Early Bronze Age population substructure in the Khabur basin: preliminary evidence from Tell Brak, Tell Arbid and Tell Barri (Syria)

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Abstract

Bioarchaeological studies of human remains examine past populations through their mortuary, biological, and socio-cultural contexts. Biological distance, or biodistance, analyses use both genetic and phenetic data to investigate biological relatedness. Biodistance studies frequently employ phenotypic characteristics, or the physical expression of genetic traits that can serve as a proxy for aDNA, to understand evolution, migration, kinship and social organisation. We used phenotypic variation in dental morphology to investigate the population history of the Khabur basin in Syria, during an important period of urbanisation in the Early Bronze Age (EBA, circa 3000-2100 BCE) that shaped the political, social, and economic history of ancient Mesopotamia.

Non-metric dental traits from three EBA sites, Tell Brak (n=77), Tell Barri (n=16) and Tell Arbid (n=17), were recorded using the Arizona State University Dental Anthropology System (ASUDAS). Individuals from the sites were compared using the Gower distance matrix and Mean Measure of Divergence (MMD) to examine the difference between sites as population groups. The results suggest that while sites were ancestrally similar, the observed heterogeneity between sites and was related to the socio-political nature of the community. The greatest variation was observed for the samples from Tell Brak and Tell Barri, the capital and the second-rank administrative centre, respectively. Although Tell Arbid showed less variability, some population segmentation was observed between different burial loci.

Keywords: ASUDAS, dental non-metric traits, dental anthropology, bioarchaeology

Résumé

Les restes humains des populations passées sont étudiés à travers des études bioarchéologiques qui prennent en compte leurs contextes funéraires, biologiques et socioculturels. Les analyses des affinités biologiques des populations humaines, utilisent à la fois des données génétiques et phénotypiques pour étudier les liens biologiques. Les caractéristiques phénotypiques, qui sont l'expression physique de traits génétiques pouvant servir de substitut à l'ADN, sont fréquemment utilisées dans les études de biodistance afin de comprendre l'évolution, les migrations, l'affinité biologique et l'organisation sociale. Dans cette étude, nous avons utilisé la variation phénotypique de la morphologie dentaire pour examiner l'histoire de la population du bassin de Khabur en Syrie, pendant une période importante d'urbanisation au début de l'âge du bronze (EBA, vers 3000-2100 av. J.-C.), qui a façonné l'histoire politique, sociale et économique de l'ancienne Mésopotamie.

Les traits dentaires non métriques de dents provenant de trois sites de l'EBA, à savoir : Tell Brak (n = 77), Tell Barri (n = 16) et Tell Arbid (n = 17), ont été enregistrés en utilisant Arizona State University Dental Anthropology System (ASUDAS). Les individus provenant de ces sites ont été comparés en utilisant la matrice de distance de Gower, et la mesure moyenne de divergence (MMD) a été utilisée pour examiner les différences entre les sites en tant que groupes de population. Les résultats suggèrent que bien que les sites présentassent des similarités ancestrales, l'hétérogénéité observée entre eux était liée à la nature sociopolitique de la communauté. Les échantillons de Tell Brak (la capitale) et Tell Barri (centre administrative de second rang), ont montré la plus grande variation. Bien que Tell Arbid ait présenté moins de variabilité, une certaine segmentation de la population a été observée entre différentes sépultures.

Mots clés : *ASUDAS, traits non-métrés dentaires, anthropologie dentaire, bioarchéologie.*

خلاصة

تتناول الدراسات البيوأثرولوجية لبقايا الإنسان تحليل مجتمعات الماضي من خلال سياقات جنائزهم وسماتهم البيولوجية والاجتماعية. تُستخدم تحليلات مسافة البيولوجية أو مسافة البيوديستانس لاستقصاء العلاقات البيولوجية باستخدام البيانات الجينية والظاهرية لفهم الصلة البيولوجية. غالبًا ما تستخدم دراسات مسافة البيوديستانس عن الصفات الجينية التي يمكن أن تكون بديلاً للحمض النووي القديم لفهم التطور والهجرة والقرابة والتنظيم الاجتماعي. قمنا باستخدام التباين الظاهري في تشريح الأسنان لاستقصاء تاريخ السكان في حوض الخابور في سوريا، خلال فترة هامة من التحضر في العصور البرونزية الأولى (حوالي 2100-3000 قبل الميلاد) التي شكلت التاريخ السياسي والاجتماعي والاقتصادي للبلاد ما بين النهرين.

تم تسجيل سمات الأسنان غير القياسية من ثلاثة مواقع في عصور البرونز الأولى، وهذه المواقع هي: تل براك (عدد العينات = 77)، تل باري (عدد العينات = 16) وتل أربيد (عدد العينات = 17) باستخدام نظام أنثروبولوجية الأسنان في جامعة ولاية أريزونا. (ASUDAS) تم مقارنة الأفراد من هذه المواقع باستخدام مصفوفة مسافة جاور، واستخدمت متوسط قياس التباين (MMD) لفحص الفرق بين المواقع كمجموعات سكانية. تشير النتائج إلى أنه في حين كانت المواقع متشابهة من الناحية الأصلية، إلا أن التباين المراقب بين المواقع كان متعلقًا بالطبيعة الاجتماعية والسياسية للمجتمع. وكان أكبر تباين مراقب في العينات من تل براك وتل باري، العاصمة والمركز الإداري من الرتبة الثانية على التوالي. على الرغم من أن تل أربيد أظهرت تباينًا أقل، إلا أنه تم ملاحظة بعض التجزئة السكانية بين مواقع الدفن المختلفة."

كلمات مفتاحية : ASUDAS - أنثروبولوجيا الأسنان - سمات الأسنان غير القياسية - علم البيوالآ

1. INTRODUCTION

The second cycle of Mesopotamian urbanisation occurred during the Early Bronze Age (EBA, circa 3000-2100 BCE) and shaped the political, social, and economic history of the region (Ur, 2010). This period of growth led to the emergence of the most well-known Sumerian city-states in southern Mesopotamia (Algaze, 2008) and the first territorial states witnessed by the historical sources in the north (Ur, 2010). The kingdom of Nagar was established circa 2600 BCE in the EBA as suggested by the presence of large-scale public buildings (Oates *et al.*, 2001). The large agglomerative site of Tell Brak served as its administrative centre and maintained connections with smaller hinterland sites for agricultural subsistence (Oates et al., 2001, Sołtysiak and Schutkowski, 2015). At the height of its power, Nagar encompassed most of the Khabur drainage in present-day NE Syria and was considered one of three major regional states, along with the kingdoms of Mari and Ebla (McMahon, 2006). Although it was conquered by the Akkadians in circa 2300 BCE, it continued for a few centuries as a minor state called Nawar before it was completely abandoned in the later 2nd millennium BCE (Oates *et al.*, 1997). EBA social networks and their reflection in local population structures, particularly from a bioarchaeological and ancestral perspective, are still relatively poorly understood. Bioarchaeological tools offer one avenue of research to better address questions related to inter-and intra-site relationships.

