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Molecular Phylogenetics and Evolution 37 (2005) 45–61
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Molecular phylogenetics and biogeography of *Lepus* in Eastern Asia based on mitochondrial DNA sequences

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Received 8 October 2004; revised 29 April 2005
Available online 28 June 2005

Abstract

In spite of several classification attempts among taxa of the genus *Lepus*, phylogenetic relationships still remain poorly understood. Here, we present molecular genetic evidence that may resolve some of the current incongruities in the phylogeny of the leporids. The complete mitochondrial cytb, 12S genes, and parts of ND4 and control region fragments were sequenced to examine phylogenetic relationships among Chinese hare taxa and other leporids throughout the World using maximum parsimony, maximum likelihood, and Bayesian phylogenetic reconstruction approaches. Using reconstructed phylogenies, we observed that the Chinese hare is not a single monophyletic group as originally thought. Instead, the data infers that the genus *Lepus* is monophyletic with three unique species groups: North American, Eurasian, and African. Ancestral area analysis indicated that ancestral *Lepus* arose in North America and then dispersed into Eurasia via the Bering Land Bridge eventually extending to Africa. Brooks Parsimony analysis showed that dispersal events followed by subsequent speciation have occurred in other geographic areas as well and resulted in the rapid radiation and speciation of *Lepus*. A Bayesian relaxed molecular clock approach based on the continuous autocorrelation of evolutionary rates along branches estimated the divergence time between the three major groups within *Lepus*. The genus appears to have arisen approximately 10.76 MYA ($\pm$ 0.86 MYA), with most speciation events occurring during the Pliocene epoch (5.65 ± 1.15 MYA $\sim$ 1.12 ± 0.47 MYA).

Keywords: Molecular phylogenetics; *Lepus*; Taxonomy; Biogeography

1. Introduction

The family Leporidae includes 11 extant genera (Angermann et al., 1990). Most genera within Leporidae are monotypic (except for *Lepus*, *Sylvilagus*, *Nesolagus*, and *Pronolagus*: Matthee et al., 2004) and pose no major taxonomic concerns. *Pronolagus* consists of more than four species of red rock hares that are restricted to Africa, but so far have not undergone a thorough taxonomic investigation (Angermann et al., 1990; Matthee and Robinson, 1996; Matthee et al., 2004; Whiteford, 1995). The New World genus, *Sylvilagus* (the cottontails), with more than 16 recognized species (Angermann et al., 1990; Chapman et al., 1992; Frey et al., 1997; Matthee et al., 2004), has close affinity with the pygmy

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doi:10.1016/j.ympev.2005.05.006

rabbit *Brachylagus* (Matthee et al., 2004). *Lepus* (the jackrabbit and hares) poses unique taxonomical problems with many irreconcilable issues (Chapman and Flux, 1990). The number of species currently recognized in *Lepus* range from 24 to 30 (Corbet and Hill, 1980; Flux and Angermann, 1990; Hoffmann, 1993). Members of the genus are characterized by similar morphological characters that contribute to the current problematic taxonomy of the group. Consequently, the various classifications have changed over the years, with no two taxonomic schemes in agreement (Flux and Angermann, 1990; Nowak, 1999; Wilson and Reeder, 1993). In this study, we follow Flux and Angermann's (1990) taxonomy and adopt a more conservative approach that takes into consideration 29 species (Flux and Angermann, 1990).

The Chinese leporids occupy a wide variety of habitats, including deciduous, boreal and temperate rain forests, prairie, and shrub-steppe. Early morphological studies divide the Chinese leporid forms into seven species (*Lepus capensis*, *L. sinensis*, *L. oiostolus*, *L. mandschuricus*, *L. timidus*, *L. yarkandensis*, and *L. hainanus*), which have been grouped into three genera, *L. capensis*, *L. oiostolus*, and *L. timidus* in the genus *Lepus* (Tate, 1947); *L. mandschuricus* in the genus *Allolagus* (Ellerman and Morrison-Scott, 1951; Loukashkin, 1943); and three species (*L. sinensis*, *L. yarkandensis*, and *L. hainanus*) in the genus *Caprolagus* (Allen, 1938; Chen, 1956; Гуреев, 1964). Recent morphological studies have raised even further questions concerning the taxonomy and evolutionary relationships of Chinese leporid species (Deng, 1960; Gao and Feng, 1964; Li and Luo, 1979; Luo, 1981; Petter, 1961; Qian et al., 1965; Shou, 1962; Wang et al., 1985; Zhu and Zhuang, 1982). Most of these studies, however, were limited because the morphological taxonomy and geographic descriptions were conducted on only one or two species. A more comprehensive study of relationships among different Chinese hare species was based on the extensive morphological comparisons of Luo (1988). He reexamined various morphometric features (body measurements and skull characters) of numerous specimens and concluded there are nine species belonging to five subgenera with distributions across China: *Lepus* (*L. timidus*, *L. sinensi*, *L. mandschuricus*, and *L. melainus*), *Eulagus* (*L. capensis*), *Proeulagus* (*L. oiostolus*), *Tarimolagus* (*L. yarkandensis*), and *Indolagus* (*L. hainanus* and *L. comus*). Of the nine hare species, *L. timidus* is distributed worldwide across North America and Eurasia, and *L. capensis* in Eurasia and South Africa. Several species (*L. hainanus*, *L. comus*, *L. oiostolus*, *L. yarkandensis*, and *L. melainus*) are endemic to and distributed in restricted regions in China (Chapman and Flux, 1990; Luo, 1988). *L. hainanus* and *L. yarkandensis* are listed as rare and endangered by the IUCN (2000) (Chapman and Flux, 1990). To date, there has been no in-depth molecular study on the evolutionary relationships among the nine Chinese leporid taxa, although some recent molecular studies are beginning to shed light on various taxonomic concerns. More recent evidence from short mtDNA sequences has provided additional information about the taxonomy of Chinese hares (Wu et al., 2000), most notably for the Yunnan hare (*L. comus*), although the fundamental and broader evolutionary, and biogeographic questions are still remain. Halanych et al. (1999) used mitochondrial DNA cytb to establish the phylogeny of 11 *Lepus* species, and concluded that the genus *Lepus* underwent an early and rapid radiation. Studies by Pierpaoli et al. (1999), Koh et al. (2001), Yamada et al. (2002), Alves et al. (2003), and Matthee et al. (2004) have shown that some mitochondrial genes, cytb, 12S, and the control region can be useful markers for determining interspecific relationships and relatively recent evolutionary events. Several researchers using molecular methods (Pérez-Suárez et al., 1994; Robinson and Osterhoff, 1983; Yamada et al., 2002) have begun to address the evolution and historical biogeography of *Lepus*, but their studies have been limited to regional species analyses. As a consequence, there is yet to be a good evolutionary picture of how *Lepus* evolved as a whole.

Our current work is an expansion of the previous studies on the molecular phylogenetics and biogeography of the leporids. Four mtDNA sequence fragments, 12S, ND4, the control region (D-loop), and cytb gene sequences were obtained from nine recognized Chinese hare species, with different individuals for each fragment (see Section 3 for detail). Our first objective was to present a phylogeny of the genus *Lepus* Worldwide based on mitochondrial DNA (mtDNA) sequences. Then, we used present molecular phylogenies to study the biogeographic history of *Lepus*. Our last objective addressed the group's ancestral distribution on a broad geographical scale. We did not attempt to change the currently accepted taxonomic classification of the genus. To do so, one needs to sample from a much wider range of localities, and with a complete synthesis of genetic and morphological characters.

2. Materials and methods

2.1. Specimen collecting and identification

Museum, hair specimens, frozen, and alcohol preserved materials representing nine morphological-based Chinese hare species were collected from 29 localities in China (Table 1 and Fig. 1). Six samples from three localities in Russia tentatively identified as *L. timidus* and *L. europaeus* were also included in this study. Fur color, body size, body weight, and geographical distribution were also used for comparative purposes. Species nomenclature for museum specimens was initially based

Table 1
Samples collected in this study

Taxa	Subspecies	Common name	Collection locality	Sample codes	Specimens type
Lepus capensis	*L. c. centrasiaticus*	Cape hare	Minqin, GanSu Prov.	g2	Tissue
	L. c. swinhoei		Yangxian, Shanxi Prov.	sh4	Tissue
	L. c. centrasiaticus		Sunite, NeimengGu Prov.	m2	Tissue
	L. c. huangshuiensis		Huzhu, Qinghai Prov.	qh1	Tissue
			Mulan, Heilongjiang Prov.	a1	Museum skin
			Tahe, Heilongjiang Prov.	a2	Museum skin
L. mandschuricus		Manchurian hare	Mulan, Heilongjiang Prov.	s7	Museum skin
			Mulan, Heilongjiang Prov.	s8	Museum skin
			Xunke, Heilongjiang Prov.	s17*	Museum skin
			Mudanjiang, Heilongjiang Prov.	dongs	Tissue
			Mudanjiang, Heilongjiang Prov.	Hubei41	Hair
L. melainus		Manchurian black hare	Xunke, Heilongjiang Prov.	s11	Museum skin
			Dedu, Heilongjiang Prov.	s14	Museum skin
L. timidus	*L. t. mordeni*	Mountain hare	Tuqiang, Heilongjiang Prov.	s5t	Museum skin
	L. t. transbaicalicus		Huzhong, Heilongjiang Prov.	xue2t	Tissue
	L. t. mordeni		Mudanjiang, Heilongjiang Prov.	dat	Tissue
	L. t. timidus		Turufan, Xinjiang Prov.	xint	Skin
	L. t. mordeni		Zhanhe, Heilongjiang Prov.	s20	Museum skin
	L. t. mordeni		Mudanjiang, Heilongjiang Prov	dong3	Tissue
L. sinensis	*L. s. sinensis*	Chinese hare	Hengyang, Hunan Prov.	HN2,	Hair
	L. s. sinensis		Zhangjiajie, Hunan Prov.	Zhang3	Hair
	L. s. sinensis		Lianjiang, Fujian Prov.	fu1	Tissue
	L. s. sinensis		Yongchun, Fujian Prov.	fu2	Tissue
	L. s. sinensis		Yongchun, Fujian Prov.	fu3	Tissue
L. comus	*L. c. comus*	Yunnan hare	Tengchong, Yunnan Prov.	t12	Tissue
	L. c. ?		Baoshan, Yunnan Prov.	b1	Hair
	L. c. peni		Zhaotong, Yunnan Prov.	zht2	Tissue
	L. c. pygmaeus		Nanjian, Yunnan Prov.	Nan5	Tissue
L. oiostolus	*L. o. oiostolus*	Woolly hare	Saka, Tibet	Tibet	Tissue
	L. o. sechenensis		Daocheng, Sichuan Prov.	s3o	Museum skin
	L. o. sechenensis		Litang, Sichuan Prov.	s5o	Museum skin
L. hainanus		Hainan hare	Bawangling, Hainan Prov.	hai1	Tissue
			Danzhou, Hainan Prov.	hai15	Tissue
			Dongfang, Hainan Prov.	hai18	Tissue
L. yarkandensis		Yarkand hare	Weili, Xinjiang Prov.	ta1	Museum skin
			Weili, Xinjiang Prov.	ta2	Museum skin
L. timidus		Mountain hare	Leningrad Prov., Russia	r1	Tissue
			Chelgabinsk Prov., Russia	r2	Tissue
			Transbaiaklia, Rep. of Buryatia, Russia	r5, 6	Tissue
L. europaeus		Brown hare	Leningrad Prov., Russia	r3	Tissue
			Transbaiaklia, Rep. Of Buryatia, Russia	r4	Tissue

Note. The * identifies the black type of the Manchurian hare. No subspecies identified mean the species has not subspecies classification.

on the name given to a sample by the researchers from whom the material was obtained. Taxa are represented by more than one specimen, usually from different localities, and assigned to the named subspecies.

