Molecular Phylogenetics and Evolution
Vol. 13, No. 2, November, pp. 227–243, 1999
Article ID mpev.1999.0619, available online at http://www.idealibrary.com on IDEAL®

The Tribal Radiation of the Family Bovidae (Artiodactyla) and the Evolution of the Mitochondrial Cytochrome *b* Gene

Alexandre Hassanin*,[1] and Emmanuel J. P. Douzery†

*Laboratoire de Préhistoire UMR 6569 CNRS and Service de Systématique Moléculaire GDR 1005 CNRS, Muséum National d'Histoire Naturelle, 75005 Paris, France; and †Laboratoire de Paléontologie, Paléobiologie et Phylogénie, Institut des Sciences de l'Evolution UMR 5554 CNRS, Montpellier, France

Received May 13, 1998; revised November 9, 1998

The nucleotide sequence of the complete mitochondrial cytochrome *b* gene has been determined and compared for 51 species of the family Bovidae and 10 potential pecoran and tragulid outgroups. A detailed saturation analysis at each codon position relative to the maximum parsimony procedure indicates that all transitions on third codon positions do not accumulate in a similar fashion: C-T are more saturated than A-G substitutions. The same trend is observed for second positions but not for first positions where A-G and C-T transitions exhibit roughly the same levels of saturation. Maximum parsimony reconstructions were weighted according to these observations. Maximum parsimony, maximum likelihood, and distance phylogenetic reconstructions all depict a major split within Bovidae. The subfamily Bovinae includes four multifurcating tribes and subtribes: Boselaphini, Tragelaphini, cattle-Bovini (*Bos* and *Bison*), and buffalo-Bovini (*Bubalus* and *Syncerus*). Its sister group is the subfamily Antilopinae, i.e., all non-Bovinae taxa, represented by seven lineages: Antilopini (including *Saiga*), Caprini *sensu lato* (i.e., Caprinae including *Pantholops*), Hippotragini, Alcelaphini, Reduncini (including *Pelea*), *Aepyceros* possibly linked to *Neotragus*, and Cephalophini possibly linked to *Oreotragus* (the suni and the klipspringer being members of a polyphyletic Neotragini). These various tribes and major lineages were produced by two noteworthy explosive radiations, which occurred simultaneously between 12.0 and 15.3 MY (Middle Miocene) in the subfamilies Bovinae and Antilopinae.

Key Words: **Bovidae; mitochondrial DNA; cytochrome *b*; phylogeny; radiation; homoplasy; saturation; substitutional bias; evolution; Pecora.**

INTRODUCTION

The family Bovidae (Mammalia, Artiodactyla, Ruminantia) is diversified, with 137 extant species classified into 45 genera (Grubb, 1993). The biogeographical distribution of bovids shows that extant groups are located mainly in Eurasia (e.g., caprines), or restricted to Africa (e.g., duikers), or span Eurasia and Africa (e.g., gazelles), but are poorly represented in America (e.g., Rocky Mountain goats) (Gentry, 1992). The bovid tribes are of uncertain phylogenetic affinities, but a conservative classification could be set as follows with the grouping of the 12 currently recognized tribes into six subfamilies (Gentry, 1992: p. 2, but see p. 29): (1) Bovinae: Bovini (cattle-like bovids), Boselaphini (nilgai and four-horned antelope), and Tragelaphini (spiral-horned antelopes); (2) Cephalophinae: Cephalophini (duikers); (3) Hippotraginae: Hippotragini (horse-like antelopes) and Reduncini (waterbuck group); (4) Alcelaphinae: Alcelaphini (hartebeest and allies); (5) Antilopinae: Antilopini (gazelles) and Neotragini (dwarf antelopes); and (6) Caprinae: Caprini (goats, sheep, and relatives), Ovibovini (muskox and takin), and Rupicaprini (chamois-like caprines). The taxonomic status of the four genera *Aepyceros* (impala), *Pantholops* (chiru), *Pelea* (vaal rhebok), and *Saiga* (saiga) remains unclear, and they are often placed in the three isolated tribes Aepycerotini, Peleini, and Saigini (saiga and chiru) (e.g. Simpson, 1945), though Gentry (1992: p. 29) suggests the inclusion of *Aepyceros* in Alcelaphinae, *Pelea* in Neotragini, and *Saiga* in Antilopini. Moreover, intertribal relationships are still surrounded by controversy. For instance, the study of morphological and ecological characters conducted by Kingdon (1997: 366) suggests a division into only two subfamilies: Bovinae (Bovini, Boselaphini, and Tragelaphini) and Antilopinae (the nine remaining tribes). This dichotomy was previously evidenced by the immunological study of Lowenstein (1986), which also suggested an association between Alcelaphini and Aepycerotini, but did not resolve the relationships among the other non-Bovinae

[1] To whom correspondence should be addressed at Service de Systématique Moléculaire GDR 1005 CNRS, Muséum National d'Histoire Naturelle, 43 rue Cuvier, 75005 Paris, France. Fax: 33 1 40 79 38 44. E-mail: hassanin@mnhn.fr.

1055-7903/99 $30.00

tribes. Kingdon (1997: 366) proposes another major split and several supratribal associations within non-Bovinae: (i) Caprini, Ovibovini, and Rupicaprini may cluster with Hippotragini, and these represent the sister group of Alcelaphini, with Antilopini basal to these three groups; (ii) Cephalophini cluster with Neotragini, and their sister group could be the Reduncini. Moreover, the definition of some tribes may be problematic: the morphological study of Gentry (1992) suggests the paraphyly of the Neotragini and the allozyme study of Georgiadis *et al.* (1990) fails to support the monophyly of Antilopini and Neotragini. The lack of resolution for the tribal relationships within Bovidae may therefore be explained by the rapid radiation of the family during the Miocene (Vrba, 1985) and the existence of numerous homoplasies in morphological characters.

Recent advances in the molecular phylogeny of bovids involved comparison of mitochondrial ribosomal RNA (rRNA) data. The sequencing of the complete 12S and 16S rRNA genes for 11 bovid species (Allard *et al.*, 1992) and studies of a larger taxonomic sample but with a reduced number of ribosomal characters (Gatesy *et al.*, 1992, 1997) both suggest that a major splitting separates Bovinae from all other extant bovids. In the latter study, the enigmatic genera *Pantholops*, *Pelea*, and *Saiga* respectively cluster with Caprinae, Reduncini, and Antilopini + Neotragini, but the position of *Aepyceros* remains unresolved. However, most of the tribal relationships remain obscure. For instance, the comparison of mitochondrial COX II sequences did not help to resolve the relationships among the three tribes described within the Bovinae (Janecek *et al.*, 1996).

The cytochrome *b* gene has proven its usefulness for resolving phylogenetic patterns among various ruminant artiodactyls during the last 20–30 million years (MY), including Pecora families (Irwin *et al.*, 1991; Chikuni *et al.*, 1995), caprine bovids (Groves and Shields, 1996; Hassanin *et al.*, 1998b), bubaline bovids (Tanaka *et al.*, 1996), and cervids (Randi *et al.*, 1998). This protein-coding gene presents some interesting characteristics for phylogenetic inferences: (1) like all mitochondrial genes, it evolves with more elevated rates than nuclear genes; (2) the positional homology is easy to establish because of its unambiguous alignment due to high conservation of sequence length across mammals (Irwin *et al.*, 1991); and (3) many data are available with respect to its structure and function (Howell, 1989; Degli Espoti *et al.*, 1993) in a phylogenetic context (Griffiths, 1997).

Here, the complete cytochrome *b* gene was sequenced for at least one representative of all extant bovid tribes and a large 62-taxa data matrix was analyzed to answer the following questions:

(1) Which cyt *b* mutational events are less prone to homoplasy and saturation, and how should they be used to reconstruct the Bovidae phylogeny?

(2) Are the traditionally recognized subfamilies and tribes natural entities? What are the phylogenetic relationships between the major lineages evidenced within Bovidae?

(3) What is the phylogenetic status of several genera whose position has been debated? This question especially concerns *Aepyceros*, *Pantholops*, *Pelea*, and *Saiga*.

(4) Is Bovidae a monophyletic family? To test this, we included representatives of the other Pecora families (Cervidae, Giraffidae, Antilocapridae), as well as an unambiguous outgroup, the Tragulidae (infraorder Tragulina).

MATERIALS AND METHODS

Data Collection

Fifty-one species of Bovidae were used in this study, including representatives of all tribes currently described (Table 1). Ten additional taxa belonging to the families Cervidae, Giraffidae, Antilocapridae, and Tragulidae were used as potential outgroups to root the bovid tree (Table 1).

Extraction, Amplification, and DNA Sequencing

Total DNAs were extracted from three sources: (i) blood, skin, and muscles, using a CTAB protocol (Winnepenninckx *et al.*, 1993); (ii) bone fragments of museum specimens (Hassanin *et al.*, 1998b); and (iii) 95% ethanol-preserved tissues maintained in the collection of the laboratory of Paleontology, Paleobiology, and Phylogeny in Montpellier (Catzeflis, 1991) following standard procedures (Sambrook *et al.*, 1989).

To determine the complete cyt *b* sequences of the various taxa listed in Table 1, two different protocols were used. With the first protocol, three to six overlapping regions of the cyt *b* gene were generated by PCR using different couples of primers (those cited in Hassanin *et al.*, 1998b; and L 15096: 5′-GAAACATGAAAYRTHGGAGT-3′). After purification from agarose gel (QIAEX II gel extraction kit; QIAGEN), both strands of the PCR products were directly sequenced using the Thermo Sequenase cycle sequencing kit (Amersham). Most of the overlapping domains were longer than 100 bp to check the authenticity of the different fragments sequenced. The use of internal specific primers and the comparison of the various overlapping amplicons avoided contaminations by exogene DNA and pseudogene sequences.

With the second protocol, a 3.0-kb segment of mtDNA spanning the cyt *b* to the first half of the 12S rDNA gene was PCR amplified and cloned in the pGEM-T (Promega) vector (Douzery and Randi, 1997). Three positive clones were isolated for each taxon, and recombinant plasmids are available upon request to E.D. The complete cyt *b* was dideoxy sequenced on both strands (Sanger *et al.*, 1977; using the Pharmacia kit), with the external L14724B primer (Irwin *et al.*, 1991) and a set

of seven internal primers. The *Kobus megaceros* DNA was extracted starting from hairs, using the Chelex procedure of Taberlet *et al.* (1993). The cyt *b* gene was then amplified using the flanking primers described by Irwin *et al.* (1991), cloned, and sequenced as described above.

Homoplasy and Saturation Analyses

The amount of homoplasy was measured through the consistency index CI (Kluge and Farris, 1969), excluding uninformative characters for each substitution type (i.e., C-T, A-G, A-C, A-T, C-G, and G-T) considered at each of the three codon positions separately (Hassanin *et al.*, 1998a). For example, to study the C-T changes, the matrix was recoded with A and G as missing data, and the corresponding most-parsimonious tree(s) and CI were computed using PAUP 3.1.1. (Swofford, 1993). The saturation of each substitution type at each codon position was then assessed graphically by plotting the pairwise number of observed differences against the corresponding pairwise number of inferred substitutions (Philippe *et al.*, 1994). This was done in a phylogenetic perspective using the matrices of "patristic distances" and "adjusted character distances" calculated by PAUP 3.1.1. For each type of change, the slope of the linear regression (S) was used to evaluate the level of saturation within the group under focus (Hassanin *et al.*, 1998a). 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 one. When the level of saturation increases, the slope decreases toward zero.

