



## Case study

## Pre-Columbian tuberculosis in Tierra del Fuego? Discussion of the paleopathological and molecular evidence



Ricardo A. Guichón<sup>a,\*</sup>, Jane E. Buikstra<sup>b</sup>, Anne C. Stone<sup>b</sup>, Kelly M. Harkins<sup>b</sup>, Jorge A. Suby<sup>a</sup>, Mauricio Massone<sup>c</sup>, Alfredo Prieto Iglesias<sup>d</sup>, Alicia Wilbur<sup>e</sup>, Florence Constantinescu<sup>f</sup>, Conrado Rodríguez Martín<sup>g</sup>

<sup>a</sup> CONICET, Laboratorio de Ecología Evolutiva Humana (FACSO, UNCPBA), Dpto. Biología (FCEyN, UNMDP), Argentina

<sup>b</sup> School of Human Evolution and Social Change, College of Liberal Arts and Sciences, Arizona State University, USA

<sup>c</sup> Dirección de Bibliotecas, Archivos y Museos (DIBAM), Museo de Historia Natural de Concepción, Chile

<sup>d</sup> Centro Universitario Puerto Natales, University of Magallanes, Chile

<sup>e</sup> University of Washington, National Primate Research Center, Seattle, WA, USA

<sup>f</sup> Poch Ambiental S.A, Renato Sánchez 3838, Las Condes, Santiago, Chile

<sup>g</sup> Instituto de Bioantropología, Museo de la Naturaleza y el Hombre, Tenerife, Islas Canarias, Spain

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## ABSTRACT

This work contributes to ongoing discussions about the nature of tuberculosis in the Western Hemisphere prior to the time of European contact. Our example, from the extreme south of South America was, at the time of our study, without firm temporal association or molecular characterization. In Tierra del Fuego, Constantinescu (1999) briefly described vertebral bone lesions compatible with TB in an undated skeleton from Myren 1 site (Chile). The remains of Myren are estimated to represent a man between 18 and 23 years old at the time of death. The objectives of this research are to extend this description, to present molecular results, to establish a radiocarbon date, and to report stable isotopic values for the remains. We provide further description of the remains, including tuberculosis-like skeletal pathology. Radiocarbon dating of  $640 \pm 20$  years BP attributes this individual to the precontact fourteenth-fifteenth centuries. Isotopic ratios for nitrogen and carbon from bone collagen suggest a mixed diet. Molecular results were positive for the rpoB quantitative PCR (qPCR) assays but negative for two independent IS6110 and IS1081 qPCR assays. Further testing using genomic methods to target any mycobacteria for specific identification are needed.

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## 1. Introduction and background

Tuberculosis (TB) is an infectious disease whose origin and co-evolutionary relationships with *Homo sapiens* are subjects of ongoing debate (Comas et al., 2013; Hershberg et al., 2008; Hershkovitz et al., 2015; Gagneux, 2012; Pálfi et al., 2015; Roberts and Buikstra, 2003; Stone et al., 2009a,b; Wirth et al., 2008). Any such discussion of human–pathogen co-evolution must consider TB's global dispersion and the variety of vectors that can transmit it. Addressing this complex scenario requires the collaboration of paleopathologists, epidemiologists, microbiologists, immunologists, bioarchaeologists, zooarchaeologists, and historians. Cold regions, with their potential for preserving bone and/or soft tissue,

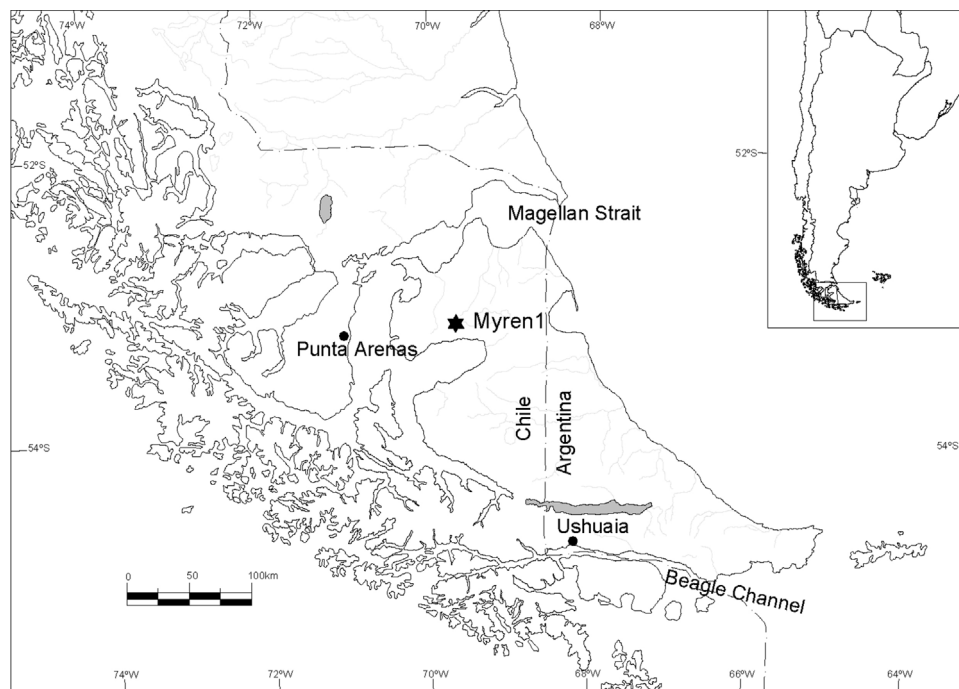
as well as preservation of ancient DNA, are especially auspicious sites for this form of paleopathological study. Our focus here is on the “Island of Tierra del Fuego” where there is preliminary bioarchaeological evidence of TB.

