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An optimal skeletal element for DNA testing: Evaluation of DNA quantity and quality from various bone types in routine forensic practice

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ABSTRACT

For human identification, the quality and quantity of DNA must be sufficient for amplification and analysis. When DNA extraction from bone tissues and teeth is required, the optimal skeletal elements should be selected as samples for DNA extraction because DNA yield differs among elements. Recently, some studies have reported that a high quantity of high-quality DNA can be extracted from the small cancellous bones of the hands and feet. In this study, we evaluated the effectiveness of small cancellous bones in the human identification of skeletal remains in routine forensic genetic casework. Cancellous bones [phalanges, (meta)carpal bones, and (meta)tarsal bones)] and the cortical bones (femur and petrous bones) and teeth, which have generally been recommended as samples, were collected from the same individuals that needed identifying using DNA analysis in our laboratory. The quantity of DNA from small cancellous bones tended to be higher than that from cortical bones, and the quality from the former was as high as that from the latter. This study showed that in routine forensic casework, the small cancellous bones of the hands and feet should be actively selected as samples for DNA testing.

1. Introduction

In human identification, DNA extraction from hard tissues and the analysis is commonly conducted in many cases, including identification of missing persons, criminal investigations, disaster victim identification (DVI), and identification of the war dead [1–4]. However, extraction from hard tissues is more challenging than that from other tissues, and the process is more complicated and time-consuming [5]. The quality and quantity of DNA preserved in skeletal elements can be influenced by the environmental conditions (temperature, humidity, pH, microbes, etc.), post mortem interval (PMI), and physical conditions (age, sex, etc.) [6–8], and DNA yield differs among body regions [9,10]. Therefore, selecting the appropriate skeletal elements according to each case is the key to the successful subsequent analyses and reduces the cost and time required for reanalysis. To date, many studies on the appropriate skeletal elements for DNA analysis, based on empirical research data, have been published [9–15], and they have shown that long bones (e.g., femur and tibia) and teeth are the optimal elements for DNA analysis [16–18], which are considered utility elements in some recommendations [19,20]. In addition, in the ancient DNA research community, a previous study reported that the petrous part of the temporal bone also preserves high DNA quality and quantity and is effective for DNA testing [21], which is established in the forensic science community [22]. Recently, some studies have suggested that a high DNA yield can be obtained from small cancellous bones, such as the carpal, metacarpal, tarsal, and metatarsal bones, and proximal phalanges [9,10,15]. They are easier to handle during sampling and preprocessing than the long bones or petrous bones, and exogenous DNA contamination can be prevented, enabling analysts to reduce experimental time and cost.

To date, some studies have investigated the optimal skeletal elements, based on quantitative data from several skeletal elements, including long bones, petrous bones, and small cancellous bones. For instance, Mundorff and Davoren [10] analyzed skeletal elements sampled from the same man (PMI = 3 – 21 years) and ranked the average DNA yield, and Zupanc et al. [9] reported empirical data obtained from three complete human skeletons of Second World War victims in Slovenia. However, to our knowledge, few studies on the optimal skeletal elements for DNA testing by comparing the traditionally recommended elements (long bones, petrous bones, and teeth) and small cancellous bones, not only from the same individual but also from

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skeletal remains that need to be identified by DNA testing as routine forensic casework, have been conducted. In casework, the skeletal remains that need to be identified by DNA testing are often affected by the physical conditions and the environmental conditions of the locations where they are found. Moreover, their PMIs are not as long as those of individuals analyzed in previous studies and are, in fact, often less than 1 year. Consequently, the conditions of the remains differ among individuals.

We focused on cases often encountered in routine forensic casework. Although their PMIs are relatively short (a few weeks or months), human identification by DNA testing is needed because the remains would have skeletonized faster than usual, largely due to external factors such as post-mortem damage by scavengers (e.g., insects, birds, and wild animals). In addition, PMIs range from a few weeks to a few years, and the locations where the skeletal remains are found are differed (i.e., inside, outdoors, mountain, etc.). As mentioned above, DNA preservation is influenced by the unique individual and environmental factors and PMIs. We emphasized that the evaluation of the efficacy of small cancellous bones of feet and hands should be conducted by analyzing the various skeletal samples encountered in routine forensic cases, as well as some previous studies. This study aimed to validate the utility of the small cancellous bones of the hands and feet for human identification using DNA analysis of routine forensic casework samples.

2. Material and methods

2.1. Skeletal samples and preparation

This study was approved by the Ethics Committee of the Shinshu University School of Medicine (Permission number: 667). We sampled the skeletal elements of 10 individuals (8 males and 2 females, 37–77 years of age, PMIs: 1 month-21 years) from forensic autopsy cases at our facility, where human identification for short tandem repeat (STR) analysis needed to be conducted. The skeletal elements sampled in this study included femur diaphyses and epiphyses; petrous bones; carpal, metacarpal, tarsal, metatarsal bones; proximal phalanges; and teeth. We could not collect the proximal phalanges of the foot in individual 9, and teeth, carpal, tarsal and metatarsal bones, proximal phalanges of the foot and femur epiphyses in individual 10, because these elements were lost at the site where they were found. (See Table 1 for more details on the samples.) For the same reason mentioned above, in finger skeletal elements (metacarpal and metatarsal bone; proximal phalanx), we could not collect all parts of a finger (i.e., I-V finger). For teeth, 2 teeth were collected in individual 1–7, 9 and one tooth were collected in individual 8.

We used a Volvere GX Dental Drill Control (Nakanishi Inc., Tochigi, Japan) with a rotatory grinding blade to grind the bone tissue surfaces, remove marks, and prevent contamination. The bone samples were washed using a neutral detergent, bi-distilled water, and ethanol and then dried for 24 h. Thereafter, the dried samples were cut into small pieces using the Volvere GX attached to a rotating saw blade. It should be noted that we sampled the epiphysis of the metacarpal and metatarsal bones. The bone pieces were crushed in 22-mL tubes using a Multi-beads Shocker® (YASUI KIKAI, Osaka, Japan). In the process of cutting and crushing the bone samples, we reduced the drilling speed as slowly as possible, and cooled the samples with liquid nitrogen because heat damage due to the high drilling speeds can negatively affect DNA yield [23].

We took preventive sufficient measures against contamination during the aforementioned sample preparations and DNA extraction and analysis. The instruments used for cutting and crushing the bone samples (rotatory blades, tubes, etc.) were washed in sodium hypochlorite and bi-distilled water and autoclaved. Other tools and the lab bench that could not be washed in sodium hypochlorite and autoclaved were wiped with DNAAWAY™ Surface Decontaminant (Thermo Fisher Scientific, Waltham, MA, USA). Moreover, we collected DNA from the examiners and obtained their DNA profiles using GlobalFiler before the sample preparations to reduce the risk of the contamination by comparing their profiles with those of the individuals being sampled.

2.2. DNA extraction and purification

For the bone samples, and not the teeth, 0.2 g of bone powder was demineralized and digested simultaneously overnight at 56 °C in 10 mL lysis buffer comprising of 0.5 M EDTA (pH 8.0) (Nippon Gene, Tokyo, Japan), Buffer ATL (QIAGEN, Hilden, Germany), 1 M 1,4-Dithiothreitol (Sigma-Aldrich, St. Louis, MO, USA), and 20 mg/mL Proteinase K (FUJIFILM Wako Pure Chemical Co. ltd, Osaka, Japan). All parts of the tooth were weighted and dissolved in the same buffer as the bone samples. Incubation was performed in a ThermoMixer® C (Eppendorf, Hamburg, Germany), shaken at 350 rpm. Subsequently, the lysate was concentrated down to 250 μL using Amicon® Ultra-15 (Merck Millipore, Burlington, MA, USA). The concentrated lysate was purified using a QIAquick Spin Column (QIAGEN), and DNA was eluted in 50 μL of EB buffer (QIAGEN), based on a previous study [24]. In the present study, we extracted DNA from the bone samples and analyzed it thrice for each element except for teeth. For teeth, DNA was extracted from each of the collected tooth samples.

2.3. DNA quantification

To determine the concentration and degradation level of human genomic DNA in all samples, a TaqMan assay was performed using a QuantStudio5 Real-Time PCR System (Thermo Fisher Scientific). The concentration of human nuclear DNA (80 bp small autosomal target, 214 bp large autosomal target) was measured using a Quantifiler™ HP DNA Quantification kit (Thermo Fisher Scientific), which includes an internal PCR control (IPC) to detect PCR inhibitors and can determine the degradation Index (DI) by dividing the concentration of the small target by that of the large target. Data analysis was performed using HID Real-Time PCR Analysis Software v1.3 (Thermo Fisher Scientific), following the user manual.

2.4. STR typing and analysis

In the samples with median values of the concentration (small target) of extracted DNA samples from each element, except for teeth in all individuals, we conducted STR analysis, and in teeth, we analyzed all samples. The extracted DNA was amplified using a GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific) with a ProFlex™ PCR System (Thermo Fisher Scientific), following the user manual. An input DNA of 1 ng was used, and the amplification was performed for 29 cycles. When a sufficient amount of DNA for STR analysis using GlobalFiler was not extracted, the maximal amount of input DNA was used, and the amplification was performed for 29 cycles. For fragment analysis, 1 μL of PCR product was added to 9.6 μL of HiDi™ formamide (Thermo Fisher Scientific) and 0.4 μL of GeneScan™ 600 LIZ® dye Size Standard (Thermo Fischer Scientific). After heating at 95 °C for 3 min and cooling on an ice pack, these samples were separated using an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific) with a POP-4 polymer in a 36-cm capillary. The data were analyzed using GeneMapper™ ID-X Software v1.6 (Thermo Fisher Scientific) with an analytical threshold of 175 relative fluorescence units.

