

**Supplementary Information**

**Phylogeny and diversity of moose (*Alces alces*, Cervidae, Mammalia) revealed by complete mitochondrial genomes**

M. Świsłocka, M. Matosiuk, M. Ratkiewicz, A. Borkowska, M. Czajkowska, P. Mackiewicz

**Table S1:** List of primer pairs used for PCR and sequencing of mitogenomes in *Alces alces*.

Primer	Primer sequence	Position	Tm [°C]	Product [bp]	Genes	Primer source
12S-FW	GGTAAATCTCGTGCCAGCCA	00295	57.3	712	<12s_rRNA>	Fajardo et al., 2007†
12S-REV	TCCAGTATGCTTACCTTGTTACGAC	01007	56.2			
00871c_F	TGCTTAGTTGAATTAGGCAATG	00872	51.3	1176	<12s_rRNA...tRNA-Val...16s_rRNA>	Matosiuk et al., 2014‡
02052c_R	AGAGAACAAGTGATTATGCTACC	02048	52.2			
01950c_F	ACCTCCAGCATAACTAGTATTGG	01945	53.7	1455	<16s_rRNA...tRNA-Leu...ND1>	Matosiuk et al., 2014‡
03402c_R	AATGGTCCTGCTGCATACTCTA	03400	55.2			
03140c_F	CTACGAGCAGTAGCTCAAACA	03138	54.1	1025	<ND1...tRNA-Ile...tRNA-Gln...tRNA-Met...ND2>	Matosiuk et al., 2014‡
04165c_R	ACAGTTCATTGGCCTGAAAATA	04163	52.5			
3910a_F	CCTTCCCGTACTAATAAACCC	03894	50.0	1519	<tRNA-Met...ND2...tRNA-Trp...tRNA-Ala...>	This study
4300a_F2	TCATCAGGCCTAATTCTACT	04279	-		<tRNA-Asn...tRNA-Cys...tRNA-Tyr...COX1>	
5430a_R	TATGCCTGCTCARGCACCAA	05413	56.0			
COX1_F	TCAGCCATTTTACCTATGTTCA	05315	51.7	826	<tRNA-Tyr...COX1>	GenBank§
COX1_R	ATRTAGCCAAARGGTTCTTTTT	06141	48.5			
06060a_F	TCTTTGGACACCCCGAAGTA	06039	55.2	991	<COX1...tRNA-Ser...tRNA-Asp...COX2>	This study
07050a_R	ATGGGGTAAGCCATATGAGG	07030	53.8			
06090a_F	TCGTAACATACTACTCAGGG	06099	50.2	1503	<COX1...tRNA-Ser...tRNA-Asp...COX2>	This study
07610a_R	ATTGTCCGTAGTACAGGC	07602	51.1			
07420a_F	GTAGATAACCGAGTCGTTCTACC	07428	54.0	1502	<COX2...tRNA-Lys...ATP8...ATP6...COX3>	This study
08930a_R	AGGCTTGAGTGGTAAAATGCTC	08930	55.4			
08750a_F	CAATAGTCCTATTAACACTTGGC	08729	51.4	819	<COX3...tRNA-Gly...ND3>	This study
09560a_R	TAATTGAGGGAGTCAGAATGC	09548	52.1			
09340a_F	AGCCCGTGTGATATTGAC	09321	55.4	1102	<COX3...tRNA-Gly...ND3...tRNA-Arg...ND4L...ND4>	This study
10440a_R	AGTGGTGTGGCTGGCTATT	10423	56.5			

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Table S1: (continued) List of primer pairs used for PCR and sequencing of mitogenomes in *Alces alces*.

