

Table S1A. List of the samples, PCR and sequence results in Lyon and Adelaide

Sample	Taxonomy		Location	Age	Age (BEAST)	Control Region						Cytb
	Morphology	Molecular				1	2	3	4	5	6	
ACAD3601	<i>Hippidion saldiasi</i>	<i>Hippidion saldiasi</i>	Chile, Tres Arroyos ^{*1}	10-13 KYBP	10,685 BP [†]	NA/3	NA/2	NA/3	NA/2	NA/2	NA/2	NA/NA
ACAD3609	<i>Hippidion saldiasi</i>	<i>Hippidion saldiasi</i>	Chile, Cueva del Medio ^{*1}	10-13 KYBP	10,785 BP [‡]	NA/2	NA/2	NA/4	NA/1	NA/2	NA/2	NA/NA
ACAD3612	<i>Hippidion saldiasi</i>	<i>Hippidion saldiasi</i>	Chile, Tres Arroyos ^{*1}	10-13 KYBP	10,685 BP [†]	NA/2	NA/2	NA/3	NA/2	NA/2	NA/2	NA/NA
ACAD3613	<i>Hippidion saldiasi</i>	<i>Hippidion saldiasi</i>	Chile, Cueva del Medio ^{*1}	10-13 KYBP	10,785 BP [‡]	NA/2	NA/2	NA/2	NA/2	NA/2	NA/2	NA/NA
ACAD5559	<i>Hippidion saldiasi</i>	<i>Hippidion saldiasi</i>	Chile, Cueva del Milodon ^{*3}	10-13 KYBP	12,451 BP [§]	6(47)/Nd	1(7)/1	8(65)/3	3(24)/Nd	3(27)/1	4(20)/Nd	Nd/NA
ACAD3615	<i>Hippidion</i>	New lineage (<i>H. devillei</i>)	Peru, Cueva Rosello (high altitude) ^{*2}	23,250±260 BP	23,250 BP ^{††}	Nd/Nd	2(9)/1	1(6)/2	1(3)/Nd	2(11)/Nd	2(16)/2	Nd/NA
ACAD3625	<i>Hippidion</i>	New lineage (<i>H. devillei</i>)	Peru, Cueva Rosello (high altitude) ^{*2}	23,250±260 BP	23,250 BP ^{††}	2(10)/Nd	1(7)/2	2(17)/3	2(11)/Nd	1(5)/1	1(7)/1	Nd/NA
ACAD3627	<i>Hippidion</i>	New lineage (<i>H. devillei</i>)	Peru, Cueva Rosello (high altitude) ^{*2}	23,250±260 BP	23,250 BP ^{††}	1(8)/1	Nd/2	Nd/2	1(1)/2	2(16)/1	8(46)/1	Nd/NA
ACAD3628	<i>Hippidion</i>	New lineage (<i>H. devillei</i>)	Peru, Cueva Rosello (high altitude) ^{*2}	23,250±260 BP	23,250 BP ^{††}	4(28)/Nd	6(47)/3	2(12)/3	3(8)/2	3(21)/2	2(16)/1	Nd/NA
ACAD3629	<i>Hippidion</i>	New lineage (<i>H. devillei</i>)	Peru, Cueva Rosello (high altitude) ^{*2}	23,250±260 BP	23,250 BP ^{††}	Nd/Nd	3(15)/Nd	2(22)/Nd	Nd/Nd	Nd/Nd	1(8)/Nd	Nd/NA
ACAD2302	<i>Equus cf. hydruntinus</i>	New lineage (Sussemione)	Russia, Khakassia, Proskuriakov Cave ^{*7}	Nd	49,755 BP (average from other 2 dates available within this lineage)	3(16)/Nd	2(9)/Nd	2(6)/1	2(6)/1	5(29)/Nd	2(15)/1	2(7)/NA
ACAD2303	<i>Equus cf. hydruntinus</i>	New lineage (Sussemione)	Russia, Khakassia, Proskuriakov Cave ^{*7}	46,287 ± 1752 BP 47,790 ± 2074 BP	46,938 BP	2(19)/Nd	2(13)/Nd	2(3)/1	3(10)/1	2(18)/Nd	4(29)/1	2(13)/NA
ACAD2305	<i>Equus cf. hydruntinus</i>	New lineage (Sussemione)	Russia, Khakassia, Proskuriakov Cave ^{*7}	49,647 ± 2253 BP 55,496 ± 5242 BP	52,571 BP	6(35)/Nd	8(42)/Nd	5(18)/1	3(19)/1	3(14)/Nd	4(8)/1	2(15)/NA
ACAD226	<i>Equus capensis</i>	<i>Equus burchelli</i>	South Africa, Wonderwerk cave ^{*8}	Nd	Not used	4(30)/Nd	3(28)/Nd	7(53)/Nd	6(35)/1	4(44)/1	3(23)/1	3(24)/Nd
ACAD227	<i>Equus capensis</i>	<i>Equus burchelli</i>	South Africa, Wonderwerk cave ^{*8}	Nd	Not used	Nd/2(10)+1	Nd/Nd	Nd/Nd	Nd/Nd	Nd/Nd	Nd/Nd	Nd/Nd
ACAD230	<i>Equus capensis</i>	<i>Equus burchelli</i>	South Africa, Wonderwerk cave ^{*8}	148 ± 120 BP	148 BP [‡]	3(36)/Nd	3(24)/2	3(26)/2	3(26)/2	3(41)/2	3(34)/1	NA/NA
ACAD236	<i>Equus capensis</i>	<i>Equus burchelli</i>	South Africa, Glen Craig rock shelter	Nd	Not used	3(26)/1	2(17)/1	2(11)/1	3(3)/2	3(23)/1	3(24)/1	5(34)/NA
CH1069	<i>Equus hydruntinus</i>	<i>Equus asinus</i>	Portugal, Gruta da Agua ^{*4}	250 ± 40 BP	250 BP	10(79)/NA	4(24)/NA	2(12)/1	2(13)/NA	2(23)/NA	4(24)/NA	4(24)/NA
CH561 (SC 81126)	<i>Equus hydruntinus</i>	<i>Equus hydruntinus</i>	Belgium, Scladina cave ^{*5}	Layer 1A	40 KYBP	4(26)/Nd	10(70)/Nd	Nd/Nd	3(12)/Nd	2(11)/Nd	3(36)/2	Nd/NA
CH28	<i>Equus hemionus</i>	<i>Equus hemionus</i>	Iran, Sagzabad ^{*6}	II-Ist mill. BC	3 KYBP	9(77)/NA	4(17)/NA	7(48)/NA	5(24)/NA	3(13)/NA	6(29)/NA	2(18)/NA
TZ9	<i>cf. Equus asinus / hydruntinus</i>	<i>Equus hydruntinus</i>	Iran, Zagheh ^{*6}	VII-VIth mill. BC	8 KYBP	7(97)/NA	9(48)/NA	Nd/NA	3(6)/NA	6(30)/NA	2(7)/NA	Nd/NA
CH562 (SC92314)	<i>Equus sp.</i>	<i>Equus caballus</i>	Belgium, Scladina cave ^{*5}	Layer 1A (30-40 KYA)	Not used	1(8)/NA	Nd/NA	Nd/NA	Nd/NA	1(8)/NA	Nd/NA	Nd/NA

