**Supplemental Material 1**

**Materials and methods: Analyzed individual**

Paiashauaia I is a primary mortuary context located in a rocky outcrop next to the mouth of the Paraná River, a few meters away from the Beagle Channel coast (**Figure 1**). The finding was the result of a prospect of human remains with a search directed at rock cracks and cavities (Piana et al., 2006). The excavation took place in January 2003 as part of the Beagle Channel Archaeological Project. The human bone remains were recovered below a layer of rocks and fine sediment, which in turn was covered by a thin shell matrix level with archaeofauna and lithic remains (Piana et al., 2006). Currently the remains of this individual are being preserved in the repository of the Museo del Fin del Mundo, Tierra del Fuego, Argentina. Permits for sample transportation and analysis were obtained from Secretaría de Cultura in 2016 (Dirección Provincial de Museos y Patrimonio Cultural de la Provincia de Tierra del Fuego, Antártida e Islas del Atlántico Sur). In addition, the sample was treated with respect and scientific rigor as established by the code of ethics for the study of human remains of the Association of Biological Anthropology of Argentina (AABA 2007 and Aranda et al. 2014).

Bioarchaeological analysis determined that the individual was a female whose age was estimated between 35 and 45 years (Suby et al., 2011). The skeleton was well preserved and anatomically articulated in an extended position with the hands superposed on the pelvis and the lower extremities together from the knees to the feet (Piana et al., 2006). The paleopathological analysis of the remains suggests the development of a systemic disease, probably related to metabolic deficiencies; hyperparathyroidism could explain the observed lesions (Suby et al., 2011). An AMS radiocarbon dating of 1504 ± 46 years BP was obtained (Suby et al., 2011). Finally, based on the analysis of stable isotopes (δ13Ccol, δ15N y δ13Capa) and Bayesian dietary models, it was inferred that the individual had a diet with a predominance of marine resources (>65%), supplemented with guanacos and plants (ca. 30%; Kochi, 2017). This paleodietary reconstruction was used to calibrate radiocarbon dating, yielding a result of AD 681- 890 (2 σ; Calib 7.0.4; Hogg et al., 2013).

An upper left incisor was taken from the human remains recovered from the burial (laboratory code: PZ3). The hypervariable region 1 and 2 of this individual's mitochondrial DNA was successfully recovered, amplified, and sequenced by Sanger in (Crespo et al., 2020). According to this study, PZ3's mitochondrial lineage was a distinctive D1g exclusively found in the Beagle Channel area.

Argentinian legislation on the analysis of human remains and particular situation for the human remains used in this work

Currently, scientific projects in Argentina that aim to analyze human remains coming from native communities are regulated by National Law n° 25.517. This law allows the communities to claim the human remains that are in museums and/or collections, while those that are not claimed can remain at the disposal of the different institutions that house them. Meanwhile, the regulatory decree 710/2010 creates the INAI (Instituto Nacional de Asuntos Indígenas) and establishes that this institution is responsible for coordinating, articulating and assisting in the monitoring and study of compliance with the directives and actions of the aforementioned law

Regarding human remains in possession of different governmental or private organizations, the INAI together with the INAPL (Instituto Nacional de Antropología y Pensamiento Latinoamericano) are in charge of carrying out the necessary surveys in order to identify such remains as native and propose their effective restitution to their community of origin, besides mediating in case of possible conflicts of interest and verifying the different scientific projects involving native communities.

The Argentine native communities that wish to make the pertinent claims and possible restitutions of human remains found in different scientific institutions have the requirement to have their legal status recognized by the state through INAI according to the National Law n° 23.302 and its regulatory decree 155/89.

The individual analyzed in the present paper was obtained in an archaeological campaign in 2003 in Estancia Remolino (Beagle Channel, Tierra del Fuego, Argentina) and ancient DNA analysis was started in 2016. At that time there was no Native community in the Beagle Channel recognized by INAI or the Argentinian National State. In the year 2021, the Yaghan Paiakoala Community was accepted as a legal entity and formally recognized by the National State, so it is not possible to present an express consent of a community to perform the analysis.

