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Research paper

The petrous bone: Ideal substrate in legal medicine?

Angéla Gonzalez[a,*], Catherine Cannet[a], Vincent Zvénigorosky[a,b], Annie Geraut[a], Guillaume Koch[c], Tania Delabarde[d], Bertrand Ludes[b,d,e], Jean-Sébastien Raul[a], Christine Keyser[a,b,e]

[a] Institut de Médecine Légale, Université de Strasbourg, Faculté de médecine de Strasbourg, France
[b] CNRS, FRE2029-BABEL, Université Paris Descartes, France
[c] Institut d'Anatomie Normale, Hôpitaux Universitaires de Strasbourg, Faculté de médecine de Strasbourg, France
[d] Institut Médico-Légal de Paris, France
[e] Institut National de la Transfusion Sanguine, Paris, France

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ABSTRACT

Over the last few years, palaeogenomic studies of the petrous bone (the densest part of the temporal bone) have shown that it is a source of DNA in both larger quantities and of better quality than other bones. This dense bone around the otic capsule has therefore been called the choice substrate in palaeogenomics. Because the practice of forensic genetics responds to different imperatives, we implemented a study aimed at (i) understanding how and why the petrous bone is an advantageous substrate in ancient DNA studies and (ii) establishing whether it is advantageous in forensic STR typing.

We selected 50 individual skeletal remains and extracted DNA from one tooth and one petrous bone from each. We then amplified 24 STR markers commonly used in forensic identification and compared the quality of that amplification using the RFU intensities of the signal as read on the STR profiles. We also performed histological analyses to compare (i) the microscopic structure of a petrous bone and of a tooth and (ii) the microscopic structure of fresh petrous bone and of an archaeological or forensic sample.

We show that the RFU intensities read on STR profiles are systematically higher in experiments using DNA extracted from petrous bones rather than teeth. For this reason, we were more likely to obtain a complete STR profile from petrous bone material, increasing the chance of identification in a forensic setting. Histological analyses revealed peculiar microstructural characteristics (tissue organization), unique to the petrous bone, that might explain the good preservation of DNA in that substrate. Therefore, it appears that despite the necessity of analysing longer fragments in forensic STR typing compared to NGS palaeogenomics, the use of petrous bones in forensic genetics could prove valuable, especially in cases involving infants, toothless individuals or very degraded skeletal remains.

1. Introduction

For over 30 years, human genetics and forensic genetics experts have been concerned with the amplification of DNA, which may be particularly fragmented, present in low quantities and /or contaminated [1–3]. The advent of the Polymerase Chain Reaction (PCR) amplification technique revolutionized the analysis of ancient and/or degraded DNA by allowing in vitro targeting of DNA fragments and obtaining several million copies [4]. Palaeogenetic studies subsequently developed, focusing on hard tissues such as bones and teeth, the substrates most represented in archaeological contexts [5,6]. In the meantime, the discovery of STRs (Short Tandem Repeats), analysed on automatic sequencers through capillary electrophoresis (CE), provided a powerful tool for the identification of individuals and the study of close relatives (kinship, paternity tests) in forensic genetics. Significant, advances have been made in palaeogenetics (now palaeogenomics) through the development of high-throughput sequencing systems [7–9]. However, despite the progress made in recent years, selecting a suitable source of endogenous DNA is still crucial to the success of ancient and/or degraded genetic analyses. DNA preservation and

* Corresponding author.
E-mail addresses: agonzalezmartin@unistra.fr (A. Gonzalez), catherine.cannet@wanadoo.fr (C. Cannet), z.vincent@LIVE.FR (V. Zvénigorosky), ageraut@unistra.fr (A. Geraut), guillaume.koch@unistra.fr (G. Koch), Tania.delabarde@interieur.gouv.fr (T. Delabarde), ludes@unistra.fr (B. Ludes), js.raul@unistra.fr (J.-S. Raul), ckeyser@unistra.fr (C. Keyser).

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Table 1
Sample overview.

Samples	Geographical origine/Site	Datation	N	Substrates
S1–S24	Altai Mogol/Saghil	5th–3rd century B.C.E.	24	9 pairs tooth/petrous bone and 13 isolated petrous bones
M1–M2	France/Marsal	4st–2nd century B.C.E.	2	2 pairs tooth /petrous bone
B1–B4	Mongolia/Burgast	3rd –1st century B.C.E.	4	4 pairs tooth /petrous bone
T	Mongolia/Xiongnu	1st century B.C.E–1st century C.E.	1	1 pair tooth /petrous bone
N1–N2	France/Niedernai	5th century C.E.	2	2 pairs tooth /petrous bone
H1–H22	Hungary	9th century C.E.	22	22 pairs tooth /petrous bone
X1–X10	–	Present day (forensic)	10	10 pairs tooth /petrous bone
			65	50 pairs tooth /petrous bone

N: number of genotyped individual. A total of 65 skeletons were sampled in this study. We have extracted DNA from one tooth and one petrous bone from all individual skeletal remains except for the Saghil site where we could only process 9 teeth and 9 petrous bone.

efficient recovery remain key issues, with taphonomy [10–12] and environmental conditions being the determinant factors [13].

In studies of skeletal remains for genetic identification, investigators often select teeth or long bones (tibiae, femora) where the compact lamellar layers of bone tissue are relatively dense allowing for a higher extraction yield than other skeletal elements. These are easily collected from mass fatality sites or in forensic cases and they represent a microenvironment particularly favourable to the preservation of the DNA thanks to the adsorption of nucleic acids on the inorganic bone phase (hydroxyapatite) [14–16], collagen fibers and other fibrous proteins [17,18]. Among these substrates, teeth appeared to yield the more satisfying results [19–21]. A study combining genetic and histological analyses demonstrated that cementum was the material which preserves DNA the longest [22]. Indeed, from a histological standpoint, cementum and bone present very similar structures [23,24].

