From bones to sediments: Ancient human DNA from open-air archaeological sites

Sawafuji, Rikai
Department of Environmental Changes, Faculty of Social and Cultural Studies, Kyushu University

Sawaura, Ryohei
Kamakura Women's University

Yokoo, Masaki
Uruma City Board of Education

Kumaki, Toshiaki
Graduate School of Humanities and Sociology, the University of Tokyo

他

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From bones to sediments: Ancient human DNA from open-air archaeological sites

Rikai Sawafuji [a,b,c,*], Ryohei Sawaura [d], Masaki Yokoo [e], Toshiaki Kumaki [f], N. August Thomasen [c], Takumi Tsutaya [b], Mikkel Winther Pedersen [c,**]

[a] Department of Environmental Changes, Faculty of Social and Cultural Studies, Kyushu University, Fukuoka, 819-0395, Japan
[b] Research Center for Integrative Evolutionary Science, The Graduate University for Advanced Studies (SOKENDAI), Kanagawa, 240-0193, Japan
[c] Centre for Ancient Environmental Genomics, Globe Institute, University of Copenhagen, 1350, Copenhagen, Denmark
[d] Kamakura Women's University, Kanagawa, 247-8512, Japan
[e] Uruma City Board of Education, Okinawa, 904-2292, Japan
[f] Graduate School of Humanities and Sociology, the University of Tokyo, Tokyo, 113-0033, Japan

A B S T R A C T

Most ancient human bones degrade and eventually disappear over time, especially in regions with acidic soils, making it challenging to obtain genetic information from past populations. As a consequence, there is currently an overrepresentation of ancient human data for certain regions of the world in contrast to others. To explore alternative sources, we analyzed ancient DNA from sediment samples collected from a burial site and a settlement site in Japan, both dating to around 1000 years ago. We found that ancient human mitochondrial DNA can be obtained from sediments surrounding bones, particularly close to rib bones, while human DNA was rarely detected in the settlement site sediments. Furthermore, the mitochondrial haplogroups identified in the sediments were identical to those from human bones, confirming the reliability of this approach. Our findings suggest that genetic information about past human populations can be directly obtained from archaeological sediments in open-air sites. This method also provides a non-destructive alternative to bones and teeth, expanding possibilities for ancient DNA research in regions where skeletal remains are poorly preserved.

1. Introduction

The analysis of ancient human DNA has revolutionized our understanding of human migration and dispersal, reshaping our historical narratives with unprecedented detail (Allentoft et al., 2015; Haak et al., 2015; Moreno-Mayar et al., 2018). However, the field remains affected by substantial biases and skewness, particularly in geographical and temporal representation. This is in part due to the disproportionate allocation of research funding and archaeological activity by the Global North. Furthermore, climatic and environmental factors contribute to this imbalance, as fossil preservation is more favorable in cooler, stable environments, thereby limiting the recovery of genetic material from regions with less suitable preservation conditions (Allentoft et al., 2012; Emmons et al., 2020; Eriksen et al., 2020a). In such regions, bones may undergo complete degradation, resulting in the absence of recoverable remains at sites. Even when skeletal remains are discovered, endogeneous DNA is often highly degraded. Consequently, ancient human DNA sampling is disproportionately concentrated in regions with more favorable preservation conditions and where research efforts are most intensive. This concentration results in a skewed understanding of human migratory and dispersal patterns, overlooking key areas where hominins would have interacted and evolved (Gokcumen and Frachetti, 2020).

In light of these challenges, the analysis of ancient human DNA from sediments has emerged as a promising alternative. For instance, ancient hominin mitochondrial DNA (mtDNA) from Denisovans and Neanderthals has been successfully recovered from sediments in Denisova Cave (Slon et al., 2017; Zavala et al., 2021) and Baishiya Karst Cave on the Tibetan Plateau (Zhang et al., 2020). Furthermore, nuclear genomes have also been extracted and analyzed from cave sediments (Gelabert et al., 2021; Vernot et al., 2021). These new approaches expand the scope of hominin ancient DNA research beyond skeletal remains, offering a novel method to investigate the history of human presence and migration (Sawafuji et al., 2024).

Although ancient human DNA in sediments holds great potential, significant challenges still limit its utility. A primary issue is identifying which specific locations and contexts within archaeological sites are most likely to yield ancient human DNA. From the few published

* Corresponding author at: Department of Environmental Changes, Faculty of Social and Cultural Studies, Kyushu University, Fukuoka, 819-0395, Japan.
** Corresponding author.
E-mail addresses: rikaisawafuji@gmail.com (R. Sawafuji), mwpedersen@sund.ku.dk (M.W. Pedersen).

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studies, the success rate of recovering human DNA from sediments seems highly variable and influenced by factors such as stratigraphic layers, mineral composition, and site-specific conditions (de-Dios et al., 2025; Massilani et al., 2022; Zavala et al., 2021). Nevertheless, more studies comparing the extent of human DNA recovery across different archaeological contexts have yet to be conducted, underscoring the need to validate and improve the success rates of sediment-based DNA analysis across diverse sites, climates and timescales.

