Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 33 (2004) 880–895
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Evolution and phylogeny of old world deer

Christian Pitra[a,*], Joerns Fickel[a], Erik Meijaard[b], P. Colin Groves[c]

[a] Department of Evolutionary Genetics, Institute for Zoo and Wildlife Research, PF 60113, D-10252 Berlin, Germany
[b] School of Tropical Biology, James Cook University, P.O. Box 6811, Cairns, Qld. 4870, Australia
[c] School of Archaeology & Anthropology, The Australian National University, P.O. Box 4, Canberra, ACT 0200, Australia

Received 6 May 2004; revised 14 July 2004
Available online 12 September 2004

Abstract

The phylogenetic pattern and timing of the radiation of Old World deer was determined based on the complete mitochondrial cytochrome *b* gene from 33 Cervinae taxa. Using rooted and unrooted phylogenies derived from distinct theoretical approaches, strong support was achieved for monophyly of the Old World deer with muntjacs as sister group as well as for the divergence of at least three distinct genera: *Rucervus*, *Dama*, and *Cervus*. The latter clade comprises what have previously been regarded as the genera or subgenera *Panolia*, *Rusa*, *Cervus*, *Sika*, and probably *Przewalskium*. Our data also consistently confirmed paraphyly of nominate *C. elaphus* and did not support the monophyly of *Axis*. We used these molecular phylogenies to assess the homoplastic evolution of morphological, geographical, ecological, and selected behavioural character state differences within the Cervinae. Reliable fossil calibrations, large molecular data sets, and improved dating methods are shaping a molecular time scale for the evolutionary radiation of Old World deer that occurred at the Miocene/Pliocene transition and is largely compatible with existing palaeontological evidence. Using node ages estimated from sequence data, we estimated an average per-lineage diversification rate of 0.51 ± 0.1 species per million years (my) over roughly the last 6 mya.
© 2004 Elsevier Inc. All rights reserved.

Keywords: *Cervinae*; Evolution; Molecular systematics; Molecular clock; Speciation; Cytochrome *b*

1. Introduction

The Cervinae (Old World deer, OW deer) form a morphologically and ecologically diverse subfamily among the family Cervidae (true deer) that inhabits a variety of terrestrial environments. Their diversity is thought to reflect several adaptive radiations. The different forms of cranial appendages (antlers; a bony inner core and coated by velvet skin cover) have attracted the interest of many biologists and palaeontologists (Darwin, 1871; Geist, 1968; Gould, 1974; Emlen, 2001). Despite the continuous accumulation of palaeontological, morphological, karyological, and behavioural data, the evolution of this group of deer has been a matter of much speculation and debate, as is reflected in uncertainties about evolutionary relationships at different taxonomic levels (Table 1). Throughout the paper we use the classification by Groves and Grubb (1987).

Previous hypotheses regarding the phylogeny of OW deer have rested primarily on morphological characters and the fossil record. As summarized by Gentry (1994), the earliest antlered deer are *Dicrocerus* and *Heteroprox*, both of which appear in the Late Orleanian in MN5 (Mammalian Neogene biostratigraphic divisions, about 17 mya), and *Euprox*, which appears in the succeeding Early Astaracian in MN6 (about 16 mya). Azanza (1993) classified *Euprox* already as belonging to the Muntiacinae, a subfamily which has been considered as the primitive stem-group of all other cervids but which she argued to be a monophyletic clade. The Muntiacinae survived in Europe until MN7/8, but then

* Corresponding author.
E-mail address: pitra@IZW-berlin.de (C. Pitra).

1055-7903/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2004.07.013

Table 1
A recent taxonomy for Old World deer (subfamily Cervinae) according to the classification by Groves and Grubb (1987), with problematic areas indicated by parenthetical questions

Family Cervidae	All extant Old World deer (Cervinae), Asian muntjacs and tufted deer (Muntiacinae), Holarctic moose and reindeer, New World odocoileines, Old World reindeer, and Asian antlerless monospecific Hydropotes (Hydropotinae)
Subfamily Cervinae	Most taxonomic schemes recognize four genera of extant Old World deer (Are the Old World deer monophyletic? Where does this group lie with regard to broader deer phylogeny?)
Genus *Cervus*	This complex group is often divided into several subgenera. (Is the genus monophyletic?)
Subgenus *Cervus* sensu lato	*Cervus elaphus* (Do red deer form a monophyletic assemblage?)
Subgenus *Rucervus*	*Rucervus schomburgki* (Is this extinct species a close ally of the barasingha?)
Subgenus *Rusa*	*R. unicolor* and *R. timorensis* (Are the sambars widely separated from the Javan rusa?)
Subgenus *Sika*	*Cervus nippon* (Are the sika and red deer sister lineages?)
Subgenus *Przewalskium*	*Cervus* (*Przewalskium*) *albirostris* (Is the rare Thorold's deer sister to all red deer?)
Genus *Elaphurus*	*Elaphurus davidianus* (What is the phylogenetic position of the enigmatic Pere David's deer?)
Genus *Dama*	*Dama dama* and *Dama mesopotamicus* (Are these two forms distinct species?)
Genus *Axis*	*Axis porcinus* (Is the hog deer allied more closely to the chital or to the Javan rusa?)

disappeared and were replaced by "*Eustylocerus*" *pierensis*, which she regarded as the earliest known member of the Cervinae. This replacement corresponds to the change from a subtropical to a more temperate climatic regime (Azanza, 1993; Azanza and Menendez, 1990).

As far as the comparative morphology of living deer is concerned, earlier behavioural and morphological studies provide divergent, fragmentary, and often purely phenetic assessments of phylogeny within the subfamily (Loomis, 1928; Geist, 1987; Bubenik and Bubenik, 1990). Groves and Grubb (1987) stated that previously accepted "formal classifications of deer were inadequate, yet through repetition have become regarded as unquestioned primary sources of knowledge." Based on the shared plesiometacarpal condition of the lateral metacarpals (the proximal parts of the second and fifth lateral metacarpals persist; Brooke, 1878), the monophyly of the Cervinae was usually not questioned (Groves and Grubb, 1987). Several dental and cranial characters, especially those of cranial appendages, were traditionally used to resolve intra-subfamilial relationships (Beninde, 1937; Bachofen-Echt, 1939; Bubenik and Bubenik, 1990; Vislobokova and Godina, 1993); yet these diagnostic characters could either be ancestral, hence phylogenetically uninformative, or prone to convergence due to ecological adaptations (Groves and Grubb, 1987; Janis and Scott, 1987), and could represent different ecomorphs (Geist, 1998; Grubb, 1993; Vrba and Schaller, 2000).

Molecular phylogenetics based on mitochondrial DNA (Miyamoto et al., 1990; Cronin, 1991; Douzery and Randi, 1997; Randi et al., 1998, 2001; Cook et al., 1999; Polziehn and Strobeck, 2002; Li et al., 2003; Ludt et al., 2004), nuclear DNA (Comincini et al., 1996) or amino acid (Cronin et al., 1996) sequence comparisons has contributed considerably to resolve evolutionary relationships among deer species at the family level (Cervidae), but these studies did not fully resolve the phylogeny of the Cervinae because they lacked many of the extant OW deer species. The present study improves these earlier phylogenies in four significant ways.

First, it is the only molecular analysis to include representatives of 32 extant OW deer taxa and the extinct Schomburgk's deer (Table 2). We have also added sequence data from 17 additional deer species to the cervine data set, including representatives of all living cervid subfamilies: Asian muntjaks (Muntiacinae), holarctic moose (Alcinae), reindeer (Rangiferinae), New World deer (Odocoileinae), Old World roe deer (Capreoleinae), and the antlerless monospecific Hydropotes (Hydropotinae), as well as musk deer (Moschidae). Thus, it inherently provides a test of patterns uncovered in previous phylogenies because the addition of taxa (especially outgroups) to a phylogenetic study can ultimately affect the polarity of character change and, consequently, the resulting topology (Poe, 1998; Zwickl and Hillis, 2002; Holland et al., 2003). Second, previous molecular studies that have addressed the timing of the cervid radiation have shortcomings that include the use of single calibration points, an unwarranted molecular clock assumption even when there is lineage-specific rate variation, and limited taxon sampling that fails to include all genera of deer. Here, we address these issues using the taxonomically most complete data set in conjunction with two fossil constraints and a recently developed approach for estimating divergence times in the absence of rate constancy. Third, because the relatively high deer diversity in modern fauna is often attributed to a burst of Pleistocene speciation (see especially Geist, 1987), we used a densely sampled phylogenetic tree and information on the relative timing of cladogenesis based on DNA sequences for quantifying

