

online Sequence Manipulation Suite, with the RNAs and control region making up the fourth and fifth partition. We ran jModelTest v0.1 (Posada, 2008) with corrected AIC to compute the most suitable substitution model for the 5-partitioned data set, unpartitioned and per partition. Outgroup taxa representing all living equids as well as three extinct lineages (Vilstrup et al., 2013) were included in all phylogenetic analyses.

We computed maximum likelihood analyses on the 5-partitioned data set using 500 thorough bootstraps and the GTR + G model in RAxML GUI 1.0 (Silvestro and Michalak, 2012). We also ran Bayesian phylogenetic inference analyses in BEAST (Drummond and Rambaut, 2007) using 300 million iterations on the 5-partitioned data set with different substitution models for each partition as computed from jModelTest. Monophyly was constrained for species with multiple individuals and the zebra clade, and we ran separate analyses with Birth and Deaths, and the Yule speciation model. Finally, we compared the control region sequence characterized in the P2_Leceia sample to the variety of haplotypes characterized in extant and ancient donkey specimens (Table 1), using median-joining haplotype network reconstruction using NETWORK v4.6 (<http://www.fluxus-engineering.com>; Bandelt et al., 1999; Forster et al., 2001; Polzin and Daneschmand, 2003) with weights between 5 and 15, using star contraction and MP correction, and epsilon = 10.

2.4.3. Results of the molecular analyses

We sequenced the near complete mitochondrial genome (13,749 bp) of sample P2_Leceia with an average coverage of 23.46× (2–178×). A total of 1545 bp of the P1_Porto sample, contemporary with P2_Leceia sample, was generated for comparison. Maximum support from both Maximum Likelihood and Bayesian analyses (Fig. 4) confirm the P2_Leceia sample is a donkey (*E. asinus*) and P1_Porto is a horse (*E. caballus*). The absence of a 28 bp deletion in the control region further rejects the Leceia sample as a hemione. Finally, a haplotype network of control region sequences from Somali and Nubian wild asses and contemporary donkeys show that the P2_Leceia sample clusters with domestic donkeys and Nubian wild asses as opposed to Somali wild ass sequences (Fig. 5).

3. Discussion

We note that it is not a requirement to sequence the complete mitogenome in order to distinguish between equids at the species level. A short segment of the hypervariable region containing the 28 bp deletion seen in *E. hydruntinus*, *E. hemionus* and *E. kiang* provide a diagnostic barcode for species identification of fossil equids (Orlando et al., 2009). However, capture-based data can provide additional information regarding the DNA damage levels of an ancient DNA extract and could help authenticating genetic data (Ginolhac et al., 2011; Jónsson et al., 2013). Therefore, it is used as a standard procedure at the Centre for GeoGenetics in Copenhagen in order to screen archeological findings for further genetic analyses. Nonetheless, the morphological and molecular analyses (Figs. 3–5) confirm with maximum support that the Leceia tooth did not originate from *E. caballus* or *E. hydruntinus*, the only two equid species known in the Pleistocene in the Iberian Peninsula. Instead, the mitochondrial genome ascribes the Leceia tooth to *E. asinus*, a donkey, suggesting a new species of equid was present in Chalcolithic Iberia. The Porto sample, of similar age, included for comparison is from *E. caballus* (Fig. 4). More specifically the Leceia sample clusters together with the domestic donkey *E. asinus* and the now extinct Nubian wild ass, *Equus africanus africanus* (Geigl and Grange, 2012) (Fig. 5). Thus, based on the mitochondrial genome analyses, the Leceia sample could belong to either a

Table 1
Control region sequences used in haplotype network.

