Ancient Mitochondrial DNA Analysis of an Iron II Burial Cave on the Slope of Tel Kiriath-Yearim

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In December 2018, the looting of an Iron II rock-cut burial cave in the village of Abu Ghosh was spotted, subsequently leading to a salvage excavation carried out by the Antiquities Theft Prevention Unit and the Jerusalem Regional Office of the Israel Antiquities Authority. Among the finds from the extensively disturbed tomb were the remains of at least ten individuals, two of which were analyzed by ancient DNA methods.

The cave is located ca. 300 m to the southwest of the site of Kiriath-Yearim (Finkelstein et al. 2021; Fig. 1). It was severely damaged several years earlier due to modern building activities and only its northeastern corner survived (Fig. 2). This corner (Fig. 3) is a small part of the original cave, which most likely served as a repository for bones and pottery vessels cleared to the side in order to make space for additional interments. The part of the cave that survived was 1.5 × 3.3 meters in size and 2 meters high (Fig. 4).

The cave's floor was level; on it three soil layers were distinguished but there was no significant difference between the finds of Layers 2 and 3. It seems that Layer 3 contained vessels *in situ*, on which items of Layer 2 were placed (Fig. 5). A pit was cut in the floor of the cave. It contained an accumulation of earth-debris, vessels and bones, although in smaller quantity than the finds on the cave's floor.

The Finds

The pottery assemblage from the cave consists of an estimated 150 vessels, most of them complete. The pottery was badly eroded and brittle, making restoration difficult. The poor state of preservation also made it difficult to trace burnish or slip, especially on the bowls and the jugs. Most of the vessels were made of reddish-brown clay with white grits, presumably originating in the local *terra rossa*. The assemblage is composed mostly of bowls (Fig. 6), jugs (Fig. 7), juglets and lamps (Fig. 8; see Table 1). Larger kraters are not present and the number of cooking vessels is insignificant. While this may appear to be a

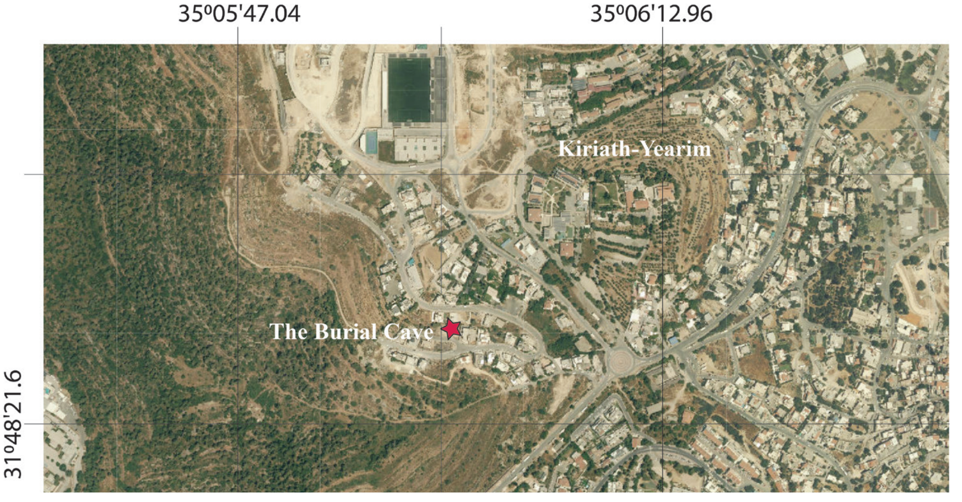

Fig 1. Location of the burial cave

Fig. 2. The burial cave, looking southeast in the direction of the mound (photograph by: Kiriath Yearim Expedition)

Fig. 3. The burial cave with the looting refuse (Courtesy of the Israel Antiquities Authority)

typical burial assemblage, the damage and looting of the cave indicates that only a small portion of the original burial was excavated. Consequently, it originally contained a far larger number of objects and ceramic vessels.[1]

Table 1: Vessel Types from the Tomb

Bowls	Jugs	Juglets	Lamps	Others
Outward folded rim: 15 Carinated with everted rim: 20 Straight wall: 10 Others: 10	Decanter: 16 Red slipped: 10 Others: 15	Elongated: 12 Small black: 6 Small beige: 8 Pyxis: 2	Rounded base: 2 Disc base: 2 High disc base: 15	Cooking pot: 2 Cooking jug: 2 Spouted jar: 3
Total: 55	**41**	**28**	**19**	**7**

1 In comparison, ca. 260 complete vessels were reported from the cave at Ketef Hinnom (Barkay 1985:298) and ca. 300 vessels at el-ʻArub (Yezerski 1997:44). Cave 1 at Motza, which had been looted, contained over 90 vessels (Negbi 1970:361).

