

Original Research Article

Postmortem Resilience of Dental Pulp Mitochondrial DNA in Disaster Victim Identification: A Cross-Sectional Casework Series from Mass Fatality Incidents in Manila, Philippines

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ARTICLE INFO

Article history

Received: 12 December 2023

Revised: 1 March 2024

Accepted: 18 March 2024

Available online: 30 April 2024

Published: 30 April 2024

Keywords

Disaster victim identification

Forensic odontology

Hypervariable control region

Mitochondrial DNA

Sanger sequencing

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DOI

10.59345/sjfm.v2i1.356

ABSTRACT

Background. Mass fatality incidents in Metropolitan Manila expose human remains to damage that defeats friction ridge analysis and antemortem dental comparison. Mitochondrial DNA (mtDNA) is present in high copy number and is maternally transmitted, so it remains informative as a lineage marker when nuclear DNA is degraded.

Objective. To quantify control-region amplification performance and maternal concordance of dental pulp mtDNA in urban disaster victim identification (DVI).

Methods. In this cross-sectional casework series conducted under Interpol DVI standards, 100 unidentified victims and 200 living maternal relatives were examined across Metropolitan Manila. Pulp was recovered by coronal sectioning or cryogenic pulverisation. Hypervariable regions 1 and 2 were amplified as reduced-size amplicons and sequenced bidirectionally against the revised Cambridge Reference Sequence. Haplogroups were assigned, and likelihood ratios were calculated using EMPOP reference data.

Results. HV1 amplified in 95.0% of victim teeth (95/100; 95% CI 88.7–98.4) and HV2 in 94.0%; 97.5% of reference kin amplified. Complete bidirectional consensus was obtained in 90.0% (90/100) and 90.0% (180/200), respectively. Molars reached complete resolution more often than anterior teeth (93.8%, 61/65 versus 73.3%, 11/15; Fisher's exact $p = 0.037$). Mandibular molars yielded approximately 4.5 times as many mtDNA copies as incisors ($(8.65 \pm 1.94) \times 10^4$ versus $(1.92 \pm 0.64) \times 10^4$ copies/ μ L; $p < 0.001$). All 90 sequenced victims showed complete maternal concordance, with likelihood ratios from 1.2×10^5 to 8.4×10^6 . Molar class, intact coronal chamber and postmortem interval ≤ 14 days were associated with complete recovery.

Conclusion. Dental pulp mtDNA sequencing is a resilient lineage-based modality supporting identification within a reconciled multidisciplinary DVI process; recovery rates are conditional on the stated eligibility criteria.

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1. Introduction

Metropolitan Manila lies at the intersection of the circum-Pacific seismic belt and the western Pacific typhoon corridor, and concentrates more than thirteen million residents across a low-lying estuarine basin that floods repeatedly during the monsoon season. Storm surge along Manila Bay, overbank flooding of the Pasig–Marikina river system, urban conflagration and rupture along the West Valley Fault each generate mass fatality events that routinely exceed the surge capacity of municipal mortuary services.¹ Population exposure is compounded by dense informal settlement in the most flood-prone districts, where housing density, constrained evacuation routes and limited health infrastructure translate hazard into mortality.² In the aftermath of such events, disaster victim identification (DVI) is not merely a technical exercise but a

humanitarian obligation and a medicolegal precondition for death certification, inheritance, insurance settlement and culturally appropriate funerary rites.³

The Interpol DVI framework distinguishes primary identifiers, which carry individualising evidentiary weight, from secondary identifiers, which are corroborative only. The primary set comprises friction ridge analysis, comparative forensic odontology and DNA profiling, and the DNA component of that set refers to autosomal short tandem repeat (STR) typing.³ Mitochondrial sequencing is a lineage marker rather than an individualising test, and is therefore properly treated as a supportive modality that becomes decisive only within a reconciled multidisciplinary identification. DNA-based identification in mass fatality settings is formally a problem of statistical kinship inference rather than simple matching, because reference material is usually supplied by surviving

relatives rather than by the decedent, and because the number of pairwise comparisons grows rapidly with incident scale.⁴ Contemporary practice therefore treats identification as an interdisciplinary reconciliation of odontological, anthropological, molecular and circumstantial evidence rather than the output of any single discipline.⁵ Visual recognition by relatives, although emotionally compelling, is explicitly excluded as a primary identifier in disasters involving disfigurement.

Tropical disaster recovery systematically disables the two non-molecular primary identifiers. Prolonged immersion in warm, debris-laden floodwater produces maceration and desquamation of friction ridge skin within days, and although advanced rehydration and postmortem ridge reconstruction techniques have improved recovery from compromised hands, they cannot restore epidermis that has fully separated.⁶ Submersion also accelerates the general decomposition sequence in ways that complicate both biological profiling and interval estimation.⁷ Immersion further degrades the molecular substrate itself: comparative analysis of skeletal material recovered from marine environments demonstrates measurable loss of genotyping success relative to terrestrial controls.⁸ Post-disaster electrical faults and ruptured fuel lines ignite structural fires that carbonise superficial tissue, while masonry collapse during seismic or wind loading produces fragmentation and commingling of remains.

Comparative forensic odontology retains exceptional value in these circumstances because dental tissues survive insults that destroy soft tissue, and because the dentition offers a large number of independently variable features.⁹ Its operational yield in multiple-victim incidents is well documented.¹⁰ Its decisive constraint, however, is dependence on antemortem records: without charts, radiographs or casts, odontology can reconstruct a biological profile but cannot individualise. Analysis of resource-constrained settings shows that fragmentary, non-standardised and geographically dispersed antemortem dental records constitute the principal barrier to odontological identification, rather than any deficiency of postmortem technique.¹¹ This constraint is acute in the Philippines, where routine dental attendance is socioeconomically stratified and where records held in private practices are frequently destroyed by the same event that killed the patient. Structured training and calibration improve postmortem charting quality,¹² and quantitative three-dimensional comparison methods are reducing subjectivity in dental matching,¹³ but neither addresses the absence of an antemortem comparator.

Forensic DNA profiling is therefore the decisive modality when conventional identifiers fail. Autosomal STR typing generates random match probabilities that are effectively conclusive when high-molecular-weight template is available, and casework series confirm that STR typing succeeds even on skeletal remains exposed to extreme environmental conditions when sampling and extraction are optimised.¹⁴ The limiting factor is template integrity. Degradation processes fragment nuclear DNA progressively, and the analytical consequences — allelic imbalance, locus dropout and uninterpretable partial profiles — scale with the length of the target amplicon.¹⁵ Methodological convergence between forensic and ancient DNA practice has clarified that recovery from compromised skeletal and dental material depends on contamination control, enzymatic access to the mineral matrix and amplicon design rather than on any single extraction chemistry.¹⁶

Thermal insult adds a distinct and severe degradation mechanism. Controlled heating of human teeth demonstrates that DNA yield and amplifiability decline sharply above approximately 200 °C, with quantifiable loss of template well before the tooth is visibly destroyed.¹⁷ Predictive modelling of incinerated teeth confirms that nuclear and mitochondrial recovery follow different trajectories, with mitochondrial targets remaining recoverable at exposures that abolish nuclear typing.¹⁸ Contemporary multidisciplinary assessment of fire-related deaths reinforces that the diagnostic value of burned remains depends on matching the analytical target to the degree of thermal alteration.¹⁹

Against this background, teeth constitute the most mechanically and chemically protected reservoir of endogenous DNA in the body. The enamel cap is approximately 96% mineral by weight, and the underlying dentine combines a dense mineral phase with a type I collagen matrix, together forming a barrier against hydrolytic fluid, microbial colonisation and radiant heat. Direct comparison of dental compartments shows that both pulp and cementum yield amplifiable DNA, with distinct performance characteristics that justify sampling strategy being matched to the state of the specimen.²⁰ Systematic comparison of nuclear DNA preservation across dental tissues confirms that compartment choice materially changes recovery from compromised teeth.²¹ Synthesis of the thermal literature likewise identifies the enclosed pulp chamber as the compartment most likely to retain typable template after heat exposure.²²

The pulp chamber houses odontoblasts, fibroblasts, endothelial cells and mesenchymal stem cells within a sealed, vascularised cavity. Its cellular content is nonetheless sensitive to the postmortem environment: a controlled experimental study of submersion in a rat model demonstrates progressive, time-dependent histological alteration of the odontoblastic layer and pulp stroma, establishing that the chamber is protected but not inert.²³ At extended postmortem intervals or after severe heating, pulpal nuclear DNA becomes too fragmented for conventional STR typing even though the tissue remains physically present, which has motivated extraction chemistries designed specifically to liberate degraded template from pulp for downstream sequencing.²⁴

Mitochondrial DNA circumvents this limitation through stoichiometry and topology. A somatic cell contains two nuclear genome copies but hundreds of mitochondria, each carrying multiple copies of the 16,569 bp circular genome, so that mitochondrial targets outnumber nuclear targets by three to four orders of magnitude; quantitative PCR assays calibrated against certified reference material make this ratio directly measurable.²⁵ The covalently closed circular topology resists exonucleolytic attack, and the double mitochondrial membrane provides an additional diffusion barrier against cytosolic nucleases. Forensic validation of control-region and whole-genome mitochondrial sequencing has formalised the analytical requirements for casework use.²⁶

