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Source: *Systematic Biology*, Vol. 49, No. 3 (Sep., 2000), pp. 515-538
Published by: Oxford University Press for the Society of Systematic Biologists
Stable URL: https://www.jstor.org/stable/2585385
Accessed: 14-04-2019 06:18 UTC

Syst. Biol. 49(3):515–538, 2000

Hidden Morphological Support for the Phylogenetic Placement of *Pseudoryx nghetinhensis* with Bovine Bovids: A Combined Analysis of Gross Anatomical Evidence and DNA Sequences from Five Genes

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Abstract.—The saola, *Pseudoryx nghetinhensis*, was unknown to science until its formal description in 1993. This endangered species is a member of the ruminant artiodactyl family Bovidae (cattle, sheep, goats, and antelopes). However, given its puzzling combination of morphological traits, the specific affinities of *Pseudoryx* within Bovidae are controversial. A preliminary genetic investigation suggested that *Pseudoryx* should be placed in the subfamily Bovinae (cattle, buffaloes, spiral-horned antelopes, and nilgai), but a recent cladistic analysis of skeletal and dental characters allied *Pseudoryx* with caprine bovids (sheep, goats, musk oxen, goat antelopes, and *Pantholops*). The morphological and molecular hypotheses differ in assigning the saola to either of the two most divergent clades of Bovidae. In this report, phylogenetic analyses of DNA sequences from five genes are used to test these alternatives. Protein coding regions, introns, and ribosomal DNAs from the nuclear and mitochondrial genomes discount the hypothesis that *Pseudoryx* is a close relative of Caprinae. Instead, combined analyses of the DNA data and published morphological evidence place *Pseudoryx* with Bovini (cattle and buffaloes), a subclade of Bovinae. In a separate analysis, the matrix of morphological characters links *Pseudoryx* with caprine bovids, but in the context of the molecular data, the gross anatomical evidence strongly supports a grouping of *Pseudoryx* with Bovinae. Surprisingly, the morphological partition provides the most character support in the combined analysis. This striking result is obscured by separate analyses of the individual data sets and the taxonomic congruence approach. [Bovidae; hidden support; *Pseudoryx*; saola; Vu Quang.]

In 1992, a distinctive new bovid species, the saola, *Pseudoryx nghetinhensis*, was discovered in the montane evergreen forests of Vu Quang, Vietnam (Dung et al., 1993) and was subsequently described from neighboring areas in Laos (Schaller and Rabinowitz, 1995). *Pseudoryx nghetinhensis* is characterized by a unique combination of morphological traits, including long, smooth, spindle-shaped horns, a large preorbital fossa, domed nasals, an elongated premolar row, frontal hollowings that extend to the base of the horn cores, and a striking pattern of white markings on a predominantly chestnut and black pelage (Dung et al., 1993, 1994; Thomas, 1994; Schaller and Rabinowitz, 1995). The new bovid does not definitively fit into any of the 13 traditional bovid tribes of Simpson (1945), most of which can be traced back to the Miocene (Savage and Russell, 1983; Vrba, 1985); therefore *Pseudoryx* may be a phylogenetic relict with no close extant relatives.

The saola is confined to the Annamite Range of South East Asia. The region is characterized by a mixture of recent and ancient taxonomic elements that in combination form a significant center of endemism (Giao et al., 1998; Surridge et al., 1999; Groves and Schaller, 2000). In the past 5 years, several new species of hoofed mammals have been discovered in the Annamites and adjacent areas. The discovery of even a single new mammalian species of large body size in the late twentieth century is a rare event. The discovery of several large mammalian species in such a small geographic area is even more remarkable. Included in the list of novel taxa from the region are several muntiacine cervids (Tuoc et al., 1994; Schaller and Vrba, 1996; Bauer, 1997; Giao et al., 1998; Groves and Dawson, in prep; Amato et al., 2000); a suid, *Sus bucculentus*, that was recently "rediscovered" (Groves et al., 1997); a large bovid that is, as yet, poorly described ("*Pseudonovibos*"; Peter and Feiler, 1994; Dioli, 1997; Nadler, 1997); and *Pseudoryx*. Phylogenetic analyses of the unique elements of the Annamite fauna should contribute to a clearer biogeographic

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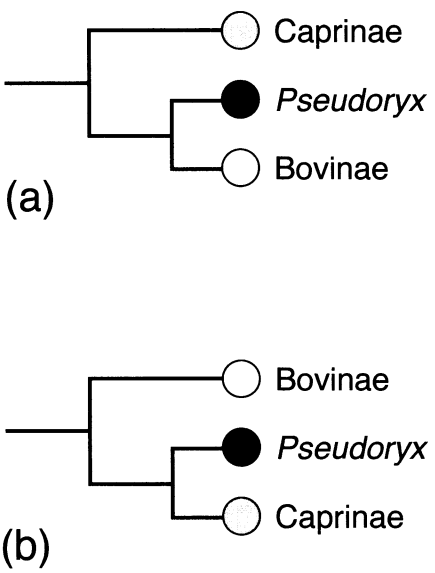

FIGURE 1. Two previous hypotheses regarding the phylogenetic relationship of *Pseudoryx* to other bovids: inferences from (a) DNA data (Dung et al., 1993) and (b) dental + skeletal data (Thomas, 1994). Shaded circles = Caprinae + *Pantholops*.

understanding of this region (e.g., Giao et al., 1998; Groves and Schaller, 2000).

In the initial description of the saola, Dung et al. (1993) presented preliminary mitochondrial (mt) cytochrome *b* evidence that placed *Pseudoryx* with Bovinae (Fig. 1a). This subfamily of bovids includes cattle (Bovini), buffaloes (Bovini), and some "antelope" tribes such as Tragelaphini and Boselaphini (Simpson, 1945). Despite some corroboration from gross anatomical evidence (Dung et al., 1994; Schaller and Rabinowitz, 1995; Groves and Schaller, 2000), the bovine affinities of the saola are controversial.

Thomas (1994) scored *Pseudoryx* for the cranial and dental characters of Gentry (1992) and concluded that the saola is the sister taxon of the bovid subfamily Caprinae (goats, sheep, goat antelopes, and musk oxen) plus the problematic genus *Pantholops*. The results of Thomas (1994) thus indicate that *Pseudoryx* is more closely related to goats and their kin than to cattle and their relatives (Fig. 1b).

In this report, phylogenetic analyses of DNA sequences from five genes—nuclear (nu) β-casein, nu κ-casein, nu α-lactalbumin, 12S mt ribosomal (r) DNA, and 16S mt rDNA—are used to determine whether *Pseudoryx* is more closely related to bovine or caprine bovids. A simultaneous/combined analysis (Kluge, 1989; Nixon and Carpenter, 1996) of the DNA data and the morphological evidence (Gentry, 1992; Thomas, 1994) ultimately is used to delineate the specific phylogenetic affinities of *Pseudoryx*. The distribution of unambiguous synapomorphies among data partitions, partitioned branch support (Baker and DeSalle, 1997), hidden branch support (Gatesy et al., 1999), and successive data set removal (Olmstead and Sweere, 1994) are used to measure corroboration and conflict among data sets in the simultaneous analysis.

MATERIALS AND METHODS

Genes and Taxa

The mt DNA of bovids accumulates nucleotide substitutions at a rapid rate (Irwin et al., 1991; Allard et al., 1992; Chikuni et al., 1995; Honeycutt et al., 1995; Groves and Shields, 1996). Because of extensive homoplasy and the rapid radiation of bovid tribes in the Miocene, analyses of mt DNA sequences have offered only weakly supported resolution of intertribal relationships within Bovidae (e.g., Allard et al., 1992; Gatesy et al., 1997). Therefore in addition to mt genes, we chose to sequence several nu loci. Relative to mt DNA, the nu genes offer fewer variable sites but (it is hoped) less saturation from multiple, overlapping substitutions.

Comparisons of published DNA sequences from bovids were used as references in choosing genes with enough informative sites for our analysis. Relatively rapidly evolving nu loci were chosen in addition to two well-characterized mt genes. The seven DNA fragments in this study are characterized by a variety of evolutionary dynamics (Gatesy et al., 1994, 1996, 1997; Chikuni et al., 1995; Cronin et al., 1996; Gatesy, 1998; Milinkovitch et al., 1998). These sequences include protein coding regions (nu β-casein exon 7 ~450 bases, nu κ-casein exon 4 ~400 bases), introns (nu κ-casein intron 4 ~400 bases, nu β-casein intron 7 ~450 bases, nu α-lactalbumin intron 1 ~450 bases), and rDNAs (12S mt rDNA ~250 bases and 16S mt rDNA ~350 bases). Mitochondrial cytochrome *b* sequences (Dung et al., 1993) are not included

in the present analysis. Apparent nu copies of mt cytochrome *b* were discovered in at least one member of Bovidae (*Boselaphus tragocamelus*; Arctander and Friis, unpublished data). Given this complication, complete mt cytochrome *b* sequence for *Pseudoryx* will be presented in a subsequent publication (Arctander et al., in prep).

Relationships among the 13 traditional bovid tribes are not well resolved (e.g., Georgiadis et al., 1990; Allard et al., 1992; Gentry, 1992; Gatesy et al., 1997), but an early split between the subfamily Bovinae and all other extant bovids is consistent with numerous studies (Kingdon, 1982; Lowenstein, 1986; Allard et al., 1992; Wall et al., 1992; Gatesy et al., 1997). Multiple representatives from each of these two basal clades were sampled.

All seven gene fragments were sequenced for a core group of 10 taxa: *Pseudoryx nghetinhensis* (tribe indeterminate), *Bos taurus* (Bovini), *Bubalus depressicornis* (Bovini), *Syncerus caffer* (Bovini), *Boselaphus tragocamelus* (Boselaphini), *Taurotragus oryx* (Tragelaphini), *Capra hircus* (Caprini), *Oryx gazella* (Hippotragini), *Kobus ellipsiprymnus* (Reduncini), and the outgroup *Antilocapra americana* (family Antilocapridae). Additional species were sequenced for some genes, and published data from GenBank also enriched the taxonomic representation. These data permitted a more fine-grained placement of *Pseudoryx* among the bovids. Table 1 includes a list of the DNA sequences sampled from the 76 taxa in this study.

DNA Extraction, Polymerase Chain Reaction (PCR) Amplification, Sequencing, and Sequence Alignment

Total DNA was extracted from fresh tissue and blood samples by standard protocols. DNA from dried skin samples of *Pseudoryx* was extracted as in Dung et al. (1993).

Primers, PCR conditions, and sequencing methods for each sequence are described below. The number of taxa sequenced for this study and the number of published sequences are indicated. All sequence alignments are available on the Internet at the home page for Systematic Biology.

12S and 16S mt rDNA.—Two new sequences and 114 published sequences (Anderson et al., 1982; Miyamoto et al., 1990; Kraus and Miyamoto, 1991; Allard et al., 1992; Gatesy et al., 1992, 1994, 1997) were used. A region of ~250 bp of 12S mt rDNA and an ~350 bp fragment of 16S rDNA were amplified by PCR and directly sequenced as in Gatesy et al. (1992, 1997). These sequences correspond to the regions surrounded by the "universal" primers of Kocher et al. (1989), Simon (1991), and Palumbi et al. (1991). The primers used were 12SA850 (5′-AAACTGGGATTAGATACCCCACTAT-3′), 12SB1270 (5′-GAGGGTGACGGGCGGTGTGT-3′), 16SA2290 (5′-CGCCTGTTTACCAAAAACAT-3′), and 16SB2860 (5′-CCGGTCTGAACTCAGATCACGT-3′). The numbers in the names refer to positions in the *Bos taurus* mt DNA sequence of Anderson et al. (1982). An additional 2000 nucleotides of mt rDNA and intervening tRNAs were available for 11 bovids and three outgroup taxa (see Table 1; Anderson et al., 1982; Miyamoto et al., 1990; Kraus and Miyamoto, 1991; Allard et al., 1992). These data were added to the total mt rDNA data set (see Gatesy et al., 1997).