Table 2
Cervide Taxa Studied and Sources of Tissues and Cytochrome *b* Sequences

Species (common name)	Origin and reference for tissues and sequences	Accession Nos.
Hydropotinae		
Hydropotes inermis (Chinese water deer)	Randi et al. (1998)	AJ000028
Muntiacinae		
Muntiacus crinifrons (black muntjac)	Li et al., unpublished	AY239042
Muntiacus muntjak vaginalis (muntjac)	Giao et al. (1998)	AF042715
Muntiacus reevesi (Chinese muntjac)	Zhang et al., unpublished	AF527537
Cervinae		
Cervus elaphus bactrianus (red deer)	Ludt et al. (2004)	AY142327
Cervus elaphus yarkandensis (red deer)	Ludt et al. (2004)	AY142326
Cervus elaphus hippelaphus (red deer)	Ludt et al. (2004)	AY244491
Cervus elaphus corsicanus (red deer)	Ludt et al. (2004)	AY244489
Cervus elaphus maral (red deer)	Ludt et al. (2004)	AY118199
Cervus elaphus barbarus (red deer)	Ludt et al. (2004)	AY118198
Cervus elaphus hispanicus (red deer)	Ludt et al. (2004)	AF489281
Cervus elaphus atlanticus (red deer)	Ludt et al. (2004)	AY070221
Cervus elaphus xanthopygus (red deer)	Ludt et al. (2004)	AY070224
Cervus elaphus kansuensis (red deer)	Ludt et al., 2004	AY070223
Cervus elaphus sibiricus (red deer)	Ludt et al. (2004)	AF423199
Cervus elaphus wallichi (red deer)	Ludt et al. (2004)	AY044861
Cervus elaphus macneilli (red deer)	Ludt et al. (2004)	AY035875
Cervus elaphus songaricus (red deer)	Ludt et al. (2004)	AY035871
Cervus elaphus canadensis (red deer)	Kuwayama and Ozawa (2000)	AB021096
Cervus nippon sichuanicus (sika deer)	Ludt et al. (2004)	AY035876
Cervus nippon yesoensis (sika deer)	Kuwayama and Ozawa (2000)	AB021095
Cervus nippon centralis (sika deer)	Kuwayama and Ozawa (2000)	AB021094
Cervus nippon mageshimae (sika deer)	Kuwayama and Ozawa (2000)	AB021092
Cervus nippon keramae (sika deer)	Kuwayama and Ozawa (2000)	AB021091
Cervus nippon pulchellus (sika deer)	Kuwayama and Ozawa (2000)	AB021090
Axis porcinus (hog deer)	Ludt et al. (2004)	AY035874
Axis axis (chital)	Tierpark-Berlin, this study	AY607040
Przewalskium albirostris (white-lipped deer)	Ludt et al. (2004)	AF423202
Elaphurus davidianus (Père David's deer)	Ludt et al., unpublished	AF423194
Rucervus schomburgki (Schomburgks deer)	this study	AY607036
Rucervus duvauceli (barasingha)	Tierpark-Berlin, this study	AY607041
Rusa unicolor cambojensis (sambar)	Ludt et al. (2004)	AF423201
Cervus eldi hainanus (Siam brow-antlered deer)	Liu et al., unpublished	AY157735
Cervus eldi thamin (Burma brow-antlered deer)	Tierpark-Berlin, this study	AY607037
Rusa timorensis macassaricus (Timor deer)	Ludt et al. (2004)	AF423200
Dama dama (European fallow deer)	Randi et al. (1998)	AJ000022
Dama mesopotamicus (Persian fallow deer)	Tierpark-Berlin, this study	AY607034
Odocoileinae		
Odocoileus hemionus (mule deer)	Hassanin and Douzery (1999)	AF091630
Odocoileus virginianus (white-tailed deer)	Tierpark-Berlin, this study	AY607035
Mazama sp. (red brocket)	Randi et al. (1998)	AJ000027
Blastocerus dichotomus (marsh deer)	Zoo Berlin, this study	AY607038
Pudu puda (pygmy deer)	Zoo Berlin, this study	AY607039
Capreoleinae		
Capreolus capreolus (roe deer)	Randi et al. (1998)	AJ000024
Capreolus pygargus (eastern roe deer)	Randi et al. (1998)	AJ000025
Alcinae		
Alces alces pfitzmayeri (moose)	Ludt et al., unpublished	AY035873
Alces alces cameloides (moose)	Ludt et al., unpublished	AY035872
Alces alces (moose)	Randi et al. (1998)	AJ000026
Rangiferinae		
Rangifer tarandus (caribou)	Randi et al. (1998)	AJ000029
Moschidae		
Moschus leucogaster (musk deer)	Su et al. (1999)	AF026889

how speciation events have changed over time. This allowed a general test of the tempo of diversification and premises that the Pleistocene was an exceptional period of speciation in deer. Finally, we attempt to clarify OW deer phylogeny in problematic areas that are relevant to the fields of both evolutionary ecology and conservation genetics (Table 1). For example, molecular phylogeny is used to decipher the evolutionary trends in ecology, cranial appendages and behaviour within the Cervinae. Besides its strict orthology, mitochondrial (mt)DNA has several features rendering it particularly suitable for the analysis of phylogenetic relationships: high copy number, apparent lack of recombination, partially high substitution rate and maternal mode of inheritance (Arnason et al., 2002). We selected the gene coding for cytochrome *b* (cyt *b*) as molecular marker to analyse phylogenetic relationships among deer because its tempo and mode of evolution is well understood, thought to be relatively constant and similar among large-bodied terrestrial mammals. The cyt *b* gene has been used in numerous studies of phylogenetic relationships among mammals and is the gene for which the most sequence information from different mammalian species is available (Johns and Avise, 1998; Castresana, 2001). The sequence variability of cyt *b* makes it most useful for the comparison of species in the same genus or family.

2. Materials and methods

2.1. DNA extraction, amplification, and sequencing

The origins of DNA samples and sequences used in this study are listed in Table 2. Representative voucher specimens for each species sequenced in this study are deposited in the Animal Tissue Collection at the Institute for Zoo and Wildlife Research in Berlin, Germany. Whole genomic DNA was extracted from blood or generative tissue of living deer using the QIAquick Tissue Kit (Qiagen, Chatsworth, CA). The specimen of Schomburgk's deer used in this study originates from the archival specimen Siam-Berlin 3 presently kept at the vertebrate collection in the Museum für Naturkunde Berlin, Germany, under the Accession No. 16032 (Mohr, 1968). Approximately 1 g tissue from the horny claw of this specimen was chosen for DNA extraction based on its good macroscopical preservation and protection against tanning procedures. The sample was ground to powder and incubated overnight in 1 ml 0.5 M EDTA, 20 µl ProteinaseK (20 mg/ml), and 20 µl 0.5 M DTT at 37 °C. The DNA was extracted using the QIAquick Tissue Kit (Qiagen, Chatsworth, CA) beginning with step 3 according to the supplier's protocol. DNA extraction was performed in an isolated pre-PCR area where no cervid DNA had previously been introduced. Appropriate controls were used in each step of the analysis, adopting the standard precautionary measurements of ancient DNA studies (Cooper and Poinar, 2000). The entire cyt *b* gene (1140 bp) was amplified using a suite of known and newly designed primers (Table 3). PCR mixtures contained 0.8 U Ampli*Taq* DNA polymerase (Perkin–Elmer), 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 µM dNTPs, 10–50 pmol of each primer and ~100 ng of DNA in a final volume of 50 µl. Reaction mixtures were subjected to the following PCR cycling protocol: 1× (94 °C for 3 min), 35× (94 °C for 15 s, 50 °C for 20 s, and 72 °C for 90 s), 1× (72 °C for 7 min). The cyt *b* gene from the museum sample (*Rucervus schomburgki*) was amplified

Table 3
Primer pairs used for amplification and sequencing

Name[a]	Sequence (5′–3′)[b]	Source
Gludg-L (14,194)	TGA CTT GAA RAA CCA YCG TTG	Kocher et al. (1989)
H-14,319	GTA GTG TAT TGC TAA GAA TAG G	This study
L-14,152	TYG GCT CYY TRC TAG GAA T	This study
H-14,491	ATG TTT CAT GTC TCT AGA A	This study
L-14,402	ACG CAA ATG GAG CAT CAA TA	This study
H-14,721	GAT AAA TGG GAG AAT AAA GTG G	This study
L-14,652	GGG TTT TTC AGT GGA TAA AGC	This study
H-14,897	TCT GGT GCG AAT AAT ACT AG	This study
L-14,868	TCT TAA TAT TAC TAG TAT TAT TC	This study
H-15,070	GGG GTA TAA GAA TTA GAA TTA G	This study
CB7u-L (15,004)	GCG TAC GCA ATC TTA CGA TCA A	Burger et al. (2004)
CB7I-H (15,146)	CTG GCC TCC AAT TCA TGT GAG	Burger et al. (2004)
L-15,112	TTC AGC CAA TGC TTA TTC TG	This study
CB6Thr-H (15,326)	TTT CAT TCT CCG RTT TAC AAG	Kocher et al. (1989)

[a] The letters L and H refer to the light and heavy strands, the numbers correspond to positions in the complete *Muntiacus muntjak* mitochondrial genome (AY225986).
[b] Degenerate sites are indicated by Y = C or T; R = A or G.

in short overlapping 150–300 bp fragments using the primers mentioned above. PCR mixtures contained 2 U FastStart*Taq* DNA polymerase (Roche), 50 mM Tris–HCl (pH 8.3), 10 mM KCl, 5 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 200 μM dNTPs, 10–50 pmol of each primer, and 1× GC-rich solution in a final volume of 50 μl. Amplification was carried out under the following reaction conditions: 1× (95 °C for 6 min), 40× (95 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s), 1× (72 °C for 7 min). Of this PCR, 5 μl were reamplified using the PCR mixture by Perkin–Elmer (see above) under the following conditions: 1× (94 °C for 4 min), 15× (95 °C for 20 s, 50 °C for 15 s, and 72 °C for 45 s), 25× (89 °C for 20 s, 50 °C for 15 s, and 72 °C for 45 s) 1× (72 °C for 7 min). PCR products were then purified (QIAquick PCR purification kit/Qiagen), bidirectionally sequenced using the Big Dye Terminator v.3.1. Cycle Sequencing kit (ABI) and analysed on an automated ABI 3100 Genetic Analyser. Sequences were deposited in the GenBank database under the accession numbers specified in Table 2. In addition to our sequences, the GenBank database was queried for the orthologues listed in Table 2.

2.2. Data analyses

Sequences of the complete cyt *b* gene from 49 putative taxa within the Cervidae were used in the analysis (Table 2). Sequences were aligned using the software ClustalX (Thompson et al., 1997), followed by visual inspection. We computed basic sequence statistics and uncorrected *p*-distances with MEGA 2.0 (Kumar et al., 1993). The empirical t_s/t_v ratios were calculated using the maximum likelihood option in PUZZLE 4.02 (Strimmer and von Haeseler, 1996), applying the default settings. We used Xia et al.'s (2002) index as implemented in DAMBE (Xia and Xie, 2001) to measure the degree of substitution saturation.

