

Supporting Information

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SI Text

DNA Extraction. Twenty-seven samples were analyzed in Lyon, and 24 in Adelaide (Fig. S1 and Table S1). Both laboratories are kept at positive air pressure, and use standard precautions (clean suits, face shields, multiple pairs of gloves), universal UV irradiation, and regular cleaning with oxidizing agents to prevent contamination. All reagents and materials are supplied DNA-free and used only once. Separate rooms are used for DNA extractions and to set up PCR experiments, and the amplification and post-PCR procedures are performed in a post-PCR laboratory located in a separate, physically remote building, to minimize the potential for contamination from PCR amplicons. The movement of researchers and materials is carefully controlled to further reduce this risk. Twelve fossils did not yield any aDNA information, despite extensive extraction and PCR attempts (Table S1B), although these samples were from equid taxonomic groups that have previously generated aDNA information with similar experimental approaches (1–4). This indicates that the DNA preservation (if any) in these samples was poor, in agreement with their high thermal age, and potential effects of recent museum chemical treatment of the Ecuadorian *E. (Amerhippus)* specimen. In addition, these samples were coextracted with samples that gave positive results, in different extraction sessions, suggesting no cross-contamination between samples has occurred.

In Lyon, the outer surface of samples was removed with a scalpel or a Dremel grinding tool, and tooth roots or bone samples were reduced to powder with a hammer in a sterile enclosed plastic bag. Decalcification of the powder was conducted at the same time as protein digestion by an overnight incubation in 5 mL of 0.5 M EDTA (pH 8.5), 0.5% *N*-lauryl-sarcosyl, and 1 mg/mL proteinase K at 37 °C. The remaining pellets were recovered and stored at 4 °C after brief spin at 2,000 rpm, and the supernatant was transferred into 20 mL binding buffer with 100 μ L silica suspension [5 M GuSCN, 25 mM NaCl, 50 mM Tris, 20 mM EDTA, 1.3% Triton-X100 (pH 4.0–5.0)]. DNA was purified by binding to the silica for 3 h at 37 °C with agitation, followed by two washings with ethanol 80%. The DNA was then eluted from the silica in 150–320 μ L of water or TE buffer [10 mM Tris, 1 mM EDTA (pH 8.0)].

In Adelaide, a Dremel drill was used to remove the surface layer of bone samples, 0.1–0.5 g of sample was pulverized using a tungsten ball bearing in a Mikro-Dismembrator (Sartorius). One mock extraction was included for every four sample extractions to assess contamination during the extraction procedure. Bone powder was decalcified overnight in 20 mL of 0.5 M EDTA (pH 8) on a rotary mixer. Subsequently, samples were digested in 1% SDS, 100 mM Tris (pH 8), 100 mM NaCl, 0.25 mg/mL proteinase K, and 7.5 mg/mL DTT overnight at 55 °C on a rotary mixer. Following digestion, the DNA was isolated by mixing the aqueous extraction solution with an equal volume of Tris-saturated phenol. After mixing for 10 min, samples were centrifuged and the aqueous layer collected. This process was repeated one additional time with phenol and one time with chloroform. DNA was further purified using Microcon Ultra-4 centrifuge filters. Approximately 200 μ L of DNA was collected.

DNA Amplification. In Lyon, single-plex PCRs were conducted in a total volume of 25 μ L using *Taq* Gold (1.25–2.5 units; Perkin-Elmer), 1 \times buffer, 2 mM MgCl₂, 1 mg/mL BSA, 250 μ M of each dNTP, 0.5–1 μ M of the different primers, and 0.5–4 μ L of the DNA extracts. PCRs were initiated by 10 min at 92 °C to activate the polymerase followed by 60 cycles of 92 °C for 40 s, 50 °C for 40 s, and 72 °C for 40 s, and a final elongation step of 10 min. For samples

with low aDNA yields, a two-round multiplex PCR approach was used to maximize the information recovered from each PCR (5). The primers were divided into two sets so that the amplification products within a set do not overlap (set 1: 15492F–16625R, 15668F–15847R, 15889F–16018R, and Cytb2L–Cytb2H; set 2: 15562F–15708R, 15788F–15945R, 15950F–16083R, and Cytb2L–Cytb2H). This amplification was diluted 16-fold, and 2 μ L of the dilution were used as template for the second round of PCR. The first-round multiplex PCRs were done in a total volume of 20 μ L using *Taq* Gold (2 units; Perkin-Elmer), 1 \times buffer, 4 mM MgCl₂, 1 mg/mL BSA, 250 μ M of each dNTP, 150 nM of each primer, and 4 μ L of the DNA extracts. Similar reagent concentrations were used for the second round of PCR, except that a single primer pair was added and 1.5 μ M of each primer and 0.25 units of *Taq* Gold were used. For each PCR round, PCR conditions were similar to those used for single-plex PCRs, except that the number of cycles was reduced to 30.

Modern *Equus grevyi* hairs obtained from the AG Zoologischer Garten in Köln were analyzed in the Lyon post-PCR laboratory, as described in Teletchea et al. (6), to provide sufficient sequence information for comparison with the ancient sequences. The complete HVR-1 sequence and 296 bases of the *cyt b* gene were determined after PCR amplification using primers 15492F–16083R and Cytb1L–Cytb2H, respectively (7), followed by cloning and the sequencing of 5–8 clones.

In Adelaide, single-plex PCRs were conducted in a final volume of 25 μ L using 1–2 units of DNA Polymerase High Fidelity Platinum *Taq* and 1 \times buffer (Invitrogen), 2 mg/mL RSA (Sigma), 2 mM MgSO₄, 250 μ M of each dNTP, 1 μ M of each primer, and 1–4 μ L of DNA. PCRs were run for 94 °C for 1 min followed by 50 cycles of 94 °C for 15 s, 50 °C for 15 s, and 68 °C for 30 s, and a final elongation step of 68 °C for 10 min. Multiplex PCRs were conducted in two sets (15492F–16625R, 15668F–15847R, 15889F–16018R and 15562F–15708R, 15788F–15945R, 15950F–16083R). The first round of the multiplex PCRs was performed in a 25- μ L reaction using 2 units of DNA Polymerase High Fidelity Platinum *Taq*, 1 \times buffer (Invitrogen), 2 mg/mL rabbit serum albumin (RSA; Sigma), 2 mM MgSO₄, 250 μ M of each dNTP, 600 nM of each primer, and 1–4 μ L of DNA. The second round of PCR was performed in single-plex reactions with 0.5 units of Hotmaster *Taq*, 1 \times buffer (Hotmaster), 200 μ M of each dNTP, 1 mg/mL BSA, 1 μ M each primer, and a 100-fold dilution of the first-round PCR product.

Sequencing. In Lyon, PCR products were cloned using the TOPO TA Cloning Kit (Invitrogen) following the manufacturer instructions. Colonies positive for insertion were screened by PCR into a 25- μ L reaction mix using universal M13 (5'-GTT TTC CCA GTC ACG ACG TTG) and REV (5'-TTT CAC ACA GGA AAC AGC TAT) primers and 35–45 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), and elongation (72 °C, 45 s). PCR products were further sequenced by a service provider (Cogenics).

In Adelaide, Big Dye sequencing reactions were performed on PCR products purified with exonuclease I and shrimp alkaline phosphatase (Fermentas). Sequencing reactions purified with Agencourt CleanSEQ were visualized on an ABI 3130xl Genetic Analyzer, and sequences were edited in Sequencher 4.7.

Sequencing Analysis. The initial, maximal length HVR-1 dataset was constructed by collapsing all identical haplotypes, leaving a total of 176 aligned sequences of 546 bp (using the horse complete mtDNA sequence as reference; GenBank accession no. X79547). As *E.*

caballus haplotypes were overrepresented in this dataset, a median-joining network was generated (8) in order to (i) select members of major haplogroups defined in Jansen et al. (9) and (ii) balance taxonomic sampling with regards to members of the hippidion lineage. The accession numbers of the sequences used were: AF014405 (hgB2), AF014408, AF014409, AF014415 (hgF1), AF014416, AF064627 (hgD2), AF072977, AF072979, AF072980 (hgD3), AF072987, AF072988 (hgC1), AF072989, AY246177, AY246225, EF597512 (hgC2), and X79547 (hgA5). The equid HVR-1 dataset consists in 554 bp (positions 15518–16063 according to the complete horse mitochondrial genome; GenBank accession no. X79547) with a final number of 75 sequences, including 16 horses, 4 NWSL (DQ007567–DQ007570), 10 *Hippidion saldiasi/principale* (DQ007560, DQ007562–DQ007564, EU030679, and 5 new samples from this study: ACAD3601, ACAD36012, ACAD3613, ACAD3628, and ACAD5559), 5 *Hippidion devillei* (samples from this study: ACAD3615, ACAD3625, and ACAD3627–3629), 4 *E. hartmannae* (AF220925–AF220927, AF220931), 4 *E. grevyi* (AF220928–AF220930, and sample O'Raye from this study), 3 Sussemionones (samples from this study: ACAD2302, ACAD2303, and ACAD2305), 2 *E. kiang* (AF220932–AF220933), 5 *E. hemionus* (AF220934–AF220937 and sample CH28 from this study), 3 *E. asinus* (AF220938, X97337, and sample CH1069 from this study), 3 *E. hydruntinus* (DQ464012 and samples CH561 and TZ9 from this study), 13 *E. quagga* (AF220916–AD220924 and AY914318, AY914321–AY914323), and 3 *E. capensis* (samples from this study: ACAD226, ACAD236, and ACAD230).

