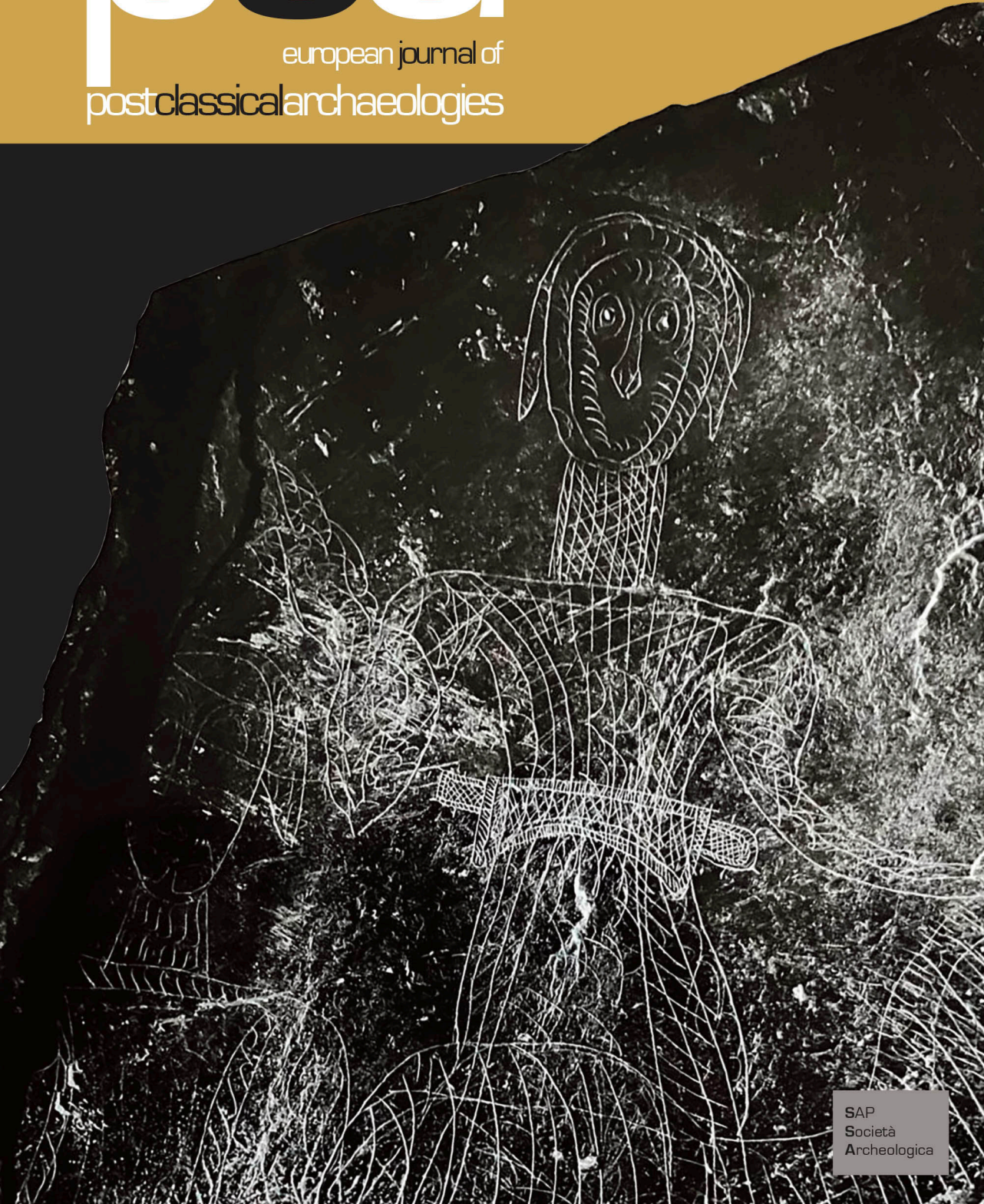


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# Dental calculus, extramasticatory tooth wear, and chronic maxillary sinusitis in individuals from San Genesio (6<sup>th</sup>-7<sup>th</sup> centuries CE), Tuscany, Italy

## 1. Introduction

The oral cavity is central to reconstructing disease, diet, activity, and environment in archaeological human remains. Teeth and adjacent structures preserve evidence of everyday subsistence, non-masticatory activities, and chronic disease. When analysed through a biocultural framework, teeth can be used to reconstruct both cultural practices and biological responses. Recently, Radini and Nikita (2023) emphasised the need to contextualise dental calculus findings with other bioarchaeological and palaeopathological data by combining microscopic and macroscopic analyses. Building on this biocultural perspective, examining multiple oral and craniofacial indicators can provide a more nuanced understanding of past human behaviours and environments. Dental calculus reflects both dietary practices and oral ecology (González-Rabanal *et al.* 2022; Radini *et al.* 2017), extramasticatory dental wear preserves habitual behaviours (Lozano *et al.* 2017; Molnar 2008, 2011; Monaco *et al.* 2022; Sperduti *et al.* 2018; Willman 2016), and chronic maxillary sinusitis indicates chronic respiratory and dental diseases (Lee *et al.* 2024; Lin *et al.* 2024; Slavin *et al.* 2005). The combined analysis of extramasticatory dental wear and dental calculus has been undertaken by some researchers (Nava *et al.* 2021; Sperduti *et al.* 2018); however, their relationship with chronic maxillary sinusitis is a relatively new line of research, with only one case study conducted (MacKenzie *et al.* 2021, p. 122).

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### 1.1. Dental calculus

Dental calculus refers to deposits of mineralised dental plaque that adhere to the surface of the teeth and accumulate over time (Lieverse 1999, p. 220; MacKenzie *et al.* 2021, p. 115). Dental plaque comprises a complex group of microbiomes that can mineralise after two weeks (Lieverse 1999, p. 220; Radini, Nikita 2023, p. 4). Dental calculus can host remnants of anything that has passed through the mouth through inhalation or ingestion, including diet, oral hygiene practices, the environment, and the use of teeth as tools (Charlier *et al.* 2010; Radini *et al.* 2017, p. 71; Radini, Nikita 2023, p. 10). During the process of mineralisation, molecules and particulate matter become embedded within the mineralised matrix (MacKenzie *et al.* 2021, p. 116). Microdebris entrapped in dental calculus can include plant remains, bast fibres, phytoliths, diatoms, starch granules, cereal chaff, pollen grains, pollen, animal hairs, charcoal, fossilised bacteria, pigment particles, and fungal spores (Radini *et al.* 2017, pp. 73–74, 2019; Radini, Nikita 2023, p. 11). In addition, particulate matter, which is an airborne pollutant such as dust or smoke that ranges between 1–100 µm, can also be found in dental calculus (MacKenzie *et al.* 2021, p. 122; Radini *et al.* 2017, p. 76).

### 1.2. Dental wear

Dental wear occurs due to attrition, abrasion, and erosion. Repeated mastication causes pressure between opposing teeth and abrasive particles that are present in the mouth, leading to a loss of enamel and eventually dentine (Hillson 2005, p. 219). Particles may be introduced into the mouth through food, occupation, and the environment (e.g., soil and atmospheric dust) (Hillson 2005, p. 219). Tooth-on-tooth contact by neighbouring and opposing teeth causes dental attrition, leading to the development of wear facets on the occlusal surface and contact sites between teeth (Hillson 1996, p. 231). Occlusal attrition is primarily caused by mastication and is therefore influenced by diet; however, tapping, bruxism, or grinding of teeth, as well as variations in enamel thickness in different teeth, can also be contributing factors (Alt, Pichler 1998, p. 387; Hillson 1996, pp. 238, 242, 2005, p. 214). Dental abrasion is a loss of surface detail without distinct wear facets that is not caused by tooth-on-tooth contact (Hillson 1996, p. 231). Dental abrasion is a plastic deformation in the form of scratches caused by contact with foreign objects and hard particles in the mouth, such as oral hygiene tools (toothbrush, toothpaste), smoking, jewellery, and blades. (Hillson 1996, pp. 231, 250). Hard particles in fluid, such as acidic foods, cause dental erosion, which is a chemical etching that dissolves the mineral content of teeth and creates plastic deformation, indentation, and fracturing (Arnadottir *et al.* 2010; Hillson 1996, p. 250).