2.5. Statistical analysis

For statistical analysis, DNA yield was expressed as ng DNA/g bone, calculated by dividing the concentration of human nuclear DNA (small target) by the mass of the digested sample. DNA quality was assessed using the DI. Additionally, to evaluate DNA quality, the percentage of the recovered alleles in STR analysis was also calculated by dividing the number of successfully detected alleles by the total number of alleles

Table 1
Detailed sample data and quantitative results.

Individual	Sex	Age	PMI	Location of discovery	DNA yield [ng DNA/g bone] and Degradation Index																			
					Carpal		Metacarpal		Proximal phalanx (Hand)		Tarsus		Metatarsal		Proximal phalanx (Foot)		Femur diaphysis		Femur epiphysis		Petrous		Tooth	
					DNA yield	DI	DNA yield	DI	DNA yield	DI	DNA yield	DI	DNA yield	DI	DNA yield	DI	DNA yield	DI	DNA yield	DI	DNA yield	DI	DNA yield	DI
1	M	37	11 months	Outdoors	17.59	1.44	8.63	1.97	4.89	1.33	11.36	1.42	26.06	3.21	47.29	1.69	1.94	2.62	4.31	1.44	5.38	3.07	0.19	4.71
					16.56	1.13	9.62	1.86	7.47	1.42	15.69	1.54	89.41	1.58	38.08	1.67	1.41	2.76	2.80	1.47	5.01	2.96	0.29	4.61
					10.66	1.25	7.45	2.09	3.80	1.56	9.85	1.42	43.78	1.68	5.97	1.41	2.61	3.39	1.76	1.59	6.83	3.34	NA	NA
2	F	64	1 year	Indoors	0.24	1.63	5.39	1.93	0.87	1.84	0.10	1.89	4.90	1.59	0.94	1.53	1.03	1.51	2.45	2.44	0.72	2.66	0.06	1.98
					0.27	2.03	9.16	1.60	0.90	1.87	0.08	2.38	5.17	1.51	0.58	1.60	1.28	1.46	2.86	2.15	0.89	3.37	0.05	1.86
					2.35	2.07	9.71	1.95	46.29	1.69	0.08	1.74	8.34	1.71	0.73	1.65	0.83	1.53	2.57	2.36	0.79	4.71	NA	NA
3	M	61	1 month	Indoors	1.26	0.77	5.43	1.11	1.18	1.10	6.06	1.07	5.30	1.18	0.36	0.66	0.73	1.26	6.66	0.92	0.49	1.55	0.03	1.76
					1.24	0.68	4.05	1.34	1.07	1.01	5.00	1.08	5.96	1.14	0.49	0.94	0.67	1.08	0.83	1.28	0.58	1.60	0.02	1.42
					1.26	0.86	2.65	1.14	3.68	1.33	5.33	1.13	5.93	0.94	0.52	0.90	0.86	1.18	1.41	1.03	0.91	2.13	NA	NA
4	M	43	1 month	Outdoors	16.92	1.17	4.05	0.99	6.41	1.16	5.44	1.09	6.73	1.05	10.80	1.31	0.70	1.38	3.05	1.42	1.05	2.09	0.07	0.73
					21.52	1.35	5.31	1.10	6.69	1.23	6.56	1.13	4.84	0.88	13.02	1.36	1.04	0.91	3.10	1.25	2.48	3.30	0.25	0.64
					14.06	1.12	6.86	1.07	9.91	1.45	7.96	0.98	7.45	1.12	7.67	1.19	0.54	1.29	3.79	1.25	2.65	3.39	NA	NA
5	M	68	1 month	Indoors	25.75	1.09	17.97	1.27	40.73	1.16	35.19	1.29	20.63	1.41	8.72	1.09	3.94	1.22	22.06	1.18	4.38	2.35	0.37	1.08
					33.15	1.11	19.12	1.26	37.55	1.14	25.65	1.19	29.56	1.47	5.07	1.07	1.71	1.63	21.01	1.14	4.17	3.70	2.10	1.11
					31.05	1.14	23.44	1.53	52.02	1.07	26.57	1.23	27.84	1.39	7.77	1.14	2.60	1.71	24.44	1.22	2.44	2.97	NA	NA
6	M	64	7 years and 10 months	Indoors	344.03	5.57	419.48	3.76	637.92	2.71	1.97	5.04	3.71	3.43	24.99	4.82	0.93	3.86	0.91	3.99	4.29	7.54	0.01	8.49
					396.97	5.62	416.96	4.16	642.45	2.75	3.08	3.34	4.35	3.05	7.90	4.95	0.36	7.50	0.87	3.98	3.87	7.73	0.01	4.69
					309.15	6.51	425.37	4.20	684.54	2.79	2.35	4.15	5.41	3.56	5.13	4.31	0.78	2.80	1.11	4.61	3.43	8.59	NA	NA
7	F	77	3 years and 1 month	Indoors	52.66	3.02	320.73	2.74	1093.31	1.60	12.43	2.57	8.56	1.70	5.17	2.36	0.75	2.56	0.58	2.38	0.68	1.87	27.51	1.06
					83.79	1.62	223.00	3.09	745.53	1.67	11.52	2.63	11.24	1.82	13.70	1.74	0.60	2.52	3.81	1.62	0.74	2.23	8.69	1.56
					69.62	2.14	199.20	2.62	510.93	2.06	11.59	2.59	12.25	1.58	12.49	1.91	0.57	2.07	2.24	2.00	1.29	2.83	NA	NA
8	M	47	11 months	Outdoors	4.79	0.79	8.99	0.93	4.93	1.05	13.64	0.95	7.37	1.15	10.26	1.08	0.36	1.15	0.91	1.47	0.76	1.47	1.70	1.00
					6.82	0.77	5.90	0.90	3.73	0.89	17.08	1.08	11.96	1.08	9.75	1.15	0.37	1.43	0.42	1.62	4.55	2.67	NA	NA
					6.63	0.79	8.93	0.97	3.74	1.00	14.75	1.11	3.97	0.59	15.57	1.15	0.36	1.17	0.63	1.53	2.26	2.39	NA	NA
9	M	57	1 year and 8 months	Indoors	29.39	1.35	133.34	1.45	742.84	1.39	8.31	1.71	249.04	1.52	NA	NA	2.58	1.99	3.66	2.22	2.64	3.03	11.52	0.92
					25.96	1.45	251.99	1.51	976.05	1.24	15.22	1.66	230.72	1.64	NA	NA	2.55	1.50	2.77	2.49	4.13	3.88	0.45	0.92
					23.75	1.49	357.23	1.06	951.38	1.27	10.70	1.35	112.24	1.25	NA	NA	2.19	1.41	3.48	2.55	1.41	3.12	NA	NA
10	M	68	21 years	Indoors	NA	NA	6.09	2.26	11.03	2.19	NA	NA	NA	NA	NA	NA	0.86	1.85	NA	NA	1.46	4.89	NA	NA
					NA	NA	5.32	2.27	20.20	2.42	NA	NA	NA	NA	NA	NA	0.82	1.53	NA	NA	1.75	5.49	NA	NA
					NA	NA	4.08	2.30	15.62	1.74	NA	NA	NA	NA	NA	NA	1.06	1.71	NA	NA	1.79	5.43	NA	NA

PMI: Post mortem Interval, NA: The samples were not able to be collected in the sites. DNA extraction and analysis were performed thrice for each element except for teeth, and for teeth, DNA extraction performed from each of the collected tooth samples.

possible for an individual.

In terms of quality and quantity of the extracted DNA, the differences between individuals and between skeletal elements were statistically estimated. Particularly, to estimate the difference in quality and quantity of the DNA between the skeletal elements in cases where the remains skeletonized faster than usual owing to the environmental conditions and the postmortem damages by scavengers, we classified the individuals according to PMIs (≤1 year or not) and analyzed the data. The time taken by remains to completely skeletonize due to scavenging could not be clearly defined as it varies with environmental conditions and scavenger type. However, in this study, we delineated this period as 1 year because the period is assumed to be anywhere between a few weeks to a few months, according to previous studies [25,26]. Moreover, to our knowledge, few studies on the optimal skeletal elements for DNA testing based on the empirical data obtained from remains whose PMIs were shorter than 1 year exist. Thus, we also wanted to analyze the data obtained from these remains, and we delineated this period. Normality was tested using the Shapiro–Wilk tests; therefore, the normality of the distribution was not assumed. Non-parametric Kruskal–Wallis tests were used to assess the differences in the quality and quantity of extracted DNA between individuals and between skeletal elements, and Steel–Dwass multiple comparison tests were used as post-hoc tests.