In addition, two outgroups were added to the equid HVR-1 dataset (GenBank accession nos. X97336 and Y07726 for *Rhinoceros unicornis* and *Ceratotherium simum*, respectively), and rooted phylogenies were constructed (Table S2). A third dataset consisting of all of the available equid sequences for the targeted 143-bp cyt *b* fragment (positions 14387–14529 according to the complete horse mitochondrial genome; GenBank accession no. X79547) was also generated, and analyzed (Fig. S2D). Finally, HVR-1 and cyt *b* datasets were merged according to the procedure described in Orlando et al. (3) (“merged consensus”; Figs. S2 E and F and S3, topologies R–V; Table S5). When available, HVR-2 sequences (from ref. 1; positions 16470–16602 according to the complete horse mitochondrial genome; GenBank accession no. X79547) were merged in a new dataset (Fig. S3, topologies R–V; Table S5).

For each dataset, the best model of molecular evolution was determined according to the AIC criterion of Modeltest using Paup 4.0b10 (10, 11) and PaupUp graphical interface (12). Maximum likelihood and Bayesian Markov chain Monte Carlo phylogenies were generated using PhyML 3.0 (following a SPR tree rearrangement procedure) (13) and MrBayes 3.12 (14), respectively. Bayesian analyses were run twice to check for convergence. The strength of the phylogenetic signal was assessed via nonparametric bootstrapping (1,000 pseudoreplicates), posterior probabilities [20 million generations, sampling frequency = 1 every 1,000 generations, burn-in value = 5,000, and aLRT (SH-like)] (15).

The topology was inconsistent with the existence of the subgenus *Hippotigris*, which comprised plains and mountain zebras, but it showed a branching of *E. hartmannae* with *E. asinus*—quite surprising even if mountain zebras do exhibit ass characters (see ref. 16, quoting Bourdelle). *E. grevyi* was found monophyletic as expected from its status in a separate subgenus (*Dolichohippus*) but nested within paraphyletic hemionids (*E. hemionus*, *E. hydruntinus*, and *E. kiang*). Therefore, supports for alternative topologies were assessed using different combinations of sequence datasets (HVR-1, Fig. S3, topologies A–Q; HVR-1+cyt *b*, Fig. S3, topologies R and S; HVR-1+HVR-2+cyt *b*, Fig. S3, topologies T–V) thanks to likelihood-based statistical tests implemented in PAUP* 4.0 (Kishino-Hasegawa and Shimodeira-Hasegawa; Table S5) (11). Finally, rooted analyses based on YR-recoded sequences were

conducted to limit possible substitution saturation due to the deep divergence of rhinos and equids ≈ 55 MYA (Table S2).

Dating. Radiocarbon dating was performed by accelerator mass spectrometry (AMS) on ACAD2303 and ACAD2305 at the University of California Irvine facility (UCIAMS). Samples were prepped for radiocarbon dating using two different methods. At the University of Colorado Laboratory for AMS Radiocarbon Preparation and Research (CURL), amino acids were purified using ion exchange chromatography (17). At the UCIAMS facility, an ultrafiltration method was used to purify collagen (18). The two dates recovered for ACAD2303 were $42,480 \pm 1,500$ (CURL 10275; CalPal $46,287 \pm 1,752$ BP) and $44,000 \pm 1,600$ (UCIAMS 57008; CalPal $47,590 \pm 2,074$ BP). The two dates recovered for ACAD2305 were $45,770 \pm 2,250$ (CURL 10284; CalPal $49,647 \pm 2,250$ BP) and $49,900 \pm 3,400$ (UCIAMS 57009; CalPal $55,496 \pm 5,242$ BP). Radiocarbon dating was performed on CH1069 (250 ± 40 BP) at Beta Analytic Radiocarbon Dating Laboratory (Miami; Beta-221672). ACAD230 was radiocarbon dated 172 ± 28 (OxA-20862; CalPal 148 ± 120 BP) by the Oxford Radiocarbon Accelerator Unit (ORAU) using an ultrafiltration method to purify collagen. Dates for hippidions were recovered from the literature (see Table S1A for details) (19–25). A date of 10,545 BP was attributed to the specimen from Cueva Lago Sofia 1 reported in Orlando et al. (4) (GenBank accession no. EU030679) according to two radiocarbon dates available for two other *Hippidion* of the same cave (26); $10,780 \pm 60$ BP and $10,310 \pm 160$ BP, AMS OXA-9319 and OXA-9504, respectively. A date of 23,250 BP was assumed to the equid material from Cueva Rosello (Peru), according to the radiocarbon dating of an *Onohippidion devillei* fossil reported in Shockey et al. (27). Finally, historical quaggas were assumed to be 100 years old, according to their extinction at the end of the 19th century.

Morphological Reanalyses: The Equid from Proskuriakov Cave. Equid fossils from this cave have been first tentatively referred to *E. cf. hydruntinus*. They seem actually to belong to two slender forms, showing slight size differences, in particular in the length of premolar protocones (Fig. S4F, Pprot). According to Simpson's ratio diagram (Fig. S4F), both are unlike extant hemiones (teeth are relatively small—Psize and Msize, and diaphyses are deep on the metatarsals (MT4). Samples ACAD2302, ACAD2303, and ACAD2305 belong to the smaller form that is similar by its general proportions to a Middle Pleistocene (*ca.* 0.6 MYA) species described at Süssenborn, Germany, as *E. altidens*. In both forms, the lower-cheek teeth differ from hemiones by deep vestibular grooves on the molars, and from *E. hydruntinus* by asymmetric double knots. They resemble the Middle Pleistocene species (Fig. S4F) found at Süssenborn and Akhalkalaki (Georgia), and referred to by Eisenmann (16) as the “Sussemionones” group. Sussemionones may have existed as early as 2 MYA in Alaska and Arizona and were supposed extinct before the Late Pleistocene (16).

Morphological Reanalyses: The Equid from Gruta de Agua, Portugal. Photographs and measurements of a badly preserved and very old skull of a small equid were kindly communicated by M.T.-A., who documented the existence of a small enigmatic equid, possibly an *E. hydruntinus*, known as the “Zebro” during the Middle Ages in Portugal (28). Morphological analysis, however, suggests that this specimen belongs to a donkey. In particular, the scatter diagram of two skull measurements shows that it plots with donkeys (Fig. S4G) and not with hemiones, as would have been expected of an *E. hydruntinus*. Therefore, as for the Cape Zebra, biomolecular analyses confirm morphological studies.

Accession Numbers for Estimating the Genetic Distance Within and Between Each Taxonomic Group (Table S3A). The dataset consisted of 1,544 sequences encompassing positions 15518 and 15818 from the

complete horse mitochondrial genome (GenBank accession no. X79547).