In addition to dental attrition, abrasion, and erosion, dental wear can also result from both intentional or unintentional processes, such as the intentional mod-

ification or mutilation of the teeth (e.g., social identity and cultural practices) (Burnett *et al.* 2023; Smith-Guzmán *et al.* 2020), unintentional modification from habitual activities (e.g., pipe smoking), and the use of teeth as tools, the so-called ‘third hand’ (Alt, Pichler 1998, pp. 388, 394; Hillson 2005, p. 215; Milner, Larsen 1991). Activities including the processing of food (e.g., shredding, stripping and peeling) (Irish, Turner 1987, 1997; Tanga *et al.* 2016; Watson, Haas 2017), sinew stripping (Brown, Molnar 1990), crafts (Bonfiglioli *et al.* 2004; Monaco *et al.* 2022; Tanga *et al.* 2016), leatherworking (Lous 1970; Monaco *et al.* 2022), plant fibre processing and basket making (Díaz-Navarro *et al.* 2023; Fidalgo *et al.* 2020; Larsen 1985; Lozano *et al.* 2017; Milner, Larsen 1991; Sperduti *et al.* 2018), wood shaping (Hylander 1973), occupations with high levels of exposure to dust pollution (e.g., quarrymen, mine workers) (Hickel 1989), and consistent probing such as the use of toothpicks for habitual, hygienic, and therapeutic reasons (Frayer, Russell 1987; Molnar 2008), have been found to cause inadvertent extramasticatory dental wear. However, caution is advised when interpreting behavioural activities from extramasticatory dental wear, whereby the incorporation of contextual archaeological evidence and other specialist analyses (e.g., dental calculus) is essential for developing robust inferences (Molnar 2010).

### *1.3. Chronic maxillary sinusitis*

Chronic respiratory diseases result from interactions between genotype and environment (Gibson *et al.* 2013, p. 30). In archaeological human skeletal remains, chronic respiratory diseases can be identified through chronic *otitis media* (Krenz-Niedbala, Łukasik 2017), periosteal reaction on the ribs (Davies-Barrett *et al.* 2021), and through chronic maxillary sinusitis, which occurs after approximately 12 weeks of inflammation of the maxillary sinuses (Cho *et al.* 2006, p. 404; Lee *et al.* 2024). Bacterial infections and viral diseases account for the majority of cases of acute sinusitis, and to a lesser extent, fungal infections (Dudde *et al.* 2023, p. 1379), whereas rhinogenic and odontogenic causes can result in chronic maxillary sinusitis (Hershberger *et al.* 2024, p. 345). The causes of rhinogenic chronic maxillary sinusitis include, but are not limited to, viral upper respiratory tract infections and consequent bacterial infections, infectious diseases, fungi, anatomical variations, asthma, and long-term exposure to environmental conditions (Raz *et al.* 2015, p. 569; Roberts 2007, p. 795; Slavin *et al.* 2005, pp. S16, S25, S35). Odontogenic chronic maxillary sinusitis occurs secondary to dental lesions (Lin *et al.* 2024, p. 5). The risk factors for chronic maxillary sinusitis include diet and nutrition, occupational activities, health events during childhood, genetics, and the environment (natural, outdoor, and indoor) (Gibson *et al.* 2013). The primary environmental agent of chronic respiratory diseases, including chronic maxillary sinusitis, is air pollution due to natural (dust, pollen, brushfires, sea spray, and vegetation) and anthropogenic (smoke, fuel



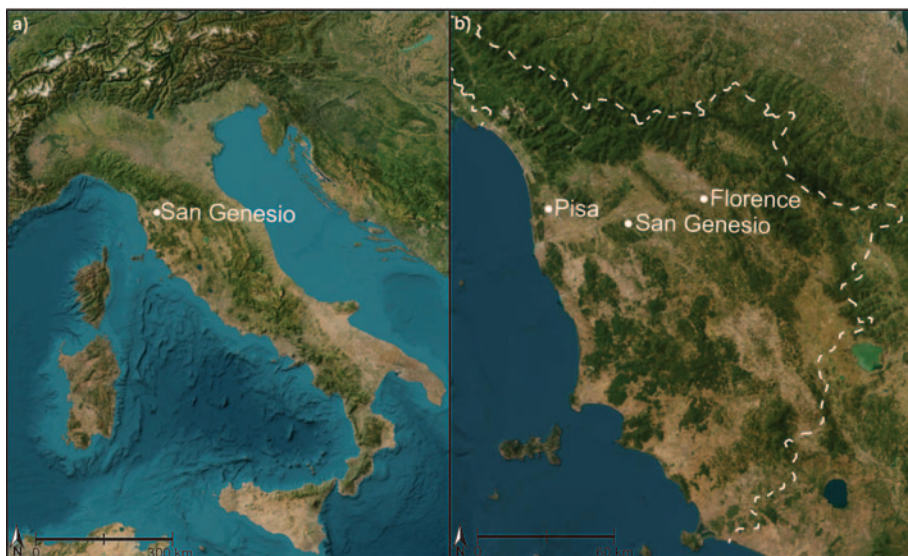


Fig. 1. a) Map of Italy indicating the location of San Genesio; (b) Map of Tuscany indicating the location of San Genesio in relation to Pisa and Florence (Basemap 'World Imagery' sourced from Esri).

fumes, industrial activities, agriculture, and biomass combustion) sources because fine particulate matter ( $<2.5 \mu\text{m}$ ) can be inhaled deep into the respiratory tract (Daellenbach *et al.* 2020, p. 414; Kim *et al.* 2015, p. 137; Lakey *et al.* 2016, p. 1; Park *et al.* 2021, p. 2).

#### 1.4. Archaeological background: vicus Wallari-borgo San Genesio

Located near San Miniato (Pisa), Tuscany, central Italy, *Vicus Wallari-borgo* San Genesio is a rural archaeological site situated along the *Via Francigena* between Pisa and Florence (fig. 1) (Cantini 2010, p. 81). The site was occupied from the 3<sup>rd</sup> century BCE, and by the 6<sup>th</sup> century CE, it had taken on a military function, likely due to the Gothic Wars (Cantini *et al.* 2017, p. 251). A large necropolis was established at the site in the middle of the 6<sup>th</sup> century CE (Cantini *et al.* 2017, p. 251). From the second half of the 6<sup>th</sup> century CE, the necropolis probably served local inhabitants, as well as people who died on the road between Pisa and Florence during the Gothic Wars and the Lombard conquest, which may have included members of the armies (Cantini *et al.* 2017, pp. 251-252). The necropolis was in use until the 13<sup>th</sup> century CE, hosting over 400 inhumations (Viva *et al.* 2022, p. 39). In the present study, 13 individuals from San Genesio, dating between the middle of the 6<sup>th</sup> and 7<sup>th</sup> centuries CE, are of interest. During this time, the site expanded to include an organised village, adopted

the Lombard place name of *Vicus Wallari* in textual sources in 715 CE, and included a religious structure dedicated to San Genesio by the end of the 7<sup>th</sup> century CE (Cantini *et al.* 2017, p. 252; Viva *et al.* 2022, p. 39).

Coinciding with the occupation of the site during the 6<sup>th</sup> and 7<sup>th</sup> centuries CE, Italy underwent a series of sociocultural and political transformations (e.g., the Gothic wars, the Justinian Plague, and the Lombard conquest of Italy), which overlapped with the climatic shifts caused by the Late Antique Little Ice Age (LALIA) (Büntgen *et al.* 2016a, 2016b, 2022; Harper 2023; McCormick *et al.* 2012; Pohl 1997; Sigl *et al.* 2015). The LALIA caused an average temperature drop of -1.54°C in the northern hemisphere (Büntgen *et al.* 2022, p. 2337) and a temperature drop of -3°C in southern Italy when compared to the climatic averages of the Roman Climate Optimum (Zonneveld *et al.* 2024, pp. 3, 5). In Tuscany, the LALIA caused increased precipitation during autumn and winter, resulting in increased palaeoflood activity (Bini *et al.* 2020; Isola *et al.* 2019; Zanchetta *et al.* 2021). The timing of site use and the central location of San Genesio position it as an ideal case study for applying a multimodal approach to investigate the everyday practices and diets adopted by a group of multicultural people during a period of climatic downturn. This study aims to evaluate the relationship between extramasticatory dental wear, dental calculus, and chronic maxillary sinusitis in 13 individuals from San Genesio (mid-6<sup>th</sup> to 7<sup>th</sup> centuries CE, Tuscany, Central Italy) in relation to cultural and occupational practices, and ecological conditions (fig. 1).

## 2. Materials and methods

### 2.1. Materials

Individuals were included in the present study if they had dental calculus on their maxillary or mandibular dentition, located on either the labial/buccal, lingual, or interproximal surface, as well as supra- and subgingival. A total of 13 individuals from San Genesio dating to the 6<sup>th</sup> and 7<sup>th</sup> centuries CE presented sufficient dental calculus for sampling.

### 2.2. Biological profile

The age at death of individuals was estimated based on morphological changes to the auricular surface of the ilium (Buckberry, Chamberlain 2002) and the pubic symphysis (Brooks, Suchey 1990), as well as the stage of dental development and eruption (AlQahtani 2010). After the complete eruption of the third molar at approximately 20 years of age, an individual was classified as an adult (Hillson 2014, pp. 29, 229).

Sex was assessed primarily based on features of the pelvis, followed by the skull (Acsádi, Nemeskéri 1970; Ferembach *et al.* 1980; Kiales 2018; Kiales, Cole 2018), and metric measurements of the humeral and femoral head in adult individuals (Stewart 1979). Individuals were assigned to their respective estimated sex categories: male, probable male, indeterminate sex, probable female, female, and unknown sex.

### 2.3. Macroscopic dental assessment

The nomenclature system for labelling teeth was employed after Hillson (1996).