Even more pronounced is the lack of studies on human DNA in sediments from open-air archaeological sites, which remain less explored compared to cave or sheltered contexts. Human DNA to date has been successfully obtained from calcite stones covering skeletal remains in a cave (Sarhan et al., 2021), as well as from sediments in caves (Gelabert et al., 2021; Massilani et al., 2022; Slon et al., 2017, 2022; Vernot et al., 2021; Zavala et al., 2021; Zhang et al., 2020) and beneath a rock shelter (Zampirolo et al., 2024). In contrast, open-air sites, which have the potential to provide a broader range of ancient human DNA, remain significantly underexplored. Given that the number of open-air sites with evidence of human activity far exceeds that of cave sites with good preservation, future studies exploring the potential of sites with poor or no bone preservation are crucial for expanding the diversity of ancient human genetic studies and our history.

Japan has a diverse climate, spanning multiple climatic regions, but high humidity and acidic soils across much of the country contribute to poor bone preservation, making ancient skeletal remains scarce (Gakuhari et al., 2020). This phenomenon is not unique to Japan but is also common in other regions where similar environmental conditions prevail, such as Southeast Asia (McColl et al., 2018). Due to these poor preservation conditions, a comprehensive understanding of prehistoric human presence and dynamics remains challenging. Despite this, Japan's wide variety of archaeological sites provides a valuable case for testing sediment-based DNA recovery techniques. Extracting DNA from sediments could offer a crucial alternative for reconstructing ancient human history and serve as a model for other regions with similar preservation challenges.

In this study, we evaluate the potential for recovering human DNA from sediments at two open-air archaeological sites in Japan, a burial site and a dwelling site, using shotgun sequencing and mitochondrial DNA target capture enrichment.

2. Materials and Methods

2.1. Sample information

Sediments were collected from two archaeological sites in Japan (Fig. 1). The first site, the Katsuren Castle site, is located in Uruma, Okinawa, and the mean annual temperature and annual precipitation (1991–2020) are approximately 23.3 °C and 2161.0 mm, respectively (Japan Meteorological Agency, 2025). It is a burial site dating back to the Gusuku period, around 1000 years ago. Human skeletons were excavated from two separate pits, both located beneath the castle wall. Four sediment samples were obtained from Pit 178, and one from Pit