In recent years however, studies have suggested that the petrous part of the temporal bone could be even more interesting than teeth as a substrate for DNA extraction [25]. It was demonstrated that the proportion of endogenous DNA (as a percentage of total DNA extracted) obtained from petrous bone is 4–16 times larger than the proportion obtained from dental elements and up to 183 times superior than the proportion obtained from other skeletal elements (ribs, metacarpal or metatarsal bones) [26]. Since then, it has been specified that it is the densest part of the otic capsule or osseous labyrinth, corresponding to the cochlea, that allows scientists to obtain greater quantities of endogenous DNA [27]. Several studies confirmed the status of the petrous bone as the substrate of choice for analyses of ancient and/or degraded DNA [28,29].

Conventional approaches used in genetic identification, usually STR-based, are not highly sensitive to the presence of exogenous DNA. They do however require the amplification of long fragments (between 70 and 450 bp), whereas Next Generation Sequencing (NGS) techniques, also called Massively Parallel Sequencing (MPS), allow sequencing from very small fragments (< 50 bp), but are highly sensitive to the presence of exogenous DNA since they do not discriminate between DNA from different sources. A study using STR analysis on archaeological samples has already suggested that DNA extracted from petrous bones is also more likely to yield complete STR profiles than DNA extracted from other substrates [30].

We endeavoured to replicate those results, apply the same comparison to forensic samples and identify the specific circumstances in which the use of a petrous bone might be advantageous. Given that both petrous bones and teeth have already been shown to yield better results than long bones [30], our study includes only the two first sample types. We also performed histological analyses to determine and illustrate the peculiar microscopic structure of the petrous bone, including the otic capsule, which has been described as a protection against pathogen intrusion [31].

Using 50 pairs of samples, each a petrous bone and a tooth from one individual exhumed in an archaeological or forensic context, we compared the RFU intensities of the amplification profiles of STR markers commonly used in forensic genetics and the number of amplified alleles. We also compared two archaeological series of more than 20 samples that had undergone very different environmental conditions since inhumation, in order to evaluate the effect of post-mortem DNA degradation on RFU intensities and the number of amplified alleles. Histological comparisons of a fresh tooth and a fresh petrous bone, as well two petrous bones collected from an archaeological and a forensic context, allow us to better identify the limits of the advantages presented by the petrous bone as a substrate for the extraction of degraded DNA.

2. Materials and methods

2.1. Samples used for genetic analyses

Genetic investigations were carried out on 65 petrous bones and 50 teeth (a total of 115 samples). Among the 65 petrous bones, 55 were from archaeological excavation sites and 10 were from forensic cases. Among the 50 teeth, 40 were from archaeological excavations and 10 were from the same forensic cases stored away from light at room temperature for more than 20 years (Table 1). The six archaeological sites were: two sites in north-eastern France (4th–2nd century B.C.E. and 5th century C.E., respectively), a Hungarian site (9th century C.E.) and three Mongolian sites (5th–3rd century B.C.E., 3rd–1st century B.C.E. and 1st century B.C.E. to 1st century C.E.).

2.1.1. Sample processing

Multirooted teeth (molars and premolars) were favoured because of a higher root area and a larger pulp volume. When selecting teeth, closed apexes were preferred. Because lesions (cracks, cavities, etc.) promote the entry of bacteria (and therefore contamination of the DNA sample) the damaged teeth were discarded. During the decontamination phase, the teeth were meticulously cleaned with a sterile compress soaked in a diluted sodium hypochlorite solution, rinsed with sterile Nuclease-Free water (Euromedex, cat. n°UW0900-A) and then dried with a sterile compress. Each face was then exposed to short-wavelength UV for 30 min. Entire teeth were then reduced to powder by cryogenic grinding (liquid nitrogen cryocrusher 6870 freezer/Mill). For each tooth, approximately 250 mg of powder were then transferred into a 1.5 ml microtube, taking care to separate the large particles (corresponding to the enamel) from the finer particles (corresponding to the other structures of the tooth). The equipment needed for cryogenic grinding (cryogenic tubes, caps and firing pins) had previously been washed with bleach, rinsed with deionized water (Euromedex, cat. no 22800-05), dried with ethanol 70%, and then exposed to a Bio-Link 254 nm irradiator (Crosslinker).

Some of the petrous bones were already isolated, having separated from the skull before or during excavation. Those who were attached to other cranial bones were separated manually. Extraction was carried out using a diamond saw mounted on a Dremel® under an extraction hood. To remove contaminants before any drilling operation, the

surface of the petrous bone was mechanically abraded 1 mm deep using a Dremel® mounted cutter. The drilling was then carried out using a ball cutter orientated towards the cochlea, the densest part of the otic capsule. The resulting fine powder was collected in a sterile cup. This original protocol/treatment of the petrous bone performed within our laboratory allows us to properly decontaminate the petrous part and yet return a near-intact petrous bone to archaeologists and anthropologists or judicial authorities. This method differs from that of Sirak and his collaborators [32], that aims to obtain bone powder without detaching the temporal bone from the skull. In the present case, the careful and precise cut allows repositioning of the petrous part once the powders have been collected.

