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ORIGINAL PAPER

Bone histology of human remains from the Late Holocene of Northwestern Patagonia, Argentina: a multidimensional taphonomic approach

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Abstract
This study presents the first histological results on human remains recovered from Late Holocene archaeological sites from Northwestern Patagonia, Argentina, from a multidimensional taphonomic perspective. The burial sites come from different geoenvironmental contexts. The main purpose of this work is to assess the influence of geoenvironmental factors on taphonomic history of human bones. The bone microstructure of eleven human femora was analyzed through thin sections and four of them also with scanning electron microscopy. The relationship of these parameters with a set of geoenvironmental variables was assessed by means of different uni-, bi-, and multivariate statistical analysis. Results indicate that the geoenvironmental contexts led to different preservation of the microstructure of skeletal remains, represented by two different patterns. Bones with the best histological preservation were associated with semi-arid to arid geoenvironmental characteristics and the worst preserved ones with subhumid to humid features. Interestingly, samples from the better-preserved pattern showed staining and microcracks, whereas bioerosion is associated with the poorly preserved ones. A weak incidence of sex and chronology was found on microstructure preservation. Finally, the effects of weathering on naturally unearthed bones were deeply destructive in a short period of time. This work highlights the power of a multidisciplinary approach to discern the taphonomical pathways in archaeological sites.

Keywords Bone preservation · Diagenesis · Biostratinomy · Geoenvironment · Microscopy · SEM

Introduction

Human bone is a primary source of information in archaeological and paleoanthropological research. Its survivability varies depending on a number of processes that take place on the surface of the ground or during and after the burial of the body over time (e.g., Collins et al. 2002; Jans 2008; Kendall et al. 2018; Reiche et al. 2011; Turner-Walker and Jans 2008). Taphonomy at the microstructural scale (histotaphonomy sensu Bell 2012) of human bone has been useful for the reconstruction of postmortem histories (e.g., Booth and Madgwick 2016; Hollund et al. 2012, 2014; 2018; Jans 2014; Kendall et al. 2018; Kontopoulos et al. 2016, 2019; Turner-Walker 2019), mostly given that some postmortem changes, like staining, inclusion, or microcracking are not visible at macroscopic level (e.g., Booth and Madgwick 2016; Garland 1993; Hanson and Buikstra 1987; Jans et al. 2002; Nicholson 1998). The deterioration pathway of archaeological bone is likely influenced by burial environment (Collins et al. 2002; Kendall et al. 2018; Nielsen-Marsh 2002; Nielsen-Marsh et al. 2007; Nielsen-Marsh and Hedges 2000; Smith et al. 2007; White and Hannus 1983). In Argentina, human bone analysis at the microscopic level has been scarcely considered in bioarchaeological research (see review in Desántolo and Bernal 2016). Recent works have analyzed the bioerosion effect as studied by scanning electron microscope in archaeological samples from a subtropical region of the country (Galligani et al. 2016, 2019), suggesting that the intensity of the bacterial attack on bone

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is related to very local conditions rather than to the edaphic zones. In Northwestern Patagonia, taphonomic research was recently approached through macroscopic bone analysis (Vazquez 2019, 2020); however, no osteohistological studies have been done in this region.

Northwestern Patagonia presents a pronounced geoenvironmental change in the west–east direction along 200 km in which two main regions are recognizable, the Andean region to the west and the extra-Andean region eastwards (Pereyra et al. 2011). Archaeologically, the peopling of this area can be traced back to 12,000 cal year B.P. with an increasing occupation after 3500 cal year B.P. (Gordón et al. 2019). Despite this, bioarchaeological record is almost nil for Early and Middle Holocene and abounds just in the Late Holocene. The space distribution of the sites is heterogeneous, likely related to permanent sources of water (Bernal et al. 2017). The area studied includes different burial environments allowing testing of the relationship between bone preservation and environmental conditions at a microstructural level.

The aim of this study is to present the first results of the histological characterization of postmortem alterations in archaeological human bones recovered from different geoenvironments from Northwestern Patagonia (Neuquén Province, Argentina) and assess the correspondence between macroscopic and microscopic states of preservation, as well as intra-bone variation, considering intrinsic and extrinsic factors (sensu Henderson 1987). Additionally, with comparative and exploratory purposes, we analyze the effects of post-diagenesis biostratinomy on the microstructure of a bone exposed to weathering for a short time (maximum 6 years) after more than 3000 years buried (Vazquez 2020). Selected archaeological sites include a wide range of geological, sedimentary, and burial settings as well as the widest time span of human remains available (Gordón et al. 2019) for a geographic and temporal area where there are no previous osteohistological studies. This topic is assessed from the viewpoint of different disciplines, a multi-faceted approach proper to improve the understanding of complex processes, enhanced by the analysis of multiple sets of variables by means of multivariate methods.

Materials and methods

Archaeological sites and bones sampled

Ten archaeological sites from Neuquén Province, Northwestern Patagonia, Argentina, were selected: Aquihuecó, Hermanos Lazcano, El Sauce, Remeco, Campo Ayoso, Sitio Grande, Caepe Malal, Cochico Márquez, Sitio Retamal, and Millaín (Fig. 1, Table 1). All of them are open-air primary burials without a funeral structure (the bodies were buried directly into the ground), except for the Remeco site in which skeletal remains were contained in cyst burials (Béguelin et al. 2017), and belong to hunter-gatherer societies of the