Phylogenetic Analyses

The complete cyt *b* sequences were aligned and analyzed using the MUST package (Philippe, 1993). The phylogenetic analyses were conducted with two taxonomic samples (tragulid outgroup included or excluded), with different weighting schemes of character-state changes (equal weighting, all transitions excluded by coding A = G and T = C, and various weighting schemes involving purines and/or pyrimidines changes), and with three methods of tree reconstruction:

(1) The maximum parsimony (MP) method (PAUP 3.1.1., Swofford, 1993) was conducted with either equal weighting or differential weighting of the character-state transformations using the consistency index (CI), the slope of saturation (S), and the product CI · S. These estimations of homoplasy were used to weight each substitution type at each of the three codon positions (Hassanin *et al.*, 1998a). After multiplication by 1000, these values (final range of weights for first, second, and third codon positions: 90 to 1000, 122 to 1000, and 36 to 773; see Table 2) were entered into the stepmatrix of PAUP 3.1.1 for weighted parsimony analysis. Support for individual branches was assessed by bootstrap percentages (BP) (Felsenstein, 1985) computed after 100 replicates (bootstrap option in PAUP 3.1.1.) and by branch support (b) (Bremer, 1988, 1994) computed by the AUTODECAY 3.0 program (Eriksson, 1995). For the differential weighting analyses, the branch supports were weighted and rescaled (b_{wr}), as proposed by Gustafsson and Bremer (1995), in order to allow comparisons with the equal-weighted branch support b. Mapping of the character-state changes on the cladograms was performed with MacClade 3.04 (Maddison and Maddison, 1992).

(2) The maximum likelihood (ML) method (Felsenstein, 1981) was conducted with the Tamura and Nei (1993) model of sequence evolution and performed by quartet puzzling (PUZZLE 2.5.1, Strimmer and von Haeseler, 1996). The robustness of nodes was estimated by reliability percentages (RP), i.e., the percentage of occurrence of the corresponding node after 1000 ML quartet puzzling steps (Strimmer and von Haeseler, 1996).

(3) The neighbor-joining (NJ) method (Saitou and Nei, 1987) was used on uncorrected percentage sequence divergences and the robustness of the phenograms was assessed by 1000 bootstrap resamplings (NJBOOT program; Philippe, 1993).

RESULTS

Authenticity of Cytochrome b Sequences and Intraspecific Polymorphism

The cyt *b* sequence of all Bovidae species begins with the ATG start codon, contains neither frameshifts nor stop codon, and terminates with an AGA or AGG stop codon, except for *Neotragus moschatus, Capra sibirica, Rupicapra rupicapra,* and *R. pyrenaica* (TAA stop codon in position 1140). Under these criteria, our 62 pecoran and tragulid nucleotide data represented true mitochondrial cyt *b* sequences. One should note that in the case of the white-bellied duiker, an AGA stop codon instead of a GGA Gly codon (positions 313–315) was detected in four of the six sequenced clones, reflecting either a *Taq* DNA polymerase error or a nonfunctional cyt *b*-like nuclear copy (Zhang and Hewitt, 1996). The consensus sequence of the two remaining cyt *b* clones of *Cephalophus leucogaster* clusters within a monophyletic Cephalophini with either a *Cephalophus monticola/C. maxwelli* clade or a *C. silvicultor/C. dorsalis/C. spadix* clade in a duiker cyt *b* study by B. J. van Vuuren and T. Robinson (unpublished results).

Downstream of the cyt *b* gene, the presence of a short spacer has been detected between the stop codon and the first nucleotide of the tRNA-Thr gene. This spacer is three to four nucleotides long: CAA (*Addax, Alcelaphus, Kobus, Oryx,* all Caprini except *Ammotragus*),

TABLE 1

Bovid Taxa Studied and Sources of Tissues and Cytochrome *b* Sequences

Species—common name	Origin, collector, and reference for tissues and sequences	Accession numbers
Bovini		
Bos taurus—domestic cow	Anderson *et al.*, 1982	V00654
Bos javanicus—banteng	Kikkawa *et al.*, 1995	D34636
	Tanaka *et al.*, 1996	D82889
Bison bison—American bison	(i) Zoo de Vincennes; F. Hergueta-Claro	AF036273
Bubalus bubalis—Asian wild water buffalo	Chikuni *et al.*, 1995	D32193
Bubalus depressicornis—lowland anoa	Tanaka *et al.*, 1996	D82890
Bubalus mindorensis—tamaraw	Tanaka *et al.*, 1996	D82895
Bubalus quarlesi—mountain anoa	Tanaka *et al.*, 1996	D82891
Syncerus caffer—African buffalo	Tanaka *et al.*, 1996	D82888
	(i) MNHN, Paris; Service de Systématique Moléculaire	AF036275
Boselaphini		
Boselaphus tragocamelus—nilgai*	(iii) T1252, Zoo de Vincennes; F. Hergueta-Claro	AJ222679
Tetracerus quadricornis—four-horned antelope	(ii) 1986-464, MNHN, Paris; Mammifères & Oiseaux	AF036274
Tragelaphini		
Tragelaphus eurycerus—bongo	(ii) 1963-953, MNHN, Paris; Mammifères & Oiseaux	AF036276
Tragelaphus imberbis—lesser kudu	(i) MNHN, Paris; Service de Systématique Moléculaire	AF036279
Taurotragus oryx—common eland	(i) MNHN, Paris; Service de Systématique Moléculaire	AF036278
Tragelaphus scriptus—bushbuck	(i) MNHN, Paris; Service de Systématique Moléculaire	AF036277
Tragelaphus spekii—sitatunga*	(iii) T1253, Zoo de Vincennes; F. Hergueta-Claro	AJ222680
Tragelaphus strepsiceros—greater kudu	(i) MNHN, Paris; Service de Systématique Moléculaire	AF036280
Alcelaphini		
Alcelaphus buselaphus—hartebeest*	(iii) T397, Zoo de Montpellier; M. Gallet	AJ222681
Damaliscus pygargus	(i) Zoo de Vincennes; F. Hergueta-Claro	AF036287
Antilopini		
Antilope cervicapra—blackbuck	(ii) 1992-618, MNHN, Paris; Mammifères & Oiseaux	AF036283
Antidorcas marsupialis—springbok	(ii) 1993-1670, MNHN, Paris; Mammifères & Oiseaux	AF036281
Gazella gazella—mountain gazelle or idmi*	(iii) T776, Zoo de Vincennes; F. Hergueta-Claro	AJ222682
Gazella granti—Grant's gazelle	Hassanin *et al.* (1998b)	AF034723
Gazella subgutturosa—goitered gazelle	(i) MNHN, Paris; Service de Systématique Moléculaire	AF036282
Neotragini		
Neotragus moschatus—suni*	(iii) T579, San Diego Zoo; O. Ryder	AJ222683
Oreotragus oreotragus—klipspringer	(i) MNHN, Paris; Service de Systématique Moléculaire	AF036288
Caprini		
Capra ibex—Alpine ibex	Hassanin *et al.* (1998b)	AF034735
Capra nubiana—Nubian ibex	Hassanin *et al.* (1998b)	AF034740
Capra sibirica—Asiatic ibex	Hassanin *et al.* (1998b)	AF034734
Ammotragus lervia—aoudad	Hassanin *et al.* (1998b)	AF034731
Hemitragus jemlahicus—Himalayan tahr	Hassanin *et al.* (1998b)	AF034733
Ovis aries—domestic sheep	Hassanin *et al.* (1998b)	AF034730
Ovis dalli—Dall's sheep	Hassanin *et al.* (1998b)	AF034728
Pseudois nayaur—barhal	Hassanin *et al.* (1998b)	AF034732
Ovibovini		
Ovibos moschatus—muskox	Groves and Shields, 1996	U17862
Budorcas taxicolor—takin	Groves and Shields, 1996	U17867
Rupicaprini		
Rupicapra rupicapra—Alpine chamois	Hassanin *et al.* (1998b)	AF034725
Rupicapra pyrenaica—Pyrenean chamois	Hassanin *et al.* (1998b)	AF034726
Capricornis crispus—Japanese serow	Chikuni *et al.*, 1995	D32191
Naemorhedus caudatus—Chinese goral	Groves and Shields, 1996	U17861
Oreamnos americanus—Rocky Mountains goat	Groves and Shields, 1996	U17863
Cephalophini		
Cephalophus leucogaster—white-bellied duiker*	(iii) T511, La Wangny; J.-P. Hugot	AJ222684
Hippotragini		
Hippotragus niger—sable antelope	(i) MNHN, Paris; F. Hergueta-Claro	AF036285
Addax nasomaculatus—addax*	(iii) T685, Zoo de Montpellier; M. Gallet and Hassanin *et al.* (1998b)	AF034722
Oryx leucoryx—Arabian oryx	(i) MNHN, Paris; F. Hergueta-Claro	AF036286
Oryx dammah—scimitar-horned oryx*	(iii) T988, MNHN, Paris; F. Hergueta-Claro	AJ222685
Reduncini		
Redunca fulvorufula—mountain reedbuck	(i) MNHN, Paris; Service de Systématique Moléculaire	AF036284
Kobus megaceros—Nile lechwe	(iii) T1612, Sigean, France; C. Bourrellis	AJ222686

TABLE 1—*Continued*

Species—common name	Origin, collector, and reference for tissues and sequences	Accession numbers
Indeterminate		
Aepyceros melampus—impala	(ii) 1996-645, MNHN, Paris; Mammifères & Oiseaux	AF036289
Pantholops hodgsonii—chiru	Hassanin *et al.* (1998b)	AF034724
Pelea capreolus—vaal rhebok	Matthee and Robinson (in press)	AF022055
Saiga tatarica—saiga	Groves and Shields, 1996	U17864
Outgroup: Tragulidae		
Tragulus napu—larger Malay chevrotain	Irwin *et al.*, 1991	X56288
Tragulus javanicus—lesser mouse deer	Chikuni *et al.*, 1995	D32189
Outgroup[a]: Antilocapridae		
Antilocapra americana—pronghorn	Irwin *et al.*, 1991	X56286
Outgroup[a]: Cervidae		
Cervus nippon—sika deer	Chikuni *et al.*, 1995	D32192
Muntiacus reevesi—Chinese muntjak	Randi *et al.*, 1998	AJ000023
Odocoileus hemionus—black-tailed deer	Irwin *et al.*, 1991	X56291
Rangifer tarandus—reindeer	Randi *et al.*, 1998	AJ000029
Hydropotes inermis—Chinese water deer	Randi *et al.*, 1998	AJ000028
Alces alces—moose	Randi *et al.*, 1998	AJ000026
Outgroup[a]: Giraffidae		
Giraffa camelopardalis—giraffe	Irwin *et al.*, 1991	X56287

Note. The classification at the tribe level is from Gentry (1992). Starting material for DNA extractions was: (i) blood, skin, or muscles; (ii) bone fragments of museum specimens; and (iii) 95% ethanol-preserved tissue. The stars denote taxa for which the complete cyt *b* sequence was determined after a cloning step following protocol 2. The cyt *b* of the other taxa was directly sequenced following protocol 1.

[a] These pecoran outgroups are potential bovid outgroups as the Bovidae monophyly is to be tested.

TAA (*Cephalophus, Gazella*), AAA (*Tragelaphus*), AAG (*Boselaphus*), ACAA (*Neotragus*), and TAAA (*Ammotragus*).

