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Morphological Changes in Dental Surfaces Suggest Health Status and Alimentary Habits in the Subjects Belonging to the Copper Age in Sardinia Island (III Millennium BC)

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Abstract: The aim of this multidisciplinary study, which involved a team of archaeologists, odontologists, biologists, is to examine a set of dental health indicators, including caries, tooth wear, and enamel hypoplasia of a III millennium BC sample from the burial site of *Scaba 'e Arriu* (Siddi, South Sardinia), in order to evaluate the dental health *status* and the diet. Our purpose is to depict a timeline of dental health in Sardinia from prehistory to the present day, starting with a focus on a Copper Age population. Caries, dental wear, and enamel hypoplasia of 259 permanent teeth were evaluated. It was not possible to assign sex and age of each tooth because of the lack of bony support, lost due to taphonomy factors. 14,7% of *Scaba'e Arriu*'s samples were affected by carious lesions, with greater involvement of the posterior teeth (12,7% against 1,9% of anterior ones). Interproximal caries was the most frequent. Dental wear affected 77,8% of the teeth, and its presence is preponderant over caries' rate. The high percentage of dental wear suggests a diet based on abrasive foods.
Further studies are necessary to delineate more accurately the *modus vivendi*, the nutrition, and, consequently, the health status of this sample.

Keywords: dental caries; copper age; dental wear; enamel hypoplasia; odontology; paleopathology; Sardinia.

1. Introduction

Teeth are excellent indicators of an individual's health, diet, and lifestyle. For this reason, in recent years, the study of teeth has been spreading within the historical-archaeological disciplines [1-6]. Teeth are often the only human material preserved unaltered in archaeological contexts since their structure is environmentally resistant and not prone to dramatic taphonomic changes. The examination of ancient teeth allows highlighting various pathological conditions [7] and diseases of variable etiology, including inflammatory odontogenic lesions [8] and benign [9,10] or malignant [11,12] neoplasms. Furthermore, since there were no dental therapies in the past, the assessment of pathologies of the orofacial district

reflects the real condition of the studied population. The international scientific literature on dental paleopathology has until now focused on the reconstruction of oral health from osteoarchaeological samples starting from the presence of caries, inflammatory odontogenic lesions, periodontal diseases, and dental wear [13]. Other studies have applied imaging diagnostics more commonly used in health check [14] to investigate pathologies that cannot be otherwise found on a sample or morphometric features in dental evolution [15]. Another object of study is represented by past dietary habits, for which are analyzed common lesions on tooth's supporting tissues, enamel and dentine [16], and isotopic [17-20], molecular [21], and palaeobotanical data [18,22]. Also, researches have been performed on tooth's macro and micro-wear [23][24] and on the dental calculus [25]. It has, indeed, recently become an important source for ancient DNA studies with various purposes, i.e., depicting the evolution of the oral microbiota [26], getting more details on the human genome [27], investigating early eating habits [28], detecting and characterizing pathogenic microorganisms that have caused historical epidemics [29], reconstructing the demographic history of ancient populations [30].

Studies on dental findings in the Italian scientific literature are mostly part of broader research that focuses on whole skeletons or mummies [31]. And the same picture can be observed in Sardinia. Except for a few but significant research [32, 33], specific multidisciplinary and diachronic studies on archaeological teeth are currently unknown.

Our samples' peculiar feature is the great genetic uniformity based on autosomal and uniparental markers and on the increasing linkage disequilibrium (LD) [34]. Since Sardinia was included in the "Human Genome Diversity" project as a reference subject in Europe, our purpose is to analyze and carry out a comparative epidemiological analysis between widely chronologically distant Sardinian samples. Nevertheless, in this paper, we present a preliminary descriptive epidemiological analysis on *Scaba 'e Arriu* samples' dental health, waiting for other historical Sardinian samples to be compared, and with the will to provide data for further researches.

We developed our research using a multidisciplinary approach, in which the skills of the odontologist, the archaeologist, the paleopathologist, and the biologists converge.

