Syst. Biol. 52(2):206–228, 2003
DOI: 10.1080/10635150390192726

Molecular and Morphological Phylogenies of Ruminantia and the Alternative Position of the Moschidae

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Abstract.—The ruminants constitute the largest group of ungulates, with >190 species, and its distribution is widespread throughout all continents except Australia and Antarctica. Six families are traditionally recognized within the suborder Ruminantia: Antilocapridae (pronghorns), Bovidae (cattle, sheep, and antelopes), Cervidae (deer), Giraffidae (giraffes and okapis), Moschidae (musk deer), and Tragulidae (chevrotains). The interrelationships of the families have been an area of controversy among morphology, palaeontology, and molecular studies, and almost all possible evolutionary scenarios have been proposed in the literature. We analyzed a large DNA data set (5,322 nucleotides) for 23 species including both mitochondrial (cytochrome *b*, 12S ribosomal RNA (rRNA), and 16S rRNA) and nuclear (κ-casein, cytochrome P-450, lactoferrin, and α-lactalbumin) markers. Our results show that the family Tragulidae occupies a basal position with respect to all other ruminant families, confirming the traditional view that separates Tragulina and Pecora. Within the pecorans, Antilocapridae and Giraffidae emerge first, and the families Bovidae, Moschidae, and Cervidae are allied, with the unexpected placement of *Moschus* close to bovids rather than to cervids. We used these molecular results to assess the homoplastic evolution of morphological characters within the Ruminantia. A Bayesian relaxed molecular clock approach based on the continuous autocorrelation of evolutionary rates along branches was applied to estimate the divergence ages between the major clades of ruminants. The evolutionary radiation of Pecora occurred at the Early/Late Oligocene transition, and Pecoran families diversified and dispersed rapidly during the Early and Middle Miocene. We propose a biogeographic scenario to explain the extraordinary expansion of this group during the Cenozoic era. [Bayesian relaxed clock; Bovidae; molecules; morphology; Moschidae; phylogeny; Ruminantia.]

One of the most spectacular aspects of mammalian evolution during the Neogene phase of the Cenozoic era was the rapid diversification and expansion of the ruminant families (Cetartiodactyla, Ruminantia). These highly specialized herbivorous even-toed ungulates were and are particularly adapted for gathering and processing plant food. They have a specific dentition characterized by the presence of an incisiform lower canine and a horny pad that replaces the upper incisors. In addition, living ruminants possess a compartmentalized stomach that serves as a chamber for fermentation of cellulose by symbiotic microorganisms.

Ruminantia is the only cetartiodactyl suborder for which living and fossil members are clearly united by an osteological apomorphy, the fusion of the cuboid and navicular bones in the tarsus (Lavocat, 1955; Romer, 1966). Today, Ruminantia constitutes one of the major groups of large mammals, with 192 species currently described (Dung et al., 1993; Grubb, 1993) and a natural distribution that covers all continents with the exceptions of Australia and Antarctica. Six extant families are traditionally recognized on the basis of characters of the cranial appendages, limbs, and dentition (e.g., Janis and Scott, 1987; Grubb, 1993; Scott and Janis, 1993). The results of all recent morphopaleontological studies agree in the designation of the family Tragulidae as the most basal branch within Ruminantia (Webb and Taylor, 1980; Bouvrain and Geraads, 1985; Janis and Scott, 1988; Vislobokova, 1990; Scott and Janis, 1993). Therefore, the basic subdivision of the Ruminantia into the infraorders Tragulina and Pecora proposed by Flower in 1883 is now widely accepted. Tragulina is represented by a single extant family, the Tragulidae, which incorporates three chevrotain or mouse deer genera of the tropical lowland forests of the Old World: *Hyemoschus* in Central Africa, *Moschiola* in India, and *Tragulus* in southeast Asia (Grubb, 1993). Pecora comprises the five living families Antilocapridae (pronghorns; a single species occurring in open countries of North America), Bovidae (cattle, sheep, and antelopes), Cervidae (deer), Giraffidae (giraffes and okapis from Africa), and Moschidae (musk deer; a single genus living in highland forests of Asia) (Janis and Scott, 1987). In modern times, Bovidae and Cervidae represent the greatest degree of taxonomic and geographical diversity among the Ruminantia, with 48 bovid genera from Africa, most of Eurasia, and North America and 16 cervid genera from mainly America and Eurasia. All Pecora exhibit cranial appendages—permanent unbranched horns for Bovidae, deciduous antlers for Cervidae, skin-covered ossicones for Giraffidae, deciduous and branched horns for antilocaprids—except *Hydropotes* (a case of secondary loss of antlers; Randi et al., 1998) and *Moschus*. These frontal appendages are probably not homologues, although they have tentatively been used to unite some pecoran families: Bovidae with Antilocapridae (O'Gara and Matson, 1975), Bovidae with Giraffidae (Hamilton, 1978), Bovidae plus Antilocapridae with Giraffidae (Gentry and Hooker, 1988), and all Pecora except living Moschidae and extinct "Gelocidae" into horned Eupecora (Webb and Taylor, 1980).

Except for the major dichotomy that separates Pecora and Tragulina, there is no consensus among morphologists for interrelationships of the living families, and

almost all possible evolutionary scenarios have been proposed in the literature. Gatesy et al. (1992:442) noted that "there are only 15 possible rooted cladograms relating the families Bovidae, Cervidae, Antilocapridae, and Giraffidae; 11 have been proposed in the literature" (Fig. 1). Janis and Scott (1988) pointed out that Ruminantia is a problematic group in phylogeny because it experienced several evolutionary radiations during the Tertiary changes from forested to more open habitats, and different families evolved the same characters in parallel. The morphological traits that characterize the extant families become unambiguously detectable in the fossil record of the Miocene epoch, with the emergence of Antilocapridae in the New World and Tragulidae, Bovidae, Cervidae, Giraffidae, and Moschidae in the Old World. However, Eocene and Oligocene deposits of North America and Eurasia already contained numerous fossil ruminants that are difficult to assign to either Tragulina or Pecora. Most of these remains have been generally included into the Hypertraguloidea, a superfamily that is either the sister group of the living Tragulidae or is the most primitive group of the Ruminantia (Vislobokova, 1998).

Several molecular investigations have been conducted on the suborder Ruminantia. The phylogenetic studies were initially performed using mitochondrial nucleotide sequences of cytochrome *b* (Irwin et al., 1991; Honeycutt et al., 1995; Montgelard et al., 1997; Randi et al., 1998; Hassanin and Douzery, 1999b; Matthee and Robinson, 1999; Su et al., 1999), ribosomal RNAs (rRNAs) (Kraus and Miyamoto, 1991; Miyamoto et al., 1993; Montgelard et al., 1997), or cytochrome *c* oxidase II (Honeycutt et al., 1995). More recent studies have involved nuclear sequences of the κ-casein (Cronin et al., 1996), β-casein (Gatesy et al., 1996), and γ-fibrinogen genes (Gatesy, 1997) and various genetically independent loci (Hassanin and Douzery, 1999a; Gatesy and Arctander, 2000; Matthee and Davis, 2001; Matthee et al., 2001). All these reports indicate that the pecoran families form a monophyletic assemblage distinct from the Tragulidae, confirming evidence from such diverse disciplines as anatomy and palaeontology (e.g., Simpson, 1945; Gentry and Hooker, 1988; Scott and Janis, 1993), ethology (Dubost, 1965), and karyology (Gallagher et al., 1996). In contrast, relationships among the pecoran families have remained an especially unresolved issue. The studies on the mitochondrial cytochrome *b* (Irwin et al., 1991; Randi et al., 1998), rRNAs (Kraus and Miyamoto, 1991), and cytochrome *c* oxidase II (Honeycutt et al., 1995) yielded an unresolved multifurcation of antilocaprids, bovids, cervids, and giraffids. Moreover, these analyses did not provide support for the monophyly of the families Bovidae or Cervidae. Kraus and Miyamoto (1991) attributed this poor resolution to the rapid radiation of the pecoran lineages over a short period of time in the Late Oligocene to Early Miocene. A nuclear marker, κ-casein, was first introduced by Chikuni et al. (1995) to analyze Pecora phylogeny and was used again with greater taxon sampling by Cronin et al. (1996). In the latter study, Antilocapridae emerged first relative to a trifurcation involving Bovidae, Cervidae, and Giraffidae, but the support for the different nodes was low. The same result was observed after combination of the mitochondrial cytochrome *b* and 12S rRNA data (Montgelard et al., 1997). The use of nuclear κ-casein and β-casein exons with mitochondrial cytochrome *b* sequences reinforced a sister-group relationship of antilocaprids relative to bovids, cervids, and giraffids and clustered *Cervus* with *Giraffa* (Gatesy et al., 1996). The association between Cervidae and Giraffidae was also recovered with nuclear γ-fibrinogen sequences in the context of the Cetartiodactyla phylogeny, but only three pecoran families were represented in that study (Gatesy, 1997). The comparison of nuclear amino acid sequences indicated that *Giraffa* and *Antilocapra* are sister groups (Miyamoto and Goodman, 1986), and the use of ribonuclease sequences confirmed that these two taxa are closer to Bovidae than to Cervidae (Beintema et al., 1988).

In all of these studies, the taxonomic sample was not appropriately determined for inferring relationships among Pecora families because (1) the biodiversity of the Bovidae and Cervidae was inadequately represented, with the exception of the studies conducted by Cronin et al. (1996) and Randi et al. (1998), (2) the isolated branch leading to *Giraffa* was not broken by the inclusion of the second living giraffid genus *Okapia*, and (3) no molecular study included the Moschidae and its single extant genus *Moschus*, with the exception of the survey of moschid species by Su et al. (1999). Concluding their study of the rapid cladogenesis among pecorans using 12S and 16S rRNA mitochondrial markers, Kraus and Miyamoto (1991:128) noted that phylogenetic hypotheses "must be further tested with comparative information, which, from a molecular perspective, requires comparable sequence data from the genus *Moschus*." However, the musk deer has never been sampled despite the reiterated claim that "molecular studies promise to be of great use in clarifying the relationships of living [pecoran] taxa; however, the usefulness of these studies at present is limited by the lack of data on *Moschus*" (Scott and Janis, 1993:300).

Two recent studies by Gatesy and Arctander (2000) and Matthee et al. (2001) partially circumvent these three problems by adding *Okapia* and a large taxon sampling for Bovidae, and for outgroups for ruminants. Moreover, these authors combined several mitochondrial and nuclear markers and showed that (1) Tragulina clearly separates from Pecora, (2) Antilocapridae is a major and earliest diverging lineage among pecorans, and (3) Bovidae and Cervidae cluster together as the sister group of Giraffidae.

We sampled at least two distant genera from each pecoran family and subfamily except for the monogeneric Moschidae and Antilocapridae. For Cervidae, we included two members of each of the three major lineages: Plesiometacarpalia and Old World and New World Telemetacarpalia (Randi et al., 1998). For Bovidae, we incorporated one representative of each of the major tribes previously identified as monophyletic (Hassanin and Douzery, 1999a, 1999b;

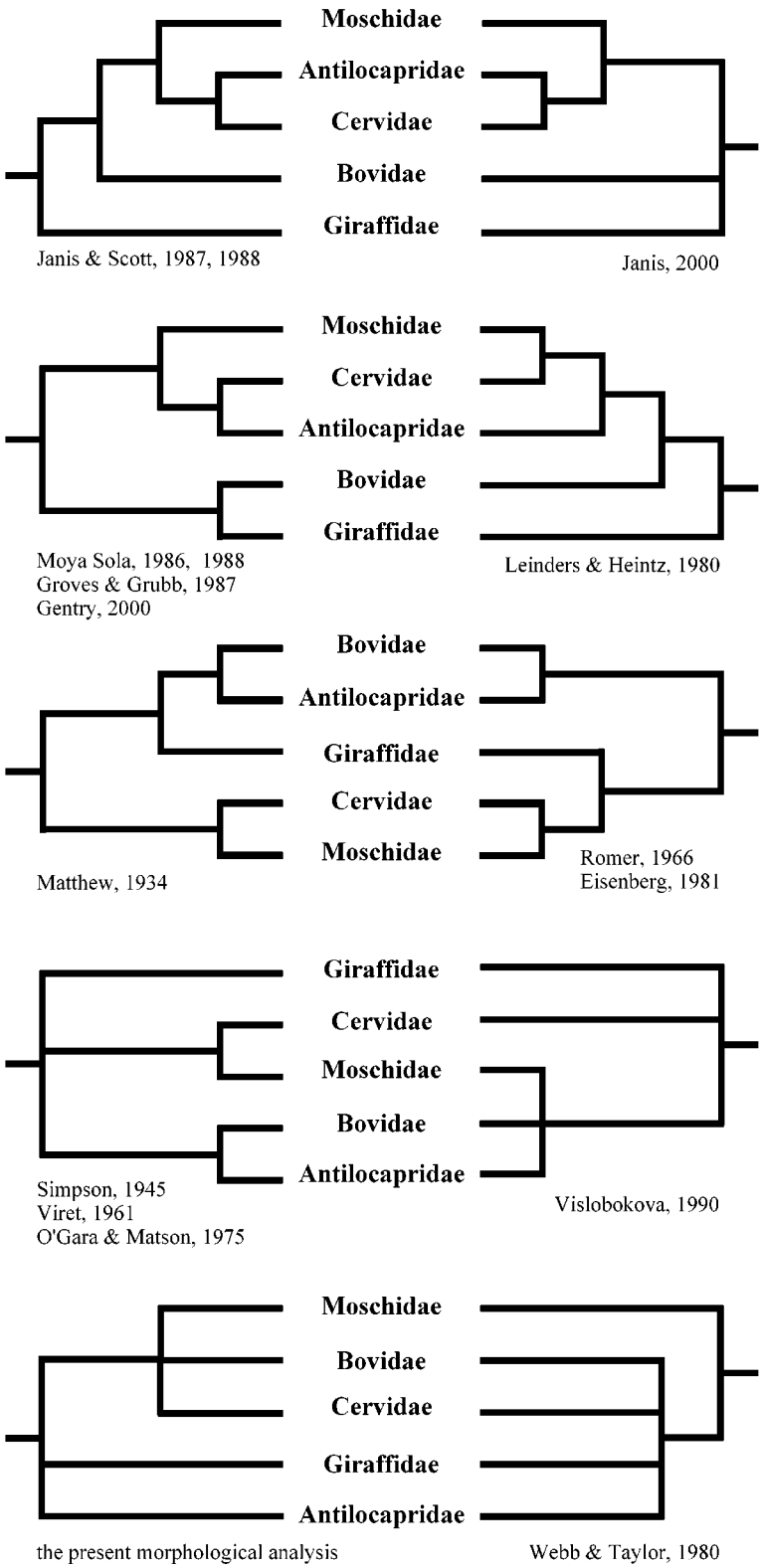

FIGURE 1. Morphological hypotheses for the interfamily relationships within Pecora.