Modern bioarchaeological research focuses on the interpretation of past human populations from skeletal remains, inclusive of their mortuary, biological, and socio-cultural contexts, to understand the biological, environmental, and social interactions that influenced past lives, including population movement and social organisation (Buikstra, 1977; Larsen, 2002). The term bioarchaeology has only been used since the 1970s (Clark, 1972) but the shift towards the current approaches for studying human skeletons began in the 1950s (Washburn, 1951) and interest in human evolution and biological relatedness began even earlier (e.g. Pearson, 1926; Pearson, 1928).

Biological distance, or biodistance, explores evolution, migration, kinship and social organisation by estimating the similarity and dissimilarity of individuals and groups (Stojanowski & Schillaci, 2006). Biodistance studies can be based on allele frequencies and haplotypes (measuring genotypic expression using ancient DNA) or phenetics, grouping organisms together according to their morphological similarity. Ancient DNA (aDNA) analysis has revolutionised biodistance studies in the field of bioarchaeology, providing insight into not only ancestry investigations but also a wide range of research areas from evolution to palaeopathology (Knapp *et al.*, 2015). Despite many recent advancements, aDNA research can be costly for large samples and requires excellent biochemical preservation and sample destruction, which may be prohibitive for some projects due to a plethora of reasons.

Phenotypic characteristics are the physical expression of genetic traits based on their interaction with environmental factors. Some traits,

such as the expression of dental characteristics, are subject to minimal environmental interaction and can serve as a proxy to examine aDNA without sample destruction. These methods assume that morphological similarity reflects underlying genetic relatedness as phenotypic expressions become more prevalent among closely related individuals (Stojanowski & Schillaci, 2006; Hefner *et al.*, 2016). Methods examining phenotypic variation offer another avenue for exploring biodistance that requires fewer resources and can be performed even where biochemical preservation is poor if macroscopic preservation of tooth crowns and roots is good.

The available methods for assessing dental morphology can be categorised based on the type of data they utilise (metric or nonmetric) (Hefner *et al.*, 2016). The data from metric methods is less reliant on observer experience and relationships within the data can be explored using a larger set of statistical tools. However, some phenotypes are not currently metrically quantifiable, and the analysis of these nonmetric skeletal and dental traits is accomplished by recording the feature either as present or absent, or on a scale to mark the degree/strength of the expression. Unlike metric measurements, nonmetric traits can often

be recorded on incomplete and fragmented material provided that the trait itself is visible.

Dental nonmetric traits record the presence and expression of ridges, tubercles, styles and cusps in tooth crowns and roots (Scott & Turner, 1988), exemplified in Fig. 1. Dental variation can be recorded in a myriad of ways (e.g., Zubov, 1977; Alt, 1991; Alt *et al.*, 1998), but the Arizona State University Dental Anthropology System (ASUDAS) has become the most widely used (Turner *et al.*, 1991; Scott and Turner, 1997; Irish, 2015; Hanihara, 2008). Though dentition is more durable than other parts of the skeleton, preservation can be affected by dental attrition/wear, pathologies (particularly ante-mortem tooth loss and caries) and taphonomic factors causing degeneration that jeopardise the structural integrity of the tooth. Traits included in ASUDAS (Tab. 1) are considered highly heritable; relatively durable against moderate wear; easy to identify and record repeatedly; and unimpacted by sexual dimorphism (Scott, 1973; Scott & Turner, 1997; Turner *et al.*, 1991; Hanihara, 1992; Hubbard *et al.*, 2015).

In the Near East, dental non-metric traits have been used to explore both intra- and inter-regional differences (Alexandersen, 1978; Bentley, 1987; Dicke-Toupin, 2012; Elias, 2016; Lovell & Haddow, 2006; Nassar, 2010, in press; Parras, 2004; Roler, 1992; Sołtysiak & Bialon, 2013; Ullinger *et al.*, 2005; Özbek, 1975). The most common statistical tool used in these studies is Mean Measure of Divergence (MMD), a robust method for identifying differences between groups (Smith, 1972; Harris & Sjøvold, 2004). Its use of frequency tables rather than individuals allows it to accommodate missing data, which is a common occurrence in archaeological data sets. Though this is extremely useful for exploring between-group differences, it does not allow for observations about how individuals cluster within groups. Furthermore, despite using grouped data, small sample sizes of recorded traits provide less powerful statistical analyses, which can limit their utility for biodistance studies. Here, we combine MMD with individual-based Glower distance matrices to explore populations using non-metric phenotypic variation, both between and within sites, in the Khabur basin, north-east Syria.

Dental non-metric data were recorded for EBA skeletons from the sites of Tell Brak (the capital city of the kingdom of Nagar), Tell Barri
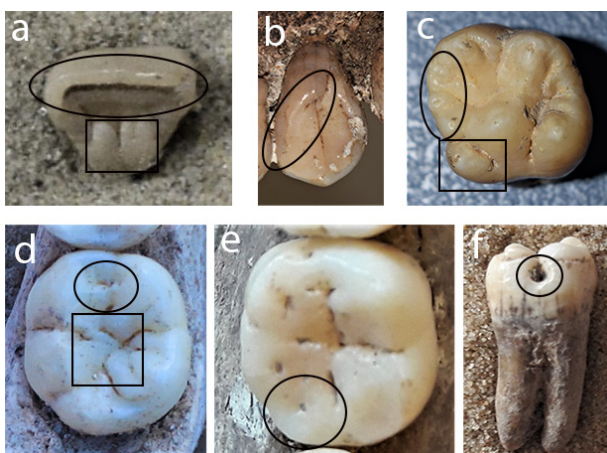
Fig. 1 – Examples of dental traits observed from the samples: a) shovelling (circle) and tubderculum dentale (square) on upper first incisor, b) mesial accessory ridge (circle), c) marginal ridge tubercles (circle) and Carabelli's cusp (square) on upper first molar, d) anterior fovea (circle) and Y-groove pattern (square) on lower first molar, e) worn but still visible cusp 5 (circle) on lower second molar, f) protostylid (circle) on lower second molar (with possible caries lesion in the pit).

