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Demography, evolution, health and cultural genocide of South Native Americans: new insights

(Demografia, evolução, saúde e genocídio cultural dos nativos do sul da América: novas descobertas)

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"We are living in times of profound contrasts. On one hand, we have the fantastic progress of science and technology, which is advancing our understanding of ourselves and the external world; but simultaneously, threats from various anti-science movements have emerged... These movements must be firmly combated. If there is one social institution that has significantly contributed to human happiness, it is science, and this fact must be clearly communicated to the general public. But science alone is not sufficient for a better future. **A profound commitment towards a world where social differences among nations and people are more equitable than they are today is necessary, leading to the goal of maximizing happiness for the greatest number of people**."

- Professor Emeritus of the UFRGS Francisco Mauro Salzano, 2018

A mi abuela Carmen, que tus abrazos sean eternos. Gracias por impulsar a esta familia a llegar lejos y soñar alto.

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Contents

Resumo

A presente Tese entrelaça dois temas centrais tendo como foco central populações indígenas americanas: (1) a dinâmica da microbiota oral, destacando suas implicações para a saúde e a história evolutiva dos organismos envolvidos; e (2) o panorama genético e demográfico das populações indígenas americanas visualizado através da análise de genomas mitocondriais (mitogenomas), salientando a importância dos estudos genéticos para essas comunidades frequentemente negligenciadas. Desse modo, os resultados estão apresentados no Capítulo 3, onde adentramos, através de uma revisão de escopo, tópicos como a coevolução dos microrganismos orais dentro do hospedeiro humano, discutindo fenômenos intrincados como a corrida armamentista e as dinâmicas de Rainha Vermelha ou Rainha Negra. Neste capítulo também é explorado o impacto da disbiose na saúde oral e sistêmica do hospedeiro, e como povos negligenciados como os indígenas americanos são duplamente impactados; diante da falta de dados sobre a sua microbiota e saúde oral, bem como de acesso facilitado aos tratamentos. Ainda, abordamos a preocupante questão da resistência aos antibióticos e os genes associados ao fenômeno, considerando as bactérias da microbiota oral, e os importantes alertas da Organização Mundial de Saúde sobre a necessidade de avançar os conhecimentos e estudos na área. Expandindo para além do ecossistema oral, o Capítulo 4 explora as dinâmicas demográficas das populações indígenas no sul do continente americano. Através da análise dos mitogenomas, postulamos que as populações miscigenadas contemporâneas desempenham um papel fundamental na preservação de linhagens mitocondriais autóctones, especialmente na região sul. Nosso abrangente banco de dados de mitogenomas lança luz sobre a diversidade genética e os padrões demográficos em todo o continente, revelando distintos padrões, considerando as populações miscigenadas e nativas do cone sul da América do Sul e aquelas nas regiões dos Andes e Amazônia. Isso sublinha a importância de examinarmos contextos geográficos e culturais diversos. Além disso,

propomos que uma forma de genocídio cultural se fez presente em algumas partes do continente, onde cruzamentos/casamentos assimétricos ocorreu, de modo que a extinção física de muitos indígenas não foi absoluta devido à integração de mulheres nas sociedades nacionais emergentes. Paralelamente a essa incorporação, um extermínio cultural total ocorreu por meio de vários mecanismos explorados em profundidade nesta Tese.

Em resumo, nossas descobertas ressaltam a relevância de estudos com populações indígenas, fornecendo uma revisão com dados valiosos sobre a complexa interação entre a microbiota oral e o hospedeiro humano, a negligência no trato com populações autóctones e a diversidade genômica, estabelecendo por fim conexões do binômio saúde/doença com reflexões antropológicas oportunas.

Palavras-chave: microbiota oral, coevolução, povos nativos americanos, genocídio cultural, mitogenomas.

Abstract

The present thesis intertwines two central themes focused on Native American populations: (1) the dynamics of oral microbiota, emphasizing its implications for the health and evolutionary history of the organisms involved, and (2) the genetic and demographic landscape of Native American populations visualized through the analysis of mitochondrial genomes (mitogenomes), highlighting the importance of genetic studies for these often overlooked communities. As a result, the findings are presented in Chapter 3, where we delve, through a scoping review, into the coevolution of oral microorganisms within the human host, discussing intricate phenomena such as the arms race and the dynamics of the Red Queen or Black Queen. In this chapter, we also explore the impact of dysbiosis on the oral and systemic health of the host, as well as how neglected peoples like Native Americans are doubly affected due to the lack of data on their oral microbiota, oral health, and easy access to treatments. Furthermore, we address the issue of antibiotic resistance and the genes associated with this phenomenon, considering oral microbiota bacteria and the significant warnings from the World Health Organization about the need to advance knowledge and studies in this area. Expanding beyond the oral ecosystem, Chapter 4 explores the demographic dynamics of indigenous populations in the southern part of the Americas. Through analyzing mitogenomes, we posit that contemporary mixed populations play a fundamental role in preserving autochthonous mitochondrial lineages, especially in the southern region. Our comprehensive database of mitogenomes sheds light on genetic diversity and demographic patterns across the continent, revealing distinct patterns among mixed and native populations in the southern cone of South America and those in the Andes and Amazon regions. This underscores the importance of examining diverse geographical and cultural contexts.

Furthermore, we propose that a form of cultural genocide occurred in some parts of the continent, where asymmetrical crossbreeding/marriages took place, resulting in the physical extinction of many indigenous people not being absolute due to the integration of women into emerging national societies. Alongside this incorporation, total cultural extermination occurred through various mechanisms explored in depth in this thesis. In summary, our findings emphasize the relevance of studies involving indigenous populations, providing a review with valuable data on the complex interaction between oral microbiota and the human host, neglect in dealing with indigenous populations, and genomic diversity, ultimately establishing connections between the health/illness paradigm and timely anthropological reflections.

Key words: Oral microbiota, co-evolution, Native Americans, cultural genocide, mitogenomes.

CHAPTER 1

INTRODUCTION

1.1Initial comment

Considering the context of the COVID-19 pandemic and the numerous demands arising from this exceptional situation, the Thesis project was occasionally interrupted for health treatment, and to support the Human and Molecular Evolution Laboratory (HMEL or LEHM in Portuguese) team in their research on the evolution of the SARS-CoV-2 strains and their ability to infect humans (Annex I: Yépez et al., 2022; Evolutionary history of the SARS-CoV-2 Gamma variant of concern (P.1): a perfect storm. *Genet Mol Biol* 45:e20210309). Nonetheless, the original Thesis project was successfully developed, alongside another one that also addressed the theme of Native American peoples. Consequently, this PhD Thesis will be structured into several sections, each addressing its distinct phases.

Recently, there has been a suggestion that the term "Native American" should be exclusively a political designation in the United States (Tsosie et al., 2020). However, in this Thesis the term will be used to refer to any indigenous person from the American continent. We acknowledge that the authors, all of whom are North Americans, may be viewed as engaging in an inadequate appropriation of the term, thus potentially unintentionally contributing to a form of neocolonialism. Additionally, the term "America" will be used to denote the American continent rather than exclusively referring to the United States of America.

1.2 Native Americans

America was the last continent occupied by humans in pre-colonial times, but its initial settlement is a long-standing topic of debate, cutting across many disciplines. Initially, it mainly involved fantasy, unscientific narratives full of prejudices, produced by the European conquerors.

Christopher Columbus and his sailors arrived at lands they initially believed to be part of the Indies, filled with hopes of uncovering the marvels described by Marco Polo. However, as time passed, Columbus became increasingly disheartened, realizing that the legendary civilization, teeming with gold riches he had envisioned, remained elusive. Unbeknownst to Columbus, this continent was home to millions of people, boasting impressive civilizations and cities that rivaled the size of Madrid (Salzano & Bortolini, 2002). Columbus's misperception of these lands ultimately gave birth to the term "Indians" to refer to the inhabitants.

A few years later, while mapping the Brazilian coastline in the service of Portugal, Amerigo Vespucci held a different perspective from Columbus. He saw the recently discovered lands as an extensive new continent. In recognition of his contribution, this newfound continent was named America after him. However, a few decades later, some opulent American civilizations, rich in gold but also in culture, succumbed to the onslaught of Pizarro and Cortez's horse-mounted conquistadors.

Concurrently with the recognition that this continent had hitherto been uncharted territory for Europeans (aside from the Vikings who had reached Greenland centuries earlier) and was inhabited by people with unfamiliar customs and languages, inquiries into its origins began to emerge.The early European conquerors also propagated a myth about the indigenous peoples, depicting them as primitive beings, not fully human, or even as human-like creatures descended from other South American primates that had never been encountered before. In

this context, the murder, extermination, slavery, and rape were deemed acceptable, even among fervent Christian conquerors and colonizers. Furthermore, these indigenous individuals were frequently exhibited as living or deceased anomalies in Western museums, circuses, and fairs, alongside other numerous native groups from various European kingdoms and empires (Santos et al., 2007).

