

# Phylogenetic Analysis of Snow Sheep (*Ovis nivicola*) and Closely Related Taxa

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## Abstract

Based on mitochondrial cytochrome *b* gene sequence analysis, the history of true sheep (*Ovis*) began approximately 3.12 million years ago (MYA). The evolution of *Ovis* resulted in three generally accepted genetic groups: Argaliforms, Moufloniforms, and Pachyceriforms. The Pachyceriforms of the subgenus *Pachyceros* comprise the thin-horn sheep *Ovis nivicola* (snow sheep), *Ovis dalli* (Dall and Stone sheep), and *Ovis canadensis* (Rocky Mountain and desert bighorn). North America wild sheep (*O. canadensis* and *O. dalli*) evolved separately from Eurasian wild sheep and diverged from each other about 1.41 MYA. Ancestral stock that gave rise to snow sheep, Moufloniforms, and Argaliforms occurred 2.3 MYA, which then gave rise to two different extant lines of snow sheep that diverged from each other about 1.96 MYA. The more recent *nivicola* line is genetically closer to the North American wild sheep and may represent a close association during the refugium when Alaska and Siberia were connected by the Bering land bridge. The earlier period of evolution of the Pachyceriforms suggests they may have first evolved in Eurasia, the oldest ancestor then giving rise to North American wild sheep, and that a *canadensis*-like ancestor most likely gave rise to *nivicola*. Cytogenetic analysis further validates that the standard diploid number for modern *nivicola* is 52.

Wild sheep of the genus *Ovis* are Holarctic, with a distribution of extant species and subspecies that span two continents. Although the fossil record has major gaps, wild sheep most likely had their beginning in Eurasia, evolving from a rupicaprine ancestor. Since the Eurasian and American wild sheep represent very distinct evolutionary entities, the American sheep, which includes the Siberia snow sheep, have been classified in the subgenus *Pachyceros* (Geist 1971; Valdez 1982).

Pachyceriforms consist of thinhorn and bighorn sheep: the snow sheep (*Ovis nivicola* spp.) and Dall (*Ovis dalli dalli*) and Stone sheep (*Ovis dalli stonei*) comprise the thinhorn sheep, while the North American Rocky Mountain and desert sheep comprise the bighorn sheep (*Ovis canadensis* spp.). The Siberian snow sheep was once referred to as an Asiatic bighorn and classified *Ovis canadensis nivicola* (Ellerman and Morrison-Scott 1966), meaning “snow dwelling.” Today *nivicola* is commonly referred to as the “snow sheep” and is very similar anatomically to the Alaskan Dall sheep (*O. dalli dalli*). Siberian snow sheep (*O. nivicola*) inhabit the most northern range of the Eurasian wild sheep, which comprises an expanse of mountain ranges in northern Russia that is larger than the lower continental United States.

There are two to six subspecies of snow sheep, depending on the classification used (Clark 1964; Ellerman and

Morrison-Scott 1966; Geist 1971; Heptner et al. 1961; Valdez 1982). Taking into consideration the six subspecies classifications, the most eastern subspecies and closest to Alaska is the Koryak (*Ovis nivicola koryakorum*). South of the Koryak, the Kamchatka sheep (*Ovis nivicola nivicola*) is found on the Kamchatka Peninsula. West and north of the Sea of Okhotsk is the Okhotsk sheep (*Ovis nivicola alleni*). Further inland and southward toward Lake Baikal is the Yablonov sheep (*Ovis nivicola potanini*). Far to the west and east of the Yenisey River is a vast area that is inhabited by the Putorian sheep (*Ovis nivicola borealis*). On the Arctic watershed and west of the Koryak range is the Yakut sheep (*Ovis nivicola hydekkeri*). However, Valdez (1982) recognizes only *nivicola* and *borealis* as valid subspecies.

There are two principal hypotheses suggesting a possible origin of the Pachyceriforms. Cowan (1940) postulated that the pachycerine sheep evolved solely within Beringia and that *O. dalli* and *O. canadensis* both evolved from a *nivicola*-like ancestor during a process of east- and southward migration, and by isolation from glacial and interglacial barriers; whereas Severtzov (1873) suggested that the pachycerine sheep arose as a consequence of “reverse migration.” Severtzov’s hypothesis suggests that the first North American wild sheep to traverse the Bering land bridge was *Ovis ammon*-like. After their

**Table 1.** Samples collected for this study

Genus	Species	Common name	Sample code	Accession no.	Localities
<i>Ovis</i>	<i>nivicola</i>	Snow sheep	1	AJ867262	Maimandzhinskii Range
			2	AJ867263	Maimandzhinskii Range
			3	AJ867264	Siglan Range of the Evreinova Cape
			4	AJ867265	Taiganos Cape
	<i>ammon</i>	Argali	M33	AJ867266	Dulan, Qinghai Province
			M23	AJ867267	Tuoli, Xinjiang
			M14	AJ867268	Tibet
			J1	AJ867269	Tibet
			J14	AJ867271	Nankazi, Tibet
			J13	AJ867270	Tibet
			a1	AJ867276	Mazongshan, Gansu Province
			a2	AJ867275	Subei, Gansu Province
			a3	AJ867274	Tashkent, Uzbekistan
			a4	AJ867273	Mazongshan, Gansu Province
			a5	AJ867272	Subei, Gansu Province
			J3	AJ867259	Bybrk, Xinjiang Province
			J17	AJ867260	Mazongshan, Gansu Province
			J16	AJ867257	Mazongshan, Gansu Province
			3gs	AJ867258	Mazongshan, Gansu Province
	<i>orientalis</i>	red sheep	J20	AJ867261	Bozda Konya, Turkey

isolation in North America by glacial ice or the interglacial Bering Strait, they evolved characteristics unique to the subgenus *Pachyceros*. A lowered sea level due to reglaciation permitted the pachycerine sheep to migrate back across the land bridge into northeastern Siberia. Sea levels then rose from glacial melt, giving rise to present day *nivicola* in Siberia with the isolation of *dalli* in Alaska. South of the snow sheep range is the largest distribution of any single species of wild sheep, the argali (*O. ammon*) of central Asia.

