus*. Notably, in the combined 12S rRNA and cytochrome *b* phylogeny, *M. muntijac* comes out as the sister taxa to the other cervines (Figure 7). Karyotyping also indicated a close taxonomic relationship between *Cervus* species and *E. davidianus* (Neitzel, 1987), irrespective of their phenotypes. In comparison, this study often showed *E. davidianus* as the sister taxa to *Cervus* (Figures 4, 5, and 7). In the lineage of the subfamily Odocoilinae, the karyotypes of *Hydropotes* and *Capreolus* are similar, except that the X

chromosome is metacentric in the latter. The mtDNA data always revealed *H. inermis and C. capreolus* as sister taxa with good bootstrap support (Figures 4 - 6). The karyotypes of the remaining odocoileines, including *Alces* and *Rangifer,* differ by the addition of a pair of metacentric autosomes and metacentric X chromosomes. In particular, Neitzel (1987) reports the retention of the ancestral karyotype in *M. gouazoubira*, but that considerable differences are evident in other *Mazama* species, especially in *M. americana*. This lends support to the frequent positioning of *M. americana* in two different clades (Figures 4 - 6) and *M. gouazoubira* phylogenetic positioning in the same clade as the other endemic South American genera (*Pudu*, *Ozoterceros*, *Blastocerus*, and *Hippocamelus*) than to the other *Mazama* species. There is also evidence that *Mazama* and *Muntiacus* form small breeding groups as part of their social behavior (Neitzel, 1987; Nowak, 1991). It is suggested that this reproductive behavior may possibly act as an isolating mechanism, which may promote not only karyotypic diversity, but also genetic and anatomical differences among species. This may explain why *M. americana* species in the analyses were often split among two clusters, as well as, the appearance of *Mazama* in at least four different clades. Hall (1981) also describes four distinct subspecies of *M. americana,* including: *M. americana cerasina*, *M. americana pandora*, *M. americana reperticia*, and *M. americana temama.* Cytogenetic work by Duarte and Giannoni (1995a and 1995b) documented unsuspected karyotypic diversity among South American deer, which indicates that there are many more sub-species of South American deer than previously suspected. Neitzel (1987) did

not accept the genera *Blastocerus* and *Ozotoceros*, but instead they were included by that author in the genus *Odocoileus*.

A review of cervid antler morphology also aids in an understanding of the phylogeny derived from the combined cytochrome *b* and rRNA data sets. The present analysis indicates that antlerless *Hydropotes* is not the plesiomorphic ancestral condition but rather that antlers have evolved only once. In this way, *Hydropotes* shows a secondary loss of these bony outgrowths, and its characteristic enlarged canines (also seen in *Muntiacus*) may have developed evolutionarily, as antlers became less important as a competitive defense mechanism. Carr (1996) reasons that the enlarged canines of *Hydropotes* and *Muntiacus* are more likely to be "atavistic convergences" on the ancestral condition, rather than the antlers of *Capreolus* not being homologous to the other cervids. This explanation is supported with the present combined phylogeny also showing *Hydropotes* and *Capreolus* as sister taxa (Figures 7 and 8). Morphologically, these sister taxa are also distinctively lacking tails, a structural characteristic that is unique to this lineage as all remaining cervids and cervid-like artiodactyls possess some form of a tail (Eisenberg, 1987).

The present phylogeny of the odocoileines indicates at least two invasions of South America by North American deer (Figure 7). Endemic South American genera (including *Ozotoceros*, *Pudu*, *Blastocerus*, *Hippocamelus,* and *M. gouazoupira*) entered the neotropics following the completion of the land bridge between North America and South America (3 MYBP) (Marshall *et al.*, 1982). A second invasion occurred which included the *Mazama* species: *M. nana*, *M. bororo* and *M. americana*. Eisenberg (1987)

and Carr (1996) further suggest a probable third invasion of neotropical *O. virginianus*. As odocoileines expanded into the southern continent, concurrent was a reduction in body size and the complexity of the antler. This is exhibited by the large body size and palmate antler patters of genera like *Alces* and *Rangifer* versus the smaller body forms and single-tined or spike antlers characteristic of *Mazama* and *Pudu* (Figure 3b). Eisenberg (1987) suggests that spike antlers may not indicate the carrying forward of a conservative character, but that these spikes may be the result of reduced selection for larger antlers due to a decrease in body size. The evolution of smaller body size and antler reduction as a result of adaptation can also be applied to *H. bisulcus*. In contrast, Carr (1996) also explains in length the alternative argument that the small-bodied *Mazama* or *Pudu* is ancestral to the Nearctic cervids; however, he dismisses this northward migration hypothesis based on karyotypic, geographic, and morphologic reasons previously discussed. Yet, it is interesting to note that fossils referred to as *Blastocerus* have been found in the southern USA (Simpson, 1928, in Kurten and Anderson, 1980). Likewise, *Navahoceros* and *Sangamona*, two extinct genera from North America, are suggested by Hershkovitz (1982) to be possible immigrants from the southern continent.

