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Syphilis in South America
A Closer Look at Pre-Contact Bolivia

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Introduction

The origin of syphilis, the apparently new venereal epidemic that swept Europe in the sixteenth century, is a longstanding question in the history of medicine.

Treponemal diseases are among the most widespread infections found in humans, and may have affected hominines since the Pleistocene (Rothschild 2005). Venereal syphilis, caused by *Treponema pallidum ssp. pallidum*, was especially dreaded because of its insidious contagion and its painful, disfiguring, and potentially lethal course. This disease was first recognized and described in Europe in the early sixteenth century AD (Fracastoro 1530). At the time, it was already thought to be a plague introduced from the New World. And, indeed, while skeletons from several pre-contact sites in the Americas show symptoms of one of the treponemal diseases, there are no unambiguous cases from Europe before 1500 AD (Roberts 1994).

Here we will present some findings from our interdisciplinary study of the Loma Salvatierra archaeological site that contribute to this discussion by further clarifying a likely origin and route of transmission of syphilis from the Old World to the New.

Heiko Prümers

Archaeology

From 2003 to 2006, the German Archaeological Institute, in collaboration with the Bolivian "Unidad Nacional de Arqueología," undertook excavations at the pre-Hispanic site of Loma Salvatierra (fig. 1) in the northern lowlands of Bolivia (Prümers 2004, 2008, 2009).

The region, called Llanos de Moxos, is one of the largest seasonally inundated savannahs in the world. It forms part of the hydrological system of the Amazon basin, but constitutes a geographic subregion on its own.

Acknowledgments
We want to thank our colleague Dr. Zuzana Obertová, who was involved in the anthropological examination of the first sample of skeletons and found the first tell-tale saber-shin tibia, with which everything started.

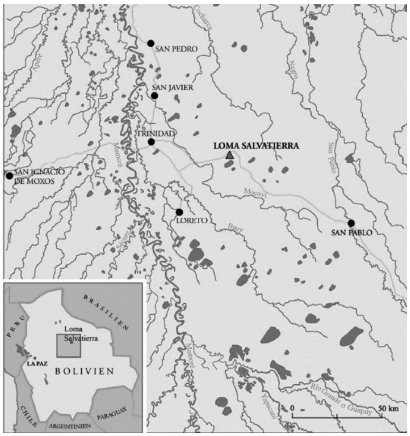


Figure 1: Location of the Loma Salvatierra site (Map: H. P. Wittersheim)

In a archaeological literature, the Llanos de Moxos are well known for their earthworks (ridged fields, canals, causeways), built in pre-Hispanic times for agricultural use, which cover extensive areas to the west of the Mamoré river (Barba 2003; Denevan 1964, 1966, 2001; Erickson 1980, 2006, 2008; Lee 1976, 1995, 1996; Lombardo 2010; Lombardo et al. 2011; Michel López 1993, 1999; Saavedra 2009; Walker 2004, 2008). Much less is known about the habitation sites and, consequently, the pre-Hispanic population of the region. Prior to our project, no survey had been carried out, none of the habitation sites had been properly mapped, and only limited excavations had been conducted at the habitation sites (Nordenskiöld 1913; Dougherty and Calandra 1981, 1981–82, 1984).

The Loma Salvatierra site is located in the southeastern part of the Llanos de Moxos, a region that is not exposed to regular flooding and therefore lacks traces of ridged field agriculture. A survey undertaken by our project over an area of 4500 km^2 revealed the existence of more than a hundred mound sites, generally situated on fluvial deposits of inactive rivers (Lombardo and Prümers 2010). The size of the mounds ranges from one to more than 20 ha, and their height ranges from two to 21 m. According to the radiocarbon dates from our excavations at Loma Salvatierra and nearby Loma Mendoza, the mounds were occupied between 500 and 1400 AD. A detailed analysis of the ceramics found at the sites allowed us to subdivide the occupation period into five phases (Jaimes Betancourt 2004, 2010; Kupferschmidt 2004) that fit well with major changes made to the outline of the platform buildings.

The center of the Loma Salvatierra site is formed by a low artificial terrace, lying in an oxbow of a palaeoriver. The major platform building (mound 1), rising to a height of 7 m, and some lower platforms (fig. 2) were built on this terrace.