The diploid chromosome number ( $2n = 52$ ) of snow sheep is unique to Holarctic wild sheep, although sampling has been limited to the inland populations north of Magadan (Nadler and Bunch 1977). All North American sheep have a  $2n$  of 54. The closest extant Eurasian wild sheep, the argali, has a  $2n$  of 56. Comparative Giemsa-banding analysis among these taxa demonstrates a highly conserved banding homology.

In this study we combined mitochondrial DNA (mtDNA) cytochrome *b* sequences and chromosomal data in order to infer the phylogenetic relationship of the Pachyceriforms and further clarify the possible mode of pachycerine evolution.

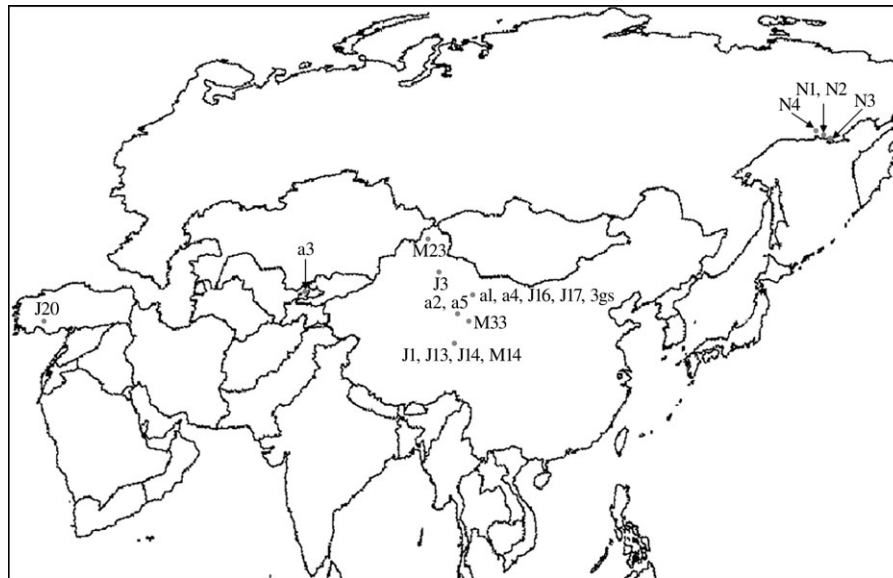
## Methods

### Sample Collecting

Table 1 and Figure 1 show the samples in this study. Four adult snow sheep rams were collected from the following locations: N 60° 49', E 152°16' (Maimandzhinskii Range); N 60° 16', E 152°21' (near Rozolmnii stream south of the Maimandzhinskii Range); N 58° 56', E 152°52' (Siglan Range off of the Evreinova Cape); and N 61° 08', E 160°02' (Taiganos Cape).

### DNA Extraction, Amplification, and Sequencing Analysis

Total genomic DNA was isolated using a modified method from tissue and old specimens (Sambrook et al. 1989; Wu et al. 2000, 2003). The entire mitochondrial cytochrome *b* gene was amplified using the universal mammalian primers L14724 and H15915 (Irwin et al. 1991) and L14841 and H15149 (Kocher et al. 1989). Polymerase chain reaction (PCR) amplifications were performed in 25 µl (total reaction volumes containing 1.5 mM MgCl<sub>2</sub>, 10 µM of each primer) of 1.0 U of *Taq* DNA polymerase in a buffer supplied by the respective enzyme manufacturer. The typical PCR cycling conditions were initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C to 56°C for 1 min, and 72°C for 1 min. A final extension for 5–10 min at 72°C was included to minimize the number of partial strands. PCR products were purified by using a DNA gel extraction kit (100 reactions; Sangon, Shanghai, China). The purified PCR product was used as template in a 10 µl cycle sequencing reaction using the cycle sequencing kit (Applied Biosystems, Foster City, CA). All specimens were sequenced completely in both directions using the PCR primers. The cycle sequencing conditions were as follows: 96°C preheat, then 25 cycles of 96°C for 15 s, 50°C for 30 s, and 60°C for 4 min. The cycle sequencing product was purified over Centriscap columns filled with 800 µl Sephadex G-50. Samples were dried and resuspended in 6 µl loading buffer solution (5:1 deionized formamide:blue dextran/EDTA, pH 8.0), denatured at 98°C for 2 min, and loaded onto acrylamide gels. Typically runs were limited to lengths of 500 bp. After tracking, gels were analyzed using ABI 377 DNA Sequencing Analysis software (version 2.0) to generate a chromatogram file. The base sequence was checked for miscalls due to bad base spacing



**Figure 1.** Approximate geographical distribution of DNA samples used in this study. The map was modified from <http://worldatlas.com/webimage/countrys/asia/asoutl.htm>.

over fluorescence of a particular dye nucleotide or masking of a C nucleotide by a G nucleotide.