For identification purposes the informative sequence lies in the non-coding control region, which contains hypervariable region 1 (HV1, nucleotide positions 16024–16365) and hypervariable region 2 (HV2, nucleotide positions 73–340). Validated control-region workflows now support routine casework at defined quality thresholds.²⁷ Because the mitochondrial genome is transmitted maternally without meiotic recombination, every maternal relative shares the victim haplotype;

multigenerational pedigree studies confirm sequence stability across two to four generations, which is precisely the property that permits distant relatives to serve as reference donors.²⁸ The same property means that a haplotype is shared by every member of a lineage, so haplotype rarity within the relevant reference population determines evidentiary weight, and population-appropriate frequency data are essential to any likelihood ratio calculation.²⁹

Despite this mechanistic rationale, empirical performance data under genuine tropical mass fatality conditions remain limited. Most published performance data derive from controlled heating experiments, temperate casework or archaeological material, none of which reproduce the combination of sustained high humidity, ambient temperatures of 28–34 °C, brackish floodwater and post-disaster fire that characterises Manila. Work in tropical settings has begun to address this gap, reporting that even circulating free DNA from decomposed cadavers can support massively parallel sequencing-based identification,³⁰ and that such alternative templates are feasible for routine forensic application. Philippine maternal lineages belong predominantly to Austronesian-associated clades whose dispersal and present-day distribution have been characterised in regional population surveys, with comparative data from neighbouring island populations further defining the regional haplogroup landscape. Whether these lineages afford sufficient discriminatory power in routine Philippine DVI casework has not been established.

This cross-sectional casework series was therefore designed to quantify the operational performance of dental pulp mitochondrial DNA under authentic urban disaster conditions. The study had three specific objectives: to compare mitochondrial recovery across anatomical tooth classes subjected to distinct real-world taphonomic insults; to identify structural and environmental correlates of complete profile recovery; and to quantify the evidentiary weight achievable with population-appropriate reference frequencies in a Philippine cohort. Collectively these objectives address whether a Sanger-based molecular odontology protocol, deliverable within the analytical capacity of a mid-resource forensic laboratory, can support identification in tropical mass fatality response.

2. Methods

2.1. Design, setting and ethical framework

This cross-sectional forensic casework series was conducted within the humanitarian and medicolegal framework of disaster victim identification. The protocol adhered to the ethical principles of the Declaration of Helsinki and to Interpol DVI operational standards for the recovery, documentation and identification of human remains after mass fatality events. Reconciliation of postmortem and antemortem data followed an interdisciplinary model in which odontological, anthropological and molecular findings were reviewed jointly before any identification was declared.⁵ Institutional ethical clearance and forensic casework oversight were granted by the Institutional Ethics and Forensic Review Board of RBH Dental School together with the collaborating municipal health authorities of Manila (protocol RBH-ERC-2021-079-DVI). Written informed consent was obtained from every living relative before reference sampling, with explicit provision for data

protection and for restriction of the genetic data to identification purposes.

2.2. Cohort and eligibility

The study cohort comprised 100 unidentified victims recovered from mass fatality incidents across Metropolitan Manila between 2021 and 2023, including typhoon-associated flooding, urban structural fires and building collapse, together with 200 living maternal reference relatives. Victims were eligible when recovered from a verified disaster scene; when taphonomic compromise precluded visual recognition, friction ridge acquisition and antemortem record reconciliation; and when at least one permanent tooth was retained in alveolar bone or recoverable from associated fragments. Because the absence of usable antemortem dental documentation is the dominant barrier to odontological identification in settings of this kind, molecular analysis was pursued in parallel with, rather than subsequent to, odontological examination.¹¹ Remains were excluded when completely skeletonised with pulpal obliteration, when the only available teeth showed carious cavitation communicating with the oral cavity, or when no putative maternal relative could be traced. Every reported recovery proportion is therefore conditional on these criteria.

2.3. Odontological examination and specimen recovery

Postmortem dental examination was performed by calibrated forensic odontologists in a dedicated negative-pressure mortuary facility. Charting followed the Interpol postmortem dental form, supported by standardised photography; structured calibration of examiners before deployment was used to reduce inter-observer variance in charting and specimen selection.¹² Postmortem computed tomography or digital periapical radiography was obtained where available to assess root morphology, pulp chamber volume and crown integrity before sampling, and quantitative comparison of dental morphology was used to support odontological assessment where antemortem imaging existed.¹³ Sampling priority was assigned by anatomical resilience: permanent first and second molars were the specimens of choice on account of their thick enamel cap, large coronal pulp chamber and multi-rooted anchorage, with premolars and canines sampled only when molars were absent or comminuted. Because this priority was not random, tooth class is not independent of specimen integrity, age or exposure.

Teeth were removed atraumatically with forceps and luxators, avoiding thermal or mechanical injury to the cervical and apical root architecture. Where the jaws were fragmented, surrounding alveolar bone was dissected with sterile low-speed trephines under continuous sterile saline irrigation. Handling followed a protocol developed for skeletal and dental specimens that avoids liquid nitrogen at the recovery stage and is therefore deployable in field mortuary conditions.³⁴ Reference material from the 200 living maternal relatives was collected as sterile buccal swabs or 5 mL EDTA venous blood, dried or refrigerated at 4 °C until extraction.

2.4. Contamination control and pulp recovery

All postmortem specimens were processed in a physically segregated pre-amplification cleanroom under positive pressure with high-efficiency particulate air filtration and scheduled ultraviolet germicidal irradiation at 254 nm. Personnel used disposable full-barrier protective equipment, double gloving and filtered pipette tips. Because laboratory consumables themselves can

introduce exogenous human DNA into low-template workflows, consumable lots were screened before use and negative controls were carried through every stage of the process. Procedural discipline followed the contamination-control standards developed for ancient and degraded skeletal material. Teeth were decontaminated externally by immersion in 10% commercial sodium hypochlorite for 15 minutes, washed in molecular-grade water, immersed in 96% ethanol for 5 minutes and ultraviolet-irradiated for 30 minutes on each surface.

Pulp was recovered by one of two protocols according to crown integrity. Teeth with intact coronal anatomy were sectioned longitudinally with a water-cooled diamond separating disc below 5,000 rpm to avoid frictional heating, after which the chamber and root canals were inspected under a stereomicroscope and the desiccated pulp and odontoblastic layer were excavated with sterile barbed broaches, Hedström files and micro-curettes. Teeth with severe coronal charring or structural fragility were pulverised whole under liquid nitrogen in a freezer mill to a particle size below 100 µm; in these specimens the template is derived from multiple dental compartments rather than from pulp alone. Selection between compartments was guided by evidence that pulp and cementum differ systematically in recoverable template,²⁰ and processing was standardised against an automated extraction workflow validated for bone and tooth specimens.

2.5. Extraction and quantification

Total DNA was extracted by an organic phenol-chloroform-isoamyl alcohol method with centrifugal micro-concentration, selected because comparative evaluation of extraction chemistries on degraded skeletal material shows organic protocols to retain an advantage for severely compromised specimens. Between 10 and 30 mg of pulp, or 200 mg of dental powder, was digested in 500 µL of lysis buffer containing 0.5 M EDTA (pH 8.0), 10 mM Tris-HCl (pH 8.0), 0.5% sodium dodecyl sulfate, 20 mM dithiothreitol and 100 µg/mL proteinase K, with agitation at 56 °C for 18 to 24 hours. Because the extent of demineralisation is itself a determinant of yield from mineralised tissue, digestion was continued to complete dissolution of the mineral phase.

An equal volume of buffered phenol-chloroform-isoamyl alcohol (25:24:1, pH 8.0) was added, vortexed and centrifuged at 14,000 × g for 10 minutes at 4 °C. The aqueous phase was re-extracted with chloroform, transferred to a 30 kDa centrifugal ultrafiltration device and washed twice with 400 µL of molecular-grade water to remove humic acids, haem and divalent cations. DNA was eluted in 30 to 50 µL of low-EDTA Tris buffer and stored at -20 °C. Extraction blanks were processed at a ratio of one blank per ten specimens.

Total DNA was quantified spectrophotometrically with assessment of A₂₆₀/A₂₈₀ and A₂₆₀/A₂₃₀ ratios and confirmed by high-sensitivity fluorometry. Mitochondrial copy number was determined separately by quantitative PCR against an 85-bp conserved fragment of the mitochondrial 16S rRNA gene, quantified against a standard curve spanning 10² to 10⁷ copies/µL. Assay calibration and the interpretation of nuclear-to-mitochondrial ratios followed published evaluations of quantification accuracy against certified reference material.²⁵

2.6. Amplification and sequencing

Amplification targeted HV1 (nucleotide positions 16024–16365, 342 bp) and HV2 (nucleotide positions 73–340, 268

bp) using reduced-size amplicon primers. Amplicon length was treated as the principal design variable because systematic evaluation of control-region amplification demonstrates that dropout in degraded specimens is driven by target length and by primer-binding-site variation rather than by input mass alone. For specimens showing failure at full amplicon length, overlapping sub-amplicons below 180 bp were substituted, following amplification and sequencing strategies developed specifically for degraded forensic material. Reactions of 25 µL contained 2.5 µL of 10× buffer, 2.5 mM MgCl₂, 0.2 mM of each deoxynucleoside triphosphate, 0.4 µM of each primer, 1.0 U of hot-start polymerase, 0.8 mg/mL bovine serum albumin and 2.0 to 5.0 µL of template.