2.2. DNA extraction, amplification, and sequencing analysis

Total genomic DNA was extracted using a modified method from the standard phenol/chloroform extraction process (Sambrook et al., 1989; Wu et al., 2000, 2003). For hair and skin specimens, the method was modified from Walsh et al. (1991) using Chelex-100 (Su et al., 1995; Wu et al., 2000). The entire mitochondrial cytb gene was amplified and sequenced using universal primers L14724 and H15915 (Irwin et al., 1991) and L14841 and H15149 (Kocher et al., 1989). Leporid specific primers (Table 2) were designed from published sequences of lagomorphs as well as from sequences we have obtained. Primer numbering is based upon the complete rabbit mitochondrial DNA (Gissi et al., 1998). One primer (L15136) specific for *Ochotona* (Yu et al., 2000) was also used. PCR amplification and cycle sequencing was carried out using the protocols described

Fig. 1. Approximate geographical distribution of DNA samples used in this study (six samples from Russia are not shown).

Table 2
Leporid specific primers used for PCR and sequencing

Sequence fragments	Primer	Sequences (5′ to 3′)
12SrRNA	SL29	cac tga aaa tgc tta gat gag cc
	SH428	act ttc gtt gtt tat ttt tgt ttg
	SL292	tag ggt tgg taa atc tcg tg
	SH756	cac tct atg ggc tac acc tt
	SL621	gag cct gtt ccg taa tcg ata
	SH1057	gta aat gaa atc tct tgg gtg taa
ND4	NL10056	tac cca ctt cac act atc at
	NH10454	tac ggg tta agg ttt ct
	NL10883	agt cct ggc agc tat tct ac
	NH11429	gcc tcg ttg ggt ggt tga t
D-loop	Thr265	cat gca tat aag cca gta
	Tdkd289	atg cat ggg gat aag gtt tt
Cytb	L15450	cca gac cta tta gga gac cca gac aac t
	H15565	cct ccg att cat gtg agt gtg tga gaa ga

in Wu et al. (2000, 2003). The different partial sequences of four sequence fragments were merged using the DNAstar package (DNASTAR, Inc.) under the Seqman active option. The sequences of the present study were aligned using Clustal W1.83 (Thompson et al., 1997). The alignment results were adjusted manually for obvious alignment errors. Cytb and ND4 genes were also translated into amino acid sequences to verify the alignments. 12S rRNA gene sequences were aligned based on published data. The control region sequences have many indels, but no repeat motif in this highly variable region. Sequences obtained in this study have been deposited in GenBank under accession control region numbers (AJ241540, AJ241609, AJ287968, AJ287969, AJ287977, AJ287978, AJ287979, AJ287980, AJ287981, AJ287982, AJ287984, AJ287985, AJ287986, and AY745088–AY745187); cytb (AJ279402, AJ279404, AJ279408, AJ279410, AJ279411, AJ279413–16, AJ279418–27, and AY745099–AY745120); ND4 (AY745121–AY745149); and 12S (AY745150–AY745187).

2.3. Phylogenetic analyses

First, we separately analyzed the four sequence fragments (D-loop, cytb, ND4, and 12S) from Chinese hare taxa using maximum parsimony (MP). For the sequence fragment combined analysis, we determined the incongruence length difference (ILD) test in PAUP v4.0b10 (Swofford, 2003) for assessing the incongruence between the sequence fragments (Farris et al., 1994, 1995). The combined sequence data set was analyzed using maximum parsimony (MP), maximum likelihood (ML) in PAUP v4.0b10 (Swofford, 2003), and Bayesian inference as implemented in MrBayes v3.04b (Huelsenbeck and Ronquist, 2001). Using the published cytb sequences

from GenBank (Alves et al., 2003; Arnason et al., 2002; Halanych et al., 1999; Halanych and Robinson, 1999; Koh et al., 2001; Matthee et al., 2004; Pierpaoli et al., 1999; Yamada et al., 2002, also see Appendix A), the combined data set of the genus *Lepus* from Worldwide was then analyzed by the above three phylogenetic methods.

Maximum parsimony analysis was performed with TBR branch swapping and 10 random taxon addition replicates under a heuristic search, saving no more than 100 equally parsimonious trees per replicate. PAUPRat (Sikes and Lewis, 2001) was used for MP analysis of the combined cytb data set because of its speed in searching large data sets. To estimate branch support on the recovered topology, non-parametric bootstrap (bt) values (Felsenstein, 1985) were assessed with PAUP 4.0b10 (Swofford, 2003). One thousand bootstrap pseudo-replicates were analyzed under a heuristic search with TBR branch swapping and 10 random taxon addition replicates.

Prior to the maximum likelihood phylogenetic analysis, Modeltest 3.06 was used to find the optimal model of DNA substitution (Posada and Crandall, 1998). However, according to Posada and Buckley's (2004) arguments, the Akaike information criterion (AIC; Akaike, 1974) is more advantageous than the hierarchical likelihood ratio test. Therefore, our phylogenetic reconstruction for maximum likelihood and Bayesian inference was based on the best-fit model, which was selected by AIC. Heuristic ML searches using TBR branch swapping (initial trees were obtained by NJ) were performed in PAUP 4.0b10 (Swofford, 2003) and PAUPRat (Sikes and Lewis, 2001). ML nodal support was estimated by using the non-parametric bootstrap (Felsenstein, 1985) and was restricted to 100 pseudo-replicates because of limited computing time.

Bayesian analyses began with random starting trees and ran for 1,000,000 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 'burn-in' generations (random points generated prior to stationarity) were defined according to the plot of an $x - y$ graph between generations and likelihood values, and then 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. Because these represent the true probabilities of the clades (Rannala and Yang, 1996), posterior probabilities greater or equal to 95% were considered significant (Leaché and Reeder, 2002). For non-parametric bootstrap analyses, 70% was used as the criterion for evidence of good support.

2.4. Historical biogeographic analyses

We used two different methods, ancestral area analysis of Bremer (1992, 1995) and the discovery-based Brook Parsimony analysis (BPA) (Brooks, 1981, 1990; Brooks and McLennan, 1991, 2001; Brooks et al., 2001), to infer the geographical location of the ancestral area and identify possible speciation, dispersal, and vicariant events in the evolutionary history of *Lepus*.

Ancestral area analyses use forward or reverse Camin–Sokal parsimony (Camin and Sokal, 1965) to optimize the area cladogram based on the phylogenetic trees. Then, the number of necessary gains and losses under the two optimizations were compared for estimating which areas were most likely parts of the ancestral area (Bremer, 1992, 1995).

The Brooks Parsimony analysis included two steps. The first step (Wiley, 1986, 1988a,b) assessed whether or not there is a single general area cladogram. The second step (Brooks, 1990) described exceptions to the general area cladogram that is capable of accounting for the complexity of speciation, dispersal, and extinction events in a historical biogeographic context. We used both methods in conjunction with Bayesian trees of the combined data sets because of the relatively high posterior probability supports. We partitioned four biogeographical regions, North America (NA), Europe (Eur), Asia (As), and Africa (Af) following the current distribution (excluding regions where some species are introduced) and fossil records (Dawson, 1981). Eight zoogeographical regions in China, Northern China (NC), Southern China (SC), Northeastern China (NeC), MengXin (MX), Southwestern China (SwC), Central China (CC), Qing-Tibet plateau (QT), and Hainan Island (HI) have been delineated according to Luo (1988) and Zhang (2002). We used ancestral area analysis to identify the geographic distribution of the ancestor of *Lepus* and their congers in China. Brooks Parsimony analysis was used to reconstruct the history of dispersal and vicariant events throughout the phylogeny of *Lepus* and Chinese congeners.

2.5. Molecular clock test and divergence time estimation

We compared log likelihood scores of trees constructed with and without the constraint of a molecular clock for the combined cytb data set with PAUP. A significant difference was observed between the likelihood scores of clock and non-clock trees, where p values were between 0.001 and 0.05. The divergence times between the major species groups were estimated through the Bayes

MCMC package, "Thornnian Time Traveller, (T3)" (ftp://abacus.gene.ucl.ac.uk/pub/T3) developed by Thorne et al. (1998), Kishino et al. (2001), Thorne and Kishino (2002), and Yang and Yoder (2003). Divergence time estimating are based on a probabilistic model that describes changes in evolutionary rate over time and involves the Markov chain Monte Carlo procedure to estimate the posterior distribution of rates and times. We followed the method of Rutschmann (2004), Hassanin and Douzery (2003), and Yang and Yoder (2003) for estimating divergence time. 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. To avoid the convergence of the MCMC algorithm, multiple independent runs were performed for the same data and same prior distributions, but with different starting points.

Calibration points were used for estimating divergence time. We used recent literature-based leporid genera divergence in the middle of Miocene from 12.08 to 17.48 MYA (Matthee et al., 2004), the absolute ages of geological periods, and chronostratigraphic references based upon the 2000 edition of the International Stratigraphic Chart (http://www.elasmo.com/refs/geotime.html). A fossil-based calibration point was from the occurrence of modern *Lepus* speciation on the North American, Europe, and Asian continents during the Pleistocene, 1.8–0.01 MYA (Dawson, 1981). The third time constraint is according to the earliest fossil record of *L. timidus* (Kurtén, 1968; Kurtén and Anderson, 1980) in Europe and North America. The last time constraint is fossil-based for modern *Lepus* species, *L. mandschuricus* in Middle Pleistocene, 0.75–0.125 MYA (Zhoukoudianian period, Tong et al., 1995) in China.