CAB, *E. caballus*. AB329587, AB329588, AB329589, AB329590, AB329591, AB329592, AB329593, AB329594, AB329595, AB329596, AB329597, AB329598, AB329599, AB329600, AB329601, AB329602, AB329603, AB329604, AB329605, AB329606, AB329607, AB329608, AB329609, AB329610, AB329611, AB329612, AB329613, AB329614, AB329615, AB329616, AB329617, AB329618, AB329619, AB329620, AB329621, AB329622, AB329623, AB329624, AB329625, AB329626, AB329627, AB329628, AF014405, AF014406, AF014407, AF014408, AF014409, AF014410, AF014411, AF014412, AF014413, AF014414, AF014415, AF014416, AF014417, AF056071, AF064627, AF064628, AF064629, AF064630, AF064631, AF064632, AF072975, AF072976, AF072977, AF072978, AF072979, AF072980, AF072981, AF072982, AF072983, AF072984, AF072985, AF072986, AF072987, AF072988, AF072989, AF072990, AF072991, AF072992, AF072993, AF072994, AF072995, AF072996, AF168689, AF168690, AF168691, AF168692, AF168693, AF168694, AF168695, AF168696, AF168697, AF168698, AF169009, AF169010, AF326635, AF326636, AF326637, AF326638, AF326639, AF326640, AF326641, AF326642, AF326643, AF326644, AF326645, AF326646, AF326647, AF326648, AF326649, AF326650, AF326651, AF326652, AF326653, AF326654, AF326655, AF326656, AF326657, AF326658, AF326659, AF326660, AF326661, AF326662, AF326663, AF326664, AF326665, AF326666, AF326667, AF326668, AF326669, AF326670, AF326671, AF326672, AF326673, AF326674, AF326675, AF326676, AF326677, AF326678, AF326679, AF354425, AF354426, AF354427, AF354428, AF354429, AF354430, AF354431, AF354432, AF354433, AF354434, AF354435, AF354436, AF354437, AF354438, AF354439, AF354440, AF354441, AF431965, AF431966, AF431967, AF431968, AF431969, AF465984, AF465985, AF465986, AF465987, AF465988, AF465989, AF465990, AF465991, AF465992, AF465993, AF465994, AF465995, AF465996, AF465997, AF465998, AF465999, AF466000, AF466001, AF466002, AF466003, AF466004, AF466005, AF466006, AF466007, AF466008, AF466009, AF466010, AF466011, AF466012, AF466013, AF466014, AF466015, AF466016, AF481232, AF481233, AF481234, AF481235, AF481236, AF481237, AF481238, AF481239, AF481240, AF481241, AF481242, AF481243, AF481244, AF481245, AF481246, AF481247, AF481248, AF481249, AF481250, AF481251, AF481252, AF481253, AF481254, AF481255, AF481256, AF481257, AF481258, AF481259, AF481260, AF481261, AF481262, AF481263, AF481264, AF481265, AF481266, AF481267, AF481268, AF481269, AF481270, AF481271, AF481272, AF481273, AF481274, AF481275, AF481276, AF481277, AF481278, AF481279, AF481280, AF481281, AF481282, AF481283, AF481284, AF481285, AF481286, AF481287, AF481288, AF481289, AF481290, AF481291, AF481292, AF481293, AF481294, AF481295, AF481296, AF481297, AF481298, AF481299, AF481300, AF481301, AF481302, AF481303, AF481304, AF481305, AF481306, AF481307, AF481308, AF481309, AF481310, AF481311, AF481312, AF481313, AF481314, AF481315, AF481316, AF481317, AF481318, AF481319, AF481320, AF481321, AF481322, AF481323, AF481324, AF481325, AF481326, AF481327, AF481328, AF481329, AF481330, AF481331, AF481332, AF481333, AF481334, AY049718, AY049719, AY049720, AY057408, AY057409, AY057410, AY057411, AY057412, AY057413, AY057414, AY057415, AY057416, AY057417, AY057418, AY057419, AY057420, AY057421, AY057422, AY057423, AY057424, AY057425, AY057426, AY057427, AY057428, AY057429, AY057430, AY057431, AY057432, AY057433, AY057434,

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GRE, *E. grevyi*. AF220928, AF220929, AF220930, GQ176427, GQ176428, GQ176429, GQ176430, GQ176431, GQ176432, O'Raye (= GQ324592).

ZEB, *E. zebra*. AF220925, AF220926, AF220927, AF220931, AY651929, AY651930, AY651931, AY651932, AY651933, AY651934, AY651935, AY651936, AY651937, AY651938, AY651939, AY651940, AY651941, AY651942, AY651943, AY651944, AY651945, AY651946, AY651947, AY651948, AY651949, AY651950, AY651951, AY651952, AY651953, AY651954, AY651955, AY651956.

HEM, *E. hemionus*. AF220934, AF220935, AF220936, AF220937, AY569551, CH28 (= GQ324612).

QUA, including *E. quagga quagga*, *E. quagga burchelli*, and *E. quagga boehmi*. AF220916, AF220917, AF220918, AF220919, AF220920, AF220921, AF220922, AF220923, AF220924, AY914318, AY914319, AY914320, AY914321, AY914322, AY914323, ACAD226 (= GQ324605), ACAD230 (= GQ324604), ACAD236 (= GQ324603).

NWSL, New World stilt-legged horses. DQ007567, DQ007568, DQ007569, DQ007570.

HIP, *Hippidion saldiasi/principale*. ACAD3601 (= GQ324593), ACAD3609 (= GQ324594), ACAD3612 (= GQ324595), ACAD3613 (= GQ324596), ACAD5559 (= GQ324597), AY152862, AY152863, DQ007560, DQ007562, DQ007563, DQ007564, EU030679.

ONO, *Hippidion devillei*. ACAD3625 (= GQ324599), ACAD3627 (= GQ324600), ACAD3628 (= GQ324601).

KIA, *E. kiang*. AF220932, AF220933, AY569539, AY569540, AY569541, AY569542.

SUS, New Noncaballine Equus Lineage (*Sussemione*). ACAD2302 (= GQ324606), ACAD2303 (= GQ324607), ACAD2305 (= GQ324608).

Accession Numbers for Estimating the Genetic Distance Within and Between Each Taxonomic Group (Table S3B). The dataset consisted of 1,866 sequences encompassing positions 15518 and 15760 from the complete horse mitochondrial genome (GenBank accession no. X79547).

CAB, *E. caballus*. AB329587, AB329588, AB329589, AB329590, AB329591, AB329592, AB329593, AB329594, AB329595, AB329596, AB329597, AB329598, AB329599, AB329600, AB329601, AB329602, AB329603, AB329604, AB329605, AB329606, AB329607, AB329608, AB329609, AB329610, AB329611, AB329612, AB329613, AB329614, AB329615, AB329616, AB329617, AB329618, AB329619, AB329620, AB329621, AB329622, AB329623, AB329624, AB329625, AB329626, AB329627, AB329628, AF014405, AF014406, AF014407, AF014408, AF014409, AF014410, AF014411, AF014412, AF014413, AF014414, AF014415, AF014416, AF014417, AF056071, AF064627, AF064628, AF064629,

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ASI, E. asinus. AF220938, AF403063, AF403064, AF403065, AF531459, AF531460, AF531461, AF531462, AF531463, AF531464, AF531465, AF531466, AF531467, AF531468, AF531469, AF531470, AF532118, AF532119, AF532120, AF532121, AF532122, AF532123, AF532124, AF532125, AF532126, AY569462, AY569463, AY569464, AY569465, AY569466, AY569467, AY569468, AY569469, AY569470, AY569471, AY569472, AY569473, AY569474, AY569475, AY569476, AY569477, AY569478, AY569479, AY569480, AY569481, AY569482, AY569483, AY569484, AY569485, AY569486, AY569487, AY569488, AY569489, AY569490, AY569491, AY569492, AY569493, AY569494, AY569495, AY569496, AY569497, AY569498, AY569499, AY569500, AY569501, AY569502, AY569503, AY569504, AY569505, AY569506, AY569507, AY569508, AY569509, AY569510, AY569511, AY569512, AY569513, AY569514, AY569515, AY569516, AY569517, AY569518, AY569519, AY569520, AY569521, AY569522, AY569523, AY569524, AY569525, AY569526, AY569527, AY569528, AY569529, AY569530, AY569531, AY569532, AY569533, AY569534, AY569535, AY569536, AY569537, AY569538, AY569539, AY569540, AY569541, AY569542, AY569543, AY569544, AY569545, AY569546, AY569547, AY666165, AY666166, AY666167, AY666168, AY666169, CH1069 (= GQ324609), DQ368497, DQ368498, DQ368499, DQ368500, DQ368501, DQ368502, DQ368503, DQ368504, DQ368505, DQ368506, DQ368507, DQ368508, DQ368509, DQ368510, DQ368511, DQ368512, DQ368513, DQ368514, DQ368515, DQ368516, DQ368517, DQ368518, DQ368519, DQ368520, DQ368521, DQ368522, DQ368523, DQ368524, DQ368525, DQ368526, DQ368527, DQ368528, DQ368529, DQ368530, DQ368531, DQ368532, DQ368533, DQ368534, DQ368535, DQ368536, DQ368537, DQ368538, DQ368539, DQ368540, DQ368541, DQ368542, DQ368543, DQ368544, DQ368545, DQ368546, DQ368547, DQ368548, DQ368549, DQ368550, DQ368551, DQ368552, DQ368553, DQ368554, DQ368555, DQ368556, DQ368557, DQ368558, DQ368559, DQ368560, DQ368561, DQ368562, DQ368563, DQ368564, DQ368565, DQ368566, DQ368567, DQ368568, DQ368569, DQ368570, DQ368571, DQ368572, DQ368573, DQ368574, DQ368575, DQ368576, DQ368577, DQ368578, DQ368579, DQ368580, DQ368581, DQ368582, DQ368583, DQ368584, DQ368585, DQ368586, DQ368587, DQ368588, DQ368589, DQ368590, DQ368591, DQ368592, DQ368593, DQ368594, DQ368595, DQ368596, DQ448878, DQ448879, DQ448880, DQ448881, DQ448882, DQ448883, DQ448884, DQ448885, DQ448886, DQ448887, DQ448888, DQ448889, DQ448890, DQ448891, DQ448892, DQ448893,

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GRE, E. grevyi. AF220928, AF220929, AF220930, AF220928, AF220929, AF220930, GQ176427, GQ176428, GQ176429, GQ176430, GQ176431, GQ176432, O'Raye (= GQ324592).

