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A SEM-based assessment of bioerosion in Late Holocene faunal bone assemblages from the southern Pampas of Argentina

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ABSTRACT

Over the last decades, research on microbial bioerosion affecting archaeological bone assemblages highlighted the fact that this is a significant factor determining the long-term survival of vertebrate hard tissues as well as the quality of the biological information retrievable from them (e.g. isotopic, genetic, histomorphological). In spite of this, information about bioerosion is still scarce or inexistent for most regions around the world. Among the likely causes of this situation are the perceived and factual technical difficulties that surround the implementation of a standard research on this subject. Taking this into account, the aim of this paper is twofold: on the one hand, to describe a protocol for the preparation of bone samples (thick sections) suitable for observation with BSE-SEM that fulfils the criteria of simplicity, low cost and effectiveness; on the other hand, to present and discuss the first results derived from the application of such protocol to artiodactyl bone samples recovered at different Late Holocene archaeological sites from the southern Pampas of Argentina. The obtained results indicate that the implemented technique was effective in terms of providing good quality information at a very low cost as measured from the resources (time and materials) invested. In addition, the results show that a significant part of the analysed specimens exhibit extensive and intensive histological alteration compatible with the action of bacteria, which is unexpected in light of the currently prevailing model about the origin and conditions of the bacterial attack on animal bones in archaeological deposits (i.e. the so-called “endogenous model”).

1. Introduction

Vertebrate bone is a composite tissue with a multiscalar hierarchical structure (Rogel et al., 2008; Turner-Walker, 2008; Weiner, 2010). Diagenetic processes affecting bone, which comprise a broad set of agents and conditions (Collins et al., 2002; Hedges and Millard, 1995; Hedges et al., 1995; Lyman, 1994; Turner-Walker, 2008; Tütken and Vennemann, 2011; Von Endt and Ortner, 1984), manifest at different structural levels. In the case of the loss of histological integrity, which is primarily noticeable at the microstructural and sub-microstructural levels (i.e. 10–500 μm; Rogel et al., 2008), the main causal factor is microbial activity that, in continental environments, comprises the destructive and transformative action of bacteria (including cyanobacteria in freshwater settings; Davis, 1997; Pesquero et al., 2010; Turner-Walker, 2012) and, allegedly, fungi (Jans et al., 2004) (For a review about the current knowledge on microbiological attack to bone in sea environments, see Bell and Elkerton, 2008). For this reason, the suite of physical and chemical changes affecting bone histology (i.e. tunnelling, destruction of localized areas of the bone microstructure, removal of the collagen, and reprecipitation of the mineral in hypermineralised areas at the edge of the areas of destruction; Hackett, 1981) is included within the general category of “bioerosion” (Booth, 2016; Hollund et al., 2014; Jans, 2008; Nielsen-Marsh and Hedges, 2000; Turner-Walker, 2012; Turner-Walker and Jans, 2008). This is one of the three diagenetic pathways identified for human and faunal bone (i.e. microbial attack or biodegradation; Smith et al., 2007), which is associated with decreasing histological preservation and increasing medium porosity (> 0.1 μm < 8.5 μm diameter; Smith et al., 2007; Turner-Walker et al., 2002), but moderate changes in mineral and collagen preservation (Smith et al., 2007).

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Microorganisms primarily alter the normal histology of bone by excavating tunnels throughout the compact tissue (Bell, 1990; Child, 1995; Garland, 1989; Hackett, 1981; Hanson and Buikstra, 1987; Hedges et al., 1995; Jackes et al., 2001; Jans, 2008; Jans et al., 2002, 2004; Turner-Walker and Jans, 2008). These tunnels, generically termed "microscopical focal destruction" or MFD (Hackett, 1981), have different morphologies that were classified by Hackett (1981) into four types: a) Wedl, b) linear longitudinal, c) budded; d) lamellate. The first type is attributed to fungi and cyanobacteria and the last three types are attributed to bacteria (Davis, 1997; Hackett, 1981; Jans, 2008; Turner-Walker, 2012; Turner-Walker and Jans, 2008; see, however, the cautionary note by Turner-Walker, 2012: 172, on the often unacknowledged difficulty of attributing organisms to structural changes). This classification was later revised by Jans (2008) who, on the basis of previous work by Davis (1997) and Trueman and Martill (2002), recognized six types of tunnelling: a) Wedl type 1; b) Wedl type 2; c) Hackett; d) linear longitudinal, e) budded; f) lamellate (for a detailed description of each type, see Jans, 2008: Fig. 1 and Table 1). Many of these features have a fine structure comprising numerous sub-micron tunnels (diameters between 400 nm and 800 nm) (i.e. sub-micron spongiform porosity; Turner-Walker et al., 2002), which are confined to discrete zones (10–40 μm across) enclosed by a hypermineralised rim (Bell et al., 1991, 1996; Jackes et al., 2001; Turner-Walker et al., 2002).

There are evidences that microstructural changes in bone due to microbial attack represent very immediate manifestations of diagenesis (Jans, 2008; Kontopoulos et al., 2016; Turner-Walker, 2012; Turner-Walker and Jans, 2008; Yoshino et al., 1991; cf. Fernández-Jalvo et al., 2010: 80), so they can be linked with burial conditions. Data from European Union countries spanning four climatic regions (Mediterranean, Continental, Maritime, and Subarctic) and different soil environments show that animal bones are less prone to be affected by bacterial attack than human bones (Jans et al., 2004). In faunal bone remains, fungal attack (inferred by the presence of Wedl tunnelling) is more common (Jans et al., 2004). These and other findings, particularly those from some observational and experimental studies (e.g. Mant, 1987; White and Booth, 2014) lead to some authors (Booth, 2016; Jans, 2008; Jans et al., 2004; Nielsen-Marsh et al., 2007; Smith et al., 2007) to propose that microbial bioerosion is controlled more by taphonomic factors associated with site usage (e.g. grave site vs. animal refuse deposit) than by the medium/long-term sedimentary environment. The so-called "endogenous model" of bioerosion (Bell et al., 1996; Booth, 2016; Child, 1995; Guarino et al., 2006; Hollund et al., 2012; Jans et al., 2004; Nielsen-Marsh et al., 2007; White and Booth, 2014), that has gained popularity in recent years, affirms that the most likely source of bone attacking bacteria in intentional and accidental burials is the gastrointestinal tract of decomposing human and animal bodies rather than the soil (see, however, Kontopoulos et al., 2016: 325 and Table 1). Most archaeological faunal bone remains enter the sedimentary deposits already disarticulated and devoid of other tissues (Lyman, 1994), then preventing the action of endogenous bacteria responsible for early diagenesis. It has been suggested that the resulting good preservation of animal bone makes it an attractive nutrient resource for saprophytic fungi present in the soil, which are dependent on certain microenvironmental conditions like the availability of oxygen and a certain level of humidity (20%) (Jans et al., 2004).

A better knowledge about microbial bioerosion in archaeological contexts is important since it has been shown that it: a) causes the loss of bone collagen and the alteration of mineral crystallinity (Child, 1995; Collins et al., 2002; Dobberstein et al., 2009; Hedges, 2002); b) promotes the introduction of bacterial and/or fungal DNA in bone (Hollund et al., 2014); c) increases bone porosity, which leads to (i) an accelerated rate of tissue decomposition (Hedges, 2002; Hedges and Millard, 1995; Hedges et al., 1995; Nielsen-Marsh and Hedges, 2000), (ii) an increment in the vulnerability of bone to other diagenetic processes (Jans et al., 2004; Smith et al., 2007), (iii) a reduction of bone strength (Turner-Walker and Parry, 1995), and (iv) an augmented susceptibility of bone to be contaminated with foreign materials (e.g. humic acids, exogenous DNA) that may cause problems in collagen and DNA extraction (Alaeddini et al., 2010; Colson et al., 1997; Gilbert et al., 2005; Van Klinken and Hedges, 1995). Due to these facts, microbial bioerosion is a significant factor determining the long-term survival of vertebrate bones as well as the quality of any preserved biological information contained within them (e.g. isotopic, genetic, histomorphological; Hollund et al., 2014; Turner-Walker, 2012).

The assessment of the degree of bone histological preservation can be accomplished by means of a variety of observational devices and techniques, including light microscopy (LM) (e.g. Booth, 2016; Fernández-Jalvo et al., 2010; Gutiérrez, 2001; Hackett, 1981; Hanson and Buikstra, 1987; Hedges et al., 1995; Hollund et al., 2012; Jans et al., 2002; Stout, 1978), transmission electron microscopy (TEM) (e.g. Ascenzi and Silvestrini, 1984; Hackett, 1981; Pesquero et al., 2010) and scanning electron microscopy (SEM), using both secondary electron images (SEI) (e.g. Arenas Alatorre et al., 2007; Barrientos, 1997; Barrientos et al., 2016; Fernández-Jalvo et al., 2010; Galligani, 2013; Grupe, 1995; Hackett, 1981; Hu et al., 2006; Morales et al., 2014; Pesquero and Fernández-Jalvo, 2014) and backscattered electron images (BSEI) (e.g. Bell, 1990; Bell et al., 1996; Fernández-Jalvo et al., 2010; Jackes et al., 2001; Jans et al., 2002; Pesquero and Fernández-Jalvo, 2014; Pesquero et al., 2010; Turner-Walker, 2012; Turner-Walker and Jans, 2008; Turner-Walker and Syversen, 2002). Undecalcified thin sections are invariably used for TEM (Hackett, 1981; Ascenzi and Silvestrini, 1984; Pesquero et al., 2010) and low-power LM work (e.g. Booth, 2016, 2017; Gutiérrez, 1998; Hedges et al., 1995; Kontopoulos et al., 2016). Undecalcified thick sections, both polished (e.g. Bell, 1990; Hackett, 1981; Turner-Walker and Jans, 2008); and unpolished (e.g. Barrientos, 1997; Barrientos et al., 2016; Hu et al., 2006), are used in almost all studies with SEM. A novel approach, tissue microarray analysis (TMA), based on the standardized comparative study of multiple decalcified and stained samples of bone, has been recently added to the literature (Barrios Mello et al., 2017), although it should be noted that there may be serious problems with using decalcified sections when looking at diagenetically altered bones since most of the interesting evidence (e.g. spongiform porosity, hypermineralisation) is lost.

