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Pre-Columbian Tuberculosis in Tierra del Fuego? Discussion of the Paleopathological and Molecular Evidence.

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1. Introduction and background

Tuberculosis (TB) is an infectious disease whose origin and co-evolutionary relationships with *H. 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. 2009, 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).

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4 In 1999, Constantinescu briefly described vertebral bone lesions compatible with TB in a
5 skeleton from the Myren 1 site (Fig. 1), excavated by the archaeologist Massone. Although
6 there was no radiocarbon dating of the human remains at that time, Massone attributed
7 this site to a pre-European contact period (Massone et al 1999). In 1995, as part of a
8 collaborative project between teams from Spain, Argentina, and Chile, the D haplogroup
9 was identified for this individual, which corresponds with haplotypes common for native
10 peoples in this region (Lalueza 1995, Lalueza et al 1997).
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14 Clinical models describe a low frequency of bone involvement as a result of disseminated
15 TB, usually associated with destructive lesions in the spinal column and appendicular
16 skeleton, and occasionally in the skull (Resnick and Niwayama 1995). New bone formation
17 on the pleural surfaces of ribs is considered suggestive but not pathognomonic for tTB
18 (Roberts and Buikstra 2003; Santos and Suby 2012).
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22 Analyses of ancient DNA have been used to investigate the causative agent of the
23 observed TB bone lesions in South America since the 1990s (Arriaza et al 1995; Salo et al
24 1994). These early studies recovered and amplified ancient DNA from the *M. tuberculosis*
25 complex (MTBC), which was inferred to have been most closely related to the TB species
26 that are found today in humans, possibly derived from an Asian strain. However, recent
27 studies from the Osmore river valley of southern Perú have identified TB in ancient South
28 American human remains most closely linked to the form that affects pinnipeds (seals and
29 seal lions), suggesting that these sea mammals served as the vector that transferred the
30 pathogen from the Old World to South America (Bos et al 2014). Studies of historic period
31 South American human remains thus become crucial for evaluating the nature of TB-
32 human co-evolution in the Western Hemisphere. The remains from Tierra del Fuego, due
33 to their location, skeletal evidence of TB, and antiquity, thus become especially important
34 in this regard. Our study was conducted to clarify the chronological placement of the
35 remains, to provide a detailed description of the remains, and to generate genetic
36 information about the pathogen.
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44 2. Archaeological context

45 By approximately 8000 BP, a land bridge connected Tierra del Fuego and the mainland
46 (McCulloch and Morello 2009), which was potentially first used by hunter-gatherers who
47 arrived at least 10,000 years ago (Massone 2003). Beginning in the 16th and extending
48 into the 18th century AD, contact between indigenous groups and Europeans was almost
49 exclusively coastal. According to documentary sources, European exploration of Tierra del
50 Fuego's interior began at the end of the nineteenth century (Borrero 1992, Fugassa and
51 Guichón 2004).
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57 The Myren 1 site is located approximately 14 km north of Inútil Bay (53 ° 14'S - 69 ° 28'W),
58 at the northern aspect of a wide glacial valley that connects this cove to the Bay of San
59 Sebastián, located in the Chilean territory north of Tierra del Fuego. Myren 1 is a large
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(approximately 200 x 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 & 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 y Luna 2009).

Sector 2, established to further explore site stratigraphy, was located 80 m east of Sector 1. It is a 31 x 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 x 1 m survey and a 2 x 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 material: including a cut guanaco epiphysis, a fragmentary stone point tip, and some red pigment. A sample of guanaco bones removed from Level III was radiocarbon dated to 870 ± 70 BP¹ (Massone et al. 1999). This date, along with the associated material culture links the site to the Selk'nam Culture (Massone *ibid*).