α-Lactalbumin intron 1.—Fourteen new sequences and one published sequence (Vilotte et al., 1987) were used. A double-stranded fragment of α-lactalbumin spanning introns 1 and 2 was amplified with primers LacII.F (5′-CCAAAATGATGTCCTTTGTC-3′) and LacIV.R (5′-GACTCACCAGTAGTTAATTC-3′) from Milinkovitch et al. (1998). Given that pseudogenes of α-lactalbumin have been detected in domesticated bovids (Soulier et al., 1989; Vilotte et al., 1991), the design of the above primers avoids the amplification of these pseudogenes (D. Irwin, pers. comm.). PCR conditions for double-stranded amplifications were 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, for 30 cycles. The double-stranded PCR product was then purified on a low-melting-point agarose gel. Single-stranded PCR product for intron 1 and the 5′ end of exon 2 was amplified from the double-stranded PCR product with asymmetric dilutions of primers LacI.R (5′-CTCACTGTCACAGGAGATGT-3′) and LacII.F from Milinkovitch et al. (1998). PCR conditions for single-stranded amplifications were 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, for 35 cycles. Single-stranded PCR products were cleaned in Millipore 30,000 NMWL filters and

TABLE 1. The 71 bovid and five outgroup taxa analyzed in this study.

Bovid tribe	Species	Data sets[a]
Tragelaphini	*Tragelaphus imberbis*	R[b] RA[b]
	Tragelaphus angasi	R[b]
	Tragelaphus scriptus[c]	M[b]
	Taurotragus oryx[c]	R[b] A BE BI KE KI
Bovini	*Bos taurus*[c]	R[b] A[b] BE[b] BI[b] KE[b] KI[b] RA[b]
	Bison bonasus[c]	KE[b] M[b]
	Bison bison bison	KE[b]
	Syncerus caffer[c]	R[b] A BE BI KE KI
	Bubalus depressicornis[c]	R[b] A BE BI KE KI M[b]
	Bubalus bubalis	KE[b]
Boselaphini	*Boselaphus tragocamelus*[c]	R[b] A BE BI KE KI M[b] RA[b]
Neotragini	*Madoqua kirki*	R[b] M[b] RA[b]
	Raphicerus campestris	R[b] BE
	Raphicerus melanotis	M[b]
	Ourebia ourebi	R[b] M[b]
Antilopini	*Antidorcas marsupialis*	R[b] A BE
	Gazella thomsoni	R[b] A RA[b]
	Gazella granti	A BE
	Gazella dorcas	M[b]
	Gazella subgutturosa	R[b]
	Antilope cervicapra	R[b] BE M[b]
	Procapra picticaudata	R[b]
Alcelaphini	*Damaliscus dorcas phillipsi*	R[b] RA[b]
	Damaliscus dorcas dorcas	R[b]
	Damaliscus lunatus jimela	R[b] M[b]
	Damaliscus lunatus lunatus	R[b] BE M[b]
	Beatragus hunteri	R[b]
	Alcelaphus buselaphus	R[b]
	Alcelaphus caama	R[b]
	Alcelaphus lichtensteini	R[b] BE
	Connochaetes taurinus	R[b] A KI
	Connochaetes gnou	R[b] BE KE
Hippotragini	*Oryx gazella gazella*[c]	R[b] KI RA[b]
	Oryx gazella callotis[c]	R[b] A BE BI KE
	Oryx dammah	R[b]
	Oryx leucoryx	R[b]
	Addax nasomaculatus	R[b]
	Hippotragus niger	R[b] BE
	Hippotragus equinus[c]	R[b] M[b]
Caprini	*Capra hircus*[c]	R[b] A BE[b] BI KE[b] KI RA[b]
	Capra aegagrus[c]	M[b]
	Hemitragus jemlahicus	R[b]
	Pseudois nayaur	R[b]
	Ovis dalli	R[b] KE[b]
	Ovis canadensis	R[b]
	Ovis aries	BE[b] KE[b]
	Ovis orientalis	M[b]
Ovibovini	*Ovibos moschatus*	R[b] BE KE[b] M[b]
	Budorcas taxicolor	M[b]
Rupicaprini	*Nemorhaedus goral*	R[b] KE[b] M[b]
	Capricornis crispus	R[b] KE[b]
	Capricornis swinhoei	KE[b]
	Capricornis sumatraensis	KE[b] M[b]
	Rupicapra rupicapra	KE[b] M[b]
	Oreamnos americanus	R[b] BE KE[b]
Reduncini	*Kobus ellipsiprymnus*[c]	R[b] A BE BI KE KI RA[b]
	Kobus megaceros	R[b]
	Kobus leche	R[b]
	Kobus kob[c]	R[b] M[b]

of the circle, the legs are in addition waved up and down several times alternately (left leg moving up while right leg moving down, and so on). The downward flick of the first leg typical of second stage display is given when the leg is high and to the side, though flicks are not done on each circle.

Specimens examined: *Galiuro Mountains*: 3 ♂, 0 ♀; males scored: High Creek 3m, 3v(6b). *Santa Teresa Mountains*: 4 ♂, 0 ♀; males scored: Cottonwood Mountain 4m, 3v(6b).

Baboquivari and Quinlan Mountains (Figs. 2i, 3g).—A form with brown and white male face but showing many features in common with the nearby gray-faced Atascosa/Pajarito/Tumacacori form. In particular, it shares the swollen carapace sides, and bare spots on the "cheeks." White band across clypeus is broad, its setae narrow and horizontally directed. Courtship similar to that of the Atascosa form.

Specimens examined: 6 ♂, 5 ♀; males scored: Kitt Peak 2m, 2v(8b); Sabino Canyon 4m, 0v.

Atascosa, Pajarito, and Tumacacori Mountains (Figs. 2k, 3i, 4a, 5a).—Clypeus covered with silvery gray scales stalked on petioles beneath the main eyes (Fig. 5a); white and yellow scales near the lateral margins. Cheeks with two or three prominent dark barren spots, and strongly swollen carapace sides. Chelicerae covered with fine hairs obliquely oriented in alternating bands so as to give a bundled appearance. The first stage of courtship is short (usually <5 seconds), with the male walking toward female in large arcs (sidling). He then engages in a second stage with vigorous leg flicking.

Specimens examined: *Atascosa and Pajarito Mountains*: ~30 ♂, ~15 ♀; males scored: Warsaw Canyon 1m, 0v; Ruby 4m, 3v(12b); Atascosa Peak 7m, 0v; Sycamore Canyon 6m, 0v. *Tumacacori Mountains*: 5 ♂, 3 ♀; males scored: Tumacacori 5m, 2v(2b). In addition, male courtship behavior was observed to be in general as expected in an additonal eight males from Atascosa Peak, Atascosa Mts. (palps not circled, sidling approach to female).

TAGCTG-3′) and CASBR3 (5′-TGAAATCY TCTTAGACCTT-3′). PCR conditions were 1 min at 94°C, 1 min at 53°C, and 1 min at 72°C, for 35 cycles. Single-stranded template was produced with the DYNABEADS protocol (Dynal), a system that utilizes biotinylated primers. Purification and sequencing of single-stranded products were as for α-lactalbumin intron 1.

All five genes exhibited length variation among species. Orthologous sequences were aligned with the parsimony-based alignment program MALIGN (Wheeler and Gladstein, 1994). Four multiple sequence alignments were executed for each of the five nu DNA fragments. The cost for opening a gap, extending a gap, and making a nucleotide substitution was varied from 1.5:1:1 to 3:2:1 to 5:4:1 to 10:9:1. Other MALIGN parameters were these: score 3, contig, quick, atbr, and iter. The mt rDNA genes were aligned as in Gatesy et al. (1997) at gap cost:nucleotide substitution cost ratios of 2:1 and 3:1. For the three nu introns and the two mt rDNAs, final alignments were chosen by tree length; alignments that implied the shortest trees (with equal character weighting) were considered optimal. For the two nu exons, the algorithmic alignments were adjusted by eye, using SeqApp 1.9a (Gilbert, 1992). Gaps were consolidated into multiples of three to maintain reading frames in these protein coding regions.

Phylogenetic Analyses

DNA.—Cladistic analyses were done with PAUP 3.1.1 (Swofford, 1993) or PAUP* 4.0.0d64 (Swofford, unpubl.). Phylogenetic searches were branch-and-bound or heuristic with 100 replicates of random taxon addition and TBR branch swapping (Swofford, 1993). All character transformations were weighted equally, and gaps were treated as missing data. Nonbovid, pecoran artiodactyls were used as outgroups (Janis and Scott, 1987; Gentry and Hooker, 1988). Additional analyses were executed in which gaps were coded as a fifth character state. This coding of gaps assigns weights to indels that are proportional to their lengths (e.g., Wheeler et al., 1993). Giribet and Wheeler (1999) have presented a simple justication for scoring single nucleotide gaps as individual character states.

The following data sets were analyzed: 12S plus 16S mt rDNA for 58 taxa, κ-casein exon 4 for 24 taxa plus κ-casein intron 4 for 11 taxa, β-casein exon 7 for 28 taxa plus β-casein intron 7 for 12 taxa, and α-lactalbumin intron 1 for 15 taxa. In addition to the above analyses, searches were performed for the 10 core taxa common to all of the DNA data sets (Table 1). The following four data sets were analyzed for the core taxa: 12S mt rDNA (for the ~250 positions common to all taxa) plus 16S mt rDNA (for the ~350 positions common to all taxa), κ-casein exon 4 plus κ-casein intron 4, β-casein exon 7 plus β-casein intron 7, and α-lactalbumin intron 1.

For the combined molecular data base, other partitioning scenarios could easily be justified. For example, the genetically linked casein genes could be combined in one data set, nu introns could be separated from nu exons, the linked mt rDNAs that encode separate RNA products could be divided, the three codon positions of β-casein and κ-casein could be separated, a distinction between mt and nu data sets could be made, and so forth. Given limitations in journal space, these additional data partitions will not be evaluated here.

Morphology.—Thomas (1994) scored five artiodactyl taxa including *Pseudoryx* for the 112 morphological characters of Gentry (1992) and added two characters: presence/absence of cranial appendages and presence/absence of upper canines. The data of Thomas (1994) were combined with the data of Gentry (1992) for 22 other bovid species (Table 1). The PAUP 3.1.1 search for the total of 27 taxa and 114 characters was as above. Characters were unordered, and all transformations were equally weighted. Cladograms were rooted with *Moschus* (Moschidae) and *Muntiacus* (Cervidae) as in Thomas (1994). An additional analysis was executed in which characters were ordered as in Gentry (1992).

Three of the 10 core taxa (Table 1)—*Pseudoryx nghetinhensis*, *Bubalus depressicornis*, and *Boselaphus tragocamelus*—were scored for morphological characters by Gentry (1992) and Thomas (1994). Additionally, five taxa scored for morphological characters are close relatives of core taxa (Table 1): *Bison bonasus* (tribe Bovini) *Tragelaphus scriptus* (tribe Tragelaphini), *Capra aegagrus* (tribe Caprini), *Hippotragus equinus*

(tribe Hippotragini), and *Kobus kob* (tribe Reduncini). A separate phylogenetic search was performed for these eight taxa and the outgroup, *Muntiacus muntjak* (family Cervidae). Characters were unordered and the phylogenetic search was as above.