Thermal cycling comprised activation at 95 °C for 11 minutes; 36 cycles of 94 °C for 30 seconds, 56 °C for 30 seconds and 72 °C for 45 seconds; and final extension at 72 °C for 10 minutes. Every batch included extraction blanks, amplification blanks and a positive control. Products were verified on 2% agarose and purified enzymatically. Recovery of assembled control-region sequence from fragmented input depends on the preprocessing applied before assembly, and read handling was standardised accordingly.

Bidirectional cycle sequencing used dye-terminator chemistry in 10 µL reactions with 25 cycles of 96 °C for 10 seconds, 50 °C for 5 seconds and 60 °C for 4 minutes, followed by resin-based purification and capillary electrophoresis on a 50 cm array with POP-7 polymer. Single-multiplex control-region typing has been shown to recover interpretable sequence from highly degraded skeletal remains, and the present workflow was configured to the same analytical tolerances. Run performance was benchmarked against internally validated control-region acceptance criteria.

2.7. Sequence interpretation and haplogroup assignment

Trace files were independently base-called and assembled by two analysts and aligned to the revised Cambridge Reference Sequence (GenBank NC_012920.1). A Phred quality threshold of Q ≥ 30 was required at every polymorphic position. Point heteroplasmy was scored when a secondary peak reached at least 15% of primary peak height in both directions, and length heteroplasmy in the HV1 and HV2 cytosine homopolymers was characterised separately; interpretation followed current appraisal of mitochondrial reporting guidelines.^{30,31} Automated heteroplasmy-calling logic developed for control-region analysis informed the thresholds applied during dual-analyst review. Population-level expectations for heteroplasmy frequency were taken from forensic survey data.

Haplogroups were assigned against PhyloTree build 16 using an automated classification tool validated for degraded and partial control-region input. Maternal concordance was evaluated by pairwise alignment of victim and reference consensus sequences across both hypervariable regions. Evidentiary weight was expressed as a likelihood ratio computed against EMPOP Southeast Asian reference frequencies with a population substructure correction of $\theta = 0.03$, in accordance with population-appropriate haplogroup frequency practice. The statistical framing of kinship-based identification followed established methodology for human remains and missing-person casework, with attention to the behaviour of likelihood ratios when sequence information is incomplete. Microhaplotype-based kinship analysis was

held in reserve for cases unresolved by mitochondrial comparison.

2.8. Statistical analysis

Analyses were performed in SPSS version 28.0 and R version 4.3.1. Continuous variables were assessed for normality by the Shapiro–Wilk test and are reported as mean $\pm$ standard deviation. Differences across tooth classes were tested by one-way analysis of variance with Tukey honestly significant difference contrasts. Categorical proportions were compared by Fisher's exact test, and exact binomial 95% confidence intervals are reported for the principal proportions. Binary logistic regression was used to examine correlates of complete profile recovery, defined as unambiguous bidirectional consensus across both HV1 and HV2; model calibration was assessed by the Hosmer–Lemeshow test. Odds ratios are reported with 95% confidence intervals, and two-tailed *p* values below 0.05 were treated as significant. Because the outcome comprised only ten failures, the multivariable model carries approximately two events per variable and its adjusted estimates are unstable; they are reported as exploratory.

3. Results

3.1. Cohort composition and taphonomic exposure

The investigated series comprised 100 severely compromised human remains recovered from mass fatality scenes across Metropolitan Manila together with 200 living maternal reference relatives, giving a total forensic cohort of 300 individuals whose sociodemographic and exposure characteristics are set out

in Table 1. Among the 100 decedents, 55 (55.0%) were male and 45 (45.0%) female, a male-to-female ratio of 1.22:1. Age ranged from 4 to 78 years with a mean of 35.8 ± 16.4 years. Young adults aged 18 to 39 years formed the largest stratum at 46 (46.0%), followed by middle-aged adults at 28 (28.0%), paediatric casualties at 14 (14.0%) and individuals aged 60 years or above at 12 (12.0%). The maternal reference cohort comprised 110 males (55.0%) and 90 females (45.0%) with a mean age of 38.2 ± 15.1 years and a range of 18 to 76 years. Figure 1 displays the age distribution and the taphonomic exposure profile of the victim cohort side by side.

Taphonomic assessment, summarised in the lower rows of Table 1 and in panel (b) of Figure 1, showed that every decedent had sustained severe postmortem compromise. Prolonged submersion in brackish, debris-laden floodwater was the dominant insult in 42 victims (42.0%). Thermal injury from post-disaster structural fire accounted for a further 34 victims (34.0%), of whom 16 (16.0%) sustained Crow–Glassman level 1 to 2 superficial to moderate charring at an estimated 100–250 °C and 18 (18.0%) sustained level 3 to 4 incineration exceeding 300 °C with destruction of facial soft tissue. Blunt crushing from masonry collapse during seismic loading or extreme wind accounted for the remaining 24 victims (24.0%). The mean postmortem interval across the whole victim cohort was 7.4 ± 3.8 days, ranging by exposure category from 4.2 ± 1.8 days in the superficially burned group to 9.4 ± 3.8 days in the submerged group. Each decedent was assigned the single dominant insult recorded at recovery, which simplifies incidents in which submersion, heating and crushing occurred in sequence.

Table 1. Sociodemographic and taphonomic profile of disaster victims and maternal reference kin.

Sociodemographic or exposure parameter	Disaster victims (n = 100)	Maternal reference kin (n = 200)	Total forensic cohort (n = 300)
Biological sex: male, n (%)	55 (55.0%)	110 (55.0%)	165 (55.0%)
Biological sex: female, n (%)	45 (45.0%)	90 (45.0%)	135 (45.0%)
Age stratum: paediatric (0–17 years), n (%)	14 (14.0%)	28 (14.0%)	42 (14.0%)
Age stratum: young adult (18–39 years), n (%)	46 (46.0%)	92 (46.0%)	138 (46.0%)
Age stratum: middle-aged (40–59 years), n (%)	28 (28.0%)	56 (28.0%)	84 (28.0%)
Age stratum: elderly (≥ 60 years), n (%)	12 (12.0%)	24 (12.0%)	36 (12.0%)
Age in years, mean $\pm$ SD (range)	35.8 ± 16.4 (4–78)	38.2 ± 15.1 (18–76)	37.4 (4–78)
Taphonomic insult: aquatic submersion (riverine and storm-surge floodwater), n (%)	42 (42.0%)	Not applicable (living kin)	42 (14.0%)
Taphonomic insult: thermal exposure, Crow–Glassman level 1–2 (100–250 °C), n (%)	16 (16.0%)	Not applicable (living kin)	16 (5.3%)
Taphonomic insult: thermal exposure, Crow–Glassman level 3–4 (300–600 °C), n (%)	18 (18.0%)	Not applicable (living kin)	18 (6.0%)
Taphonomic insult: blunt crushing and structural masonry collapse, n (%)	24 (24.0%)	Not applicable (living kin)	24 (8.0%)
Postmortem interval in days, mean $\pm$ SD	7.4 ± 3.8	Not applicable (living kin)	7.4 ± 3.8

Notes: Percentages for the total cohort are calculated against *n* = 300. Taphonomic insult categories follow Interpol disaster victim identification nomenclature and the Crow–Glassman burn scale; each victim was assigned the single dominant insult recorded at recovery.

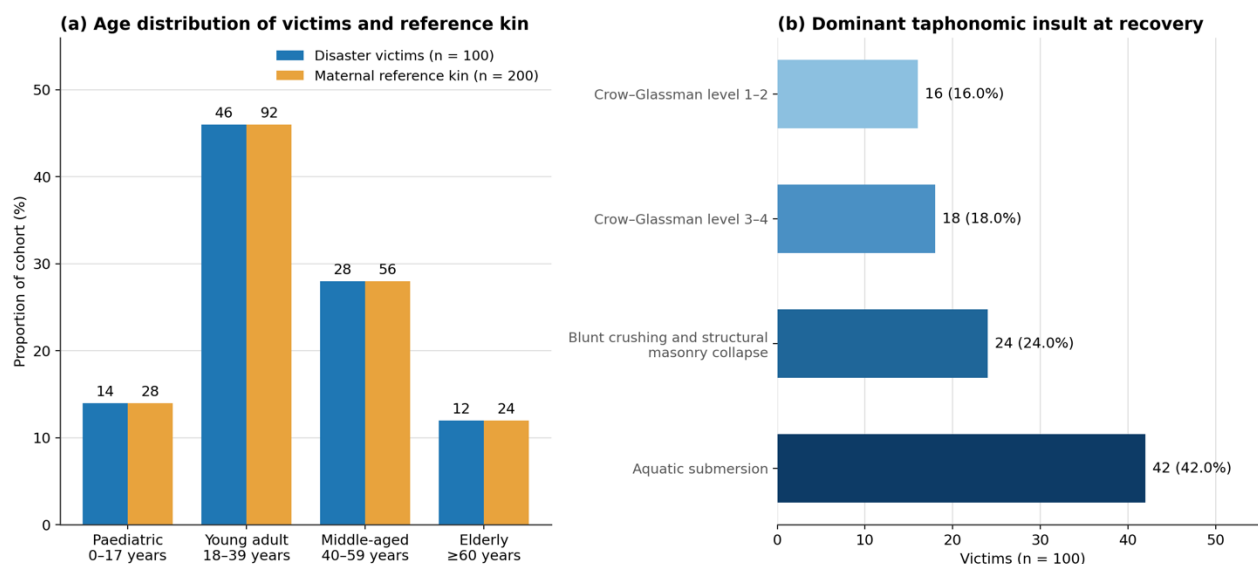


Figure 1. Sociodemographic composition and taphonomic exposure of the Manila disaster cohort. (a) Age distribution of the 100 disaster victims and 200 maternal reference relatives, with counts above each bar. (b) Dominant taphonomic insult recorded at recovery. Values correspond to Table 1.