Although more than 500 years have passed since the arrival of Christopher Columbus, and now studies on their origins have a scientific nature, Native Americans continue to be disrespected in their rights and needs as will be seen in items of this Thesis.

1.3 Scientific studies and the settlement of America: demography and evolution

This item does not aspire to serve as a comprehensive review of the subject concerning the colonization of America and its ramifications for the evolutionary and demographic trajectory of Native Americans. Rather, its sole objective is to furnish a theoretical framework on the subject, thereby enhancing comprehension of the results generated from the present PhD Thesis.

A well-deserved recognition for the studies of Professor Franciso Mauro Salzano from the 1950s, who led the LEHM team for several decades, brought light to the completely unknown genetic panorama of Native Americans. Such studies were largely based on data on blood systems and protein polymorphisms, in addition to epidemiological data and rare genetic diseases (see examples in Salzano & Callegari-Jacques, 1988). For example, the fission-fusion concept regarding hunter-gatherers from the American continent was identified from these studies in collaboration with North American geneticist James Neel (see revision in Salzano, 2009). More recently, genetic data based on DNA information, including the efforts of the LEHM research team, have been used to rescue this history at a level of

accuracy probably unimaginable just a few years ago (Bortolini, 2019). This new set of genetic data has helped to raise the level of knowledge on many topics related to Native Americans, including where and how they were dispersed throughout the continent.

Based on genetic and archeological data sets, the current consensus is that the human arrival into the American continent occurred probably through one or more streams of migration of hunter-gatherers from Northeast Asia and Siberia between ~26 and 12 thousands of years ago (from now on ka) (probably in the latter half of that range) using the Beringian land bridge (Figure 1) (Bortolini et al., 2003; Llamas et al., 2016; Braje et al., 2017; Brandini et al., 2018; Gnecchi-Ruscone et al., 2019; Pena et al., 2020; Bisso-Machado & Fagundes, 2021). This migration entailed a prolonged isolation phase in Beringia (also known as the Beringian Standstill) spanning potentially thousands of years (~2-9 ka), preceding the settlers' ingress into the continent (Tamm et al., 2007; Gonzáles-José et al., 2008; Bortolini et al., 2014; Llamas et al., 2016; Braje et al., 2017; Brandini et al., 2018; Pinotti et al., 2019; Gnecchi-Ruscone et al., 2019).

In addition to this well-known Siberian-Beringian component, a Harvard team with help of the LEHM, showed that Native Americans have a striking genetic affinity with contemporary Austromelanesians. This unprecedented genetic signal was linked to a potential "ghost" ancestral population called by the authors as *Ypykuéra* (or 'population Y') (Skoglund et al., 2015; Castro e Silva et al., 2022). *Ypykuéra* means "ancestor" in the Tupi language.

The migration into the American continent consequently led to significant genetic and cultural differentiation among groups, with multiple events of genetic exchange occurring between various ancient populations thereafter (Ramallo et al., 2013; Bisso-Machado & Fagundes, 2021). Furthermore, González-José- et al (2007) and Bortolini et al. (2014) suggested that the biological and cultural characteristics of the first Americans, which began to emerge, in part, during a standstill period in Beringia, were reshaped by recurrent

trans-Beringian/circum-Arctic gene flow and local population dynamics (already within the American continent). It is worth noting that this recurrent process of trans-Beringian/circum-Arctic gene flow influenced initial indigenous biological and cultural characteristics, especially in North America.

Although the Asian origin of Native Americans has been confirmed, discarding other possible origins (via trans-Pacific or North Atlantic), subsequent timings, number of founder events, and diffusion processes within the American regions are still debatable (Gnecchi-Ruscone et al., 2019). For example, the Clovis archaeological culture is the most broadly distributed archaeological tradition in North America and was until recently considered the earliest. However, a number of sites in both North and South America pre-date Clovis sites, indicating that Clovis hunters were not the earliest migrants to America (O'Rourke & Raff., 2010; Willerslev & Meltzer, 2021). Several pre-Clovis archeological sites such as Monte Verde in Chile, have raised the possibility of human presence in the southern cone of South America, by 14–15 ka or even earlier, during the final Pleistocene, which would mean that the spread of Paleo-Indians throughout the American continent would have occurred very rapidly which have been confirmed by several genetic studies (Bortolini et al., 2003; Battaglia et al., 2013; Arias et al.,2018; Brandini et al.,2018).

In recent years, archeological evidence has indeed shown that people occupied North America prior to Clovis and findings at multiple sites in South America have confirmed that Paleo-Indians not only had already spread through the southern subcontinent, but had also colonized extreme high-altitude Andean environments at the time in which the Clovis culture was developing in North America (Brandini et al., 2018).

Several authors have proposed some possible routes of entry into the continent, one through the Pacific Coast (16 - 15 ka) following a Pacific Rim coastal corridor rich in aquatic and terrestrial resources (Llamas et al., 2016; Braje et al., 2017; Brandini et al., 2018;

Gnecchi-Ruscone et al., 2019; Bisso-Machado & Fagundes, 2021; Willerslev & Meltzer, 2021) and other through the ice-free corridor between the Cordilleran and Laurentide ice sheets, although the latter has recently been challenged by geological evidence, and by ancient DNA (aDNA) from both fossil bison and lake sediments (Pedersen et al., 2016; Heintzman et al., 2016; Willerslev & Meltzer, 2021). Some northern populations seem to have remained in the north of the continent for a long time, but at some point in the Holocene they may have moved even further north, since they are now found in Alaska and The Yukon (Mendes et al, 2020; Willerslev & Meltzer, 2021).

Later, after the initial entry, there were successive internal splits. Demographic modeling using aDNA suggests a latter split between Northern Native American and Central/Southern Native American populations dated 17.5-14.6 ka which likely occurred south of the ice. The dispersal patterns of both groups, once south of the continental ice sheets, diverged considerably (Mendes et al, 2020; Willerslev & Meltzer, 2021).

