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XRM imaging for non-destructive age at death estimation of the incinerated teeth from the Motya Tophet (Sicily, 6th century BC)

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***Abstract* – The study of odonto-skeletal remains of immature individuals provides important information on the cultural significance of childhood in past societies. The Tophets – i.e., distinct burial areas for infant cremated remains found in several Phoenician-Punic colonies in the Mediterranean area – offer an ideal case study to test the potential of alternative analysis approaches on cremated remains. This study presents a non-destructive methodology based on X-ray microscopy (XRM), aimed to analyze a sample of cremated deciduous teeth from the Tophet of Motya, providing information on the demographic profile and age-at-death classes of the buried individuals. The results are consistent with previous studies on remains from other Tophets and highlight the importance of using non-invasive techniques to collect and analyze data that are useful for the interpretation of burial practices reserved for newborns and infants in the Phoenician world.**

I. INTRODUCTION

In bioarchaeological research, the study of odonto-skeletal remains of immature individuals and the estimation of their age-at-death provide biocultural insights for understanding past human societies [1]. In particular, the reconstruction of infant and juvenile mortality rates, the observation of various mourning rituals and funeral practices specific for them, and in general, the treatment of their remains provide important insights on the cultural significance of the childhood period and the social relations between adults and children in different civilizations [2]. Various ethnographic and archaeological studies show that certain populations buried immature individuals in different places from the adults, grouping infant and juvenile burials in dedicated areas [3, 4].

Despite their controversial nature and debates over their exact function [5-7], the Tophets represent an ideal case study. Found in Phoenician-Punic cities around the Mediterranean area, Tophets are distinct burial areas, located in city marginal areas, intended for the deposition of fetal, newborn or infants, whose remains were cremated and placed in cinerary urns [8].

Due to the destructive nature of the burning process, anthropological analysis of cremated remains can often be challenging and usually provide limited results [9, 10]; however, the consequent fragmentation and frailty of these materials may provide an opportunity to test the potential of alternative data collection and analysis approaches.

A. Age-at-death estimation: the case of the tophet infants

The most accurate method for estimating age at death in immature individuals is based on the histological evaluation of dental development through growth markers, formed during the enamel matrix deposition of

deciduous teeth and the eruption of the first permanent molar [11, 12]. Concerning the Tophet infants, Schwartz and colleagues [13] have successfully performed dental histology and ascertained the actual survival for at least 10-15 days post-partum on a sample of 24 cremated teeth from the Tophet of Carthage, out of a total of 50 deciduous incisors [6, 13]. Despite these promising results, analyses with conventional histology involve a certain degree of sample destruction and obviously are not reproducible; moreover, in the case of cremated remains, the poor preservation of the dental crowns could complicate the cutting procedure as well as the correct orientation of the sections.

Although in recent years the use of synchrotron radiation microtomography imaging (SR-μCT), considered a structurally non-destructive technique, enabled high-resolution virtual histology [14-16], its application in routine and extensive analyses is undermined by the limited access to facilities and the expensive cost.

Imaging based on X-ray microscopy (XRM) could positively solve some of the problems mentioned above. Recently applied to a wide range of materials – such as cultural heritage [17, 18], batteries [19], and biological samples [20] – X-ray microscopy allows visualizing the internal architecture of an object, providing information on its microstructure with sub-micrometric spatial resolution. Like microtomographic techniques, XRM can extract a 3D model of the sample from its two-dimensional scans, which can be virtually handled without physical alteration [21-23].

In the present work, an alternative, viable and non-destructive methodology is applied, using XRM imaging technique to virtually reconstruct the volumes of cremated deciduous teeth from the Tophet of Motya Island (Sicily, Italy), estimating an age at death interval by regression equations to the linear measurements obtained on the virtual sections from the three-dimensional models.

B. Archaeological context: the Motya Tophet

Motya, in the center of the Marsala Lagoon (TP), on the western cusp of Sicily, was a Phoenician port city founded around 800 BC, for four centuries one of the most flourishing centers in the central Mediterranean. The archaeological explorations, begun by Joseph Whitaker and then conducted by Sapienza University of Rome in collaboration with the BB.CC.AA Superintendency of Trapani and the G. Whitaker Foundation from 1964 to today have, among other monuments, identified and excavated the Sanctuary called Tophet (Figure 1), dedicated in the Phoenician colony, as in other relatively few centers of the Mediterranean, to house the cremated remains of children who died in perinatal age and, not infrequently, also of small animals, in urns made of vases [24-26]. In the Phoenician world of the West and in North Africa, Sicily, and Sardinia, where the Tophets are documented, these specific sepulchral sanctuaries, which also include shrines