#### Sequences Alignment, Statistical Analysis, and Substitution Saturation Test

The different partial sequences were merged using the DNASTar package (DNASTar, Inc., Madison, WI) under the Seqman option. The sequences were aligned by ClustalX (Thompson 1997) and the alignment results were adjusted manually for obvious alignment errors. Mega3.0 (Kumar et al. 2001) was used for statistical analysis. Substitution saturation was tested by DAMBE (version 4.2.8) (Xia 2000; Xia and Xie 2001; Xia et al. 2003).

#### Phylogenetic Analysis

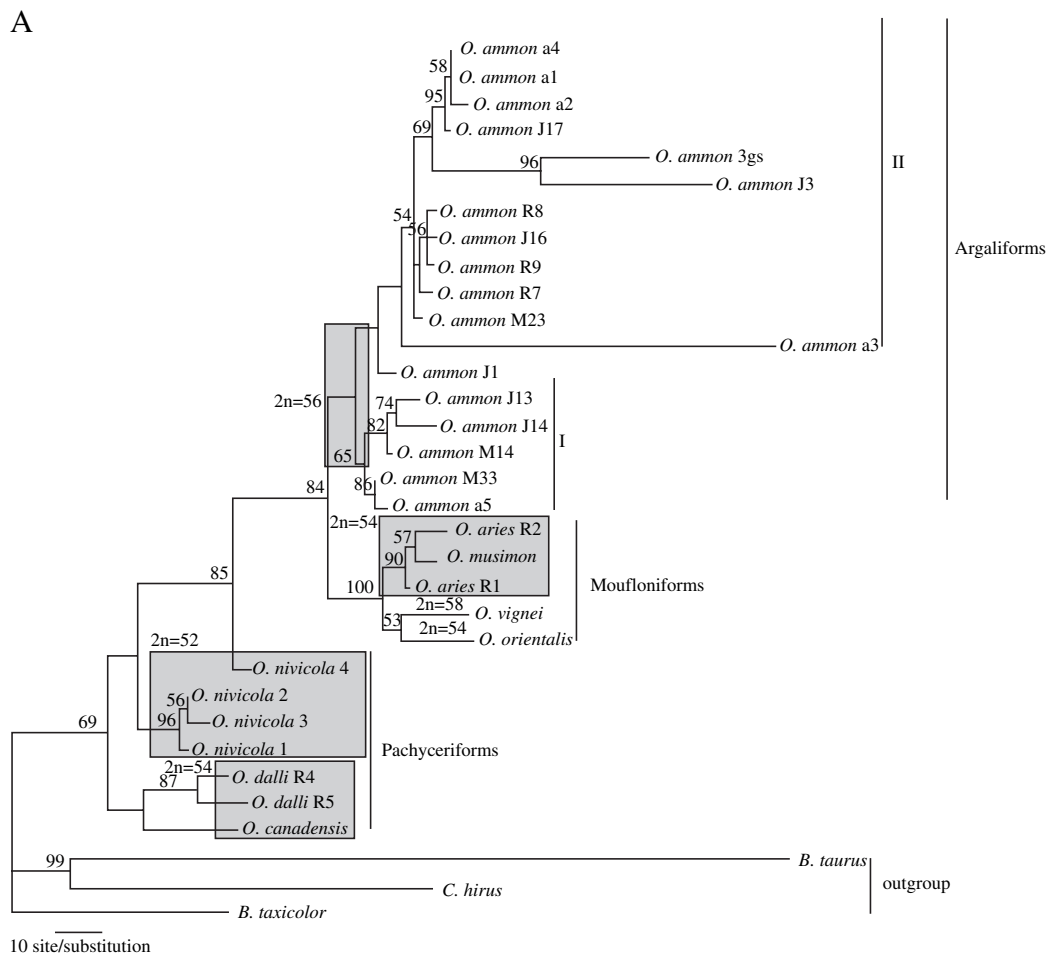
Unweighted maximum parsimony (MP) (Hasegawa 1985) was estimated using PAUP 4.10b (Swofford 2003) running

heuristic searches with tree bisection-reconnection (TBR) branch swapping and 10 random addition replicates. Nodal support was estimated using the nonparametric bootstrap (Felsenstein 1985) limited to 1000 pseudoreplicates for MP. The domestic cow (*Bos taurus*, AY526085), domestic goat (*Capra hircus*, AB004072), and takin (*Budorcas taxicolor*, U17867) were used as the outgroup. Table 2 shows the published sequence data for our present phylogenetic analysis.

Prior to the maximum-likelihood phylogenetic analysis, Modeltest 3.06 was used in order to find the optimal model of DNA substitution for maximum-likelihood construction (Posada and Crandall 1998). The substitution model was selected by both hierarchical likelihood ratio tests (HLRTs) (Huelsenbeck and Rannala 1997) and the Akaike information criterion (AIC) (Akaike 1974). Maximum-likelihood settings from the best-fit model for the different datasets with different parameters were selected by HLRT in Modeltest 3.06 (Posada and Crandall 1998). Heuristic maximum-likelihood

**Table 2.** Published sequences for present phylogenetic analysis

Genus	Species	Common name	2n	Accession no.	Sample code	Reference
<i>Bos</i>	<i>taurus</i>	Domestic cow	60	AY526085		Chung HY and Ha JM (unpublished)
<i>Budorcas</i>	<i>taxicolor</i>	Takin	52	U17867		Groves and Shields (1996)
<i>Capra</i>	<i>hircus</i>	Domestic goat	60	AB004072		Takada et al. (1997)
<i>Ovis</i>	<i>aries</i>	Domestic sheep	54	D84205	R1	Arai K, et al. (unpublished)
					R2	Irwin et al. (1991)
	<i>canadensis</i>	Bighorn sheep	54	U17859	R3	Groves and Shields (1996)
					R4	Hassanin A, et al. (1998)
	<i>dalli</i>	Dall sheep	54	AF034728, U17860	R5	Groves and Shields (1996)
					R6	Hassanin A, et al. (1998)
	<i>vignei</i>	Urial	58	AF034729	R7	Hiendleder et al. (2002)
	<i>ammon</i>	Argali	56	AF242349, AF242350, AF034727	R8	Hiendleder et al. (2002)
					R9	Hassanin A, et al. (1998)
	<i>musimon</i>	Mouflon	54	D84203	R10	Arai K, et al. (unpublished)