Gatesy and Arctander, 2000; Matthee et al., 2001), i.e., Bovini, Boselaphini, Tragelaphini, Antilopini, Aepycerotini, Alcelaphini, Caprini s.l., Cephalophini, Hippotragini, and Reduncini. We attempted to break the long Pecora ancestral segment by adding a tragulid species. This ruminant ingroup, Tragulina + Pecora, was rooted by one cetacean (Balaenopteridae) and one ancodontan (Hippopotamidae) because these two taxa are considered the closest living relatives of Ruminantia (Irwin and Arnason, 1994; Gatesy et al., 1996; Gatesy, 1997, 1998; Montgelard et al., 1997; Shimamura et al., 1997).

We sequenced molecular markers that could have experienced different mutational and selective constraints during cetartiodactyl evolution, i.e., mitochondrial and nuclear markers belonging to two different genomes with different rates and patterns of evolution and protein-encoding, ribosomal, and noncoding markers with different selective pressures. Because they have been previously used to decipher the cetartiodactyl phylogeny, the following protein-encoding markers were used: the mitochondrial cytochrome *b* (*Cyb*) gene (e.g., Irwin et al., 1991; Irwin and Arnason, 1994; Stanley et al., 1994; Tanaka et al., 1996; Montgelard et al., 1997; Hassanin et al., 1998b; Randi et al., 1998; Hassanin and Douzery, 1999b; Matthee and Robinson, 1999) and the nuclear κ-casein exon 4 (*κCas*) (Chikuni et al., 1995; Cronin et al., 1996; Gatesy et al., 1996). The ribosomal markers were the mitochondrial 12S and 16S rRNAs (e.g., Miyamoto et al., 1989; Kraus and Miyamoto, 1991; Allard et al., 1992; Gatesy et al., 1992, 1997; Montgelard et al., 1997), and the non-coding markers were the nuclear promotor segment of the lactoferrin gene (*Lf*), the 3′ untranslated region of the aromatase cytochrome P450 gene (*Cyp*) (Pitra et al., 1997; Hassanin and Douzery, 1999a), and intron 2 of the α-lactalbumin gene (*αLAlb*).

The aims of this study were (1) to evaluate the contribution of seven mitochondrial and nuclear markers (*Cyb*, 12S rRNA, 16 rRNA, *αLAlb*, *Cyp*, *κCas*, and *Lf*) and their combination (5,322 characters) to decipher the phylogeny of all ruminant Pecora families, including Moschidae; (2) to compare the phylogenetic pictures inferred from molecular and morphological characters; (3) to determine the time frame for Pecora evolution under a Bayesian relaxed molecular clock assuming an Early Miocene (16.4–23.8 million years ago [MYA]) a priori divergence of both bovid and cervid families; and (4) to build a biogeographic scenario explaining the current distribution of ruminants.

Material and Methods

Taxon Sampling

Twenty-three taxa were analyzed in this study (Table 1). Two taxa, *Balaenoptera* (family Balaenopteridae) and *Hippopotamus* (family Hippopotamidae), were used as outgroups to root the ruminant tree. These outgroup taxa were chosen because molecular investigations have shown that hippos and cetaceans are closely related to the ruminants (Gatesy, 1997; Shimamura et al., 1997). All extant families of the suborder Ruminantia were represented in this study: the Tragulidae with *Tragulus*, the Antilocapridae with *Antilocapra*, the Giraffidae with *Giraffa* and *Okapia*, the Moschidae with *Moschus*, the Cervidae with 6 species, and the Bovidae with 10 species. For the Cervidae, we incorporated at least one representative of each of the major clades of Randi et al. (1998): *Cervus* (Cervinae), *Muntiacus* (Muntiacinae), *Capreolus* and *Hydropotes* (Alcini + Hydropotinae + Capreolini clade), and *Odocoileus* and *Rangifer* (Odocoileini + Rangiferini clade). For the Bovidae, we integrated one representative of each of the major lineages of Hassanin and Douzery (1999a, 1999b): *Aepyceros* (Aepycerotini), *Bos* (Bovini), *Boselaphus* (Boselaphini), *Cephalophus* (Cephalophini), *Damaliscus* (Alcelaphini), *Gazella* (Antilopini), *Hippotragus* (Hippotragini), *Ovis* (Caprini s.l.), *Redunca* (Reduncini), and *Tragelaphus* (Tragelaphini).

DNA Sequencing

Total DNA was extracted from blood, hair, skin, muscles, or other soft tissues following the protocols described by Sambrook et al. (1989) or Winnepenninckx et al. (1993) and from bone fragments of museum specimens using the procedure of Hassanin et al. (1998b).

The entire *Cyb* gene was amplified by the polymerase chain reaction (PCR) with the primers described by Hassanin et al. (1998b) and Hassanin and Douzery (1999b). For *Moschus*, a 3.0-kilobase (kb) fragment spanning the *Cyb* to the first half of the 12S rRNA was amplified and cloned as described by Douzery and Randi (1997). The complete *Cyb* was sequenced in four clones using a set of primers described by Irwin et al. (1991). The amplification of the complete 12S rRNA gene was performed as described by Hassanin and Douzery (1999a). For *Hippopotamus, Okapia*, and *Moschus*, a 2.8-kb fragment spanning the complete 12S to 16S rDNA was amplified with primers R1 (5′-AAAGCAAGGCACTGAAAATGCCTAGA-3′) and S1 (5′-TGGCAGATCTCGGTAATTGCATAA-3′) annealing in the $tRNA^{Phe}$ and $tRNA^{Leu}$, respectively, using 30 cycles of denaturation (94°C, 45 sec), annealing (50°C, 45 sec) and elongation (72°C, 210 sec), and *Taq* polymerase from A.T.G.C. The efficiency of PCRs was strongly increased using DMSO at a 5% final concentration. PCR products were cloned in the pGEM-T vector (Promega) using the *Escherichia coli* JM109 competent cells for transformations. Recombinant plasmids were purified from three, five, and one positive clone for hippo, okapi, and musk deer, respectively, and DNA sequencing was conducted on both strands using [α ^{35}S]dATP and the ^{T7}Sequencing mixes kit (Pharmacia Biotech). For *Giraffa, Capreolus, Rangifer, Hippotragus*, and *Redunca*, a 2.0-kb fragment spanning the end of the 12S to the complete 16S rDNA was amplified with primers 27′ (5′-TATACCGCCATCTTCAGCAAAC-3′) and S1 and directly sequenced using [α ^{33}P]ddNTP and the Thermo Sequenase radiolabeled terminator cycle Sequencing kit (Amersham).

TABLE 1. Taxonomy according to Grubb (1993) for the species used in this study. The accession numbers and corresponding references (numbers in parentheses[a]) for all sequences used in this study are provided for the mitochondrial cytochrome *b* (*Cyb*), 12S rRNA (12S), and 16S rRNA (16S) and the nuclear α-lactalbumin intron 2 (*αLAlb*), aromatase cytochrome P450 (*Cyp*), lactoferrin promotor (*Lf*), and κ-casein exon 4 (*κCas*).

Species	Common name	*Cyb*	12S	16S	*αLAlb*	*Cyp*	*Lf*	*κCas*
Giraffidae								
Giraffa camelopardalis	giraffe	AY121992 (1)	AY121986 (1)	AY122046 (1)	AY122015 (1)	AY122007 (1)	AY122041 (1)	U37516 (2)
Okapia johnstoni	okapi	AY121993 (1)	AY121987 (1)	AY122044 (1)	AY122016 (1)	AY122008 (1)	AY122042 (1)	AY121996 (1)
Moschidae								
Moschus moschiferus	musk deer	AY121995 (1)	AY121988 (1)	AY122045 (1)	AY122033 (1)	AY122009 (1)	AY122043 (1)	AY121997 (1)
Antilocapridae								
Antilocapra americana	pronghorn	AF091629 (3)	AF091706 (3)	M55540 (4)	AY122014 (1)	AF091666 (3)	AF091694 (3)	U37515 (2)
Cervidae								
Cervus elaphus	red deer	AJ000021 (5)	AF091707 (3)	M35875 (4)[b]	AY122017 (1)	AF091667 (3)	AF091636 (3)	U37505 (2)
Muntiacus reevesi	Chinese muntjak	AJ000023 (5)	M35877 (6)	M35877 (6)	AY122018 (1)	AY122010 (1)	AY122037 (1)	U37509 (2)
Odocoileus hemionus	black-tailed deer	AF091630 (3)	AF091708 (3)	M35874 (4)[c]	AY122022 (1)	AF091665 (3)	AF091637 (3)	U37360 (2)
Rangifer tarandus	reindeer	AJ000029 (5)	AY121989 (1)	AY122047 (1)	AY122019 (1)	AY122005 (1)	AY122038 (1)	U37503 (2)
Hydropotes inermis	Chinese water deer	AJ000028 (5)	M35876 (6)	M35876 (6)	AY122020 (1)	AY122006 (1)	AY122039 (1)	Douzery and Randi, unpubl.
Capreolus capreolus	European roe deer	AJ000024 (5)	AY121990 (1)	AY122048 (1)	AY122021 (1)	no accession number (7)	AY122040 (1)	U37363 (2)
Bovidae (Bovinae)								
Bovini								
Bos taurus	domestic cow	V00654 (8)	V00654 (8)	V00654 (8)	AY122023 (1)	Z32741 (9)	L19985 (10)	X14908 (11)
Boselaphini								
Boselaphus tragocamelus	nilgai	AJ222679 (12)	M86494 (13)	M86494 (13)	AY122024 (1)	AF091672 (3)	AF091642 (3)	AF030331 (14)
Tragelaphini								
Tragelaphus imberbis	lesser kudu	AF036279 (12)	AF091697 (3)	M86493 (13)	AY122025 (1)	AF091677 (3)	AF091649 (3)	AF030330 (14)
Bovidae (Antilopinae)								
Caprini								
Ovis aries	domestic sheep	AF034730 (15)	AF091699 (3)	AF010406 (16)	AY122026 (1)	AF091685 (3)	AF091651 (3)	X51822 (17)
Hippotragini								
Hippotragus niger	sable antelope	AF036285 (12)	AF091709 (3)	AY122049 (1)	AY122027 (1)	AF091684 (3)	AF091652 (3)	AY122001 (1)
Alcelaphini								
Damaliscus pygargus	blesbok	AF036287 (12)	M86499 (13)	M86499 (13)	AY122028 (1)	AF091683 (3)	AF091653 (3)	AY122002 (1)
Antilopini								
Gazella granti	Grant's gazelle	AF034723 (15)	AF091700 (3)	M86501 (6)[d]	AY122029 (1)	negative PCR	AF091654 (3)	AY122003 (1)
Cephalophini								
Cephalophus dorsalis	bay duiker	AF091634 (3)	AF091701 (3)	M86498 (6)[e]	AY122030 (1)	AF091682 (3)	AF091655 (3)	AY122000 (1)
Reduncini								
Redunca fulvorufula	mountain reedbuck	AF036284 (12)	AF091704 (3)	AY122050 (1)	AY122031 (1)	AF091681 (3)	AF091658 (3)	AY121999 (1)
Aepycerotini								
Aepyceros melampus	impala	AF036289 (12)	M86496 (13)	M86496 (13)	AY122032 (1)	AF091680 (3)	AF091659 (3)	AY121998 (1)
Tragulidae								
Tragulus javanicus	lesser Malay chevrotain	AY121994 (1)	AY121991 (1)	M55536 (18)[f]	AY122013 (1)	negative PCR	AY122034 (1)	D14381 (19)
Balaenopteridae								
Balaenoptera physalus	fin whale	X61145 (20)	X61145 (20)	X61145 (20)	AY122011 (1)	AY122004 (1)	AY122035 (1)	U53888 (21)
Hippopotamidae								
Hippopotamus amphibius	hippo	Y08813 (18)	Y08810 (18)	AJ010813, unpublished	AY122012 (1)	negative PCR	AY122036 (1)	U53889 (21)

[a]1 = this paper; 2 = Cronin et al. (1996); 3 = Hassanin and Douzery (1999a); 4 = Kraus and Miyamoto (1991); 5 = Randi et al. (1998); 6 = Miyamoto et al. (1990); 7 = Pitra et al. (1997); 8 = Anderson et al. (1982); 9 = Vanselow and Fürbass (1995); 10 = Seyfert et al. (1994); 11 = Alexander et al. (1988); 12 = Hassanin and Douzery (1999b); 13 = Allard et al. (1992); 14 = Ward et al. (1997); 15 = Hassanin et al. (1998b); 16 = Hiendleder et al. (1998); 17 = Furet et al. (1990); 18 = Montgelard et al. (1997); 19 = Chikuni et al. (1995); 20 = Arnason et al. (1991); 21 = Gatesy et al. (1996).

[b]Interspecific chimera with *Cervus unicolor*.

[c]Interspecific chimera with *Odocoileus virginianus*.

[d]Interspecific chimera with *Gazella thomsoni*.

[e]Interspecific chimera with *Cephalophus maxwelli*.

[f]Interspecific chimera with *Tragulus napu*.

Partial sequences of the nuclear gene encoding aromatase cytochrome P450, i.e, positions 2,992–3,185 of the *Bos taurus* sequence (accession Z32741), were acquired using the oligonucleotides determined by Pitra et al. (1997). The promotor segment of the lactoferrin-encoding gene, i.e., positions 322-647 of the *Bos taurus* sequence (accession L19985), was generated using the primers given by Hassanin and Douzery (1999a). Exon 4 of the κ-casein gene (positions 84–485 of exon 4 of *Bos taurus* sequence, accession X14908) was obtained using the primers designed by Ettore Randi (unpubl.). Intron 2 of the α-lactalbumin gene (*αLAlb*) was amplified using the primer 5′-ATCTGTAACATCTCCTGTGA-3′ positioned in exon 2 and the primer 5′-TCAGTAAGRTCATCATCCAG-3′ located in exon 3. Both strands of all amplicons were directly sequenced using the Thermo Sequenase cycle sequencing kit (Amersham). The sequences have been deposited in the EMBL/GenBank/DDBJ databases under the accession numbers specified in Table 1.