Trait	Recorded	Abbreviation
Labial Curvature	Score	LC
Shoveling	Score	S
Double-shoveling	Score	DS
Interruption groove	Present/Absent	IG
Tuberculum dentale	Score	TD
Radical number	Score	R
Pegged or reduced incisor	Score	UI2V
Mesial accessory ridge	Score	MAR
Distal accessory ridge	Score	DAR
Premolar accessory ridges	Score	PAR
Accessory cusps	Present/Absent	AC
Metacone size	Score	M
Hypocone size	Score	H
Cusp 5	Score	C5 UM
Carabelli cusp	Score	CC
Parastyle	Score	PA
Enamel extensions	Score	EE
Upper premolar root number	Count	RN UP
Upper molar root number	Count	RN UM
Odotome	Present/Absent	O
Tome's root	Score	TR
Lower premolar lingual cusp number	Count	CN
Anterior fovea	Score	AF
Groove pattern	x/y/+	GP
Cusp 5	Present/Absent	C5 LM
Cusp 6 size	Score	C6
Cusp 7 size	Score	C7
Deflecting wrinkle	Score	DW
Distal trigonid crest	Score	TR
Mid-trigonid crest	Score	MTC
Protostylid	Score	PR
Lower canine root number	Count	RLC
Lower molar root number	Count	RN LM1

Tab. 1 – ASUDAS traits used in the analysis.

(ancient Kahat) and Tell Arbid (ancient name unknown) (Fig. 2). We hypothesise that the population of the capital city of Nagar would be more heterogenous than the populations of provincial towns within the kingdom, thus reflecting population movement linked to site size and status in the inter-site network.

2. MATERIAL

Tell Brak, also known as ancient Nagar/Nawar, is situated not far away from the confluence of two major Khabour River tributaries, Wadi Jaghjagh and Wadi Radd. The site, which covered more than 60 ha at its height during the EBA, was excavated between 1976-2010 by a team from the University of Cambridge led by Joan and David Oates (Oates *et al.*, 2001). These excavations uncovered 161 human burials dated to the EBA, including 81 adults, and explored three additional contexts with commingled bones from at least 44 individuals (Sołtysiak, 2009). Most human remains from Tell Brak were found in Areas FS and TC in the eastern part of the site and dated to the period when the kingdom of Nagar was a part of the Akkadian empire, or the period following the fall of Akkadian rule when it was re-established as the small Hurrian kingdom of Nawar.

Tell Barri, a second-rank administrative centre in the Nagar kingdom, is located 10 km north of Tell Brak along the Wadi Jaghjagh. Following the fall of Nagar, the site remained a regional power as the capital city of a small independent state (Pecorella, 2008). Tell Barri was excavated between 1980-2010 by an Italian team directed by Paolo Emilio Pecorella (University of Florence) and then by Raffaella Pierobon-Benoit (University of Naples) (Pierobon Benoit, 2013). In total, 25 skeletons dated to the EBA were found representing the periods before and after the Nagar kingdom became part of the Akkadian empire (Sołtysiak, 2008).

Tell Arbid is located farther north, near the former northern border of the kingdom of Nagar. The site was most likely originally a part of the small kingdom of Nabada (modern Tell Beydar), which was conquered at some point by the kings of Nagar. Excavated by two Polish teams directed by Piotr Bieliński (University of Warsaw) and Rafał Koliński (Adam Mickiewicz University in Poznań),
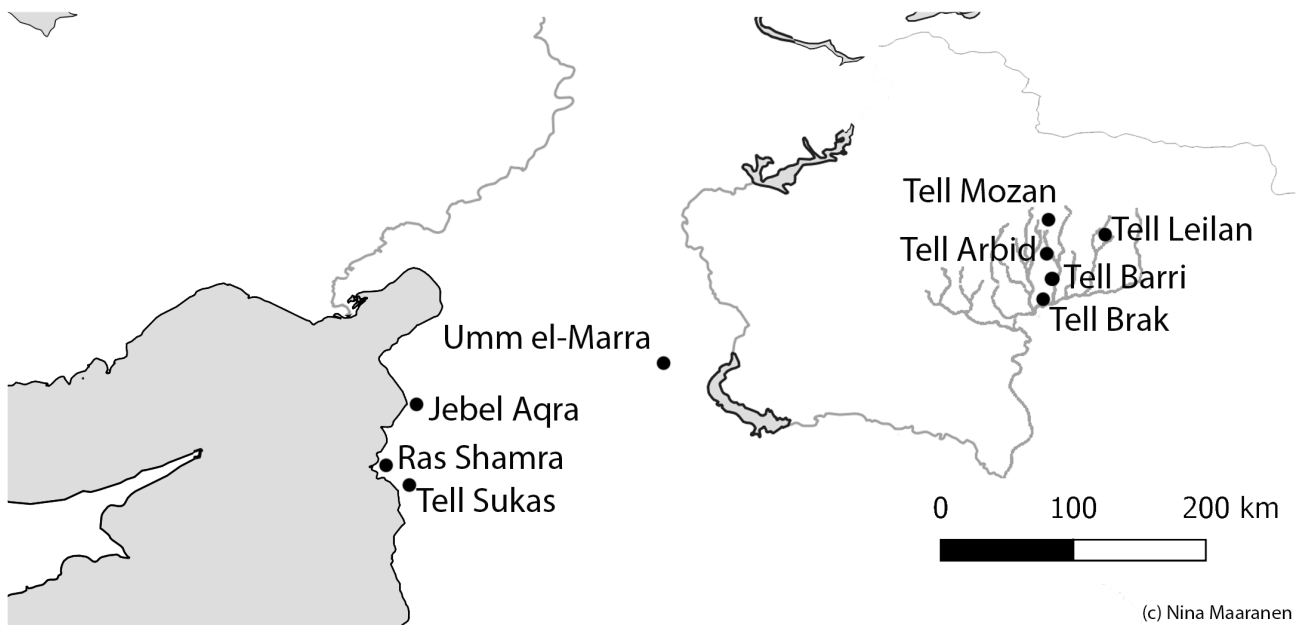
Fig. 2 – Map of the Khabur basin, Syria.

the site yielded 115 individuals dated to the Early Bronze Age (circa 2600 BCE to the end of the 3rd millennium BCE) when many small independent regional kingdoms arose after the fall of the Akkadian empire (Sołtysiak, 2010; Sołtysiak & Koliński, 2011).

