CATALOGUE

Classification of an equine skull in the Science Museum of Coimbra, Portugal, by means of multivariate and qualitative analysis

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Abstract

The study aimed at classifying an equine skull stored in *Museu da Ciência da Universidade de Coimbra*, Coimbra (Portugal) on the basis of its morphology using multivariate analyses. A visual appraisal had revealed that it was not from a horse. Nineteen cephalic measurements were obtained and compared with available data of horses of different groups (poneys, trait and saddle), equine hybrids, Przewalski's horse (*Equus przewalskii*), wild and domestic asses, and quaggas (*Equus quagga*). Multivariate analysis plus head profile allowed us to assign the skull to a mare hinny -the hybrid between a jenny (female donkey) and a stallion (male horse). The research highlights the possibilities of categorization of equid skulls according to morphometry but with a need to consider qualitative traits, as head profiles. But more such studies are needed to be conducted to establish clearly differences between mules and hinnies, especially among sympatric populations.

Keywords

Equids, hinny, mule, quagga.

PENSOFT

Introduction

All living species of equids belong to the genus *Equus,* which is divided into two taxa: the caballine taxa, subgenera *Equus* (*Equus*), which includes domesticated horses and Przewalski's horses, and non-caballine taxa, subgenera *Equus* (*Asinus*), which comprises the different asses and zebra species (Bennett 1980; Heck et al. 2018). Within the caballine taxa, the Przewalski's horses likely represent the sister-taxon to the extinct wild ancestor of domesticated horses (Heck et al. 2018).

Equids biodiversity has been characterized using morphological measurements (Zhu et al. 2014; Salamanca-Carreño et al. 2020). Living species of *Equus* can be differentiated by a number of morphological characters (Bennett 1980). Morphological description is an essential component of breed characterization that can be used to physically identify, describe and recognize a breed, and also to classify livestock breeds into broad categories (Sañudo 2009). Historically, morphological studies, especially of the skull, were the major source of data used to characterize breeds (Sañudo 2009). Horse skull has been the most extensively studied bone for establishing taxonomic grouping at a breed level (Aparicio 1944). Crania are also the most commonly used skeletal elements in population studies because they are known to be more genetically driven and less affected by environmental factors. Given their biometric nature, cephalic measurements and indices allow comparisons between breeds from very distant geographical areas, and permit research into the distinctiveness of breeds based on cephalic evaluation (Sañudo 2009).

This study sought to examine skull measurements of a rare equine skull, numbered MCUC, ZOO.0004465, stored in the collection of Zoology of the *Museu da Ciência da Universidade de Coimbra* in Coimbra (Portugal) (Fig. 1), by means of multivariate analyses via principal component and discriminant analyses. It is a complete and well- preserved skull (cranium and mandible) belonging to an old female. The skull was already at the Museum in 1931, because it is listed in a catalogue. It presents a long and marked celoid (concave) face with a completed permanent series with no canines. Occlusal surfaces of teeth are very worn with a marked rostral tail on PM1. Mandible lacks M1, probably due to an infectious process as bone at this level is laterally deformed. Specimen is relatively recent because it presents intact nasal cartilages. Cause of death was unknown. It was detected accidentally during a photographic session of ancient cattle skulls in January 2020. It was classified simply as *Equus caballus*, with no associated information of origin, collector and antiquity, but it seemed clear it was neither a horse nor a donkey, according to a visual detailed appraisal (Barone 1999) in a visit first author did there in 2020.

Figure 1. Biometrical characters used in the comparison of different equid species and breeds. See table 1 for identification of morphological features.

Material and methods

Data collection

Measurements were taken for specimen MCUC, ZOO.0004465 and adult Araucan skulls (n=10, from the osteological virtual collection of the Department of Animal Science of the University of Lleida) following standard procedures and anatomical reference points as described by Eisenmann (1980). Araucan horse is a local breed from the "Llanos" in Arauca, East Colombia. A data set of 19 measurements were

collected for each specimen. Measurements were obtained from digital pictures using the Digimizer software (© MedCalc Software) by second author. Measurements on MCUC, ZOO.0004465 were performed three times and averages obtained. The rest of data were obtained from Eisenmann (Eisenmann 1980; Eisenmann and Baylac 2000) and included Plains zebras (*Equus burchellii*) were subdivided into two of different described subspecies: Grant's (*Equus quagga boehmi* Matschie, 1892) and Burchell's zebra (*Equus quagga burchellii* Gray, 1824). "Trait" group included draft horses such as Boulonnais and Percheron, and others of unknown breed. "Poneys" group included Islandais, Shetland and Welsh, and others of unknown breed. Mules and hinnies were considered purely as "hybrids" in the consulted source. Saddle horses included Arabian (n=13) and Araucan. A total of 238 specimens were finally studied:

Grant's zebra (*Equus quagga boehmi*)	6
Burchell's Zebra (*Equus quagga burchellii*)	14
Trait horses (different breeds)	15
Domestic donkeys (unknown breeds)	50
Saddle horses (different breeds)	23
Przewalski's horse (*Equus przewalskii*)	46
Asian wild Ass (*Equus hemionus*)	24
Poneys (different breeds)	15
African wild Ass (*Equus asinus*)	8
Hybrids (mules and hinnies)	31
MCUC, ZOO.0004465	**1**

Statistical analyses

First, a NPMANOVA was done using Euclidean distances for all values. Then, we used a Canonical Variate Analysis (CVA) with log-transformed values. CVA attempts to distinguish between groups based on studied measurements. Finally, we performed a hierarchical cluster analysis using Ward's algorithm and a k-clustering test. Clustering systems assign objects into groups (called clusters) so that objects (cases) from the same cluster are more similar to each other than objects from different clusters. Data were subjected to these analyses using PAST v. 2.17c (Hammer et al. 2001).

Results

Numerical data for MCUC, ZOO.0004465 appear in table 1. NPMANOVA reflected statistical differences of our specimen with saddle horses, Przewalski's horses and Asian wild asses (F=71.42; 9,999 permutation rounds; Table 2). All these 4 groups were then deleted for ulterior analysis. CVA appears in Fig. 2, which showed a clear position of skull MCUC, ZOO.0004465 to the hybrid group. This result would indicate that this specimen would be a hybrid specimen. Clustering analysis with

poney and trait group assigns (cophenetic correlation=0.828) the specimen in an intermediate position (Fig. 3). K-clustering to partite these groups observations into 3 clusters assigned all poneys and trait horses correctly, but 2 mules appeared into the former and 16 into the latter, e.g. only 13 mules were "correctly" assigned. MCUC, ZOO.0004465 was assigned to this latter.

Discussion

Sometimes, the diagnosis of species based on external qualitative characters can lead to confusion, especially in groups with high levels of morphological similarity and/or variability, as in *Equidae*. Skull quantitative characters took into account the nature and extent of variability of these characters.

According to Hanot's results (Hanot and Bochaton 2018), equine hybrids are strongly morphologically variable. In regard of our study, hybrid specimens displayed three differentiated groups, we call elipometrical ("poney-like"), eumetrical (medium body size) and hypermetrical (trait horse-like") traits. Our study reflects that the

Table 1. Numerical data for MCUC, ZOO.0004465 specimen. Values in mm.

1.	Facial breadth	134.57
2.	Frontal breadth	174.64
3.	Bizygomatic breadth	174.94
4.	Cranial breadth	99.22
5.	Breadth of the supra-occipital crest	54.42
6.	Anterior ocular line	365.62
7.	Posterior ocular line	152.26
8.	Muzzle breadth at the posterior border of I3	73.41
8 bis.	Least muzzle breadth (between the crests)	57.98
9.	Greatest length	516.10
10.	Height of the infra-orbital bar	13.18
11.	Height of the external auditive meatus	14.41
12.	Antero-posterior orbital diameter	59.05
13.	Dorso-ventral orbital diameter	56.44
14.	Facial height in front of P2	77.69
15.	Facial height between P4 and M1	100.01
16.	Facial height behind M3	130.65
17.	Length of the naso-incisive notch	143.02
18.	Cheek length	155.44

specimen numbered MCUC, ZOO.0004465 from Coimbra belonged unambiguously to a hybrid domestic equine female, and probably of an eumetrical type.