We carried out four types of phylogenetic analyses to investigate evolutionary relationships: (i) neighbour-joining (NJ) as implemented in MEGA 2.0 (Kumar et al., 1993), (ii) maximum parsimony (MP) using PAUP* 4.0b10 (Swofford, 2001), (iii) maximum-likelihood (ML) using the program PUZZLE 4.02 (Strimmer and von Haeseler, 1996), and (iv) Bayesian inference (BI) of phylogeny as implemented in MrBayes 2.0 (Huelsenbeck and Ronquist, 2001). For the NJ analyses, distances were calculated applying Tamura and Nei's (1993) method using an α parameter of gamma distribution calculated by maximum likelihood. Non-parametric bootstrap analyses (Felsenstein, 1985) with 1000 pseudo-replicates were performed to obtain estimates of support for each node of the NJ trees. For MP analyses, we excluded constant and uninformative sites, weighted all characters and character transformations equally, and used the TBR branch swapping option. Because exhaustive and branch and bound analyses resulted in prohibitively long computation times, we used the heuristic search option with 1000 replicates of random sequence addition. Statistical support for recovered nodes was assessed using non-parametric bootstrap analysis with 1000 pseudo-replicates. For the ML analyses, we used likelihood ratio tests and the computer application MODELTEST 3.06 (Posada and Crandall, 1998) to determine the best suited model of sequence evolution. The best-fit model selected by MODELTEST for the cervine data set was the general time-reversible model (Rodriguez et al., 1990) with an allowance for invariant sites and a discrete gamma distribution (six categories) for among-site rate variation under the hierarchical likelihood ratio test method. Heuristic ML searches were performed with 10 replicates of random sequence addition and TBR branch swapping. ML bootstraps employed 100 iterations. The robustness of the ML phylogenies was also assessed by the number of times the group appeared after 1000 ML puzzling steps (Strimmer and von Haeseler, 1996) under the TN93 model (Tamura and Nei, 1993) of sequence evolution using PUZZLE 4.02. The substitution model of evolution found by MODELTEST and four incrementally heated chains were used (default temperature). The starting tree was chosen randomly and the analysis was run for 10^6 generations, with Markov chain sampling every 100 generations, resulting in 10,000 sampled trees. To insure that the Markov chain procedure did not become trapped in local optima, we used the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) algorithm with default setting implemented in MrBayes 2.0. Stationarity was assumed to have been reached when the likelihoods of the sample points reached a stable equilibrium (Huelsenbeck and Ronquist, 2001). Trees generated prior to stationarity were discarded as "burn-in." These cases correspond to the first 7.0% of the sampled trees. We ran the analyses twice, using different random starting seeds, in order to evaluate the congruence of the likelihood values (Huelsenbeck et al., 2002). To calculate the posterior probability of each node, a 50% majority-rule consensus tree was constructed from the remaining trees using PAUP*. The trees from the two Bayesian analyses were identical and the posterior probabilities for clades were almost identical.

For our comparative cervid groups we used representatives of the different cervid subfamilies (listed in Table 2). These were not outgroups as such, because they were not used to polarize the dataset, but as tests for general models of cervid relationships. The selection criterion was that they fell outside the distinct OW deer clade under scrutiny. Musk deer (*Moschus leucogaster*) served as outgroup. The latter taxon was selected for its close evolutionary relationship with the Cervidae based on molecular phylogenetic comparisons (Su et al., 1999; Hassanin and Douzery, 2003).

A likelihood ratio test for rate constancy (Felsenstein, 1988) was performed using PUZZLE 4.02, where the likelihood of the ML tree was compared to the likeli-

hood of the same tree with the constraint of a strict molecular clock. Because the rate heterogeneity among lineages was highly significant, we dated the nodes by using the nonparametric rate smoothing (NPRS) method of Sanderson (1997). This method estimates rates and divergence times by using a criterion that maximizes the autocorrelation of rates within clades. The ML tree with optimized branch lengths using PAUP* 4.0 was transformed into an ultrametric tree by using the NPRS algorithm implemented in the software TREEEDIT (version 1.0 α 4-61, August 2000, written by Andrew Rambaut and Mike Charleston and available at http://evolve.zoo.ox.ac.uk/software/TreeEdit/main.html). This approach does not assume a molecular clock, but assumes that rates of change tend to be similar between adjacent branches on the tree. It produces an ultrametric tree by minimizing the sum of squared changes in rate between ancestor and descendant branches across the tree. To transform relative time to absolute ages we calibrated the tree using dates from the fossil record. To compute error estimates for the ages inferred from the cyt *b* gene, we reapplied the NPRS procedure to 50 bootstrapped matrices obtained by resampling the data using PHYLIP 3.573c (Felsenstein, 1993). All absolute ages of the chronostratigraphic references were taken from the 1999 Geological Time Scale of the Geological Society of America (www.geosociety.org/science/timescale/timescl.pdf).

A lineages–time plot (Harvey et al., 1994) was constructed by plotting the log-transformed number of lineages against the age of the node (as estimated above). Under a constant birth–death model of diversification, if rates of speciation are uniform throughout the history of the clade, the lineages–time plot is expected to be a straight line of slope b–d (where b is the speciation rate and d is the extinction rate). Significant departure towards negative values means that the internal nodes are relatively too close to the root, whereas positive values mean that nodes are relatively too close to the tips, compared to the constant speciation rate model. To test the expectation of a rapid increase in the rate of species diversification in deer during the Pleistocene, we used the methods described by Paradis (1997) and implemented in the computer program DIVERSI version 0.2 (E. Paradis, Univ. of Montpellier, France, http://www.isem.univ-montp2.fr/ppp/phylogenie/ParadisHom.php). DIVERSI 0.2 provides Akaike information criteria (AIC) for the fit of the divergence times to each of three models. The model with the smallest AIC is selected. Model A assumes a constant rate of speciation throughout time, model B allows to vary through time, and model C assumes two different rates of diversification before and after a breakpoint in time. Based on the expectation of the Pleistocene speciation model, the breakpoint for model C was set to 1.8 mya. If an increase in the rate of diversification during the Pleistocene is projected, rejection of model A in favor of model B or model C is expected. If any increase in the rate of diversification occurred after 1.8 mya, AIC should favour model C over model B.

Finally, we listed geographical, ecological, morphological and selected behavioural character state differences for major cervine taxa (dataset available at http://arts.anu.edu.au/grovco) and traced them through the phylogeny uncovered by the molecular dataset using MacClade version 3.08 (Maddison and Maddison, 1999). This will allow to improve the understanding of the diversification and deployment of the *Cervinae*, leading to the proposal of a new taxonomic arrangement.

3. Results

3.1. Test for monophyly of OW deer

In all phylogenetic analyses of deer cyt *b* sequences, OW deer species formed a moderately to strongly supported monophylum with respect to the other cervid taxa (statistical support in trees: NJ 74%, MP 51%, ML 79%, and BI 99%, respectively). To illustrate this result obtained using different phylogenetic methods, a Bayesian 50% majority consensus tree is shown based on cyt *b* sequences from 49 cervid taxa (Fig. 1). In addition to cervine monophyly (clade III), BI placed the muntjacs as sister group of the OW deer (clade IV) with a posterior probability value of 96%. The remaining species also formed a monophyletic, although weakly (60%) supported assemblage (clade I). This topology corresponds to the anatomical feature (conditions of the lateral digits) based division into two monophyletic lineages, the telemetacarpalian (clade I) and the plesiometacarpalian (clade II), although basal splits within the family Cervidae are poorly resolved by other analytical methods. In brief, a basal multifurcation into five lineages (Cervinae, Muntiacinae, Alcinae, Odocoileinae, and Capreolinae plus Hydropotinae), emerged consistently under the MP, ML, and NJ algorithm (not shown). Within these topologies, the species *Rangifer tarandus* had an uncertain phylogenetic position. The antlerless Chinese water deer grouped solidly with the Capreolinae. Consequently, phylogenetic relationships among traditionally recognized cervid subfamilies could not be validated unambiguously based on the cyt *b* data set. Thus, we subsequently focused on the evolution of monophyletic species groups within OW deer.

3.2. Sequence variation, saturation and phylogenetic information content

Out of the 1140 bp of the cyt *b* gene sequenced, 338 sites were variable among OW deer of which 247 sites were phylogenetically informative. Codon variation

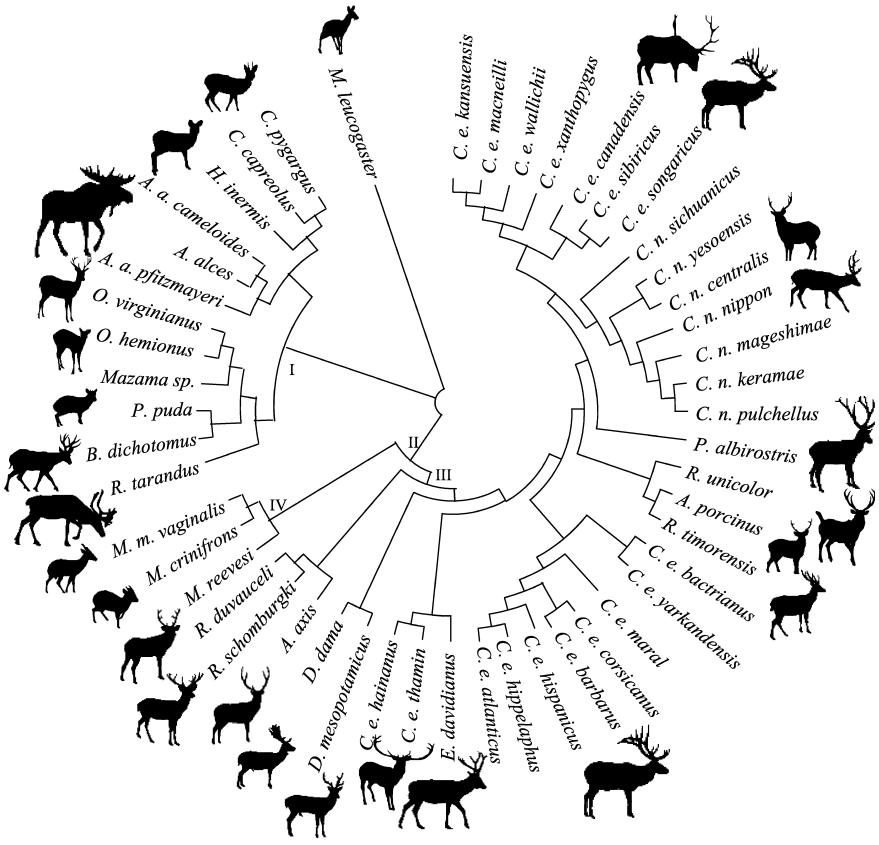

Fig. 1. Topology of the intrafamilial relationships among deer (Cervidae). The 50% majority rule consensus tree is based on the Bayesian MCMC sample of 10,000 trees inferred from the complete cytochrome *b* data set. The model of site substitution was the GTR model with rate parameters of 1.13(A–C), 15.23(A–G), 0.88(A–T), 0.91(C–G), 23.84(C–T), and 1.00(G–T), a proportion of invariable sites of 0.2515, and a gamma distribution shape parameter of $a = 0.5736$. The split into two distinct lineages, the telemetacarpalian (I) and plesiometacarpalian (II) deer is shown. The rooting point of the tree, however, remains unresolved. Bayesian analysis identified monophyletic Old World deer (III) with muntjacs (IV) as sister group.

was most pronounced at first (16.9%) and third (73.2%) positions, which accounted for 90.1% of the overall variation. Most phylogenetically informative mutations were silent (i.e. synonymous), however, nonsynonymous substitutions were inferred at 55 of the 380 codons. Uncorrected nucleotide sequence divergence among cervine taxa ranged from 0.09% to 12.5% (mean 6.4%). Mean divergences among the group of OW deer and all other true deer as well as the far outgroup (*Moschus leucogaster*) were 12.5% and 15.1%, respectively.