**Figure 2.** (A) Majority rule tree from two equally parsimonious trees. The numbers on the branch indicate bootstrap values and  $2n$  indicates chromosome number. (B) The 50% majority consensus tree is recovered from Bayesian trees sampled during four independent Bayesian analyses under the TrN + I + G model for DNA substitution. The pP is shown above every branch on the BI tree. The values below the branch are maximum-likelihood bootstrap support. The bold Italian numbers are indicated on the corner of topology corresponding to those in Table 4. Bayesian analysis and maximum likelihood resulted in the same topology. (C) Unrooted MP consensus tree was from the four unweighted trees. Three major taxonomic groups were recognized: Pachyceriforms that include *O. nivicola*, *O. dalli*, and *O. canadensis*; Moufloniforms that comprise *O. vignei*, *O. orientalis*, *O. musimon*, and *O. aries*; and the Argaliform, *O. ammon*, which consists of two subclades.

searches using TBR branch swapping (initial trees were obtained by neighbor joining) were performed using PAUP 4.0b10 (Swofford 2003). Maximum-likelihood nodal support was estimated using the nonparametric bootstrap (Felsenstein 1985), limited to 100 pseudoreplicates because of computing time. The application of Modeltest 3.06 to the dataset resulted in two different substitution models. The Tamura and Nei (1993) model with invariable sites and gamma distribution (TrN + I + G) was chosen by HLRT and the GTR + I + G model was chosen by AIC.

Bayesian analysis was implemented with MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001) in a likelihood framework. We used the same molecular model as for the maximum likelihood analysis. Model parameters (base pair frequencies, substitution rate matrix) were treated as unknown variables to be estimated by MrBayes. Analyses

began with random starting trees and ran for 2,000,000 generations, with Markov chains sampled every 100 generations. Multiple Bayesian searches using Metropolis-coupled Markov chain Monte Carlo sampling were conducted. One cold and three heated Markov chains, applying MrBayes default heating values ( $t = 0.2$ ), were used in the analysis. The “burnin” generations (random points generated prior to stationarity) were discarded and subsequent generations were used to form the posterior probability distribution. The analysis was conducted twice using identical settings to ensure that the Bayesian analyses were not trapped in local optima (Huelsenbeck and Bollback 2001; Leaché and Reeder 2002). The remaining trees from both analyses were used to create a majority rule consensus tree, where the percent of samples recovering the same clade represent the posterior probability of that clade.