On top of mound 1, low platforms are arranged in a U-shape, enclosing a space that opens to the northwest. The site is delimited by a polygonal causeway running at a distance of

approximately 100 m from the central terrace and enclosing an area of 21 ha. To the south are canals, ponds, and dykes, which were most probably used to regulate and/or capture water from the savannah in the south.

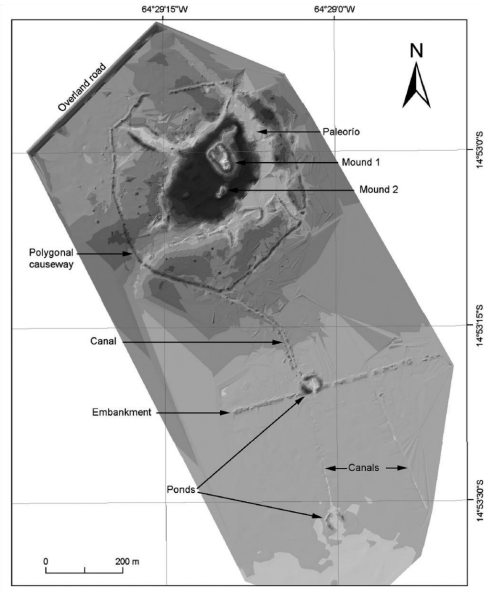


Figure 2: Elevation map of the Loma Salvatierra (Graphic: H. Prümers)

Excavation units were placed at different parts of the site. Two of them, located on top of the major platform building (mound 1), revealed a complex stratigraphy, with the events that formed it corresponding to phases 3 to 5. Only a few graves were found here, but in a very good state of preservation. The ceramic found in this excavation unit was of "high quality" throughout the sequence, indicating that the top of mound 1 might have been reserved for special purposes or was perhaps the residence of the "élite."

The excavation unit located on the terrace to the south of mound 1 revealed contexts from all five phases of the sequence. Large amounts of ceramic fragments and animal bones were found in the refuse layers, as well as some graves. Instead of fancy ceramics, domestic wares prevailed, and a high number of spindle whorls and bone tools suggest that the people living here passed much of their time working. So the area corresponds best to what one would call a "domestic area."

An area of more than 200 m^2 was excavated in mound 2, an L-shaped platform located to the south of mound 1. Almost fifty graves were uncovered here, and only shallow refuse layers were encountered. These refuse layers are probably the result of funerary rites, although they might also result from a short-lived occupation of the mound.

The graves found across the excavation units were astonishingly diverse in the deposition of the body and its orientation. Seated bodies, and some buried face down, are among the most unusual examples. The intriguing pattern of different positions and orientations is clearly visible in the plan of the graves found in mound 2 (fig. 3).

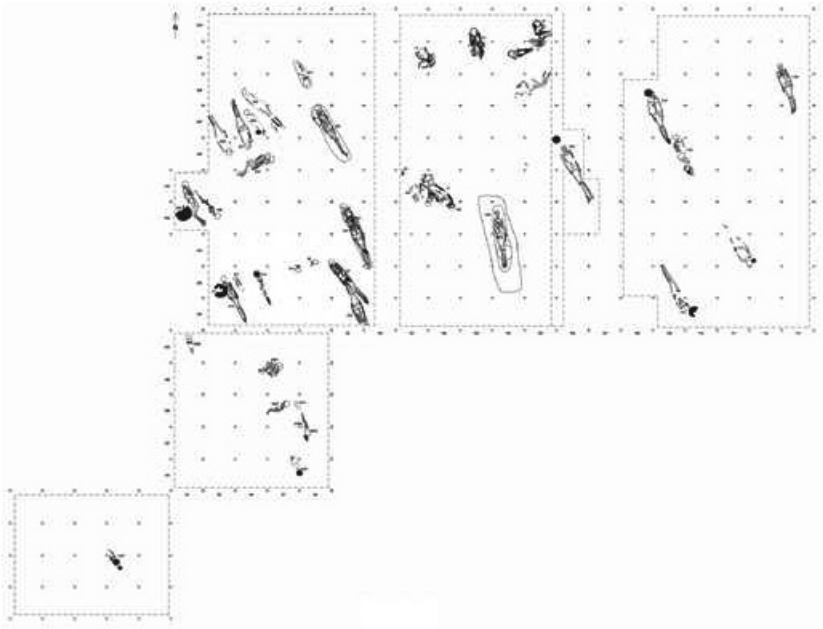


Figure 3: Plan of the graves found in Mound 2, Loma Salvatierra (Graphic: H. Prümers)

Some graves overlie older ones, clearly indicating that they do not correspond to a single point in time. In determining the chronological order of the burials, we faced two major problems. Firstly, the burial pits were, with very few exceptions, invisible, due to the fact that the fill had the same texture and color as the surrounding soil. Secondly, most of the graves had no offerings at all that would have helped in their dating. Nevertheless, most of them could be ascribed to one of the five occupation phases. The plot in figure 4 illustrates some of the changes detectable in burial practice.