Phylogenetic Analyses

Sequences were aligned using the MUST package (Philippe, 1993). Indels were coded according to Barriel (1994), with introduction of I and D character states and question marks representing the methodological consequences of gap coding. The alignment of 12S rRNA and 16S rRNA sequences was refined using the secondary structure model for mammals (Springer and Douzery, 1996; Schnare et al., 1996). All regions with ambiguities for DNA alignment were excluded from the analyses. Alignments with indels have been deposited in the EMBL nucleotide database under the following accession numbers: ALIGN_000484 (*Cyp*), ALIGN_000487 (12S), ALIGN_000488 (*κCas*), ALIGN_000489 (*αLAlb*), ALIGN_000490 (*Lf*), and ALIGN_000491 (16S). To benefit from the maximum number of molecular characters, the different data sets were combined.

The phylogenetic analyses were primarily performed by rooting with *Balaenoptera* and *Hippopotamus* (sample I = 23 taxa). To test the stability of the pecoran tree topology, a subsequent analysis was conducted excluding the two most diverging outgroup taxa. It included only genera of the suborder Ruminantia, with *Tragulus* used as outgroup (sample II = 21 taxa).

Maximum parsimony analyses.—The maximum parsimony (MP) analysis (PAUP 3.1.1.; Swofford, 1993) was conducted with either equal weighting or differential weighting of the character-state transformations using the product of CI × S (CI = consistency index, S = slope of saturation) (Hassanin et al., 1998a, 1998b). For each substitution type (i.e., A to G, C to T, A to C, A to T, C to G, and G to T), the amount of homoplasy was measured through the CI and the saturation was assessed graphically by plotting the pairwise number of observed differences against the corresponding pairwise number of inferred substitutions. The saturation analysis was performed using the matrices of patristic distances and adjusted character distances calculated by PAUP 3.1.1. The slope of the linear regression (S) was then used to evaluate the level of saturation. The pairwise genetic and patristic distances are not independent because they reflect a shared evolutionary history of the taxa compared, but the linear regression provides a good measure of the saturation intensity. When no saturation is observed, the slope of the linear regression is equal to 1. When the level of saturation increases, the slope decreases toward zero. The CI and S values were calculated for the complete sequences for the three noncoding nuclear markers (*Lf*, *αLAlb*, and *Cyp*), separately for the three codon positions for the coding markers (*Cyb* and *κCas*) to take into account the selective constraints, and separately for stems and loops of the secondary structure for 12S rRNA (Springer and Douzery, 1996) and 16S rRNA (Schnare et al., 1996) genes to take into account the functional constraints. Searches for the shortest tree(s) were performed using default options but with 100 replicates of the random stepwise addition of taxa. The reliability of the nodes was assessed by bootstrap percentages (BP; Felsenstein, 1985) and by branch support (b or b_r) (Bremer, 1988). The bootstrap values were computed after 1,000 replicates of the closest stepwise addition of taxa. The Bremer analysis was conducted using topological constraints with 100 replicates of the random stepwise addition of taxa. For the differential weighting analyses, the branch support values were rescaled (b_r) with respect to the equally weighted tree length (Gustafsson and Bremer, 1995). Searches under topological constraints were used to measure how long a tree must be before a given group of taxa becomes monophyletic. The number of additional steps was rescaled with respect to the equally weighted tree to allow comparisons with the b and b_r values. DeBry (2001) cautioned that decay indices must be interpreted in light of branch lengths and that low values can be meaningless in terms of support.

Maximum likelihood and Bayesian analyses.—A standard maximum likelihood (ML) approach was first conducted as an alternative to the MP approach because ML is known to be less sensitive to potential long-branch attraction artifacts, to take into account the underlying molecular evolutionary process, and to statistically compare competing hypotheses (Swofford et al., 2001; Whelan et al., 2001). ML analyses with PAUP* 4.0b8 (Swofford, 1998) were conducted under the general time reversible model (GTR; Yang, 1994) with among-site substitution rate heterogeneity described by a gamma distribution with eight categories (Γ_8; Yang, 1996a) and a fraction of sites (INV) constrained to be invariable. ML parameters were optimized after heuristic search on a neighbor-joining (NJ) starting tree using tree bisection-reconnection (TBR) branch swapping. Reliability of nucleotide-derived trees was estimated by BP values (Felsenstein, 1985) computed with PAUP* using the optimal ML parameters, with NJ starting trees and a number of TBR branch swapping rearrangements limited to 1,000 per replicate.

To account for the combination of markers with contrasted molecular properties, i.e., nuclear versus mitochondrial and protein coding or ribosomal versus

noncoding, a partitioned ML analysis was conducted. Twelve partitions were distinguished in the original data set according to the structural and functional properties of the markers: codon positions 1 (380 nucleotides [nt]), 2 (380 nt), and 3 (380 nt) for *Cyb*, loops (457 nt) and stems (446 nt) of the 12S rRNA, loops (857 nt) and stems (643 nt) of the 16S rRNA, codon positions 1 (125 nt), 2 (125 nt), and 3 (126 nt) for κ*Cas*, *Lf* (a single partition because of its promotor nature; 354 nt), and α*LAlb* (a single partition because of its intron nature; 502 nt), yielding a total of 4,775 sites. *Cyp* was not used because three taxa (*Hippopotamus*, *Tragulus*, and *Gazella*) were not represented for this marker. For each partition, one independent GTR + Γ_8 model was defined to reflect both within- and between-marker differences in the process of evolution. The likelihoods of alternative phylogenetic hypotheses were then computed using PAML version 3.1 (Yang, 1997). We used the method of Yang (1996b) to combine multiple sequence data: Each of our 12 sequence partitions was analyzed with its own estimates of the GTR + Γ_8 model and with proportional estimates of branch lengths from one partition to another. To evaluate alternative hypotheses for the location of the root of the pecoran subtree, the NucML program (MOLPHY 2.3; Adachi and Hasegawa, 1996) was used to write all the 105 bifurcating trees connecting the five Pecora families, starting from the following enforced topology: (*Balaenoptera*, *Hippopotamus*(*Tragulus*(Antilocapridae, Giraffidae, Moschidae, Bovidae, Cervidae))). To limit the number of possible trees, the subtopology within each family was constrained according to the highest likelihood trees reconstructed from the concatenated matrix of characters. The pecoran families were respectively represented by *Antilocapra*, *Giraffa* + *Okapia*, *Moschus*, ((*Aepyceros*((*Cephalophus*(*Ovis*(*Damaliscus* + *Hippotragus*)))(*Gazella* + *Redunca*)))(*Boselaphus*(*Bos* + *Tragelaphus*))), and (((*Capreolus* + *Hydropotes*)(*Odocoileus* + *Rangifer*))(*Cervus* + *Muntiacus*)). This choice seems justified for well supported nodes (e.g., Bovinae) but more controversial for weaker nodes (e.g., *Bos* + *Tragelaphus*). The exploration of the tree space was therefore approximated, and it is difficult to predict how much the constraints on poorly supported nodes affected the ranking of the different phylogenetic alternatives according to their log-likelihood. However, this tree space limitation under PAML was the only way to compute 105 log-likelihoods under the complex combination of independent GTR + Γ_8 models for 12 different gene partitions.

The likelihoods of the 105 trees were then evaluated by partitioned ML, with a simultaneous estimation of 12 × (5 [GTR] + 1 [Γ_8]) + 11 (proportionality rates between partitions)) + 43 (branch lengths) = 126 independent parameters for each topology. The nonparametric test of Kishino and Hasegawa (1989) was conducted with the conservative Shimodaira and Hasegawa (1999) correction for multiple tree comparisons (KH-SH test) to evaluate the significance of differences in log-likelihood between the tree with the highest likelihood and the 104 alternative topologies. The ratios between the difference in log-likelihoods of the best (B) and evaluated (E) trees (δ = lnL[B] − lnL[E]) and the standard error (σ) of this difference, and the confidence P_{SH} values have been computed by PAML 3.1 under the 12 GTR + Γ_8 models previously defined.

Phylogenetic analyses were also performed using the Bayesian inference (Huelsenbeck et al., 2001). This new approach evaluates the posterior probability of a tree given the character matrix, i.e., the probability that the tree is correct. The posterior probability is obtained after combining the prior probabilities of a tree and of the data with the likelihood of the data given that tree. The Bayesian approach combines the advantages of defining an explicit probability model of character evolution and of obtaining a rapid approximation of posterior probabilities of trees through the use of the Markov chain Monte Carlo (MCMC) approach. The likelihood model chosen is always GTR + Γ_8, with partition-specific rates. All analyses were conducted using MrBayes 2.1 (Huelsenbeck and Ronquist, 2001), with five independent Markov chains (one cold chain and four incrementally heated chains) run for 200,000 metropolis-coupled MCMC generations, with tree sampling every 20 generations and burn-in after 5,000 trees.

Molecular Dating

Ages of divergence between the pecoran clades have been estimated by the Bayesian relaxed molecular clock approach developed by Thorne et al. (1998) and Kishino et al. (2001) in the software DIVTIME 5b. This approach combines the advantages of relaxing the molecular clock with a continuous autocorrelation of substitution rates over evolutionary time and of allowing the simultaneous use of several calibration references. As time constraints, we considered the first splits within Bovidae (Miyamoto et al., 1993) and within Cervidae (Ginsburg, 1988) to have occurred in the Early Miocene, i.e., between 16.4 and 23.8 MYA. All absolute ages of the geological periods and chronostratigraphic references were taken from the 1999 Geological Time Scale of the Geological Society of America (www.geosociety.org/science/timescale/timescl.pdf).

The dating procedure involved two steps. First, the program ESTBRANCHES estimated branch lengths and the variance-covariance matrix from the concatenated nucleotide data set (4,775 sites; i.e., the same data matrix as used for the partitioned ML analysis). The F84 nucleotide substitution model was the only one implemented for DNA under ESTBRANCHES and included the following parameters (as estimated from PAML 3.1): A = 32.9%, C = 24.2%, G = 17.7%, and T = 25.2% for the base composition; 3.45 for the rate parameter; and $\alpha = 0.22$ for the shape of the Γ_8 distribution that corresponds to eight discrete categories with rates of 0.00004, 0.0026, 0.01696, 0.07533, 0.23829, 0.63007, 1.57486, and 5.46250.

Second, after pruning the outgroup taxa (*Balaenoptera* and *Hippopotamus*), the program DIVTIME estimated the prior and posterior ages of divergence between ruminants, their SDs, and the 95% credibility intervals ($CredI_{95\%}$). The Markov chain was sampled 10,000 times

with 100 cycles between each sample and burn-in after 100,000 cycles. The following prior distributions were adopted: 40 MYA (SD = 20 MYA) for the expected time between tip and root if there had been no constraints on node times, 0.008 (SD = 0.004) substitutions per site per million years for the rate at root node, 0.025 (SD = 0.025) for the parameter that controls the amount of rate autocorrelation per million years, and 65.0 MYA for the highest possible number of time units between tip and root. Because of the uncertainty about the location of the root of the pecoran subtree, molecular datings were conducted on two topologies, one with Antilocapridae as sister of the remaining pecorans and one with Antilocapridae + Giraffidae as sister group of Moschidae + Bovidae + Cervidae. To check for convergence of MCMC analyses, two independent runs were performed for the same data and same prior distributions but with different starting points, one at a randomly selected state and the other at a potentially better state.

Morphological Analyses

Forty-eight morphological characters were analyzed to infer phylogenetic relationships among the five pecoran families Antilocapridae, Bovidae, Cervidae, Giraffidae, and Moschidae. The family Tragulidae was used as an outgroup because all recent morphological studies have shown its placement as sister taxon of Pecora (e.g., Webb and Taylor, 1980; Bouvrain and Geraads, 1985; Gentry and Hooker, 1988; Janis and Scott, 1988; Vislobokova, 1990; Scott and Janis, 1993). Most of the characters used in analyses came from the studies performed by Janis and Scott (1987), Gentry and Hooker (1988), and Scott and Janis (1993). All the character states were coded using the following assumptions (Table 2): All the ruminant families were considered monophyletic, and all the character states examined were considered the ancestral states within each of the six ruminant families. For Bovidae and Cervidae, ancestral states were estimated by using consensus topologies derived from previous molecular investigations (Randi et al., 1998; Hassanin and Douzery, 1999a, 1999b; Matthee and Robinson, 1999; Matthee and Davis, 2001). A question mark indicates that the primitive character state was not determined, and a dash indicates that the character was not applicable. All question marks and dashes were treated as missing character states for the parsimony analyses.

