

A HORSE CULTURALLY SHAPED AS A DONKEY.
aDNA ANALYSIS ON EQUID REMAINS FROM KHIRBET AL-BATRAWY:
ON HORSES, DONKEYS AND TRADE
IN THE EARLY BRONZE AGE SOUTHERN LEVANT

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This study investigates the role and management of equids in the Early Bronze Age Southern Levant, with a focus on Khirbet al-Batrawy. It presents the results from an ancient DNA (aDNA) analysis conducted by the Molecular Anthropology/Paleogenetic Unit of the Laboratory of Anthropology in the University of Florence, on a horse specimen (Equus ferus caballus Linnaeus, 1758) from the site, offering new insights on the region's early presence of horses. As horses are quite rare in the archaeological record of the Southern Levant, especially prior the Late Bronze Age (1550-1200 BC) and the advent of chariot warfare, the aDNA analysis contributes to the current debate on horse presence, management and domestication in the Southern Levant, prompting a reflection on Khirbet al-Batrawy's frequency of equid remains, including horses and donkeys, in relation to the site's significance as a trade hub, and the possible socio-economic role of these equids within the community.¹

Keywords: Khirbet al-Batrawy; Early Bronze Age; Southern Levant; equids; aDNA

1. INTRODUCTION

This paper presents the results of the ancient DNA analysis (aDNA) conducted on an equid specimen (KB.16.B.2), retrieved during the 12th season (2016) of excavations and restorations at the Early Bronze Age (EBA)² site of Khirbet al-Batrawy, carried out by «La Sapienza» Expedition to Jordan, under the aegis of the Department of Antiquities of the Hashemite Kingdom of Jordan (DoA). The specimen was identified as a horse, prompting a discussion regarding the presence of these animals in the early urban society of Khirbet al-Batrawy, and the broader issue of the role and management of equids, encompassing horses and donkeys, found in extensive numbers at this site.

As equids became increasingly integrated into the economic and social systems of the emerging urban centres of the EBA Southern Levant, this subject has gathered significant scholarly attention in recent studies. A particular focus has been placed on the domestic donkey (*E. africanus asinus* Linnaeus, 1758, henceforth *E. asinus*), its domestication, economic role, and its symbolic and ritual significance.³ Conversely, research on horses, albeit decisively vast, has been comparatively limited for the Levant, with many

¹ Paragraphs §§ 1.-2. are authored by Lorenzo Nigro; §§ 3.-3.1., 4. by Cecilia Ripamonti; § 3.2. by David Caramelli and Alessandra Modi.

² In the present paper the authors shall follow the EBA chronology (3500-2000 BC) proposed by Nigro 2019; Nigro *et al.* 2019. For alternative dates see Regev *et al.* 2012; 2014; Höflmayer *et al.* 2017.

³ Rossel *et al.* 2008; Greenfield - Shai - Maeir 2012; Greenfield *et al.* 2015; 2020; 2021; 2022; Shai *et al.* 2016; Mitchel 2018; Milevski - Horwitz 2019.

contributions addressing the complex issue of the domestication process in the wider area of the Ancient Near East, and the problematic tracing of the domestication event.⁴

Difficulties lie on the one hand in discerning the wild (*Equus ferus* Boddaert, 1785, henceforth *E. ferus*) from the domesticated horse (*Equus ferus caballus* Linnaeus, 1758, henceforth *E. caballus*), and on the other hand in the relatively obscure presence of *E. caballus* in the Levantine archaeological record prior to the Late Bronze Age (1550-1200 BC) and the advent of chariot warfare.⁵

Although further data are still needed, sites like Khirbet al-Batrawy suggest that equids were highly valued within this society and contribute to a growing body of knowledge on their management and purposes and of the interactions between humans and animals. Additionally, the recent aDNA analysis adds a further piece to the scant record of horses in the EBA Southern Levant.

2. EQUIDS IN THE CONTEXT OF THE FAUNAL ASSEMBLAGES OF EARLY BRONZE AGE SOUTHERN LEVANT

During the 3rd millennium BC, the taxonomic assemblages of Southern Levant display a prominence of sheep and goats, followed by cattle, equids, a low representation of pigs, and little emphasis on hunted animals. Although these taxa appear to be present at most sites, with the rise of the earliest urban communities in the EBA,⁶ urban and rural settlements show differential patterns in their taxonomic proportions and display diachronic changes during the period.⁷

At the beginning of the 3rd millennium BC, no clear trend or defined distinction between urban and rural sites is yet visible, although the general focus appears to be on the management of sheep and goats. In the EB II, urban sites show a slight decrease in sheep, goats, and equids, compared to rural communities, in favour of a higher percentage of cattle and pigs. In the EB III, as this pattern continues, urban areas show a markedly increased representation of equids, encompassing mainly donkeys (§ 2.1.) and to a considerably lesser extent horses (§ 2.2.), indicating increasing interactions between humans and equids due to the growing involvement of these animals within the socio-economic framework of the Southern Levant.

⁴ Ducos 1968; 1970; Bökönyi 1972; Zarins 1978; Uerpmann 1990; Anthony 1991; Clutton-Brock 1992; Grigson 1993; 2012; Vila 2006; Olsen 2006; Outram *et al.* 2009; Shev 2016.

⁵ See on the matter: Yadin 1963; Amadasi Guzzo 1965.

⁶ See on the matter: de Miroschedji 2018; Greenberg 2019, 70-135; Nigro 2023a; 2023b with previous bibliography.

⁷ For an in-depth discussion see Gaastra - Greenfield - Greenfield 2020, 14.

2.1. *Donkeys in the Early Bronze Age Southern Levant*

Although it has been suggested that donkeys began to be domesticated as early as the Ghassulian period,⁸ their domesticated status appears to be fully expressed only by the EBA, when the increase in faunal remains of *E. asinus* is coupled with a marked decrease in their size compared to earlier specimens.⁹

In EBA contexts, remains of domesticated donkeys reach an average of *ca.* 10% of the total number of animal bones, with sites like Afridar (Ashkelon)¹⁰ and Bâb edh-Dhrâ',¹¹ yielding higher percentages, up to 20% and 13% of the total respectively, possibly related to the specific role played by these settlements as trade centres.¹²

The growing involvement of donkeys in the EBA Levantine society is also demonstrated by an increased diffusion of clay figurines representing equids, and by the widespread tradition of ritual donkey interments.