2.1.2. DNA extraction and amplification

From bone and tooth powders (approximately 250 mg), DNA extraction was performed during an all-night incubation at 50 °C with an extraction buffer consisting of EDTA (0.5 M), DTT (1 M) and proteinase K (20 mg/mL). To increase the efficiency of DNA extraction a decalcification step was performed (incubation overnight with EDTA 0.5 M). The extracted DNA was then purified on silica columns and then concentrated [33]. Two independent DNA extractions were completed from each sample. STR analysis was performed using a commercial human genetic identification kit, the GlobalFiler® PCR (Thermo Fisher Scientific) amplification kit, following the supplier's recommendations. It allows the simultaneous analysis of 24 genetic markers, including 21 autosomal markers (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S13), two Y chromosome markers (DYS391, Y indel) and a sex determination marker (amelogenin gene). The sizes of the fragments amplified using this kit range from 70 to 450 bp. PCR amplification reactions were performed using a thermocycler (Biometra). We followed the manufacturers' protocols, except for the number of PCR cycles, which was increased from 29 to 32 for the archaeological sample. The amplified products were then analysed using an automatic 3500 Genetic Analyzer (Applied Biosystems) using polymer POP7. The DNA profiles were analysed using GeneMapper®v4.1 software. The analytical threshold for allelic calls was set at 50 RFU, in accordance with our internal validation guidelines. At least two independent extractions underwent amplification for each sample and consensus DNA profiles were established by retaining the alleles that had been typed at least twice for each sample [Online Materials Table S1].

2.1.3. Precautions taken to avoid contamination

This work was carried out in rooms dedicated to the study of degraded DNA. The pre-PCR and post PCR laboratories are located on different floors. The pre-PCR laboratory was strictly dedicated to ancient DNA, with positive pressure and UV light irradiations. Very strict rules were followed, between each manipulation; benches and supplies were cleaned with bleach, ultrapure water and DNA away (Dominique Dutscher, cat. no 038188) and placed under UV light. The manipulators wore appropriate equipment: overshoes, a facial mask, a mobcap, a lab coat and gloves. The genetic profile of all the people in contact with the samples was established and compared with the DNA profiles of ancient specimens. For each sample (tooth or petrous bone), multiple DNA extractions and PCR amplifications were performed and negative controls were included in each experiment (one extraction or amplification blank for every four samples).

2.1.4. Statistical analysis

The quality of DNA amplification was measured for each substrate using the peak RFU (Relative Fluorescence Units) intensities of the amplified alleles for all 24 markers [Online Materials Table S2]. For the following analyses, we retained the results of only one multiplex amplification for each sample, the most successful (that is to say the amplification yielding the most allele calls and, when comparing profiles with identical numbers of allele calls, the highest RFU intensities).

Alleles were ranked by size from shortest to longest into 5 intervals: < 120 bp, $120-180$ bp, $180-240$ bp, $240-300$ bp and > 300 bp and the RFU intensity of homozygotes (only one peak) was divided by two [Online Materials Table S3]. Some markers encompass alleles belonging to more than one interval.

For all 100 paired samples (with one tooth and one petrous bone from 50 individuals), the number of markers successfully amplified for each STR profile was also compared. Markers were divided into two categories: "short", if all alleles were less than or equal to 240 bp in length and "long", if all alleles were more than 240 bp in length. This 240 bp demarcation was chosen to exclude as few markers as possible. For this test, typed alleles of the marker D21S391 fell into both categories and were thus distributed in one or the other [Online Materials Table S4a].

We tested the difference in mean RFU intensities between size intervals using Student t-tests. Differences were considered significant for p-values < 0.05. We tested the difference in the number of short or long markers successfully amplified from tooth or petrous bone using a paired t-test with the same significance threshold. Statistical tests were performed using in-house R scripts [34].

2.2. Samples used for histological analyses

Histological sections were carried out on (i) a fresh tooth and a fresh petrous bone in order to compare the microscopic structure of these two organs and (ii) two undated archaeological petrous bones in order to analyse microscopic tissue degradation within these structures.

2.2.1. The fresh dental sample

The human dental sample was collected from a patient undergoing a conventional wisdom tooth extraction. It was therefore a left superior third molar (tooth N28). The freshly extracted tooth was immediately fixed in 10% buffered formalin (pH = 7.2) for 6 weeks to stabilise tissue structures. At the end of the fixation, the tooth was sagittally halved in two segments by means of a diamond saw mounted to a Dremel® to expose the pulp and root canal. Both dental fragments were then decalcified in 10% EDTA (pH 7.2). Decalcification time was 27 days. After completion of the decalcification, both fragments were dehydrated using increasing graded series of ethyl alcohol, cleared in xylene and impregnated with melted paraffin, in a vacuum infiltrating processor (VIP Tissue-Tek 6, Sakura®). Each dental sample was embedded separately in one block of paraffin wax by means of an embedding station (Tissue Tek III of Sakura®). Paraffin with a high melting point of $62-64$ °C (Ref: 19304-01, EMS, Philadelphia) was retained to provide a more solid matrix to the tooth samples and consequently easier sectioning. Serial sections of 5 µm thickness were cut with a motorized microtome (Jung Autocut, Leica®) and stained with (i) hematoxylin and eosin (H&E) to assess the general morphology and (ii) Picrosirius (PS) using Sirius red F3B [C.I. 32782] as a dye for the study of collagen network viewed under polarised light [35–37]. All stained slides were examined with a light and polarised microscope (Axiophot, Zeiss®) connected to an AxioCam MRc5 camera (Zeiss®). Sections were also scanned using the PathScan® Touch software (Excilone) connected to an Axiophot microscope (Zeiss®) with a CMOS camera.