RESULTS

MP Analyses

MP analyses were based on CI × S weighting of the character-state changes because this weighted approach allows the definition of different stepmatrices to reflect the contrasted evolutionary properties within (e.g., different codon positions) and between (e.g., mitochondrial versus nuclear) markers. When MP analyses based on the CI × S weights are conducted with *Balaenoptera* and *Hippopotamus* included as outgroups, one most-parsimonious tree of 1,675,124 steps was recovered (Fig. 2). All members of the suborder Ruminantia cluster together (BP = 100; b_r = +199), with *Tragulus* clearly separated from the other ruminant genera (BP = 100; b_r = +100). Within Pecora, the families Bovidae, Cervidae, and Giraffidae are monophyletic with high bootstrap and branch support values (respectively, BP = 100/100/100; b_r = +28/+91/+74), and *Antilocapra* appears to branch first with respect to all other lineages (BP = 81; b_r = +9). The families Bovidae, Cervidae, and Moschidae cluster together (BP = 85; b_r = +12), with *Moschus* unexpectedly closer to bovids (BP = 95; b_r = +16) than to cervids (residual BP = 1; rescaled additional steps = +16). Two markers individually support the

TABLE 2. Morphological character[a] matrix (a = 0 or 1; b = 1 or 2; ? = unknown; - = not applicable).

```
                    1111111111222222222233333333334444444444
                    1234567890123456789012345678901234567890123456789012345678
TRAGULIDAE          0001100000a000000000000000000---------1000100000a
ANTILOCAPRIDAE      01?1120011100111210000a0110011200111 0?0011011 0010
GIRAFFIDAE          010a12101100101121111001a0011100000020a110111111
CERVIDAE            0110010011110111110a0101111111110000 11aa110111011
MOSCHIDAE           11?1120111a101111100 1a1110110---------10110111011
BOVIDAE             0111120a110a0111b10aaa01aa1111001001 10a110111011
```

[a]Soft anatomy: 1 = abdominal musk gland; 2 = ruminating stomach with four chambers (including a well-developed omasum); 3 = cardiac orifice of rumen more ventrally situated; 4 = gall bladder; 5 = ileocecal gland; 6 = placenta diffuse (0) or cotyledonary with few (1) or many (2) cotyledons; 7 = extensible tongue; 8 = females with four (0) or two (1) mammae. Postcranial: 9 = fibular facet on calcaneum with proximal concavity large, distal concavity small; 10 = astragalus with proximal and distal portions parallel; 11 = anterior longitudinal metatarsal gully including a bridged distal end; 12 = posterior metatarsal tuberosity; 13 = cubonavicular facet very flat and broad; 14 = internal cuneiform independent; 15 = metatarsals II and V lost; 16 = metacarpals III and IV fused from proximal end for most or entire length; 17 = metacarpals II and V incomplete (1) or complete loss of lateral digits (2); 18 = complete distal metapodial keels; 19 = limbs much elongated; 20 = number of sacral vertebrae: 5(0), 4(1); 21 = number of ribs: ≤13 (0), 14 (1), ≥15 (2). Cranial: 22 = double lacrimal orifice; 23 = tympanohyal vagina fully enclosed; 24 = auditory bulla hollowed internally; 25 = anteorbital (ethmoidal) vacuity; 26 = lacrimal fossa (a depression in the lacrimal bone that contains the preorbital gland); 27 = foramen ovale large; 28 = postglenoid process absent; 29 = cranial appendages; 30 = cranial appendages present in postorbital position (1) or supraorbital position (2); 31 = branched bone core; 32 = deciduous bone core; 33 = bone core with keratinous sheath; 34 = deciduous horn sheath; 35 = forked horn sheath; 36 = cranial appendages only in males; 37 = cranial appendages are formed from an outgrowth of the frontal bone (1) or from a dermal ossification center (2). Dental: 38 = long and curved upper canines; 39 = upper molar entostyle; 40 = upper molar metastyle; 41 = upper M3 metaconule large; 42 = upper molar lingual cingulum; 43 = anterior cingulum on lower molar; 44 = metaconid on lower premolars simplified; 45 = metastylid on the lower molars; 46 = lower canine bilobed; 47 = postentocristid complete on the lower molars; 48 = ectostylids on the lower molars.

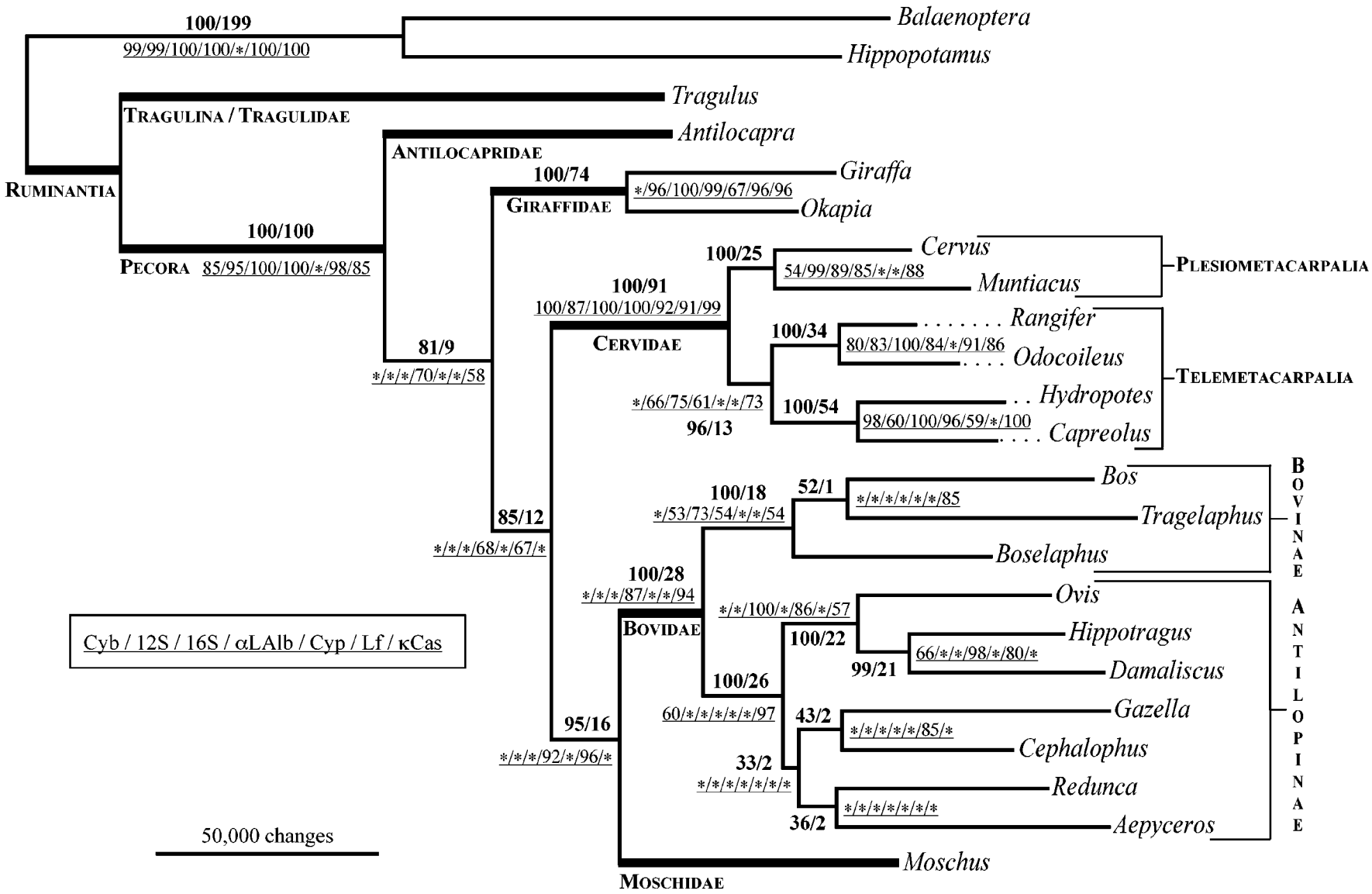

FIGURE 2. Maximum parsimony tree reconstructed from the combination of the seven markers. Weighted parsimony analysis was based on the product of homoplasy × saturation indices (length = 1,675,124 steps). Bootstrap percentages/rescaled branch support values found with the combination of the seven markers are indicated in bold. Underlined values are bootstrap percentages (>50%) obtained independently on the seven different markers: *Cyb*/12S/16S/*αLAlb*/*Cyp*/*Lf*/*κCas* (*Cyb* = cytochrome *b*; 12S = 12S rDNA, 16S = 16S rDNA; *Cyp* = cytochrome oxidase P450; *Lf* = lactoferrin, *αLAlb* = α-lactalbumin; *κCas* = κ-casein). Asterisk indicates that the node was not supported by the bootstrap analysis (<50%). Thick branches indicate higher taxa corresponding to the order, suborders, and families.

position of *Moschus* close to the Bovidae: *αLAlb* (BP = 92) and *Lf* (BP = 96). Within the family Cervidae, there are two major clades: the Plesiometacarpalia, which associates *Cervus* with *Muntiacus* (BP = 100; $b_r = +25$), and the Telemetacarpalia (BP = 96; $b_r = +13$), which includes two clades: *Rangifer* + *Odocoileus* (BP = 100; $b_r = +34$) and *Hydropotes* + *Capreolus* (BP = 100; $b_r = +54$). Within Bovidae, two major clades previously named Bovinae and Antilopinae sensu lato were recovered with high support (respectively, BP = 100/100; $b_r = +18/+26$). The tribes Caprini (*Ovis*), Alcelaphini (*Damaliscus*), and Hippotragini (*Hippotragus*) are strongly associated (BP = 100; $b_r = +22$) with Hippotragini and Alcelaphini as sister taxa (BP = 99; $b_r = +21$). When MP analyses were performed excluding the more distant genera *Balaenoptera* and *Hippopotamus* and keeping only *Tragulus* as an outgroup, three most-parsimonious trees of 1,322,933 steps were recovered (data not shown). The topology of these trees is identical to that of the tree in the previous analysis, except for lack of resolution in the branching pattern of *Aepyceros*, *Cephalophus*, *Gazella*, and *Redunca*. All interfamily nodes were again recovered but with higher support: the basal position of *Antilocapra* (BP = 97), the clade Cervidae + *Moschus* + Bovidae (BP = 95), and the association of *Moschus* with the Bovidae (BP = 96).

When MP analyses were performed with equal weighting of the character-state transformations, two most-parsimonious trees of 5,687 steps were recovered. The interfamily relationships are identical to those from the weighted MP analyses except for the unresolved position of *Moschus*, which appears associated with either Bovidae (BP = 45) or Cervidae (BP = 43) (data not shown). When MP analyses were performed excluding the more distant genera *Balaenoptera* and *Hippopotamus* and using only *Tragulus* as the outgroup, two most-parsimonious trees of 4,832 steps were recovered (data not shown). The topology of these trees is identical to that of the tree in the previous analysis. *Moschus* is allied with either Bovidae (BP = 40) or Cervidae (BP = 36).

ML and Bayesian Phylogenetic Analyses

Different nucleotide substitution models implemented under both PAUP* and PAML were compared. The log-likelihood (lnL) of the best tree was −35848.43 under HKY85, −35543.16 under TN93, and −35360.37 under GTR. Incorporation of the gamma distribution of substitution rate heterogeneity among sites yielded −31 980.92 under GTR + Γ_8 and −31978.43 after adding a fraction of invariable sites. All these log-likelihood increases were significant ($P < 0.05$) under likelihood ratio tests comparing simpler to more complex models. Therefore, the single GTR + Γ_8 + INV model best explained our combination of 7 markers and was used in subsequent analyses.

The highest likelihood and maximum posterior probability phylograms (Fig. 3) are mostly in agreement with the most well-supported nodes of the MP analyses and recovered with high support (BP > 98; Bayesian posterior probability [PP_B] = 1.00) the following clades and their subclades: Pecora; Giraffidae; Cervidae, Plesiometacarpalia, Telemetacarpalia, Old World Telemetacarpalia, and New World Telemetacarpalia; Bovidae, Bovinae, Antilopinae, Caprini + Alcelaphini + Hippotragini, and Alcelaphini + Hippotragini. The Moschidae appears robustly associated with Bovidae (BP = 94; $PP_B = 1.00$), and this group then branches with Cervidae (BP = 71 ; $PP_B = 0.99$). One topology disagreement between MP and ML or Bayesian analyses concerns the location of Antilocapridae. MP suggests that Antilocapridae is the sister group of the remaining pecoran families, but ML suggests an alternative hypothesis with Antilocapridae weakly clustering with Giraffidae (BP = 52 ; $PP_B = 0.57$). Residual BP = 46 and $PP_B = 0.43$ define a basal position of *Antilocapra* among pecorans.

When the closer outgroup *Tragulus* was used instead of the more distant *Balaenoptera* and *Hippopotamus*, all the clades previously identified were recovered, including the sister-group relationship between Moschidae and Bovidae (BP = 92 ; $PP_B = 1.00$) and their association with Cervidae (BP = 71; $PP_B = 1.00$). However, *Antilocapra* is in the basal position with respect to all other Pecora (BP = 77; $PP_B = 1.00$), as seen in MP analyses.

Differences in replacement patterns and rates between mitochondrial and nuclear sequences can lead to biased phylogenetic results (Whelan et al., 2001) and could hinder us from using the powerful approach to analyze the seven markers as a concatenated whole. The simultaneous use of protein-encoding (*Cyb*, *κCas*), ribosomal (12S and 16S rRNAs), and noncoding (*αLAlb*, *Cyp*, *Lf*) sequences may even exacerbate this problem. Thus, we conducted a partitioned ML evaluation of the phylogenetic relationships among Antilocapridae, Giraffidae, Cervidae, Moschidae, and Bovidae. To reach this goal, the KH-SH test was used to evaluate the null hypothesis stating that all 105 trees compared, including the ML tree, are equally good explanations of the data. Because the use of a fraction of invariable sites is not implemented in PAML and to homogenize the models used across the different partitions, a GTR + Γ_8 model was attributed to each of the 12 character partitions.

