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Morphological Convergence in *Hippidion* and *Equus* (*Amerhippus*) South American Equids Elucidated by Ancient DNA Analysis

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Abstract. Unusual equids named hippidions inhabited South America for more than 2 MY (million years). Like many other animals they succumbed to the worldwide climatic change that occurred 10 KY (thousand years) ago and completely disappeared during the great late Pleistocene megafaunal extinction. According to fossil records and numerous dental, cranial, and postcranial characters, *Hippidion* and *Equus* lineages are known to have diverged prior to 10 MY. Some equid bones from Rio Verde and Ultima Esperanza (Patagonia, Chile) dating back to the late Pleistocene period (8–13 KY) have been identified as *Hippidion saldiasi*, while a few teeth have been assigned to *Equus*. Six samples of those remains have been obtained from the Zoological Museum of Amsterdam for ancient DNA analysis to try to place *Hippidion* in the evolutive tree of Perissodactyla. Two samples of *Hippidion* and one sample of *Equus* yielded 241–394 bp of the mtDNA control region and 172–296 bp of the cytochrome *b* gene. Unexpectedly, all the sequences clustered deep inside the *Equus* genus, casting doubt on the initial identification of the bones. For paleontologists, one of the striking and classical diagnostic characters of *Hippidion* is their extremely short and massive metapodials, a probable locomotory adaptation to the Andine steep slopes. However, our DNA analysis reveals that a very *Hippidion*-like metapod might also have been possessed by another South American equid, i.e., *Equus* (*Amerhippus*), an interpretation supported by complementary anatomical observations. This adaptive convergence between members of the two South American equid genera may lead paleontologists to limb bone misidentification.

Key words: Ancient DNA — *Equus* (*Amerhippus*) — *Hippidion* — Equids — Ultima Esperanza — mtDNA control region

Introduction

According to the correspondence of the sixteenth-century explorers, horses were unknown in the New World at the time of European settlement, and until the mid-nineteenth century, no one thought that equids had ever lived in the New World. During the voyage of the Beagle, however, Darwin (1836) collected numerous specimens of extinct mammals from Bahia Blanca (now in Argentina). Among them, Owen recognized an equid that he described as *Equus curvidens* in 1869 (MacFadden 1997), thus providing evidence that equids had existed in the New World in the recent past. Soon afterward, caves in Brazil and

Sequence data from this article have been deposited at the EMBL/GenBank Data Libraries under accession Nos. AY152859–AY152863.

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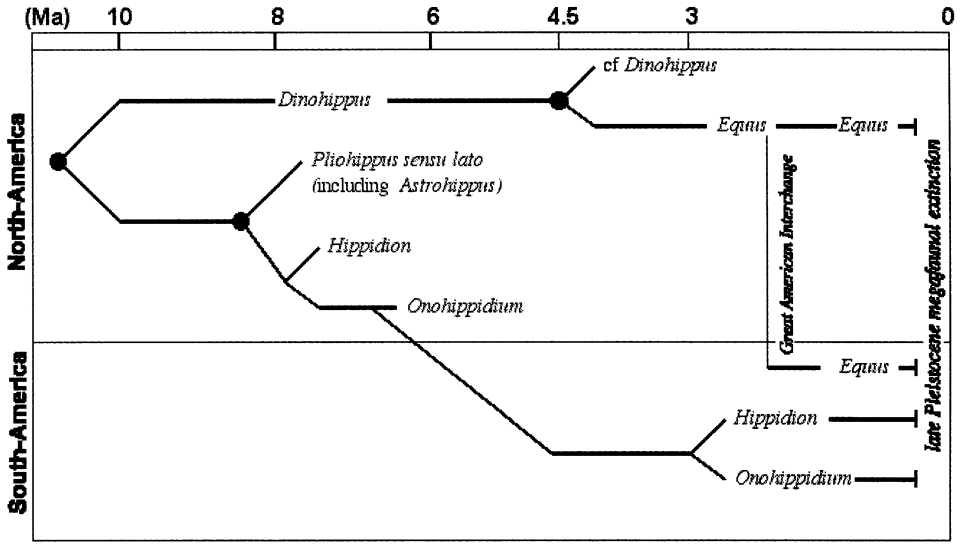


Fig. 1. Relationships between *Equus* and *Hippidion*: the paleontological model (after MacFadden 1997). According to morphological data, the most recent common ancestor of *Equus* and *Hippidion* would date back prior to 10 MY ago.

Bolivia yielded new equid fossils and subsequently many pleistocene *Equus* species have been described in Southern America, from Ecuador to Central Argentina, although often from isolated teeth or small fragments with poor taxonomic value (Boule and Thevenin 1920; Prado and Alberdi 1994).

The South American equiforms were still obviously closely related to the various extant horse-like animals. On the contrary, members of the genus *Hippidion* and other hippidiforms look very different than members of the genus *Equus* and other equiforms. Hippidiforms and equiforms coexisted until their extinction during the late Pleistocene megafaunal extinction, around 10 KY (thousand years) ago. Hippidiforms are characterized by a skull with an extremely deep nasoincisival notch associated with unusually long nasal bones and by very short and massive metapodials (Sefve 1912; Boule and Thevenin 1920). Paleontological analyses suggest that hippidiform horses most probably descended from *Pliohippus*, while equiforms (e.g., species of the *Plesippus*, *Allohippus*, and *Equus* genera) are related to *Dinohippus*, the two parallel lines having started to diverge about 10 MY ago (MacFadden 1997) (Fig. 1).

Ancient DNA has now become a valuable tool for the study of the phylogenetic relationships between extinct and extant species (Hofreiter et al. 2001a). The very first ancient DNA study concerned the Equidae family and revealed the close relationship between the quagga (*Equus quagga*) and the plains zebra (*Equus burchelli* [Higuchi et al. 1984, 1987]). More recently, the comparison of 355 bp of the mtDNA control region (CR) from 16 ancient horse specimens to 191 extant domestic horses shed light on the process of horse domestication (Vila et al. 2001). Given (*i*) that mtDNA has already been used to address the relationships between extant equids (Ishida et al. 1995; Oakenfull et al. 2000) and (*ii*) the availability of mtDNA CR region for all of the extant *Equus* species and cytochrome *b* sequences for three of them (Xu et al. 1996; Kavar et al. 1999; Kim et al. 1999; Bowling et al. 2000), we decided to investigate the relationships between the *Hippidion* and the *Equus* genera by targeting 394 bp in the mtDNA CR and 296 bp in the cytochrome *b* (cytb) genes using DNA from ancient *Hippidion* specimens.