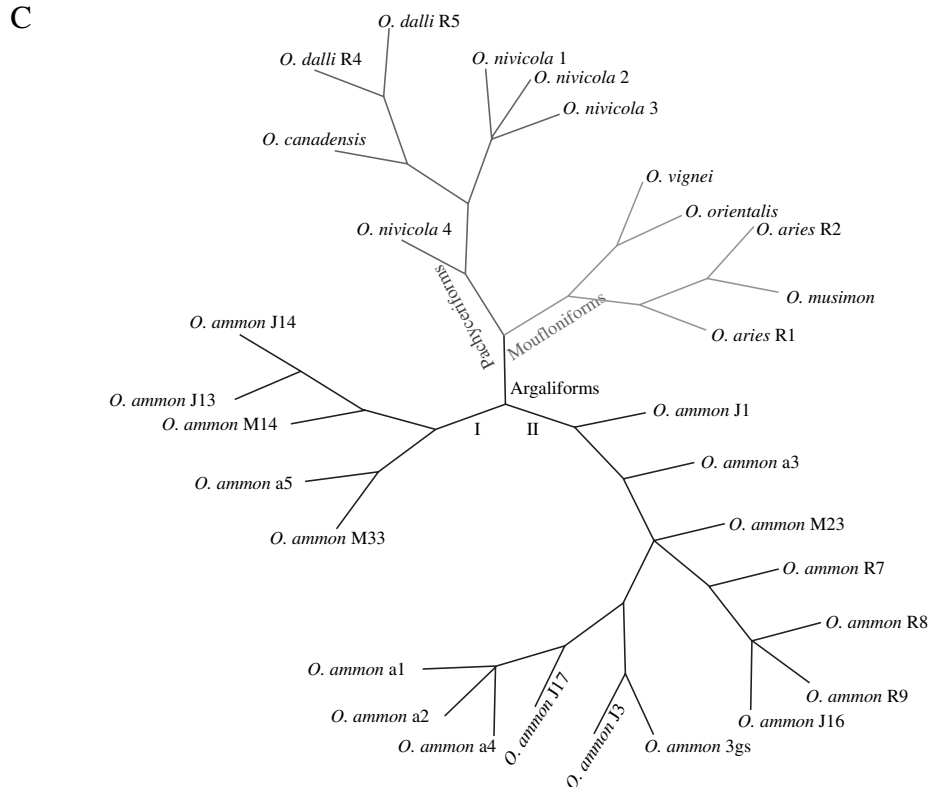
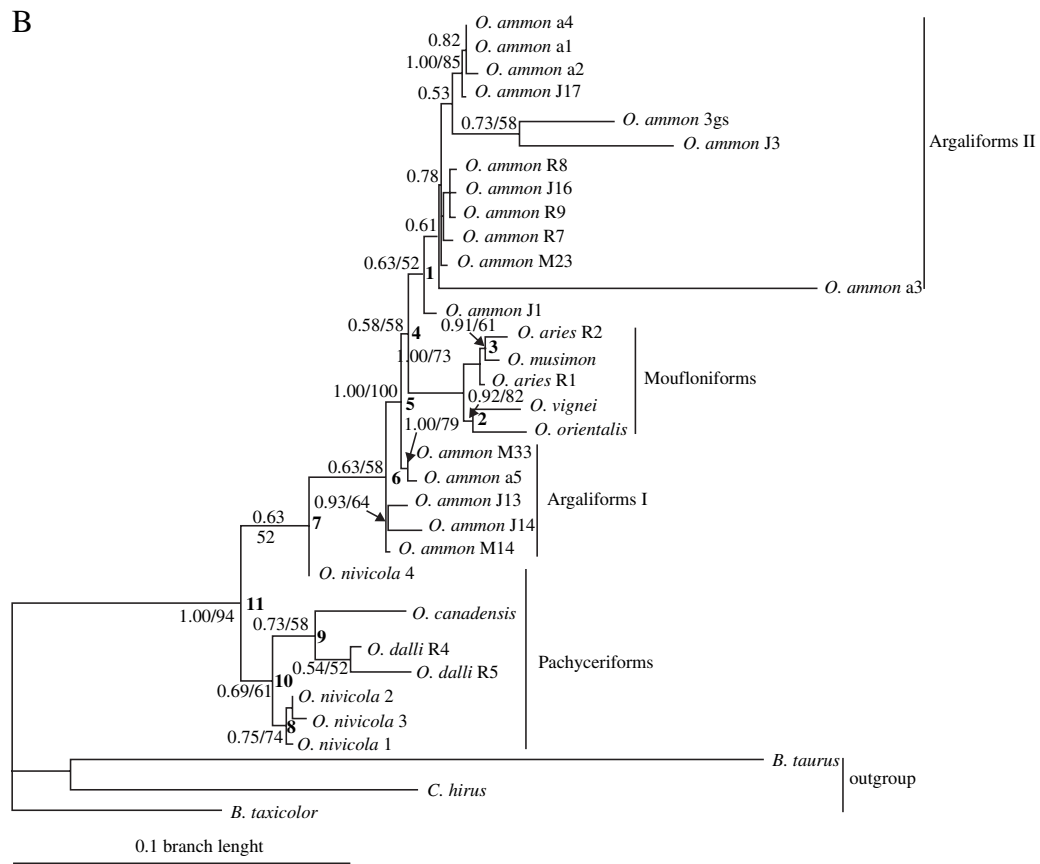


Figure 2. Continued.



## Estimating Divergence Time

The molecular clock hypothesis was tested through maximum-likelihood construction with and without an enforcing clock. We applied Kishino-Hasegawa (Kishino and Hasegawa 1989) and Shimodaira-Hasegawa (Shimodaira and Hasegawa 1999) tests as used in PAUP for the difference between the two maximum-likelihood trees. The test of the molecular clock revealed a significant deviation from rate consistency (diff  $-\ln L = 82.736$ ,  $P < .05$ ). Therefore we used the Bayes MCMC package "Thornian Time Traveller" (T3) (<ftp://abacus.gene.ucl.ac.uk/pub/T3>), developed by Thorne et al. (1998), Thorne and Kishino (2002), and Yang and Yoder (2003), which is based on a probabilistic model to describe the change in evolutionary rate over time and conducts the Markov chain Monte Carlo procedure to estimate the posterior distribution of rates and times. To obtain the mean for posterior divergence time, we used six input values for the mean of the prior distribution of the root of the ingroup tree based on the literature or fossil records. A primary calibration is based on the first opening of the Bering Strait (Marincovich and Gladenkov 1999). The absolute ages of geological periods and chronostratigraphic references were based on the 2000 edition of the International Stratigraphic Chart (<http://www.elasmo.com/refs/geotime.html>). The second time constraint, 2.5 million years ago (MYA), was the divergence of the genus *Ovis* (Thenius and Hofer 1960). We selected the divergence time within the genus *Ovis*, 1.8–0.75 and 0.125–0.01 MYA, as the third and fourth pairs time constraint. Markov chain Monte Carlo analyses were run for 1,000,000 generations after a burn-in of 100,000 generations to allow Markov chains to approach stationarity before states were sampled; chains were sampled every 100 generations (Hassanin and Douzery 2003; Yang and Yoder 2003).

## Cytogenetic Analysis and Chromosome Evolution

Chromosome preparations were prepared from fibroblast cultures. G-banding followed the procedures of Wang and Federoff (1972) and the description of karyotypes was based on the format of Ansari et al. (1999), ISCN DB 2000 (2001), and Menscher et al. (1989). We used MacClade 3.08 (Maddison and Maddison 1997) to investigate the phylogenetic distribution of chromosome numbers in the genus *Ovis*. The chromosome data were traced onto the majority rule parsimonious tree.