3. METHODS

3.1. Data collection

Non-metric traits were scored for permanent teeth from a total of 110 EBA individuals from the three sites (77 from Tell Brak, 17 from Tell Arbid, and 16 from Tell Barri). Thirty-two non-metric dental traits were recorded using the ASUDAS by a single observer (JW) to minimise inter-observer error (Tab. 1) and ASUDAS dental casts were used to produce standardised scoring (Turner *et al.*, 1991). All permanent teeth, both right and left, were scored for each trait.

The samples from each site included large numbers of subadults with very little or no wear on the permanent dentition. Any traits that were obscured by wear, damage, or dental caries, were not scored. In cases where trait scores differed bilaterally for the same individual, the higher score was used (following Irish, 2005; Turner & Scott, 1977). To reduce noisy variation in the statistical analysis for LP2 cusp numbers, scores were recorded by tooth and then combined as proposed by Scott and Irish (2017).

All age-at-death and biological sex categories were pooled for statistical analysis.

3.2. Statistical analysis

Biodistance analysis was conducted in R environment (R Core Team, 2020). Inter-trait correlation can produce biased outputs for most distance matrices, including MMD and Gower distance matrices. To avoid bias, traits exhibiting a strong correlation were excluded, based on Kendall's tau-b rank correlation coefficients (τ-b$\geq$0.5).

For MMD (Harris & Sjøvold, 2004; Sjøvold, 1977; Smith, 1972), ASUDAS scores were dichotomised as present (1) or absent (0) in the frequency table. The dichotomisation thresholds or breakpoints are set by the investigator. These breakpoints are not commonly set from presence (grade 1) but from a higher score that excludes weak expressions to differentiate between individuals exhibiting strong trait expressions. This procedure decreases inter- and intra-observer error by omitting weaker expressions that might be difficult to see, but it also decreases variation in the data and hides some of the variation between individuals within samples. Here, we use the breakpoints recommended by prior research (Scott & Irish, 2017).

Small sample sizes and traits that do not contribute to variation can create bias in the

| | total | | 5 traits (Gower) | |
	Teeth n	Individuals	Teeth n (before imputation)	Individuals
Arbid	192	17	28	7
Barri	161	16	17	5
Brak	399	77	43	12

Tab. 2 – **Total of individuals and teeth used in the MMD and Gower distance matrix analysis.**

MMD distance matrix. To avoid this issue, traits with less than 10 total observations were excluded. The analysis was conducted in the R package AnthropMMD (Santos, 2018) using the Anscombe angular transformation. The program was used to drop traits that did not contribute to variation (overall mean divergence or MD ≤0). The resulting distance value increases with dissimilarity and the difference between groups is considered statistically significant (p≤0.05) when they are over two standard deviations (2 SD) apart.

The non-dichotomised ASUDAS scores were used to create a Gower distance matrix (Gower, 1971), which measures differences between observations by computing distances between pairs and then combining them into a single value per record-pair. The values fall between 0 and 1, the latter indicating maximal dissimilarity (Maechler *et al.*, 2019). Gower distances were calculated with the package |cluster| (Maechler *et al.*, 2019) where additional transformations are specified using the type of argument, such as binary values. Before producing the Gower distance matrix, missing values had to be minimised to produce a reliable imputed data set for further analysis. Traits and individuals were omitted until missing data remained below 30%. Missing values were then imputed using predictive mean matching (PMM), a hot deck method that draws pre-existing values, from the package |mice| (van Buuren & Groothuis-Oudshoorn, 2011).

Because the Gower distance matrix measures individuals, we can use PERMANOVA and PERMDISP tests for further statistical analysis. These are permutational tools designed for non-Euclidean data to compare differences in group mean locations and directions (PERMANOVA)

as well as dispersion (PERMDISP). PERMANOVA can also compare the spread of values, but PERMDISP is more sensitive to changes in dispersion. We used the functions |adonis| and |betadisper| from the package |vegan| (Oksanen *et al.*, 2019). The function |betadisper| conducts a principal coordinate analysis (PCoA) on the distance matrix that can be used to visualise the data in a two-dimensional graph. Though PCoA is like Principal Component Analysis (PCA), its output properties are not identical to its parametric counterpart. Non-Euclidean data can produce negative vectors (coordinate points) and eigenvalues. Negative eigenvalues can be corrected for in most packages (including |vegan|), but the coordinate points/vectors can remain negative as the ordination process scatters the points around the origin.

4. RESULTS

Tab. 2 shows sample numbers of the three EBA data sets. Despite a sufficient sample of individuals, the number of recorded traits per site was low (Fig. 3, Supp. Tab. 1). The following common abbreviations are used for teeth in the text: U=upper; L=lower; I=incisor; C=canine; P=premolar; and M=molar. For instance, UM2 indicates upper (U) second molar (M2).

4.1. MMD

Due to low intra-site sample sizes, the near-by sites of Tell Arbid and Tell Barri were pooled together to compare Tell Brak with its hinterland

Fig. 3 (opposite) – **Descriptive plot showing the distribution of individual scores between sites.**