3. Material and methods

The skeleton Myren 1 (# 30477 curated at the Center for the Study of Southern Man, Instituto de la Patagonia, Universidad de Magallanes, Punta Arenas, Chile) was excavated in 1998. A complete inventory of the remains was conducted, as well as an overall assessment of preservation. Sex estimation was based upon pelvis and skull morphology (Buikstra and Ubelaker 1994). Age at death was estimated by observing the pubic symphysis (Brooks and Suchey 1990) and auricular surface (Lovejoy et al 1985, Falys et al 2006). The AMS radiocarbon dating was performed at the Center for Applied Isotope Studies at the University of Georgia, Athens, Georgia. Sample preparation for isotopic analyses followed standard protocols and procedures (Ambrose 1990). Isotopic analyses

¹ Beta 128091 Measured C14 Age 880±70 BP; C13/C12 Ratio -19.0‰ o/oo; Conventional C14 Calibrated results (2 sigma, 95% probability): cal AD 965 to 1220 (Cal BP 985 to 730)

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4 were carried out at the Stable Isotope Ratio Facility for Environmental Research (SIRFER)
5 at the University of Utah, Salt Lake City, Utah.
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8 Recording pathological lesion of the skeleton followed the criteria suggested by
9 (Aufderheide and Rodriguez Martin (1998), Ortner (2003), Santos and Roberts (2006),
10 Baker et al (1999), Pálfi et al (2012) and Haas et al (2000) for TB. Abnormal bone
11 formation, bone destruction, and lesion distribution were assessed.
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14 Figure 1. Location of Myren 1 in The “Isla Grande” of Tierra del Fuego.
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17 Figure 2. Myren 1, Sector 1. (Massone & Jackson 1998).
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20 **Destructive analyses:** The aDNA bone sample from Myren was processed at Arizona
21 State University’s (ASU) laboratory designated for ancient DNA work and following
22 protocols to prevent contamination as is standard in the field (e.g. Cooper and Poinar,
23 2000, Paabo et al. 2004). The ancient DNA laboratory contains a class 10,000-HEPA
24 filtered clean room. All personnel wear appropriate personal protective equipment,
25 including a coverall with hood, facemask, shoe covers, double gloves and a face shield.
26 The clean room is UV irradiated after use and every night as well as cleaned with bleach to
27 reduce the potential for contamination by exogenous DNA. The ancient and modern DNA
28 laboratories at ASU conform to a unidirectional workflow to eliminate contamination of
29 post-PCR products into the clean room environment; this includes all personnel,
30 equipment and reagents.
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34 A portion of the vertebral body adjacent to a lesion was excised and rinsed to remove
35 surface contaminants with 1.5% sodium hypochlorite and sterile water. The bone was
36 pulverized in a SPEX ceramic mill. 50-200 mg of powder were left overnight in extraction
37 buffer, 0.5M EDTA and Proteinase K. The sample was purified alongside extraction blanks
38 with a modified silica protocol (Rohland and Hofreiter 2007).
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42 The MTBC includes several closely related “species” or ecotypes: *M. tuberculosis*, *M.*
43 *canettii*, *M. bovis*, *M. microti*, *M. africanum*, and *M. pinnipedii* (Bos et al 2014). Four
44 quantitative PCR (qPCR) assays to test for the presence and quantity of a single-copy and
45 two multi-copy regions within the MTBC DNA were designed using the primers and probes
46 listed in Table 1 (Harkins et al. 2015). The assays target two nearby regions in the beta
47 subunit of the RNA polymerase gene, referred to here as rpoB-1 and rpoB-2, and the
48 multi-copy mobile insertion elements, IS6110 and IS1081, which are considered to be
49 exclusive to the members of the MTBC (Dziadek et al. 2001, Thierry et al. 1990, Eisenach
50 et al. 1990, van Soolingen et al. 1993), although evidence suggests that some mobile
51 elements are present in other mycobacteria (Kent et al. 1995). Nevertheless, these loci
52 have been used to detect tuberculosis in ancient DNA studies (e.g. Salo et al. 1994; Taylor
53 et al. 2007, Müller et al. 2014). Specifically, the IS6110 and rpoB-1 primers/probe sets
54 used here were designed recently and described in Klaus et al. (2010) and Harkins et al.
55 (2015). All qPCR assays employed TaqMan® hydrolysis probe chemistry, which hybridizes a
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probe to a target sequence within the amplicon, releasing fluorescence proportional to the amount of starting DNA template. This process increases the assay's specificity compared to other quantitation technologies, which detect any amplifiable double-stranded DNA, including primer-dimers and non-specific product. The fluorescence signal is recorded as it crosses a threshold during the linear phase at a given cycle number, called the cycle threshold (Ct). The assay's efficiency is determined by testing a dilution series of DNA with known starting copy numbers, called a standard curve. Theoretically, a PCR efficiency of 1, or 100%, would result in a doubling of product for each cycle, reflected in a slope of the standard curve at -3.32. In other words, sample dilutions that differ by 10-fold should cross the Ct ~3.32 cycles apart.