DNA + morphology.—Miyamoto (1985) and Kluge (1989) have presented arguments for combining molecular and morphological characters in simultaneous phylogenetic analyses (Nixon and Carpenter, 1996). Simultaneous analyses permit scrutiny of molecular and morphological evidence according to identical criteria for homology (e.g., Patterson, 1982).

The morphological data of Gentry (1992) and Thomas (1994) were combined with DNA sequence data from five genes. Because of the uneven taxonomic sampling among data sets, many characters were scored as missing in the combined data matrix. Some closely related species in the molecular and morphological data sets were equated to make composite terminal taxa. The monophyly of these hybrid terminals was assumed. The PAUP 3.1.1. search for the total of 62 taxa and 5602 characters was heuristic, with 100 random taxon addition replicates and TBR branch swapping. All character transformations were weighted equally, gaps in the DNA alignments were coded as missing data, and morphological characters were unordered.

A second combined analysis was done for the 10 core taxa. This core matrix was characterized by a lower percentage of missing data (per taxon) relative to the combined matrix of 62 taxa above. Data from the five genes and morphology were merged. Closely related taxa in the molecular and morphological matrices were equated (Table 1)—that is, the bovine cattle (*Bos taurus* and *Bison bonasus*), the tragelaphines (*Taurotragus oryx* and *Tragelaphus scriptus*), the hippotragines (*Oryx gazella* and *Hippotragus equinus*), the caprine goats (*Capra hircus* and *Capra aegagrus*), the reduncines (*Kobus ellipsiprymnus* and *Kobus kob*), and the outgroup taxa (*Antilocapra americana* and *Muntiacus muntjak*). The phylogenetic search was branch-and-bound, all character transformations were weighted equally, gaps in the DNA alignments were coded as missing data, and morphological characters were unordered. Additional searches were executed to test the stabilities of the combined analyses. Transition substitutions were ignored (i.e., transversion parsimony), or gaps were coded as a fifth character state, or both.

Base Composition and Transition/Transversion Bias

For the 10 core taxa (Table 1), nucleotide base compositions of the seven DNA fragments were calculated by using MacClade 3.04 (Maddison and Maddison, 1992). Estimates of the transition/transversion bias for each DNA fragment also were calculated with MacClade 3.04. For each DNA alignment, nucleotide substitutions were optimized onto the total evidence topology for core taxa by parsimony, and the numbers of transition and transversion substitutions were recorded. All most-parsimonious optimizations were incorporated into the estimates by using the "equivocal cycling" option. Each character optimization was given equal weight in determinations of the relative number of transitions versus transversions for a given data set (Maddison and Maddison, 1992).

Nodal Stability and Support

Bootstraps and branch support.—The relative stability of nodes was assessed by bootstrap percentages (BP; Felsenstein, 1985) and branch support (BS; Bremer, 1988, 1994). In each bootstrap analysis, informative characters were resampled with replacement from the original data set, and bootstrap replicate data sets the same size as the original were assembled (Felsenstein, 1985). The number of bootstrap iterations ranged from 100 to 1,000, depending on the number of equally parsimonious topologies for a particular data set. Each bootstrap replicate involved a heuristic parsimony search with simple taxon addition and TBR branch swapping (Swofford, 1993).

BS scores for selected nodes were also estimated with PAUP. For a particular data set and a particular node, BS is the minimum number of character steps for that data set on the shortest topologies that do not contain that node, minus the minimum number of character steps for that data set on the shortest topologies that do contain that node. Note that with this definition, BS can be positive, zero, or negative (in contrast to Bremer,

1994). If the node of interest is supported by a given data set, BS is positive. If the node is not supported by a given data set, BS is negative or zero (see Gatesy et al., 1999). BS was estimated by using the "constraints" command of PAUP and branch-and-bound or heuristic searches. Heuristic parsimony searches for these analyses included at least 10 random taxon addition replicates with TBR branch swapping. Because of the complexity of some data sets, BS scores at some nodes may be overestimates.

Interdependence of stabilities for different nodes.—BS measures the stability of a particular node to relaxation of the parsimony criterion (Bremer, 1988, 1994; Davis, 1993). BS scores for individual nodes are calculated independently, but the stabilities of different nodes in a minimum length topology may not be additive (see Faith and Ballard, 1994). By calculating BS for several nodes simultaneously, the interaction of stabilities for different nodes can be assessed. For a given data matrix, linked branch support (LBS; Gatesy, in press) for a set of supported nodes is the length of the shortest topology that lacks all of those nodes, minus the length of the shortest topology that contains all of those nodes. If there is no homoplasy in a matrix of binary characters, BS scores are additive. LBS for each set of nodes is equal to the sum of BS scores for nodes in that set. If homoplasy is present in a data matrix, the distribution of character incongruence determines whether LBS for a set of nodes is less than, equal to, or more than the sum of BS scores for nodes in that set (Gatesy, in press).

LBS can be scaled to the amount of BS to make LBS scores for different sets of nodes more comparable. The LBS index (LBSI; Gatesy, in press) for a set of supported nodes is

$$\frac{\text{(LBS for those nodes)} - \text{(the largest BS score among those nodes)}}{\text{(sum of BS scores for those nodes)} - \text{(the largest BS score among those nodes).}}$$

If LBS for a set of nodes is less than the sum of BS scores for those nodes, then the stability to relaxation of the parsimony criterion is not additive for those nodes and LBSI is <1. If LBS for a set of nodes is equal to the sum of BS scores for those nodes, BS scores are additive, and LBSI = 1. If LBS for a set of nodes is greater than the sum of BS scores for those nodes, then the collapse of one node makes the simultaneous collapse of another node more costly, there is a stabilizing effect in the cladogram, and the LBSI is >1.

For the combined analysis of 10 core taxa (Table 1), LBS scores and LBSIs were determined for each pair of nodes supported by the combined analysis (gaps treated as missing data, all characters unordered, and all character transformations given equal weight). Minimum tree lengths for topologies that simultaneously lack two supported nodes were determined with the "constraints" and "filter" options of PAUP. All PAUP searches were branch-and-bound.

Distribution of conflict and support among data sets.—For the 10 core taxa (Table 1), the incongruence length difference (ILD; Mickevich and Farris, 1981) test was used to assess the null hypothesis of congruence among data sets (Farris et al., 1994). Six ILD tests were performed. Each of the five individual data sets (12S rDNA + 16S rDNA, β-casein, κ-casein, α-lactalbumin, or morphology) was compared with the sum of the characters from the other four data sets. Also, a division of the combined data set into the five individual data sets was tested. To establish a null distribution for each test, 999 random data partitions were generated, and ILDs were calculated for each ILD replicate with PAUP* (Swofford, unpubl.). In all ILD tests, uninformative characters were excluded, and searches were heuristic with simple taxon addition and TBR branch swapping. $P = 0.05$ was taken as the threshold for significance.

Corroboration among the five individual data sets also was estimated for the core group of 10 taxa (Table 1). This was done in four ways. First, taxonomic congruence (Nelson, 1979) was assessed; that is, strict consensus trees derived from each of the five data sets were compared to quantify topological similarity. Second, for each node supported by the simultaneous analysis of core taxa, unambiguously optimized synapomorphies derived from each of the five data sets were noted. The "list of apomorphies" option of PAUP* was used to diagnose clades. Third, partitioned branch support (PBS; Baker and DeSalle, 1997) was calculated for each data set and each node supported by the

combined analysis of all five data sets. For a particular combined data set, a particular node, and a particular data partition, PBS is the minimum number of character steps for that partition on the shortest topologies for the combined data set that do not contain that node, minus the minimum number of character steps for that partition on the shortest topologies for the combined data set that do contain that node. If there are multiple equally short topologies, then tree lengths are averaged (Baker and DeSalle, 1997).

PBS offers a simple means for assessing support rendered by different data sets within a simultaneous analysis framework. The method permits the detection of hidden conflicts and support (e.g., Barrett et al., 1991) that are not obvious from separate analyses of each data set. Furthermore, because characters are allowed to interact in simultaneous analysis, the relative weight of evidence from each data set is taken into account. Within a simultaneous analysis framework, a positive PBS score indicates that a given data set provides net positive support for that particular node over the alternative relationships in the shortest tree(s) without the given node; a negative PBS score shows that a data set favors the shortest tree(s) without the given node over the minimum length solution(s); and a PBS score of zero indicates the indifference of a given data set at that node. The sum of PBS scores at a particular node equals BS at that node (Baker and DeSalle, 1997).

Fourth, the data set removal index (DRI; Gatesy et al., 1999) was calculated for each node supported by the combined analysis. The DRI is analogous to the clade stability index of Davis (1993). For a particular node and a particular combined data set, the DRI is the minimum number of data set removals required to collapse that node. A node that is not supported by the combined data set has a DRI of zero. A node that collapses with the removal of only one data set has a DRI of one. For a combined data set in which each component data set and each combination of data sets support the node of interest, the DRI is equal to the number of data sets that compose the combined data set. A high DRI indicates that character support for a particular node is distributed among many data sets. Calculation of the DRI also identifies particular data sets or combinations of data sets that are critical for the resolution of particular nodes (Gatesy et al., 1999). PBS and DRIs were determined as in Gatesy et al. (1999).

Hidden nodal support and conflict.—The interaction of different data sets in simultaneous analysis often implies hidden character support and conflicts (Barrett et al., 1991; Chippindale and Wiens, 1994; Olmstead and Sweere, 1994). For a particular set of data partitions and a particular node, hidden character support can be defined as increased support for the node of interest in the simultaneous analysis of all data partitions relative to the sum of support for that node in the separate analyses of each partition. For a particular set of data partitions and a particular node, hidden conflict can be defined as decreased support for the node of interest in the simultaneous analysis of all data partitions relative to the sum of support for that node in the separate analyses of the various data partitions.

Hidden support and conflicts can be quantified with a variation of BS, hidden branch support (HBS; Gatesy et al., 1999). For a particular combined data set and a particular node, HBS is the difference between BS for that node in the simultaneous analysis of all data partitions and the sum of BS scores for that node from each data partition. For a particular combined data set and a particular node, a positive HBS score indicates that more hidden support than hidden conflict emerges at that node in simultaneous analysis. A negative HBS score indicates more hidden conflict than hidden support.

HBS can be partitioned among the various data sets in the combined analysis. For a particular combined data set, a particular node, and a particular data set, partitioned hidden branch support (PHBS; Gatesy et al., 1999) is the difference between PBS at that node for that data set and BS at that node for that data set. For a particular node, the sum of PHBS scores for the various data partitions equals the HBS at that node.

Hidden support can also be defined in terms of synapomorphy. For a particular combined data set and a given clade supported by that combined data set, hidden synapomorphy (HS; Gatesy et al., 1999) is the number of unambiguous synapomorphies for that clade in the simultaneous analysis of the combined data set, minus the sum of unambiguous synapomorphies for that clade

in the separate analyses of individual data partitions. The contribution of a particular data partition to HS for a given clade is the number of unambiguous synapomorphies for that clade from that partition in simultaneous analysis, minus the number of unambiguous synapomorphies for that clade from that partition in separate analysis (Gatesy et al., 1999).

For the simultaneous analysis of five data sets and 10 core taxa, HBS, PHBS, HS, and the distribution of HS among data sets were calculated for each node in the minimum length topology as in Gatesy et al. (1999).

TABLE 2. Ranges of base composition and the transition to transversion ratio (TI/TV) for each gene fragment for core taxa.

