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FIRST RECORD OF TUBERCULOSIS LESIONS IN ZOOARCHAEOLOGICAL SAMPLES OF OTARIID PINNIPEDS. NEW ASPECTS OF THE PRE-EUROPEAN ORIGIN OF HUMAN TUBERCULOSIS IN SOUTH AMERICA AND DISSEMINATION MECHANISMS OF *Mycobacterium pinnipedii* IN THE SOUTHERN HEMISPHERE

PRIMER REGISTRO DE LESIONES TUBERCULOSAS EN MUESTRAS ZOOARQUEOLÓGICAS DE PINNÍPEDOS OTÁRIDOS. NUEVOS ASPECTOS DEL ORIGEN PRE-EUROPEO DE LA TUBERCULOSIS HUMANA EN AMÉRICA DEL SUR Y MECANISMOS DE DISEMINACIÓN DEL *Mycobacterium pinnipedii* EN EL HEMISFERIO SUR

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Abstract

In 2003 Mycobacterium pinnipedii was described as responsible for producing tuberculosis (TB) in living otariid pinnipeds from Argentina and Australia. It is the only member of marine origin within the Mycobacterium tuberculosis Complex (MTBC), which also affects other domestic and wild mammals, and humans. Based on several pre-Columbian records of human tuberculosis in South America, in 2010-2011 a new hypothesis about the origin of this zoonosis through otariid pinnipeds arose. In 2014, this hypothesis was confirmed based on the study of ancient DNA from three mummies (700-1,000 years BP) of the Chiribaya culture (Peru). Since there were no records of TB bone lesions in zooarchaeological samples of otariid pinnipeds from South America and the rest of the world, our study aimed at examining zooarchaeological samples of pinnipeds from coastal sites of the Beagle Channel (Tierra del Fuego, Argentina), being the oldest Túnel I (6,400-4,300 years BP). A total of 4,138 vertebrae were analyzed, of which 0.46 % showed lesions compatible with TB. In addition, we propose a new hypothesis on possible mechanisms of Mycobacterium pinnipedii dissemination that would explain the *transmission routes to the different otariid pinniped species of the Southern Hemisphere. Mycobacterium pinnipedii is one of the most aggressive mycobacteria of the MTBC and of high risk for humans.*

Key words: *zooarchaeology; pinniped paleotuberculosis; Tierra del Fuego; Middle-Late Holocene.*

Resumen

En 2003 fue descripto Mycobacterium pinnipedii como responsable de producir tuberculosis (TB) en pinnípedos otáridos de Argentina y Australia. Esta especie es la única de origen marino del Mycobacterium tuberculosis Complex (MBTC), que afecta también a otros mamíferos domésticos, silvestres y humanos. Debido a numerosos registros de tuberculosis humana precolombina en Sudamérica, surge en 2010-2011 una nueva hipótesis sobre el origen de esta zoonosis a través de los pinnípedos otáridos. En 2014 esta hipótesis fue confirmada a través de estudios de ADN antiguo de tres momias (700-1.000 años AP) de la cultura Chiribaya (Perú). En virtud de no existir registros de TB ósea en muestras zooarqueológicas de pinnípedos de Sudamérica y del resto del mundo, nuestro estudio tuvo como finalidad examinar muestras zooarqueológicas de pinnípedos otáridos de sitios costeros del Canal Beagle (Tierra del Fuego, Argentina), siendo el más antiguo Túnel I (6.400-4.300 años AP). Se analizó un total de 4.138 vértebras, de las cuales el 0,46 % mostró lesiones compatibles con TB. Complementariamente, se propone una nueva hipótesis sobre posibles mecanismos de diseminación de Mycobacterium pinnipedii, planteando las posibles rutas de transmisión a las diferentes especies de pinnípedos otáridos del Hemisferio Sur. Mycobacterium pinnipedii es una de las micobacterias más agresivas del MBTC y de mayor riesgo para los humanos.

Palabras clave: *zooarqueología; paleotuberculosis en pinnípedos; Tierra del Fuego; Holoceno Medio-Tardío.*

Introduction

The different species of mycobacteria responsible for TB have been grouped in the so-called *Mycobacterium tuberculosis* Complex (MTBC). The genus *Mycobacterium* presumably originated over 150 million years ago (Daniel 2006), but the evolutionary age of the species responsible for TB in humans and domestic animals are still controversial. It has even been recently suggested that the MTBC would be older than previously believed, and that Neanderthals were possibly infected (Donoghue 2017; Houldcroft & Underdown 2016).

According to researches by several specialists, the age of the Most Recent Common Ancestor (MRCA) of TB was estimated in 50.000 to 150.000 years (Brites & Gagneux 2015; Brites *et al.* 2018; Comas *et al.* 2015; Gagneux 2018; Hershkovitz *et al.* 2015; Masson *et al.* 2015). Since then, the dissemination of TB occurred by clonal expansion among human communities, giving rise to seven lineages. The study of

Comas *et al.* (2013), support that one of the ancient phylogeographic lineages of TB emerged about 67.000 years ago, coinciding with the first stage of modern human out of Africa dispersal.

Until the late nineteenth and early twentieth centuries, TB was exclusively linked to two pathogenic species: *Mycobacterium tuberculosis,* whose primary host is the human species, and *Mycobacterium bovis*, whose primary host is domestic cattle. The species within the MTBC have undergone numerous taxonomic and nomenclatural changes over time. Studies carried out a few years ago defined the MTBC as comprising at least nine species of the genus *Mycobacterium*, of which eight has primary hosts of the terrestrial environment and only one a primary hosts of the marine environment. However, recent phylogenomic studies suggest taxonomic and nomenclatural changes for several MTBC members, modifying their category of species to infrasubspecific categories as "variant" of *Mycobacterium tuberculosis* (Riojas *et al.* 2018).

Tuberculosis in the marine environment was discovered at the end of the 20th century in Australia and Argentina, and it was responsible for producing TB in marine mammals (Bastida *et al.* 1999; Bernardelli *et al.* 1996; Cousins 1987, 1995; Cousins *et al.* 1990; Forshaw & Phelps 1991). These records expanded the knowledge about the characteristics of the mycobacteria that affected six species of pinnipeds of the family Otariidae of the Southern Hemisphere, both from Oceania (*Neophoca cinerea*, *Arctocephalus pusillus doriferus* and *Arctocephalus forsteri*) (Cousins 1987, 1995; Forshaw & Phelps 1991; Thompson *et al.* 1993; Woods *et al.* 1995), and from South America and the Subantarctic Islands (*Otaria flavescens*, *Arctocephalus australis* and *Arctocephalus tropicalis*) (Bastida *et al.* 1999; Bernardelli *et al.* 1996; Lucero *et al.* 2019; Rodríguez & Bastida 1993). This discovery led to a new scenario in the knowledge of TB due to the incorporation of marine species as primary hosts in this zoonosis (Bastida *et al.* 1999; Bernardelli *et al.* 1996; Cousins 1995; Cousins *et al.* 1993).