3. Results

3.1. Sequence statistical results

The complete cytb (1140 bp) and 12S (960 bp) rDNA gene sequences, and partial ND4 (524 bp) and control region (554 bp) sequences were determined, respectively, after removing ambiguous regions from the alignment. Several of the museum skin samples did not yield full-length sequences with our primers. Level of sequence variation based on uncorrected pairwise distance for the four sequence fragments were different from each other and summarized in Table 3, which were calculated using Mega3 (Kumar et al., 2004). Sequences divergence ranged from 2.3 to 16.4% in the control region, 3.5–12.7% in cytb, 0.2–4.5% in 12SrDNA, and 2.6–14.2% in ND4. Hainan hare (*L. hainanus*) had the smallest intraspecific sequence divergence for the four sequence fragments, respectively (0–0.5%). Manschurian hare and Manschurian black hare (*L. melainus*) were the least diverged species pair (0.2–2.6%) except for cytb sequence data. The smallest distance between *L. capensis* and *L. timidus* was observed for the cytb gene. The second least diverged species pair was the Chinese Cape hare (*L. capensis*) and Mountain hare (*L. timidus*). The sequence divergences ranged from 0.9 to 4.4%.

For the combined cytb data set from 24 morphological character-based *Lepus* species, the average maximum likelihood distance based on the selected optimal model is 10.7%. *L. arcticus* and *L. othus* had the least divergence (0.1%), while South Africa *Lepus* taxa and some of taxa from China had the greatest divergence, with a range of 11.4–28.6% (ML distance not shown). The nucleotide composition is typical for mammalian values (Irwin et al., 1991) with a low proportion of guanines (10.9–17.9%) in the overall composition.

3.2. Phylogeny overview

The incongruence length difference (ILD) tests for the four sequence fragments (D-loop, ND4, 12S, and cytb) and three regions combined (ND4, 12S, and cytb) showed a significant conflict between these fragments ($P = 0.001 < 0.05$). However, we still combined the four sequence fragments because of the following reasons: (1) more and more published studies show that multiple sequence data sets can be combined when incongruence is detected (Cunningham, 1997; Darlu and Lecointre, 2002; Xiao et al., 2005; Yoder et al., 2001). (2) Four sequence fragments were linked on a single mtDNA molecule. Therefore, the phylogenetic relationships among Chinese hare taxa were based first on single-sequence fragment analysis. Although the maximum parsimony trees were different from each other, all four sequence fragments consistently supported sister relationships between *L. comus* and *L. oiostolus* (topologies not shown).

We combined the four sequence fragments for inferring the phylogenetic relationships among the nine Chinese hare species. Maximum parsimony analysis recovered four trees with the 1953 tree length for the combined data set of four mitochondrial sequence fragments. Fig. 2 illustrates a 50% majority rule consensus tree with bootstrap values and posterior probabilities above or under the branch. Maximum likelihood and Bayesian analysis yielded the same topology (Fig. 2). The best model for maximum likelihood analysis in the combined data set is "TrN + I + G" with the following parameter setting: Base = (0.3156 0.2840 0.1345 0.2659), Nst = 6, Rmat = (1.0000 8.7249 1.0000 1.0000 10.7290), Rates = gamma Shape = 0.4719, and Pinvar = 0.3981. A single tree ($-\ln L = 13892.258$) was recovered with a topology similar to the MP analysis with only the species group, *L. comus* and *L. oiostolus*, which exchanged the position with *L. hainanus.* Nine morphological, character-based, Chinese hare species

Table 3
Uncorrected pairwise distance (P-distance) among the nine Chinese hare species based on the four sequence fragments separately

Taxa	1	2	3	4	5	6	7	8	9
L. capensis	**0.03** (CR)								
	0.009 (Cytb)								
	0.002 (12S)								
	0.006 (ND4)								
L. mandschuricus	0.094	**0.029**							
	0.097	**0.072**							
	0.009	**0.004**							
	0.138	**0.017**							
L. sinensis	0.122	0.106	**0.008**						
	0.097	0.106	**0.019**						
	0.022	0.02	**0**						
	0.081	0.115	**0.006**						
L. melainus	0.1	0.023	0.112	**0.006**					
	0.126	0.048	0.117	**0.007**					
	0.007	0.002	0.016	**0**					
	0.14	0.026	0.116	**0.033**					
L. timidus	0.097	0.045	0.102	0.042	**0.056**				
	0.035	0.089	0.089	0.119	**0.028**				
	0.009	0.005	0.02	0.003	**0.006**				
	0.044	0.114	0.074	0.119	**0.044**				
L.comus	0.152	0.14	0.143	0.138	0.132	**0.051**			
	0.084	0.113	0.102	0.126	0.091	**0.03**			
	0.026	0.023	0.027	0.02	0.023	**0.005**			
	0.085	0.133	0.083	0.139	0.082	**0.021**			
L. oiostolus	0.12	0.103	0.124	0.111	0.099	0.101	**0.042**		
	0.074	0.103	0.082	0.117	0.073	0.058	**0.008**		
	0.028	0.027	0.028	0.023	0.026	0.019	**0.001**		
	0.079	0.113	0.067	0.116	0.076	0.05	**0.01**		
L. hainanus	0.164	0.145	0.139	0.139	0.134	0.136	0.141	**0.001**	
	0.096	0.119	0.086	0.127	0.099	0.097	0.081	**0.002**	
	0.04	0.038	0.041	0.036	0.037	0.034	0.039	**0**	
	0.089	0.115	0.08	0.119	0.078	0.09	0.075	**0.005**	
L. yarkandensis	0.141	0.133	0.137	0.134	0.128	0.136	0.139	0.15	**0.059**
	0.091	0.079	0.091	0.075	0.085	0.094	0.076	0.101	**0.023**
	0.028	0.027	0.033	0.026	0.027	0.031	0.036	0.045	**0.003**
	0.127	0.077	0.122	0.081	0.119	0.142	0.124	0.125	**0.078**

Note. The bold values on the diagonal indicated sequence diversity within species. Values on the first line were the distance from the control region sequences (CR), the second for cytb (cytb), the third for 12S rDNA (12S), and the fourth for ND4 sequences (ND4).

fell into three clades: *L. comus* and *L. oiostolus* formed clade A with high bootstrap support (0.88 for BI, 100% for MP and ML). Clade B comprises six species: *L. sinensis*, *L. timidus*, *L. capensis*, *L. melainus*, *L. yarkdensis*, and *L. mandschus*. Clade B could possibly be separated into two subclades. *L. sinensis* would represent the first subclade, and the other five species the second subclade. However, the nodal support for such subclade division is weak (0.54 for BI, ML, and MP bootstrap values <55%). *L. hainanus* formed clade C with high nodal support (1.00 for BI, 100% for MP and ML, Fig. 2). The species that were geographically adjacent to each other were the most phylogenetically related except for *L. yarkdensis* that has a closer relationship with *L. melainus* and *L. mandschus*, albeit a farther geographical distance.

The combined cytb data set included 146 sequences that belong to nine genera of the family Leporidae; 24 morphological character-based species were included in the genus *Lepus* (Table 1 and Appendix A). A best-fit model (TrN + I + G) selected by AIC in Modeltest Version 3.06 are as follows: base frequencies (0.3054 0.3634 0.0924 0.2388), proportion of invariable sites = 0.4374, and γ distribution shape parameter = 0.8964. Three phylogenetic methods gave similar tree topologies (Fig. 3). All *Lepus* taxa fell into three species groups that correspond to their geographical distribution, namely, North American, Eurasian, and African. The North American group included four species, *L. americanus*, *L. callotis*, *L. alleni*, and *L. californicus*, and consisted of the earliest offshoot in ML and Bayesian analysis,

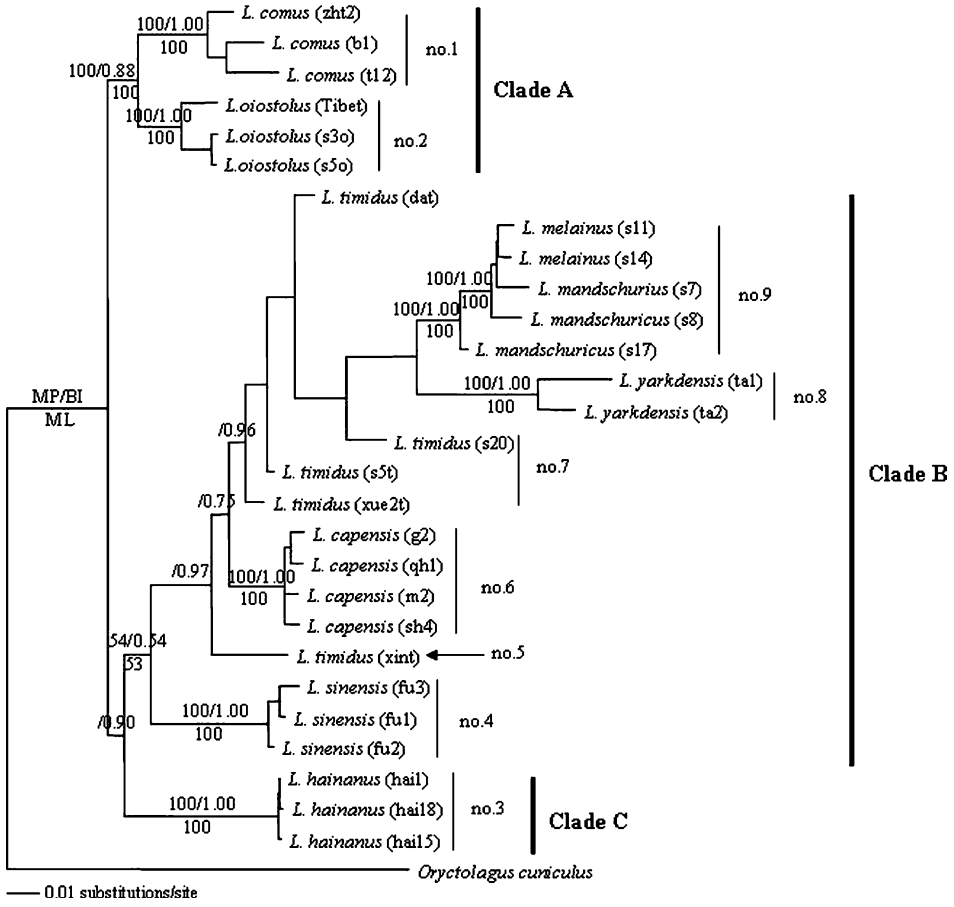

Fig. 2. Fifty percent majority consensus tree of the four gene fragments combined based on Bayesian inference. Phylogenetic analyses are rooted with *Oryctolagus cuniculus*. The branch lengths are shown to scale. A maximum parsimony 50% majority tree is from four trees with 1959 steps (consistency index = 0.5641, retention index = 0.7458, rescaled consistency index = 0.4207). Bootstrap values (1000 replicates) are shown above the branches (MP). Bayesian analysis is based on the 100,0000 generations replicates, with the posterior possibilities indicated above the branch (BI). Maximum-likelihood tree is recovered ($-\ln L = 13582.25882$) using Likelihood settings from best-fit model (TrN + I + G) selected by hLRT in Modeltest Version 3.06 and are as follows: Base frequencies (A = 0.3156, C = 0.2840, G = 0.1345), Proportion of invariable sites = 0.3981, γ distribution shape parameter = 0.4719. Maximum-likelihood bootstrap values are based on 100 replicates because of computation time are shown under the branches (ML). The values with a line following taxa correspond to the species number in Fig. 4A.