Gene fragment	%A	%C	%G	%T	TI/TV
12S rDNA	35–38	21–25	16–19	22–25	5.5
16S rDNA	33–35	21–23	19–20	23–25	4.6
α-Lactalbumin	17–24	23–26	13–14	37–45	2.2
β-Casein exon	21–23	34–36	16–18	25–26	2.1
β-Casein intron	32–34	15–17	16–17	34–35	1.5
κ-Casein exon	32–34	27–30	15–17	22–24	1.6
κ-Casein intron	38–39	13–15	11–13	35–37	1.7

RESULTS AND DISCUSSION

Separate Analyses of Molecular and Morphological Data Sets

Miyamoto and Fitch (1995) argued that congruent topologies derived from many independent, uniquely evolving loci offer compelling evidence for phylogenetic relationships. Unfortunately, distinctions between different DNA data sets are not always so clear-cut (Kluge and Wolf, 1993). Regardless, a broad selection of mt and nu genes clearly represents a better sampling of the genetic material than information from any single gene.

In this study, diverse DNA sequences from both the nu and mt genomes were used to test previous hypotheses of bovid phylogeny (Fig. 1a and b). Base composition varies widely among the different sequences (Table 2). For example, the average percentage of cytosine ranges from 0.14 to 0.36. The two mt rDNA genes have broadly similar base compositions. However, regions of the tightly linked nu casein genes, κ and β (Threadgill and Womack, 1990), show a range of compositional bias: The β-casein exon 7 sequences are G + C rich, whereas the remaining casein sequences are A + T–biased (Table 2).

Transition/transversion ratios estimated by parsimony are greatest in the two mt genes (4.6–5.5). The nu DNA data sets are less extreme, their ratios ranging from 1.5 to 2.2 (Table 2). Besides having greater transition/transversion ratios, the mt data evolve at a faster overall rate relative to the nu data. Moreover, the average rates of nucleotide substitution, insertion, and deletion are all greater in the mt rDNA genes than in the three nu genes (results not shown). The mt genes also have lower ensemble consistency indices (Kluge and Farris, 1969) and retention indices (Farris, 1989) than any of the nu genes for the core group of 10 taxa. These differences in evolutionary dynamics could influence phylogenetic results for the mt and nu data sets.

Perhaps not surprisingly, topologies derived from separate analyses of the different DNA data sets show a variety of results (Figs. 2–5). However, none of the molecular matrices supports an especially close relationship between *Pseudoryx* and Caprinae, as Thomas (1994) had proposed (Fig. 1b). All of the genes except α-lactalbumin intron 1 (Figure 5) are consistent with the placement of *Pseudoryx* within Bovinae (Figs. 2–4), as suggested by Dung et al. (1993) (Fig. 1a). For each DNA sequence, the cost of assigning the saola to Bovinae or Caprinae + *Pantholops* is shown in Figure 6. From 2 to 18 extra nucleotide substitutions per DNA data set are required to group *Pseudoryx* with the caprines.

The precise phylogenetic position of *Pseudoryx* relative to other members of Bovinae varies from data set to data set. The mt rDNA data weakly support a *Pseudoryx* + *Boselaphus* (Boselaphini) clade (Fig. 2); κ-casein groups *Pseudoryx* with the cattle, *Bos taurus* and *Bison* (Fig. 3); and the strict consensus tree for β-casein (Fig. 4) shows an unresolved polytomy among *Pseudoryx* and members of Bovini (*Bos*, *Bubalus*, and *Syncerus*). κ-Casein and β-casein agree in placing *Pseudoryx* closer to Bovini than to other members of Bovinae (tribes Tragelaphini and Boselaphini).

The dental and skeletal characters, however, are more consistent with the conclusions of Thomas (1994), shown in Figure 1b, than with the results of Dung et al. (1993),

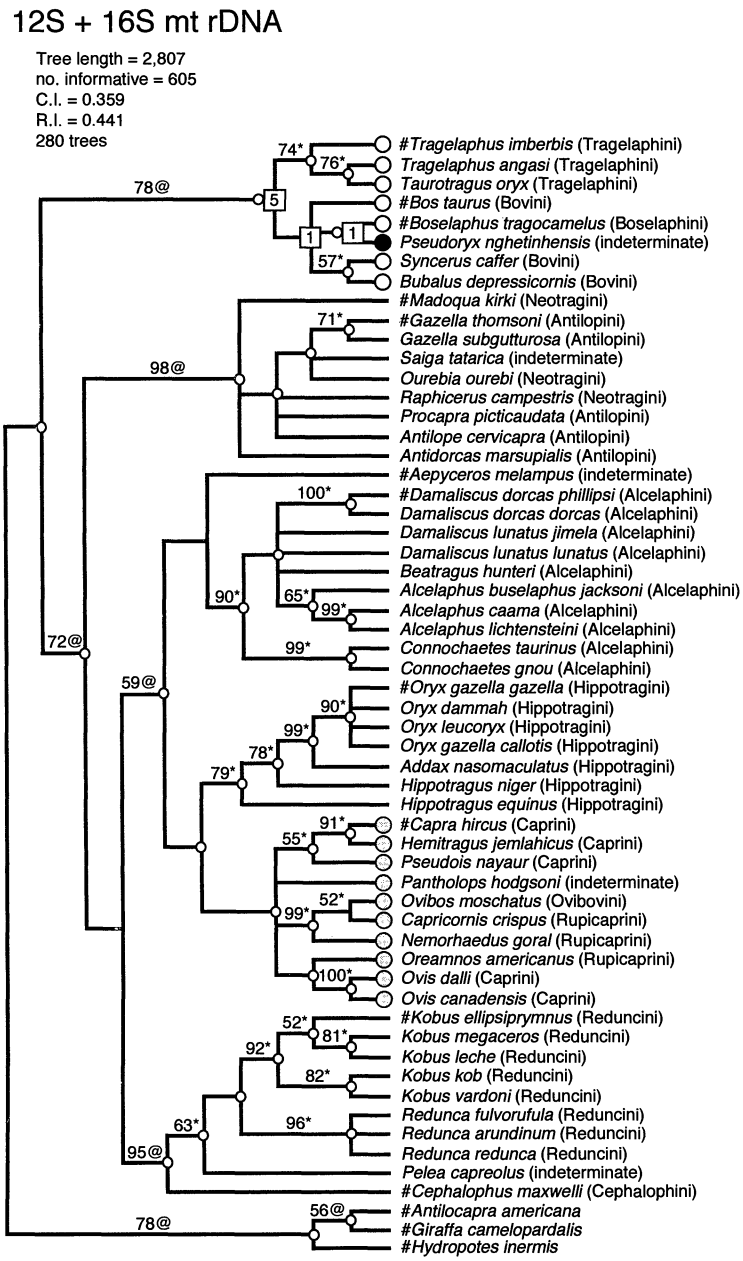

FIGURE 2. Strict consensus tree derived from minimum length topologies for the 12S + 16S mt rDNA data set. Gaps were treated as missing data. Small open circles at internal nodes mark clades that were also supported when gaps were considered as a fifth character state. Taxa that have data for the full 2,805-bp alignment are preceded by #. Bootstrap percentages (BP) >50% for an analysis of these taxa are above internodes and are followed by @. BP >50% for an analysis of the 600 bp of the rDNA alignment that are common to all taxa are above internodes and are followed by *. Branch support (BS) scores for selected nodes are inside rectangles. Large open circles at termini mark members of Bovinae, shaded circles mark members of Caprinae + *Pantholops*, and the solid circle identifies *Pseudoryx*. Tribal affinities of bovids are shown in parentheses. The consensus is rooted with nonbovid artiodactyl sequences. Minimum tree length is 2,807, the number of informative characters 605, the consistency index disregarding uninformative characters (C.I.) = 0.359, the retention index (R.I.) = 0.441, and the number of equally parsimonious topologies (trees) is 280. Branch lengths are not proportional to the number of nucleotide substitutions on each branch.

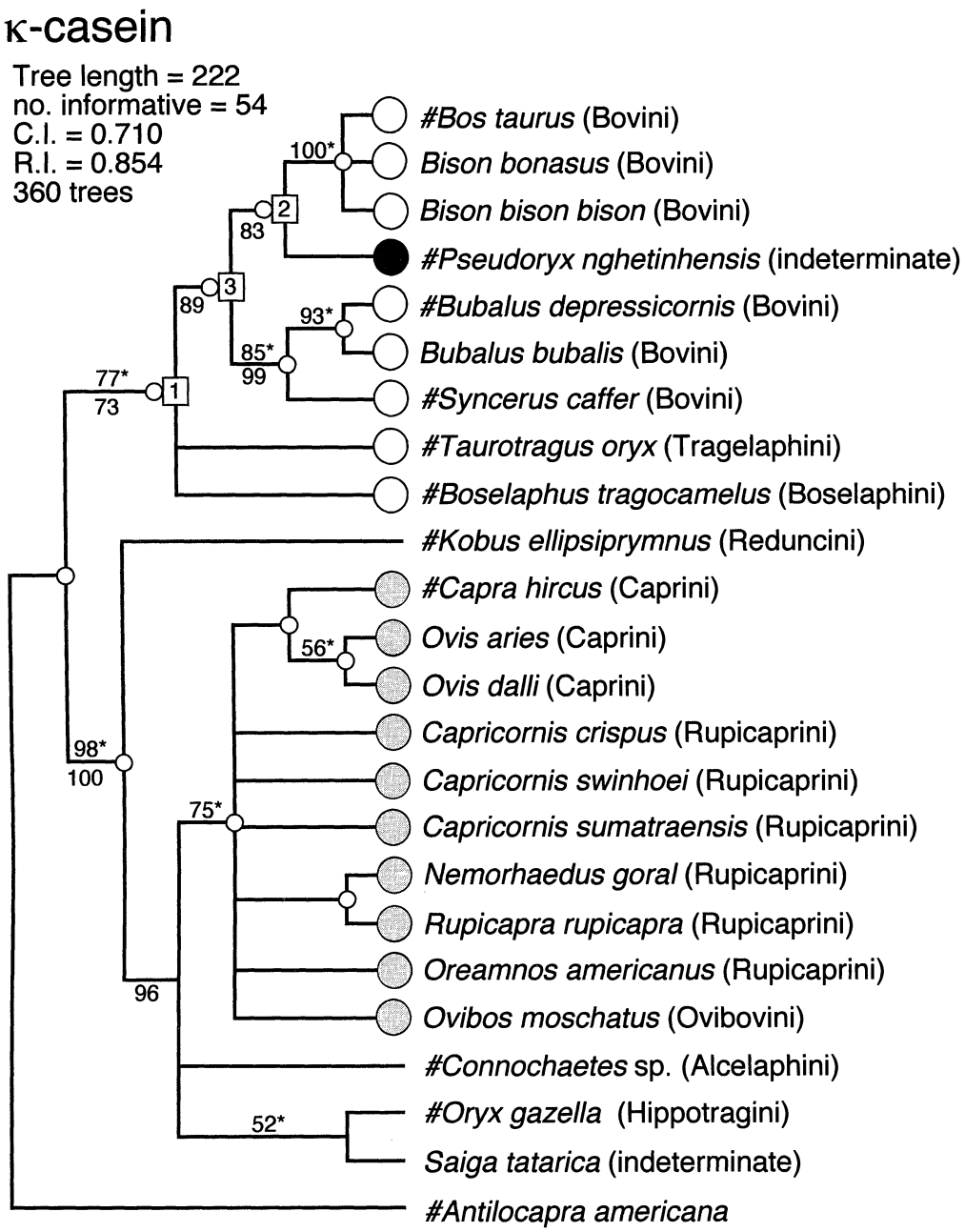

FIGURE 3. Strict consensus tree derived from minimum length topologies for the κ-casein data set. Taxa that have data for κ-casein intron 4 are preceded by #. BP >50% for an analysis of these taxa (κ-casein intron 4 plus κ-casein exon 4) are below internodes. κ-Casein exon 4 sequences were sampled for all taxa in the figure. BP >50% for an analysis of the κ-casein exon 4 sequences are above internodes and are followed by *. Maximally 10,000 trees were saved in each bootstrap replicate for the analysis of κ-casein exon 4. See Figure 2 for explanation of other symbols and statistics.