The first studies carried out on these pinnipeds indicated that the isolated mycobacteria had characteristics compatible with both *Mycobacterium tuberculosis* and *Mycobacterium bovis.* On this basis, an international and interdisciplinary team was set up to study the subject, applying the genetic techniques available at that time. As a result of these studies, a new species, *Mycobacterium pinnipedii*, was identified and described (Cousins *et al.* 2003). This new species is also one of the most aggressive mycobacteria of the MTBC and constitutes a potential health risk for humans, as well as for wild and domestic mammals that can be in contact with pinnipeds infected with TB (Bastida *et al.* 2010; Jurczynski *et al.* 2011; Kiers *et al.* 2008; Lacave *et al.* 2009; Loeffler *et al.* 2014; Moser *et al.* 2008; Thompson *et al.* 1993).

Regarding the arrival of TB in South America, until the end of the 20th century, it was considered that this disease had been introduced by the European conquerors in the sixteenth century, in spite of the fact that there were archaeological records of human bone remains with lesions compatible with TB corresponding to periods long before their arrival. During the 1970s, pre-contact human remains with clear indications of TB lesions were identified for the first time in Peru (Allison *et al.* 1973). From then on, numerous cases based on osteological and/or molecular methods were also described in different regions of South America (Arriaza *et al.* 1995; Arrieta *et al.* 2011; Constantinescu 1999; García Guraieb 2006; Guichón *et al.* 2015; Klaus *et al.* 2010; Salo *et al.* 1994; Sotomayor *et al.* 2004; Wilbur & Buikstra 2006). These records continuously generated uncertainties about the origin and mechanism of TB dissemination in South America (Daniel 2000; Gómez i Prat & Mendonça de Souza 2003).

Along the Holocene, hunter-gatherers in the Atlantic and Pacific coasts of southern South America depended upon pinnipeds as one of their main trophic resources with high energy value (Bastida *et al.* 2007; Bastida & Rodríguez 2009; Jessup 1990a, 1990b; Legoupil 1989; Martinoli 2017; Muñoz 2011; Orquera & Piana 1999b; Panarello *et al.* 2006; Schiavini 1993; Zangrando 2014; Zangrando *et al.* 2011; Zangrando *et al.* 2014). Taking into account this trophic habit, Bastida *et al*. (2010, 2011) presented a new highly feasible hypothesis, which proposed the entry of pre-Columbian TB into South America through wildlife and in particular, through otariid pinnipeds. This hypothesis was later confirmed by the study of ancient DNA of *Mycobacterium pinnipedii* isolated from the spinal column of Peruvian mummies belonging to the Chiribaya culture of southern Peru; radiocarbon analyses indicated ages between 1028 and 1280 years AD (Bos *et al.* 2014). It should be noted that part of the population of the Chiribaya culture inhabited coastal sectors and based its subsistence upon intertidal invertebrates, marine fish and pinnipeds (Jessup 1990a, 1990b; Lozada & Buikstra 2005; Rostworowski 1975; Sandweiss *et al.* 1989).

The possible dissemination mechanisms of *Mycobacterium pinnipedii* along the Southern Hemisphere were unknown, although it was suspected that *Arctocephalus tropicalis* could be a key species in this process (Bastida *et al.* 1999, 2007, 2011). As before the present study there were no records of TB in pinnipeds of archaeological coastal sites of South America and the rest of the world, we developed our research in zooarchaeological assemblages from the northern coast of the Beagle Channel (Tierra del Fuego, Argentina). This coastal area was inhabited by marine hunter-gatherer-fishers with an efficient technology aimed at the exploitation of pinnipeds since 6400 years BP (7200 cal. years BP) (Martinoli 2017; Orquera & Piana 1999a, 1999b; Schiavini 1993; Zangrando *et al.* 2011).

The main objective of our study is to present the results of an extensive review of the zooarchaeological collection at the Centro Austral de Investigaciones Científicas (CADIC-CONICET), in order to identify bone lesions in otariid pinnipeds compatible with tuberculosis and discuss the implications of this disease for human populations of South America in pre-contact times. Finally, we propose a new hypothesis on *Mycobacterium pinnipedii* dissemination in the Southern Hemisphere and mechanisms of contagion between different otariid pinniped species.

Study area

The Beagle Channel runs west–east between the southern coast of the Isla Grande de Tierra del Fuego and the Navarino and Hoste Islands. This channel has approximately 180 km long and 4 to 7 km wide. The region is characterized by a mountainous geography and an irregular coast, which varies in topography between high-slope rocky seashores on the western section and low-slope coastlines to the east (Figure 1).

The mean annual temperature is 6.5°C. The terrestrial environment is characterized by woods of *Nothofagus* (*N. betuloides* and *N. pumilio*) located between the coastline and 600 m above sea level, and by a variety of faunal taxa. Important populations of marine mammals and a broad taxonomical diversity of birds, fish and mollusks inhabit coasts and seascapes.