Homoplasy and Saturation Levels for Each Type of Substitution

The comparison of the graphics of saturation and CI values for all substitution types reveals several combinations of patterns according to mutational event and codon position (Fig. 1 and Table 2). Substitution types can be strongly homoplastic, and they display fully saturated plots (e.g., C-T 3: $S < 0.5$ with an intermediate r^2), or highly dispersed plots (e.g., C-T 1, A-G 1, and C-T 2), or moderately dispersed plots (e.g., A-G 3, A-C 3, and A-T 3). Reciprocally, the profiles of saturation can be perfectly linear ($S > 0.8$–0.9 with a high r^2) with relatively high levels of homoplasy and a small number of informative sites (e.g., A-C 1, A-T 1, and A-G 2), or with low levels of homoplasy and either a great (e.g., C-G 3 and G-T 3) or a small (e.g., C-G 1, G-T 1, and all Tv in second positions) number of informative sites. The comparison of the raw CI and S values indicates that the amounts of homoplasy and the levels of saturation for a given type of substitution are the greatest in third positions where the overall lowest CI and S are found (Table 2). Only one exception must be noted: the A-G transitions in first positions are slightly more homoplastic but much more saturated than the same substitution type in third positions. Second positions of codons are less subject to homoplasy and saturation than the two other positions, even if C-T events remain highly affected by multiple substitution events.

Whatever the codon positions, transitions (Ti) are much more homoplastic and saturated than transversions (Tv). Among Ti, C-T substitutions are more homoplastic than A-G substitutions at every codon position. The same trend is observed for the level of saturation, except for Ti in first positions for which A-G are more saturated than C-T. Among Tv, the substitution types which involve A rather than G contain larger amounts of homoplasy and are more strongly saturated (Table 2).

The Question of Bovid Monophyly

When the topologies are rooted with *Antilocapra*, the bovids are monophyletic, with Cervidae as sister group and the giraffe more basal (Figs. 2 and 3). The phylogenetic relationships between Cervidae depict three major clades: Cervinae + Muntiacinae, Odocoileini + Rangiferini, and Hydropotini + Alcini, therefore indicating the paraphyly of antlered deers. The evolutionary implications of these results and more detailed studies of Cervidae phylogenetics may be found elsewhere (Douzery and Randi, 1997; Randi *et al.*, 1998). When the topologies are rooted with the more distant outgroup *Tragulus*, the phylograms show that *Antilocapra* and *Giraffa* are basal relative to three unresolved clades: Bovinae, Antilopinae (*sensu* Kingdon), and Cervidae (data not shown).

Maximum Parsimony Phylogenetic Analyses

In this study, we used two different approaches to estimate the level of homoplasy of the various substitution types: one uses CIs whereas the other uses graphs of saturation. These complementary approaches reveal patterns of homoplasy (Hassanin *et al.*, 1998a,b) and, therefore, we prefer to apply CI · S products for weighting the MP analyses, rather than CI or S values alone.

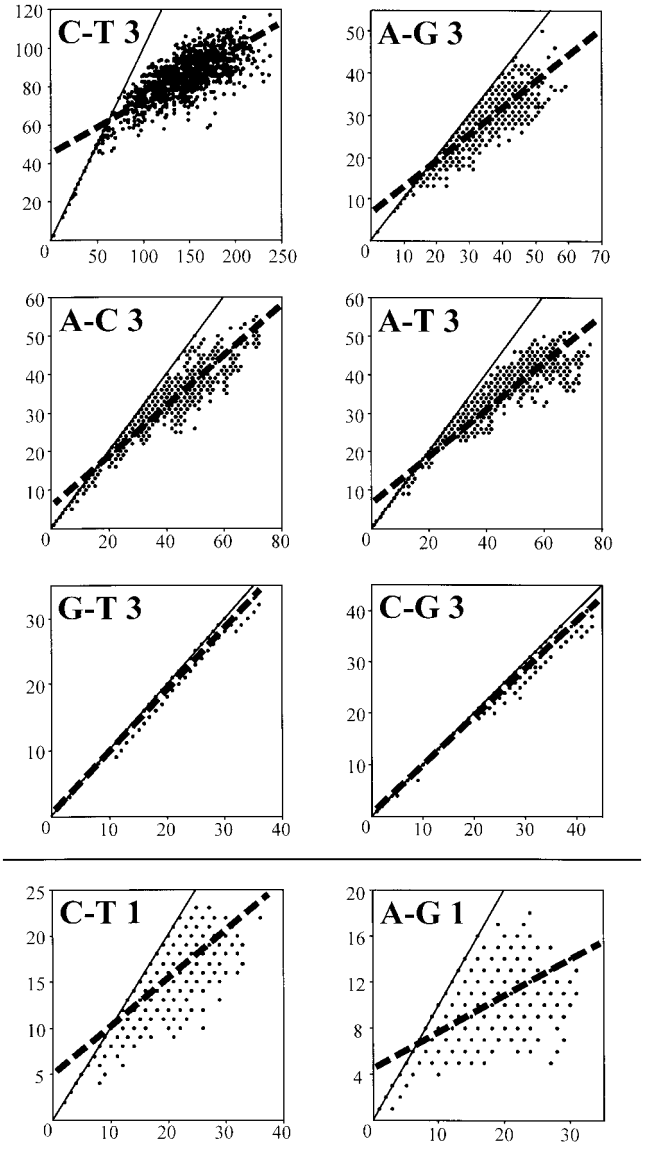

FIG. 1. Detailed study of saturation for all substitution types in third codon positions (i.e., C-T 3, A-G 3, A-C 3, A-T 3, C-G 3, and C-G 3) and only for Ti in first codon positions (i.e., C-T 1 and A-G 1) of the cytochrome *b* of 52 bovid taxa. Graphs were obtained by plotting the pairwise number of observed differences (in ordinate) against the corresponding pairwise number of inferred substitutions (in abscissa). The linear regression, used to evaluate the level of saturation, corresponds to the dashed line. The continuous line represents the area of equal numbers of observed and inferred changes, i.e., the theorical situation for which no saturation is observed.

TABLE 2

Number of Informative Sites, Amount of Homoplasy Measured through the Consistency Index (CI) Excluding Uninformative Sites, Intensity of Saturation Evaluated by the Slope of the Linear Regression (S), and Products (CI · S) for Each Substitution Type at Each of the Three Codon Positions

	Informative sites	Amount of homoplasy (CI)	Level of saturation: the slope S (r^2)[a]	Product (CI · S)
First positions				
C-T	56	**0.247**	0.520 (0.657)	0.128
A-G	36	0.286	**0.314** (0.449)	**0.090**
A-C	18	0.562	0.870 (0.921)	0.489
A-T	17	0.630	0.835 (0.947)	0.526
C-G	5	1.000	1.000 (1.000)	1.000
G-T	7	0.875	0.992 (0.998)	0.868
Second positions				
C-T	27	**0.293**	**0.416** (0.451)	**0.122**
A-G	4	0.667	0.967 (0.954)	0.645
A-C	0	1.000	1.000 (1.000)	1.000
A-T	1	1.000	1.000 (1.000)	1.000
C-G	1	1.000	1.000 (1.000)	1.000
G-T	1	1.000	1.000 (1.000)	1.000
Third positions				
C-T	217	**0.130**	**0.274** (0.621)	**0.036**
A-G	133	0.296	0.628 (0.764)	0.186
A-C	110	0.426	0.652 (0.909)	0.278
A-T	104	0.486	0.610 (0.885)	0.296
C-G	41	0.745	0.932 (0.991)	0.694
G-T	34	0.829	0.932 (0.989)	0.773

Note. The following values correspond to the study of a data matrix including only taxa of the family Bovidae (ingroup). The lowest values of CI, S, and CI · S are indicated in boldface.

[a] S is the slope of the linear regression and r^2 its correlation coefficient.

The MP analysis produced one most-parsimonious tree of 502,368 steps when the CI · S weights were used (Fig. 2). Bootstrap and branch support analyses strongly support the monophyly of the following tribes: Boselaphini (BP = 100, b_{wr} = +28.9), Tragelaphini (BP = 100, b_{wr} = +19.8), Hippotragini (BP = 100, b_{wr} = +18.5), Alcelaphini (BP = 99, b_{wr} = +25.2), Antilopini (BP = 95, b_{wr} = +13.5), and Reduncini (BP = 83, b_{wr} = +10.5). Moreover, the *Saiga* appears sister group of all Antilopini (BP = 81, b_{wr} = +8.1), suggesting the existence of an enlarged tribe Antilopini. *Pelea* joins *Kobus* and *Redunca* (BP = 79, b_{wr} = 12.6), suggesting the existence of an enlarged tribe Reduncini. The tribe Bovini is splitted into a cattle-Bovini clade (BP = 100, b_{wr} = +35.8) and a buffalo-Bovini clade (BP = 100, b_{wr} = +14.0). The monophyly of Bovini is supported by a residual BP = 28 and involves 1.4 additional steps. The Neotragini appears polyphyletic, as *Neotragus* and *Oreotragus* do not cluster together (residual BP < 1, additional steps = +28.3). The suni actually groups with the impala (BP = 62, b_{wr} = +11.7) and the klip