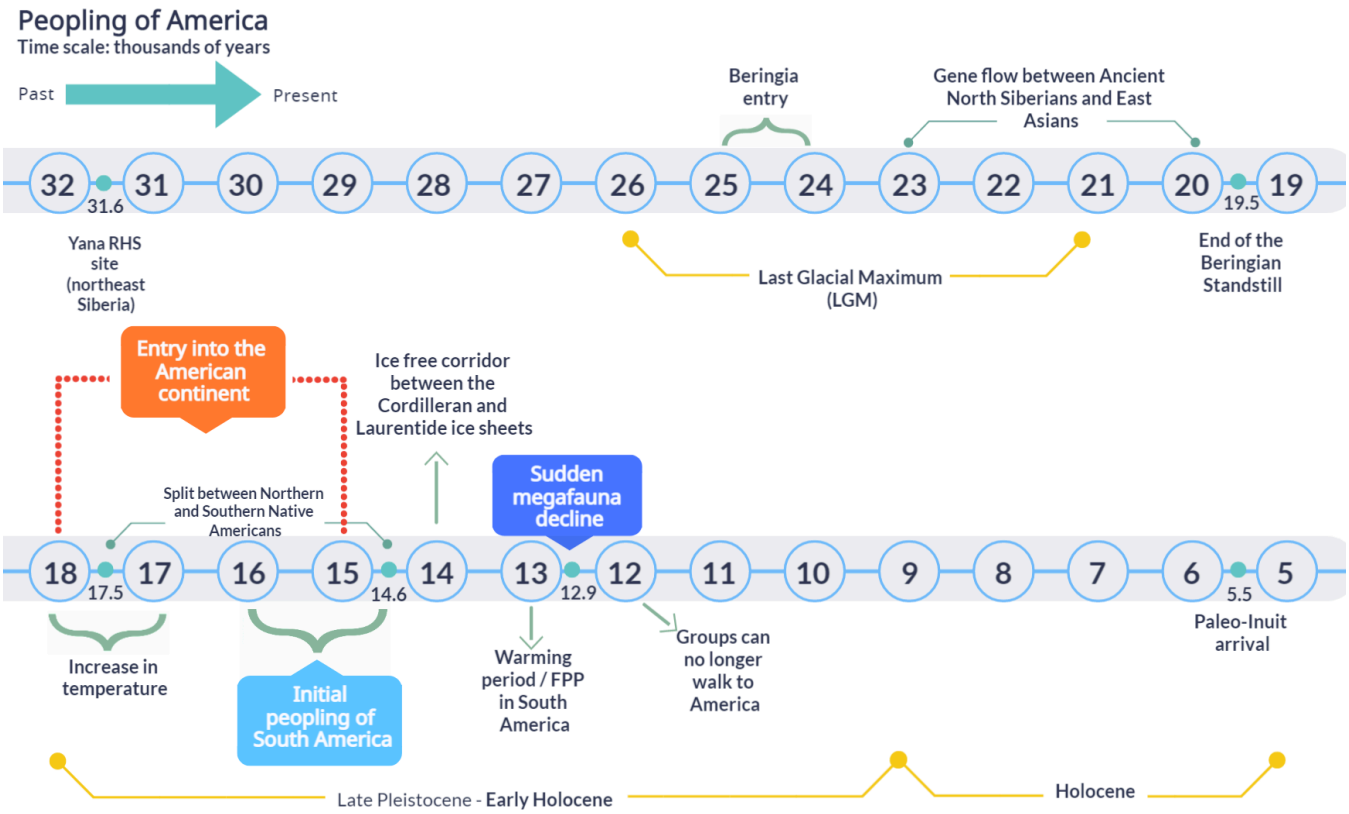

Figure 1. Diagram illustrating the key events in the peopling of the American continent. Dates are approximations based on both genetic and archaeological studies (Brandini et al., 2018; Gomez-Carballa et al.,2018; Pinotti et al., 2019; Pires et al., 2020; Prates et al., 2020; Prates et al., 2021; Stewart et al., 2021).

1.4 Unique Attributes of South America

Despite some differing opinions (Willerslev & Meltzer, 2021), the dispersal across South America has been considered relatively rapid, occurring over a period of about 1,500 years (Bortolini et al., 2003; Battaglia et al., 2013; Arias et al.,2018; Brandini et al.,2018; Mendes et al, 2020; Motti et al., 2023), evidenced in the genetic closeness between individuals who lived at approximately the same time (10 ka) in extremely distant places in North and South America (Pinotti et al., 2019).

By the time that the migration wave arrived the northern latitudes of South America,

Native Americans split following two possible routes of dispersion: the Pacific and the Atlantic Coast (Gómez-Carballa et al., 2018; Mendes et al., 2020). Data from mitochondrial DNA (mtDNA; the particularities of this genetic system of exclusively maternal inheritance will be seen in the item 1.5 “mtDNA and estimation of effective sample size” later in this Thesis) indicates that Native Americans would already be in Ecuador and Peru between 16-14 ka, a finding that fits with archeological evidence attesting to human presence at the Monte Verde (Chile) site at least 14.5 ka (Brandini et al., 2017). Regarding Y chromosome data, Pinotti et al. (2019) provided key insights into the genetic history and migration patterns of indigenous populations across the American continent. The authors identified several independent lineages believed to be founders, including the widespread Q-M3, the rarer Q-CTS1780, the very rare C3-MPB373 in South America, and possibly C3-P39/Z30536 in North America. The study estimates a Beringian Standstill duration, of either 2.7 or 4.6 ka depending on the model used, followed by a southward migration about 19.5 ka. Notably, the research highlights a star-like expansion of the Q-M848 lineage within Q-M3, commencing approximately 15 ka in the America, leading to substantial spatial structure in South America. The study suggests that male population structure was already evident in South America, in agreement to the significant heterogeneity in South American archaeological traditions, contrasting with the relatively uniform Clovis tradition in North America during the same period (Pinotti et al., 2019).

Over a period of approximately 14,000 years, significant environmental changes occurred, starting with the climatic transition from the cold and dry Pleistocene to the warmer and wetter Holocene (see timeline in Figure 1), which impacted indigenous settlements in South America. During this period, ancient South Americans experienced demographic, evolutionary, and cultural transformations, likely influenced by the transition from a hunter-gatherer lifestyle to improved forms of agriculture, with a notable emphasis on

developments in the Andes region (Salzano & Bortolini, 2002; Pena et al., 2020).

This transition also allowed people to live in hostile ecosystems, with different selective forces. For example, the Andean Altiplano, characterized by high incidence of UV light, low concentration of free oxygen in the atmosphere and large thermal amplitude during a day, has been occupied continuously since the late Pleistocene, ~12 ka, which places the Andean natives as one of the most ancient populations living at high altitudes, enough time for specific adaptations (Jacovas et al., 2018). For instance, the LEHM team has discovered a unique genetic repertoire for adaptation to high altitudes among individuals who have resided in the Andes since pre-Columbian times (Jacovas et al., 2015, 2018, 2021).

The great distances that the first settlers of South America traveled in their initial movement increased the chances of isolation and divergence, leading to the formation of small, isolated groups, typical of hunter-gatherers which in turn led to a particular pattern of diversity, which contrasts with those in more populated areas in the regions of great empires with populous and well-organized cities before the arrival of the European conquerors (*e.g.*, Inca, Aztec) (Salzano & Bortolini, 2002). The emergence of these complex societies structured in the form of empires was only possible through domestication of plants (such as potatoes) and animals (like camelids), which supported a sedentary way of life.

Hunter-gatherer societies are characterized by their limited gene exchange between different groups, which leads to substantial genetic drift due to their relatively small population sizes. As a consequence, this typically results in reduced genetic diversity within each population and significant genetic differentiation among neighboring groups. In contrast, agricultural societies, such as those in the Andes, with larger population sizes and sedentary lifestyles, experience greater gene flow and a decreased influence of genetic drift. Consequently, these populations often exhibit higher genetic diversity within their own groups and lower genetic differentiation among distinct populations (Tarazona-Santos et al.,

2001; Dos Santos et al., 2009; Reales et al., 2017) (Figure 2). More recent studies correlating genetic diversity among Native American peoples with geography, environment, and cultural factors, also found that populations in western South America show greater homogeneity compared to those in the east (De Oliveira et al 2023).

Furthermore, some authors, utilizing broader and robust genetic datasets, demonstrated a distinct East/West genetic structure within the Andes, corroborating the long-established notion of a geographical barrier separating Andeans from Amazonians (Gnecchi-Ruscone et al., 2019; De Oliveira et al 2023).

On one hand, the limited gene flow and reduced population sizes in the Amazonian area may be related with its location; both the dense vegetation and the enormous linguistic diversity throughout the basin may have limited population size and kept communities isolated (Lewis et al., 2007; Dos Santos et al., 2009). On the other hand, beyond the largest and most intricate societies, Andes is the home to one of the continent's most prevalent indigenous language families (Quechua), an important sign of relative homogeneity, at least at the linguistic level. As highlighted above, linguistic barriers are important limiters of gene flow, and can perpetuate ethnic-cultural segregations and thus shape specific genetic patterns.

Gómez-Carballa et al. (2018) postulated that indigenous populations residing in the Andes not only exhibited limited genetic exchange with those to the East of South America such as Amazonian populations, but also had minimal contact with those living further to the south, with a few exceptions. The Amazon forest may have also served as a geographical barrier to gene flow between the Pacific and Atlantic expansion waves, facilitating the emergence of the classic diversity pattern illustrated in Figure 2. Moreover, the complex dynamics of hunter-gatherer societies encompass various processes, including fission-fusion dynamics (Neel & Salzano, 1967; Salzano, 2009; Ramallo et al., 2013), demographic expansions (Castro e Silva et al., 2020; Ramallo et al., 2013), and differentiation linked to

cultural practices (Hünemeier et al., 2012b).

For some authors, this unique pattern of genetic diversity makes it difficult to fully understand how migration occurred within South America (Lewis et al., 2007), while for others, it is reflecting the nature of the dispersal throughout the continent. For instance, the pattern mentioned above (Figure 2) may have arisen during the initial peopling of South America, with Andean and Amazonian populations originating from separate migrations at different timeframes. (Lewis et al., 2007; Dos Santos et al., 2009).

Conversely, some authors propose that Amazonian populations have been involved in recent gene flow with each other and across ecogeographic domains (Barbieri et al., 2019). Additionally, Castro e Silva et al. (2022) suggested that no distinct genetic division between the Andes and the Amazonia can be discerned, concluding that a gradient of genetic variation exists along a west-east axis. These genetic findings pose a conflict with the conventional perspective of small and isolated groups throughout all human history in the East region, particularly in Amazonia.

More recent archaeological and ethnographic discoveries have also challenged the idea of small and isolated groups, as there are signs of the existence of large interconnected Amazonian communities (Levis et al., 2018; de Souza et al., 2018; Moraes & Neves, 2019; Silva et al., 2021; Peripato et al., 2023; Schmidt et al., 2023). Despite these suggestions, the difference remains notably pronounced between the Native American peoples who developed a sedentary lifestyle supporting large empires, and those from the lowland areas (such as Amazonia, Gran Chaco, the Brazilian savanna known as Cerrado, and Patagonia), where agriculture never ceased to be incipient. In this way, the general pattern of genetic diversity, as visualized in Figure 2, seems to reflect more accurately the evolutionary, demographic and cultural history of the South Native Americans.