In addition, differences in the quantity of DNA extracted from the cortical bones (femur diaphyses and petrous bones) and the cancellous

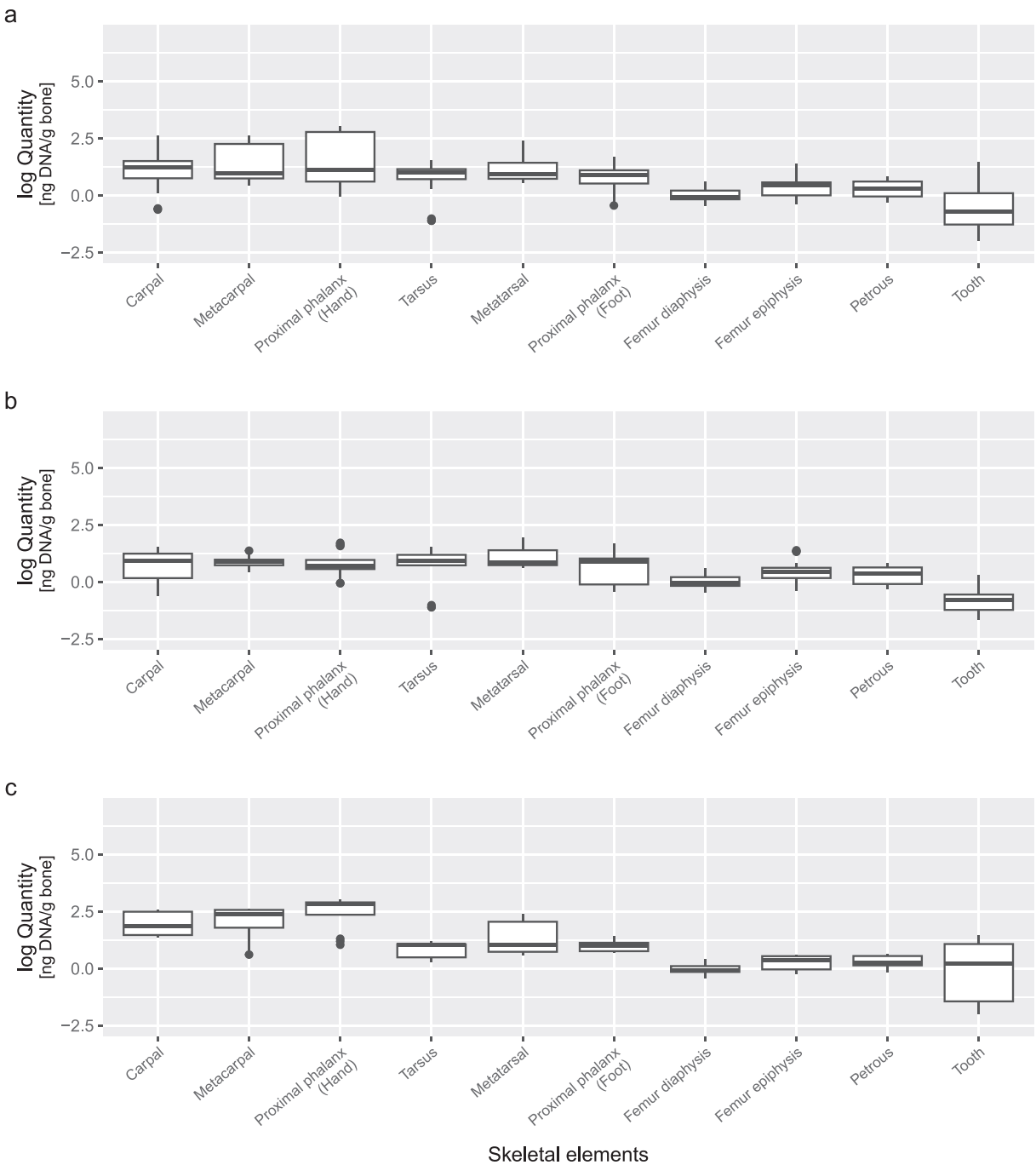

Fig. 1. A comparison of DNA yield between skeletal elements in all individuals (a), the individuals whose PMIs were ≤ 1 year (b), and PMIs were > 1 year (c). DNA yield [ng DNA/g bone] was calculated by dividing the concentration of human nuclear DNA (small target) by the mass of the digested sample, and the vertical axis was transformed into a common logarithm.

bones (femur epiphyses; carpal, metacarpal, tarsal, metatarsal bones; and proximal phalanges) according to PMIs and age were assessed. As mentioned above, we classified the individuals according to PMIs (≤1 year or others) and analyzed the relationship between PMIs and DNA quantity. Furthermore, individuals were classified according to age (≤60 years old or others), and the relationship between age and DNA quantity was analyzed since the decrease in trabeculae with age increases considerably at 60 years old and above, as suggested by a previous study [27]. Normality was also tested using the Shapiro–Wilk tests. When the normality of the distribution was not assumed, the Wilcoxon Signed-rank tests were used to assess the differences between the two groups in terms of PMIs (≤1 year or others) and between those in term of age (≤60 years old or others). When the normality of the distribution was assumed, Student's *t*-test was used. Statistical analyses were performed using R software (v4. 3. 1; R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was considered at $p < 0.05$.

3. Results

Detailed information on each individual, and the quantity and quality data are listed in Table 1. A comparison of the quantity and quality results between the skeletal elements is shown in Figs. 1 and 2, respectively, and a comparison between the individuals is shown in

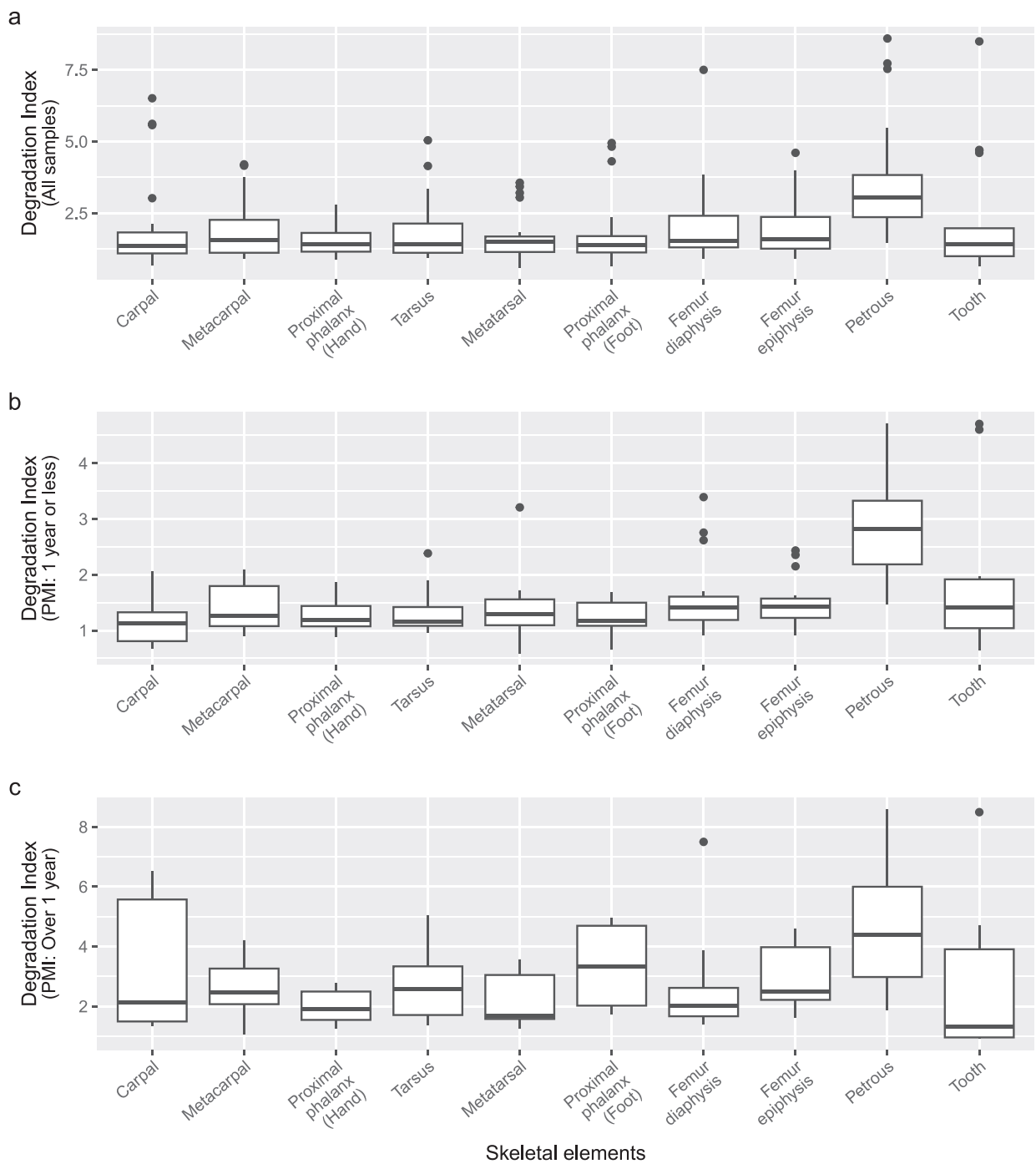

Fig. 2. A comparison of DNA quality (Degradation Index) between skeletal elements in all individuals (a), the individuals whose PMIs were ≤ 1 year (b), and PMIs were > 1 year (c).