The clay figurines, mostly found in funerary contexts, attest to the use of the donkey both as a pack and a riding animal.¹³ These beasts are in fact depicted either free-standing, burdened by two containers on their backs, or equipped with saddles and bridles,¹⁴ whose use has been confirmed by zooarchaeological analyses carried out on EB III donkey remains from Tell eṣ-Şâfi/Gath.¹⁵ Although infrequently these figurines are also attested carrying a rider, as seen at Khirbet ez-Zaraqon.¹⁶

Hence these animals appear to have been mostly employed to transport people and commodities, being thus a possible asset for the intra- and inter-regional trade network established in the Southern Levant during the EBA.¹⁷ Texts and iconographic sources from Old Kingdom Egypt supports this assumption, with high numbers of donkeys raised and used in the exchanges between Egypt and the surrounding territories, from Nubia to the Sinai and the Levantine coast.¹⁸ Further evidence is also provided by the Egyptian outposts installed along the Abu Ballas desert route towards the Libyan territories, where stations with a number of jars for the refreshment of traders have been identified together with watering and feeding facilities for donkeys, whose bones have been found along the trail.¹⁹

Furthermore, the evidence provided by the analyses of dental isotopes on the sacrificed donkeys from Tell eṣ-Şâfi/Gath, that were ritually interred beneath a courtyard, indicate

⁸ Grigson 2012.

⁹ Horwitz - Tchermov 1989; Milevski 2009; Milevski - Horwitz 2019. The high number of donkeys suggest they were well integrated within the socio-economic landscape of Southern Levant, while their reduced size indicates that they were possibly living in an anthropogenic environment or were subject to selective breeding (Milevski - Horwitz 2019, 107).

¹⁰ Whitcher 1999; Whitcher-Kansa 2004.

¹¹ Finnegan 1976; 1979.

¹² Milevski - Horwitz 2019, 101, tab. 4.1.

¹³ On the matter of donkey figurines see: Milevski 2005, figs. 24-26; 2011; Milevski - Horwitz 2019, 108, tab. 4.2.

¹⁴ Hizmi 2004.

¹⁵ Greenfield *et al.* 2018.

¹⁶ Al-Ajlouny *et al.* 2012, 106, fig. 5.

¹⁷ Ilan - Sebbane 1989; Milevski 2005; 2009; 2011; Nigro 2014b.

¹⁸ Sowada 2009; 2018.

¹⁹ The main period of use of the Abu Ballas trail, that crossed the vast terrain between Dakhla and the Gilf Kebir; is during the late Old Kingdom and the (early) First Intermediate Period (Förster 2007).

that the specimens were born and raised in Egypt and only later in life arrived in EB III Canaan.²⁰ These results provide proof of a direct movement of animals between the two regions and again corroborate the existence of a substantial exchange network involving donkey caravans,²¹ as portrayed, albeit later, by the 12th Dynasty wall paintings of the Egyptian tomb of Beni Hasan.²²

Donkeys were therefore involved in the trading network to transport commodities, but whether they were themselves the actual commodity to be exchanged, as in the case of the highly valuable *kungas*, - a possible cross between female domestic donkey and male hemippes²³ -, which Eblaite texts record as being purchased by Tell Mardikh/Ebla from Tell Brak/Nagar,²⁴ is still up for debate.

Either way, the donkey's attainment of a particular role within the Levantine society is further expressed through the spread of ritual burials of equids,²⁵ whose complete or partial skeletons have been found at several sites, Tell Zakariya/Azekah,²⁶ Tell es-Sakan,²⁷ Lod²⁸ and Tell eš-Šâfi/Gath.²⁹ Equids burials are attested across a vast area, from Predynastic and Early Dynastic Egypt to late Early Dynastic Mesopotamia³⁰ onwards.³¹ Interments are characterised by two different kinds of burials: a) simple pits devoid of any other good, as it is for the Levant, associated with architectures or standing alone as is the case for the donkey burial unearthed at Abu Tbeirah, Southern Iraq;³² b) interments associated with (human) élite burials, like the striking funerary equipment of Queen Dusigu, who was accompanied in the afterlife by a silver and gold decorated chariot led by two equids.³³ From the *kungas* hybrids found in the élite mortuary complex of Umm el-Marra,³⁴ to the

²⁰ Greenfield *et al.* 2020.

²¹ At Tell eš-Šâfi/Gath was also found a Nile Valley goat, possibly suggesting that livestock might have been traded as a commodity (Arnold *et al.* 2016; 2018; Greenfield *et al.* 2020, 386). It might also be possible that goats and donkeys were not part of the traded goods but simply served the merchants, following and/or being part of the caravans during the journey.

²² Cohen 2015.

²³ Bennett *et al.* 2022, 6.

²⁴ Dolce 2014.

²⁵ For a summary on the intentional interments of articulated donkeys in the Early and Middle Bronze Age see: Prell 2019; Sapir-Hen 2020, 86-87.

²⁶ Sapir-Hen - Gadot - Lipschits 2017.

²⁷ A complete skeleton of a male donkey between 6-9 years of age was found within the walls of an EB III house (de Miroschedji *et al.* 2001, 97, fig. 9).

²⁸ Yannai 2008.

²⁹ The female donkeys from Tell eš-Šâfi/Gath were prematurely slaughtered as a ritual offering when the residential neighborhood was being constructed and renewed and are to be interpreted as being foundation deposits (Greenfield - Shai - Maeir 2012; Greenfield *et al.* 2015; 2020; 2021; 2022).

³⁰ Zarins 1986.

³¹ For a comprehensive overview see: Way 2011.

³² Alhaique *et al.* 2019.

³³ Biga 2007-2008, 260-261.

³⁴ Schwartz *et al.* 2006, 620, note 75; Weber 2008; recent analyses have been carried out on the animal and human interments from Umm el-Marra, see: Stantis *et al.* 2023. At Umm el-Marra, as at Tell Brak, Tell Mozan, and Tell Madhur, ritual equid burials were also found in association with dogs (Wygnańska 2017, 145, 148, fig. 1).

equid sacrifices discovered in front of tomb PG1054 in the Royal Cemetery of Ur,³⁵ this tradition perseveres until the 2nd and 1st millennium BC.

Since the 3rd millennium BC onwards donkeys in the Near East thus become burden, riding, and draught animals for agriculture and long-distance trade, status indicators and are finally even involved in warfare, as seen e.g. in the Standard of Ur.