For the first, second, and third codon positions of the cervine cyt *b* sequences, the values for the index of substitution saturation I_{ss} (Xia et al., 2002) are 0.0334, 0.0065, and 0.1946, respectively. Given 34 OTUs and a sequence length of 380 bp, the critical $I_{ss.c}$ value is 0.6887 for a symmetrical true tree, and 0.4098 for an asymmetrical one. Both values are significantly greater than the observed I_{ss} values at all three codon positions rendering them far from saturation.

3.3. OW deer trees

All phylogenetic analyses yielded similar topologies (only the NJ tree is shown in Fig. 2). The statistical support for individual nodes, detailed according to analytical approach, is given in Table 4. The following description of phylogenetic relationships among cervine species begins with the inferred root of the tree and proceeds upwards to the more derived taxa.

Muntjacs are preferred in the NJ and BI trees as the sister group to the Cervinae, in agreement with Groves (1974), but in contrast to Azanza (1993) who placed them as sister group to Cervinae + Odocoileinae. The three outgroup species, *M. crinifrons*, *M. vaginalis*, and *M. reevesi*, are differentiated from the ingroup taxa by corrected genetic distances ranging from 0.123 to 0.154.

Starting at the base of the Cervinae phylogeny (Fig. 2), we observed a split between *Axis axis*, *Rucervus schomburgki*, and *Rucervus duvaucelii* on one hand, and the remaining Cervinae on the other. The former clade is not strongly supported (61–78% according to different methods), whereas the clade containing the remaining Cervinae has very strong MP support, but less by other methods. Within this basal group, *R. schomburgki* and *R. duvaucelii* are clearly sister species, with all methods giving 100% estimates of statistical support. Our results did not support a sister relationship between *A. axis* and

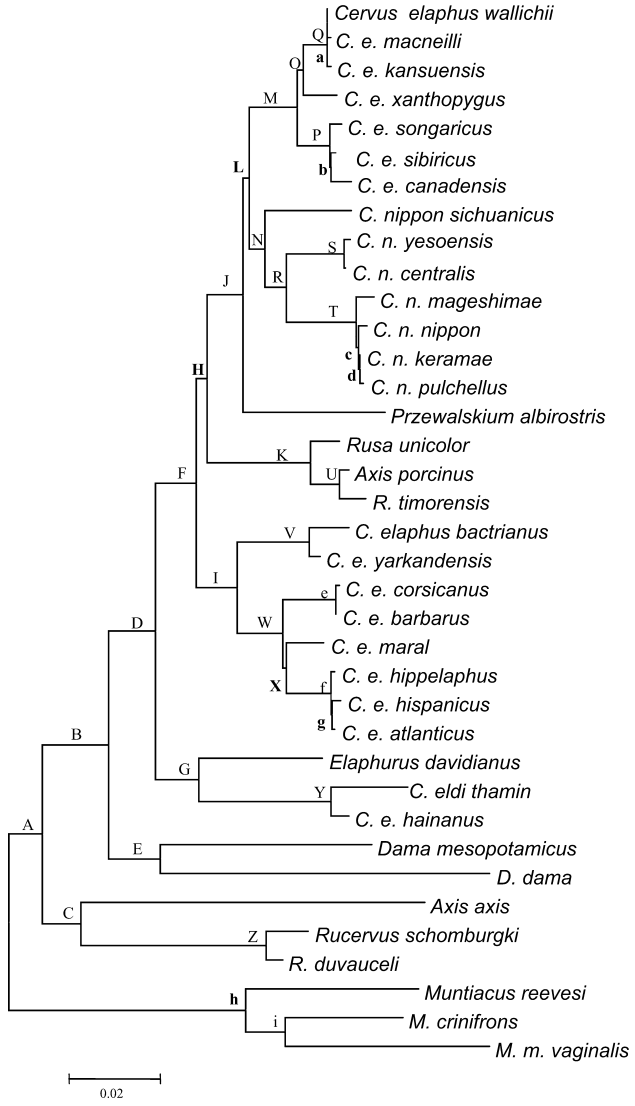

Fig. 2. Neighbor-joining tree showing relationships among OW deer mitochondrial cyt *b* lineages. The model of site substitution was the TN93 model. The MP-, ML-, and BI-topologies were similar. Support values for nodes, detailed according to analytical approach are listed in Table 4. Nodes indicated by bold letters are not supported by at least one out of four reconstruction methods.

Table 4
Statistical support for the internal nodes (A–i) shown in Fig. 2

Node	NJ	MP	ML	ML puzzle	BI
A	100	100	100	88	100
B	84	100	70	79	93
C	63	62	61	78	70
D	89	86	69	87	93
E	70	52	66	93	72
F	91	85	74	77	100
G	96	71	79	93	92
H	68	83	*	*	*
I	95	95	95	87	100
J	97	94	92	86	100
K	100	100	100	99	100
L	52	66	52	*	71
M	100	99	96	75	100
N	71	79	93	60	100
O	69	59	62	78	69
P	100	100	97	100	100
Q	99	99	100	83	100
R	83	79	86	60	90
S	100	100	100	65	100
T	100	100	100	88	100
U	98	98	83	100	100
V	100	100	100	99	100
W	99	95	95	91	100
X	42	*	*	75	*
Y	100	100	100	99	100
Z	100	100	99	84	100
a	70	68	*	85	*
b	81	*	*	96	*
c	81	*	*	82	*
d	67	29	*	69	*
e	100	100	100	99	100
f	100	100	100	87	100
g	58	*	*	69	*
h	100	100	*	*	100
i	77	60	57	95	51

Bootstrap values were obtained by different tree constructing methods, neighbour joining (NJ), maximum parsimony (MP), and maximum-likelihood (ML), from 1000 replications for nucleotide sequences. The posterior probability in percent was obtained by Bayesian inference (BI). Asterisk indicates that the node was not supported by the reconstruction method.

the hog deer *Axis porcinus*, indicating that the genus *Axis* is paraphyletic. Based on morphometric analyses, Meijaard and Groves (2004) remarked on the clear craniometric differences between the two subgenera of *Axis*.

All phylogenetic methods generated a monophyletic *Dama* clade and confirmed the two species of fallow deer, *D. dama* and *D. mesopotamica*, as sister species but with estimates of statistical support for this grouping being between 52% and 93%.

An unexpected finding within the cervine phylogeny was the strong support (71–96%) for a sister taxon relationship between Père David's deer *Elaphurus davidianus* and Eld's deer *C. eldi* (96% NJ, 93% ML, 92% BI). We will return to this unexpected finding later in the paper.

The next most derived clade constituted the genus *Cervus* comprising the putative subgenera *Cervus* (*sensu lato*), *Rusa*, *Sika*, and *Przewalskium*. In this clade, most relationships were highly resolved, but the basal division into a clade consisting of *C. elaphus* (*sensu stricto*), and a clade combining a rusa and hog deer group with a sika, wapiti and white-lipped deer group, was not at all strongly supported. Within the *Rusa* clade, the relationship between the hog deer *Axis* (or *Hyelaphus*) *porcinus* and the Timor deer *Rusa timorensis* was unexpected, but well supported in all phylogenetic analyses; their sister species is *Rusa unicolor*.

Red deer (*Cervus elaphus*) are divided into four distinct and monophyletic subspecific groups. The first group includes six subspecies from Europe (*C. e. hippelaphus*, *C. e. corsicanus*, *C. e. atlanticus*, *C. e. hispanicus*),

northern Africa (*C. e. barbarus*), and from Caucasia, Turkey and northern Iran (*C. e. maral*). The second group includes two subspecies from middle Asia (*C. e. bactrianus* and *C. e. yarkandensis*). These two groups form a well-supported monophylum. The third group includes two subspecies from Siberia (*C. e. songaricus* and *C. e. sibiricus*) and one from North America (*C. e. canadensis*), together referred to as wapiti. The fourth group includes four subspecies from China and Tibet (*C. e. wallichi*, *C. e. macneilli*, and *C. e. kansuensis* [often referred to as shou], and *C. e. xanthopygus*). The latter two groups form a compact, well-supported clade with sika (*C. nippon*). The white-lipped or Thorold's deer *Przewalskium albirostris* is equally part of this clade, constituting the sister species to a wapiti/shou/sika clade, but this division is not strongly supported.

3.4. Evolution of the cervinae

The MacClade optimization based on the phylogeny shown in Fig. 2 suggests the following evolutionary scenario for the Cervinae:

Node A. The cervine morphotype can be reconstructed on the assumption of parsimony. The primitive cervine was probably tropical in distribution, it certainly inhabited woodland or open country, not closed forest, and it probably lived in eastern Eurasia, or possibly India (not in western Eurasia or Southeast Asia). The antlers were built on the 3-point plan. The beam was anterior, continued by A2 following the Groves and Grubb (1987) nomenclature. The brow tine, characteristic of the Cervinae/Muntiacinae clade (Groves and Grubb, 1990) stood at a very obtuse angle to the beam. Above this, the P2 tine emerged from the beam at an acute angle. The plesiomorphic cervine lacked strongly developed head-pole and tail-pole display. Though moderately sexually dimorphic (the male's weight was 1.35 to 1.4 times that of the female), it lacked sexual dichromatism, had a long tail, little or no rump patch, and no flaring of the rump hairs. The mane was short. Canines were present, moderately developed (less than in muntjac). There were deep face glands. It was spotted when young, and spots may have survived, but in muted form, into maturity. The female in oestrus urinated with a slightly curved back. The male uttered a deep roaring vocalization in the rut, and sought tending bonds with the females, rather than trying to gather a harem. The dominance posture was head-high.