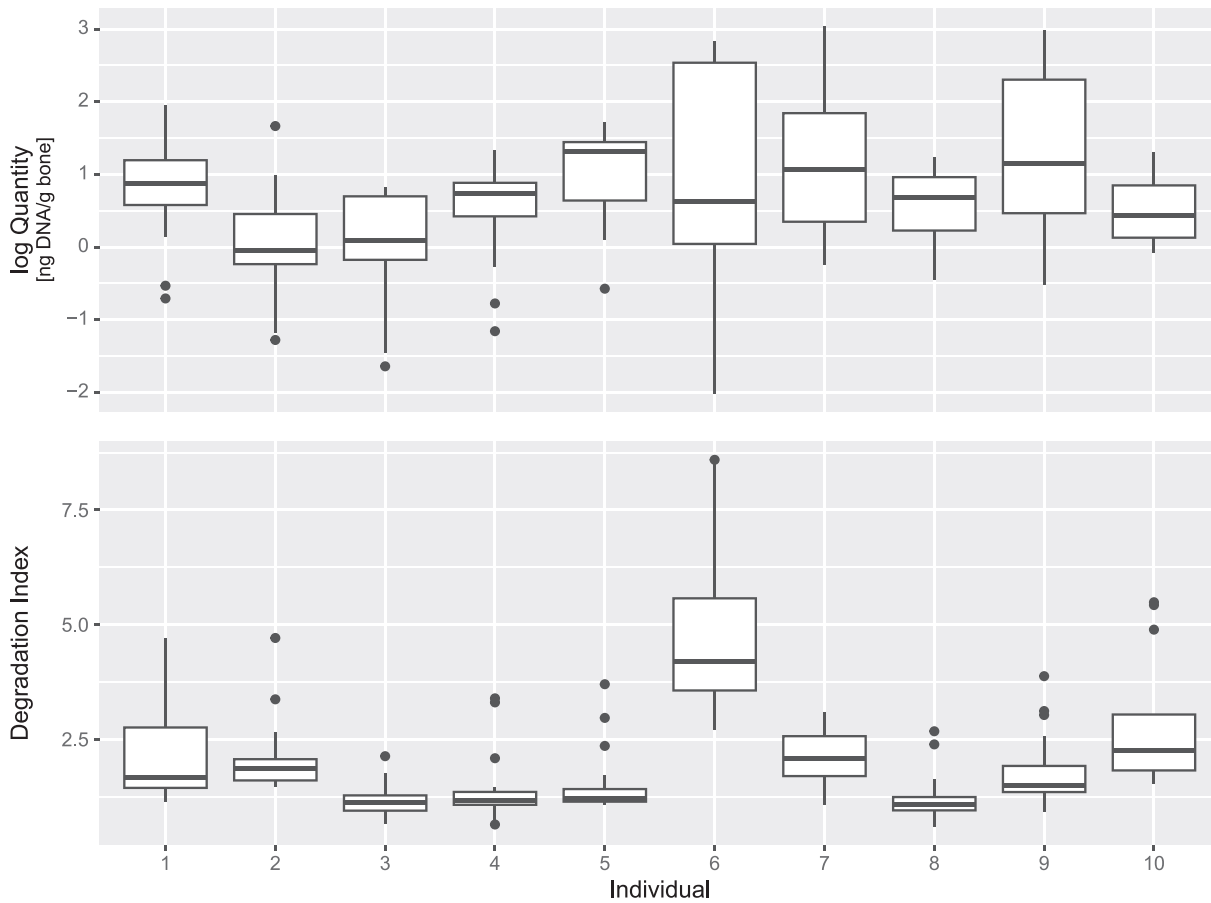

Fig. 3. A comparison of DNA yield [ng DNA/g bone] (the upper figure) and the quality (Degradation Index) (the lower figure) between individuals. DNA yield [ng DNA/g bone] was calculated by dividing the concentration of human nuclear DNA (small target) by the mass of the digested sample, and the vertical axis was transformed into a common logarithm.

Fig. 3. The *p*-values of these comparisons by Steel–Dwass multiple comparison tests are presented in Table S1–3. In the cortical and cancellous bones, the relationships between DNA quantity and PMIs, and between quantity and age are shown in Fig. 4 and Fig. 5, respectively. Data from the quantification in this study are shown in Table S4.

3.1. DNA quantity

In all individuals, cancellous bones (carpal, metacarpal, tarsal, and metatarsal bones and proximal phalanges of the hand and foot) had higher DNA yields than cortical bones (femur diaphyses and petrous bones) and teeth ($p < 0.05$; Fig. 1a). However, cortical bones and teeth did not significantly differ ($p > 0.05$). Notably, the DNA yield from the femur epiphysis was significantly higher than that from the diaphysis ($p < 0.05$). In individuals whose PMIs were 1 year or less (Fig. 1b), the cancellous bones had a significantly higher DNA yield than that of femur diaphysis and teeth ($p < 0.05$). In cancellous bones, only metacarpal bones and tarsal bones had a significantly higher DNA yield than that of petrous bones ($p < 0.05$); the other small cancellous elements and petrous bones did not significantly differ ($p > 0.05$). Additionally, no significant differences were observed among cancellous bones ($p > 0.05$). In individuals whose PMIs were ≥ 1 year (Fig. 1c), the small cancellous bones had a significantly higher DNA yield than that of the femur and petrous bones ($p < 0.05$); however, although there was no significant difference between tarsal and petrous bones, tarsal bones tended to have higher DNA yields.

Fig. 4 illustrates the comparison of the DNA yield between the individuals whose PMIs were ≤ 1 year and those whose PMIs were > 1 year in the three skeletal element types, namely, cancellous bones (hand), including carpal and metacarpal bones and proximal phalanges of the hand; cancellous bones (foot), including tarsal and metatarsal bones and proximal phalanges of the foot; femur epiphyses; and cortical bones, including femur diaphyses and petrous bones. Normality of distribution was not assumed for cancellous bones (foot and hand), and the Wilcoxon signed-rank tests were used to assess the differences. The results showed that in cancellous bones (hand), DNA yield in the individuals whose PMIs were > 1 year was significantly higher than in those whose PMIs were ≤ 1 year (Wilcoxon: $p < 0.05$), but in cancellous bones (foot), no significant difference was observed between the individuals whose PMIs were > 1 year and whose PMIs were ≤ 1 year (Wilcoxon: $p > 0.05$). In cortical bones, the normality of the distribution was assumed, and Student's *t*-test was used; the difference between the two groups was not significant (*t*-test: $p > 0.05$).

Fig. 5 shows the comparison between the individuals aged ≤ 60 years and those who were > 60 years old, in the three skeletal element types. Normality of distribution in the cancellous (hand and foot) and cortical bones was not assumed. In cancellous (hand) and cortical bones, the difference between individuals aged ≤ 60 years and those aged > 60 years old was not significant (Wilcoxon: $p > 0.05$). In contrast, in cancellous bone (foot), DNA yield in the younger individuals was higher than that in older individuals (Wilcoxon: $p < 0.05$).

As shown in Fig. 3 and Table S3 (Steel–Dwass *p*-values), differences between individuals significantly differed.

3.2. DNA quality

In this study, we assessed DNA quality based on the DI. The DI data for each sample are listed in Table 1. According to the data for all