Antler evolution in the Old World Cervinae being the ancestral condition (Geist, 1971; Eisenberg, 1987, Carr 1996), are indicative of the two or three tined antlers seen in *Munitacus*, *Axis*, and *Elaphurus*. This primitive condition has been modified to multi-tined patterns in New World genera such as *Odocoileus*. The more complex multi-tined antler of *Cervus* may have arisen in parallel (Carr, 1996). The trend then as summarized

by Carr (1996) was historically towards more complicated antler patterns as seen in the Old World genera, then movement towards antler simplification and size reduction in the New Word genera (e.g. odocoileines) and concurrent simplification and later increasing tine numbers in antler development among the cervines.

In addition to Carr (1996), previous molecular studies to some degree also support the revised cervid phylogeny presented here. Cronin (1991) investigated twelve cervids based on restriction endonuclease site maps of mtDNA. Although, Cronin's cladistic analysis resulted in somewhat different phylogenetic relationships within each group than those that are presented here, in contrast to my study, Cronin found the odocoileines and cervines to be monophyletic. Similarly to my study, *Mazama* occurred with *Odocoileus*, but in contrast, *Capreolus* was more closely related to this latter genus than *Rangifer* and *Alces*. Cronin did propose that the phenetic analyses may better suggest phylogenetic relationships among taxa. Also, it is important to note that *Hydropotes* was not included in this study and therefore one could expect different topologies.

Irwin *et al.* (1991) investigated several species of artiodactyls with 1140-bp mitochondrial cytochrome *b* sequences. *O. h. columbianus* and *Dama dama* were the only deer studied, and these cervids did not occur as sister taxa, as would be expected under a monophyletic cervid origin (Figure 8). Carr (1996) however, suggests that Irwin *et al.*'s (1991) suggestion that cervids are non-monophyletic was a result of "artifacts" due to the absence of close relatives in the analyses so that parsimony did not reflect accurate phylogenetic relationships. A reconstruction of the data set of Irwin *et al.* (1991) by Carr (1996) using more than two cervid taxa, showed a monophyletic phylogeny. Greenslade

(1998), using 401-bp cytochrome *b* sequences, identified four mtDNA assemblages among populations of *Odocoileus* in western North America, with *O. h. columbianus* representing the ancestral lineage. In the present study, the mule deer and white-tailed deer occurred as the sister to the black-tailed deer (Figure 7).

An investigation by Miyamoto *et al.* (1993), studying 2.7 kbp of mitochondrial rRNA and tRNA gene sequences suggested that *C. (Rusa) timorensis* and *M. muntiacus* were sister taxa, in comparsion to *H. inermis* and *O. virginianus* which were also sequenced. However, without the inclusion of other cervine genera it is not possible to ascertain if these two cervines are truly monophyletic subfamilies. Emerson and Tate (1993) studied *Axis*, *Elaphurus*, *Dama* and four species of *Cervus* using protein electophoresis. Emerson and Tate (1993) found *C. (Rusa) timorensis* was not grouped with the other *Cervus* species; but, instead was clustered with *Axis* and *Dama*. A re-analysis of this data set in Carr (1996) using *Dama* as the outgroup to the other cervines resulted in *Cervus* displaying a monophyletic topology. This revised analysis is more consistent with the *Cervus* phylogeny presented here.

The molecular data presented in this thesis suggests an alternative biogeographic hypothesis to Webb (2000). Notably, while Webb's work investigates a broad range of morphological comparisons, it was not quantitative and the author explained it was only a "preliminary phylogenetic hypothesis" of Odocoileinae relationships. Webb's arrangement of the telemetacarpalian deer into two tribes, with the Odocoileini (*Mazama*, *Ozoterceros*, *Blastocerus*, and *Odocoileus*), and the Rangiferini (*Rangifer*, *Hippocamelus*, and *Pudu*), suggests a polyphyletic origin of the endemic South American

genera (Figure 1). Instead the implication from the current data set is that endemic South American genera (*Ozoterceros*, *Blastocerus*, *Hippocamelus*, and *Pudu)* as well as one South American species of *Mazama* (*M. gouazoupira*) are a monophyletic lineage, whereas *Odocoileus* is more closely related to the remaining species of Central and South American *Mazama* (*M. americana*, and *M. nana*, and *M. bororo*) (Figure 8). The shared derived character sets used by Webb to distinguish between the two tribal diverging nodes on the cladogram for Rangiferini and Odocoileini include antler morphology and the loss of upper canines, respectively. Attempts to define genera by morphology have been complicated by various factors such as intraspecific variation of antler morphology (Smith *et al.*, 1983; Ullrey, 1983; Scott and Janis, 1987; Cronin, 1993), and the presence or absence of upper canines in *Mazama* (Nowak, 1991). Thus, while morphological characteristics maybe useful in determining phylogenetic relationships, resulting preliminary phylogenies may not be entirely accurate if all the available evidence is not taken into consideration.

