



# First evidence of *Equus asinus* L. in the Chalcolithic disputes the Phoenicians as the first to introduce donkeys into the Iberian Peninsula



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## ABSTRACT

Two equid species have been documented in the Pleistocene of the Iberian Peninsula, the horse *Equus caballus*, and the Eurasian hemione *Equus hydruntinus*. While the former survived the Holocene–Pleistocene until now, the timing for the extinction of the latter is unclear. Scarce, fragmented archaeological remains assess the presence of small equids living in the Holocene of Iberia. Those could possibly correspond to the Eurasian hemione although unambiguous morphological identification is often not possible. With the find of an equid tooth from Leceia, a Chalcolithic fortified site in Portugal, and using both morphological and mitochondrial genome analyses, we demonstrate for the first time the presence of a new equid species in Holocene Iberia, namely a donkey (*Equus asinus*). Radiocarbon dating of the tooth to Cal 2340–2130, and 2080–2060 BC with 95% probability, demonstrates that donkeys were present in Iberia well before the arrival of Phoenicians in the first quarter of the first millennium BC (900–750 years BC), which were considered so far as the first who introduced donkeys in the region.

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## 1. Introduction: the past presence of equids in the Iberian Peninsula

During the Late Pleistocene, the horse, *Equus caballus* is well-represented in the fossil record of the western part of the Iberian Peninsula. A geographic subspecies, *Equus caballus antunesi* (Cardoso and Eisenmann, 1989), mainly characterized by more slender metapodials than other wild horses of the same period, constitutes a significant part of the assemblages. This morphotype was characterised from limestone caves of the Portuguese Estremadura dated from 30,000 until almost 15,000 years BP (Cardoso, 1993, 1996) and constituted the source of post-Pleistocene wild horses from the Late Mesolithic shell-middens of Muge (Tagus valley).

The continuous presence of *E. caballus* in the Iberian peninsula (Morales Muñiz et al., 1998) is also attested from the Late Mesolithic

in the shell-middens of Muge, since the pioneer work of Francisco Pereira da Costa (Costa, 1865, Pl. 6, n.º 6), recently confirmed by Detry (2007) with scarce remains in the shell-middens of the Sado valley (Arnaud, 1987, p. 61) and in other sites of the same period in the Guadiana valley – Barca do Xerez de Baixo (Valente, 2008), and in the estremaduran region – Forno da Telha (Rowley-Conwy, 1993) to the Chalcolithic, with unambiguous first phalanx biometric measurements at several sites (Cardoso, 1995a), well known idols with a natural anthropomorphic shape engraved in horse first phalanxes, and alimentary remnants found in settlements.

Besides *E. caballus*, a small equid is recorded in the Late Pleistocene of Portugal and more generally, all over southern Europe. Morphological features identified in the upper cheek teeth found in one karstic cave near Lisboa, Pedreira das Salemas, assigned them to *Equus hydruntinus* (Cardoso, 1995b). This presently extinct species was never domesticated and reached the Crimea and Iran, where it was known until the Iron Age (Orlando et al., 2006). It is disputable however whether it survived until the Chalcolithic in the Iberian Peninsula, where scarce, fragmented remains often preclude unambiguous diagnosis. In fact, the bone material attributed to *E. hydruntinus* of some Chalcolithic settlements of the southern part of the

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peninsula (Uerpmann, 1976) returned a Medieval radiocarbon age, post-dating the global extinction time for this species, and consequently were revised to donkey (*Equus asinus*) (Von den Driesch, 2000). This does not necessarily imply that all material identified as *E. hydruntinus* by Uerpmann belong to domestic donkey of historical times. In fact, A. v. d. Driesch admits that some pieces may belong to this species, like one calcaneum from Cueva de Carigüela, which was found in a layer under dated horse bones, suggesting that this species was still alive in the Iberian Peninsula at the beginning of the 5th millennium BC. H.-P. Uerpmann omits the occurrences of some pieces of this or other similar small non-caballine equids in some Chalcolithic Portuguese settlements: as is the case of the fortified sites of Vila Nova de S. Pedro, Azambuja, Lisbon District, where H. Breuil had identified, besides *E. caballus*, rare remains of an asinine (Jalhay and Paço, 1945), and Monte da Tumba, eventually attributed to *E. hydruntinus* (Antunes, 1987).

The time of extinction of *E. hydruntinus* in the Iberian Peninsula has also been a matter of debate. For some authors, the Portuguese name “zebro” or “zebra”, equivalent to the Spanish name “encebro” could correspond to *E. hydruntinus*; the presence of a wild equid in the Iberian Peninsula is registered both in medieval documents from the 12–16th century, as well as in toponymy (Nores and Liesau, 1992; Antunes, 2006). Quoting the geographical distribution of the references to this animal, both authors presented a map which shows the last reference is in the southeast, a semi-arid region where the species survived until its extinction. In fact, the leather was very appreciated for shoes (Oliveira de Oliveira Marques, 1987; p. 28, 51, 225) and even for the manufacture of shields for war (Antunes, 2006), supporting venatory activities, and also to prevent the destruction of crops by this herd living animal. The correlation between *E. hydruntinus* and the “zebro”, though attractive, has been rejected by ancient DNA analyses that assigned the remains purported to have been the last representative of this species, to donkeys (Orlando et al., 2009). Therefore, the “zebro” were most likely domestic donkeys (*E. asinus*) turned feral, a possibility proposed by C. Liesau, during several stages of the historic record, related to epidemic diseases, which conduced to the abandonment of the fields (pers. comm., 5 March 2013).