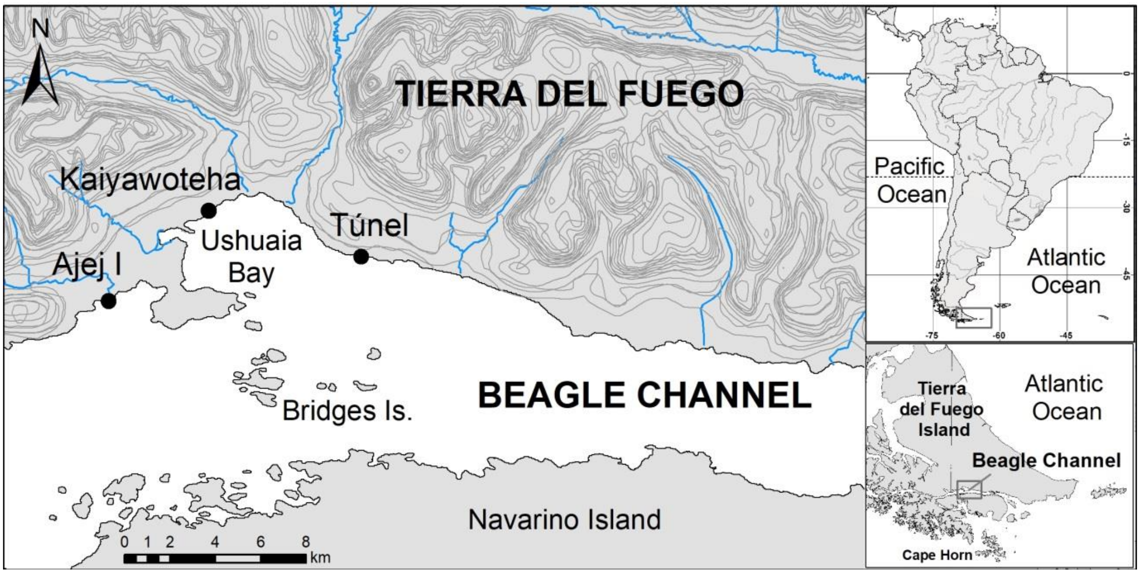

Figure 1. Archipelago of Tierra del Fuego and archaeological sites.

The Beagle Channel was inhabited by hunter-gatherer populations adapted to the marine environment from 6400 radiocarbon years BP to the 19th century. The coastal archaeology of this region is characterized by shell midden formations, which provide evidence of a predominant consumption of marine resources and lithic, bone, and shell technology (Gusinde 1986; Orquera & Piana 1999a, 2009). Two species of pinnipeds are abundant in the southern tip of South America: the South American fur seal (*Arctocephalus australis*) (Figure 2a) and the South American sea lion (*Otaria flavescens*) (Figure 2b). However, zooarchaeological assemblages of the Beagle Channel are widely dominated by *Arctocephalus australis* (Orquera & Piana 1999a; Schiavini 1993).

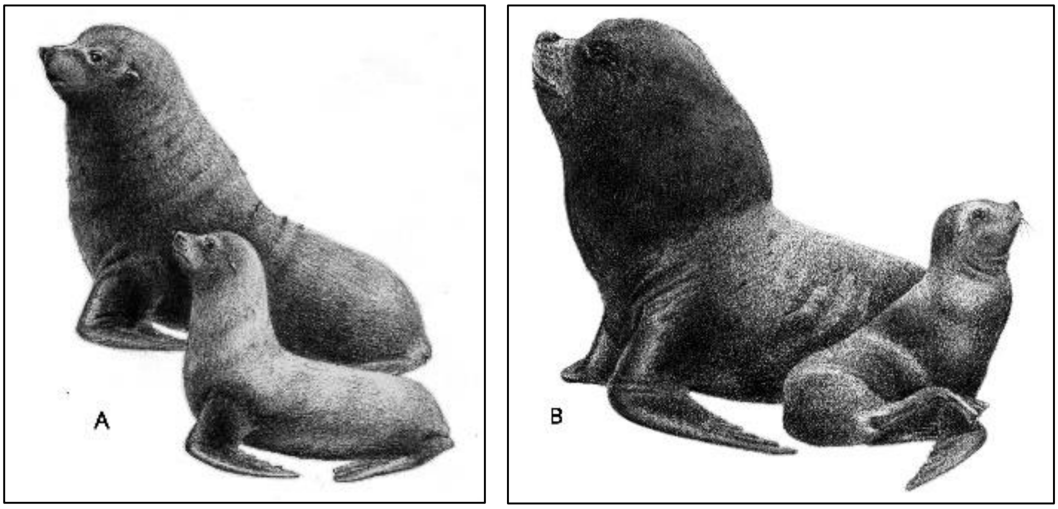

Figure 2. a) South American Fur Seal (*Arctocephalus australis*) (J.L. Vázquez Mazzini).
b) South American Sea Lion (*Otaria flavescens*) (J.L. Vázquez Mazzini).

Zooarchaeological assemblages

In this paper we analyze zooarchaeological assemblages from four archaeological sites: Second Component of Túnel I, Túnel II, Ajej I and Kaiyawoteha II. These sites are located at different coastal settings near Ushuaia city (Figure 1). The Second Component of Túnel I encompasses settlements between 6400 and 4300 radiocarbon years BP (Orquera & Piana 1999a). The other three sites correspond to the Late Holocene: Túnel II presents radiocarbon ages of 1140±90 BP and 1120±90 BP (Orquera & Piana 1999a), while Ajej I presents ages of 1400±90 and 1270±180 years BP (Piana *et al.* 2008), being Kaiyawoteha II the later site with 730±45 years BP.

Archaeological deposits at the Beagle Channel generally maintain good conditions of bone preservation due to two main factors: 1. Formation processes of these deposits normally imply high accumulation rates of shells, there being relatively small lapses of exposure of bone material at the surface (Orquera & Piana 1992), and 2. Conditions of pH and humidity are stable in these sediments (Linse 1992; Orquera & Piana 2000, 2001).

Materials and methods

The study of zooarchaeological bone samples from otariids was carried out at the Laboratory of Anthropology of CADIC-CONICET. The studied samples came from the four sites indicated in Figure 1, and the largest number of analyzed bone remains corresponds to Túnel I and II. *Arctocephalus australis* was the marine mammal species most exploited by hunter-gatherer-fishers, and therefore the one most represented in archaeological sites (Martinoli 2018; Orquera & Piana 1999a; Orquera *et al.* 2012; Schiavini 1993).

The search for TB bone lesions was focused on vertebrae, since bone modifications produced by this disease appear mainly in the spine. We analyzed a total of 4,138 vertebrae, most of them correspond to juvenile and adult males. Vertebrae with lesions compatible with TB were selected and prepared for diagnostic imaging studies. Digital Rx, 3D External Computed Tomography and thin cuts of vertebral bodies were obtained (Philips Equipment, Brilliance 64 CT Scanner and Siemens Equipment, Scope Power).

Regarding the transmission routes and probable dissemination mechanisms of *Mycobacterium pinnipedii*, a first hypothesis was formulated based on information from vagrant specimens of *Arctocephalus tropicalis* (Figure 3). These specimens were frequently recorded in the South American coasts and throughout the Southern Hemisphere since 1977 to 2016 (Castello & Pinedo 1977; Jefferson *et al.* 2015; Prado *et al.* 2016; among many other publications).