In conclusion, while the present 12S rRNA study substantially aided the subfamilial resolution of the family Cervidae by complementing the existing cytochrome *b* data set, several outstanding questions at different taxonomic levels still remain. While lending support to several of the relationships within the Cervidae, this study has also raised many questions regarding the current classification and systematics of this family. The evolutionary relationships of South American cervids was augmented with the addition of the expanded taxa list to included *H. bisulcus, B. dichotomus* and several species of *Mazama* (*M. nana*, *M. bororo*, and *M. gouazoupira*), supporting a dual

paraphyletic origin of the neotropical genera. However, if researchers are to arrive at a consensus as to the appropriate taxonomy and systematics of this family, further examination of the relationships among and within the cervids is required.

In particular, the phylogenetic analyses presented in this thesis are based on a single maternally-inherited molecule, whereas the nuclear gene products are biparentally inherited. In order to determine accurately the evolutionary history of cervids (including the South American taxa), sufficient allelic DNA sequences at several nuclear loci are required to generate a gene tree that would be independent of the mtDNA phylogeny. To date, such an extensive nuclear study has not been conducted.

REFERENCES

Ballinger, S. W., Blankenship, L. H., Bickham, J. W., and Carr, S. M. 1992. Allozyme and mitochondrial DNA analysis of a hybrid zone between white-tailed deer and mule deer (*Odocoileus*) in west Texas. Biochemical Genetics **30**: 1-11.

Bartlett, S. E., and Davidson, W. S. 1991. Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome *b* genes. Can. J. Fish. Aquat. Sci. **48**: 309-317.

Brook, V. 1878. On the classification of the Cervidae with a synopsis of the existing species. Proceedings of the Zoological Society of London **1878**: 883-928.

Bubenik, A. B. 1983. Taxonomy of Pecora in relation to morphophysiology of their cranial appendages. In: Antler Development. R. D. Brown (Ed.). Caesar Kleberg Wildlife Research Institute. Texas. pp. 163-185.

Bubenik, A. 1990. Epigenetical, morphological, physiological, and behavioral aspects of evolution of horns, pronghorns, and antlers. In: Horns, Pronghorns, and Antlers. A. Bubenik and G. Bubenik (Eds.). Springer-Verlag. New York. pp. 3-113.

Cabot, E. L., and Beckenbach, A. T. 1989. Simultaneous editing of multiple nucleic acid sequences with ESEE. Comput. Appl. Biosci. **52**: 233-234.

Carr, S. M. 1996. Molecular Systematics of New and Old-World deer: implications for taxonomy, biogeography, and the evolution of antlers. Unpublished Manuscript.

Carr, S. M., Ballinger, S. W., Derr, J. N., Blankenship, L. H., and Bickham, J. W. 1986. Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west Texas. Proc. Natl. Acad. Sci. USA. **83**: 9576-9580.

Carr, S. M., and Hughes, G. A. 1993. Direction of introgressive hybridization between species of North American deer (*Odocoileus*) as inferred from mitochondrial-cytochrome-*b* sequences. J. Mamm. **74**: 331-342.

Cathy, J. C., Bickham, J. W., and Patton, J. C. 1998. Introgressive hybridization and nonconcordant evolutionary history of maternal and paternal linages in North American deer. Evolution **52**: 1224-1229.

Chomezynski, P., and Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Analyt. Biochem. **162**: 156-159.

Cronin, M., Vyse, E., and Cameron, D. 1988. Genetic relationships between mule deer and white-tailed deer in Montana. J. Wildlife Management **52**: 320-328.

Cronin, M. 1991. Mitochondrial DNA phylogeny of deer (Cervidae). J. Mamm. **72**: 1270-1279.

Cronin, M. 1992. Intraspecific variation in mitochondrial DNA of North American cervids. J. Mamm. **73**: 70-82.

Cronin, M. A. 1993. Mitochondrial DNA in wildlife taxonomy and conservation biology: cautionary notes. Wildlife Society Bulletin. **21**:339-348.

Díaz, N. I., and Smith-Flueck, J. 2000. The Patagonian Huemul. A Mysterious Deer on the Brink of Extinction. Literature of Latin America. Buenos Aires. 150 pp.

Duarte, J. M. B., and Giannoni, M. L. 1995a. Cytogenetic analysis of the marsh deer, *Blastocerus dichotomus* (Mammalia, Cervidae). Brazil. J. Genetics **18**: 245-248.

Duarte, J. M. B., and Giannoni, M. L. 1995b. Cytogenetic analysis of the pampas deer, *Ozotoceros bezoarticus* (Mammalia, Cervidae). Brazil. J. Genetics **18**: 485-488.