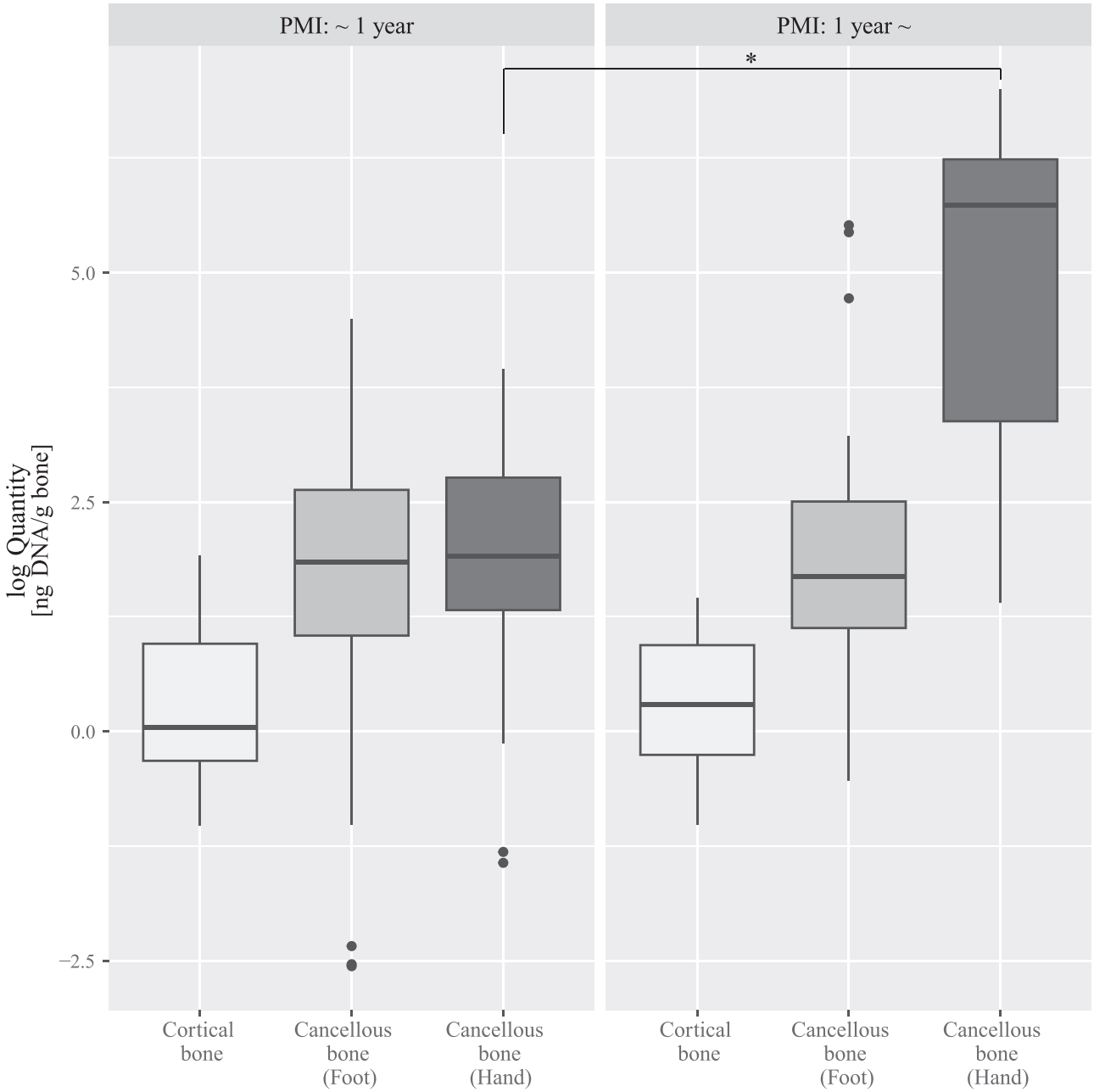

Fig. 4. A comparison of the DNA yield between the individuals whose PMIs were ≤ 1 year (the left figure) and those whose PMIs were > 1 year (the right figure) in the three skeletal types. Cortical bone includes the femur diaphysis and petrous bones, Cancellous bone (Foot) includes tarsal and metatarsal bones and proximal phalanges of the foot; femur epiphyses, and Cancellous bone (Hand) includes carpal and metacarpal bones and proximal phalanges of the hand. *p < 0.05.

individuals in Fig. 2a, cancellous bones (carpal, metacarpal, tarsal, metatarsal bones and the proximal phalanges of the hand and foot), femur, and teeth did not significantly differ ($p > 0.05$). Additionally, no significant differences were observed between the femur diaphyses and epiphyses or between the cancellous bones ($p > 0.05$). In contrast, in petrous bones, DI was significantly higher than that of the other elements ($p < 0.05$), implying lower quality. In individuals whose PMIs were ≤ 1 year, skeletal elements except for petrous bones did not significantly differ in terms of DI; however, in petrous bones, the DI was higher than that in the other elements (Fig. 2b). Fig. 2c shows the DI in individuals whose PMIs were > 1 year. Significant differences were found between the proximal phalanges of the hand and petrous bones ($p < 0.05$); however, the other elements did not significantly differ.

In the comparison of individuals (Fig. 3), some individuals exhibited differences, particularly individual 6, whose DI was higher than that of the other individuals.

3.3. PCR inhibitors

In this study, we detected PCR inhibitors based on the Ct values of Internal PCR controls (Table S1). The Ct values of all samples were lower than 30, and the PCR reactions of all samples were not inhibited. Even if some inhibitors were present, they hardly affected the PCR reaction.

3.4. STR analysis

STR analysis was performed using GlobalFiler; in all individuals, their DNA profiles matched among all elements, and we found no contamination in this analysis. Table 2 and S5 illustrate the number of recovered alleles and their ratios for each sample. The markers of the

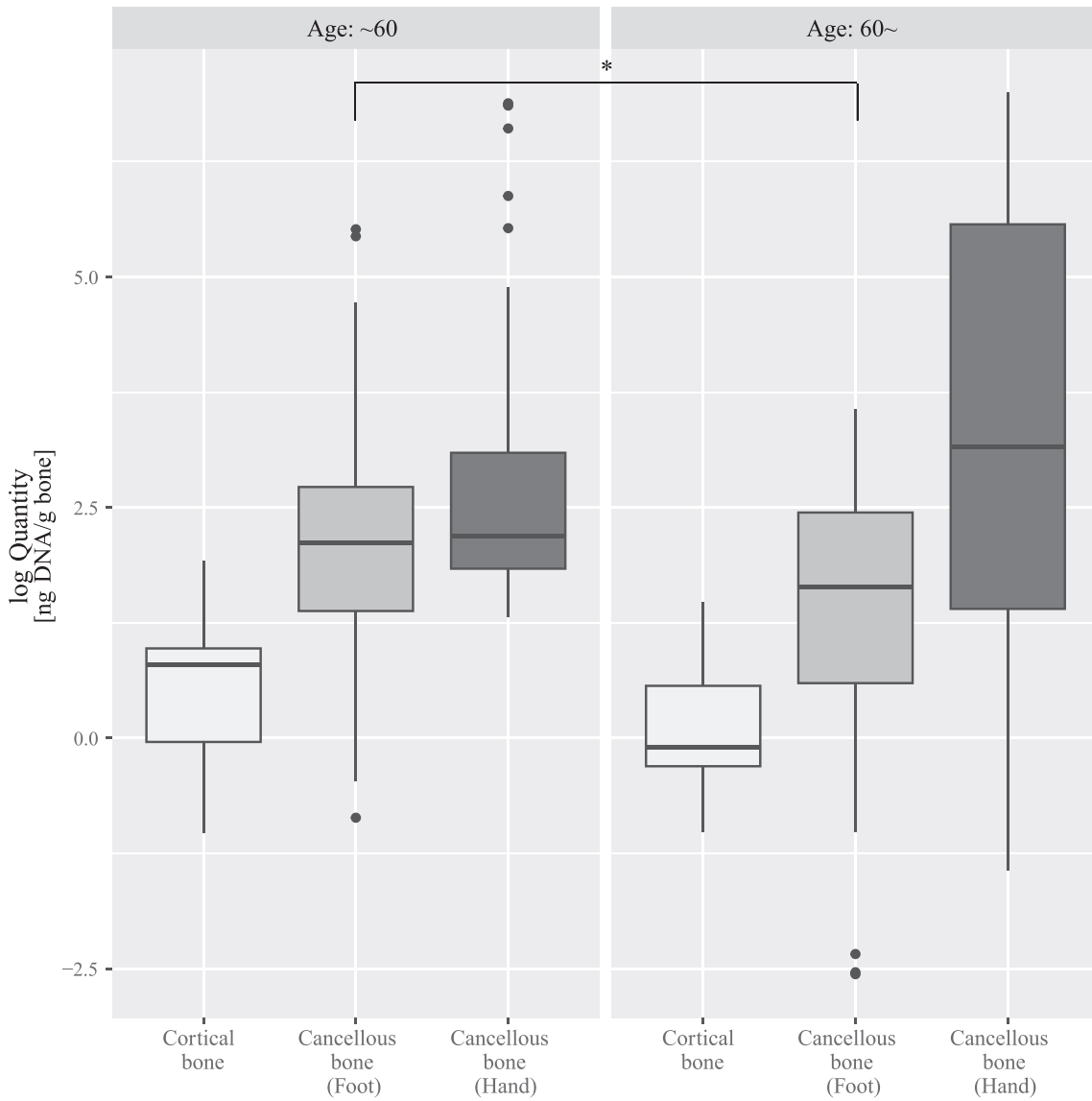

Fig. 5. A comparison of the DNA yield between the individuals aged ≤ 60 years and those who were > 60 years old in the three skeletal element types. Cortical bone includes the femur diaphysis and petrous bones, Cancellous bone (Foot) includes tarsal and metatarsal bones and proximal phalanges of the foot; femur epiphyses, and Cancellous bone (Hand) includes carpal and metacarpal bones and proximal phalanges of the hand. *$p < 0.05$.

unrecovered alleles are detailed in Table 2. In individuals 5 and 9, full profiles were detected for all elements; however, in the other individuals, some alleles were unrecovered for some markers. When insufficient DNA was extracted, alleles were not detected for many of the markers. In samples with low DNA quality (large DI), the alleles of some markers with relatively long amplicon sizes were not recovered. As suggested by the data for individual 6, the lower the DNA quality, the lower the number of recovered alleles. In some samples, although the quality and quantity of DNA were high, full profiles were not detected (e.g., foot proximal phalanx of individual 1).

4. Discussion

In DNA extraction for human identification using DNA testing, long bones such as the femur and tibia, the petrous part of the temporal bones (petrous bones), and teeth have been the optimal elements for DNA testing [19,20,28,29]. Recent studies have reported that small cancellous bones have good DNA yield, and their utility for DNA testing has been proven [9,10,14]. In the present study, we sampled the small cancellous bones of the hands and feet, the cortical bones and teeth from the same individual in routine forensic casework, in which the environmental and physical conditions and PMIs vary by case, and assessed the efficacy of cancellous bones of hands and feet in routine forensic genetics identification by comparison with cortical bones.