We computed the log-likelihood of the 105 possibilities of connecting the five pecoran families into a bifurcating subtree, rooted by *Balaenoptera*, *Hippopotamus*, and *Tragulus*. These $15 \times 7 = 105$ rootings arise because there are 15 possible unrooted five-taxon trees, each with seven potential locations for the root. The best rooting, i.e., the one yielding the highest likelihood, is along the branch leading to *Antilocapra* + Giraffidae (Fig. 4). The 104 other topologies were ranked by increasing level of rejection by the KH-SH test (P_{SH} values ranging from 0.93 to 0.004). Among them, 74 are rejected ($P_{SH} < 0.05$). The 30 other topologies correspond to only five pecoran subtrees among 15 possible. In these five subtrees, Moschidae were never associated with either Antilocapridae or Giraffidae, providing further evidence for the tight affinities of *Moschus* with bovids and cervids (Fig. 4). Moreover, we observed that the first rootings

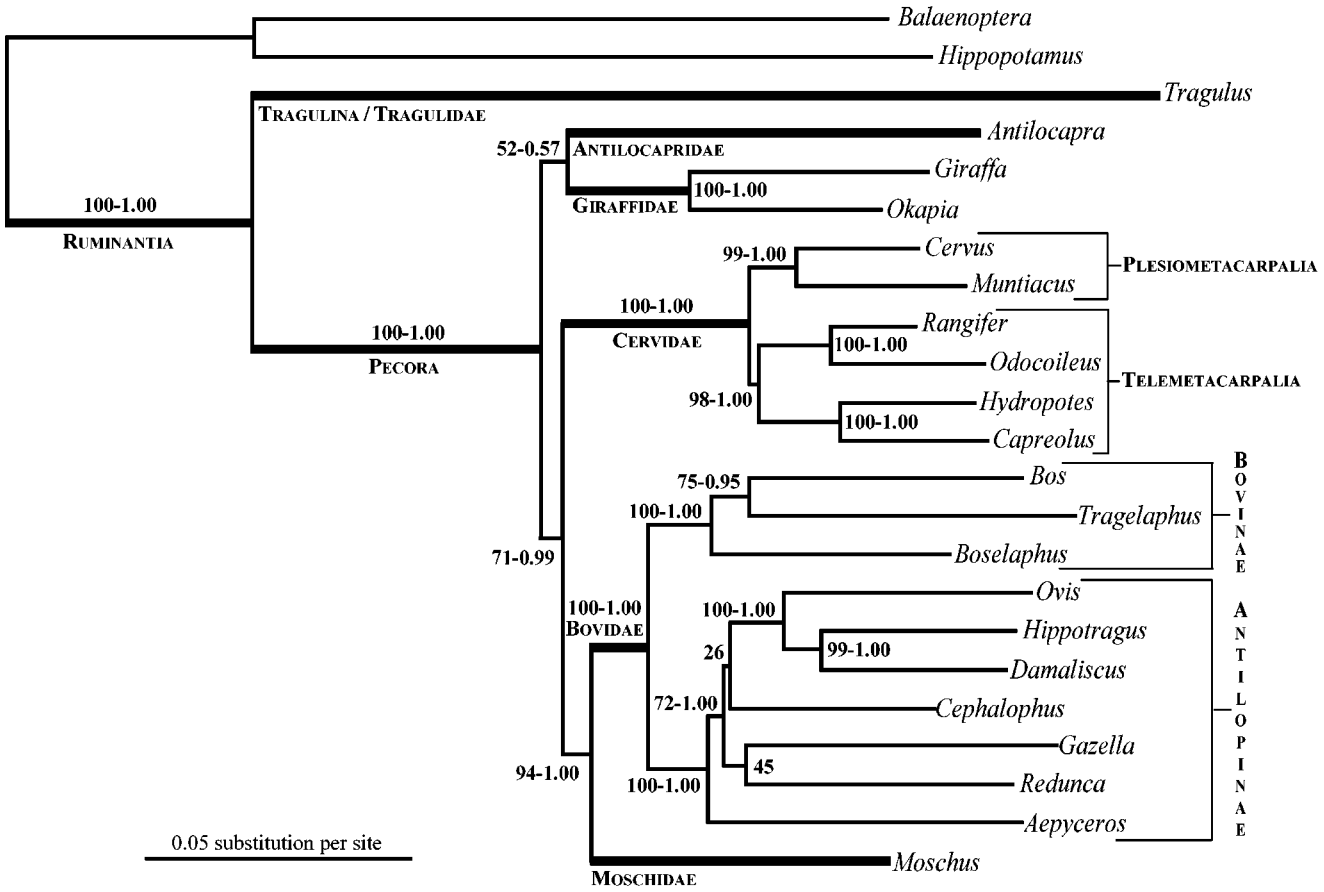

FIGURE 3. Maximum likelihood tree reconstructed from the combination of the seven markers. This highest likelihood tree (lnL = −31978.43) has been reconstructed under the following model of sequence evolution: GTR with rate parameters of 2.47 (A to C), 7.71 (A to G), 1.74 (A to T), 0.75 (C to G), 20.35 (C to T), and 1.00 (G to T), 14% invariable sites, and a gamma rate heterogeneity of $\alpha = 0.33$. The maximum posterior probability tree of the Bayesian approach displays an identical topology. ML bootstrap percentages/posterior probabilities are given for each node. Thick branches indicate higher taxa corresponding to the order, suborders, and families.

breaking the Moschidae–Bovidae clade were numbers 12 and 13 ($P_{SH} = 0.34$). Owing to the conservative behavior of the KH-SH test (Shimodaira, 2002), the latter P_{SH} value should also be viewed as a reasonably good indication of the phylogenetic affinity between Moschidae and Bovidae. This hypothesis is supported by the fact that all rootings separating the musk deer from bovids were rejected by the Kishino-Hasegawa (1989) test ($P_{KH} < 0.05$).

Molecular Estimates of Divergence Ages

The Bayesian relaxed molecular clock method of Thorne et al. (1998) and Kishino et al. (2001) was used to estimate the divergence ages within the pecoran subtree. Two calibration constraints also were simultaneously used: 16.4–23.8 MYA for the first split within bovids and within cervids. Comparison of the prior and posterior divergence ages show that these distributions are different for most nodes, with narrower credibility intervals for posterior ages (Table 3), indicating that much information regarding node times is attributable to the concatenated markers. If we had observed that prior and posterior distributions were about the same, then most molecular dating information would seem to be coming from the priors rather than the data.

The a priori calibration constraints were set to the Early Miocene (16.4–23.8 MYA) for both Cervidae and Bovidae, i.e., for the split between *Cervus* + *Muntiacus* and the other cervids and the split between *Boselaphus* + *Bos* + *Tragelaphus* and the other bovids. Whereas the divergence of the two families was assumed to be contemporary, posterior estimates of the divergence ages for cervids and bovids appear stuck to the lower bound ($CredI_{95\%} = 16.4–19.0$ MYA; Table 3) and upper bound ($CredI_{95\%} = 20.4–23.8$ MYA), respectively, of the Early Miocene, suggesting that the evolutionary radiation of bovids occurred before that of cervids.

The difficulty in finding the root of the pecoran subtree is illustrated by the short time intervals between subsequent divergences. Pecorans diversified between 26.0 and 35.6 MYA, whereas antilocaprids and giraffids appear between 23.9 and 32.5 MYA and bovids, moschids, and cervids diverged 25.1–30.3 MYA during the Late Oligocene (cf $CredI_{95\%}$; Table 3). The split between Moschidae and Bovidae occurred between 23.6 and

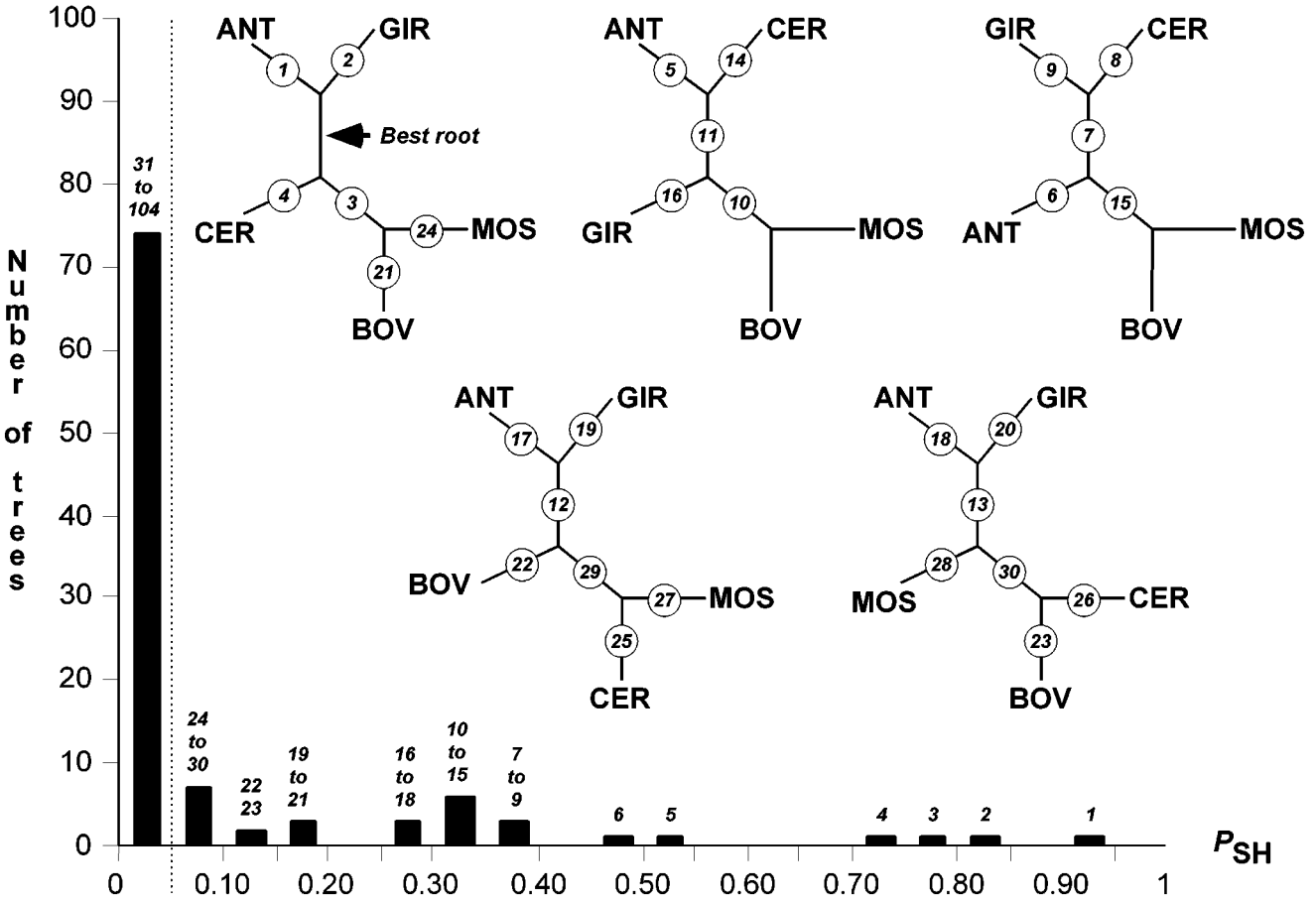

FIGURE 4. Confidence levels of the conservative Shimodaira–Hasegawa test for the location of the root of the pecoran subtree under a 12-partition ML analysis. The 105 possibilities of rooted trees for relationships among the five pecoran families were explored and ranked according to their increasing level of rejection (i.e., circled numbers on the trees and numbers on histogram bars correspond to decreasing probability values [P_{SH}]). For the computation of log-likelihoods, each of the 12 character partitions had its own GTR + Γ_8 model. Relative to the highest likelihood tree, 74 rooting possibilities were statistically rejected. The 30 remaining root positions that were not rejected are located on the five illustrated unrooted subtrees. Among them, Moschidae is never directly associated with either Antilocapridae or Giraffidae. The vertical dotted line corresponds to the $P_{SH} = 0.05$ rejection level. For the present data, a probability value of 0.30 for the Shimodaira–Hasegawa test corresponds to a P value of 0.05 for the Kishino–Hasegawa test. ANT, BOV, CER, GIR, and MOS are Antilocapridae, Bovidae, Cervidae, Giraffidae, and Moschidae, respectively.

28.5 MYA during the Late Oligocene. All subsequent cladogenesis within giraffids, cervids, and bovids then occurred during the Early and Middle Miocene (Table 3). The SDs for splitting ages ranged from 1.4 to 4.0 MYA for the two most basal dichotomies among ruminants and from 0.7 to 1.7 MYA for cladogenic events within pecorans.

The use of two different reference topologies because of the ambiguous location of the pecoran root seems not to have affected the posterior distributions of divergence ages by >0.1 MYA for divergence within the Bovidae + Moschidae + Cervidae clade and by >0.9–2.7 MYA for Giraffidae and the base of the ruminant subtree (Table 3).

Analysis of Morphological Characters

Of 48 total characters, only 12 were parsimony informative for the relationships among pecoran families (highlighted sites, Table 2): position of the cardiac orifice of rumen (character 3), bridge on the metatarsal gully (11), presence of posterior metatarsal tuberosity (12), fusion of the internal cuneiform (14), loss of lateral metacarpals (17), number of ribs (21), size of the foramen ovale (27), presence of cranial appendages (29), bone core covered by keratinous sheath (33), cranial appendages only in males (36), long and curved upper canines (38), and presence of metastylid on the lower molars (45).

Of 105 trees evaluated, 6 most-parsimonious trees of 55 steps were retained (CI excluding uninformative characters = 0.684, retention index = 0.539). The differences between the six MP trees involve the position of *Moschus*, which appears close to either Bovidae or Cervidae or basal with respect to Bovidae and Cervidae, and the position of *Antilocapra*, which appears either basal within Pecora or as sister taxon of the clade composed of Bovidae, Moschidae, and Cervidae. The strict consenus (Fig. 1) is congruent with the molecular analyses: Cervidae, Moschidae, and Bovidae are enclosed together (b = +1) and this result is supported by a single exclusive synapomorphy, a large foramen ovale (27).

TABLE 3. Molecular dating of the main splitting events within Pecora. The Bayesian relaxed molecular clock approach of continuous autocorrelation of evolutionary rates along branches developed by Thorne et al. (1998) and Kishino et al. (2001) was used. Two calibration points allowed the estimate of absolute divergence ages: the first splits within Bovidae and within Cervidae were constrained to occur in the Early Miocene, i.e. 16.4–23.8 million years ago (MYA). Values correspond to the prior and posterior divergence ages ±1 SD, calculated according to two slightly different topologies: *Antilocapra* as sister group to Giraffidae or in the basal position among pecorans. The 95% credibility intervals ($CredI_{95\%}$) for prior and posterior divergence ages are given in parentheses. The corresponding geologic time scale is also given. The following posterior distributions were obtained (values for the *Antilocapra* + Giraffidae topology): 44.3 (SD = 3.5, $CredI_{95\%}$ = 37.6–51.7) MYA for the expected time between tip and root, 0.0078 (SD = 0.0009, $CredI_{95\%}$ = 0.0061–0.0097) substitutions per site per million years for the rate at root node, and 0.0039 (SD = 0.0021, $CredI_{95\%}$ = 0.0012–0.0094) for the parameter that controls the amount of rate autocorrelation per million years.

