



## Short communication

## Titanic's unknown child: The critical role of the mitochondrial DNA coding region in a re-identification effort<sup>☆</sup>

Rebecca S. Just<sup>a,1</sup>, Odile M. Loreille<sup>a,1</sup>, J. Eldon Molto<sup>b</sup>, D. Andrew Merriwether<sup>c</sup>, Scott R. Woodward<sup>d,2</sup>, Carney Matheson<sup>e</sup>, Jennifer Creed<sup>f</sup>, Stacey E. McGrath<sup>c</sup>, Kimberly Sturk-Andreaggi<sup>a</sup>, Michael D. Coble<sup>a</sup>, Jodi A. Irwin<sup>a</sup>, Alan Ruffman<sup>g</sup>, Ryan L. Parr<sup>f,\*</sup>

<sup>a</sup> Armed Forces DNA Identification Laboratory, Armed Forces Institute of Pathology, 1413 Research Blvd., Rockville, MD 20850, USA

<sup>b</sup> University of Western Ontario, Department of Anthropology, London, Ontario, N6A 5C2 Canada

<sup>c</sup> Binghamton University, Department of Anthropology, P.O. Box 6000, Binghamton, NY 13902, USA

<sup>d</sup> Brigham Young University, Provo, UT 84602, USA

<sup>e</sup> Lakehead University, Department of Anthropology, 955 Oliver Road, Thunder Bay, Ontario, P7B 5E1 Canada

<sup>f</sup> Genesis Genomics Inc., 290 Munro Street, Ste. 1000, Thunder Bay, Ontario, P7A 7T1 Canada

<sup>g</sup> Geomarine Associates Ltd., P.O. Box 41, Stn. M, Halifax, Nova Scotia, B3J 2L4 Canada

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## ABSTRACT

This report describes a re-examination of the remains of a young male child recovered in the Northwest Atlantic following the loss of the Royal Mail Ship *Titanic* in 1912 and buried as an unknown in Halifax, Nova Scotia shortly thereafter. Following exhumation of the grave in 2001, mitochondrial DNA (mtDNA) hypervariable region 1 sequencing and odontological examination of the extremely limited skeletal remains resulted in the identification of the child as Eino Viljami Panula, a 13-month-old Finnish boy. This paper details recent and more extensive mitochondrial genome analyses that indicate the remains are instead most likely those of an English child, Sidney Leslie Goodwin. The case demonstrates the benefit of targeted mtDNA coding region typing in difficult forensic cases, and highlights the need for entire mtDNA sequence databases appropriate for forensic use.

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### 1. Introduction

One of history's greatest non-wartime maritime disasters was the sinking of the RMS *Titanic* on April 15, 1912. The accident resulted in the death of 1497 people; only 712 of the 2209 individuals aboard the *Titanic* survived [1]. Within a few days of the disaster the White Star

Line dispatched a ship on a body-recovery mission [2] and on Sunday April 21, 1912 the crew of the *Mackay-Bennett* recovered the body of "a child of 2 or 3 years, a boy" (body No. 4) [2–4]. When the young boy went unidentified and unclaimed, the crew of the *Mackay-Bennett* arranged a funeral service and had a headstone dedicated "to the memory of an unknown child" placed on his grave in the Fairview Lawn Cemetery in Halifax, Nova Scotia [4].

The remains of the "Unknown Child" were long believed by some to be those of Gösta Leonard Pålsson, a 2-year-old Swedish boy. The association was made on the basis of several pieces of information: the age of the child as estimated by the crew of the *Mackay-Bennett*; a hand-written notation reading "Paulson child?" in the description of the child's body; eye witness accounts of the Pålsson child being washed overboard before the *Titanic* sank; and the recovery of the body of Alma Pålsson, Gösta's mother, with the tickets of all four of her children still in her pocket [2,4]. By request of the Pålsson family, and in coordination with the *Titanic* Ancient DNA Project, the remains of the unknown child were exhumed in May 2001 to investigate the child's identity [4].

<sup>☆</sup> Certain commercial equipment, instruments, and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the United States Department of Defense or the United States Department of the Army, nor does it imply that any of the materials or equipment identified are necessarily the best available for the purpose. The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

\* Corresponding author. Tel.: +1 807 768 4501; fax: +1 807 346 8105.

E-mail address: [Ryan.Parr@genesigenomics.com](mailto:Ryan.Parr@genesigenomics.com) (R.L. Parr).

<sup>1</sup> These authors contributed equally to this work.

<sup>2</sup> Current address: Sorenson Molecular Genealogy Foundation, 2480 South Main Street, Suite 200, Salt Lake City, UT 84115, USA.

The exhumation recovered only four small skeletal elements: a 6 cm shard of “poorly preserved” bone, and three teeth [5]. Presumed to be too degraded for nuclear DNA (nDNA) analysis, a mitochondrial DNA (mtDNA) hypervariable region 1 (HV1; base positions 16,024–16,365, numbered according to the revised Cambridge Reference Sequence (rCRS) [6,7]) profile was generated for the bone fragment and the only dental remains containing dentin. The concordant profile from the skeletal remains was compared to HV1 sequences from maternal relatives of the Pålsson child and did not match. Subsequently, maternal references were obtained for additional male children under the age of 3 who perished aboard the *Titanic*. The haplotype of the skeletal remains was consistent with the HV1 sequence of the references for two of these children: Eino Viljami Panula and Sidney Leslie Goodwin. In addition to the mtDNA analysis, an examination of the developmental features of the teeth recovered from the unknown child's grave estimated the age of the boy at 9–15 months. On the basis of the HV1 sequence data, the odontological evidence, and the ages of Eino Panula and Sidney Goodwin at the time of death (13 and 19 months, respectively), the child was identified as the Panula boy [5]. Despite publication of that conclusion in 2004, however, uncertainty about the identity of the unknown child lingered due to the HV1 match to the Goodwin references, an examination of *Titanic* artifacts at the Maritime Museum of the Atlantic (Halifax, Nova Scotia) which suggested that the shoes of the unknown child would have been too large for 13-month-old Eino Panula, and the identification decision having been based on an imprecise age determination.