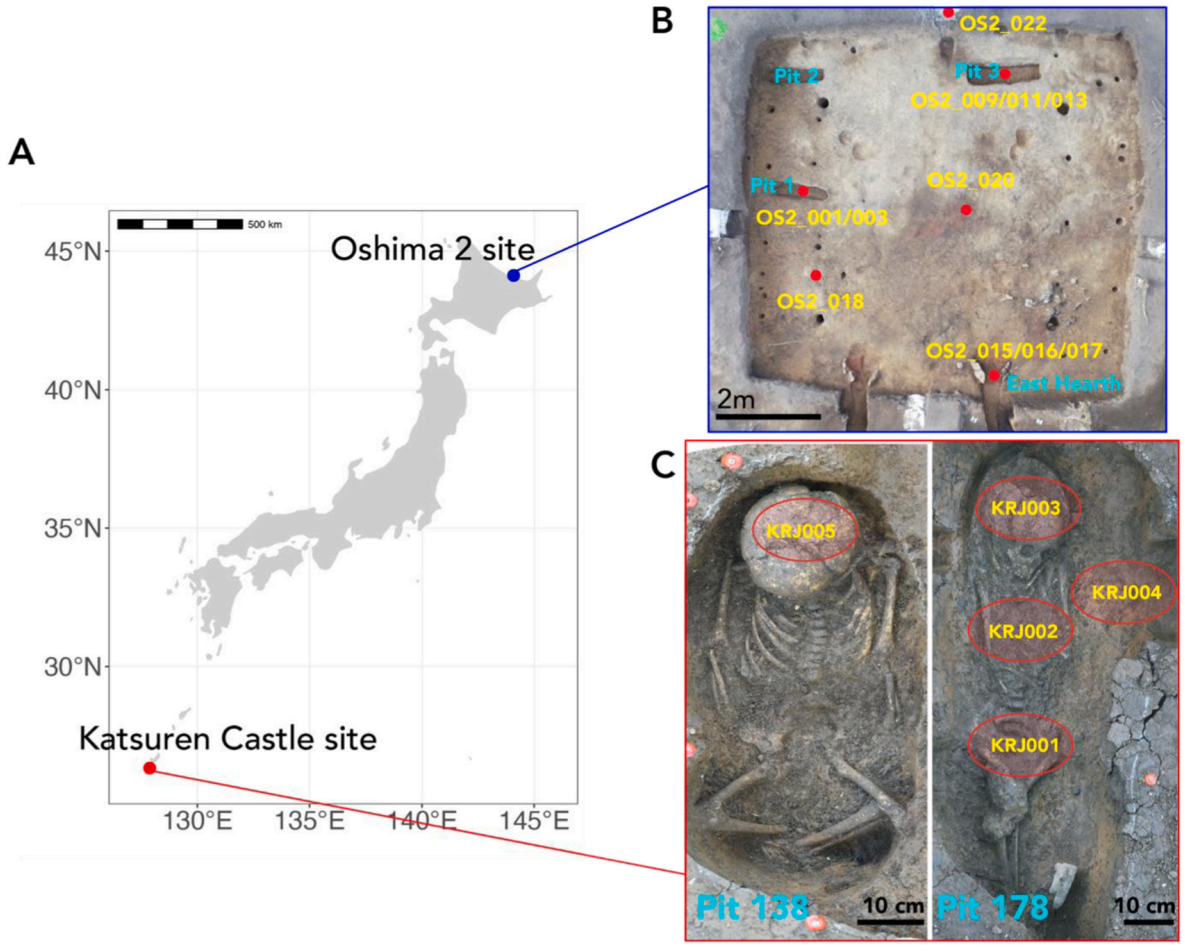

Fig. 1. Geographic locations of the study sites and sampling points. A) Maps showing the locations of the two archaeological sites analyzed in this study. B) The Oshima 2 ste, a dwelling site, Hokkaido. C) The Katsuren Castle site, a burial site, Okinawa. Red dots and circles indicate the locations of collected sediment samples. Yellow text labels indicate sample IDs, while blue text labels represent archaeological features. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

138 (Table S1). Additionally, rib bones from each individual buried in these pits were also sampled to verify whether the haplogroup of mtDNA from the sediment is identical to that from bone.

The second site, the Oshima 2 site, is located in Kitami, Hokkaido, and the mean annual temperature and annual precipitation (1991–2020) are approximately 6.4 °C and 774.8 mm, respectively (Japan Meteorological Agency, 2025). It is a dwelling site dating back to the 11th to 12th century in the Satsumon period. The date of this site is estimated from the chronology of pottery types and radiocarbon dating from another dwelling in the same site. We took 11 sediment samples from pits, an oven, floors and outside the dwelling (Fig. 1, Table S2).

The pH of the sediment samples was measured using a Takemura Denki Seisakusho "DM-2" colorimetric soil pH tester (range: pH 4.0–10.0) by directly applying reagent to the sediment samples and comparing colour change against the standard chart. The pH of the samples from the Katsuren Castle site and from the Oshima 2 site was 6.5 and 5.5, respectively.

2.2. Radiocarbon measurement

Radiocarbon measurement was carried out by Palynosurvey Co., Ltd. at Yamagata University on the two human skeletal individuals from Pit 138 and Pit 178 of the Katsuren Castle site. Collagen was extracted from a left rib and the left tibia for skeletons from Pit 138 and 178, respectively, based on the method described in Tsutaya et al. (2017). Carbon and nitrogen stable isotope ratios were measured to estimate the marine reservoir effect using an elemental analyzer-isotope ratio mass spectrometry (EA-IRMS). Radiocarbon concentration was measured using an accelerator mass spectrometry (AMS). The radiocarbon age was calibrated against atmospheric and marine calibration curves IntCal20 and Marine20 (Heaton et al., 2020; Reimer et al., 2020) using the software OxCal, version 4.4 (Bronk Ramsey, 1995). We assumed that the contribution of carbon derived from marine products was 20 ± 10% based on the results of the stable isotope analysis (Table S4). By considering the local marine reservoir effect ($\Delta R = -143 \pm 33$, recalculated with Marine20) around Okinawa Island (Yoneda et al., 2007), the 95% confidence intervals of the calibrated age were 1157–1279 cal AD for both skeletons (Table S4).

Radiocarbon measurement was carried out by Paleo Labo Co., Ltd. on three charred woods from the Oshima 2 site (Table S5). Charred wood samples were treated with the acid-base-acid method described in Hajdas et al. (2017) with slight modifications. Briefly, samples were washed with acetone and sequentially treated with 1.2 M HCl, 1.0 M NaOH, and 1.2 M HCl. Radiocarbon concentration for the resultant graphite samples was measured using an accelerator mass spectrometry (AMS) at Paleo Labo. The radiocarbon age was calibrated against an atmospheric calibration curve IntCal20 (Reimer et al., 2020), using the software OxCal, version 4.4 (Bronk Ramsey, 1995). The 95% confidence intervals of the calibrated age were middle 11th to early 13th centuries AD for all three samples (Table S5). The radiocarbon measurement was applied to the charred wood samples without a final growth ring, so the measurement results may have been affected by the tree rings that formed in older years. Therefore, the true age of the tree's death or the site occupation would be slightly more recent than the obtained radiocarbon ages.