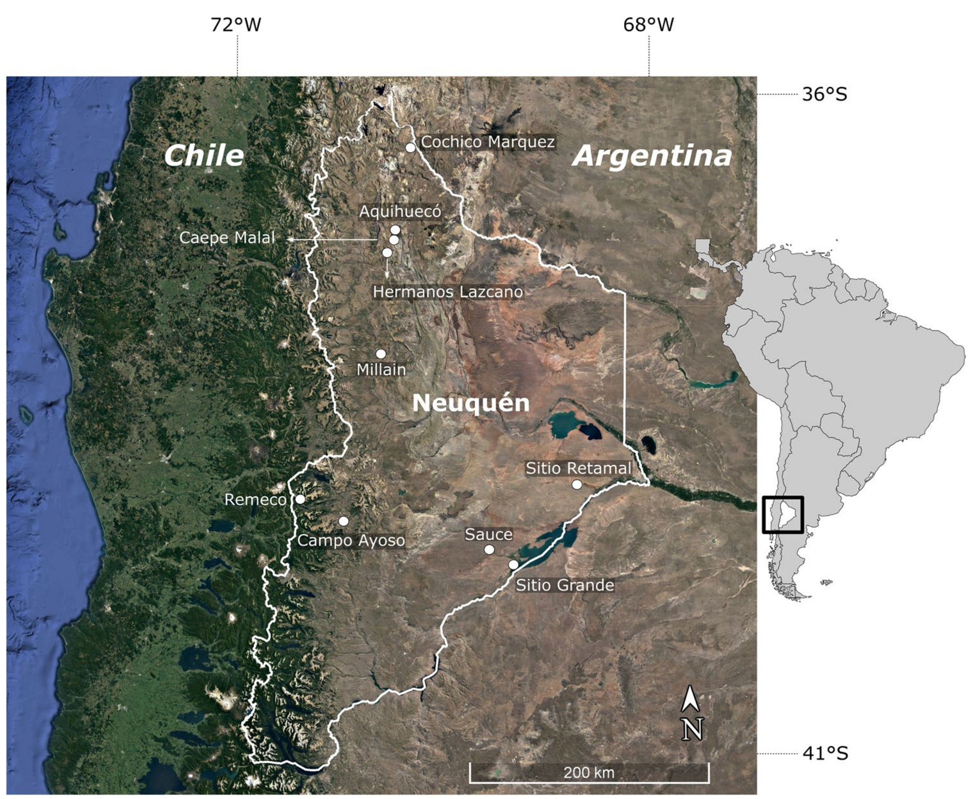


Fig. 1 Distribution of archaeological sites with human burials in Neuquén Province, Northwestern Patagonia, from which histological bone samples were analyzed (white dots)

Table 1 Characterization of the archaeological sites and bone samples analyzed. The time period assigned to each sample is shown in brackets in the second column

Site	Acronym	Chronology (time period)	Subregion	Placement	Sex	Age	Femur laterality	Macroscopic taphonomy characterization	Preservational scoring (Gordon & Buikstra, 1981)	Reference
Aquihuecó	Aqh	4700–3200 (1)	Subhumid of plains, hills, and mountainous areas	Aeolic sand-dune	Female	Medium adult	Right	Unbroken complete femur. Presence of calcium carbonate deposits on the entire surface	1	Della Negra and Novellino 2005; Della Negra et al. 2009; Gordón et al. 2019
	AqhSup				Undetermined	Adult	Right	Unbroken complete femur. Slight fissures parallel to the main axis of the bone. Decolorated	2	
Caepe Malal	CM	200–300 (3)	Subhumid of plains, hills, and mountainous areas	Alluvial fan	Female	Medium adult	Right	Unbroken complete femur. Presence of root marks	1	Hajduk and Biset 1991; Hajduk et al. 2000
Campo Ayoso	Cay	Post-contact (3)	Subhumid of plains, hills, and mountainous areas	No information	Female	Young adult	Right	Broken complete femur. Slight loss of osseous tissue in both epiphyses and the entire cortical lamina. Root marks	2	Bernal et al. 2017; Vazquez 2020
Cochico Márquez	CoM	2449 ± 34 (1)	Plateau arid	No information	Undetermined	Adult	Right	Broken and incomplete femur. More than 50% of bone loss. Severe deterioration of the cortical surface	3	Gordón et al. 2019; Bernal et al. 2017
El Sauce	Sau	No information (2)	Plateau arid	No information	Female	Adult	Right	Unbroken complete femur. Some root marks	1	Bernal et al. 2017; Vazquez 2020

Table 1 (continued)

Site	Acronym	Chronology (time period)	Subregion	Placement	Sex	Age	Femur laterality	Macroscopic taphonomy characterization	Preservational scoring (Gordon & Buikstra, 1981)	Reference
Sitio Grande	SG	670 ± 40; 890 ± 80 (2)	Plateau arid	Fluvial bar	Male	Medium adult	Left	Broken and incomplete femur. Epiphyses absent. Fissured and flaked	3	Della Negra and Novellino 2002; Pérez et al. 2009
Hermanos Lazcano	HL	4016 ± 33 (1)	Mountain arid	Alluvial sediments	Undetermined	Adult	Right	Broken and incomplete femur. More than 50% of bone loss. Severe deterioration of the cortical surface	3	Della Negra et al. 2014; Gordón et al. 2019
Millaín	Mil	Post-contact (3)	Mountain arid	No information	Undetermined	Adult	Right	Unbroken complete femur. Some root marks	1	Bernal et al. 2017; Vazquez 2020
Remeco	Rco	Eighteenth century (3)	Wet mountainous	Ancient high-level terrace	Probably female	Young adult	Right	Broken complete femur. Severe loss of osseous tissue from the cortical lamina. Small-diameter roots observed into cancellous bone. The femur shows extensive surface corrosion	3	Béguelin et al. 2017; Vazquez 2020

Table 1 (continued)

Site	Acronym	Chronology (time period)	Subregion	Placement	Sex	Age	Femur laterality	Macroscopic taphonomy characterization	Preservational scoring (Gordon & Buikstra, 1981)	Reference
Sitio Retamal	SR	190 ± 60 (3)	Plateau arid	No information	Male	Young adult	Right	Broken complete femur. Slight loss of osseous tissue in both epiphyses and the entire cortical lamina. Root marks. Femur is somewhat bleached and has reddish sediment deposits	2	Pérez et al. 2009; Bernal et al. 2017

Late Holocene period (ca. 4600 years BP to post-contact period; Gordón et al. 2019). We selected sites geographically distributed in different geoenvironmental regions across the province. The bone sample, mainly its size, was tied to local regulations related to respect for native people and preservation of heritage.