Sample numbers for maxillary teeth
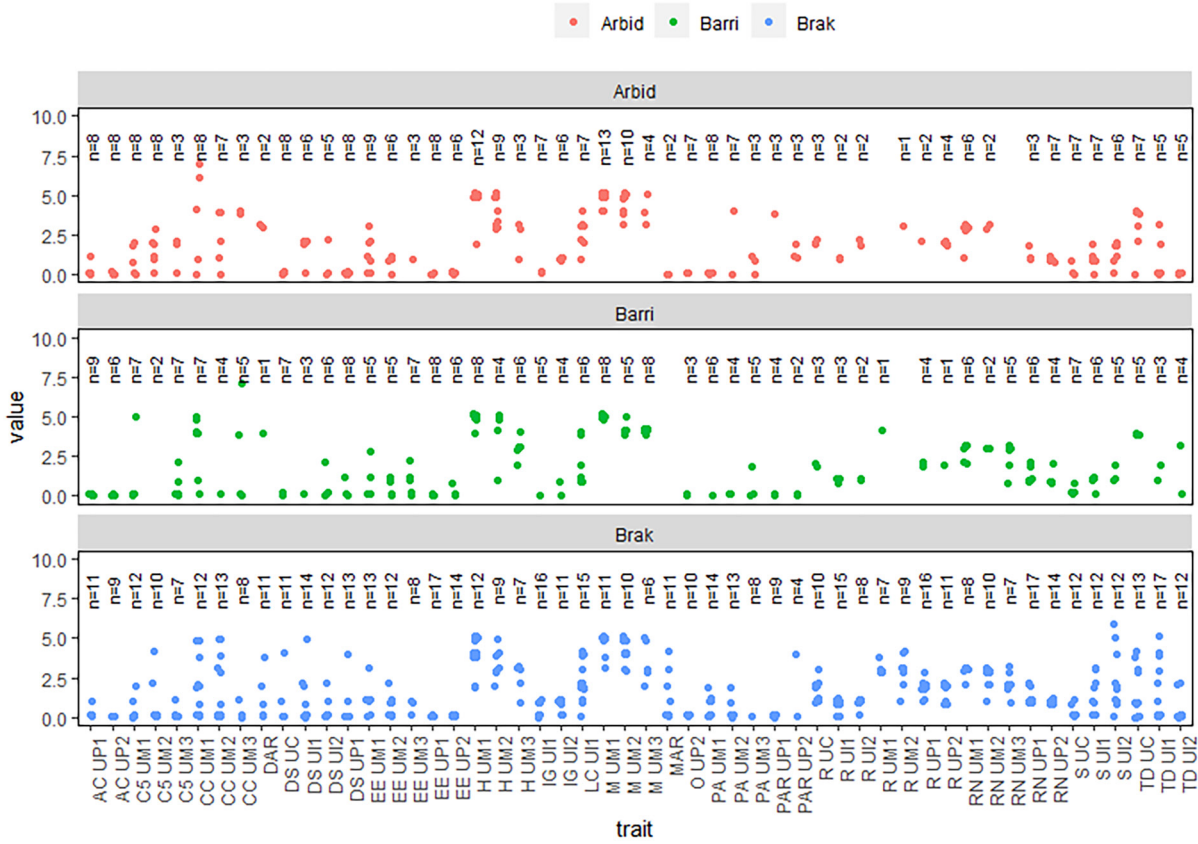
Sample numbers for mandibular teeth
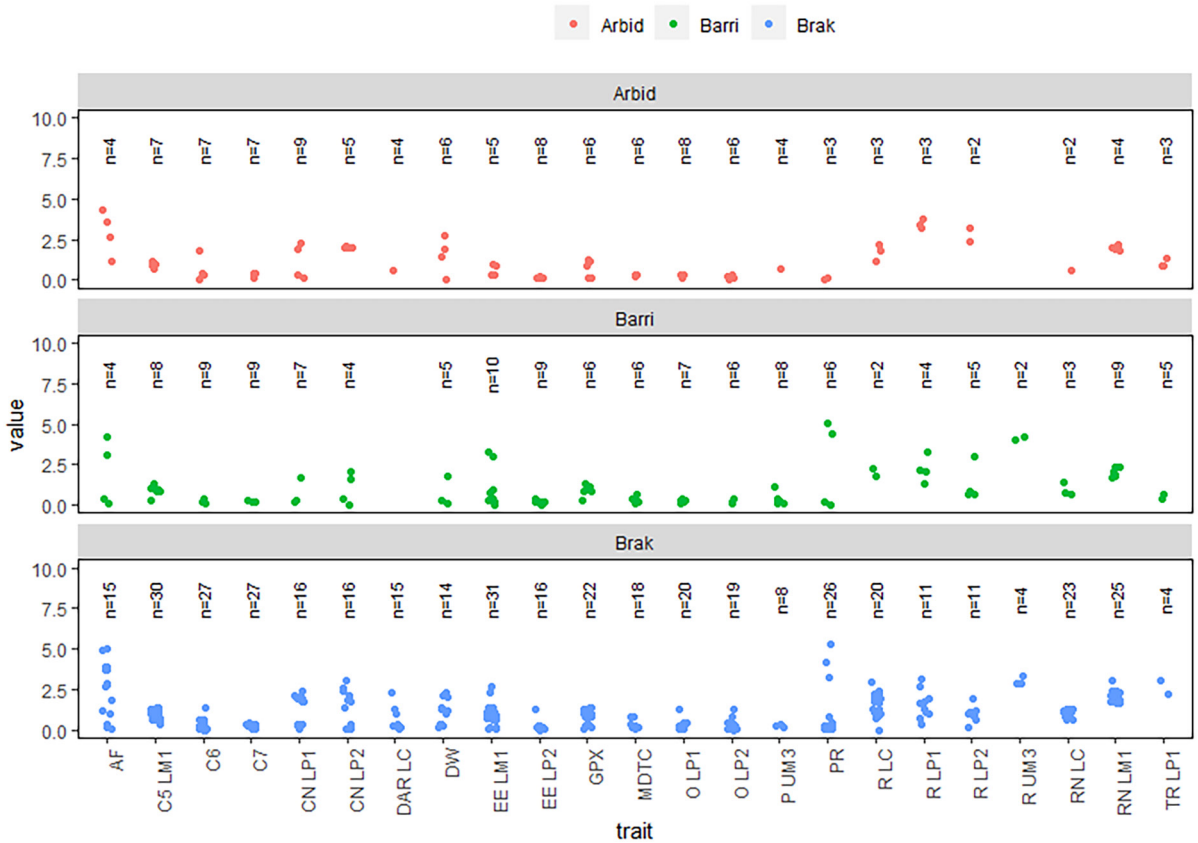
(Tab. 3). Traits were dichotomised according to recommendations (Scott and Irish, 2017). Though 16 traits had more than 10 observations (and would have thus been sufficient for a statistical analysis), only two traits, UI1 shoveling and UM1 enamel extension, showed any positive variation in the data set (Supp. Tab. 1). Traits that do not count for variation (i.e., have mean divergence values equal to zero or lower), were excluded as they bias the analysis. When conducting the MMD analysis with these traits, the results did not indicate a significant difference between Tell Brak and its hinterland (MMD=0.170, SD=0.154, p=0.12). In other words, out of 16 traits only two

|	BP	N hinterland	N Brak	Freq hinterland	Freq Brak
DS UI1	2	8	14	3	3
LC UI1	2	12	15	8	10
S UI1	2	12	12	1	4
IG UI2	1	10	11	5	7
TD UI2	2	9	12	1	2
DAR UC	2	3	11	3	4
AC UP1	1	16	11	1	1
PAR UP2	2	5	4	1	1
RN UP1	2	8	17	4	3
C5 UM1	2	14	12	3	1
CC UM1	2	14	12	7	6
EE UM1	1	13	13	4	1
PA UM1	1	13	14	0	1
H UM2	3+	12	9	12	8
RN UM2	3	4	10	4	7
M UM3	3.5+	11	6	10	2
P UM3	1	11	8	2	0
RN LC	2	5	23	0	0
TR LP1	4	7	4	0	0
CN LP2	2	8	16	6	8
O LP2	1	11	19	0	2
AF LM1	2	8	15	5	10
C5 LM1	1	14	30	13	28
C6 LM1	1	15	27	1	3
C7 LM1	2	15	27	0	0
DW LM1	2	11	14	3	3
GP LM1	X	11	22	7	13
MTC LM1	1	11	18	1	2
PR LM1	1	8	26	2	4
RN LM1	3	12	25	0	1

Tab. 3 – Trait frequencies according to breakpoints (BP).

