Your horse is a donkey! Identifying domesticated equids from Western Iberia using collagen fingerprinting

Supplementary Information

The raw data has been uploaded to the following locations for open access:

- MS/MS Data:
  - DOI: doi:10.25345/C5T727K8H
  - ProteomeXchange through MassIVE: PXD035509
  - MassIVE Record Number: MSV000089943
- ZooMS Spectra:
  - DOI: 10.5281/zenodo.6878868
  - Zenodo Record Number: 6878868

Also uploaded with the ZooMS Spectra to Zenodo under the same DOI are:
1. Aligned FASTA files for the mature peptides of *col*1α1 and *col*1α2 of all sequences used in the manuscript.

Included in this Supplemental Information PDF

Supplemental Figures

Figure S1: MS/MS examples for the distinguishing chymotryptic peptide in horse and donkey

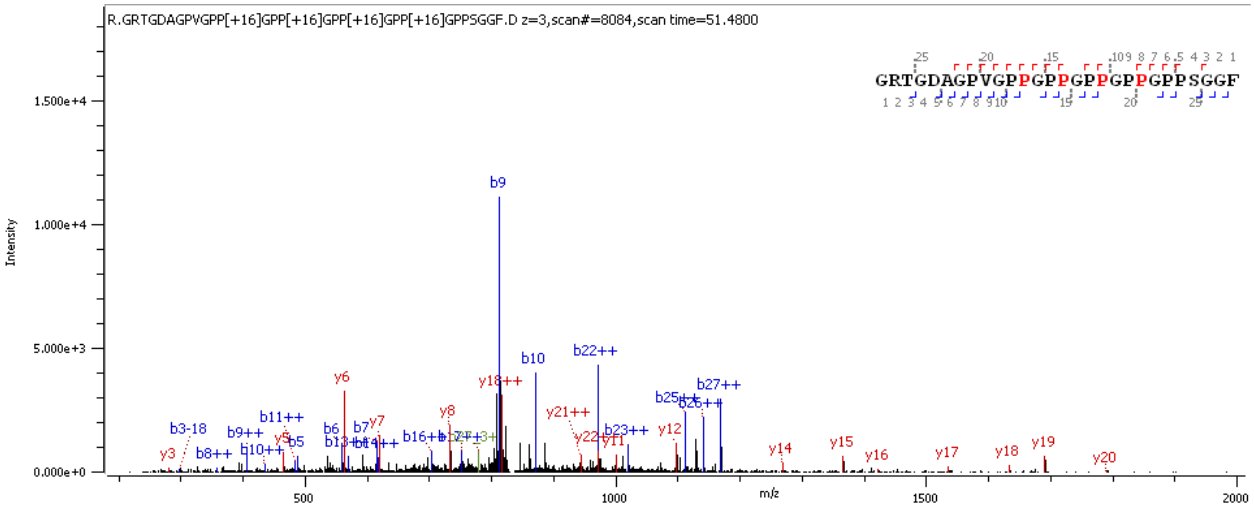
Supplemental Tables

Table S1: Sample list of all archaeological and taxonomic reference samples analysed in this study.
Table S2: List of published collagen markers for species from the Equidae family.
Table S3: Number of proteins in proteome search and coverage of collagen for confirmation search digested with chymotrypsin.

SI Figure 1: MS/MS Sequence Identification of Biomarkers

The MS/MS spectra of the peptides for the diagnostic marker COL1A1 991-1018 from horse (LARC.265) and donkey (LARC.1498). Both peptides have 4 proline oxidations.