3.2. Odontological anatomy, pulp preservation and nucleic acid yield

One hundred permanent teeth were recovered and classified into five anatomical groups, with morphometric and molecular yield data presented in Table 2. Mandibular first and second molars were the most frequently available specimen at 40 teeth (40.0%), followed by maxillary molars at 25 (25.0%), premolars at 20 (20.0%), canines at 8 (8.0%) and incisors at 7 (7.0%). Anatomical class exerted a substantial effect on recoverable pulp mass, with one-way analysis of variance yielding $F(4, 95) = 39.3, p < 0.001$. Mandibular molars yielded the greatest mean desiccated pulp mass at 14.8 ± 3.2 mg, exceeding premolars at 8.6 ± 2.1 mg, canines at 6.4 ± 1.8 mg and incisors at 4.8 ± 1.4 mg (Tukey $p < 0.001$ for each contrast). Maxillary molars yielded 13.2 ± 2.9 mg, which did not differ significantly from mandibular molars ($p = 0.160$).

Nucleic acid recovery showed a similar pattern across tooth classes, as the third and fourth data columns of Table 2 show and as Figure 2 illustrates. Fluorometric quantification gave mean total DNA yields of 0.42 ± 0.08 ng/mg for mandibular molars, 0.38 ± 0.07 ng/mg for maxillary molars, 0.26 ± 0.05 ng/mg for premolars, 0.19 ± 0.04 ng/mg for canines and 0.14 ± 0.03 ng/mg for incisors, with $F(4, 95) = 48.7, p < 0.001$. Quantitative PCR against the mitochondrial 16S rRNA locus reproduced this ordering with a steeper gradient, $F(4, 95) = 55.2, p < 0.001$. Mandibular molars contained $(8.65 \pm 1.94) \times 10^4$ copies/ μ L and maxillary molars $(7.42 \pm 1.65) \times 10^4$ copies/ μ L, approximately 4.5 times and 3.9 times the $(1.92 \pm 0.64) \times 10^4$ copies/ μ L recovered from incisors (Tukey $p < 0.001$). Because copy number is expressed per microlitre of eluate rather than per milligram of pulp, and mandibular molars supplied about three times the input mass of incisors, this ratio is not independent of the input denominator; the normalised total DNA yield shows a 3.0-fold molar-to-incisor difference.

Table 2. Odontological anatomy, pulp-chamber preservation and nucleic acid yield across sampled tooth classes.

Anatomical odontological class	Sampled, n (%)	Pulp mass, mg (mean $\pm$ SD)	Total DNA yield, ng/mg (mean $\pm$ SD)	mtDNA copy number, $\times 10^4$ copies/ μ L (mean $\pm$ SD)
Mandibular first and second molars (multi-rooted)	40 (40.0%)	14.8 ± 3.2	0.42 ± 0.08	8.65 ± 1.94
Maxillary first and second molars (tri-rooted)	25 (25.0%)	13.2 ± 2.9	0.38 ± 0.07	7.42 ± 1.65
Premolars, mandibular and maxillary (bi-rooted)	20 (20.0%)	8.6 ± 2.1	0.26 ± 0.05	4.18 ± 1.12
Canines, maxillary and mandibular (single root)	8 (8.0%)	6.4 ± 1.8	0.19 ± 0.04	2.85 ± 0.88
Incisors, central and lateral (uniradicular)	7 (7.0%)	4.8 ± 1.4	0.14 ± 0.03	1.92 ± 0.64
One-way ANOVA across the five classes	$n = 100$	$F(4, 95) = 39.3, p < 0.001$	$F(4, 95) = 48.7, p < 0.001$	$F(4, 95) = 55.2, p < 0.001$

Notes: Pulp mass denotes desiccated pulpal tissue recovered per tooth. Mitochondrial copy number was determined by quantitative PCR against the conserved mitochondrial 16S rRNA locus and is expressed per microlitre of eluate, so it is not normalised to input mass. Between-class differences were tested by one-way analysis of variance with Tukey honestly significant difference post-hoc contrasts.

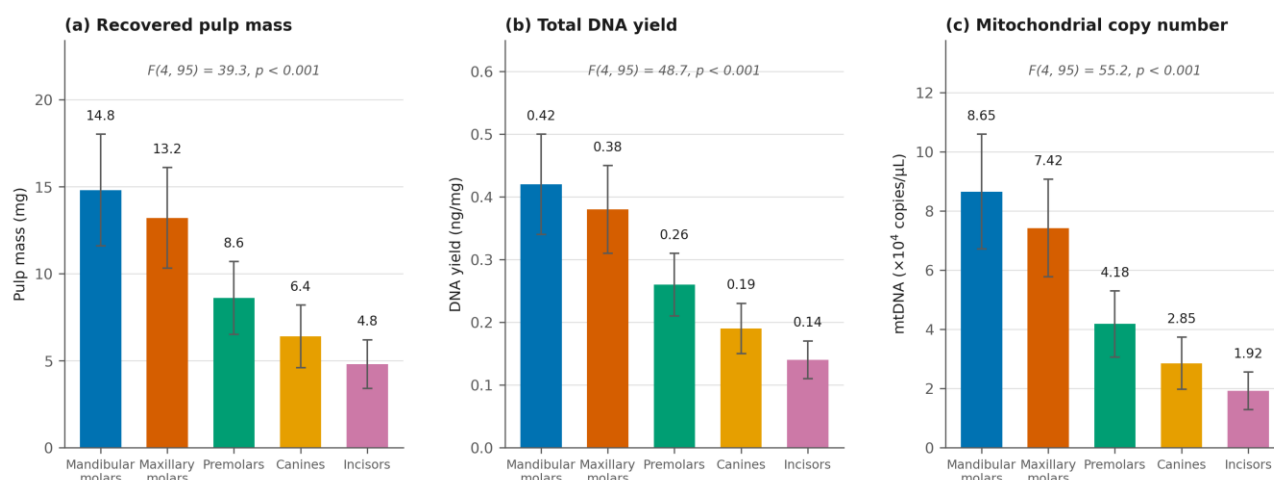


Figure 2. Odontological yield across anatomical tooth classes. (a) Recovered pulp mass, (b) total DNA yield per milligram of pulp and (c) mitochondrial copy number by quantitative PCR. Bars show the mean and error bars one standard deviation; group sizes range from 7 to 40 specimens, so the dispersion shown for the smallest classes is imprecise. The one-way analysis of variance statistic is inset in each panel. Values correspond to Table 2.

3.3. Amplification performance across taphonomic exposure regimes

Amplification of both hypervariable regions was robust across the compromised cohort, with results stratified by exposure category in Table 3 and displayed in Figure 3. HV1 amplified successfully in 95 of 100 victim specimens (95.0%; 95% CI 88.7–98.4) and HV2 in 94 of 100 (94.0%; 87.4–97.8); among the 200 living reference donors amplification succeeded in 195 (97.5%; 94.3–99.2). Teeth from victims with superficial thermal exposure amplified in all 16 cases (100.0%; 79.4–100.0) at both loci. Teeth recovered after prolonged submersion, at a mean postmortem interval of 9.4 days, amplified in 40 of 42 cases (95.2%; 83.8–99.4) at both loci, indicating that the mineralised crown and apical seal had substantially excluded waterborne hydrolytic and microbial activity from the chamber. Remains subjected to structural crushing amplified in 23 of 24 cases (95.8%; 78.9–99.9).

Under Crow–Glassman level 3 to 4 incineration, where facial soft tissue had been wholly consumed, dental pulp yielded amplifiable template in 16 of 18 cases (88.9%; 65.3–98.6) for HV1 and 15 of 18 (83.3%; 58.6–96.4) for HV2, the lowest values in the series and the only category in which the two loci diverged. Complete amplification failure was restricted to five teeth: two severely charred incisors with crown fragmentation exposing the chamber directly to flame, two comminuted teeth with root fracture, and one molar recovered after 21 days in stagnant warm floodwater with putrefactive invasion through an open apical foramen. Grouped by anatomy, molars amplified in 63 of 65 specimens (96.9%) against 13 of 15 (86.7%) for canines and incisors combined, a difference that did not reach statistical significance (Fisher's exact $p = 0.158$); premolars occupied an intermediate position at 19 of 20 (95.0%).

Table 3. Hypervariable control-region amplification and sequencing resolution across taphonomic exposure regimes.