Materials and Methods

Samples

The fossils are presently part of the Kruimel collection of the Zoological Museum of Amsterdam. J.H. Kruimel, a student in Biology, traveled to Patagonia in 1908–1909. He bought the limb bones and the teeth during the same voyage but from distinct localities: the teeth originate from Rio Verde peat deposits dated 8000 years BP, in the Fitz-Roy Channel, 70 km north of Punta Arenas (Chile), while the limb bones come from a cave near Ultima Esperanza (270 km north of Punta Arenas). Deposits from caves in the vicinity have been radiocarbon dated to between 8.5 and 13 KY (Alberdi and Prieto 2000) and even as recently as about 5 KY (Alberdi et al. 1987). Kruimel subsequently brought his fossils back to London, where Smith-Woodward referred the limb bones to *Onohippidium saldiasi*, i.e., to the genus *Hippidion* (Alberdi et al. 1987), and the teeth to *Equus argentinus*. For our purpose, this Patagonian material looked most suitable since fossil bones in this area may be accompanied by pieces of skin, hairs, and tendons, showing evidence of environmental conditions more propitious to DNA preservation than in the tropical caves from which other *Hippidion* samples have also been retrieved. A metatarsal, a metacarpal, and three phalanges have been recorded under collection No. ZMA20104 (labeled, respectively, 01.20.104, 02.20.104, 03.20104-a, 03.20.104-b, and 03.20.104-c). Lastly, an

upper left first molar referred to *Equus* (E.1.20.103) under collection No. ZMA20103 has been sampled. For bone sampling, the superficial part of the bone was discarded to avoid any contamination that could have occurred during the storage in the museum. Thus, a total of five *Hippidion* and one *Equus* samples was analyzed.

DNA Extraction and Amplification

Extraction and amplification procedures were conducted in separate rooms with specific facilities devoted to ancient DNA work as described by Hänni et al. (1994) and Orlando et al. (2002). Sample 01.20.104 (0.8 g) was coextracted in a first extraction session with two historic human bones. Sample 02.20.104 (0.6 g) was coextracted in a second extraction session with two other historic human bones. The three phalanges (03.20.104-a, 03.20.104-b, and 03.20.104-c) were extracted (about 0.7 g for each) 3 months later in a third session; finally, the tooth sample of *Equus* (E.1.20.103) was extracted alone still 1 month later (1.1 g). An extraction blank was included in each session to ensure that no contamination occurred. After reduction of the samples to powder in a sterile enclosed plastic bag, an overnight decalcification and protein digestion was conducted at 55°C with agitation (0.5 *M* EDTA, pH 8.5, 1–2 mg/ml proteinase K, 0.5% *N*-lauryl Sarcosyl). The pellets were removed by 10 min of centrifugation (800 rpm) and the supernatants were further purified by three steps of centrifugation in a mixture of phenol/chloroform/isoamyl (25:24:1; 1200 rpm, 15 min). The aqueous phase was then concentrated in 100 µl of distilled water by means of Centricon-30 columns (Amicon).

mtDNA CR was targeted using the three primer couples described by Vila et al. (2001), as they were specially defined to amplify short fragments from ancient samples of equids (164, 194, and 197 bp obtained with L1H1, L2H2, and L3H3 primers, respectively; Fig. 2a). Using the Perissodactyla cytb sequences from GenBank, we designed two couples of equid-specific primers to retrieve short overlapping fragments in the cytb gene (187 bp for cytb1L 5′CTAATTAAAATCATCAATC and cytb1H 5′ATA ATTCATCCGTAGTTA, 209 bp for cytb2L 5′AACTGCCTT CTCATCCGTCA and cytb2H 5′AAAAGTAGGATGATTCCA AT). PCR reactions were carried out in a total volume of 50 or 100 µl. PCR conditions were as follows: 10 U of *Taq* Gold polymerase (Perkin–Elmer), 2–3 m*M* $MgCl_2$, 1 mg/ml BSA, 250 µ*M* of each dNTP, and 300 ng of each primer. DNA was amplified in an Eppendorf PCR Mastergradient apparatus following heat activation of the *Taq* (92°C, 10 min) and 50 cycles of denaturation (92°C, 60 s), annealing (48–50°C, 60 s), and elongation (72°C, 45 s). Depending on the sample, 0.5–2 µl of ancient extract was added to avoid an inhibition of the *Taq* polymerase activity. Three independent blanks were carried out for each set of PCR experiment as reported by Loreille et al. (2001). On the four human bones coextracted with 01.20.104 and 02.20.104, none of the attempts to amplify DNA with the equid-specific primers yielded any PCR product, suggesting that no cross-contamination occurred during our extraction sessions.

Cloning and Sequencing

PCR products were cloned into bacterial vectors using the Topo TA cloning kit (Invitrogen) following the manufacturer's instructions. Plasmids were purified using the QIAprep spin miniprep kit (QIAGEN) and the sequences of both strands were obtained on a Megabace^{1000} automatic capillary sequencer (Amersham). Up to 13 clones per amplification product were sequenced. In total, 143 clones of 27 PCR products were sequenced. The final sequence of each sample was deduced from the consensus sequence of its different clones.