Primer	Primer sequence	Position	Tm [°C]	Product [bp]	Genes	Primer source
10270a_F 11660a_R	CAATTCCACAACACTCACAGCCTA AGCAGTTCTTGCGTGCTTTT	10248 11642	55.0 55.7	1394	<ND4...tRNA-His...tRNA-Ser>	This study
11466c_F 13062c_R	CACGAGAAAATGCCCTCATATCA ATCGAGTTGTCCTAAGAGTGC	11465 13060	54.9 56.2	1595	<ND4...tRNA-His...tRNA-Ser...tRNA-Leu...ND5>	Matosiuk et al., 2014‡
12965c_F 14224c_R	CAACGCCTGAGCCCTCTTA TGGGAGATCAATGAATGCGTT	12963 14222	56.7 54.4	1259	<ND5...ND6...tRNA-Glu...cytb>	Matosiuk et al., 2014‡
ML103 ML104	GACTAATGATATGAAAAACCATCGTTG TTGTTCTTCATCTCTGGTTTACAAGAC	14108 15352	52.8 55.0	1244	<tRNA-Glu...cytb...tRNA-Thr>	Chikuni et al., 1995¶
15150a_F 15470a_R	CACTCACATGAATCGGAGGA AAGGTTCTTGAATTTTGTGGAA	15157 15472	53.9 50.9	315	<cytb...tRNA-Thr...tRNA-Pro...d-loop>	This study
LGL283 ISM015	TACACTGGTCTTGTAAC TGGCCCTGTAGAAAGAAC	15319 15997	46.0 51.5	678	<tRNA-Thr...tRNA-Pro...d-loop>	Hundertmark et al., 2002#
15840a_F 00320a_R	ATGCGTATCCCGTCCATTAG GTGGCTGGCACGAGATTTAC	15840 00315	54.1 56.3	893	<d-loop...tRNA-Phe...12s_rRNA>	This study
16290a_F 16410a_R	TCCTTATATAGTTACCACTA CTGCGCTTAAATAGTTACCTTG	16293 16406	43.3 51.9	113	<d-loop>	This study

†Fajardo V., González I., Lopez-Calleja I., Martín I., Rojas M., Hernández P.E., García T., Martín R., 2007. Identification of meats from red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*) using polymerase chain reaction targeting specific sequences from the mitochondrial 12S rRNA gene. *Meat Science* 76: 234-240.

‡Matosiuk M., Sheremetyeva I.N., Sheremetyev I.S., Saveljev A.P., Borkowska A., 2014. Evolutionary neutrality of mtDNA introgression: evidence from complete mitogenome analysis in roe deer. *J. Evol. Biol.* 27: 2483-2494.

§GenBank acc. num. GQ336878.1 PCR primers.W

¶Chikuni K., Mori Y., Tabata T., Saito M., Monma M., Kosugiyama M., 1995. Molecular phylogeny based on the kappa-casein and cytochrome b sequences in the mammalian suborder Ruminantia. *J. Evol. Biol.* 41: 859866.

# Hundertmark K.J., Shields G.F., Udina I.G., Bowyer R.T., Danilkin A.A., Schwartz C.C., 2002. Mitochondrial phylogeography of moose (*Alces alces*): late pleistocene divergence and population expansion. *Mol. Phyl. Evol.* 22: 375-387.

## Supplementary Information

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**Table S2:** List of ungulate species and GenBank accession numbers of their mitogenomic sequences used in phylogenetic analysis.