but not in the MP reconstruction which split North American species group into two clades with relatively high node support at the very basal position of the genus (84%). The Africa species group includes two *Lepus* species from South Africa, *L. capensis* and *L. saxatilis* with high posterior probability (0.98) in BI. This group is the second earliest offshoot. The Eurasian species group is the largest clade and includes all hare species from Asia and Europe (Fig. 3). Three subclades are recognized in this group. The Japanese hare (*L. brachyurus*) is the earliest subclade (I). *L. hainanus* and *L. sinensis* from China, *L. granatensis* and *L. europaeus* from Europe form subclade II. *L. hainanus* is sister to *L. europaeus*, and *L. granatensis* is also sister to these two species. The placement of *L. sinensis* and *L. granatensis*, however, were different in the ML and MP analyses. Subclade III consists of two sister Chinese species, *L. comus* and *L. oiostolus* with relatively high bootstrap support (81% in MP and ML) and posterior probability (1.00 in BI), and the *timidus* group classified by Tate (1947). The *timidus* group consists of three Chinese species *L. yarkandensis*, *L. mandschuricus*, and *L. melainus*; *L. timidus* from wider geographical regions of Asia and Europe; two European species *L. castroviejoi* and *L. corsicanus*; *L. coreanus* from Korea; and *L. othus*, *L. townsendii*, and *L. arcticus* from North America. However, MP and ML analyses place more species in this subclade. Overall, the three phylogenetic methods used in this study strongly support the genus *Lepus* as monophyletic in origin.

3.3. Biogeography

Reconstructing the distributional history of *Lepus* showed that this genus originated in North America or Asia. Interestingly, when we considered Asia as three regions (China, Japan, and Korea), the results strongly support a North American origin.

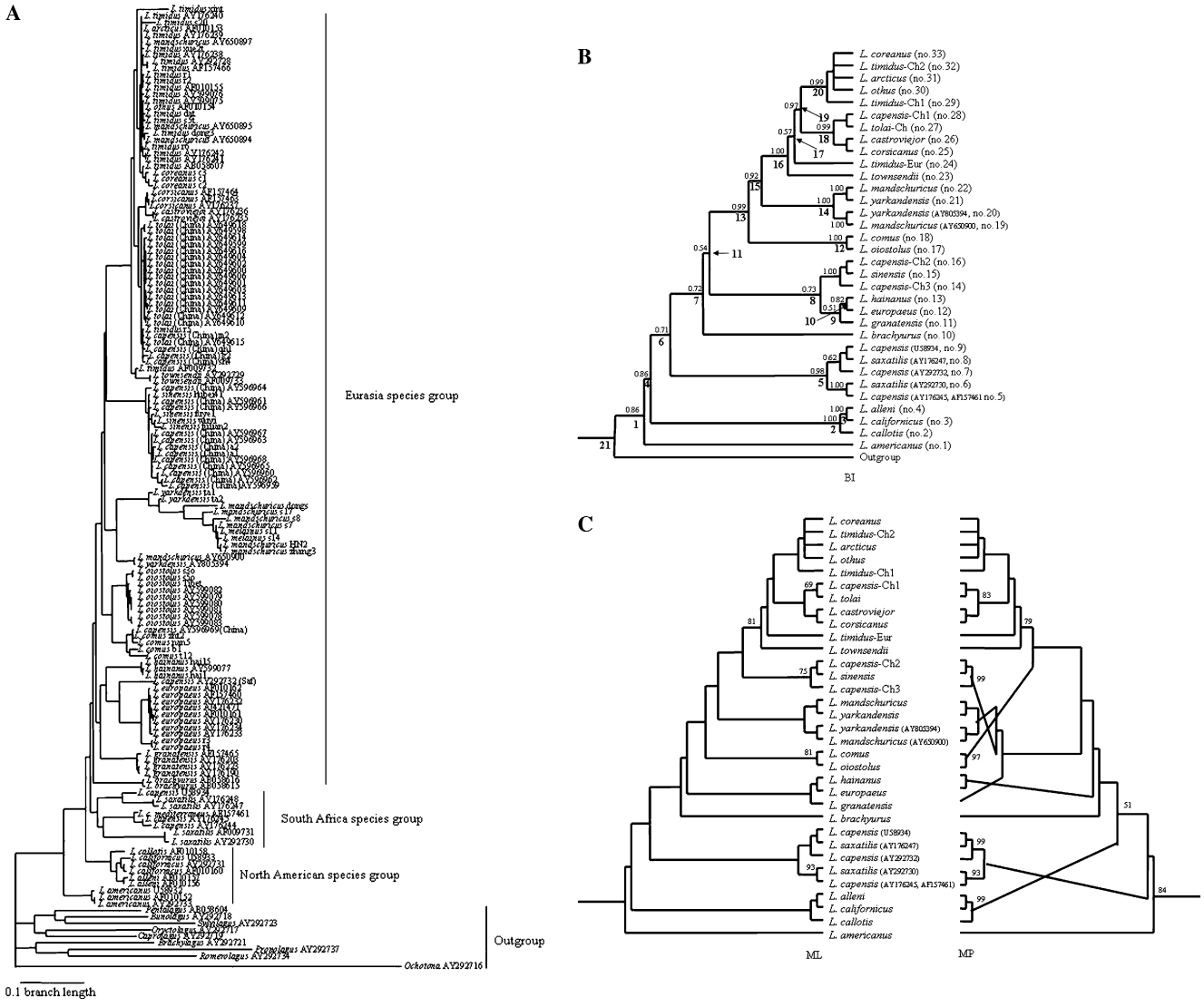

Fig. 3. Combined analyses of cytb data set. (A) The 50% majority consensus tree is recovered from Bayesian inference with maximum likelihood setting under the GTR + I + G model for DNA substitution. (B) The simply BI tree inferred from the combined analyses of cytb data. The pP is shown above every branch on this tree. Bold numbers under the branch are corresponding to those in Table 4. The numbers in the bracket are indicated following taxa corresponding to the species number in Fig. 4B. (C) The simply MP and ML trees. Bootstrap values (1000 replicates) are shown above the branches.

Primary BPA analyses indicate that hare speciation in China resulted from peripheral isolation and vicariance with post-speciation dispersal. Four peripheral isolations [*L. comus* (no. 1), *L. oiostolus* (no. 2), *L. hainanus* (no. 3), and *L. sinenesis* (no. 4)] and one vicariance with four post-speciation dispersals occurring during the evolutionary history of the Chinese hare (Fig. 4A).

Primary BPA for the combined cytb data set produce polytomy area cladograms and support a vicariant relationship between North American, European, Asian, and African hares. This led us to duplicate these areas for secondary BPA (Brooks, 1990; Brooks and McLennan, 1991; Brooks et al., 2001). Secondary BPA using PAUPv4.0b10 produces one parsimonious area cladogram with a CI of 100%. Vicariant events with subsequent dispersal into four continents by the ancestor of *Lepus* explain the current distribution of *Lepus*. The analyses of the combined cytb infers that both dispersal and vicariance played important roles in the early diversification of *Lepus*. At the same time, the Bering land bridge also contributed to the evolutionary history of *Lepus*. Secondary BPA indicates peripheral isolates and vicariance with post-speciation dispersal were major factors in the distribution of *Lepus* (Fig. 4B).

3.4. Dating main phylogenetic events

We used the Bayesian topology of average-branch-length consensus reconstructed from the combined data set of cytb for dating phylogenetic events because of the

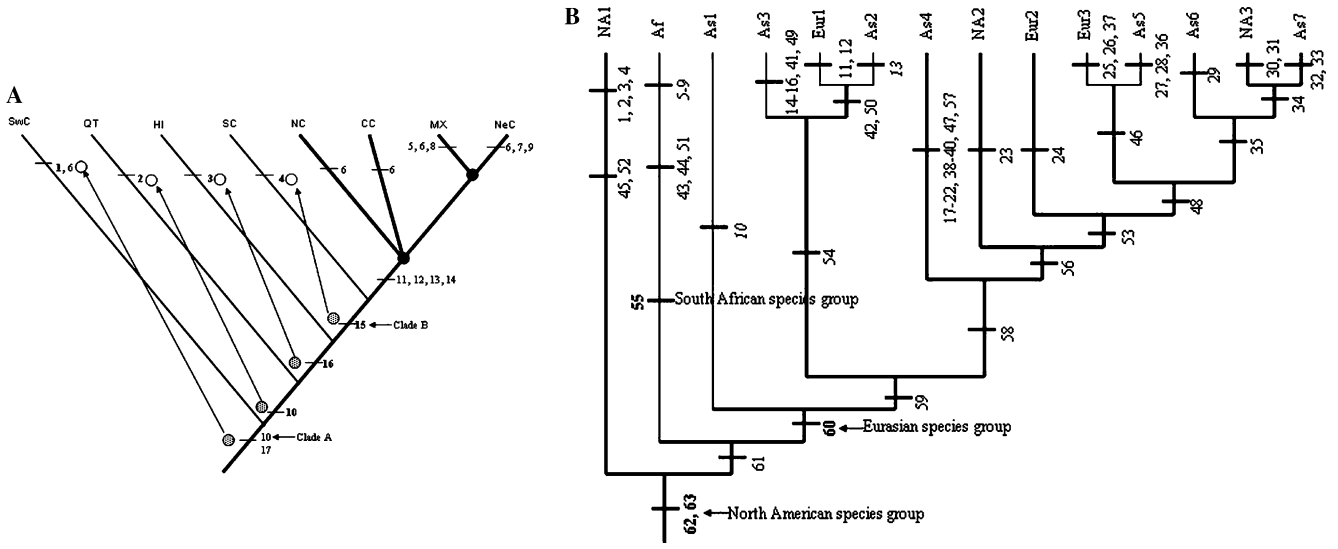

Fig. 4. (A) Primary BPA area cladogram outlines the speciation picture of the Chinese hare. Bold lines indicate the backbone of vicariant speciation. Solid circle, vicariance. Spotted circle (ancestor)+open circle (peripheral isolate), instance of peripheral isolates speciation. Numbers accompanying slash marks refer to species (from Fig. 2). SwC, Southwestern China; QT, Qing-Tibet Plateau; HI, Hainan Island; SC, Southern China; NC, Northern China; CC, Central China; MX, NeiMeng-XinJiang and NeC, Northeastern China. (B) Secondary BPA area cladogram of the genus *Lepus* based on the combined cytb data. Slim lines indicate the backbone of vicariant speciation. Italic bold number indicate peripheral isolates speciation from ancestor. Numbers accompanying slash marks refer to species (from Fig. 3B). NA, North America; As, Asia (in this study, Asia includes Japan, Korea and China); Eur, Europe; Af, South Africa.