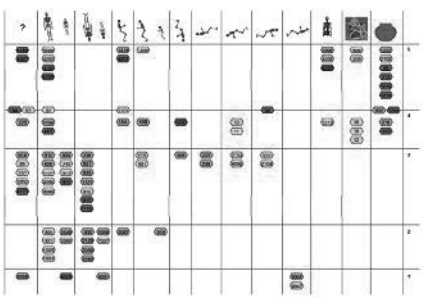


Figure 4: Distribution of different burial types per occupation phase.

Extended burials with the head to the southeast are present only in phases 2 and 3; burials of children with ceramic plates covering the head are restricted to the early phases as well; burials oriented east-west are present in phases 3 and 4; and urn burials, mostly children, make their appearance in phase 4, along with the seated and face-down burials mentioned above.

An outstanding grave, dating to phase 2, was found in the center of mound 2 at a depth of 3 m. It contained the extended skeleton of an adult man who died at the age of approximately thirty-five (fig. 5).

Figure 5:
Grave 1005, Loma Salvatierra. This grave was outstanding for its location at the centre of mound 2 and for the rich grave goods it contained, including copper discs.

Four jaguar tusks and three pendants of blue stone, once part of a collar, were found on the corpse's chest. More than five thousand shell beads were recovered from the torso region, some still aligned in up to seven different strings. The left wrist housed a bracelet of forty-seven carefully worked and polished bone segments. His forehead was covered by a copper disc that probably formed part of a headdress made of organic materials, and thus not preserved. He also wore ear ornaments consisting of smaller copper discs with round segments of armadillo shell on the reverse, and a lip plug made of green stone (amazonite).

To learn more about the 123 individuals whose graves were unearthed in Loma Salvatierra, an interdisciplinary project, funded by the DFG (German Research Foundation), was started in 2009. Some results of this project, which included radiocarbon dating, isotope analysis, and genetics, as well as osteological studies, are presented below.

Martin Trautmann and Iris Trautmann

Anthropology

Material

Tropical climates are notoriously bad for the preservation of skeletal material. Mineral-poor soil, heavy rainfall, and abundant plant growth cause a rapid deterioration of the bones' mineral structure; they usually vanish completely in a matter of decades. Nonetheless, most of the skeletons found at the Loma Salvatierra site are fairly well preserved, allowing for extensive anthropological and archaeometric study. The position of the graves, deep in the compact clay of the tell-like hill, probably protected the bones from elutriation by rainwater and annual flood, and from destruction by plant roots.

With around 123 skeletons, the Loma Salvatierra site has possibly the largest and best-preserved collection of a pre-contact population in the Amazon region. The skeletons show a mean completeness of 33 percent. Losses were mainly caused by disturbance, rather than erosion. In most cases, the bone matrix is strong and undamaged with only slight surface alterations.

Receiving a DFG grant allowed us to supplement our osteological analysis with more costly physical and chemical methods. Our aim was a complete anthropological assessment of the material, including demography, metric documentation, kinship analysis by anatomical variants as well as aDNA, diet and lifestyle reconstruction, palaeopathology, and mobility behavior.

The C-14 dating of twenty-one selected skeletons was very important, providing us with a sound chronological frame for the study. The dead were interred between the fifth and thirteenth centuries AD, predominantly between circa 650 and 1150. Around 1000 AD, there seems to have been a phase of reduced activity and, perhaps, population: possibly a crisis in the history of the settlement. Although contemporary to the neighboring Tihuanaco culture of the Andean highlands, the region had its own cultural character, with particular ways of adapting to the unfavorable environment.

Population Characteristics

The unearthed sample seems representative of a complete, natural population. Spread over the entire period of the settlement's existence, the sex ratio is fairly balanced at fifty-one males to forty-six females; individuals of all ages, from the newborn to the elderly, are represented. Subadult mortality is well within expectations; a somewhat increased mortality of female children may indicate preferential treatment of male offspring. The relative number of very elderly people seems rather low, perhaps because of a slightly above-average mortality rate in all age groups.