Trait	Overall MD
S UI1	0,18
EE UM1	0,16
O LP2	-0,02
C5 UM1	-0,04
PA UM1	-0,06
C6 LM1	-0,09
C7 LM1	-0,09
C5 LM1	-0,1
RN LM1	-0,1
IG UI2	-0,12
GPx LM1	-0,12
AC UP1	-0,13
DW LM1	-0,14
MTC LM1	-0,14
LC UI1	-0,14
CC UM1	-0,15

Tab. 4 – Overall mean divergence (MD) of each trait available for the MMD analysis. Only traits with positive overall MD were used to avoid bias.

showed any variation between sites and even in this case the divergence created by them was not significant.

4.2. Biodistance analysis from the Gower distance matrix

Inter-trait correlation test indicated LM1 protostylid and trigonid crest were correlated. The latter was excluded as it showed less variation in the data set. Missing values were minimised to ensure reliability of imputation. This was conducted by excluding traits and individuals with high numbers of missing values until the frequency of missing data was no more than 30%. This left 8 traits: UI1 shoveling (S UI1) and labial convexity (LC UI1), UP accessory cusp (AC UP1), UM1 cusp 5 (C5 UM1) Carabelli cusp (CC UM1), parastyle (PA UM1) and enamel extension (EE UM1) and LM1 cusp 6 (C6 LM1). Three traits showed little (i.e., only one positive observation) to no variation (AC UP1, C5 UM1, C6 LM1) and were excluded from further analysis. The imputation was conducted on 5 traits from 25 individuals.

PERMDISP and PERMANOVA tests were conducted to compare sites. There was no statistically significant difference in dispersion of values between sites (PERMDISP df=2, SS=0.0221, MS=0.0110, F=1.766, p=0.214). Group means were not significantly different between sites (PERMANOVA df=2, SS=0.4647, MS=0.2323, F=1.0758, R2=0.0929, p=0.424). Due to the sample sizes, these results are susceptible to type 2 error and should be regarded tentatively.

Group clustering and differences were plotted using principal coordinates and their distances from group centroids acquired from the PERMDISP test. While not significantly different, dispersion of values appears smaller for Tell Arbid when compared to Tell Barri and Tell Brak for the same time period. The overlap of these groups, visible in Fig. 4, is considerable.
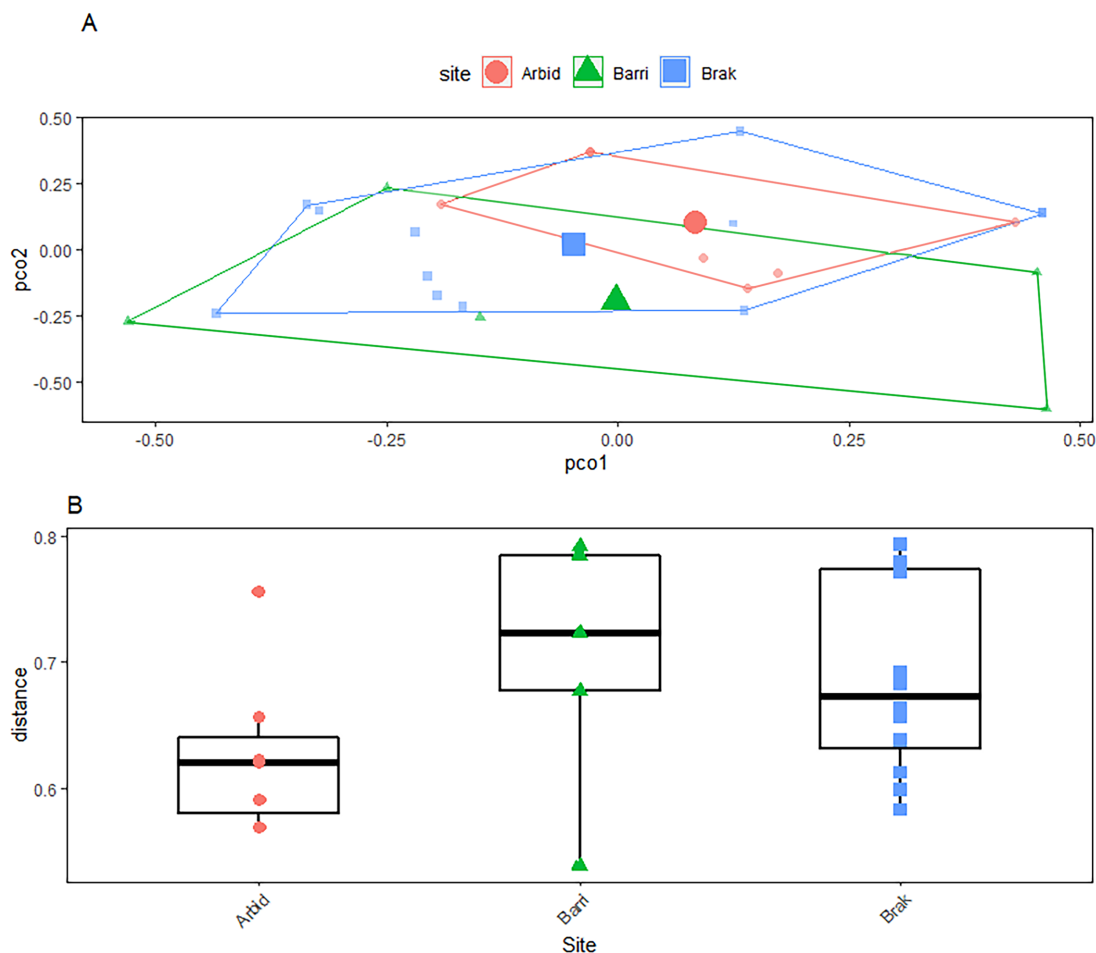

Fig. 4 – Dispersal of values between sites along principal coordinates (A) and their distance from group centroid (B).

5. DISCUSSION AND CONCLUSION

Available cuneiform documents (mainly from Ebla and Mari) mention diplomatic relations between North Mesopotamian kingdoms and discuss marriages between regional dynasties that involved the movement of people between the capital cities (Oates *et al.*, 2008). These people either belonged to the court of new, incoming princesses or they were artisans sent as the result of political treaties (McMahon, 2006). After the Akkadian conquest, the new elites likely settled in the capital city rather than in the marginal areas of the kingdom. As a result, we hypothesised that the EBA population in the Nagar capital, modern Tell Brak, would be more heterogenous than contemporary populations in its provincial towns, represented here by Tell Arbid and Tell Barri.