Table S1B. List of the negative samples analyzed

Sample	Taxonomy (morphology)	Location	Age
ACAD229	<i>Equus capensis</i>	South Africa, Wonderwerk cave ^{*8}	Unknown
ACAD3610	<i>Hippidion</i>	Peru, Cueva de Los Chingues [*]	Unknown (Pleistocene)
ACAD3614	<i>Hippidion</i>	Peru, Cueva Rosello [‡]	Unknown (Pleistocene)
ACAD3626	<i>Hippidion</i>	Peru, Cueva Rosello [‡]	Unknown (Pleistocene)
ACAD3632	<i>Hippidion</i>	Chile, Betecsa-1 [*]	Unknown (Pleistocene)
CH29 (S17)	<i>Equus asinus</i>	Iran, Sagzabad [‡]	II-Ist mill. BC
CH30 (TZ19)	<i>E. hemionus onager / kulan</i>	Zagheh, Iran [‡]	VII-VIth mill. BC
CH844	<i>Equus (Amerhippus) andium</i>	Ecuador, Punin Chimborazo [‡]	Unknown (Pleistocene)
CH845	<i>Equus (Amerhippus) andium</i>	Ecuador, Punin Chimborazo [‡]	Unknown (Pleistocene)
CH846	<i>Equus (Amerhippus) andium</i>	Ecuador, Punin Chimborazo [‡]	Unknown (Pleistocene)
CH847	<i>Equus (Amerhippus) andium</i>	Ecuador, Alangesi-Quito Pichincha [‡]	Unknown (Pleistocene)
CH848	<i>Equus (Amerhippus) andium</i>	Ecuador, Punin Chimborazo [‡]	Unknown (Pleistocene)
CH849	<i>Equus (Amerhippus) andium</i>	Ecuador, Punin Chimborazo [‡]	Unknown (Pleistocene)

Above slash: number of independent PCR products analyzed in Lyon and number of clones sequenced to deduce the final consensus sequence (in brackets). Below slash: total number of independent PCR products sequenced at the ACAD. F1-F6: short overlapping fragments encompassing 546bp of the horse mtDNA HVR-I, described in [S4] (for some amplifications of the new hippidion lineage, primers 15562F and 15945R have been modified in 5'-CATCCCTATARTGCCCTATGTAC and 5'-TGTTAGGCATGGGCTGATTAGTC). ^{*1} provided by CEHA (Instituto de la Patagonia, Universidad de Magallanes); ^{*2}

provided by the Peru Natural History Museum. ^{*4} provided by Miguel Telles Antunes. ^{*5} provided by Dominique Bonjean. ^{*3} provided by Maite Alberdi. ^{*6} provided by Marjan Mashkour. ^{*7} provided by provided by Patrick Wrinn, Sergei Vasil'ev, and Nikolai Ovodov. ^{*8} provided by David Morris and Peter Beaumont. Dates used for BEAST molecular dating analyses are reported and relied either on radiocarbon dates or archeological context (see suppltext, Dating). † estimated after Borrero [S20] and Massone and Prieto [S23] (AMS; OXA-9247 10,685±70 BP), Stern (1990) [S22] and Massone (1991) [S21] that dated the volcanic ash around 12,480 years BP. ‡ average estimated after Nami and Nakamura (1995) [S24] (NUTA-1811 AMS and NUTA-2331 AMS et 10,710±100 and 10,860±160 BP, respectively). § average estimated after Borrero [S18] (BM-1209, BM-728, LU-794, LP-49 at 13,183±202 BP, 12,984±76 BP, 13,260±115, 10,377±481 BP, respectively). †† estimated from [S26]. Nd: Not determined. NA: Not Attempted.