Scientific name	English name	GenBank acc. num.
<i>Axis axis</i>	Chital	JN632599.1
<i>Axis porcinus</i>	Indian hog deer	JN632600.1
<i>Blastocerus dichotomus</i>	Marsh deer	JN632603.1
<i>Bos taurus</i>	Koren native cattle	NC_006853.1
<i>Capreolus capreolus</i>	European roe deer	JN632610.1
<i>Capreolus pygargus</i>	Siberian roe deer	NC_025271.1
<i>Cervus albirostris</i>	Thorold's deer	JN632690.1
<i>Cervus canadensis songaricus</i>	Tian Shan wapiti	HQ191429.1
<i>Cervus elaphus</i>	Red deer	NC_007704.2
<i>Cervus hanglu yarkandensis</i>	Yarkland deer	GU457435.1
<i>Cervus nippon yesoensis</i>	Yezo sika deer	AB210267.2
<i>Dama dama</i>	Fallow deer	JN632629.1
<i>Dama mesopotamica</i>	Persian fallow deer	NC_024819.1
<i>Elaphodus cephalophus</i>	Tufted deer	DQ873526.1
<i>Elaphurus davidianus</i>	Pere David's deer	JN632632.1
<i>Hippocamelus antisensis</i>	Taruca	JN632646.1
<i>Hydropotes inermis</i>	Water deer	NC_011821.1
<i>Mazama americana</i>	Red brocket	JN632656.1
<i>Mazama gouazoupira</i>	Gray brocket	JN632658.1
<i>Mazama nemorivaga</i>	Amazonian brown brocket	NC_024812.1
<i>Mazama rufina</i>	Dwarf red brocket	JN632661.1
<i>Moschus berezovskii</i>	Forest musk deer	NC_012694.1
<i>Muntiacus crinifrons</i>	Black muntjac	AY239042.1
<i>Muntiacus feae</i>	Fea's muntjac	NC_041100.1
<i>Muntiacus muntjak</i>	Southern red muntjac	AY225986.1
<i>Muntiacus putaoensis</i>	Leaf muntjac	NC_036430.1
<i>Muntiacus reevesi</i>	Reeves' muntjac	AF527537.1
<i>Muntiacus vaginalis</i>	Northern red muntjac	MK050505.1
<i>Muntiacus vuquangensis</i>	Large-antlered muntjac	NC_016920.1
<i>Odocoileus hemionus</i>	Mule deer	JN632670.1
<i>Odocoileus virginianus</i>	White-tailed deer	HQ332445.1
<i>Ovis aries</i>	Domestic sheep	NC_001941.1
<i>Ozotoceros bezoarticus</i>	Pampas deer	JN632681.2
<i>Panolia eldii</i>	Eld's deer	JN632697.1
<i>Pudu mephistophiles</i>	Northern pudu	JN632691.1
<i>Pudu puda</i>	Southern pudu	JN632692.1
<i>Rangifer tarandus</i>	Rein deer	AB245426.1
<i>Rucervus duvaucelii</i>	Brasingha	JN632696.1
<i>Rusa alfredi</i>	Philippine spotted deer	JN632698.1
<i>Rusa timorensis</i>	Javan deer	JN632699.1
<i>Rusa unicolor</i>	Sambar	NC_031835.1

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**Table S3:** Substitution models and partitions applied for the Cervidae mitogenomic set.

Software	Substitution model	Partitions
IQ-TREE	TIM2+F+I+Γ4	atp6.1, atp8.1, cyt.1, nd2.1, nd4.1, nd5.1, rRNA 12s, tRNA-Asp, tRNA-His, tRNA-Thr, tRNA-Val
	TIM3+F+I+Γ4	atp6.2, cyt.2, nd1.2, nd2.2, nd3.2, nd4.2, nd4L.2, nd5.2
	TN+F+I+Γ4	atp6.3, atp8.3, cox1.3, cox2.3
	TPM2u+F+R3	atp8.2, tRNA-Gly, control region
	TNe+I+Γ4	cox1.1
	HKY+F+R2	cox1.2, cox2.2, cox3.2, nd6.2, tRNA-Met
	TIM3e+I+Γ4	cox2.1, cox3.1, nd1.1, nd3.1, nd4L.1, tRNA-Asn, tRNA-Leu1
	TN+F+R4	cox3.3, cyt.3, nd1.3, nd2.3, nd3.3, nd4.3, nd4L.3, nd5.3
	TPM2u+F+I+Γ4	nd6.1, tRNA-Ala, tRNA-Gln, tRNA-Glu, tRNA-Pro, tRNA-Ser1
	HKY+F+I+Γ4	nd6.3
	TIM2+F+I+Γ4	rRNA 16s, tRNA-Arg, tRNA-Cys, tRNA-Ile, tRNA-Leu2, tRNA-Lys, tRNA-Phe, tRNA-Ser2, tRNA-Trp, tRNA-Tyr
MrBayes	mixed+I+Γ5	nd4.1, nd2.1, atp6.1, nd3.1, tRNA-Thr, cyt.1
	mixed+I+Γ5	atp6.2, nd4.2, nd3.2
	mixed+I+Γ5	atp6.3
	mixed+Γ5	atp8.1
	mixed+I+Γ5	atp8.2
	mixed+I+Γ5	atp8.3
	mixed+I+Γ5	cox1.1
	mixed+I	cox2.2, cox1.2
	mixed+I+Γ5	cox2.3, cox1.3
	mixed+I+Γ5	cox2.1
	mixed+I+Γ5	nd4L.1, cox3.1, tRNA-Asn
	mixed+I+Γ5	cox3.2, tRNA-Met, tRNA-Leu1
	mixed+I+Γ5	nd3.3, nd1.3, cox3.3, nd4L.3, nd4.3
	mixed+I+Γ5	cyt.2, nd4L.2, nd1.2
	mixed+I+Γ5	cyt.3
	mixed+Γ5	nd1.1
	mixed+I+Γ5	nd2.2, nd5.2
	mixed+Γ5	nd2.3
	mixed+I+Γ5	rRNA 12s, nd5.1
	mixed+I+Γ5	nd5.3
	mixed+I+Γ5	nd6.1
	mixed+I+Γ5	nd6.2
	mixed+I+Γ5	nd6.3
	mixed+I+Γ5	tRNA-Gly, rRNA 16s, tRNA-Trp
	mixed+I+Γ5	tRNA-Gln, tRNA-Ser1, tRNA-Pro, tRNA-Ala, tRNA-Glu
	mixed+I+Γ5	tRNA-Phe, tRNA-Arg
	mixed+I+Γ5	tRNA-Asp, tRNA-Ser2, tRNA-Val, tRNA-His
	mixed+Γ5	tRNA-Cys, tRNA-Lys
mixed+I+Γ5	tRNA-Ile, tRNA-Tyr, tRNA-Leu2	
mixed+I+Γ5	control region	