2. Materials and Methods

Samples come from the collective burial of *Scaba 'e Arriu* (Siddi, South Sardinia province, (Figure 1.A), which architecture (Figure 1.B) is known as *domu de janas* (house of fairies) in Sardinian language, and date between Late Neolithic and Copper Age. The tomb was built in Late Neolithic period (4000-3300 BC) and reused in the later Copper Age by people of *Filigosa-Abealzu*'s archaeological *facies* (3300-2700 BC, phase A) and Monte Claro's (2700-2200 BC, phase B) culture. Analyzed samples belong to phase A. Archaeological teeth were found devoid of their anatomical support because the burial was repurposed, resulting in bone deterioration. Moreover, no skeletons with anatomical connections were found. Therefore, it wasn't possible to assign sex nor a specific range of age to every sample. We recommend already published work by Usai *et al*., 2008 [35] for other archaeological details.

The samples are currently stored in the osteological laboratory of Villanovaforru's Archaeological Museum. For our study, we selected 259 permanent teeth classified, as shown in Table 1.

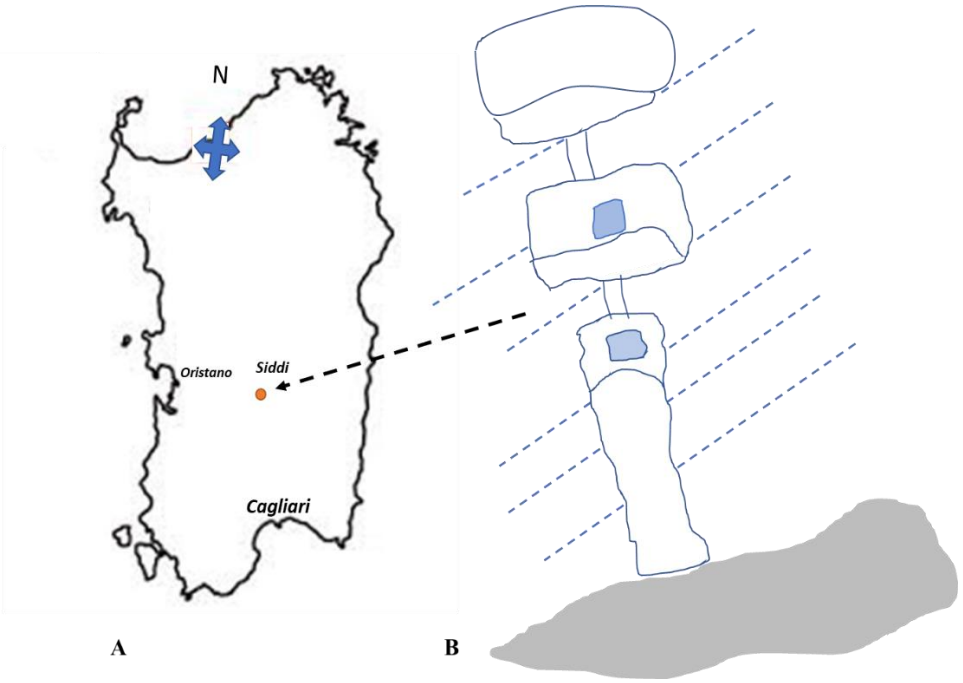

Figure 1. A) Geographical position and B) detail with axonometry of *Scaba 'e Arriu*'s tomb (image of axonometry from [35]). Graphic editing by Carlo Murtas.

Table 1. Tooth type distribution of *Scaba 'e Arriu*'s sample (phase A), based on lower and upper teeth.

Tooth type	Lower arch	Upper arch
Incisors	20	20
Canines	17	23
First premolars	20	14
Second premolars	20	15
First molars	17	21
Second molars	36	19
Third molars	8	9

We considered teeth that were not altered by external factors, and they were examined using clinical procedures. Dental analyzes were performed with artificial lighting, using the following personal protective equipment to avoid any type of contamination: sterile latex gloves, surgical masks, protective glasses, and disposable gowns. Due to the lack of anatomical connections, the whole analysis was unfortunately run without any subdivision by sex and/or age.