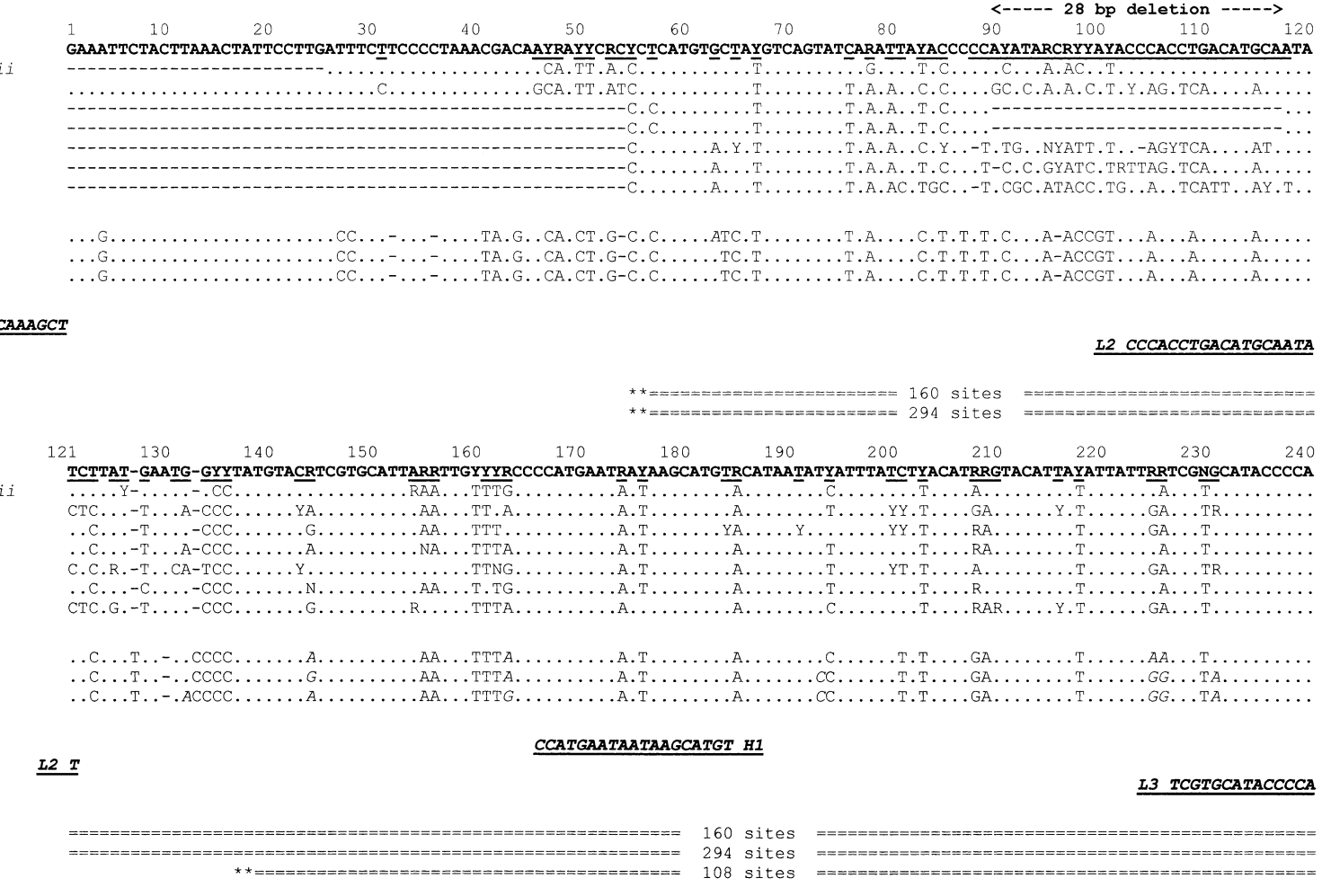
Sequence Analyses

mtDNA CR and cytb sequences of Perissodactyla species were retrieved from GenBank. Given computation time necessities, not all the mtDNA CR reported to date for horses could be used. Thus, to summarize all their genetic diversity, we chose the most divergent sequences for each defined horse mtDNA CR haplogroup (Ishida et al. 1994; Xu et al. 1996; Lister et al. 1998; Kim et al. 1999; Oakenfull and Ryder 1998; Kavar et al. 1999; Oakenfull et al. 2000; Vila et al. 2001). All mtDNA CR sequences retrieved from ancient horses were taken into account to be able to compare the *Hippidion* diversity to horse diversity in the past. Our mtDNA CR data set accounts for 16 extant (HgA—AF326661, AF326647; HgB—AF072989, AF014411; HgC—AF168694, AF072988, AF168696, AF326662; HgD—D23665, AF326659, AF326660, AF326664; HgE—D14991, AF072986; HgF—AF326637, AF056071) and 17 ancient (AF326668–AF326686, AY049720) horses, 2 *E. przewalskii* (AF05878, AF072995), 2 *E. asinus* (X97337, AF220938), 4 *E. hemionus* (AF220934–AF220937), 2 *E. kiang* (AF220932, AF220933), 3 *E. grevyi* (AF220928–AF220930), 9 *E. burchelli* (AD220916–AF220924), 4 *E. zebra* (AF220925–AF220931), and 2 rhinos used as outgroups (*Ceratotherium simum* [Y07726] and *Rhinoceros unicornis* [X97336]). The *E. zebra* sequence reported by Ishida et al. (1995) under accession number S79878 was excluded from our data set, as it obviously corresponds to an *E. caballus* contamination. Our cytb data set includes three extant horses (NC_001640, D32190, D82932 [Xu and Arnason 1994; Ishida et al. 1996]), seven *E. asinus* (X97337, AF380130–AF380135 [Xu et al. 1996]), and one *E. grevyi* (X56282 [Irwin and Kocher 1991]). Sequences were aligned manually using the Seaview software (Galtier et al. 1996). All the phylogenies were computed with the Phylo_win program (Galtier et al. 1996). In cytb phylogenetic analyses, the trees were rooted with the five members of the Rhinocerotidae family (accession Nos.: *Ceratotherium simum*, NC_001808; *Diceros bicornis*, X56283; *Rhinoceros unicornis*, NC_001779; *Rhinoceros sondaicus*, AJ245725; and *Dicerorhinus sumatrensis*, AJ245723 [Irwin and Kocher 1991; Xu et al. 1996; Xu and Arnason 1997; Tougard et al. 2001]) and two members of the Tapiroidae family (*Tapirus indicus*, AF145734; and *Tapirus terrestris*, AF056030). Single-gene or concatenated-gene phylogenetic analyses were done. To avoid an exponential increase in sequences in the concatenated data set, we retained only the five most divergent mtDNA CR sequences for *E. caballus* (AF326659, AF326664, D23665, AF326678, AF326674); they were combined with the two cytb sequences of three that are different (NC_001640, D82932). The two *E. przewalskii* mtDNA CR sequences (AF055878 and AF072995) were also concatenated with the same cytb sequences since it has been shown by independent loci that *E. przewalskii* is a subset of the *E. caballus* species (Ishida et al. 1995; Vila et al. 2001). Finally, all the sequences of *E. grevyi* and *E. asinus* of the mtDNA CR data set have been concatenated with their only corresponding sequence in the cytb data set. Distances were corrected according to the Kimura two-parameter (K2) model. Neighbor-joining, maximum parsimony, and maximum likelihood (TI/TV = 2.0, one rate category, no heterogeneity of substitutions between sites) methods were used. The robustness of the branching was estimated by 1000 bootstrap replicates, except for likelihood analyses where 100 replicates were performed. The Kishino–Shimodoira–Hasegawa test was performed with the DNAML program of the PHYLIP 3.6b package (Felsenstein 1993).