ZEB, E. zebra. AF220925, AF220926, AF220927, AF220931, AY651929, AY651930, AY651931, AY651932, AY651933, AY651934, AY651935, AY651936, AY651937, AY651938, AY651939, AY651940, AY651941, AY651942, AY651943, AY651944, AY651945, AY651946, AY651947, AY651948, AY651949, AY651950, AY651951, AY651952, AY651953, AY651954, AY651955, AY651956.

HEM, E. hemionus. AF220934, AF220935, AF220936, AF220937, AY569551, CH28 (= GQ324612).

QUA, including E. quagga quagga, E. quagga burchellii, and E. quagga boehmi. AF220916, AF220917, AF220918, AF220919, AF220920, AF220921, AF220922, AF220923, AF220924, AY914318, AY914319, AY914320, AY914321, AY914322, AY914323, EU650488, EU650489, EU650490, EU650491, EU650492, EU650493, EU650494, EU650495, EU650496, EU650497, EU650498, EU650499, EU650500, EU650501, EU650502, EU650503, EU650504, EU650505, EU650506, EU650507, EU650508, EU650509, EU650510, EU650511, EU650512, EU650514, EU650515, EU650516, EU650517, EU650518, EU650519, EU650520, EU650521, EU650522, EU650523,

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HIP, Hippidion saldiasi/principale. ACAD3601 (= GQ324593), ACAD3609 (= GQ324594), ACAD3612 (= GQ324595), ACAD3613 (= GQ324596), ACAD5559 (= GQ324597), AY152862, AY152863, DQ007560, DQ007562, DQ007563, DQ007564, EU030679.

ONO, Hippidion devillei. ACAD3625 (= GQ324599), ACAD3627 (= GQ324600), ACAD3628 (= GQ324601).

KIA, E. kiang. AF220932, AF220933, AY569539, AY569540, AY569541, AY569542.

SUS, New Noncaballine Equus Lineage (Sussemione). ACAD2302 (= GQ324606), ACAD2303 (= GQ324607), ACAD2305 (= GQ324608).

List of Accession Numbers for Estimating the Genetic Distance Within and Between Each Taxonomic Group (Table S3C). The dataset consisted of 1,878 sequences encompassing positions 15518 and 15606 from the complete horse mitochondrial genome (GenBank accession no. X79547).

CAB, E. caballus. 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ASI, *E. asinus*. AF220938, AF403063, AF403064, AF403065, AF531459, AF531460, AF531461, AF531462, AF531463, AF531464, AF531465, AF531466, AF531467, AF531468, AF531469, AF531470, AF532118, AF532119, AF532120, AF532121, AF532122, AF532123, AF532124, AF532125, AF532126, AY569462, AY569463, AY569464, AY569465, AY569466, AY569467, AY569468, AY569469, AY569470, AY569471, AY569472, AY569473, AY569474, AY569475, AY569476, AY569477, AY569478, AY569479, AY569480, AY569481, AY569482, AY569483, AY569484, AY569485, AY569486, AY569487, AY569488, AY569489, AY569490, AY569491, AY569492, AY569493, AY569494, AY569495, AY569496, AY569497, AY569498, AY569499, AY569500, AY569501, AY569502, AY569503, AY569504, AY569505, AY569506, AY569507, AY569508, AY569509, AY569510, AY569511, AY569512, AY569513, AY569514, AY569515, AY569516, AY569517, AY569518, AY569519, AY569520, AY569521, AY569522, AY569523, AY569524, AY569525, AY569526, AY569527, AY569528, AY569529, AY569530, AY569531, AY569532, AY569533, AY569534, AY569535, AY569536, AY569537, AY569538, AY569543, AY569544, AY569545, AY569546, AY569547, AY666165, AY666166, AY666167, AY666168, AY666169, CH1069 (= GQ324609), DQ368497, DQ368498, DQ368499, DQ368500, DQ368501, DQ368502, DQ368503, DQ368504, DQ368505, DQ368506, DQ368507, DQ368508, DQ368509, DQ368510, DQ368511, DQ368512, DQ368513, DQ368514, DQ368515, DQ368516, DQ368517, DQ368518, DQ368519, DQ368520, DQ368521, DQ368522, DQ368523, DQ368524, DQ368525, DQ368526, DQ368527, DQ368528, DQ368529, DQ368530, DQ368531, DQ368532, DQ368533, DQ368534, DQ368535, DQ368536, DQ368537, DQ368538, DQ368539, DQ368540, DQ368541, DQ368542, DQ368543, DQ368544, DQ368545, DQ368546, DQ368547, DQ368548, DQ368549, DQ368550, DQ368551, DQ368552, DQ368553, DQ368554, DQ368555, DQ368556, DQ368557, DQ368558, DQ368559, DQ368560, DQ368561, DQ368562, DQ368563, DQ368564, DQ368565, DQ368566, DQ368567, DQ368568, DQ368569, DQ368570, DQ368571, DQ368572, DQ368573, DQ368574, DQ368575, DQ368576, DQ368577, DQ368578, DQ368579, DQ368580, DQ368581, DQ368582, DQ368583, DQ368584, DQ368585, DQ368586, DQ368587, DQ368588, DQ368589, DQ368590, DQ368591, DQ368592, DQ368593, DQ368594, DQ368595, DQ368596, DQ448878, DQ448879, DQ448880, DQ448881, DQ448882, DQ448883, DQ448884, DQ448885, DQ448886, DQ448887, DQ448888, DQ448889, DQ448890, DQ448891, DQ448892, DQ448893, DQ448894, DQ448895, DQ448896, DQ448897, DQ448898, DQ448899, DQ448900, DQ448901, DQ448902, DQ448903, DQ448904, DQ448905, DQ448906, DQ448907, DQ448908, DQ448909, DQ448910, DQ448911, DQ448912, DQ448913, DQ448914, DQ448915, DQ448916, DQ448917, DQ448918, DQ448919, DQ448920, DQ448921, DQ448922, DQ448923, DQ448924, DQ448925, DQ448926, DQ448927, DQ448928, DQ448929, DQ448930, DQ448931, DQ448932, DQ448933, DQ448934, DQ448935, DQ448936, DQ448937, DQ448938, DQ448939, DQ448940, DQ448941, DQ448942, DQ448943, DQ448945, DQ448946, DQ448947, DQ448948, DQ448949, DQ448950, DQ448951, DQ448952, DQ448953, DQ448954, DQ448955, DQ448956, DQ448957, DQ448958, DQ448959, DQ448960, DQ448961, DQ448962, DQ448963, DQ448964, DQ448965, DQ448966, DQ448967, DQ448968, DQ448969, DQ448970, DQ448971, DQ448972, DQ448973, DQ448974, DQ448975, DQ448976, DQ448977, DQ448978, DQ448979, DQ448980, DQ448981, DQ448982, DQ448983, DQ448984, DQ448985, DQ448986, DQ448987, DQ448988, DQ448989, DQ448990, DQ448991, DQ448992, DQ448993, DQ448994, DQ448995, DQ448996, DQ448997, DQ448998, DQ448999, DQ449000, DQ449001, DQ449002, DQ449003, DQ449004, DQ449005, DQ449006, DQ449007, DQ449008, DQ449009, DQ449010, DQ449011, DQ449012, DQ449013, DQ449014, DQ449015, DQ449016, DQ449017, DQ449018, DQ449019, DQ449020, DQ449021, DQ449022, DQ449023, EF056020, EF056021, EF056022, EF056023, EF056024, EF056025, EF056026, EF056027, EF056028, EF056029, EF056030, EF056031, EF056032, EF056033, EF056034, EF056035, EF056036, EF056037, EF056038, EF056039, EF056040, EF056041, EF056042, EF056043, EF056044, EF056045, EF056046, EF056047, EF056048, EF056049, EF056050, EF056051, EF056052, EF056053, EF056054, EF056055, EF056056, EF056057, EF056058, EF056059, EF056060, EF056061, EF056062, EF056063, EF056064, EF056065, EF056066, EF056067, EF056068, EF056069, EF056070, EF056071, EF056072, EF056073, EF056074, EF056075, EF056076, EF056077, EF056078, EF056079, EF056080, EF056081, EF056082, EF056083, EF056084, EF056085, EF056086, EF056087, EF056088, EF056089, EF056090, EF056091, EF056092, EF056093, EF056094, EF056095, EF056096, EF056097, EF056098, EF056099, EF056100, EF056101, EF056102, EF056103, EF056104, EF056105, EF056106, EF056107, EF056108, EF056109, EF056110, EF056111, EF056112, EF056113, EF056114, EF056115, EF056116, EF056117, EF056118, EF056119, EF056120, EF056121, EF056122, EF056123, EF056124, EF056125, EF056126, EF056127, EF056128, X97337.