Despite of the importance that a deep knowledge about the state of preservation of bones has in regional archaeological studies and the relatively long history of research on histological modification in ancient bone—that slowly but significantly increased after the pioneering work by Marchiafava et al. (1974) and Hackett (1981) [in fact, the earliest references to microscopical alteration of ancient bone by boring microorganisms are the works by Wedl, 1864 and Roux, 1887, but the truly systematic study of microbial bioerosion started in the 1970s and accelerated after the early 1990s, as a survey of the published literature shows]—, information about bioerosion is still scarce or non-existent for most regions around the world, even for those geographic areas in which archaeological taphonomy is a well-established practice (e.g. Patagonia). While different reasons may explain this situation in particular cases, one of the most likely general causes of the lack of popularization of research about bone bioerosion is the perceived and factual technical difficulties that surround its implementation. In effect, most of the technical procedures involved—particularly those aimed at obtaining polished thin or thick sections suitable for observation with LM, TEM or SEM—are time and resource consuming, require high levels of expertise and, above all, entail the access to appropriate facilities that are often unavailable in most archaeological labs (e.g. Turner-Walker and Mays, 2008). In order to make feasible the processing of a significant number of samples by specialized and unspecialized users alike, a simple, inexpensive, and effective standardized procedure is needed. Taking this into account, the aim of this paper is twofold. On the one hand, to describe a protocol for the pretreatment of bone samples suitable for observation with SEM, either using SEI or BSEI, that fulfil the above mentioned criteria of simplicity, low cost (measured in terms of time and materials), and effectiveness (evaluated in terms of quality and reliability of the information retrieved), which are basic components of the functional value of a product or procedure (e.g. Woodruff, 1997). On the other hand, to present and discuss the results derived from the application of such protocol to samples recovered at different Late Holocene archaeological sites from the southern Pampas of Argentina, a region for which there are at least five antecedents in the literature about microscopical assessment of bioerosion (one on human bone and four on faunal bone), in all cases on assemblages from earlier time periods (i.e. Pleistocene, Early and Middle Holocene) (Barrientos, 1997; Gutiérrez, 1998, 2001; Gutiérrez et al., 2001; Tomassini et al., 2010). It is expected that the content of this paper will encourage both taphonomist and unspecialized archaeologist to engage in a more comprehensive description of the state of preservation of bone samples from different environmental settings and inferred taphonomic histories. This information is crucial to get a more complete understanding of the variation spectrum of bioerosion in relation to different combinations of intrinsic and extrinsic factors influencing bone histological integrity in archaeological deposits on a worldwide basis.

2. Materials and methods

2.1. Study area and sampled sites

The study area (Fig. 1) is located in the southern portion of the Humid Pampas (approximately 38°13′S and 62°43′W at the center of the map of Fig. 1). It comprises the northwest portion of Ventania, the highest hilly range of the Pampas, and the plains that extend southwards, including the basin of the Chasicó creek. The Humid Pampas is a grassland/steppe biome comprising a flat to slightly undulating surface landscape in east-central Argentina (Morrone, 2001). This area presents a temperate, almost dry climate, with predominant winds from the northeast, north and northwest, a mean maximum temperature between 21.1 °C and 23.7 °C and a mean minimal temperature between 6.8 °C and 9.9 °C, with winter frost and occasional snowfall, the latter particularly on the hills of Ventania. Precipitations, which are primarily under the control of eastern winds from the Atlantic Ocean (Schäbitz, 2003), are scarce (between 751.8 and 331 mm/year) gradually diminishing towards west and south.

Mollisol is the dominant soil order in the area (sub-orders Udolls and Ustolls) (Liu et al., 2012; Moscatelli and Pazos, 2000; SAGyP-INTA, 1990; for a detailed description, see INTA, 2015; Panigatti, 2010). They are developed on the aeolian Quaternary sediments that cover the plains (i.e. Pampean loess; Frenguelli, 1955; Teruggi, 1957), with materials formed by debris of weathered rocks and significant amounts of volcanic glass, product of the eruption of Andean volcanoes (Moscatelli and Pazos, 2000). A prominent feature of Pampean soils is the presence of a $CaCO_3$ enriched horizon that sometimes qualifies as petrocalcic horizon (Moscatelli and Pazos, 2000; Soil Survey Staff, 1999). The natural vegetation and partially the soil fauna have been deeply modified in the areas long utilised for crop production and cattle grazing. In the southwestern extreme of the area, in a dryer transitional zone with northeastern Patagonia, there are Entisols (Moscatelli and Pazos, 2000). According to another classification, soils are Phaeozem around Ventania, Kastanozem in the middle valley of the Chasicó creek, and Areonosol in the distal part of that water course (Moscatelli and Pazos, 2000).

The sampled archaeological sites—La Montaña 1 (LM1) (Catella, 2014; Oliva, 2017), Laguna de Puán 1 (LP1) (Oliva, 2017; Oliva et al., 1991a, 1991b), Laguna Los Chilenos 2 (LLChi2) (Barrientos et al., 1997; Catella, 2014; Oliva, 2017), San Martín 1 (SM1) (Catella, 2014; Oliva, 2017; Oliva et al., 1991a, 1991b, 2010), and Laguna Chasicó 1-2-3 (LCha1-2-3) (Catella, 2014)—are located in different environmental settings (Fig. 1; Table 1). All the selected elements, long bones of Artiodactyla including guanaco (*Lama guanicoe*) and Pampean deer (*Ozotocerus bezoarticus*), come from archaeological contexts of Late Holocene age (i.e. last 3000 ^{14}C years BP) (Table 2). (No specimen in the sample has been unambiguously determined as *O. bezoarticus*; however, it is likely that among the bones determined at the order level, i.e. Artiodactyla, some correspond to this species, particularly those of lesser size).

2.2. Sampling procedure

A total number of 50 samples were selected for this exploratory study (LM1 = 10; LP1 = 11; LLChi2 = 10; SM1 = 10; LCha1-2-3 = 9). With the aid of a handle mini drilling machine provided with a cutting wheel, a small sample of cortical bone (6 mm × 6 mm × shaft thickness) was extracted from each selected element (Fig. 2). Due to curation policy issues, the sampling procedure was carefully planned to be minimally invasive: only fragmented elements were selected and, in each case, the cuts for extracting the sample started at a pre-existing fracture front; after producing the longitudinal cuts corresponding to the lateral sides of the sample, a fresh transversal fracture was induced and then prepared to be observed with a BSE-SEM (see below). Previous to the extraction of the samples, each bone element was photographed

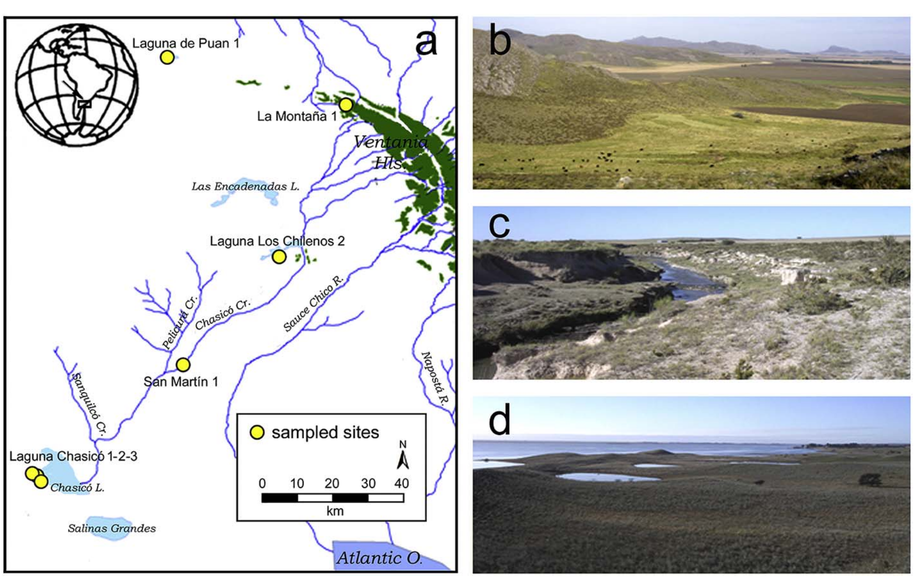

Fig. 1. Geographical location of the sampled archaeological sites and represented environments. a) Map of the study area with major physiographic features and sampling locations; b) piedmont; c) fluvial terraces; d) lakeshore.

Table 1
Descriptive variables of the sampled sites and their respective bone assemblages.