Results

Authentification Criteria

Although a large number of PCR amplifications was attempted (34 to 56 per sample), only three among


A1
<----- 28 bp deletion ----->
1 10 20 30 40 50 60 70 80 90 100 110 120
Equus caballus GAAATTCTACTTAAACTATTCCTTGATTTCTTCCCCTAAACGACAAYRAYYCRCYCTCATGTGCTAYGTCAGTATCARATTAYACCCCCAYATARCRYYAYACCCACCTGACATGCAATA
Equus prwewalskii
Equus asinus
Equus hemionus
Equus kiang
Equus burchelli
Equus grevyi
Equus zebra
Ancient samples
E.1.20.103
01.20.104
03.20.104-b
Primers
L1 GCCATCAACTCCCAAAGCT
L2 CCCACCTGACATGCAATA
Sites used
** 160 sites
** 294 sites
121 130 140 150 160 170 180 190 200 210 220 230 240
Equus caballus TCTTAT-GAATG-GYYTATGTACRTCGTGCATTARRTTGYYYRCCCCATGAATRAYAAGCATGTRCATAATATYATTTATCTYACATRRGTACATTAYATTATTRRTCGNGCATACCCCA
Equus prwewalskii
Equus asinus
Equus hemionus
Equus kiang
Equus burchelli
Equus grevyi
Equus zebra
Ancient samples
E.1.20.103
01.20.104
03.20.104-b
Primers
CCATGAATAATAAGCATGT H1
L2 T
L3 TCGTGCATACCCCA
Sites used
160 sites
294 sites
108 sites