The bone sample consists of 11 human femora from adult individuals of both sexes, unearthed one from each site, and, for comparative purposes, we included an additional femur found on the sandy surface of the Aquihuecó site (AqhSup). Aquihuecó is the biggest hunter-gatherer burial site in Patagonia dated around 5000 cal year B. P. (Gordón et al. 2019). It was first explored in 1997, and since 2003, it has been systematically excavated. In 2003 fieldwork, some bones had been naturally released from the containing sediment and remained on the surface. These bones were exposed to weathering for a maximum of 6 years due to geoenvironmental conditions (semi-arid climate and erosive action of winds) that produced the natural exhumation of them (Della Negra and Novellino 2005).

All bones showed different states of macroscopic preservation, following preservational scoring proposed by Gordon and Buikstra (1981). None of them presented apparent macroscopic bone pathologies. For the sake of performing statistical analysis, the sample was assigned to three consecutive time periods, mainly according to dating (Gordón et al. 2019) and to dietary and cultural criteria (Gordón et al. 2017; Gordón and Novellino 2017, see Supplementary Text S_1). Table 1 details information on the archaeological sites and the studied bone samples.

Thin sections and restoration treatment

Thin bone sections were prepared following the protocol proposed by Chinsamy and Raath (1992) and modified by Navarro et al. (2018) at Paleohistological Laboratory of Museo Provincial Carlos Ameghino (Cipolletti, Argentina). To avoid the loss of morphological information, the femora were measured, photographed, and tomographed before cutting. For sampling, 10-mm-thick blocks from the midshaft were obtained. Samples were obtained using a precision saw. A mold and cast of each sample were made and used to replace the same in its respective femur. Samples obtained were embedded in two-component epoxy resin (resin DICAST LY 554 and hardener DICURE HY 554). Once the resin was completely hardened (after 24 to 36 h), the face that would remain adhered to the frosted glass slide (the one closest to the center of the diaphysis) was polished. An automatic grinder was used for polishing. Regarding the impregnation and mounting of the sample, the resin used was DISCAST 867 (different from that applied to the mentioned protocol), since it presented lower viscosity, improving the adhesion between bone and slide, in a shorter time and avoiding to a greater extent the subsequent detachment of the sample at the time of final grinding. Samples were grinded with 400 granulometry silicon carbide to obtain a homogeneous finish without micro-grooves that hinder later observation (Navarro et al. 2018).

Histological assessment

Thin bone sections were examined under a petrographic microscope ZEISS AXIO Imager.A2m with normal and polarized transmitted light at a magnification ranging from × 25 to × 100. The microstructures analyzed comprise lamellae, canaliculi, osteocyte lacunae, Haversian canals, Volkmann's canals, and inner and outer circumferential lamellae. The degree of histological preservation was established through a score, ranging from 0 (unpreserved) to 3 (well-preserved), to each histological structure in the sampled bones (Table 2). Microstructure preservation score (MPS) was designed for this study (see Supplementary Text S_2). Scores of these variables were summed up per sample to summarize overall microstructure preservation. Microcracks were recorded as presence/absence values. Birefringence intensity was assessed as 0 (absent), 0.5 (reduced), and 1 (normal) (Jans et al. 2002). Microbial action was assessed employing the Oxford Histological Index (OHI; Hedges et al. 1995), and the other alterations (cracking, staining, generalized destruction) with the General Histological Index (GHI; Hollund et al. 2012). All observations were registered in four sectors of the femoral shaft (anterior, posterior, medial, and lateral) and were conducted by the same person, one thin section per turn.

Additionally, four bones (from Remeco, El Sauce, Aquihuecó, and Hermanos Lazcano sites) were polished and coated with carbon for assessment through scanning electron microscope (SEM) ZEISS EVO MA15 with an energy dispersive spectrometer attached (SEM–EDS), at Laboratorio de Microscopía Electrónica y Difractometría de Rayos X at Instituto de Investigación en Paleobiología y Geología (CONICET—Universidad Nacional de Río Negro). Elemental spot and scan assessments were performed to determine chemical composition and zonation.

Geoenvironmental assessment

Geoenvironment was assessed for each archaeological site through different sets of variables (Table S_1). Climatic information includes average annual temperature, maximum temperature, minimum temperature, average annual precipitation, and type of weather variables. The first four were obtained from a climate model (climate-data.org, 2020), through the precise or approximate geolocation of the site. The type of weather was taken from Pereyra et al. (2011). Sedimentological evidence consists of physical (texture and sorting) and chemical (pH, organic matter, P, and $CaCO_3$) analysis of the sediments surrounding the skeleton. Physical characterization was done under a vibratory sieve shaker using sieves of different diameters to retain grains from gravel to loam-clay size. If the sample was wet, it was dried prior to sieving with a laboratory stove. Each grain size weight was calculated proportional to the total weight of the sample. Chemical analysis was performed at Laboratorio de Servicios Agrarios y Forestales, Neuquén Province, Argentina. These sediment samples ($n = 28$) correspond to seven archaeological sites. Three remaining sites, Campo Ayoso, Millaín, and El Sauce, lack this data given that they are no longer accessible for different reasons after the excavation of the remains (e.g., nowadays lay under buildings). Positional data of the sites (i.e., meters above sea level (masl) and horizontal and vertical distance to the river) was compiled using Google Earth Pro software. Finally, type of vegetation, soil, and geomorphology were obtained by Pereyra et al. (2011).

Statistical analysis

Histological data was standardized ((x-average)/standard deviation). Comparisons of histological variables between sites were performed with one-way permutation multiple ANOVA (PERMANOVA), with Manhattan distance, followed by post hoc pairwise comparisons (Anderson 2001; Hammer and Harper 2006). Non-metric

Table 2 Preservation categories according to the scope of observation of microstructures

Score	Description	Exemplary images in Fig. S_1
0	Microstructure is not identifiable or absent	b, i
1	Microstructure is present but hardly observable	h, i, j
2	Microstructure is present and moderately observable	d, e, g
3	Microstructure is perfectly observable, similar to modern bone	a, c, d, g

multidimensional scaling (nMDS) was calculated, with Manhattan distance, for two dimensions (coordinates) for the set of histological variables that reflect the state of preservation (Legendre and Legendre 2012). The plot was interpreted as the ordination of cases (every four sectors of each thin section) according to their micro-taphonomic preservation (taphonomic ordination). For further analysis, we used each nMDS coordinate as the new taphonomic variables. To evaluate the relationship between every geoenvironmental variable and the taphonomic ordination, we used either the Spearman rank correlation (for ordinal and quantitative variables) or non-parametric ANOVA, a.k.a. Kruskal–Wallis (for qualitative variables). We used the same nMDS coordinates as taphonomic variables to evaluate differences in sex, age (Kruskal–Wallis), and chronological values (Spearman rank correlation). In all cases, null hypotheses were rejected with p-values lower than 0.05 (Hammer and Harper 2006; Zar 2010). All statistical analyses were performed using PAST 4.01 software (Hammer et al. 2001).