Site	Environment	Altitude (masl)	Soil order	Soil suborder	Mean pH	Soil salinity	Probability of erosion	^{14}C Age (years AP)	NISP	MNE	Bones with cutmarks
LP1	Piedmont/ lakeshore	220	Mollisol	Ustol	6.7	Low	Low	3330 ± 100	62	13	9
LM1	Piedmont	410	Mollisol	Udol	6.5	Low	Low	700 ± 40	59	15	1
LLChil2	Lakeshore	230	Mollisol	Ustol	9.0	Low	Low	2323 ± 50	143	22	38
LCha1-2-3	Lakeshore	-20	Entisol	Psament	9.4	High	High	n.d.	56	9	1
SM1	Fluvial terrace	115	Mollisol	Ustol	9.0	Low	High	2526 ± 50	582	146	37

and its general state of macroscopic preservation was assessed (i.e. good, regular, bad) on the basis of surface and structural modifications like weathering, root etching, fracturing/cracking, geological abrasion, and solution pitting or surface bone corrosion (Fernández-Jalvo et al., 2010; Gutiérrez, 2001; Lyman, 1994).

2.3. Sample pretreatment

The complete process, aimed at obtaining clean and dry samples for BSE-SEM examination, is illustrated in Fig. 2. It proceeded as follows:

Table 2
Bone samples: identification and analysed variables.

Sample N°	Site	Taxon	Bone element	Macroscopic state	OHI	ZSPHMB	MaPo	Me/MiPO	Hyph	AHCB
20	LCha1-2-3	Artiodactyla	Long bone shaft	2	4	0	1	0	0	1
21	LCha1-2-3	*Lama guanicoe*	Metapodial	1	5	0	0	0	0	1
22	LCha1-2-3	Artiodactyla	Long bone shaft	2	4	0	0	0	0	1
23	LCha1-2-3	Artiodactyla	Long bone shaft	2	3	0	1	1	0	1
24	LCha1-2-3	Artiodactyla	Long bone shaft	2	4	0	0	0	0	0
40	LCha1-2-3	Artiodactyla	Long bone shaft	2	4	0	0	1	0	2
41	LCha1-2-3	Artiodactyla	Long bone shaft	2	2	1	1	1	0	2
42	LCha1-2-3	Artiodactyla	Long bone shaft	2	4	1	0	1	0	1
44	LCha1-2-3	Artiodactyla	Long bone shaft	1	0	1	1	1	0	1
5	LLChi2	Artiodactyla	Long bone shaft	2	4	0	1	0	0	1
6	LLChi2	Artiodactyla	Long bone shaft	2	0	1	1	1	0	0
10	LLChi2	*Lama guanicoe*	Humerus	2	2	0	1	1	0	1
11	LLChi2	*Lama guanicoe*	Metatarsal	2	0	1	1	1	1	0
12	LLChi2	Artiodactyla	Long bone shaft	2	2	0	1	1	0	0
16	LLChi2	*Lama guanicoe*	Long bone shaft	2	4	0	1	0	0	0
17	LLChi2	Artiodactyla	Long bone shaft	2	4	0	1	0	0	0
18	LLChi2	*Lama guanicoe*	Radioulna	1	1	0	1	1	0	1
19	LLChi2	Artiodactyla	Long bone shaft	2	1	0	1	1	0	1
45	LLChi2	*Lama guanicoe*	Long bone shaft	1	0	1	1	1	0	0
26	LM1	Artiodactyla	Long bone shaft	0	0	0	1	1	1	1
27	LM1	Artiodactyla	Long bone shaft	0	0	1	1	1	0	2
28	LM1	Artiodactyla	Long bone shaft	0	0	1	1	1	0	1
29	LM1	Artiodactyla	Long bone shaft	0	0	1	1	1	0	2
30	LM1	Artiodactyla	Long bone shaft	0	0	1	1	1	0	1
46	LM1	Artiodactyla	Long bone shaft	1	0	1	1	1	0	2
47	LM1	Artiodactyla	Long bone shaft	0	2	1	1	1	0	0
48	LM1	Artiodactyla	Long bone shaft	1	0	1	1	1	0	1
49	LM1	*Lama guanicoe*	Tibia	1	0	1	1	1	0	1
53	LM1	Artiodactyla	Long bone shaft	1	3	1	0	1	0	2
3	LP1	*Lama guanicoe*	Tibia	2	5	0	0	0	0	1
4	LP1	*Lama guanicoe*	Long bone shaft	2	4	0	1	0	0	1
13	LP1	*Lama guanicoe*	Humerus	1	4	0	0	0	0	0
14	LP1	*Lama guanicoe*	Femur	1	4	0	0	1	0	1
15	LP1	*Lama guanicoe*	Metacarpal	0	3	0	0	1	1	1
31	LP1	*Lama guanicoe*	Metapodial	2	4	0	0	1	0	1
32	LP1	*Lama guanicoe*	Humerus	2	4	0	0	1	0	1
33	LP1	*Lama guanicoe*	Tibia	1	4	0	0	1	0	1
34	LP1	Artiodactyla	Long bone shaft	1	4	0	0	1	0	1
35	LP1	Artiodactyla	Long bone shaft	1	4	0	0	1	0	2
56	LP1	Artiodactyla	Long bone shaft	2	1	1	1	1	0	1
2	SM1	*Lama guanicoe*	Metapodial	2	5	0	0	0	0	0
7	SM1	*Lama guanicoe*	Metapodial	2	3	0	1	0	0	0
8	SM1	*Lama guanicoe*	Metapodial	2	2	1	1	1	0	0
9	SM1	*Lama guanicoe*	Tibia	2	4	0	1	0	0	0
25	SM1	Artiodactyla	Long bone shaft	2	5	0	0	0	0	0
36	SM1	*Lama guanicoe*	Metatarsal	2	4	1	0	1	0	0
37	SM1	Artiodactyla	Long bone shaft	1	4	1	1	1	0	2
38	SM1	*Lama guanicoe*	Tibia	2	4	0	0	1	0	2
39	SM1	Artiodactyla	Long bone shaft	1	4	0	0	1	0	1
1	SM1	*Lama guanicoe*	Metapodial	2	2	0	1	0	0	1

Macroscopic State: 0 (bad), 1 (regular), 2 (good); OHI (Hedges et al., 1995); MaPo, Me/MiPO, PAHMB, and Hyph: 0 (absent), 1 (present); AHCB: 0 (unaltered), 1 (regularly preserved), 2 (significantly altered).

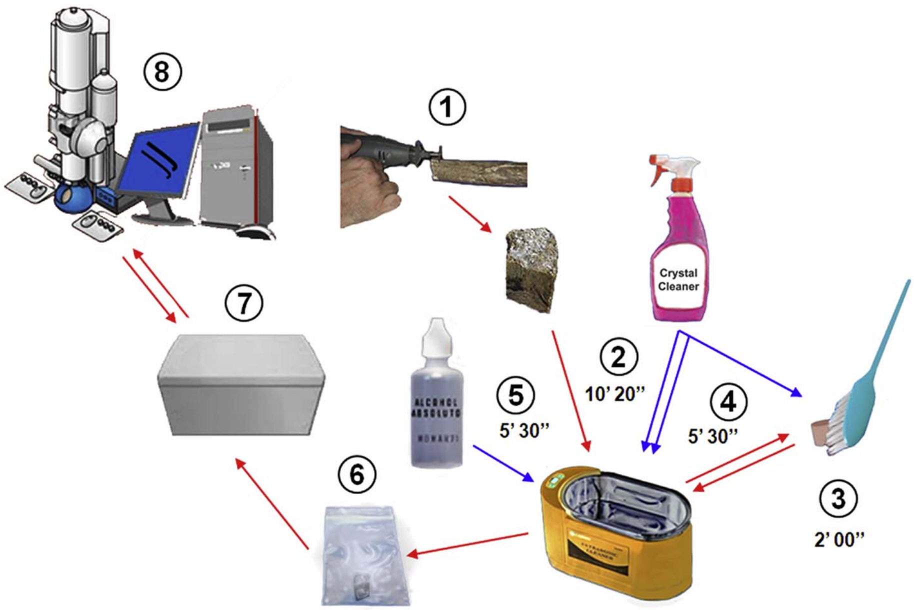

Fig. 2. Different steps of the sampling, cleaning, and observational procedures followed in this research. Encircled numbers indicate the sequential order of actions, and small numbers the time involved in specific actions: 1) bone thick section cutting; 2–5) sample cleaning; 6–7) sample storage; 8) BSE-SEM analysis.

1) Each sample was immersed in a vial filled with a commercial liquid crystal cleaner—whose constituents are water, propylene glycol n-butyl ether (pnb), alkyl polyglycosides (apg), anionic surfactant, sodium citrate, perfume, ammonium hydroxide, colorant, and preservative—; after that, the vial was put inside a portable ultrasonic contact lens cleaner (frequency 45 kHz) filled with water for 10 min and 30 s;
2) Subsequently, the sample was gently brushed with a soft brush for 2 min;
3) Then, the sample was again put into the vial filled with the crystal cleaner for another 5 min and 30 s round of ultrasonic vibration;
4) In the next step, the sample was rinsed with absolute alcohol and then put inside a clean vial filled with the same product for a 5 min and 30 s round of ultrasonic vibration;
5) Finally, the sample was put inside of a small open Ziploc bag in order to let the alcohol to evaporate; when the sample was dry, the bag was closed and stored in an expanded polystyrene container.

It should be noted that archaeological bone is a very variable material whose response to any experimental action depends, to a great extent, on its particular state of preservation. For this reason, the proposed protocol should not be followed mechanically, but introducing slight variations (e.g. time of exposition of each sample to physical agents like ultrasonic vibration or brushing) in relation to the state of each sample or set of samples in order to keep the procedure effective and innocuous to bone.