2.2.2. The fresh petrous bone sample

The human temporal bone sample was taken from body donated to science, through our partnership with the laboratory of anatomy of the Faculty of Medicine of Strasbourg. Once the petrous part of the temporal bone was extracted and carefully cleaned by a medical examiner, it was fixed in 10% buffered formalin (pH = 7.2) for 6 weeks. At the end of the fixation, the isolated petrous bone was cut according to a sagittal plane using a diamond saw mounted on a Dremel® to expose the otic capsule. The two fragments exposing the three parts of the petrous bone (cochlea, vestibule and semi-circular canals) were decalcified in

Kristensen's solution (formic acid and sodium formate). This solution was prepared in the laboratory according to the procedure described in the literature [38]. This method of decalcification uses a weak acid and is therefore less damaging. Decalcification time was 18 days. After completion of the decalcification, the following histoprocessing (dehydration, embedding, cutting and staining) was similar to the one used to process the tooth. One additional staining was performed, i.e. Safranin O / Fast green (SO/FG) to highlight cartilage [39].

2.2.3. The two undated archaeological petrous bones samples

The first petrous bone came from a temporal bone that was already detached from the skull and stored in a dark room at room temperature between 10–15 °C for more than 20 years. The second petrous bone was extracted using a diamond saw mounted on a Dremel® from a more recent skull that had been stored at room temperature for about 10 years. Both were undated archaeological skeletal remains and were stored in a dry cardboard box at room temperature between 10 °C and 15 °C. These two petrous bones were fixed in 10% buffered formalin (pH = 7.2), for 6 weeks and 8 weeks, respectively. They were subsequently decalcified in 10% EDTA (pH 7.2). Decalcification time was 30 days for both samples. After completion of the decalcification, the following histoprocessing (dehydration, embedding, cutting and staining) was similar to the one used to process the fresh petrous bone.

3. Results

3.1. Amplification is more reliable using DNA extracted from petrous bones

For the three first allele size intervals (up to 240 bp in length, "short" fragments), the amplification of DNA from petrous bones consistently yields higher mean RFU intensities than the amplification of DNA from teeth (*p*-values: 5.142e−07, 3.313e−06 and 1.51e−04) (Fig. 1; Table 2). This is not the case for longer fragments (over 240 bp), for which mean RFU intensity does not significantly differ between DNA extracted from teeth and DNA extracted from petrous bones. This result stands when comparing only archaeological teeth to archaeological petrous bones, or only forensic teeth to forensic petrous bones (Table 2).

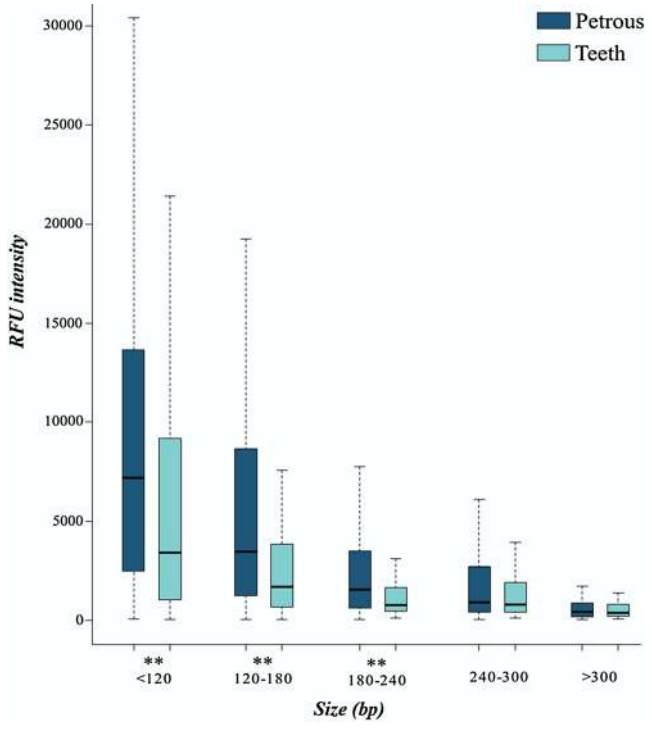

Fig. 1. Distribution of the average RFU intensities for the 50 petrous bone/teeth pairs, **p-value inferior to 0.05 for a student t-test: mean RFU intensity for DNA extracted from petrous bones is significantly superior to mean RFU intensity for DNA extracted from teeth.

3.2. The degradation of DNA in petrous bones is subject to environmental factors

We compared 24 petrous bones from one Mongolian necropolis-Saghil (5th–3rd century B.C.E.) and 22 from one Hungarian necropolis (9th century C.E.). The first is situated in the Altai region of Mongolia which is generally cold and extremely arid, with mild summers. The second necropolis is situated in more temperate Hungary, which is significantly warmer and more humid.

We show that samples from the first site consistently yield better quality DNA for all five size intervals (Table 2), although, in both cases, longer fragments are amplified at lower RFU intensities than shorter fragments. This highlights the discrepancies in preservation between different archaeological sites, that are not necessarily linked to the age of the samples since, in this case, the older samples yielded better quality DNA. Rather it is environmental conditions that have the greater influence over preservation, as previously described and expected in such samples [40,41].

3.3. More complete profiles are obtained using DNA from petrous bones

Paired samples (one petrous bone, one tooth) from 50 individuals were used to compare the effectiveness of DNA extracted from both substrates in delivering complete autosomal STR profiles. Comparing numbers of amplified STR loci, we show that DNA extracted from petrous bones more often yields "successful allelic calls" (i.e. expected heterozygous alleles are observed) for markers relying on long fragments (over 240 bp, p-value: 0.02473) and for markers relying on shorter fragments (< 240 bp, p-value: 0.03974) [Online Materials Table S4b). This results in more complete STR profiles when using petrous bones, especially when teeth do not permit the amplification of longer fragments. Based on the multiple amplifications from independent DNA extracts of the same individual, we did not observe any discrepancy in allelic calls between substrates, although some heterozygotes could be mistaken for homozygotes when one allele had not been amplified using DNA extracted from teeth.