relatively well-resolved phylogenetic relationships. Applying several time calibrations based on the fossil record and recovered topology, the origin of *Lepus* dates to10.76 MYA (±0.86 MYA, Table 4). We also compared the divergence time by estimating with and without the genus divergence time calibration. The results were no obvious differences. The comparison only widened the range of divergence time. As emphasized by

Table 4
Bayes estimates of divergence times with 95% credibility intervals

Node	Divergence time without genus time constraint						Divergence time with genus time constraint					
	Prior divergence time			Posterior divergence time			Prior divergence time			Posterior divergence time		
	Date	SD	95% CI	Date	SD	95% CI	Date	SD	95% CI	Date	SD	95% CI
1	2.515	0.761	(1.486, 4.395)	3.287	1.051	(1.916, 5.933)	4.002	0.818	(2.418, 5.702)	5.649	1.152	(3.504, 8.097)
2	0.793	0.335	(0.334, 1.613)	1.036	0.452	(0.436, 2.150)	1.321	0.476	(0.519, 2.397)	1.865	0.672	(0.751, 3.415)
3	0.486	0.226	(0.178, 1.039)	0.635	0.303	(0.232, 1.391)	0.794	0.331	(0.283, 1.588)	1.121	0.469	(0.406, 2.246)
4	1.766	0.688	(0.932, 3.667)	2.31	0.949	(1.222, 5.030)	3.111	0.905	(1.311, 4.799)	4.391	1.279	(1.869, 6.787)
5	1.377	0.594	(0.686, 3.089)	1.802	0.818	(0.898, 4.185)	2.493	0.816	(0.963, 4.022)	3.518	1.153	(1.369, 5.697)
6	1.584	0.654	(0.828, 3.457)	2.072	0.902	(1.077, 4.725)	2.851	0.882	(1.139, 4.444)	4.024	1.247	(1.624, 6.301)
7	1.408	0.599	(0.732, 3.157)	1.843	0.826	(0.958, 4.294)	2.567	0.825	(0.99, 4.032)	3.623	1.167	(1.406, 5.721)
8	1.203	0.53	(0.613, 2.782)	1.575	0.731	(0.802, 3.797)	2.213	0.743	(0.82, 3.548)	3.124	1.05	(1.178, 5.04)
9	1.109	0.497	(0.554, 2.601)	1.451	0.684	(0.726, 3.513)	2.043	0.7	(0.752, 3.324)	2.884	0.99	(1.074, 4.712)
10	0.94	0.433	(0.454, 2.222)	1.231	0.595	(0.592, 3.004)	1.733	0.62	(0.612, 2.925)	2.446	0.877	(0.881, 4.172)
11	1.293	0.563	(0.675, 2.971)	1.693	0.775	(0.877, 4.030)	2.377	0.787	(0.89, 3.788)	3.355	1.113	(1.276, 5.373)
12	0.71	0.32	(0.340, 1.629)	0.928	0.439	(0.445, 2.188)	1.296	0.473	(0.464, 2.253)	1.83	0.669	(0.659, 3.182)
13	1.017	0.456	(0.527, 2.353)	1.331	0.627	(0.690, 3.220)	1.887	0.662	(0.679, 3.122)	2.663	0.936	(0.974, 4.445)
14	0.698	0.334	(0.336, 1.684)	0.914	0.457	(0.442, 2.298)	1.297	0.499	(0.438, 2.292)	1.83	0.706	(0.628, 3.258)
15	0.856	0.401	(0.440, 2.058)	1.121	0.551	(0.577, 2.797)	1.605	0.594	(0.556, 2.714)	2.266	0.841	(0.794, 3.886)
16	0.096	0.007	(0.078, 0.106)	0.124	0.005	(0.113, 0.130)	0.088	0.009	(0.071, 0.104)	0.123	0.005	(0.112, 0.13)
17	0.088	0.01	(0.066, 0.103)	0.114	0.01	(0.089, 0.128)	0.079	0.011	(0.055, 0.099)	0.111	0.012	(0.08, 0.128)
18	0.065	0.015	(0.033, 0.090)	0.084	0.019	(0.044, 0.115)	0.057	0.016	(0.023, 0.085)	0.08	0.021	(0.032, 0.115)
19	0.08	0.012	(0.053, 0.099)	0.103	0.013	(0.073, 0.124)	0.07	0.014	(0.04, 0.094)	0.099	0.017	(0.058, 0.123)
20	0.065	0.016	(0.031, 0.091)	0.085	0.019	(0.042, 0.116)	0.054	0.018	(0.016, 0.085)	0.076	0.024	(0.022, 0.114)
21	4.965	1.22	(2.986, 7.760)	6.488	1.72	(3.857, 10.544)	7.624	0.679	(6.279, 8.943)	10.76	0.86	(9.82, 13.031)

Node numbers correspond to those in Fig. 3B. The time unit is million of years. The standard deviation (SD) is given for each node.

Hassanin and Douzery (2003) and Matthee et al. (2004), time constraints are very important for obtaining divergence time ranges.

4. Discussion

4.1. Taxonomic consideration in the genus Lepus

To define species or subspecies in the genus *Lepus*, we used the concept of DNA-based taxonomy (Blaxter, 2004; Tautz et al., 2002, 2003). We also followed the premise of Johns and Avise (1998) and Castresana (2001) that expected levels of divergence should be quantified at all taxonomic levels. However, DNA-based taxonomy is still contentiously debated, particularly on "whether one gene fits all" (Moritz and Cicero, 2004). Therefore, we approached *Lepus* taxonomy with the idea that systematic clarification needs to integrate molecular with traditional morphological characters. All phylogenetic analyses, either different data sets or different phylogenetic methods, strongly supported sister relationships between *L. comus* and *L. oiostolus* with high node support (Figs. 2 and 3). The well-supported sibling species have a ML sequence divergence of 5.14–7.93% (mean 6.75%). These values were used as a measure for interspecies differentiation.

In our first attempt at resolving some of the inconsistancies in *Lepus* taxonomy, we looked at various *Lepus* taxa in the Eurasian species group. The taxonomic classification of *L. timidus*, *L. arcticus*, and *L. othus* has been a source of considerable debate for a long time (Baker et al., 1983). Corbet (1978) listed *L. arcticus* and *L. othus* as a subspecies of the Eurasian *L. timidus*. Dixon et al. (1983) tentatively agreed, but Hall (1981) and Jones et al. (1986) continues to recognize the specific distinction of *L. arcticus* and *L. othus*. In this study, the phylogenetic results link the two taxa with the Eurasian *L. timidus* group with high bootstrap values (84% in MP) and strong posterior probability for BI (1.00). The ML distance between the two taxa and the Eurasian *L. timidus* group is 0.007. Our results strongly suggest that the two taxa should be a single circumpolar species and recognized as *L. timidus* (Halanych et al., 1999).

The genetic distances and branching patterns of the phylogenetic trees confirm that the Japanese hare *L. brachyurus* is a distinct species differing from the other Eurasian *Lepus* species by 10.8–13.8% sequence divergence. One Chinese hare species, *L. mandschuricus*, was once recognized as a subspecies of the Japanese hare, although we could not establish a close relationship with the phylogenetic conclusions of Ellerman and Morrison-Scott (1951), Angermann (1966, 1983), Corbet (1978), Luo (1988), Flux and Angermann (1990), and Hoffmann (1993). We do support the phylogenic approach of Yamada et al. (2002) that *L. brachyurus* should be considered a distinct and separate species.

For a number of years taxonomists have treated *L. europaeus* as a subspecies of *L. capensis*, thereby giving *capensis* one of the largest mammalian ranges (Flux and Angermann, 1990). However, more recent opinions have shifted and they now are regarded as two separate species (Angermann, 1983; Corbet, 1986; Corbet and Hill, 1986; Meester et al., 1986). Our results from ML distance comparison, strongly support two distinct species. Based upon morphological character comparisons, *L. granatensis* is a species distinct from *L. capensis*, *L. castroviejoi*, and *L. europaeus* (Bonhomme et al., 1986; Palacios, 1976, 1983). Corbet (1986) has tentatively accepted this taxonomic arrangement, but other authorities (Angermann, 1983; Corbet and Hill, 1986; Flux, 1983; Schneider and Leipoldt, 1983) do not considered *castroviejoi* or *granatensis* to be more than a subspecies. The Italian hare (*L. corsicanus*) was initially considered a separate species by Winton (1898), then, was included in *L. europaeus* (Ellerman and Morrison-Scott, 1951; Flux and Angermann, 1990; Wilson and Reeder, 1993). However, based on recent morphological and molecular findings *L. corsicanus* should be considered as a separate species (Palacios, 1996; Pierpaoli et al., 1999; Riga et al., 2001). Considering the genetic distance and the phylogenetic relationships between *L. europaeus* and *L. granatensis* (10.4%), we recommend they be recognized as separate species. *L. corsicanus* from Italy and *L. castroviejoi* from Northwest Spain have a sister relationship, but are still included in the *timidus* group. The ML distance between the two taxa, and between them and the other *timidus* group is 0.013 and 0.023–0.034, respectively. We suggest that the two taxa be considered as *L. timidus* or as two subspecies of *L. timidus*, which is contrary to the phylogenetic conclusions of Alves et al. (2003) and Pierpaoli et al. (1999) where they are treated as distinct species.

The Korean hare (*L. coreanus*) is recognized as a distinct species (Hoffmann, 1993; Jones and Johnson, 1965; Thomas, 1906) and has supported by the molecular study of Koh et al. (2001). Ellerman and Morrison-Scott (1951) and Corbet (1978), however, consider the Korean hare as a subspecies of the Chinese hare (*L. sinensis*). Our phylogenetic analyses include five species from eastern Asia near the Korean Peninsula and the results indicate that *L. coreanus* has close affinity with *L. timidus* from Japan. The genetic distance between *coreanus* and other *timidus* taxa range from 0.018 to 0.041. Our analyses do not support the classification of Koh et al. (2001), and therefore we suggest that *L. coreanus* is neither a subspecies of *L. sinensis* nor a valid species, and as a matter of fact we consider *L. coreanus* as *L. timidus*.