However, in the year 2021, two of the members of this manuscript (Dr. Cristian Marcelo Crespo and Dr. Francisco Zangrando) had several approaches that culminated in a meeting with 3 community representatives, where the objectives of the project, the scope and limitations of the molecular markers and studies carried out and the molecular information available in the area in terms of current and pre-Hispanic populations were explained to them. The 3 representatives were very pleased and with the intention of continuing with the work carried out, since they expressed that the information provided by the ancient DNA studies is very valuable in terms of the possibility of giving identity to human remains from different archaeological contexts in the Beagle Channel area.

**Laboratory protocol**

Contamination Prevention

To avoid the potential contamination of ancient DNA samples, DNA extraction and the ancient DNA library preparation were carried out in the ancient DNA laboratory clean room facility (Universidad Maimonides, Ciudad de Buenos Aires, Argentina). This laboratory has physically separated rooms for the different stages of the analysis of archaeological samples. The cleaning and powder of the sample was carried out in a special area, while DNA extraction and the library preparation were carried out in another area which is physically isolated. All surfaces and non-disposable working materials used for working with *aDNA* were cleaned with a 10% commercial bleach solution (approximately 0.55 p/v sodium hypochlorite) and/or 70% ethanol. In addition, all rooms and equipment used were irradiated with UV light for 45 minutes prior to use in order to crosslink organic molecules (such as nucleic acids and proteins) and reduce the risk of contamination. All procedures were carried out using appropriate clothing previously irradiated with UV light: dust coat to cover the body, cap to cover the hair, mask to cover the nose and mouth, and double pair of disposable gloves.

The subsequent steps, the library amplification, bait preparation and capture, were carried out at the Instituto Nacional de Tecnología Agropecuaria (INTA, Argentina) laboratories. The laboratory has equipment for exclusive use, with appropriate facilities for the construction of libraries, including DNA-free hood for PCR mixtures. All surfaces and non-disposable working materials used for working with *aDNA* were cleaned with a 10% commercial bleach solution (approximately 0.55 p/v sodium hypochlorite). All procedures were carried out using appropriate clothing: dust cover, chinstrap and disposable gloves.

Sample preparation and DNA extraction

In order to remove possible sample surface contamination, we followed a strict decontamination protocol according to previous recommendations (Barta et al., 2013; Dissing et al., 2008; Kemp and Smith, 2005; Malmström et al., 2007). The tooth was washed with 6% sodium hypochlorite in a sterile Falcon tube for 15 minutes. Sample was then rinsed three times with molecular biology quality water and irradiated with UV light for a minimum time of 45' on each side.

The sample was powdered by mechanical grinding as previously reported by (Carnese et al., 2010). About 70-80mg of powder were decalcified and the proteins digested with Proteinase K (Crespo et al., 2020). An organic extraction with phenol:chloroform:isoamyl alcohol (25:24:1) was then performed on the digested solution following recommendations of (Hummel, 2003) and (Carnese et al., 2010). Supernatant was transferred to a new tube and DNA was purified with AccuPrep® PCR Purification kit (Bioneer) and concentrated using AMICON® Ultra Centrifugal Filters30K (Millipore), following manufacturer's instructions.

Library preparation

The ancient DNA library was constructed in the ancient DNA laboratory clean room facility (Universidad Maimonides) using the NEBNEX Ultra II DNA Library Prep Kit for Illumina (#E7645S) according to manufacturer’s instructions. DNA fragmentation was not performed.

Ancient DNA was end repaired and dAtailed using the Ultra II end repair module (NEB). NEBNext adaptors for Illumina were ligated using the NEBNext Ultra II Ligation Master Mix (**Table S1**). The sample was treated with USER enzyme (NEB) and the adaptor-ligated DNA was purified with AMPureXP Beads (Beckman Coulter) without size selection. The adaptor-ligated DNA was enriched by 15 cycles of PCR using the NEBNext Ultra II Q5 Master Mix and NEBNext primers for Illumina, followed by a cleanup with AMPureXP Beads.