2.4. Observation

The samples were observed with a scanning electron microscope (SEM) JEOL JSM 6360 LV belonging to the *Servicio de Microscopía Electrónica de Barrido de la Facultad de Ciencias Naturales y Museo de la Universidad Nacional de La Plata* (SEM Facility, Natural Sciences and Museum Faculty, National University of La Plata, Argentina). The instrument is equipped with a secondary electron (SE) detector and a backscattered electron (BSE) detector. After several tests with both detectors (Barrientos et al., 2016), the observations were finally made with the BSE-SEM in two of its possible modes: compositional and shadow. The magnification of the BSEI taken typically ranged between 25 × and 700 ×, although some images at a higher magnification (up to 1600 ×) were also obtained. In all cases, the samples were observed without coating. Depending on the particularities of each sample, the images were obtained at a variable pressure (1–97 Pa), with an accelerating voltage of 10 keV, and a variable working distance (between 14 and 24 mm). When necessary, additional observations with a FEI Quanta 200 SEM coupled to an energy dispersive X-ray detector (EDAX) were made at the *Laboratorio de Investigaciones de Metalurgia Física* "*Ing. Gregorio Cusminsky*" *(LIMF)*, *Facultad de Ingeniería de la Universidad Nacional de La Plata* (Physical Metallurgy Research Laboratory "Ing. Gregorio Cusminsky", Engineering Faculty, National University of La Plata, Argentina).

2.5. Diagenesis assessment

Microbial bioerosion was assessed qualitatively and quantitatively. In the first case, BSEI were closely inspected in search for different types of MFD (both Wedl and non-Wedl; Hackett, 1981; Jans, 2008), as well as for other features characteristic of the sedimentary microenvironment and burial conditions like pyrite framboids (Turner-Walker, 1998a, 1998b, 2009, 2012; Turner-Walker and Jans, 2008), mineral infillings of natural bone pores (Fernández-Jalvo and Andrews, 2016), microorganisms (e.g. bacteria; Jackes et al., 2001) or part of microorganisms (e.g. fungal structures like hyphae, fruiting bodies, and spores; Jans, 2008). In the second case, degree of bioerosion was assessed using the standard Oxford Histological Index (OHI) (Hedges et al., 1995; Millard, 2001). Additionally, five other variables were recorded: 1) presence/absence of macroporosity (≥ 5 μm) (MaPo); 2) presence/absence of meso and microporosity (≤ 5 μm) (Me/MiPO); 3) presence/absence of discrete zones of spongiform porosity enclosed by a hypermineralised border (ZSPHMB); 4) degree of alteration of the Haversian canal border (AHCB) (i.e. unaltered, regularly preserved, significantly altered); 5) presence/absence of fungal hyphae (Hyph) (Fig. 3).

2.6. Statistical analysis

Basic descriptive statistics were computed for the different variables recorded as well as nonparametric rank-order correlation coefficient (Spearman R, $\alpha = 0.01$) for the comparison between the values of OHI and the degree of macroscopic preservation of each sample.

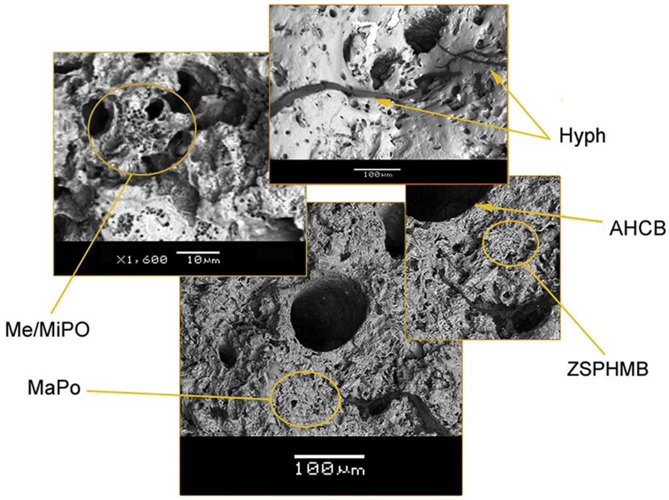

Fig. 3. Examples of the five variables recorded describing different aspects of histological modification attributable to microbial activity: macroporosity (≥ 5 µm) (MaPo); meso and microporosity (≤ 5 µm) (Me/MiPO); discrete zones of spongiform porosity enclosed by a hypermineralised border (ZSPHMB); degree of alteration of the Haversian canal border (AHCB); fungal hyphae (Hyph).

3. Results

3.1. Qualitative analysis

Only three types of MFD were found: linear longitudinal, budded, and lamellate. In those specimens with extensive alteration of normal histology (OHI ≤ 3), many discrete zones of spongiform appearance enclosed by a hypermineralised border were found with both, sub-micron and above-micron porosity (Fig. 4a and b). No Wedl tunnels were detected, although hyphae occupying Haversian canals were identified in three specimens: sample 11 (LLChil2), sample 15 (LP1), and sample 26 (LM1) (Fig. 5a). In one specimen (sample 37, SM1), a group of small spheres with a granular-textured surface were detected inside of a Haversian canal, likely corresponding to pyrite framboids (Fig. 5b). In two specimens—sample 9 (SM1) and sample 38 (SM1)—crystals of different morphology (regularly elongated and angular in sample 9 and amorphous in sample 38) were detected in the inner margin of Haversian and Volkmann's canals (Fig. 6a and b). In both cases, EDAX spectra suggest a calcium carbonate composition (e.g. calcite). In some crystals, minor quantities (At.% < 1.5) of elements other than C, O, and Ca were found (e.g. Al, Fe).

3.2. Quantitative analysis

Fig. 7a shows that, in the total sample, the most represented values of the OHI are 4 (moderately well preserved microstructure) and 0 (absence of recognizable microstructural features others than Haversian canals). Intermediate values of the index are less represented. At the level of individual sites, there are clear differences in histological preservation patterns. In Fig. 7b, the frequencies are represented as percentages of two groups of index values: G1 (OHI ≥ 4), representing general good histological integrity and G2 (OHI ≤ 3), representing general bad histological preservation. The best preserved assemblages are those from LP1, SM1, and LCha1-2-3 and the poorest preserved those from LM1 and LLChi2. A moderate but significant positive correlation between the values of OHI and the degree of macroscopic preservation was found (Spearman R = 0.41; $p < 0.01$).

4. Discussion

From a technical point of view, the results of this exploratory study are satisfactory and encouraging since they show that the sampling procedure, the pretreatment protocol, and the selected observational technique were effective in terms of providing good quality information at a very low cost as measured from the resources (time and materials) invested. It seems that the suite of procedures described in this paper is a good choice for a study aimed at obtaining a first rapid evaluation of the general state of microstructural preservation of one or more bone assemblages. On the basis of such evaluation, samples requiring a more detailed study can be selected for further examination with other techniques (e.g. BSE-SEM observation of polished thick sections, EDAX analysis).

Regarding the sampled assemblages, there is no apparent relationship between the degree of bone microstructural preservation and the kind of landscape setting from which the bones come from. The two worst preserved assemblages, LM1 and LLChil2, come from very different environments (piedmont and lakeshore, respectively); in a similar way, the three best preserved groups of bones—LP1, SM1, and LCha1-2-3—were recovered at equally disparate settings (piedmont/lakeshore, fluvial terrace and lakeshore, respectively). At the same time, there is an internal diversity in the degree of bone preservation at each site: while two sites, LM1 and LP1, present a clear tendency either to destruction or preservation, the other three do not exhibit such an obvious pattern. The causes of the observed differences are still unknown and they will be the subject of future investigations.

One major finding is that some observations are consistent with the inferred taphonomic history of the involved assemblage. This is the case with the bones from San Martín 1, a site located in a fluvial terrace in the middle basin of the Chasicó creek. In this site, most of the guanaco bones as well as other archaeological materials were found in small depressions filled with a dark, muddy sediment containing numerous snail shells belonging to the genera *Austroborus*, *Biomphalaria*, *Heleobia*, and *Succinea* that inhabit freshwater, low energy environments (Oliva et al., 1991a, 1991b). On the basis of a taphonomic study of this assemblage, Oliva et al. (2010) concluded that the water had played an important role in the selection and accumulation of skeletal parts after site abandonment (for a detailed taphonomic and zooarchaeological analysis of this site, see Morales, 2015). This archaeological assemblage presents a fairly good state of preservation of bone macro and microstructure (Figs. 6 and 7; Table 2). The most remarkable microscopic

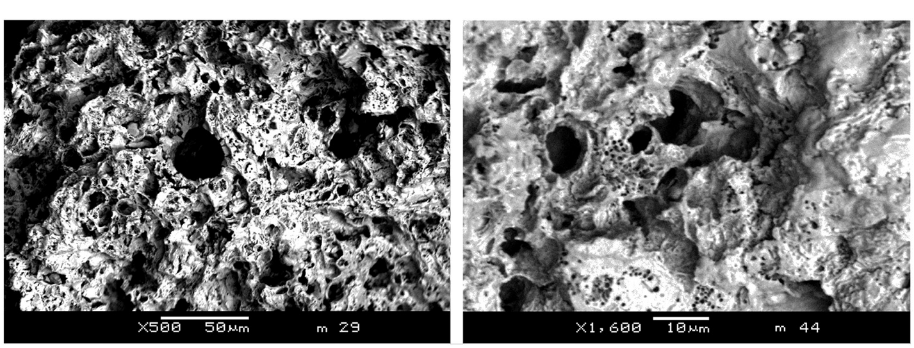

Fig. 4. Backscatter electron images (BSEI) of bone samples exhibiting intensive histological alteration (OHI = 0): a) sample 29 from La Montaña 1 (LM1) (500 ×), with presence of macro and mesoporosity as well as many discrete zones of spongiform appearance enclosed by a hypermineralised border; no histological structures are identifiable with the exception of Haversian canals with severely altered borders; b) sample 44 from Laguna Chasicó 1-2-3 (LCha1-2-3) (1600 ×), with several areas with both, submicron and above-micron porosity.