The long-term goal of our research program is to explore the molecular nature of TB in Tierra del Fuego, before and after the arrival of Europeans. Importantly, during the late 19th and during the 20th century, with the development of the first urban centers in southern Patagonia, TB was the leading cause of death in the Rio Grande valley, as recorded for Ushuaia in Argentina and Punta Arenas in Chile (Casali, 2013).

In 1999, Constantinescu briefly described vertebral bone lesions compatible with TB in a skeleton from the Myren 1 site (Fig. 1), excavated by the archaeologist Massone. Although there was no radiocarbon dating of the human remains at that time, Massone attributed this site to a pre-European contact period (Massone et al., 1999). In 1995, as part of a collaborative project between teams

\* Corresponding author.

E-mail address: [guichon2012@gmail.com](mailto:guichon2012@gmail.com) (R.A. Guichón).



**Fig. 1.** Location of Myren 1 in “Isla Grande” of Tierra del Fuego.

from Spain, Argentina, and Chile, the D haplogroup was identified for this individual, which corresponds with haplotypes common for native peoples in this region (Lalueza-Fox, 1995; Lalueza-Fox et al., 1997).

Clinical models describe a low frequency of bone involvement as a result of disseminated TB, usually associated with destructive lesions in the spinal column and appendicular skeleton, and occasionally in the skull (Resnick and Niwayama, 1995). New bone formation on the pleural surfaces of ribs is considered suggestive but not pathognomonic for tTB (Roberts and Buikstra, 2003; Santos and Suby, 2012).

Analyses of ancient DNA have been used to investigate the causative agent of the observed TB bone lesions in South America since the 1990s (Arriaza et al., 1995; Salo et al., 1994). These early studies recovered and amplified ancient DNA from the *Mycobacterium tuberculosis* complex (MTBC), which was inferred to have been most closely related to the TB species that are found today in humans, possibly derived from an Asian strain. However, recent studies from the Osmore river valley of southern Peru have identified TB in ancient South American human remains most closely linked to the form that affects pinnipeds (seals and seal lions), suggesting that these sea mammals served as the vector that transferred the pathogen from the Old World to South America (Bos et al., 2014). Studies of historic period South American human remains thus become crucial for evaluating the nature of TB–human co-evolution in the Western Hemisphere. The remains from Tierra del Fuego, due to their location, skeletal evidence of TB, and antiquity, thus become especially important in this regard. Our study was conducted to clarify the chronological placement of the remains, to provide a detailed description of the remains, and to generate genetic information about the pathogen.

## 2. Archaeological context

By approximately 8000 BP, a land bridge connected Tierra del Fuego and the mainland (McCulloch and Morello, 2009), which was potentially first used by hunter–gatherers who arrived at least 10,000 years ago (Massone, 2003). Beginning in the 16th and

extending into the 18th century AD, contact between indigenous groups and Europeans was almost exclusively coastal. According to documentary sources, European exploration of Tierra del Fuego’s interior began at the end of the nineteenth century (Borrero, 1992; Fugassa and Guichón, 2004).

The Myren 1 site is located approximately 14 km north of Inútil Bay ( $53^{\circ}14'S$ – $69^{\circ}28'W$ ), at the northern aspect of a wide glacial valley that connects this cove to the Bay of San Sebastián, located in the Chilean territory north of Tierra del Fuego. Myren 1 is a large (approximately  $200 \times 150$  m) archaeological site, positioned on the eastern shore of Banty lagoon. In 1998, Massone and Jackson noted four areas with concentrations of cultural material. A few years earlier, farm workers had found a skull located on the eastern side of the lagoon, 38 m from the edge of the water (now called Sector 1). Partially buried human remains also were discovered in a deposit of silt near the lagoon (Sector 1), within a flat area near the bay where the herbaceous cover had been partially eroded (Fig. 2). Massone recovered long bones, phalanges, teeth, and fragments of ribs and vertebrae scattered on the silt surface within a radius of up to 20 m surrounding the buried remains. Both the surface scatter and the buried remains appear to be from the same individual. The skeleton was removed due to its progressive deterioration and dispersal due to a very active erosion front located near the edge of the lagoon. The skeletal remains discovered *in situ* was in a flexed position, with the upper part oriented towards the southeast. No cultural remains associated with the interment were discovered (Massone and Jackson, 1998). The skull, recovered in 1986, thought to be associated with the same individual, had been culturally modified in the tabular erect style (Guichón, 1994; Berón and Luna, 2009).

Sector 2, established to further explore site stratigraphy, was located 80 m east of Sector 1. It is a  $31 \times 19$  m flat, irregularly shaped, raised surface with a noticeable concentration of stone artifacts and remains of a guanaco exposed on the eroded silt surface and in the cuts adjacent to the raised area. In January 1999, a  $1 \times 1$  m survey and a  $2 \times 2$  m excavation next to the survey region were carried out. In this sector the excavations exposed four natural silt strata containing small freshwater snails. Only Stratum III, situated between 45 and 75 cm below the surface, contained cultural mate-

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