Ancient samples often contain substances co-extracted with the endogenous DNA (King et al. 2009). Co-extractors negatively affect PCR efficiency (Wilson 1997), which can be determined from the slope of the standard curve. Inhibition in ancient samples is evaluated by including 10-fold dilutions, here 1:10 and 1:100, and examining deviations from the expected Ct values, or ΔCq . 90-110% efficiency, or a slope of -3.58 to -3.10 of the standards is required to examine the ancient sample results. In the clean room, the reaction was prepared and qPCR plate loaded with 2× Taqman Universal PCR Mastermix, primers, probe and 2ul sample and sample dilutions in triplicate, each in a 20ul reaction; the plate remained covered during the addition of the standard 10-fold serial dilutions, which were added in the post-PCR DNA lab. Standard curves for absolute quantitation were created by diluting DNA from *Mycobacterium tuberculosis* H37rv with known copy numbers (1 to 1e05 copies/μl).

The Myren sample was also sent to the Paleogenetics Laboratory at the Institute for Archaeological Sciences (IAS) at the University of Tübingen, Germany, where targeted enrichment and sequencing (MiSeq 2000) were performed and reported in Bos et al. (2014), Table S1. Quantitative PCR results are discussed below.

Table 1: qPCR primer and probe sequences (final concentrations).

4. Results

4.1. Preservation:

The Myren 1 skeleton was fairly complete (Fig. 3), although the upper section of the vertebral column, the right arm and some bones of the hands and feet were not recovered. Macroscopically, bone preservation is very good to excellent, with only minor post-depositional alteration of the 4th thoracic vertebrae and the left first rib.

Figure 3. Surviving elements of Myren 1.

4.2. Sex and age of death. This individual's estimated sex was male, through observations of pelvic and cranial morphology (Buikstra and Ubelaker 1994). Age-at-death was estimated to have been between 18 and 23 years, due to incomplete fusion of long bone

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4 epiphyses (head and greater trochanter of the femora and the proximal tibiae) and the
5 iliac crests. Also evaluated were the pubic symphyses (phases 1-2 of Brooks and Suchey
6 1990) and auricular surfaces coincident with Figure 6: A & B (Lovejoy et al, 1985).
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9 4.3. Pathological changes.