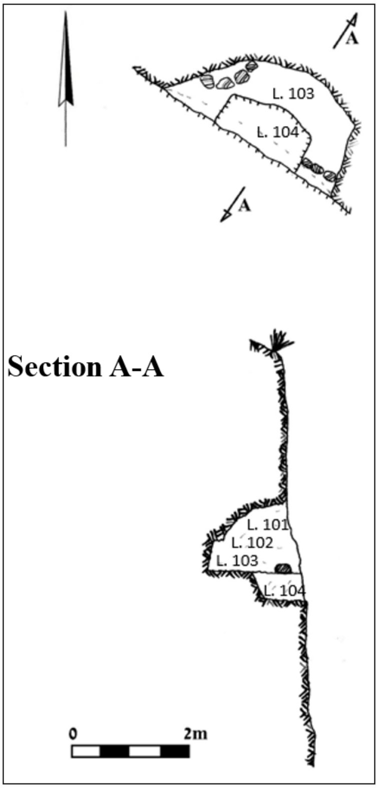

Fig. 4. Plan and section of the remaining part of the burial cave (Courtesy of the Israel Antiquities Authority)

Bowls constitute the most frequent vessel type in the assemblage (n=55; Table 1). These bowls consist of three main types:

a. Bowls with an out-folded rim, which are the most common bowl-type in Iron IIB–C Judah. The bowls found in the cave better resemble vessels of this type found in the 701 BCE destruction layers (City of David, Strata 12–11—De Groot and Bernick-Greenberg 2012:Fig. 4.2: 3–5, Type 8b1; Lachish, Level III—Zimhoni 2004b:Fig. 26.3: 16–20; Motza, Stratum IV—Greenhut and De Groot 2009:Fig. 3.19: 10–12; Caves I and VII at Motza—Negbi 1970:Figs. 3: 2; 5: 1) than those which appear in the 586 BCE destruction layers.[2]

2 Bowls of the same type, dating from the second half of the 7th to the beginning of the 6th centuries BCE, are generally smaller and have a disc base (Freud 2016:Table 16.1:B5 and references therein).

Fig. 5. Pottery and bones in Layer 2 (Courtesy of the Israel Antiquities Authority)

b. Carinated bowls with an everted or upturned rim. Such bowls most commonly appear in Iron IIB strata (City of David, Stratum 12—De Groot and Bernick-Greenberg 2012:Fig. 4.20: 8–13, with a red slip; Lachish, Level III—Zimhoni 2004b:Figs. 26.3: 15, 26.18: 6; Motza Stratum V—Greenhut and De Groot 2009:Fig. 3.11: 7, 9, 11; Cave I at Motza—Negbi 1970:Fig. 3: 3–5).
c. Straight-walled bowls in varying sizes, typical of Iron IIB (City of David, Stratum 12—De Groot and Bernick-Greenberg 2012:Fig. 4.19: 4; Lachish Level III—Zimhoni 2004b:Fig. 26.3: 4–5).

Other less frequent types are miniature bowls of various shapes and a bowl with a bar handle and knob, more common in Iron IIA than in Iron IIB (e.g., City of David, Strata 15–14—De Groot and Bernick-Greenberg 2012:Fig. 5.17: 8; Lachish, Levels IV–III—Zimhoni 2004a:Figs. 25.52: 14, 25.55: 7; 2004b:26.27: 2; Cave I at Motza—Negbi 1970:Fig. 3: 1).

Forty-one jugs were found. These mostly consisted of decanters with elongated bodies widening toward the base, typical of Iron IIC; one decanter has a rounded body, more common in Iron IIB. Other jugs, of various shapes, include elongated, cylindrical or rounded bodies, some with a spout and some with trefoil rims. At least ten of the jugs are red, slipped, although this number was probably higher as the slip likely faded, due

Fig. 6. Bowls and a chalice (photograph by: Sasha Flit, Courtesy of the Kiriath Yaerim Expedition)

Fig. 7. Cooking vessels and jugs
(photograph by: Sasha Flit, Courtesy of the Kiriath Yaerim Expedition)

to post-depositional processes.