2.3. DNA extraction, library construction, capture enrichment and sequencing

Approximately 150 mg of either sediment or powdered bone were extracted for DNA using the method described by Rohland et al. (2018). All libraries were constructed using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs) following the manufacturer's protocol. Libraries were amplified with 12 PCR cycles using Pfu Turbo Cx. Human mtDNA capture was performed using the myBaits Mito (Daicel Arbor Biosciences) following the manufacturer's protocol v5.01. Sequencing was performed on the Novaseq and iSeq platforms for both shotgun sequencing and target capture sequencing. The raw sequencing data have been deposited at the European Nucleotide Archive (ENA) and are publicly available (Project PRJEB87497). The corresponding accession numbers for each sample are provided in Table S6. The sequence datasets, both shotgun and target-capture sequencing, were mapped against the human genome. We used fastp (Chen et al., 2018) to trim the adapters and low-quality sequences, and to merge pair-end reads with 11bp overlap. Sequences after these steps were aligned to the human hg38 reference genome (GRCh38.p13) and human reference mtDNA genome (rCRS) using bwa (Li and Durbin, 2009). Duplicate reads were removed using Picard's MarkDuplicates (http://broadinstitute.github.io/picard/). Subsequently, reads assigned to human mtDNA were extracted using SAMtools (Danecek et al., 2021), and haplogroups were determined using haplocart (Rubin et al., 2023) and haplogrep (Schönherr et al., 2023). We used DamageProfiler (Neukamm et al., 2021) to check the damage patterns of ancient DNA as an authentication and contamMIX (Fu et al., 2013) and Schmutzi (Renaud et al., 2015) to estimate the level of contamination. To evaluate the complexity of human DNA recovered from sediments and bone samples at the burial site, we estimated library complexity using preseq (lc_extrap).

2.4. Microbial community analysis

Microbial taxonomic profiles were generated following the workflow described by Fernandez-Guerra et al. (2023) using the aMAW-taxonomy pipeline for shotgun sequencing data from all samples. Briefly, quality-controlled reads were first extended using Tadpole (BBTools package) (Bushnell, 2014) and were dereplicated with VSEARCH (Rognes et al., 2016). The dereplicated and extended reads were then mapped with Bowtie2 (Langmead and Salzberg, 2012) against a custom reference database including NCBI Refseq (chroloplast, mitochondria, archaea), the complete GTDB v207 (Parks et al., 2022), and high-quality IMG/VR v4 genomic data (Camargo et al., 2023). Duplicate reads were removed with Picard's MarkDuplicates (http://broadinstitute.github.io/picard/), and mapped reads were reassigned and filtered using filterBAM (https://github.com/genomewalker/bam-filter). Taxonomic assignments and post-mortem DNA damage estimates were performed using Bayesian inference implemented in metaDMG (Michelsen et al., 2022) in both local and LCA modes. We then inferred source proportions using SourceTracker2 (Knights et al., 2011; McGhee et al., 2020). Species-level profiles from our samples of the burial site (the Katsuren Castle) were used as sinks, and we selected the same source datasets used by de-Dios et al. (2025) and Zampirolo et al. (2024), respectively, as sources. SourceTracker2 was run with default parameters.

We performed Principal Coordinate Analysis (PCoA) to examine the relatedness of the microbial compositions across the samples. The species-level abundance table was filtered by removing taxa present in fewer than 10% of samples to avoid the influence of extremely rare taxa. The remaining counts were converted to relative abundances (%) by dividing each sample's counts by its total read count. The normalized abundance table was used to calculate Bray–Curtis dissimilarities using the vegan package in R (v.4.3.0). PCoA was then performed with the cmdscale function in base R, and the first two axes were visualized using ggplot2 and ggrepel. The heatmap was generated using pheatmap and included only taxa with estimated DNA damage $\geq$3% to minimize the influence of possible modern DNA contamination.

3. Results

Five sediment samples were collected at a burial site, Katsuren castle, located in Okinawa, which dates to the mid-12th to early 13th century during the Gusuku period (Fig. 1). Here, two burials with skeletal remains were found beneath the castle's wall. One sediment sample from inside the skull was collected from Pit 138. From Pit 178, four sediment

samples were obtained: one from inside the skull, one from sediment around the hip bones, one from sediment around the rib bones, and one from an area outside the pit (Fig. 1, Table S1). Additionally, one rib bone was collected from each individual to compare with the results obtained from the sediments. The second site is a dwelling site known as Oshima 2, which dates to the 11th to 12th century during the Satsumon period and is located in Hokkaido (Fig. 1). We collected eleven sediment samples from different contexts within the site (Table S2). DNA was extracted from all samples and sequenced using Illumina NovaSeq and iSeq platforms (see Methods). We performed a combination of shotgun sequencing (between 12447839 and 85971229 number of reads) and targeted capture enrichment for human mtDNA (between 70064 and 2174014 number of reads) to investigate the feasibility of determining human mtDNA haplogroups from sediment samples in open-air archaeological sites.

3.1. Recovery of human mitochondrial DNA from sediments

Haplogroups were successfully identified from sediment samples at the burial site after mitochondrial DNA target capture (Fig. 2, Table S3). Ancient DNA authenticity was confirmed by assessing ancient DNA damage patterns such as thymine misincorporation from cytosine deamination (Fig. S1) and by analyzing the distribution of read length (Fig. 2a) and damaged reads (PMD score >2) across the reference genome (Fig. 2b). We also estimated the level of mitochondrial contamination, which was below 10% in all samples (Table S3). Haplogroups identified in the sediment samples matched those from the bone remains from the same pits: M7a1b1 from Pit 138 and D4a1 from Pit 178. Specifically, one sediment sample from each pit yielded mtDNA haplogroups. In contrast, sediment samples from the dwelling site did not yield sufficient human mtDNA for haplogroup determination or damage pattern analysis, even after capture enrichment (Fig. S2).