Information about external kinship is difficult to obtain, but analysis of the aDNA remains of eighteen individuals put them all in mitochondrial haplogroup C, which is mostly found today in northeastern South America: for example, in northern Brazil and Guyana. Moreover, historical sources report that the former inhabitants of the Beni region were Arawak-speaking tribes, who can still be found in the north of the continent and in the Caribbean. Our data indicate uninterrupted habitation of the Loma Salvatierra site,

perhaps with an influx of new settlers around 1000 AD, but with no general replacement of the earlier population. The settlement died out after the early fourteenth century.

Subsistence

Information about diet and subsistence strategies is the foundation for all further reconstruction of lifestyle and living conditions. The examined skeletons show typical signs of dental disease closely related to the regular consumption of sticky, high-carbohydrate foods. This type of food—usually grain, or starchy tubers—is cariogenic, and an exceedingly high rate of dental decay is present: almost two or three times that of most mixed agricultural premodern populations. Dental abrasion is also high and of a type characteristic of high cereal consumption and caused by the abrasive grit in flour ground in stone or ceramic mills.

The analysis of nitrogen and carbon isotopes indicates an important role for plant food sources, especially C4 plants, such as maize. Maize was documented at the site by the analysis of starch residues in pottery and by burnt seeds. Animal food sources, such as game and fish, seem to have been a dietary staple until circa 1000 AD. The tall stature of the Loma Salvatierra people, compared with genetically close populations, hints at a stable, high-quality food supply. Later on, isotope data as well as a decline in mean body height indicate a decrease in the availability of animal food sources and maize in the later settlement phases after 1000 AD.

Palaeopathology

Skeletons usually show a certain number of stress markers related to physical workload. The Loma Salvatierra population shows an overall high degree of degenerative joint disease, and most individuals also possess strongly developed marks on their bones caused by muscular activity. A handful of individuals seems only slightly affected by heavy work, and, since they also have less dental abrasion (and therefore perhaps higher quality food), it would be logical to identify them as members of a privileged group.

Of special interest is the uncommon absence of physical trauma caused by interpersonal violence. Although the settlement hill has structures that could be fortifications, we found very few injuries, and those were more typical of accidents than violence. The Spanish labeled the Arawak tribes as “peaceful.” Perhaps they were right?

There is no clear evidence of malnutrition: although stress markers suggestive of arrested development phases during childhood—such as cribra orbitalia and enamel hypoplasia—are frequent, the generally tall and robust stature of the subjects indicates a sufficient diet. Therefore, we assume that the stress markers were caused by periods of illness. The Loma Salvatierra population was clearly exposed to a multitude of infectious diseases. For example, the skulls of five children show signs of heavy periostitis (inflammation of the connective tissue surrounding the bones) with plaques of woven bone on the interior side of the skull: probably a symptom of infectious meningitis.

Figure 6-9: Skeletal symptoms of treponemal infection

Up to twenty-five individuals show patches of reaction to inflammation on their long bones, especially the tibiae. Although there are many causes for periosteal woven bone growth, the symptoms we found here are peculiar to treponemal infection.

There are multiple active and healed foci of periostitis with woven bone growth and vascularisation, extensive excrescences, and, in some cases, bloated diaphyses with rough thickening of the compacta and spongy filling of the medulla in tibiae, fibulae, ulnae, femora, and humeri.

We also observed a sabre-shin deformation with thickened anterior compacta in the tibiae of at least two individuals, and short and slightly barrel-shaped upper and lower first deciduous incisors, with a central notch (Hutchinson's sign), in one case.

Although these symptoms are pathognomonic for treponemal infections, they are not specific to a particular one. In humans, there are four different afflictions caused by four closely related pathogens (Petzold 2005):

T. pallidum ssp. pallidum: venereal syphilis
T. pallidum ssp. pertenue: non-venereal yaws
T. pallidum ssp. endemicum: non-venereal bejel
T. carateum: pinta

Pinta, found in Central and Southern America, is a mild disease of the skin and does not affect the skeleton; it may be therefore ruled out as cause of the observed symptoms. Bejel was common in arid regions of the Old World, as in the Near East, North Africa, and even Bosnia. Yaws, in contrast, is found in warm and humid regions of Africa, Asia, Oceania, and the Americas. Syphilis, which is exclusively a sexually transmitted disease, seems to prefer temperate zones; but today it can be found globally (Idsøe and Guthe 1967).