Twenty-eight juglets were found. Of these, 12 are elongated dipper juglets, the most common type in Iron IIB–C Judah (City of David, Stratum 12–10—De Groot and Bernick-

Greenberg 2012: 72, Jt1c, Fig. 4.4: 2; Lachish, Levels III–II—Zimhoni 2004b: Figs. 26.4: 13–17, 26.53: 2). Other types of juglets are mainly black juglets with a rounded body and base and a handle extending from the rim or directly below the rim to the shoulder. These are also typical of Iron IIB (City of David, Stratum 12—De Groot and Bernick-Greenberg 2012:Fig. 4.44: 20; Cave I at Motza—Negbi 1970:Figs. 4: 8–9; 5: 11).

Nineteen oil lamps were found, with flat and high disc base. The flat lamp is an earlier type, while the high disk base is more common during Iron IIC. These types appear together in Stratum 12 at the City of David (De Groot and Bernick-Greenberg 2012:Fig. 4.9: 1–4), as well as in Burial Cave 5 at Tell Beit Mirsim (Ben-Arieh 2004:Fig. 2.81: 17–20).

Chronologically, the earliest vessel types in the assemblage are the bowl with a bar handle and knob, the spouted jug and the flat lamp. These are more typical of Iron IIA, but are also found in Iron IIB contexts. The other vessels uncovered are typical of Iron IIB and Iron IIC. The jug with wide neck and trefoil rim is more typical of Iron IIC, as are most of the decanters. The cylindrical jug has an exact parallel dated to Iron IIC. The black juglets are primarily common in burials, but are also found in settlement strata; they continue an earlier tradition, but in their present shape are typical of Iron IIB–C. The spouted jar is typical of the Iron IIB and is not found in later periods. The number of lamps with a high disk base, typical of the Iron IIC, is much larger than the other two

Fig. 8. Juglets (Photo: Sasha Flit, Courtesy of the Kiriath Yaerim Expedition)

types (Table.1). In light of these observations, the cave was presumably in use in the Iron IIB and the early Iron IIC, i.e., ca. 750–650 BCE.

SKELETAL REMAINS

A large amount of bones were piled on the cave's floor (Fig. 5). The bones were studied as a group, since it was impossible to separate them into individual interments during the excavation. Some of the bones were in a relatively good state of preservation, while others were badly damaged. Some teeth were also preserved. The skeletal remains were studied on-site. The bones were investigated in order to establish the Minimum Number of Individuals (MNI) buried in the excavated part of the cave, as well as for sex and age determination. The age-at-death of infants and children was estimated according to long-bone measurements (Ubelaker 1989), while adolescents were aged by epiphyseal closure stages (Johnston and Zimmer 1989). Adult age-at-death was estimated by tooth attrition rate (following Hillson 1993:176–201) and cranial suture closure stages (Hershkovitz et al. 1997). Sex of adult individuals was determined by morphology and measurements of the femur, tibia and humerus (Bass 1987), as well as by cranial and mandibular morphology (Bass 1987).

The high density of the bones in the cave and the disturbed burial position of most of the individuals indicate the presence of numerous individuals and a long period of usage, as reflected in the pottery assemblage. The bones represent a minimum of ten individuals (Table 2). Of these, six were adults—three males and three females—and four were children, including two babies, one infant, and an adolescent. The small sample did not allow for a comprehensive demographic analysis. Children were the most vulnerable in ancient communities, with a 40% mortality rate until the age of 10 (Coale and Demeny 1966; Ubelaker 1974; Paine and Boldsen 2002; Eshed et al. 2004), which is consistent with the remains found at Kiriath-Yearim.

Table 2: Summary of Individuals in the Cave, by Sex and Age

No.	Adult male	Adult female	Subadult	Age (years)
1	1			30< (35-45)
2		1		30<
3			1	10-17
4		1		19<30
5	1			15<
6		1		15<
7	1			19<
8			1	0-0.5

No.	Adult male	Adult female	Subadult	Age (years)
9			1	0.5-1.5
10			1	1.5-2.5
Summary	**3**	**3**	**4**	

DNA ANALYSIS

Two right petrous bones from different individuals, denoted herein as I14918 and I14919, were analyzed (Figs. 9–10). In order to verify the chronological attribution (i.e., dismiss the possibility of intrusive materials), a small amount of material from I14918 was sent for 14C dating. The obtained dates, calibrated using OxCal 4.4.2 (Bronk Ramsey 2009) and IntCal20 (Reimer et al. 2020), span the period between 739–409 calBCE (2430±20 BP, PSUAMS-8559). This fits well with the ceramic dating, and places the analyzed bones in the timeframe of the Iron IIB–C.