10 In a previous paper, Constantinescu (1999) mentioned that bone characteristics of lower
11 thoracic and the first lumbar vertebrae of this skeleton could be suggestive of having been
12 affected by TB, although detailed descriptions or images were not presented. Our observation
13 showed irregular and asymmetrical hypervascularization on the antero-lateral aspect of the bodies
14 of T6 to T10 vertebrae, with small resorptive foci (less than 3 mm) and cortical remodeling (Fig. 4).
15 Similar hypervascularized areas are present on the left side of the bodies from T11 to L1. The end
16 plates of the bodies and the neural arches are not affected. No collapsed vertebrae typical of TB
17 were identified, although the body of L1 shows a 15-degree anterior compression of the body,
18 which could have resulted in slight angular kyphosis. The vertebra, as can be seen in Figure 5,
19 showed reduction of the trabecular matrix, probably due to osteopenia, mentioned by
20 Constantinescu (1999).
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26 No non-specific lesions associated with TB were found in other bones, including on the
27 articular surfaces of long bones (Aufderheide and Rodriguez Martin 1998; Ortner 2003), dactylitis
28 (Teo and Peh 2004), periosteal reactions of rib ends (Kelly and Micozzi 1984; Roberts et al. 1998;
29 Mays et al. 2002; Santos and Roberts 2006) and symmetrical new bone formation in long bones
30 (Assis *et al.* 2011). Endocranial lesions, mentioned as possibly related to tuberculous meningitis
31 (*e.g.* Roberts and Buikstra 2003; Giacon, 2008; Pálfi et al. 2012) could not be studied, although
32 facial bones that in some cases could be affected, were normal in this skeleton.
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37 Hypervascularization of vertebral bodies has been suggested to be associated with TB in
38 skeletons from archaeological sites and in documented collections (Baker 1999; Haas *et al.* 2000;
39 Pálfi *et al.* 2012). As stated by Pálfi *et al.* (2012:9) vertebral hypervascularization, endocranial
40 lesions and rib and long bone periosteal reactions may be useful for diagnosis of TB, but especially
41 when these lesions are all associated together. However, vertebral hypervascularization is also
42 associated with the normal developing spine in non-adult and young adult individuals (Baker 1999;
43 Roberts and Buikstra 2003; Pálfi *et al.* 2012).
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47 Other pathological conditions that could affect vertebral bodies, mainly brucellosis,
48 trauma and neoplasia, have to be considered in a differential diagnosis of TB (*e.g.* Aufderheide and
49 Rodriguez Martín 1998; Ortner 2003; Pálfi *et al.* 2012). Vertebral hypervascularization identified in
50 this skeleton seems not to be related to trauma and bone tumors. On the other hand, it is similar
51 to vertebral lesions attributed to brucellosis described by Mutolo *et al.* (2012), although no lysis of
52 the antero-superior rim of the vertebral bodies described in this disease was observed (Curate
53 2006; Mays 2007).
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57 Thus, in the young individual studied here with no other bone lesions suggestive of TB, the
58 observed vertebral hypervascularization could be the result of the normal developing spine,
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4 although infectious diseases as TB or brucellosis cannot be completely discarded as causative.
5 Other methods, such as molecular analyses (see below), are needed to discuss its etiology.
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9 Figure 4. Thoracic 11th (above), 12th (middle) and Lumbar 1st (below) vertebrae of the
10 skeletons from the Myren 1 site, showing circumferential resorptive areas on the left
11 antero-lateral surfaces of the bodies.
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14 Figure 5. Radiographic images of T11, T12 and L1 of the skeletons from Myren 1. No lytic
15 lesions were observed. These vertebrae were apparently affected by osteopenia. Anterior
16 compression of L1 is clearly visible (below right).
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19 4.4. Dating:

20 An AMS radiocarbon date of the remains (2005, Center for Applied Isotope Studies,
21 University of Georgia) of 640 ± 20 years BP (Caliber Rev 7.0.2 Calibration data set:
22 Marine/So Hem. Two Sigma Ranges Cal AD 1445: cal AD 1504) indicates that these
23 remains predate direct European contact with the indigenous people of Tierra del Fuego.
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27 4.5. Diet. The results obtained from SIRFER, in collaboration with other work in progress,
28 suggest a possible mixed diet (d15N col 16.8 and d13C col -15.7). These values must be
29 considered within the context of the resources available in this region, such as guanaco
30 (*Lama guanicoe*) and sea lions (*Otaria flavescens*), which have values of d13C al -21 and -8
31 respectively (Borrero et al 2001, Barberena et al .2009). The values for the Myren
32 individual are within an expected range for a hunter-gatherer diet in this area.
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36 4.6. Biomolecular identification:

37 Four qPCRs designed to target MTBC were used to test for the presence of TB DNA.
38 Multiple replicates of the sample showed the presence of rpoB in both the rpoB-1 and
39 rpoB-2 assays; however, neither IS1081 nor IS6110 were detected during the qPCR
40 analyses. The rpoB-1 and rpoB-2 qPCR analyses displayed an overall slope of -3.29 and -
41 3.34, respectively, with the coefficient of correlation (R_2) for the standard curves being >
42 0.99. All non-template controls were negative. An undiluted (1:1) extract was tested for
43 rpoB-1 on three separate dates wherein every replicate (total 9 of 9) recorded a signal, as
44 well as three replicates each at 1:10 and 1:100 dilutions. In the rpoB-2 assay, the signal
45 was recorded for two (of two) 1:1 replicates. Only one of three 1:10 replicates recorded a
46 signal, thus resulting in a negative result for this dilution, but it is interesting to note that
47 the replicate recorded a higher quantity and Ct value than all undiluted extracts. No
48 replicates recorded a signal at 1:100.
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54 These qPCR data, presented in Table 2 and shown in Figure 6, suggest inhibition or
55 primer/probe mismatch in the Myren sample extract, which not only affects efficient
56 reactions but also renders the estimates of starting molecules unreliable. For ancient
57 samples, inhibition is not necessarily surprising. Inhibition can be detected by changes in
58 the threshold cycle or slope as compared to an uninhibited reaction or unexpected ΔC_q
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values in a dilution series. The potential inhibition in the Myren sample is demonstrated by a consistent difference across qPCR plates in the slant of the amplification curves, as compared to those of the standards or other positive ancient samples (Fig. 6). Additionally, within the rpoB -1 assay, sample dilutions of the extract showed presence of the gene regions at the same or higher Ct values than the undiluted extract, further supporting the presence of inhibition. Although only one of three 1:10 replicates recorded a signal for rpoB-2, it occurred higher than the undiluted replicates. Nevertheless, the qPCR assays detect low quantities of the conserved region of rpoB. Unfortunately, the final amplicon size for the rpoB-1 and rpoB-2 is 66 and 70 bp, hindering our ability to successfully Sanger sequence the small products. Without sequence data for the qPCR product, we must then consider the concerns of relying on an evolutionarily conserved element such as the rpoB gene (Stadhouders et al. 2010, Yao et al. 2006). Despite its homology in many organisms, rpoB gene sequence-based identification has been shown to be an effective tool to characterize emerging nontuberculous mycobacteria (NTM) and mycobacterial infections because of the high amount of sequence diversity harbored within parts of the gene (Adekambi et al. 2006). However, to target the correct species accurately, primer/probe design must take into account the ever-increasing number of genome projects that produce new sequence data for previously unknown environmental bacteria, including species of NTM.

An additional challenge to molecular paleopathology is that other pathogenic species outside of the MTBC can cause clinical symptoms resembling tuberculosis, namely *M. kansasii* and *M. avium*. In fact, in tests of our assays, these two species were detected by our rpoB-1 primer/probe, at low levels; with an input of ~1E05 known starting copies/ul of *M. kansasii* or *M. avium* DNA, approximately 40 and 8 copies were recorded, respectively, underestimating the known value by 99%. These results indicate the rpoB-1 primer/probe set can record signals from closely-related NTM. The rpoB-2 assay, however, was specific to the MTBC. Given what we observe with mismatches in an alignment between the reference genome and other mycobacterial species *within* the complex present at the 3' end of the primers and probe, any sequence variability or damage at these sites in the ancient template could explain the late, non-exponential qPCR signal. If rpoB sequence information for the Myren sample became available, the high intra- and interspecies variability within the rpoB gene could help refine the classification as a member of the MTB complex, or as one of the pathogenic species causing tuberculosis like clinical outcomes (Adekambi et al. 2003). Independently, DNA from the Myren bone sample was extracted at the Paleogenetics lab at the IAS in Tübingen, Germany, and enriched via in-solution capture targeting five gene regions within the *M. tuberculosis* genome. The capture, product was then sequenced; the capture results are reported in Table S1 of Bos et al. (2014). The capture sequence reads map to < 50% of each targeted region, with a fluctuating range of coverage across each gene, from 0 to over 100x. These coverage values did not meet the threshold criterion used for full genome capture, indicating that the Myren sample does not have adequate DNA preservation. Unfortunately, if an ancient tuberculosis-causing pathogen is present, it is difficult to discern endogenous pathogen DNA from other endogenous but contaminant (myco)bacteria DNA that may have

hybridized to the probes during enrichment. Improvements in methods may allow further analysis and independent confirmation of the presence of MTBC DNA.