2.2. Horses in the Early Bronze Age Southern Levant

The controversial issue of horse domestication, as outlined above, has been the focal point of several studies. Difficulties lie with the fact that this was a multi-stage process, with early management practices leaving no trace in the genetic record.³⁶ Moreover, domestication seem to have occurred across multiple regions,³⁷ including the Kazakh steppe³⁸ and the Carpathian Basin,³⁹ ultimately achieving the lineage of modern domestic horses in the Pontic-Caspian area by the 3rd millennium BC.⁴⁰

Meanwhile, delineating the presence in the Southern Levant appears even more challenging (fig. 1). Inferences were made suggesting that domesticated specimen appeared in the Negev desert⁴¹ already by the Chalcolithic period, specifically at Shiqmin,⁴² Grar,⁴³ and Cave 4 of Shoham (North).⁴⁴ However, although these remains fall within the size range of domesticated horses, this assumption has not reached full consensus, and the debate on the appearance of the domesticated horse in the Southern Levant remains open.⁴⁵

The record concerning EBA horses is somewhat more substantial, albeit their domestication status remains again uncertain. At Arad, a metacarpus, larger than the 18th

³⁵ Zarins 1986.

³⁶ For a review on the existing evidence see Niskanen 2023.

³⁷ The domestication process might have taken place in different areas where there were wild specimen and pastoralist communities already familiar with the concept of breeding cattle and sheep (Grigoriev 2023, 181). One of the earliest pieces of evidence for equids bones of caballine size was found in the 6th millennium BC site of Tappeh Zagheh, in north-western Iran. However, due to the difficulty in distinguishing between *E. caballus* and wild *E. przewalskii*, as well as the early dating of the site, scholars have not accepted this as evidence of domestication at such an early stage (Mashkour 2003, 134). Instead, it has been interpreted as evidence of the continued presence of wild equids in the region (Shev 2016, 129).

³⁸ Anthony - Brown 1998; Outram *et al.*, 2009; 2021, *contra* Taylor - Barrón Ortiz 2021.

³⁹ Kanne 2022.

⁴⁰ Librado *et al.* 2021. The horse domestication process in this region seems to have naturally began earlier, but we do not know how much earlier (Niskanen 2023, 55).

⁴¹ Grigson 1993.

⁴² Above the floor of an underground room was discovered a humerus with a proximal breadth of 92.6 mm (Levy *et al.* 1991), consistent with that of a horse. Furthermore, osteopathic wear associated with draft-labor were identified on the metacarpals attributed to *E. caballus* (Grigson 1993).

⁴³ Gilead 1989.

⁴⁴ The Late Chalcolithic burial Cave 4 contained several equids remains, mostly *E. asinus*, and some belonging to larger specimens, possibly *E. africanus*, the wild donkey, or *E. caballus*. Specifically, a distal metapodial from context L202, B2043 had a breadth measurement (Bd) of 47.9 mm, comparable to that of a horse. The remains however were found just below the topsoil, rendering their attribution less certain (Horwitz 2007).

⁴⁵ See also Wapnish 1997; Levine 1999; Olsen 2006.

Dynasty domesticated specimen from Soleb,⁴⁶ was tentatively identified as belonging to a domesticated horse.⁴⁷

Similarly, Afridar (Ashkelon), which yielded one of the most extensive EB I faunal assemblages of the Southern Levant, displays numerous equid remains that include predominantly domesticated donkeys along with a few larger specimens consistent with caballine size.⁴⁸ Additional evidence is provided by Area B of Ras el-'Ain/Aphek, where the remains of one *E. caballus* were identified in an EB I layer.⁴⁹

In contrast to the evidence available for donkeys, EBA figurines that can be attributed with certainty to horses are seemingly absent, yet, evidence of a horse burial is documented at EB I Tell Jenin.⁵⁰ Here, a double equid interment has been unearthed, comprising an incomplete *E. caballus* stallion, recognised for its impressive dimensions, and a donkey.⁵¹ The absence of butchery marks on either skeleton, and their near-complete articulation dismiss the idea that they might have been consumed. Instead, this evidence supports the inference that this burial was intentional. The donkey-horse interment might suggest them fulfilling a similar ideological role within this community, arising from the similar purpose they played, perhaps that of transport.

Overall, the scarcity of horse remains attested in the EBA Southern Levant, makes Khirbet al-Batrawy a key site in the current debate on equids and horses specifically.

[LN]

3. THE CASE STUDY OF KHIRBET AL-BATRAWY

Over twenty years of work by «La Sapienza» Expedition to Jordan⁵², under the aegis of the Department of Antiquities of the Hashemite Kingdom of Jordan (DoA),⁵³ have brought to light the 3rd millennium BC fortified city⁵⁴ of Khirbet al-Batrawy.⁵⁵ Located in the Upper Wadi az-Zarqa' Valley, this EBA site reveals a continuous urban occupation spanning from the Early Bronze Age II (Batrawy II period, EB II, 3000-2700 BC) to the end of the Early Bronze IIIB (Batrawy IIIB period, EB IIIB, 2500-2300 BC).⁵⁶ Excavations uncovered at

⁴⁶ Shev 2016, 132.

⁴⁷ Davis 1976. The identification as a horse was not fully supported by scholars, see Lernau 1978, 86.

⁴⁸ Witcher-Kansa 2004.

⁴⁹ The identification of the EBA remains at Ras el-'Ain/Aphek as domesticated horse is, like for Afridar (Ashkelon), still debated, as these faunal remains might represent the persistence of a pocket of feral horses in the area (Shev 2016). Further horse remains were uncovered at Ras el-'Ain/Aphek in Areas and B, yet date to the Middle Bronze Age (2000-1750 BC) (Kochavi 1977, 15; Hellwing 2000).

⁵⁰ Al-Zawahra - Ezzughayyar 1998, 132.

⁵¹ Grigson (2012, 186) does not agree with this interpretation, identifying the specimen with a mule rather than a horse.

⁵² Nigro 2006a; 2006b; 2007; 2008a; 2008b; 2009a; 2009b; 2010a; 2010b; 2010c; 2011; 2012a; 2013a; 2013b; 2014a; 2016a; 2016b; 2017; 2020; 2022; 2024; Nigro ed. 2006; 2008; 2010; 2012; Nigro - Sala 2007; 2009; 2010; 2011; 2012; 2013; Nigro - Sala - Polcaro 2008; Nigro - Gallo 2022; Gallo *et al.* 2023.

⁵³ Updated results of excavations and restorations at Khirbet al-Batrawy and the complete bibliography are available at the expedition's website: <https://sites.google.com/uniroma1.it/sapienzatojordan/home-page>.

⁵⁴ Alternatively, the term "walled towns", coined for the EB II-III fortified settlements by R.T. Schaub (1982, 67) was used for Khirbet al-Batrawy by L. Nigro (2017, 1).

⁵⁵ Lat. 32° 05' 218" N. Long. 36° 04' 237" E. The site is registered in the MEGA Jordan project as n. 7411.