Dental calculus deposits were scored on a three-scale system based on their quantity: grade one is a mild deposit (minimal deposit in a line), grade two is a moderate deposit (covering <50% of the tooth surface), and grade three is a severe deposit (covering >50% of the tooth surface) after Brothwell (1981).

Dental wear on the maxillary and mandibular occlusal surfaces was macroscopically scored according to the schema of Smith (1984). Third molars were excluded from analyses due to axial inclination (Hillson 1996, p. 238). The mandibular and maxillary dentitions were scored, and average scores of  $\geq 0.5$  were rounded up. Scores of 1 and 2 indicated little to no wear, scores of 3 and 4 indicated moderate wear, scores of 5 and 6 indicated heavy wear, and scores of 7 and 8 indicated extreme wear.

Five parameters were considered for assessing extramasticatory dental wear: lingual surface attrition of the maxillary anterior teeth (LSAMAT), excessive occlusal load, chipping, grooving, and notching.

LSAMAT refers to progressive extramasticatory dental wear of the lingual surface of the anterior teeth of the maxilla without lingual or labial occlusal wear of the adjacent mandibular teeth (Turner, Machado 1983, p. 126). LSAMAT could only be assessed in individuals with maxillary and mandibular anterior dentition.

Excessive occlusal load was analysed on premolar and molar teeth, of which the occlusal surface presents little to no enamel, and often this wear is oblique, according to Molnar (2008, p. 424).

Antemortem chipping of the dental enamel with or without dentine, located on the labial, buccal, lingual, or interproximal edges of the teeth, was recorded as per the three-grade scale of Bonfiglioli *et al.* (2004, p. 449). Grade one was a crack or enamel flake of <0.5 mm, grade two was an irregular square lesion of <1 mm, and grade three was a crack that involved both the enamel and dentine and was greater than 1 mm (Bonfiglioli *et al.* 2004, p. 449).

Grooving refers to tubular troughs that occur on the occlusal surface of the dentition and run in either a mesiodistal or linguolabial/buccal direction, and are assessed using light microscopy (stereo zoom microscope MAHR SM 150, magnification 7X-45X) (Larsen 1985, p. 394; Monaco *et al.* 2022, p. 3). Interproximal grooving typically occurs parallel to the cemento-enamel junction (CEJ) of the

teeth and can extend into the crown and root in a linguolabial direction (Bonfiglioli *et al.* 2004, p. 449).

Notching is defined as an indentation on the occlusal and incisal edge of a tooth, which can extend across the entire surface, is broader than deep, has a smooth and polished appearance through enamel and dentine, runs in a vestibulo-lingual direction, and is oriented transversely or perpendicular to the mesiodistal direction (Bonfiglioli *et al.* 2004, p. 449). The three-grade scale of Bonfiglioli *et al.* (2004, p. 449) was used to assess notching: grade one is a slight indentation exclusively in enamel, grade two is an evident indentation that is wider and deeper with polished dentine, and grade three is an equally deep and wide depression with heavily polished dentine.

In addition to extramasticatory dental wear, antemortem tooth loss (AMTL) was scored as present or absent. Dental diseases, including dental caries (Doro Garetto *et al.* 1991) and periapical voids (abscesses, granulomas, and cysts) (Ogden 2008), were also recorded. Periodontal disease was scored according to the system of Ogden (2008), whereby healthy alveolus with no disease is scored as one when the alveolar margin meets teeth at a knife-edged occlude angle; mild periodontal disease is scored as two when the alveolar margin is blunt and flat-topped with a slightly raised rim; moderate periodontitis is scored as three, the alveolar margin is rounded and porous, with a trough of 2–4 mm depth; and severe periodontal disease is scored as four, whereby the alveolar margin is highly raised and porous, with an irregular trough and/or funnel >5 mm depth.

#### *2.4. Dental calculus sampling and analyses*

Dental calculus sampling, extraction, and decontamination were performed following published protocols (Fiorin, Cristiani 2023; Radini *et al.* 2017; Sabin, Fellows Yates 2020), with some modifications. The initial sampling of calculus from the teeth was conducted at the Division of Paleopathology, University of Pisa, in a thoroughly sanitised space, following the recommendations described by Velsko *et al.* (2017). The sampling preference was for supragingival dental calculus on the anterior teeth, on either the labial, lingual, or interproximal surfaces; deposits were selected based on their size. Calculus ranging from 0.003–0.038 grams was removed using a sterile scalpel on aluminium foil, transferred into sterile 1.5 mL Eppendorf tubes, and then weighed using a KERN EWJ 300–3H microbalance. The surfaces, tools, and nitrile gloves were sterilised using ethanol between each sample. The origin, colour, and weight of the calculus samples were recorded, and the samples were transferred to the DANTE Laboratory of Sapienza University of Rome for specialist analyses.

Concerning the dental calculus analyses, all procedures were performed in dedicated clean areas that were physically separated from spaces used for modern botanical research under strict environmental monitoring at the DANTE

Laboratory of Sapienza University of Rome. Cleaning took place on specific days, separate from the experimental work, and only after thorough sanitisation of all surfaces to prevent contamination. Before each analysis, the bench surfaces were cleaned with soap and ethanol, covered with film foil, and clean starch-free nitrile gloves were worn at all times. Before these steps, the dental calculus samples were weighed using an Ohaus Explorer® E11140 precision balance (capacity: 0.0001 g). The dental calculus analysed consisted of larger flakes and small quantities of tiny fragments.

Decontamination, that is, the removal of any soil residues and other contaminants from the sample, was carried out under a stereomicroscope (ZEISS Discovery V20, 10X-170X) on a Petri dish previously washed and sterilised with soap, alcohol, and boiling water. The operation was performed using brushes with synthetic bristles, a sterile surgical blade made of carbon steel, or an acupuncture needle sterilised with ethylene oxide, combined with a small amount of hydrochloric acid (1.5 M HCl) and ultrapure water (H<sub>2</sub>O Merck Millipore). After cleaning, the sample was subjected to three washing cycles in a Thermo Fisher microcentrifuge and finally dried in a Narbetherm muffle furnace (30°-1300°C) set at 40°C. Subsequently, the dental calculus was dissolved in hydrochloric acid (1.5 M HCl), and each step was followed by agitation on a rolling mixer for the tubes. Once completely dissolved, the vial contents were mounted on rectangular glass slides using a 50:50 solution of glycerol and ultrapure water and covered with a square glass coverslip.

Microdebris embedded in the calculus matrix were analysed using a transmitted Zeiss Imager2 cross-polarised microscope with magnifications ranging from 100X to 630X. Furthermore, control samples from clean working tables and dust traps were collected and analysed as references to monitor and exclude potential modern contamination. This practice is routinely carried out in the DANTE Laboratory, even during periods when no archaeological analysis is conducted, to better understand the flow of contamination over seasons. We did not retrieve any debris morphologically similar to any of the remains found in the environmental control samples. Only a few non-diagnostic starch granules were observed in the laboratory dust samples.

The micro-remains observed in the analysed samples were classified according to their nature and morphological characteristics as plant remains (e.g., starch granules, plant fibres, wood, and charcoal), fungal spores, or animal remains (e.g., fish scales). Where possible, these categories were further subdivided into smaller subgroups for taxonomic identification purposes. To identify archaeological starch granules, a modern reference collection of over 300 plants native to the Mediterranean region and Europe was used in conjunction with published literature. The other micro-residues (plant fibres, fish remains, fungi, and wood) were identified by comparison with an experimental reference collection stored in the DANTE laboratory. The microcharcoal particles were cate-



gorised based on previous research on dental calculus (Hardy *et al.* 2016; MacKenzie *et al.* 2021).

### 2.5. Chronic maxillary sinusitis

Chronic maxillary sinusitis could be assessed in adult individuals with at least one right or left maxillary sinus of >50% preservation, including the sinus floor, and either the medial or lateral wall (Riccomi *et al.* 2021, p. 42). The osseous features of chronic maxillary sinusitis were assessed macroscopically using the criteria of Boocock *et al.* (1995) and Merrett and Pfeiffer (2000), including spicules, remodelled spicules, pitting, and lobules. Thickening of the sinus walls followed the definition of Casa *et al.* (2025). As chronic maxillary sinusitis of rhinogenic and odontogenic origins can co-occur, cases of chronic maxillary sinusitis were not differentiated by apparent aetiology in the present study.

## 3. Results

Dental wear and calculus were analysed in seven males, five females, and one individual of indeterminate sex (tab. 1). Deposits of dental calculus were most frequently mild and affected both the maxillary and mandibular dentition (fig. 2; tab. 1). Moderate deposits of dental calculus occurred more frequently on the mandibular dentition (fig. 2), although one severe case was observed on the maxillary dentition (tab. 1).