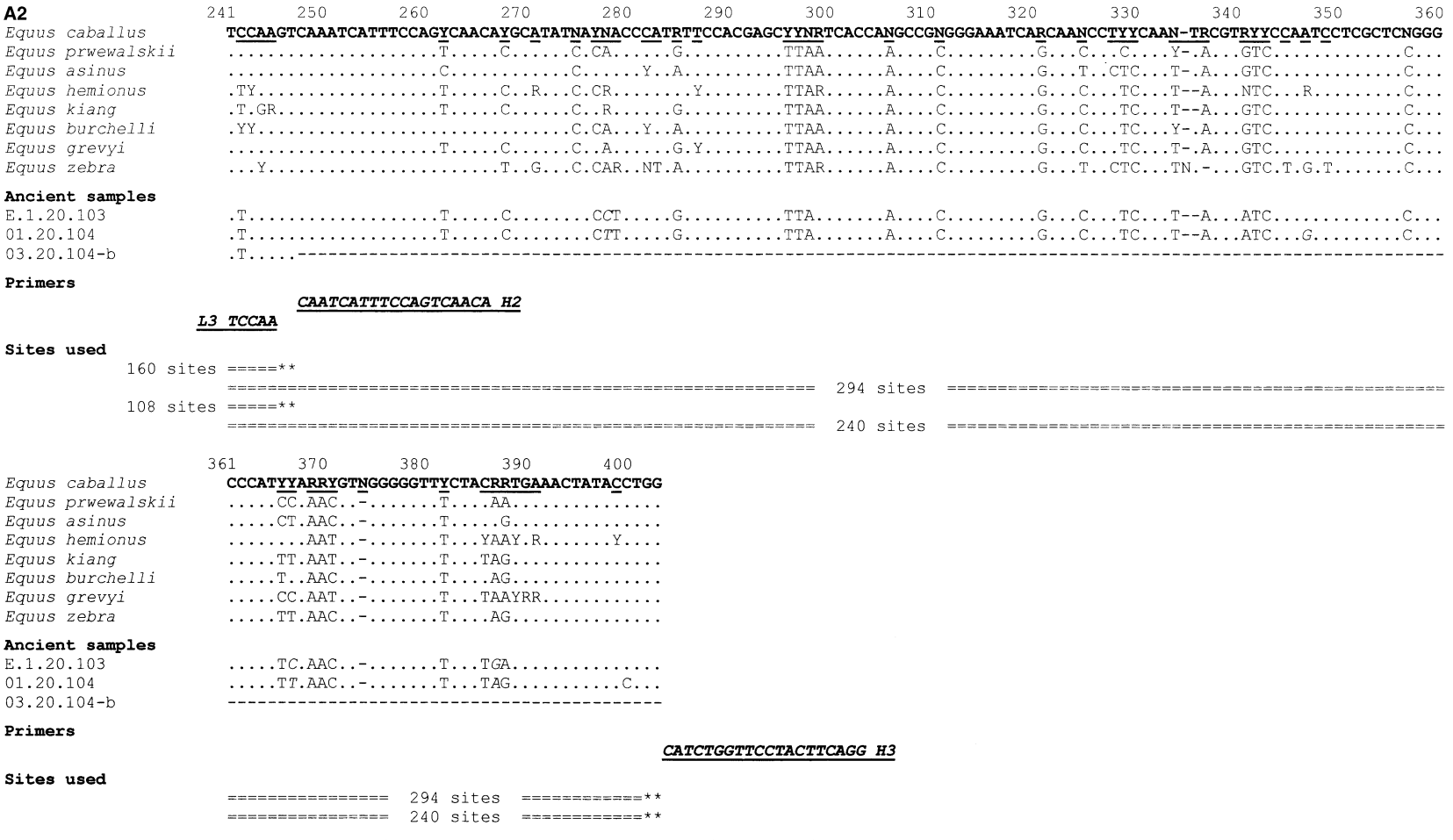


Fig. 2. Alignments. **A** mtDNA CR alignment between ancient and extant equids. **B** cytb alignment between the ancient samples and extant Perissodactyla. The polymorphism observed between the different individuals of the same species is summarized: Y = C or T, R = A or G, N = A, C, G, T, or gap. The polymorphic sites between extant equids sequences are *underlined*. The polymorphic sites between the three ancient sequences are *italicized*.

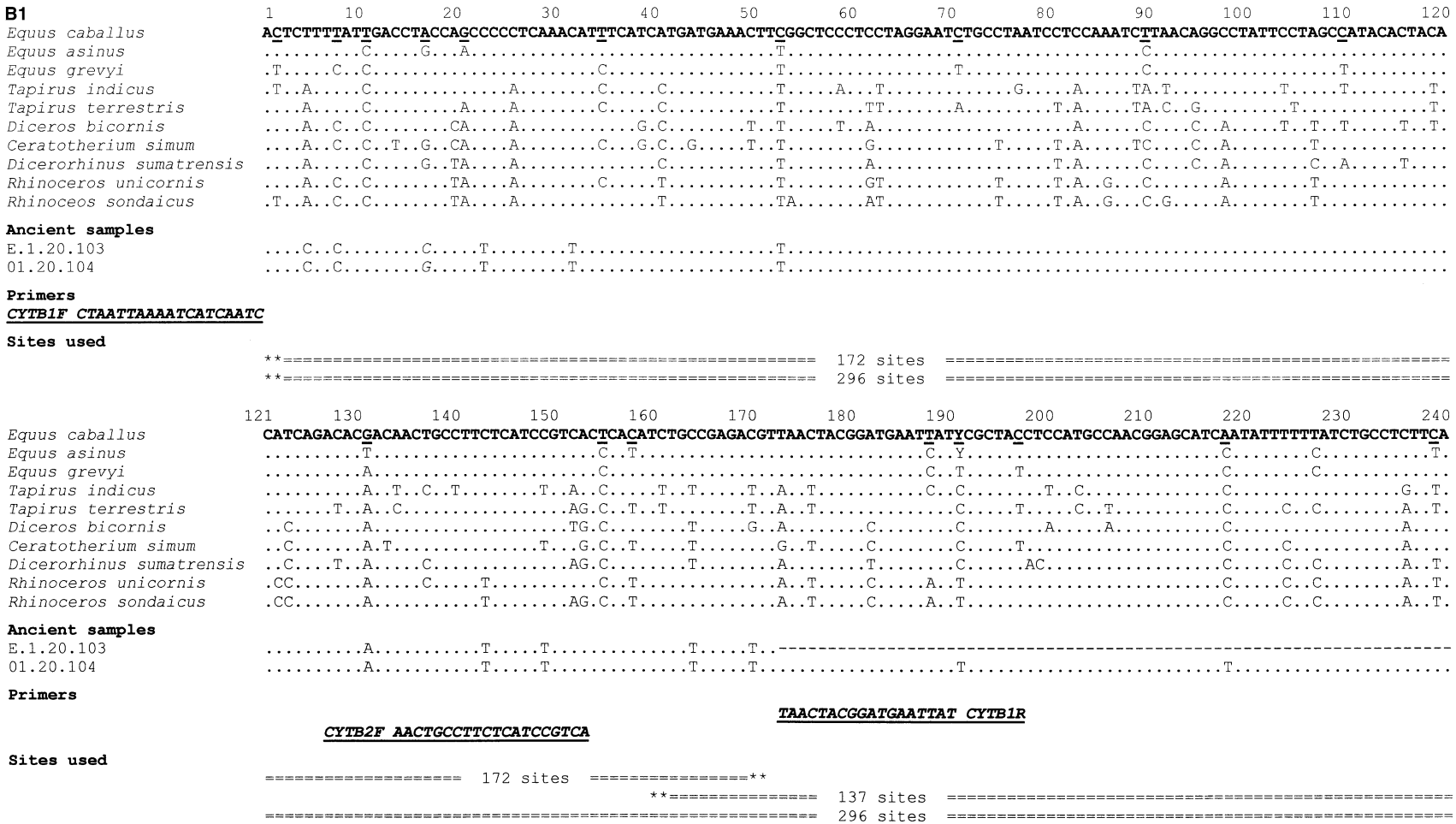


Fig. 2. Continued.

Fig. 2. Continued.

the six fossils analyzed yielded ancient DNA (the metatarsal 01.20.104—394 and 296 bp for mtDNA CR and cytb, respectively; the upper molar E.1.20.103—394 and 172 bp for mtDNA CR and cytb, respectively; the second phalanx 03.20.104-b—241 bp in the mtDNA CR: Fig. 2a and b). Each PCR fragment was independently amplified two or three times for samples 01.20.104 and E.1.20.103. For sample 03.20.104-b, we succeeded in amplifying the first CR fragment (L1H1 primers) 5 times, but only once for the second (L2H2 primers), although 29 amplifications were attempted. The final sequences were deduced from the sequences of 143 clones of 27 PCR products in order to avoid any damage-induced polymorphism. Each nucleotide was thus determined in an unambiguous way except for sample E.1.20.103, where either a C or a T is found in two positions (positions 116 and 128; Fig. 2b). But since (i) a C was scored at those positions for sample 01.20.104 thanks to three independent amplifications, (ii) C is conserved among all equids, and (iii) no intraspecific variation affects those positions in equids, C was legitimately chosen as the right base.