Duarte, J. M. B. (Ed.). 1997. Biologia e Conservaçao de Cervídeos Sul-Americanos: *Blastocerus*, *Ozotoceros* e *Mazama*. FUNEP. Jaboticabal. 238 pp.

Eisenberg, J. F. 1987. The evolutionary history of the cervidae with special reference to the south american radiation. In: Wemmer, C. M. (Ed.). Biology and Management of the Cervidae. Smithsonian Institution Press. Washington, D. C. pp. 60-64.

Ellerman, J. R. and Morrision-Scott, T. C. S. 1966. Checklist of Palearctic and Indian Mammals 1758 to 1946. 2nd Ed. Trustees of the British Museum. London. pp. 1-810.

Emerson, B. C. and Tate, M. L. 1993. Genetic analysis of evolutionary relationships among deer (subfamily Cervinae). J. Hered. **84**: 226-273.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. **17**: 368-376.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**: 783-791.

Frid, A. 2001. Habitat use by the endangered huemul (*Hippocamelus bisulcus*), cattle, snow, and the problem of multiple causes. Biol. Conserv. **100**:261-267.

Gavin, T. A. and May, B. 1988. Taxonomic status and genetic purity of Columbian white-tailed deer. J. Wildlife Management **52**: 1-10.

Geist, V. 1966. The evolution of horn-like organs. Behaviour **27**: 12-214.

Geist, V. 1971. The relation of social evolution and dispersal in ungulates during the Pleistocene, with emphasis on the Old World deer and the genus *Bison*. Quaternary Res. **1**:283-315.

Geist, V. 1998. Deer of the World: Their Evolution, Behavior, and Ecology. Stackpole Books. Pennsylvania. 421 pp.

Gentry, A. W. and Hooker, J. J. 1988. The phylogeny of the Artiodactyla. In: Benton, M. J. (Ed.). The Phylogeny and Classification of the Tetrapods Vol. 2. Clarendon Press. Oxford. pp. 235-267.

Greenslade, A. D. 1998. Inter- and Intraspecific Phylogenography of North American *Odocoileus* Deer Based on Mitochondrial DNA Sequences. M.Sc. Thesis, Department of Biology, Memorial University of Newfoundland, St. John's.

Groves, C. P. and Grubb, P. 1987. Relationships of living deer. In: Wemmer, C. M. (Ed.). Biology and Management of the Cervidae. Smithsonian Institution Press. Washington, D. C. pp. 21-59.

Hall, E. R. 1981. The Mammals of North America. Vol. II. John Wiley & Sons. New York. pp.1097-1098.

Hamilton, W. R. 1978. Cervidae and Palaeomerycidae. In: Maglio, V. J. and Cooke, H. B. S. (Eds.). Evolution of African Mammals. Harvard University Press. Cambridge. pp. 496-508.

Hassanin, A. and Douzery, E. J. P. 2003. Molecular and morphological phylogenies of Ruminantia and the alternative position of the Moschidae. Syst. Biol. **52**: 206-228.

Hershkovitz, P. 1982. Neotropical Deer (Cervidae). Part 1. Pudus, genus *Pudu* Gray. Feldfiana Zool. n. s. no. 11. pp. 1-86.

Hillis, D. M., Moritz, C., and Mable, B. K. 1996. Molecular Systematics 2nd Ed. Sinauer Associates Inc. Massachusetts. pp. 205-247 and 321-381.

Hughes, G. A., and Carr, S. M. 1993. Reciprocal hybridization between white-tailed deer (*Odocoileus virginianus*) and mule deer (*O. hemionus*) in western Canada: evidence from serum albumin and mtDNA sequences. Can. J. Zool. **71**: 524-530.

Irwin, D. M., Kocher, T. D., and Wilson, A. C. 1991. Evolution of the cytochrome *b* gene of mammals. J. Mol. Evol. **32**: 128-144.

Janis, C. M. 1988. New ideas in ungulate phylogeny and evolution. Trends. Ecol. Evol. **3**: 291-297.

Kessing, B., Croom, H., Martin, A., McIntosh, C., McMillan, W.O., and Palumbi, S. 1989. The Simple Fools Guide to PCR. University of Hawaii. Honolulu.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X., and Wilson, A. C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA **86**: 9196-6200.

Kurten, B., and Anderson, E. 1980. Pleistocene Mammals of North America. Columbia University Press. New York. pp. 1-442.

Lister, A. M. 1987. Diversity and evolution of antler form in quaternary deer. In: Wemmer, C. M. (Ed.). Biology and Management of the Cervidae. Smithsonian Institution Press. Washington, D. C. pp. 81-98.

Marshall, L. D., Webb, S. D., Sepkoski, J. J., and Raup, D. M. 1982. Mammalian evolution and the great American interchange. Science **215**:1351-1357.

McKenna, M. C., and Bell, S. K. 1997. Classification of Mammals Above the Species Level. Columbia University Press, New York.