	Ages (MYA)				
	Antilocapra + Giraffidae		*Antilocapra* basal		
Divergences between taxa	Prior	Posterior	Prior	Posterior	Epoch
Tragulina/Pecora	33.0 ± 7.5 (22.4–51.9)	44.3 ± 3.6 (37.6–51.7)	35.8 ± 8.0 (23.8–55.6)	46.3 ± 4.0 (39.1–54.8)	Middle Eocene
Radiation of Pecora	29.4 ± 6.2 (20.7–45.1)	28.9 ± 1.4 (26.0–31.7)	32.4 ± 7.0 (22.2–49.2)	31.6 ± 2.0 (27.9–35.6)	Early Oligocene
Antilocapridae/Giraffidae	19.5 ± 8.2 (4.5–36.7)	27.0 ± 1.6 (23.9-30.1)			Late Oligocene
Giraffidae/(Cervidae + Moschidae + Bovidae)			29.0 ± 5.7 (20.7–43.2)	29.4 ± 1.5 (26.4–32.5)	Early Oligocene
Giraffa/Okapia	9.6 ± 7.4 (0.3–26.6)	19.0 ± 1.6 (16.0–22.3)	14.5 ± 8.9 (0.8–32.8)	19.9 ± 1.7 (16.7–23.2)	Early Miocene
Cervidae/(Moschidae + Bovidae)	25.7 ± 4.5 (19.2–36.9)	27.7 ± 1.3 (25.1–30.1)	25.5 ± 4.3 (19.2–36.3)	27.8 ± 1.3 (25.1–30.3)	Late Oligocene
Cervidae (constraints: 16.4–23.8 MYA)	20.1 ± 2.1 (16.6–23.6)	17.2 ± 0.7 (16.4–19.0)	20.1 ± 2.1 (16.6–23.6)	17.2 ± 0.7 (16.4–19.0)	Early Miocene
Cervus/Muntiacus	10.1 ± 6.0 (0.5–21.1)	13.8 ± 0.9 (12.1–15.7)	10.0 ± 5.9 (0.5–20.8)	13.7 ± 0.9 (12.1–15.7)	Middle Miocene
Hydropotes/Capreolus	6.7 ± 4.8 (0.2–17.3)	10.3 ± 0.9 (8.6–12.3)	6.6 ± 4.8 (0.2–17.4)	10.3 ± 0.9 (8.6–12.2)	Late Miocene
Rangifer/Odocoileus	6.6 ± 4.8 (0.2–17.3)	11.5 ± 0.9 (9.8–13.5)	6.7 ± 4.9 (0.2–17.4)	11.5 ± 0.9 (8.6–12.2)	Middle/Late Miocene
(*Hydropotes* + *Capreolus*)/(*Rangifer* + *Odocoileus*)	13.4 ± 5.0 (3.1–21.6)	16.2 ± 0.8 (14.9–18.1)	13.4 ± 5.0 (3.1–21.5)	16.2 ± 0.8 (14.9–18.0)	Early/Middle Miocene
Moschidae/Bovidae	22.8 ± 3.5 (17.7–31.3)	26.1 ± 1.2 (23.6–28.3)	22.6 ± 3.4 (17.6–30.7)	26.2 ± 1.2 (23.6–28.5)	Late Oligocene
Bovidae (constraints: 16.4–23.8 MYA)	19.8 ± 2.1 (16.6–23.5)	22.6 ± 0.9 (20.4–23.8)	19.8 ± 2.1 (16.6–23.5)	22.6 ± 0.9 (20.4–23.8)	Early Miocene
Bovinae	13.2 ± 4.9 (3.1–21.4)	19.3 ± 1.1 (17.0–21.2)	13.2 ± 4.8 (3.3–21.4)	19.3 ± 1.1 (17.0–21.3)	Early Miocene
Antilopinae	16.5 ± 3.3 (9.0–22.2)	19.7 ± 1.0 (17.4–21.5)	16.5 ± 3.3 (9.1–22.3)	19.6 ± 1.0 (17.4–21.4)	Early Miocene
Hippotragus/Damaliscus	3.3 ± 2.8 (0.1–10.3)	13.1 ± 1.1 (11.0–15.3)	3.3 ± 2.8 (0.1–10.5)	13.1 ± 1.1 (11.0–15.2)	Middle Miocene
Ovis/(*Hippotragus* + *Damaliscus*)	6.5 ± 3.6 (1.0–14.3)	15.1 ± 1.1 (13.0–17.2)	6.6 ± 3.6 (1.0–14.4)	15.1 ± 1.1 (12.9–17.2)	Middle Miocene

DISCUSSION

Monophyly of the Pecora

The five living pecoran families, Antilocapridae, Bovidae, Cervidae, Giraffidae, and Moschidae, are classically unified as higher ruminants, or pecorans, and are distinguished from tragulids by numerous morphological characters (Janis and Scott, 1987). This distinction between Tragulina and Pecora is here confirmed by our DNA data, with *Tragulus* (Tragulidae) diverging first with respect to all other ruminant families. The monophyly of Pecora, here represented by an exhaustive sampling of the five living families, is highly supported in terms of bootstrap and Bayesian support (100, whatever the method applied), and 23 autapomorphies were found for this clade, including three diagnostic indels (deletion of T at position 401 of the 12S rRNA; insertion of A at position 222 of the *Lf*, and a deletion of nine nucleotides [GTAGGGCYA] at position 98 of the *Lf*). This huge number of exclusive synapomorphies probably reflects the long divergence time between the Tragulina/Pecora split (44.3–46.3 MYA) and the pecoran radiation (28.9–31.6 MYA), which spans a minimum of 12.7 million years as here estimated by the Bayesian relaxed molecular clock.

Monophyly of the Pecoran Families and Subfamilies

All our molecular results strongly support the monophyly of all the families for which several representatives are available: Bovidae, Cervidae, and Giraffidae ($BP > 98$, $PP_B = 1.00$). Many molecular autapomorphies characterize these families: 5 for Bovidae, 16 for Giraffidae, and 17 for Cervidae. The most striking molecular signatures are in Giraffidae, with two long deletions (6 nucleotides [ATAAGC] at position 401 of *αLAlb* and

8 nucleotides [GCCCCAGG] at position 113 of *Lf*), and Cervidae, with two insertions (A at position 760 of 16S rRNA and T at position 7 of *Cyp*) and one large deletion of 16 nucleotides (CATAAAAGGCAACAGG at position 381 of *αLAlb*). Two distinct clades, previously proposed on the basis of the structure of the metacarpals, were recovered within the family Cervidae: the Plesiometacarpalia (*Cervus* + *Muntiacus*; BP = 99–100, $PP_B = 1.00$), which are morphologically characterized by the retention of both distal and proximal parts of the metacarpals (of digits II and V), and the Telemetacarpalia (*Rangifer* + *Odocoileus* + *Hydropotes* + *Capreolus*; BP = 96–98, $PP_B = 1.00$), in which the metacarpals are greatly reduced with only the distal parts remaining. This distinction is also supported by signatures such as a deletion of 12 nucleotides for the Telemetacarpalia (TAATACCCTGTA at position 259 of *αLAlb*). Within Bovidae, two major clades previously named Bovinae and Antilopinae sensu lato (Hassanin and Douzery, 1999a, 1999b) were recovered with high support (BP = 97–100, $PP_B = 1.00$) but without diagnostic signatures. Although some workers have placed *Antilocapra americana* within Bovidae (e.g., O'Gara and Matson, 1975), our results confirm its family status in the monotypic family Antilocapridae (e.g., Simpson, 1945) because it appears clearly outside of Bovidae. Similarly, the genus *Moschus*, represented by the living musk deer species, has traditionally been regarded as constituting the subfamily Moschinae within Cervidae (e.g., Simpson, 1945), but a number of authors have suggested that *Moschus*, should be placed in its own family within the superfamily Cervoidea (e.g., Gray, 1821; Brooke, 1878; Flerov, 1952; Webb and Taylor, 1980; Groves and Grubb, 1987). Our molecular tree supports the family status of Moschidae because *Moschus* was not grouped within Cervidae.

Interfamily Relationships within the Pecora

Morphology.—The Pecora are generally recognized as a monophyletic group, but their interfamily relationships are poorly understood, and recent morphological studies have produced numerous conflicting hypotheses (Fig. 1). Many of the characters used for pecoran phylogeny are known to be homoplastic (Scott and Janis, 1993), and depending on the authors, different character states can be defined for each of the various families. Given that these problems are especially critical in MP analyses, the definition and/or the value of several characters traditionally used for pecoran taxonomy should be examined.

Cranial appendages have been widely used in the reconstruction of pecoran phylogeny. Webb and Taylor (1980) proposed the term Eupecora to designate all the Pecora characterized by the presence of cranial appendages, i.e., all families except the Moschidae. However, the absence of cranial appendages in *Moschus* can be alternatively interpreted as a secondary loss, exactly as demonstrated for *Hydropotes* in Cervidae (Randi et al., 1998). Other authors have allied bovids with antilocaprids because both share horns composed of a bone core covered with a keratinous sheath (O'Gara and Matson, 1975). Nevertheless, deciduous keratinous sheaths are also encountered in some specimens of *Okapia* (Frechkop, 1955), which is why we coded this character as unknown in the ancestor of Giraffidae (character 33, Table 2). Bovids have also been grouped with giraffids because they are supposed to share a similar developmental pathway with a separate epiphyseal os cornu (Frechkop, 1955). Nonetheless, the supposed homology between bovid horns and giraffid ossicones appears to be based on the erroneous assumption that a separate ossified os cornu is routinely formed in bovids. In bovids, as in cervids, the cranial appendages are formed from an outgrowth of the frontal bone, whereas in giraffids these appendages are formed from a dermal ossification center (Janis and Scott, 1987). Unfortunately, the developmental condition in antilocaprids has not been determined.

Simplified and high-crown or hypsodont cheek teeth has traditionally been used to group Antilocapridae with Bovidae whereas has been used to group Giraffidae with Cervidae the possession of low-crown or brachyodont cheek teeth with many accessory styles and ribs (e.g., Romer, 1966). Although the height of cheek teeth has been widely used in ruminant phylogeny, Janis and Scott (1987) considered this character to be without taxonomic value because it has evolved in parallel many times within herbivorous mammals (e.g., Proboscidea, Rhinocerotidae, Equidae, Suidae, and Cameleidae). Moreover, this character is problematic because of the ambiguity in its definition. For example, *Moschus* and *Hydropotes* were considered hypsodont by Gentry and Hooker (1988) but brachyodont by Scott and Janis (1993). Furthermore, hypsodonty is variable within Bovidae, some species being more brachyodont than others. Considering these problems, hypsodonty was not included in our morphological character data matrix.

The possession of two lacrimal orifices situated on the orbital rim has been used to associate Antilocapridae with Cervidae (Leinders, 1979; Leinders and Heintz, 1980). The number of lacrimal orifices is a very ambiguous character since it is highly variable in bovids—with one, two or even three orifices—and since it may even be variable within a species. For example, one or two orifices are found for *Antilocapra americana* and *Moschus moschiferus* (Scott and Janis, 1987; personal observations).

The association of Antilocapridae with Cervidae and Moschidae was also proposed on the basis of the presence of a closed metatarsal gully (Leinders, 1979; Leinders and Heintz, 1980; Janis and Scott, 1987; Scott and Janis, 1987). The mode of fusion of the metatarsal gully does not include a bridged distal end in bovids and giraffids, whereas a closed metatarsal gully is present in cervids and antilocaprids (Leinders and Heintz, 1980). This character is not constant in *Moschus*; an open gully was observed in one specimen by Janis and Scott (1987). This character is also variable in Tragulidae; the metatarsal gully of *Tragulus* is open and that of *Hyemoschus* is closed.

The complete loss of side toes has been used to unite Antilocapridae with Bovidae, but it does not constitute

a real synapomorphy. Actually, the complete loss of side toes is encountered in antilocaprids and some bovid species but also in giraffids. In bovids, the second and fifth digits are generally not completely lost because of the retention of proximal remnants (dewclaws). Because the loss of lateral digits can be functionally associated with increasing cursoriality in open habitats, this character is expected to be homoplastic for ruminant phylogeny (Scott and Janis, 1993) and has apparently evolved in parallel many times within cetartiodactyls (e.g., Camelidae and Tayassuidae).

The retention of a gall bladder has been used to unite Antilocapridae with Bovidae (e.g., O'Gara and Matson, 1975; Gentry and Hooker, 1988), but this organ is also present in Tragulidae, Moschidae, and some Giraffidae (Frechkop, 1955). Its absence is probably a derived character of the Cervidae that has also been acquired independently by some giraffids (Groves and Grubb, 1987).

We built a new morphological matrix (Table 2) using the two following assumptions for the coding of character states: (1) all the six ruminant families were considered monophyletic and (2) all the character states examined here were considered the primitive states within each of the families. Our analysis based on 48 morphological characters revealed that only two of these characters were phylogenetically informative for establishing interfamily relationships within Ruminantia. The results suggest that Antilocapridae and Giraffidae emerged first with respect to a clade composed of the families Bovidae, Moschidae, and Cervidae (Fig. 1). Although this topology is in perfect agreement with our molecular trees, the nodes are poorly supported and only one unambiguous synapomorphy supports the grouping of bovids, moschids, and cervids: the presence of a large foramen ovale (character 27, Table 2). Although this character was implicitly coded as discontinuous by Scott and Janis (1993), and in our data matrix, this character is continuous and is therefore of less interest because its coding can be variable depending on the observer.

Molecular phylogeny: Moschus + *Bovidae*.—The living musk deer (*Moschus*) was regarded as a member of the family Cervidae (e.g., Simpson, 1945; Viret, 1961; Eisenberg, 1987; Nowak, 1991) because it possesses a cervidlike closed metatarsal gully. Because of the absence of antlers and the possession of sabrelike upper canines, the Moschinae were often considered a basal offshoot within the Cervidae, as it was assumed for *Hydropotes* (Chinese water deer), which also lacks antlers and possesses sabrelike upper canines. Contrary to *Hydropotes*, *Moschus* exhibits several features of the soft anatomy, that are supposed to be primitive with respect to all living cervids: a gall bladder, an ileocecal gland, an intestine with three and a half (as opposed to two and a half) colic coils, and a placenta with many (rather than few) cotyledons. For these reasons, *Moschus* is more generally included in its own family, the Moschidae. Because it lacks the cervid feature of a double lacrimal orifice, Leinders (1979) and Leinders and Heintz (1980) considered *Moschus* to be less closely related than *Antilocapra* to cervids. Webb and Taylor (1980) considered all living Pecora except the Moschidae (the Eupecora) to be united by the presence cranial appendages.