## Results

### Statistical Sequences and Substitution Saturation Results

The complete cytochrome *b* (1140 bp) was sequenced for 20 individuals that included three species of the genus *Ovis*. The alignment sequences have 268 variable sites and 134 sites are parsimony informative. Net sequence divergence ranged from 0.14% to 11.94% in the genus *Ovis*. The nucleotide composition is typical for mammalian values (Irwin et al. 1991), with a low proportion of guanines (12.9%) in the over-

all composition. Substitution saturation tests, based on Xia et al. (2003), revealed little saturation (results not shown). Therefore the dataset used in this study was suitable for phylogenetic analysis.

## Phylogenetic Analysis and Divergence Time Within the Genus *Ovis*

Three phylogenetic methods (maximum parsimony, maximum likelihood, and Bayesian) resulted in similar tree topology (Figure 2) with or without outgroups, with the taxa of wild sheep separating into three major genetic groupings. The Pachyceriforms include *O. nivicola*, *O. dalli*, and *O. canadensis*. The Moufloniforms comprise *O. vignei*, *O. orientalis*, and *O. musimon*. The domestic sheep (*O. aries*) arose from Moufloniform stock. The Argaliforms consist of only one species, *O. ammon*, which has evolved into two subclades. Only the Moufloniforms, however, had high bootstrap support greater than 50% (maximum parsimony 100%, Bayesian 1.00). Divergence time estimating shows that all wild sheep shared a common ancestor about 3.12 MYA. The North America wild sheep, *O. canadensis* and *O. dalli*, diverged from each other about 1.412 MYA (Table 3). The snow sheep (*O. nivicola*) began to diverge about 2.30 MYA. The genetic divergence within snow sheep, however, is only about 1.25 MYA. One of the snow sheep sampled (sheep no. 4) was very different from the other snow sheep (nos. 1–3), with a genetic distance between 2.8% and 6.04% in comparison with other *Ovis* species.

## Cytogenetic Data

Cytogenetic analysis could only be performed on snow sheep rams 2 and 3. Both rams had a  $2n = 52$  and a karyotype consisting of 4 pairs of biarmed and 21 pairs of acrocentric autosomes, a large acrocentric X and a minute biarmed Y chromosome. Giemsa banding permitted pairing of chromosomes. The banding patterns of the largest pair of biarmed chromosomes were similar to those of the largest biarmed chromosomes in all wild and domestic sheep of the genus *Ovis*. The second largest biarmed pair is similar to the second largest pair of all wild and domestic sheep, with a  $2n = 54$ , but absent in the argali (*O. ammon*). The third largest biarmed pair is similar to the third largest for all wild and domestic sheep, with a  $2n = 54$ , and similar to the second largest biarmed pair in the argali karyotype. The fourth largest biarmed pair is unique to *nivicola* and absent in all other species of *Ovis*.

## Discussion

The evolution and speciation of *Ovis* has been problematic, and the problem lies primarily in the measurements used. Some investigators use the classical concept of morphologic differences, while others use biological approaches that stress chromosomal and molecular uniqueness. As a result, the taxonomy of *Ovis* and its relatives remains unsettled. The results of this study are no less unsettling, but add a new perspective to the possible evolution of *Ovis*.

**Table 3.** Bayes estimates of divergence times (including 95% credibility intervals)

Node	Divergence time (millions of years ago)					
	Prior divergence time distribution			Posterior divergence time distribution		
	Date	SD	95% CI	Date	SD	95% CI
1	0.272	0.070	(0.151, 0.421)	1.092	0.227	(0.651, 1.525)
2	0.305	0.074	(0.175, 0.458)	1.225	0.230	(0.758, 1.631)
3	0.094	0.048	(0.034, 0.221)	0.378	0.181	(0.145, 0.859)
4	0.118	0.056	(0.049, 0.269)	0.473	0.210	(0.210, 1.049)
5	0.340	0.077	(0.202, 0.495)	1.366	0.223	(0.883, 1.722)
6	0.381	0.080	(0.231, 0.539)	1.530	0.208	(1.025, 1.790)
7	0.485	0.104	(0.286, 0.690)	1.960	0.399	(1.257, 2.878)
8	0.308	0.175	(0.019, 0.656)	1.254	0.724	(0.072, 2.762)
9	0.347	0.135	(0.098, 0.615)	1.412	0.569	(0.406, 2.646)
10	0.566	0.139	(0.288, 0.824)	2.304	0.600	(1.217, 3.635)
11	0.765	0.101	(0.544, 0.937)	3.118	0.510	(2.521, 4.402)

Node numbers correspond to those in Figure 2B.

The time unit is millions of years.

The standard deviation (SD) is given for each node.