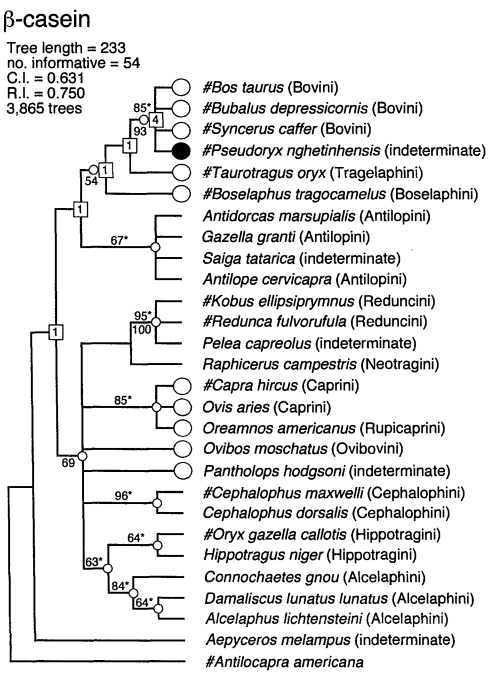

FIGURE 4. Strict consensus tree derived from minimum length topologies for the β-casein data set. Taxa that have data for β-casein intron 7 are preceded by #. BP >50% for an analysis of these taxa (β-casein intron 7 plus β-casein exon 7) are below internodes. β-Casein exon 7 sequences were sampled for all taxa in the figure. BP >50% for an analysis of the β-casein exon 7 sequences are above internodes and are followed by *. Maximally 10,000 trees were saved in each bootstrap replicate for the analysis of β-casein exon 7. See Figure 2 for explanation of other symbols and statistics.

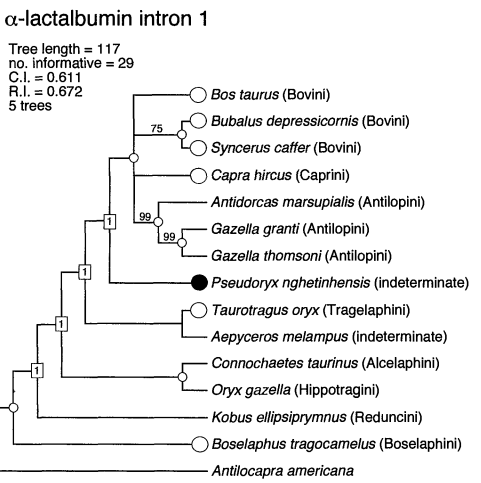

FIGURE 5. Strict consensus tree derived from minimum length topologies for the α-lactalbumin intron 1 data set. See Figure 2 for explanation of symbols and statistics.

shown in Figure 1a. The morphological consensus tree puts the saola closer to caprines than to bovines (Fig. 7). However, the specific position of *Pseudoryx* relative to Caprinae and *Pantholops* is not identical to Thomas' (1994) hypothesis, which placed *Pseudoryx* in a basal position. The shortest topology in our reanalysis aligns *Pseudoryx* with *Capricornis*, the serow (tribe Rupicaprini). Other caprines are more distantly related to *Pseudoryx*, and *Pantholops* is grouped as the sister taxon of Caprinae + *Pseudoryx* (Fig. 7).

Five nodes in the strict consensus tree separate *Pseudoryx* from the nearest bovine taxon, *Tragelaphus scriptus* (Fig. 7). The sum of BS scores for these five nodes is +6, but the BS scores are not additive. The LBS for this set of nodes is only +2 and the LBSI is 0. Because of the linked instabilities of adjacent nodes, the morphological topology is like a house of cards. For the morphological data, only four extra character steps are required to move *Pseudoryx* from within Caprinae to a monophyletic Bovinae. This cost is trivial relative to the molecular evidence from multiple loci that supports a close relationship between *Pseudoryx* and Bovinae (Fig. 6).

Character Conflict and Taxonomic Congruence Among Data Sets

Although the various data partitions are characterized by a variety of evolutionary tempos and modes (e.g., Table 2; Gatesy et al., 1994, 1996; Gatesy, 1998; Milinkovitch et al., 1998), ILD tests show that character incongruence between any one data set and the remaining character evidence is not significant. P values for these five comparisons range from 0.276 for the morphological data set to 0.680 for α-lactalbumin. Similarly, the ILD for a partitioning of the combined matrix into five data sets (ILD = 15) is not extreme relative to ILDs derived from random divisions of the combined data set into five character subsets ($P = 0.440$).

The ILD tests suggest limited character conflicts among data sets. However, there is little topological congruence among strict consensus trees derived from each of the five character subsets (Fig. 8). For the core group of 10 taxa, only two clades, *Syncerus* + *Bubalus* and *Capra* + *Kobus* + Hippotragini, are supported as many as three

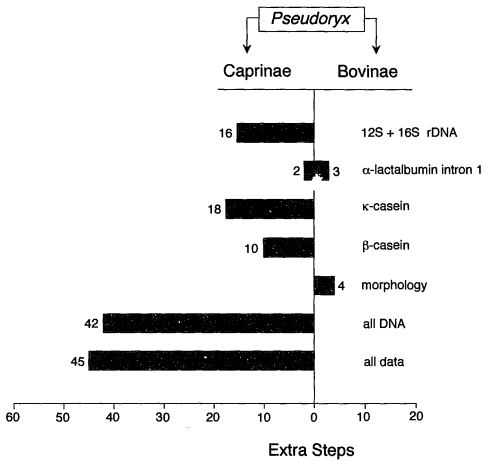

FIGURE 6. The cost in numbers of extra character transformations for the phylogenetic placement of *Pseudoryx* with Bovinae (Bovini + Tragelaphini + Boselaphini) or with Caprinae (Caprini + Rupicaprini + Ovibovini) plus *Pantholops*. For each data set, the cost in extra steps was determined by using the "constraints" command in PAUP 3.1.1. Gaps were treated as missing data. For the morphological data, characters were unordered. All DNA = the combination of all four DNA data sets for the 10 core taxa; all data = the combination of all four DNA data sets plus the morphological evidence for the 10 core taxa (Table 1).

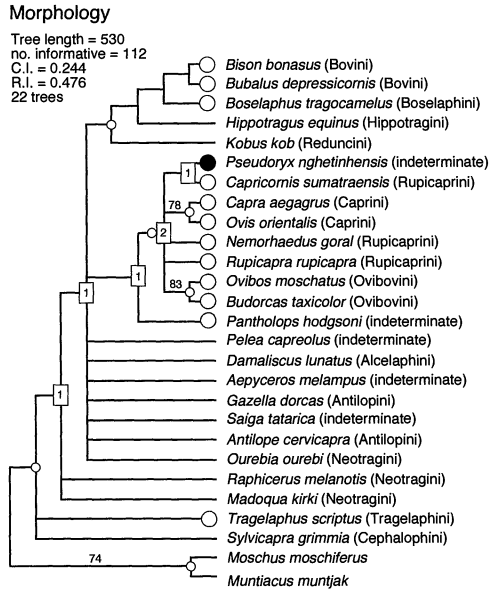

FIGURE 7. Strict consensus tree derived from minimum length topologies for the morphological data set (Gentry, 1992; Thomas, 1994). Characters were unordered. Small open circles at internal nodes mark clades that were also supported when characters were ordered. See Figure 2 for explanation of other symbols and statistics.

times in the five separate analyses; no other groupings are replicated in the majority of the individual searches. In part, the lack of taxonomic congruence may be a result of the limited number of informative characters in some data sets (Fig. 8). A more precise placement of the saola requires the combination of the various data sets in simultaneous phylogenetic analyses.

Combined Analyses

The weakness of the morphological result is illustrated by the combined analyses of the molecular and morphological data (Figs. 9 and 10). The weight of the DNA evidence overwhelms the few morphological characters that suggest a link between caprines and *Pseudoryx*, and *Pseudoryx* aligns with Bovinae as in most of the purely molecular searches. The cost of grouping *Pseudoryx* with Caprinae is an extra 45 steps in the analysis of core taxa (Fig. 6) and an extra 44 steps in the analysis of 59 bovid taxa (Fig. 10). For both combined data sets, the cattle lineage (*Bos* + *Bison*) is the closest relative of *Pseudoryx*, with buffaloes (*Bubalus* + *Syncerus*), Boselaphini, and Tragelaphini being successively more distantly related. Most of these relationships show moderate support according to BS and BP scores, but stability increases if individual gaps are treated as a fifth character state (Fig. 9). All relationships favored in the analysis of 10 core taxa are robust to increased taxonomic sampling (Fig. 10), but the *Pseudoryx* + cattle clade is especially weakly supported (BS = 1). When only transversion substitutions are considered, this clade is not favored, and *Pseudoryx* groups with the buffaloes.

Within the context of the combined data set for core taxa (Fig. 9), some of the morphological characters of Gentry (1992) and Thomas (1994) are consistent with the interpretation of *Pseudoryx* as a member of Bovini. The presence of horns in females, closure of the ethmoidal fissure, and an increase in the exposure of the mastoid unambiguously support the *Pseudoryx* + Bovini clade. Uncompressed horn cores, the absence of keels on horn cores, and small auditory bullae unambiguously support a close relationship between *Bison* and *Pseudoryx* on the total data tree.

The combined analysis of 62 taxa (Fig. 10) reiterates most of the conclusions of Gatesy et al. (1997) regarding higher-level bovid relationships. Phylogenetic analyses of 12S and

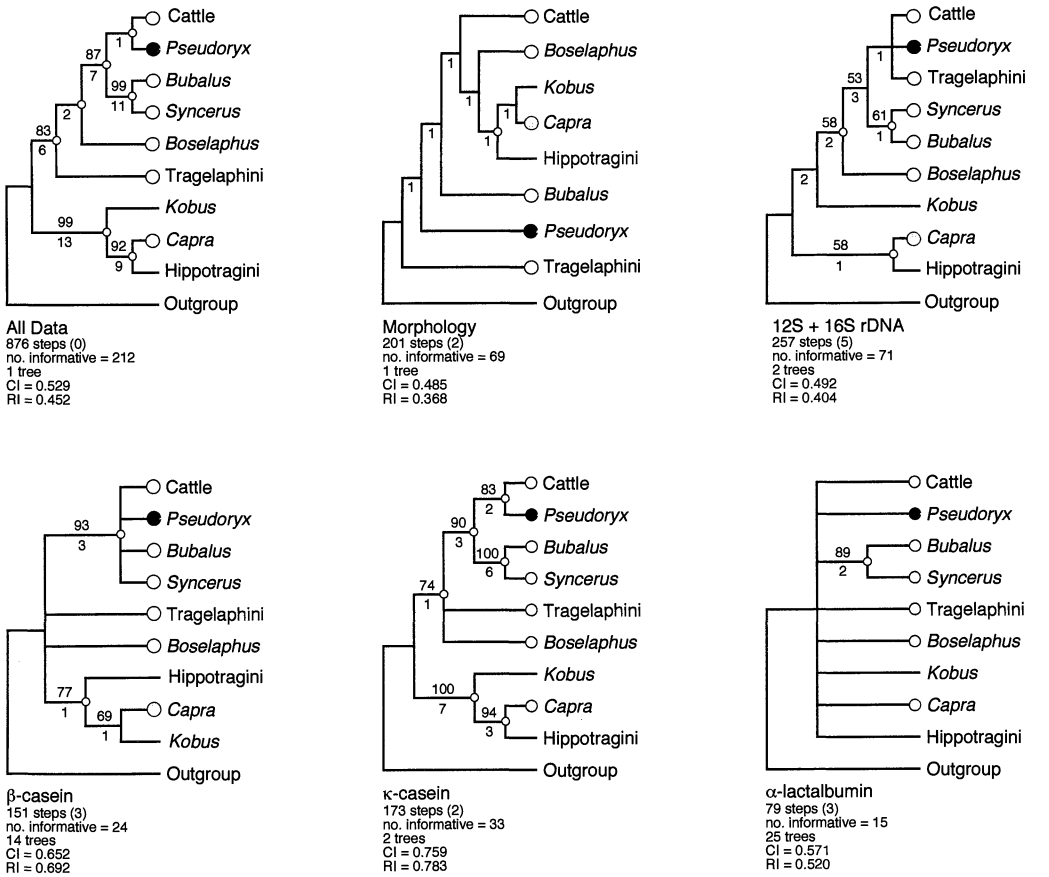

FIGURE 8. Strict consensus trees of minimum length topologies for the core group of 10 taxa (Table 1) for each of the five individual data sets and for the combined analysis of all five data sets. Gaps were treated as missing data, and morphological characters were unordered. Small open circles at internal nodes mark clades consistent with the simultaneous analysis of all five data sets. BP >50% are above internodes and BS scores are below internodes. The number of extra steps required to fit a given data set to the total data tree is shown in parentheses to the right of the minimum number of character steps for that data set. See Figure 2 for explanation of other symbols and statistics.