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**Table S4:** Substitution models and partitions applied for the *Alces* mitogenomic set.

Software	Substitution model	Partitions
IQ-TREE	HKY+F+I	atp6.1, nd2.1, nd4.1, nd5.1, rRNA 12s, rRNA 16s, tRNA-Gly, tRNA-Phe, tRNA-Ser2, tRNA-Val
	HKY+F	atp6.3, atp8.3, cox1.3, cox2.3, cox3.3, cyt.3, nd1.3, nd2.3, nd3.3, nd4.3, nd4L.3, nd5.3
	K2P	cox1.1, cox3.1, cyt.1, nd1.1, nd3.1, nd4L.1, tRNA-Cys, tRNA-Met
	F81+F	nd5.2
	HKY+F HKY+F+I	nd6.3, tRNA-Ala, tRNA-Gln, tRNA-Glu, tRNA-Pro control region
MrBayes	mixed	atp6.1
	mixed	nd4.3, cox1.3, atp6.3
	mixed	nd1.3, cox2.3, atp8.3
	mixed	nd4L.1, tRNA-Cys, cox1.1
	mixed	cox3.1, tRNA-Met, cyt.1, nd1.1
	mixed	cox3.3, nd5.3, nd3.3
	mixed	cyt.3, nd2.3, nd4L.3
	mixed+I	tRNA-Phe, nd2.1, rRNA 12s, tRNA-Ser2
	mixed	nd3.1
	mixed+I	tRNA-Gly, tRNA-Val, nd4.1, rRNA 16s, nd5.1
	mixed	nd5.2
	mixed	nd6.3
	mixed	tRNA-Glu, tRNA-Ala, tRNA-Pro, tRNA-Gln
mixed+I+Γ5	control region	

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**Table S5:** Substitution models and partitions applied for two mitogenomic set in molecular dating in BEAST.