Library concentration was measured using Qubit 2.0 Fluorometer in combination with Qubit dsDNA HS Assay Kit (both Invitrogen), and were analysed on Fragment Analyzer using High Sensitivity NGS Fragment Analysis Kit (both Agilent Technologies).

Bait preparation

DNA was extracted from the blood of an H haplogroup donor and the complete mitochondrial DNA was amplified in two separate PCR reactions with two primer pairs (L13232/H5230 and L5120/H13393, **Table S1**) using Long Amp DNA Polymerase (NEB #M0323) according to the manufacturer's protocol. PCR products were purified from gel (QIAquick, gel extraction kit, QIAGEN) and DNA concentration was measured using Qubit 2.0 Fluorometer in combination with Qubit dsDNA HS Assay Kit.

In order to obtain fragments around 500 bp size, the PCR products were sheared using the Bioruptor sonication system (Diagenode). The sample was diluted to recommended DNA concentration following the manufacturer’s instructions and sonicated by 12 cycles of 15 seconds sonicated / 90 seconds rest at low intensity. The DNA fragmentation was checked by agarose gel electrophoresis.

The fragmented DNA was pooled at equimolar concentration, concentrated by vacuum, and purified with AMPure XP Beads. The DNA concentration was measured using Qubit 2.0 Fluorometer in combination with Qubit dsDNA HS Assay Kit.

The fragmented DNA was denatured and 3’ labelled with Biotin-dUTP (SIGMA) and the enzyme Terminal Transferase (TdT, NEB; Templeton et al., 2013). Reactions were performed at 50uL final volumes according to the manufacturer’s instructions: 10pmol of 3’ ssDNA ends of sonicated mitochondrial probe DNA, 0.1mM Biotin-16-dUTP (SIGMA), and 40U Terminal Transferase enzyme (New England Biolabs). The reaction was performed at 37°C for 60min followed by 70°C for 10 min. The labelled probe was purified with AMPure XP beads and the concentration was measured using Qubit 2.0 Fluorometer in combination with Qubit dsDNA HS Assay Kit.

The purified 3’ biotin labelled probes were incubated in solution with Streptavidin-coupled Dynabeads® Magnetic Beads M-280 (Invitrogen; Maricic et al., 2010): 500 ng of denatured 3’ labelled probe was combined with 5 ul of M-280 magnetic beads, according to the manufacturer’s instructions. The mix was incubated under rotation in BWT buffer (1x) at room temperature for 20 minutes, then was washed 2 times with BWT buffer (5mM Tris-HCl pH8.0; 1M NaCl; 0.5mM EDTA pH 8.0; 0.05% Tween-20) and finally suspended in TET (10mM Tris-HCl pH8.0; 1mM EDTA pH 8.0; 0.05% Tween-20) buffer until the hybridization mix was prepared.

Mitochondrial DNA capture

Capture was performed in solution by incubating the *aDNA* library with the bait-coated beads.

Two micrograms of the indexed library were denatured in the hybridization mix (2xSSC; 0.5% SDS; 13ng/ul BSA; 2uM blocking oligo mix), added to the washed bait-coated beads and then incubated at 55°C in a hybridization oven (following Maricic et al., 2010 with minor changes).

After 48 hours, the bead complex (Library-probe-beads) was subjected to successively increasing stringency washed using decreased salt and increased temperature: 2x saline sodium citrate (SSC)/0.1% sodium dodecyl sulphate (SDS) at 37°C for 1 min; 2x SSC/0.1% SDS at 42°C for 10 min; 1x SSC/0.1% SDS at 42°C for 10 min and 0.5x SSC/0.1% SDS at 42°C for 10 min.