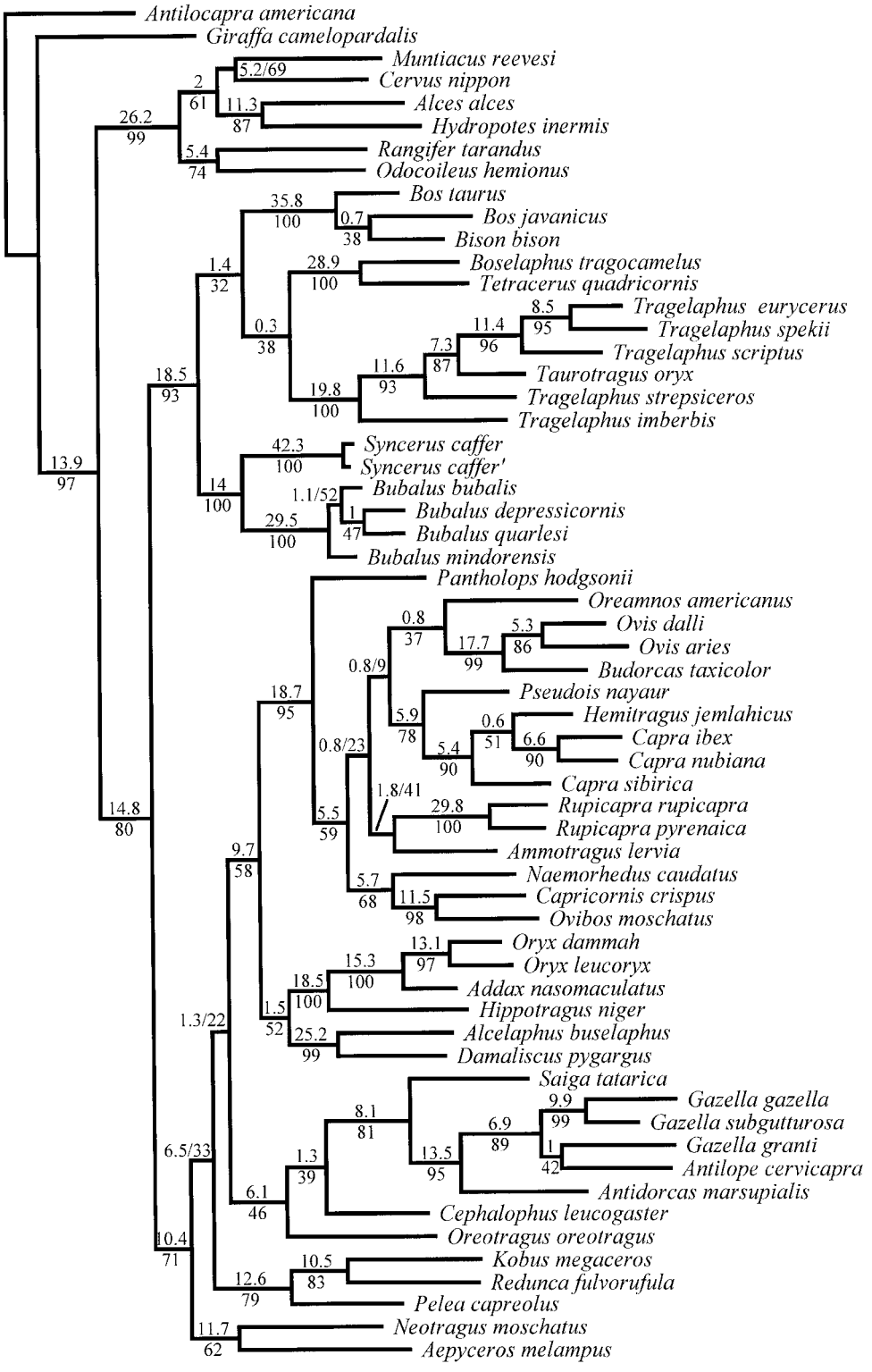

FIG. 2. Phylogram of the most parsimonious tree obtained from a differential weighting scheme based on the product of the homoplasy and saturation estimators (CI.S) and rooted by *Antilocapra*. Its length is 502,368 steps. The bootstrap percentages after 100 replicates and the weighted and rescaled branch supports (b_{wr}) are indicated below and above branches, respectively. The use of the differential weighting based on either CI or S values yielded similar topologies, except for some very weakly supported branchings: Bovini monophyly, *Bos* monophyly with respect to *Bison*, relative position of *Bubalus* species, and *Gazella* monophyly with *Antilope* as sister group. Moreover, *Cephalophus* and *Oreotragus* were sister genera and clustered with a clade where Reduncini *s. l.* was sister tribe of Alcelaphini–Hippotragini–Caprini *s. l.* A similar topology was obtained after the standard equal weighting procedure except for the positions of *Taurotragus*, *Aepyceros* and *Neotragus*, Tragelaphini, and Caprini *s. l.* (see text). One should note that there is no phylogenetic signification for the "antelope" term, as the spiral-horned antelopes (Tragelaphini) belong to the subfamily Bovinae, whereas the true antelopes (Antilopini) and the dwarf antelopes (Neotragini) belong to the subfamily Antilopinae.

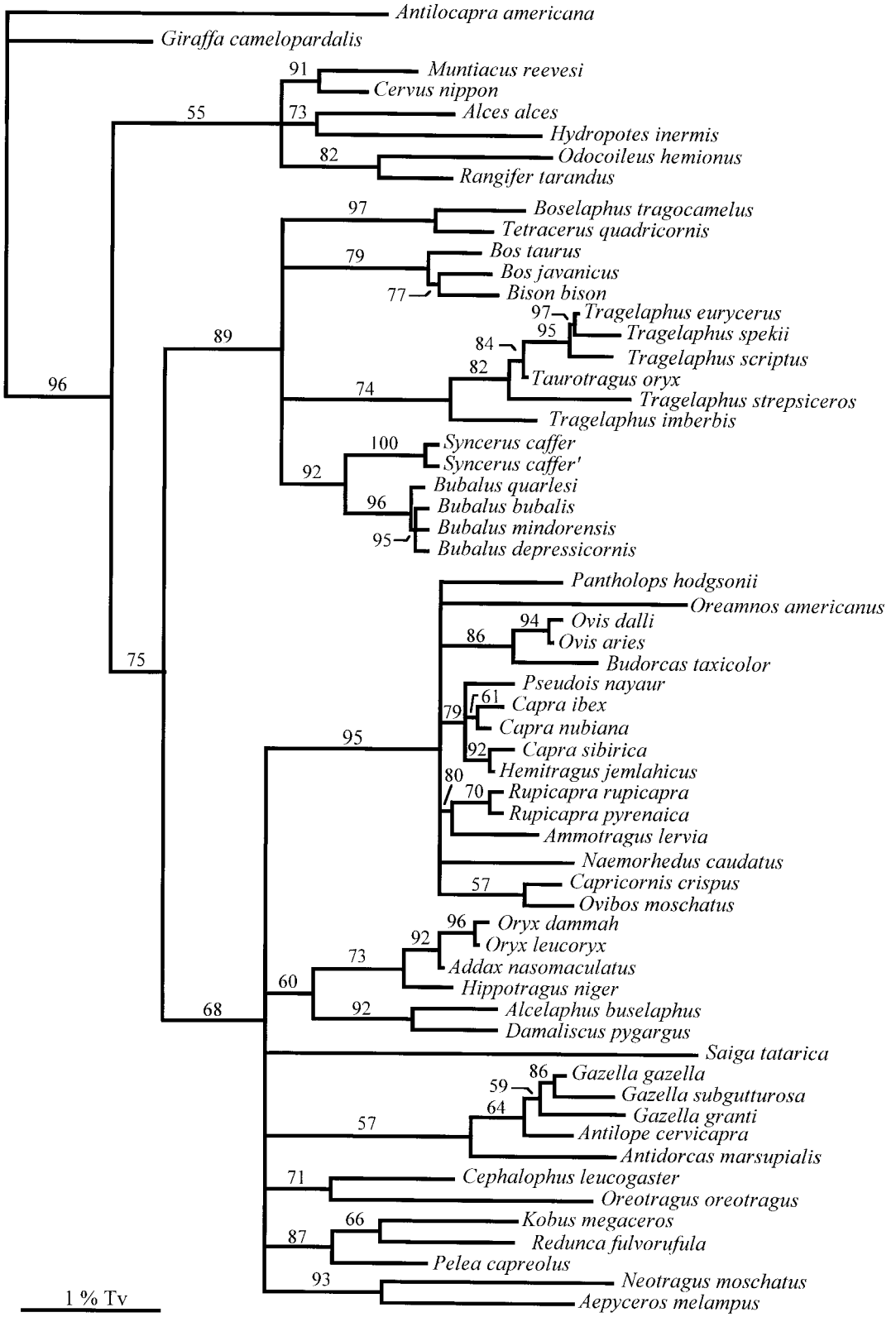

FIG. 3. Maximum-likelihood phylogram reconstructed from the transversions of the cyt *b* for 60 pecoran taxa and rooted by *Antilocapra*. The topology corresponds to the most likely tree (lnL = 4464 ± 219) reconstructed from the Tv only after 1000 quartet puzzling steps. Branch lengths are proportional to the expected number of changes under the Tamura and Nei (1993) model of sequence evolution. Reliability percentages are indicated and depict the robustness of each node.

springer clusters with the Antilopini *s. l.* (residual BP = 39, b_{wr} = +1.3) or with the white-bellied duiker (BP = 46, additional steps = 1.3). The three tribes currently recognized within the subfamily Caprinae are artificial. The muskox groups with the serow (BP = 98, b_{wr} = +11.5) and the takin clusters with the sheep (BP = 99, b_{wr} = +17.7), involving the polyphyly of the tribe Ovibovini.

At the subfamilial level, only two of the six subfamilies classically recognized (Gentry, 1992) appear monophyletic (Fig. 2): (1) Bovinae, i.e., Boselaphini, Tragelaphini, cattle-Bovini, and buffalo-Bovini (BP = 93, bwr = +18.5) and (2) Caprinae, including *Pantholops* and all the representatives of the three artificial tribes Caprini, Ovibovini, and Rupicaprini (BP = 95, b_{wr} = +18.7). In contrast, the monophylies of Hippotraginae and Antilopinae are not supported and require respectively a huge number of additional steps: 17.2 and 34.9 (including *Saiga* within Antilopinae) or 46.8 (excluding *Saiga*). New relationships between tribes are, however, suggested: Alcelaphini may be sister group of Hippotragini (BP = 52, b_{wr} = +1.5) and these two tribes may cluster with Caprinae (BP = 58, b_{wr} = +9.7).

The MP tree depicts a major basal split within Bovidae (Fig. 2). First, the Bovinae clade includes four multifurcating tribes or subtribes: cattle-Bovini (*Bos* and *Bison*), buffalo-Bovini (*Bubalus* and *Syncerus*), Boselaphini, and Tragelaphini. Second, its sister group is the Antilopinae clade (BP = 71, b_{wr} = +10.4) according to the subfamilial sense of Kingdon (1997). Antilopinae are represented by a minimum of four multifurcating lineages: (1) Antilopini *sensu lato* (including *Saiga*) possibly linked to *Oreotragus* and Cephalophini; (2) Reduncini *sensu lato* (including *Pelea*); (3) *Aepyceros* + *Neotragus;* and (4) Caprini *sensu lato* possibly linked to Hippotragini and Alcelaphini. Here, the term Caprini *s. l.* is used to group together the three artificial caprine tribes (Caprini *sensu stricto,* Ovibovini, and Rupicaprini) and *Pantholops.* This term is preferred to "Caprinae," which brings a subfamilial sense and does not fit our Bovinae/Antilopinae distinction.

When all nucleotide changes were equally weighted, five most-parsimonious trees were obtained (length = 4058 steps; CI = 0.215; CI excluding uninformative characters = 0.196; RI = 0.435), and one robust node conflicted with the results obtained after the three differential-weighted (CI · S, C, or S) MP analyses (data not shown). *Taurotragus* actually groups with *Tragelaphus strepsiceros* (BP = 73, b = +3), whereas this genus appears in basal position with respect to *Tragelaphus scriptus, T. spekii,* and *T. euryceros* in the MP analyses based on the CI · S weights (BP = 87, b_{wr} = +7.3), CI weights (BP = 65, b_{wr} = +3.6), or S weights (BP = 51, b_{wr} = +1.1). Three weakly supported nodes were also conflictual: Bovidae and Bovinae were polyphyletic due to sister group relationship of Tragelaphini to Cervidae; *Aepyceros* and *Neotragus* were separated, with the impala clustering with *Oreotragus* and the suni grouping with Reduncini *s. l.;* and Hippotragini and Alcelaphini did not cluster with Caprini *s. l.* Moreover, the equal weighting yielded a poor support for most tribes: Reduncini *s. l.* (BP = 32, b = +2), Antilopini *s. l.* (BP = 36, b = +1), Hippotragini (BP = 55, b = +5), and Caprini *s. l.* (BP = 71, b = +6).

Maximum-Likelihood Phylogenetic Analyses

When the ML analysis is conducted on all events, i.e., the more saturating Ti are included with an estimated Ti/Tv ratio of 3.6, the most likely tree (lnL = −20,676 ± 606) exhibits the same topology as the MP tree of Fig. 2, except for three conflicting nodes: (1) *Taurotragus* clusters with *Tragelaphus strepsiceros;* (2) *Aepyceros* does not associate with *Neotragus;* and (3) *Pantholops* clusters with *Rupicapra* (data not shown). Otherwise, a lack of resolution at the RP = 50 threshold is observed for some nodes (e.g., *Muntiacus* and *Odocoileus* within Cervidae or the Antilopini within Bovidae). One should note that all sequences but those of *Hydropotes* and *Rangifer* do not differ significantly in nucleotide composition from the frequency distribution of the ML model (Tamura and Nei, 1993) on the basis of a 5% level χ^2 test. The Chinese water deer and reindeer cyt *b* sequences actually exhibit a slight overall deficit of C and an excess of T.

When the ML analysis is conducted on transversional events only, i.e., the more saturating Ti are excluded from all codon positions, the topology of the most likely tree (lnL = −4464 ± 219) is similar to the previous ML tree and is presented in Fig. 3. The removal of Ti does not change the phylogenetic relationships except for five nodes: (1) *Taurotragus* clusters with *Tragelaphus euryceros/T. spekii/T. scriptus* (RP = 84); (2) *Neotragus* groups with *Aepyceros* (RP = 93); (3) *Gazella granti* clusters with the two other *Gazella* species (RP = 59); (4) *Hemitragus* shifts to *Capra sibirica* (RP = 92); and (5) the position of *Pantholops* within Caprinae is unresolved. Moreover, a better reliability is measured for the majority of the nodes. For example, the monophyly of the following groups is supported by higher RP: Bovidae (75 vs 63), Bovinae (89 vs 62), Antilopinae (68 vs 57), and *Addax* + *Oryx* (92 vs 51).