This report details the genetic analyses performed on the remains following the earlier identification of the unknown child as Eino Panula, and includes mtDNA data from the maternal references of all six male children under the age of 3 years who were lost when the *Titanic* sank. Although minor contamination was evident in the dental remains, sequence data from additional portions of the mtDNA control region (CR) and coding region revealed two sequence differences in comparison to the Panula reference, and established that the rare mtDNA haplotype recovered from the remains was instead consistent with the Goodwin references. These new data therefore indicate that the unknown child is most likely Sidney Leslie Goodwin.

## 2. Materials and methods

An overview of the materials and methods is included in this section; experimental procedures are detailed more completely in the [Supplementary Material](#).

A 6 cm fragment of poorly preserved ulna and three primary teeth: #55 (a maxillary right second primary molar), #73 (a mandibular left primary cuspid) and #84 (a mandibular right first primary molar) were used for analyses. Well-preserved dentin was only present in the pulp chamber of the first primary molar #84 [5]. DNA was extracted from either one or both sources of nucleic acids (bone and/or dentin) at multiple laboratories (Genesis Genomics, Binghamton University, Brigham Young University and the Paleo-DNA Laboratory at Lakehead University) between 2001 and 2004. DNA was extracted exclusively from dentin at the Armed Forces DNA Identification Laboratory (AFDIL) in 2007, as no bone material remained for re-extraction. Blood or buccal samples previously collected from maternal relatives of the six candidate children were extracted at both Genesis Genomics and at the AFDIL.

MtDNA CR amplification of the skeletal remains targeted HV1 and HV2 fragments ranging from 126 to 271 base pairs (bp) in length. The HV1 sequences obtained from the skeletal material prior to the initial identification publication in 2004 [5] were evaluated by re-testing in 2007–2008, and the HV2 data were generated in the years following the 2004 publication.

Amplification and sequencing of the reference samples targeted the entire CR.

Y-chromosome short tandem repeat (Y-STR) typing was performed for the dentin extract using the AmpF $\ell$ STR $^{\text{®}}$  Yfiler $^{\text{TM}}$  PCR Amplification Kit (Applied Biosystems, Foster City, CA) according to the modified protocol described in Refs. [8–10]. Two amplifications were performed, using 1 and 3  $\mu$ l of extract respectively, due to limited extract volume.

The first mtDNA coding region analyses consisted of SNaPshot $^{\text{®}}$  (Applied Biosystems) typing of 19 coding region single nucleotide polymorphisms (SNPs; multiplexes A and F; [11–13]). To identify additional sequence differences between the Panula and Goodwin references that could be targeted for further typing, the reference extracts were anonymized and sequenced for a portion of the mtDNA coding region (approximately 3500 bp, between positions 8164 and 11,600). Reference sample and dentin extracts were subsequently SNP-typed for nucleotide position 9923 using a 109 bp amplicon and the amplification products were sequenced to confirm the SNP typing results.

Likelihood ratios (LRs) were generated using haplotype frequencies, which were then included in the calculation of a posterior probability according to Bayes theorem as applied to alternate hypotheses [14]:

$$\frac{Pr(H_0)}{Pr(H_a)} \times \frac{Pr(E|H_0)}{Pr(E|H_a)} = \frac{Pr(H_0|E)}{Pr(H_a|E)}$$

where  $H_0$  represents the maternal relation hypothesis and  $H_a$  represents the unrelated hypothesis. Calculations assumed 16.67% as the prior probability (on the basis of only six male children under the age of 3 years having been lost in the *Titanic* disaster).

## 3. Results and discussion

Entire CR sequences were obtained from maternal references for all six male children (Table 1). Where more than one maternal reference was tested for a single child, the data obtained from the maternal relatives matched. All of the references except for the Goodwin and Panula families were excluded on the basis of HV1 data, and the later HV2 sequencing confirmed these exclusions (Table 1). The Goodwin references differed from the rCRS at nucleotide positions 263 (G) and 315 (C insertion); the Panula references differed from the rCRS at the same positions and additionally at position 146 (excluding HV2 polycytosine length variants).

Sequence data obtained from the unknown child bone extract amplifications covered nucleotide positions 16,223–16,390 in HV1 and 35–267 in HV2. Sequence data obtained from the dentin extracts covered positions 16,009–16,390 in HV1 and 35–273 in HV2 (Table 1). In both cases, the sequence data from the unknown child matched the Goodwin child's references. The Y-STR typing did not produce any reliable results (data not shown); this was likely due to both a high degree of DNA degradation and the extremely limited quantity of dental material available (which severely restricted the number of amplifications and the input extract volume).

Despite independent extraction in multiple laboratories accustomed to handling highly degraded human remains, some of the unknown child dentin extracts exhibited evidence of contamination. The HV1 data was mixed at nucleotide positions 16,126, 16,294, 16,296, and 16,304 (Fig. S1 and Table S1, [Supplementary Material](#)); no mixtures were observed in the HV2 data. All but one of the HV1 amplicons in which a mixture was observed resulted in similar minor component signals (estimated at 5–15%) with peak height ratios reproduced in duplicate amplifications. These data provide some support for linkage of the major and (separately) the

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