The petrous bones were processed in a dedicated ancient DNA cleanroom at Harvard Medical School. The powder was generated following a technique that uses a dental sandblaster to systematically locate, isolate and clean the cochlea (Pinhasi et al. 2019). The DNA was extracted following well-established protocols (Dabney et al. 2013; Korlević et al. 2015; Rohland et al. 2018). Libraries (one for each sample) were prepared in a dual-indexed single-stranded mode (Gansauge et al. 2017; Gansauge et al. 2020). In order to reduce the rate of characteristic ancient DNA damage, the libraries were treated with uracil-DNA glycosylase (UDG) (Rohland et al. 2015), originating from *E. coli* (USER enzyme from NEB). DNA libraries were sequenced on an Illumina instrument following in-solution enrichment for sequences overlapping the mitochondrial genome, and were bioinformatically processed as previously described (Fu et al. 2015; Mathieson et al. 2015; see also the custom processing pipeline at https://github.com/DreichLab/ADNA-Tools). Authenticity of the generated DNA data was satisfactory, assessed by examining C-to-T substitutions at the end of the sequenced fragments (Rohland et al. 2015) and mitochondrial DNA consensus sequence (Fu et al. 2013a).

mtDNA haplogroups were determined by aligning data (Li et al. 2009) of sufficient quality to the RSRS consensus genome (Behar et al. 2012). Following a construction of mitochondrial consensus sequences, haplogroups were determined using HaploGrep2 2.1.15 (Weissensteiner et al. 2016) and Phylotree 17 (https://www.phylotree.org). The match rate was estimated using contamMix 1.0-12 (Fu et al. 2013b).

In what follows, only information pertaining to matrilineal mtDNA haplogroup is provided. An investigation of data from the whole genome will be the subject of a

Fig. 9: Right petrous bone prior to sampling, Individual I14918 (Courtesy of the Kiriath Yaerim Expedition)

Fig. 10: Right petrous bone prior to sampling, Individual I14919 (Courtesy of the Kiriath Yaerim Expedition)

separate study as the analysis for this is complex and not yet complete. A summary of the results can be found in Table 3. The mtDNA coverage is good. The mtDNA haplogroups are different, reflecting unrelated maternal lineages.

Table 3: Summary of Basic Information for Individuals I14918 and I14919

ID	Skeletal element	Date (14C or pottery dictated)	mtDNA haplogroup	mtDNA coverage
I14918	Right petrous	739–409 calBCE (2430±20 BP, PSUAMS-8559)	T1a9	193.7
I14919	Right petrous	750–650 BCE	H87	156.2

The uniparental haplogroups can be compared with previously published individuals, including modern and ancient DNA, utilizing information from Allen Ancient DNA Resource (AADR) V50.0 (https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data). In the following, no low coverage or potentially contaminated individuals are mentioned; in cases where several family members were published, the sample with the highest number of covered SNPs is shown.

The mtDNA haplogroups are certainly of interest. As shown in Table 4, haplogroup T1a9, observed in Individual I14918, has close counterparts in the published ancient DNA literature. Indeed, its ancestral haplogroup T1a can be seen in the sixth–fifth millennia BCE in southeastern Europe. Individuals possessing a sister haplogroup T1a2 can be seen in ʻAin Ghazal in Jordan already during PPNB, ca. 8000 BCE. These two facts may indicate a rather ancient source of T1a, perhaps in Anatolia or the Levant. Later instances of sub-haplogroups of T1a include a Chalcolithic individual from Pki'in in Israel; A Bronze Age individual from Shahr-i Sokhta in Eastern Iran; and Middle Bronze Age Levantine individuals from Alalakh and Megiddo. After 1000 BCE, we can observe a larger geographic spread, including (apart from Kiriath-yearim) the Baltics, the Ural Mountains, the central Mediterranean and northern Europe. An exact match to T1a9 can only be detected in an individual (PCA0093) excavated in an late Iron Age Polish site attributed to the Goths (Stolarek 2019). However, the Goths in this period are certainly connected to Crimea, and thus with the Mediterranean Sea, so perhaps this fact is not as surprising as it seems.

Table 4: Comparison with previously published material: mtDNA haplogroups closest to T1a9 ~ (sub-haplogroups of T1a) – their oldest attestations, as well as all attestations of ancestral haplogroup T1a. Individuals with haplogroups T1a9 are marked in red.