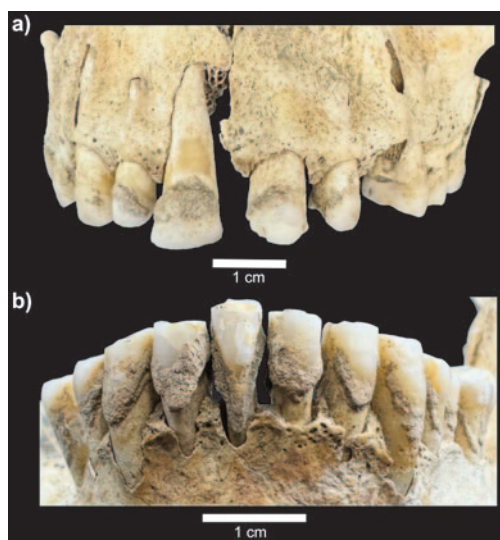


Fig. 2. Moderate deposits of dental calculus on the labial surface of the (a) maxillary and (b) mandibular dentition of US 13022.

Context and individual ID	Age at death	Sex	Dental calculus deposit		Dental diseases	Occlusal attrition score	Extramasticatory dental wear						AMTL/teeth and observable alveoli: %		Teeth			
			Mx	Md			LSA MAT	Excessive occlusal	Chipping		Grooves		Notching		Mx	Md	Mx	Md
									Mx	Md	Mx	Md	Mx	Md				
US 26159 SK297	37.86± 13.08 yrs	M?	1	1	Mild periodontal disease; superficial CEJ dental caries	3	3	X	0	0	X	X	0	0	0/16	0/16	0	0
US 32441 SK143	47±2.82 yrs	M	1	1	Mild periodontal disease; superficial crown and destructive CEJ and root dental caries	2	2	0	0	0	0	0	0	0	0/13	3/16, 18.75%	0	RM2, RM3, LM3
US 21083	51.41± 14.47 yrs	F	1	1	Mild periodontal disease; superficial and perforating crown and CEJ dental caries	6	6	X	X	X	X	0	0	X	0/14	1/15, 6.66%	0	LM3
US 13020	45.6± 10.4 yrs	M?	cba	1	Mild periodontal disease; destructive crown, CEJ and root dental caries	NR	6	NR	X	X	0	X	0	0	0/1	6/16, 37.50%	NR	LRM1, LRM2, LRM3, LLM1, LLM2, LLM3
US 13022	51.41± 14.47 yrs	F	1	2	Mild periodontal disease; destructive crown, CEJ and root dental caries; periapical abscess	5	4	X	X	X	X	0	0	0	2/15, 13.33%	4/16, 25.00%	LM2, LM3	RM1, RM2, RM3, LM3
US 32564 SK186	Adult	I	1	1	Mild periodontal disease; superficial, dentine, and destructive crown and CEJ dental caries	6	3	X	X	0	X	X	0	0	1/13, 7.69%	1/14, 7.14%	RM3	RM1
US 3178 SK206	15.5 yrs	F?	1	1	Superficial crown dental caries	2	2	0	0	0	X	X	0	0	0/13	1/12, 8.33%	0	RM3
US 32730 SK230	Adult	M?	1	2	Superficial crown dental caries	5	4	X	X	0	X	X	0	0	0/11	5/16, 31.25%	0	RM1, RM3, LM1, LM2, LM3
US 2100 SK31	37.86± 13.08 yrs	F	1	2	Dentine and destructive crown and CEJ dental caries	2	1	NR	0	0	0	X	0	0	0/16	0/16	0	0
US 2103 SK32	51.41± 14.47 yrs	F	1	2	Mild periodontal disease; dentine crown, and destructive crown, CEJ and root dental caries	5	3	X	0	0	X	X	0	0	7/13, 53.84%	4/16, 25.00%	0	RM1, RM2, LP4, LM1
US 32505 SK167	45.6± 10.4 yrs	M?	3	2	Dentine crown and CEJ, and superficial root dental caries, periapical abscess	3	2	NR	0	0	0	0	0	0	0/3	2/16, 12.50%	0	RM2, RM3
US 26078 SK60	37.86± 13.08 yrs	M	1	1	Mild periodontal disease; superficial CEJ and destructive crown and CEJ dental caries; periapical abscesses	5	3	X	X	0	X	X	0	0	4/16, 25.00%	0/16	RM1, RM2, LM1, LM2	0
US 11012 SK20	51.41± 14.47 years	M	1	0	Dentine crown and CEJ dental caries	NR	5	NR	NR	X	X	X	0	0	0/1	9/15, 60.00%	NR	RM1, RM2, RM3, RC, RI2, RI1, LP4; LM1, LM3

Tab. 1 (previous page). Summary of dental diseases, occlusal dental wear, and extramasticatory dental wear in individuals sampled for dental calculus.

NR=not recordable; N/A=not applicable; Mx = maxilla; Md = mandible; CEJ=cementoenamel junction; L=left; R=right; I=incisor; C=canine; PM=premolar; M=molar.

### *3.1. Macroscopic dental assessment*

Assessment of dental wear on the occlusal surface revealed three individuals with light wear, one with light-moderate wear, one with moderate wear, five with moderate-heavy wear, and three with heavy wear (tab. 1). Seven individuals had a discrepancy between the attrition scores of the maxillary and mandibular dentition, with higher average scores for the maxillary dentition (tab. 1). Notably, one individual (US 26159 SK297) displayed irregular wear facets on the maxillary and mandibular molars, which were characterised by a rough texture (fig. 3).

LSAMAT dental wear was present in seven individuals, comprising three males, three females, and one individual of indeterminate sex (tab. 1, fig. 4). Excessive occlusal load of the molars and premolars occurred in seven individuals, comprising four males, two females, and one individual of indeterminate sex (tab. 1). Six individuals presented excessive occlusal load on the maxillary teeth and four on the mandibular dentitions (tab. 1). One individual (US 13020) presented excessive oblique wear of the mandibular anterior dentition (fig. 5).

Antemortem chipping was found in 11 individuals; grade one chipping was present in 11 individuals, grade two in five individuals, and no individuals exhibited grade three (tab. 1). Grooves were not observed in any of the individuals in

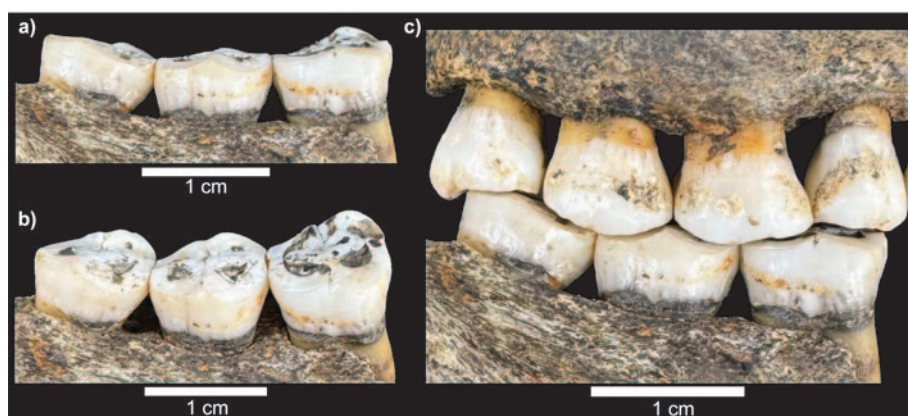


Fig. 3. US 26159 SK297 (a) Lateral view and (b) superolateral view of right mandibular molars with irregular wear facets and rough texture, (c) Occlusion of maxillary and mandibular dentition.

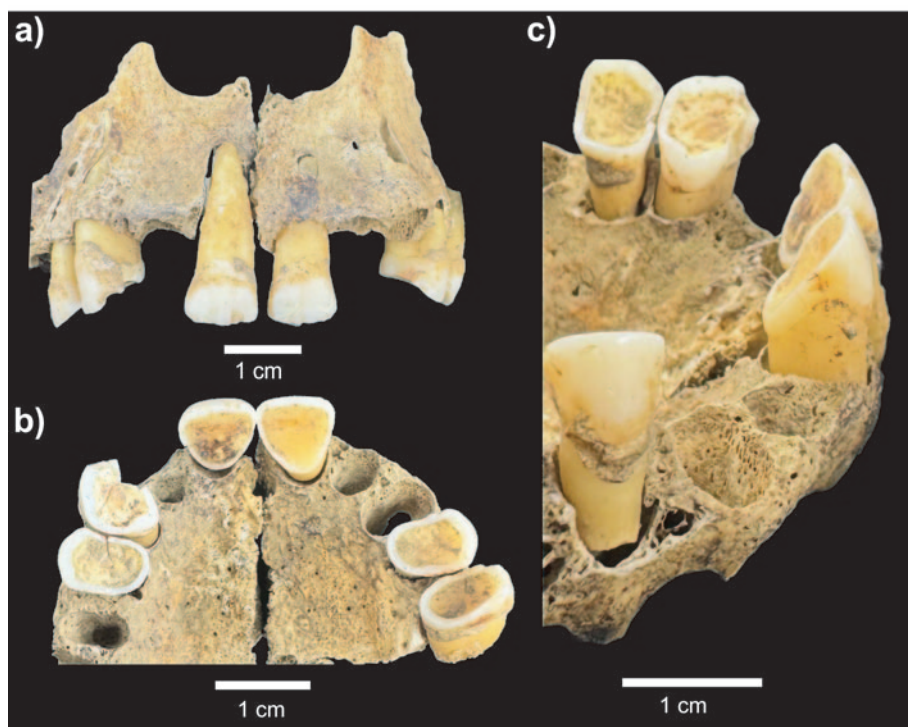


Fig. 4. US 32564 SK186 maxillary dentition exhibiting LSAMAT dental wear pattern on anterior teeth (a) labial view, (b) occlusal view, and (c) occlusal view of the left side.