We do not consider the nine Chinese hare species recovered as a monophyletic. The phylogenetic relation-

ships among the various hare taxa were not largely consistent with the morphological studies of Luo (1988). The Manchurian hare (*L. mandschuricus*) has an uncertain taxonomic assignment. Sowerby (1923, 1933) and Loukashkin (1943) recognized it as a separate species, while Ellerman and Morrison-Scott (1951) considered it as a subspecies of the Japanese hare because of similarities in teeth characters and cranial measurements. Given the inferred phylogenetic relationships and the mean ML distance between the Manchurian hare and Japanese hare (0.197), we support species status. Our data also indicates that the Manchurian hare has a close phylogenetic relationship with the Yarkand hare (*L. yarkandensis*). The Manchurian black hare (*L. melainus*) has been cited in the literature as a new species based upon the morphological characters of having a long tail, short ears and its entire body covered with black fur (Li and Luo, 1979; Luo, 1981). Not surprising, our phylogenetic analyses show that the Manchurian black hare commingled with the black type of Manchurian hare and Manchurian hare, and formed a clade with 100% bootstrap values and 1.00 posterior probability support. The mean sequence divergence between the Manchurian hare and Manchurian black hare is only 0.03, which is within the levels of species divergence and is far below the extent of interspecies divergence. We suggest that the three forms be treated as a single species. Whether they be considered a valid subspecies or not will require additional sampling.

The Chinese Cape hare (*L. capensis* or *L. tolai*) is most closely allied with the *timidus* group or *L. sinensis* with strong support (100% for MP and ML, 1.00 for BI). The mean ML distance between some of *L. capensis* or *L. tolai* in China and the *timidus* group is 0.02–0.041. The values fall within the realm of species divergence, as well as between some of *L. capensis* taxa in China and *L. sinensis* values (0–0.031). Based on distance comparisons and phylogenetic relationships, we conclude that the Cape hare (*L. capensis* or *L. tolai*) does not exist in China as a unique taxon, or that the Cape hare has been replaced by *L. timidus* during evolutionary processes. Another plausible conclusion is that *L. sinensis* might have wider geographical distribution than previous thought. This is an area where more samples over vaster geographical localities need to be collected before a definitive conclusion can be made.

In addition, the results from the ML distance comparisons hint that there might be two or more new hare species in the Chinese Xinjiang Prov. Here again, to delineating new species, adequate sampling must be followed with appropriate molecular markers and reliable morphometric characters.

The ML distance among North American hare taxa, (*L. californicus*, *L. alleni*, and *L. callotis*) range from 0.028 to 0.041. Whether or not the hares represent three distinct species will require further sampling combined with studies using traditional morphological characters (Halanych et al., 1999). When comparing the ML distance (0.038–0.044) between *L. townsendii* and other arctic species, our results are consistent with the morphological and molecular studies of Gureev (1964) and Halanych et al. (1999), which suggest that *L. townsendii* belongs to the arctic species and should be considered *L. timidus*.

The geographically adjacent African samples were genetically similar. The genetic distance among the African taxa in this study ranged from 8.76 to 15.07%. The mean ML distance between *L. capensis* from South Africa and *L. capensis mediterraneus* from Sardinian and Moroccan is 10.5%, and the range of distance is consistent with the findings of Palacios (1989) and Alves et al. (2003). The genetic distance between *L. capensis* (U58934) and *L. saxatilis* (AF009731 and AY292730) from South Africa is 9.96%, and 12.71% between *L. saxatilis* from South Africa and *L. saxatilis* from Mozambique. A strong case can be made for treating the two taxa as individual species. Given a 6.64% distance divergence between *L. capensis* (U58934) from South Africa and *L. saxatilis* from Mozambique, and that a subclade was recovered in the phylogenetic analyses, we suggest that *L. saxatilis* from Mozambique may very well be sister to *L. capensis* (U58934) from South Africa.

4.2. Lepus evolutionary biogeography

The earliest lagomorphs have been found in central Asia (Dawson, 1981), and early leporids were widespread in both the Old and New Worlds before *Lepus* evolved. Based on the fossil record, Hibbard (1963) suggested a North America origin for *Lepus*, and from there radiated to other continents. Halanych and Robinson (1999) suggested that *Lepus* experienced rapid radiation during speciation based on mtDNA analysis using characters of short branch lengths and basal clades with low bootstrap support. Matthee et al. (2004) suggested that all modern leporid genera occurred during the Miocene (between 14 and 8 MYA). The theory that ancestral *Lepus* dispersed from North America through Asia into Africa is more recent (Matthee et al., 2004). Based upon our analysis of geographic distributions among *Lepus* species, e.g., the ancestral area analysis (Bremer, 1992, 1995), we support the theory that ancestral *Lepus* is of North America origin. The oldest nodes in our molecular phylogeny are North American, which infers that the oldest speciation events took place in North America. Our molecular clock calibration dates speciation events at approximately 5.65 MYA (Table 4). There were, however, many other subsequent speciation events occurring outside of North America giving rise to various extent populations of leporids. Our histori-

cal biogeographic analyses suggest that the presence of *Lepus* species outside of the origin area is the result of dispersal followed by subsequent speciation within focal areas (Fig. 4B). Our results are consistent with fossil records and the recent molecular conclusions of Halanych and Robinson (1999). Moreover, North America as the center for early *Lepus* speciation agrees with the timing of geologic events. The relaxed molecular clock based on the Bayesian method indicates that species divergence within *Lepus* occurred as early as 5.65 MYA, with radiation and speciation occurring about 1.45–4.02 MYA (Table 4), a conclusion previously reached by Yamada et al. (2002). Most speciation events in *Lepus* occurred during the early Pliocene and extended through the Pleistocene. This was at a time when the Bering land bridge connected North America with Asia during two time periods. According to Marincovich and Gladenkov's (1999), the initial opening of the Bering Strait occurred during Late Miocene (11.2–7.1 MYA) or at the earliest Pliocene epochs (5.3–3.6 MYA). The land bridge provided adequate opportunities for dispersal and subsequent speciation (Austin et al., 2003). Even though the timing of this event is debatable, it most likely happened during the Miocene with periodic fluctuations in sea levels resulting in the opening and closing of the Bering Strait (Sher, 1999). The second connection between the North America and Asia through the Strait occurred during the Pleistocene (1.5–1.0 MYA, Austin et al., 2003). Divergence leading to speciation in *Lepus* is well within the realm of time of the Bering Strait closing.

Due to the lack of chromosomal diversity within *Lepus* (Azzaroli Puccetti et al., 1996; Robinson et al., 1983) and hybridization between more distantly related species, Halanych et al. (1999) concluded that isolation mechanisms (geographic, behavioral or ecological) contributed to *Lepus* speciation. Yamada et al. (2002) concluded that speciation within *Lepus* occurred in the early Pliocene (4–5 MYA) when taking into account that the split within *Lepus* occurred 12–16 MYA. The secondary BPA results (Fig. 4B) indicate that the pattern of speciation was the results of vicariance and peripheral isolation. Because of vicariant events, at least two African species (*L. saxatilis* and *L. capensis*) occurred about 3.52 MYA (range 1.37–5.7 MYA). Therefore, we suggest that the wide geographical distribution of *Lepus* is the result of subsequent dispersals that eventually lead to the evolution of various adaptations upon isolation. North American and Asian *Lepus* taxa had several opportunities to come into contact and genetically mix through the Bering land bridge. The dispersal route for biotic exchanges was eventually interrupted in the Late Pleistocene-Holocene (0.01 MYA), following the retreat of large ice sheets (Austin et al., 2003). The secondary BPA infers that *Lepus* taxa exchange between North America and Asia through the second terrestrial connection mainly occurred within the *timidus* group. This conclusion is in agreement with Halanych et al. (1999) that "some *Lepus* species invaded North America because of a secondary interchange among continents."

Ancestral *Lepus* dispersed onto the European and Asian continents about 4.02 MYA (± 1.25 MYA). In Japan, *L. brachyurus* arose from post-speciation dispersal events before 3.62 MYA (± 1.17 MYA). This is in agreement with the conclusion of Yamada et al. (2002) that the ancestor of *L. brachyurus* dispersed into Japan during the Pliocene. The conclusion is also supported by the phylogenetic tree topologies in this study (Fig. 3), where *L. brachyurus* is the earliest offshoot in the Eurasian species group. Speciation of Chinese *Lepus* resulted from post-speciation dispersal and peripheral isolate speciation with the uplifting of the Tibet Plateau (Dong et al., 1995) and the isolation of Hainan Island from the mainland (Fig. 4A). According to geologic evidence, the Hainan Island did not join with the mainland until the early Pleistocene (1.8 MYA), although speciation of *L. hainanus* had already occurred by ~2.45 MYA (± 0.88 MYA). Since speciation had occurred before isolation of the Hainan Island from the mainland, and based upon the results of the secondary BPA, *L. hainanus* appears to have arisen from post-speciation dispersal events and not from isolation, i.e., vicariance. In contrast, the speciation of *L. yarkandensis* is the result of peripheral isolate speciation about 0.64 MYA (± 0.26 MYA). During that time there was the uplifting of the Qing-Tibet Plateau (first time occurred 3.4 MYA and the second 2.5 MYA) in Northern China contributing to the formation of natural geographical barriers and alterations in the environment. Consequently, morphological diversification resulted from strong environmental selective pressures that gave rise to the sibling species *L. comus* and *L. oiostolus* in Southwestern China about 2.66 MYA (± 0.94 MYA). On the European continent, post-speciation dispersal events gave rise to *L. europaeus* and *L. granatensis* about 2.45 MYA (± 0.88 MYA) and 2.88 MYA (± 0.99 MYA), respectively. The *timidus* group has a more complex speciation pattern due to various dispersal events across the Bering land bridge during the Pleistocene. The estimated divergence time of 0.12 MYA and secondary BPA for the *timidus* group are in agreement with the land-bridge event.

5. Conclusions

This study confirms that *Lepus* is a monophyletic genus comprising three species groups and that the ancestral stock is of North America origin. Speciation events in *Lepus* were the result of post-speciation dispersal and peripheral isolate speciation. *Lepus* experi-

enced a relatively rapid radiation into Asia, Europe, and Africa resulting in *L. saxatilis* and *L. capensis* in Africa and *L. brachyurus* in Asia. The current widespread distribution of *L. capensis* in China is due to misinformation and taxonomic errors and neither to an exceptional dispersal pattern, nor to a broad adaptation to varying environments. However, to unequivocally decipher the relationships between all *Lepus* taxa with currently recognized species names, this study should be considered as the beginning point for a much larger and more comprehensive investigation. Such an undertaking would necessitate collecting larger numbers of specimens for morphological and genetic analysis.

The mtDNA sequence data set suggest that the number of species currently recognized is a gross overestimate of actual number of extent species. Many currently named taxa are not valid species. Furthermore, assigning definitive diagnostic morphological characters to the current species of *Lepus* has been compromised by the fact that many of the currently recognized species actually represent more than one species or subspecies.