The captured library was eluted by heating at 80°C for 15 min, purified with AMPure XP beads and DNA concentration was measured using Qubit 2.0 Fluorometer in combination with Qubit dsDNA HS Assay Kit.

Sequencing

The captured library was diluted and sequenced by synthesis with MiSeq Reagent Kit v2 (2x150). Sequencing services using MiSeq (Illumina) were performed at INTA, Consorcio Argentino de Tecnología Genómica (CATG) funded by grant MinCyT PPL 2011 004 Genómica, AECID PCI\_ARG109 A1/041041/11 and INTA.

**Bioinformatic Analysis** (in this section, the options and parameters used are detailed with this typography)

Processing and mapping of sequenced reads and variant calling

The quality of the Illumina reads was evaluated with FastQC (v0.10.1). Reads were trimmed and end-clipped to a PHRED score of 30 using Cutadapt (v1.16; Martin 2011), setting 24 bases for quality cutoff from 5’ and 3’ ends (-q 24,24) and discarding reads shorter than 36 bases (-m 36). The parameters for each option were chosen based on the characteristics of the readings obtained from the sequencing.

The paired-end reads were then merged using AdapterRemoval (v2.3.3; Schubert et al. 2016) when overlaping at least 23 bases (--minalignmentlength 23) and using a conservative merging algorithm (--collapse-conservatively).

The reads were then mapped to the revised Cambridge Reference Sequence (rCRS; Andrews et al. 1999) using Burrows Wheeler Aligner (BWA; v0.7.10; Li and Durbin 2009). BWA-MEM (mem) algorithm was chosen since it is optimized for reads between 70bp to 1Mbp. Uncollapsed reads were mapped as paired-end, and then collapsed reads were mapped as single end.

The outputs were converted to BAM files (view), sorted (sort) and then merged to a single bam file (merge) using Samtools (v1.7; Li and Durbin 2009). In addition, one 3' nucleotide was trimmed in the bam file with bamUtil (v1.0.15; Jun et al. 2015; trimBam -R 1 -c).

Mapping statistics were calculated with Bamtools (v2.5.1; Barnett et al. 2011; stats) and Samtools (depth).

Duplicates were marked and discarded using Picard-tools (v2.18; <http://broadinstitute.github.io/picard/>; MarkDuplicates VALIDATION\_STRINGENCY=LENIENT), and a bam file was generated with mapped reads only using Bamtools (split -mapped). The *aDNA* damage patterns were assessed using MapDamage2 (v2.0; Jónsson et al. 2013).

Variant calling was performed using BCFtools (v1.9; Danecek and McCarthy 2017; Narasimhan et al. 2016; mpileup, and then call -mv --ploidy 1 -Ob). The information yielded by the Sanger sequences and obtained for HVR1 in Crespo et al. 2020 was incorporated into variant calling by manually increasing the variant depth by two units or increasing the base quiality to 30.

Finally, variant filtering was made also using BCFtools with the following parameters: for both reference site and variation site, base quality was set to >=30 with at least two reads covering the variation site (filter -i 'DP>=2 && MQ>=40 && QUAL>=30'). SNPs located less than 10 bp away from indels were removed to avoid false positive SNP.

Construction of the consensus mitogenome

To identify positions with depth <2, awk was used to filter the file generated with Samtools (depth -a). Those positions were treated as missing data ("N"). The latter file together with the final VCF were used to construct a consensus genome of the individual PZ3 using BCFtools (consensus -m). The mitochondrial haplogroup was determined by using HaploGrep2 (v2.2; Weissensteiner et al. 2016).

**References cited**

AABA (2007). Declaración de la Asociación de Antropología Biológica Argentina en relación con la ética del estudio de restos humanos. Available at: [www.fcnym.unlp.edu.ar/aaba](http://www.fcnym.unlp.edu.ar/aaba).