Results

All sampled bones showed some degree of modification including alteration of bone microstructures, bioerosion, staining, or microcracks and displayed different preservation patterns. The results of the histological analyses are shown in Table 3.

Figure 2 presents the results from the nMDS ordination of the sites according to the histological variables (OHI and GHI are not included here). For a better interpretation, histological variables were projected in the nMDS ordination plot. Vector's lengths are associated with the degree of correlation of the variables with respect to both nMDS coordinates. To assess which histological variables contributed to the ordination structure, non-parametric correlations for Spearman ranges between each coordinate and the histological variables were calculated (Table 4). Except for birefringence, the remaining histological variables are significantly correlated with coordinate 1, i.e., all the points on the left side of the plot present higher values of those histological variables, opposite for cases on the right side. Coordinate 2 showed a significant correlation with the inner cortical layer, outer cortical layer, microcrack, and birefringence. Sites spread separated from each other, with almost no overlapping, as can be seen in Fig. 2. PERMANOVA comparison among sites on the standardized matrix is highly significant ($p=0.000$). Multiple comparisons showed that all sites differ from each other with the exception of four pairs: AqhSup-CoM, Rco-Cay, Rco-Mil, and HL-CM. Regarding the comparison of a buried bone (Aqh) and the bone found on the surface (AqhSup) from the same site, notably, they differ significantly ($p=0.029$) and spread well apart in the ordination plot. The arrangement shows a group of four sites (Aqh, SR, SG, and Sau) towards the lesser values of coordinate 1, displaying the best preservation according to the analyzed variables. A poorly preserved group is placed on the right side of the plot. HL, CM, Rco, Mil, and Cay are slightly separated from CoM and AqhSup in coordinate 2.

The areas of the midshaft in each sample (posterior, anterior, medial, and lateral) are closely grouped, indicating no differential intra-bone preservation. One exception to this pattern is the posterior sector of AqhSup sample, which stands apart from the other three sectors in the ordination plot (indicated with an arrow in Fig. 2).

The distribution of groups of sex, age, and chronology in the nMDS plot (not shown) does not show any recognizable pattern. However, in further analysis, we found that sex ($p=0.001$) and chronology ($p=0.001$) were significantly different for coordinate 2.

Geoenvironmental variables showed some degree of association with bone preservation (Table 5). pH, average annual temperature, minimum annual temperature, maximum annual temperature, precipitation, and altitude show significant correlations with coordinate 1. Also, pH, organic material, sorting, maximum temperature, and precipitation variables show significant correlations with coordinate 2. None of the comparisons made among coordinate scores for the different qualitative variables (geomorphology, type of weather, vegetation, and type of soil) resulted statistically significant. Nevertheless, some of the p-values are marginally significant ($0.05<p<0.10$), and thus, it is expected that they might be more significant in a larger sample.

Significant differences were found in GHI for coordinates 1 and 2 ($p=0.000$ and $p=0.034$, respectively); in OHI differences appear only with coordinate 1 ($p=0.000$).

According to Gordon and Buikstra categories (1981), macroscopic preservation is weakly associated with microscopic preservation across the samples (Coord1: $r=0.342$ $p=0.023$; Coord2: $r=0.304$ $p=0.045$).

Regarding SEM analysis, the Remeco sample showed intensifying corrosion from outside to inside, which in turn showed an outwards decrease of P and Ca elements in EDS-SEM analysis (Fig. 3a). In the Aquihuecó sample, incipient calcareous permineralization within Haversian canals was identified. Cavities and pores bear evidence of filling from surrounding sediment (i.e., silicon peaks; Fig. 3b), like the Hermanos Lazcano sample (Fig. 3c). The last sample was also affected by bacterial attack (microscopical focal destruction as described by Hackett, 1981) as shown in Fig. 4. The El Sauce sample did not show any diagenetic alteration at SEM

Table 3 Histotaphonomical database of the analyzed variables. See sample names in Table 1