⁵⁶ On the matter of chronology see note 2.

least two major episodes of destruction that shaped the site's history and created a coherent and well-defined stratigraphy: an earthquake around 2700 BC and a fire around 2300 BC, which marked the end of the urban centre.⁵⁷ Following a period of abandonment, a rural village (EB IV, Batrawy IV, 2200-2000 BC) arose amidst the ruins of the former city.⁵⁸

The development and flourishing of the site was largely favoured by its advantageous setting, as Khirbet al-Batrawy arose on top of a steep mound that dominated the Wadi az-Zarqa' river ford, in a strategic position that allowed control over access to exploitable resources within the surrounding landscape, like freshwater and cultivable land,⁵⁹ and offered a commanding view of a pivotal crossroad where the EBA caravan routes that traversed the Southern Levant towards Egypt and Mesopotamia intersected.⁶⁰

Several imported luxury items, often of Egyptian origin or Egyptianising appearance, were discovered in the multi-functional building known as the "Palace of the Copper Axes"⁶¹ at Khirbet al-Batrawy in Area B South.⁶² These artefacts serve as a compelling testimony to the commercial prowess of this EBA Jordanian city. Among the notable finds is a bear paw, found in Pillared Hall L.1040.⁶³ An animal native of regions extending from the Northern Levant to the Taurus, that underscores the city's access to distant goods, as do the copper axes *cachette* found nearby,⁶⁴ the composition of which points to mining compounds located in the Wadi 'Araba or Anatolia, that again highlights this community's ability to engage in long-distance trade and secure access to valuable copper ores. Additional evidence include a four-string necklace made of gemstones from Hall L.1110, whose likely poles of extraction were to be found in the Red Sea, the Arabian Peninsula, Egypt or Anatolia;⁶⁵ two Egyptian schist palettes recovered in the Entrance Hall L.1100, and Pillared Hall L.1040;⁶⁶ and the amazonite gemstone, whose precise provenance remains uncertain.⁶⁷ Overall, these artefacts not only illustrate Khirbet al-Batrawy's extensive trade connections but speak of its distinctive relationship with the Pharaonic Kingdom of the 5th - 6th Dynasty.⁶⁸

⁵⁷ See on the matter Sala 2014a.

⁵⁸ On the matter see: Nigro 2021; Sala 2012.

⁵⁹ Nigro 2011; 2017.

⁶⁰ Nigro 2013a; 2014b.

⁶¹ Albeit the definition of "Palace of the Copper Axes" is the one preferred by the authors, it has not reached full consensus among scholars. The "Palace of the Copper Axes" or Building B from Khirbet al-Batrawy does not fit within the criteria proposed by P. de Miroschedji (2019, 160-161) to identify palatine buildings; however, its belonging to a distinctive category rather than a simple but enlarged dwelling, has been recognized by de Miroschedji (2019, 175) himself, who suggests interpreting it as an "elite residence". The alternative term "non-residential building" is also used, see e.g. D'Andrea 2021, 33.

⁶² Nigro ed. 2010; Nigro 2014b; Sala 2014b; Nigro *et al.* 2020.

⁶³ Nigro 2014c.

⁶⁴ Nigro 2010a; 2015.

⁶⁵ Nigro 2012b.

⁶⁶ For the Egyptian green schist palette recovered from Entrance Hall L.1100 see Nigro *et al.* 2020; Nigro - Gallo 2022, 172, fig. 13; for the one collected in Pillared Hall L.1040, see Nigro 2014b, 47, fig. 13; Sala 2014b, 69.

⁶⁷ The amazonite gemstone had reached Khirbet al-Batrawy either through an Egyptian route or a Russian one (Nigro *et al.* 2020).

⁶⁸ Sala 2014b; Nigro *et al.* 2020.

In this thoroughly explored archaeological context several archaeozoological remains were sampled and studied, revealing a relatively high number of equids, a possible further testimony of the site's vocation as a caravan city. Among these, during the 2016 season an equid specimen (KB.16.B.2) of which the uncinat and scaphoid of the right carpus, and the third phalanx were recognized, was chosen to undergo an aDNA analysis (fig. 2), allowing the determination of the species and sex (§ 3.2.).

3.1. *The equids at Early Bronze Age Khirbet al-Batrawy*

The faunal assemblage of Khirbet al-Batrawy is predominantly composed of ovicaprids, which represent by far the most frequent taxon throughout the EBA. Additional taxa include cattle, equids, and a few suids, encompassing both the domestic pig and the wild boar. Notably, the latter, alongside gazelles, bezoar goats and aurochs, represent the sole hunted animals. Dogs, are attested rarely, mostly through gnaw marks.⁶⁹

In the EB II-III A, equids represent the third most common taxa after sheep, goat and cattle, yet faunal remains belong almost exclusively to donkeys. In the EB III B, horses appear in the faunal assemblage alongside donkeys, and equids in general rise decisively in numbers.

Horse remains are indeed mostly found in the destruction layers of the city, caused by the dramatic conflagration that took place at the end of EB III B.⁷⁰ A minimum number of two individuals (MNI) was found in Area A on the *khirbat* summit; at least one other was retrieved in Area B South in the “Palace of the Copper Axes”, and one more in the abandonment layers above the EB III B destruction. At least another specimen was discovered in Area B North, along the Main Inner Wall, the innermost of four lines of fortifications.⁷¹ This specimen was found close to postern L.160 where excavations also identified a pierced stone, possibly used to tie animals at the entrance of the city.⁷²

A further sample, namely KB.16.B.2 was again collected from Area B North. The latter, constituted by the uncinat and scaphoid of the right carpus, and the third phalanx of an equid, was retrieved while excavating the EB III B filling F.834 (square BjIII) which, when the city was finally destroyed, accumulated between the northern wall W.837 of Bastion T.830 and Outer Wall W.155; respectively the second and third line of the quadruple fortification system.⁷³ The sample was first examined on the field,⁷⁴ then analysed by the Laboratory of Anthropology of the University of Florence, where the remains were found to belong to the same adult male, which through the aDNA analysis of the phalanx, the better-preserved bit, was identified as belonging to the *Equus caballus* species.

⁶⁹ For a comprehensive study on the faunal remains of Khirbet al-Batrawy, see: Alhaique 2008; 2012; Alhaique - di Fede 2010.

⁷⁰ Gallo 2014; Nigro 2016b.

⁷¹ Nigro ed. 2012; Nigro 2016b; Nigro - Gallo 2022.

⁷² Nigro ed. 2012, 52.

⁷³ Nigro 2016a, 138. For a summary on the development of the fortification system of Khirbet al-Batrawy, see (Nigro - Gallo 2022).