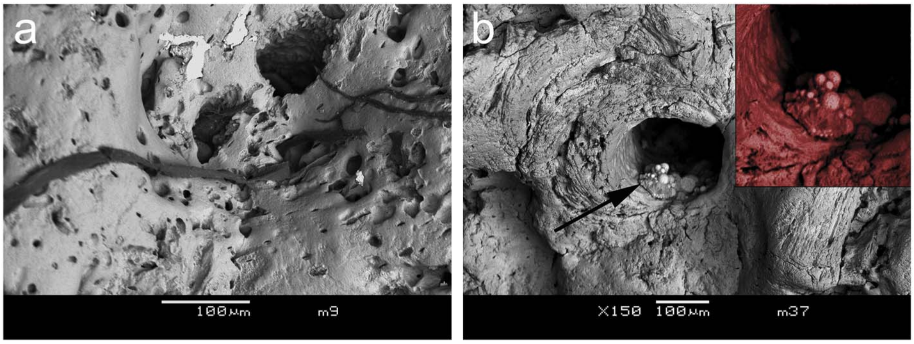

Fig. 5. Backscatter electron images (BSEI) of bone samples exhibiting the presence of foreign materials originated in the burial environment: a) Sample 9 from San Martín 1 (SM1) (250 ×) with hyphae emerging from Haversian canals; b) Sample 37 from San Martín 1 (SM1) (150 ×) with pyrite framboids (arrow) inside a Haversian canal; in the enlarged coloured image, the typical granular-textured surface can be observed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

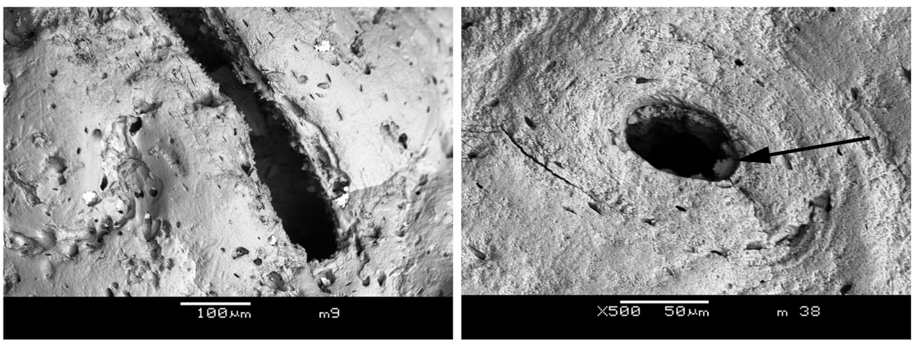

Fig. 6. Backscatter electron images (BSEI) of bone samples showing the growth of several crystals inside natural pores: a) Sample 9 from San Martín 1 (SM1) (200 ×) with many elongated and angular crystals along the inner border of a Volkmann's canal; b) Sample 38 from San Martín 1 (SM1) (500 ×) with amorphous crystals inside a Haversian canal. In both cases, the degree of histological preservation is remarkable (OHI = 4).

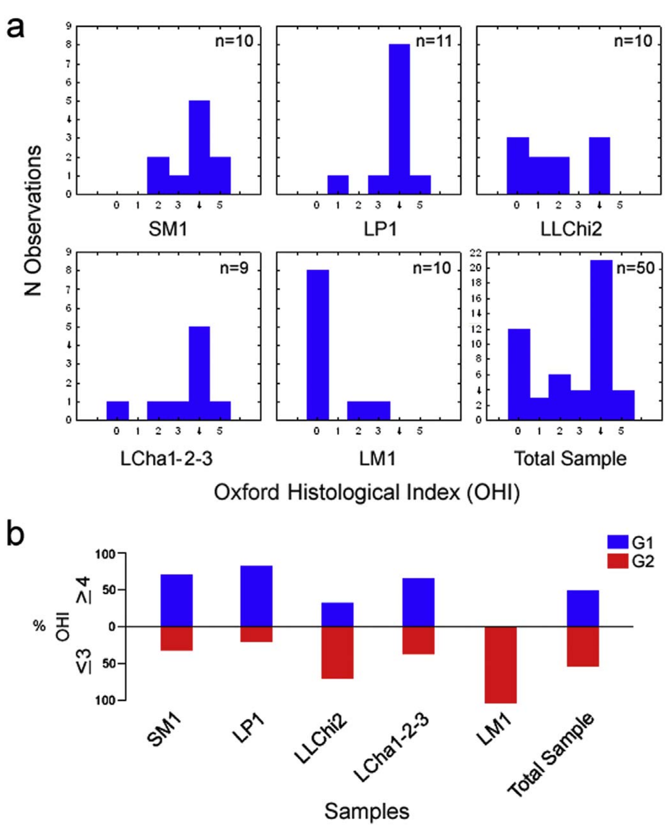

Fig. 7. a) Categorised histograms showing the frequency distributions of OHI values for each of the sampled sites and for the total sample; b) the frequency distributions of OHI values for each site are represented as percentages of two groups of index values: G1 (OHI ≥ 4), representing general good histological integrity and G2 (OHI ≤ 3), representing general bad histological preservation.

features detected are the presence of pyrite framboids and crystals inside the natural bone pores (Figs. 5b, 6a, and b). The relative absence of microbial tunnelling and the presence of framboids in the natural porosity suggest that bones were buried in waterlogged sediments shortly after the death of the animals (Turner-Walker, 2012: 179), which is compatible with the taphonomic history inferred for the assemblage.

Approximately 50% of the bone samples present extensive and/or intensive microstructural damage consisting in isolated and coalescent MFD (linear longitudinal, budded, and lamellate), with many discrete zones of spongiform appearance—with both sub-micron and above-micron porosity—enclosed by a hypermineralised rim (Hackett, 1981; Jans, 2008). While it is currently difficult to unambiguously relate different forms of bioerosion with specific agents (Turner-Walker, 2012), the pattern of the MFD and the dimensions of the diagenetic pores identified point to bacteria as the most likely causal factor (Jackes et al., 2001; Jans, 2008; Turner-Walker et al., 2002). The apparent absence of Wedl tunnelling—whose correct identification may have been impeded or made difficult by the tridimensional information contained in the images—also points to bacteria rather that fungi as the likely destructive/transformative agent. Whereas fungal action cannot be completely ruled out since hyphae were found intimately associated with bone microstructures—although the degree to which such finding is not a post-excavation artefact should be assessed in the future—, it is noteworthy the mention by Jans (2008: 401) of the fact that fungal structures like hyphae are regularly found in archaeological bone and that its presence is not necessarily associated with destruction of the bone microstructure, suggesting that fungi may use bone both as a source of nutrients and as a medium.

These findings are contrary to the expectations derived from the endogenous model of bacterial attack on bone (Bell et al., 1996; Booth, 2016; Child, 1995; Guarino et al., 2006; Jans et al., 2004; Nielsen-Marsh et al., 2007; Hollund et al., 2012; White and Booth, 2014) that predicts that animal bones, by virtue of its entry into archaeological deposits in a disarticulated, often broken way and already free from other tissues, are less susceptible than human bones of being invaded by bacteria, whose main source are the guts of the decomposing body. The results of this preliminary study are compatible with an exogenous model in which, at least in some environments or particular burial conditions like those represented in this sample, the bacteria likely responsible of bone bioerosion are those present in soils (Balzer et al., 1997; Dixon et al., 2008; Fernández-Jalvo et al., 2010; Grupe and Dreses-Werringloer, 1993; Grupe et al., 1993).

Regarding microbial bioerosion on mammal bones, the results of this study are indicative of the impact of such process in a temperate dry environment (i.e. the predominant climatic conditions in the study area during the last 3000 years; Tonello and Prieto, 2010). The three other studies on bioerosion available for the Pampas that consider animal bone assemblages buried under different climatic and environmental conditions, present a rather diverse picture. The study by Tomassini et al. (2010) on a paleontological sample of Late Pleistocene mammals (most of them megamammals) naturally buried in an ancient floodplain environment (Playa del Barco site) under cooler and presumably dryer conditions, indicate the absence of extensive microbial attack on bones, but the regular presence of recrystalised apatite, manganese oxides and calcium carbonate crystals inside natural pores. The study by Gutiérrez (1998, 2001) on a sample of bones of Middle Holocene age (humid and warmer conditions) from a floodplain environment (Paso Otero 1 site, Quequén Grande river basin) indicates a generally good state of preservation of the assemblage (≈70% of bones with a relatively well preserved histology), although no indication is given about the nature of the microbial alteration found in the less well preserved segment of the sample (≈30%) [Some images published by Gutiérrez (1998: Figs. 3.5 and 3.6), however, suggest the presence of Wedl tunnelling coexisting with non-Wedl MFD]. Finally, the comparative study by Gutiérrez et al. (2001) of different sites from the Quequén Grande floodplain (Paso Otero 1, 3, and 5; Early and Middle Holocene) shows that, in terms of the mean values of the histological index utilised by the authors—that differ from the OHI used in this study (Gutiérrez, 1998)—, there are some slightly differences in the histological preservation of the investigated assemblages but within a general picture of a low degree of histological alteration.