With the exception of pinta, all treponematoses show the same set of symptoms, albeit with varying degrees of occurrence. Only syphilis can be transmitted to the fetus *in utero* and interferes with dental growth, causing the so-called dental stigmata (notched incisors typical of congenital syphilis). The following table compares the frequency of certain symptoms of these three treponematoses with the findings of the Loma Salvatierra population:

	syphilis	bejel	yaws	Loma Salvatierra
periostitis	+	+	+	+
ulcerous lesion	+	(+)	(+)	+
gumma	+	(+)	–	–
caries sicca	+	(+)	(+)	–
medullary closing	+	(+)		+
sabershin-tibia	+	+	+	+
gangosa	+	(+)	+	–
dental stimata	+	–	–	(+)

Table 1: Symptoms of treponematoses

Table 2: Epidemiological characteristics of treponematoses

	syphilis	bejel	yaws	Loma Salvatierra
% of pop. w/ symptoms	5%	25%	33%	6.5 - 20%
# of affected bone parts	<3 elements	<3 elements	>3 elements	>3 elements
usually affected bone parts	tibia, frontal bone, ulna, ribs	tibia, frontal bone, ulna, ribs	tibia, ulna, hand, frontal bone	tibia, femur, ulna, fibula

Although we had some convincing symptoms of treponematosis, we could not arrive at a differential diagnosis with a final degree of certainty due to a considerable overlap in symptoms and the rather atypical picture we got from the Loma Salvatierra finds. Therefore, following Rothschild (2005), we also assessed population epidemiology parameters in the hope of further clarity.

Again, the pattern we encountered was not typical for any of the three infections specifically. The age distribution was also inconclusive: we found nine possible cases in infants up to five years old; only one in a subadult between five and twenty; and fifteen cases in adults over twenty years old. As observed in modern populations, syphilis is usually contracted primarily by sexually active juvenile or adult individuals and only rarely passed on as a congenital infection; yaws, on the other hand, is transmitted by skin contact, the first infection usually occurring at an age of six to ten years (Rothschild and Rothschild 1995).

Since the macroscopic findings alone were not convincing with regard to an unambiguous differential diagnosis, we decided to try for a palaeogenetic proof of the pathogen (DeMelo et al. 2010).

Sandra Lösch and Carsten Pusch

Palaeogenetics

Even in fresh sample material, the identification of *T. pallidum* via polymerase chain reaction (PCR) is not without problems. Bouwman and Brown 2005 showed that ancient samples were useless in their hands and concluded that it is generally impossible to detect treponemal DNA. Due to the DNA degradation in ancient samples, it becomes more difficult to obtain positive PCR results from extracted DNA, even when the morphological symptoms clearly indicate advanced stage III treponematosis.

However, we were able to recover and specifically amplify the polymerase A gene of *T. pallidum* from skeleton samples of the Loma Salvatierra site independently in separate aDNA laboratories in Tübingen (C. Pusch, M. Ball, N. Gaultier) and Munich (A. Nerlich, S. Lösch).

Sampling and Preparation

Our investigation used selected samples of thirty individuals from the burial site for testing.

Negative control samples were also obtained from individuals of this population who showed no symptoms of a treponemal infection. All samples were obtained after excavation, during the subsequent thorough examination of the skeletal remains.

Undamaged teeth—mostly molars—were extracted from the jaw and used as sample material. All necessary precautions to avoid contamination were taken (Roberts and Ingham 2008). Sampling was performed under strictly sterile conditions in laboratories dedicated to work with ancient DNA; all involved were wearing clean clothes, sterile gloves, and masks in an environment uncontaminated by modern samples from patients affected by syphilis. Samples were prepared and sent directly from sample suppliers to cooperating laboratories.

To eliminate contamination, all bone surfaces were first cleaned with a 0.5% sodium hypochlorite solution, and then the outer surface was removed mechanically by sterile instruments. After that, the bones were irradiated with UV-light with a wavelength of 255 nm. Material was then taken from the dentine inner part next to the pulpa cavity. This was then used to produce a homogeneous bone powder using a mixer mill.

Other precautions against contamination were also taken, including a strict separation of work areas between sample handling, DNA extraction, PCR set-up, and post-PCR procedures using different laboratories. Fresh sterile filter tips were routinely used, and surfaces and equipment in contact with sample tubes were cleaned before and after each assay. The results were replicated independently in the Munich laboratories. Here, a different methodological approach was used.