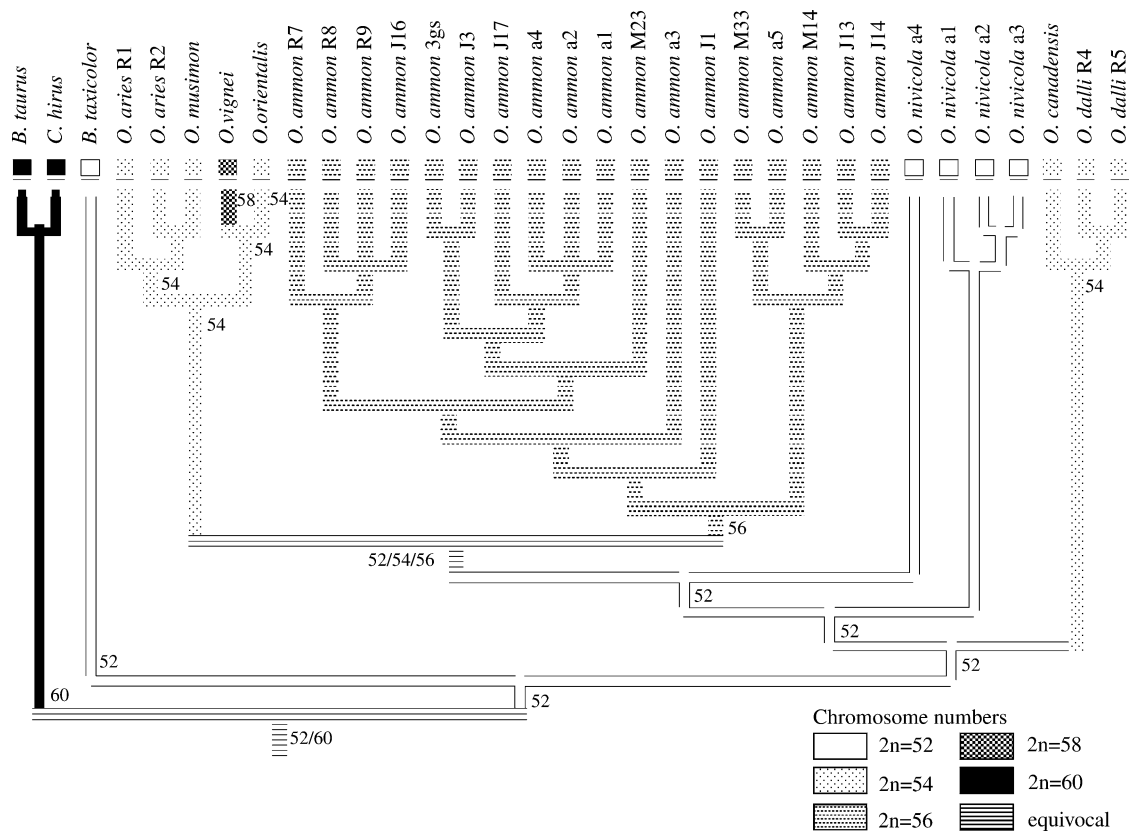
Wild sheep are Holarctic, with a wide distribution in the Palearctic (temperate Eurasia) and Nearctic (temperate New World regions) (Clark 1964; Valdez 1982). They occur in several European countries (as introduced stock from the islands of Corsica and Sardinia) and range from Cyprus through the Middle East (excluding northern Africa), Turkestan, the arid and semiarid regions of the Indian subcontinent, central Asia, and Siberia into North America, where they are limited to the mountainous regions of Alaska, western Canada, and the western United States through Baja California and northwestern Mexico.

The tree topology generated from the mtDNA sequence data confirms that wild sheep have evolved into three major recognizable genetic groups: Argaliforms, Moufloniforms, and Pachyceriforms. These groups have also been somewhat distinguished on the basis of anatomical, chromosomal, and habitat preference [see Valdez (1982) for a description of life-history patterns]. Modern forms of wild sheep have adapted to two different types of terrain. According to Schaller (1977), the Pachyceriforms are stockily built, designed for life in mountainous regions near cliffs, whereas the Argaliforms and Moufloniforms are agile, built for speed and endurance in a more open, rolling habitat. Central to the question on the evolution of wild sheep is where and when the process began. Based on the mtDNA evidence of this study, *Ovis* first appeared much earlier than the Pliocene/Pleistocene epoch (1.5–2.5 MYA). *Ovis-Capra*-like ancestral stock separated into two unique lineages about 3.118 MYA. The primitive *Capra* line gave rise to two genera, modern-day forms of *Capra* and *Budorcas* (takin). Phenotypically the takin is much different from extant *Capra* sp. and is currently classified in the tribe Ovibovini, whereas *Capra* and *Ovis* are classified in the tribe Caprini. Caprines include five extant genera: *Ammotragus* (aoudad), *Capra* (goat), *Hemitragus* (tahr), *Ovis* (sheep), and *Pseudois* (bharal or blue sheep).

The fossil record of *Ovis* is poor because conditions for fossilization are not optimal in montane regions, and recur-

ring glaciation often ground over terrain inhabited by sheep. There is, however, enough evidence to support the hypothesis that *Ovis* was part of the mammal fauna that flooded into Europe and central Asia about 2.5 MYA. Old World fossil *Ovis* suggests they occurred first in the late Villafranchian of Europe and Asia and then sporadically afterwards in the Pleistocene fossil record (Thenius and Hofer 1960). Early Pleistocene ancestral sheep were fairly large, with an Argaliform-like sheep evolving in eastern or central Asia. A smaller Moufloniform-like sheep evolved in western Asia or Europe in the late Pleistocene. Ox-sized giants, *Megalovis*, have also been found in the Villafranchian of Europe and Asia (Kurten 1968). According to Herre and Kesper (1953), large sheep were present in Eurasia during the early and middle Pleistocene, only to be replaced by smaller sheep toward the end of the Ice Age. Wild *Ovis* first appeared in the North American fossil record during the Illinoian glacial period and are found in several localities near Fairbanks, Alaska (Guthrie 1968). Guthrie (1968) considered Alaskan *O. dalli* as conspecific with Siberian *O. nivicola*. All fossil sheep found thus far in North America are assignable to the subgenus *Pachyceros*, and differentiation of *Pachyceros* from *Ovis* probably occurred in the Barringtonian region of interglacial warm zones prior to their migration southward from Alaska to western Canada and the United States, Baja California, and northwestern Mexico.