We also analyzed the shotgun sequencing data from sediments and bones. Samples with determinable haplogroups displayed clear damage signatures when mapping to the human nuclear and mitochondrial genome using shotgun datasets, while samples without identifiable haplogroups did not (Fig. 3, Fig. S2). However, shotgun sequencing alone yielded insufficient reads for haplogroup determination, sex

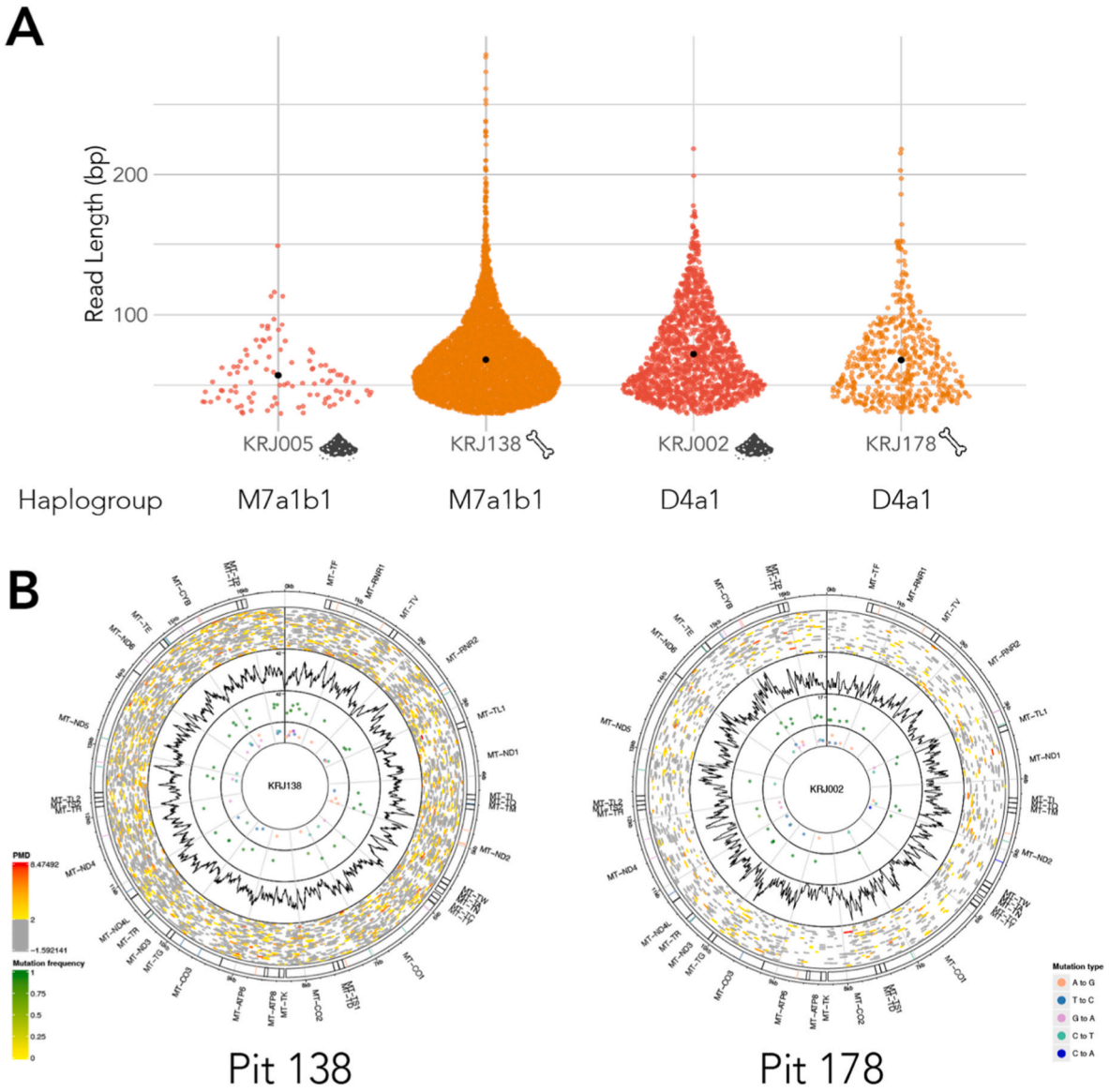

Fig. 2. Read length and haplogroup of human mitochondrial DNA. A) Read lengths of mitochondrial DNA and their haplogroups. Sample names and corresponding haplogroups are indicated. B) Circular maps of the human mitochondrial DNA from bone (KRJ138) and sediment (KRJ002) samples. From outer to inner rings: gene annotations, mapped reads with PMD scores, coverage depth, positions of nucleotide substitutions, and substitution types.