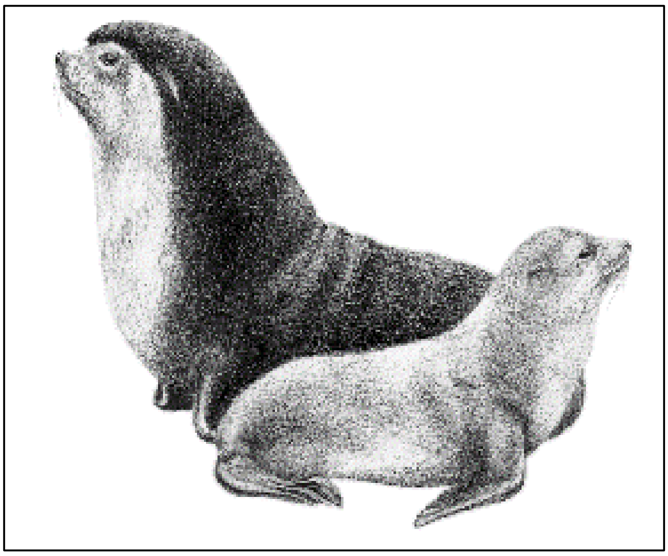

Figure 3. Subantarctic Fur Seal (*Arctocephalus tropicalis*) (J.L. Vázquez Mazzini).

On the other hand, we used published data on molecular markers (mtDNA) of vagrant specimens from the South-West Atlantic, in order to determine the origin of these specimens and their possible routes of dispersal from the rookeries at Gough, Crozet, Macquarie, Amsterdam, Saint Paul, and Prince Edward islands, located north of the Antarctic Convergence of the Atlantic, Pacific and Indian Oceans (Jefferson *et al.* 2015; Machado Ferreira *et al.* 2007; Wynen *et al.* 2000).

Results

The vertebrae from Túnel I, Trench XIII, Layer D19 to D/F showed the highest number of lesions related to TB. From the total studied bone samples, only 0.46 % of *Arctocephalus australis* vertebrae showed lesions compatible with TB. We studied the most representative lesions of TB (0.14 %) further by diagnostic imaging (Table 1). We also found non-infectious vertebra alterations, such as osteophytes, in 2 % of the studied bone samples.

	Archaeological Sites			
	Túnel I	***Túnel II***	***Ajej I***	***Kaiyawoteha II***
Analyzed vertebrae	3,482 (84.1 %)	337 (8.1 %)	52 (1.2 %)	267 (6.4 %)
Vertebrae with severe lesions	6 (0.14 %)	___	___	___
Vertebrae with partial lesions	4 (0.10 %)	___	___	___
Vertebrae with minor lesions	8 (0.19 %)	___	___	1 (0.03 %)

Table 1. Number and percentage of vertebrae studied in each archeological site (Beagle Channel, Argentina) and vertebrae with lesions compatible with TB (0.46 % of n = 4,138 analyzed).

The thoracic vertebrae XI, XII and XIII of the most affected individual were completely fused, forming a single rigid unit (ankylosis). On the other hand, the articulation between vertebrae X and XI presented a clear angulation that moved away from the normal horizontal axis of the spine (Figure 4a) as human xiphosis. The thoracic vertebra XII was massively reabsorbed, showing a process of destruction (osteolysis) and bone reabsorption; only the upper part of the spinous process was evident (Figure 4b). On the anterior surface of the vertebral body XI there was also important bone destruction, forming a very big cavity (Figure 4c).

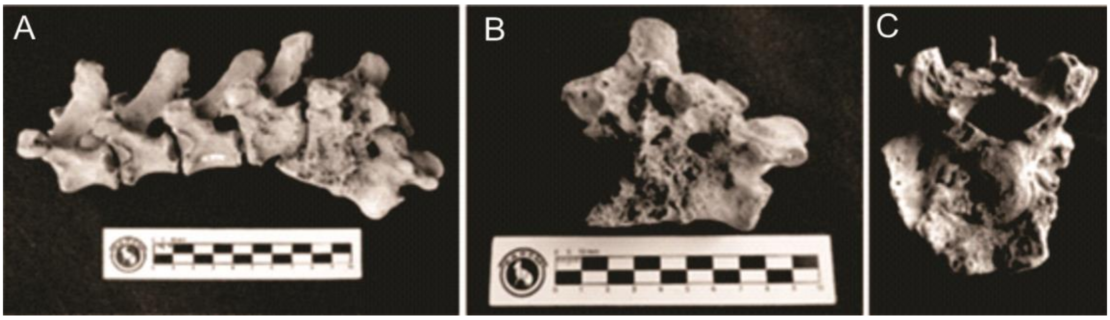

Figure 4. a) Thoracic vertebrae VII to XIII of *Arctocephalus australis* with severe lesions compatible with TB. b) Ankyloses of vertebrae XI, XII and XIII. Vertebra XII massively reabsorbed. c).Vertebra XI with big cavity in the anterior surface.

Digital Rx images showed rarefaction of the vertebral bodies due to a decrease in bone density (osteopenia), destruction of intervertebral discs and ankyloses processes. The presence of small marginal osteophytes, secondary to spondylosis, was visualized in the thoracic vertebrae VII, VIII, IX and X, suggesting that they belonged to an adult specimen. Thoracic vertebrae XI, XII and XIII presented a marked alteration in their morphology, while thoracic vertebra XI had an important and irregular depression of its anterior articular surface. Vertebrae XI, XII and XIII were massively fused with a clear resorption of vertebra XII (Figure 5). The 3D Computed Tomography (CT) allowed us to obtain external images of the affected vertebrae (Figure 6).

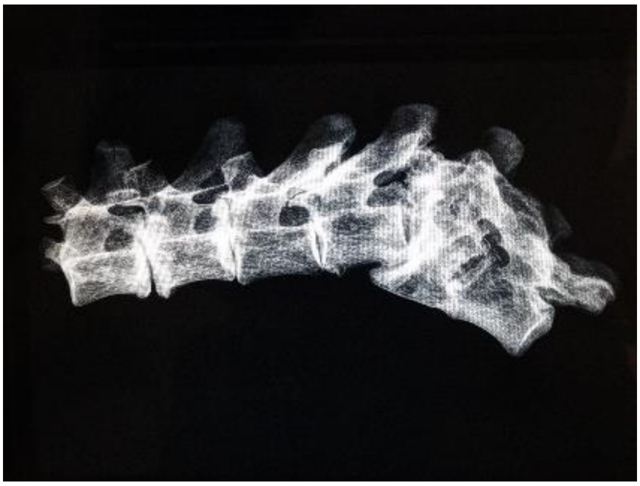

Figure 5. Rx of thoracic vertebrae VII to XIII of *Arctocephalus australis*. Vertebrae XI, XII and XIII with collapse of vertebral bodies and evident angling of the spine (xiphosis).