To summarize, the MP tree reconstructed with differential weighting using the CI and S values (Fig. 2) is congruent with the ML tree based on Tv only (Fig. 3) and the NJ tree based on Tv only (data not shown). However, three topological differences appear with a weak to strong support for the nodes in conflict. (1) *Cephalophus* and *Oreotragus* cluster together in ML (RP = 71) and NJ trees (BP = 35), whereas two topologies are in conflict in the case of the MP analysis: the klipspringer is sister group of a clade comprising the

white-bellied duiker and the Antilopini *s. l.* in the MP tree (BP = 39, b_{wr} = 1.3), whereas the clade *Cephalophus* + *Oreotragus* is supported in the bootstrap analysis by a higher value (BP = 46). (2) The monophyly of *Gazella* is evidenced by the ML (RP = 59) and NJ (BP = 50) analyses, whereas *Gazella granti* and *Antilope cervicapra* cluster together in the MP tree (BP = 42) even if the monophyly of *Gazella* cannot be rejected (residual BP = 42). (3) *Capra sibirica* and *Hemitragus jemlahicus* cluster together with ML (RP = 92) and NJ (BP = 78) analyses, but *Capra sibirica* originates first before *Hemitragus* and the other *Capra* species in the MP tree (BP = 51). However, a similar value of branch support (residual BP = 46) agrees with the topology obtained by the ML and NJ methods. Moreover, the ML did not provide reliable resolution for the position of the genera *Naemorhedus*, *Pantholops*, and *Saiga*, and the tribes Alcelaphini and Hippotragini. In contrast, the MP and NJ approaches cluster the goral with the serow and muskox (BP = 68 and 60, respectively), place the chiru basalmost within Caprinae (BP = 59 and 75), group the saiga with Antilopini (BP = 81 and 57), and associate Alcelaphini and Hippotragini with Caprinae (BP = 58 and 51).

Evolutionary Rates of the Cytochrome b and Divergence Dates Among Bovidae

The family Bovidae seems to originate in the Early Miocene. The minimum age of bovids divergence is actually 18 MY, as indicated by the European and Indian location of *Eotragus*, the first typical bovid documented in the fossil record (Ginsburg and Heintz, 1968). Thus, the age of bovid emergence is postulated at 20 MY (Miyamoto *et al.*, 1993). We applied this calibration point to estimate the rate of Ti + Tv or Tv only accumulation in the overall cyt *b*, without distinction of codon position, and using the ML estimates of branch lengths (Fig. 3). If all mutational events are considered, a mean evolutionary rate of 0.63 ± 0.07% Ti + Tv/MY/lineage is found for the family Bovidae represented by 51 species. If the saturating Ti are excluded, a mean rate of 0.13 ± 0.02% Tv/MY/lineage is calculated. These rates represent mean values computed on the complete cyt *b* and do not depict the rate heterogeneities existing between the three codon positions.

The dates of divergence of the main bovid lineages were then calculated using local molecular clocks based on Tv only. To reduce the impact of rate heterogeneities on the time estimations, the rates were actually calculated for each different lineage. They range from 0.08% Tv/MY/lineage for cattle-Bovini to 0.16% Tv/MY/lineage for Antilopini, Tragelaphini, and *Aepyceros* + *Neotragus*. The estimations of divergence times deduced after these rates are summarized in Table 3. The first cladogenesis at 20 MY during the Early Miocene produces two major bovid groups: the Bovinae (Bovini, Boselaphini, and Tragelaphini) and the Antilopinae (all the other extant tribes). Then, our cyt *b* data suggest that Bovinae and Antilopinae experienced a contemporaneous and rapid tribal radiation in the Middle Miocene. Four Bovinae lineages arose between 12.0 and 14.3 MYA, and six Antilopinae lineages arose between 13.6 and 15.3 MYA (Figs. 2 and 3, Table 3). Subsequent cladogeneses occur at the tribe and genus levels in the Late Miocene, between Miocene and Pliocene, and in the Early Pliocene (Table 3).

TABLE 3

Estimation of the Divergence Dates for Various Bovidae Taxa Based on the Rate of Accumulation of Tv for all Codon Positions of the Cyt *b*

Taxon	Divergence time (MY)	Geological time scale
Antilopinae	13.6–15.3	Middle Miocene
Bovinae	12.0–14.3	Middle Miocene
Alcelaphini + Hippotragini	10.6–11.3	Late Miocene
Reduncini including *Pelea*	9.0–10.8	Late Miocene
Aepyceros/*Neotragus*	9.0–9.6	Late Miocene
Cephalophus/*Oreotragus*	8.4–11.8	Late Miocene
Kobus + *Redunca*	5.7–8.4	Late Miocene
Buffalo-Bovini (i.e., *Bubalus*/*Syncerus*)	6.9–7.7	End of Late Miocene
Antilopini	4.6–5.8	Miocene/Pliocene
Tragelaphini	4.7–6.6	Miocene/Pliocene
Caprini *s. l.*	2.8–7.0	Miocene/Pliocene
Alcelaphus + *Damaliscus*	3.6–5.4	Early Pliocene
Hippotragini	3.6–5.4	Early Pliocene
Boselaphini	3.3–4.8	Early Pliocene
Cattle-Bovini (i.e., *Bos*/*Bison*)	3.3–4.8	Early Pliocene
Antilope + *Gazella*	1.9–3.4	Late Pliocene
Oryx + *Addax*	0.6–2.9	Plio-Pleistocene

Note. The branch length estimates of the tree reconstructed by maximum-likelihood (Fig. 3) were used. Only Tv on first, second, and third positions of the codon were considered, and the removal of Ti was justified by their saturated pattern of accumulation during Pecora evolution. The calibration date was chosen at 20 MY for the divergence of Bovidae, yielding a mean rate of 0.13 ± 0.02% Tv/MY/lineage. To reduce the impact of rate heterogenities on the time estimations, the rates of Tv accumulation were calculated for each lineage.

DISCUSSION

Molecular Evolution of the Bovid Cytochrome b

The cyt *b* is a protein-coding gene and its evolution is confined to a limited number of nucleotide sites and character-state transformations because the gene product should remain functional. Consequently, the largest part of the phylogenetic signal corresponds to synonymous substitutions which are free to accumulate with time. As previously observed (e.g., Irwin *et al.*, 1991), the highest levels of homoplasy and saturation are measured at third codon positions (Table 2) for which all Ti and most of Tv are synonymous. At these positions, C-T transitions are more affected by multiple

substitutions than A-G transitions. Moreover, Tv involving A are more homoplastic and saturated than those involving G, reflecting the biases in base composition observed in third positions (T: 15%, C: 37%, A: 44%, G: 4%). These results, which confirm previous observations (Hassanin *et al.*, 1998a), show clearly that mutational constraints are different depending on the nucleotide change involved.

The majority of amino acid replacements involve residues such as Ile, Met, Thr, Val, and Ala which are easily permutable because they do not disrupt the properties of the molecule (Hassanin *et al.*, 1998a). For A-G transitions, it is striking to measure a similar amount of homoplasy and a higher level of saturation for first positions (CI = 0.286 and S = 0.314) compared to third positions (CI = 0.296 and S = 0.628). In this case, nonsynonymous substitutions (i.e., A-G 1) may consequently be less reliable for phylogenetic analyses than synonymous substitutions (i.e., A-G 3). Our hypothesis is that most of the A-G substitutions in first positions are weakly constrained and other types of substitutions (i.e., C-T 1, A-C 1, A-T 1, C-G 1, and G-T 1) are more constrained in first than in third positions. In other words, the mutational possibilities should be more limited in first positions. Three arguments support this hypothesis: (1) the amount of homoplasy and level of saturation measured for C-T 1, A-C 1, A-T 1, C-G 1, and G-T 1 are always lower than the corresponding substitution types in third position (Table 2); (2) the number of informative multistate sites involving A, G, and one of the two pyrimidines relative to the total number of informative sites for A-G is lower in first (6/36 = 0.17) than in third positions (51/133 = 0.38); and (3) A-G transitions at some first positions are poorly constrained because the concomitant change in amino acid (i.e., Ile-Val, Ile-Met, and Thr-Ala) involve residues which are biochemically very similar.

In second positions of the cyt *b*, the mutational possibilities are mainly limited to C-T transitions (Hassanin *et al.*, 1998a) like all other mitochondrial protein-encoding genes (Naylor *et al.*, 1995). It is interesting to note that second position, like first position, substitutions involve the replacement of the same amino acid residues (Ala, Ile, Met, Thr, and Val). For these two codon positions, the number of variable sites is low and strongly limited to the homoplastic and saturated A-G and C-T transitions (respectively for first and second positions). Moreover, in third codon positions the nonsynonymous substitutions are not numerous. Consequently, phylogenetic reconstructions using the cyt *b* amino acid sequences are based on a small number of informative sites which are strongly affected by homoplasy and we suspect therefore that such analyses are less reliable than those using the corresponding nucleotide sequences.

Our analyses of homoplasy and saturation resulted in a weighting procedure in which each of the six kinds of substitutions at each codon position has received a homoplasy- and saturation-related weight. This procedure, described in Hassanin *et al.* (1998; 1998a,b), is quite different from other approaches which have also used the Consistency Index for weighting, such as those of Farris (1969: successive weighting) or Goloboff (1993: searching fittest trees, with character fits being a concave function of homoplasy).

Phylogeny of the Family Bovidae

When the family Antilocapridae is used as outgroup, the monophyly of Bovidae relative to Giraffidae and Cervidae is well supported by MP (BP = 80, b_{wr} = +14.8) and ML based on Tv only (RP = 75) phylogenetic analyses. This suggests that the horns appeared once during the history of the family and that the possession of permanent and unbranched frontal appendages covered with a keratin sheath represents a true synapomorphy uniting the Bovidae (Janis and Scott, 1988). However, when Tragulidae are added to root the tree, the bovid monophyly is not evidenced: Antilocapridae and Giraffidae appear in basal position with respect to a multifurcation comprising Cervidae, Bovinae, and Antilopinae. In that case, the lack of signal toward Bovidae monophyly may be attributed to the distant position of the tragulid outgroup and the subsequent saturation of substitutions for Pecora/Tragulina comparisons. Additional data are required to unambiguously solve the question of the Bovidae monophyly.