Fig. 1. View of the island of Motya from the North-West; in the foreground its Tophet. Copyright: Archaeological Expedition to Motya, Sapienza University of Rome.

and other installations linked to the funerary cult, including numerous carved steles with facades of temples that frame figures of divinities or sacred symbols, represent an identity and distinctive element of the West Phoenician communities [27]. In the case of Motya, the Tophet carefully excavated by Antonia Ciasca and re-explored by the still active mission of Sapienza, has returned more than two thousand urns and as many stelae. The study of the burnt remains preserved in the urns has for just over a century animated the debate on the nature and meaning of Tophets [6, 7], since some of the ancient sources have led scholars to believe that children were offered in ritual killings. The study of the human remains preserved in the urns, the development of new methods of analysis and, above all, greater precision regarding the age of the incinerated, could help to collect new data useful for the interpretation of what currently remains an incineration necropolis expressly dedicated to infants.

II. MATERIALS AND METHODS

A sample of 15 crowns of deciduous incisors, upper and lower regardless of the side, from five different cinerary urns (MT93.139/1579, MT93.29, MT93.143, MT93.153, MT93.49) dated the second half of the 6th century BC, from the Tophet of Motya (Sicily, Italy) is selected for the present study (Table 1).

The urns were not sealed; the inner portions of the fill returned best-preserved remains while the bottom and surface layers contained the most fragmentary ones All material was passed through a stack of sieves with mesh sizes from 3 to 0.5 mm. The most important specimens were retrieved with precision cross-action tweezers characterized by low elastic force in order not to damage the fragile remains. The urns contained burnt human bones and teeth, in one case (MT93.49) also animals. Among the few bone fragments, in a poor state of preservation, portions of long bones, skull, ribs, hearing bones, vertebral arches and clavicles were recovered in each urn. MT93.143 urn only yielded pelvic girdle bones (right and left female ischium, right male ischium), however it was not possible to assign these remains to a particular individual or pair them with the teeth found. Only the dental remains were sufficiently intact, but due to their fragility it was in any case impractical to carry out accurate caliper measurements.

Table 1. Study sample. Ldi2 = lower lateral deciduous incisor, Udi1 = upper central deciduous incisor, Udi2 = upper lateral deciduous incisor.

Tooth type	ID sample	N tot
Ldi2	01, 03, 34, 35	4
Udi1	06, 12, 13, 32, 41	5
Udi2	04, 05, 11, 21, 31, 33	6

A. XRM acquisition and volumetric reconstruction

To acquire the scans in the least stressful way, a dedicated sample holder was designed, and 3D printed. Each sample was scanned in high resolution, using a Zeiss Xradia Versa 610 X-ray microscope operating in phase absorption, available at the Research Center on Nanotechnology Applied to Engineering (CNIS) which is part of the open Infrastructure for "Advanced Tomography and Microscopies (ATOM)" of Sapienza University of Rome. Scans were acquired with pixel sizes of 11 µm and 13 µm, exposure time of 1.5 seconds and 4x optical magnification. The three-dimensional reconstructions were imported into the image analysis software Dragonfly Pro 2020.1 (ORS, Canada) to extract enamel and dentine volumes by manual segmentation. A volumetric dataset and a 3D model, suitable for virtual handling and measurement, were then derived for each tooth under analysis.

B. Virtual sectioning and data processing

The reconstructed crown volume for each tooth was imported into Dragonfly Pro 2020.1 and rotated until the cervical plane was parallel to the horizontal XY plane of the Cartesian coordinate system. The enamel was sectioned by identifying the bucco-lingual plane, according to the protocol described by Benazzi and colleagues [28], to generate a two-dimensional image. The sections were then imported into ImageJ software (NIH, USA), where the crown length (CL, mm) was measured by drawing a line from the highest point of the enamel cap to the cervical plane (Figure 2).

The gestational age in years (GA) was calculated by applying the following regression equations, specific to unknown sex infant individuals of Mediterranean origin, from the crown length of the deciduous incisors [29]:

$$GA\ (Udi1) = 0.411 \times e^{0,120x} \quad (1)$$

$$GA\ (Udi2) = 0.462 \times e^{0,123x} \quad (2)$$

$$GA\ (Ldi2) = 0.492 \times e^{0,118x} \quad (3)$$

where e is Euler's number and x = CL. Following the values reported by Irurita and colleagues [29], the lower and upper ranges are likewise calculated. Considering a gestational period of 40 weeks, i.e., 0.75 years, the post-natal age (PNA) in years was obtained by subtracting this value from the gestational age.