3.4. Histology of DNA preservation in teeth

Most of the volume of the tooth is made up of dentine, as shown on the sagittal section of the left superior third molar (Fig. 2A). Dentine is mostly composed of type I collagen (Fig. 2B) and observation in polarised light shows that these collagen fibers are grossly parallel (Fig. 2B1). Cementum (an entirely mineralised matrix) is also constituted of type I collagen fibers, oriented parallelly to the axis of the root (Fig. 2B2). Neither dentine nor acellular cementum are vascularised or innervated. Nucleated cells are mainly visible in root or crown pulp. There is a high concentration of odontoblasts at the pulp-dentine junction (Fig. 2A1) and cementoblasts can also be observed in cellular cementum (Fig. 2A2).

3.5. Histology of DNA preservation in petrous bones

Distinct histological structures within the petrous bone are highlighted on a sagittal section (Fig. 3A). These structures are especially visible around the cochlea (Fig. 3B), where a protective shell divides two regions [Online Materials Figure S1]. The region bordering the cavities of the inner ear (cochlea and semi-circular canals) is a type I collagen matrix, rich in nucleated cells, inside which under-coloured areas can be observed. Safranine-O/Fast green (SO/FG) indicates that these areas are made up of cartilaginous tissue. Observation under

Table 2
Comparative analysis of the quality of DNA extracted from petrous bones and teeth, of archaeological and/or forensic origin.

	< 120 bp	120 – 180 bp	180 – 240 bp	240 – 300 bp	> 300 bp
All petrous bones/teeth	5.142e – 07*	3.313e-06*	0.000151*	0.09526	0.1671
Archeological petrous bones/teeth	0.001464*	0.001933*	0.003069*	0.3351	0.1672
Forensic petrous bones/teeth	2.018e – 07*	2.175e – 05*	0.008736*	0.03866*	0.7509
Petrous bones Saghil/Hungary	8.49e – 13*	4.199e – 12*	1.923e-08*	0.008345*	9.951e – 10*

* *p*-value inferior to 0.05.

polarised light shows the different organisation of collagen fibers: unorganised in the border region of the inner ear and parallel inside the protective shell segregating that region from the rest of the petrous bone. The unorganised border region is also devoid of secondary osteons, which suggests the absence of vascularisation [Online Materials Figure S2].

3.6. The impact of environmental conditions on petrous bone microstructure

We performed histological analyses on a "degraded" petrous bone and a "very degraded" petrous bone, in order to observe the state of the structures previously described after degradation. The sagittal section of the "degraded" bone (Fig. 4, Online Materials Figure S3) shows the same organisation, with a protective shell surrounding the border region of the inner ear. Because of autolysis, cell nuclei cannot be observed in this degraded sample [Online Materials Figure S4].

The sagittal section of the "very degraded" sample [Online Materials Figure S5] shows the same signs of degradation (absence of cell nuclei) inside the border region of the inner ear [Online Materials Figure S6], and further signs outside of that region. Although the presence of the protective shell and the orientation of collagen fibers are unchanged [Online Materials Figure S7], bone tissue outside the protective shell has undergone significant bioerosion.

4. Discussion

4.1. The protective shell around the otic capsule preserves it from degradation

The comparison of histological structures in a degraded petrous bone and a very degraded petrous bone showed that, although there is significant bioerosion around the otic capsule, histologically distinct regions can still be observed: a protective shell segregates an area of hyper-mineralised bone tissue where collagen fibers are unorganised [42]. Safranine-O/Fast green also highlighted the presence of glycoproteins (constituents of the cartilaginous matrix) within this protected area (the border region of the inner ear), even in very degraded petrous bone. Since the degradation of glycoproteins is normally a rapid process, their persistence at this stage is another indication of the isolation of the otic capsule from other skeletal elements [Online Materials Figure S7].

4.2. The conditions of degradation in petrous bones vary between archaeological sites

Physical, biological and chemical alterations of skeletal remains are the determining factors in the long-term preservation of DNA [10,12]. Cold and dry environments are the most favourable as previously shown [40,41].

The comparison of STR amplification results in a 9th century C.E. Hungarian necropolis (temperate climate) and a 5th–3rd century B.C.E. Mongolian necropolis-Saghil (cold and dry climate) confirmed this issue. Across all allelic size intervals, petrous bone material from the second site yields higher mean RFU intensities. This demonstrates that whatever advantage exists in preferring petrous bones to teeth as primary substrates for DNA amplification, there are important discrepancies between different petrous bones. Some samples might be too degraded for reliable analysis. It should be noted however, that teeth undergo the same degradation process under the same conditions.

4.3. The absence of vascularisation prevents exogenous contamination

As shown on the sagittal section of the petrous bone, the border region of the cochlea and the semi-circular canals contains chondrocyte residue. These components are typical of immature bone. Moreover, secondary osteons cannot be observed in that region. Since secondary osteons are centred around blood vessels, innervated and intervene in the development and remodelling of bone tissue, this observation is consistent with limited vascularisation and the absence of remodelling, as observed in previous studies [27]. Without blood supplied to this region, the otic capsule is preserved from the introduction of some microorganisms, while dental pulp is densely vascularised and has been shown to contain greater microbial diversity [31]. The border region of the inner ear is therefore relatively protected from exogenous contamination, both because of its anatomical isolation and its particular histological structure.

4.4. Palaeogenomic methods rely on short endogenous fragments

In NGS approaches used in palaeogenomics, DNA libraries are created using all the DNA available in a sample, human or otherwise. DNA is recovered from microorganisms that occupied the sample before death, colonised it after death and/or participated in its degradation. Although this exogenous DNA is eliminated by the NGS methodology, the selection of suitable samples influences the quantity of DNA recovered.