Horse: m/z 2497



Donkey m/z 2511

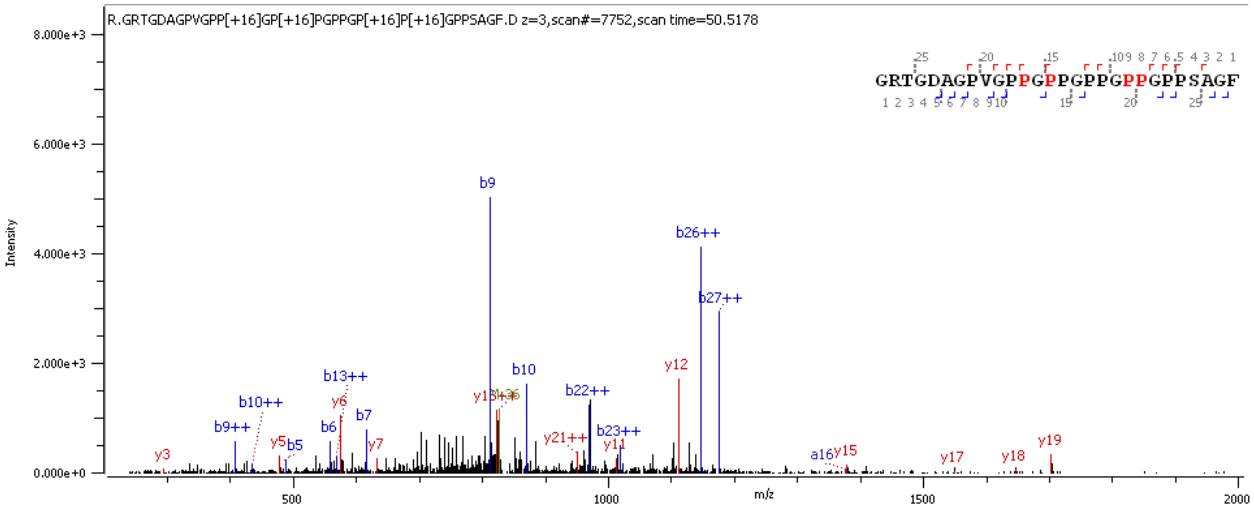


Table 1: Sample list of all archaeological and taxonomic reference samples analysed in this study.