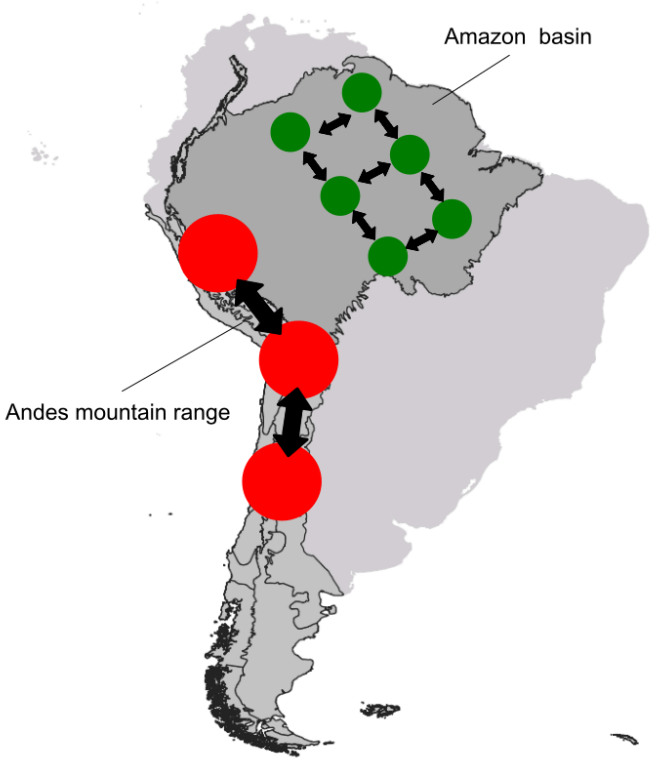

Figure 2. Diagram illustrating the comparison of genetic structure variability between the Andes (red) and Amazonian (green) autochthonous populations.

Circle sizes roughly indicate the relative effective sizes of populations representing the region. Arrow sizes denote gene-flow levels (Modified from Tarazona-Santos et al., 2001). In hunter-gatherer societies typically there is a low rate of gene flow between populations and significant impact of genetic drift due to relatively small population sizes. This often results in reduced intra-population genetic variability and high genetic differentiation between neighboring populations. In contrast, agricultural societies, with larger population sizes and greater sedentary lifestyles, exhibit higher gene flow and a reduced importance of genetic drift. Consequently, these populations often display greater genetic variability within their own populations and lower genetic differentiation between distinct populations.

Although the populations in the Andes have been more extensively studied (Gómez-Carballa et al., 2018; Harris et al., 2018; Barbieri et al., 2019; Gnecchi-Ruscone et

al., 2019; Jacovas et al., 2015, 2018, 2021), populations from lowland areas are generally less represented, despite the efforts of research groups whose results have already been mentioned above. This underrepresentation limits a better understanding because inferences about the genetic structure of an entire region are based on data from a limited number of populations. Similarly, studies focused on sub-regions face significant limitations when examining past population movements on a finer scale (Barbieri et al., 2019). Consequently, there is a gap in genetic information regarding the role of the Amazonian populations and others from eastern South America in the continent's settlement, as well as in other evolutionary and demographic events regarding Native Americans.

Availability of genetic data in certain geographic regions across the American continent, such as the Pacific coast, eastern Brazil (Amazonia), and the Southern Cone, presents significant sampling challenges. These challenges arise from substantial reductions in population sizes and diversity, a consequence of the population collapse induced by European contact (Castro e Silva et al., 2022; Motti et al., 2023). Other factors such as difficulty in accessing populations due to their isolation, and difficulties in legal approval to access indigenous samples also limit studies.

Nevertheless, recent endeavors have been directed at deepening our understanding in the subject. For example, Castro e Silva et al. (2020), using genomic data, demonstrated that Pre-Columbian migration from the Amazon to the northeast coast gave rise to Tupí coastal populations, while a single migration southward was responsible for the emergence of the Guaraní people in Brazil and Paraguay (Mendes et al., 2020). Consequently, the exploration of the prehistory of regions like present-day Uruguay becomes exceedingly intricate. Lindo and colleagues (2022) proposed that the ancestral population of ancient individuals in Uruguay might have originated from a migration closer to the Atlantic coast, potentially distinct from the migrations leading to modern Amazonian Indigenous populations in Brazil.

They also identified shared ancestry with an ancient sample from Panama.

According to Willerslev and Meltzer (2021), population continuity can be found before the arrival of Europeans in many regions during the Holocene. While there was continued movement and admixture of populations, it happened on a smaller spatial scale than in earlier periods. For example, Motti et al. (2023) revealed mtDNA continuity in the Southern Pampa region of Argentina extending back at least 7 ka. The Pampa region emerged as one of the earliest populated areas in South America during the Pleistocene/Holocene transition. The Pampa is characterized by vast grassy plains, and it has a notable concentration of archaeological sites in the early period, particularly between 13-11 ka.

1.5 mtDNA and estimation of effective sample size

As mitogenomes (entire mitochondrial genome) from Native American individuals will be employed for original analysis in the Thesis, further elaboration on the specifics of this genome and its characteristics will be provided in the present section.

In studying the complex scenario of the American population mosaic an important parameter to understand the various patterns of diversity is the effective population size (Ne) defined as the size of an ideal Wright-Fisher population that goes through the same amount of random drift as the population considered (Charlesworth, 2009;). Methods developed within the framework of coalescent theory have been successfully used to estimate historical patterns of population size from the genealogy of the DNA sequences (Perez et al., 2016). Based on our understanding of the initial peopling, migrations, timing, and genetic structure of the continent's first settlers, we can discern the signatures that these demographic patterns have left in both present-day and archaeological Native and Admixed populations. This involves analyzing the variation of Ne over time in an attempt to reconstruct past population

dynamics. Utilizing coalescence theory, a potent method for extracting historical information from DNA sequences, provides valuable insights into the intricate history of these populations (Stewart, 2015), especially from uniparental markers such as the mtDNA.

As a single maternally inherited locus with no recombination and high mutation rates (Mitchell et al., 2014; Amorin et al., 2019), the mitochondrial DNA has been proved as a great tool to trace the female line of descent of a population in order to test historical, archeological, linguistic and anthropological hypotheses through the phylogeographic approach, which combines the phylogenetic tree with geographic information and a molecular clock. This type of approach led to the proposal in the 1980s that modern humans arose in Africa and dispersed recently around the rest of the world (Cann et al., 1987). Likewise, in America the main contribution to the reconstruction of Native American genetic origin and history before and after the extensive admixture with Europeans and Africans after 1492 has been provided by uniparental systems, such as Y chromosome and mtDNA, as already mentioned and cited in previous sections.

Human mitochondrial DNA is a histone-free, circular, double-stranded DNA molecule found within the mitochondria organelle. It consists of approximately 16,569 base pairs (bp) and encompasses 37 intronless genes, characterized by limited or even absent intergenic sequences, with the sole exception being one regulatory region (1118 bp), the only non-coding region of mtDNA corresponding to the D-Loop displacement loop (Habbane et al., 2021) (Figure 3) . The mtDNA encodes 22 tRNAs, 2 rRNAs, and 13 proteins that are part of the respiratory chain complexes. Mitochondrial DNA strands have different densities due to different G+T base composition. The heavy (H) strand encodes more information, with genes for two rRNAs (12S and 16S), twelve polypeptides and fourteen tRNAs, while the light (L) strand encodes eight tRNAs and one polypeptide (Mitchell et al, 2014; Amorim et al, 2019 and Genetics Home Reference). However, this genome encodes a small fraction of

mitochondrial proteins, while the remaining proteins, including those needed for replication of the mitogenome and formation of the organelle, are encoded by nuclear DNA (nDNA) (Fernández, 2006).