Table 1: Primer and probe sequences used in qPCR screening of skeletal samples

Table 2: qPCR results for rpoB-1 and rpoB-2 of Myren extracts and dilution series.

Figure 6. qPCR amplification plots. A. rpoB-1 assay, depicting standards (green), inhibited Myren samples and dilutions (purple), and two uninhibited ancient samples (brown and blue). NTCs=yellow. B. rpoB-1 assay depicting late amplification of all Myren samples and dilutions (blue) with standards (purple). C. rpoB-2 assay, Myren= blue. Green=standards.

5. Discussion and Conclusions

The extreme south of the American continent has only recently entered conversations about the presence of TB among indigenous peoples prior to direct contact with Europeans (Gómez et al 2003). The results presented here are discussed with regard to the possible presence of tuberculosis in humans during the 14th and 15th centuries in this region. The geographically closest evidence of TB in a pre-contact skeleton is recorded in remains recovered from the Salitroso-Basin Inn site in Santa Cruz province, Argentina, 650 km north and dated to between 1224-1380 cal. AD (Goñi et al. 2003/2005; Garcia Guraieb 2006). Locating other possible evidence of pre-contact TB in Patagonia with better preservation is the future agenda. This is important in relationship to other pre-Columbian evidence, e.g. the TB genomes sequenced from the Osmore River Valley, Peru (Bos et al. 2014). At present, it is assumed that this represents the form identified in other South and North American contexts (Roberts and Buikstra 2003; Stone et al 2009). Its relationship to TB documented in the region during the late 19th and early 20th centuries in death certificates from the Salesian Mission in Rio Grande, Tierra del Fuego will be the subject of further inquiry (García Laborde et al 2010). Other hypothesized sources of TB infection for these groups of hunter-gatherers of southernmost South America also need to be explored (Bastida et al 2011).

Two decades ago TB in several otariid pinniped species in Australia and Argentina was first diagnosed (Bernaldelli et al 1996 and Bastida et al 1999). In 2003, molecular studies determined that pinniped TB was caused by a new species described as *Mycobacterium pinnipedii*, which is now included in the MTBC. This new species could have been responsible for TB in humans and in wild and domestic animals from prehistoric and historic Tierra del Fuego, although its membership in the MTBC should have resulted in strong signals for all three of the qPCR tests performed here. The distribution and mobility of hunter-gatherers and their prey, including ethnographic, archaeofaunal, and ecological data will be considered to assess the source and antiquity of this type of TB, which could be caused by other types of mycobacteria, including *M. kansasii* and *M. avium*. Its possible relationship to the disease spectrum of other hunter-gatherers in specific geographical regions will also be examined. Finally, further study using targeted enrichment and next-generation sequencing could shed light on the relationship between the causative pathogen and modern mycobacteria.

As emphasized in the introduction, discussions of the nature and spread of infectious diseases such as TB in the Americas requires a consideration of pan-continental patterning and rigorous tests of alternative hypothesis concerning the nature of ancient TB and its relationship to more recent epidemics. This study represents a first step in addressing these issues from the far south of the Western Hemisphere.

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

Figure 2. Myren 1 Sector 1. (Massone & Jackson 1998).

Figure 4. Thoracic 11th (above), 12th (middle) and lumbar 1st (below) vertebrae of Myren 1 site, showing circumferential resorptive areas on left anterior-lateral surfaces of the bodies.

Figure 5. X-ray images of T11, T12 and L1 of Myren 1. No lytic lesions were observed in the images. These vertebrae were apparently affected by osteopenia and anterior compression of L1 is clearly visible (below right).

Figure 6. qPCR amplification plots. A. rpoB-1 assay, depicting standards (green), inhibited Myren samples and dilutions (purple), and two uninhibited ancient samples (brown and blue). NTCs=yellow. B. rpoB-1 assay depicting late amplification of all Myren samples and dilutions (blue) with standards (purple). C. rpoB-2 assay, Myren= blue. Green=standards.