Data set	Substitution model	Partitions
Cervidae	GTR+I+Γ5	nd4.1, nd2.1, atp6.1, nd3.1, tRNA-Thr, cyt.1
	GTR+I+Γ5	atp6.2, nd4.2, nd3.2
	GTR+I+Γ5	atp6.3
	TrN+Γ5	atp8.1
	HKY+I+Γ5	atp8.2
	HKY+I+Γ5	atp8.3
	TrNef+I+Γ5	cox1.1
	HKY+I	cox2.2, cox1.2
	TIM+I+Γ5	cox2.3, cox1.3
	TrNef+I+Γ5	cox2.1
	SYM+I+Γ5	nd4L.1, cox3.1, tRNA-Asn
	HKY+I+Γ5	cox3.2, tRNA-Met, tRNA-Leu1
	TIM+I+Γ5	nd3.3, nd1.3, cox3.3, nd4L.3, nd4.3
	TIM+I+Γ5	cyt.2, nd4L.2, nd1.2
	TrN+I+Γ5	cyt.3
	GTR+Γ5	nd1.1
	GTR+I+Γ5	nd2.2, nd5.2
	TrN+Γ5	nd2.3
	GTR+I+Γ5	rRNA 12s, nd5.1
	TrN+I+Γ5	nd5.3
	GTR+I+Γ5	nd6.1
	HKY+I+Γ5	nd6.2
	HKY+I+Γ5	nd6.3
	GTR+I+Γ5	tRNA-Gly, rRNA 16s, tRNA-Trp
	GTR+I+Γ5	tRNA-Gln, tRNA-Ser1, tRNA-Pro, tRNA-Ala, tRNA-Glu
	HKY+I+Γ5	tRNA-Phe, tRNA-Arg
	TrN+I+Γ5	tRNA-Asp, tRNA-Ser2, tRNA-Val, tRNA-His
	TVM+Γ5	tRNA-Cys, tRNA-Lys
GTR+I+Γ5	tRNA-Ile, tRNA-Tyr, tRNA-Leu2	
GTR+I+Γ5	control region	
Alces	TrN	atp6.1
	HKY	nd4.3, cox1.3, atp6.3
	K81uf	nd1.3, cox2.3, atp8.3
	TrN	nd4L.1, tRNA-Cys, cox1.1
	K80	cox3.1, tRNA-Met, cyt.1, nd1.1
	TrN	cox3.3, nd5.3, nd3.3
	HKY	cyt.3, nd2.3, nd4L.3
	TIM+I	tRNA-Phe, nd2.1, rRNA 12s, tRNA-Ser2
	TrNef	nd3.1
	TrN+I	tRNA-Gly, tRNA-Val, nd4.1, rRNA 16s, nd5.1
	F81	nd5.2
	TrN	nd6.3
	TrN	tRNA-Glu, tRNA-Ala, tRNA-Pro, tRNA-Gln
	HKY+I+Γ5	control region

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**Table S6:** Molecular dating of the main splitting events within Cervidae for two approaches assuming the normal or lognormal distribution prior for the age of the crown Cervidae. The values are given in Mya.