Acknowledgments

The authors are grateful to the individuals and institutions that provided DNA samples (including, Xiang Yang, Aihua Liu, Ruifang Wang, Dingqi Rao), and Ms. Shifang Wu, Shikang Gou, and Chunling Zhu, for their expert technical assistance. We sincerely give thanks to the two anonymous reviewers who offered very useful suggestions, which greatly improved the content of the manuscript. We give thanks to Dr. Thorne, J.L. and Prof. Yang, Z. for their kind help on the divergence time estimating. We thank Drs. Ryder and Gagneux for their comments on the original manuscript. This work was supported by the State Key Basic Research and Development Plan (2003CB415105), the Chinese Academy of Sciences, the program for Key International S and T Cooperation Project of China (2001CB711103), Natural Science Foundation of Yunnan Province, and National Natural Science Foundation of China to Y.P.Z.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2005.05.006.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19, 716–723.

Allen, G.M., 1938. The Mammals of China and Mongolia, Natural History of Central Asia, vol. 10 (in two parts). New York, Am. Mus. Nat. Hist. (Pt. 1), pp.1–660.

Alves, P.C., Ferrand, N., Suchentrunk, F., Harris, D.J., 2003. Ancient introgression of *Lepus* timidus mtDNA into *L. granatensis* and *L. europaeus* in the Iberian Peninsula. Mol. Phylogenet. Evol. 27, 70–80.

Angermann, R., 1966. Beitr ge zur Kenntnis der Gattung *Lepus* (Lagomorpha, Leporidae). I. Abgrenzung der Gattung *Lepus*. II. Der taxonomische Status von *Lepus* brachyurus Temminck und Lepus mandshuricus Radde. Mitteilungen aus dem Zoologische Museum in Berlin 42, 127–335.

Angermann, R., 1983. The taxonomy of Old World *Lepus*. Acta Zool. Fennica 174, 17–21.

Angermann, R., Flux, J.E.C., Chapman, J.A., Smit, A.T., 1990. Lagomorph classification. Rabbits, hares and pikas: status conservation action plan. In: Chapman, J.A., Flux, J.E.C. (Eds.), International Union for Conservation of Nature and Natural Resources. Gland, Switzerland.

Arnason, U., Adegoke, J.A., Bodin, K., Born, E.W., Esa, Y.B., Gullberg, A., Nilsson, M., Short, R.V., Xu, X., Janke, A., 2002. Mammalian mitogenomic relationships and the root of the eutherian tree. Proc. Natl. Acad. Sci. USA 99, 8151–8156.

Austin, J.D., Lougheed, S.C., Moler, P.E., Boag, P.T., 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biol. J. Linn. Soc. 80, 601–624.

Azzaroli Puccetti, M.L., Corti, M., Scanzani, A., Civitelli, M.V., Cappana, E., 1996. Karyotypes of two endemic species of hare from Ethiopia, *Lepus habessinicus* and *L. starcki* (Lagomorpha, Leporidae). A comparison with *L. europaeus*. Mammalia 60, 223–230.

Baker, A.J., Eger, J.L., Peterson, R.L., Manning, T.H., 1983. Geographic variation and taxonomy of arctic hares. Acta Zool. Fennica 174, 45–48.

Blaxter, M.L., 2004. The promise of a DNA taxonomy. Philos. Trans. R. Soc. Lond. B 359, 669–679.

Bonhomme, F., Fernandez, J., Palacios, F., Catalan, J., Machordon, A., 1986. Charact'erisation du complex d'esp'eces du genre *Lepus* en Espagne. Mammalia 50, 495–506.

Bremer, K., 1992. Ancestral areas: a cladistic reinterpretation of the center of origin concept. Syst. Biol. 41, 436–445.

Bremer, K., 1995. Ancestral areas: optimization and probability. Syst. Biol. 44, 255–259.

Brooks, D.R., 1981. Hennig's parasitological method: a proposed solution. Syst. Zool. 30, 229–249.

Brooks, D.R., 1990. Parsimony analysis in historical biogeography and coevolution: methodological and theoretical update. Syst. Zool. 39, 14–30.

Brooks, D.R., McLennan, D.A., 1991. Phylogeny, Eecology and Behavior: A Research Program in Comparative Biology. University of Chicago Press, Chicago.

Brooks, D.R., McLennan, D.A., 2001. A comparison of a discovery-based and an event-based method of historical biogeography. J. Biogeogr. 28, 757–767.

Brooks, D.R., Van Veller, M.G.P., McLennan, D.A., 2001. How to do BPA, really. J. Biogeogr. 28, 345–358.

Camin, J.H., Sokal, R.R., 1965. A method for deducing branching sequences in phylogeny. Evolution 19, 311–326.

Castresana, J., 2001. Cytb phylogeny and the taxonomy of great apes and mammals. Mol. Biol. Evol. 18, 465–471.

Chapman, J.A., Flux, J.E.C., 1990. The introduction and overview in Rabbits, Hares and Pikas, status survey and conservation action plan rabbits, hares and pikas: status conservation action plan. In: Chapman, J.A., Flux, J.E.C. (Eds.), International Union for Conservation of Nature and Natural Resources. Gland, Switzerland.

Chapman, J.A., Cramer, K.L., Dippenaar, N.J., Robinson, T.J., 1992. Systematics and biogeography of the New England cottontail,

Sylvilagus transitionalis (Bangs, 1895), with the description of a new species from the Appalachian Mountains. Proc. Biol. Soc. Washington 105, 841–866.

Chen, J.Sh., 1956. The Fauna of Vertebrata in Taiwan. Taiwan Commerce Publishing Company, p. 376.

Corbet, G.B., 1978. The mammals of the Palaearctic region: A taxonomic review. British Museum (Natural History), London, 314.

Corbet, G.B., Hill, J.E., 1980. A World list of mammalian species. Br. Mus (Nat. Hist.), 196–199.

Corbet, G.B., 1986. Relationships and origins of European lagomorphs. Mammal Rev. 16, 105–110.

Corbet, G.B., Hill, J.E., 1986. A world list of mammalian species, second ed. British Museum (Natural History), London, 254.

Cunningham, C.W., 1997. Can three incongruence tests predict when data should be combined? Mol. Biol. Evol. 14, 733–740.

Darlu, P., Lecointre, G., 2002. When does the incongruence length difference test fail? Mol. Biol. Evol. 19, 432–437.

Dawson, M.R., 1981. Evolution of modern leporids. Myers, K., MacInnes, C.D. (Eds.), In: Proceedings of the World Lagomorph Conference University of Guelph Press, Ontario, pp. 1–8.

Deng, Q.X., 1960. Living habit and prevention and cure of Woolly hare (*L.oiostolus*). J. Zool. 3, 136–139.

Dixon, K.R., Chapman, J.A., Willner, G.R., Wilson, D.E., Lopez-Forment, W., 1983. The New World jackrabbits and hares (genus *Lepus*)-2. Numerical taxonomic analysis. Acta Zool. Fennica 174, 53–56.

Dong, G., Wang, G., Chen, H., Yan, M., Jin, J., Wang, Y., 1995. The formation and evolution of the deserts in China and their relation to the uplifting of Qinghai-Tibet Plateau. In: "Qinghai-Tibetan Plateau and Global Variations" China Society of the Qinghai-Tibetan Plateau Research (Eds.), China Meteorological Press, Beijing, pp. 13–29.

Ellerman, J.R., Morrison-Scott, T.C.S., 1951. Check list of Palaearctic and Indian mammals, 1758–1946. Br. Mus. (Nat. Hist), 419–445.

Farris, J.S., Käallersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–319.

Farris, J.S., Käallersjö, M., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.

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

Flux, J.E.C., 1983. Introduction to taxonomic problems in hares. Acta Zool. Fennica 174, 7–10.

Flux, J.E.C., Angermann, R., 1990. The hares and jackrabbits. In: Chapman, J.A., Flux, J.E.C. (Eds.), Rabbits, Hares and Pikas: Status Conservation Action Plan. International Union for Conservation of Nature and Natural Resources. Gland, Switzerland, pp. 61–94.

Frey, J.K., Fisher, R.D., Ruedas, L.A., 1997. Identification and restriction of the type locality of the Manzano Mountains cottontail, *Sylvilagus cognatus* Nelson, 1907 (Mammalia: Lagomorpha: Leporidae). Proc. Biol. Soc. Washington 110, 329–331.

Gao, Y.T., Feng, Z.J., 1964. The study of *Lepus oiostolus* subspecies in China. Acta Zootaxonomica Sinica 1, 19–29.

Gissi, C., Gullberg, A., Arnason, U., 1998. The complete mitochondrial DNA sequence of the rabbit, *Oryctolagus cuniculus*. Genomics 50, 161–169.

Gureev, A.A., 1964. Zaitzeobrazniye (Lagomorpha). Fauna SSSR 3, 276.

Hall, E.R., 1981. The Mammals of North America, vol. 1, second ed. John Wiley and Sons, New York, NY, p. 181.

Halanych, K.M., Demboski, J.R., Van Vuuren, B.J., Klein, D.R., Cook, J.A., 1999. Cytb phylogeny of North American hares and jackrabbits (*Lepus*, Lagomorpha) and the effects of saturation in outgroup taxa. Mol. Phylogenet. Evol. 11, 213–221.

Halanych, K.M., Robinson, T.J., 1999. Multiple substitutions affect the phylogenetic utility of cytb and 12S rDNA data: examining a rapid radiation in leporid (Lagomorpha) evolution. J. Mol. Evol. 48, 369–379.

Hassanin, A., Douzery, E.J.P., 2003. Molecular and morphological phylogenies of ruminants, and the alternative position of the Moschidae. Syst. Biol. 52, 206–228.

Hibbard, C., 1963. The origin of the P3 pattern of *Sylvilagus*, *Caprolagus*, *Oryctolagus*, and *Lepus*. J. Mammal. 44, 1–15.

Hoffmann, R.S., 1993. Order Lagomorpha. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference, second ed. Smithsonian Inst. Press, Washington, DC, pp. 807–827.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Huelsenbeck, J.P., Bollback, J.P., 2001. Empirical and hierarchical Bayesian estimation of ancestral states. Syst. Biol. 50, 351–366.

IUCN 2000. IUCN Red list of Threatened Animals. IUCN, Gland..

Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the Cytb gene of mammals. J. Mol. Evol. 32, 128–144.

Johns, G.C., Avise, J.C., 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytb. Mol. Biol. Evol. 15, 1481–1490.

Jones, J.K., Johnson, D.H., 1965. Synopsis of the lagomorphs and rodents of Korea. University of Kansas Publications, Museum of Natural History. 16, pp. 357–407.

Jones, J.K., Carter, J.D.C., Genoways, H.H., Hoffmann, R.S., Rice, D.W., Jones, C., 1986. Revised Checklist of North American Mammals North of Mexico. Occasional Papers, The Museum, Texas Tech University, 107, pp. 1–22.