GRE, *E. grevyi*. AF220928, AF220929, AF220930, GQ176427, GQ176428, GQ176429, GQ176430, GQ176431, GQ176432, O'Raye (= GQ324592).

ZEB, *E. zebra*. AF220925, AF220926, AF220927, AF220931, AY651929, AY651930, AY651931, AY651932, AY651933, AY651934, AY651935, AY651936, AY651937, AY651938, AY651939, AY651940, AY651941, AY651942, AY651943, AY651944, AY651945, AY651946, AY651947, AY651948, AY651949, AY651950, AY651951, AY651952, AY651953, AY651954, AY651955, AY651956.

HEM, *E. hemionus*. AF220934, AF220935, AF220936, AF220937, AY569551, CH28 (= GQ324612).

QUA, including *E. quagga quagga*, *E. quagga burchellii*, and *E. quagga boehmi*. AF220916, AF220917, AF220918, AF220919, AF220920, AF220921, AF220922, AF220923, AF220924, AY914318, AY914319, AY914320, AY914321, AY914322, AY914323, AY914324, EU650488, EU650489, EU650490, EU650491, EU650492, EU650493, EU650494, EU650495, EU650496, EU650497, EU650498, EU650499, EU650500, EU650501, EU650502, EU650503, EU650504, EU650505, EU650506, EU650507, EU650508, EU650509, EU650510, EU650511, EU650512, EU650513, EU650514, EU650515, EU650516, EU650517, EU650518, EU650519, EU650520, EU650521, EU650522, EU650523, EU650524, EU650525, EU650526, EU650527, EU650528, EU650529, EU650530, EU650531, EU650532, EU650533, EU650534, EU650535, EU650536, EU650537, EU650538, EU650539, EU650540, EU650541, EU650542, EU650543, EU650544, EU650545, EU650546, EU650547, EU650548, EU650549, EU650550, EU650551, EU650552, EU650553, EU650554, EU650555, EU650556, EU650557, EU650558, EU650559, EU650560, EU650561, EU650562, EU650563, EU650564, EU650565, EU650566, EU650567, EU650568, EU650569, EU650570, EU650571, EU650572, EU650573, EU650574, EU650575, EU650576, EU650577, EU650578, EU650579, EU650580, EU650581, EU650582, EU650583, EU650584, EU650585, EU650586, EU650587, EU650588, EU650589, EU650590, EU650591, EU650592, EU650593, EU650594, EU650595, EU650596, EU650597, EU650598, EU650599, ACAD226 (= GQ324605), ACAD230 (= GQ324604), ACAD236 (= GQ324603), ACAD227 (GU062887).

NWSL, New World stilt-legged horses. DQ007567, DQ007568, DQ007569, DQ007570.

HIP, Hippidion saldiasi/principale. ACAD3601 (= GQ324593), ACAD3609 (= GQ324594), ACAD3612 (= GQ324595), ACAD3613 (= GQ324596), ACAD5559 (= GQ324597), AY152862, AY152863, DQ007560, DQ007562, DQ007563, DQ007564, EU030679.

ONO, Hippidion devillei. ACAD3625 (= GQ324599), ACAD3627 (= GQ324600), ACAD3628 (= GQ324601).

KIA, E. kiang. AF220932, AF220933, AY569539, AY569540, AY569541, AY569542.

HYD, E. hydruntinus. CH561 (= GQ324610), DQ464012, TZ9 (= GQ324611).

SUS, New Noncaballine Equus Lineage (Sussemione). ACAD2302 (= GQ324606), ACAD2303 (= GQ324607), ACAD2305 (= GQ324608).

List of Accession Numbers for Median-Joining Network Analyses (Fig. S2B). Four hundred and sixty-seven sequences, positions 15518–15842 from the complete horse mitochondrial genome (GenBank accession no. X79547).

ASI, E. asinus. CH1069 (= GQ324609), AF220938, AF403063, AF403064, AF403065, AF531459, AF531460, AF531461, AF531462, AF531463, AF531464, AF531465, AF531466, AF531467, AF531468, AF531469, AF531470, AF532118, AF532119, AF532120, AF532121, AF532122, AF532123, AF532124, AF532125, AF532126, AY569462, AY569463, AY569464, AY569465, AY569466, AY569467, AY569468, AY569469, AY569470, AY569471, AY569472, AY569473, AY569474, AY569475, AY569476, AY569477, AY569478, AY569479, AY569480, AY569481, AY569482, AY569483, AY569484, AY569485, AY569486, AY569487, AY569488, AY569489, AY569490, AY569491, AY569492, AY569493, AY569494, AY569495, AY569496, AY569497, AY569498, AY569499, AY569500, AY569501, AY569502, AY569503, AY569504, AY569505, AY569506, AY569507, AY569508, AY569509, AY569510, AY569511, AY569512, AY569513, AY569514, AY569515, AY569516, AY569517, AY569518, AY569519, AY569520, AY569521, AY569522, AY569523, AY569524, AY569525, AY569526, AY569527, AY569528, AY569529, AY569530, AY569531, AY569532, AY569533, AY569534, AY569535, AY569536, AY569537, AY569538, AY569543, AY569544, AY569545, AY569546, AY569547, AY666165, AY666166, AY666167, AY666168, AY666169, DQ368497, DQ368498, DQ368499, DQ368500, DQ368501, DQ368502, DQ368503, DQ368504, DQ368505, DQ368506, DQ368507, DQ368508, DQ368509, DQ368510, DQ368511, DQ368512, DQ368513, DQ368514, DQ368515, DQ368516, DQ368517, DQ368518, DQ368519, DQ368520, DQ368521, DQ368522, DQ368523, DQ368524, DQ368525, DQ368526, DQ368527, DQ368528, DQ368529, DQ368530, DQ368531, DQ368532, DQ368533, DQ368534, DQ368535, DQ368536, DQ368537, DQ368538, DQ368539, DQ368540, DQ368541, DQ368542, DQ368543, DQ368544, DQ368545, DQ368546, DQ368547, DQ368548, DQ368549, DQ368550, DQ368551, DQ368552, DQ368553, DQ368554, DQ368555, DQ368556, DQ368557, DQ368558, DQ368560, DQ368561, DQ368562, DQ368563, DQ368564, DQ368565, DQ368566, DQ368567, DQ368568, DQ368569, DQ368570, DQ368571, DQ368572, DQ368573, DQ368574, DQ368575, DQ368576, DQ368577, DQ368578, DQ368579, DQ368580, DQ368581, DQ368582, DQ368583, DQ368584, DQ368585, DQ368586, DQ368587, DQ368588, DQ368589, DQ368590, DQ368591, DQ368592, DQ368593, DQ368594, DQ368595, DQ368596, DQ448878, DQ448879, DQ448880, DQ448881, DQ448882, DQ448883, DQ448884, DQ448885, DQ448886, DQ448887, DQ448888, DQ448889, DQ448890, DQ448891, DQ448892, DQ448893, DQ448894, DQ448895, DQ448896, DQ448897, DQ448898, DQ448899, DQ448900, DQ448901, DQ448902, DQ448903, DQ448904, DQ448905, DQ448906, DQ448907, DQ448908,

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HEM, E. hemionus. CH28 (= GQ324612), AF220934, AF220935, AF220936, AF220937.

KIA, E. kiang. AF220932, AF220933.

GRE, E. grevyi. AF220928, AF220929, AF220930, O'Raye (= GQ324592).

SUS, New Noncaballine Equus Lineage. ACAD2302 (= GQ324606), ACAD2303 (= GQ324607), ACAD2305 (= GQ324608).

ZEB, E. hartmannae. AF220925, AF220926, AF220927, AF220931.

List of Accession Numbers for Median-Joining Network Analyses (Fig. S2C). Four hundred eighty-one sequences, positions 15518–15808 from the complete horse mitochondrial genome (GenBank accession no. X79547).

ASI, E. asinus. CH1069 (= GQ324609), AF220938, AF403063, AF403064, AF403065, AF531459, AF531460, AF531461, AF531462, AF531463, AF531464, AF531465, AF531466, AF531467, AF531468, AF531469, AF531470, AF532118, AF532119, AF532120, AF532121, AF532122, AF532123, AF532124, AF532125, AF532126, AY569462, AY569463, AY569464, AY569465, AY569466, AY569467, AY569468, AY569469, AY569470, AY569471, AY569472, AY569473, AY569474, AY569475, AY569476, AY569477, AY569478, AY569479, AY569480, AY569481, AY569482, AY569483, AY569484, AY569485, AY569486, AY569487, AY569488, AY569489, AY569490, AY569491, AY569492, AY569493, AY569494, AY569495, AY569496, AY569497, AY569498, AY569499, AY569500, AY569501, AY569502, AY569503, AY569504, AY569505, AY569506, AY569507, AY569508, AY569509, AY569510, AY569511, AY569512, AY569513,

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HEM, *E. hemionus*. CH28 (= GQ324612), AF220934, AF220935, AF220936, AF220937.