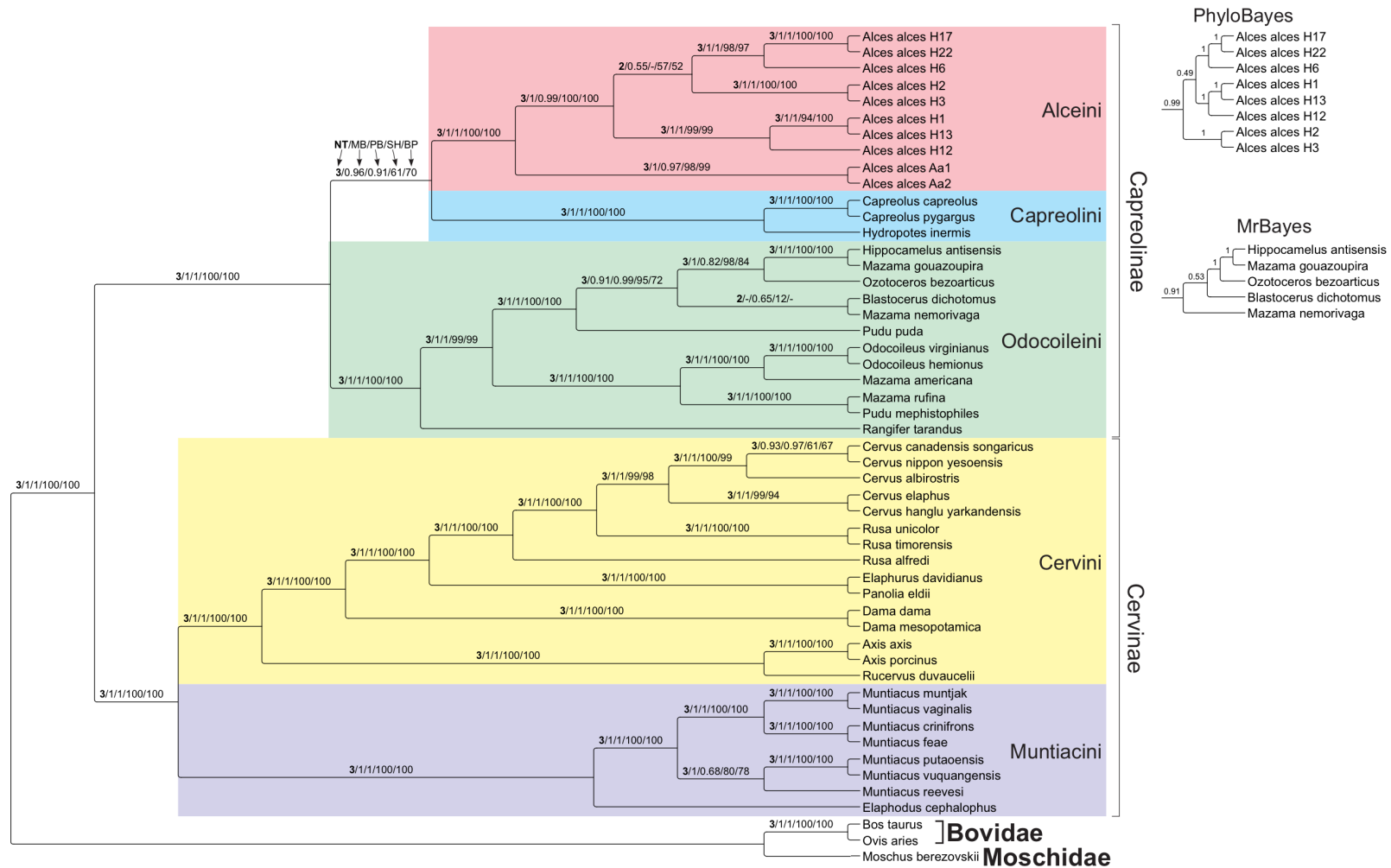
Split	Normal distribution			Lognormal distributionB		
	Man	Man	95 %HP	Man	Man	95 %HP
Cervidae–(Moschidae+Bovidae)	22.04	21.88	17.79 to 26.69	25.67	25.57	21.73 to 29.82
Moschidae–Bovidae	19.21	19.09	14.59 to 24.08	21.99	21.97	16.85 to 27.15
Cervinae–Capreolinae	13.38	13.25	11.09 to 15.92	16.28	16.16	14.91 to 17.9
Cervinae	10.72	10.61	8.98 to 12.67	12.67	12.63	10.87 to 14.56
Muntiacini	9.33	9.23	7.66 to 11.22	11.00	10.97	9.15 to 12.89
Muntiacus	3.83	3.79	3.00 to 4.76	4.57	4.54	3.69 to 5.48
Cervini	6.51	6.44	5.20 to 7.94	7.73	7.68	6.44 to 9.10
Cervus	2.25	2.22	1.76 to 2.77	2.65	2.64	2.16 to 3.18
Capreolinae	12.11	12.00	9.97 to 14.45	14.67	14.59	13.11 to 16.38
Odocoileini	9.01	8.93	7.33 to 10.84	10.75	10.71	9.24 to 12.35
Alceini–Capreolini	11.52	11.42	9.43 to 13.80	13.95	13.89	12.33 to 15.67
Capreolini	7.48	7.42	5.69 to 9.43	8.99	8.97	6.99 to 11.05
Alceini	0.45	0.44	0.31 to 0.61	0.54	0.53	0.37 to 0.73

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**Figure S7:** The consensus of trees obtained in three approaches for the Cervidae set. Numbers at the nodes, in the order shown, correspond to: the number of the trees that contained a given node (NT), posterior probabilities estimated in MrBayes (MB) and PhyloBayes (PB) as well as support values obtained by the approximate likelihood ratio test based on a Shimodaira–Hasegawa-like procedure (SH) and bootstrap method (BP) calculated in IQ-TREE. Subtrees with different topology than the consensus are also shown.