None of the laboratories had previous contact with treponemal DNA of either recent or historic origin. Furthermore, extraction protocols of aDNA and work-up of the samples were different in the two laboratory sets, further ruling out mere carry-over or handling contamination.

Methods of Amplification and Identification of Treponemal DNA

DNA was extracted from all samples according to Scholz and Pusch (1997), purified in a number of subsequent steps, and then subjected to PCR. Some of the crude DNA extracts turned out to be inhibitory, which somewhat correlated with the extracts' color.

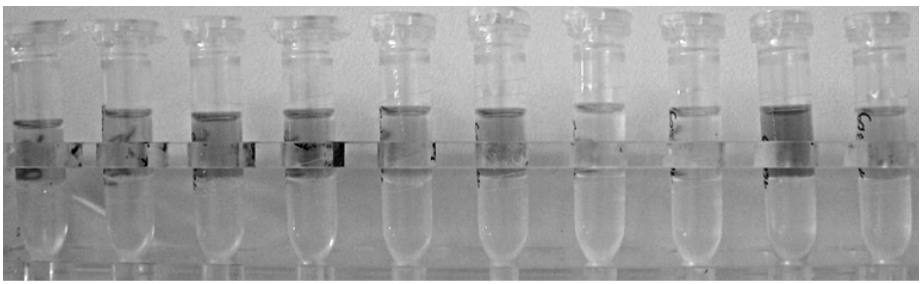


Figure 10: Exemplary extraction of ancient DNA from 10 LoSa specimens. Chloroform phase is at the bottom, the aqueous phase showing a spectrum of colors contains nucleic acids.

In these cases, further purification and/or sample dilution prior to PCR was required. The target of the two-step amplification was the gene encoding for the polymerase A of *T. pallidum*. Sanger sequencing of the assays yielding PCR bands of the correct size confirmed that the amplification was specific to the species *T. pallidum.*

Firstly, in order to track the extraction efficiency and to rule out inhibition of the PCR reaction, the presence of DNA was investigated by analysis of the presence of a cytoplasmic human multicopy ß-actin-gene (Nakajima-Iijima et al. 1985). Negative PCR controls were always included. We carried out 40 cycles with an annealing temperature of 60°C using the Veriti 96-w Thermal Cycler (Applied Biosystems, Darmstadt).

For detection of *T. pallidum*, a heminested PCR protocol (Behrhof et al., 2007) was performed with a total volume of 25 µl for each sample, utilizing FastStart Polymerase (Roche). Cycling conditions for the first round of PCR were set with five minutes at 95°C, followed by forty cycles of thirty seconds each at 95°C, thirty seconds at 53°C, thirty at

72 °C, followed by seven minutes at 72 °C. For the subsequent nested PCR, the same conditions were observed, but applying thirty-six cycles. The nested primers locate to the polymerase A coding region of *Treponema pallidum*. We used the primers TPmod-R1, TPmod-F1, and TPmod-F2 according to Behrhof and coworkers (2007). TPmod-R1 and Tpmod-F1 yielded an amplification product of 150bp, and TPmod-R1 and TPmod-F2 finally resulted in a treponemal-specific amplification product of 125bp.

Samples from seven individuals repeatedly provided a positive amplification product of *Treponema pallidum* DNA of the expected size of 125 bp.

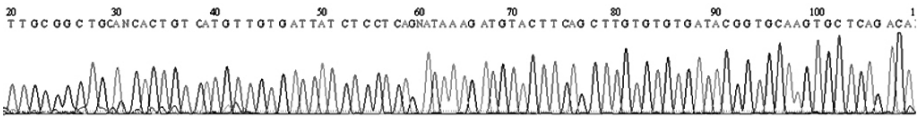


Figure 11:
Electropherogram showing the region of the polymerase A gene of *T. pallidum* from sample LoSa0018. The highlighted segment covers the region of the DNA sequence deposited under accession number CP001752.1 in NCBI nucleotide collection.