KIA, *E. kiang*. AF220932, AF220933.

GRE, *E. grevyi*. AF220928, AF220929, AF220930, AF220928, AF220929, AF220930, GQ176427, GQ176428, GQ176429, GQ176430, GQ176431, GQ176432, O'Raye (= GQ324592).

SUS, *New Noncaballine Equus Lineage*. ACAD2302 (= GQ324606), ACAD2303 (= GQ324607), ACAD2305 (= GQ324608).

ZEB, *E. hartmannae*. AF220925, AF220926, AF220927, AF220931.

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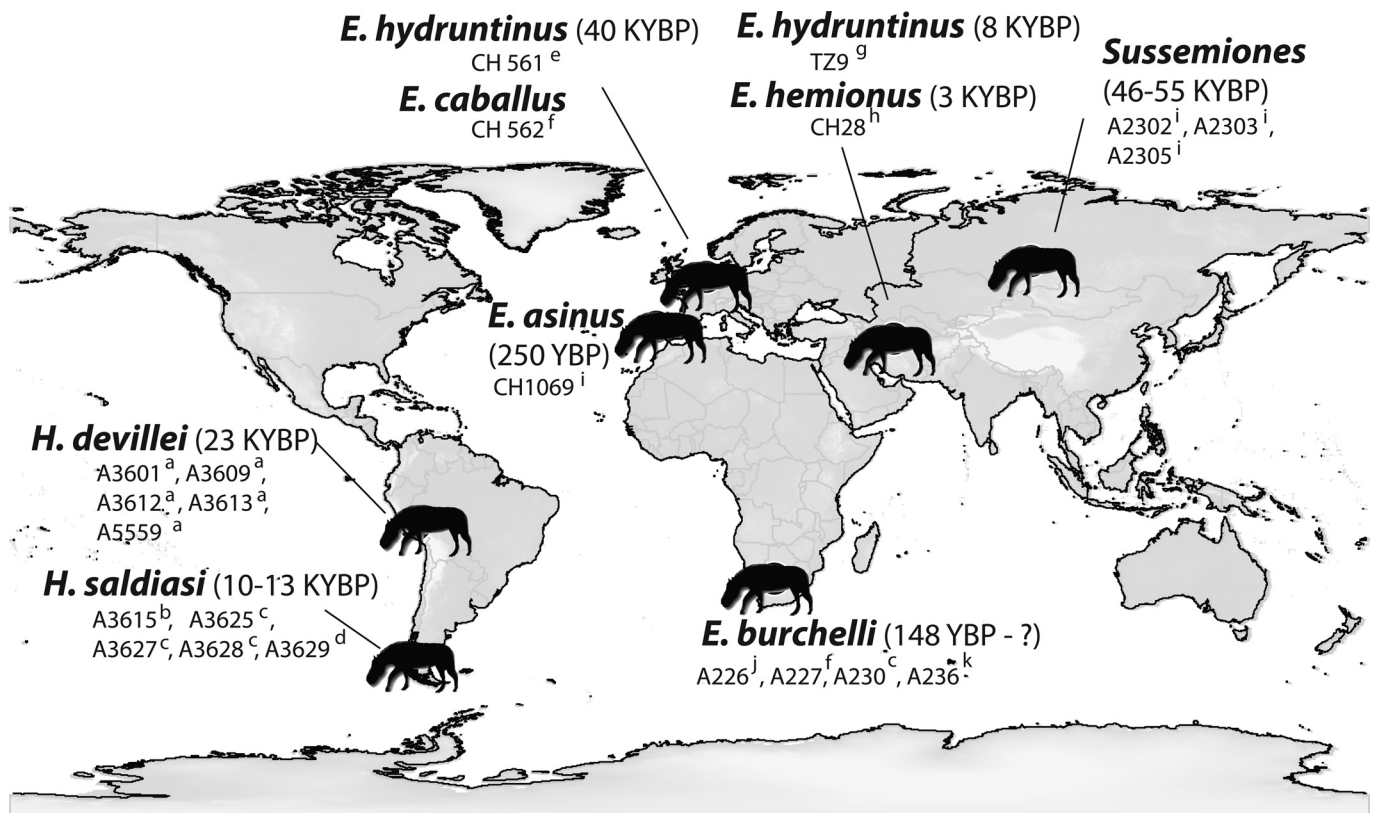


Fig. S1. World map showing samples unique to this study with associated radiocarbon dates and a superscript designating the amount of sequence data. Superscript letter denotes control region base pairs/cytochrome *b* base pairs: (a) 542 bp/-, (b) 476 bp/-, (c) 543bp/-, (d) 336bp/-, (e) 400 bp/-, (f) 87 bp/-, (g) 401 bp/-, (h) 516 bp/143 bp, (i) 545 bp/143 bp, (j) 543 bp/143 bp, (k) 544 bp/143 bp.

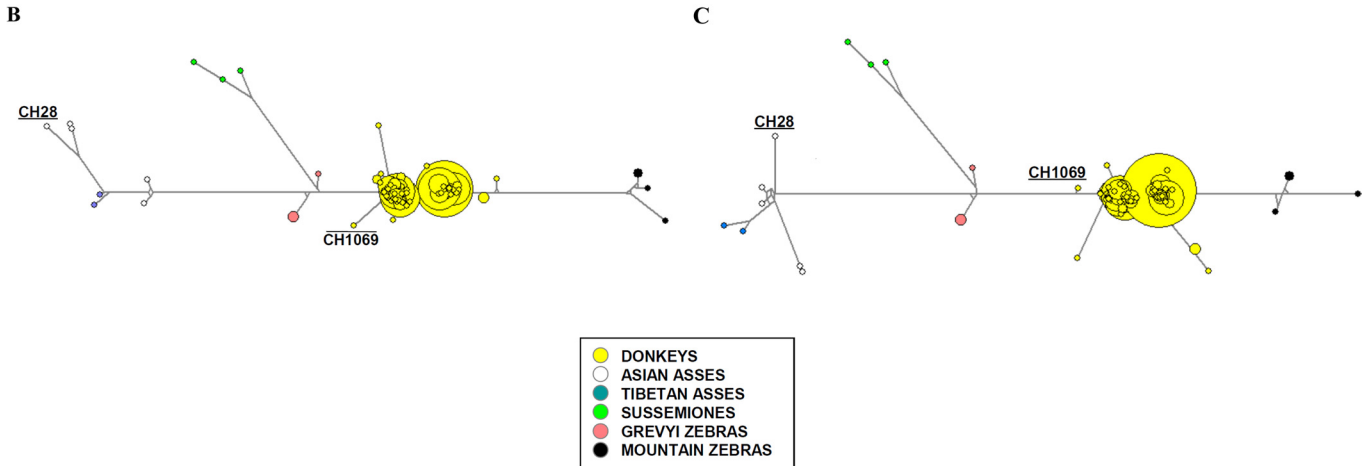
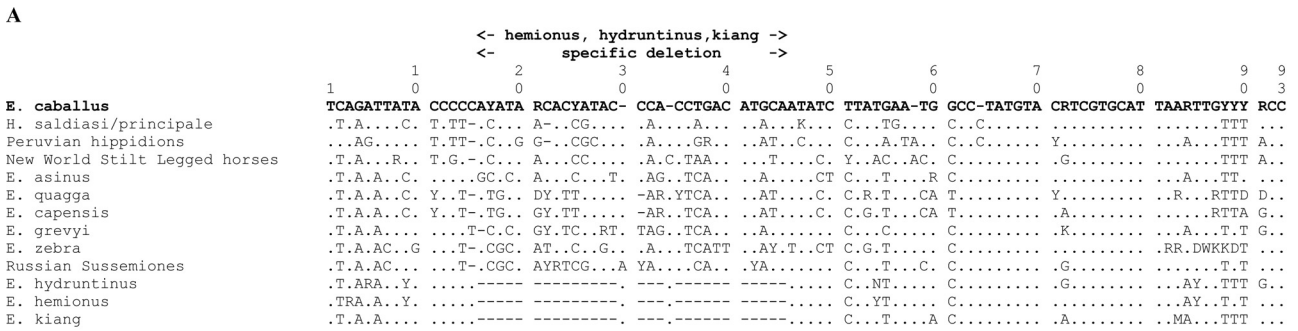