⁷⁴ The third phalanx measured: GB 4.57cm, GL 4.62 cm, BF 3.35 cm, LF 1.26 cm, HP 3.84 cm, Ld 3.22 cm. Uncinat: GB 2.99 cm. Scaphoid: GB 3.05 cm. Identification of the species was established on the field with Davis 1980; Barone 1980; for the measurements, Boessneck - von den Driesch 1976.

The remarkably high frequency of equids at Khirbet al-Batrawy deviates from what is observed in most other contemporary southern Levantine sites.⁷⁵ While this taxon generally shows an increase during the EBA, its presence is in fact relatively modest when compared to the total assemblage of faunal remains.⁷⁶

Khirbet al-Batrawy's equids also frequently bear butchering marks related to disarticulation and meat removal, as well as signs of marrow extraction that strongly suggests their consumption and use for the manufacture of bone tools. Similar is the case of EB III Tell eṣ-Şâfi/Gath where, in stark contrast to the articulated donkeys chosen for sacrifice, loose equid bones were found on floors and above them, showing traces of butchering again suggestive of their consumption.⁷⁷

The culling pattern of Khirbet al-Batrawy's specimens, which encompasses juvenile and adult/senior individuals, and the pathologies detected on a horse tibia,⁷⁸ further indicate that these animals might have been exploited for their secondary products, transport, and traction power. While there is extensive evidence of an intra- and inter-regional equid-based trade (§ 2.1.) in which this site seemingly participated, clear proof to support the use of equids as a pulling force and their employment in agricultural pursuits is lacking from Levantine contexts. Textual and iconographic evidence may however be found in Mesopotamia, such as the notorious Standard of Ur in which equids are seen pulling wagons, while their use in threshing is attested in Egypt by Old Kingdom tombs and cemeteries.⁷⁹

[CR]

3.2. Ancient DNA of an equid: methods and results

Genetic analysis was conducted on the third phalanx of the equid specimen KB.16.B.2 collected at Khirbet al-Batrawy, following specific protocols designed for recovering and analysing aDNA, taking into account the peculiar characteristics of this molecule (i.e. high degree of degradation and contamination with exogenous DNA).⁸⁰ Moreover, all the experimental steps were carried out in dedicated clean laboratory facilities to minimize contamination risks.

The sample was initially decontaminated removing the outer layer of the bone by brushing and then irradiated by ultraviolet light (254 nm) for 30 minutes. Fifty milligrams of powder were collected from the densest part of the bone using a dentist microdrill with

⁷⁵ The percentage of identified equid specimen (NISP) found at Khirbet al-Batrawy (encompassing domesticated donkeys, horses and unidentified equids) amounts to 7.0% of all the taxa in the EB II, rising to 12.45% in the EB IIIA, and reaching up to 26.1% in the EB IIIB (for the specific percentage of each species see Alhaique 2008, 351, tab. X; 2012, 359, tab. IX). Similar high percentages of equids are found only in the EBA sites of Afridar and Bab ed-Dhra' (§2.1.); while other contemporary EB III Jordan sites like Tall al-Handaqq South show that taxa besides sheep, goats and cattle make up only 7% of the total remains, with only few fragmented equids remains belonging to domestic donkeys and wild onagers (Price - Makarewicz - Chesson 2018, 85).

⁷⁶ Milevski - Horwitz 2019, Appendix.

⁷⁷ Greenfield *et al.* 2021; 2022, 35.

⁷⁸ Alhaique 2012, 345.

⁷⁹ El-Menshawey 2009, 51-52.

⁸⁰ Orlando *et al.* 2021.

disposable tips. An optimized protocol has been adopted for the extraction and recovery of the ultrashort DNA fragments.⁸¹ Thirty microliters of the extract were subsequently used for Illumina library construction following a custom double-stranded and double-indexing protocol.⁸² A partial uracil–DNA–glycosylase (UDG) treatment was applied⁸³ for reducing the rate of deamination. After qualitative and quantitative evaluation with Agilent TapeStation (D1000 kit), library was pooled with other samples and sequenced on Illumina NovaSeq 6000 in paired-end mode (2x50+8+8).

Sequencing data were demultiplexed and sorted according to the individual sample barcodes, obtaining 8,864,010 raw reads, that were subsequently pre-processed and filtered for sequencing quality and fragments length using a bioinformatics approach specifically developed for aDNA analysis.⁸⁴ Merged reads longer than 30 base pair (bp) were aligned on the *Equus caballus* reference genome (EquCab2) using BWA v.0.6.2.⁸⁵ After removing PCR duplicates with DeDup v0.12.1,⁸⁶ 1,164 equine reads were identified, showing poor DNA preservation and a small amount of endogenous DNA content (0.144%). For genomic DNA authentication, post-mortem damage patterns were quantified using mapDamage2.0.⁸⁷ The average fragment length of the sequenced DNA molecules was 37 bp, while C-to-T misincorporation patterns at the 5' end of the molecules was approximately 2%. Considering the reduction of damages in the terminal bases from partial UDG-treatment, these data are compatible with ancient DNA degradation.

Zonkey pipeline⁸⁸ was used for taxonomic identification and sex attribution of the sample. Mapped reads were compared with a reference panel consisting of ~36.5 million autosomal sites and complete mitochondrial sequences of nine wild and domesticated equids and processed as described in Schubert *et al.* 2017. Principal Component Analyses generated with EIGENSOFT “SmartPCA”⁸⁹ (fig. 3), estimation of ancestry components performed with ADMIXTURE⁹⁰ (fig. 4) and the phylogenetic analysis of mitochondrial DNA (fig. 5) have confirmed that the third phalanx analysed belongs to purebred horse (*Equus caballus* species). This result was also confirmed when transitions were excluded, pointing out the robustness of the taxonomic identification against post-mortem DNA damage. The genetic sex was estimated by calculating the X chromosome-to autosomal coverage ratio, revealing that the phalanx belongs to a male specimen (fig. 6).

[DC-AM]

⁸¹ Dabney *et al.* 2013.

⁸² Meyer - Kircher 2010.

⁸³ Rohland *et al.* 2015.

⁸⁴ Peltzer *et al.* 2016.

⁸⁵ Li - Durbin 2009.

⁸⁶ Peltzer *et al.* 2016.

⁸⁷ Jónsson *et al.* 2013.

⁸⁸ Schubert *et al.* 2017.

⁸⁹ Price *et al.* 2006.

⁹⁰ Alexander - Novembre - Lange 2009.

4. CONCLUSIONS

The study of the equid remains from Khirbet al-Batrawy, particularly the ancient DNA analysis of specimen KB.16.B.2, provides significant insights into the role and management of equids in the urban communities of Early Bronze Age Southern Levant.