The consensus of our present phylogenetic analyses based on 60 pecoran taxa (Fig. 4) indicates that extant bovids represent the product of a main split which gave rise to one Bovinae clade and one Antilopinae clade (Antilopini, Alcelaphini, Caprini *s. l.*, Cephalophini, Hippotragini, Neotragini, and Reduncini). This basal dichotomy is upheld by two exclusive synapomorphic Ti in the codon 123 (Table 4), which unambiguously define the Antilopinae and involve an amino acid replacement in the middle of the third transmembrane helix of the cyt *b* protein according to the Eight Domain Model (Howell, 1989). However, in the popular mind, Bovidae were divided into three groups: bovines (oxen and relatives), ovines (sheeps, goats, and others), and antilopes (all the other bovids) (cited in Simpson, 1945: 270). Schlosser (1904) criticized this longstanding view and introduced a distinction between Boodontia (Bovini, Boselaphini, Tragelaphini, Cephalophini, Hippotragini, and Reduncini) and Aegodontia (the other tribes), mainly based on dental characters (but see the morphological and paleontological discussion by Thomas, 1984: 263–266). The dichotomy between Bovinae species and non-Bovinae species, which invalidates the Aegodontia/Boodontia concept, has already been suggested by marked chromosomal differences (Buckland and Evans, 1978; Gallagher and Womack, 1992), immunological distances (Lowenstein, 1986), and mitochon-

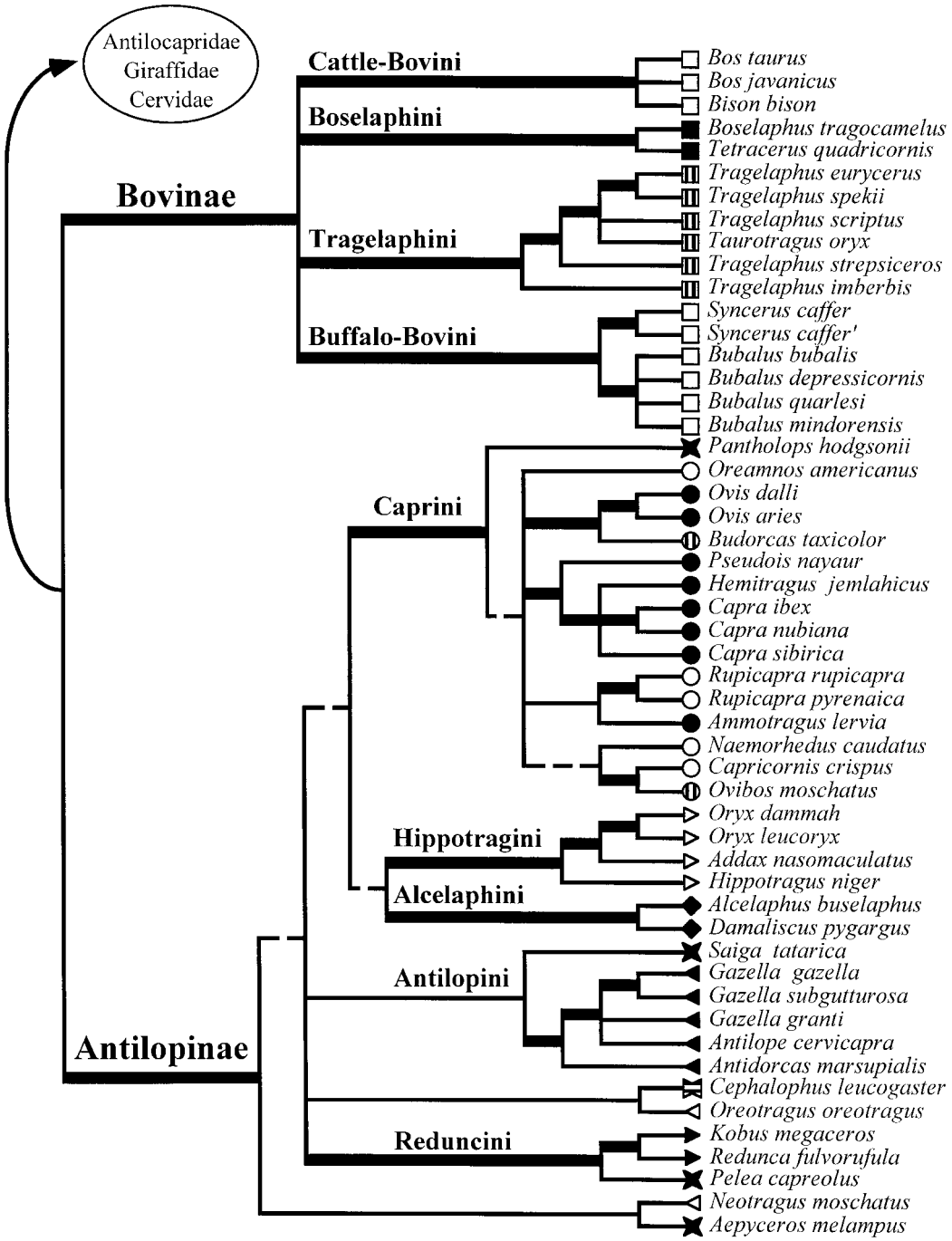

FIG. 4. Consensus tree of the MP, ML, and NJ analyses based on a differential weighting of transitions and transversions (CI · S weights for MP, and transversions only for ML and NJ methods). Three categories of nodes are identified depending on their robustness: ▬ nodes strongly supported by the three methods; — nodes strongly supported by at least one of the three methods; and - - - nodes moderately supported by the three methods. Each taxon is labeled by an icon corresponding to its classification in one of the subfamilies and tribes currently recognized (Gentry, 1992): Subfamily Bovinae: □ Tribe Bovini, ■ Tribe Boselaphini, ▥ Tribe Tragelaphini; Subfamily Cephalophinae: ▤ Tribe Cephalophini; Subfamily Hippotraginae: ▷ Tribe Hippotragini, ▶ Tribe Reduncini; Subfamily Alcelaphinae: ◆ Tribe Alcelaphini; Subfamily Antilopinae: ◀ Tribe Antilopini, ◁ Tribe Neotragini; Subfamily Caprinae: ● Tribe Caprini, ◍ Tribe Ovibovini, ○ Tribe Rupicaprini; Subfamily indeterminate: ★ Tribe indeterminate.

TABLE 4

Summary of the Cytochrome *b* Strictly Exclusive Molecular Synapomorphies Defining Some of the Bovidae Clades Evidenced in the Present Study, after Sequence Comparison of Two Tragulids, One Giraffid, One Antilocaprid, Six Cervids, and 52 Bovids

Clades defined by exclusive synapomorphies	Nucleotide substitution	Position (gene)	Amino acid replacement	Position (protein)
Antilopinae clade	G → A	367	Val → Thr	123
(i.e., all non-Bovinae bovids)	T → C	368	Val → Thr	123
Boselaphini (*Boselaphus* + *Tetracerus*)[a]	T → G	190	Ser → Ala	64
Buffalo-Bovini clade				
(*Bubalus* + *Syncerus*)	G → C	40	Val → Leu	14
Cattle-Bovini clade (*Bos* + *Bison*)	R → Y	921	Met → Ile	304
	A → C	927	No	309
Hippotragini	A → T	570	Homoplastic	190
(*Oryx* + *Addax* + *Hippotragus*)	T → C	917	Leu → Ala[b]	306
Genus *Oryx*	A → T	1017	No	339
(here *O. dammah* + *O. leucoryx*)				
Reduncini (*Redunca* + *Kobus*)	A → T	180	Homoplastic	60
Alcelaphini (*Alcelaphus* + *Damaliscus*)	A → C	591	No	197
Cephalophus + *Oreotragus*[c]	C → A	328	Autapomorphic[d]	110
Within Antilopini: *Gazella* + *Antilope*	C → A	522	No	174
Within Caprini *s. l.*: *Ovis* + *Budorcas*	A → C	876	No	292
Capra sibirica + *Hemitragus*	G → A	292	Val → Ile	98

Note. Only the exclusive nucleotide substitutions and amino acid replacements are indicated, i.e., those representing a molecular "signature" for the clade under study. The low number of exclusive synapomorphies is the consequence of the high number (62) of taxa examined, leading to a high frequency of homoplasy occurrence. The diagnostic nucleotide substitutions are either synonymous (no amino acid replacement) or involve diagnostic, autapomorphic, or homoplastic replacements. Sites of the cytochrome *b* gene are numbered from nucleotides 1 to 1140, and sites of the cytochrome *b* protein are numbered from amino acids 1 to 379.

[a] The same analysis identifies an exclusive morphological synapomorphy for this clade: supraorbital foramina placed forward and widely apart.

[b] An autapomorphic Ala → Thr replacement occurred subsequently in *Oryx dammah*.

[c] The cladistic analysis of the 112 morphological characters listed by Gentry (1992) reveals that two character states, initially coded as primitive, may actually constitute two exclusive synapomorphies for this clade: an enlarged back half of molar M^3 (possibly acquired in *Dorcatragus* by convergence) and a smaller foramina ovalia.

[d] Leu → Met for *Cephalophus* and Leu → Ile for *Oreotragus*.

drial ribosomal gene comparisons (Gatesy *et al.*, 1997). On the basis of differences concerning thermoregulation, gland structures, and horn types, Kingdon (1997: 346–347) distinguishes these two major groups and suggests that a continental separation over 20 million years ago might have marked their first divergence, with Bovinae and Antilopinae evolving respectively in Eurasia and Africa.

The Tribal Radiation of Bovinae

The subfamily Bovinae comprises four multifurcating major clades (Fig. 4): Boselaphini, two Bovini clades (cattle-Bovini and buffalo-Bovini), and Tragelaphini. This multifurcation either may reflect the lack of resolving power for the cyt *b* marker at this level of the bovid tree or could evidence an explosive radiation among Bovinae, therefore precluding the accumulation of molecular synapomorphies in the cyt *b* during successive cladogeneses.

Boselaphini are clearly monophyletic, and this contradicts the morphological analysis of Gentry (1992), which concludes an association of *Boselaphus* with *Bison* rather than with *Tetracerus*. Boselaphines are defined by an exclusive synapomorphic Tv involving an amino acid replacement in our cyt *b* data, and morphology supports this result with supraorbital foramina placed forward and widely apart (Table 4). However, the lack of resolution of the branching order among the four lineages prevents checking for the postulated basal position of boselaphines among Bovinae suggested by the fossil record (e.g., Gentry, 1990: 204).

A clear distinction between cattle (*Bos* and *Bison*) and buffaloe (*Bubalus* and *Syncerus*) has been previously noted within Bovini by morphological and molecular studies (Groves, 1981; Wall *et al.*, 1992; Janecek *et al.*, 1996; Pitra *et al.*, 1997). Our cyt *b* data confirm this deep dichotomy, providing three diagnostic Tv for these clades (Table 4), but do not evidence a robust signal toward the Bovini monophyly. Moreover, the paraphyly of the genus *Bos* due to the inclusion of *Bison* is suggested but not strongly supported, despite a limited taxonomic specific representation for *Bos*, in accordance with previous mitochondrial DNA studies (Miyamoto *et al.*, 1993; Janecek *et al.*, 1996) but discordant from the nuclear DNA study of Pitra *et al.* (1997).

Within buffaloes, the anoa (*Bubalus depressicornis*) robustly groups with other *Bubalus* species, which conforms with morphology and paleontology (Geraads, 1992), but contradicts its sister group relationship to the nilgai as suggested by the molecular data of Pitra *et al.* (1997).

Tragelaphini are monophyletic and the lesser kudu (*Tragelaphus imberbis*) is the basalmost species. This cyt *b* result contrasts with the paleontological view of Gentry (1978) in which *T. imberbis* is the sister group of *T. strepsiceros*. The genus *Tragelaphus* appears paraphyletic due to the inclusion of *Taurotragus,* and this questions the distinct generic status given to the eland. *Taurotragus* should be better included within *Tragelaphus,* following Georgiadis *et al.* (1990) and Gatesy *et al.* (1997). One should note that transitional events saturate for comparisons involving *T. strepsiceros, T. imberbis,* and *T. oryx* (Fig. 1 and data not shown). The consequence of this saturation is that *Taurotragus* robustly groups either with *T. strepsiceros* when all substitutions are equally weighted or with *T. scriptus, T. spekii,* and *T. euryceros* when Tv are favored relative to Ti by differential weighting (Figs. 2 and 3). A detailed analysis of this situation reveals that in the MP tree with equal weighting of Ti and Tv, the *Taurotragus/ Tragelaphus strepsiceros* association is defined by 17 synapomorphies (mean CI of the 17 substitutions: 0.137 ± 0.103), of which 14 are third codon position C-T changes. These events have been identified as the most homoplastic and saturated among bovids (Fig. 1 and Table 2). So, the clustering of the common eland with the greater kudu may be artefactual, as it relies on very noisy substitutions. If we constrain the grouping of the common eland with the bongo–sitatunga–bushbuck clade, 16 synapomorphies will define the ancestral segment (mean CI of the 16 substitutions: 0.236 ± 0.175). Among them, 15 are Ti, of which 9 are C-T and 6 are A-G events (including 2 first and 2 second codon position substitutions). This branching pattern is therefore defined by mutations less subject to homoplasy and saturation and could better describe the phylogenetic position of *Taurotragus oryx.*

The Tribal Radiation of Antilopinae

The Antilopinae clade, i.e., all non-Bovinae bovids, includes seven major lineages: Caprini *s. l.,* Hippotragini, Alcelaphini, Antilopini *s. l.,* Cephalophini + *Oreotragus,* Reduncini *s. l.,* and *Aepyceros* + *Neotragus.* Among them, three tribes are clearly monophyletic and defined by diagnostic substitutions (Table 4): Alcelaphini (*Alcelaphus, Damaliscus*), Hippotragini (*Hippotragus, Addax,* and *Oryx*), and Reduncini (*Redunca, Kobus*).