Caries, dental wear, and enamel hypoplasia were evaluated. The presence of caries was assessed by naked eyes and subsequently using a dental explorer Hu-Friedy EX23/66. The carious lesion was considered as a clear loss of dental tissue or cavitation and recorded based on four scoring criteria: 1 superficial (caries in enamel), 2 moderate (caries in dentin and enamel undermined), 3 severe (pulp involvement), 4 destructive (death of pulp and possible periapical infection). Caries's severity was measured by using Hu-Friedy PCPUNC 15 periodontal probe.

Dental wear's severity was assessed by naked eyes and using Smith's qualitative scale [36] (developed from the study of hunter-gatherers and farmers populations), which assign a point scale to each tooth, from 0 (the stage of an unworn tooth) to 8 (the stage of a severely compromised tooth), based on the amount of exposed enamel and dentin. We divided the scale as follows: low severity (scores 1-2) from a polished crown or small facets to moderate cusp removal, moderate severity (scores 3-5) from full cusp removal, and lines of visible dentin to

large areas of dentinal exposure, high severity (scores 6-8) from coalesced areas of dentinal exposure to severe or complete loss of the crown.

Enamel hypoplasia was recorded as a furrowed defect of enamel on the tooth's crown, which is the most common type. Nevertheless, the defect could also appear like a pit or a plane [37]. For each tooth, we calculated the number of hypoplastic grooves. To record the linear defects and their distances from the cementoenamel junction, we used a digital caliper and a magnifying glass 3-4x. However, this method requires to be strengthened with a scanning electron microscope analysis [38] and histological studies [39]. In this study, we only present preliminary data about global frequencies of affected teeth without focusing on each hypoplastic event's age range.

Statistical computations were run by using the Chi-Square test concerning the frequencies of caries and dental wear. The result was considered significant if the p-value was lower than 0.05. Tests were run on the open-access website *Social Science Statistics* (https://www.socscistatistics.com/).

3. Results and Discussion

14,7% of *Scaba 'e Arriu*'s sample was affected by carious lesions, especially in the posterior and inferior areas (Table 2), and the interproximal region (Figure 2) is the most evolved one (Table 3).

Table 2. Frequencies of carious teeth of the *Scaba 'e Arriu*'s sample (phase A), based on inferior, superior, anterior, and posterior dental arches and total amount affected teeth. The number of observable teeth is 259, while the number of affected teeth is 38.

Dental arches	N° of involved teeth	Percentages of carious teeth
Inferior	23	8,9%
Superior	15	5,8%
Anterior	5	1,9%
Posterior	35	12,7%
Total amount of carious teeth	38	14,7%

Table 3. Position of carious lesions of the *Scaba 'e Arriu*'s sample (phase A), calculated on the number of observed lesions, which is 45. The number of carious teeth doesn't equal the number of affected teeth, of which some have multiple lesions.

Position of carious lesions	N° of involved teeth	Percentages of carious lesions
Occlusal	7	15,6
Interproximal	26	57,8
Lingual	4	8,9
Buccal	2	4,4
Neck	4	8,9
Root	1	2,2
Interproximal and incisal angle	1	2,2

Figure 2. Interproximal caries on an upper second molar.

The severity of the carious lesions is mostly moderate (Table 4).

Table 4. Severity of carious lesions of the *Scaba 'e Arriu*'s sample (phase A), calculated on the number of observed lesions, which is 45. The number of carious teeth doesn't equal the number of affected teeth, of which some have multiple lesions.

Severity of carious lesions	N° of carious lesions	Percentages of carious lesions
Superficial	15	33,3
Moderate	23	51,1
Severe	7	15,6
Destructive	0	0

Among 256 observable teeth, 201 (77,8%) showed dental wear (Figure 3). All dental wear stages have been observed, but scores 6 and 7 have a low frequency, while stage 8 is absent (Table 5). Moderate severity is the most frequent class (39,9% of total), but low severity almost equals it (36,7%).

Table 5. Severity of dental wear of the *Scaba 'e Arriu*'s sample (phase A), calculated on the number of observable teeth, which is 256.