Primer/Probe	Final concentration	Sequence (5'-3')	Amplicon size (bp)
IS1081_F	900 nM	<i>GCACTCCATCTACGACCAG</i>	84
IS1081_Probe	300 nM	<i>6FAM-ATTGGGCAACAACCTGATTCGGCGTCG-TAMRA</i>	
IS1081_R	900 nM	<i>GGGAGTTTGTCTGGTCAGAG</i>	
IS6110_F	50 nM	<i>GGGTAGCAGACCTCACCTATGTG</i>	63
IS6110_Probe	300 nM	<i>6FAM-ACCTGGGCAGGGTT-MGBNFQ</i>	
IS6110_R	300 nM	<i>CGGTGACAAAGGCCACGTA</i>	
rpoB_F	50 nM	<i>GGTCGCCGCGATCAAG</i>	66
rpoB_Probe	300 nM	<i>6FAM-CTCAGCTGGCTGGTG-MGBNFQ</i>	
rpoB_R	300 nM	<i>GGGTTGTTCTGGTCCATGAATT</i>	
rpoB2_F	300 nM	<i>CAACGTCGAGGTGCTATCG</i>	70
rpob2_Probe	300 nM	<i>FAM-TCGCCGCACCGTCACT-NFQMGB</i>	
rpob2_R	300 nM	<i>CTCCAGGTCCTCGTCCTCA</i>	

Table 1 : Primer and probe sequences used in qPCR screening of skeletal samples

Assay	Locus	Lab Sample Name	Dilutio n Factor	Quantity Copies/ul	Mean Copies/ul	Mean Copies X Dilution	Quantity Copies Std Dev	Positive replicates	Ct Value	Ct Std Dev	Ct Mean	Cq	
aTBassay8	rpoB1	AD216.1	1	0.058000047	0.110536	0.11053572	0.07570621	3 of 3	40.63561	0.960075	39.88925	> 3.227	
aTBassay8	rpoB1	AD216.1	1	0.19731332					38.806175				
aTBassay8	rpoB1	AD216.1	1	0.076293774					40.225975				
aTBassay11	rpoB1	AD216.1	10	1.0434722	1.620302	16.203026	0.71972317	3 of 3	34.79895	0.62125	34.25308		
aTBassay11	rpoB1	AD216.1	10	1.3905947					34.383213				
aTBassay11	rpoB1	AD216.1	10	2.4268408					33.57707				
aTBassay18	rpoB1	AD216.2	1	2.0081918	1.550058	1.5500581	0.647899	2 of 2	31.914865	0.624106	32.35617		
aTBassay18	rpoB1	AD216.2	1	1.0919243					32.797485				
aTBassay18	rpoB1	AD216.2	10	0.27564484	0.175781	1.7578085	0.09255814	3 of 3	34.7916	0.787973	35.58311		
aTBassay18	rpoB1	AD216.2	10	0.09287314					36.367496				
aTBassay18	rpoB1	AD216.2	10	0.15882461					35.59023				
aTBassay18	rpoB1	AD216.2	100	0.11141992	0.070585	7.058489	0.04080977	3 of 3	36.10375	0.970045	36.96132		> 1.378
aTBassay18	rpoB1	AD216.2	100	0.029800422					38.014133				
aTBassay18	rpoB1	AD216.2	100	0.07053434					36.766056				
aTBassay18	rpob2	AD216.2	1	0.001929223	0.002331	0.002331	0.0005682	2 of 2	42.93358	0.3555576	42.68216		> -1.868
aTBassay18	rpob2	AD216.2	1	0.002732785					42.430744				
aTBassay18	rpob2	AD216.2	10	0		0	1 of 3	Undetermined					
aTBassay18	rpob2	AD216.2	10	0		0		Undetermined					
aTBassay18	rpob2	AD216.2	10	0.008372532		0.08372532		40.8139					
aTBassay18	rpob2	AD216.2	100	0		0	0 of 3	Undetermined					
aTBassay18	rpob2	AD216.2	100					Undetermined					
aTBassay18	rpob2	AD216.2	100					Undetermined					

Table 2. qPCR results for rpoB-1 and rpoB-2 of Myren extracts and dilution series.