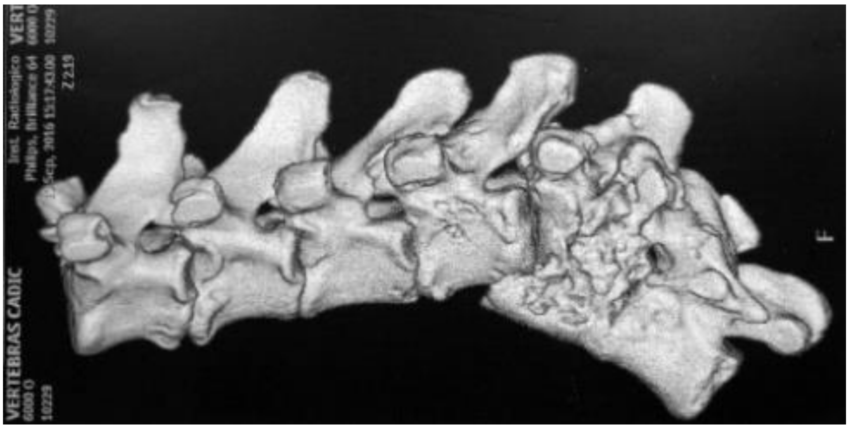

Figure 6. 3D Computed Tomography showing external lesions in thoracic vertebrae of *Arctocephalus australis*.

While CT sagittal cross sections images clearly showed the collapse of the vertebral bodies, destruction and absence of intervertebral discs. The bodies of the fused thoracic vertebrae XI, XII and XIII were collapsed, losing their normal morphology. Cortical were thinned and the posterior wall of the XI vertebral body protrudes into the neural canal due to vertebral collapse. Laminae were thickened due to secondary bone remodeling as a consequence of chronic inflammatory changes. All these lesions produced a reduction in the diameter of the neural canal (stenosis) (Figures 7a and 7b). Caries in the trabecular bone at the level of the vertebral bodies were also observed.

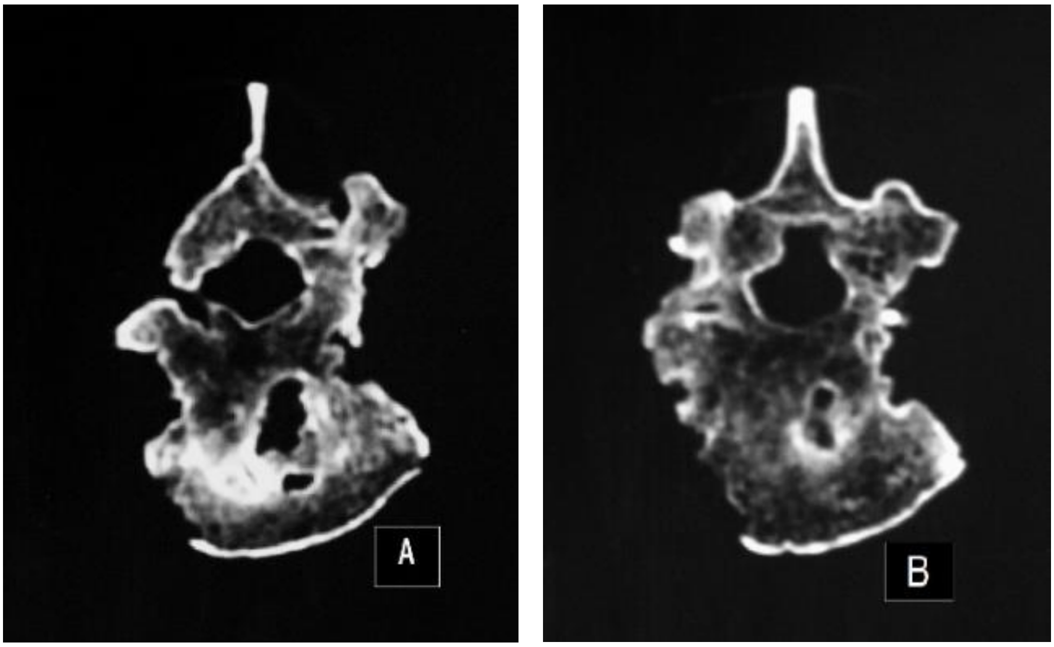

Figure 7. a-b) Computed Tomography sagittal cross section of thoracic vertebra XI of *Arctocephalus australis* showing severe bone destruction and secondary bone remodeling.

Regarding the role of *Arctocephalus tropicalis* in TB disease, the spatial distribution analysis of vagrant specimens shows clear coincidences with the dynamic of Southern Hemisphere surface ocean currents and the winds associated with them. In this way, *A. tropicalis* becomes the *nexus* species with the rest of the southern latitude otariids, being also the seal species with the greatest geographical displacement of the world.

Discussion

Zoonoses and implications for human populations in the past

In Argentina the incidence of TB in modern specimens of *Otaria flavescens* and *Arctocephalus australis* is similar in both species and does not exceed 2 % in soft tissue lesions, without considering the incidence in juvenile individuals and pups (Loureiro 2014). In Uruguay, including pups and all age population classes of both species, the incidence of TB is higher, ranging between 5.7 % and 9.8 % in soft tissues, also including individuals presenting no symptoms of the disease (Arbiza *et al*. 2012; Castro Ramos *et al*. 2006). After TB was discovered during the 1980s in local otariid pinnipeds from Argentina and Uruguay, the disease was also recorded in a vagrant specimen of *Arctocephalus tropicalis* stranded on the northern coast of Buenos Aries Province (Argentina) (Bastida *et al.* 1999). Years later new TB cases in vagrant *A. tropicalis* individuals, both on the northern coast of the Province of Buenos Aires and in Patagonian coasts were also diagnosed.