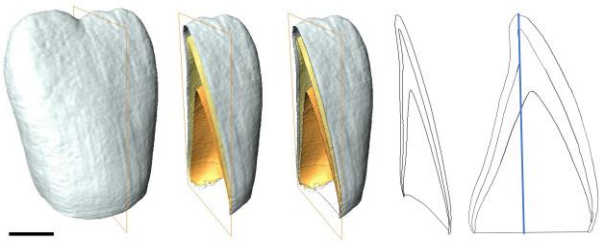

Fig. 2. Virtual sectioning procedure; blue line = CL. Scale bar = 1mm.

III. RESULTS

Crown length distribution (Figure 3) and demographic profile (Figure 4) for each tooth in the sample from the Motya Tophet are shown. Although the equations provide three ranges, the lowest values are assumed to be non-significant for age at death assessment in this specific sample, since due to the cracking on the enamel surface, the resulting data are likely to be underestimated. Therefore, in specimens 01, 06, 13, 04, 05, 11, 21, 31 and 33 the mean values, expressed in weeks after birth, are considered; for the others upper ones are taken, as 3D models proved that their crowns are damaged at the cervical line. The observed size ranges in Figure 3 did not differ significantly between central (2.98-4.59 mm), lateral maxillary incisors (3.18-5.02 mm) and lateral mandibular incisors (3.01-4.76 mm), suggesting a similar age-at-death estimate for all investigated individuals.

In Table 2, the resulting data are grouped according to the pre- and post-natal life stages reported in Scheuer and Black [30]. The demographic profile shows that most incisors (N = 8) belonged to individuals presumably born or stillborn during the 37th or 38th weeks of gestation. Therefore, according to currently accepted definitions [30], these samples are classified as perinatal (from 37-42 weeks gestation). Crown lengths of samples identified as infant (N = 3), i.e., belonging to individuals who lived more than 4 weeks of extra-uterine life, estimate an age at death of maximum 6 weeks post-partum. Similarly, samples (N = 3) indicated as neonates were probably born at the end of 40 gestational weeks and survived for about 20 days. A single sample (ID = 32), whose PNA is negative in every estimated range, is classified as either preterm (less than 37 weeks of life in utero) as well as spontaneously aborted or stillborn during the 34th gestational week.

Table 2. Distribution of age at death. Preterm (<37 weeks), perinatal (around birth), neonate (first 4 weeks after birth), infant (birth to end of 1st year of life).

	N tot	ID sample
Preterm	1	32
Perinatal	8	34, 06, 12, 13, 04, 05, 31, 33
Neonate	3	03, 35, 41
Infant	3	01, 11, 21

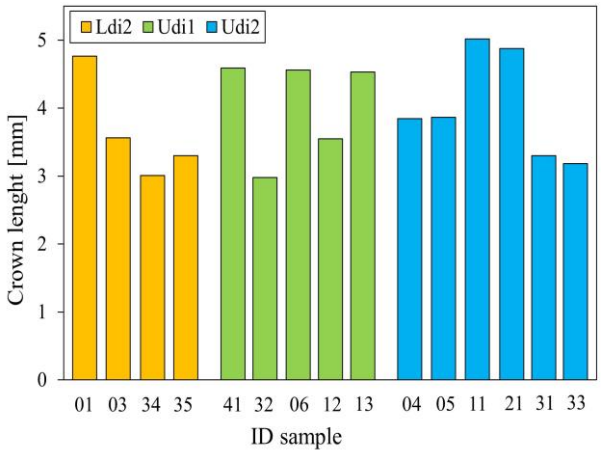

Fig. 3. Crown length distribution by tooth type. Ldi2 = lower lateral deciduous incisor, Udi1 = upper central deciduous incisor, Udi2 = upper lateral deciduous incisor.

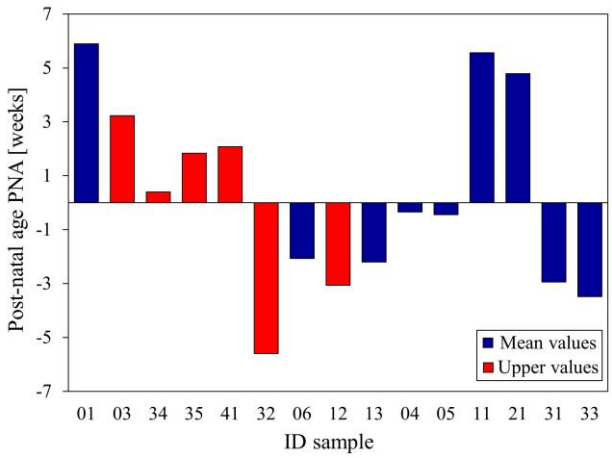

Fig. 4. Demographic profile according to Irurita and colleagues [29] method expressed in weeks after birth. The zero post-natal age PNA value corresponds to birth. Negative values refer to pre-partum weeks.