The present morphological analysis indicates that *Moschus* is allied with the families Bovidae and Cervidae. This result confirms the view of several authors who have noted that the musk deer exhibits a mixture of bovid and cervid characters (Leinders and Heintz, 1980). However, the molecular placement of *Moschus* as the sister group of bovids rather than cervids is unexpected, because morphologists have never proposed this evolutionary possibility. Phylogenetic analyses using a large sample of pecoran species for *Cyb* sequences have already suggested that *Moschus* could be more closely related to Bovidae than Cervidae (Hassanin, 1999), but these relationships were not robustly supported. Here, the association of *Moschus* with Bovidae is supported by (1) Bayesian, ML, and MP analyses, (2) the separate analyses of two nuclear markers (MP, Fig. 2; *Lf*: BP = 92 and *αLAlb*: BP = 96), and (3) the analysis combining seven markers (BP = 95, Fig. 2; $BP_{ML} = 94$, $PP_B = 1.00$, Fig. 3). The log-likelihoods of the best tree under models with a single partition versus 12 partitions are −31980.15 and −30322.09, respectively. Therefore, the 12-partition model better describes the evolution of the seven markers ($P < 0.001$ under a likelihood ratio test), and the phylogenetic results observed (i.e., the sister group relationship between Moschidae and Bovidae) does not reflect the use of an oversimplified ML model (Whelan et al., 2001).

Moreover, the position of *Moschus* close to Bovidae remains strongly supported when phylogenetic relationships are reconstructed using a less divergent outgroup, such as *Tragulus*. Two diagnostic molecular signatures characterized the grouping of *Moschus* with Bovidae (C → T, *Lf*, position 128; and C → G, *αLAlb*, position 80), whereas no signature was detected for *Moschus* considered either sister to Cervidae or basal to the clade joining bovids and cervids. From a paleontological point of view, the case of *Hispanomeryx* might reflect the close relationships between *Moschus* and Bovidae; this Miocene genus of Spain, which does not possess cranial appendages, has been alternatively included in Moschidae (Morales et al., 1981) and Bovidae (Moyà-Solà, 1986).

Hoplitomeryx, from the late Miocene of Italy, seems crucial for a better understanding of the interrelationships between Cervidae, *Moschus*, and Bovidae. This endemic genus of the island fauna of Monte Gargano was placed in a new family, Hoplitomerycidae, on the basis of two unusual properties of its skull (Leinders, 1983): (1) it possesses five cranial appendages, i.e., one in medial position on the nasal bone and four in supraorbital position on the frontal bone, and (2) it bears daggerlike upper canines, whereas the presence of large upper canines is always correlated with the absence of cranial appendages in all extant pecorans. Leinders (1983:41) described the cranial appendages of *Hoplitomeryx*: "they show typical features of horncores as present in the Bovidae and they almost certainly once were covered by a keratine sheath." Janis and Scott (1987:57) reported that "the deep grooving of the horn cores of *Hoplitomeryx* is

reminiscent of the condition of bovid horn cores with a permanent keratin sheath, rather than the spongy texture of the antilocaprid horn core." Nevertheless, Leinders concluded that horns developed independently in Bovidae and placed Hoplitomerycidae as the sister family of Cervidae because they share a double lacrimal orifice and a closed metatarsal gully. However, these two characters are highly variable in taxa other than cervids. For instance, specimens of *Moschus* have either one or two lacrimal orifices, and the gully on the anterior side of metatarsal III/IV is either open or closed. These two characters are therefore very doubtful for linking *Hoplitomeryx* with Cervidae. In contrast, there is no evidence of parallel evolution of bovidlike horns. Hence, we suggest that Hoplitomerycidae is closer to Bovidae than to Cervidae. *Hoplitomeryx* differs from Bovidae in having five rather than two (most bovids) or four (*Tetracerus quadricornis*) horns and by the presence of large sabrelike upper canines. Males of *Moschus* and *Hydropotes*, which are the sole extant pecorans that lack cranial appendages, use their upper canines as weapons in intraspecific combat (Grzimek, 1968) and probably as means of defense against predators. On the basis of the absence of antlers, *Hydropotes* was considered as the sister group of all living antlered cervids (e.g., Groves and Grubb, 1987; Janis and Scott, 1987), but recent molecular analyses have clearly shown that *Hydropotes* lost the antlers secondarily (Randi et al., 1998; this study). The development of large upper canines may be therefore interpreted as a secondary adaptation for intraspecific fighting. Similarly, the acquisition of sabrelike upper canines in *Moschus* may have occurred after the loss of horns from a bovidlike ancestor, i.e., one without large upper canines. Small upper canines have been found in Miocene bovids, e.g., *Protragocerus labidotus* (Gentry, 1970). In addition, vestiges of upper canine alveoli can be seen in some extant genera of Bovidae (e.g., *Tetracerus, Cephalophus, Oreotragus*), and the occasional occurrence of rudimentary upper canines has been recorded in various species (e.g., *Neotragus pygmaeus, Ourebia ourebi, Gazella granti, Rupicapra rupicapra*) (Dekeyser and Derivot, 1956; Gentry, 1970). However, *Hoplitomeryx* is unique among extant and fossil ruminants because it possesses a large set of weapons, with five horns and large upper canines. Leinders (1983) suggested that this remarkable adaptation could have developed as a consequence of insularity to protect against large birds of prey, which were the exclusive predator of *Hoplitomeryx*.

Molecular phylogeny: basal position of Antilocapridae and Giraffidae.—Our DNA results suggest two possibilities about the phylogenetic position of antilocaprids and giraffids. First, MP analyses suggest a basal position for *Antilocapra*, in agreement with previous molecular investigations that suggested the first divergence of Antilocapridae within Pecora (BP = 75–81) (Cronin et al., 1996; Montgelard et al., 1997; Gatesy and Arctander, 2000; Matthee et al., 2001). Two autapomorphies that appear to be transversions support the placement of Giraffidae with Cervidae, *Moschus*, and Bovidae (T → A [G for *Giraffa*], 16S, position 541; and T → G, *κCas*, position 314). Second, ML analyses suggest the grouping of *Antilocapra* with the Giraffidae. Two autapomorphies, again transversions, define this association (A → T, 16S, position 586; and A → C [T for *Giraffa*], *Cyb*, position 897).

The ML conclusion of the phylogenetic analysis of the six markers, described as a combination of 12 partitions, is that two competing phylogenetic hypotheses for the radiation of Pecora families are observed. The first one, supported by the MP and some ML analyses is that Antilocapridae is the first offshoot among Pecora, followed by Giraffidae, and then the three remaining families. The second one is that Antilocapridae and Giraffidae are sister groups and represent the sister clade of Bovidae, Cervidae, and Moschidae. The conservative SH test does not discriminate ($P_{SH} = 0.93$) between these alternative rootings of the pecoran subtree (Fig. 4).

Despite the fact that the relative position of Antilocapridae and Giraffidae is not resolved, it seems clear that both families diverged before the divergence of the families Bovidae, Cervidae, and Moschidae. This result is supported by all of our analyses, and three nuclear DNA signatures are diagnostic for the clade uniting bovids, moschids, and cervids (C → T, *Cyp*, position 199; G → C, *αLAlb*, position 281; and C → T, *Lf*, position 276). This result is also corroborated by our morphological analysis (Fig. 1). The single morphological synapomorphy uniting Cervidae, Moschidae, and Bovidae is the presence of a large foramen ovale. Other poorly explored characters tend to give more credit to this clade. With regard to the stomach anatomy, the entrance of the oesophagus to the rumen is more ventrally positioned in bovids and cervids (Hofmann, 1973) than in the giraffe (*Giraffa*). Unfortunately, the position of the oesophagus in *Antilocapra, Okapia*, and *Moschus* is not known. The major volatile compounds in the interdigital glands of *Antilocapra* are clearly different from those discovered in various species of Bovidae and Cervidae (Wood, 2001). These biochemical differences may also reflect the distinctness of bovids and cervids with respect to antilocaprids. Interdigital gland compounds have not been determined for Moschidae and Giraffidae.

Paleontology and Biogeography

What are the diagnostic characters of Ruminantia in the fossil record?—Numerous recent molecular analyses have shown that the order Cetartiodactyla is composed of five major groups: Tylopoda (camels and llamas), Suina (pigs and peccaries), Hippopotamidae, Cetacea, and Ruminantia. There is a strong consensus among molecular systematists to unite Cetacea with Hippopotamidae, to associate this clade with Ruminantia, and to consider Tylopoda as the first offshoot among Cetartiodactyla (e.g., Graur and Higgins, 1994; Irwin and Arnason, 1994; Gatesy et al., 1996, 1999; Gatesy, 1997; Montgelard et al., 1997; Shimamura et al., 1997, 1999; Nikaido et al., 1999; Madsen et al., 2001; Murphy et al., 2001a, 2001b). These molecular results therefore invalidated the grouping of Tylopoda and Ruminantia into Selenodontia, as assumed by many paleontologists

(e.g., Lavocat, 1955; Romer, 1966; Webb and Taylor, 1980; Scott and Janis, 1987; Gentry and Hooker, 1988). In this context, all the skeletal characters used to unite these two suborders must be considered to have evolved in parallel, probably as the consequence of similar ecological constraints.

Ruminants and tylopods are terrestrial herbivores and feed primarily on fibrous vegetation. They independently acquired a complex stomach with multiple chambers that permits rumination, i.e., they are able to digest cellulose because of a symbiotic relationship with microorganisms. The dentition of ruminants also reflects the evolutionary trends toward an herbivorous diet. The cheek teeth are selenodont (and not bunodont as in Suina and *Hippopotamus*), and there is a spacious diastema between labial teeth (incisors and canine) and cheek teeth (molars and premolars). Although the latter characters evolved in parallel in tylopods, ruminants are easily distinguishable by two dental synapomorphies: the upper incisors are absent and replaced by a horny pad, and the lower canine is incisiform and contiguous with the three incisors. Moreover, the upper canine is absent or its presence is sexually dimorphic, i.e., large and curved in males and smaller in females (in Tragulidae, Moschidae, *Muntiacus*, and *Hydropotes*).

Evolution of the limb skeleton of Ruminantia parallels changes in stomach anatomy and dentition. Ruminants and tylopods live in more open habitats than do other cetartiodactyls, and as a result they have developed convergent adaptations to better cursoriality: Lateral digits II and V are either absent or reduced, and in the hind feet metatarsals III and IV are always fused into a canon bone. However, ruminants possess two derived limb characteristics: in the tarsus the navicular and cuboid are fused, and in the carpus the magnum and trapezoid are fused.

The most recent common ancestor of all extant ruminants is expected to display the following diagnostic features: (1) cubonavicular, (2) fusion of the magnum and trapezoid, (3) absence of upper incisors, and (4) inclusion of the lower canine in the incisor row. All other characters must be regarded with caution to signal the first occurrence of Ruminantia in the fossil record.

Middle Eocene origin of ruminants.—In the Middle/Late Eocene, a peak of herbivore diversity occurred for mammals in Europe, Asia, and North America, reflecting the diversification of selenodont cetartiodactyls, perissodactyls, rodents, and lagomorphs. This epoch was characterized by increasing seasonal dryness that favored the development of more open, less forested habitat than occurred earlier in the Eocene (Janis, 2000b).

In agreement with our mean molecular estimation of 44.3–46.3 MYA for the Tragulina/Pecora split (Fig. 5), the fossil record indicates that ruminant traits appeared suddenly in the Middle–Late Eocene of the Northern Hemisphere: Amphimerycidae in Europe (*Amphimeryx*, France), Archaeomerycidae in Central Asia (*Archaeomeryx*, Mongolia), and Leptomerycidae (*Leptomeryx*) and Hypertragulidae (*Hypertragulus* and *Simimeryx*) in North America (Webb and Taylor, 1980; Vislobokova, 1997; Webb, 1998). All members of these families have a cuboid fused with the navicular (Viret, 1961; Geraads et al., 1987; Gentry and Hooker, 1988; Scott and Janis, 1993) and incisiform lower canines (Gentry and Hooker, 1988). Leptomerycidae are however more derived than other families; contrary to Amphimerycidae, Archeomerycidae, and Hypertragulidae, they do not possess upper incisors, and contrary to Hypertragulidae, they present a fusion of the trapezoid and magnum. Leptomerycidae alone possess all four diagnostic features expected in the common ancestor of all extant ruminants. The close relationships of Leptomerycidae with Ruminantia has been supported by cladistic analyses; they have been hypothesized as sister taxa to either Tragulina (Geraads et al., 1987) or Pecora (Janis and Scott, 1987, Gentry and Hooker, 1988; Scott and Janis, 1993).

At the beginning of the Middle Eocene, Europe and Asia were separated from Africa, and North America was seperated from South America. The rifting of the North Atlantic cut off the dispersal routes between Europe and North America, and in parallel the sea level rose and seas invaded much of Siberia (Raven and Axelrod, 1974; McKenna, 1983; MacFadden, 1992). This inundation created a significant oceanic barrier to faunal interchange, and as a consequence the mammalian faunal similarity between Palaearctica and Nearctica decreased dramatically. This divergence is illustrated by early perissodactyls, for which there was a split resulting in Equidae in North America and Palaeotheriidae in Eurasia (MacFadden, 1992). A similar vicariant separation may be inferred also for fossils related to ruminants at this time, with Hypertragulidae and Leptomerycidae in North America, Amphimerycidae in Europe, and Archaeomerycidae in Asia.

First occurrence of Pecora in the early oligocene of Eurasia.—The major division of Ruminantia between Pecora and Tragulina is widely accepted among morphologists and molecular systematists, with Pecora including Antilocapridae, Bovidae, Cervidae, Giraffidae, and Moschidae and Tragulina including only Tragulidae. However, there is no consensus among paleontologists for establishing the phylogenetic affinities of the fossils from Eocene and Oligocene deposits. Therefore, it seems particularly important to define the morphological differences between extant Pecora and Tragulina.