Dental wear severity	Stage	N	%
Healthy teeth	**0**	55	21,4
Low severity (from polished crown or small facets to moderate cusp removal)	**1**	42	16,4
	2	52	20,3
Moderate severity (from full cusp removal and lines of visible dentin to large areas of dentinal exposure)	**3**	45	17,6
	4	26	10,2
	5	31	12,1
High severity (from coalesced areas of dentinal exposure to severe or complete loss of crown)	**6**	2	0,8
	7	3	1,2
	8	0	0

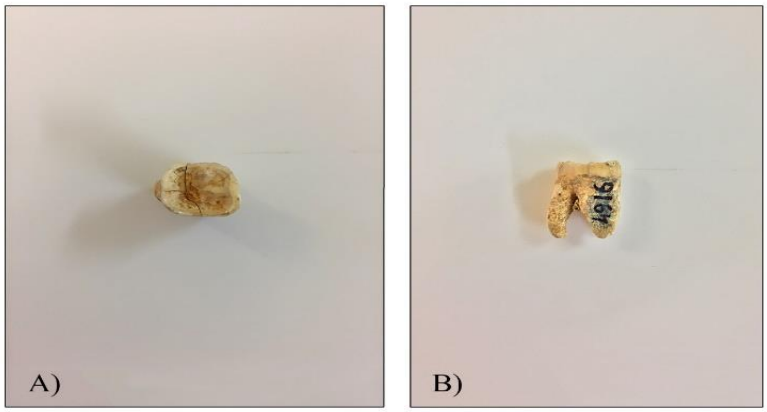

Figure 3. Occlusal dental wear with moderate severity (cusp removal and large dentinal exposure areas) on a second upper molar. A) occlusal view, B) interproximal view.

Table 6 compares the rates of caries and wear among anterior/posterior and maxillary/mandibular teeth: in each comparison, the dental wear score is always preponderant over caries' one.

Table 6. Comparison among values of dental wear and caries of the *Scaba 'e Arriu*'s sample (phase A).

	Inferior teeth		Superior teeth		Anterior teeth		Posterior teeth	
	N°	%	N°	%	N°	%	N°	%
Dental wear	110	42,9	88	34,3	59	23	139	54,3
Dental caries	23	8,9	15	5,7	5	1,93	33	12,7

11,7% of teeth were affected by enamel hypoplasia (Table 7). The furrowed type was visible only.

Table 7. Global frequencies of enamel hypoplasia of the *Scaba 'e Arriu*'s sample (phase A). We only were able to report the furrowed or linear type.

N° of hypoplastic events	N° of observable teeth	N° of affected teeth	Percentage of affected teeth
51	249	30	12%

Dental elements are more affected by wear than caries; thus, it is possible that *Scaba 'e Arriu*'s samples were involved in a more traumatic process, other than a cariogenic one, due to food texture was probably tougher and contained coarse particles, making chewing heavy. Therefore, dental wear is probably connected not only with the chewing but also with the food's consistency, while caries are mostly related to the food's quality. It should be stressed that "wear" is an umbrella term for several phenomena, which includes several types of lesions, and that we weren't able to perform a microscopic observation of the patterns, which would have been a more accurate analysis [40]. Tooth wear has a multifactorial etiology, and it usually occurs with two or more combined mechanisms. It includes attrition (caused by the contact between occlusal or incisal surfaces), abrasion (caused by the contact between tooth's surface and a foreign object), and erosion (a caused by acid and chelating agents, which promote the loss of hard tissue and accelerate attrition or abrasion processes), but more specific mechanisms, patterns, and causes can be found [41]. Tooth wear could be due to extra-chewing factors such as bruxism, GERD (gastroesophageal reflux disease), eating disorders, the use of teeth as tools, etc. [42]. In the past, there was probably more abrasion and/or attrition than erosion. The latter seems to be specific to the modern age: recent studies recorded a high presence of erosive wear, especially in children and adolescents, and that occurrence of erosion is increasing. The reason could be found in acid-rich foods and drinks, which were less consumed in the past. Erosive wear is also occupationally related: airborne acid reached the teeth of industrial workers. Indeed, a clear change of oral health direction has occurred between past and present, the latter being an actual major public health problem [43].