On one side, the identification of the specimen as a male equid belonging to the *Equus caballus* species adds valuable evidence to the ongoing debate on the presence and early management of horses in the region, especially as research is still unfolding and looking at the scarcity of reliable remains in the Southern Levant throughout the Early Bronze Age. On the other, the successful recovery and analysis of aDNA from KB.16.B.2 phalanx highlights the potential of genetic studies to refine species identification in archaeozoological research and the need for further interdisciplinary approaches.

While the scarcity of comparable data limits broader conclusions, the peculiar archaeological context and faunal assemblage of Khirbet al-Batrawy allow a tentative interpretation on the purpose of this horse, which might have served a similar function to the other numerous equids, donkeys specifically, identified at this urban centre. Indeed, the findings emphasise Khirbet al-Batrawy's role as a regional hub, with equids serving multiple purposes, including transport, trade, and possibly constituting a dietary resource, with residual raw materials (bones, skin) used for crafts and the production of tools. The high frequency of equid remains, the butchery marks observed on them, and their use in secondary products reflect a distinctive pattern of equid utilisation compared to other contemporary sites. Moreover, the site's strategic location and extensive trade networks, as attested by the several imported luxury goods, align with the interpretation of equids as an integral asset of medium and long-distance trade, and therefore fundamental within the socioeconomic framework of this community. Although, ritual interments of equids, one of the most remarkable piece of evidence supporting the peculiar role attained by these animals in the EBA society, are notably lacking from Khirbet al-Batrawy; a fragmentary animal clay figurine tentatively to be interpreted as an equid (KB.05.B.51)⁹¹ (fig. 7) has been recovered during past season of excavations, providing an additional, albeit little, insight into the symbolic role achieved by these animals within this community.

Given the relatively high frequency of horses at the site and the similar marks recognized on their bones, it may be thus tentatively suggested that the inhabitants of Khirbet al-Batrawy perceived and managed their equids in a similar manner, utilizing horses for purposes (meat, labour, transport, travelling, etc.), akin to those well-documented for donkeys in the Ancient Near East. The double burial at Tell Jenin may lend support to this hypothesis, as the donkey and horse seem to have been ideally associated by being buried in a joint interment.

⁹¹ The figurine was discovered alongside a grinding stone and a fragment of arsenical copper within refuse pit P.130, which was positioned against the inner face of the Main Inner Wall. These artifacts are attributed to the Batrawy III layers, which the pit itself had cut through, dating them to the EB III (Nigro ed. 2006, 168, pl. XIX; Montanari 2012, 419, fig. 22). Initially, the clay figurine was interpreted as representing an onager. However, given the absence of onager remains at Batrawy, it is now more plausible to identify the figurine as a more generic equid. Shallow markings on its right side with a slight protuberance at the center may be reminiscent of a figurine from Arad that has been interpreted as carrying two containers (Amiran 1978, pl. 117:6), however the state of preservation of the Batrawy's figurine makes this interpretation only tentative.

Albeit additional research is mandatory, these findings not only provide a window into the human-animal interactions of this early urban society but represent a further stepping stone for future multidisciplinary studies on the matter of horse appearance, domestication and the socio-economic and symbolic purposes of equids both at Khirbet al-Batrawy and in the Southern Levant.

[CR]

DATA AVAILABILITY

The datasets generated and analysed during the current study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1003267.

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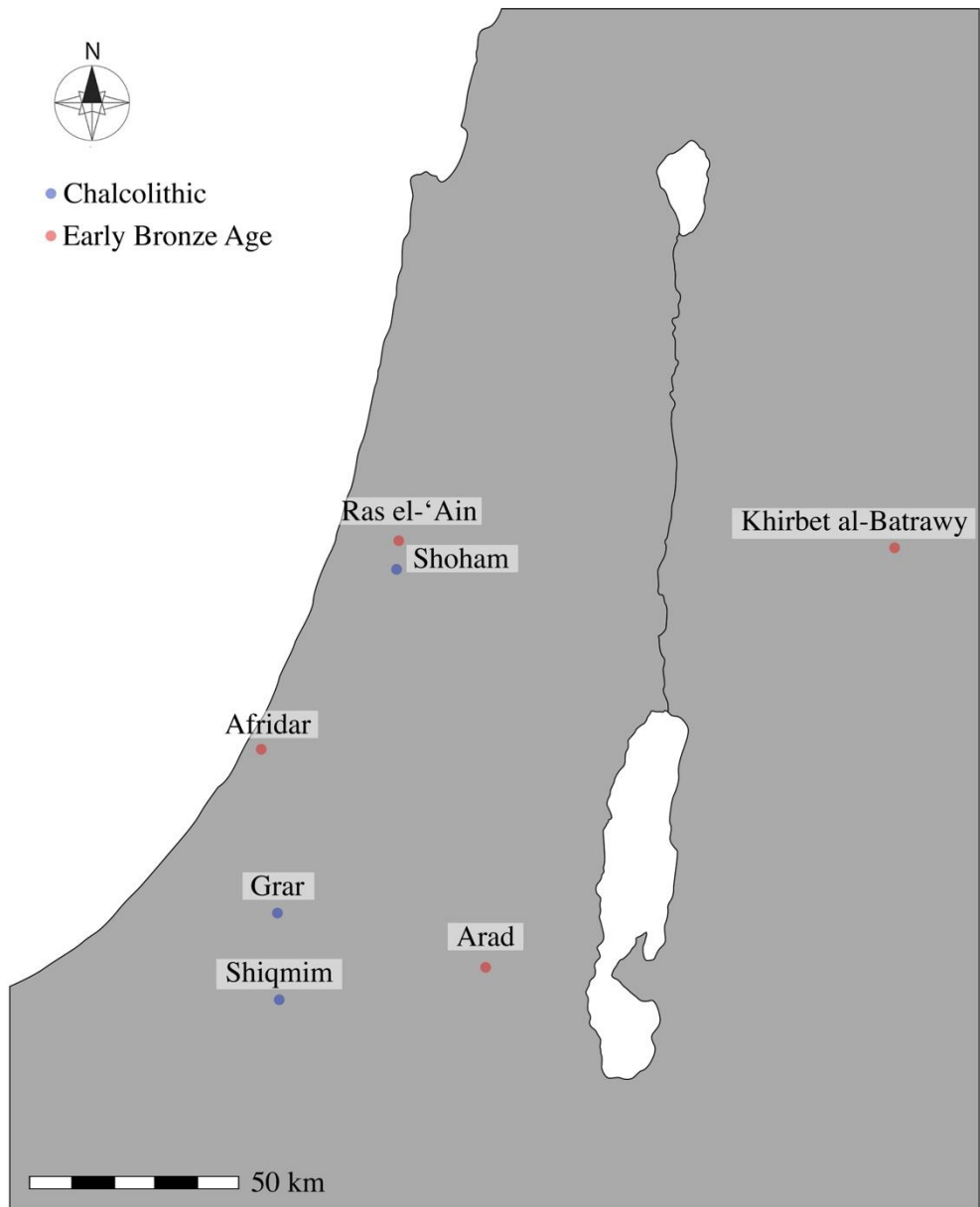


Fig. 1 - Map of the Southern Levant with the Chalcolithic and Early Bronze Age horse remains mentioned in the text.