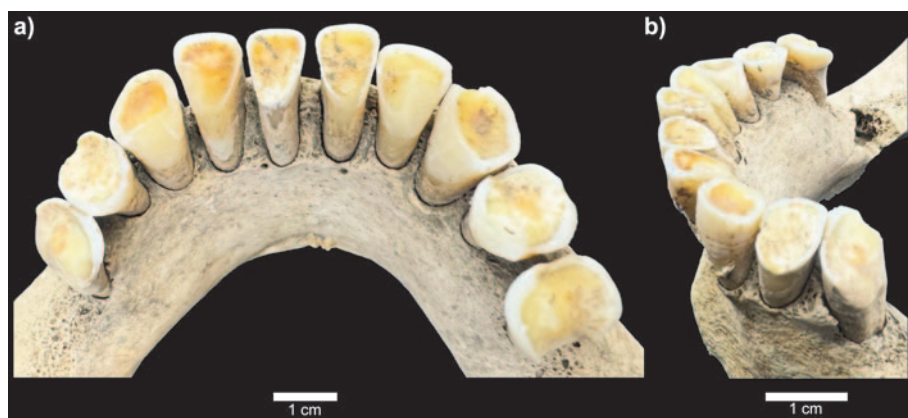


Fig. 5. US 13020 mandibular dentition with oblique wear pattern on anterior dentition (a) occlusal view and (b) occlusal view of left side.

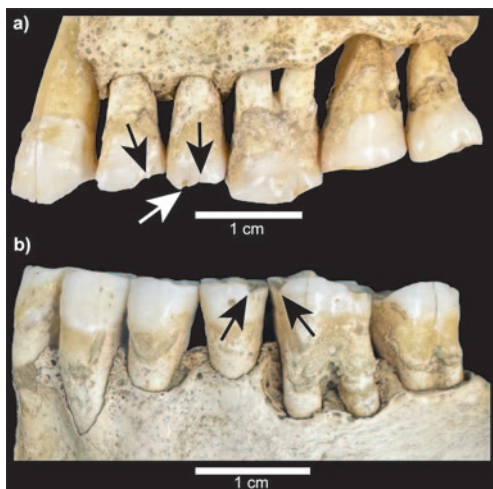


Fig. 6. Individual US 21083 (a) left maxilla with notching at the occlusal edge of P3 and P4 (black arrows) and chipping on the P4 incisal edge (white arrow); (b) left side of the mandible with notching at the occlusal edge of the interproximal surface between P4 and M1 (black arrows).

the present study. One individual, US 21083, exhibited notching of the edge of the occlusal surface of the left P3 and P4 (fig. 6a), as well as in the mandibular dentition, squared notches crossing the interproximal surface between the left P4 and M1, and between M1 and M2 (fig. 6b).

AMTL primarily affected molar teeth and was found in 11 individuals, including four maxillary and 10 mandibular dentitions (tab. 1). The frequency of AMTL per observable dentition and alveoli ranged between 7.7%-53.8% of the observable maxillary dentition and between 6.66%-60% of the mandibular dentition (tab. 1). Eight individuals exhibited mild periodontal disease, while lesions of dental caries occurred in 12 individuals, and three individuals had periapical abscesses (tab. 1).

### 3.2 Dental calculus

Table 2 presents the archaeological dental calculus that was preserved and identified in the analysed samples (fig. 7).

#### *Starch granules*

Starch granules were recovered from five samples. Based on morphology, distribution, appearance, and other features observable under cross-polarised transmitted light, two distinct morphotypes were identified in the samples analysed in this study. To avoid misinterpretation, granules smaller than 5  $\mu\text{m}$  (transient starches) were excluded, as they are not diagnostic (Haslam 2004). Morphotype I starch granules belong to the Triticeae tribe (subfamily Pooideae, family Poaceae), whereas Morphotype II starch granules are attributable to the Paniceae tribe (subfamily Panicoideae, family Poaceae). The Paniceae and Triticeae



Context and individual ID	Age at death	Sex	Type I Triticeae	Type II Panicoideae	Fish	Wood	Indeterminate	Other
US 26159 SK297	37.86±13.08 yrs	M?						3FI
US 32441 SK143	47±2.82 yrs	M	1				1	AN; CH; 6FI; 4FU
US 21083	51.41±14.47 yrs	F	1				1	FI
US 13020	45.6±10.4 yrs	M?	1		1	1		FI
US 13022	51.41±14.47 yrs	F				1		FI
US 32564 SK186	Adult	I		1				CH
US 3178 SK206	15.5 yrs	F?						
US 32730 SK230	Adult	M?						BA; 2CH; 2FI; AN
US 2100 SK31	37.86±13.08 yrs	F		1				CH
US 2103 SK32	51.41±14.47 yrs	F			1	2		
US 32505 SK167	45.6±10.4 yrs	M?						FI
US 26078 SK60	37.86±13.08 yrs	M				4		2CH; FI
US 11012 SK20	51.41±14.47 yrs	M				2		3FI; FU

Tab. 2. Details of the plant and animal micro-debris found in the archaeological dental calculus sample from San Genesio (Ch=Charcoal; FI=Fibres; FU=Fungi; AN=Animal Tissue; BA=Bacteria).

tribes both belong to the Poaceae (Gramineae) family, which comprises flowering plants commonly known as cereals, as well as many other herbaceous species. The Triticeae tribe, within this large family, includes cereals such as wheat (*Triticum*), barley (*Hordeum*), and rye (*Secale*), which are characterised by their particular shapes, sizes, and distribution of starch granules. In contrast, the Paniceae tribe includes species such as common millet (*Panicum*) and millets of the genus *Setaria*, which are typical small-grained cereal species. Although these groups differ in starch morphology and taxonomy, they are closely related within the same botanical family (Torrence, Barton 2006).

*Morphotype I.* Starch granules of this type were found in three individuals: US 32441 SK143, US 21083, and US 13020 (fig. 7a). Optical microscopy observations revealed that the starch granules were predominantly round or sub-oval in two dimensions and lenticular in three dimensions. They exhibited a characteristic bimodal size distribution, comprising large lenticular A-type granules and numerous small spherical B-type granules with smooth, regular contours. The granules occur as single granules (US 21083, [fig. 7a]) or loosely clustered (US

32441 SK143 [fig. 7d]), with A-type granules typically  $>20\text{ }\mu\text{m}$  and B-type granules typically  $<10\text{ }\mu\text{m}$ . In A-type granules, the hilum is central to slightly eccentric, and the granules show a typical extinction cross in polarised light. Growth lamellae may be visible in the larger A-type granules, whereas B-type granules are generally featureless at this scale. This description allows the identification of diagnostic features that are useful for taxonomic classification, thanks to the granules' size, shape and morphology (Geera *et al.* 2006; Henry *et al.* 2011; Henry, Piperno 2008; Stoddard 1999; Yang, Perry 2013). These first-type starches are consistent with the Triticeae tribe.

*Morphotype II.* This type of starch granule was identified in two individuals (US 32564 SK186 and US 2100 SK31 [fig. 7b]). It exhibits specific characteristics, including a polyhedral shape, a central hilum, a symmetrical extinction cross, and dimensions ranging from 16 to 21  $\mu\text{m}$ . Its slightly larger-than-average dimensions (typically 3–15  $\mu\text{m}$ ) could result from cooking or processing, which may cause swelling. These diagnostic characteristics are typical of the Paniceae tribe, and particularly the small-grained cereals of the Panicoideae subfamily, including several domesticated millet species (Henry *et al.* 2009, 2011). The morphology and size of the granules in our modern reference collection are consistent with *Setaria italica*, though there is some overlap with closely related species, such as *Panicum miliaceum* and wild *Setaria* spp. This necessitates caution in species-level identification, especially when granules are poorly preserved or few in number, as in this study.

#### *Unidentified starch*

One unidentified starch with a circular shape was observed in sample US21083. No visible lamellae were present, and the hilum was centric. Under polarised light, the extinction cross was clearly defined with four arms. Given these features, this starch morphology could occur in multiple plant species, and its taxonomic attribution therefore remains indeterminate.

#### *Fungi*

Microscopic examination of dental calculus revealed several fungal structures, including spores, dispersed within the matrix of two individuals (US 32441 SK143 and US 11012 SK20). However, the morphological characteristics were partially obscured by diagenetic alteration and wall fragmentation, which prevented the observation of diagnostic features such as ornamentation, wall thickness, and germination structures (Hardy *et al.* 2016; Radini *et al.* 2017). The state of preservation appears to have been influenced by post-burial depositional conditions and the oral environment, including pH fluctuations during tar formation. Consequently, it was not possible to make a reliable taxonomic attribution.