We evaluated the efficacy for DNA testing by comparing skeletal elements based on extracted DNA quality and quantity. Our results suggest that small cancellous bones of hands and feet are appropriate elements for DNA testing in routine forensic casework samples, as some previous studies have shown their usefulness in human identification for relatively long PMI remains [9,10,14]. We compared the DNA yield between individuals whose PMIs were ≤ 1 year and those whose PMIs were > 1 year and found that the DNA yield from the cancellous bones was higher than that from cortical bones, regardless of the PMIs. While DNA yield from the individuals whose PMIs were > 1 year was higher than that from the individuals whose PMIs were ≤ 1 year in only the cancellous bones of the hands, DNA yield from the shorter PMI individuals was as high as that from longer PMI individuals in the other elements. Therefore, PMIs may slightly affect DNA yield, particularly in individuals whose PMIs are relatively short, to the extent of a few weeks to a few years, as in the individuals analyzed in this study. DNA yield is

Table 2
Undetected loci in STR analysis using GlobalFiler kit.

Individual	Elements	Recovered alleles (%)	Undetected loci
1	Ploximal phalanx (Foot)	90.7	TPOX, DYS391, SE33
	Tooth 1	79.1	CSF1PO, D21S11, DYS391, D7S820, SE33, D12S391, D2S1338
	Tooth 2	79.1	CSF1PO, D18S51, DYS391, D13S317, D7S820, SE33, D12S391, D2S1338
2	Petrous	85.4	CSF1PO, TPOX, D7S820, SE33, D2S1338
	Tarsus	73.2	vWA, CSF1PO, TPOX, D21S11, D18S51, D2S441, TH01, SE33, D12S391
3	Petrous	97.7	TPOX
	Tooth 1	88.6	D16S539, TPOX, DYS391, SE33
	Tooth 2	90.9	D16S539, D7S820, SE33
4	Tooth 2	65.0	D16S539, CSF1PO, TPOX, D18S51, DYS391, D7S820, SE33, D2S1338
6	Petrous	73.2	CSF1PO, TPOX, D18S51, D7S820, SE33, D12S391, D2S1338
	Proximal phalanx (Foot)	92.7	DYS391, D2S1338
	Tarsus	92.7	TPOX, DYS391, D2S1338
	Tooth 1	43.9	D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, DYS391, D19S433, TH01, D7S820, SE33, D1S1656, D12S391, D2S1338
	Tooth 2	39.0	vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, DYS391, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338
7	Femur epiphysis	88.1	CSF1PO, TPOX, DYS391, SE33, D2S1338
	Metacarpal	97.6	TPOX
	Petrous	83.3	CSF1PO, TPOX, D7S820, SE33
	Tarsus	97.6	CSF1PO
8	Petrous	97.6	TPOX
10	Femur diaphysis	97.6	TPOX
	Petrous	90.5	CSF1PO, TPOX, D18S51

assumed to depend on several factors, including environmental and physical conditions. Cancellous bones had high DNA yields in the current study because some cells, except osteocytes, were thought to be contributed to the high DNA quantity of these elements, as suggested by the previous studies [9,15]. The cells that exist in bone tissue include osteocytes, osteoblasts, and osteoclasts, with osteocytes accounting for approximately 90–95 % [30,31], and their lifespan being up to approximately 25 years [32]; therefore, many of them are stable for a long time in bone tissues. Accordingly, during DNA extraction from bone tissues, the endogenous DNA of osteocytes is generally thought to be extracted. Bone tissues extracellular matrix components consist of organic materials, such as type I collagen (85–90 %) and proteoglycan, and inorganic materials, such as hydroxyapatite, mainly composed of calcium, phosphorus, and osteocytes, which exist in lacunes surrounded by the extracellular matrix [33]. According to a previous study, the number of the lacunes in cortical bones is larger than that in cancellous bones [34]. If DNA extracted from bone tissues is obtained largely from osteocytes, cortical bones may have a higher DNA yield; however, the DNA yield from cancellous bones was higher than that from the cortical bones in the current study. This was likely due to the influence of hematopoietic tissues and cells remaining between the trabeculae, as suggested in a previous report [34]. Additionally, bone cells, except for osteocytes, namely osteoblasts and osteoclasts, which mainly exist on the surface of the bone matrix, are likely to influence the higher DNA yield because the number of osteoblasts and osteoclasts remaining in cancellous bones is thought to be larger than that in cortical bones. This is because the surface area of the bone matrix in cancellous bones, which mainly consists of cancellous parts, is larger than that in cortical bones [33,35]. Collectively, considering these points, the main reason for the greater DNA yield from cancellous bones in the present study was thought to be the effects of hematopoietic tissues and cells, osteoblasts, and osteoclasts remaining between the trabeculae and attached to the surface of the bone matrix. Moreover, compared with the previous studies [9,10,14], the PMIs of the samples analyzed in our study were shorter-PMIs were approximately a few years to a few decades in the previous studies but the PMIs of almost all samples proximately a few weeks to a few years in our study. Thus, more hematopoietic tissues, cells, osteoblasts, and osteoclasts were likely to remain in the cancellous bones in the current study than in the previous studies, and their effects were thought to be more significant.

Another reason for the greater DNA yield from cancellous bones is that microbes also likely influence DNA preservation, as pointed out previously [15,36]. In general, microbes tend to promote DNA degradation and negatively impact DNA preservation, regardless of whether they are indigenous microbes or environmental [6–8]. Previous studies have suggested that microbial enzymatic activity decomposes bone tissues, resulting in low DNA preservation [37,38]. Some microbes produce collagenase and decompose collagen, a component of bone tissue. In addition, many soil microbes can produce DNA nucleases that deteriorate DNA [39,40]. However, considering previous studies, it should be noted that microbes do not necessarily negatively affect DNA preservation. Previous research on metagenomic sequence data from skeletal samples using next-generation sequencing methods showed that bacterial species differed between individuals and between body elements. For instance, while *Dermacoccaceae* is relatively abundant in the foot, wherein DNA quantity is higher, the relatively abundance of *Clostridium*, which produces collagenase, is related to lower skeletal DNA quantity [41]. Although the microbial effect of improving DNA preservation has not been clarified, microbial biofilms contribute to greater DNA preservation, probably because they minimize the environmental changes in bone tissues that play an important role as a culture medium. A previous study reported that microbial biofilms were observed in cancellous bones by scanning electron microscopy [42].

Incidentally, the body parts with a high DNA yield are anatomically distant from the gut, where microbes (e.g., *Clostridium*) contributing to low yield are relatively abundant [15,41]. A higher quantity of DNA was extracted from the skeletal elements of the feet than from the torso, partly because the former parts are anatomically more distant from the gut than the latter; therefore, the microbial impact on DNA preservation was relatively small. Thus, in the present study, the quantity of DNA from cancellous bones (metacarpal bones, metatarsal bones, etc.) was larger than that from the cortical bones (femur diaphysis and petrous bones). Considering the tendency of microbes to colonize cancellous bones more than cortical bones [7,43], microbes were likely to have a causal role in good DNA preservation and higher DNA yield from the cancellous bones. In addition, the cancellous bones analyzed in our study were from the extremities, distant from the torso; therefore, DNA preservation and yield may have been better because microbes did not significantly affect preservation. However, while a study suggested that microbial bone erosion occurs over a short period, another study implied that it takes a long time to erode bone tissues [44,45]. Of the samples analyzed in the current study, particularly those whose PMIs were relatively short (≤1 year), mainly due to post-mortem damage by scavengers such as insects, the possibility that microbial influence was not significant should be considered. Therefore, the effect of

hematopoietic tissues and cells, osteoblasts and osteoclasts between trabeculae was thought to be relatively larger than the microbial impact in individuals with short PMIs. On the other hand, considering that the DNA yield from the cancellous bones did not decrease as PMIs increased in this study, it is plausible that microbes also play a role in affecting DNA preservation, particularly in individuals with longer PMIs.

Because of the high DNA yield from the cancellous bones, the effect of the remaining cells between trabeculae and the impact of microbes were suggested, and they were thought to explain why DNA quantity from the femur epiphyses was larger than that from the femur diaphyses. In previous studies comparing DNA yields from the diaphyses and epiphyses of metacarpal and metatarsal bones and femur, the epiphyses had a higher DNA yield than that of the diaphyses, and the two aforementioned reasons were also suggested as an explanation [36,46].

Regarding the relationship between DNA yield and individual age, particularly in the cancellous bone of the feet, the higher the age, the lower the quantity of DNA. A relationship between bone remodeling activity and DNA quantity is thought to exist. As suggested in a previous study, foot bone remodeling occurs more actively than that of other body parts because foot bones are weight-bearing [15]; thus, many hematopoietic cells, osteoblasts, and osteoclasts, which play a major role in bone remodeling, are present on foot bones and thus contribute to the high DNA yield. As age increases, bone remodeling declines; for example, previous studies have suggested that the number of trabeculae in the epiphysis of long bones such as femur and tibia, decreases with age [47,48]. Therefore, in the older individuals analyzed in this study, the decline of bone remodeling likely led to a lower DNA yield in the cancellous bones of the feet.