Regarding the studied zooarchaeological material of Beagle Channel sites, our data indicate that the TB lesions observed macroscopically in bone samples is 0.46 % and correspond exclusively to *Arctocephalus australis*. Until now there is no record of paleotuberculosis bone lesions in *Otaria flavescens* samples from Beagle Channel sites. Three main causes could be related with this fact: 1. Very low presence of *Otaria flavescens* bone remains in the archaeological deposits at the Beagle Channel; 2. Lower density population of *Otaria flavescens* in relation to that of *Arctocephalus australis*, the dominant pinniped species of Tierra del Fuego, and 3. Hunting techniques from canoes that required pinnipeds of smaller size.

All bone lesions compatible with TB in the zooarchaeological material analyzed correspond exclusively to thoracic vertebrae, which together with the first lumbar vertebrae are generally the most affected by this disease in different groups of mammals (Balázs *et al.* 2015; Holloway *et al.* 2011; Holloway *et al.* 2013). The highest incidence of TB bone lesions in the spine is due to the fact that the vertebrae are extremely vascularized, which facilitates bacterial colonization of bones through the blood flow. These lesions could be differentiated from similar bone lesions caused by other diseases (neoplasms, osteomyelitis, brucellosis, etc.), in the same way that Arrieta *et al.* (2014) could differentiate TB lesions in human archaeological bones. However, Luna *et al.* (2017) mention that the non-specificity of various bone pathologies generally it is impossible to make an accurate differential diagnosis.

Regarding the studies of ancient mycobacterial DNA of the MTBC, it is important to mention that molecular studies do not always provide reliable results for paleopathological diagnoses, which depend not only on the preservation state of the bone sample, but also on contamination by environmental bacteria that could have colonized *post-mortem* bone remains. These bacteria can be non-tuberculous mycobacteria and be responsible for false positives of TB (Eisenhofer & Weyrich 2019; Guichón *et al.* 2015; Müller *et al.* 2016). In these and other cases, it is convenient to resort to a combination of mycolic, mycocerotic, micoliphenic and phthiocerol biomarker assays (Lee *et al.* 2015; Thi-Nguyen-Ny Tran *et al.* 2011; Wilbur *et al.* 2009). It is also important to mention that the excess of exposition of TB lesions of bone samples to radiation by Computed Tomography or Rx studies can inactivate the ancient DNA for its genetic study and give false negatives (Donoghue 2017).

The airway is the most frequent entry route of the MTBC mycobacteria to the host. Based on our pinniped necropsies of modern specimens, the respiratory system is affected initially, followed by the lymph nodes, and important organs such as the liver, kidneys and heart, which may be also involved (Bastida *et al.* 1999; Bernardelli *et al.* 1996). Another route of entry is the sputum that the individual swallows, also sneezing close to another individual and rubbing noses between individuals.

As a final process of pulmonary TB, after several years, the bone system can also be compromised, mainly the vertebral column, as in Pott's disease or xiphosis in humans (Holloway *et al.* 2013; Klaus *et al.* 2010; Roberts & Buikstra 2003; Vergara Santos *et al.* 2015). This form of bacillary infection in aquatic and diving species, such as pinnipeds, would not allow the animals to reach the end of the bone lesion process, as the lung condition affects diving, a vital behavior through which pinnipeds access their preys, basically fish and squid. For this reason, we estimate that bone lesions in pinnipeds could only occur when the mycobacteria enter through the bloodstream directly into the bone tissue. A feasible way of entry could be through the frequent injuries that occur during the struggles that adult male pinnipeds maintain during the reproductive season.

As we indicated at the beginning of this article, cases of TB have been identified in human remains in different regions of South America through osteological and/or molecular descriptive methods, for both agro-pastoral societies (Allison *et al.* 1973; Arriaza *et al.* 1995; Sotomayor *et al.* 2004; Arrieta *et al.* 2011; Salo *et al.* 1994) and hunter-gatherer societies (Constantinescu 1999; García Guraieb 2006; Guichón *et al.* 2015). It should be noted that all these cases are restricted to the Late Holocene (last 2000 years). The results in the present paper suggest that TB infection could have occurred at least 6400 years BP (7200 years cal. AP) in the southern extreme of South America. This argument is feasible due to the high infectious-contagious capacity of *Mycobacterium pinnipedii* that could have affected the hunter-gatherers who captured, processed and consumed pinnipeds since the Middle Holocene (Bastida *et al.* 2011; Martinoli 2017; Muñoz 2011; Orquera & Piana 1999a, 2009; San Román 2008; Schiavini 1993; Zangrando 2014). Moreover, humans could transmit the infection to others due to close contact, household types and rigorous environmental conditions of the Fuegian Archipelago.

Bioarchaeological records of the Middle Holocene are very scarce in the southern tip of South America, so that we are unable to evaluate the incidence of TB in hunter-gatherer populations. However, the presence of TB has been molecularly confirmed in a pre-contact human individual from the Northern Tierra del Fuego for the Late Holocene, although the MTBC species could not be defined (Guichón *et al.* 2015).

Another aspect to consider is the physiographic and environmental characteristics of the Fuegian Archipelago, which provides favorable conditions for the feeding of marine mammals, as well as for the refuge of diseased or physically exhausted pinnipeds. Precisely these individuals would be the ones of easier capture by coastal hunter-gatherers, and this would increase the possibility of TB infections from pinnipeds.

Hypothesis about dissemination of Mycobacterium pinnipedii

When TB was detected for the first time in wild pinnipeds from Australia and Argentina, it was assumed that the disease was transmitted by terrestrial species affected by *Mycobacterium bovis* or *M. tuberculosis*. Based on this assumption, two hypotheses emerged on the origin of TB in marine mammals.

One of them stated that the animals were infected with *Mycobacterium bovis* by coastal cattle (Cousins *et al.* 1993). The other hypothesis speculated on the alternative that humans could have transmitted TB to otariid pinnipeds. This hypothesis was based on the possibility that pinnipeds scavenged on tuberculous human bodies thrown into the sea from vessels, as it was habitual until the 19th century (Cousins *et al.* 1993); however, there are no references of such trophic behavior in pinnipeds (Bastida & Rodríguez 2009). Both hypotheses were discarded years later, when it was discovered that TB in pinnipeds was produced by a new TB species named *Mycobacterium pinnipedii* (Cousins *et al.* 2003).

Regarding the dissemination process of *Mycobacterium pinnipedii* in the oceanic environment, there were many unknowns and only preliminary progress has been made (Bastida *et al.* 1999, 2011). In order to understand this process in the Southern Hemisphere, it is necessary to consider some biological and ecological features of *Arctocephalus tropicalis*, as well as some oceanographic aspects affecting its movements.