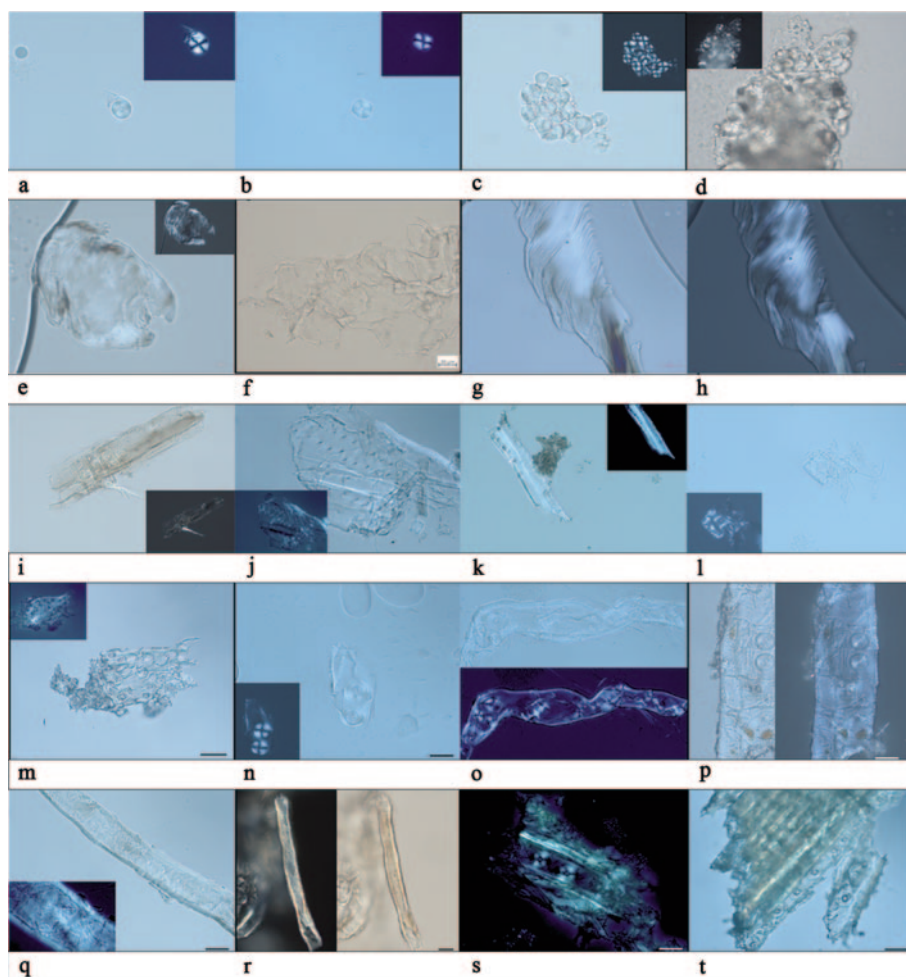


Fig. 7. Archaeological micro-remains were identified in individuals from the San Genesio site together with the experimental reference collection (black-framed photos). (a) Starch granule of *Triticum* from US 21083; (b) Starch granule of *Setaria* from US 2100 SK31; (c) Experimental starch grains of *Setaria italica*; (d) Starch granule of *Triticum* from US 32441 SK143; (e) Fish scale from US 13020; (f) Experimental ground fish scale; (g) Fish scale from US 2103 SK32; (h) Fish scale from US 2103 SK32 (polarised light); (i) Wood micro-remains from US 13022; (j) Oak wood from US 11012 SK20; (k) Plant tissue from US 11012 SK20; (l) Willow wood from US 2103 SK32; (m) Experimental oak wood (*Quercus* spp.) fragment under transmitted light microscopy, showing large vessels and associated tissue organisation typical of hardwood anatomy. Scale bar: 20  $\mu$ m; (n) Apple wood from US 2103 SK32; (o) Apple wood from US 2103 SK32; (p) Experimental apple wood fragment under transmitted light microscopy, showing vessels and parenchyma typical of hardwood anatomy. Scale bar: 20  $\mu$ m; (q) Willow wood from US 26078 SK60; (r) Experimental longitudinal view of a fibre from apple wood (*Malus domestica*) under transmitted light microscopy. Polarised light (left) and brightfield (right) reveal thickened cell walls characteristic of hardwood fibres. Scale bar: 20  $\mu$ m; (s) Pine wood from US 2103 SK32; (t) Experimental pine wood (*Pinus* spp.) fragment under transmitted light microscopy, showing aligned tracheids and characteristic softwood structure. Scale bar: 20  $\mu$ m.

### *Plant fibres*

Among the plant micro-remains, some fibres were very long and, in some cases, were still embedded in dental calculus. Some of these fibres have specific characteristics that allow identification under polarised light. However, most of the fibres present in the tartar lacked sufficient diagnostic characteristics for reliable taxonomic identification. Among those that could be described, flax (*Linum* sp.; US 32441 SK143 and US 11012 SK20) is characterised by a narrow lumen and an S-pattern of cellulose microfibrils, whereas hemp (*Cannabis* sp.; US 13020, US 32441 SK143 and US 26159 SK297) shows a narrow lumen and a Z-pattern of cellulose microfibrils (Lukesova, Holst 2024). Some fibres in the analysed samples were recognised as compatible with flax and hemp.

### *Wood*

In dental calculus, wood micro-remains recovered from the site, several of which showed clear signs of anthropic modification, were identified. This identification was based on a detailed microscopic analysis, including the observation of cell structure and arrangement, the presence of tracheids or vessels, and the type of pitting (simple or bordered pits) (Pallardy 2008; Wheeler *et al.* 2007). These analyses suggest that the wood fragments mostly derive from *Salix* spp. (willow wood, US 2103 SK32 [fig. 7l] and US 26078 [fig. 7q]), *Malus* spp. (apple wood, US 2103 SK32 [figs. 7n-o]), *Quercus* spp. (oak wood, US 11012 SK20 [fig. 7j]) and *Pinus* spp. (pine wood, US 2103 SK32 [fig. 7s]). These taxa are all compatible with regional vegetation and palaeoenvironmental reconstructions (Santeramo 2014, p. 65). However, it should be noted that the microscopic identification of such small wood fragments rarely allows for precise taxonomic classification. Therefore, the results must be regarded as preliminary and require confirmation through additional analyses.

### *Fish scale*

Two fragments of mineralised animal tissue were recovered from dental calculus matrix (US 13020 [fig. 7e] and US 2103 SK32 [figs. 7g-h]) (Cristiani *et al.* 2018). Under 400X magnification, the fragments displayed a compact, translucent structure with a smooth yet subtly contoured external surface. Fine curvatures and micro-reliefs are visible, which, based on experimental comparison with Chondrichthyes (cartilaginous fish) and Osteichthyes (bony fish) scales, may correspond to the ridges or growth rings characteristic of fish body scales. The fragments showed a dense, birefringent appearance under cross-polarised light, consistent with mineralised dermal tissue, suggesting preservation of the original scale microstructure despite the small size of the fragment (Sabbah *et al.* 2021; Zylberberg *et al.* 1988). Given the observed morphology and structural features, the most plausible interpretation is an attribution to Osteichthyes, although caution is warranted due to the fragmentary nature.

### *Charcoal remains*

Charcoal remains were dark in colour and had angular edges, displaying no birefringence when observed in transmitted polarised light and were found in five individuals (US 32441 SK143, US 32564 SK206, US 32730 SK230, US 2100 SK31, US 26078 SK60).

### *Animal tissue*

The animal tissue was found inside the dental calculus (US 32441 SK143, US 32730 SK230). However, their morphological and structural characteristics did not permit a precise taxonomic classification. These micro-remains have a fibrous consistency and a lamellar structure that is reminiscent of dermal or cutaneous tissues (e.g. skin or animal membranes) (Linossier *et al.* 1996). However, their state of preservation and the absence of diagnostic markers mean that it is impossible to determine their origin with certainty.

### *Bacteria*

Aggregates of bacteria were observed in the calcified matrix of dental calculus (US 32730 SK230). In several cases, these aggregates were microcolonies whose morphology was consistent with that of the bacteria present in the oral microbiota (Saini *et al.* 2011): they were either spherical or rod-shaped. While it was possible to recognise the general morphology, taphonomic alterations and preservation limitations prevented further characterisation at species or functional group level.

## *3.3 Chronic maxillary sinusitis*

A total of eight individuals out of 12 had at least one maxillary sinus of >50% completion preserved for assessment of chronic maxillary sinusitis. Two females and one male exhibited chronic maxillary sinusitis (fig. 8).

## *3.4. Extramasticatory dental wear, dental calculus, and chronic maxillary sinusitis*

Seven of the nine individuals with extramasticatory dental wear had fibrous inclusions in their dental calculus. Among them, one individual (US 21083) exhibited LSAMAT, excessive occlusal load, chipping, and notching, while another (US 13022) showed LSAMAT, excessive occlusal load, and chipping, in combination with chronic maxillary sinusitis (tabs. 1, 3). Five individuals with wood inclusions in dental calculus coincided with extramasticatory dental wear (tab. 3). Charcoal remnants were found in the dental calculus of four individuals: three of these had extramasticatory dental wear, and none presented chronic maxillary sinusitis (tab. 3).