16S mt rDNA (Gatesy et al., 1997) suggested the following groups: Bovinae, all non-Bovinae, Caprinae (Caprini + Ovibovini + Rupicaprini) + *Pantholops*, Hippotragini + Caprinae + *Panthalops*, Reduncini + *Pelea* + Cephalophini, Reduncini + *Pelea*, and Antilopini + Neotragini + *Saiga*. The bovid tribes Tragelaphini, Reduncini, Hippotragini, and Alcelaphini also were supported in most analyses of the mt rDNA alone (Gatesy et al., 1997). The addition of three nuclear genes and morphological evidence to the mt rDNA data set did not perturb these relationships (Fig. 10).

Linked Branch Support and Linked Branch Support Indices

LBS and LBSIs for each pair of nodes favored by the combined analysis are shown in Figure 11. Most pairs of nodes have LBSIs <1. In eight cases, the collapse of one node permits the collapse of another node at no extra cost (LBSIs = 0). LBSIs are <1 for 14 of 21 nodal pairs (Fig. 11). The low LBS and LBSIs for the combined topology show that the stabilities of different nodes are not additive. Therefore, the combined cladogram is not as robust as BS scores for individual nodes might suggest. For example, Bovinae including *Pseudoryx* (node E, Fig. 11) collapses in the strict consensus of trees that are as many as six steps beyond minimum length, and Hippotragini + *Capra* (node G, Fig. 11) collapses in the strict consensus of trees that are as many as nine steps beyond minimum length. If the stabilities of these nodes were additive, LBS would be 15, but both nodes are lacking in a single topology

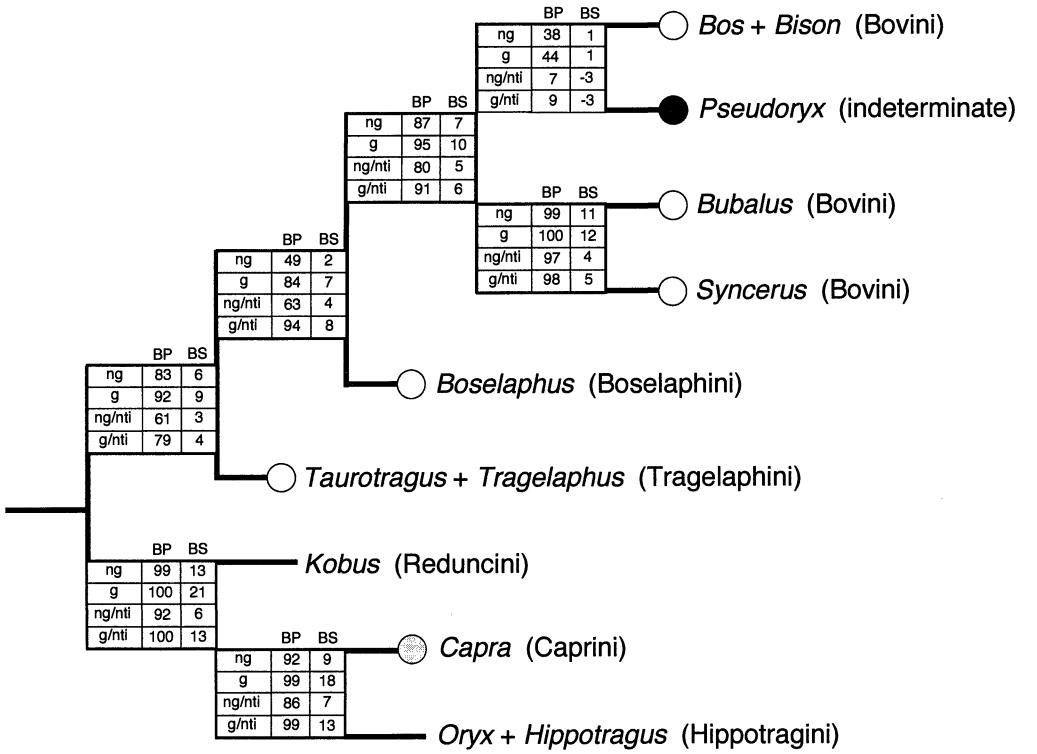

FIGURE 9. Minimum length topology for the simultaneous analysis of four DNA data sets and morphology for the core group of 10 taxa (Table 1). Morphological characters were unordered. Indices of clade stability for four weighting schemes—ng = nogaps, g = gaps treated as a fifth character state, ng/nti = no gaps and no transition substitutions, g/nti = gaps treated as a fifth character state and no transitions—are shown at internodes See Figure 2 for explanation of other symbols and statistics.

that is only nine steps longer than minimum length (LBS = 9, LBSI = 0; Fig. 11).

In contrast, five pairs of nodes that include node B ("buffaloes," Fig. 11) have LBSIs ≥ 1. For example, BS for node B is 11, and BS for node G is 9. LBS for nodes B and G (21) exceeds the sum of BS scores for these nodes (20), so the LBSI is >1 (1.11; Fig. 11). In terms of additional character steps, the optimal cladogram is resistant to the simultaneous loss of these two nodes.

Corroboration Among Data Sets in Separate and Simultaneous Analyses

Some authors have suggested that corroboration among data sets is critical for assessing phylogenetic support and accuracy (e.g., Miyamoto and Fitch, 1995). Others have argued that data sets are arbitrary but character congruence is of utmost importance (Kluge, 1989; Kluge and Wolf, 1993). We propose that an assessment of the distribution of support and conflict among data sets can only lead to a better understanding of the combined character evidence.

For example, if five data sets are included in a combined phylogenetic analysis, and if most of the support is concentrated in only one of the five data sets, a careful reanalysis of the one critical data set is warranted. Are characters in the pivotal data set dubious (e.g., is positional homology questionable)? Is that data set odd in some way (e.g., is it characterized by extreme base composition bias or rate variation among lineages)? Furthermore, do the other four data sets contradict the critical data set, or are they equivocal? Is there hidden support in the four data sets that do not individually support the total data topology? All of these questions can be addressed within a simultaneous analysis framework.

PBS scores for the various data sets are a good indication of the distribution of

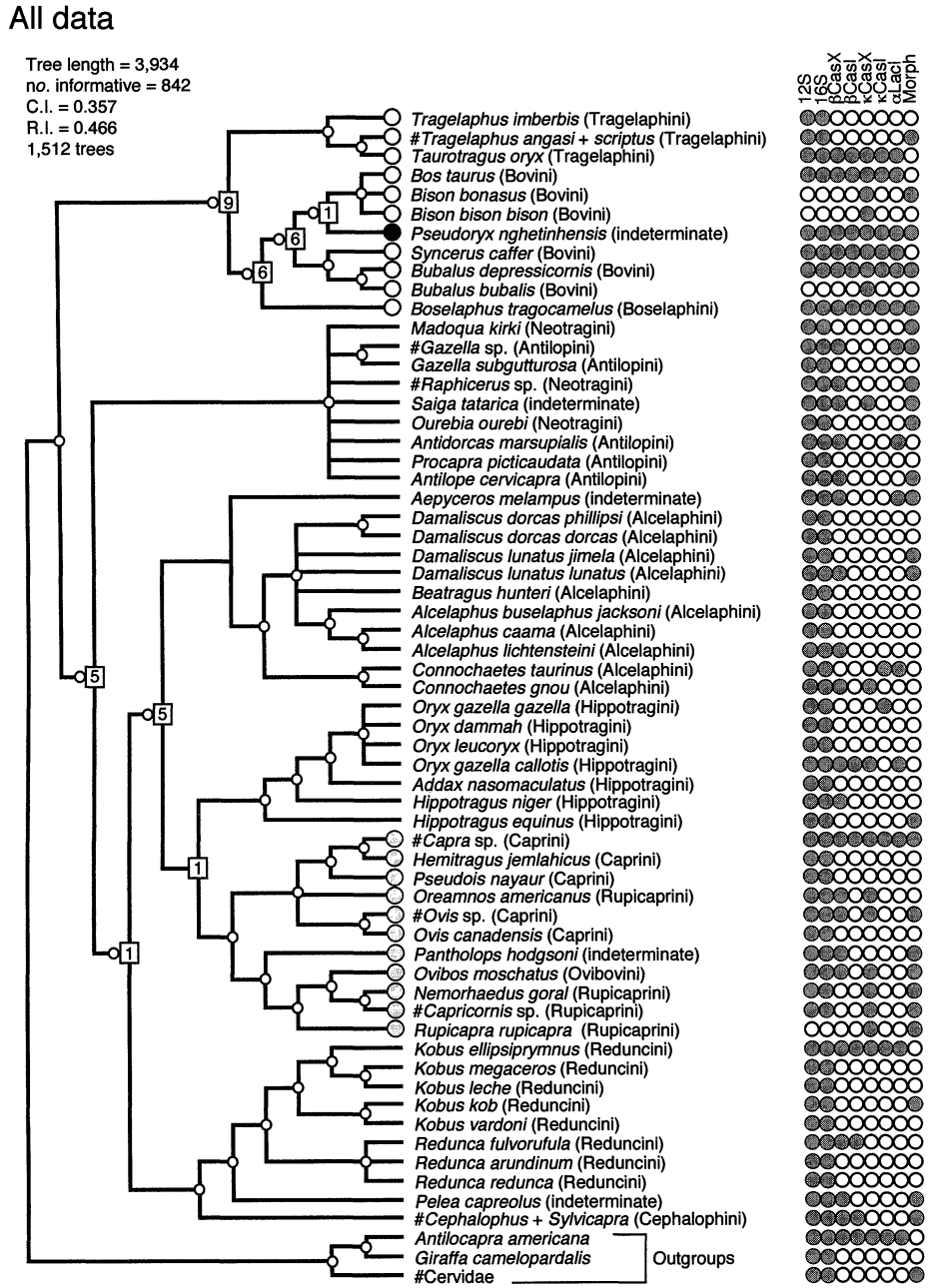

FIGURE 10. Strict consensus of minimum length topologies for the simultaneous analysis of four DNA data sets and morphology for 62 bovid taxa. Gaps were treated as missing data, and morphological characters were unordered. Data sets sampled for each taxon are indicated by shaded circles to the right of each taxon (Morph = morphology, 12S = 12S mt rDNA, 16S = 16S mt rDNA, βCasI = β-casein intron 7, βCasX = β-casein exon 7, κCasI = κ-casein intron 4, κCasX = κ-casein exon 4, αLacI = α-lactalbumin intron 1). Composite terminal taxa (preceded by #) are *Tragelaphus angasi* + *T. scriptus*: 12S + 16S = *T. angasi*, Morph = *T. scriptus*; *Gazella sp.*: 12S + 16S + αLacI = *G. thomsoni*, βCasX = *G. granti*, m = *G. dorcas*; *Raphicerus sp.*: 12S + 16S + βCasX = *R. campestris*, Morph = *R. melanotis*; *Capra sp.*: 12S + 16S + βCasX + βCasI + κCasX + κCasI + αLacI = *C. hircus*, Morph = *C. aegagrus*; *Ovis sp.*: 12S + 16S + κCasX = *O. dalli*, βCasX = *O. aries*, Morph = *O. orientalis*; *Capricornis sp.*: 12S + 16S + κCasX = *C. crispus*, Morph = *C. sumatraensis*; *Cephalophus* + *Sylvicapra*: 12S + 16S + βCasX + βCasI = *C. maxwelli*, Morph = *S. grimmia*; and Cervidae: 12S + 16S = *Hydropotes inermis*, Morph = *Muntiacus muntjak*. See Figure 2 for explanation of other symbols and statistics.