Mendez, E. 1984. Mexico and Central America. In: Halls, L. K. (Ed.). White-Tailed Deer Ecology and Management. Stackpole Books. Pennsylvania. pp. 513-524.

Miller, S. D., Rottman, J., Raedeke, K. J., and Taber, R. D. 1983. Endangered mammals of Chile: status and conservation. Biol. Conserv. **25**: 335-52.

Miyamoto, M. M., Kraus, F., Lapis, P. J., Tanhauser, S. M., and Webb, S. D. 1993. Mitochondrial DNA phylogenies within Artiodactyla. In: Szalay, F. S., Novacek, M. J., McKenna, M. C. (Eds.). Mammal Phylogeny. Springer-Verlag. New York. pp. 268-302.

Miyamoto, M. M., Kraus, F., Ryder, O. A. 1990. Phylogeny and evolution of antlered deer determined from mitochondrial DNA sequences. Evolution. **87**:6127-6131.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press. New York. pp. 287-326.

Neitzel, H. 1987. Chromosome evolution of Cervidae karyotypic and molecular aspects. In: Obe, G. and Basler, A. (Eds.). Cytogenetics. Springer-Verlag. Berlin. pp. 91-112.

Nowak, R. M. 1991. Walker's Mammals of the World. 5th Ed. Vol. II. The Johns Hopkins University Press. Baltimore. pp. 1303-1499.

Redford, K. H. and Eisenberg, J. F. 1992. Mammals of the Neotropics. The Southern Cone. Vol. 2. Chile, Argentina, Uruguay, and Paraguay. The University of Chicago Press. Chicago. pp. 1-7, 229-252, and 420-424.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406-425.

Scott, K. M. and Janis, C. M. 1987. Phylogenetic relationships of the Cervidae, and the case for a superfamily "Cervoidea." In: Biology and Management of the Cervidae, E. M. Wemmer (Ed.). Washington, D. C. Smithsonian Institution Press. pp. 3-20.

Simpson, R. E. 1983. Artiodactyls. In: Order and Families of Recent Mammals of the World. S. Anderson and J. K. Jones (Eds.). Texas. Caesar Kleberg Wildlife Research Institute. pp. 365-387.

Simpson, C. D. 1984. Artiodactyls. In: Anderson, S. and Jones, J. K. Orders and Families of Recent Mammals of the World. John Wiley & Sons. New York. pp. 563-587.

Smith-Flueck, J. 2000. The current situation of the Patagonian huemul. In: Díax, N. I. and Smith-Flueck, J. The Patagonian Huemul: A Mysterious Deer on the Brink of Extinction. Literature of Latin American. Buenos Aires. pp. 67-145.

Smith-Flueck, J. M. and Flueck, W. T. 1995. Threats to the huemul in the southern Andean Notofagus forests. In: Bissonette, J. A. and Krausman, P. R. (Eds.). Integrating people and Wildlife for a Sustainable Future. Proceedings of the First International Wildlife Management Congress. The Wildlife Society. Bethesda. pp. 402-405.

Smith-Flueck, J. M. and Flueck, W. T. 1997. A survey of a huemul population in the province of Rio Negro, Argentina. Mastozoologia Neotropical. **4**:25-33.

Smith-Flueck, J. M. and Flueck, W. T. 2001. Natural mortality patterns in an endangered Andean cervid population in southern Argentina: the huemul (*Hippocamelus bisulcus*). European Journal of Wildlife Research and Management. **47**:178-188.

Smith, M. H., Chesser, R. K., Cothran, E. G. and Johns, P. E. 1983. Genetic variability and antler growth in a natural population of white-tailed deer. In: Antler Development, R. D. Brown (Ed.). Texas, Caesar Kleberg Wildlife Research Institute. pp.365-387.

Swofford, L. 1998. PAUP: Phylogenetic analysis using parsimony, version 4.0d63 computer program distributed by the author. The Smithsonian Institution, Washington, D.C.

Tamura, K., and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. **10**: 512-526.

Ullrey, U. 1983. Nutrition and development in white-tailed deer. In: Antler Development, R. D. Brown (Ed.). Texas, Caesar Kleberg Wildlife Research Institute. pp. 49-59.

Webb, S. D. 2000. Evolutionary history of New World Cervidae. In: Vrba, E. S. and Schaller, G. B. (Eds.). Antelopes, Deer, and Relatives. Yale University Press. New Haven. pp. 38-64.

Webb, S. D. and Taylor, B. E. 1980. The phylogeney of hornless ruminants and a description of the cranium of *Archaeomeryx*. Bull. Amer. Mus. Nat. Hist. **167**: 117-158.

Whitehead, G. K. 1993. The Whitehead Encyclopedia of Deer. Swan Hill Press. UK. pp. 467.

Wilson, D. E. and Reeder, D. M. (Eds). 1993. Mammal Species of the World, A Taxonomic and Geographic Reference. 2nd Ed. Smithsonian Institution Press in association with the American Society of Mammalogists. Washington. pp. 389-392.