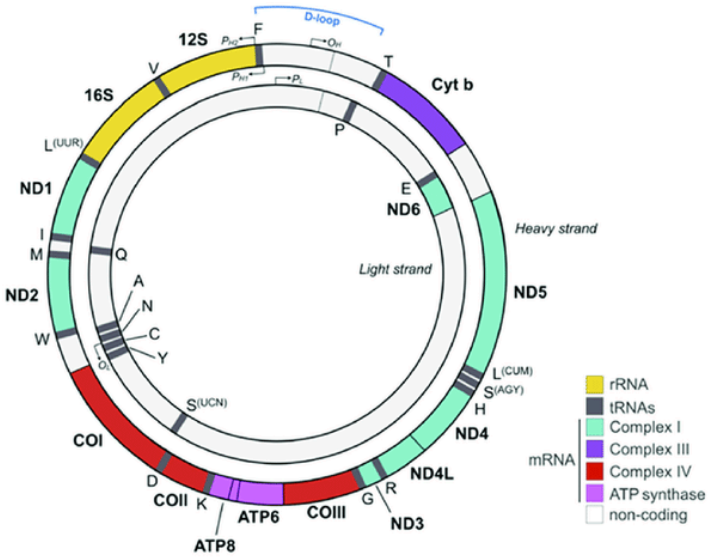

Figure 3. Map of the human mitochondrial DNA. From Hoffmann & Spengler (2018).

The mtDNA sequence determines the individual's haplotype which is defined by the different base pairs relative to the revised Cambridge Reference Sequence (rCRS; (https://www.ncbi.nlm.nih.gov/nuccore/NC_012920.1) mtDNA sequence (Andrews et al., 1999). The collection of similar haplotypes defined by the combination of single nucleotide polymorphisms (SNPs) in mtDNA inherited from a common ancestor defines an haplogroup which was formed as a result of the sequential accumulation of mutations through maternal lineages (Mitchell et al., 2014; Amorin et al., 2019). Due to population migration, distinct lineages of mtDNA are associated with major continental groups, including African, European, Native American/Asian, and Oceanic/Aboriginal Australian (Mitchell et al., 2014). Most of the sequence variation between individuals is found in two segments of the control region: the hypervariable region 1 (HV1, positions 16,024 to 16,365) and in the hypervariable region 2 (HV2, positions 73 to 340) (Amorin et al., 2019).

The nomenclature used for the mtDNA essentially identifies and reports the differences relative to the rCRS. This nomenclature primarily entails a system that identifies the sequence in which mutations occur, thereby defining lineages (haplotypes and haplogroups). By employing phylogeography strategies, one can determine the geographic distributions of genealogical lineages. This procedure is analogous to the nomenclature used for lineages within the non-recombinant region of the Y chromosome, a genetic system of exclusively paternal inheritance.

Overall, the majority of individuals from sub-Saharan regions are categorized into one of the main haplogroups that diverged from macro haplogroup L: L0, L1, L2, L3, L4, L5, and L6. On the other hand, more than 90% of the individuals of European and United States-European descendents are categorized into 10 main haplogroups: H, I, J, K, M, T, U, V, W, and X. The rCRS is classified as the European haplogroup H2a2a (Bandelt et al., 2013), while the named "mitochondrial Eve" as L (Cann et al., 1987). The main haplogroups found in individuals from Asian populations are haplogroups M and N (Amorin et al., 2019).

Regarding Native Americans haplogroups, initially, just four founding haplogroups (from A to D) were identified. Currently, it is widely accepted that all Native American mitochondrial DNA can be traced back to five major haplogroups: A, B, C, D, and X. Achilli et al. (2008) considered four "pan-American haplogroups" A2, B2, C1, and D1, since they are distributed across the entire continent. Due to the sequencing of mitogenomes, the overall number of recognized maternal founding lineages of Asian/Beringian origin has gone from just four to 16, allowing to increase the phylogenetic resolution. More specifically, Indigenous American mtDNA belongs to various sub-haplogroups, including A2a to A2k, B2a to B2d, C1b, C1c, C1d, D1b, D1c, and D1d. Less common Native American haplogroups, such as C4c, D2a, D3, D4h3, and X2a, have also been identified in various studies (Achilli et al., 2008; Bisso-Machado et al., 2012; Bisso-Machado & Fagundes, 2022),

and the extremely rare (X2g and D4e1) or generally restricted to the populations of the arctic and subarctic regions of North America. Some of the less common haplogroups (C4c, and X2a) might have arrived from Beringia with the first indigenous groups who entered the continent by alternative migration routes, whereas others (A2a, A2b, D2a, and D3) may have came from much later arrivals (Tamm et al., 2007; de Saint Pierre et al., 2012 and Brandini et al., 2018).

According to de Saint Pierre and colleagues (2012), with the analyses of mitogenomes, the studies of Native American mtDNA variation have entered the final phase of phylogenetic refinement: the dissection of the founding haplogroups into clades that arose in America during and after human arrival and spread. Ages and geographic distributions of these clades provide novel clues on the colonization processes of the different regions of America. Furthermore, it is considered that the Asian and Beringian original lineages also gave rise to other derivatives *in situ* during the colonization process. Therefore, the complete mitochondrial genome allows to identify phylogeographic relationships between populations closely related (Stewart, 2015; Arias et al., 2018). In addition, new analysis tools, including tip-based calibration methods that use the age of ancient samples to estimate the rate at which differences between sequences accumulate, have greatly improved the accuracy of estimations, especially using mtDNA data (Rieux et al., 2014; Miller et al., 2018).

Several authors have evaluated the changes in population sizes of different groups of populations in South America through Bayesians approaches and mtDNA data sets (Llamas et al., 2016; Arias et al., 2018; Barbieri et al., 2019; Brandini et al., 2018; Tavares et al., 2019; Garcia et al., 2021). Some of these studies include archeological mitogenomes, mainly demonstrating that both current and ancient South American populations carry signs of a major demographic event, the entrance of the first settlers to the continent. Therefore, this kind of approach allows to disentangle demographic patterns interesting to evaluate

hypotheses such as migrations, isolations and population replacements, both pre and post colonial events.

Since mtDNA haplogroups can be identified with geographic regions of origin, ancient maternal ancestries can be identified in admixed populations. For example, the pioneering study by Sergio Pena and research team showed that 39%, 33% and 28% of the mitogenome present in a representative sample of Brasilians could be identified as from European, Native American and African origins, respectively (Alves-Silva et al., 2000). They identified 10 haplogroups of European origin, with H being the most frequent (44% among those mtDNA of European origin), while the African haplogroup L3e is the most prevalent (30% among those of African origin) (Alves-Silva et al, 2000).

1.6 Colonization and admixture: The population declined in South America following the Conquest

- 1.6.1 Quick overview within South America

The history of America, from the arrival of the first humans, is marked by successful colonization events, differentiated technological development, tragedies, and genocides, resulting in specific genetic signatures some of which were already mentioned (Tarazona-Santos et al., 2001; Ramallo et al., 2013; Bisso-Machado & Fagundes, 2021; Castro e Silva et al., 2020). Furthermore, particular morphological (Gonzáles-José et al., 2008; Bortolini et al., 2014; Hünemeier et al., 2012a), and cultural patterns (Hünemeier et al., 2012b; Reales et al., 2018, Castro e Silva et al., 2022) across the American continent are also observed.

The territorial occupation of the southern portion of the American continent by foreign people began in the 15th century when Spanish conquerors and colonizers first met American's autochthonous communities. The exact count of indigenous when Europeans arrived is still debated, with estimates ranging from 10 to 100 millions (Salzano & Bortolini, 2002; Adhikari et al., 2017) (Figure 4). Sadly, many of these different populations and groups disappeared during the colonization process (De Oliveira et al., 2023).