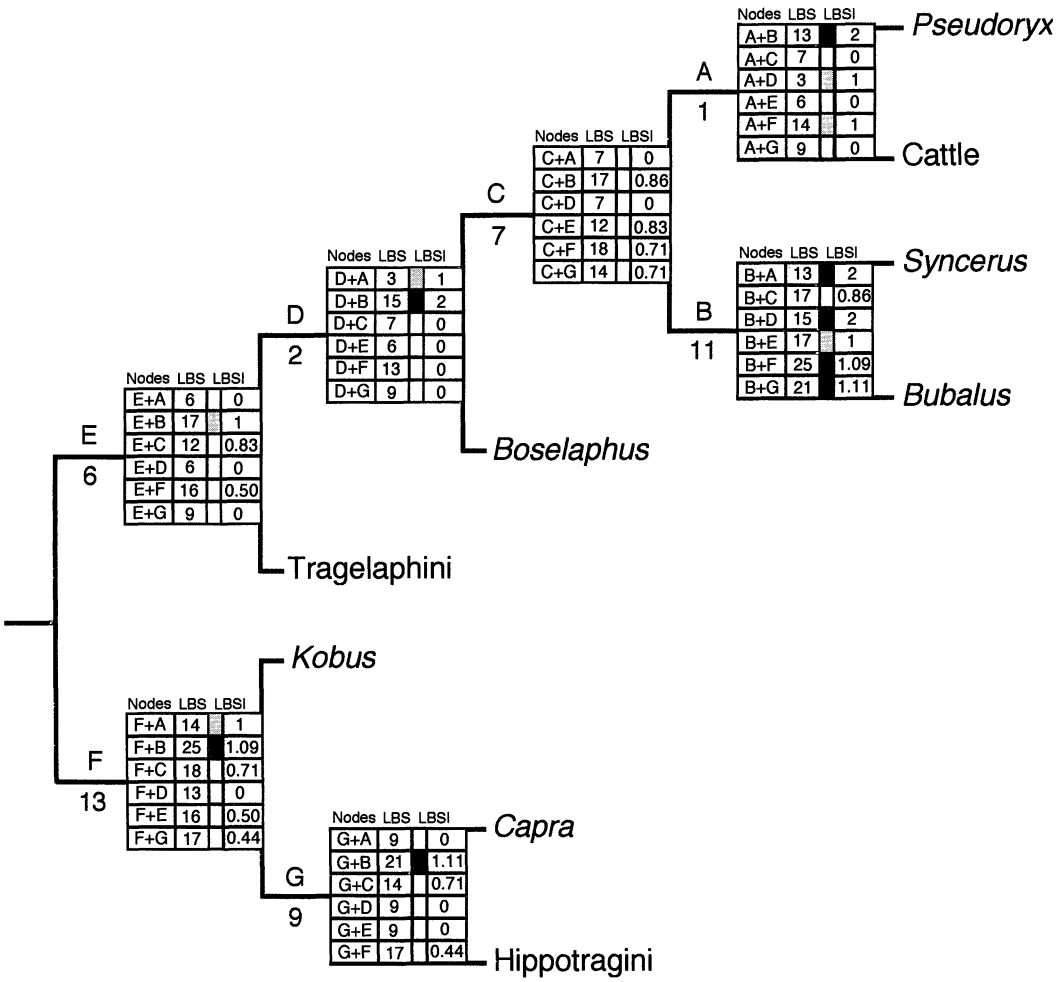

FIGURE 11. LBS and LBSIs for each pair of nodes supported by the simultaneous analysis of five data sets for the 10 core taxa. Clades are labeled A–G above internodes, and BS is given below internodes. LBSI scores >1 are marked by solid boxes to the left, LBSIs <1 are marked by open boxes to the left, and LBSIs = 1 are marked by shaded boxes to the left.

character support and conflict among data sets in simultaneous analysis. Unlike taxonomic congruence, PBS accounts for hidden support and conflicts that emerge in simultaneous phylogenetic analyses of multiple data sets (Baker and DeSalle, 1997). If hidden character support emerges with the combination of various data sets in simultaneous analysis, corroboration among data sets in the combined analysis is oftentimes greater than the corroboration among data sets recorded in the taxonomic congruence approach (Gatesy et al., 1999).

For the most part, this is the case for the combined cladogram of *Pseudoryx* and kin. Figure 12 shows the influence of the various data sets in the simultaneous analysis. For four of the seven nodes supported by the combined data set, corroboration among data sets is greater in the simultaneous analysis (Fig. 12) than in comparisons of topologies derived from separate analyses of the five individual data sets (Fig. 8). For example, *Capra* + Hippotragini is favored by two of the separate analyses, but three PBS scores are positive at this node. Similarly, only two of the individual data sets favor Bovini including *Pseudoryx*, but four data sets support this group in the simultaneous analysis.

At six of seven nodes in the combined tree (Fig. 12), simultaneous analysis demonstrates less conflict among data sets than is

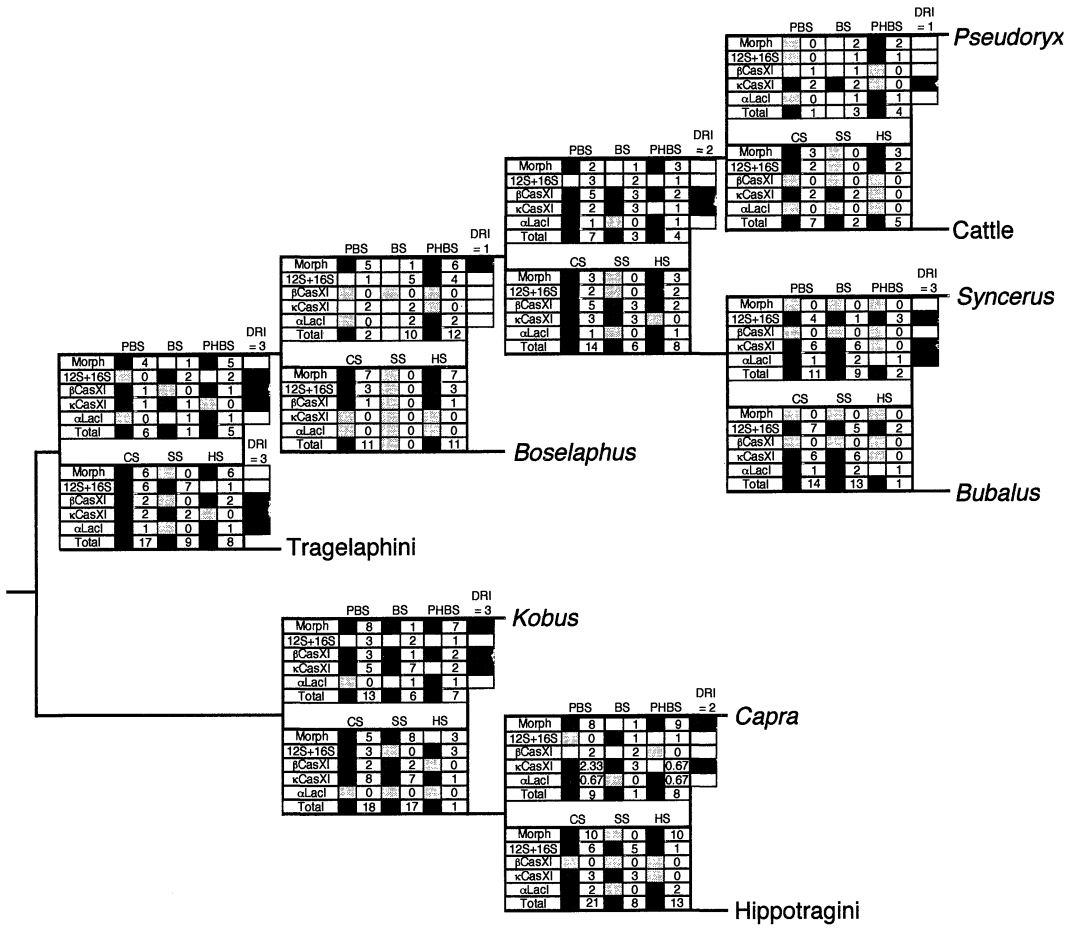

FIGURE 12. The distribution of character support among data sets in simultaneous analysis, the influence of data set removal on clade stability, and hidden support/conflict. The minimum length topology for the four DNA data sets and morphology for the core group of 10 taxa is shown. Gaps were treated as missing data, and morphological characters were unordered. For each of the five data sets (abbreviations as in Fig. 10) and each node, partitioned branch support (PBS), branch support for the separate analysis of that data set (BS), partitioned hidden branch support (PHBS), the data set removal index (DRI), the number of unambiguous synapomorphies in combined analysis (CS), the number of unambiguous synapomorphies in separate analysis (SS), and hidden synapomorphy (HS) are shown. Also, at each node, the sum of PBS scores (= BS in the simultaneous analysis), the sum of BS scores for the separate analyses of the five data sets, the sum of PHBS scores (= HBS), the sum of CS, the sum of SS, and the sum of HS are shown. For PBS, BS, PHBS, CS, SS, and HS, positive scores are marked by solid boxes to the left, negative scores are marked by white boxes to the left, and scores of zero are marked by shaded boxes to the left. The critical combinations of data set removals that collapse a particular node in the DRI analysis are indicated in columns of solid boxes below the DRI score for that node. For example, the DRI for *Syncerus* + *Bubalus* is 3. The node is stable to the removal of any single data set or any combination of two data sets. However, the removal of 12S + 16S, κCasXI, and αLac1 collapses this node. The removal of any other combination of three data sets does not collapse *Syncerus* + *Bubalus*. The removal of two different groups of three data sets collapses Bovinae including *Pseudoryx*.

suggested by separate analyses of individual data sets. For example, the cattle + *Pseudoryx* clade is weakly contradicted by four data sets in separate analyses. Yet, only one of these data sets, β-casein, conflicts with this clade in simultaneous analysis. The other three "conflicting" data sets—morphology, α-lactalbumin, and mt rDNA—are actually neutral and have PBS scores of zero at this node (Fig. 12).