Taphonomic exposure category	Sub-cohort, n	Postmortem interval, days (mean ± SD)	HV1 amplification, n (%)	HV2 amplification, n (%)	Complete bidirectional consensus, n (%)
Aquatic submersion (riverine and storm-surge floodwater)	42	9.4 ± 3.8	40 (95.2)	40 (95.2)	38 (90.5)
Thermal exposure, Crow–Glassman level 1–2 (100–250 °C)	16	4.2 ± 1.8	16 (100.0)	16 (100.0)	16 (100.0)
Thermal exposure, Crow–Glassman level 3–4 (300–600 °C)	18	5.1 ± 2.1	16 (88.9)	15 (83.3)	15 (83.3)
Blunt crushing and structural masonry collapse	24	7.6 ± 3.2	23 (95.8)	23 (95.8)	21 (87.5)
Whole victim cohort	100	7.4 ± 3.8	95 (95.0)	94 (94.0)	90 (90.0)

Notes: HV1 denotes hypervariable region 1 (nucleotide positions 16024–16365, 342 bp amplicon); HV2 denotes hypervariable region 2 (nucleotide positions 73–340, 268 bp amplicon). Complete consensus requires unambiguous bidirectional resolution across both regions. Exact binomial 95% confidence intervals for the whole-cohort values are 88.7–98.4 (HV1), 87.4–97.8 (HV2) and 82.4–95.1 (complete consensus).

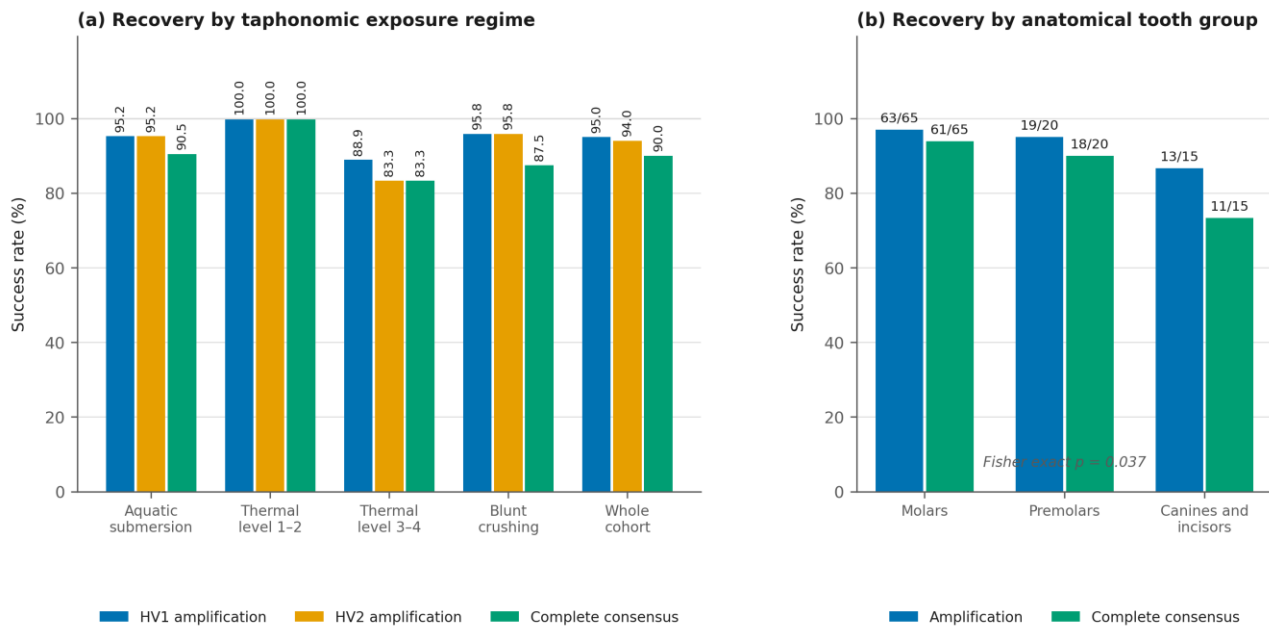


Figure 3. Amplification and sequencing performance. (a) HV1 amplification, HV2 amplification and complete bidirectional consensus stratified by taphonomic exposure regime, with the whole-cohort result at right. (b) The same outcomes grouped by anatomical tooth class, annotated with the underlying counts. Values correspond to Tables 2 and 3.

3.4. Sequencing resolution, sequence quality and heteroplasmy

Bidirectional capillary sequencing produced complete, unambiguous consensus across both HV1 and HV2 in 90 of 100 victim specimens (90.0%; 82.4–95.1) and in 180 of 200 reference donors (90.0%; 85.0–93.8). Partial profiles resolving only one hypervariable region, predominantly the shorter HV2 amplicon, were obtained in 5 victims (5.0%), while the remaining 5 (5.0%) failed at the amplification stage. The difference between tooth groups was evident at the sequencing stage: molars achieved complete resolution in 61 of 65 specimens (93.8%; 85.0–98.3) against 11 of 15 (73.3%; 44.9–92.2) for canines and incisors, a difference that was statistically significant on Fisher's exact testing ($p = 0.037$), with premolars intermediate at 18 of 20 (90.0%). The interval around the anterior-tooth estimate is wide, and the contrast rests on four failures in fifteen specimens. Sequence quality metrics and heteroplasmy findings among the 90 fully sequenced victims are detailed in Table 4.

Electropherogram review showed a mean Phred quality score of 42.6 ± 4.8 across called positions, exceeding the $Q \geq 30$ acceptance threshold, with well-resolved peak morphology and no detectable dye-terminator artefact. Point heteroplasmy was identified in 8 of the 90 sequenced victims (8.9%; 3.9–16.8), distributed across three recognised transition hotspots: nucleotide position 16093 in 3 individuals, position 16189 in 3, and position 214 in 2, as Figure 4 sets out alongside the corresponding quality distribution. In every one of these 8 cases the identical heteroplasmic state was observed in the matched maternal relative at closely comparable peak ratios, which is consistent with maternal transmission rather than postmortem damage or polymerase error. Length heteroplasmy was correspondingly common, affecting the HV1 cytosine tract at positions 16184–16193 in 22 individuals (24.4%; 16.0–34.6) and the HV2 tract at positions 303–315 in 48 individuals (53.3%; 42.5–63.9); all length variants were concordant between victim and relative.

Table 4. Sequence quality and heteroplasmy characterisation among successfully sequenced victims.

Sequence-quality or heteroplasmy parameter	Victims sequenced (n = 90)	Proportion, % (95% CI)	Forensic interpretation
Mean Phred quality score across called positions	42.6 $\pm$ 4.8	Not applicable	Exceeds the $Q \geq 30$ acceptance threshold at every polymorphic site
Point heteroplasmy at np 16093 (Y: C/T)	3	3.3 (0.7–9.4)	Concordant with the matched maternal relative in every instance
Point heteroplasmy at np 16189 (Y: C/T)	3	3.3 (0.7–9.4)	Concordant with the matched maternal relative in every instance
Point heteroplasmy at np 214 (R: A/G)	2	2.2 (0.3–7.8)	Concordant with the matched maternal relative in every instance
Point heteroplasmy, any position	8	8.9 (3.9–16.8)	Shared heteroplasmy supports maternal kinship
Length heteroplasmy, HV1 C-tract (np 16184–16193)	22	24.4 (16.0–34.6)	Stutter in the cytosine homopolymer; concordant with kin
Length heteroplasmy, HV2 C-tract (np 303–315)	48	53.3 (42.5–63.9)	Stutter in the cytosine homopolymer; concordant with kin

Notes: Denominators are the 90 victims for whom complete bidirectional consensus was obtained. Proportions are given with exact binomial 95% confidence intervals. Point heteroplasmy was scored when the secondary peak reached at least 15.0% of primary peak height in both sequencing directions. IUPAC ambiguity codes: Y = C/T, R = A/G.

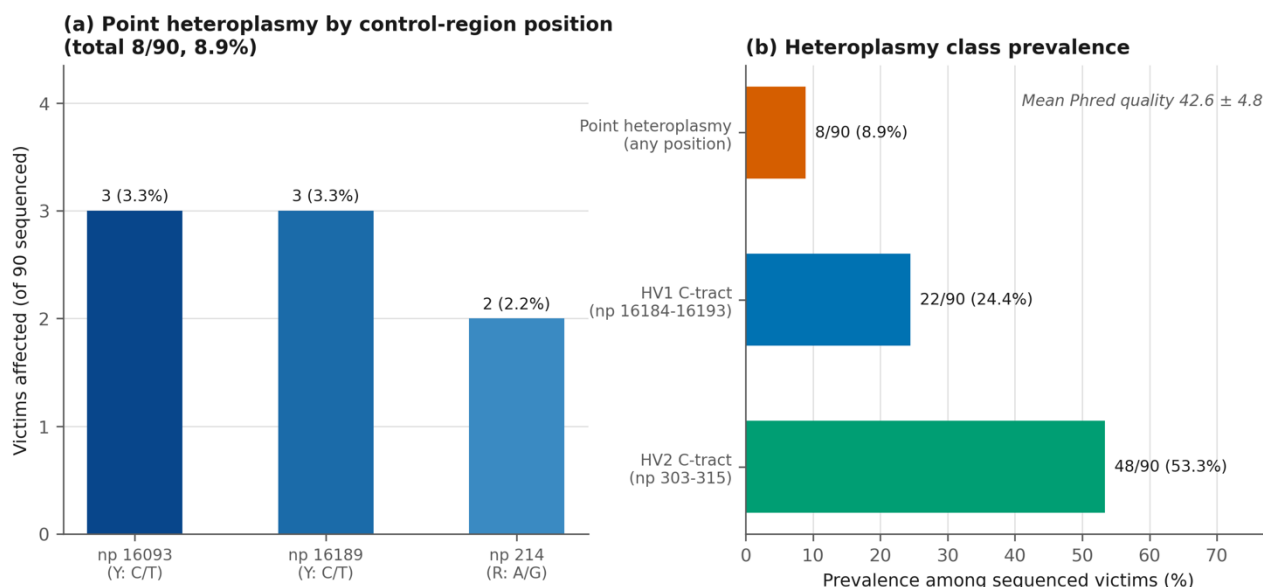


Figure 4. Sequence quality and heteroplasmy among the 90 fully sequenced victims. (a) Point heteroplasmy by control-region position. (b) Prevalence of each heteroplasmy class. Every heteroplasmic state was concordant with the matched maternal relative.