From the differences between the consensus sequence and the sequences of the clones of each PCR product, DNA damage-induced errors were counted. G/C→A/T substitutions account for the majority of the substitutions (75/130 = 57.7%). Such substitutions are incorporated by *Taq* polymerase when faced with deaminated C, which is prevalent in ancient DNA molecules (Hofreiter et al. 2001). On the contrary, G/C→T/A substitutions, which could appear due to G oxidation (Lindahl 1993), occur about 3% (4/130). This pattern is perfectly consistent with the pattern generally observed for ancient sequences (Hofreiter et al. 2001b; Orlando et al. 2002).

Evidence against the occurrence of cross-contamination includes the following: (i) each PCR blank (three per experiment) remained negative during all the amplifications; (ii) three of the six samples did not yield any DNA; (iii) each sample was extracted in independent extraction sessions well separated in time; (iv) whereas amplification of the three mtDNA CR fragments succeeded on both sample 01.20.104 and sample E.1.20.103, sample 03.20.104-b did not yield the third mtDNA CR fragment; (v) sample 03.20.104-b yielded neither the first nor the second cytb fragment, whereas samples 01.20.104 and E.1.20.103, respectively, did; and (vi) each sample yielded a unique final sequence.

Base composition and substitution patterns are similar to those observed in homologous mtDNA sequences of extant equids (Xu et al. 1996; Xu and Arnason 1997). In particular, substitutions occurred most frequently at sites shown to be polymorphic among equids (underlined in Fig. 2), neither gap nor stop codon disrupts the cytb-coding frame and no

Table 1. Bootstrap support for major phylogenetic nodes[a]

			Bootstrap support of node			
			A	B	C	D
NJ (K2-corrected distances)						
108 (CR)			72	—	—	nd
240 (CR)			100	64	6	nd
137 (cytb)			88	nd	nd	71
172 (cytb)			96	100	nd	71
296 (cytb)			100	nd	nd	96
416 (combined)			100	100	nd	53
540 (combined)			100	nd	nd	87
MP	Steps	Informative sites				
108	107	27	—	—	—	nd
240	241	62	100	—	56	nd
137	80	29	—	nd	nd	61
172	112	44	84	100	nd	76
296	182	67	96	nd	nd	93
416	185	80	100	100	nd	57
540	226	94	100	nd	nd	84
ML	In(likelihood)					
108	−725.5		70	—	—	nd
240	−1701.5		100	—	54	nd
137	−585.2		87	nd	nd	68
172	−766.1		88	100	nd	69
296	−1279.9		99	nd	nd	97
416	−1535.4		100	100	nd	—
540	−1901.6		100	nd	nd	75

[a]The definition of nodes A, B, C, and D is indicated in Fig. 3. NJ, MP, and ML: neighbor-joining, maximum parsimony, and maximum likelihood, respectively. Boostrap values less than 50% are not reported (noted with a dash). nd, stands for not determined.

substitution affects the second codon position. Thus, our ancient sequences cannot correspond to nuclear insertions of mtDNA origin.

Phylogenetic Analyses

The sequences from samples 01.20104 and E.1.20.103 share 172 bp in the cytb (Fig. 2b). Whether we choose these 172 sites (first fragment, cytb1F-R primers) or the 137 (second fragment, cytb2F-R primers) or 296 (two cytb fragments) sites retrieved from sample 01.20.104, the ancient samples cluster inside a group including *E. caballus*, *E. przewalskii*, *E. asinus*, and *E. grevyi*. The bootstrap supports are better than 84%, regardless of the phylogenetic method (NJ, MP, ML; Table 1). For CR sequences, the rhino sequences used as outgroups share 108 sites with our three ancient sample sequences and 240 with the 01.20.104 and E.1.20.103 samples (Fig. 2a). Our CR data set includes all the extant species of the genus *Equus*. Once again, all ancient samples cluster among the different species of the genus *Equus* and the monophyly of the clade is strongly supported (Fig. 3a and b, Table 1). This topology is significantly better than the alternative topology where node B is placed before node A (Kishino–Shimodoira–Hasegawa test: difference likelihood, −45.3; SD = 18.8215). Thus, we can confidently conclude that our ancient samples were members of the genus *Equus*. In CR phylogenies, all extant species appear as monophyletic, except *E. hemionus*. Our ancient sequences are most closely related to two sequences of *E. hemionus kulan*; the 28 bp deleted in the sequences of both *E. hemionus* and *E. kiang* individuals are, however, present on our ancient sequences (Fig. 2a).

The situation is similar for phylogenetic analyses constructed with the two genes concatenated; the clustering of the ancient samples inside the group composed of the extant species of the genus *Equus* receives maximal bootstrap support (Fig. 3b, Table 1).

Discussion

Fossils of Ultima Esperanza were originally described by Sefve (1912) as *Onohippidium* (*Parahipparion*) *saldiasi*, more recently revised as *Hippidion saldiasi* (Alberdi and Prado 1993), and are currently believed to be the only equid present in that area (Alberdi and Prieto 2000). The teeth have been referred to *Equus* by Smith-Woodward, but the determination has