In palaeogenomics, substrates containing a high proportion of endogenous DNA are preferred in order to obtain genomes with sufficient coverage. The endogenous/ exogenous ratio is therefore a decisive factor. For these reasons, the petrous bone is a choice substrate for palaeogenomics: (i) its rapid formation *in utero* and isolation [43] favour the good preservation of DNA; (ii) this anatomical isolation also protects the otic capsule from exogenous contamination (more vascularised bone is more susceptible to the introduction of microorganisms); (iii) the petrous bone is not remodelled during life and presents a high concentration of osteocytes [44]; (iv) large quantities of DNA, even fragmented, are adapted to NGS sequencing techniques, that focus on short fragments. On the contrary, PCR-based techniques generally rely on fragments longer than 70 bp [45]. Although this technique is equally reliable, in the case of highly degraded and fragmented DNA it becomes less effective.

4.5. Forensic genetics rely on the recovery of complete STR profiles

The endogenous DNA yield of the otic capsule is not the main benefit of using petrous bones in forensic investigations. Exogenous contamination is in fact less of an issue when amplifying human-specific markers that cannot be found in microbial DNA sequences. Contamination by exogenous human DNA (during analysis or handling) is a more pressing issue.

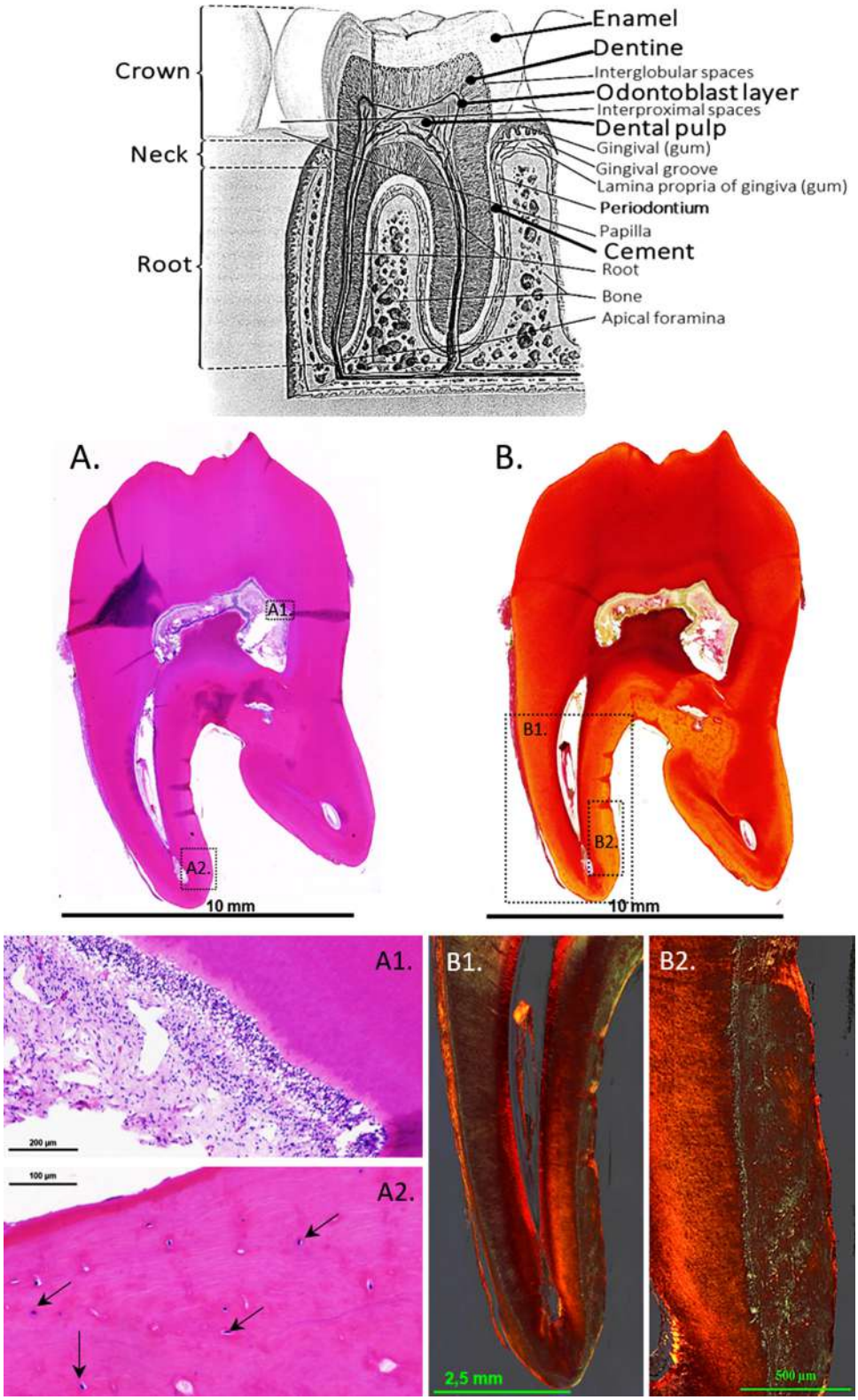

Fig. 2. Histomorphological analysis of a freshly extracted tooth, Top, presentation of a section of human mandibular molar and its support structures. *According to Franck H. Netter, Atlas of Human Anatomy, 5th edition, 2011, p57.* A. Histological section of a superior third molar cut according to a sagittal plane. A1. A higher magnification of the pulp-dentine junction shows blue points corresponding to the nuclei of the odontoblasts. A2. A higher magnification at the cementum level shows blue points (*black arrows*) corresponding to the nuclei of the cementoblasts. B. The collagen fibers are stained red. B1. Observed under polarised light the collagen fibers are grossly parallel and run at a right angle to the long axis of the root. B2. At the cementum level, the collagen fibers are thinner and run parallel to the long axis of the root.