## 2. The findings of the Chalcolithic fortified site of Leceia, Oeiras, Lisbon district

### 2.1. General archaeological characteristics of the settlement

The fortified site of Leceia is situated in the top of the right slope of the Barcarena valley, about 4 km from the confluence with the Tagus estuary. During the 20 years of excavations, it was defined as a large and complex defensive system, occupying about 11,000 m<sup>2</sup>, organized in three successive defensive lines, and articulated with the local topography, delimited in space by the two other sides corresponding to an escarpment of cretaceous reefal limestones. It was possible to relate the stratigraphical sequence with the described cultural expression through the successive constructional phases that were identified, and with the absolute chronology obtained for the sequence (Cardoso, 2000, 2008, 2011). The results may be summarized as follows:

- Layer 2 = Third Cultural Phase (Full/Late Chalcolithic of the Estremadura) = Fifth Constructive Phase, second half of the third millennium BC;
- Layer 3 = Second Cultural phase (Early Chalcolithic of the Estremadura) = Second to Fourth Constructive Phases, circa 2800/2900–2600/2500 cal BC;

- Layer 4 = First Cultural Phase (Late Neolithic of the Estremadura) = First Constructive Phase = second half of the fourth millennium to the beginning of the third millennium BC.

The first prehistoric occupation is represented by the building structures of purely habitational nature, which belong to the First Phase of Construction. The following constructive phases show that there was an organized and preconceived occupation of the space with the erection, in a single stroke in the Second Constructive Phase, of a complex fortification. The internal coherence of the restorations and changes themselves, made during the two following constructive phases, still within the Initial Chalcolithic, confirm that evidence. The placing of the constructions shows that there were preoccupations of a proto-urban nature. There are paths, some paved and others not, with steps in one of them to surmount the southern slope of the settlement, and paths that serve as main lines of communication, both within and outside the fortified space. There is also a large intra-mural open-air area paved with stone slabs for people and goods to be gathered into, perhaps in times of conflict. In the Full Chalcolithic, the decadence of the building effort is evident. In this cultural phase the buildings are almost exclusively dwellings. In many instances the fortification walls have already been abandoned and, in some places, even ruined.

### 2.2. The archaeological context and the absolute chronology

It is in one context of the Full Chalcolithic that, during the 2002 campaign, an equid tooth was collected, the main object of this study. The finding is situated in the exterior of the EX Bastion (Fig. 1), a structure connected to the first defensive line of the fortification (Fig. 2). Due to the scarcity of equid remains in the settlement and, even more so, of small equids, some of them were selected for radiocarbon analysis by AMS, but only the piece in question is contemporaneous with the last occupation of the site (Full/Late Chalcolithic: Beta – 260293–3790 ± 40 BP, or 2340–2130 and 2080–2060 cal BC, with 95% probability, by using INTCAL 04 Radiocarbon Age Calibration (Radiocarbon, Vol. 46, nr. 3, 2004).

In fact, there are some limb bones (phalanges) and several teeth of horse (*E. caballus*) from Leceia, but all of the ones that were analysed by the radiocarbon AMS method were more modern than the Chalcolithic occupation of the site, which is consistent with the historical record of the site, that it was occupied for farming activities during medieval and modern times.

### 2.3. Morphology and biometry

The tooth, a left M/3, is incomplete along the distal border, presents medium wear, and is characterised by its small dimensions, which immediately suggests a non-caballine equid (Fig. 3). In fact, the DMD (length, longitudinal diameter), although incomplete, would not be greater than 20 mm, and the DMD (breadth, transversal diameter), is 12 mm. The morphology of the enamel folds confirms it as an asinine, and the small ectoflexid, which does not penetrate into the double knot, excludes *E. hydruntinus*. This analysis revealed the possible presence of a new wild or domestic asinine, possibly the donkey, in the second part of the third millennium in the occident of Iberia. This finding contradicts current models suggesting that donkeys have been introduced by the Phoenicians in the first quarter of the first millennium BC (900–750 years BC), along the littoral establishments, from which the species spread out occupying soon after all of the Iberian Peninsula, before the roman conquest (Altuna, 1980; Altuna and Mariezkurrena, 1986; Pereira et al., 2006). We therefore decided



**Fig. 1.** General plan of the fortified settlement of Leceia; the location of the finding is signalled by a star. After J. L. Cardoso.

to confirm the taxonomic status of the tooth by ancient DNA molecular analyses.