The DRI offers information on the distribution of character support among data sets in simultaneous analysis, showing which data sets are critical for the resolution of

particular nodes. For the simultaneous analysis of bovids, *Syncerus* + *Bubalus*, Bovinae including *Pseudoryx*, and *Kobus* + *Capra* + Hippotragini are most stable to the removal of data sets (DRIs = 3). The remaining nodes collapse with the removal of only one or two of the five data sets (Fig. 12). The DRI analysis suggests that κ-casein is critical in the simultaneous analysis. Removal of this data set has implications for DRIs at six of the seven supported nodes (Fig. 12).

The spread of synapomorphies among data sets can also be used to assess the support among data sets in simultaneous analysis (Kluge, 1989). In the combined cladogram for core taxa, six nodes are supported by unambiguously optimized synapomorphies from the morphological character set, at least one nu DNA data set, and the mt rDNA data set. All five data sets provide unambiguous character support for Bovini including *Pseudoryx*, and for Bovinae including *Pseudoryx*. In contrast, only seven synapomomorphies distributed among three data sets provide clear-cut support for the cattle + *Pseudoryx* node (Fig. 12). The morphological matrix provides the most unambiguous synapomorphies in the combined analysis (Table 3).

TABLE 3. Summed character support for each of the five data sets in separate and combined analyses.

Data set[a]	CS[b]	SS[c]	HS[d]	PBS[e]	BS-C[f]	PHBS[g]	BS-S[h]
Morph	34	8	26	27	−5	32	6
12S + 16S	29	17	12	−3	−6	3	10
βCasXI	10	5	5	6	1	5	5
κCasXI	24	23	1	16.33	20	−3.67	22
αLacI	5	2	3	2.67	−3	5.67	2
Total	102	55	47	49	7	42	45

[a] Abbreviated as in Figure 10.
[b] Unambiguously optimized synapomorphies in the combined analysis of 10 core taxa.
[c] Unambiguously optimized synapomorphies in separate analysis of the 10 core taxa for clades supported by the combined analysis.
[d] Hidden synapomorphy in the combined analysis of the 10 core taxa.
[e] Partitioned branch support in the combined analysis of 10 core taxa.
[f] Branch support for relationships supported by the combined analysis of the 10 core taxa.
[g] Partitioned hidden branch support in the combined analysis of the 10 core taxa.
[h] Branch support for relationships supported by that data set for the 10 core taxa.

Hidden Character Support and Conflicts

The hallmark of hidden support is the emergence of clades in combined analysis that are not supported by any of the separate analyses (Barrett et al., 1991; Chippindale and Wiens, 1994). One clade, *Boselaphus* + Bovini including *Pseudoryx*, is not resolved in any of the five separate analyses of core taxa (Fig. 8). The sum of BS scores for the clade in these separate analyses is −10 (Fig. 12). Nonetheless, this grouping appears in all of the combined analyses of the five data sets (Fig. 9).

For the core taxa, phylogenetic analysis of the morphological data set yields only one group that is consistent with the simultaneous analysis of all five data sets (Fig. 8). However, in the context of the molecular data, the skeletal and dental evidence provides positive support at five nodes according to PBS and at six nodes according to the distribution of unambiguous synapomorphies (Fig. 12). Much hidden support is contained in the morphological data set. For this partition, HS is +26, and the sum of PHBS scores is +32 (Table 3). BS totals only +6 for groups supported by the separate analysis of the morphological matrix. Therefore, for this data set, the PHBS in simultaneous analysis is more than fivefold greater than BS in separate analysis (Table 3)! Less than one-third of the informative characters from the combined data set reside in the morphological partition (Fig. 8). Because of extensive PHBS, however, the morphological evidence provides more than one-half of the PBS in combined analysis (Table 3). This critical support is basically invisible in the topologically discrepant results for the morphological matrices (Figs. 7 and 8). When the anatomical evidence is analyzed in isolation from the DNA characters, the sum of BS for nodes supported by the combined data set is just −5 (Table 3).

All five data sets are characterized by a net positive HS in the combined analysis, four data sets have a net positive PHBS, HBS and HS are net positive at each of the seven nodes in the total data tree, and for six of these nodes, HBS is greater than the sum of BS in separate analyses (Fig. 12; Table 3). For clades supported by the combined character set, HBS (+42) accounts for almost 86% of the BS in the combined analysis, and HS (+47) for clades in the total data topology rivals the number of unambiguous synapomorphies for these groups in the five separate analyses (+55). The sum of HBS in the simultaneous

analysis (+42) also approaches the sum of BS scores for clades supported by separate analyses of the individual data sets (+45; Figs. 8 and 12 and Table 3).

The DRI analysis uncovered some of the hidden support in the combined data set. For example, only two data sets support Bovinae in separate analyses (Fig. 8), but the DRI at this node is 3. Three data sets, morphology, β-casein, and α-lactalbumin, do not individually support Bovinae, but combining these three data sets in simultaneous analysis does support Bovinae (Fig. 12). This cryptic phylogenetic information is obscured by separate analyses of the individual data sets.

Weak hidden character conflicts are also apparent in the combined analysis. Negative PHBS emerges at five of seven nodes supported by the combined analysis of 10 core taxa, and negative HS is present at three nodes (Fig. 12). Hidden conflicts are most apparent in the mt rDNA data set, which is characterized by four negative PHBS scores and one negative HS score. κ-Casein is the only data set with net negative PHBS (Table 3) and negative PHBS scores at 3 nodes (Fig. 12). These hidden conflicts hint that support among data sets for a particular node can be overestimated as well as underestimated by the taxonomic congruence approach. The influence of hidden support and hidden conflict cannot be assessed unless data sets are combined in simultaneous analysis (Barrett et al., 1991; Chippindale and Wiens, 1994; Nixon and Carpenter, 1996).

Summary of Support for the Phylogenetic Position of Pseudoryx

In all combined analyses of the core taxa (Fig. 9), *Pseudoryx* groups within Bovinae and is not associated with Caprinae. The cost in extra character steps for grouping *Pseudoryx* with caprines is substantial (Fig. 6). Little of the molecular evidence supports a close association between *Pseudoryx* and Caprinae (Figs. 2–5). Additionally, in the context of the four molecular data sets, the morphological characters offer overwhelming support for the clustering of *Pseudoryx* within Bovinae (Fig. 12).

Relationships supported by the 10 core taxa are not rearranged with increased taxonomic sampling (Fig. 10). However, the specific relationships between *Pseudoryx* and other Bovinae are not all robustly supported. *Pseudoryx* + cattle is especially weakly supported: BS is 1; BP is <50%; only κ-casein supports this node in separate analyses (BS = +2); and in simultaneous analysis according to PBS (PBS = +2), the removal of only one data set collapses this node; three LBSIs are <1; only seven unambiguous synapomorphies are apparent for the clade in the simultaneous analysis; and transversion parsimony of the core taxa does not support this node (Figs. 3, 9, 10, 11, and 12). However, although only one data set supports this node in simultaneous analysis according to PBS, only one data set, β-casein, weakly contradicts this node (PBS = −1). All other data sets are neutral with respect to a grouping of cattle + *Pseudoryx* (PBS = 0, Fig. 12). *Boselaphus* + Bovini including *Pseudoryx* is also weakly supported. Most of the BS for this node is derived from hidden support in the morphological data set (PHBS = +6, HS = +7). Among the molecular data sets, only β-casein and mt rDNA provide unambiguous synapomorphies for this clade in simultaneous analysis (Fig. 12).

Bovini including *Pseudoryx* and Bovinae including *Pseudoryx* are more solidly supported by the data. Bovinae excluding *Pseudoryx* is a traditionally recognized clade that has been recovered in several previous analyses of bovid phylogeny (Simpson, 1945; Kingdon, 1982; Lowenstein, 1986; Allard et al., 1992; Wall et al., 1992; Gatesy et al., 1997). Although only two data sets support Bovinae in separate analyses of the core taxa (Fig. 8), the node is well supported in simultaneous analysis. Because of hidden support that emerges with the combination of data sets, the clade is stable to the removal of any two data sets and has no negative PBS scores. Character changes in all five data sets support the node in simultaneous analysis (Fig. 12). Cattle + buffaloes + *Pseudoryx*, a revised Bovini, is not as stable as Bovinae to data set removal, but has a slightly higher BS. The morphological characters and the nu DNA data sets provide positive PBS at this node, and all five data sets offer unambiguously optimized synapomorphies in the simultaneous analysis (Fig. 12).

All combined phylogenetic analyses place *Pseudoryx* with the tribe Bovini (cattle and buffaloes), a subclade of the subfamily Bovinae (see Simpson, 1945). Most combined analyses align *Pseudoryx* with cattle to the exclusion of true buffaloes

(*Syncerus* + *Bubalus*). However, given the weak support for a cattle + *Pseudoryx* clade, we suggest that *Pseudoryx* be classified as Bovini *incertae sedis*. Phylogenetic analyses that include fossil bovines will help to clarify the relationships of *Pseudoryx* to other Bovini (e.g. Geraads, 1992; Groves and Schaller, 2000). Alternatively, a more basal positioning of *Pseudoryx* in bovid phylogeny is not definitively rejected by the current data base; *Pseudoryx* may lie outside the extant diversity of Bovini. For the core group of 10 taxa, only two additional character steps are required to group buffaloes and cattle to the exclusion of *Pseudoryx*, only seven extra steps are required to group *Pseudoryx* with Tragelaphini, and only eight extra steps are required to group cattle, buffaloes, and *Boselaphus* to the exclusion of *Pseudoryx*. Because character support among nodes is not additive (Fig. 11), relationships within Bovinae are unstable.

CONCLUSION

Combined phylogenetic analyses of morphological characters and data from five genes clearly reject a close relationship between *Pseudoryx* and Caprinae. Instead, simultaneous analyses support the placement of *Pseudoryx* among Bovinae (Fig. 1a, data from Dung et al., 1993). Specifically, an association between *Pseudoryx* and Bovini is supported. These results contradict conclusions based solely on skeletal and dental evidence (Thomas, 1994), but these same morphological data provide extensive hidden support in simultaneous phylogenetic analyses (Fig. 12; Table 3). Separate and combined analyses of the bovid data sets demonstrate that both hidden support and hidden conflict are critical in determining the robustness of particular relationships. The distribution of support among data partitions can be underestimated or overestimated by the taxonomic congruence approach. Further phylogenetic studies of Bovinae and, in particular, Bovini are required to test some of the more tenuous relationships suggested by this study.

NOTE ADDED IN PROOF

Hassanin and Douzery (1999) recently published an analysis of four genes that also supports a grouping of *Pseudoryx* with cattle. Combined analysis of our data set and the three genes unique to Hassanin and Douzery (1999) also supports *Pseudoryx* + cattle. Hassanin, A., and E. Douzery. 1999. Evolutionary affinities of the enigmatic saola (*pseudoryx nghetinhensis*) in the context of the molecular phylogeny of Bovidae. Proc. R. Soc. Lond. B. 266:893–900.

ACKNOWLEDGMENTS

We thank K. B. Pederson for α-lactalbumin sequences, P. Friis for much laboratory assistance and support, and D. Swofford for use of test versions of PAUP*. A. de Queiroz and C. Hayashi made useful comments on various stages of the manuscript. O. Ryder, K. Hecker, G. Amato, and G. Schaller provided DNA or tissue samples that were critical for this study. The staff of the University of Arizona automated sequencing facility and the Laboratory of Molecular Systematics and Evolution aided in data collection. This work primarily was funded by the Center for Tropical Biodiversity, Copenhagen, Denmark (Danish National Science Foundation, #11-0390). Additional funding was from National Science Foundation grant DEB-9509551 awarded to J.G.

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Received 23 September 1998; accepted 23 March 1999
Associate Editor: M. Westneat