Mandibular teeth appear to be more markedly affected by caries than the maxillary ones. Therefore, this may be due to a little adequate cleaning system, while in the superior dental arch, we have the washing action of the parotid gland, which carries out an anti-cariogenic function through the production of saliva. Moreover, the food tends to stand on the mandibular area because of gravity: without a correct cleaning system, this only promotes caries' formation.

The prevalence of interproximal caries may be due to lack of a "dental floss" and, consequently, to the accumulation of food residues and a bacterial film's development. Moreover, the abrasive function of food may have avoided the occurrence of caries on occlusal surfaces, slowing and eventually halting the cariogenic demineralization process.

The main problem of our study is that we haven't a full picture of our sample yet: we couldn't assess the age and sex of every sample, so we don't know how much these factors may or may not have influenced the development of the dental pattern that we observed.

An interesting comparison can be made between *Scaba 'e Arriu's* samples with the ones from another Sardinian burial site, called *Mont'e Prama*, which dates to the Iron Age (1100-800 B.C.) [33]. In the latter, the severity of dental wear was not high, even in the older individuals, and the isotopic analysis showed that the population has fed on cooked and softened foods, poor in-ground cereals, and rich in animal proteins. Also, 4,8% of the teeth' total showed caries, which was generally small and occlusal: that is, a completely different and opposite pattern compared to our samples. Thanks to the isotopic investigations carried out on

Scaba 'e Arriu's bone collagen [44], it was found that the diet was mainly based on the consumption of vegetable products such as cereals and legumes and, scarcely, of animal proteins (swine). These data, combined with inadequate oral hygiene, justify the percentage of carious lesions (14,7%), which seems to be high for the prehistoric world [13, 33].

The greater presence of tooth wear in the *Scaba 'e Arriu*'s sample is hypothetically due to a diet rich in more abrasive foods, but also the grinding process of cereals using millstones, which release abrasive residues in food as confirmed by other bio-archaeological studies [45].

Finally, enamel hypoplasia is a condition frequently found in ancient populations [46], as they were more exposed to nutritional (such as malnutrition, avitaminosis) and infectious (prolonged infections, intestinal parasitosis) stresses. *Scaba 'e Arriu*'s hypoplasia frequency is not high compared to those of other ancient populations. Therefore, it's hypothesized a diet rich in vitamins.

The tomb of *Scaba 'e Arriu* is quite interesting because two communities from two different times choose to set up a home for their dead in the same place. Indeed, it would be interesting to compare the oral status of phases A and B, with the aim to find out any differences among caries', dental wear's, and enamel hypoplasia's rates. The aforementioned isotopic study on bone collagen was run on bones from each phase. It showed an increase in animal protein consumption from phase A to phase B. That comparison would be important not only in the archaeological field, concerning any cultural or sociological reason behind nutritional choices but also in the biological and pathological branches that focus on nutritional mechanisms and consequences.

4. Conclusions

The oral health pattern study showed that *Scaba 'e Arriu*'s samples had a high percentage of dental wear, that's preponderant over caries' one. This could indicate both a diet based on abrasive foods and production tools, such as an ancient millstone, which releases mineral residues on food during the grinding process. Indeed, nutrition may have played a decisive role in outlining this picture. More detailed research on dental wear's patterns could tell us more about food choices and food processing tools.

In a previous study [47], we compared our Copper Age cohort with present-day teeth, showing a dramatic difference between the two samples: modern samples showed a significantly higher incidence of caries and a lower one of dental wear, each one related to a diet rich in processed carbohydrates and soft food. Furthermore, the absence of linear enamel hypoplasia in present-day teeth highlights the absence of nutritional or infectious stress during the period of dental ontogenesis. Nevertheless, these are preliminary studies: for instance, it would be interesting to compare levels of the *Streptococcus mutans*, which is an important etiologic agent in dental caries, among samples of *Scaba'e Arriu*'s phases A and B, of *Mont'e Prama,* and the modern age, in order to verify the decreasing tendency of caries' frequency observed from our studies. Further multidisciplinary researches, which should especially converge archaeological, microscopic, and biomolecular elements, will give us more specific data.

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Conflicts of Interest

The authors declare no conflict of interest.

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