Fig. S2. Identifying extinct and extant equids: alignment, network and phylogenetic analyses. (A) Identifying extinct and extant equids with a short mtDNA HVR-1 fragment. For each taxonomic group, the 95% consensus of sequences present in the dataset used in Table S3C is presented. (B) Median-joining network among asses, sushsemiones, donkeys, and Grevy's zebras based on mitochondrial control region sequences. The dataset consisted of 467 sequences encompassing positions 15518 and 15842 from the complete horse mitochondrial genome (GenBank accession no. X79547). All sites were equally weighted. The sample CH1069 does not appear within the *E. hemionus* cluster (as would have been expected for a member of the *E. hydruntinus* lineage) but as a donkey. (C) Median-joining network among asses, sushsemiones, donkeys, and Grevy's zebras based on mitochondrial control region sequences. The dataset consisted of 481 sequences encompassing positions 15518 and 15808 from the complete horse mitochondrial genome (GenBank accession no. X79547). All sites were equally weighted. The sample CH1069 does not appear within the *E. hemionus* cluster (as would have been expected for a member of the *E. hydruntinus* lineage) but as a donkey. (D) Molecular phylogeny (HKY+I) of the 143-bp cytochrome *b* gene fragment alone. The numbers above the branches correspond to node bootstrap values, approximate likelihood ratio tests (aLRT SH-like), and Bayesian posterior probabilities. The support for nodes is reported only if greater than 50%. The sequences recovered in this study are highlighted in gray. Sequence accession numbers are reported for HVR-1 and *cyt b*, respectively, except for sequences obtained from complete mitochondrial genomes (noted with an asterisk). (E) Molecular phylogeny of the merged dataset (HVR-1, excluding positions 15518–15577 because of alignment uncertainty, and *cyt b*, with one global model of molecular evolution: GTR+I+I). The numbers above the branches correspond to node bootstrap values, approximate likelihood ratio tests (aLRT SH-like), and Bayesian posterior probabilities. The support for nodes is reported only if greater than 50%. The sequences recovered in this study are highlighted in gray. Sequence accession numbers are reported for HVR-1 and *cyt b*, respectively, except for sequences obtained from complete mitochondrial genomes (noted with an asterisk). (F) Molecular phylogeny of the merged dataset (two dataset partition, with two different models of molecular evolution: control region HVR-1: HKY+I+I; *cyt b*: HKY+I). The alignment is exactly the same as for Fig. S2E. The phylogenetic was recovered thanks the Bayesian Markov chain Monte Carlo method using two different models of molecular evolution for the two genes considered (HVR-1: HKY+I+I; *cyt b*: HKY+I). The numbers above branches correspond to the Bayesian posterior probabilities. The support for nodes is reported only if greater than 50%. The sequences recovered in this study are highlighted in gray. Sequence accession numbers are reported for HVR-1 and *cyt b*, respectively, except for sequences obtained from complete mitochondrial genomes (noted with an asterisk).

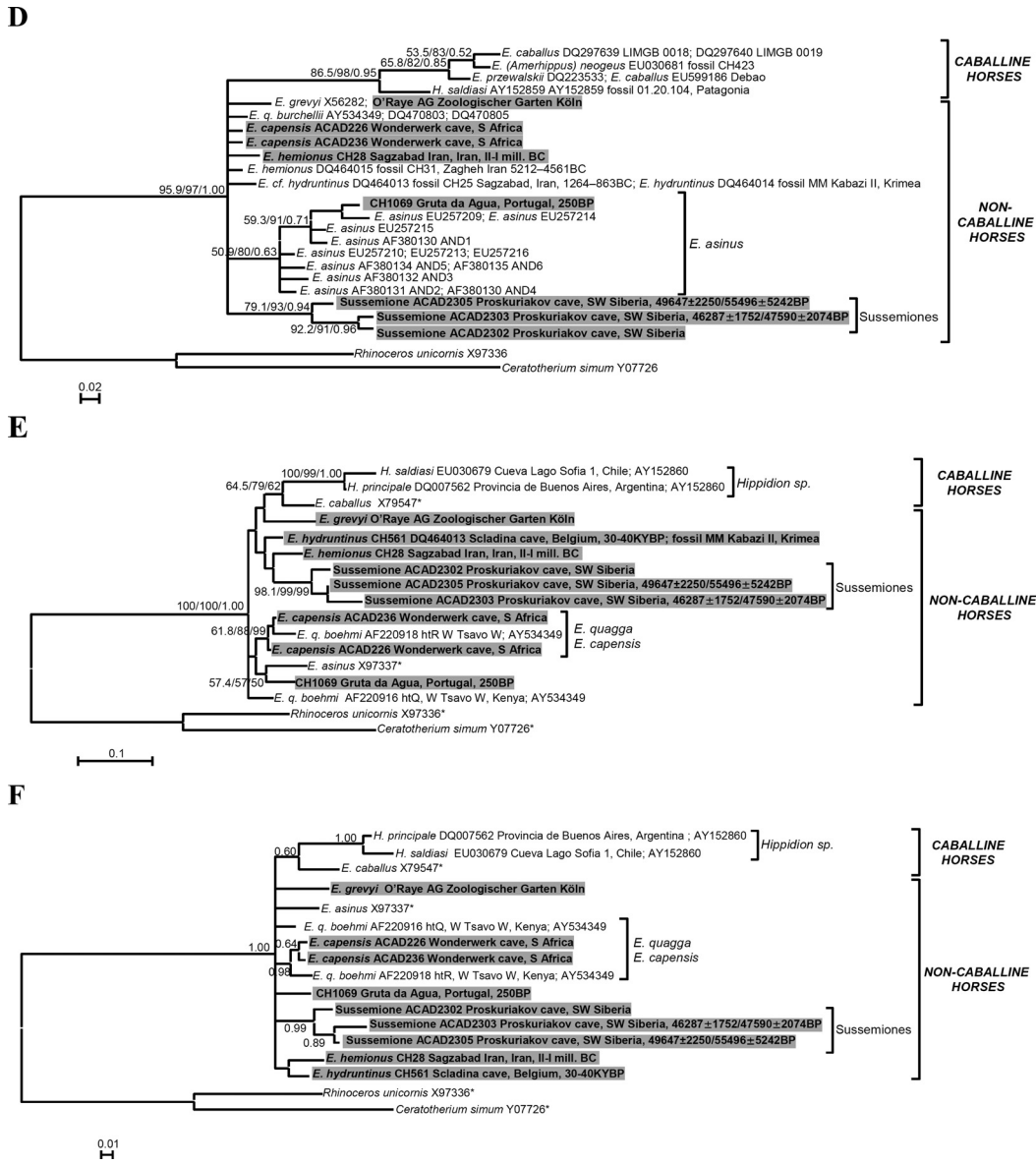


Fig. S2. Continued.