Forensic analysis still relies mainly on STR genotyping, although other markers (e.g. SNP, mitochondrial sequences) do have specific applications. Because the usefulness of databases resides in comparing an STR profile with reference samples, crime-scene samples or samples from unidentified corpses, more complete STR profiles are more likely to lead to successful forensic investigations [46].

Our results indicate that more complete profiles (more loci amplified) are obtained using DNA extracted from petrous bone than DNA extracted from teeth. We showed that this is due to the better amplification of longer fragments in the first substrate. Complete profiles can however be obtained using teeth when they are well-preserved. This implies that, in very degraded skeletons, selecting petrous bones for DNA extraction could be a simple way to increase the probability of obtaining complete STR profiles and limit allelic drop-out.

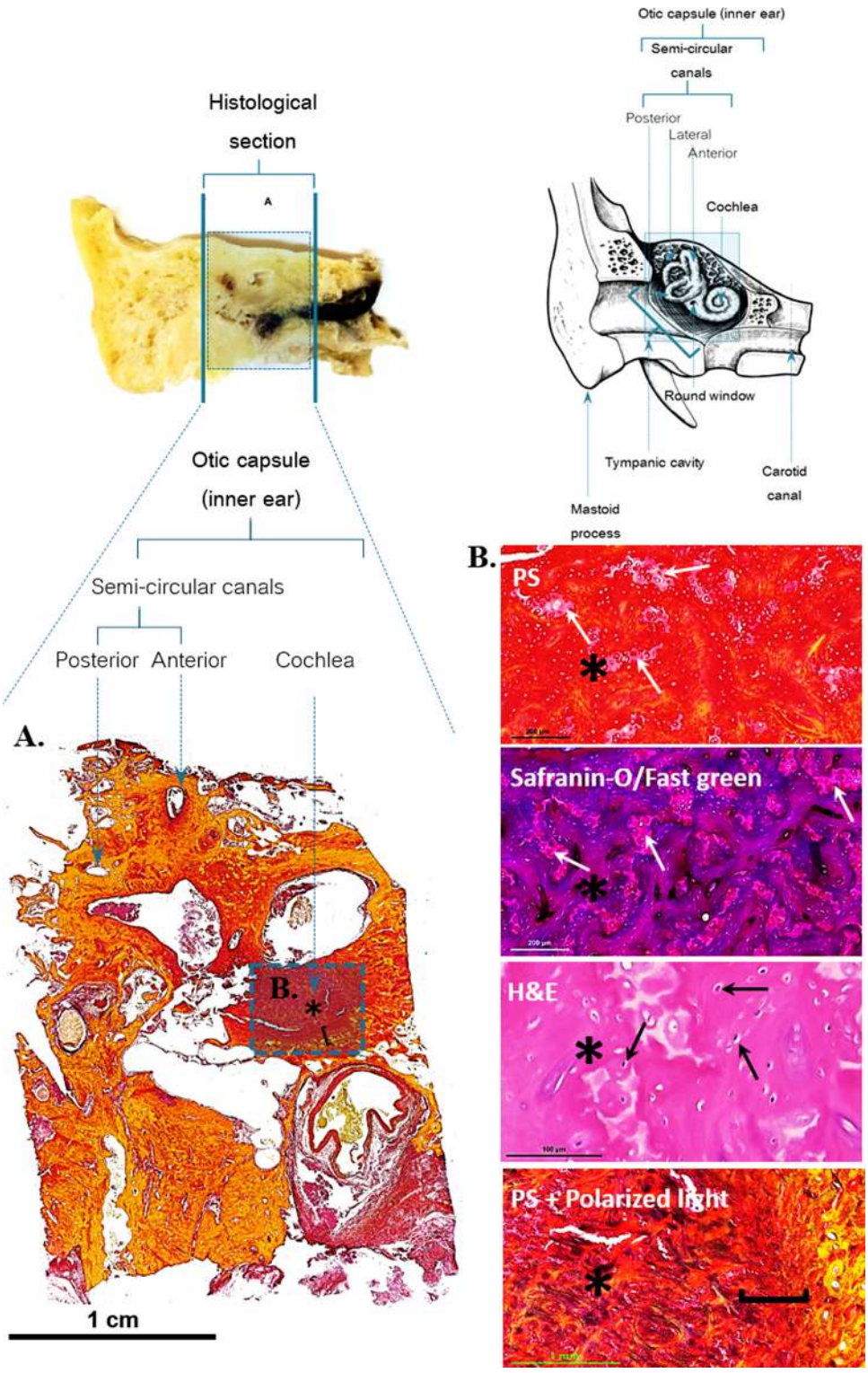

Fig. 3. Histomorphological analysis of a freshly petrous bone sample, Top left, sagittal section of the fresh petrous bone. Top right, shape and situation of the otic capsule, *according to Legent F, Perlemuter L, Vandenbrouck C. Anatomy notebooks O. R. L., 2nd edition.1968 -1975 Masson et Compagnie, Editeurs, Paris*. A. Histological section including the otic capsule (*square*) and a peculiar bone structure (asterisk). B. The peculiar structure stained with picrosirius (PS) shows under-colored areas (*white arrows*). The safranin-O/Fast green demonstrates the presence of cartilage at the level of under-colored areas (*white arrows*). Higher magnification of the same structure with Hematoxylin and Eosin (H&E) shows the presence of many blue colored nuclei (*black arrows*). The picrosirius stained area observed under polarised light (PS + polarised light) demonstrated the presence of collagen fibers with no particular orientation whereas the fibers constituting the protective shell are grossly parallel (*bracket*).