All modern families of Pecora are associated with more open habitats than are Tragulidae, which are exclusively found in overgrown tropical forests in southeast Asia and Africa. Consequently, limb features of Pecora reveal better adaptation to cursoriality: (1) third and fourth metacarpals are completely fused into a canon bone, whereas in tragulids they are either unfused (African tragulids) or partially fused (Asian tragulids); (2) the astragalus is compact and has parallel sides; and (3) there are complete distal metapodial keels (Frechkop, 1955; Scott and Janis, 1993). In addition, tragulids are small, with a shoulder height that ranges from 20 to 35 cm, whereas pecorans are generally much larger: 81–104 cm for antilocaprids, 150–370 cm for giraffids (*Okapia*: 150–170 cm; *Giraffa*: 250–370 cm), 50–61 cm for moschids,

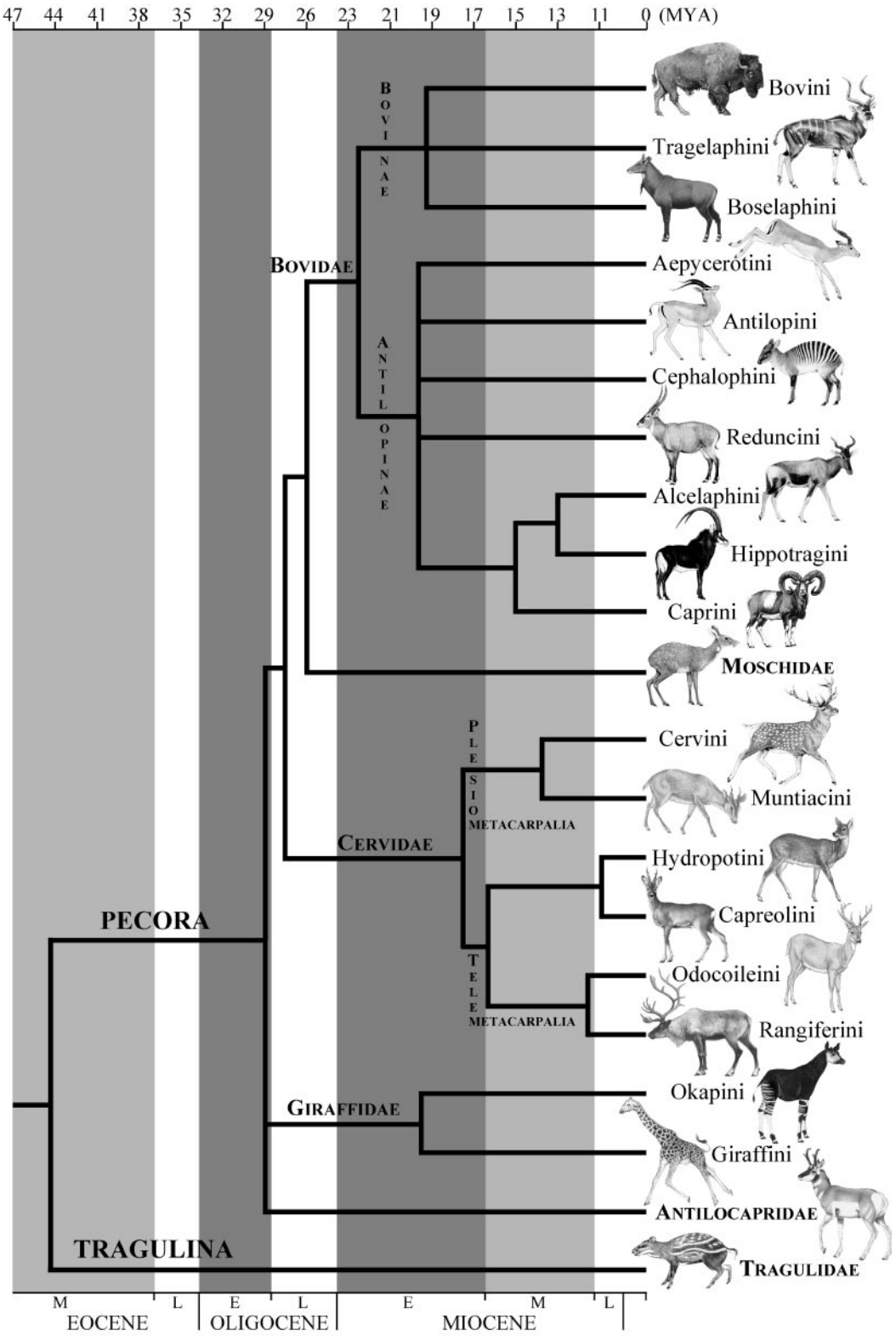

FIGURE 5. Consensus phylogenetic tree from the MP, ML, and Bayesian analyses and a time scale for ruminant evolution as inferred from a Bayesian relaxed molecular clock approach. Only the nodes strongly supported by the three methods (BP > 70, PPB > 0.99) are indicated in this consensus.

24–220 cm for bovids, and 25–190 cm for cervids (Nowak, 1991). Small pecorans are represented by bovid and cervid species secondarily adapted to forests or areas of dense vegetation, e.g., dwarf antelope in Africa (24–41 cm), muntjacs in Asia (40–80 cm), or pudus in South America (25–43 cm). All extant pecorans except *Moschus* and *Hydropotes* have cranial appendages. However, the presence of cranial appendages in the common ancestor of modern pecorans is not certain; several authors have suggested that horns, antlers, and ossicones are not homologous (e.g., Janis and Scott, 1987; Gentry and Hooker, 1988; Scott and Janis, 1993).

The Eocene/Oligocene boundary at around 34 MYA is correlated with the expansion and diversification of grasslands, when the warm and damp environment of the Eocene changed to colder and dryer Oligocene conditions (Prothero and Heaton, 1996; Meng and McKenna, 1998). During the Late Eocene and Oligocene, the number of small herbivores declined severely, whereas a significant increase was recorded in medium-size animals, almost entirely represented by tylopods, equids, and ruminants in North America (Janis, 2000b). Our molecular estimation indicates that the evolutionary radiation of Pecora occurred at the Early Oligocene (Fig. 5) between 28.9 and 31.6 MYA, i.e., just after the Eocene/Oligocene transition referred as the Grande Coupure (Stehlin, 1909). This estimation is in accordance with the fossil record, since the first occurrence of medium-size mammals unambiguously related to Pecora is in Eurasia with *Eumeryx* in the Early Oligocene of Mongolia about 32 MYA and *Dremotherium* in France at approximately 29–30 MYA (Blondel, 1997; Vislobokova, 1997). *Dremotherium* appears more derived than *Eumeryx* because it possesses all characteristics of modern pecorans, i.e., a canon bone in the four limbs, complete distal metapodial keels (not in *Eumeryx*), and a compact and parallel-sided astragalus (Scott and Janis, 1993).

After the Middle Eocene, Beringia became the dominant Holarctic intercontinental connection between North America and Eurasia. The Bering land bridges periodically emerged during times of low sea level, permitting sporadic intercontinental exchanges between North America and Asia. On the basis of the available fossil record, extant ruminants originated in the Middle/Late Eocene of North America, with the first appearance of Leptomerycidae. Because pecorans emerged in Europe during the Oligocene, it must be assumed that their common ancestor crossed the Bering Strait from North America to Asia before this period, perhaps at the Eocene/Oligocene boundary, which was probably characterized by a major sea level regression due to severe cooling (Prothero and Heaton, 1996).

Evolutionary radiation and dispersion of the pecoran families during the Miocene and Plio-Pleistocene.—The greatest radiation of Pecora occurred during the Miocene, when much of the Earth's forest habitats were replaced by grasslands because of the widespread cooling and drying of the climate. The Miocene was also an important epoch for dispersal events between Africa, Eurasia, and North America. The characters defining all extant families of Pecora appeared in the Early Miocene (Gentry, 1994; Gentry et al., 1999).

The Antilocapridae are endemic to North America, and the sole extant species is the pronghorn (*Antilocapra americana*). This family was firstly recorded in the Early Miocene with diverse genera included into the subfamily Merycodontinae, but species closely related to the pronghorn and placed into the subfamily Antilocaprinae flourished in the Middle/Late Miocene (Janis and Manning, 1998). The origin of this family may lie in Eurasia, because there are no pecorans in North America prior to the Early Miocene. Paleoclimatological studies indicate that the Early Miocene was a relatively cold time and presumably a time of low sea level, which opened up the previously inundated land bridges of Beringia (Opdyke, 1990). At about 24 MYA, horses dispersed from North America to Eurasia (MacFadden, 1992). The earliest entry of Antilocapridae into North America from Eurasian ancestors may have been at the same time.

The Giraffidae are today confined to Africa, where they are represented by only two genera, *Giraffa* and *Okapia*. The ossicones and bilobed lower canines are two autapomorphic features of extant giraffids. Bilobed lower canines were found in the Early Miocene of Africa in genera such *Climacoceras* and *Canthumeryx* (Hamilton, 1978; Geraads, 1986). However, some possible ossicone fragments from *Lorancameryx* were found in more ancient deposits in the Early Miocene of Spain (Morales et al., 1993). These findings suggest that giraffids originated in Europe in the Early Miocene and dispersed rapidly in Africa. This hypothesis is supported by the fact that Africa was isolated from Eurasia until the Early Miocene and by the highly probable Eurasian origin of pecorans. Giraffids may have entered Africa during a period of low sea level, at about 21 MYA (Van der Made, 1999), after which they split off in the African–Arabian–Indian faunal realm and enjoyed a certain early success. Our molecular results suggest that the split between *Giraffa* and *Okapia* occurred at that time (Fig. 5), between 19.0 and 19.9 MYA. Some giraffids later dispersed to the rest of Eurasia, but they declined with the diversification of bovids later in the Miocene (Gentry, 1994).

The Cervidae are today found in America, Eurasia, and North Africa. The possession of antlers in males is a diagnostic feature for this family. Antlers appeared in the Early Miocene of Eurasia with *Procervulus*, *Ligeromeryx*, *Dicrocerus*, *Lagomeryx*, and *Stephanocemas* (Eisenberg, 1987; Gentry, 1994; Gentry et al., 1999). Molecular datings then indicate that most of the major splits within cervids took place during the Early and Middle Miocene (Fig. 5). Later, Cervidae colonized North America in the Early Pliocene, and at the Pliocene completion of the Isthmus of Panama they entered South America, where they experienced a dramatic adaptative radiation (Eisenberg, 1987). Later they made marginal penetrations into northern parts of Africa, and they entered India at the end of the Pliocene, at about 2.5 MYA (Barry and Flynn, 1989).

The Moschidae are now confined in Asia, but numerous fossils from Oligocene and Miocene deposits of Eurasia, America, and Africa have been included in this

family on the basis of three major characters: small to medium body size, absence of cranial appendages, and presence of long upper canines. However, these characters are not diagnostic for Moschidae because they are also encountered in the living cervid *Hydropotes* and in most ancient fossil pecorans. Accordingly, it is difficult to establish the first occurrence of moschids in the fossil record. The use of a Bayesian relaxed molecular clock with the combination of nuclear and mitochondrial markers suggests that moschids originated before the Oligocene/Miocene transition at 26.1–26.2 MYA (Fig. 5).

The Bovidae have a very large distribution; they are found on all continents except Australia, Antarctica, and South America. In the fossil record, they are easily identified by the presence of horn cores. These typical appendages appeared with *Eotragus* in the Early Miocene of western Europe and Pakistan at about 18 MYA and thereafter in Africa (Vrba, 1985; Barry and Flynn, 1989; Gentry, 1994). According to our molecular estimations, the split between Antilopinae and Bovinae took place during the beginning of the Early Miocene, around 22.6 MYA, and may be explained by a vicariant barrier of sea level change and/or development of an arid belt between Africa and Eurasia (Hassanin and Douzery, 1999b). These two subfamilies simultaneously flourished during the Early Miocene, around 19.3–19.7 MYA, with the appearance at this time of all extant tribes of the family (Fig. 5). Among antilopines, the splits separating Caprini, Alcelaphini, and Hippotragini occurred during the Middle Miocene, around 15.1 MYA. During the Plio-Pleistocene, three caprine genera (*Oreamnos*, *Ovibos*, and *Ovis*) dispersed to North America by crossing the Bering Strait, but none of them entered in South America.

CONCLUSIONS

Molecular analyses of seven mitochondrial and nuclear markers have shown that families Bovidae, Cervidae and Moschidae are closely related, with the musk deer as the sister group of bovids rather than cervids. However, the relative phylogenetic affinities of Antilocapridae and Giraffidae remain unsettled. Morphological characters currently used in the literature exhibit too much homoplasy to be powerful for inferring phylogenetic relationships among extant ruminant families. The evolutionary radiation of Pecora occurred at the Early/Late Oligocene transition as estimated from a Bayesian relaxed molecular clock approach, and pecoran families diversified and dispersed rapidly during the Early and Middle Miocene.

ACKNOWLEDGMENTS

A.H. thanks Christiane Denys, Jean Deutsch, and Simon Tillier for laboratory facilities, Jacques Cuisin, Jean-Marc Pons, Francis Renoult, Daniel Robineau, and Michel Tranier for providing access to the MNHN collections, and Denis Geraads, Grégoire Métais, and Salvador Moya-Sola for help with the bibliography. E.J.P.D. thanks François Catzeflis for introducing him in the Pecora world. We both thank Philippe Chardonnet and Bertrand des Clers for tissues of *Aepyceros melampus*, *Gazella granti*, *Redunca fulvorufula*, and *Tragelaphus imberbis*, Eric Pasquet for *Cephalophus dorsalis* skin, Jean-Claude Thibault for *Ovis aries* blood samples, Francoise Hergueta-Claro for *Hippotragus niger* and *Damaliscus pygargus* cells, Jaap Beintema, Amélie Bonnet, Jean-Yves Dubois, Francoise Hergueta-Claro, and David Irwin for *Giraffa camelopardalis* and *Okapia johnstoni* tissue and DNA, Anne Collet for *Balaenoptera physalus* tissues, Dan Gallagher, Arlene Kumamoto, and Jim Womack for *Tragulus javanicus* DNA, Conrad Matthee and Bettine Jansen van Vuuren for *Hippotragus niger* DNA, Knut Roed for *Rangifer tarandus* DNA, Diana R. Reynolds for *Hippopotamus amphibius* tissues, Harry Scherthan for *Muntiacus reevesi* cells, and Antoine Sempéré for *Moschus moschiferus* tissues. We also acknowledge Chris Simon, Jeffrey Thorne, Hirohisa Kishino, and one anonymous reviewer for their helpful comments and suggestions on the manuscript. This work has been supported by funds from the TMR Network Mammalian Phylogeny (contract FMRX-CT98-022) of the European Community, the Genopole Montpellier Languedoc-Roussillon, and the Action Bioinformatique inter-EPST of the CNRS to E.J.P.D. and represents contribution no. 2002-047 of the Institut des Sciences de l'Evolution de Montpellier (UMR 5554-CNRS).

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First submitted 22 July 2002; reviews returned 6 October 2002; final acceptance 23 November 2002
Associate Editor: Jeff Thorne