#### 2.4. Molecular analyses

##### 2.4.1. DNA extraction, amplification and sequencing

We drilled and extracted the tooth sample which we named P2\_Leceia (415 mg) and for comparison a second tooth P1\_Porto (a left M/2 collected from Porto das Carretas, a Chalcolithic site in the south of Portugal, near the Guadiana river, and dated to around the same time as the Leceia sample, the second half of the third millennium BC) 185–332 mg were obtained from three extractions by

a silica-based extraction protocol as in Orlando et al. (2009) with appropriate extraction controls in an ancient DNA laboratory at the Centre for GeoGenetics, Copenhagen, Denmark. We then built the DNA extractions into indexed blunt-end libraries using the NEB-Next DNA Library Prep Master Mix set for 454 (Biolabs, ref:E6070s) and amplified the libraries as in Vilstrup et al. (2013). The first 50  $\mu$ l PCR amplification consisted of 8–10 cycles and included the full 25  $\mu$ l DNA library, while the second consisted of four parallel PCRs consisting of 5  $\mu$ l library each amplified for 10–12 cycles and purified on the same MinElute column. Half of the best amplified and purified libraries were directly shotgun sequenced on Illumina HiSeq2000 and the other half was capture enriched individually



Fig. 2. External view of the Bastion EX, corresponding to the location of the finding. Photo by J. L. Cardoso.

with a blank using BioT/B adapted baits as in Vilstrup et al. (2013). Post capture, the samples were amplified by two consecutive PCRs, the first 15 cycles in a 50  $\mu$ l amplification followed by 8 cycles of three parallel 25  $\mu$ l PCRs in the second amplification, purified on the same column using the same PCR conditions as in Vilstrup et al. (2013). The captured libraries were pooled in equimolar ratios together with other indexed samples on 1 lane on a Hiseq2000 sequencing platform (Illumina).

We trimmed our reads using a minimal read length of 25 bp in AdapterRemoval (Lindgreen, 2012) and mapped them against full mitochondrial equid genomes (Vilstrup et al., 2013) using BWA (Li and Durbin, 2009), disabling the seed and relaxing the edit distance

(Schubert et al., 2012). Reads that mapped to multiple positions and which had a mapping quality score  $<25$  were discarded using SAMtools (Li et al., 2009). Finally, we removed sequence duplicates using MarkDuplicates from the Picard package (<http://picard.sourceforge.net>). Final alignments were visually corrected for potential local misalignments and the final sequence was called using a minimum base coverage of 2 and over 50% sequence match among reads.

#### 2.4.2. Phylogenetic and topological analyses

We partitioned the protein coding genes of the mitogenomes into first, second and third codon positions using Stothard's (2000)

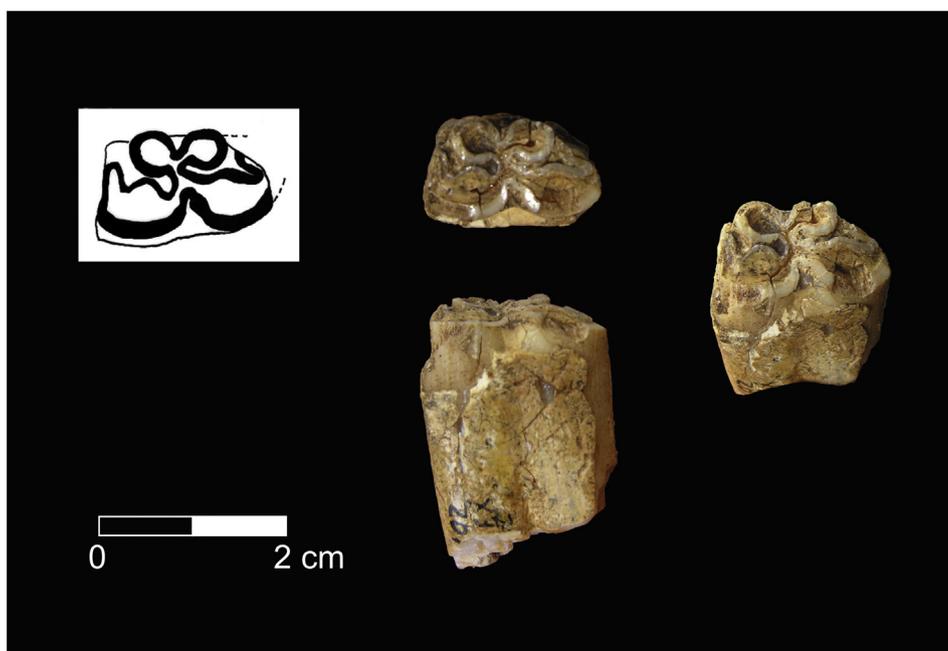


Fig. 3. M/3 of *Equus asinus* of Leceia: morphology of the enamel in occlusal view and different views of the same exemplar. Drawn by B. L. Ferreira; photos by J. L. Cardoso.