Sample ID	Lab Code	Site	Country	Time Period	Skeletal Element	Morphological Id.	ZooMS Id.
TRAOF100	HZ145	Troia	Portugal	Roman	Left Femur	*Equus asinus*	*Equus asinus*
TRAOF101	HZ146	Troia	Portugal	Roman	Left Scapula	*Equus asinus*	*Equus asinus*
TRAOF102	HZ147	Troia	Portugal	Roman	Mandible	*Equus asinus*	*Equus asinus*
TRAOF104	HZ149	Troia	Portugal	Roman	Pelvis	*Equus asinus*	*Equus asinus*
TRAOF105	HZ150	Troia	Portugal	Roman	Rib	*Equus* sp.	*Equus asinus*
TRAOF107	HZ152	Troia	Portugal	Roman	Long bone fragment	*Equus* sp.	*Equus asinus*
RDA.19.EQ1	HZ156	Rua do Anjos	Portugal	Roman	Molar	*Equus* sp.	*Equus asinus*
RDA.19.EQ2	HZ157	Rua do Anjos	Portugal	Roman	Molar	*Equus* sp.	*Equus caballus*
RDA.19.EQ3	HZ158	Rua do Anjos	Portugal	Roman	Mandible	*Equus* sp.	*Equus caballus*
RDA.19.EQ4	HZ159	Rua do Anjos	Portugal	Roman	Metapode	*Equus* sp.	*Equus caballus*
RDA.19.EQ5	HZ160	Rua do Anjos	Portugal	Roman	Metapode	*Equus* sp.	*Equus caballus*
RDA.19.EQ7	HZ161	Rua do Anjos	Portugal	Roman	Radius	*Equus* sp.	*Equus caballus*
RDA.19.EQ8	HZ162	Rua do Anjos	Portugal	Roman	Pelvis	*Equus* sp.	*Equus caballus*
RDA.19.EQ9	HZ163	Rua do Anjos	Portugal	Roman	Astragalus	*Equus* sp.	*Equus asinus*
RDA.19.EQ10	HZ164	Rua do Anjos	Portugal	Roman	Femur	*Equus* sp.	*Equus caballus*
LCB.15.EQ19	HZ153	Largo do Coutador	Portugal	Late Antiquity	Metapode	*Equus* sp.	*Equus asinus*
LCB.15.EQ18	HZ154	Largo do Coutador	Portugal	Late Antiquity	Molar	*Equus* sp.	*Equus asinus*
LCB.15.EQ17	HZ155	Largo do Coutador	Portugal	Late Antiquity	Molar	*Equus* sp.	*Equus asinus*
RNA63EQ11	HZ165	Rua Nova do Almada 63	Portugal	Roman Imperial	Molar	*Equus* sp.	*Equus caballus*
RNA63EQ12	HZ166	Rua Nova do Almada 63	Portugal	Roman Imperial	Incisor	*Equus* sp.	*Equus asinus*
BPLX.246	HZ167	Banco de Portugal	Portugal	Roman	Cranium	*Equus* sp.	*Equus asinus*
H4.1070.1	HZ143	Los Morrones 11	Spain	Iron Age	Radius	*Equus caballus*	*Equus caballus*
H4.1070.2	HZ144	Los Morrones 12	Spain	Iron Age	Radius	*Equus caballus*	*Equus caballus*
H4.1075.3	PHD1075.3	Los Morrones 11	Spain	Iron Age	Radius	*Equus caballus*	*Equus caballus*
TRSLOF100	HZ140	Torre Sal	Spain	Iberian	Radius	*Equus caballus*	*Equus caballus*
TRSLOF103	PHDTSOF100	Torre Sal	Spain	Iberian	Radius	*Equus caballus*	*Equus caballus*
MJV.1	HZ121	Horta da Torre	Portugal	Late Roman	Right Radius	*Equus caballus*	*Equus caballus*
MJV.2	HZ122	Horta da Torre	Portugal	Late Roman	Right Metacarpus	*Equus caballus*	*Equus caballus*
MJV.3	HZ123	Cacela - Poço Antigo	Portugal	Late Medieval Islamic	Calcaneum R	*Equus* sp.	*Equus caballus*
MJV.5	HZ125	Cacela - Largo Fortaleza	Portugal	Late Medieval Islamic/Christian	Upper Incisor 1 or 2 R (root)	*Equus* sp.	*Equus asinus*
MJV.7	HZ127	Oficina Senhor Carrilho	Portugal	Medieval Islamic	Metapodial	*Equus* sp.	*Equus caballus*
MJV.11	HZ131	Castillo de Aracena	Spain	Medieval Islamic	Scapula R	*Equus* sp.	*Equus caballus*
MJV.12	HZ132	Castillo de Aracena	Spain	Late Medieval Islamic/Christian	Scapula L	*Equus* sp.	*Equus asinus*
MJV.13	HZ133	Castillo de Aracena	Spain	Late Medieval Islamic/Christian	Scapula R	*Equus* sp.	*Equus caballus*
MJV.14	HZ134	Rua da Sé	Portugal	Medieval Islamic	Ulna L	*Equus caballus*	*Equus caballus*
MJV.15	**HZ135**	**Rua da Sé**	**Portugal**	**Medieval Islamic**	**Humerus R**	***Equus*** sp.	***Equus asinus***
MJV.16	HZ136	Convento das Bernardas	Portugal	Late Modern (18/19th century)	Cranium	*Equus caballus*	*Equus caballus*
MJV.17	HZ137	Cerro da Vila	Portugal	Roman Imperial	Metacarpus L	*Equus* sp.	*Equus asinus*
MJV.18	HZ138	Cerro da Vila	Portugal	Roman Imperial	Ulna R	*Equus* sp.	*Equus asinus*
MJV.19	HZ139	Cerro da Vila	Portugal	Roman Imperial	Maxillar	*Equus caballus*	*Equus caballus*
LARC.265	PHD265	Minho	Portugal	Modern/Reference	Vertebra	*Equus caballus*	*Equus caballus*
LARC.238	PHD238	Minho	Portugal	Modern/Reference	Nasal conchae	*Equus caballus*	*Equus caballus*
LARC.2324	PHD2324	Minho	Portugal	Modern/Reference	Scapula	*Equus caballus*	*Equus caballus*
LARC.1498	PHD1498	Baixo Alentejo	Portugal	Modern/Reference	Vertebra	*Equus asinus*	*Equus asinus*
LARC.2000	PHD2000	Trás-os-Montes	Portugal	Modern/Reference	Vertebra	*Equus asinus*	*Equus asinus*
LARC.2313	PHD2313	Trás-os-Montes	Portugal	Modern/Reference	Vertebra	*Equus asinus*	*Equus asinus*