3.5. Maternal concordance, haplogroup distribution and evidentiary weight

Pairwise alignment established complete sequence identity across both hypervariable regions between victim and putative maternal relative in all 90 successfully sequenced cases, with no exclusions and no ambiguous comparisons. Representative profiles spanning the range

of taphonomic insults, together with their diagnostic polymorphisms and assigned haplogroups, are catalogued in Table 5. The 10 victims who could not be resolved mitochondrially, owing to amplification or sequencing failure, were subsequently referred for osteological profiling and contextual property association; because property association is a secondary identifier, no identification was declared on that basis alone.

Table 5. Exemplar victim mitochondrial profiles, control-region polymorphisms and haplogroup assignment.

Victim accession and dental specimen	Taphonomic insult and postmortem interval	Control-region polymorphisms (rCRS nucleotide positions)	Assigned haplogroup	Maternal reference and concordance
MNL-004, mandibular first molar (FDI 36)	Brackish aquatic submersion, 8 days	16189C, 16217C, 16261T, 73G, 263G, 315.1C	B4a1a	Biological mother and sister; full concordance
MNL-017, maxillary second molar (FDI 17)	Thermal charring at approximately 400 °C, 4 days	16140C, 16189C, 16274A, 73G, 150T, 263G	B5a	Maternal aunt; full concordance
MNL-039, mandibular second molar (FDI 47)	Alluvial mudflow entombment, 14 days	16223T, 16298C, 16362C, 73G, 263G, 309.1C	E1a1a	Maternal cousin; full concordance
MNL-062, maxillary first premolar (FDI 14)	Structural concrete collapse, 6 days	16223T, 16295T, 16304C, 73G, 150T, 263G	M7c3c	Biological mother; full concordance
MNL-088, mandibular first molar (FDI 46)	Putrefactive decomposition at 35 °C, 18 days	16129A, 16223T, 16311C, 73G, 195C, 263G	F1a1a	Biological brother; full concordance

Notes: Nucleotide positions are numbered against the revised Cambridge Reference Sequence (GenBank NC_012920.1); haplogroups were assigned against PhyloTree build 17. FDI denotes the Fédération Dentaire Internationale two-digit tooth notation.

Haplogroup assignment across the 90 identified victims reproduced the expected Philippine maternal landscape, as Table 6 records and Figure 5 depicts. Haplogroup B4a1a was the most frequent lineage at 28 victims (31.1%), followed by E1a1a at 22 (24.4%), M7c3c at 18 (20.0%), F1a1a at 12 (13.3%) and B5a at 10 (11.1%). Likelihood ratios computed against EMPOP Southeast Asian reference

frequencies exceeded 1.0×10^5 in every case, spanning 1.2×10^5 to 8.4×10^6 overall. Evidentiary weight varied with lineage frequency: the common B4a1a haplogroup produced a median likelihood ratio of 2.4×10^5 , whereas the comparatively rare B5a lineage produced a median of 3.1×10^6 , an order of magnitude higher.

Table 6. Mitochondrial haplogroup distribution and forensic likelihood ratios among identified victims.

Mitochondrial haplogroup	Victims identified, n (%)	Diagnostic control-region motif	Median likelihood ratio	Likelihood-ratio range
B4a1a	28 (31.1%)	16189C, 16217C, 16261T	2.4×10^5	1.2×10^5 to 6.8×10^5
E1a1a	22 (24.4%)	16223T, 16298C, 16362C	5.6×10^5	2.8×10^5 to 1.4×10^6
M7c3c	18 (20.0%)	16223T, 16295T, 16304C	9.2×10^5	4.1×10^5 to 2.6×10^6
F1a1a	12 (13.3%)	16129A, 16223T, 16311C	1.8×10^6	7.5×10^5 to 4.2×10^6
B5a	10 (11.1%)	16140C, 16189C, 16274A	3.1×10^6	1.1×10^6 to 8.4×10^6
All identified victims	90 (100.0%)	Not applicable	Not applicable	1.2×10^5 to 8.4×10^6

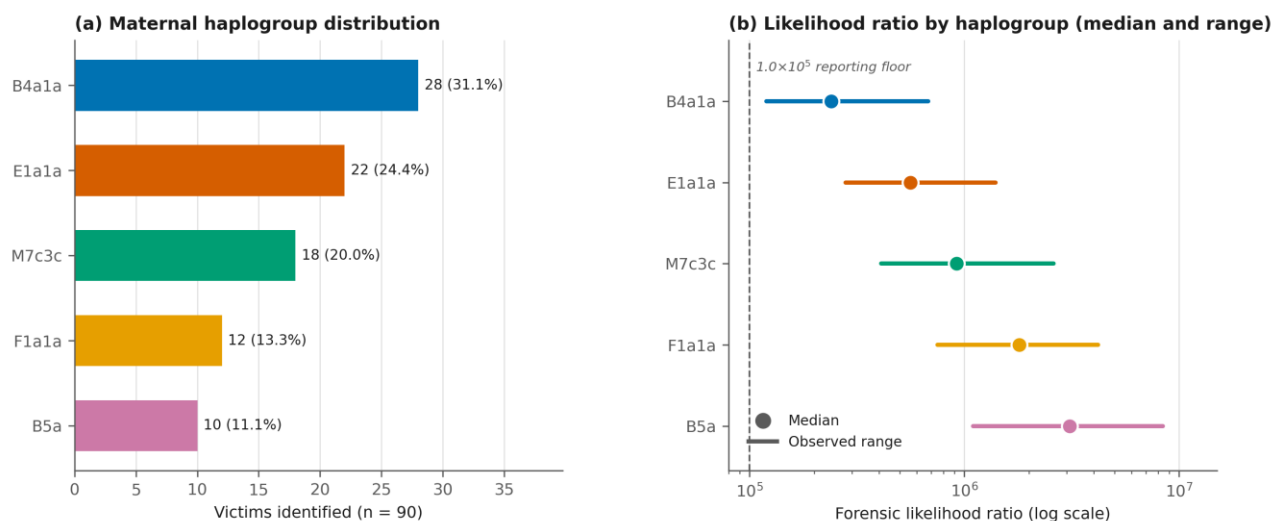


Figure 5. Maternal lineage composition and evidentiary weight. (a) Haplogroup distribution among the 90 identified victims. (b) Median and observed range of the forensic likelihood ratio by haplogroup on a logarithmic scale, showing the inverse relationship between lineage frequency and evidentiary weight.

3.6. Correlates of complete profile recovery

Univariable and multivariable logistic regression for complete bidirectional consensus is presented in Table 7, with adjusted effect sizes displayed as a forest plot in Figure 6. Three variables remained associated with the outcome after mutual adjustment. Molar tooth class showed the largest estimate, with an adjusted odds ratio of 4.12 (95% CI 1.85–9.18, $p = 0.001$), followed by intact coronal pulp-chamber integrity at 3.65 (95% CI 1.62–8.22, $p = 0.002$) and a postmortem interval of 14 days or less at 2.84 (95% CI 1.34–6.02, $p = 0.006$). Absence of severe charring was significant univariably at an unadjusted odds

ratio of 2.18 (95% CI 1.05–4.52, $p = 0.036$) but not after adjustment (1.92, 95% CI 0.88–4.18, $p = 0.102$). Terrestrial recovery, compared with aquatic submersion, was not statistically associated with complete recovery in either analysis (adjusted 1.28, 95% CI 0.60–2.75, $p = 0.524$). Model calibration was acceptable (Hosmer–Lemeshow $p = 0.784$), although this test alone does not establish adequate calibration. With ten outcome events and five covariates the estimates are unstable and should be read as exploratory; the pattern nonetheless suggests that specimen anatomy and structural integrity are more closely associated with recovery than the nature of the environmental insult.

Table 7. Univariable and exploratory multivariable logistic regression for complete mitochondrial profile recovery.

