Paleoparasitologic, paleogenetic and paleobotanic analysis of XVIII century coprolites from the church La Concepción in Santa Cruz de Tenerife, Canary Islands, Spain

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We present the results of a paleoparasitologic, paleogenetic and paleobotanic analysis of coprolites recovered during the excavation of the church La Concepción in Santa Cruz de Tenerife. Coprolites (n = 4) were rehydrated and a multidisciplinary analysis was conducted. The paleobotanic analysis showed numerous silicates, seeds and fruits of the family Moraceae. In the paleoparasitologic study, Ascaris *sp. eggs (n = 344) were identified. The paleogenetic results confirmed the* Ascaris *sp. infection as well as the European origin of human remains. These findings contribute to our knowledge of ancient helminthes infections and are the first paleoparasitological record of* Ascaris *sp. infection in Spain*.

Key words: paleoparasitology - *Ascaris* sp. - paleobotany - coprolite - paleogenetic - Canary Islands - ancient DNA

La Concepción was the major church of the ancient village of Santa Cruz de Tenerife in the centuries following the Spanish conquest. The church was not only the most important in the region, but also served as the community's cemetery. Following the Catholic liturgy, the dead were buried in the church foundation, a practice which began in the XVI century and persisted until the end of the XVIII century. The location of La Concepción near the seashore led to the relatively poor preservation of skeletal remains, but some archaeological studies have been possible (Gámez-Mendoza 2004, Arnay-de-la-Rosa et al. 2009). During the excavation, both bones and coprolites were recovered, which allowed us to conduct a multidisciplinary analysis.

Samples of coprolites (n = 4) were removed from tomb 146 (Fig. 1A), which contained a young female, whose sex was determined by cranial characteristics and whose age at death was determined by the absence of dental attrition (Ubelaker 1989). Coprolites were rehydrated by immersion in 0.5% aqueous trisodium phosphate for 72 h, as described by Callen and Cameron (1960) (Fig. 1B). Seeds of fruits belonging to the family Moraceae were clearly visible in the coprolite. Microscopic investigation was performed using the methods described by Danielson and Reinhard (1998). This revealed numerous phytoliths derived from the silicated external envelopes of crop grains (silica skeletons) and starch grains corresponding to vegetables in the family Triticeae, which includes wheat and barley, indicating the presence of cereals in the diet. The paleoparasitologic investigation was conducted by microscopic analysis prior to the spontaneous sedimentation (Lutz 1919) of rehydrated coprolite solutions. We examined 20 slides for the presence of parasite eggs at magnifications of 100X and 400X. The results revealed the presence of *Ascaris* sp. eggs (Fig. 1C). The eggs measured 57.92-70.1 µm in length and 47.14-49.25 µm in width (n = 344).

To ensure that authentic original sequences were analyzed by paleogenetic analysis, precautions to prevent contamination by modern DNA were followed as described by Drancourt and Raoult (2005). The preparation of sample, aDNA extraction and polymerase chain reaction (PCR) were performed at the Laboratory of Paleogenetics, Oswaldo Cruz Institute/Oswaldo Cruz Foundation (IOC/FIOCRUZ). Electrophoresis, cloning, sequencing and sequence analysis were conducted at the Laboratory of Molecular Genetics of Microorganisms, IOC/FIOCRUZ. These two laboratories are physically distant from each other. The surface of the coprolites was removed, samples were irradiated with ultraviolet light and the aDNA was extracted as described by Iñiguez et al. (2003, 2006). The sediments of nine coprolites were submitted for molecular investigation. Human and *Ascaris* sp. targets were analyzed by procedures described by Fernandes et al. (2008) and Leles et al. (2008), respectively. For *Ascaris* sp. analysis, the primers of 18S ribosomal DNA (rDNA) gene were used and others were designed based on the NADH dehydrogenase subunit 1 (*nad*1) and cytochrome c oxidase subunit 1 (*cox*1) genes (Table). PCR products were directly sequenced in both directions using a 3100 Automated DNA Sequencer (Ap-

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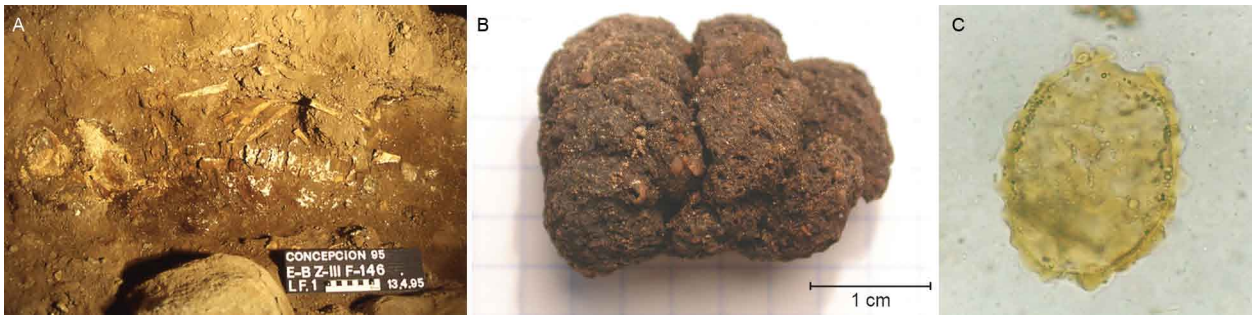

Fig. 1A: skeletal remains of a young female dated from XVIII century buried in La Concepción church, Canary Islands, Spain; B: coprolite sample from the human remains; C: *Ascaris* sp. egg (66.10 µm x 47.96 µm) recovered after rehydration of the coprolite.