Note:

The entry in bold was formally identified as an equid but presumed to be horse since all the other equids from the same context were adult horses. But ZooMS identification revealed it to be a donkey.

Table 2: List of published collagen markers for species from the Equidae family.

Scientific Name	COL1A1 508-519	COL1A1 586-618	COL1A1 586-618 (+16)	COL1A2 978-990	COL1A2 978-990 (+16)	COL1A2 484-498	COL1A2 502-519	COL1A2 292-309	COL1A2 793-816	COL1A2 454-483	COL1A2 757-789	COL1A2 10-42	References
Equus grevyi	1105.6	2883.4	2899.4	1182.6	1198.6	1427.7	1550.8	1649.8	2145.1	2820.4	2983.4	2999.4	Welker Frido et al. (2016)
Equus quagga	1105.6	2883.4	2899.4	1182.6	1198.6	1427.7	1550.8	1649.8	2145.1	2820.4	2983.4	2999.4	Welker Frido et al. (2016)
Equus caballus	1105.6	2883.4	2899.4	1182.6	1198.6	1427.7	1550.8	1649.8	2145.1	2820.4	2983.4	2999.4	Welker Frido et al. (2016); Buckley et al. (2009); Buckley and Collins (2011); Kirby et al. (2013); Buckley et al. (2017)
Equus asinus	1105.6	2883.4	2899.4	1182.6	1198.6	1427.7	1550.8	1649.8	2145.1	2820.4	2983.4	2999.4	Welker Frido et al. (2016)
Equus hemionus khur	1105.6	2883.4	2899.4	1182.6	1198.6	1427.7	1550.8	1649.8	2145.1	2820.4	2983.4	2999.4	Welker Frido et al. (2016)
Equus hemionus hydruntinus	1105.6	2883.4	2899.4	1182.6	1198.6	1427.7	1550.8	1649.8	2145.1	2820.4	2983.4	2999.4	Welker Frido et al. (2016)
Equus caballus	1105.6	2883.4	2899.4	1182.6	1198.6	1427.7	1550.8	1649.8	2145.1	2820.4	2983.4	2999.4	Welker Frido et al. (2016)

Note:
The nomenclature of the markers follow the scheme recommended in Brown et al. (2021).

Table 3: Number of proteins in proteome search and coverage of collagen for confirmation search digested with chymotrypsin.

Sample	Taxonomic ID	Sample Type	# proteins [a]	COL1A1 best hit [b]	% COV [b]	COL1A2 best hit [b]	% COV [b]
LARC.265	*Equus caballus*	Modern	7	*Equus caballus*	93	*Equus caballus*	95
LARC.1498	*Equus asinus*	Modern	6	*Equus asinus*	78	*Equus asinus*	89

[a] Filters: 2 or more unique peps, log prob > 3, run against database of SwissProt™, horse proteome, donkey proteome.
[b] from the semi-specific Byonic™ runs with the limited database.

References

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