Predictor in the forensic recovery model	Unadjusted OR (95% CI)	Univariable p-value	Adjusted OR (95% CI)	Multivariable p-value
Molar tooth class (vs premolar, canine or incisor)	3.85 (1.78–8.34)	$p = 0.001$	4.12 (1.85–9.18)	$p = 0.001$
Intact coronal pulp-chamber integrity	3.42 (1.58–7.41)	$p = 0.002$	3.65 (1.62–8.22)	$p = 0.002$
Postmortem interval ≤ 14 days (vs > 14 days)	2.65 (1.28–5.48)	$p = 0.008$	2.84 (1.34–6.02)	$p = 0.006$
Absence of severe charring (vs ≥ 300 °C exposure)	2.18 (1.05–4.52)	$p = 0.036$	1.92 (0.88–4.18)	$p = 0.102$
Terrestrial recovery (vs aquatic submersion)	1.45 (0.72–2.94)	$p = 0.301$	1.28 (0.60–2.75)	$p = 0.524$

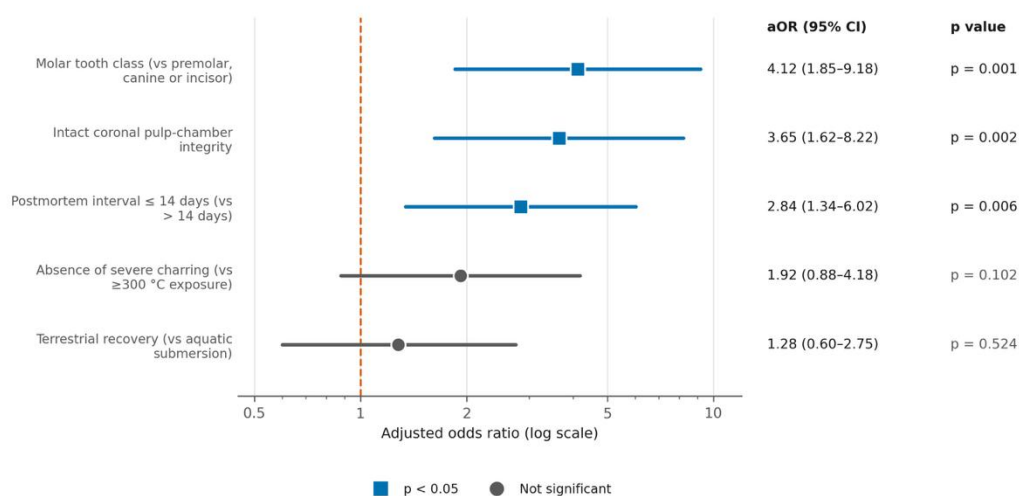


Figure 6. Adjusted odds ratios with 95% confidence intervals for complete mitochondrial profile recovery. Square markers denote predictors significant at $p < 0.05$ after mutual adjustment; the dashed line marks the null.

4. Discussion

This casework series indicates that dental pulp mitochondrial DNA sustains a high rate of sequence recovery under authentic tropical mass fatality conditions, with 95.0% HV1 amplification, 90.0% complete bidirectional resolution and complete maternal concordance in every resolved case. The result is operationally consequential because the cohort was assembled precisely from those victims for whom conventional primary identification had already failed, although the eligibility and exclusion criteria mean that these proportions are conditional on a specimen that retained recoverable pulp, an intact seal against the oral environment and a traceable maternal relative. In such circumstances DNA-based identification becomes a kinship inference problem whose tractability depends on obtaining interpretable sequence from the decedent,⁴ and the present data indicate that the dentition supplies that sequence reliably even when the rest of the body cannot.

The molecular basis of this resilience lies in the physical architecture of the tooth. Enamel is approximately 96% mineral by weight and conducts heat poorly, while peritubular dentine sclerosis progressively seals the tubular network against fluid ingress; together these create a compartment that is thermally insulated and hydraulically isolated. Controlled heating experiments demonstrate that measurable DNA loss in teeth begins above roughly 200 °C,¹⁷ yet in the present series pulp from remains exposed to 300–600 °C still amplified in 88.9% of cases, which is consistent with a steep internal thermal gradient across the enamel and dentine shell. Predictive modelling of incinerated teeth has likewise shown that mitochondrial targets remain recoverable across exposures that abolish nuclear typing,¹⁸ and synthesis of the thermal literature identifies the enclosed chamber as the compartment that retains typable template longest.²² No nuclear comparator was analysed in the present series, so the contrast with nuclear DNA rests on the cited literature rather than on paired measurements reported here.

Anatomical class was the dominant modifiable determinant of success. Molars yielded three times the pulp mass of incisors and a higher mitochondrial copy number, and converted that advantage into a higher rate of complete resolution (93.8% versus 73.3%, $p = 0.037$), with molar class showing the largest estimate in the multivariable analysis. Two mechanisms plausibly contribute. Multi-rooted molars possess larger coronal chambers and multiple divergent canals, providing redundant reservoirs of odontoblastic and stromal remnants; and they are anatomically sheltered within deep alveolar bone and overlying musculature, whereas anterior teeth are directly exposed to radiant heat and blast overpressure. The finding that dental compartment selection materially alters recovery from compromised teeth is consistent with systematic comparison of nuclear preservation across dental tissues,²¹ and with direct comparison of pulp and cementum as alternative targets.²⁰ These findings support prioritising permanent molars when suitable specimens are available.

Amplicon design was the second decisive methodological factor. Postmortem hydrolytic and oxidative processes reduce template to fragments that fall below the length required by conventional nuclear STR multiplexes, so that recovery becomes a function of target length rather than of input mass. Systematic evaluation of control-region amplification confirms that dropout in degraded

specimens is driven principally by amplicon length and primer-binding-site variation, and strategies developed specifically for degraded forensic material show that reduced-size and overlapping sub-amplicon designs recover sequence that full-length designs cannot. The 342 bp and 268 bp targets used here, with sub-180 bp substitution for the most severely charred specimens, recovered complete sequence from 90.0% of victims. Robust performance on highly degraded skeletal material using comparable single-multiplex control-region approaches supports the generalisability of this design.²²

The maternal mode of mitochondrial transmission confers an operational advantage that is particularly valuable in disasters affecting whole households. Where entire nuclear families perish together, first-degree reference donors may be unavailable, and autosomal kinship inference weakens rapidly with genealogical distance. Because the mitochondrial genome passes essentially unchanged down the maternal line, any maternal relative is an informative donor; multigenerational pedigree analysis confirms haplotype stability across two to four generations.²⁸ In this series complete concordance was achieved in all 90 resolved cases, including those in which the only traceable relative was a maternal cousin or aunt. The same property limits individualising power, because every member of a lineage shares the haplotype, and adventitious sharing between unrelated individuals in a population dominated by a few clades is foreseeable; mitochondrial agreement therefore supports a shared maternal lineage and must be reconciled with anthropological, odontological or contextual evidence before an identification is declared. The corresponding statistical framework for kinship-based identification of human remains is well established, and microhaplotype panels remain available for cases that mitochondrial comparison alone cannot resolve.

Evidentiary weight in this cohort was governed by lineage frequency rather than by sequence quality. Haplogroups B4a1a, E1a1a and M7c3c together accounted for 68 of 90 identified victims (75.6%), a distribution consistent with the Austronesian-associated maternal landscape of the archipelago described in regional dispersal studies³⁰ and in comparative surveys of neighbouring island populations.^{31–33} Because mitochondrial haplotypes are shared among maternal relatives, likelihood ratios cannot approach the random match probabilities of large autosomal multiplexes. The inverse relationship between lineage frequency and likelihood ratio observed in Table 6 underlines why population-appropriate reference frequencies are indispensable, since applying a global database to a Philippine cohort would systematically misstate the strength of evidence.²⁹ Attention to likelihood ratio behaviour under incomplete sequence information remains warranted where only partial profiles are available.¹⁹

Heteroplasmy proved to be an asset rather than an obstacle. Point heteroplasmy in 8.9% of sequenced victims falls within the range reported in forensic population surveys,¹⁶ and in every instance the variant state was shared with the maternal relative at comparable peak ratios. Under prevailing interpretive practice a shared heteroplasmic position strengthens rather than undermines an identification, provided the analyst does not treat the ambiguity as a mismatch; current appraisal of mitochondrial reporting guidelines emphasises exactly this point.⁴ Dual-analyst review with explicit calling thresholds, informed by automated heteroplasmy-detection logic, prevented any erroneous exclusion in this

series. The high prevalence of length heteroplasmy in the HV2 cytosine tract, affecting 53.3% of individuals, similarly required structured handling rather than exclusion of the affected region.

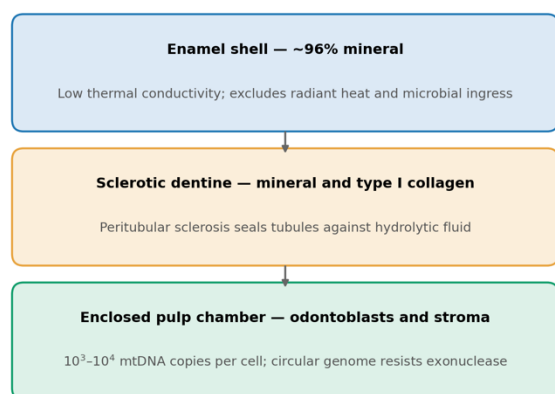
Figure 7 consolidates these findings into an integrated molecular odontology architecture for archipelagic DVI. The schematic sets the physical protection afforded by the enamel and dentine shell alongside the analytical cascade that exploits it, running from odontological triage and molar-priority sampling through decontamination and

organic micro-extraction to reduced-size amplification, bidirectional sequencing and population-calibrated likelihood ratio reporting. Panel (b) is conceptual: the thermal decay curves are illustrative of the published contrast between nuclear and mitochondrial targets and were not measured in this study. The architecture is deliberately specified around capillary electrophoresis rather than massively parallel sequencing, because the former is already installed in most mid-resource forensic laboratories in the region and requires no additional capital investment to deploy during a surge.