Aranda, C., Barrientos, G., & Del Papa, M. C. (2014). Código deontológico para el estudio, conservación y gestión de restos humanos de poblaciones del pasado. *Revista Argentina de Antropología Biológica, 16*(2), 111-113.

Barta, J. L., Monroe, C., & Kemp, B. M. (2013). Further evaluation of the efficacy of contamination removal from bone surfaces. *Forensic Science International*, *231*(1–3), 340–348.

Carnese, F. R., Mendisco, F., Keyser, C., Dejean, C. B., Dugoujon, J. M., Bravi, C. M., Ludes, B., & Crubézy, E. (2010). Paleogenetical study of pre-Columbian samples from Pampa Grande (Salta, Argentina). *American Journal of Physical Anthropology*, *141*(3), 452–462. https://doi.org/10.1002/ajpa.21165

Crespo, C. M., Cardozo, D. G., Tessone, A., Vázquez, M., Kisielinski, C., Arencibia, V., Tackney, J., Zangrando, A. F., & Dejean, C. B. (2020). Distribution of maternal lineages in hunter-gatherer societies of the southern coast of Tierra del Fuego, Argentina. *American Journal of Physical Anthropology*, *March*, 1–12. https://doi.org/10.1002/ajpa.24107

Dissing, J., Kristinsdottir, M. A., & Friis, C. (2008). On the elimination of extraneous DNA in fossil human teeth with hypochlorite. *Journal of Archaeological Science*, *35*(6), 1445–1452.

Hogg, A. G., Hua, Q., Blackwell, P., Niu, M., Buck, C., Grilderson, T., Heaton, T., Paalmer, J., Reimer, P., Reimer, R., Turney, C., & Zimmermann, S. (2013). SHCal13 Southern Hemisphere calibration, 0-50,000 years cal. BP. *Radiocarbon*, *55*(4), 1889–1903.

Hummel, S. (2003). *Ancient DNA Typing. Methods, strategies and applications* (New York). Springer.

Jun, G., Wing, M. K., Abecasis, G. R., & Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome research*, *25*(6), 918-925.

Kemp, B. M., & Smith, D. G. (2005). Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. *Forensic Science International*, *154*(1), 53–61.

Kochi, S. (2017). Paleodietas en cazadores-recolectores del canal Beagle durante el Holoceno tardío. *Intersecciones En Antropología*, *18*(3), 329–340.

Malmström, H., Svensson, E. M., Gilbert, M. T. P., Willerslev, E., Götherström, A., & Holmlund, G. (2007). More on contamination: the use of asymmetric molecular behavior to identify authentic ancient human DNA. *Molecular Biology and Evolution*, *24*(4), 998–1004.

Maricic, Tomislav, Mark Whitten, and Svante Pääbo (2010). Multiplexed DNA Sequence Capture of Mitochondrial Genomes Using PCR Products. *PLoS ONE* 5 (11): 9–13. <https://doi.org/10.1371/journal.pone.0014004>.

Piana, E., Tessone, A., & Zangrando, F. (2006). Contextos Mortuorios En La Región Del Canal Beagle… Del Hallazgo Fortuito a La Búsqueda Sistemática. *Magallania (Punta Arenas)*, *34*(1), 103–117. https://doi.org/10.4067/s0718-22442006000100007

Schubert, M., Lindgreen, S. & Orlando, L (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Res Notes* **9,** 88. https://doi.org/10.1186/s13104-016-1900-2

Suby, J. A., Zangrando, A. F. J., & Piana, E. (2011). Exploraciones osteológicas de la salud de las poblaciones humanas del canal Beagle. *Relaciones de La Sociedad Argentina de Antropología, XXXVI*, *XXXVI*, 249–270.

Templeton, J. E. L., Brotherton, P. M., Llamas, B., Soubrier, J., Haak, W., Cooper, A., & Austin, J. J. (2013). DNA capture and next-generation sequencing can recover whole mitochondrial genomes from highly degraded samples for human identification. *Investigative Genetics*, *4*(26), 1–13.