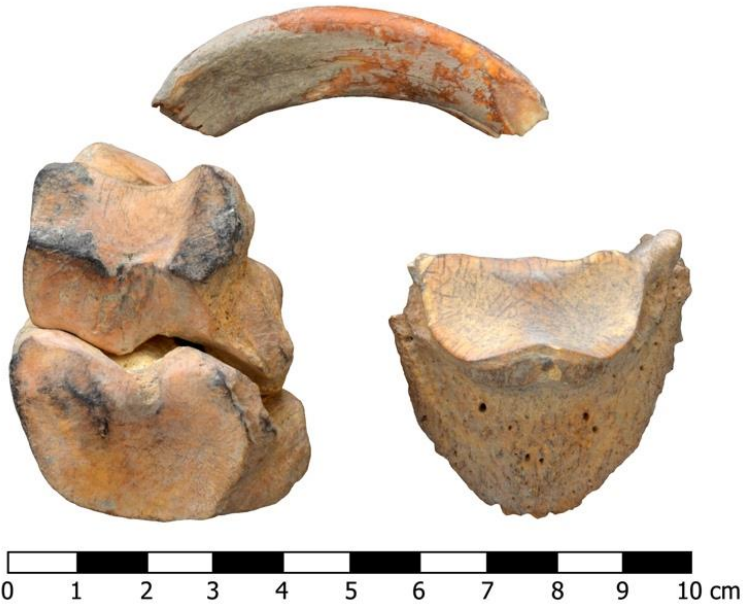


Fig. 2 - The sample KB.16.B.2 found in the destruction layer F.834 in Area B North of Khirbet al-Batrawy.

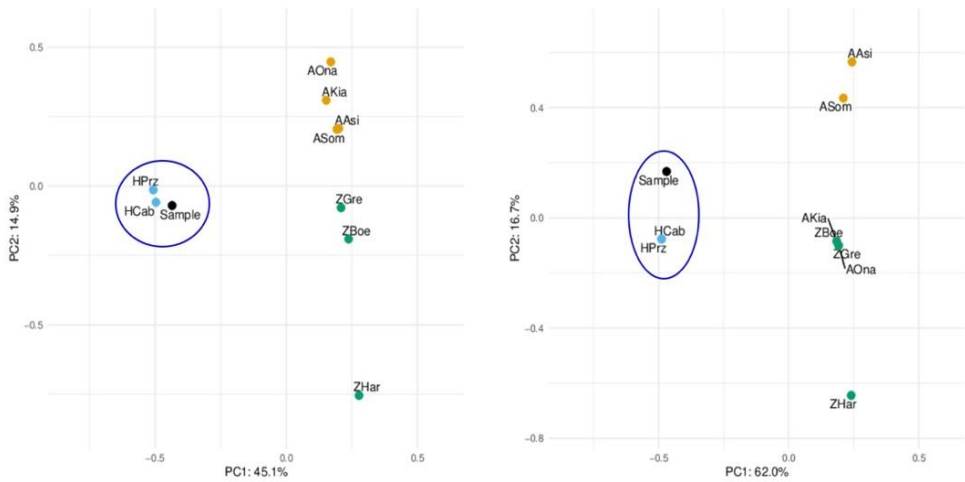


Fig. 3 - PCA (Principal Component Analysis) plot of species analysis of the sample KB.16.B.2 (carried out using SmartPCA v16000, from the EIGENSOFT toolkit).

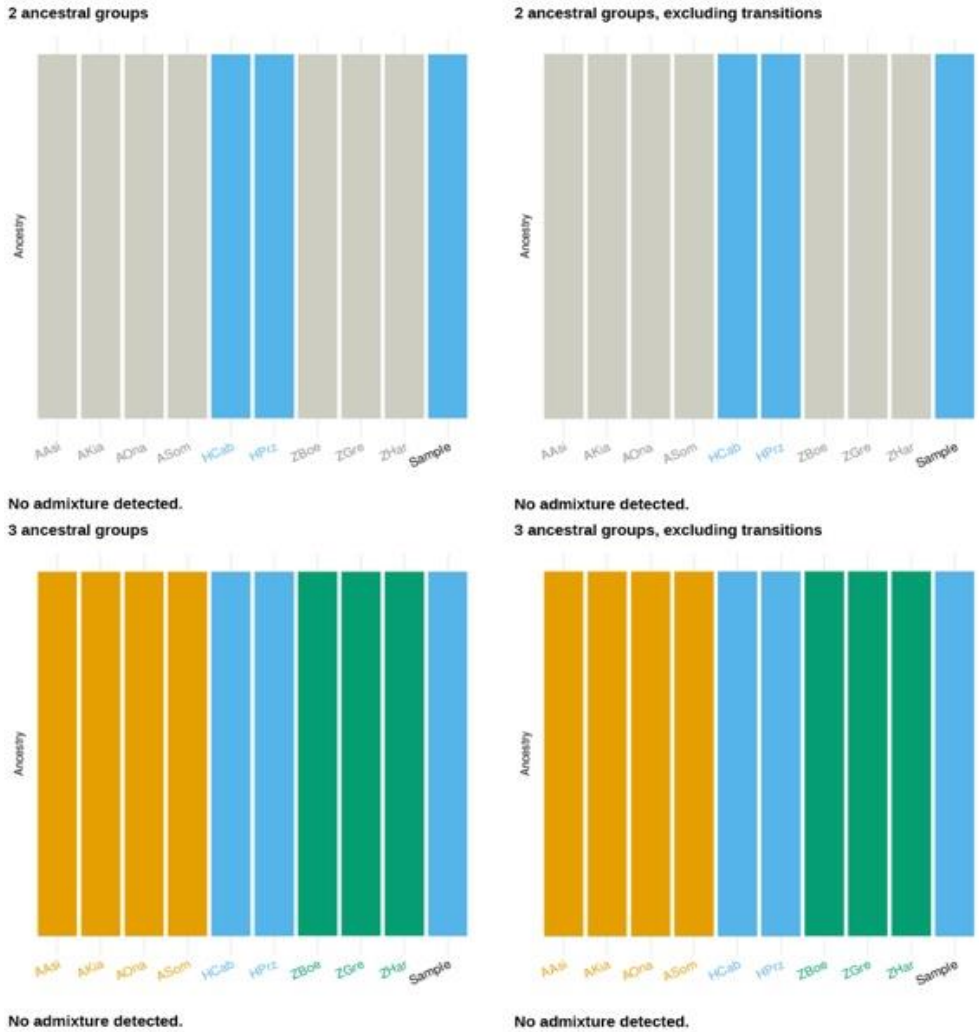
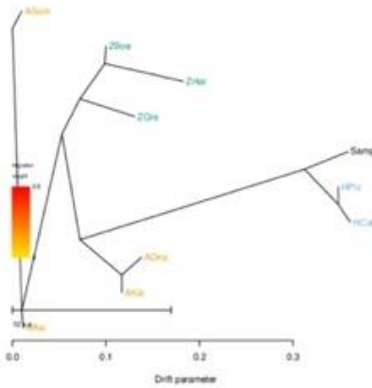