Node C. Support for this node is week to moderate (62–78% under different schemes) and there are no clear synapomorphies uniting *Axis axis* and *Rucervus*, with the possible exception of a slight tail reduction. The two subclades are both highly autapomorphic. *R. duvaucelii* has more complex antlers in which the angle between A2 and P2 has become a right angle and it has lost its face glands. *A. axis* has retained the simple antlers which were further simplified by closing up the angle between the brow tine and P1. It has retained its juvenile spots throughout life, its rump hairs flare as a signal and it has lost its canines. The association of *R. schomburgki* with *R. duvaucelii* is perhaps no surprise, because it has generally been placed in the genus or subgenus *Rucervus* along with *R. duvaucelii* and *C. eldi* (Ellerman and Morrison-Scott, 1951). One author (Giles, 1937) even proposed to reduce it to a subspecies of *R. duvaucelii*, although Pocock (1943) went in the opposite direction and described numerous cranial features differentiating Schomburgk's deer (based on a single available skull) from other rucervine species and assigned it to a new genus, *Thaocervus*.

Node B. In the present analysis, the clade containing the other Cervinae (the vast majority) is well supported, but morphological synapomorphies are few. The clade is of temperate origin, probably in temperate East Asia. Parsimony clearly indicates that the antlers were 4-point, but the only other synapomorphy in the display apparatus may be the female's deep-crouch urination posture.

Node E. The two species of fallow deer (*Dama*) form a specifically West Eurasian clade. The numerous apomorphies of the clade include the continuation of the beam as P2 (not as A2), a developed rump-patch, a lengthened tail used in display-urination, retention of spots in adult life, loss of canines, and gross sexual dimorphism with the male being twice as heavy as the female.

Node D. The non-*Dama* clade remained in temperate East Eurasia and was defined by the innovation of a harem mating strategy. Correlated with such strategy the antlers were more complex, more robust, usually with more tines or more rugosity, or both, and P3 pointing backwards.

Node G. This unites Père David's deer (*Elaphurus davidianus*) with the tropical Southeast Asian Eld's deer (*C. eldi thamin* and *C. e. hainanus*) which have in the past almost invariably been associated with *R. duvaucelii* in a genus or subgenus *Rucervus* (Ellerman and Morrison-Scott, 1951). Only Pocock (1943) gave them separate genera; he revived a 19th-century generic name, *Panolia*, for Eld's deer. We will discuss the status of Père David's deer below.

Node F. This well-supported node contains red deer, wapiti, sika, white-lipped deer, hog deer, sambar, and rusa, a highly diverse group which nonetheless has some detectable synapomorphies: reduced canines, reduced face glands, and antlers in which P2 continues the beam. The ancestral species was a temperate-zone inhabiting plesiomorphic sister species to the tropical Eld's deer.

Node I is the ancestor of the *Cervus elaphus* (*sensu stricto*: red deer) group, which differentiated in West Eurasian temperate woodlands. *C. elaphus* has large complex antlers in which the angle between the brow

tine (A1) and the beam (P1) is reduced to a right angle, while that between A3 and P3 has opened up to a right angle. The head-pole is further differentiated by the development of a mane in the male and the rump-pole is concomitantly specialized by the development of a prominent rump patch. Most (not all) taxa are characterized by the presence of a bez tine (A2) just above the brow tine and a "crown" at the tip. Those lacking the bez tine are the Corsico-Sardinian and North African taxa (*Cervus elaphus corsicanus* and *C. e. barbarus*). Deer were probably introduced to Corsica and Sardinia by humans (Vigne and Alcover, 1985). There are however, claims of Corsican red deer (*Cervus elaphus rossii*) from the Middle and Late Pleistocene (cited in Di Stefano and Petronio, 2002; but we have not seen the description of the fossils). The lack of a bez tine in Corsico-Sardinian red deer and North African deer, combined with the fact that the mtDNA clade uniting them has 99–100% support in all methods used, points strongly to North Africa as their source. Although, if the claims of Pleistocene deer on Corsica are upheld, it could be the other way around, i.e. Barbary deer developed after being brought by humans to Africa from Corsica/Sardinia. The absence of the bez tine in these small deer is most parsimoniously regarded as a loss, rather than to assume an independent acqusition by both the European mainland red deer (*maral*, *hippelaphus*, etc.) and the Central Asian *bactrianus/yarkandensis* clade.

Node H. This node is poorly supported and no morphological synapomorphies can clearly be associated with it. We conclude that the descendants of node F underwent a very rapid diversification into a tropical group (node K) and two temperate groups, the western one (node I) and the eastern one (node J).

Node K. This groups sambar *Rusa unicolor* and rusa *R. timorensis* with hog deer *Axis* [or *Hyelaphus*] *porcinus*. Such association has not been proposed before, but has nonetheless 99–100% statistical support. There are numerous synapomorphies defining it. The antlers are simple and the most parsimonious interpretation is that they are reduced to three points. This is associated with the tropical habitat, typically in heavy cover (sambar live in dense forest, hog-deer in thick grassland and undergrowth; only rusa are glade-loving grazers). They are coarse-coated deer which lost rump patch and mane (though this was redeveloped in the only open-habitat species, *R. timorensis*). They lost most traces of spots and reduced their sexual dimorphism (the male weighs less than 1.35 times the female) with the exception, again, of *R. timorensis*. They mate with tending bonds, lack deep rutting vocalizations and possess face glands. All these features are expected in tropical deer: these are heavy-cover species, which the cervine ancestor was not. The representative for open grazing, *R. timorensis*, having descended from a *unicolor/porcinus*-like ancestry, has adapted to its habitat in quite a different manner to the temperate red deer and wapiti.

Node J. This unites white-lipped or Thorold's deer (*Przewalskium albirostris*), the sika group (node N), and a group of deer which have hitherto been regarded as giant East Asian and North American forms of Red deer (node M). We propose the latter to be named the wapiti group, which in analogy to the sika group (node N) should be awarded species status as *C. canadensis*. as was also proposed by Randi et al. (2001) and Ludt et al. (2004). The name *canadensis* was introduced in 1777—far earlier than *songaricus* or *sibiricus* (1873 in both cases). The descendants of node J are temperate like those of node I, and Asian like those of node K. Like the descendants of node I, they have large and complex antlers. The basic antler form is probably that of *P. albirostris*. It has likely been reduced in the sika group but further complexified in the wapiti group, which has the most developed head-pole/rump-pole system of all cervids. All descendants of node J have manes and rump patches, mate with a harem system and have a head-low dominance posture. *P. albirostris* has a short tail and large rump patch. Sika (*Cervus nippon*) retains white spots in adulthood, at least seasonally, has sexual dichromatism, very strong sexual dimorphism (the male weighs twice as much as the female) and flaring rump hair. The members of the wapiti group parallel *C. elaphus* (*sensu stricto*) in one idiosyncratic feature, namely the development of a bez tine. This is such a striking addition to the basic antler that it had seemed certain that the deer possessing it had to be sister taxa, even conspecific (Ellerman and Morrison-Scott, 1951; Dolan, 1988). But the present study indicates that the bez tine must have developed in parallel in these groups.

A special case is the Père David's deer *Elaphurus davidianus* (node G). *Elaphurus* shares derived morphological, karyotypic, behavioural, and genetic characters with species that are otherwise not directly related, i.e. *C. elaphus*, *C. schomburgki*, *C. unicolor*, *C. nippon*, and *C. eldi*, which raises the possibility that the *Elaphurus*-lineage (there are several extinct species of *Elaphurus*) is of hybrid origin (see Meijaard and Groves, 2004). The hybridization, which likely occurred in the Late Pliocene or earlier (this being the date of the earliest fossils of the Père David's deer lineage, according to Taru and Hasegawa, 2002) involved both a rucervine and an elaphine parent. On the basis of our new data we are now in a position to substantiate this hypothesis. Inferring from the mitochondrial data the female parent was obviously *eldi* or a very close ancestral relative, as was also suggested by the results of a molecular phylogenetic study by Randi et al. (2001). Like Eld's deer, Père David's deer is adapted to swampy habitat, its antlers lack a bez tine, the beam is continued by A2, P3 points backwards and A3 branches. It has no rump patch, deep face glands and fairly large canines. The young are spotted

and the tail is long. The male parent was likely *C. canadensis* or an ancestral relative as suggested by the following arguments. Like wapiti, *E. davidianus* lives in a temperate climate, A3 and P3 are nearly at right angles, its dominance posture is head-low, the mane development is intermediate and females urinate with a crouch. Like both putative parents, Père David's deer has heavy, complex antlers, but lacks a brow tine which is unique among the Cervinae. It retains two primitive cervine conditions: a very long tail and the rutting male's vocalization, which is a deep roar.

3.5. Unequal rates of change and dating cervid divergences

Our phylogram for OW deer (Fig. 2) clearly portrays violation of a molecular clock, with interspersed long and short branches. A general-clock-like behavior was also rejected because the constrained and unconstrained analyses were significantly different in a likelihood ratio test of the OW deer (without clock: $-\ln = 6098.72$, with clock: $-\ln = 6275.15$, $P < 0.005$) and of the NW deer lineage (without clock: $-\ln = 5870.02$, with clock: $-\ln = 5905.57$, $P < 0.005$). Because the tests of rate heterogeneity among lineages were significant, we dated the nodes by using a tree-based methodology (Sanderson, 1997) that relies on fossil calibration of nucleotide substitution rates. Assignment of fossils to appropriate nodes followed guidelines discussed in Magallon and Sanderson (2001). To circumvent problems associated with employing a single calibration point to calculate rates of nucleotide substitution and subsequent estimation of divergence time, minimal age estimates were assigned to two nodes within cervid phylogeny. The oldest known New World deer fossil is *Eocoileus gentryorum* from sediments of the upper Bone Valley Formation in Florida, dated in the late Hemphillian age of the Pliocene, about 5.0 mya (Webb, 2000). The second fossil-calibrated node is the Muntiacinae/Cervinae