Sample	Sector	Lamellae	Canaliculi	Osteocyte lacunae	Haversian canals	Volkmann's canals	Outer circumferential lamellae	Inner circumferential lamellae	MPS	Microcr	Biref	OHI	GHI	Alteration
Aqh	Posterior	3	3	3	3	3	0	2	74	0	0.5	5	3	Staining
Aqh	Lateral	3	3	3	3	3	1	2		0	0.5			Staining
Aqh	Anterior	3	3	2	3	3	3	2		0	0.5			Staining
Aqh	Medial	3	3	3	3	3	3	2		0	0.5			Staining
AqhSup	Posterior	2	1	2	2	1	0	0	12	0	0.5	5	0	
AqhSup	Lateral	1	0	0	1	0	0	0		0	0.5			
AqhSup	Anterior	0	0	0	1	0	0	0		0	0			
AqhSup	Medial	0	0	0	1	0	0	0		0	0			
CM	Posterior	0	0	0	1	0	1	2	16	0	0	5	0	
CM	Lateral	0	0	0	1	0	1	2		0	0			Staining
CM	Anterior	0	0	0	1	0	1	2		0	0			
CM	Medial	0	0	0	1	0	1	2		0	0			
Cay	Posterior	0	0	0	1	1	2	3	27	0	0	2	0	Bioerosion
Cay	Lateral	0	1	1	1	1	0	3		0	0			
Cay	Anterior	0	0	0	1	1	2	3		0	0			
Cay	Medial	0	0	0	1	1	1	3		0	0			
CoM	Posterior	0	0	0	1	0	0	0	5	0	1	2	0	Bioerosion
CoM	Lateral	0	0	0	1	0	0	0		0	0			
CoM	Anterior	0	0	0	1	1	0	0		0	1			
CoM	Medial	0	0	0	1	0	0	0		0	0.5			Bioerosion
Sau	Posterior	3	2	3	3	3	3	3	75	1	1	5	4	
Sau	Lateral	3	2	3	3	3	3	3		1	1			
Sau	Anterior	3	2	3	3	3	2	2		1	1			
Sau	Medial	2	2	3	3	3	2	2		1	1			
SG	Posterior	3	2	3	3	3	2	2	63	1	1	5	3	Staining
SG	Lateral	3	2	3	3	0	2	2		0	1			
SG	Anterior	3	2	3	3	0	2	1		0	1			Staining
SG	Medial	3	1	3	3	2	2	2		1	1			
HL	Posterior	0	0	0	1	1	1	1	16	0	0	1	0	Bioerosion
HL	Lateral	0	0	0	1	1	1	1		0	0			Bioerosion
HL	Anterior	0	0	0	1	1	1	1		0	0			Bioerosion
HL	Medial	0	0	0	1	1	1	1		0	0			Bioerosion
Mil	Posterior	1	0	1	1	0	1	2	22	1	1	3	1	Bioerosion
Mil	Lateral	0	0	0	1	0	1	2		1	0			Bioerosion
Mil	Anterior	1	0	1	1	0	1	2		1	0			Bioerosion
Mil	Medial	1	0	1	1	0	1	2		1	0.5			Bioerosion

Table 3 (continued)

Sample	Sector	Lamellae	Canaliculi	Osteocyte lacunae	Haversian canals	Volkmann's canals	Outer circumferential lamellae	Inner circumferential lamellae	MPS	Microcr	Biref	OHI	GHI	Alteration
Rco	Posterior	1	0	1	1	1	0	2	25	1	0.5	3	1	Staining; bioerosion
Rco	Lateral	1	0	1	1	1	3	3		1	0.5			Staining; bioerosion
Rco	Anterior	0	0	0	1	0	1	2		1	0			Staining; bioerosion
Rco	Medial	0	0	0	1	0	2	2		0	0			Staining; bioerosion
SR	Posterior	3	3	3	3	3	1	1	65	0	1	5	4	Staining
SR	Lateral	3	2	3	3	3	1	1		1	1			Staining
SR	Anterior	3	2	3	3	3	1	1		0	1			Staining
SR	Medial	3	2	3	3	3	1	1		1	1			Staining

MPS microstructure preservation score, *Microcr* microcracks, *Biref* birefringence, *OHI* Oxford Histological Index, *GHI* General Histological Index

observation. No evidence of chemical composition replacement was observed in any of the samples.

Discussion

The multivariate ordination analysis shows two clearly separated groups (Fig. 2), one located to the left of the graph, characterized by well-preserved histology, and the other one to the right, with poor histological preservation. While in the "well-preserved" group the mean MPS (Table 3) is 69.25, a value of 17.57 was obtained in the "poorly preserved" group. These differences are visually very well marked (see Supplementary Information, Fig. S_1). Moreover, we detected statistically significant associations of extrinsic variables to the preservation patterns.

A noteworthy result reveals that preservation shows little variation within the same bone, and such variability is lesser than the variability between bones that come from different archaeological sites. PERMANOVA results show that the multivariate histological characterization differs between sites in mostly all comparisons.

The absence of secondary burials limited the possibility of a significantly changed burial environment and therefore of contradicting preservation factors (e.g., climatic, geologic) during their taphonomic trajectories (i.e., from their burial until they were found). According to different studies (Fernández et al. 2012; De Porras 2017), most of these agents were nearly constant during the time comprised in our samples, the last 5000 years, and most like current geoenvironmental conditions.

Samples in the group characterized by well histological preservation are associated with semi-arid (Aqh) to arid (Gde, Sau, SR) climate (average annual temperature between 12 and 14 °C and rainfall below 500 mm), alkaline soils with well-sorted sediments, and poor organic matter content, located between 300 and 1000 masl. Samples in these groups include GHI scores of 3 and 4, mostly normal birefringence, and occurrence of only some microcracks (Table 6). Sedimentary filling and incipient calcareous permineralization were observed in two samples of this set. The prevalent good size sorting of the substrate of these archaeological sites would have favored fluid transport through the sediment, which facilitated the deposition of sand grains in the holes, pores, and vascular channels as well as precipitation of $CaCO_3$.

It is notable that Aquihuecó, being the oldest site in this group (more than 3000 years old), does not show critical differences in histological preservation with younger samples. This suggests that under the geoenvironmental conditions described for samples in this group, histological deterioration is slow. Collins et al. (2002) identified three initial scenarios for bone diagenetic history, (1)