Sample name	Genbank ID	Reference
Ea_YN01F4	AF531462	Lei et al., 2007
Ea_XJ02F3	AF532120	Lei et al., 2007
Ea_LZ2F5	AF532125	Lei et al., 2007
Ea_GZ02F6	AF532126	Lei et al., 2007
Eas_AP012271	AP012271	Goto et al., 2011
Ea_ER04	AY569465	Beja-Pereira et al., 2004
Ea_RU01	AY569471	Beja-Pereira et al., 2004
Ea_AL17	AY569473	Beja-Pereira et al., 2004
Ea_AS01	AY569477	Beja-Pereira et al., 2004
Ea_AZ01	AY569484	Beja-Pereira et al., 2004
Ea_BE10	AY569485	Beja-Pereira et al., 2004
Ea_BF07	AY569487	Beja-Pereira et al., 2004
Ea_CH02	AY569493	Beja-Pereira et al., 2004
Ea_HU07	AY569499	Beja-Pereira et al., 2004
Ea_KE01	AY569503	Beja-Pereira et al., 2004
Ea_KO03	AY569504	Beja-Pereira et al., 2004
Ea_LI05	AY569505	Beja-Pereira et al., 2004
Ea_MA01	AY569507	Beja-Pereira et al., 2004
Ea_ML01	AY569509	Beja-Pereira et al., 2004
Ea_MO01	AY569511	Beja-Pereira et al., 2004
Ea_RO17	AY569517	Beja-Pereira et al., 2004
Ea_SA11	AY569519	Beja-Pereira et al., 2004
Ea_SPI3	AY569522	Beja-Pereira et al., 2004
Ea_SU01	AY569523	Beja-Pereira et al., 2004
Ea_TK26	AY569527	Beja-Pereira et al., 2004
Ea_UE34	AY569528	Beja-Pereira et al., 2004
Ea_CR02	AY569531	Beja-Pereira et al., 2004
Ea_GH08	AY569532	Beja-Pereira et al., 2004
Ea_EG36	AY569534	Beja-Pereira et al., 2004
Ea_ET10	AY569536	Beja-Pereira et al., 2004
Ea_H6	AY569543	Beja-Pereira et al., 2004
Ea_H7	AY569544	Beja-Pereira et al., 2004
Eas_H8	AY569545	Beja-Pereira et al., 2004
Eas_H9	AY569546	Beja-Pereira et al., 2004
Eas_H10	AY569547	Beja-Pereira et al., 2004
Ea_JMS8	AY666167	Lei et al., 2007
Ea_JMS26	AY666168	Lei et al., 2007
Ea_JM14	DQ368536	Lei et al., 2007
Ea_JM80	DQ368538	Lei et al., 2007
Ea_MG16	DQ368560	Lei et al., 2007
Ea_MG25	DQ368562	Lei et al., 2007
Ea_QY14	DQ368573	Lei et al., 2007
Ea_TH31	DQ368585	Lei et al., 2007
Ea_YPGZ58	DQ448935	Chen et al., 2006
Ea_YPXZ02	DQ449009	Chen et al., 2006
Ea_YPXZ14	DQ449021	Chen et al., 2006
Eaa_PCM54	HM622629	Kimura et al., 2011
Eas_NHML1886	HM622631	Kimura et al., 2011
Eaa_NHML1904	HM622632	Kimura et al., 2011
Eaa_BSZM1952	HM622634	Kimura et al., 2011
Eaa_BSZM1963	HM622635	Kimura et al., 2011
Eaa_RMCA31155	HM622636	Kimura et al., 2011
Eas_WA6	HM622641	Kimura et al., 2011
Eas_WA7	HM622642	Kimura et al., 2011
Eas_WA21	HM622653	Kimura et al., 2011
Eas_WD6	HM622659	Kimura et al., 2011
Eas_WD8	HM622660	Kimura et al., 2011
Eas_WD12	HM622663	Kimura et al., 2011
Eas_ErA4	HM622668	Kimura et al., 2011
Ea_X97337	X97337	Xu et al., 1996
Ea_P2_Leceia	XXXXXXXX	This study

Ea = *Equus asinus*, Eaa = *Equus africanus africanus*, Eas = *Equus africanus somaliensis*.