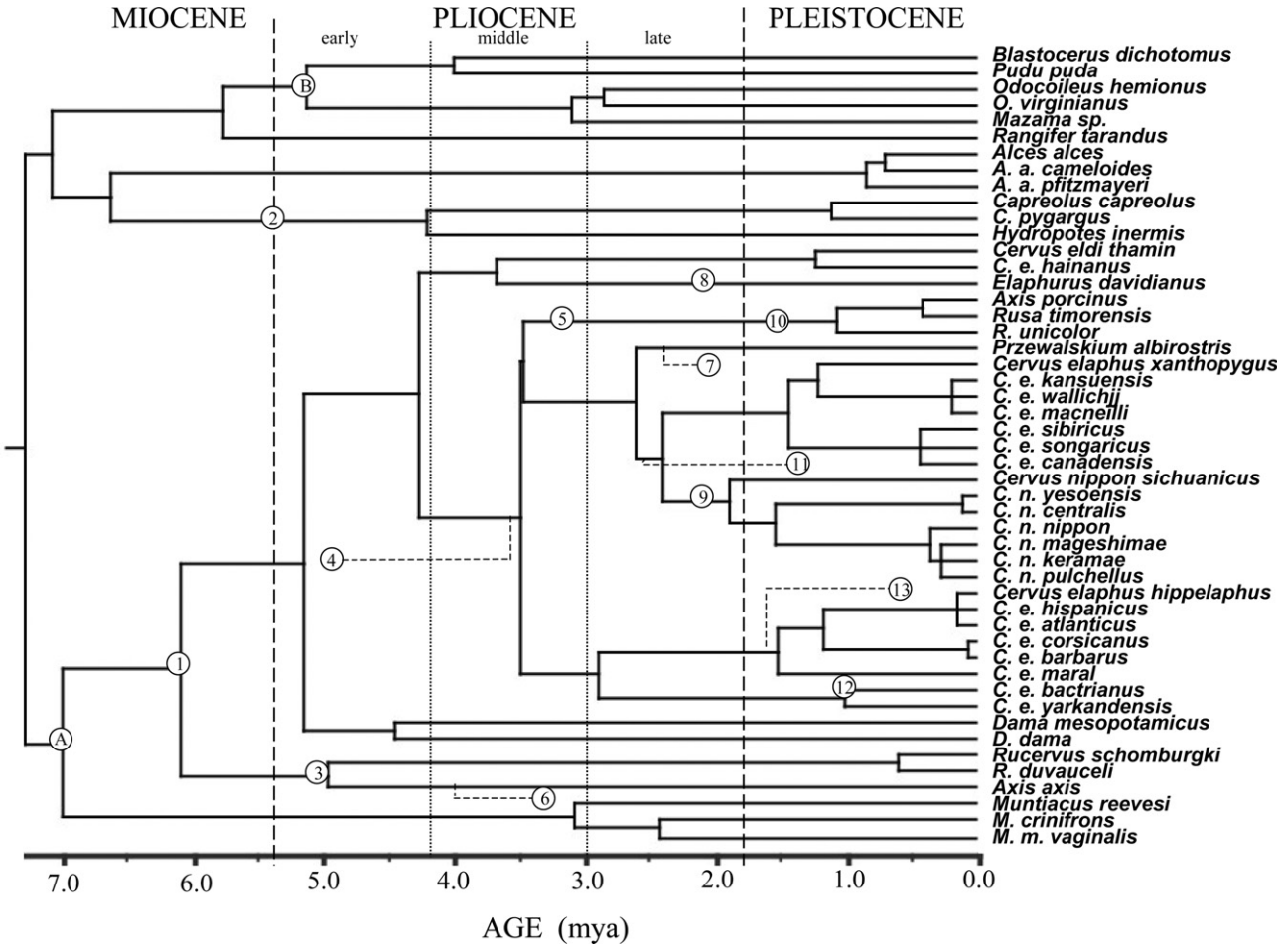

Fig. 3. Fossil-constrained phylogram (chronogram) based on Fig. 1. The node ages were estimated according to Sanderson's (1997) nonparametric rate smoothing (NPRS) method using TreeEdit and PAUP*. The fossil-calibrated nodes are the Muntiacinae/Cervinae split (A) and the origin of the New World Odocoileinae (B) constrained to have a minimum age of 7.0 and 5.0 my, respectively. Positions of numbered circles mark the age of fossils with their postulated lineage affinity (dotted line) as explained in the discussion section. Geological time scale is given in millions of years ago. As noted in the text, these ages may be somewhat too recent, but we are confident of their proportionality to one another.

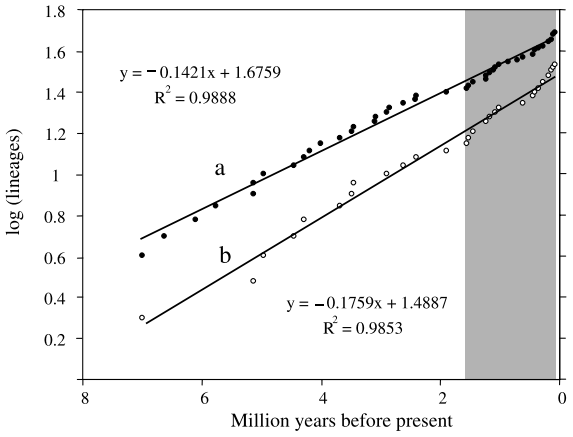

Fig. 4. Lineages–time plot for the radiation of deer using NPRS dates from Fig. 3. Graph (a) is the plot for all species sampled in our study, graph (b) includes OW deer taxa only. Regression line parameters are given and the Pleistocene epoch is shaded.

split. The oldest fossil remains assignable to this node appear in the Miocene deposit of Lufeng in China. The age of this fossil bed is 7.0 mya (Han, 1985) and was used as the minimal age estimate for the node that includes the most recent common ancestors (MRCA) of the two lineages. Despite uncertainty in assigning minimal age estimates to nodes in the NPRS phylogeny using the fossil record, the two calibration points independently resulted in very similar age estimates (Fig. 3). For example, estimation of divergence times using both calibrations points separately yielded a mean age estimate of 5.92 ± 0.49 mya ($CI_{95\%}$: 5.78–6.06) for the MRCA of the extant species of OW deer and of 6.87 ± 0.42 mya ($CI_{95\%}$: 6.73–7.00) for the NW deer, respectively. The rate of nucleotide substitution was estimated by linear regression as 0.0257 substitutions/site/my/lineage ($r = 0.95$), giving a pairwise rate of 5.14% per million years.

3.6. Rate of species diversification

To study the rate of diversification of lineages within deer, we used the lineages-through-time (LTT) plot approach (Harvey et al., 1994). Using the ultrametric cladogram (Fig. 3) obtained for estimating node ages (see above), we plotted the logarithm of the cumulative number of lineages against the absolute age of each node. The resulting LTT plot is shown in Fig. 4. Considering both the entire Cervidae data set (line a) and a subset of all OW deer (line b), the patterns observed are that of a roughly linear increase on the semi-log plot without any significant upturn in the slope towards Pleistocene. These findings were confirmed by comparing the data under three different models of diversification rates. The favoured model with the lowest AIC (91.961) was the constant speciation rate model when compared to alternative models that involved varying diversification rates through time (AIC: 93.882), or a different rate of diversification before and after the onset of Pleistocene (AIC: 93.957). When only OW deer species were included in the analysis (line b), the per-lineage net diversification rate from the NPRS tree was 0.514 ± 0.099 species per million years. This is comparable to diversification rates observed from fossil evidence during the radiations of Neogene horses (Hulbert, 1993). Moreover, the histogram of the distribution of reconstructed speciation events (Fig. 4) does not show an upward slope of the graph in the Pleistocene and thus does not support Geist's (1987) suggestion, according to which extant deer species originated mostly in the Pleistocene Epoch.

4. Discussion

Within the Cervinae, there is widespread homoplasy caused by climatically similar conditions. A cervine deer that enters a temperate region will develop large and complex display organs, typically based on the head-pole/rump-pole strategy. This extends in the case of the node F clade (Fig. 2) to the development of a bez tine in two independent lineages. This is presumably connected to rutting seasonality. Deer that remained tropical mostly retained a year-round or weakly seasonal breeding regime, an undifferentiated rump-pole and simple, stabbing antlers.

4.1. Integration of molecular and fossil data

In this study, using different tree reconstruction methods, more than one (namely two) calibration point, and the NPRS method we minimized errors of topology, calibration, and rate heterogeneity among lineages in order to explore the correspondence between the fossil record and our molecular-based age estimates (Fig. 3). The following checks of accuracy of divergence time estimates include minimum ages estimated conservatively by the first appearance of fossils referable to one of the constituent OW deer lineages. We realize of course, that the fossil record inevitably contains real gaps and incorporates many biases. The following numbered paragraphs refer to the accordingly numbered circles in Fig. 3.

(1) Di Stefano and Petronio (2002) named the Late Miocene *Cervocerus novorossiae* as the most primitive member of the Cervinae. While conflicting with the conclusions of Azanza (1993), this is much more consistent with the DNA, except that it would probably push back somewhat the date of the basal cervine node. Azanza's (1993) schema probably relates rather to the earliest radiations of the antlered Cervidae as a whole.

(2) If indeed the Asian genus *Procapreolus* is ancestral to *Capreolus*, its estimated age of Late Miocene-Early

Pliocene (Zdansky, 1925) would suggest that our nodes are in the right order of magnitude. It is unclear, however, whether the species *Procapreolus latifrons*, aged at ~6–5.5 mya, is or is not also ancestral to *Hydropotes*. We think probably not: *Procapreolus* has short 3-pointed antlers which are scarcely developed and usually lack tracks, crest, and pearls. The angle between the brow tine and the beam is always strongly acute, while the b span and the pedicles are very long. The pliomericine fold is present, even if sometimes scarcely developed. Molars are columnar and with weak interlobal styles (Di Stefano and Petronio, 2002).

(3) Di Stefano and Petronio (2002) named *Axis shansius*, which dates back to ~5 mya, as the earliest fossil of the lineage leading to *Axis axis*.

(4) Di Stefano and Petronio (2002) identify Early/Middle Pliocene *Cervus magnus* as the very first 4-point deer, i.e. part of the *elaphus*-like group. The implications are that the node that includes the *elaphus*/Wapiti/*nippon*/*Rusa*/*Hyelaphus* group needs to be pushed back.

(5) Di Stefano and Petronio (2002) reported that *Rusa elegans* (Middle Pliocene of Shaanxi) is "very close" to *R. unicolor*, as are the Pliocene European fossils referred to as *Pseudodama*. This is considerably earlier (about twice as early) than in the present DNA study. But the *unicolor* antler is doubtless the primitive one for the entire *unicolor*/*porcinus*/*timorensis* clade, so we can suppose that *elegans* could be anywhere along it, not necessarily on the actual *unicolor* clade.

(6) *A. lyra* of the European Mid/Late Pliocene follows *A. shansius* (Di Stefano and Petronio, 2002).

(7) Di Stefano and Petronio's (2002) assignment of late Pliocene *R. hilzheimeri* to the *albirostris* clade is in excellent agreement with our DNA dating.