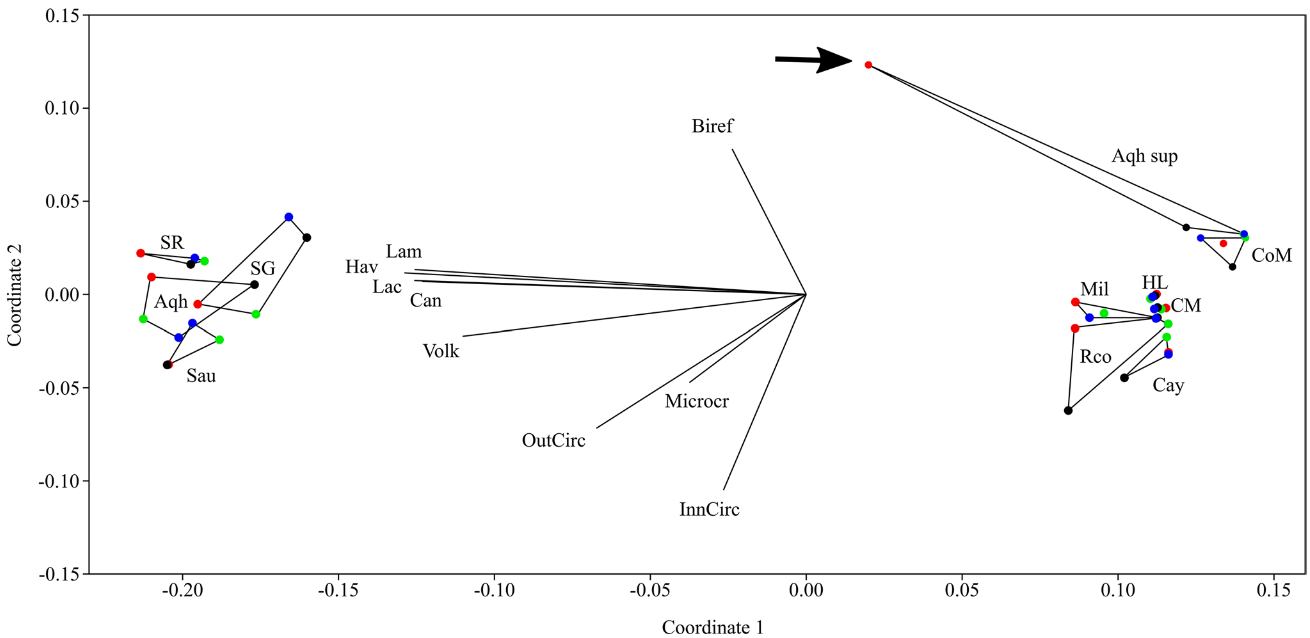


Fig. 2 Bivariate plot resulting from a non-metric multidimensional scaling analysis (nMDS). Each point on the plot corresponds to each sector of a thin bone section analyzed (anterior, blue; posterior, red; lateral, black; medial, green). They are linked by convex hulls per sample site. Vectors correspond to each histological variable, and their relative lengths are proportional to the magnitude of the correlation with the coordinates. Notice that the point corresponding to the posterior sector of the AqhSup thin section (indicated by an arrow) departs from the other points of the same bone. References: Biref, birefringence; Lam, lamellae; Hav, Haversian canals; Lac, osteocyte lacunae; Can, canaliculi; Volk, Volkmann's canals; InnCirc, inner circumferential lamellae; OutCirc, outer circumferential lamellae; Microcr, microcracks

Table 4 Non-parametric Spearman rank correlations between each coordinate and the histological variables

Histological variables	Coordinate 1		Coordinate 2	
	r	p	r	p
Lamellae	−0.886	0.000	0.026	0.867
Canaliculi	−0.86	0.000	0.018	0.907
Osteocyte lacunae	−0.909	0.000	−0.058	0.709
Haversian canals	−0.849	0.000	0.05	0.747
Volkmann canals	−0.764	0.000	−0.254	0.097
Outer circumferential lamellae	−0.503	0.001	−0.593	0.000
Inner circumferential lamellae	−0.305	0.044	−0.867	0.000
Microcrack	−0.391	0.009	−0.432	0.003
Birefringence	−0.119	0.441	0.651	0.000
GHI	−0.823	0.000	−0.32	0.034
OHI	−0.54	0.000	0.17	0.271

Table 5 Spearman's correlation between each coordinate of the histological ordination and geoenvironmental variables

Geoenvironmental variables		Coordinate 1		Coordinate 2	
		r	p	r	p
Chemical	pH	−0.481	0.005	0.392	0.026
	OM	0.171	0.349	−0.581	0.000
	P	−0.017	0.926	−0.23	0.205
	$CaCO_3$	0.075	0.683	0.03	0.872
Textural	Texture	0.196	0.282	0.107	0.558
	Sorting	−0.259	0.152	0.596	0.000
Climatic	AAT	−0.666	0.000	0.184	0.233
	MinT	−0.384	0.01	0.292	0.055
	MaxT	−0.331	0.028	0.429	0.004
	AAP	0.432	0.003	−0.321	0.034
Other geoenvironmental	HdW	0.046	0.768	0.113	0.467
	VdW	0.295	0.052	−0.027	0.861
	masl	0.511	0.000	0.096	0.536

slow (chemical) loss of protein is likely in environments that promote the stability of the mineral phase and where bacterial attack is limited, resulting in a slow process of chemical degradation. This leads to good preservation of remains, including microstructure; (2) rapid chemical deterioration of the mineral phase (dissolution), contrary to the previous one, occurs in environments that are not conducive to the mineral phase remaining stable. If the deterioration is followed by mineral replacement, it favors the survival of the remains, but with poorly preserved histology; if not, it leads to bone loss; (3) rapid deterioration of the organic phase by biodegradation. It occurs in environments in which the attack of bacteria on bones is intense, optimized by near-neutral pH values. This alternative, which begins with the consumption of proteins by bacteria, culminates in the destruction of the bone. Aqh pattern can be paralleled to pathway 1 due to the presence of well-preserved histology without alteration by bioerosion. Pathway 3 of this model could be the case of HL and CoM (OHI 1 and 2, respectively) that, although sharing similar geoenvironmental characteristics with the well-preserved cluster, they are the worst preserved group due to bacterial attack. There are other samples such as Cay, Mil, and CM that are from even more arid areas and are poorly preserved by bioerosion. Although bioerosion would leave the bone more vulnerable to the effects of other diagenetic agents, these sites, which are the oldest in the sample (2000–4000 years old), show nevertheless almost the same quality of preservation as younger (post-contact) samples of the "badly preserved" group. This argument reinforces the idea that the semi-arid to arid geoenvironments would slow down the histological deterioration.