Version ID	Publication	Mean BP*	Group ID (main analysis: no ending=1240k; SG=shotgun; mtDNA=mtDNA only)	mtDNA haplogroup
I1700_ published	Lazaridis et al. 2016	10,050	Jordan_'AinGhazal_PPNB	T1a2
I1415_ published	Lazaridis et al. 2016	9,827	Jordan_'AinGhazal_PPNB	T1a2
I2529_ published	Mathieson et al. 2018	7,610	Bulgaria_N	T1a
I1882	Lipson et al. 2017	7,050	Hungary_MN_LBK	T1a
I0447_ published	Lipson et al. 2017	6,700	Hungary_LN_Tisza	T1a
I3708	Mathieson et al. 2018	6,500	Greece_Peloponnese_N	T1a
I10274	Novak et al.2021	6,050	Croatia_C_Lasinja	T1a1
I1182	Harney et al.2018	5,950	Israel_Pki'in_C	T1a+152

Version ID	Publication	Mean BP*	Group ID (main analysis: no ending=1240k; SG=shotgun; mtDNA=mtDNA only)	mtDNA haplogroup
I11476	Narasimhan et al. 2019	4,600	Iran_BA1_ShahrISokhta	T1a3
I10264	Agranat-Tamir et al. 2020	3,750	Israel_Megiddo_MLBA	T1a
ALA026	Skourtanioti et al. 2020	3,627	Turkey_Alalakh_MLBA	T1a
I10097	Agranat-Tamir et al. 2020	3,500	Israel_Megiddo_MLBA	T1a
s19_X02_1.SG	Saag et al 2019	2,937	Estonia_BA.SG	T1a1b
MJ-42.SG	Järve et al. 2019	2,604	Russia_EasternScythian_SouthernUrals.SG	T1a1d
I14918	**This study**	**2,477**	**Israel_KJ_IA**	**T1a9**
R131.SG	Antonio et al. 2019	1,850	Italy_Imperial.SG	T1a12
PCA0093	**Stolarek 2019**	**1,700**	**Poland_IA_Gothic.mtDNA**	**T1a9**
VK147.SG	Margaryan et al. 2020	1,010	England_Viking.SG	T1a1q
VK435.SG	Margaryan et al. 2020	975	Sweden_Viking.SG	T1a5a
vik_nuf002.SG	Krzewińska et al. 2018	900	Sweden_Viking.SG	T1a1j
VK535.SG	Margaryan et al. 2020	700	Italy_Medieval.SG	T1a5

Date in BP in years before 1950 CE (OxCal mu for a direct radiocarbon date, and average of range for a contextual date).

To date, haplogroup H87, observed in Individual I14919, has no analogs in the published ancient DNA literature. However, the exact haplogroup was observed in modern populations of Basques (Young 2009), Tunisian Arabs (Elkamel et al. 2018) and Iraqis (Shneewer et al. 2015). This may provide an indication of a Mediterranean or Near Eastern, perhaps Arabian Peninsula source of the haplogroup. It should also be noted that Behar et al. (2012) date the appearance of H87 to 5678.6 BP. However, with a standard deviation (SD) of 5583, this estimate is highly non-specific.

SUMMARY

This short report supplies basic information regarding an extensively looted burial cave found close to the site of Kiriath-Yearim. The finds in the cave indicate that it was in use during the Iron IIB and early Iron IIC (ca. 750–650 BCE). At least 10 individuals were accounted for, although it is clear that this and similar caves would have been used for many more burials. Two individuals underwent genetics analysis. Our analysis concentrated on matrilineal (mtDNA haplogroups) markers. In comparison to other published material, the haplogroups appear to be of a broadly Near Eastern geographic span and possibly rather ancient origin. This may cautiously hint at local origins of the two individuals; the matrilineal markers might have been different in case of a major gene flow from, e.g., Egypt. mtDNA haplogroup T1a9 may have been present in the Near East in PPN, while H87 may have its roots in the Arabian Peninsula. Moreover, we can cautiously observe that their ancestral, cladal and sub-haplogroups continued to exist, mainly in the Near East and the Mediterranean for many millennia, with geographically proximate examples in sites such as ‘Ain Ghazal in PPNB, Chalcolithic Pki’in and Middle–Late Bronze Megiddo and Alalakh. These haplogroups persist through to the present day.

As noted above, this report did not explore the challenging wealth of whole genome data obtained from these individuals, which will be provided in a subsequent publication. The excellent quality of the data also suggests that non-cranial osteologic materials from other individuals mentioned above may yield beneficial paleogenomic data. Future attempts on these samples might be worthwhile despite the overall low rate of preservation of DNA in skeletal materials from ancient Israel.

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