domestic or wild donkey. Studies using mitochondrial DNA suggests that domesticated donkeys originated primarily from the extinct Nubian wild ass and ancestors of the extant Somali wild ass (Kimura et al., 2011; Rossel et al., 2008). The radiocarbon date obtained for the Leceia tooth rejects the first introduction to the Iberian Peninsula by the Phoenicians in the 8–9th century BC. As donkeys were domesticated in northeast Africa or/and potentially the Middle East around 5000 years ago (Beja-Pereira et al., 2004), more than 750 years before the age of the Leceia tooth, our sample

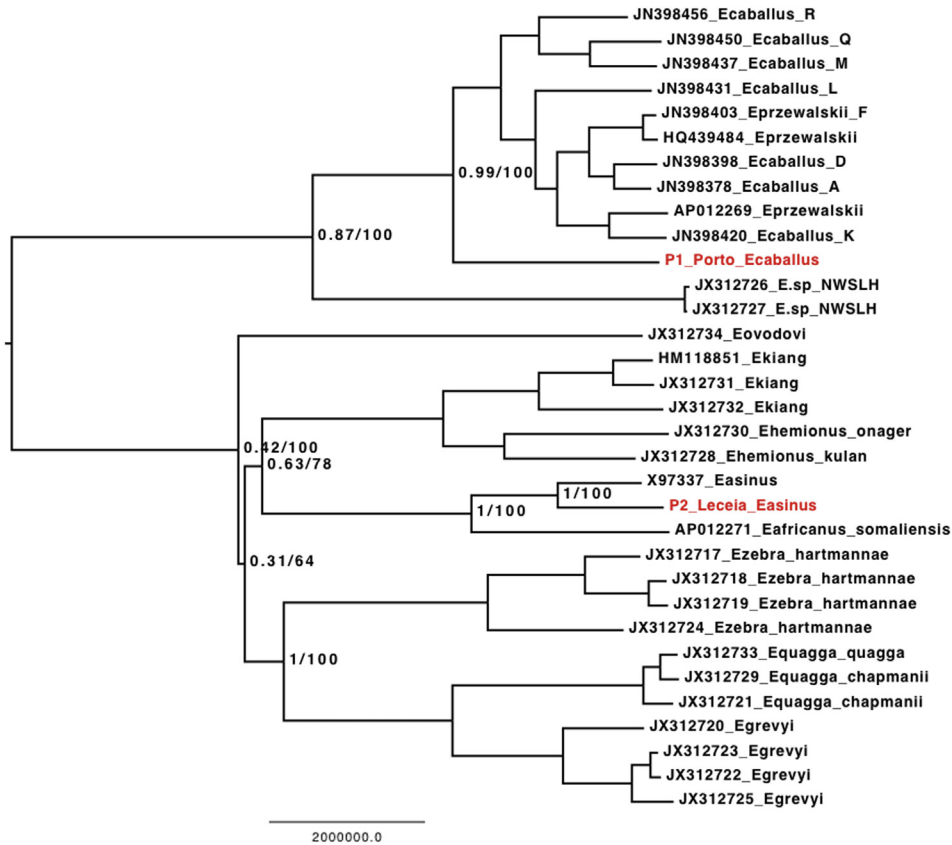


Fig. 4. Phylogenetic tree with Bayesian posterior probabilities (decimal) and Maximum Likelihood bootstrap support (percentage) based on the partitioned analyses in RaxML (GTR + G) and BEAST.

could equally represent a wild individual, or a donkey from the early domestication process. The hypothesis that this piece represents a trophy or a symbolic artefact is not confirmed by the presence of use wear, or any morphological modification in their shape or transformation of their surface.