Due to the fact that some differences in the impact of bioerosion on bone assemblages can be found within a same region under different climatic regimes and environmental conditions and that there are archaeological and experimental clues that, at least, some of the assumptions, results, and interpretations of the pioneering large-scale study by Jans et al. (2004), Nielsen-Marsh et al. (2007), and Smith et al. (2007) on a European Union sample are not universally valid (Fernández-Jalvo et al., 2010; Kontopoulos et al., 2016; this study), an expansion of taphonomically oriented research on bone bioerosion allowing for the conformation of a larger and inclusive database with a worldwide coverage is urgently needed.

5. Concluding remarks

This paper represents the first contribution of an ongoing research aimed at the attainment of two main objectives: 1) to develop a simple, economic, and effective procedure enabling the processing of a significant number of bone samples suitable for observation with BSE-SEM, and 2) to assess the differential impact of microbial bioerosion in five Late Holocene samples of artiodactyls bones from different sites and environments from the southern Pampas of Argentina. In both cases, our preliminary results are encouraging since (i) the relationship between the costs of the chosen methodology, measured in terms of time and materials, and the effectiveness of the procedure, measured in terms of the quality of the retrieved information is, at this point, satisfactory albeit perfectible; (ii) intra- and inter-site variation in histological preservation was found, with an unexpected preponderance of damage attributable, at least at this stage of the research, to bacterial attack.

From a methodological point of view, the next steps in this research will include: 1) the enhancement of the techniques of data recording, particularly through a more systematic and randomized selection of the observation fields in each specimen, which would allow for a more consistent estimation of the magnitude of the diagenetic alteration on an individual basis; 2) the development of simple techniques for surface polishing, which would likely increase the chances of detecting features of diagenetic interest.

From an empirical point of view, the subsequent steps will include: 1) the enlargement of sample size, particularly through the inclusion of more faunal assemblages from similar and different environments within the study area, which would allow to get a more complete knowledge about the variability in histological preservation at the regional level; 2) the inclusion of human samples in order to make comparisons about the kind and magnitude of microbial bioerosion affecting different taxa; 3) an in-depth analysis of the local conditions that would explain the patterned and unpatterned variation in bone histological preservation found at the site level.

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References

Alaeddini, R., Walsh, S.J., Abbas, A., 2010. Forensic implications of genetic analyses from degraded DNA—a review. Forensic Sci. Int. Genet. 4, 148–157. http://dx.doi.org/10.1016/j.fsigen.2009.09.007.

Arenas Alatorre, J.A., Sánchez Pérez, S., Escalona, A., Sterpone, O., Zorrilla, C., Gómez Serrano, A., 2007. Diagénesis en huesos humanos de la época colonial del estado de Hidalgo, México. Estudios Antropol. Biol. 13 (1), 361–380.

Ascenzi, A., Silvestrini, G., 1984. Bone-boring marine microorganism, an experimental investigation. J. Hum. Evol. 13, 531–536. http://dx.doi.org/10.1016/s0047-2484(84)80006-8.

Balzer, A., Gleixner, G., Grupe, G., Schmidt, H.L., Schramm, S., Turban-Just, S., 1997. In vitro decomposition of bone collagen by soil bacteria: the implications for stable isotope analysis in archaeometry. Archaeometry 39, 415–429. http://dx.doi.org/10.1111/j.1475-4754.1997.tb00817.x.

Barrientos, G., 1997. Nutrición y Dieta de las Poblaciones Aborígenes Prehispánicas del Sudeste de la Región Pampeana. (Unpublished doctoral dissertation) Facultad de Ciencias Naturales y Museo. Universidad Nacional de La Plata, La Plata.

Barrientos, G., Leipus, M., Oliva, F., 1997. Investigaciones arqueológicas en la laguna Los Chilenos, (provincia de Buenos Aires). In: Berón, M., Politis, G. (Eds.), Arqueología Pampeana en la Década de los '90. Museo de Historia Natural de San Rafael, San Rafael, pp. 115–125.

Barrientos, G., Sarmiento, P., Galligani, P.E., 2016. Evaluación de la diagénesis ósea mediante el uso de microscopía electrónica de barrido (MEB): aproximaciones analíticas aplicables a muestras arqueológicas. Rev. Argent. Antropol. Biol. 18 (2), 1–13. http://dx.doi.org/10.17139/raab.2016.0018.02.03.

Barrios Mello, R., Regis Silva, M.R., Seixas Alves, M.T., Evison, M.P., Guimarães, M.A., Arrabaca Francisco, R., Dias Astolphi, R., Sadayo Miazato Iwamura, E., 2017. Tissue microarray analysis applied to bone diagenesis. Sci. Rep. 7, article number 39987. http://dx.doi.org/10.1038/srep39987.

Bell, L.S., 1990. Palaeopathology and diagenesis: a SEM evaluation of structural changes using backscattered electron imaging. J. Archaeol. Sci. 17, 85–102. http://dx.doi.org/10.1016/0305-4403(90)90016-x.

Bell, L.S., Elkerton, A., 2008. Unique marine taphonomy in human skeletal material from the medieval warship the Mary Rose. Int. J. Osteoarchaeol. 18, 523–535. http://dx.doi.org/10.1002/oa.952.

Bell, L.S., Boyde, A., Jones, S.J., 1991. Diagenetic alteration to teeth in-situ illustrated by backscattered electron imaging. Scanning 13, 173–183. http://dx.doi.org/10.1002/sca.4950130204.

Bell, L.S., Skinner, M.F., Jones, S.J., 1996. The speed of post mortem change to the human skeleton and its taphonomic significance. Forensic Sci. Int. 82, 129–140. http://dx.doi.org/10.1016/0379-0738(96)01984-6.

Booth, T.J., 2016. An investigation into the relationship between funerary treatment and bacterial bioerosion in european archaeological human bone. Archaeometry 58 (3), 484–499. http://dx.doi.org/10.1111/arcm.12190.

Booth, T.J., 2017. The rot sets in: low-powered microscopic investigation of taphonomic changes to bone microstructure and its application to funerary contexts. In: Errickson, D., Thompson, T. (Eds.), Human Remains: Another Dimension. The Application of Imaging to the Study of Human Remains. Academic Press, London, pp. 7–28. http://dx.doi.org/10.1016/B978-0-12-804602-9.00003-5.

Catella, L., 2014. Movilidad y Utilización del Ambiente en Poblaciones Cazadoras-Recolectoras del Sur de la Región Pampeana: La cuenca del Arroyo Chasicó como Caso de Estudio. (Unpublished doctoral dissertation) Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata.

Child, A.M., 1995. Microbial taphonomy of archaeological bone. Stud. Conserv. 40, 19–30. http://dx.doi.org/10.2307/1506608.

Collins, M.J., Nielsen-Marsh, C.M., Hiller, J., Smith, C.I., Roberts, J.P., Prigodich, R.V., Wess, T.J., Csapò, J., Millard, A.R., Turner-Walker, G., 2002. The survival of organic matter in bone: a review. Archaeometry 44, 383–394. http://dx.doi.org/10.1111/1475-4754.t01-1-00071.

Colson, I.B., Bailey, J.F., Vercauteren, M., Sykes, B.C., Hedges, R.E.M., 1997. The preservation of ancient DNA and bone diagenesis. Anc. Biomol. 1 (2), 109–117.

Davis, P.G., 1997. The bioerosion of bird bones. Int. J. Osteoarchaeol. 7, 388–401. http://dx.doi.org/10.1002/(SICI)1099-1212(199707/08)7:4 < 388::AID-OA357 > 3.0.CO;2-H.

Dixon, R.A., Dawson, L., Taylor, D., 2008. The experimental degradation of archaeological human bone by anaerobic bacteria and the implications for the recovery of Ancient DNA. In: Greenblatt, C.L., Spigelman, M., Cipollaro, M., Nerlich, N.G., Witas, H.W. (Eds.), The Proceedings of the 9th International Conference on Ancient DNA and Associated Biomolecules, Pompeii, pp. 1–10.

Dobberstein, R.C., Collins, M.J., Craig, O.E., Taylor, G., Penkman, K.E.H., Ritz-Timme, S., 2009. Archaeological collagen: why worry about collagen diagenesis? Archaeol. Anthropol. Sci. 1, 31–42. http://dx.doi.org/10.1007/s12520-009-0002-7.

Fernández-Jalvo, Y., Andrews, P., 2016. Atlas of Taphonomic Identifications: 1001 + Images of Fossil and Recent Mammal Bone Modification. Vertebrate Paleobiology and Paleoanthropology Series Springer, Dordrecht. http://dx.doi.org/10.1007/978-94-017-7432-1.

Fernández-Jalvo, Y., Andrews, P., Pesquero, M.D., Smith, C., Marín-Monfort, D., Sánchez, B., Geigl, E.M., Alonso, A., 2010. Early bone diagenesis in temperate environments. Part I: Surface features and histology. Palaeogeogr. Palaeoclimatol. Palaeoecol. 288, 62–81. http://dx.doi.org/10.1016/j.palaeo.2009.12.016.