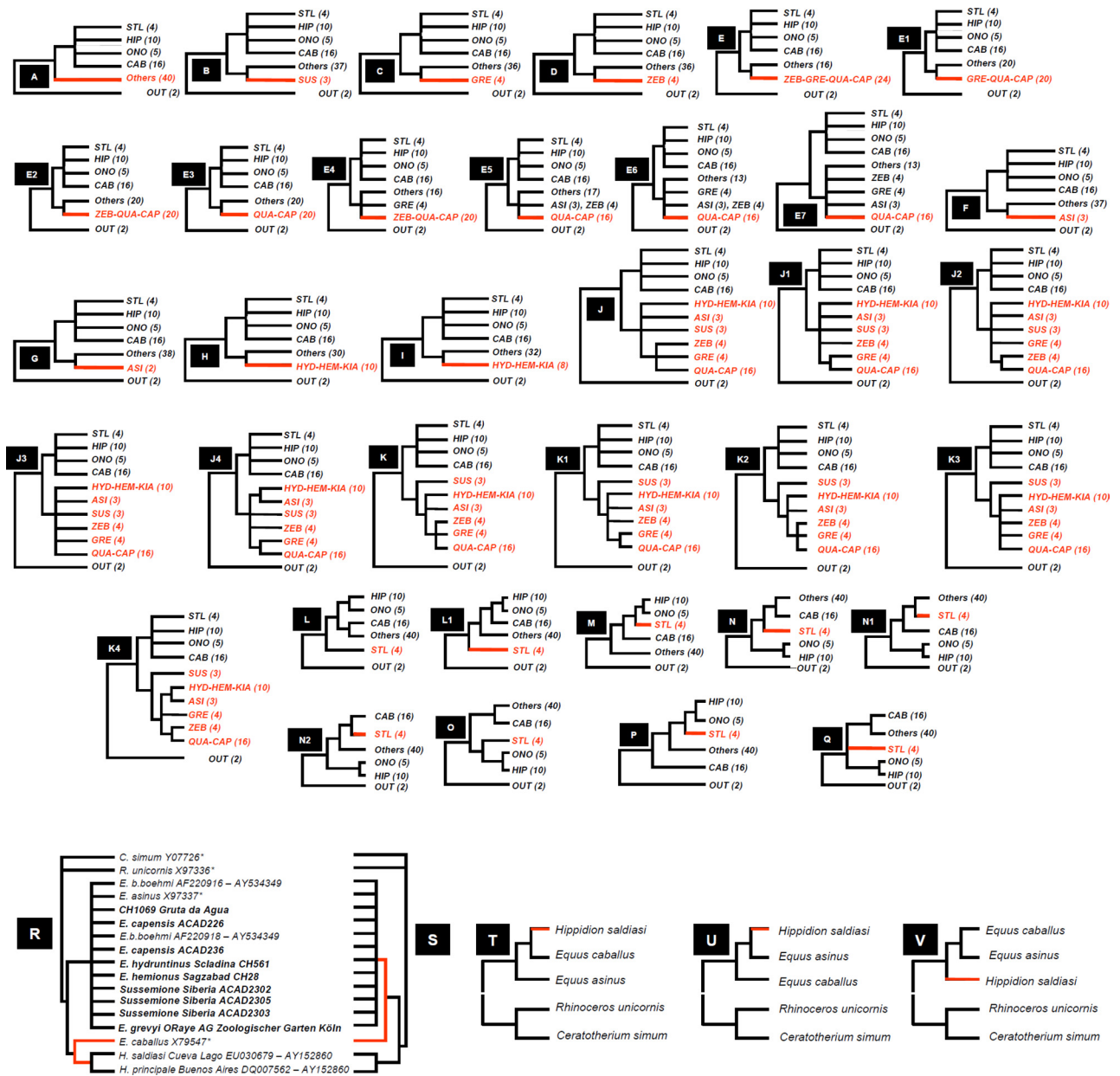


Fig. S3. Alternative topologies used for assessing phylogenetic relationships with Kishino-Hasegawa and Shimodeira-Hasegawa tests.

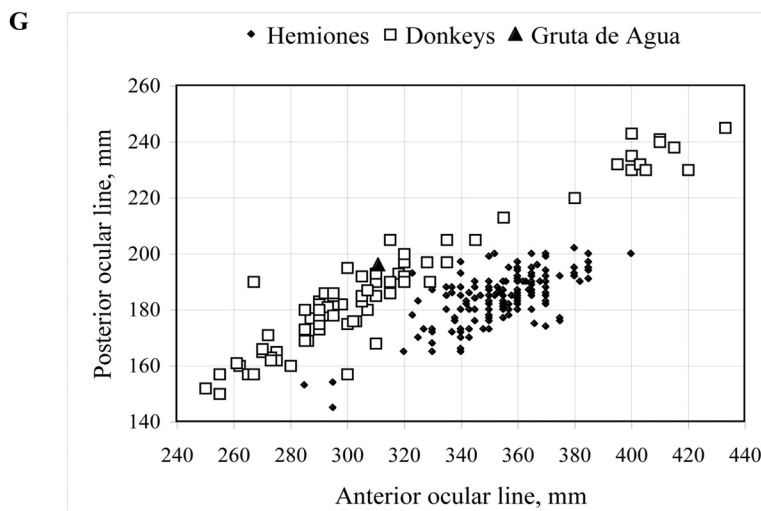
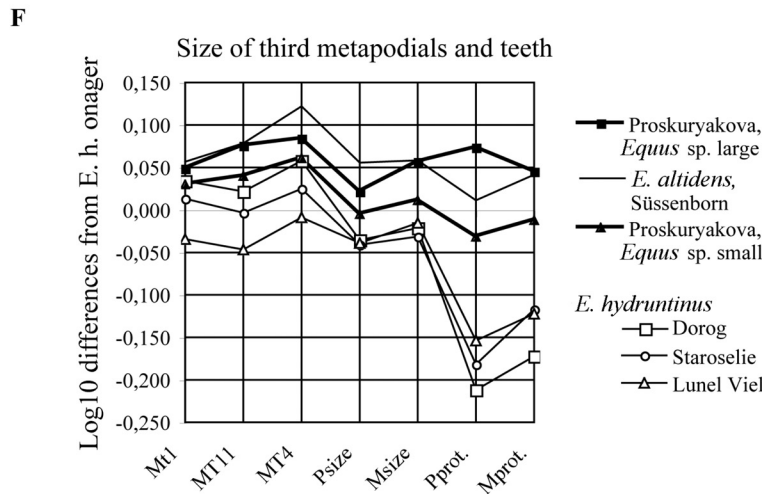
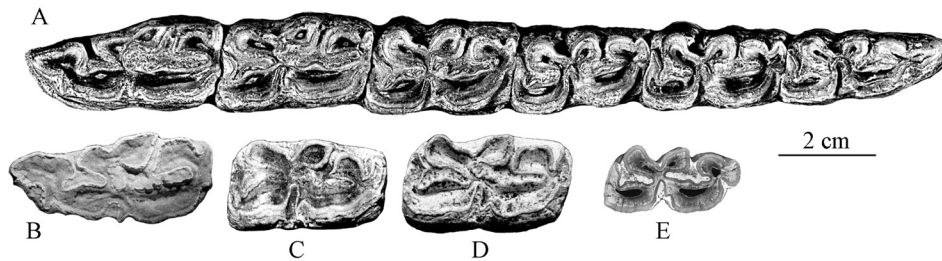


Fig. 54. Morphological analyses of Proskuryakova and Gruta de Agua specimens. (A–E) Comparison of lower-cheek teeth of Sussemionos and Proskuryakova specimens. Lower-cheek series from Proskuryakova (A). Lower P2 from Akhalkalaki, no. 1302 (B). Lower P from Süssenborn, S 5169 (C). Lower P from Süssenborn, S 1368 (D). Sectioned lower M from Süssenborn, S 6436 (E). (F) Ratio diagrams comparing third metatarsals length (Mt1), distal articular breadth (MT11), and diaphysis depth (MT4); size of upper premolars (P) and molars (M); and protocone length of upper premolars (Pprot.) and molars (Mprot.) in extant *E. hemionus onager* = reference, and *E. hydruntinus* (Dorog, Staroselie, Lunel-Viel). Measurements in millimeters of third metatarsals length MT1, distal articular breadth MT11, and diaphysis depth MT4; of occlusal size (occlusal length + occlusal breadth)/2 of upper premolars P and molars M; and protocone length of upper premolars (Pprot.) and molars (Mprot.). Number of specimens are provided in brackets. *E. hemionus onager*: MT1 = 247.5 [16]; MT11 = 37.4 [16]; MT4 = 25.3 [16]; PL + 1/2 = 25.6 [47]; ML + 1/2 = 23.0 [48]; Pprot. = 11.4 [47]; Mprot. = 11.0 [48]; *E. hydruntinus*, Dorog: MT1 = 267.7 [3]; MT11 = 39.3 [4]; MT4 = 28.9 [7]; PL + 1/2 = 23.6 [2]; ML + 1/2 = 21.9 [3]; Pprot. = 7.0 [2]; Mprot. = 7.4 [3]; *E. hydruntinus*, Staroselie: MT1 = 255.1 [12]; MT11 = 37.1 [20]; MT4 = 26.8 [12]; PL + 1/2 = 23.3 [7]; ML + 1/2 = 21.4 [17]; Pprot. = 7.5 [7]; Mprot. = 8.4 [17]; *E. hydruntinus*, Lunel Viel: MT1 = 228.7 [14]; MT11 = 33.6 [15]; MT4 = 24.8 [14]; PL + 1/2 = 23.4 [11]; ML + 1/2 = 22.2 [14]; Pprot. = 8.0 [11]; Mprot. = 8.3 [14]; Proskuryakova, *Equus sp.* Large: MT1 = 276.8 [1]; MT11 = 44.5 [1]; MT4 = 30.7 [1]; PL + 1/2 = 26.9 [6]; ML + 1/2 = 26.2 [3]; Pprot. = 13.5 [6]; Mprot. = 12.2 [3]; Proskuryakova, *Equus sp.* Small: MT1 = 265.3 [5]; MT11 = 41.0 [6]; MT4 = 29.1 [5]; PL + 1/2 = 25.3 [5]; ML + 1/2 = 23.6 [4]; Pprot. = 10.6 [5]; Mprot. = 10.7 [4]; *E. altidens*, Süssenborn: MT1 = 282.0 [1]; MT11 = 44.8 [5]; MT4 = 33.5 [4]; PL + 1/2 = 29.1 [25]; ML + 1/2 = 26.3 [19]; Pprot. = 11.7 [24]; Mprot. = 12.1 [19]. (G) Scatter diagram of skull measurements (anterior/posterior ocular line) in hemionos, donkeys, and the Gruta de Agua specimen.

Other Supporting Information Files

[Table S1 \(PDF\)](#)

[Table S2 \(PDF\)](#)

[Table S3 \(PDF\)](#)

[Table S4 \(PDF\)](#)

[Table S5 \(PDF\)](#)