Regarding DNA quality, represented by the DI, skeletal elements did not significantly differ, except for petrous bones; however, individuals exhibited differences. The extent of DNA degradation is thought to be influenced by individual physical conditions (age, sex, etc.), PMIs, and environmental conditions (temperature, humidity, pH, etc.) [6,8]. In addition, we analyzed samples from individuals under varying physical and environmental conditions, but the effects of PCR inhibitors were limited. In general, many materials characteristics of the remains and environment are considered PCR inhibitors [49]; however no superior PCR inhibitor was detected in a specific skeletal element in the current study.

In this study, DNA extraction was performed three times for each element, excluding teeth. Variations were observed among the three quantitative values of DNA yield in some elements, particularly in finger bones such as proximal phalanx. This variation is likely due to the differing conditions of each of the 5 fingers (I-V) on the left and right sides, suggesting that DNA yields may differ among fingers (I-V). Notably, previous studies have suggested significant differences in DNA yield among finger skeletal elements, such as metatarsals, even within the same individuals [9,10]. In our study, it was impossible to perform DNA extraction three times from the same finger (e.g., solely from the "2nd" proximal phalanx), which is also a limitation of this study, because we focused on routine forensic casework samples. Collecting all skeletal elements, particularly finger skeletal elements such as proximal phalanx, is not always feasible due to their small size, and these elements are often lost at the site where they were found.

In the present study, the quantity of DNA from cancellous bones was greater than that from the cortical bones and teeth, and its quality was sufficient to analyze STR for human identification as was that from cortical bones; however, small cancellous bones cannot always be sampled in forensic casework because of their size. These bone elements are small, and wild animals often carry them away. Additionally, when several remains are buried in the same location (e.g., a mass grave), their skeletal samples are likely to be mixed, making it difficult to identify these samples by individual. However, in routine forensic casework, which is the focused of this study, the small cancellous bones of the hands and feet are often sampled from skeletal remains, and particularly in cases where skeletal remains are found indoors, these elements are more often collected. Moreover, a higher DNA yield was expected because of the relatively short PMIs, as discussed above. In addition, the cancellous bones of the hands and feet are smaller than cortical bones; therefore, these elements are easier to process (easier washing and sampling, no cutting necessary) than long bones such as the femur and petrous bones. The risk of contamination is also minimized, and the experimental time and cost are reduced; therefore, DNA analysis and profile generation are expedited. Thus, we suggest that the small cancellous bones of the hands and feet be actively selected for human identification using DNA analysis, besides long bones and teeth. Considering that the quantity of DNA from the femur epiphysis was higher than that from the femur diaphysis in this study, selecting the epiphysis is desirable when long bones, such as femur, are used for DNA analysis. Additionally, in cases of DVI, where DNA profiles need to be generated as soon as possible for several missing persons, small cancellous bones are likely to be suitable for DNA analysis because they enable shorter experimental times. In many studies on DVI cases, the effective utilization of small cancellous bones for DNA analysis has been reported [4,9,50,51]. The International Society for Forensic Genetics guidelines recommend multi-sampling to avoid re-sampling [19]; therefore, it is thought that sampling small cancellous bones is optimal for DNA analysis when skeletal elements other than long bones and teeth, which are generally recommended as suitable elements, are collected in DVI cases.

5. Conclusions

This study demonstrated the potential of using the small cancellous bones of the hands and feet for DNA testing in routine forensic casework, owing to the high quality and quantity of DNA from these elements. In routine forensic casework samples whose physical conditions, PMIs, and environmental conditions vary, DNA extraction from small cancellous bones (carpal, metacarpal, tarsal, and metatarsal bones and proximal phalanges) for DNA testing may be equal to or more suitable than that from cortical bones (femur and petrous bones) and teeth, which have generally been recommended as optimal elements for DNA testing, as well as from relatively longer PMI samples as analyzed in previous studies. For forensic human identification, when there is no choice but to extract DNA from skeletal elements and if sampling cancellous bones from skeletal remains is possible, these elements should be actively used as samples for DNA testing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.legalmed.2024.102415.

References

[1] E.I. Zavala, J.T. Thomas, K. Sturk-Andreaggi, J. Daniels-Higginbotham, K. K. Meyers, S. Barrit-Ross, A. Aximu-Petri, J. Richter, B. Nickel, G.E. Berg, T. P. McMahon, M. Meyer, C. Marshall, Ancient DNA methods improve forensic DNA profiling of Korean War and World War II unknowns, Genes (Basel) 13 (2022) 1–19, https://doi.org/10.3390/genes13010129.