Frenguelli, J., 1955. Loess y limos pampeanos. In: Serie Técnica y Didáctica del Museo de La Plata 7. pp. 84–88.

Galligani, P.E., 2013. Tafonomía de los Entierros Humanos del Sitio Río Salado-Coronda II. (Unpublished undergraduate dissertation) Facultad de Humanidades y Artes. Universidad Nacional de Rosario, Rosario.

Garland, A.N., 1989. Microscopical analysis of fossil bone. Appl. Geochem. 4, 215–229. http://dx.doi.org/10.1016/0883-2927(89)90021-8.

Gilbert, T.P.M., Rudbeck, L., Willerslev, E., Hansen, A.J., Smith, C., Penkman, K.E.H., Prangenberg, K., Nielsen-Marsh, C.M., Jans, M.E., Arthur, P., Lynnerup, N., Turner-Walker, G., Biddle, M., Kjølbye-Biddle, B., Collins, M.J., 2005. Biochemical and physical correlates of DNA contamination in archaeological human bones and teeth excavated at Matera, Italy. J. Archaeol. Sci. 32, 785–793. http://dx.doi.org/10.1016/j.jas.2004.12.008.

Grupe, G., 1995. Preservation of collagen in bone from dry, sandy soil. J. Archaeol. Sci. 22, 193–199. http://dx.doi.org/10.1006/jasc.1995.0021.

Grupe, G., Dreses-Werringloer, U., 1993. Decomposition phenomena in thin-sections of excavated human bones. In: Grupe, G., Garland, A.N. (Eds.), Histology of Ancient Human Bone: Methods and Diagnosis. Springer, Berlin, pp. 27–36.

Grupe, G., Dreses-Werringloer, U., Parsche, F., 1993. Initial stages of bone decomposition: causes and consequences. In: Lambert, J.B., Grupe, G. (Eds.), Prehistoric Human Bone. Archaelogy at the Molecular Level. Springer, Berlin and Heidelberg, pp. 257–274. http://dx.doi.org/10.1007/978-3-662-02894-0.

Guarino, F.M., Angelini, F., Vollono, C., Orefice, C., 2006. Bone preservation in human remains from the Terme del Sarno at Pompeii using light microscopy and scanning electron microscopy. J. Archaeol. Sci. 33, 513–520. http://dx.doi.org/10.1016/j.jas.2005.09.010.

Gutiérrez, M.A., 1998. Taphonomic Effects and State of Preservation of the Guanaco (*Lama guanicoe*) Bone Bed From Paso Otero 1 (Buenos Aires Province, Argentina). (Unpublished Master's dissertation) Texas Tech University, Lubbock. https://ttu-ir.tdl.org/ttu-ir/handle/2346/15865 (accessed 27 Jan 2017).

Gutiérrez, M.A., 2001. Bone diagenesis and taphonomic history of the Paso Otero 1 bone bed, Pampas of Argentina. J. Archaeol. Sci. 28, 1277–1290. http://dx.doi.org/10.1006/jasc.2000.0648.

Gutiérrez, M.A., Martínez, G.A., Nielsen-Marsh, C.M., 2001. Alteración diagenética y preservación diferencial de los conjuntos óseos de la localidad arqueológica Paso Otero (Provincia de Buenos Aires, Argentina). Estud. Geol. 56, 291–299. http://dx.doi.org/10.3989/egeol.00565-6145.

Hackett, C.J., 1981. Microscopical focal destruction (tunnels) in exhumed human bones. Med. Sci. Law 21, 243–265. http://dx.doi.org/10.1177/002580248102100403.

Hanson, D.B., Buikstra, J.E., 1987. Histomorphological alteration in buried human bone from the Lower Illinois Valley: implications for palaeodietary research. J. Archaeol. Sci. 14, 549–563. http://dx.doi.org/10.1016/0305-4403(87)90038-0.

Hedges, R.E.M., 2002. Bone diagenesis: an overview of processes. Archaeometry 44, 319–328. http://dx.doi.org/10.1111/1475-4754.00064.

Hedges, R.E.M., Millard, A.R., 1995. Bones and groundwater: towards the modelling of diagenetic processes. J. Archaeol. Sci. 22, 155–164. http://dx.doi.org/10.1006/jasc.1995.0017.

Hedges, R.E.M., Millard, A.R., Pike, A.W.G., 1995. Measurements and relationships of diagenetic alteration of bone from three archaeological sites. J. Archaeol. Sci. 22, 201–209. http://dx.doi.org/10.1006/jasc.1995.0022.

Hollund, H.I., Jans, M.M.E., Collins, M.J., Kars, H., Joosten, I., Kars, S.M., 2012. What happened here? Bone histology as a tool in decoding the postmortem histories of archaeological bone from Castricum, The Netherlands. Int. J. Osteoarchaeol. 22 (5), 537–548. http://dx.doi.org/10.1002/oa.1273.

Hollund, H.I., Jans, M.M.E., Kars, H., 2014. How are teeth better than bone? An investigation of dental tissue diagenesis and state of preservation at a histological scale (with photo catalogue). Int. Archaeology 36http://dx.doi.org/10.11141/ia.36.7. (accessed 22 Nov 2016).

Hu, Y.W., He, D.L., Dong, Y., Wang, C.S., Gao, M.K., Lan, Y.F., 2006. Linear scanning analysis of prehistoric human bones in Xigongqiao site, Tengzhou, Shandong province by use of SEM-EDS. Spectrosc. Spectr. Anal. 26, 1179–1182.

INTA, 2015. Carta de Suelos de la Provincia de Buenos Aires. http://anterior.inta.gov.ar/suelos/cartas/index.htm (accessed 23 Oct 2016).

Jackes, M., Sherburne, R., Lubell, D., Barker, C., Wayman, M., 2001. Destruction of microstructure in archaeological bone: a case study from Portugal. Int. J. Osteoarchaeol. 11, 415–432. http://dx.doi.org/10.1002/oa.583.

Jans, M.M.E., 2008. Microbial bioerosion of bone - a review. In: Wisshak, M., Tapanila, L. (Eds.), Current Developments in Bioerosion. Springer, Berlin and Heidelberg, pp. 397–413. http://dx.doi.org/10.1007/978-3-540-77598-0_20.

Jans, M.M.E., Kars, H., Nielsen-Marsh, C.M., Smith, C., Nord, A., Earl, N., 2002. In situ preservation of archaeological bone: a histological study within a multidisciplinary approach. Archaeometry 44, 343–352. http://dx.doi.org/10.1111/1475-4754.t01-1-00067.

Jans, M.M.E., Nielsen-Marsh, C.M., Smith, C., Collins, M., Kars, H., 2004. Characterisation of microbial attack on archaeological bone. J. Archaeol. Sci. 31, 87–95. http://dx.doi.org/10.1016/j.jas.2003.07.007.

Kontopoulos, J., Nystrom, P., White, L., 2016. Experimental taphonomy: post-mortem microstructural modifications in *Sus scrofa domesticus* bone. Forensic Sci. Int. 266, 320–328. http://dx.doi.org/10.1016/j.forsciint.2016.06.024.

Liu, X., Burras, C.L., Kravchenko, Y.S., Duran, A., Huffman, T., Morras, H., Studdert, G., Zhang, X., Cruse, R.M., Yuan, X., 2012. Overview of Mollisols in the world: distribution, land use and management. Can. J. Soil Sci. 92, 383–402. http://dx.doi.org/10.4141/cjss2010-058.

Lyman, R.L., 1994. Vertebrate Taphonomy. Cambridge University Press, Cambridge.

Mant, A.K., 1987. Knowledge acquired from post-war exhumations. In: Boddington, A., Garland, A.N., Janaway, R.C. (Eds.), Death, Decay and Reconstruction: Approaches to Archaeology and Forensic Science. Manchester University Press, Manchester, pp. 65–78.

Marchiafava, V., Bonuci, L., Ascenzi, A., 1974. Fungal osteoclasia: a model of dead bone resorption. Calcif. Tissue Res. 14, 195–210. http://dx.doi.org/10.1007/BF02060295.

Millard, A., 2001. The deterioration of bone. In: Brothwell, D., Pollard, A.M. (Eds.), Handbook of Archaeological Sciences. Wiley, Chichester, pp. 637–647.

Morales, N., 2015. Estudio de modificaciones de la superficie ósea en restos faunísticos del sector sur del Área Ecotonal Húmedo-Seca Pampeana. El sitio San Martín 1 como caso de estudio. Comechingonia 19 (2), 323–345.

Morales, N., Catella, L., Barrientos, G., Oliva, F., 2014. Evaluación de un modelo predictivo acerca de las probabilidades diferenciales de preservación ósea en diferentes sectores de la cuenca del arroyo Chasicó, Región Pampeana Argentina. In: Paper Presented at the 12th International Conference of Archaeozoology, San Rafael, Argentina.

Morrone, J.J., 2001. Biogeografía de América Latina y el Caribe, first ed. M & T-Manuales y Tesis SEA, vol. 3 Sociedad Entomológica Aragonesa, Zaragoza, Spain.

Moscatelli, G., Pazos, M.S., 2000. Soils of Argentina: nature and use. In: Kheoruenromne, I., Theerawong, S. (Eds.), Proceedings of International Symposium in Soil Science: Accomplishments and Changing Paradigm Towards the 21th Century and IUSS Extraordinary Council Meeting, Bangkok, pp. 81–92.