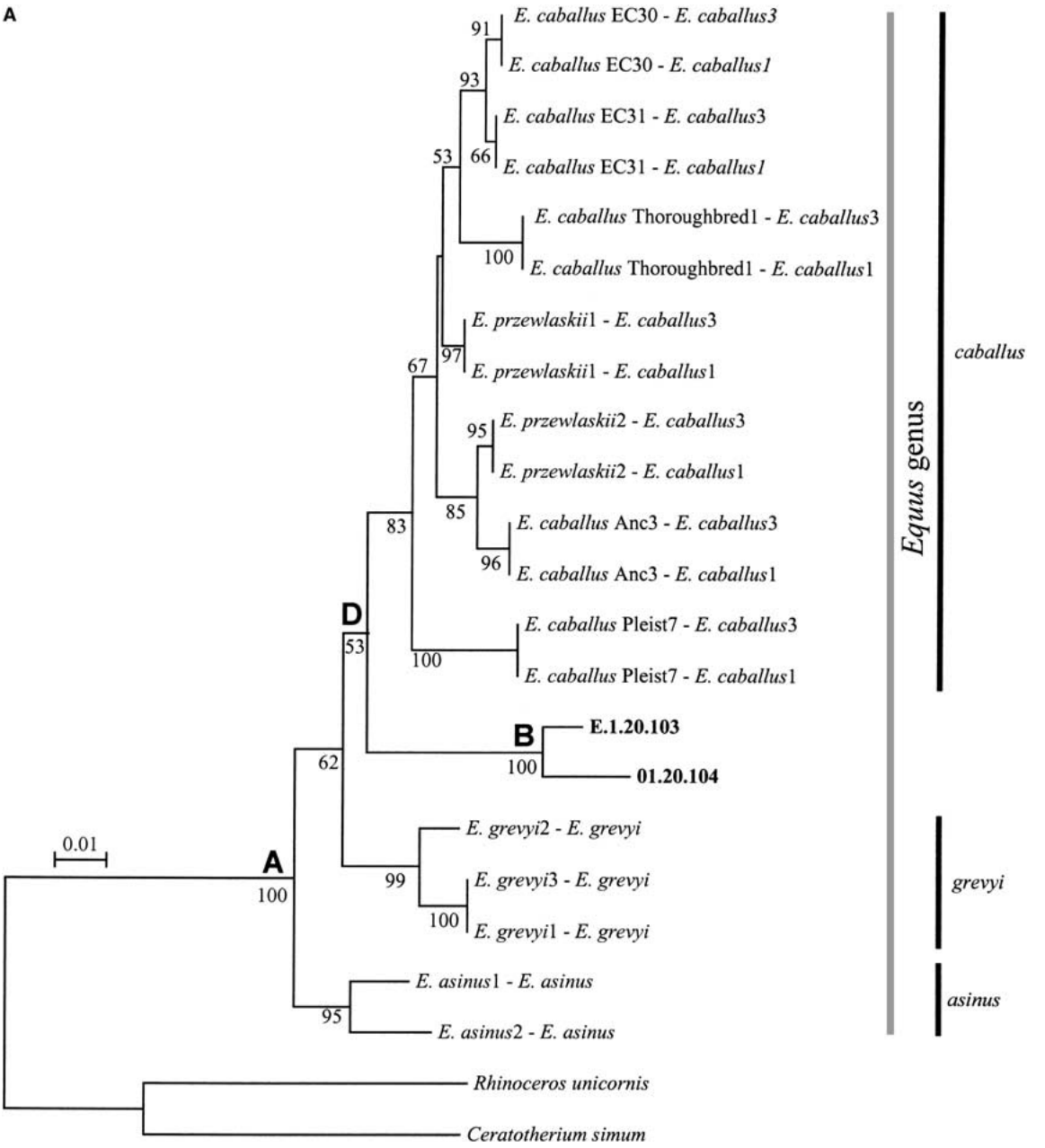


Fig. 3. Phylogenetic position of the ancient samples within the Perissodactyla order. **A** Neighbor-joining tree of the mtDNA CR sequences; 240 sites were used. **B** Neighbor-joining tree of the concatenated sequences of mtDNA CR and cytb; 416 sites were used. On each branch, the first name refers to the mtDNA CR sequence retained for the concatenated analysis, whereas the second refers to the cytb one. Our ancient sequences are reported in *boldface* characters. Distances were corrected with the Kimura two-parameter model. One thousand bootstrap replicates were performed. Bootstrap support values less than 50% are omitted.

never been published and the assigning of the bones, at least, to hippidiform equids was beyond doubt at the time we started the study. Major dental, cranial, and postcranial characters defining hippidiforms and distinguishing them from equiform horses include (i) a short protocone for the first and second upper molar crown, (ii) an extremely deep nasoincisival notch associated with characteristic very long nasal bones, and (iii) short and massive third metapodials (Boule and Thevenin 1920; Alberdi and Prado 1993; MacFadden 1997).

The phylogenetic origin of the South American hippidiform horses is not problematic since an abundance of morphological characters points to an origin in the *Pliohippus* of North America. For instance, both the dorsal and the ventral preorbital fossae are clear synapomophies of *Pliohippus* and *Onohippidium*, the most primitive hippidiforms (MacFadden 1997). Likewise, several dental, cranial, and postcranial synapomorphies relate the equiform horses to another descendant of *Pliohippus*, *Dinohippus*, which arose during the late Miocene (10 MY). Thus, the divergence