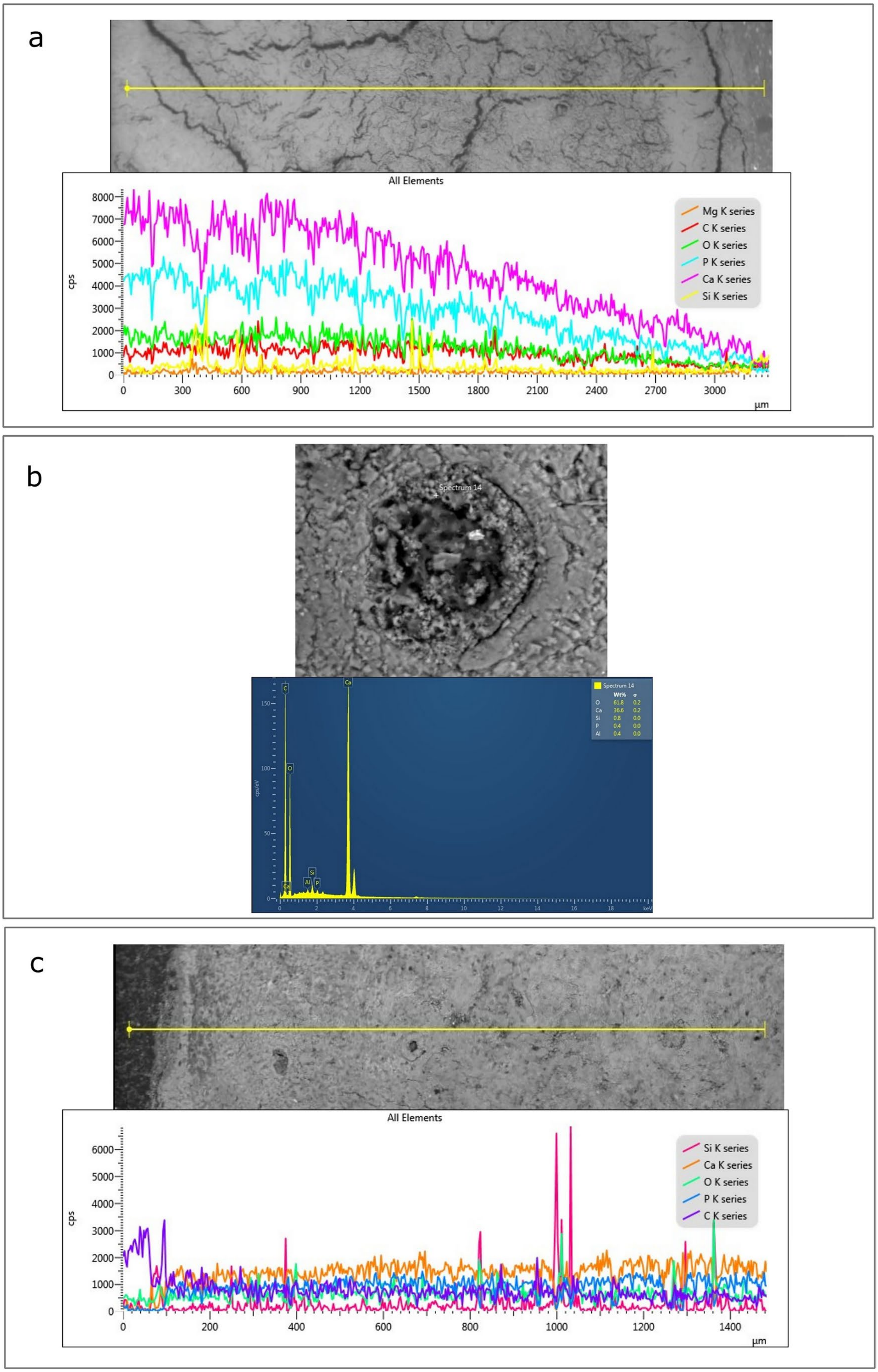
The remaining seven samples that share a poor preservation come from sites characterized by wetter climate, lower average annual temperatures, neutral to acid soils with a higher percentage of organic matter, and a higher altitudinal position compared to the previous group. GHI values are between 0 and 1, birefringence is reduced, or absent, and microstructural features are difficult or impossible to discern. All but two (AqhSup and CM) are altered by bacterial attack. In this group, it seems that bioerosion was so pervasive as to eliminate or blur all other microtaphonomic evidence.

Under the conditions that characterize the second group, bones would not be likely to survive for a long time, especially if they were affected by bacterial attack. Although the effect of bacterial attack is conspicuous in this group, the SEM analysis of the Rco sample reveals loss of the mineral component of the bone (bone dissolution, Fig. 3a). This rapid chemical deterioration of the mineral phase (pathway 2 of Collins et al. 2002) is expected in environments that are not conducive for the stability of mineral phase since hydroxyapatite increases its solubility as pH decreases (Mays 2010) and is consistent with the acid nature of substrate from which these bones were recovered. However, in the Rco sample, bioerosion was also observed (pathway 3 of rapid (microbial) loss of protein; Collins et al. 2002). As suggested in previous contributions (Collins et al. 2002; Nielsen-Marsh and Hedges 2000; Turner-Walker 2008), mineral dissolution could enable degradation by bacterial activity. Regarding the corrosion reported by SEM, the available evidence is not enough to discern if bone degradation is mainly due to bacterial bioerosion or geoenvironmental factors.

The occurrence of staining (a typical feature from bones of the well-preserved group) is usually linked with soil properties (Hollund et al. 2012; Kendall et al. 2018; Turner-Walker 2008). Sediments of Sitio Retamal and Sitio Grande burials are slightly reddish, and bones unearthed from them have similar features in their surfaces, as was observed in the bone at a macroscopic level on SR (Table 1) and could be responsible for the microscopic staining.