Nielsen-Marsh, C.M., Hedges, R., 2000. Patterns of diagenesis in bone I: the effects of site environments. J. Archaeol. Sci. 27, 1139–1150. http://dx.doi.org/10.1006/jasc.1999.0537.

Nielsen-Marsh, C.M., Smith, C.I., Jans, M.M.E., Nord, A., Kars, H., Collins, M.J., 2007. Bone diagenesis in the European Holocene II: taphonomic and environmental considerations. J. Archaeol. Sci. 34, 1523–1531. http://dx.doi.org/10.1016/j.jas.2006.11.012.

Oliva, F., 2017. Investigaciones Arqueológicas en el Sector Centro-occidental de la Sierra de la Ventana y la Llanura Adyacente. (Unfinished doctoral dissertation) Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata.

Oliva, F., Gil, A., Roa, M., 1991a. Recientes investigaciones en el sitio San Martín 1, Partido de Puan, Pcia de Bs As. Shincal X Congreso Nacional de Arqueología Argentina 3 (3), 135–139.

Oliva, F., Moirano, J., Saghessi, M., 1991b. Estado de las investigaciones en el sitio Laguna de Puan 1. Boletín del Centro 2, 127–138.

Oliva, F., Catella, L., Morales, N., 2010. Análisis de los procesos de formación actuantes en el sitio San Martín 1, cuenca media del arroyo Chasicó, Provincia de Buenos Aires. In: Bárcena, J.R., Chiavazza, H. (Eds.), Arqueología Argentina en el Bicentenario de la Revolución de Mayo. Actas del XVII Congreso Nacional de Arqueología Argentina. 5. FFyL, UNCu e Instituto de Ciencias Humanas Sociales y Ambientales, Conicet, Mendoza, tomo, pp. 1805–1810.

Panigatti, J.L., 2010. Argentina 200 Años, 200 Suelos. INTA, Buenos Aires.

Pesquero, M.D., Fernández-Jalvo, Y., 2014. Bioapatite to calcite, an unusual transformation seen in fossil bones affected by aquatic bioerosion. Lethaia 47, 533–546. http://dx.doi.org/10.1111/let.12079.

Pesquero, M.D., Ascaso, C., Fernández-Jalvo, Y., Alcalá, L., 2010. A new taphonomic bioerosion in a Miocene lakeshore environment. Palaeogeogr. Palaeoclimatol. Palaeoecol. 295, 192–198. http://dx.doi.org/10.1016/j.palaeo.2010.05.037.

Rogel, M.R., Qiu, H.J., Ameer, G.A., 2008. The role of nanocomposites in bone regeneration. J. Mater. Chem. 18, 4233–4241. http://dx.doi.org/10.1039/b804692a.

Roux, W., 1887. Uber eine Knochen lebende gruppe von faderpilzen (Mycelites ossifragus). Z. Wiss. Zool. 45, 227–254.

SAGyP-INTA, 1990. Atlas de Suelos de la República Argentina. Escala 1:500.000 y 1:1.000.000. Proyecto PNUD Arg-85/019, Vols. I and II. Instituto de Evaluación de Tierras, Buenos Aires.

Schäbitz, F., 2003. Estudios polínicos del Cuaternario en las regiones áridas del sur de Argentina. Rev. Mus. Cienc. Nat. Bernardino Rivadavia Nueva Serie 5, 291–300.

Smith, C.I., Nielsen-Marsh, C.M., Jans, M.M.E., Collins, M.J., 2007. Bone diagenesis in the European Holocene I: patterns and mechanisms. J. Archaeol. Sci. 34, 1485–1493. http://dx.doi.org/10.1016/j.jas.2006.11.006.

Soil Survey Staff, 1999. Soil Taxonomy. A Basic System of Soil Classification for Making and Interpreting Soil Surveys, 2nd edition. Natural Resources Conservation Service. U.S. Department of Agriculture Handbook 436, Washington D. C.

Stout, S.D., 1978. Histological structure and its preservation in ancient bone. Curr. Anthropol. 19, 601–604. http://dx.doi.org/10.1086/202141.

Teruggi, M., 1957. The nature and origin of Argentine loess. J. Sediment. Petrol. 27, 322–332. http://dx.doi.org/10.1306/74D706DC-2B21-11D7-8648000102C1865D.

Tomassini, R.L., Montalvo, C.I., Manera, T., Oliva, C., 2010. Estudio tafonómico de los mamíferos pleistocenos del yacimiento de Playa del Barco (Pehuen Có), provincia de Buenos Aires, Argentina. Ameghiniana 47 (2), 137–152. http://dx.doi.org/10.5710/AMGH.v47i2.5.

Tonello, M.S., Prieto, A.R., 2010. Tendencias climáticas para los pastizales pampeanos durante el Pleistoceno tardío-Holoceno: estimaciones cuantitativas basadas en secuencias polínicas fósiles. Ameghiniana 47, 501–514. http://dx.doi.org/10.5710/AMGH.v47i4.7.

Trueman, C.N.G., Martill, D.M., 2002. The long-term survival of bone: the role of bioerosion. Archaeometry 44, 371–382. http://dx.doi.org/10.1111/1475-4754.t01-1-00070.

Turner-Walker, G., 1998a. The West Runton fossil elephant: a pre-conservation evaluation of its condition, chemistry and burial environment. Conservator 22, 26–35. http://dx.doi.org/10.1080/01410096.1998.9995124.

Turner-Walker, G., 1998b. Pyrite and bone diagenesis in terrestrial sediments: evidence from the West Runton fresh water bed. Bull. Geol. Soc. Norfolk 48, 3–26.

Turner-Walker, G., 2008. The chemical and microbial degradation of bones and teeth. In: Pinhasi, R., Mays, S. (Eds.), Advances in Human Palaeopathology. John Wiley and Sons, West Sussex, pp. 3–29. http://dx.doi.org/10.1002/9780470724187.ch1.

Turner-Walker, G., 2009. Degradation pathways and conservation strategies for ancient bone from wet, anoxic sites. In: Huisman, H.D.J., Straetkvern, K. (Eds.), Proceedings of the 10th Triennial Meeting of the ICOM-CC Working Group for Wet Organic Archaeological Materials. Nederlandse Archeologische Rapporten (NAR), Amsterdam, pp. 659–675.

Turner-Walker, G., 2012. Early bioerosion in skeletal tissues: persistence through deep time. Neues Jahrb. Geol. Palaeontol. Abh. 265, 165–183. http://dx.doi.org/10.1127/0077-7749/2012/0253.

Turner-Walker, G., Jans, M.M.E., 2008. Reconstructing taphonomic histories using histological analysis. Palaeogeogr. Palaeoclimatol. Palaeoecol. 266, 227–323. http://dx.doi.org/10.1016/j.palaeo.2008.03.024.

Turner-Walker, G., Mays, S., 2008. Histological studies on ancient bone. In: Pinhasi, R., Mays, S. (Eds.), Advances in Human Palaeopathology. John Wiley and Sons, West Sussex, pp. 121–146. http://dx.doi.org/10.1002/9780470724187.ch7.

Turner-Walker, G., Parry, T.V., 1995. The tensile strength of archaeological bone. J. Archaeol. Sci. 22, 185–191. http://dx.doi.org/10.1006/jasc.1995.0020.

Turner-Walker, G., Syversen, U., 2002. Quantifying histological changes in archaeological bones using BSE-SEM image analysis. Archaeometry 44, 161–168. http://dx.doi.org/10.1111/1475-4754.t01-1-00078.

Turner-Walker, G., Nielsen-Marsh, C.M., Syversen, U., Kars, H., Collins, M.J., 2002. Submicron spongiform porosity is the major ultra-structural alteration occurring in archaeological bone. Int. J. Osteoarchaeol. 12, 407–414. http://dx.doi.org/10.1002/oa.642.

Tütken, T., Vennemann, T.W., 2011. Fossil bones and teeth: preservation or alteration of biogenic compositions? Palaeogeogr. Palaeoclimatol. Palaeoecol. 310, 1–8. http://dx.doi.org/10.1016/j.palaeo.2011.06.020.

Van Klinken, G.J., Hedges, R.E.M., 1995. Experiments on collagen-humic interactions: speed of humic uptake, and effects of diverse chemical treatments. J. Archaeol. Sci. 22, 263–270. http://dx.doi.org/10.1006/jasc.1995.0028.

Von Endt, D.W., Ortner, D.J., 1984. Experimental effects of bone size and temperature on bone diagenesis. J. Archaeol. Sci. 11, 247–253. http://dx.doi.org/10.1016/0305-4403(84)90005-0.

Wedl, C., 1864. Ueber einen im Zahnbein und Knochen keimenden Pilz. Mineral. Biol. Erdkunde 50, 171–193.

Weiner, S., 2010. Microarchaeology: Beyond the Visible Archaeological Record. Cambridge University Press, Cambridge.

White, L., Booth, T.J., 2014. The origin of bacteria responsible for bioerosion to the internal bone microstructure: results from experimentally-deposited pig carcasses. Forensic Sci. Int. 239, 92–102. http://dx.doi.org/10.1016/j.forsciint.2014.03.024.

Woodruff, R.B., 1997. Customer value: the next source for competitive advantage. J. Acad. Mark. Sci. 25, 139–154. http://dx.doi.org/10.1007/BF02894350.

Yoshino, M., Kimijima, T., Miyasaka, S., Sato, H., Seta, S., 1991. Microscopical study on estimation of time since death in skeletal remains. Forensic Sci. Int. 49, 143–158. http://dx.doi.org/10.1016/0379-0738(91)90074-S.