(8) The earliest fossils of *Elaphurus* date back to the Late Pliocene (~3–2 mya) (Taru and Hasegawa, 2002), agreeing well with the present study.

(9) *C. nippon* fossils were first found in the Late Pliocene deposits of Europe (Thenius and Hofer, 1960 in Geist, 1998). This agrees well with the first divergence of the *nippon* clade (Fig. 2, node N) in the Late Pliocene.

(10) *A. lydekkeri* Dubois, 1908, a fossil species from Java dating back to ~1.5 mya (cited in Di Stefano and Petronio, 2002), could be related to *Axis axis* but is probably part of the *Hyelaphus* clade (Meijaard and Groves, 2004). This does not appear to be congruent with our phylogeny as it would push back the *porcinus*/*timorensis* node by ~1.1 my. On the other hand, we could make the case that the *Hyelaphus* morphology is plesiomorphic for the entire *Rusa*, so that the *A. lydekkeri* date might represent any point along the stem of the hog deer/sambar/rusa clade; this proposal, however, would not be compatible with the one made under (5), above, and we are unable to resolve the conflict at the moment.

(11) Di Stefano and Petronio (2002) identified *Cervus grayi* as the latest common ancestor to *Cervus nippon* and a lineage leading to the temperate *C. elaphus* (*sensu stricto*) and the wapiti group. According to them these two lineages split during the late Villafranchian (~1.3 mya), which is ca. 1 my later than suggested by the present mtDNA phylogeny. The DNA data of this and *all* other studies are quite clear about the non-monophyly of the *elaphus* group; we therefore take *C. grayi* as denoting the last common ancestry of the sika- and wapiti group.

(12) According to Di Stefano and Petronio (2002), *C. bactrianus* is a relict of the acoronate red deer that lived in Europe in the Early Pleistocene (Galerian, about 1 mya or a bit less).

(13) The first coronate (i.e. real *C. elaphus*) deer occur in the Late Galerian (about 0.5 mya). This seems consistent with the present phylogeny. At the same time, there are wapiti in "central-eastern Asia."

In conclusion, reliable fossil calibrations, large molecular data sets, and improved dating methods are shaping a molecular time scale for the evolution of deer that is largely compatible with existing palaeontological evidence. A few discrepancies may be caused by insufficient correction for rate heterogeneity among OW deer lineages, a too-recent dating of the Muntiacinae/Cervinae split, or a combination of these factors.

4.2. Taxonomic conclusions

The demarcation of genera is still a rather arbitrary affair, but recently it has been maintained that it is high time to adopt an objective standard; and that this standard should be time-depth (Goodman et al., 1998; Avise and Glenn, 1999; Groves, 2001a,b; Meijaard, 2003). These authors argued that separation by at least 7 mya (Goodman et al., 1998), or by 5 mya (Groves, 2001a,b; Meijaard, 2003), should be a requisite for recognizing a clade as a genus. Accordingly, in the chronogram of the Cervidae (Fig. 3) only two primary clades (telemetacarpals and plesiometacarpals) would meet the 7 mya criterion. If these two groups were to be assigned to genus level, then six genera within the former and eight in the latter would have to be downgraded to subgenus level or sunk altogether. Furthermore, the Muntiacini and Cervini would consist of one genus each, unless future studies show a time depth of >7 mya. Azanza (1993) suggested an earlier split between Muntiacini and Cervini; we have indicated above, however, that Azanza's (1993) schema relates rather to an earlier radiation amongst the most primitive, simple-antlered deer as a whole. A more workable taxonomy for the Cervinae, using the 5 mya criterion, would assign clades to genus level that separated before the end of the Miocene. Consequently, in the clade containing *R. schomburgki*, *R. duvaucelii*, and *Axis axis*, the former two

species should be assigned to the genus *Rucervus*. The genus *Axis* should be retained as such, but it excludes *Hyelaphus* (also see Meijaard and Groves, 2004). *Dama* diverged ~5.1 mya, just after the start of the Pliocene, sufficiently early to assign genus status to *Dama.* The remaining species should all be assigned to the genus *Cervus*. If required, the Eld's Deer clade (~4.2 mya) could be separated as subgenus *Panolia* and Père David's Deer as subgenus *Elaphurus*, but all other species should be retained in the nominotypical subgenus *Cervus*.

As far as species are concerned, there are likely more species in the Cervinae than have been customarily recognized. This is most obvious in the *elaphus*, sika and wapiti groups (Fig. 2). In the *elaphus* clade (descended from node I), the Central Asian *bactrianus/yarkandensis* subclade (node V) almost certainly represents a species distinct from *C. elaphus* (descendants from node W). Detailed study, however, is needed for verification and to elucidate the morphological differences between the two. Although genetic differences exist between them, it is not clear that the two are different at the morphological level (though the photos in Dolan (1988) certainly do look somewhat different). If they are to be combined, the name *Cervus yarkandensis* Blanford, 1892 has eight years' priority over *C. bactrianus* Lydekker, 1900. As descendants of node W, the North African/Corsico-Sardinian clade too almost certainly represents a species distinct from *C. elaphus* (node F), although detailed studies would be necessary to fully document this. The prior name for such a species would be *Cervus corsicanus* Erxleben, 1777, which has over half a century priority over the name *C. barbarus* Bennett, 1833.

Following the outline from the *elaphus* clade, in the sika clade (node N), geographical and genetic separation would warrant the introduction of at least three species concordant with the three subclades descendent from node N.

In the wapiti group (node M), the true wapiti (node P) inhabit North America and Central Asia (Tianshan, Altai, Great Khingan). Despite such wide distribution and fragmentation into numerous isolates, they form a single clade in the present study. Flerov (1952) did not separate them even at subspecies level, while Dolan (1988) acknowledged how alike they are, though he regarded them as forming several different subspecies. In concordance with Randi et al. (2001) and Ludt et al. (2004) we propose to assign them species status as *Cervus canadensis*. The deer from the southern and eastern rim of the Tibetan plateau (often called "shou," node Q) form a species separate from true wapiti and should be assigned species status too (*Cervus wallichi*; G. Cuvier, 1823). Genetically, there were only marginal differences among these deer. Groves (2003) argued that *C. hanglu* from Kashmir (not available in the present study) and *C. wallichii* from Bhutan, Sikkim and neighbouring parts of Tibet are good species; they share an idiosyncratic antler form with a mid-beam angulation, and a "muted" form of wapiti apomorphies, but are otherwise distinct from each other. The relationships of the remainder to these two, and to each other, are not so clear; the names *macneilli* Lydekker, 1909 ("Sichuan border of Tibet"), *kansuensis* Pocock, 1912 ("Thirty miles southeast of Tao-chow, Kansu") and *alashanicus* Bobrinskoi and Flerov, 1935 (Alashan Ridge) are available, but study of samples of known origin would be necessary to elucidate exactly which name refers to which population; variation in overall colour, rump patch shape, and antler (some populations seem to have the shou type, others the wapiti type) suggests that there is likely to be more than one taxon in this Sichuan/Gansu region. The final taxon which we would like to suggest as distinct species is *C. xanthopygus* from the Russian Far East (Amur basin) and neighbouring parts of China. We find it preferentially associated in a clade with the shou group; although this assemblage is admittedly not strongly supported (node O), *C. xanthopygus* is certainly not part of the true wapiti clade. Morphologically, it is strongly differentiated and both Flerov (1952) and Dolan (1988) recognize its distinctiveness, despite the fact that its range appears to insert between Asian and American populations of true wapiti. The Amur basin is a region where Siberian and Chinese faunas meet, and has well-differentiated taxa of both.

Given that different species of deer hybridize freely in the wild (Groves and Grubb, 1987), we would like to raise the possibility (to be tested in the future) that there might exist some hybrid taxa among the contemporary OW deer. *Elaphurus davidianus* is such a species that may well have resulted from an ancient introgressive hybridization event between parent species which are widely unrelated. Pitra et al. (2002) and more generally Seehausen (2004), discussed the role of ancient hybridization between closely related parents in diversification processes and postulated its likely occurrence in periods of adaptive radiation. We point out that in this study there are several poorly supported nodes which are difficult to distinguish from true polytomies. Future studies using nuclear DNA markers might profitably examine whether one or more of the species or clades arising from near-polytomies are of hybrid origin.

Acknowledgments

The authors thank Anke Schmidt for technical assistance. We would also thank the editor and two anonymous reviewers for their helpful comments and suggestions.

References

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.

Avise, J.C., Glenn, C.J., 1999. Proposal for a standardized temporal scheme of biological classification for extant species. Proc. Natl. Acad. Sci. USA 96, 7358–7363.

Azanza, B., 1993. Sur la nature des appendices frontaux des cervidés (Artiodactyla, Mammalia) du Miocêne inférieur et moyen. Remarques sur leur systématique et leur phylogénie. C. R. Acad. Sci. Paris II 316, 1163–1169.

Azanza, B., Menendez, E., 1990. Los ciervos fósiles del éogeno español. Paleontol. Evol. 23, 75–82.

Bachofen-Echt, A., 1939. Die fossilen Geweihe von Cervus elaphus and ihre Beziehungen zu den lebenden Formen. Paleontol. Zeit. 21, 68–76.

Beninde, J., 1937. Der Merkmalswert des Hirschgeweihs für die paläntologische Systematik der Gattung Cervus. Paleontol. Zeit. 19, 52–56.

Brooke, V., 1878. On the classification of the Cervidae, with a synopsis of the existing species. Proc. Zool. Soc. Lond. 1878, 883–928.

Bubenik, G.A., Bubenik, A.B., 1990. Horns, Proghorns, and Antlers: Evolution, Morphology, Physiology and Social Significance. Springer, New York.

Burger, J., Rosendahl, W., Loreille, O., Hemmer, H., Eriksson, T., Götherström, A., Hiller, J., Collins, M.J., Wess, T., Alt, K.W., 2004. Molecular phylogeny of the extinct cave lion *Panthera leo spelaea*. Mol. Phylogenet. Evol. 30, 841–849.

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

Cronin, M.A., 1991. Mitochondrial-DNA phylogeny of deer (Cervidae). J. Mammal. 72, 553–566.

Cronin, M.A., Stuart, R., Pierson, B.J., Patton, J.C., 1996. Kappa-casein gene phylogeny of higher ruminants (Pecora, Artiodactyla). Mol. Phylogenet. Evol. 6, 295–311.