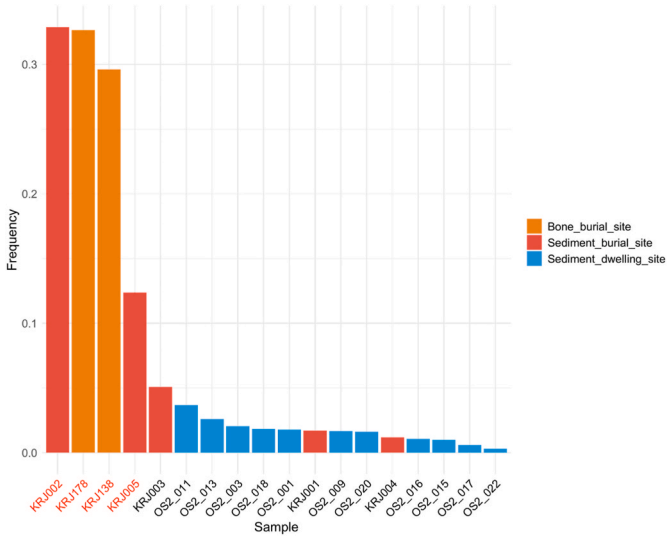

Fig. 3. Damage degree of shotgun reads mapped to human genome. The y-axis indicates the frequency of cytosine-to-thymine misincorporations at the 5′ ends of DNA reads from sediment and bone samples. The color of each bar represents the sample category (orange: bone samples from the burial site; red: sediment samples from the burial site; blue: sediment samples from the dwelling site). Red text labels indicate samples from which mitochondrial haplogroups were determined. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

determination, or PCA, as all shotgun libraries at the burial sites reached saturation by ~10 million reads regarding human DNA (Fig. S3). Targeted capture significantly improved mtDNA recovery, increasing coverage depth 30- to 200-fold in samples with identifiable haplogroups, with the greatest increase observed in sample KRJ002 (Fig. S4).

3.2. Bacterial DNA degradation and microbial community composition

To assess DNA degradation, we analyzed the bacterial DNA fraction from the shotgun datasets generated from sediments and bones. Most samples, except for KRJ001 and KRJ004, showed highly fragmented bacterial DNA, with average fragment lengths below 60 bp (Fig. S5). KRJ001 and KRJ004 from the burial site contained relatively longer DNA fragments (>100 bp), suggesting possible intrusion by modern bacteria. Although all samples contained bacterial taxa with damage patterns (Fig. S6), these ancient taxa were more prevalent at the burial site. To estimate source composition, we applied SourceTracker2 to sediments and bones from the burial site, but more than 90% of inferred sources were classified as “unknown” (Fig. S7). Principal Coordinate Analysis (PCoA) of the microbiomes indicated that microbial communities were similar within the same site and material type (bone or sediment). (Fig. 4). The sediment sample around the rib bones (KRJ002) showed microbial communities plotted in an intermediate position between the bone and sediment clusters at the burial site. The heatmap of microbial communities from samples at the burial site showed that although the sample-level clustering did not clearly separate bone and sediment samples, taxa-level clustering tended to group bone-associated taxa (upper part of the heatmap) and sediment-associated taxa (lower part of the heatmap) together (Fig. 5).

4. Discussion

We identified human mitochondrial haplotypes (M7a1b1 and D4a1) from sediments surrounding the bones at a burial site, whereas only a very small amount of mitochondrial DNA was recovered from the dwelling site sediments, which was insufficient to determine haplotypes.

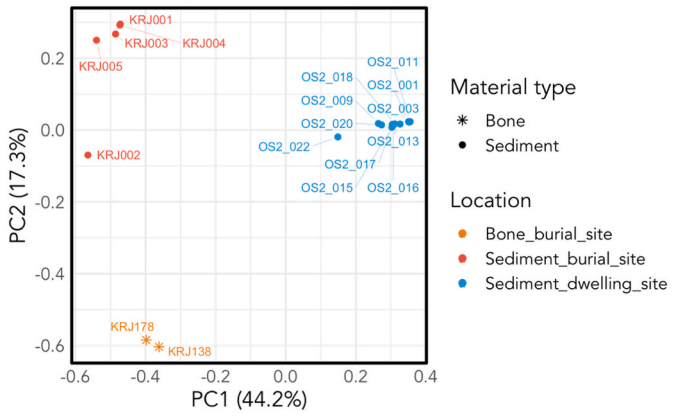

Fig. 4. Principal Coordinate Analysis (PCoA) of microbial community composition based on Bray–Curtis dissimilarity. Colors indicate sampling context (orange: bone samples from the burial site; red: sediment samples from the burial site; blue: sediment samples from the dwelling site), and shapes represent material type (crosses: bone; circles: sediment). Axes correspond to the first two principal coordinates, with the percentage of variance explained shown in parentheses. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Regarding mitochondrial haplotypes, M7a and D4 were predominant during this period in Okinawa (5 out of 15 individuals each) (Shinoda et al., 2013), and the frequencies of M7a1 and D4a1 are the highest in the modern Okinawan population compared with neighboring regions (14% and 4%, respectively) (Tanaka et al., 2004). The haplotypes we identified are in line with these previous findings, reinforcing the validity of our results.

This study presents two significant findings: (1) ancient human mtDNA can be successfully recovered from open-air archaeological sites, and (2) the abundance of human mtDNA varies considerably based on the site's location and context. This highlights that human activity at an archaeological site does not necessarily ensure the recovery of ancient DNA or ancient human DNA from sediments.

Our results mark an early foundational step in exploring human DNA recovery from open-air archaeological contexts. By recovering human mtDNA from both bones and sediments and identifying matching haplogroups with characteristic degradation patterns, we provide a robust framework for future sediment-based human DNA analysis. These findings highlight the potential to extend human DNA analysis to a broader range of open-air sites, providing a foundation for broader applications.