Post-diagenesis biostratinomy impacts

The skeletal remains of the Aquihuecó site prevailed into Late Holocene sandy deposits for more than 3000 years until wind action caused the denudation of the sand-dune. Bone remains became exposed to weathering that provoked their disarticulation and dispersal (Della Negra and Novellino 2005; Della Negra et al. 2009; Gordón et al. 2019). AqhSup is a femur collected from the surface, in the vicinity of the burial area. The bone was subaerially exposed (i.e., under the realm of the biostratinomy) for no longer than 6 years, the time of the previous visit to the site. Contrasting with its microscopic deterioration, which places this sample together with the poorly preserved ones in the multivariate ordination, the macroscopic preservation of this sample is exceptionally well. However, Aqh sample (the not-naturally exhumed), which shared most of its history with AqhSup, stands in the group of histologically well-preserved bones and significantly departs from the latter in the ordination. Evidence shows that after 6 years of weathering, bone histology of this sample decayed to the point that the microstructures are not recognizable and the bone organic and inorganic components are totally altered (GHI = 0, Table 3). The birefringence in AqhSup was slightly reduced compared to Aqh sample, and the effect of biostratinomy did not lead to the appearance of microcracks for the time lapse of the exposure (Table 3). The posterior sector of this sample shows better preservation than the other three (Fig. 2, Table 3), possibly because the bone laid on that side over the ground, allowing some isolation from the weathering in that area. Despite the fact that wind action is considered a predominant erosion factor on the sandy surface where skeletons were buried, it is unlikely that a bone such as a femur (with low sphericity) has been constantly rotated, exposing its four sides for the same time period. Another fact that results from this natural experiment is that histological degradation took a few years for the previously diagenized bone, and hence, it can be inferred that total destruction would take a few more years. In this way, the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cps
8000
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Spectrum 14
Wt% σ
O 61.8 0.2
Ca 36.6 0.2
Si 0.8 0.0
P 0.4 0.0
Al 0.4 0.0
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◂**Fig. 3** SEM analysis. Backscattered image and EDS spectrum **a** line scan of Remeco sample indicates a decrease of P and Ca elements from the medial portion to the outer cortical layer; **b** single-point spectrum into a Haversian canal of Aquihuecó sample shows peaks of C, Ca, and O; **c** Hermanos Lazcano line scan shows filled cavities and pores with silicon grains

temporal window opened from the moment an archaeological bone is visible until its complete destruction is possibly very short. This has consequences on the finding of archaeological human remains, especially in regions or periods with low density of population.

Conclusions

This study documents the first report on the microscopic preservation of archaeological human bones from Northwestern Patagonia, Argentina, as well as the geoenvironmental impact on its diagenetic taphonomic trajectories. Small sample sizes and missing data preclude conclusive statements but permitted us identifying patterns and proposing future lines of investigation. This approach was facilitated through a multidisciplinary viewpoint and enhanced by powerful multivariate analysis that allows a better understanding of the taphonomic processes. In this way, we found a clear pattern of preservation, defined by two situations: a well preserved one from semi-arid to arid geoenvironmental context, and a worse preserved one, from a wetter region. In this sense, geoenvironmental characteristics could be considered major factors of microscopical preservation. However, bioerosion, another key factor in bone preservation according to many authors (Fernández-Jalvo et al. 2010; Jans et al. 2004; Kendal et al. 2018; Nielsen-Marsh et al. 2007; Smith et al. 2007; Turner-Walker 2019), did not show a clear association with the preservation pattern. This is an interesting fact that should be deeply explored in the future, expanding the SEM analysis to the whole sample.

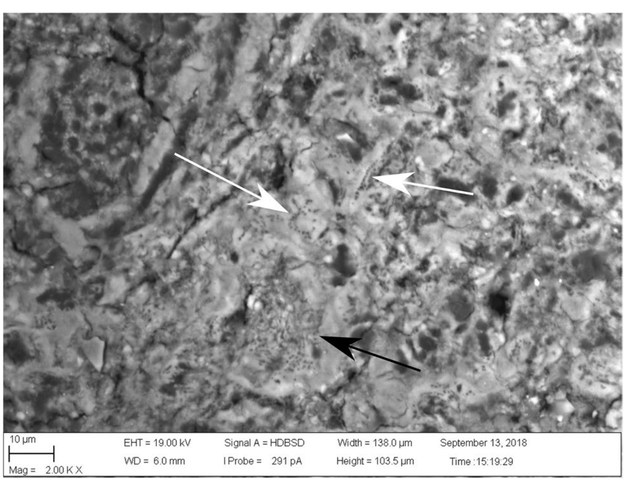

Fig. 4 SEM microphotograph. Bone section of HL detail of bacterial attack (small pores indicated by white arrows and thin channels indicated by black arrow)

Macroscopic preservation does not always match microscopic preservation. The samples analyzed in this work suggest that in semi-arid to arid climates, the post-diagenesis biostratinomy has a severe effect on bone histology in a short time period. An apparently very well-preserved bone found on the surface was extremely damaged at the histological level. It was also observed that histological alterations can occur rapidly during early diagenesis mainly linked to microbial attack. These observations are important considering the lack of human remains in this windy region for the earliest population periods (Gordón et al. 2019). This work fills the need of taphonomic information to improve demographic models for Patagonia, such as Perez et al. (2016a, 2016b) that are mostly based on theoretical taphonomic corrections. The fast destruction of human bones exposed to weathering described here might enrich models of human remains survival, distinguishing time of bone destruction in open-air sites that is different from rockshelter or cave sites, as pointed out by Gordón et al. (2019).

Microstructural analyses on human bones have been shown to be very important to understand their taphonomic histories. Histological and electron microscopic techniques in addition to the multivariate statistical approach allowed us to assess the effect of geoenvironmental context on microstructure of bone. The knowledge of the preservation pattern will be enriched by the incorporation of intrinsic variables such as mineral density, which we hope to include in future contributions. We are aware of the fact that our selection criterion of samples did not discard differences in DMO or non-visible diseases. However, our results are extremely useful for describing the taphonomical pattern as a first histological approach in Patagonian human archaeological samples. Further analysis of samples from different environments will lead to a deeper comprehension of the taphonomic histories and the pattern of bioarchaeological record in Patagonia.

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Table 6 Synthesis of histotaphonomic traits of the two preservation patterns detected and their association with geoenvironmental variables

		Good histological preservation	Poor histological preservation
Geoenvironmental characteristics	pH	Alkaline soils	Neutral to acid soils
	Organic matter	Low content	High content
	Sorting	Well-sorted sediments	Badly sorted sediments
	Average annual temperature (ºC)	12–14	8–12
	Precipitation (per year)	< 500 mm	500–1000 mm
	Altitude (masl)	300–1000	> 1000
Histotaphonomic traits	GHI	3–4	0–1
	OHI	5	1—2—3—5
	Microcracks	Moderately present	Slightly present
	Staining	Moderately present	Slightly present
	Birefringence	Mostly normal	Reduced or absent

Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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