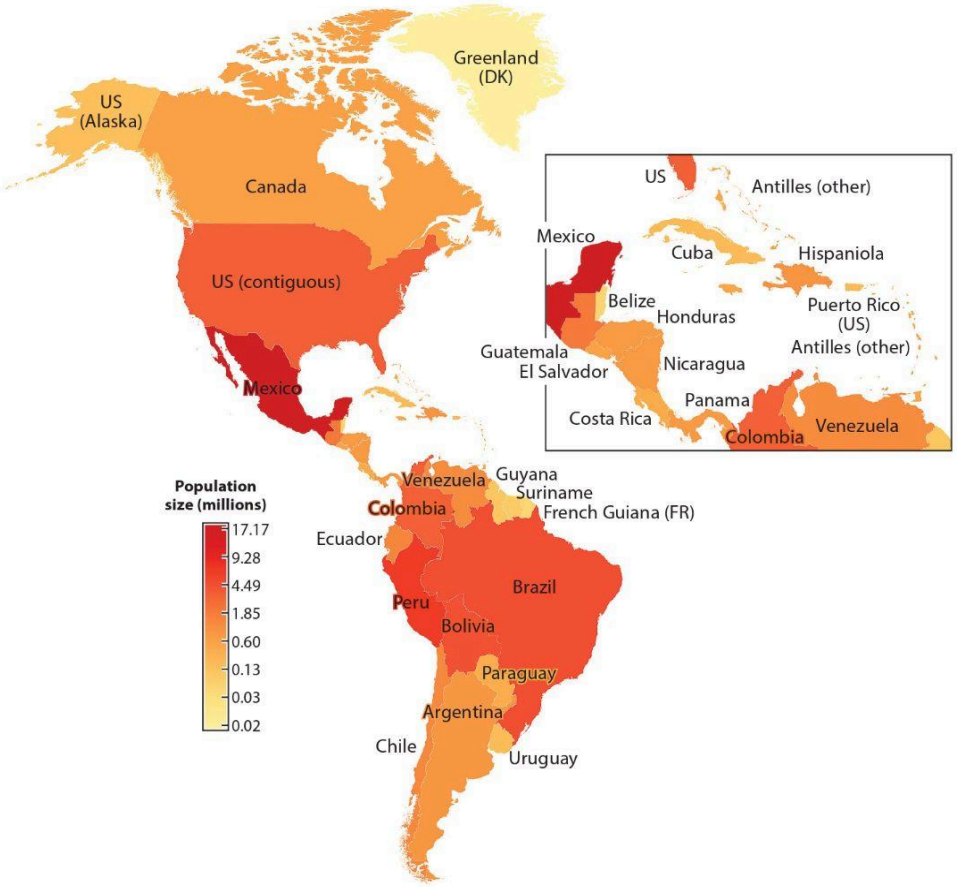

Figure 4. Estimated size of the Native American population at the time of Columbus's initial arrival on the continent.

Population estimates are categorized by country, based on current borders. It's important to note that the actual population density varied geographically, irrespective of contemporary political borders. The authors based their estimations mostly on Denevan (1992), using geographic and anthropological data. The populations of most of the Antilles and the island of Hispaniola (shared by Haiti and the

Dominican Republic) have been combined. The respective country associated with each American dependency is indicated in parentheses (DK for Denmark, FR for France, and US for the United States). From Adhikari et al., 2017.

In the contemporary Latin American region, the indigenous population comprises approximately 58 million individuals, constituting about 10% of the entire continent's population. It is crucial to acknowledge that these proportions might be significantly underestimated due to potential underreporting. Overall, the indigenous community is characterized by diversity, encompassing for instance, around 400 distinct linguistic groups (Cabeza de Baca et al., 2020).

It is widely acknowledged that the current American populations resulted from a blend of Native Americans, who had been residing there for an extended period, along with two other continental populations—Europeans and Africans (Salzano & Bortolini, 2002; Ruiz-Linares et al., 2014; Salzano & Sans, 2014; Pena et al., 2020; De Oliveira et al., 2023). The admixture of Native Americans, Europeans, and Africans, occurring with significant frequency, fostered extensive genetic and cultural intermingling on the continent, especially in Latin America. This is particularly significant given the considerable diversity among Native American populations across the Americas and the diverse backgrounds of African individuals. Both of these factors significantly contribute to the remarkable genetic diversity and variable level of admixture observed in the American continent today (Adhikari et al., 2017; De Oliveira et al., 2023).

The history of this admixture began with interbreeding between European settler men, primarily from Portugal and Spain, and local indigenous women. Subsequently, individuals from sub-Saharan Africa were brought to the continent through the slave trade during the 16th to 19th centuries, with their history in America marred by violence, including various forms of sexual abuse. As a result, the formation of mestizo (also known as admixed)

societies also encompasses the African genetic component, particularly from women, at least during the initial centuries of American colonial history.

From that time onward, conflicts arose against Native Americans, leading to the enslavement of various populations for forced labor. Despite the prohibition of Native American slavery in Spanish colonies in 1542 and Portuguese colonies in 1570, certain outlying regions persisted in maintaining such practices (De Oliveira et al., 2023).

The colonization and blending of populations exhibit notable variations across different regions, influenced by a multitude of factors including geography, the timing and scale of population movements, and diverse social dynamics. These factors collectively contribute to distinctive patterns of genetic diversity throughout the American continent. While common demographic forces have shaped the overall narrative, the extent of admixture has been significantly influenced by various social factors that differed across regions. Notably, historical studies underscore differences in the social characteristics of European colonization areas, suggesting their potential impact on the frequency of population blending (Adhikari et al., 2017).

For instance, when examining different geographical regions in Brazil alongside other Latin American countries, Northeast Brazil, Cuba, and Venezuela stand out for having the highest African ancestry, while Southeast/South Brazil, along with Argentina and Uruguay, exhibit the highest European ancestry. In a broader context, the autosomal biparental Native American ancestry in Brazil is comparatively low when contrasted with countries like Mexico, Guatemala, Peru, and Bolivia, which boast the highest Native American ancestry levels in Latin America (for a more recent review on Brazilian ancestry see Pena et al., 2020). Caribbean islands present a higher proportion of sub-Saharan African ancestry, as demonstrated by studies from Bahamas, Cuba, Jamaica, Haiti, Dominican Republic, Puerto Rico, Dominica, St. Lucia, Barbados, St. Vincent, Grenada, and Trinidad and Tobago; Central

America, Andean regions, and Ecuador have populations with high Native American ancestry and Brazil, Paraguay, Uruguay, Argentina, and Chile present high proportions of European ancestry (De Oliveira et al., 2023).

- 1.6.2 Asymmetry of matings

Similar to many conquerors, the Spanish and Portuguese positioned themselves at the top tiers of the social hierarchy in their American colonies, particularly concerning the enslaved population at the bottom. This granted male colonizers preferential access to colonized females through various means, including physical coercion and sexual abuse, social influence, or voluntary marriages and sexual relationships (Cabeza de Baca et al., 2020). Additionally, the biased immigration of Iberian males to America during the initial phases of colonization unfolded within a context of pronounced dominance over Native American men. This dominance, in turn, played a significant role in fostering early and extensive intermingling between Iberian men and Native American women—a pattern extensively documented, especially among noticeable Spanish and Portuguese conquistadors (Salzano & Bortolini, 2002; Adhikari et al., 2017).

The majority of the genetic studies with mtDNA and the Y-chromosome data supports this sex-biased contributions, corroborating the historical archives of the marital unions happening throughout the regions of colonial Latin America (Salzano & Bortolini, 2002; Cabeza de Baca et al., 2020). The genetic outcome of this asymmetry in the nuclear genomes of many Brazilians is a mosaic, characterized by mtDNA and Y chromosomes with distinct phylogeographical origins (Alves-Silva et al., 2000; Marrero et al., 2007; Pena et al., 2020). However, despite this general pattern, there are extensive regional variations.

In the Southern Cone of South America, particularly in Chile, Argentina, and Uruguay, the perceived European ancestry holds dominance. Uruguay, in particular, boasts the highest perceived frequency of European ancestry in the Americas, standing at approximately 81%. Across various regions, it appears that natives have, to some extent, been supplanted by other populations, with the replacement being nearly complete in areas like the Antilles. Notably, indigenous populations still constitute significant proportions in regions where the pre-Columbian population size was most substantial, such as Mesoamerica and the Central Andes. Across most of Ibero-America, natives seem to have been succeeded by a population of mixed ancestry (Adhikari et al., 2017).

As the colonial era progressed, the Spanish strategically settled in regions already densely populated by natives, aiming to exploit them as a labor force and extract tribute, thereby facilitating the process of admixture. Following independence in the nineteenth century, Ibero-American nations gradually dismantled colonial regulations governing interethnic relations and slavery, aligning with the republican ideal of citizen equality. In the post-independence era, Ibero-American states adopted diverse approaches towards admixture. There were instances where admixture was positively valued and actively encouraged, often seen as a crucial aspect of national identity. Conversely, some Ibero-American states pursued initiatives to ‘whiten’ their populations by promoting additional European immigration, a strategy that found notable success in the Southern Cone and Brazil (Adhikari et al., 2017). Specifically, in Brazil there was the official policy of whitening, with incentives during the 19th century for the arrival of European settlers.

Taking into account the high frequency of Native American mtDNA lineages in admixed populations resulting from the initial Latin American mating pattern (indigenous women with European men) and the relatively short evolutionary time frame of the admixture process (500 years), it has been suggested that current South American admixed populations

serve as a reservoir for Native American matrilineal genetic lineages, including those of extinct autochthonous peoples (Marrero et al., 2007). The LEHM team discovered that some mixed populations from Uruguay and southern Brazil (American Southern cone) carried in their mitochondrial lineages of native origin, demographic signs that, for example, better represented the peopling of America, than the lineages that are present in today native populations of the same region, where the signal was completely eroded (Tavares et al., 2019).