Even though *Arctocephalus tropicalis* is not a species exhibiting migratory behavior, it is characterized by having a high number of vagrant individuals, which travel the longest distances known for otariid pinnipeds, in some cases exceeding 16,000 km (Machado Ferreira *et al.* 2007). In this way, individuals of this key species affected with TB could have reached the South American coasts and diverse regions of the Southern Hemisphere, taking advantage of the surface ocean currents and widening the geographic range of the species (Bastida *et al.* 1999, 2007; Campos *et al.* 1995; Reid *et al.* 1977; Rodríguez *et al.* 1995; Sudre *et al.* 2013; Zanre & Bester 2011).

The breeding colonies of this species are found on the islands Tristan da Cunha/Gough, Prince Edward/Marion, Crozet, Amsterdam/Saint Paul and Macquarie, forming the Subantarctic belt of islands located near the Antarctic Convergence of the Atlantic, Indian and Pacific Oceans (Figure 8) (Bester 1980, 1981, 1987, 1990; Bester *et al.* 2003).

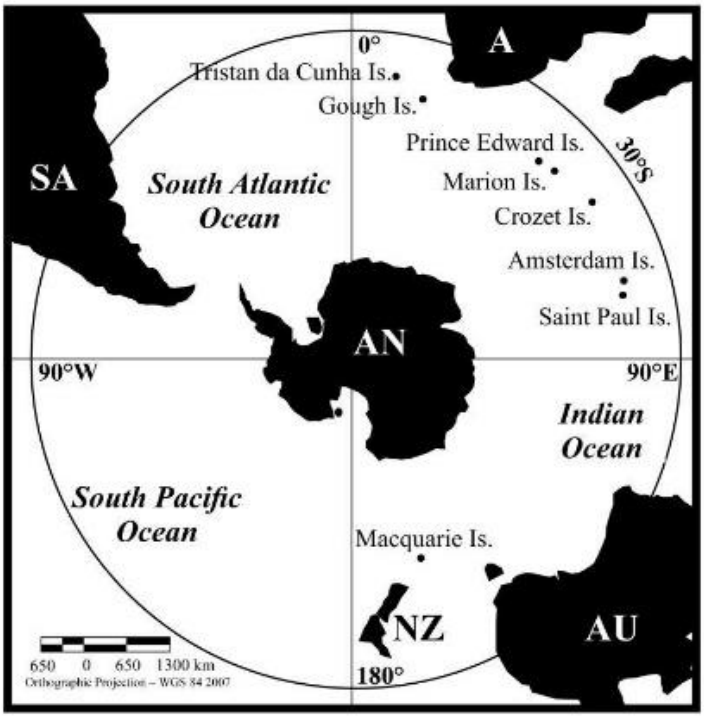

Figure 8. Breeding island colonies of *Arctocephalus tropicalis* in the Southern Hemisphere.

The most important breeding colonies are those of Gough Island, Prince Edward islands and Amsterdam Island (Bester 1990; Bester *et al.* 2003), which concentrate 95 % of the population of *Arctocephalus tropicalis*. The current population exceeds 400,000 individuals (Bester *et al.* 2003, 2006; Hofmeyr *et al.* 2017).

Vagrant specimens of this species have been recorded from the south of the Antarctic Convergence in South Georgia Islands, South Shetland Islands and even in the Antarctic Continent, as well as in the South Island of New Zealand, in Australia and Tasmania, in Mauritius and Rodrigues Islands, in Madagascar, in the Comores Islands, in Tanzania, South Africa, Angola, Gabon, Brazil, Uruguay, Argentina, Chile and Juan Fernández Island (Jefferson *et al.* 2015).

As already mentioned, the presence of vagrant specimens of *Arctocephalus tropicalis* on the South American coast is related to the main southern surface currents, both the South Atlantic Anticyclone vortex and the Western Ocean Drift. These currents constitute the main dispersion routes of the vagrant specimens of *Arctocephalus tropicalis* to South America, South Africa, Australia and New Zealand (Bastida *et al.* 1999, 2010, 2011; Bastida & Rodríguez 2009; Rodríguez *et al.* 1995; Shannon *et al.* 1973). The presence of vagrant specimens is recorded mainly during the winter and spring of the Southern Hemisphere.

The presumption that the increase of vagrant specimens of *Arctocephalus tropicalis* is a direct consequence of the sealer exploitation ending by the 20th century is no longer valid. Recent archaeological studies indicate the presence of this species in areas away from its breeding colonies, such as the archaeological sites in southern Brazil

(1200 years BP) (Volkmer de Castilho & Simões-Lopes 2008), and in Argentina (Santa Cruz) (between 1290 and 1170 years BP) (Castro *et al.* 2010). In other words, there were always a certain number of vagrant individuals able to travel very long distances from their breeding colonies in *A. tropicalis* populations, even before the inception of commercial exploitation of this species.

The genetic studies of Wynen *et al.* (2000) and Machado Ferreira *et al.* (2007) allowed confirming the colonies of origin of the vagrant specimens, relating the molecular profiles of these specimens with the genetic profiles of the populations in their reproductive areas. The vagrant specimens of our region, which includes Brazil, Uruguay, Argentina and Chile, come from three genetically identifiable reproductive units that, geographically, correspond to the following Subantarctic islands: (1) Gough, (2) Amsterdam and (3) Marion, Macquarie and Crozet (MMC) (Figure 8).