In general, based on bone measurements, larger sized specimens are attributed to wild asses and smaller individuals to domestic (Rossel et al., 2008), however size cannot be deduced from teeth samples. Kimura et al. (2011) also found little mitochondrial genetic diversity between wild and domestic donkeys and put this

down to wild individuals still being present during the domestication process, something also defined in dogs, deer, pigs and horses. This also proposed that the domestication process continued over a prolonged time with new wild recruitments (Kimura et al., 2011; Rossel et al., 2008).

Significant amount of ivory artefacts from elephant tusks are found in a wide variety of archeological sites in South Portugal (Schuhmacher et al., 2009; Schuhmacher and Banerjee, 2012), including the settlement of Leceia. This trade (Schuhmacher et al., 2009; Cardoso and Schuhmacher, 2012), particularly evident

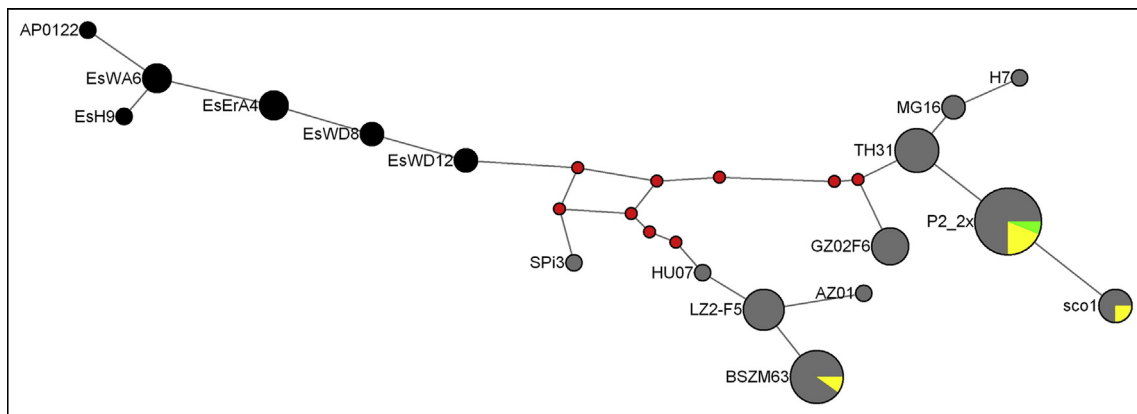


Fig. 5. Haplotype network of 17 different haplotypes of *Equus asinus* (grey), *Equus africanus africanus* (yellow) and *Equus africanus somaliensis* (black). The Leceia sample is highlighted in green and median vectors are in red. Branch length indicates the number of mutation events. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

since the first half of the third millennium BC, with the occurrence in the second half of this millennium of bell beakers, Palmela points, and tonged copper daggers in the Moroccan territory (Harrison and Gilman, 1977; Poyato Holgado and Hernando Grande, 1988; Bokbot, 2005), could be accompanied by the introduction from Northern Africa of other products, like domestic donkeys. Alternatively, should the Chalcolithic ivory pieces be of an Asian elephant (*Elephas maximus*) origin that other trade routes than the North African one could have resulted in the introduction of the domestic donkey in Iberia.

4. Conclusions

In this study, we combine morphological and molecular analyses and demonstrate the presence of a donkey, *E. asinus*, in the second half of the third millennium BC in Iberia, which could be a domestic descendant of the now extinct Nubian wild ass (*E. africanus africanus*). We dismiss our Leceia donkey as from the Phoenician introduction of domestic donkeys in the 8–9th century BC. The earlier introduction of this species in the Iberian Peninsula could possibly be related to the ivory trade between North Africa and Iberia during the Chalcolithic. Our findings suggest that domestic donkeys were introduced into the Iberian Peninsula approximately 1500 years earlier than previously considered.

Author contributions

JLC conceived the study, collected the samples, and performed the archaeological and chronological discussion, JTV and LO performed the molecular experiments and phylogenetic analyses; LO contributed material, reagents, and methods; VE conducted the morphological analyses; JLC and JTV wrote the paper, with significant input from VE and LO.

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