The mtDNA sequence data suggests that all extant wild sheep arose from ancestral stock 3.118 MYA (Figure 3). The early *Ovis* prototype eventually gave rise to North American wild sheep (*O. canadensis* and *O. dalli*), and the two species began to diverge about 1.412 MYA. A branch that led to the rise of modern-day snow sheep arose about 1.25 MYA. However, the Moufloniforms and Argaliforms diverged from ancestral stock about 1.23 to 0.06 MYA and 1.53 MYA, respectively. Snow sheep no. 4 is genetically distinct from snow sheep nos. 1–3, and most likely represents isolation at an early time, long before the dissolution of the



**Figure 3.** Majority rule parsimonious tree obtained for the genus *Ovis* using mtDNA cytochrome *b* gene sequences onto which chromosome numbers have been mapped using MacClade 3.08 (Maddison and Maddison 1997).

Bering land bridge, which genetically isolated the North American wild sheep from the Siberian snow sheep.

The ancestral *Ovis* karyotype had 60 chromosomes, which is still maintained in *Capra* (Nadler and Bunch 1977). The G-band karyotype of *Capra* has served as a reference to identify specific acrocentric chromosomes involved in the evolution of extant *Ovis* and their respective karyotypes. The first biarmed chromosome observed in present-day *Ovis* arose from the fusion of acrocentrics 1 and 3 and is maintained in the urial (*O. vignei*,  $2n = 58$ ) and all true sheep with  $2n = 56$ , 54, and 52. The second in sequence arose from acrocentrics 2 and 8 and is still maintained in the argali (*O. ammon*,  $2n = 56$ ) and all true sheep with  $2n = 54$  and 52. The third resulted from the fusion of acrocentrics 5 and 11, resulting in the  $2n = 54$  karyotype, and is maintained in the mouflons (*O. musimon* and *O. orientalis*) and all North American wild sheep (*O. canadensis* and *O. dalli*) and the snow sheep with a  $2n = 52$ . The most recently evolved *Ovis* karyotype arose from acrocentrics 9 and 19, and is exclusive to the snow sheep (*O. nivalis*) (Bunch 1978; Bunch and Nadler 1980; Bunch et al. 1976, 2000; Menscher et al. 1989; Nadler and Bunch 1977; Nadler et al. 1973) (Figure 3). The  $2n = 52$  karyotype of *O. nivalis* most likely occurred after disruption of the Bering land bridge 12,000 years ago (Korobitsyna et al. 1974), even though mtDNA differentiation between *nivalis* and *dalli* began much earlier.

Fusions of acrocentric chromosomes are common in many mammalian taxa, including Bovidae (Wurster and Benirschke 1968), and have played a major role in karyotype changes within wild sheep and domestic sheep and cattle (Appels et al. 1998). Cases of nonrandom fusions have been reported in cattle and in humans (Appels et al. 1998; Brüere et al. 1974). There is evidence that acrocentric fusions in wild sheep are nonrandom from a monophyletic origin (Bunch and Nadler 1980; Nadler and Bunch 1977). Only when the larger acrocentrics fused did fusion occur with the smaller chromosomes. The G-band patterns of chromosomal similarity in *Ovis* compared to diverse karyotypes of *Pseudois* (blue sheep) and *Hemitragus* (tahr) argue for a monophyletic origin of *Ovis*. However, mtDNA data suggest in some cases that the evolution of the *Ovis* karyotype was polyphyletic, and that the fission of biarmed chromosomes as well as the fusion of acrocentric chromosomes was involved. Figure 3 illustrates that the  $2n = 58$  karyotype of *O. vignei* may have arisen from  $2n = 54$  stock, which requires that a fission process involving two different pairs of biarmed chromosomes was involved. The fact that centric fusion and fission have been documented in a wide variety of plants and animals leads one to assume that the autosomal acrocentrics in *Ovis* can unite with, or dissociate from, each other without impairment; although there are no chromosome studies suggesting that fission is, or has been,



a means of karyotype change in extant populations of wild sheep.

It appears that once a new Robertsonian translocation arises within a wild sheep population, there appears to be prezygotic selection for the lower chromosome number. Support for this hypothesis is based on the chromosomal composition of two wild sheep populations in Iran and an experimental wild sheep hybrid population at Utah State University. In the Iranian wild sheep population, only  $2n = 54$  and  $55$  were typed; whereas, theoretically, a  $2n$  of  $56$  should occur in one of four offspring from  $2n = 55 \times 2n = 55$  crosses (Valdez et al. 1978). Analysis of progeny from controlled breeding of mouflon/argali hybrids ( $2n = 55$ ) resulted in a much higher than expected number of offspring with  $2n = 54$ .

In this study we were only able to karyotype two (nos. 2 and 3) of the four snow sheep sampled. Sheep no. 2 was sampled west of the region of the sheep sampled in the Korobitsyna et al. (1974) study. Sheep no. 3 was sampled east of Magadan, along the coast of the Sea of Okhotsk. More than likely these two sheep were of the same subspecies (*O. nivicola alleni*) sampled by Korobitsyna et al. (1974). Geist (1971) hypothesized that the Kamchatka snow sheep (*O. nivicola nivicola*) most likely maintains the  $2n = 54$  karyotype as found in all North American wild sheep. Unfortunately we were unable to karyotype sheep no. 4 (Evreinova Cape), which could have resolved this issue. The cape is proximate to the Kamchatka snow sheep range. Evidence based on the mtDNA sequence data suggests sheep no. 4 was genetically different from the sheep sampled in proximity to Magadan and therefore could very well retain the ancestral karyotype of  $2n = 54$ , as hypothesized by Geist (1971). Until the Kamchatka sheep is karyotyped, the  $2n = 52$  karyotype should still be considered typical for the snow sheep, as is the  $2n = 54$  karyotype for all the North American sheep.

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