Kishino, H., Thorne, J.L., Bruno, W.J., 2001. Performance of a divergence time estimation method under a probabilistic model of rate evolution. Mol. Biol. Evol. 18, 352–361.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Koh, H.S., Chun, T.Y., Yoo, H.S., Zhang, Y.P., Wang, J.X., Zhang, M.H., Wu, C.H., 2001. Mitochondrial cytb gene sequence diversity in the Korean hare, *Lepus coreanus* Thomas (Mammalia, Lagomorpha). Biochem. Genet. 39, 417–429.

Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5, 150–163.

Kurtén, B., 1968. Pleistocene Mammals of Europe. Weidenfeld and Nicholson, London, UK.

Kurtén, B., Anderson, E., 1980. Pleistocene Mammals of North America. Columbia University Press, New York.

Leaché, A.D., Reeder, T.W., 2002. Molecular systematics of the Eastern Fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and bayesian approaches. Syst. Biol. 51, 44–68.

Li, Zh.Y., Luo, Z.X., 1979. A new species: *Lepus melainus*. The transaction of northeast Forest University, 2, pp. 71–81.

Loukashkin, A.S., 1943. On the Hares of Northern Manchuria. J. Mammal. 24, 75–81.

Luo, Z.X., 1981. Classification of *Lepus capensis* in China. Acta Theriologica Sinica 1, 149–151.

Luo, Z.X., 1988. The Chinese hare. China forestry publishing Beijing.

Matthee, C.A., Robinson, T.J., 1996. Mitochondrial DNA differentiation among geographical populations of *Pronolagus rupestris*, Smith's red rock rabbit (Mammalia: Lagomorpha). Heredity 76, 514–523.

Matthee, C.A., Vuuren, B.J.V., Bell, D., Robinson, T.J., 2004. A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. Syst. Biol. 53, 433–447.

Marincovich, J.L., Gladenkov, A.Y., 1999. Evidence for an early opening of the Bering Strait. Nature 397, 149–151.
Meester, J.A.J., Rautenbach, I.L., Dippenaar, N.J., Baker, C.M., 1986. Classification of southern African mammals. Transvaal Museum Monograph 5, 1–359.
Moritz, C., Cicero, C., 2004. DNA barcoding: promise and pitfalls. PLoS Biol. 2, e354.
Nowak, R., 1999. Walker's Mammal of the World, fifth ed. The Johns Hopkins University Press, Baltimore and London.
Palacios, F., 1976. Descripcin de una especie de liebre (*Lepus castroviejoi*), end ica de la Cordillera Cantrica. Dona Acta Vertebrata 3, 205–225.
Palacios, F., 1983. On the taxonomic status of the genus *Lepus* in Spain. Acta Zool. Fennica 174, 27–30.
Palacios, F., 1989. Biometric and morphologic features of the species of the genus *Lepus* in Spain. Mammalia 53, 227–264.
Palacios, F., 1996. Systematics of the indigeneous hares of Italy traditionally identified as *Lepus europaeus* Pallas, 1778 (Mammalia: Leporidae). Bonner Zoologische Beitr[mk]age 56, 59–91.
Petter, P.F., 1961. Elément dune revision des Lievres europeèns et asiatiques du sous-genre *Lepus* Zeitschr. Saugetierk 26, 30–40.
Pérez-Suárez, G., Palacios, F., Boursot, P., 1994. Speciation and paraphyly in western Mediterranean hares (*Lepus castroviejoi*, *L. europaeus*, *L. grantensis,* and *L. capensis*) revealed by mitochondrial DNA phylogeny. Biochem. Genet. 32, 423–436.
Pierpaoli, M., Riga, F., Trocchi, V., Randi, E., 1999. Species distinction and evolutionary relationships of the Italian hare (*Lepus corsicanus*) as described by mitochondrial DNA sequencing. Mol. Ecol. 8, 1805–1817.
Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.
Qian, Y.W., Zheng, B.L., Guan, G.X., Zhang, J., Wang, X., Shen, X.Y., 1965. The Bird and Animal in the South of Xinjiang. Scientific publishing Company.
Rannala, B., Yang, Z., 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J. Mol. Evol. 43, 304–311.
Riga, F., Trocchi, V., Randi, E., Toso, S., 2001. Morphometric differentiation between the Italian hare (*Lepus corsicanus* De Winton, 1898) and the European Brown hare (*Lepus europaeus* Pallas, 1778. J. Zool. Lond. 253, 241–252.
Robinson, T.J., Osterhoff, D.R., 1983. Protein variation and its systematic implications for the South African Leporidae (Mammalia: Lagomorpha). Anim. Blood Groups Biochem. Genet. 14, 139–149.
Robinson, T.J., Elder, F.F.B., Chapman, J.A., 1983. Karyotype conservatism in the genus *Lepus* (order Lagomorpha). Can. J. Genet. Cytol. 25, 540–544.
Rutschmann, F., 2004. Bayesian molecular dating using PAML/multidivtime. A Step-by-step Manual. University of Zurich, Switzerland.
Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A laboratory manual, second ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Schneider, E., Leipoldt, M., 1983. DNA relationship within the genus *Lepus* in SW Europe. Acta Zool. Fennica 174, 31–33.
Sikes, D.S., Lewis, P.O., 2001. PAUPRat: a tool to implement Parsimony Ratchet searches using PAUP*, beta software, version 1.
Sher, A., 1999. Traffic lights at the Beringian crossroads. Nature 397, 103–104.
Sowerby, A.de C., 1923. The naturalist in Manchuria. Tientsin Press Ltd., China, 2, p. 191.
Sowerby, A.de.C., 1933. The rodents and lagomorphs of China. China J. 19, 189–207.
Shou, Zh.H., 1962. Chinese Economic Fauna. Scientific publishing company, pp. 92–109.
Su, B., Keri, M., Wang, W., Jiang, X.L., Wang, Y.X., Woodruff, D., Zhang, Y.P., 1995. Molecular phylogeny of Chinese concolor gibbons (Subgeneus *Nomascus*) using noninvasive DNA genotyping. In: Xia, W.P., Zhang, Y.Z. (Eds.), Primate Research and Conservation. China Forestry publishing House, Beijing, p. 481.
Swofford, D.L., 2003. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods), Version 4.0b10. Sinauer Associates, Sunderland, MA.
Tate, G.H.H., 1947. Mammals of Eastern Asia. MacMillan, New York.
Tautz, D., Arctander, P., Minelli, A., Thomas, R.H., Vogler, A.P., 2002. DNA points the way ahead in taxonomy. Nature 418, 479.
Tautz, D., Arctander, P., Minelli, A., Thomas, R.H., Vogler, A.P., 2003. A plea for DNA taxonomy. Trends Ecol. Evol. 18, 70–74.
Thomas, O., 1906. Descriptions of new mammals from Mount Ruwenzori. Ann. Mag. Nat. Hist. ser. 7 18, 136–147.
Thompson, J.D., Gibson, T.J., Plewniak, F., Mougin, F.J., Higgins, D.G., 1997. The Clustal X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
Thorne, J.L., Kishino, H., Painter, I.S., 1998. Estimating the rate of evolution of the rate of molecular evolution. Mol. Biol. Evol. 15, 1647–1657.
Tong, Y.Sh., Zheng, Sh.H., Qiu, Zh.D., 1995. Cenozoic mammal ages of China. Vertebrata Palasiatica 33, 290–314.
Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51, 689–702.
Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex-100 as a medium for simple extraction of DNA for PCR based typing from forensic material. Biotechniques 10, 506–513.
Wang, Y.X., Luo, Z.X., Feng, Z.J., 1985. Taxonomic revision of Yunnan hare *Lepus comus* G. Allen with description of two new subspecies. Zool. Res. 6, 101–109.
Wiley, E.O., 1986. Methods in vicariance biogeography. In: Hovenkamp, P. (Ed.), Systematics and Evolution: Matter of Diversity. University of Utrecht Press, Utrecht, pp. 283–306.
Wiley, E.O., 1988a. Vicariance biogeography. Ann. Rev. Ecol. Syst. 19, 513–542.
Wiley, E.O., 1988b. Parsimony analysis and vicariance biogeography. Syst. Zool. 37, 271–290.
Wilson, D.E., Reeder, D.A.M., 1993. Mammal Species of the World a Taxonomical and Geographic Reference, second ed. Smithsonian Inst. Press, Washington, London.
Winton, D.W.E., 1898. On the hares of Western Europe and North Africa. Ann. Mag. Nat. Hist. London 1, 149–158.
Whiteford, C.E.M., 1995. Molecular phylogeny of the genus *Pronolagus* (Mammalia: Lagomorpha) and the use of morphological and molecular characters in the delineation of *P. rupestris*. MSc. Thesis, University of Pretoria.
Wu, C.H., Li, H.P., Wang, Y.X., Zhang, Y.P., 2000. Low genetic variation of the Yunnan Hare (*Lepus comus* Allen 1927) as revealed by mitochondrial CytbGene sequences. Biochem. Genet. 38, 149–155.
Wu, C.H., Zhang, Y.P., Bunch, T.D., Wang, S., Wang, W., 2003. Mitochondrial control region sequence variation within the argali wild sheep (*Ovis ammon*): evolution and conservation relevance. Mammalia 67, 109–118.
Xiao, H., Chen, S.Y., Liu, Z.M., Zhang, R.D., Li, W.X., Zan, R.G., Zhang, Y.P., 2005. Molecular phylogeny of *Sinocyclocheilus* (Cypriniformes: Cyprinidae) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. (in press).
Yamada, F., Takaki, M., Suzuki, H., 2002. Molecular phylogeny of Japanese Leporidae, the Amami rabbit Pentalagus furnessi, the

Japanese hare *Lepus brachyurus*, and the mountain hare *Lepus timidus*, inferred from mitochondrial DNA sequences. Genes Genet. Syst. 77, 107–116.

Yang, Z., Yoder, A.D., 2003. Comparison of likelihood and Bayesian methods for estimating divergence times using multiple gene loci and calibration points, with application to a radiation of cute-looking mouse lemur species. Syst. Biol. 52, 705–716.

Yoder, A.D., Irwin, J.A., Payseur, B.A., 2001. Failure of the ILD to determine data combinability for Slow Loris phylogeny. Syst. Biol. 50, 408–424.

Yu, N., Zheng, C.L., Zhang, Y.P., Li, W.H., 2000. Molecular systematics of Pikas (Genus *Ochotona*) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 1, 85–95.

Zhang, R.Z., 2002. Geological events and mammalian distribution in China. Acta Zool. Sinica 48, 141–153.

Zhu, X.M., Zhuang, H.B., 1982. Cape hare's domestication (*L. capensis*). Wild Animal 3, 42–43.

Гуреев, А.А., 1964. Фауна С.С.С.Р., Млекопитающие, Зайцеобразные (lagomorpha). Издательство ≪Наукоа≫ Москва.