Comincini, S., Sironi, M., Bandi, C., Giunta, C., Rubini, M., Fontana, F., 1996. RAPD analysis of systematic relationships among the Cervidae. Heredity 76, 215–221.

Cook, C.E., Wang, Y., Sensabaugh, G., 1999. A mitochondrial control region and cytochrome *b* phylogeny of sika deer (*Cervus nippon*) and report of tandem repeats in the control region. Mol. Phylogenet. Evol. 12, 47–56.

Cooper, A., Poinar, H.N., 2000. Ancient DNA: do it right or not at all. Science 289, 1139.

Darwin, C., 1871. The Descent of Man, and Selection in Relation to Sex, second ed. Murray, London.

Di Stefano, G., Petronio, C., 2002. Systematics and evolution of the Eurasian Plio-Pleistocene tribe Cervini (Artiodactyla, Mammalia). Geol. Romana 36, 311–334.

Dolan, J.M., 1988. A deer of many lands: a guide to the subspecies of Red Deer *Cervus elaphus* L.. Zoonooz 62 (10), 4–34.

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

Ellerman, J.R., Morrison-Scott, T.C.S., 1951. Checklist of Palaearctic and Indian Mammals, 1758 to 1947. British Museum (Natural History) Trustees, London.

Emlen, D.J., 2001. Costs and the diversification of exaggerated animal structures. Science 291, 1534–1536.

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

Felsenstein, J., 1988. Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. 22, 521–565.

Felsenstein, J., 1993. PHYLIP: Phylogeny Inference Package. University of Washington, Seattle, WA.

Flerov, K.K., 1952. Fauna of USSR: Mammals. 2. Musk Deer and Deer. Moscow, Leningrad: Academy of Sciences of USSR (English translation for National Science Foundation, Washington, DC, and Israel Program for Scientific Translations).

Geist, V., 1968. Horn-like structures as rank symbols, guards and weapons. Nature 220, 813–814.

Geist, V., 1987. On speciation in Ice Age mammals, with special reference to cervids and caprids. Can. J. Zool. 65, 1067–1084.

Geist, V., 1998. Deer of the World, Their Evolution, Behavior, and Ecology: Stackpole Books.

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

Giao, P.M., Tuoc, D., Dung, V.V., Wikramanayake, E.D., Amato, G., Arctander, P., MacKinnon, J.R., 1998. Description of *Muntiacus truongsonensis*, a new species of muntjac (Artiodactyla: Muntiacidae) from Central Vietnam, and implications for conservation. Anim. Conserv. 1, 61–68.

Giles, F.H., 1937. The riddle of *Cervus schomburgki*. J. SIAM Soc. (Nat. Hist. Suppl.) 11, 1–34.

Goodman, M., Porter, C.A., Czelusniak, J., Page, S.L., Schneider, H., Shoshani, J., Gunnell, G., Groves, C.P., 1998. Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence. Mol. Phylogenet. Evol. 9, 585–598.

Gould, S.J., 1974. The origin and function of bizarre structures: Antler size and skull size in the Irish elk, *Megaloceros giganteus*. Evolution 8, 191–200.

Groves, C.P., 1974. A note on the systematic position of the Muntjac (Artiodactyla, Cervidae). Z. Saugetierk 39, 369–372.

Groves, C.P., 2001a. Time and taxonomy. Ludus Vitalis 9 (15), 91–96.

Groves, C.P., 2001b. Primate Taxonomy. Smithsonian Institution Press, Washington, DC.

Groves, C.P., 2003. Taxonomy of ungulates of the Indian subcontinent. J. Bombay Nat. Hist. Soc. 100, 341–362.

Groves, C.P., Grubb, P., 1987. Relationships of living deer. In: Wemmer, C.M. (Ed.), Biology and Management of the Cervidae. Smithsonian Inst. Press, Washington, DC.

Groves, C.P., Grubb, P., 1990. Muntiacidae. In: Bubenik, G.A., Bubenik, A.B. (Eds.), Horns, Pronghorns and Antlers Horns, Proghorns, and Antlers: Evolution, Morphology, Physiology and Social Significance. Springer, New York.

Grubb, P., 1993. Order Artyodactyla. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World. A Taxonomic and Geographic Reference, second ed. Smithsonian University Press, Washington, DC.

Han, D., 1985. Preliminary study of the fossils of the order Artiodactyla from the ancient fossil locality of Lufeng: Renlixue Xuebao. Acta Anthropol. Sinica 4, 4–54.

Harvey, P.H., May, R.M., Nee, S., 1994. Phylogenies without fossils. Evolution 48, 523–529.

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.

Holland, B.R., Penny, D., Hendy, M.D., 2003. Outgroup misplacement and phylogenetic inaccuracy under a molecular clock—a simulation study. Syst. Biol. 52, 229–238.

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

Huelsenbeck, J.P., Larget, B., Miller, R.E., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51, 673–688.

Hulbert Jr., R.C., 1993. Taxonomic evolution in North American Neogene horses (subfamily Equinae): the rise and fall of an adaptive radiation. Paleobiology 19, 216–234.

Janis, C.M., Scott, K.M., 1987. The interrelationships of higher ruminant families with special reference on the members of the Cervoidea. Am. Mus. Novit. 2893, 1–85.

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

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, 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.

Kumar, S., Tamura, K., Nei, M., 1993. MEGA*: Molecular Evolutionary Genetics Analysis, Version 1.02. Pennsylvania State University, University Park.

Kuwayama, R., Ozawa, T., 2000. Phylogenetic relationships among european red deer, wapiti, and sika deer inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 15, 115–123.

Li, M., Tamate, H.B., Wei, F.-W., Wang, X.-M., Masuda, R., Sheng, H.-L., Ohtaishi, N., 2003. Phylogenetic relationships among deer in China derived from mitochondrial DNA cytochrome *b* sequence. Acta Theriol. 48, 207–219.

Loomis, F.B., 1928. Phylogeny of the deer. Am. J. Sci. 16, 531–542.

Ludt, J.C., Schroeder, W., Rottmann, O., Kuehn, R., 2004. Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). Mol. Phylogenet. Evol. 3, 1064–1083.

Maddison, W.P., Maddison, D.W., 1999. MacClade: Analysis of Phylogeny and Character Evolution, Version 3.08. Sinauer Associates, Sunderland, MA.

Magallon, S., Sanderson, M.J., 2001. Absolute diversification rates in angiosperm clades. Evolution 55, 1762–1780.

Meijaard, E., 2003. Solving mammalian riddles. A reconstruction of the Tertiary and Quaternary distribution of mammals and their palaeoenvironments in island South-East Asia. Department of Anthropology and Archaeology, Australian National University, Canberra, Australia.

Meijaard, E., Groves, C.P., 2004. Morphometrical relationships between South-east Asian deer (Cervidae, tribe Cervini): evolutionary and biogeographic implications. J. Zool. 263, 179–196.

Miyamoto, M.M., Kraus, F., Ryder, O.A., 1990. Phylogeny and evolution of antlered deer determined from mitochondrial DNA sequences. Proc. Natl. Acad. Sci. USA 87, 6127–6131.

Mohr, E., 1968. Haltung und Zucht des Schomburgk-Hirsches, *Rucervus schomburgki* Blyth, 1863. D. Zool. Garten (NF) 36, 34–57.

Paradis, E., 1997. Assessing temporal variations in diversification rates from phylogenies: estimation and hypothesis testing. Proc. R. Soc. Lond. B 264, 1141–1147.

Pitra, C., Hansen, A.J., Lieckfeldt, D., Arctander, P., 2002. An exceptional case of historical outbreeding in African sable antelope populations. Mol. Ecol. 11, 1197–1208.

Pocock, R.I., 1943. The larger deer of British India. Part II. J. Bombay Nat. Hist. Soc. 43, 553–572.

Poe, S., 1998. Sensitivity of phylogeny estimation to taxonomic sampling. Syst. Biol. 47, 18–31.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Polziehn, R.O., Strobeck, C., 2002. A phylogenetic comparison of red deer and wapiti using mitochondrial DNA. Mol. Phylogenet. Evol. 22, 342–356.

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

Randi, E., Mucci, N., Claro-Hergueta, F., Bonnet, A., Douzery, E.J.P., 2001. A mitochondrial DNA control region phylogeny of the Cervinae: speciation in *Cervus* and implications for conservation. Anim. Conserv. 4, 1–11.

Rodriguez, F., Oliver, J.F., Marin, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142, 485–501.

Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14, 1218–1231.

Seehausen, O., 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. 19, 198–207.

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

Su, B., Wang, Y.-X., Lan, H., Wang, W., Zhang, Y., 1999. Phylogenetic study of complete cytochrome b genes in musk deer (genus *Moschus*) using museum samples. Mol. Phylogenet. Evol. 12, 241–249.

Swofford, D.L., 2001. PAUP*: phylogenetic analysis using parsimony (* and Other Methods). Sinauer Associates, Sunderland, MA.

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

Taru, H., Hasegawa, Y., 2002. The Plio-Pleistocene fossil mammals from the Kasumi and Tama Hills. Mem. Nat. Sci. Mus. 38, 43–56.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 15 (25), 4876–4882.

Vigne, J.-D., Alcover, J.A., 1985. Incidences des relations historiques entre l'homme et l'animal dans la composition actuelle du peuplement amphibian, reptilian et mammalian desîles de Méditerranée occidentale. Actyes 110^{e} Cong. Nat. Soc. Savantes, Montpellier, Sci. II, 79–91.

Vislobokova, I.A., Godina, A.Y., 1993. Application of the structural analysis of frontal appendages for determining the systematic position of fossil higher ruminants. Paleontol. J., 108–123.

Vrba, E.S., Schaller, G.B., 2000. Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics, and Conservation. Yale University Press, New Haven.

Webb, D., 2000. Evolutionary history of New World Cervidae. In: Vrba, E.S., Schaller, G.B. (Eds.), Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics, and Conservation. Yale University Press, New Haven.

Xia, X., Xie, Z., 2001. DAMBE: software package for data analysis in molecular biology and evolution. J. Hered. 92, 371–373.

Xia, X., Xie, Z., Salemi, M., Chen, L., Wang, Y., 2002. An index of substitution saturation and its application. Mol. Phylogenet. Evol. 26, 1–7.

Zwickl, D.J., Hillis, D.M., 2002. Increased taxon sampling greatly reduces phylogenetic error. Syst. Biol. 51, 588–598.