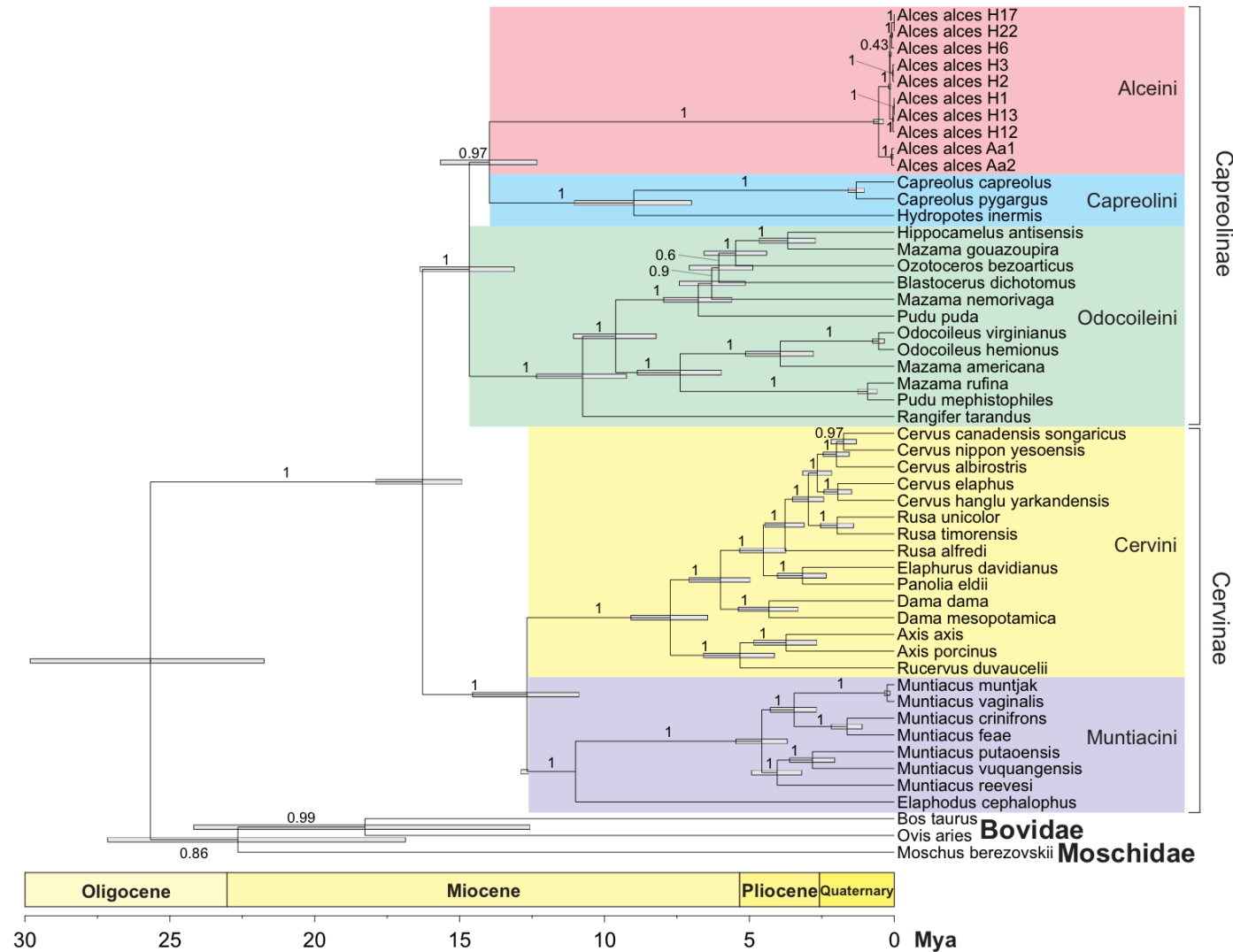


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**Figure S9:** The chronogram for the Cervidae set assuming the lognormal distribution prior for the age of the crown Cervidae. Posterior probabilities are shown at nodes. Bars correspond to 95 % HPD range. The average, median and 95 % HPD of main splitting events were included in Table S6.



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**Figure S10:** The chronogram for the *Alces* set. Posterior probabilities are shown at nodes. Bars correspond to 95 % HPD range. MIS means Marine isotope stages. The  $\delta^{18}\text{O}$  curve was compiled from several sources depending on four time slices: 0–9750 yrs. BP from NGRIP1 (Rasmussen et al., 2014; Seierstad et al., 2014), 9770–10,630 yrs. BP from NGRIP2 (Rasmussen et al., 2014; Seierstad et al., 2014), 10,650–56,050 yrs. BP from Combined Cariaco and Greenland Ice Core Chronology 2005 (GICC05) (Cooper et al., 2015) and 123,000–5,320,000 yrs. BP from the benthic curve by Lisiecki and Raymo (2005). This tree was obtained assuming for the age of the root 95 % confidence interval 374–725 kya as obtained in the dating for the Cervidae set assuming the lognormal distribution prior for the age of the crown Cervidae.