4.1. Contextual variation in human DNA recovery

The recovery of human DNA varied depending on the archaeological context. Burial sites are likely the most promising, as ancient human mtDNA was recovered from sediments surrounding the bones, particularly near the rib bones. This aligns with a previous finding from a cave site (Sarhan et al., 2021) and suggests that the presence of decomposing remains contributes significantly to human DNA recovery in sediments.

In contrast, human DNA was rarely detected in sediments from the dwelling site. This is likely because the primary candidates of human DNA resources in this context are hair, skin and sweat, which are typically present in low quantities and may not yield enough DNA for ancient human DNA analysis. Additionally, limited direct contact between inhabitants and the ground due to footwear use may have further reduced the deposition of human DNA. Further studies are required to confirm the absence of human DNA in sediments at dwelling sites and explore other contexts such as latrines and middens, which have yielded DNA in previous studies (Sabin et al., 2020; Søe et al., 2018). Such contexts may offer valuable DNA sources, especially if methods like microsampling or other improvements can isolate DNA from individual

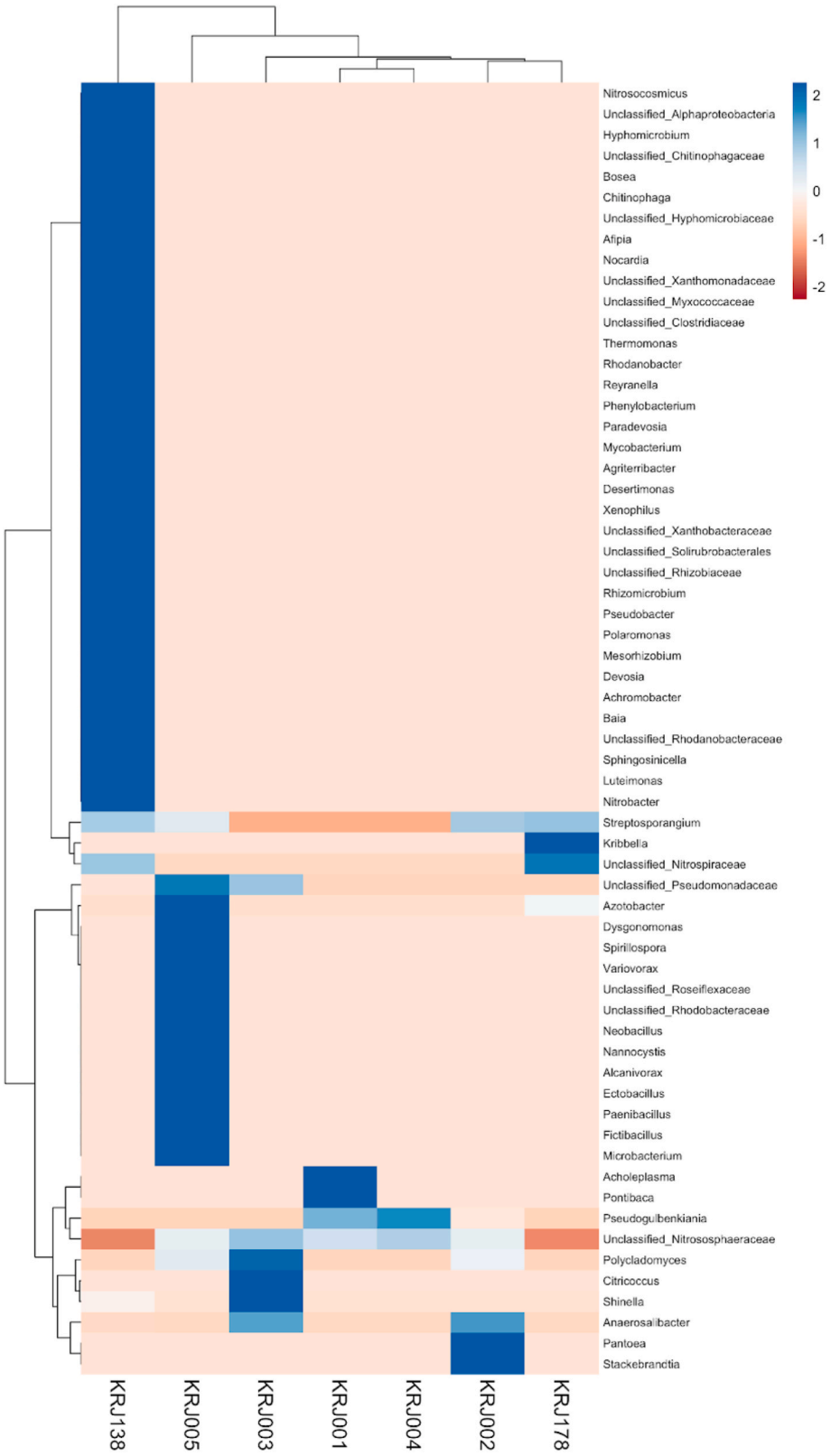

Fig. 5. Heatmap of microbial communities from samples at the Katsuren Castle. Only taxa with estimated DNA damage ≥3% were used in the analysis.

contributors.