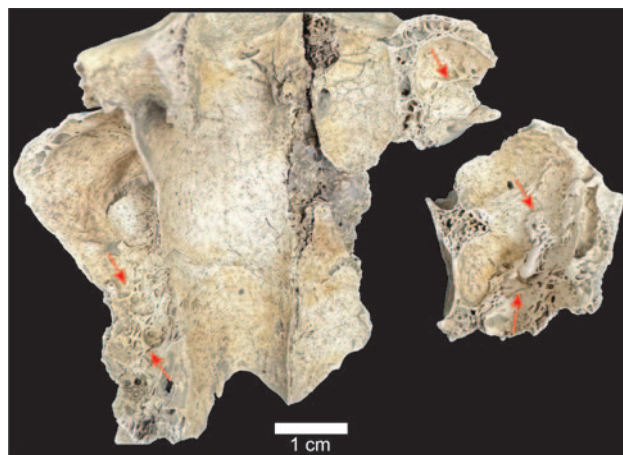


Fig. 8. Chronic maxillary sinusitis in individual US 13022; osseous bone changes include spicules, remodelled spicules and thickening of the sinus walls.

Context and individual ID	Age at death	Sex	Extramasticatory dental wear	Dental calculus inclusions	Chronic maxillary sinusitis
US 26159 SK297	37.86±13.08 yrs	M?	Present	Fibres	Absent
US 32441 SK143	47±2.82 yrs	M	Absent	Animal tissue, charcoal, fibres, fungi, starch	Present
US 21083	51.41±14.47 yrs	F	Present	Fibres, starch	Present
US 13020	45.6±10.4 yrs	M?	Present	Fibres, starch, fish, wood	NR
US 13022	51.41±14.47 yrs	F	Present	Fibres, wood	Present
US 32564 SK186	Adult	I	Present	Charcoal, starch	Absent
US 3178 SK206	15.5 yrs	F?	Absent	No data	N/A
US 32730 SK230	Adult	M?	Present	Charcoal, fibres, animal tissue	NR
US 2100 SK31	37.86±13.08 yrs	F	Absent	Charcoal, starch	Absent
US 2103 SK32	51.41±14.47 yrs	F	Present	Fish, wood	Absent
US 32505 SK167	45.6±10.4 yrs	M?	Absent	Fibres	NR
US 26078 SK60	37.86±13.08 yrs	M	Present	Fibres, charcoal, wood	Absent
US 11012 SK20	51.41±14.47 yrs	M	Present	Fibres, fungi, wood	NR

Tab. 3. Summary of extramasticatory dental wear, dental calculus inclusions, and chronic maxillary sinusitis (NR=not recordable; N/A=not applicable).

## 4. Discussion

Thirteen individuals from San Genesio, dating to the second half of the 6<sup>th</sup> and 7<sup>th</sup> centuries CE, had preserved dental calculus for sampling and analysis. Among them, nine individuals exhibited extramasticatory dental wear, and three presented osseous changes consistent with chronic maxillary sinusitis. The majority of individuals presented dental calculus deposits of mild quantity, though five individuals presented moderate deposits, and one individual presented a severe deposit. The dental attrition scores ranged from light to heavy wear, which were largely age-related, and more attrition occurred on the maxillary dentition compared to the mandibular teeth. The LSAMAT dental wear pattern was

present in roughly half of the observable individuals, and notably, one anomalous individual had an oblique wear pattern of the mandibular anterior dentition, although the maxillary dentition could not be observed. Almost all individuals had antemortem chipping of the teeth, with mild chipping in 11 and moderate chipping in five individuals. No individuals had grooving; however, one individual presented notching of the premolars and molars. Concerning oral pathologies, the majority of individuals exhibited AMTL, which primarily affected the molar teeth, as well as dental caries and mild periodontal disease, while a few individuals had periapical abscesses. Dental calculus inclusions consisted of wheat and barley starch grains, accompanied by plant fibres, microfragments of wood, charcoal and fungal spores. Dental calculus inclusions of plant fibres, wood, and charcoal coincided with the occurrence of extramasticatory dental wear. Notably, dental calculus inclusions of plant fibres coincided with the occurrence of extramasticatory dental wear (LSAMAT, excessive occlusal load, chipping, and notching) and chronic sinusitis in two individuals, and in one case, dental calculus included animal tissue, charcoal, fibres, fungi, and starch, and the individual did not have extramasticatory dental wear but did have chronic maxillary sinusitis. There is a lack of directly comparative data for the present study, with one study evaluating dental calculus and activity-induced dental modifications from the Eneolithic to early Bronze Age site of Gricignano d'Aversa, Campania, southern Italy (Sperduti *et al.* 2018). Only one other study has evaluated dental calculus in combination with chronic maxillary sinusitis (MacKenzie *et al.* 2021).

During the 6<sup>th</sup> and 7<sup>th</sup> centuries CE in Italy, there was a shift in subsistence practices from crop-centred toward local systems, and the emergence of an agro-sylvan pastoral economy, which, in addition to C<sub>3</sub> plants, increasingly incorporated C<sub>4</sub> cereals, which were previously used modestly, such as millet (Riccomi *et al.* 2020, p. 2). Stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) levels of 11 individuals from San Genesio, including seven individuals from the present study (US 21083, US 13020, US 13022, US 2100 SK31, US 2103 SK32, US 26078 SK60, US 11012 SK20), indicate the use of C<sub>3</sub> foods (e.g., wheat, barley, rye) and the increasing integration of C<sub>4</sub> resources such as millet (Riccomi *et al.* 2020, pp. 12, 14). Starch granules embedded in the dental calculus of individuals from San Genesio confirm the consumption of both C<sub>3</sub> and C<sub>4</sub> cereals, the former including wheat and barley (tab. 2), and the latter exhibiting evidence of cooking or processing of millet. The pattern of dental attrition, characterised by increasing wear with age, in individuals from San Genesio, corresponds with diets from agricultural societies (Smith, 1984). The occurrence of higher scores of dental attrition in the maxillary dentition compared to mandibular dentition likely reflects both the use of teeth as tools, particularly involving the anterior maxillary dentition, as well as the high rate of AMTL of molar teeth.

With further consideration of diet, the incorporation of fish scales, likely from a species of bony fish, into dental calculus attests to their consumption at San Gen-

esio during the 6<sup>th</sup> and 7<sup>th</sup> centuries CE. The site is located close to the junction of the Arno and Elsa rivers, which served as a local freshwater source (Cantini *et al.* 2017, p. 252), and lead weights used for fishing nets have been documented at the site (Cantini 2010). Extramasticatory dental wear from San Genesio could indicate the production of fishing nets from plant fibres and/or animal sinew (Larsen 1985; Littleton *et al.* 2013). Although grooves are absent in individuals from San Genesio, one individual did present dental notching, and excessive occlusal wear and LSAMAT were found in most individuals in the present study.

LSAMAT dental wear has been primarily found in populations where archaeological and ethnographic evidence attest to the shredding, peeling, and processing of plant fibres for the production of nets, baskets, cordage, and textiles (Irish, Turner 1987, 1997; Littleton *et al.* 2013; Turner, Machado 1983). Typically, LSAMAT is associated with carbohydrate-rich plants such as manioc (Irish, Turner 1987) and tule roots (Turner, Machado 1983). Identifiable plant fibres found in the dental calculus of individuals from San Genesio include flax (*Linum* sp.) and hemp (*Cannabis* sp.), both of which are known to have been used for crafting in past societies (Díaz-Navarro *et al.* 2023) and have been found in dental calculus (MacKenzie *et al.* 2021). Excessive occlusal wear of the molars has also been linked with the processing of fibres and sinews by pulling them through clenched teeth, as well as by chewing (Molnar 2008, p. 429). This could also explain the high level of AMTL, specifically affecting the molar teeth in the current sample (Molnar 2011). Chewing of rough fibres, as well as rope twisting and weaving, could account for the unusual occlusal wear found on the molars of US 26159 SK297 (fig. 3), which exhibited a rough texture indicating the presence of abrasive agents and the continued introduction of gritty fibres into the mouth, and coincided with the presence of fibres embedded in dental calculus. Fibre processing is also a risk factor for the development of chronic maxillary sinusitis, and in the present study, fibres were found in the calculus of the three individuals with chronic maxillary sinusitis. In particular, the production of textiles such as rugs, clothing, and rope using hemp is an occupational risk factor for chronic maxillary sinusitis (Sundaresan *et al.* 2015).