As predicted, the results indicate that Tell Brak and Tell Barri, the capital and the second-rank administrative centre, had the widest dispersal of values (Fig. 4). Tell Brak experienced extensive growth during the Late Chalcolithic (LC) period, with new settlements emerging on several tells around the main site (Tell Majnuna, Tell Temmi) due to immigration from settlements within approximately 4 km of the city (McMahon, 2020). The EBA populations of Tell Brak, Barri and Arbid were not distinct from one another based on the PERMANOVA or MMD analyses, suggesting the populations were ancestrally similar. This agrees with the archaeological evidence demonstrating similarities in material culture between the sites (Rutkowski, 2019, Smogorzewska, 2012). The evidence from Tell Barri and Tell Arbid also reflect their positions in the Nagar administrative network, complementing previous bioarchaeological research. An isotope study conducted using individuals from all three sites indicated stable subsistence and diet during the EBA with very little inter-site variability, indicating a uniform and resilient economy irrespective of site rank (Sołtysiak & Schutkowski, 2015; Sołtysiak and Fernandes, 2021). Unlike Tell Brak and Tell Barri, Tell Arbid experienced a smaller influx of people as a low-rank peripheral administrative centre (Fig. 4B). There was likely a strong local subsistence system overseen by a central administration that organized the exchange of resources during the EBA and Middle Bronze Age (MBA) (Sołtysiak & Schutkowski, 2015).

Some observations were made for the individuals from Tell Barri and Tell Arbid based on the PCoA plot (Fig. 5). Many of the Tell Barri skeletons used in the Gower distance analysis show a high frequency of carious lesions, clustering somewhat close together (Fig. 4A, Fig. 5A). Skeletons 1302 and 1515 belonged to an upper-class segment within the society as evidenced by rich grave goods, while individuals 1526 and 1554 were buried near a temple that was built using a plan that was not common in the north of Mesopotamia, but typical for the south, suggesting some movement of people from that direction (Sołtysiak, 2014). Although individuals 1302 and 1515 are not separated by the greatest distance in the two-dimensional principal coordinate plot, they do represent the largest and smallest distances from the group centroid, respectively and attribute to the high overall variation for Tell Barri. At Tell Arbid, the clustering of skeletons buried in Area SD (eastern slope of the site) and Area W (southern slope of the site) indicates that local population segmentation may have taken place and was manifested through burial location.

Due to the low sample numbers, particularly for the Gower distance analysis, these results are tentative. The distortion produced by sample sizes is evidenced by the single data point from Tell Barri (Fig. 4B) that, while not considered an outlier, expands the site dispersal extensively. This example underscores one challenge posed by small samples where the range of individual variation can often be seen more readily at the population level. For instance, the higher dispersal of Tell Brak values could be due to its larger sample number. The small sample sizes from Tell Arbid and Tell Barri were pooled for the MMD analysis for this reason; however, the combination of the sites could potentially obscure local variation. Furthermore, biodistances acquired from dental non-metric traits seem to reflect overall genetic distances, but these distances may not be as useful for highly admixed populations (Delgado *et al.*, 2019). Another recent study has shown that Gower coefficients may be more reliable indicators of biological kinship when used on metric rather and non-metric dental data (Stojanowski & Hubbard 2017).

With these caveats in mind, the visual observation of the individuals did, however, form patterns that support archaeological and textual evidence. Dental non-metric traits indicated that while sites were ancestrally similar, the observed heterogeneity between sites and was related to the socio-political nature of the community. The results complemented both archaeological and
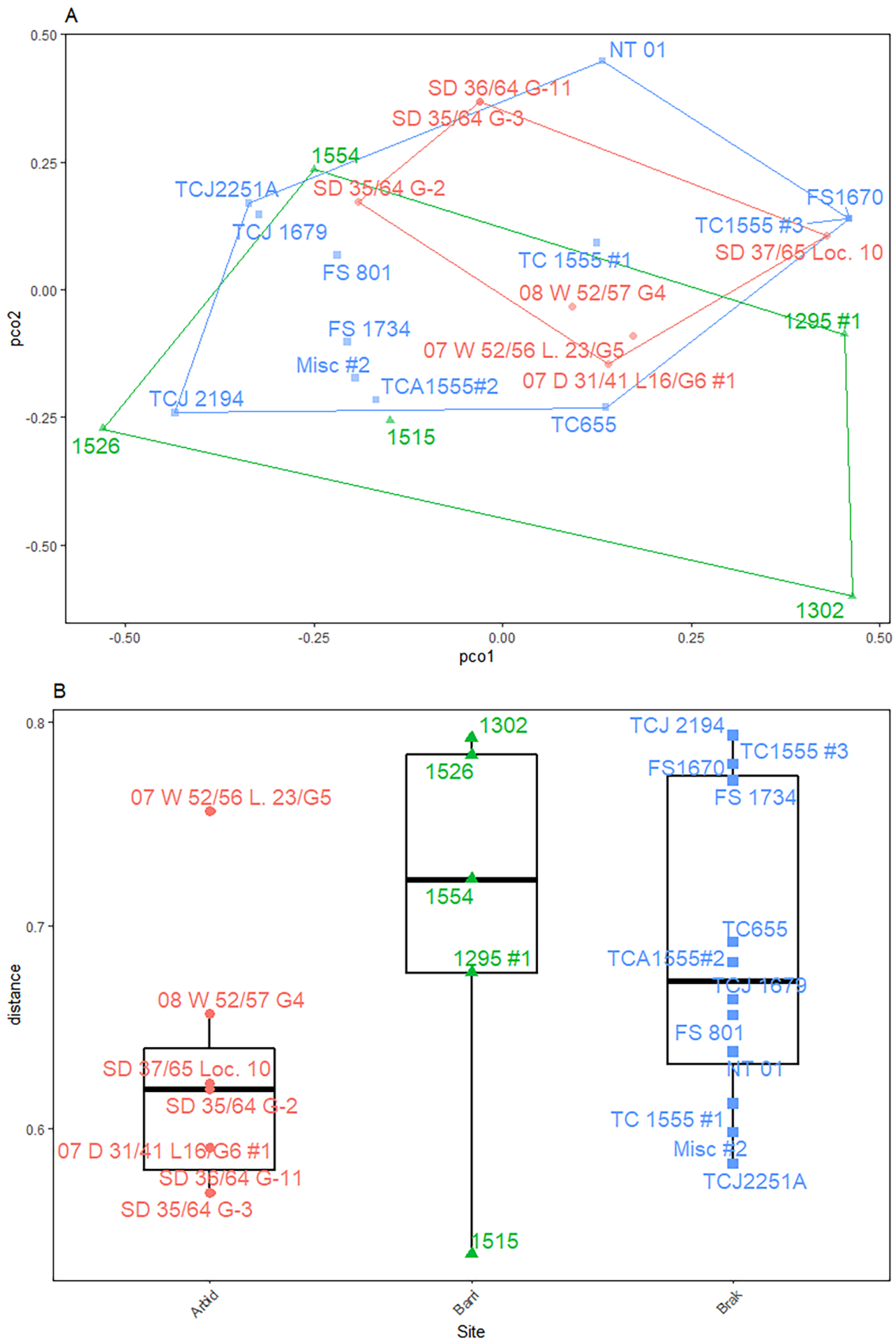
Fig. 5 – Copy of the Fig. 4 with labels for individuals.