Furthermore, these and other authors, utilizing various mtDNA datasets from studies involving both contemporary and archaeological Native South American populations, have demonstrated a robust expansion signal dating back to approximately 17-15 ka, alongside a more recent demographic contraction coinciding with the continent's initial peopling and the arrival of Europeans, respectively (Llamas et al, 2016; Arias et al., 2018a; Barbieri et al., 2019; Brandini et al., 2018; Tavares et al., 2019). This implies that studies present varying demographic patterns in mtDNA lineages for the continent. The fundamental question remains: is the observed pattern in the native lineages of the southern cone mixed populations, found in both urban and rural areas, a more accurate representation of major Native American historical events compared to the lineages discovered in natives of the same region, consistently observed across other geographical regions?

This discussion will be extended in the Chapter 4 of present Thesis.

1.7 Cultural genocide

- 1.7.1 Genocide of Southern American Natives

In the past 500 years, a significant portion of the pre-Columbian diversity of native american peoples and their history has been lost. The colonization process led to the near-complete disappearance of many indigenous groups and their languages (Pena et al., 2020). The European colonization of the American continent involved the occupation of native territories, the imposition of European cultural elements, and the displacement and cultural diminution of local populations (Mogollón Olivares et al., 2020). Estimates suggest that around 90% of the Native American population succumbed following the arrival of Europeans. The severity of this population collapse was notably acute in regions where the pre-Columbian native population was relatively small, leading to the virtual eradication of natives from those areas (e.g., in the Antilles). For instance, among the major Caribbean islands, the native Taino Indians were almost extinct by 1550 (Livi-Bacci , 2006 and Adhikari et al., 2017). A year after Columbus landed, a policy of indigenous slavery was implemented within the settlement (Salzano & Bortolini, 2002), which led to a virtual (though unintended) genocide of the so-called Taino (Arawak) people (Cabeza de Baca et al., 2020).

Another example that can be cited is found in the Southern cone of the continent. The Charrua indigenous people, which actually comprises a community of people who speak Pampean languages, disappeared in the Pampa region in both Uruguay and the southernmost Brazilian state, Rio Grande do Sul, in the 19th century. However, their cultural heritage is acknowledged within the admixed people of these same regions, the so-called Gaucho. Marrero et al. (2007) discovered Charrua mtDNA lineages among urban and rural populations along the border between Brazil and Uruguay, suggesting that Charrua genetic heritage also defines the Gaucho mestizo people.

As already mentioned, the Charrua ethnic group has been considered virtually extinct since the 19th century. However, more recently, some groups scattered across the State of Rio Grande do Sul and Uruguay have started to assert their affiliation with this ethnic group (see report at https://www.bbc.com/portuguese/brasil-45947432).

The autochthonous population decline has been ascribed to a multitude of intricate factors, encompassing biological elements (the introduction of new diseases), environmental aspects (infrastructure destruction, deforestation, and the impact of European-imported livestock), political forces (wars, loss of liberty and autonomy), social dynamics (community dislocation, forced migration), economic influences (alterations in production patterns, labor exploitation, and confiscation), and demographic shifts (abduction of women, separation of couples, and migration) (Livi-Bacci , 2006).

As articulated by Livi-Bacci in 2006, these factors can be classified into three categories including the "confiscation of labor," "diffusion of disease," and the "atrocities of Conquest." The first factor pertains to forced labor, primarily non-legally enslaved individuals engaged in activities like transportation, construction, and gold prospecting. The second factor involves the introduction of new diseases from Europe, with their impact more pronounced in densely populated areas compared to smaller, scattered, or isolated populations. Some of this novel transmissible pathogens included viruses responsible for diseases like Measles, Influenza, Chickenpox, Smallpox, and Mumps, along with bacteria causing Scarlet Fever, Typhoid Fever, Typhus, Pertussis (Whooping Cough), Cholera, Leprosy, Diphtheria, Bubonic Plague, and certain sexually transmitted diseases. Introduced new strains were able to proliferate due to the lower genetic diversity of native strains and triggered widespread epidemics that resulted in the loss of millions of lives (Adhikari et al., 2017; Cabeza de Baca et al., 2020). Additionally, the slave trade facilitated the introduction of yellow fever and other diseases from Africa to the Americas (Cabeza de Baca et al., 2020).

Finally, the "Conquest's atrocities" encompass the direct consequences of killings, mutilations, rape, abduction, and pillaging on the conquered population, along with the loss of traditional autonomy and the resulting social and economic upheaval.

However, as mentioned earlier, the extent to which these factors impacted various native populations varied significantly across the continent. In certain instances, such as the already reported peoples of the Caribbean islands or the South American Pampa, the system was so profoundly disrupted by European intrusion that the population was decimated even before the onset of the first epidemic. In cases like the missions of Paraguay, the demographic system endured, and the population grew despite recurrent and deadly epidemics of new diseases (Livi-Bacci, 2006).

Between these extremes, a range of situations existed. The Caribbean case extends beyond the Greater Antilles to include the islands of pearls off the coast of Venezuela, the coasts of the mainland called Castilla de Oro, and other gold-yielding areas. The extermination of the natives in this region was swift, completed within just a few decades. Another impactful scenario includes the low-lying coastal areas of the Gulf of Mexico and the Pacific coast of Peru, where depopulation occurred much more rapidly than in the plateau of Mesoamerica or at the high altitudes of the Andes (Livi-Bacci, 2006).

While these factors led to a swift decline in the numbers of Native American peoples, it is important to note that the losses were characterized by a gender bias. The calamities through American lands resulted in a significant reduction in males of reproductive age. In the case of Brazil, where the Portuguese settlers initially spread along the coastline, the relentless demand for labor in the plantation system and conflicts with natives gradually pushed the indigenous populations into the interior. Despite the introduction of African labor through the slave trade, colonists organized expeditions to hunt for indigenous labor in the interior, further depleting native societies. Because indigenous male populations declined as a

result of disease, genocide, and warfare, indigenous females were left with fewer viable indigenous mates (Cabeza de Baca et al., 2020). Enslaved or indentured Indigenous often faced conditions that hindered reproduction, and native women were assimilated into the European reproductive pool. As has already been highlighted throughout this Thesis, this pattern of expulsion and depletion of human resources was replicated in various other regions across the continent.

For instance, in Peru, European men significantly outnumbered women (seven to one), leading external forces to integrate females into the prevailing patriarchal structure. During the 19th century, nation-states aimed for unity but eroded indigenous and African cultures through forced assimilation or extermination by the emerging Latin American elites, mainly of European descent. For lower-class women, such as natives/mestizas, extramarital affairs, often involving immediate rape or extended concubinage, were common. European males, despite being fewer, controlled resource-rich environments, leading some indigenous females to mate with them. Additionally, enslaved African women were also subjugated, resulting in extramarital children. Post-independence, as native men dwindled, indigenous females actively sought to increase Native American numbers (Cabeza de Baca et al., 2020).

Regarding the Gaucho people, Marrero et al. (2007) found that the indigenous heritage, as indicated by the matrilineal sequences (mtDNA) of indigenous origin, including some from the Charrua, was 52%, whereas the presence of indigenous Y chromosomes was only at 5% (the remaining 95% Y chromosome being typical of Spanish and Portuguese men), illustrating a significant asymmetry. In other words, indigenous men had limited opportunities to leave a substantial genetic legacy in certain regions, thus making a smaller contribution to the genetic pool of emerging national admixed populations.

Considering major urban areas (Porto Alegre city, capital of the southernmost Brazilian state, Rio Grande do Sul), even in individuals identified as “white” (where

non-European heritage is not detected by visible phenotypic traits) the presence of indigenous mtDNA sequences is 21% (Guerreiro et al., 2009). “Whites” represent 73.6% - or 981,251 individuals - of the residents of Porto Alegre according to the IBGE Census 2022 (https://cidades.ibge.gov.br/brasil/rs/porto-alegre/panorama). Taking into account the data from Guerreiro et al. (2009), there could be at least 206,000 Indigenous mtDNA genomes in the city of Porto Alegre.

In light of genome-wide data from 5,825 admixed Brazilian individuals, the LEHM team and the Pompeu Fabra University team, reconstructed populations that mimic the Native American groups residing in the 16th century around our sampling locations. This genetic reconstruction followed a local ancestry analysis of the admixed Brazilian populations, indicating genetic profiles influenced by the Tupi or Je-speaking ancestral contributions. Additionally, there was evidence indicating a reduction in the genetic diversity of non-admixed Native American groups following European contact, in contrast to the reconstructed populations. This suggests that the admixed Brazilian population serves as a reservoir for preserving Native American genetic diversity (Màs-Sandoval et al., 2019).