APPENDIX I

Figure 9. DNA sequences variation in a 410-bp region of the mitochondrial 12S rRNA gene of 40 Ungulate samples (38 Artiodactyls and 2 Perissodactyls), obtained in this study. 21 species of cervids are represented (31 individuals).

C. elaphus canadensis	GCCTAGCCTTAAACACAAATAGTT--ATGTA---AACAAGACTGTTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
Cervus duvauceli	GCTTAGCCTTAAACACAAATAGTT--ATATA---AACAAAACTACTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
Cervus (Rusa) unicolor	GCCTAGCCTTAAACACAAATAGTT--GTGTA---AACAAAACTATTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
Elaphurus davidanus	GCCTAGCCTTAAACACAAATAGTT--GTGTA---AACAAAACTATTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
Axis axis D33	GCTTAGCCTTAAACACAAATAGTT--ATATA---AACAAAACTATTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
Axis axis	GCCTAGCCTTAAACACAAATAGTT--GTATA---AACAAAACTATTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
Muntiacus muntjac	GCCTAGCCCTAAACACAAATAGTT--TCCACA--AACAAAACTATTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
Odocoileus virginianus	GCTTAGCCCTAAACATAAATAGTT--ATATA---AACAAAACTATTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
O. hemionus columbianus	GCTTAGCCCTAAACATAAATAGTT--ATATA---AACAAAACTATTCGCCAGAGTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA
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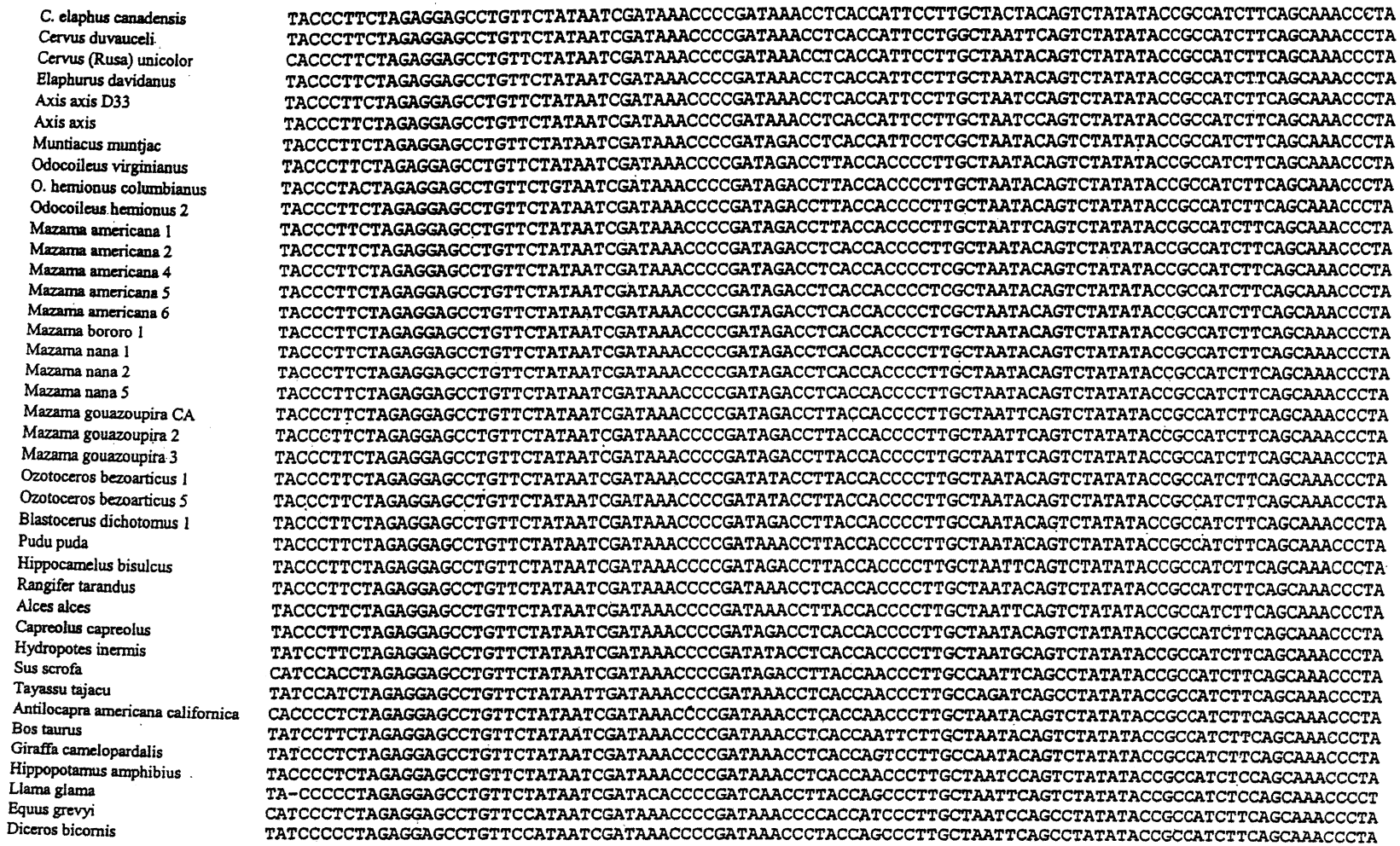
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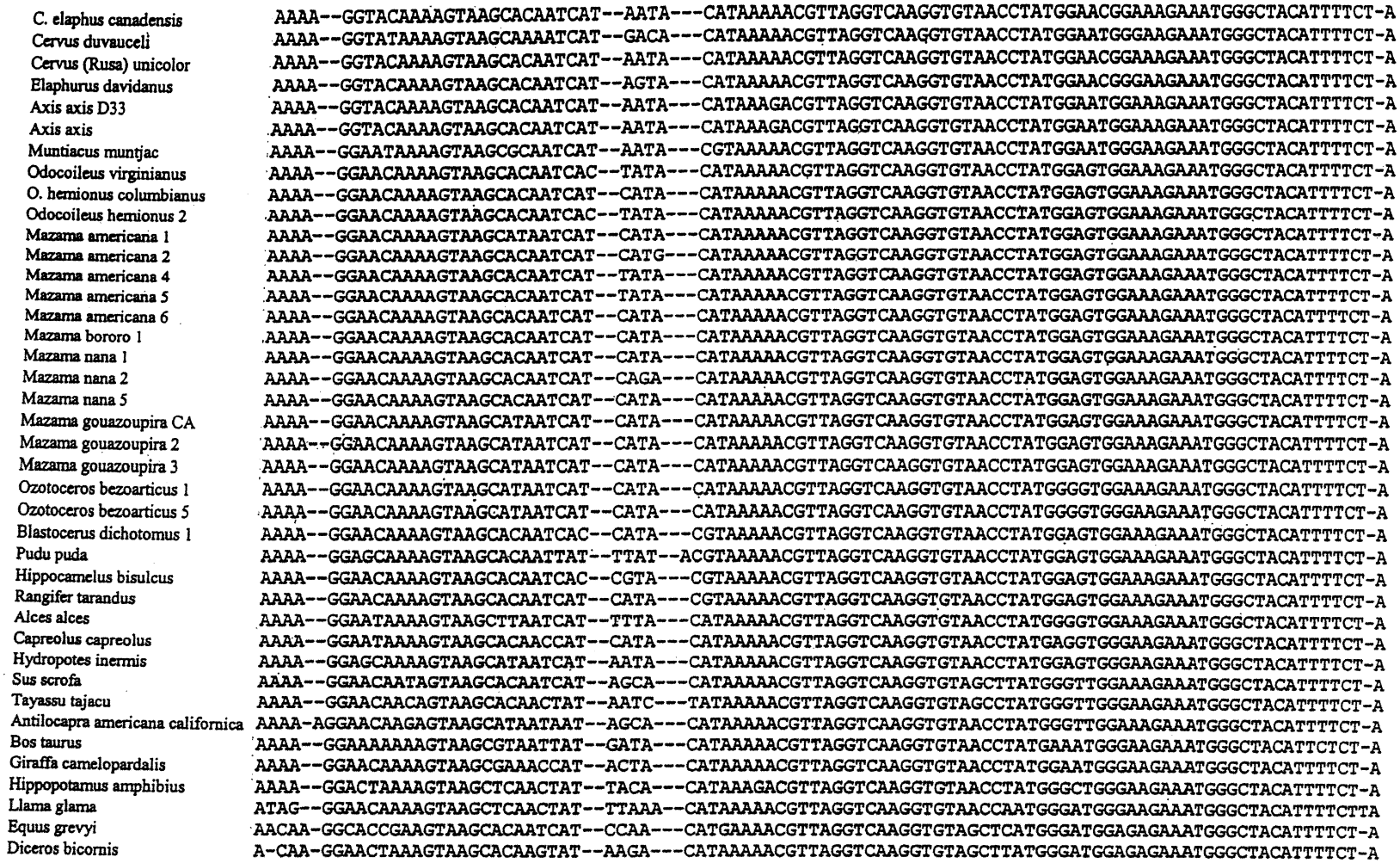
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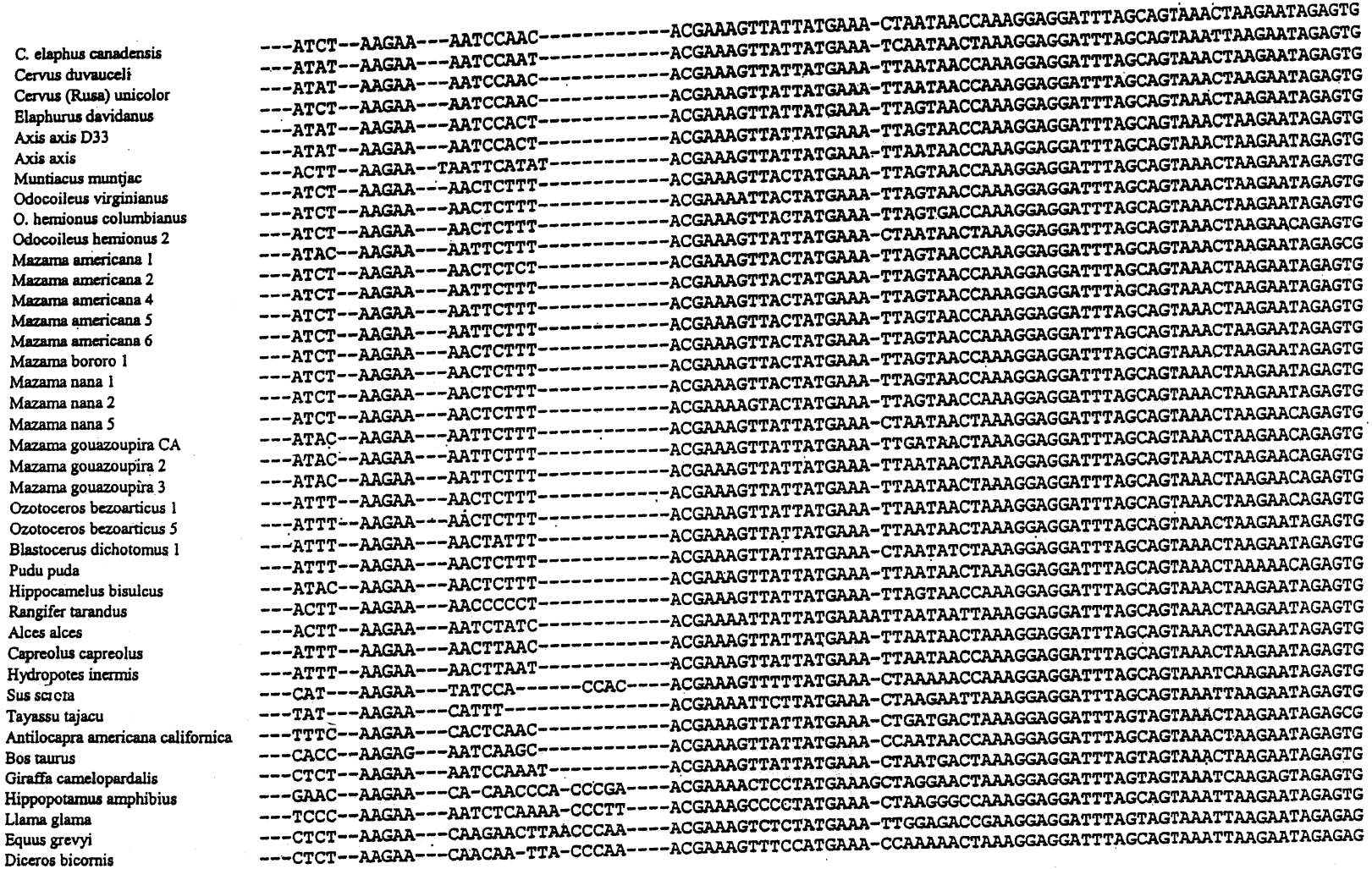
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Cervus duvauceli
Cervus (Russa) unicolor
Elaphurus davidanus
Axis axis D33
Axis axis
Muntiacus muntjac
Odocoileus virginianus
O. hemionus columbianus
Odocoileus hemionus 2
Mazama americana 1
Mazama americana 2
Mazama americana 4
Mazama americana 5
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Mazama gouazoupira CA
Mazama gouazoupira 2
Mazama gouazoupira 3
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Blastocerus dichotomus 1
Pudu puda
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Rangifer tarandus
Alces alces
Capreolus capreolus
Hydropotes inermis
Sus scrofa
Tayassu tajacu
Antilocapra americana californica
Bos taurus
Giraffa camelopardalis
Hippopotamus amphibius
Llama glama
Equus grevyi
Diceros bicornis

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Cervus duvauceli	CTTAATTGAATTAGGCCATGAAGCACGC
Cervus (Rusa) unicolor	CTTAGTTGAATTAGGCCATGAAGCACGC
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Capreolus capreolus	CTTAGTTGAATTAGGCAATGAAGCACGC
Hydropotes inermis	CTTAGTTGAACTAGGCAATGAAGCACGC
Sus scrofa	CTTGATTGAATAAGGCCATGAAGCACGC
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Antilocapra americana californica	CTTAGTTGAATTAGGCTATGAAGCACGC
Bos taurus	CTTAGTTGAATTAGGCCATGAAGCACGC
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