An alternative explanation for the occurrence of LSAMAT in a high frequency of individuals at San Genesio could be the processing of animal skin for leather-making (Lous 1970; Monaco *et al.* 2022) or the preparation of animal sinews for crafting and utilitarian purposes (Pechenkina *et al.* 2002, p. 29). This theory is supported by the structure of the animal tissue found in the dental calculus, which is reminiscent of animal skin or membranes. In fact, preserved animal tissue was found in the dental calculus of at least one individual with LSAMAT (US 32730 SK230), though it could not be taxonomically identified, and could suggest both consumption and use of animal tissue for crafts. The high occurrence of antemortem chipping in the dentitions of the individuals from San Genesio could have been caused by chewing on abrasive foods such as nuts, bones, and fruit stones; however, as the frequency of chipping is so high, it probably oc-

curred during occupational activities such as fibre processing, leather making, or holding objects, among others (Bonfiglioli *et al.* 2004; Tanga *et al.* 2016).

The discovery of wood residues in the dental calculus of individuals from San Genesio, which are not consumed but can be inhaled during occupational or domestic activities, provides insights into both the activities practised, diet, and environmental conditions at the site. Inhaled or ingested wood dust can be incorporated into dental calculus (MacKenzie *et al.* 2021, p. 122; Radini *et al.* 2017, pp. 75-76). Individuals at San Genesio may have been exposed to wood dust through contact with the natural environment, as well as through activities such as carpentry, artefact manufacture, and building and construction (MacKenzie *et al.* 2021, p. 122). The teeth could have been used as a tool for stripping, processing, or as pliers (Scott 1997), and holding a wooden stick between the occlusal surfaces of the teeth could account for the sole individual (US 21083) who presented notching of the molars and premolars (Monaco *et al.* 2022, p. 7). In addition, it cannot be excluded that wood chewing sticks were used for oral hygiene; however, further investigation of dental microwear is necessary to explore this possibility (MacKenzie *et al.* 2021; Radini *et al.* 2017, p. 77). Wood dust is a known respiratory irritant, and occupational exposure has been found to increase the chances of developing chronic maxillary sinusitis by 45-75% (Clarhed *et al.* 2020, p. 600). One individual (US 13022) from San Genesio presented wood inclusions in dental calculus in combination with chronic maxillary sinusitis and the use of teeth as tools. The presence of *Quercus robur*, typically found in humid forests, is identified alongside potential traces of species such as willows and poplars, consistent with local hydromorphic conditions (SANTERAMO 2014, p. 65). In contrast, the presence of apple wood residues indicates the cultivation of fruit in the surrounding area.

Microfragments of charcoal incorporated into the dental calculus of five individuals from San Genesio attest to their exposure to smoke and fire, likely for everyday domestic, occupational, and cultural activities, and possibly natural brush fires. Charcoal could have been incorporated into dental calculus through ingestion, such as through accidental integration into food or through cooking practices (e.g., charred food), as well as the smoking of food for preservation (Buckley *et al.* 2021; Cristiani *et al.* 2018, p. 5; Radini *et al.* 2017, p. 11). Regarding San Genesio, one individual (US 32441 SK143) had chronic maxillary sinusitis and charcoal in their dental calculus, and notably, no evidence of extramasticatory dental wear. Theoretically, the temperature drop caused by the LALIA could have influenced people to spend more time indoors to keep warm and thus increased potential exposure to smoke from hearths and indoor fires, particularly due to poor ventilation indoors, which has been linked to chronic maxillary sinusitis (Clarhed *et al.* 2018, 2020; Panhuysen *et al.* 1997; Roberts 2007); however, regular exposure to fires both inside and outside can also account for the presence of charcoal in dental calculus.

Fungi, including spores, were embedded in the dental calculus of two individuals from San Genesio, of which one had extramasticatory dental wear (US 11012 SK20), and another chronic maxillary sinusitis (US 32441 SK143). The integration of fungi into archaeological dental calculus has been attributed to both the ingestion of mushrooms (Hardy *et al.* 2016; Radini *et al.* 2017) and the unintentional ingestion or inhalation of spores from the environment (Hardy *et al.* 2016). For example, Afonso-Vargas and colleagues (2015) interpret the integration of fungal spores into the dental calculus of individuals from the 18<sup>th</sup>-century Canary Islands as the result of the consumption of contaminated maize. In the case of San Genesio, although the type of fungi could not be taxonomically identified, the incorporation of fungi into dental calculus could reflect the consumption of edible mushrooms, but more likely of spoiled food at the site such as grains – for example, one individual (US 32441 SK143) had both fungi and type I starch granules integrated into dental calculus (tab. 2). An additional avenue of integration of fungi into calculus could also be exposure to spores during everyday life. In this period, the LALIA caused increased precipitation and humidity in Tuscany (Bini *et al.* 2020; Isola *et al.* 2019; Zanchetta *et al.* 2021), which would have created the ideal environment for the growth of fungal spores. In addition, food storage practices, poor hygiene, and damp surfaces could have promoted the accumulation of fungal spores and mould (MacKenzie *et al.* 2021, p. 121). It has been postulated that the cooccurrence of fungal spores in dental calculus with chronic maxillary sinusitis could infer an aetiological influence (MacKenzie *et al.* 2021); however, clinical cases of fungal chronic sinusitis have predominantly been linked to *Aspergillus* spp. (Raz *et al.* 2015), which has seldom been identified in archaeological dental calculus. Therefore, any association between the presence of fungal spores in dental calculus and chronic maxillary sinusitis at San Genesio is speculative.

The inclusion of bacterial remains consistent with that of the oral microbiota in the dental calculus of one individual (US 32730 SK230) has the potential to provide valuable insights into past microbial communities and confirm the role of dental calculus as a long-term biological archive (Adler *et al.* 2013; Saini *et al.* 2011; Warinner *et al.* 2015). Future research on oral microbiota should use biomolecular techniques such as aDNA and proteomics to investigate the potential occurrence of oral pathogens in dental calculus derived from individuals from San Genesio.

#### 4.1. Limitations

In some individuals, either the maxillary or mandibular dentition was not present or could not be assessed, which limited evaluation of the LSAMAT dental wear pattern. The sample size of the present study was too small to evaluate sex or age at death variations. Poor preservation of the maxillary sinuses limited the number of individuals that could be assessed for chronic maxillary sinusitis.



## 5. Conclusion

This study highlights the value of integrating dental calculus, extramasticatory wear, and chronic maxillary sinusitis evidence to reconstruct daily life and health in early medieval Tuscany. At San Genesio, cereals such as wheat, barley, and millet formed dietary staples, and were supplemented by freshwater fish. Plant fibres, wood, and charcoal embedded in dental calculus, together with LSAMAT and excessive occlusal wear, indicate fibre- and wood-working practices that employed the teeth as a 'third hand'. These activities were not only utilitarian but also occupational hazards, exposing individuals to inhaled particulates and increasing risks of respiratory conditions. Chronic maxillary sinusitis in three individuals likely reflects an interaction of dietary, environmental, and occupational stressors, including fibre and wood dust, indoor smoke, and potentially, fungal spores, exacerbated by the damp climate of the LALIA. Although small sample size and poor preservation limit broad generalisations, the findings highlight the oral cavity as a key nexus where subsistence, craft, and environment intersect. This integrative approach shows how diet, work, and climate jointly shaped health in communities adapting to political and ecological upheavals. Future biomolecular research will refine our understanding of diet, craft practices, and oral microbiomes, deepening insights into biocultural adaptation in 6<sup>th</sup> and 7<sup>th</sup> centuries CE Italy. Incorporating this combined framework into future research will enable for more robust and nuanced reconstructions of past human diet, behaviour, and health.

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## Abstract

This study examines dental calculus, extramasticatory wear, and chronic maxillary sinusitis in 13 individuals from San Genesio, central Italy, dating to the second half of the 6<sup>th</sup> and 7<sup>th</sup> centuries CE. Microdebris in calculus revealed wheat, barley, millet, fish, fibres, wood, charcoal, and fungi. The patterns of LSAMAT and chipping suggest fibre and wood processing using teeth as a third hand. In three individuals, chronic maxillary sinusitis coincided with fibre and wood inclusions, highlighting occupational and environmental risks. The results highlight the biocultural interplay of subsistence, work, and the environment during the Late Antique Little Ice Age in post-Classical Tuscany.

**Keywords:** non-alimentary dental wear, micro-debris, occupational activities, environment, LALIA.

*Questo studio esamina il tartaro dentale, l'usura extramasticatoria e la sinusite mascellare cronica in 13 individui provenienti da San Genesio, nell'Italia centrale, risalenti alla seconda metà del VI e VII secolo d.C. I microdetriti presenti nel tartaro hanno rivelato la presenza di grano, orzo, miglio, pesce, fibre, legno, carbone e funghi. I modelli di LSAMAT e scheggiatura suggeriscono la lavorazione di fibre e legno utilizzando i denti come terza mano. In tre individui, la sinusite mascellare cronica coincideva con inclusioni di fibre e legno, evidenziando rischi occupazionali e ambientali. I risultati mettono in luce l'interazione bioculturale tra sussistenza, lavoro e ambiente durante la Piccola Era Glaciale tardoantica nella Toscana post-classica.*

**Parole chiave:** usura dentale non alimentare, micro-detriti, attività occupazionali, ambiente, LALIA.

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