Mules and hinnies are hybrids that combine traits of their equid parents - donkeys (*Equus asinus*) and horses (*Equus caballus*) - although phenotypic traits have been not much studied or understood (Mclean 2016; McLean et al. 2019), although like most equine hybrids, the traits of the sire are generally more predominant. In fact, because mules vary so much in genetic makeup, they do not make good subjects for research (Adams et al. 2013); therefore, there exists an important lack of morphological data, and practically nothing on the differentiation between mules and hinnies. Unluckily no skull data for each hybrid type is available on consulted bibliography, so it would seem not possible to assign skull MCUC, ZOO.0004465 to be from a mule - an hybrid between a mare (female horse) and a jackass - or to a hinny - hybrid between a jenny (female donkey) and a stallion (male horse) - just based on numerical traits

But we can add qualitative information. In Portugal there are two local asinine breeds, the *Burro da Graciosa*, in the archipelago of the Azores, and the *Burro da Miranda*, from Algarve (Porter et al. 2016). This latter presents a straight profile. Among local horse Portuguese breeds (Lusitano, Garrano, Sorraia and *Pónei da Terceira*), Lusitano is a saddle cirtoid horse. Garrano is a breed from the mountainous regions of the Minho and Trás-dos-Montes, with an average withers height of 128 cm for females and 130 cm for males (mature poneys measure less than 150 cm at the withers). The Garrano horse has mostly orthoid profile. Sorraia, the other elipometrical local breed, with a convex head profile, seems also to be discarded as parenteral line. *Pónei da Terceira* is from Açores Islands.

Table 2. Results for NPMANOVA. There were statistical differences of our specimen with saddle horses, Przewalski's horses and Asian wild asses (significative *p* values in bold).
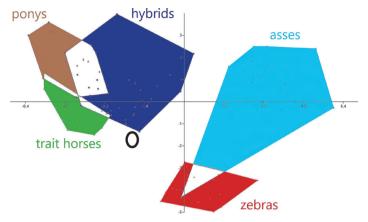
	Grant's zebra	Burchell's zebra	Trait	Donkey	Saddle	Przewalski's horse	Asian wild Ass	Poneys	African wild Ass	Hybrids
Burchell's	0.4773									
Trait	0.0001									
Donkey	0.1406	0.0308	0.0001							
Saddle	0.0001	0.0001	0.0001	0.0001						
Przewalski's horse	0.0001	0.0001	0.0001	0.0001	0.0001					
Asian wild Ass	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001				
Poneys	0.0001	0.0001	0.0001	0.0012	0.0001	0.0001	0.0001			
African wild Ass	0.0652	0.0051	0.0001	0.9927	0.0002	0.0001	0.0001	0.0001		
Hybrids	0.0357	0.0021	0.0001	0.0001	0.1144	0.0001	0.0001	0.0001	0.0006	
MCUC, ZOO.0004465	0.1406	0.0668	0.0618	0.3800	**0.0449**	**0.0205**	**0.0393**	0.0593	0.2206	0.254

Figure 2. Canonical Variate Analysis (axes 1 and 2) for studied groups: zebras (n=20), asses (n=58), poneys (n=15), trait horses (n=15), hybrids (n=31) and specimen MCUC, ZOO.0004465 (black circle), which showed a clear position close to the hybrid group.

The head of a hinny is said to resemble that of a horse more than it does a mule's (Aparicio 1944). The marked celoid head profile would correspond to the male line. Thus it seems very plausible to consider hybrid MCUC, ZOO.0004465 as a cross between a stallion of celoid profile (male line) and a jenny (female line), that is, to be
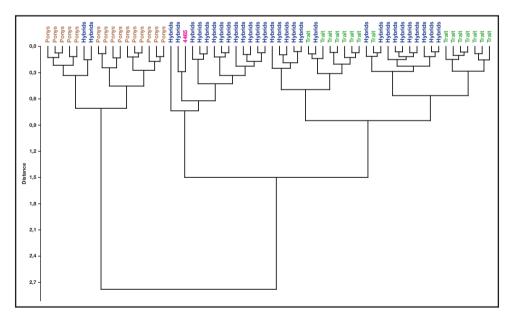

Figure 3. Clustering analysis of specimen MCUC, ZOO.0004465 with poney and trait group assigns (cophenetic correlation=0.828). The specimen appeared in an intermediate position between poneys' and trait horses' clusters (left and right sides, respectively).

a mare hinny, but the type for the male parenteral line remains unknown. As growth potential of equine offspring is influenced by the size of the dam's womb, MCUC, ZOO.0004465 was bigger than her male parent. In fact, in some areas of Portugal, as Miranda, there are yet many hinnies used in the communities for ploughing potatoes and helping harvest grapes (Mclean 2016), so during 30's hinnies had to be more spread.

Acknowledgements

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