Among Caprini *s. l.* (i.e., Caprinae plus *Pantholops*), the chiru places in basal position with respect to other caprine genera for which only three clades are evidenced (Figs. 2 and 3): (1) *Ovis* + *Budorcas* (see Table 4); (2) *Pseudois* + *Hemitragus* + *Capra;* and (3) *Naemorhedus* + *Capricornis* + *Ovibos.* These results confirm previous observations which concluded that a rapid cladogenesis exists within this tribe (Hassanin *et al.,* in press).

Our analyses group together Alcelaphini and Hippotragini, supporting the point of view of Simpson (1945) and Gentry (1992) based on the study of morphological characters. A phyletic closeness for alcelaphines and hippotragines has also been proposed by Gallagher and Womack (1992) by comparison of the X chromosome. Moreover, the cyt *b* data suggest that Caprini *s. l.,* Hippotragini, and Alcelaphini could have shared a direct common ancestor, confirming some cytogenetic (Buckland and Evans, 1978; Claro *et al.,* 1995) as well as morphological and ecological observations (Kingdon, 1997: 366).

Neotragini, represented here by *Neotragus* and *Oreotragus,* clearly appear polyphyletic. The klipspringer is close to the only cephalophine considered in the present study, the white-bellied duiker. The smaller foramina ovalia, coded as a primitive state by Gentry (1992), could actually be a derived state representing an exclusive synapomorphy among bovids defining the *Oreotragus*/Cephalophini clade.

The suni surprisingly clusters with the impala but three characteristics let us suppose a possible effect of long branch attraction (Felsenstein, 1978): (1) no exclusive synapomorphy characterizes this clade; (2) the branch support remains contrasted with a low BP (62) and a high b_{wr} (+11.7) (Fig. 2); (3) the group stands in basalmost position within the Antilopinae. The basal position of *Neotragus* and *Aepyceros* with respect to other Antilopinae may also suggest that they are the extant representatives of two primitive lineages.

Within the Antilopini, the blackbuck appears closely related to the gazelles, which confirms the conclusions given by cytogenetic (e.g., Gallagher and Womack, 1992), osteological (Groves, 1985), and alloenzyme data (Vassart, 1994), and indicates that *Antilope* and *Gazella* should be treated as congeneric. An exclusive Tv synapomorphy reinforces this result (Table 4). The springbok is the sister group of the *Gazella–Antilope* assemblage, and this agrees with their classical branching within the Antilopini (Gentry, 1992).

The questions related to the phylogenetic position of the odd genera *Pelea, Pantholops,* and *Saiga* receive robust answers. *Pelea* appears as the sister taxa of all reduncines in our analyses, confirming previous morphological and molecular data (Simpson, 1945; Gatesy *et al.,* 1997). *Pantholops* and *Saiga* were placed by Simpson (1945) in the subfamily Caprinae while other authors group them with the Antilopini (von Roy, 1958, and Schwartz, 1937; in Gentry, 1992). Our cyt *b* data cluster the saiga with *Gazella, Antilope,* and *Antidorcas* in an enlarged tribe Antilopini, and the chiru with caprines in the tribe Caprini *s. l.,* in agreement with

morphological (Gentry, 1992) and ribosomal DNA (Gatesy *et al.*, 1997) analyses.

The relationships among the various lineages of Antilopinae remain rather unresolved, but a first step in the knowledge of the evolution of this subfamily is the identification of close phylogenetic affinities between three tribes, with Caprini *s. l.* being sister group of Alcelaphini plus Hippotragini. Additional investigations based on other mitochondrial and nuclear molecular markers may clarify the phylogeny of this predominantly African clade.

Chronology of Bovidae Evolution

A mean rate of 0.13 ± 0.02%/MY/lineage was found for Tv accumulation in the cyt *b* of Bovidae. This rate is of the same order of magnitude as the 0.14% Tv/MY found among Bovidae in the mitochondrial 12S and 16S (Allard *et al.*, 1992). The bovid substitution rate, calibrated on 20 MY for the emergence of the family, leads us to identify three main episodes of cladogeneses during the evolution of the group.

A first major split might have taken place in the Early Miocene as a consequence of a continental separation: the Bovinae remain in Eurasia and the Antilopinae develop in Africa (Gentry, 1994; Kingdon, 1997). The cyt *b* phylogenetic picture (Figs. 2 and 3) is congruent with this view, as the extant Bovinae are represented mainly in Eurasia (Boselaphini, cattle-Bovini, *Bubalus*) and the more basal lineages of Antilopinae are restricted to Africa (Aepycerotini, Neotragini, Reduncini *s. l.*, Cephalophini).

The second episode resulted in an explosive tribal radiation during the Middle Miocene and gave rise to the majority of extant Bovinae and Antilopinae tribes. These molecular conclusions are in full agreement with the study of paleoecological trends of the late Cenozoic terrestrial ecosystems, which indicates that during 16.0 to 13.5 MY, bovids (and muroid rodents) radiated independently in Southern Asia and Africa (Behrensmeyer *et al.*, 1992: 482). The timing of the Bovidae radiation is notably well documented in the Siwalik formations of India and Pakistan, where a Middle Miocene transition to large mammal assemblages dominated by bovids is observed, as a result of a process of diversity increase occuring largely through immigration rather than *in situ* speciation (Barry *et al.*, 1991).

The third phase corresponded to a period of radiation at the Miocene/Pliocene boundary (Late Miocene and Early Pliocene: Table 3). This period was marked by an important global climate change promoting the spread of grasslands and the evolution of bovids adapted to a savanna-type habitat (Cerling *et al.*, 1997). This scenario agrees with the paleontological data that show the emergence of Aepycerotini, Alcelaphini, Hippotragini, Reduncini, and Tragelaphini in Africa, the diversification of Boselaphini in Asia, the appearance of Bovini in Eurasia and its subsequent immigration into Africa, and the expansion of Antilopini in Africa and Eurasia (Vrba, 1985; Gentry, 1994). Our cyt *b* data also imply that Alcelaphini, Caprini *s. l.* and Hippotragini diverged during this time either in Africa or in Eurasia. This is in contradiction with the view of Vrba (1985), which proposes that Caprini *s. l.* appeared for the first time in sub-Saharan Africa near 14 MY ago but is in agreement with the opinion of Gentry (1994), which suggests a more recent origin (Late Miocene). Subsequent diversifications in the tribes Antilopini and Hippotragini occurred during the Plio-Pleistocene, with the emergence of new genera such as *Antilope* and *Gazella*, or *Oryx* and *Addax* (Table 3).

Three main phases of Bovidae evolution were evidenced by the cyt *b* sequence comparisons, and, as evidenced by the paleontology, they imply that different bovid groups experienced several migrations between Eurasia and Africa. The Bovinae fossil record suggests a common South Asian origin for the subfamily. The radiation of Boselaphini, Bovini, and Tragelaphini during the Middle Miocene (Fig. 3, Table 3) indicates that tragelaphines migrated in Africa where they have been distinct for at least 15 MY and the later separation between African and Asian buffaloes indicates a subsequent migration in Africa (Kingdon, 1997). The Antilopinae probably began to evolve in Africa from the very earliest immigrants from Eurasia. Some of them were always restricted to Africa, such as Cephalophini, but subsequent migrations in Eurasia could have given rise to Caprini *s. l.* during the Mio-Pliocene or to the specialized species *Saiga tatarica*.

CONCLUSION

Our study has proven that the cyt *b* gene is a suitable phylogenetic marker to reconstruct the Bovidae phylogeny, but the use of differential weighting schemes seems to be an unescapable prerequisite in order to reduce the impact of the highly homoplastic and saturated events on phylogenetic inferences. The position of *Pantholops*, *Pelea*, and *Saiga* is unambiguously established while *Aepyceros* remains a problematic genus even if our results suggest a basal position, close to *Neotragus*, with respect to other Antilopinae.

The family Bovidae is shown to be monophyletic with two major clades: the Bovinae and the Antilopinae. Two contemporaneous periods of explosive cladogeneses seem to occur in the Middle Miocene, producing most of the extant tribes which underwent a subsequent radiation in the Late Miocene/Early Pliocene. As a result, no clear relationships have been determined among the various Antilopinae and Bovinae lineages except a possible link between Caprini *s. l.* and the sister tribes Alcelaphini and Hippotragini. The brevity of the Middle Miocene radiations within the two subfamilies must be advanced to explain this lack of resolution.

ACKNOWLEDGMENTS

The bovid samples were kindly provided by Nico Avenant (South Africa), Carine Bourrellis (Sigean, France), Philippe Chardonnet (Paris, France), Jacques Cuisin (Paris, France), Bertrand Des Clers (Paris, France), the late Marcel Gallet (Montpellier, France), Françoise Hergueta-Claro (Vincennes, France), Jean-Pierre Hugot (La Wangny, Gabon), Jean-Marc Pons (Paris, France), Oliver Ryder (San Diego, CA), and Michel Tranier (Paris, France). We are especially indebted to Bettine Jansen van Vuuren, Conrad Matthee, Terry Robinson, and Josephine Birungi for kindly providing us with the *Pelea* cytochrome *b* sequence and phylogenetic data prior to publication. Jean-Jacques Jaeger, Guillaume Lecointre, Jean Sudre, Simon Tillier, and two reviewers provided useful comments and criticism. We thank Céline Bonillo and Annie Tillier for their contribution to this work. Laboratory equipment was partially funded by the "Service Commun de Biosystématique de Montpellier," and E.D. gratefully acknowledges the help of François Catzeflis for laboratory facilities, for curating the Mammals Tissue Collection, and for helpful discussions. This work was supported by the "Groupement de Recherche et d'Etude des Génomes" (G.R.E.G.; décision N° 82/94 of 19 July 1994). This is contribution N° 98-093 of the Institut des Sciences de l'Evolution de Montpellier (UMR 5554-CNRS).

REFERENCES

Allard, M. W., Miyamoto, M. M., Jarecki, L., Kraus, F., and Tennant, M. R. (1992). DNA systematics and evolution of the artiodactyl family Bovidae. *Proc. Natl. Acad. Sci. USA* **89:** 3972–3976.

Anderson, S., De Bruijn, M. H. L., Coulson, A. R., Eperon, I. C., Sanger, F., and Young, I. G. (1982). Complete sequence of bovine mitochondrial DNA. Conserved feature of the mammalian mitochondrial genome. *J. Mol. Evol.* **156:** 683–717.

Barry, J. C., Morgan, M. E., Winkler, A. J., Flynn, L. J., Lindsay, E. H., Jacobs, L. L., and Pilbeam, D. (1991). Faunal interchange and Miocene terrestrial vertebrates of southern Asia. *Paleobiology* **17:** 231–245.