[2] I. Zupanič Pajnič, M. Obal, T. Zupanc, Identifying victims of the largest Second World War family massacre in Slovenia, Forensic Sci. Int. 306 (2020) 110056, https://doi.org/10.1016/j.forsciint.2019.110056.
[3] L. Chaitanya, I.Z. Pajnič, S. Walsh, J. Balažic, T. Zupanc, M. Kayser, Bringing colour back after 70 years: Predicting eye and hair colour from skeletal remains of World War II victims using the HIrisPlex system, Forensic Sci. Int. Genet. 26 (2017) 48–57, https://doi.org/10.1016/j.fsigen.2016.10.004.
[4] A.Z. Mundorff, E.J. Bartelink, E. Mar-cash, DNA preservation in skeletal elements from the World Trade Center disaster : Recommendations for mass fatality management, J. Forensic Sci. 54 (2009) 739–745, https://doi.org/10.1111/j.1556-4029.2009.01045.x.
[5] O.M. Loreille, T.M. Diegoli, J.A. Irwin, M.D. Coble, T.J. Parsons, High-efficiency DNA extraction from bone by total demineralization, Forensic Sci. Int. Genet. 1 (2007) 191–195, https://doi.org/10.1016/j.fsigen.2007.02.006.
[6] K.E. Latham, J.J. Miller, DNA recovery and analysis from skeletal material in modern forensic contexts, Forensic Sci. Res. 4 (2019) 51–59, https://doi.org/10.1080/20961790.2018.1515594.
[7] C. Raffone, M. Baeta, N. Lambacher, E. Granizo-Rodríguez, F. Etxeberria, M.M. de Pancorbo, Intrinsic and extrinsic factors that may influence DNA preservation in skeletal remains: a review, Forensic Sci. Int. 325 (2021) 110859, https://doi.org/10.1016/j.forsciint.2021.110859.
[8] R. Alaeddini, S.J. Walsh, A. Abbas, Forensic implications of genetic analyses from degraded DNA-A review, Forensic Sci. Int. Genet. 4 (2010) 148–157, https://doi.org/10.1016/j.fsigen.2009.09.007.
[9] T. Zupanc, E. Podovšovnik, M. Obal, I. Zupanič Pajnič, High DNA yield from metatarsal and metacarpal bones from Slovenian Second World War skeletal remains, Forensic Sci Int. Genet. 51 (2021) 102426, https://doi.org/10.1016/j.fsigen.2020.102426.
[10] A. Mundorff, J.M. Davoren, Examination of DNA yield rates for different skeletal elements at increasing post mortem intervals, Forensic Sci. Int. Genet. 8 (2014) 55–63, https://doi.org/10.1016/j.fsigen.2013.08.001.
[11] C. Finaughty, L.J. Heathfield, V. Kemp, N. Márquez-Grant, Forensic DNA extraction methods for human hard tissue: a systematic literature review and meta-analysis of technologies and sample type, Forensic Sci. Int. Genet. 63 (2023) 102818, https://doi.org/10.1016/j.fsigen.2022.102818.
[12] T. Benedik Bevc, L. Božič, E. Podovšovnik, T. Zupanc, I. Zupanič Pajnič, Intra-bone nuclear DNA variability and STR typing success in Second World War 12th thoracic vertebrae, Forensic Sci. Int. Genet. 55 (2021) 102587, https://doi.org/10.1016/j.fsigen.2021.102587.
[13] L. Božič, T. Benedik Bevc, E. Podovšovnik, T. Zupanc, I. Zupanič Pajnič, Comparison of DNA preservation between ribs and vertebrae, Int. J. Leg. Med. 136 (2022) 1247–1253, https://doi.org/10.1007/s00414-022-02860-8.
[14] I. Zupanič Pajnič, J. Inkret, T. Zupanc, E. Podovšovnik, Comparison of nuclear DNA yield and STR typing success in Second World War petrous bones and metacarpals III, Forensic Sci. Int. Genet. 55 (2021) 102578, https://doi.org/10.1016/j.fsigen.2021.102578.
[15] A.L. Emmons, J. Davoren, J.M. DeBruyn, A.Z. Mundorff, Inter- and intra-individual variation in skeletal DNA preservation in buried remains, Forensic Sci. Int. Genet. 44 (2020) 102193, https://doi.org/10.1016/j.fsigen.2019.102193.
[16] A. Miloš, A. Selmanović, L. Smajlović, R.L. Huel, C. Katzmarzyk, A. Rizvić, T. J. Parsons, Success rates of nuclear short tandem repeat typing from different skeletal elements, Croat Med. J. 48 (2007) 486–493.
[17] A. Alonso, S. Andelinović, P. Martín, D. Sutlović, I. Erceg, E. Huffine, L.F. de Simón, C. Albarrán, M. Definis-Gojanović, A. Fernández-Rodriguez, P. García, I. Drmić, B. Režić, S. Kuret, M. Sancho, D. Primorac, DNA typing from skeletal remains: Evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples, Croat Med. J. 42 (2001) 260–266.
[18] F.X. Ricaut, C. Keyser-Tracqui, E. Crubézy, B. Ludes, STR genotyping from human medieval tooth and bone samples, Forensic Sci. Int. 151 (2005) 31–35, https://doi.org/10.1016/j.forsciint.2004.07.001.
[19] M. Prinz, A. Carracedo, W.R. Mayr, N. Morling, T.J. Parsons, A. Sajantila, R. Scheithauer, H. Schmitter, P. M. Schneider, DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI), 1 (2007) 3–12. 10.1016/j.fsigen.2006.10.003.
[20] ICMP, Standard Operating Procedure for Sampling Bone and Tooth Specimens from Human Remains, pp. 1–19, 2015.
[21] C. Parker, A.B. Rohrlach, S. Friederich, S. Nagel, M. Meyer, J. Krause, K.I. Bos, W. Haak, A systematic investigation of human DNA preservation in medieval skeletons, Sci. Rep. 10 (2020) 18225, https://doi.org/10.1038/s41598-020-75163-w.
[22] S.M. Edson, A.F. Christensen, S.M. Barritt, A. Meehan, M.D. Leney, L.N. Finelli, Sampling of the cranium for mitochondrial DNA analysis of human skeletal remains, Forensic Sci. Int. Genet. Suppl. Ser. 2 (2009) 269–270, https://doi.org/10.1016/j.fsigss.2009.09.029.
[23] C.J. Adler, W. Haak, D. Donlon, A. Cooper, Survival and recovery of DNA from ancient teeth and bones, J. Archaeol. Sci. 38 (2011) 956–964, https://doi.org/10.1016/j.jas.2010.11.010.
[24] S. Amory, R. Huel, A. Bilic, O. Loreille, T.J. Parsons, Automatable full demineralization DNA extraction procedure from degraded skeletal remains, Forensic Sci. Int. Genet. 6 (2012) 398–406, https://doi.org/10.1016/j.fsigen.2011.08.004.
[25] S.R. Bucheli, J.A. Bytheway, S.M. Pustilnik, J. Florence, Insect successional pattern of a corpse in cooler months of subtropical southeastern Texas, J. Forensic Sci. 54 (2009) 452–455, https://doi.org/10.1111/j.1556-4029.2008.00970.x.
[26] Y. Wang, M.Y. Ma, X.Y. Jiang, J.F. Wang, L.L. Li, X.J. Yin, M. Wang, Y. Lai, L. Y. Tao, Insect succession on remains of human and animals in Shenzhen, China, Forensic Sci. Int. 271 (2017) 75–86, https://doi.org/10.1016/j.forsciint.2016.12.032.
[27] H. Chen, S. Shoumura, S. Emura, Y. Bunai, Regional variations of vertebral trabecular bone microstructure with age and gender, Osteoporos Int. 19 (2008) 1473–1483, https://doi.org/10.1007/s00198-008-0593-3.
[28] A. Gonzalez, C. Cannet, V. Zvénigorosky, A. Geraut, G. Koch, T. Delabarde, B. Ludes, J.S. Raul, C. Keyser, The petrous bone: ideal substrate in legal medicine? Forensic Sci. Int. Genet. 47 (2020) 102305 https://doi.org/10.1016/j.fsigen.2020.102305.
[29] D. Higgins, J.J. Austin, Teeth as a source of DNA for forensic identification of human remains: a review, Sci. Justice 53 (2013) 433–441, https://doi.org/10.1016/j.scijus.2013.06.001.
[30] R. Florencio-Silva, G.R.D.S. Sasso, E. Sasso-Cerri, M.J. Simões, P.S. Cerri, Biology of bone tissue: Structure, function, and factors that influence bone cells, Biomed Res. Int. 2015 (2015) 421746, https://doi.org/10.1155/2015/421746.
[31] G.Y. Rochefort, S. Pallu, C.L. Benhamou, Osteocyte: The unrecognized side of bone tissue, Osteoporos Int. 21 (2010) 1457–1469, https://doi.org/10.1007/s00198-010-1194-5.
[32] A.G. Robling, F.M. Hinant, D.B. Burr, C.H. Turner, Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts, J. Bone Miner. Res. 17 (2002) 1545–1554, https://doi.org/10.1359/jbmr.2002.17.8.1545.
[33] B. Clarke, Normal bone anatomy and physiology., Clin. J. Am. Soc. Nephrol. 3 Suppl 3 (2008) 131–139. 10.2215/CJN.04151206.
[34] J.M. Andronowski, A.Z. Mundorff, I.V. Pratt, J.M. Davoren, D.M.L. Cooper, Evaluating differential nuclear DNA yield rates and osteocyte numbers among human bone tissue types: A synchrotron radiation micro-CT approach, Forensic Sci. Int. Genet. 28 (2017) 211–218, https://doi.org/10.1016/j.fsigen.2017.03.002.
[35] S.M. Ott, Cortical or trabecular bone: what's the difference? Am. J. Nephrol. 47 (2018) 373–375, https://doi.org/10.1159/000489672.
[36] J. Inkret, E. Podovšovnik, T. Zupanc, G. Haring, I.Z. Pajnič, Intra-bone nuclear DNA variability in Second World War metatarsal and metacarpal bones, Int. J. Leg. Med. 135 (2021) 1245–1256, https://doi.org/10.1007/s00414-021-02528-9.
[37] M.J. Collins, C.M. Nielsen-Marsh, J. Hiller, C.I. Smith, J.P. Roberts, R.V. Prigodich, T.J. Wess, J. Csapò, A.R. Millard, G. Turner-Walker, The survival of organic matter in bone: a review, Archaeometry 44 (2002) 383–394, https://doi.org/10.1111/1475-4754.t01-1-00071.
[38] A.M. Child, Towards and understanding of the microbial decomposition of archaeological bone in the burial environment, J. Archaeol. Sci. 22 (1995) 165–174, https://doi.org/10.1006/jasc.1995.0018.
[39] J. Antheunisse, DNA decomposition by soil microorganisms, Antonie Van Leeuwenhoek 37 (1971) 258–259, https://doi.org/10.1007/BF02218491.
[40] J. Antheunisse, Decomposition of nucleic acids and some of their degradation products by microorganisms, Antonie Van Leeuwenhoek 38 (1972) 311–327, https://doi.org/10.1007/BF02328101.
[41] A.L. Emmons, A.Z. Mundorff, S.W. Keenan, J. Davoren, J. Andronowski, D. Carter, J.M. Debruyn, Characterizing the postmortem human bone microbiome from surface-decomposed remains, PLoS One 15 (2020) e0218636.
[42] T.G. Kaye, G. Gaugler, Z. Sawlowicz, Dinosaurian soft tissues interpreted as bacterial biofilms, PLoS One 3 (2008) e2808.
[43] A. Götherström, M.J. Collins, A. Angerbjörn, K. Lidén, Bone preservation and DNA amplification, Archaeometry 44 (2002) 395–404, https://doi.org/10.1111/1475-4754.00072.
[44] N. Procopio, C.A. Mein, S. Starace, A. Bonicelli, A. Williams, Bone diagenesis in short timescales: insights from an exploratory proteomic analysis, Biology (basel). 10 (2021), https://doi.org/10.3390/biology10060460.
[45] R.E.M. Hedges, Bone diagenesis: an overview of processes, Archaeometry 44 (2002) 319–328, https://doi.org/10.1111/1475-4754.00064.
[46] T.C. Antinick, D.R. Foran, Intra- and inter-element variability in mitochondrial and nuclear DNA from fresh and environmentally exposed skeletal remains, J. Forensic Sci. 64 (2019) 88–97, https://doi.org/10.1111/1556-4029.13843.
[47] H. Chen, X. Zhou, S. Shoumura, S. Emura, Y. Bunai, Age- and gender-dependent changes in three-dimensional microstructure of cortical and trabecular bone at the human femoral neck, Osteoporos Int. 21 (2010) 627–636, https://doi.org/10.1007/s00198-009-0993-z.
[48] H. Chen, Y. Washimi, K.Y. Kubo, M. Onozuka, Gender-related changes in three-dimensional microstructure of trabecular bone at the human proximal tibia with aging, Histol. Histopathol. 26 (2011) 563–570. 10.14670/HH-26.563.
[49] R. Alaeddini, Forensic implications of PCR inhibition - A review, Forensic Sci. Int. Genet. 6 (2012) 297–305, https://doi.org/10.1016/j.fsigen.2011.08.006.
[50] S.T.G. Ferreira, H.H. Kuser, R.G. Garrido, A. Trindade-Filho, K.A. Paula, M. F. Galvão, A.V. Moraes, Floods and mudslides in the State of Rio de Janeiro and a plane crash in the Brazilian Amazon rainforest: a study of two different experiences in disaster victim identification (DVI), Forensic Sci. Int. Genet. Suppl. Ser. 3 (2011) 516–517, https://doi.org/10.1016/j.fsigss.2011.10.005.
[51] S.T.G. Ferreira, R.G. Garrido, K.A. Paula, R.C. Nogueira, E.S. Oliveira, A.V. Moraes, Cartilage and phalanges from hallux: Alternative sources of samples for DNA typing in disaster victim identification (DVI). A comparative study, Forensic Sci. Int. Genet. Suppl. Ser. 4 (2013) e366–e367, https://doi.org/10.1016/j.fsigss.2013.10.186.