Fig. 4 - Admixture proportions estimated plots. There have been used the following default parameters: Aasi Ass NonCaballine E. a. asinus Male Willy; Akia Ass NonCaballine E. kiang Female KIA; Aona Ass NonCaballine E. h. onager Male ONA; ASom Ass NonCaballine E. a. somaliensis Female SOM; Hcab Horse Caballine E. caballus Male FM1798; HPrz Horse Caballine E. przewalskii Male SB281; Zboe Zebra NonCaballine E. q. boehmi Female BOE; Zgre Zebra NonCaballine E. grevyi Female GRE; Zhar Zebra NonCaballine E. z. hartmannae Female HAR.

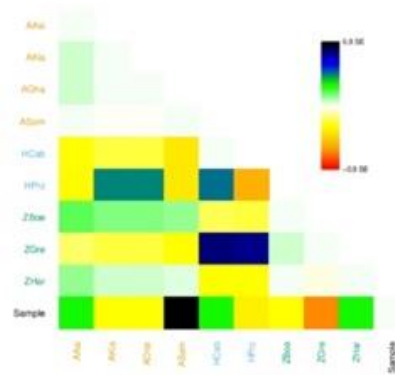
Including transitions

Edges = 0

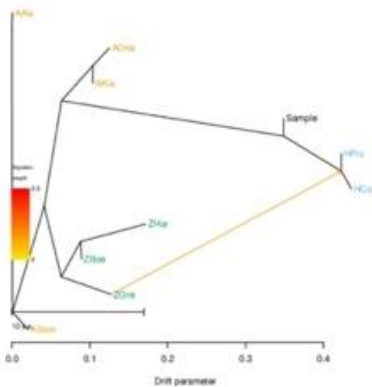


Variance explained by model = 0.992506.

Residuals



Edges = 1



Variance explained by model = 0.994256.

Residuals

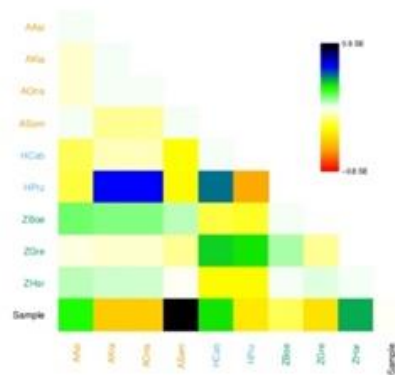


Fig. 5 - Detection of population mixture analysis plots of the sample KB.16.B.2, using TreeMix v1.12; parameters were -k 0; -global; and supervised estimation using ancestral groups listed in the references above (fig. 4).

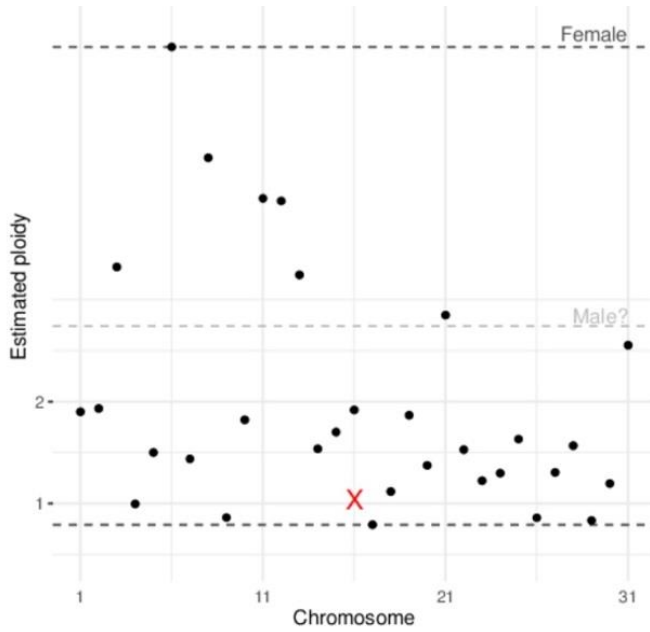


Fig. 6 - Plot of chromosomes analysis of the sample KB.16.B.2.

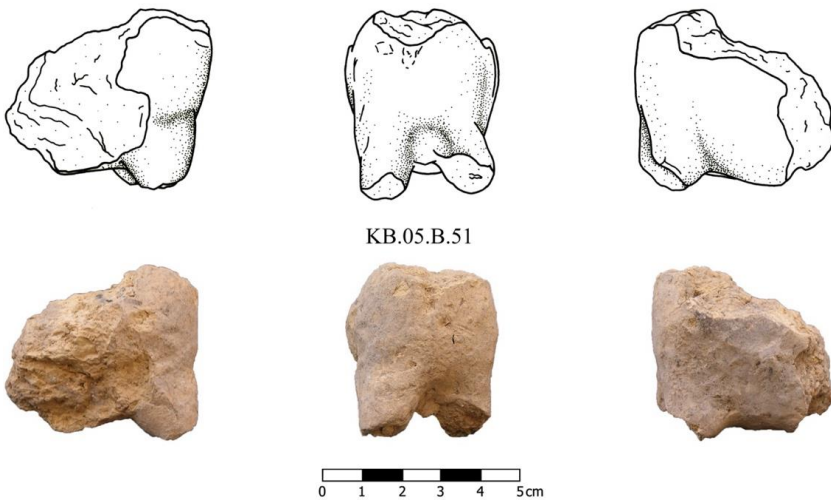


Fig. 7 - The animal figurine KB.05.B.51 found at Khirbet al-Batrawy.