(a) Protective architecture and analytical cascade

External taphonomic load

Submersion 42% · Thermal 34% · Crushing 24%



Analytical cascade

Molar-priority triage → hypochlorite decontamination → organic micro-extraction
→ reduced-size HV1/HV2 amplification → bidirectional sequencing
→ EMPOP-calibrated likelihood ratio

(b) Thermal resilience of the two genomes (schematic)

Conceptual curves; not measured in this study

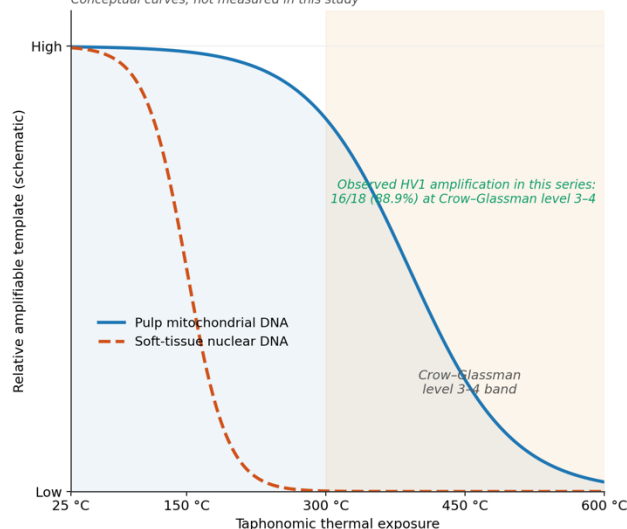


Figure 7. Integrated molecular odontology architecture. (a) The layered mineral barrier that isolates the pulp chamber from external taphonomic load, and the analytical cascade applied to it. (b) Conceptual schematic contrasting the thermal decay of soft-tissue nuclear DNA and of pulp mitochondrial DNA. The curves are illustrative of the published contrast between the two targets and were not measured in this study; the observed casework amplification rate is shown for orientation only and is not a measured fraction of retained template.

Several operational implications follow. The first concerns mortuary triage. Sampling in disaster deployments is frequently performed by general recovery personnel without odontological calibration, which produces crown fracture, root avulsion and preferential harvesting of accessible anterior teeth that carry the least pulp. Structured calibration measurably improves charting and specimen selection,¹² and the present data quantify what is lost when it is absent. Establishing dedicated odontological triage units equipped with low-speed saws, luxators and portable radiography, and embedding pulp sampling within the standard Interpol postmortem form, would protect both specimen quality and chain of custody. This is especially important where antemortem dental records are scarce, since the tooth then functions solely as a molecular specimen rather than as a comparative one.¹¹

The second implication concerns aquatic recovery. That 95.2% of submerged teeth amplified after a mean of 9.4 days, and that individual specimens remained typable at 18 days, qualifies the working assumption that flood recovery is futile after the first week. Comparative analysis of skeletal material from marine environments does document real degradation attributable to immersion,⁸ and submersion measurably alters the decomposition trajectory,⁷ but the intact periodontal attachment and apical seal evidently restrict microbial access to the coronal chamber over this interval. Experimental study of submerged teeth confirms that pulpal change is progressive and time-dependent rather than immediate,²³ which is consistent with the kinetics the present

amplification data imply. Recovery teams in flood-inundated districts should therefore continue to collect dental specimens well beyond the period in which soft-tissue identification has ceased to be viable.

The third implication concerns analytical platform choice. Capillary sequencing is accessible and cost-effective but cannot resolve low-level heteroplasmy below roughly 10 to 15%, nor deconvolute mixtures arising from commingling. Massively parallel sequencing addresses both limitations and extends analysis to the whole mitochondrial genome, with validated forensic workflows now available,²⁶ demonstrated efficiency gains from long-fragment amplification strategies, and internally validated control-region implementations in routine use. Tropical-setting work has further reported that circulating free DNA from decomposed cadavers can support such workflows,³⁰ a template class whose broader forensic feasibility is now established.³¹ Migration to these platforms is the logical trajectory once capital and bioinformatic capacity permit; the present protocol is offered as a defensible interim standard, not as an endpoint.

Contamination control deserves explicit emphasis in any low-template protocol. Demonstration that laboratory consumables themselves can introduce exogenous human DNA establishes that reagent and plasticware screening is a substantive analytical control rather than a formality, and the discipline developed in ancient DNA practice provides the appropriate procedural template.¹⁶ Extraction and amplification blanks at defined ratios, physical separation of pre- and post-amplification workspaces, and dual-

analyst review together constitute the minimum defensible configuration for casework whose conclusions will be tested in court. Laboratory accreditation status, participation in external proficiency testing and the documented chain of custody from recovery site to sequencer are equally material to medicolegal scrutiny and should accompany any operational deployment of this protocol.

4.1. Limitations

This study has limitations that bound its conclusions. It was conducted at a single institution within one metropolitan region, so the haplogroup frequencies and the resulting likelihood ratios are specific to the Philippine population and cannot be transferred to other archipelagic settings without local reference data. The reported recovery proportions are conditional on eligibility criteria that excluded remains with pulpal obliteration, carious teeth communicating with the oral cavity and decedents without a traceable maternal relative, each of which is correlated with the outcome; the yield against the full recovered denominator is therefore lower than the conditional figures reported here. Analysis was oriented to permanent dentition, and paediatric victims with mixed or primary dentition, who constitute a substantial share of disaster mortality, were not characterised separately. Age-related pulp chamber obliteration was not analysed as a determinant of recovery. No nuclear comparator was tested in parallel. Taphonomic exposure was classified by the single dominant insult recorded at recovery, and postmortem intervals were estimated from incident timelines rather than measured directly. The multivariable model carries approximately two events per variable and its adjusted estimates are exploratory. Finally, the 10 unresolved victims were referred for non-molecular assessment, so the series cannot speak to the performance of alternative molecular targets in precisely those specimens where pulp mitochondrial analysis failed; petrous bone and cementum comparisons would be informative in that subgroup.

Future work should extend this framework in three directions: prospective paediatric cohorts to establish age-specific degradation models; direct comparison of pulp, cementum and petrous bone within the same decedent to define an evidence-based sampling hierarchy for archipelagic DVI; and field evaluation of rapid, deployable amplification at mortuary stations to compress the interval between recovery and identification. Each addresses a constraint that the present data identify rather than resolve.

5. Conclusion

This investigation provides evidence of the postmortem recoverability, amplification robustness and maternal concordance of dental pulp mitochondrial DNA in mass disaster victim identification across Metropolitan Manila. Where friction ridge skin has been destroyed and antemortem dental records are unavailable, the tooth functions as a protected biological compartment that retains amplifiable mitochondrial template through submersion, incineration and structural crushing. Multi-rooted posterior molars delivered greater pulp mass, total DNA yield and mitochondrial copy number than anterior teeth, and a higher rate of complete sequencing resolution, which supports prioritising permanent molars in mass fatality sampling protocols.

Bidirectional capillary sequencing of reduced-size amplicons resolved 90.0% of severely degraded victims

with complete maternal concordance across the prevailing Austronesian and Southeast Asian haplogroups. Molar class, intact coronal chamber integrity and a postmortem interval within 14 days were associated with complete profile recovery in exploratory modelling. Because the mitochondrial control region is a lineage marker, these results support its use as a supportive modality within a reconciled multidisciplinary identification rather than as a stand-alone primary identifier. Embedding a standardised molecular odontology protocol within national and international DVI frameworks would allow even the most severely compromised disaster victims to be identified and returned to their families.

Declarations

Use of generative artificial intelligence. None. The author declares that no generative artificial intelligence tool was used in the conception, research, analysis, drafting, revision or preparation of this manuscript.

Author contributions. MV: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review and editing, Project administration. The author has read and approved the final version of the manuscript and agrees to be accountable for all aspects of the work.

Funding. None. This work received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest. The author declares no competing financial, personal or professional interests in relation to this work.

Ethical approval. The protocol and all postmortem forensic procedures were approved by the Institutional Ethics and Forensic Review Board of RBH Dental School (clearance RBH-ERC-2024-079-DVI), together with the collaborating municipal health authorities of Manila, in accordance with Interpol disaster victim identification standards and the Declaration of Helsinki. Written informed consent was obtained from every participating living relative.

Consent for publication. Not applicable. All individual forensic genetic data were de-identified and are reported under alphanumeric accession codes.

Data availability. The anonymised mitochondrial consensus sequences, electropherogram trace files and haplogroup assignment matrices are available from the corresponding author on reasonable academic request.

Acknowledgements. The author thanks the forensic odontologists, mortuary technicians and municipal disaster response personnel of Metropolitan Manila whose work made this identification programme possible.

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