5. Conclusion

Histological investigations have shown the isolation of the otic capsule favours its preservation from exogenous contamination. Its atypical structure, with immature bone that is not remodelled and less vascularised than tooth pulp, also contributes to maintaining a high proportion of endogenous DNA. The protective shell itself limits bioerosion and the physical degradation of the tissue around the inner ear.

Genetic analyses have shown that DNA extracted from the petrous bones yields better results than DNA extracted from teeth: (i) short fragments are amplified at higher RFU intensities and (ii) long fragments are more likely to be amplified. The first result (along with the absence of exogenous contaminants) made the petrous bones the preferred sample for palaeogenomic studies. The second result implies that it yields complete STR profiles more often than teeth. This is an indication that petrous bones could also be the ideal substrate for forensic genetics investigations.

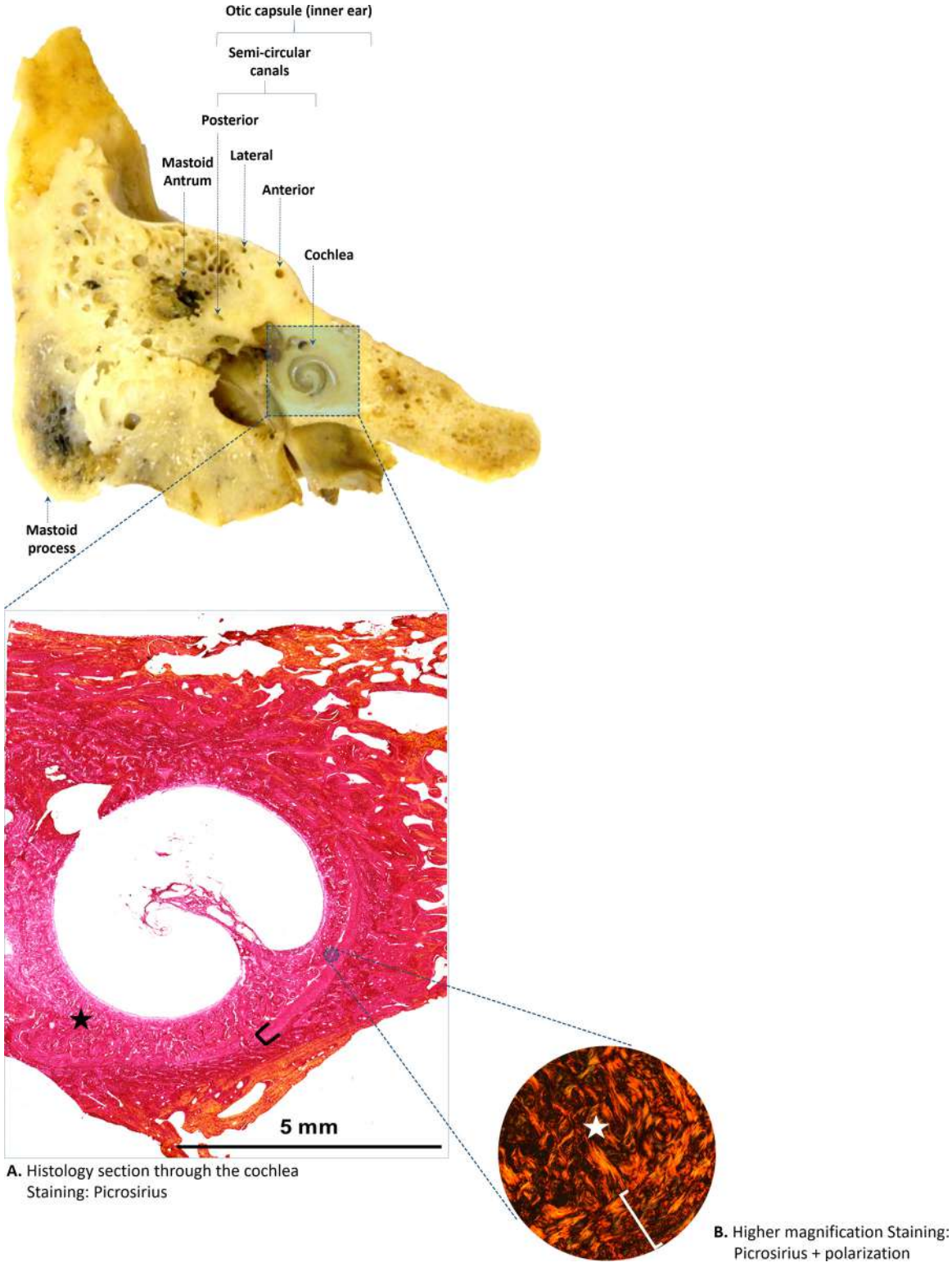

Fig. 4. Histology section through the cochlea, A. Hypermineralized bone with interlaced collagen bundles with no predominant orientation (star) and absence of bone remodelling enclosed in a thick layer of lamellar bone with collagen fibers running parallel (bracket). B. Higher magnification Staining: Picrosirius + polarization. Collagen fibers constituting the protective shell are grossly parallel (bracket) whereas other fibers have no particular orientation (star).

Although the use of the petrous bone should not be systematic (teeth can also provide complete STR profiles), it could be especially advantageous when studying very degraded skeletons, for example after carbonisation of the corpses or in particularly deleterious taphonomic conditions, or for toothless individuals. Finally, as forensic genetics incorporate Next Generation Sequencing (NGS) approaches, using petrous bones could become as beneficial as it has been to palaeogenomics, for the same reasons.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigen.2020.102305.

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