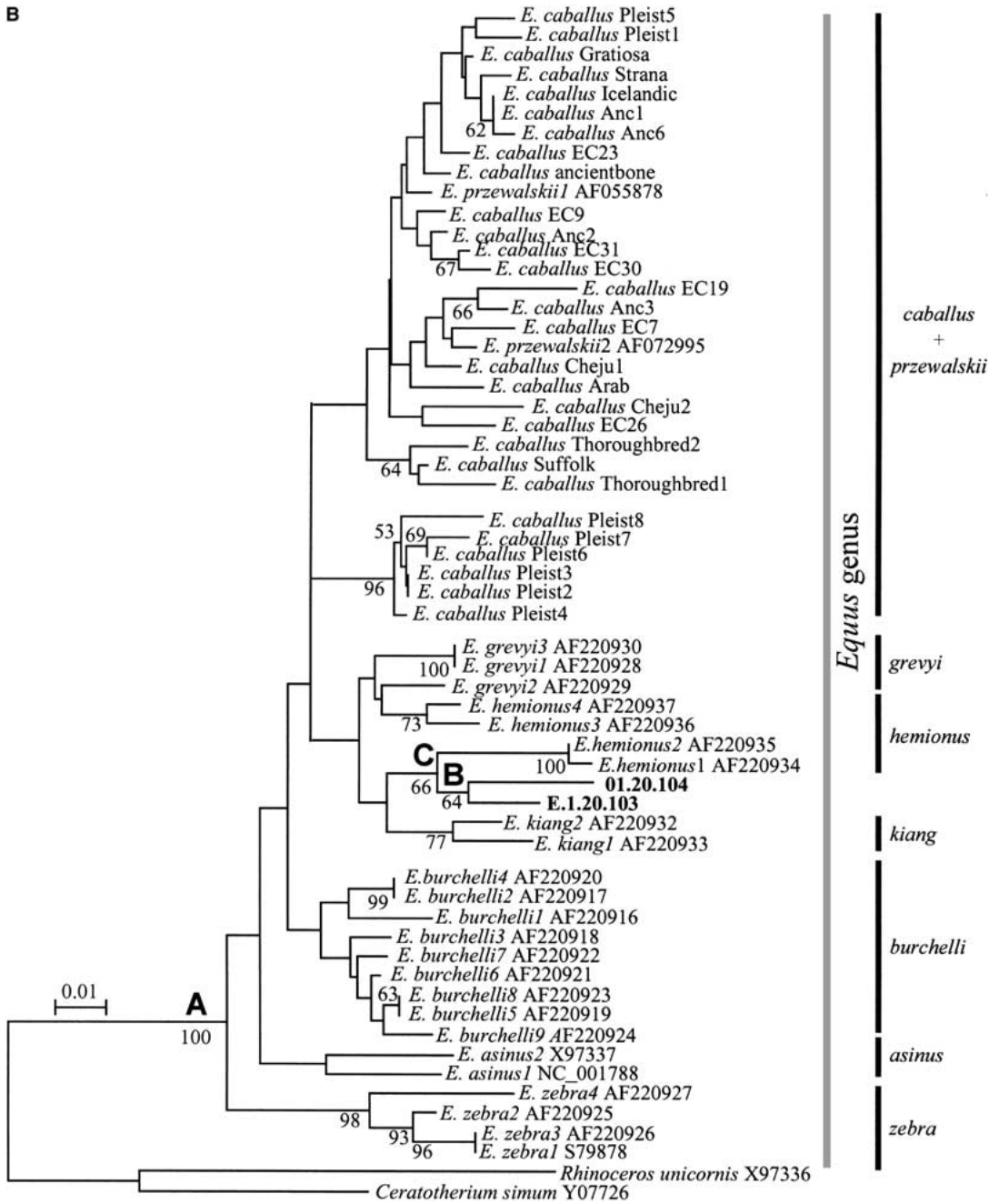


Fig. 3. Continued.

between equiform and hippidiform lineages dates back prior to 10 MY, long before the emergence of the first *Plesippus* (3.7 MY; Fig. 1 [MacFadden 1997]), let alone before the emergence of the first *Equus* (about 2 MY [Oakenfull et al. 2000; Eisenmann and Baylac 2000]). Yet, according to ancient DNA data, our "*Hippidion saldiasi*" was a member of the *Equus* genus, as (i) all phylogenetic analyses significantly cluster the ancient samples well among the modern *Equus* species, and (ii) molecular clock assumptions estimate the emergence of the species between 0.6 and 1.8 MY (calibration point 0.7 MY for the emergence of *E. burchelli* after Oakenfull et al. [2000]).

Given this discrepancy, we decided to look in more detail at the morphological characters of the studied samples. Compared to measurements on *Equus* (*Amerhippus*) second phalanges from Peru collected by one of us (V.E.), the measurements of *Hippidion* second phalanges kindly provided by M.T. Alberdi seem to indicate a greater proximal flatness. Our phalanx (03.20104-b) from Ultima Esperanza is rather deep, as in *Equus* (*Amerhippus*). The upper cheek teeth of *Hippidion* are said to have rather low and incurvated crowns and short oval protocones (Boule and Thevenin 1920; MacFadden 1997). Our upper first molar (E.1.20.103) presents an ovoid but

not very short (10-mm) protocone, and a crown of moderate curvature and height (63 mm): it could thus belong to an *Equus*, as indeed already noted by Smith-Woodward. The third metatarsal (01.20.104) is very short (179 mm), robust, and extremely wide in its distal part (55 mm at the tuberosities). It is almost identical in all its dimensions to the metatarsals preserved at La Plata (Chile) and referred by Sefve (1912) to *Hippidion saldiasi*; but at the same time, it lacks the second, posterior, facet for the cuboid, another characteristic of hippidiforms according to Hoffstetter (1952). We do not know whether this facet is present on the La Plata specimens.

In summary, we believe that the analyzed samples may well have belonged to *Equus* and not to *Hippidion* because; (i) the second phalanx (03.20.104-b) looks like a normal *Equus* phalanx, (ii) the second facet for the cuboid is lacking, while said to be present on third metatarsals of hippidiform horses, (iii) the morphology of the molar is typical not of *Hippidion* but of *Equus*, (iv) all ancient sequences cluster in a monophyletic group, and (v) it would explain the phylogenetic DNA clustering within the *Equus* genus. In the absence of lower incisors, there is no telling whether the lack of infundibula on which Hoffstetter based the subgenus *Amerhippus* in 1950 is shared by our taxon. Accordingly, the South American range of *Equus* should now be greatly extended: believed to stop south of Buenos Aires and Santiago do Chili (Alberdi and Frassinetti 2000), it now reaches southern Patagonia. At the same time, our data bring into question the placement of the southernmost equid fossils into the genus *Hippidion* by previous workers. A thorough revision is obviously necessary, but several observations seem to point to the coexistence of both genera. The first phalanx 03.20.104-a (no DNA retrieved) looks like a typical *Hippidion*; there are two second phalanges from Ultima Esperanza that seem very different from ours, according to measurements published by Sefve (1912).

Our ancient DNA analysis has revealed that some South American equid fossils are not correctly classified at the generic level. The fact that both hippidiform horses and *Equus* (*Amerhippus*) exhibit a pronounced distal limb shortening, probably due to a convergent adaptation to life in a sloped habitat, most probably explains the misidentification in fossils. Whatever the case, since the equid family has been, for more than a century, a famous model to describe processes of evolution (Gould 1994), our findings show that, at least in South America, some of the fossil determinations need revision.

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