Behrensmeyer, A. K., Damuth, J. D., DiMichele, W. A., Potts, R., Sues, H.-D., and Wing, S. L. (1992). "Terrestrial Ecosystems through Time: Evolutionary Paleoecology of Terrestrial Plants and Animals," The Univ. of Chicago Press, Chicago.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42:** 795–803.

Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10:** 295–304.

Buckland, R. A., and Evans, H. J. (1978). Cytogenetic aspects of phylogeny in the Bovidae. I. G-banding. *Cytogenet. Cell. Genet.* **21:** 42–63.

Catzeflis, F. M. (1991). Animal tissue collections for molecular genetics and systematics. *Trends Ecol. Evol.* **6:** 168.

Cerling, T. E., Harris, J. M., MacFadden, B. J., Leakey, M. G., Quade, J., Eisenmann, V., and Ehleringer, J. R. (1997). Global vegetation change through the Miocene/Pliocene boundary. *Nature* **389:** 153–158.

Chikuni, K., Mori, Y., Tabata, T., Saito, M., Monma, M., and Kosugiyama, M. (1995). Molecular phylogeny based on the k-casein and cytochrome *b* sequences in the mammalian suborder Ruminantia. *J. Mol. Evol.* **41:** 859–866.

Claro, F., Hayes, H., and Cribiu, E. P. (1995). Identification of p and q arms of the blesbok (*Damaliscus dorcas phillipsi,* Alcelaphinae) RBG-banded chromosomes with comparison to other wild and domestic bovids. *Cytogenet. Cell. Genet.* **70:** 268–272.

Degli Espoti, M., De Vries, S., Crimi, M., Ghelli, A., Patarnello, T., and Meyer, A. (1993). Mitochondrial cytochrome *b:* Evolution and structure of the protein. *Biochim. Biophys. Acta* **1143:** 243–271.

Douzery, E., and Randi, E. (1997). The mitochondrial control region of Cervidae: Evolutionary patterns and phylogenetic content. *Mol. Biol. Evol.* **14:** 1154–1166.

Eriksson, T. (1995). AutoDecay version 3.0. Distributed by the author. Department of Botany, University of Stockholm, Sweden.

Farris, J. S. (1969). A successive approximations approach to character weighting. *Syst. Zool.* **18:** 374–385.

Felsenstein, J. (1978). Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* **27:** 401–410.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* **17:** 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.

Gallagher, D. S., and Womack, J. E. (1992). Chromosome conservation in the Bovidae. *J. Hered.* **83:** 287–298.

Gatesy, J., Yelon, D., Desalle, R., and Vrba, E. (1992). Phylogeny of the Bovidae (Artiodactyla, Mammalia), based on mitochondrial DNA sequences. *Mol. Biol. Evol.* **9:** 433–446.

Gatesy, J., Amato, G., Vrba, E., Schaller, G., and DeSalle, R. (1997). A cladistic analysis of mitochondrial ribosomal DNA from Bovidae. *Mol. Phylogenet. Evol.* **7:** 303–319.

Gentry, A. W. (1978). Bovidae. *In* "Evolution of African Mammals" (V. J. Maglio and H. B. S. Cooke, Eds.), pp. 540–572. Harvard Univ. Press, Cambridge, MA.

Gentry, A. W. (1990). Evolution and dispersal of african Bovidae. *In* "Horns, Pronghorns, and Antlers" (G. A. Bubenik and A. B. Bubenik, Eds.), pp. 195–233. Springer-Verlag, New York.

Gentry, A. W. (1992). The subfamilies and tribes of the family Bovidae. *Mammal. Rev.* **22:** 1–32.

Gentry, A. W. (1994). The Miocene differentiation of Old World Pecora (Mammalia). *Historical Biol.* **7:** 115–158.

Georgiadis, N. J., Kat, P. W., Oketch, H., and Patton, J. (1990). Allozyme divergence within the Bovidae. *Evolution* **44:** 2135–2149.

Geraads, D. (1992). Phylogenetic analysis of the tribe Bovini (Mammalia: Artiodactyla). *Zool. J. Linn. Soc.* **104:** 193–207.

Ginsburg, L., and Heintz, E. (1968). La plus ancienne antilope d'Europe, *Eotragus artenensis* du Burdigalien d'Artenay. *Bull. Mus. Hist. Nat.* **40:** 837–842.

Goloboff, P. A. (1993). Estimating character weights during tree search. *Cladistics* **9:** 83–91.

Griffiths, C. S. (1997). Correlation of functional domains and rates of nucleotide substitution in cytochrome *b*. *Mol. Phylogenet. Evol.* **7:** 352–365.

Groves, C. P. (1981). Systematic relationships in the Bovini (Artiodactyla, Bovidae). *Z. Zool. Syst. Evolutionsforsch.* **19:** 264–278.

Groves, C. P. (1985). An introduction to the gazelles. *Chinkara* **1:** 4–16.

Groves, P., and Shields, G. F. (1996). Phylogenetics of the Caprinae based on cytochrome *b* sequence. *Mol. Phylogenet. Evol.* **5:** 467–476.

Grubb, P. (1993). Family Bovidae. *In* "Mammal Species of the World. A Taxonomic and Geographic Reference" (D. E. Wilson and D. M. Reeder Eds.), pp. 393–414. Smithsonian Inst. Press, Washington.

Gustafsson, M. H. G., and Bremer, K. (1995). Morphology and phylogenetic interrelationships of the Asteraceae, Calyceraceae, Campanulaceae, Goodeniaceae, and related families (Asterales). *Am. J. Bot.* **82:** 250–265.

Hassanin, A., Lecointre, G., and Tillier, S. (1998a). The "evolutionary signal" of homoplasy in protein-coding gene sequences and its consequences for *a priori* weighting in phylogeny. *C. R. Acad. Sci.*, Sér. III **321:** 611–620.

Hassanin, A., Pasquet, E., and Vigne, J.-D. (1998b) Molecular systematics of the subfamily Caprinae (Artiodactyla, Bovidae) as deter-

mined from cytochrome *b* sequences. *J. Mammal. Evol.,* 5: 217–236.

Howell, N. (1989). Evolutionary conservation of protein regions in the proton-motive cytochrome *b* and their possible roles in redox catalysis. *J. Mol. Evol.* **29:** 157–169.

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

Janecek, L. L., Honeycutt, R. L., Adkins, R. M., and Davis, S. K. (1996). Mitochondrial gene sequences and the molecular systematics of the artiodactyl subfamily Bovinae. *Mol. Phylogenet. Evol.* **6:** 107–119.

Janis, C. M., and Scott, K. M. (1988). The phylogeny of the Ruminantia (Artiodactyla Mammalia). *In* "The Phylogeny and Classification of the Tetrapods (Vol. 2: Mammals)" (M. J. Benton, Ed.), pp. 273–282. Clarendon, Oxford.

Kikkawa, Y., Amano, T., and Suzuki, H. (1995). Analysis of genetic diversity of domestic cattle in east and Southeast Asia in terms of variations in restriction sites and sequences of mitochondrial DNA. *Biochem. Genet.* **33:** 51–60.

Kingdon, J. (1997). "The Kingdon Field Guide to African Mammals," Academic Press, London.

Kluge, A. G., and Farris, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst. Zool.* **18:** 1–32.

Lowenstein, J. M. (1986). Bovid relations based on serum immunology. *S. Afr. J. Sci.* **82:** 77–78.

Maddison, W. P., and Maddison, D. R. (1992). MacClade, version 3.04. Sinauer, Sunderland, MA.

Matthee, C. A., and Robinson, T. J. Cytochrome *b* phylogeny of the family Bovidae: Resolution within the Alcelaphini, Antilopini, Neotragini, and Tragelaphini. *Mol. Phylogenet. Evol.,* in press.

Miyamoto, M. M., Kraus, F., Laipis, P. J., Tanhauser, S. M., and Webb, S. D. (1993). Mitochondrial DNA phylogenies within Artiodactyla. *In* "Mammal Phylogeny: Placentals" (F. S. Szalay, M. J. Novacek, and M. C. McKenna, Eds.), pp. 268–281. Springer Verlag, New York.

Naylor, G. J. P., Collins, T. M., and Brown, W. M. (1995). Hydrophobicity and phylogeny. *Nature* **373:** 565–566.

Philippe, H. (1993). MUST: a computer package of management utilities for sequences and trees. *Nucleic Acids Res.* **21:** 5264–5272.

Philippe, H., Sörhannus, U., Baroin, A., Perasso, R., Gasse, F., and Adoutte, A. (1994). Comparison of molecular and paleontological data in diatoms suggests a major gap in the fossil record. *J. Evol. Biol.* **7:** 247–265.

Pitra, C., Fürbass, R., and Seyfert, H.-M. (1997). Molecular phylogeny of the tribe Bovini (Mammalia: Artiodactyla): Alternative placement of the Anoa. *J. Evol. Biol.* **10:** 589–600.

Randi, E., Mucci, N., Pierpaoli, M., and Douzery, E. (1998). New phylogenetic perspectives on the Cervidae (Artiodactyla) are provided by the mitochondrial cytochrome *b* gene. *Proc. R. Soc. London,* series B **265:** 793–801.

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

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:** 5463–5467.

Schlosser, M. (1904). Die fossilen Cavicornia von Samos. *Beitr. Paläont. Geol. Österreich-Ungarns* **17:** 21–118.

Simpson, G. G. (1945). The principles of classification and a classification of mammals. *Bull. Am. Mus. Nat. Hist.* **5:** 1–350.

Strimmer, K., and von Haeseler, A. (1996). Quartet puzzling: A quartet maximum-likelihood method for reconstructing tree topologies. *Mol. Biol. Evol.* **13:** 964–969.

Swofford, D. L. (1993). PAUP 3.1.1: Phylogenetic analysis using parsimony. Computer program distributed by the Illinois Natural History Survey, Champaign.

Taberlet, P., Mattock, H., Dubois-Paganon, C., and Bouvet, J. (1993). Sexing free-ranging brown bears *Ursus arctos* using hairs found in the field. *Mol. Ecol.* **2:** 399–403.

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

Tanaka, K., Solis, C. D., Masangkay, J. S., Maeda, K.-I., Kawamoto, Y., and Namikawa, T. (1996). Phylogenetic relationship among all living species of the genus *Bubalus* based on DNA sequences of the cytochrome *b* gene. *Biochem. Genet.* **34:** 443–452.

Thomas, H. (1984). Les Bovidae (Artiodactyla: Mammalia) du Miocène du sous-continent indien, de la péninsule arabique et de l'Afrique: Biostratigraphie, biogéographie et écologie. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **45:** 251–299.

Vassart, M. (1994). "Evolution et Diversité Génétique chez les Gazelles (*Gazella*); Apports de l'Électrophorèse des Protéines, de la Cytogénétique et des Microsatellites." Thèse de l'Université Paris XI Orsay.

Vrba, E. S. (1985). African Bovidae: Evolutionary events since the Miocene. *S. Afr. J. Sci.* **81:** 263–266.

Wall, D. A., Davis, S. K., and Read, B. M. (1992). Phylogenetic relationships in the subfamily Bovinae (Mammalia: Artiodactyla) based on ribosomal DNA. *J. Mamm.* **73:** 262–275.

Winnepenninckx, B., Backeljau, T., and Dewachter, R. (1993). Extraction of high molecular weight DNA from molluscs. *Trends Genet.* **9:** 407.

Zhang, D.-X., and Hewitt, G. M. (1996). Nuclear integrations: Challenges for mitochondrial DNA markers. *Trends Ecol. Evol.* **11:** 247–251.