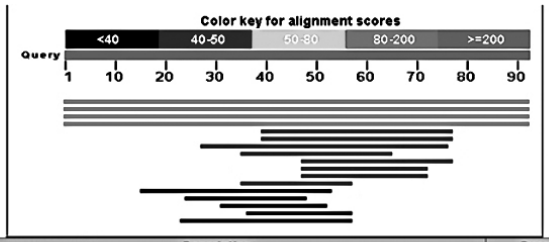
Successful replication tests on one sample were performed in Munich under different conditions: A 50 µL reaction mix was used with 2.5 µL of DNA extract, hot start Taq polymerase (1.25 U), negative and blank extraction PCR controls were always included. We carried out forty cycles of a touch-down PCR, starting annealing at 64 °C, and reducing the temperature after the first five cycles by 0.1 °C every further cycle using the Veriti 96-w Thermal Cycler (Applied Biosystems, Darmstadt).

PCR products were detected on 2 % agarose gels after staining with ethidium bromide and visualisation under UV light. Purification of the PCR products in solution after amplification was performed with "High Pure PCR Product Purification Kit" (Roche, Penzberg Germany), and gel purification of DNA fragments was performed with "NucleoSpin Extract II" (Macherey-Nagel, Germany). DNA sequence integrity of the obtained PCR products was determined by Sanger sequencing employing the initial PCR primers.

Database analyses were done utilizing the BlastN algorithms at the NCBI platform.

Results

The sequencing of the PCR products resulted in a 100% coverage and a 100% identity of our sequence to the polymerase A gene of *T. pallidum* deposited in the NCBI nucleotide collection with blastN. This result was also replicated in Munich.



Accession	Description	Query coverage	Max ident
CP001752.1	Treponema pallidum subsp. pallidum str. Chicago, complete genome	100%	100%
CP000805.1	Treponema pallidum subsp. pallidum SS14, complete genome	100%	100%
AE000520.1	Treponema pallidum subsp. pallidum str. Nichols complete genome	100%	100%
U57757.1	Treponema pallidum PolA gene, partial cds	100%	100%
CP001357.1	Brachyspira hyodysenteriae WA1, complete genome	41%	86%
AF038544.1	Serpulina hyodysenteriae DNA polymerase I (polA) gene, complete cds	41%	86%
AJ238762.1	Rickettsia helvetica ftsY (partial), polA, dnaE (partial) and hicB genes and metK pseudo	53%	79%

Figure 12: Blast results against NCBI nucleotide collection with blastN

The data for the polymerase A gene from different subspecies of *T. pallidum* are not yet represented in the databases. Our genetic findings point to *T. pallidum ssp. pallidum*, which is causative for syphilis; but we cannot exclude the possibility that, for example, the closely related disease yaws, caused by *T. pallidum ssp. pertenue*, shows the same nucleotide sequence in the targeted PCR amplicon. Therefore, ongoing research will focus on the known SNPs of different treponemal strains and substrains for clarification of the exact pathogen and its evolutionary context in history.

Furthermore, we suggest that good results can only be expected with samples from individuals suffering from stage I or II treponematosis, but not manifest stage III showing the more obvious morphologically diagnostic osseous symptoms. This is presumably because treponematoses are refractory to PCR amplification, since the treponemal titer is obviously far too low in the respective individuals (Behrhof et al. 2007). Higher titers are known from the early stages of syphilis corresponding to stage I and II. In other words, bones with an obvious pathology of treponematosis would be less useful in ancient DNA analyses.

Conclusions

The excavations at the pre-contact settlement site of Loma Salvatierra in lowland Bolivia turned out to be an archaeological source of the highest importance. It became evident that large, independent, specialized, and complex societies existed in the Amazonian basin, contemporary to the Andean civilizations, showing a high degree of adaptation to a challenging environment.

Even in the first millennium AD, these populations were plagued by an insidious disease: venereal syphilis, which caused a global epidemic starting in the Age of Exploration, about 1500 AD. Our results demonstrate conclusively that the pathogen *Treponema pallidum ssp. pallidum* was already present in populations that settled northeastern South America, and presumably also the Caribbean.

In accordance with Harper et al. (2008), who reconstructed a phylogenetic tree of Treponema, we suggest that an American branch of yaws may have developed into an early strain of syphilis. The close vicinity of warm, humid conditions to cool and arid ones in the Amazonian-Andean transition zone would have been the perfect stimulus for the pathogen's adaptive changes. After 1300 AD, when the Llanos de Moxos were deserted, the disease could have spread—presumably through migrating Arawak-speaking tribes—towards the Caribbean.

This model would strongly support the hypothesis that Columbus's crew contracted the disease from contact with native peoples and brought it to Europe, from whence it spread worldwide.

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