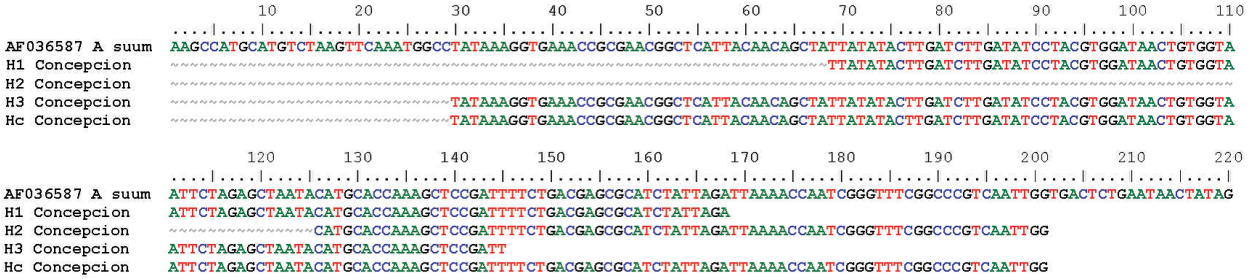

Fig. 2: alignment of *Ascaris* sp. 18S RNA gene segments recovered from human coprolite material. H1-H3 corresponded to *Ascaris* sp. aDNA segments generated from combined primers. Hc corresponds to the consensus aDNA 18S ribosomal DNA sequence. Reference sequence AF036587 belongs to *Ascaris suum*.

TABLE

Primers used to amplify *Ascaris* sp. of ancient DNA samples

Primer	Primer sequence	PCR product size	References
18S rRNA			
Asc6	5'-CGAACGGCTCATTACAAC0AG-3'	123 bp	Loreille et al. (2001)
Asc7	5'-TCTAATAGATGCGCTCGTC-3'		
Asc8	5'-ATACATGCACCAAAGCTCCG-3'	99 bp	Loreille et al. (2001)
Asc9	5'-GCTATAGTTATTCAGAGTCACC-3'		
Asc10	5'-CCATGCATGTCTAAGTTCAA-3'	147 bp	Loreille et al. (2001)
Asc11	5'-CARAAAWTCGGAGCTTTGGT-3'		
cox1			
As-Co1F	5'-TTTTTTGGTCATCCTGAGGTTTAT-3'	199 bp	Peng et al. (2005)
COX1R	5'-GCCCGAGAGTCAAGATCCAT-3'		Designed in this study.
COX1F	5'-GGATCTTGACTCTCGGGCTTA-3'	248 bp	Designed in this study.
As-Co1R	5'-ACATAATGAAAATGACTAACAAC-3'		Peng et al. (2005)
nad1			
NAD1F	5'-CTCCTCTGAATTCTTCGGAAA-3'	152 bp	Designed in this study.
NAD1R	5'-CAGAAAACCCAATCAAACACA-3'		

PCR: polymerase chain reaction.

plied Biosystems, Life Technologies, Foster City, CA, USA). The nucleotide sequences were analyzed with Lasergene© SeqMan v.7.0.0 (DNASTAR, Madison, WI, USA) and Bioedit v.5.0.9 (Department of Microbiology, North Carolina State University, USA) software. The human mtDNA sequence was compared to the mtDNA database from GenBank and from laboratory staff. We successfully recovered a human mtDNA HVS-I region (16209-16356) and the European origin of the sample was confirmed based on the signatures of HVSI se-

quence (16224, 16311) revealed by haplogroup K. *Ascaris* sp. PCR products from *nad*1 and *cox*1 were obtained, but the sequencing analysis revealed unspecific amplicons. However, the 18S rDNA target was useful in yielding aDNA fragments corresponding to the *Ascaris* sp. gene in two different sediments. The assembly of aDNA fragments allowed the authentication of the 18S rDNA amplicons (Fig. 2).

According to Gonçalves et al. (2003), *Ascaris* sp. eggs have been found in 39 European archaeological sites from 11 countries, excluding Spain. In this study we showed for the first time, by paleoparasitologic and paleogenetic analysis, that the occurrence of ascariasis in ancient times includes the Canary Islands, Spain. Despite the identification of human remains and human mtDNA haplotype recovery, we could not identify the species of *Ascaris* sp. parasite as *Ascaris lumbricoides*, the species of human origin, because the 18S rDNA gene is a genetic marker that discriminates only by genus. Nuclear and mitochondrial genes have been proposed to discriminate species of *Ascaris*, but the genetic definition between these closely related forms remains unclear (Anderson 2001, Leles et al. 2010). The first molecular paleoparasitologic study of *Ascaris* sp. reported the recovery of aDNA from a concentrate of parasite eggs from the Middle Age site Place d'Armes in Namur (XIV century) (Loreille et al. 2001). Later, a sample from Walraversijde, Belgium (XVI century) was used as a positive control in paleogenetic analyses and showed an aDNA *cyt b* gene segment that was directly from coprolites (Leles et al. 2008). In this study, we confirm the ability of this methodology to recover aDNA without the prior concentration of parasite eggs (Iñiguez et al. 2003, 2006, Leles et al. 2008). This paleoanalysis, based on an interdisciplinary approach, is the first of its kind to be performed in the Canary Islands.

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