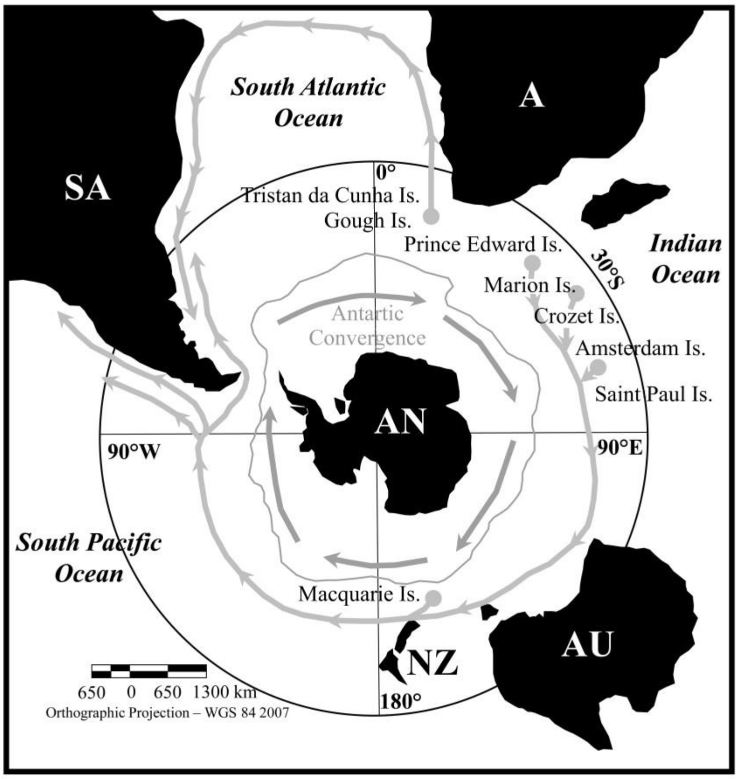

Figure 9. Dissemination of vagrant specimens of *Arctocephalus tropicalis* in the Southern Hemisphere. Breeding colonies of origin and displacement routes related to main ocean surface currents.

Arctocephalus tropicalis travel from their breeding colonies to the north, using the anticyclone system of the South Atlantic. Thus, Subantarctic fur seals are deflected by the surface current to South Africa and move northward through the Benguela Current along the Atlantic African coast, until reaching the Equatorial South Current that moves from east to west. Following the branch of this current that moves southward, vagrant's individuals can reach the northern coast of Brazil (Velozo *et al.* 2009; Vigário 2010) and keep moving further south (Figure 9). Thus, these specimens can arrive in the coasts of Uruguay and Argentina, where the Brazilian Current meets the Malvinas/Falklands Current, forming a confluence area and a subtropical front in the north of Patagonia. Based on these new studies, it was possible to confirm the dispersal process of vagrant specimens of *A. tropicalis* (Bastida & Rodríguez 2009; Bastida *et al.* 1999; Rodríguez *et al.* 1995).

Other vagrant specimens that arrived in the South American coasts would come from the Amsterdam Island and the MMC group, located at longer distances (Machado Ferreira *et al.* 2007). Those specimens move along the Western Circumpolar Drift that runs clockwise around the Antarctic Continent, travelling distances that exceed 16,000 km to the coasts of Brazil. Along this route, vagrant specimens can also interact with individuals in rookeries of other pinnipeds in the coasts of Australia, New Zealand and the islands and coasts of Chile and Peru. In addition to these dispersal routes, the distribution of vagrant specimens across the Indian Ocean may also occur (Jefferson *et al.* 2015) (Figure 9).

The dispersion routes of vagrant specimens of *Arctocephalus tropicalis* were based on the currently available knowledge of the surface ocean currents of the Southern Hemisphere. This was possible thanks to the oceanic research initiated in the 1970s, based on the use of drift cards and oceanographic drifting buoys (Shannon *et al.* 1973). Recently, sophisticated remote sensors have been used, producing excellent models of oceanic dynamics (Sebille *et al.* 2018).

Another important aspect to consider in the dissemination of TB is that vagrant specimens of *Arctocephalus tropicalis* may also interact with individuals of other pinniped species in the Southern Hemisphere, concentrated in reproductive and non-reproductive colonies in high densities, facilitating TB transmission to these other species (Bastida & Rodríguez 2009; Bastida *et al.* 1999, 2011). In South America, *A. tropicalis* can occasionally associate to *Otaria flavescens* and *Arctocephalus australis* in rookeries along both the Atlantic and the Pacific coasts.

In the Pacific Ocean, *Arctocephalus tropicalis* is related to the colony of *Arctocephalus philippii* in the island of Juan Fernández (Torres & Aguayo 1984). In New Zealand and Australia *A. tropicalis* also interacts with *Arctocephalus forsteri*, *Neophoca cinerea*, *Phocarctos hookeri* and *Arctocephalus pusillus doriferus*. Furthermore, in South Africa, *A. tropicalis* is related to *Arctocephalus pusillus pusillus*, and in the Subantarctic Belt with *Arctocephalus gazella* (Bastida *et al.* 1999, 2007; Jefferson *et al.* 2015). The interactions of *A. tropicalis* with other pinniped species have been so close that it has even hybridized with several of them, such as *Arctocephalus gazella*, *Arctocephalus forsteri* and *Arctocephalus philippii*, achieving hybrid specimens that can reach sexual maturity and reproduce successfully (Jefferson *et al.* 2015) (Figure 9).

Considering all these factors, we hypothesize that *Arctocephalus tropicalis* was a key species in the process of dissemination and contagion of TB in the Southern Hemisphere, and in turn, this argument facilitates the understanding of cases of pre-contact TB in South America and other southern regions (Bastida *et al.* 2010, 2011; Bos *et al.* 2014).

Final remarks

Several studies have reported bone lesions compatible with TB in human remains at different archaeological contexts of the Late Holocene before the arrival of Europeans in South America. The disease was identified both based on bone lesions and on molecular studies in several pre-contact South American societies. More recently, it was possible to identify the ancient DNA of *Mycobacterium pinnipedii* isolated from the vertebral column of Peruvian mummies dated to 1000 years BP and belonging to the Chiribaya culture, and related with the exploitation of otariid pinnipeds.

The present paper provides first evidence of lesions compatible to TB in bone remains of pinnipeds of Tierra del Fuego with dates of approximately 6400 years BP (7200 years cal. BP). This record suggests that TB could have been transferred to human populations in the southern tip of South America in the Middle Holocene through the practice of capture and consumption of these marine mammals. In turn, the evidence shown here supports the hypothesis about the dissemination of *Mycobacterium pinnipedii* in the Southern Hemisphere through vagrant specimens of *Arctocephalus tropicalis* and the interaction of this species with different austral otariid pinnipeds.

Tuberculosis records in pinnipeds of both the Atlantic and Pacific coasts of South America, as well as TB bone lesions in human remains, would indicate two main areas of probable dissemination of this zoonosis in the Argentine territory. On the one hand, an austral income through Tierra del Fuego and the Patagonian coasts, where there are numerous pinniped colonies, species that have been traditionally consumed by hunter-gatherer-fishers and, on the other hand, an entry from the north of Argentina, associated to the penetration of the Inca Empire into our territory and Incas acting as disseminators of this pre-existing zoonosis in the South America Pacific coast.

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