IV. DISCUSSION

A. Regression equations for Motya Tophet infants

Odontometric methods for estimating age at death from fetal period to the first year of life [reviewed in 31] are generally derived from the assessment of dental developmental stages and the correlation of age with a set of teeth measurements, from which several regression equations have been developed [11, 29, 31, 32]. Many studies state that different populations may vary in dental developmental rates negatively affecting these techniques, recommending that only specific methods designed or tested in similar study populations should be used [e.g., 3, 6, 28, 32, 34]. Following this guideline, the equations proposed by Irurita and colleagues [29] are the ones that best fit the infant sample from Motya, as they are developed from a sample between 5 months of gestation and 6 years from the Granada osteological collection (Spain, 20th century) [35], specific to each tooth type and allow the estimation of the age of fetal and newborn individuals of Mediterranean origin, even if their sex is unknown. However, a study by Petrone and colleagues [36] states that these equations could underestimate chronological age by approximately 3 weeks if applied to a sample that includes preterm individuals, which could show a discrepancy between dental and chronological age. Considering the diversity of the sample tested and making a correction to the age intervals as indicated, it still results that most of the specimens fall into the neonatal category (N = 7).

B. Bioarchaeological perspective on Tophets fuction

Historiological and archaeological evidence-based interpretations of Tophet function, such as Greco-Roman accounts, votive images, tomb markings and the presence of animal remains commingled with human ones, describe these cemeteries as dedicated areas for the burial of sacrificial victims, identified as individuals in their early childhood, whose bodies were cremated and placed in urns [5, 37].

In response to this hypothesis, human remains preserved in more than 800 urns, found in Tophets of the Phoenician colonies of Hadrumentum and Carthage in Tunisia, Sulci in Sardinia and Motya island, have been analyzed applying standard multifactorial protocols for anthropological and forensic analyses [ref. in 6]. Based on the developmental stage of teeth and bones, and on the measurement of certain discriminating skeletal districts for age at death estimation, these studies found that the predominant part of the sample considered was composed of preterm, peri- and neonatal age individuals [rev. in 6] which, in any case, had not survived the two-week post-partum lifespan. Considering the small number of specimens, the distribution of age at death in the present study (Figure 4) agrees with these conclusions, as most of the individuals investigated fall into the perinatal category, thus presumably born at term (37th-40th week of gestation) and deceased or stillborn. Furthermore, the crown lengths measured on the virtual sections fall within the size ranges identified for most of the 1st and 2nd deciduous incisors reported in Smith and colleagues [37] from the inhumates in the Carthage Tophet.

C. Brief notes on tooth-crown shrinkage

One of the discussion topics in the Tophet cremated remains study concerns their possible alteration in morphology and structure caused by the burning process [6, 7, 34, 37]. Bone and teeth react differently to heat, and as dental enamel contains significantly less organic matter than bone, it is less prone to deformation, which would not visibly affect the overall morphology or state of crown formation [34]. In the specific case of the crowns of developing and unerupted teeth – as in perinatal and newborn individuals from the Motya Tophet – the surrounding bone protects the tissue from the direct effects of heat, which causes relatively little cracking compared to that observed in the crowns of erupted teeth

[6]. Therefore, even though the study sample was fragile and showed enamel and cervical line cracking in six specimens, the measurements obtained are regarded as valid; having also considered the general state of crown preservation when choosing values for age at death estimates.

V. CONCLUSIONS

This study represents the first attempt to determine the age at death on a sample of cremated deciduous teeth from the Motya Tophet using a non-destructive method applying specific regression equations for individuals from the Mediterranean area. The use of XRM allowed the virtual analysis of a sample of fragile and precariously preserved cremated infant teeth, enabling the measurement of the crowns with a completely non-destructive method. The application of advanced, non-invasive, and easily accessible analytical techniques demonstrates to be of primary importance not only for preserving remains, whatever the reasons, but also in cases where it is difficult to use measuring instruments.

In agreement with previous studies regarding these burial sites, the presence of remains belonging to individuals deceased in prenatal and perinatal ages agrees with the interpretation that considers Tophets as dedicated necropolis for premature and newborn infants who, regardless of the cause of death, always represent the most fragile segment of the population, contributing to the understanding of funerary practices dedicated to the infant and juvenile portion of the Phoenician-Punic populations of the Mediterranean.

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