4.2. Insights from microbial analysis

Microbial community analysis provided additional insight into taphonomic processes. PCoA revealed a tendency toward differing bacterial compositions at the burial site compared to other sediment samples (Fig. 4). The bacterial community in sediments around the rib bones (KRJ002) showed an intermediate pattern between that of bones and sediments, suggesting that sediment DNA in this context reflects

decomposition from human remains. This finding indicates the dominant role of human tissues as a DNA source and underscores the need for further taphonomic studies to refine interpretations of DNA preservation and degradation. The heatmap analysis showed that the taxa-level clustering tended to group bone-associated taxa (upper part of the heatmap) and sediment-associated taxa (lower part of the heatmap) together. Notably, *Streptosporangium* was abundant in samples (both bones and sediments) containing human DNA and showed high levels of DNA damage (7–11%). Previous studies have also reported that *Streptosporangium* is abundant in human bone and in calcite deposits covering human bones (Eriksen et al., 2020b; Philips et al., 2017; Sarhan et al., 2021). These findings suggest that this taxon is associated with the taphonomy of human remains. This result is also consistent with the necrobiome framework (Benbow et al., 2019), which posits that decomposition processes of human remains can alter the surrounding sediment microbiome, with potential implications not only for ancient DNA but also for future forensic applications.

4.3. Implications for screening and ethical Considerations

This study highlights the potential of sedimentary ancient DNA (sedaDNA) as a valuable tool for screening ancient human DNA. Advances in DNA recovery from non-destructive materials like chewing gum (Jensen et al., 2019), dental calculus (Ozga et al., 2016; Ziesemer et al., 2019), and coprolites (Hagan et al., 2020) demonstrate the feasibility of extracting DNA from sediments. On one hand, such sediment-based recovery methods enable rapid screening during excavations without damaging human remains, supporting heritage preservation, and addressing some ethical concerns related to destructive analyses.

On the other hand, this approach introduces new ethical challenges, particularly when working with materials from contexts with sensitive historical, cultural, and social implications (Der Sarkissian et al., 2021). The lack of clear regulations or guidelines for handling ancient metagenomes compounds these concerns. Researchers must adopt responsible practices, engaging with stakeholders - including local communities and indigenous groups (Alpaslan-Roodenberg et al., 2021; Johnson et al., 2024) - to define project goals, manage sample storage, and communicate results transparently. Collaboration, transparency, and education on ethical practices must remain central to ancient metagenomic studies to ensure ethical and sustainable research. As a final note, it is also important to acknowledge that, although sedaDNA analyses, especially when combined with targeted enrichment, can yield authentic mitochondrial and even nuclear sequences (Gelabert et al., 2021), their success rate remain lower than those typically achieved from ancient DNA recovered directly from human bones.

4.4. Limitations of the study

This study examined sediments from only two archaeological sites dating to approximately 1000 years ago, located in geographically distinct regions. Overall climate conditions, such as lower temperatures and lower humidity, are generally more favorable for DNA preservation at the dwelling site in northern Japan (Hokkaido), as noted in the Materials and Methods. However, soil pH at the dwelling site was lower than at the burial site, and multiple environmental parameters, including pH, chemistry, sediment type, and microbial activity, can influence DNA preservation in complex ways. Therefore, environmental differences between the two sites cannot be fully disentangled, and their respective contributions to DNA preservation remain uncertain.

The microbial DNA profiles also showed contrasting patterns between the sites: microbial reads from the dwelling site exhibited lower damage and shorter fragment lengths, whereas those from the burial site showed higher damage and also included longer fragments (Figs. S5 and S6). The annual mean temperatures differ substantially between the two sites (6.4 °C at the dwelling site and 23.3 °C at the burial site). Considering these climatic conditions, the observed patterns may indicate generally better DNA preservation at the dwelling site and possible modern bacterial influence at the burial site. However, they could also result from rapid degradation of ancient microbial DNA at the dwelling site, leading to an overrepresentation of more recent, less damaged sequences. Thus, interpretations on microbial damage patterns should be treated with caution.

Ideally, future studies should compare multiple sites of similar geographic and climatic settings but with differing archaeological contexts. Such designs would allow more robust assessment of how environmental and taphonomic factors influence the preservation of human and microbial DNA in sediments.

The coverage of the mitochondrial data obtained was insufficient for detailed kinship analysis or fine-scale haplogroup resolution. Additionally, our results are based solely on mtDNA; future work should aim to include nuclear DNA and sex determination.

CRediT authorship contribution statement

Rikai Sawafuji: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ryohei Sawaura:** Writing – review & editing, Resources, Data curation. **Masaki Yokoo:** Writing – review & editing, Resources, Data curation. **Toshiaki Kumaki:** Writing – review & editing, Resources, Data curation. **N. August Thomasen:** Writing – review & editing, Visualization. **Takumi Tsutaya:** Writing – review & editing, Writing – original draft, Formal analysis. **Mikkel Winther Pedersen:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Conceptualization.

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 data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2026.106537.

Data availability

The raw sequencing data have been deposited at the European Nucleotide Archive (ENA) and are publicly available (Project PRJEB87497). The corresponding accession numbers for each sample are provided in Table S6.

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