other bioanthropological studies conducted on these samples, underlining the importance of multidisciplinary approaches in contemporary archaeological investigations. Modern bioarchaeological research can elucidate even smaller data sets by focusing on qualitative interpretation. In this case, biodistance analysis provided insight into the population history of the Khabur basin during a period of intense urbanisation.

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Supp. Tab. 1 – Raw data from Early Bronze Age Tell Brak, Tell Barri and Tell Arbid.

Column headers (left to right): Site | Skeleton | Site2 | R_UM1 | M_UM2 | H_UM2 | CS_UM2 | CC_UM2 | PA_UM2 | EE_UM2 | RN_UM2 | R_UM2 | M_UM3 | H_UM3 | CS_UM3 | CC_UM3 | PA_UM3 | EE_UM3 | RN_UM3 | R_UM3 | P_UM3 | DAR_LC | R_LC | RN_LC | RN_LP1 | R_LP1 | EE_LP1 | O_LP1 | CN_LP1 | TR_LP1 | RN_LP2 | R_LP2 | EE_LP2 | O_LP2 | CN_LP2 | AF_LM1 | GPx_LM1 | DW_LM1 | MOTC_LM1 | PR_LM1 | CS_LM1 | G6_LM1 | C7_LM1 | EE_LM1 | RN_LM1

Site	Skeleton	Site2
Brak	TCI519 #2	Brak
Brak	TCG639	Brak
Brak	FS1794	Brak
Brak	TCI655	Brak
Brak	TCI670	Brak
Brak	TCI693 Mixed	Brak
Brak	TCI519 #3	Brak
Brak	TCI854	Brak
Brak	TCI633	Brak
Brak	UA45	Brak
Brak	TCI519 #3	Brak
Brak	No Tag Box #2	Brak
Brak	TCI699	Brak
Brak	703.Gor 703.16	Brak
Brak	TCI251 D	Brak
Brak	TCI643 #2	Brak
Brak	TCI643 #3	Brak
Brak	TCI251 #3	Brak
Brak	FS80	Brak
Brak	TCI251 A	Brak
Brak	TCA1555#2	Brak
Brak	TCI555#1	Brak
Brak	TCI1	Brak
Brak	TCI186	Brak
Brak	TCI519 #1	Brak
Brak	TCI555 #3	Brak
Brak	TCI1679	Brak
Brak	FS1375 MUC	Brak
Brak	Misc #1	Brak
Brak	FS1957	Brak
Brak	TC 675	Brak
Brak	FS801	Brak
Brak	TCI1251 C	Brak
Brak	TCI 254 #2	Brak
Brak	TCI214 Mixed	Brak
Brak	TCI 251 #4	Brak
Brak	TCI 207	Brak
Brak	TCI 251 #6	Brak
Brak	TCH 1338	Brak
Brak	TCI 251 #5	Brak
Brak	TCI 251 #5	Brak
Brak	TC194 #0	Brak
Brak	Misc #2	Brak
Brak	Misc #2	Brak
Brak	Misc #2	Brak
Brak	TCI 207 #6	Brak
Brak	TCI 251 #1	Brak
Brak	TCI 214 #3	Brak
Brak	TCI 207 #8	Brak
Brak	TCI 214 #1	Brak
Brak	NT 01	Brak
Brak	TCI 207 #4	Brak
Brak	TCI 214 #2	Brak
Brak	TCI 219S	Brak
Brak	FS327	Brak
Brak	FS1374 N.Mx.1 Md B	Brak
Brak	FS1794	Brak
Brak	TCI 207 Mixed	Brak
Brak	TCI 207 Mixed	Brak
Brak	TCI 207 Mixed	Brak
Brak	FS1374 Mx.2	Brak
Brak	FS801	Brak
Brak	FS1374 md A	Brak
Brak	TCI 251 B	Brak
Brak	TCI 207 #3	Brak
Brak	TCI 207 #9	Brak
Brak	Tags missing???	Brak
Brak	NT 1/10	Brak
Brak	TCI 2194	Brak
Brak	NT 02	Brak
Brak	TCI 2207 #7	Barri
Barri	813	Barri
Barri	1295 #1	Barri
Barri	1515	Barri
Barri	1526	Barri
Barri	1554	Barri
Barri	1302	Barri
Barri	1298	Barri
Barri	K9 GA1-0-14	Barri
Barri	1307	Barri
Barri	K17 /A-B 1 ST 44	Barri
Barri	1066	Barri
Barri	K9 G A1 -0-14 B	Barri
Barri	1267	Barri
Barri	1515	Barri
Barri	1526	Barri
Barri	813	Barri
Barri	1218	Arbid
Arbid	1295 #1	Arbid
Arbid	SL 38/56 G-1 "W Layer"	Arbid
Arbid	D 29/41G-1	Arbid
Arbid	SD 36/64 G-2	Arbid
Arbid	08 W52/56 L-23/G5	Arbid
Arbid	08 W52/55 Loc_33 G2	Arbid
Arbid	08 W 52/55 Loc_ 28 G1	Arbid
Arbid	08 W 52/57 G4	Arbid
Arbid	07 D 31/41 L15/G4	Arbid
Arbid	07 D 31/41 L16/G6 #1	Arbid
Arbid	07 D 31/41 L19/G3	Arbid
Arbid	SD 36/60 G-3	Arbid
Arbid	SD 36/64 G-3	Arbid
Arbid	SD 36/64 G12	Arbid
Arbid	SD 36/64 Loc_10	Arbid
Arbid	SD 36/64 G12	Arbid
Arbid	W D III P Grave	Arbid