As a whole, in American countries with a colonial history characterized by asymmetry, the predominant transmission of indigenous genetic heritage occurred through native women. Despite the disappearance of indigenous populations and traditional cultures in regions like the south of Brazil, the substantial legacy of indigenous genetics persists. Furthermore, the assimilation of indigenous women into emerging South American societies resulted in the erosion of their culture—a phenomenon viewed as a manifestation of cultural genocide, despite their physical survival.

- 1.7.2 Legal and theoretical background

The term genocide marked a significant departure from the longstanding tradition in international law. Initially, the focus was primarily on crimes against individuals rather than groups. In the 1940's the polish lawyer Raphael Lemkin introduced the term "genocide," deriving it from the Greek word "genos" (tribe, race) and the Latin "cide" (analogous to terms like homicide and fratricide). He outlined the "techniques of genocide" across eight domains: political, social, cultural, economic, biological, physical, religious, and moral (Luck, 2018). Within the realm of international law, at first, within the concept of genocide, the cultural dimension was envisioned as a method or form of genocide. This term signifies the deliberate destruction of both tangible elements, such as places of worship, and intangible aspects, such as language, within cultural structures (Bilsk and Klagsbrun, 2018).

The term cultural genocide is characterized by diverse interpretations across academic, legal, and historical contexts. Originating from Lemkin's delineation of the term "genocide," it initially encompassed a broad spectrum of deliberate actions aimed at the destruction of tangible and intangible elements within cultural structures across various domains. Upon its introduction, the concept sparked controversy and remains contentious, as it has never been officially defined, acknowledged, or codified by governments worldwide despite its significance (Luck, 2018). Notably, the Convention on the Prevention and Punishment of the Crime of Genocide (CPPCG) or Genocide Convention, adopted by the United Nations (UN) General Assembly on December 9th of 1948 during its third session that became effective on January 12th of 1951 (https://www.un.org/en/genocideprevention/documents/atrocity-crimes/Doc.1_Convention%20on%20the%20Prevention%20and%20Punishment%20of%20the%20Crime%20of%20Genocide.pdf), omits cultural genocide from its provisions. This omission is attributed to the

convention's limited definition, which solely encompasses physical and biological aspects (Bilsk and Klagsbrun, 2018).

The Truth and Reconciliation Commission of Canada (TRC) provides a more specific definition of cultural genocide in the context of Canadian indigenous genocide history as:

> The destruction of those structures and practices that allow the group to continue as a group. States that engage in cultural genocide set out to destroy the political and social institutions of the targeted group. Land is seized, and populations are forcibly transferred and their movement is restricted. Languages are banned. Spiritual leaders are persecuted, spiritual practices are forbidden, and objects of spiritual value are confiscated and destroyed. And, most significantly to the issue at hand, families are disrupted to prevent the transmission of cultural values and identity from one generation to the next (2023).

Most scholars embrace a broad definition of genocide, where some strictly interpret it as acts intending to destroy groups, holly or partially, in accordance with the Genocide Convention from the UN. Others adopt a more extensive view, encompassing actions like preventing ethnic groups from practicing traditional customs, forced resettlement, denying essential resources like food relief, health assistance and development funds and destroying habitats utilized by the populations (Luck, 2018).

The legal gap surrounding the term cultural genocide has profound implications for addressing cultural atrocities. The absence of a universally agreed-upon definition impedes the recognition and prosecution of cultural genocide as a distinct crime. The limited scope of the Genocide Convention, which focuses on physical and biological aspects, sidelines the destruction of cultures. Consequently, this gap challenges the international community's ability to hold perpetrators accountable for cultural atrocities and hinders efforts to prevent such crimes. The complexities of cultural genocide necessitate a more inclusive legal

framework that addresses both tangible and intangible dimensions, ensuring justice for affected communities and promoting the preservation of cultural diversity worldwide.

In here we will distinguish "Cultural genocide" or "ethnocide" from physiscal genocide, and remark the nature of this particular type of genocide as a process in which populations are deprived of the right to practice their own religions, customs, and/or speak their languages by nation-states most likely in an assimilation context. According to Hitchcock and Twedt (2009), ethnocide is differentiated from genocide, focusing on the destruction of cultures rather than the physical extermination of people. It can significantly impact the well-being of indigenous societies, potentially leading to a loss of morale and diminished resilience as people may become dispirited to the point of lacking the desire to survive. Cultural genocide frequently occurs in the context of state-imposed educational programs, modernization initiatives, and nation-building efforts. While not as shocking as physical genocide, ethnocide can also have profound consequences on the well-being of indigenous societies.

Distinguishing physical and cultural genocide is complex; for example assimilation policies in the United States, along with disparate legal treatment, significantly affected Native Americans' well-being (Chalk & Jonassohn, 1990). In settler societies globally, including the American continent, Australia, and South Africa, indigenous peoples endured substantial suffering due to disease, starvation, and related physical and cultural stresses (Hitchcock and Twedt, 2009). Cultural genocide involves intentionally weakening and eventually destroying the cultural values of feared out-groups. This is achieved through systematic or systemic actions, whether intentional or unintentional, that erode or undermine the integrity of the culture and values defining that particular group (Kingston, 2015).

Many indigenous communities have experienced various forms of violent physical harm throughout the course of their colonization, and the ongoing cultural erosion represents

just one aspect of a more extensive continuum. According to indigenous scholar Taiaiake Alfred, “relationships founded on hatred and violence and a culture founded on lies to assuage the guilt or shame of it all” (Kingston, 2015). Both victims and perpetrators persist in denying their shared history and its moral implications. Approaching this situation through the lens of cultural genocide provides a framework to examine accountability for historical and ongoing injustices, with the aim of progressing towards equality (Kingston, 2015).

One important way in which cultural genocide can manifest is through biocultural assimilation in the context of settler colonialism. Settler colonialism is a term used to describe a situation where colonizers enter and take control of a territory with the intention of replacing the existing society with their own society on a permanent basis. This typically involves displacing, marginalizing and/or neglecting the indigenous or autochthonous population and imposing the culture, values, and institutions of the colonizers (To a review of this subject see Veracini, 2010; Careya & Silverstein, 2020).

This complex context indicates that within settler-colonial societies, assimilation programs can function as a mode of elimination by seeking to integrate indigenous peoples into the prevailing culture and eradicate their unique cultural and biological identities (Wolfe, 2006), as this form of colonialism aims at the permanent replacement of existing societies. As well, the ongoing absence of a standardized definitions impedes international efforts to recognize, prevent, and address cultural genocide, impacting the well-being of indigenous societies. Urgent action is needed to establish a comprehensive and inclusive definition, fostering international cooperation and justice for affected communities.

1.8 Indigenous health

- 1.8.1 Indigenous people, neglected people

The repercussions of the colonization of the American continent have profoundly impacted various communities, notably indigenous groups, as discussed in detail in the item 1.7.1 “Genocide of Southern American Natives”. We will use the term indigenous as defined by the World Health Organization (WHO):

> Heirs and practitioners of unique cultures and ways of relating to people and the environment. These peoples also strive to maintain their social, cultural, economic, and political characteristics that are distinct from those of the dominant societies in their surroundings. Despite their cultural differences, indigenous peoples worldwide share common issues related to the protection of their rights as distinct peoples. Furthermore, indigenous peoples have been seeking recognition of their identities, way of life, and the right to traditional lands, territories, and natural resources for years, but throughout history, their rights have consistently been violated. Indigenous peoples today are among the most disadvantaged and vulnerable groups in the world. The international community now recognizes that special measures are needed to protect their rights and preserve their distinct cultures and ways of life. (https://www.un.org/development/desa/indigenouspeoples/about-us.html).

The enduring socioeconomic effects of colonial history have created disparities, with one notable aspect being the correlation of wealth with European ancestry (Adhikari et al., 2017). Presently, vulnerable populations, including descendants of formerly enslaved individuals and Native Americans, contend with the enduring consequences of history and challenging living conditions (Arango-Isaza et al., 2023; De Oliveira et al., 2023). The lasting effects of structural discrimination significantly shape the contemporary landscape for indigenous and African-descendant peoples in Latin America. The power dynamics

stemming from political, economic, and cultural influences perpetuate pre-existing structures of inequality inherited from colonialism, forming what is termed as neocolonialism (Arango-Isaza et al., 2023)

Despite noticeable advancements in recent decades, indigenous communities continue to face disproportionate levels of poverty, marginalization, and limited access to essential resources such as employment, education, and healthcare (Adhikari et al., 2017; Arango-Isaza et al., 2023). According to the WHO, 86% of indigenous people work in the informal economy and are almost three times more likely to live in extreme poverty, with their average life expectancy being around 20 years lower than that of the non-indigenous population living in the same country or region (https://stories.undp.org/10-things-we-all-should-know-about-indigenous-people)

Indigenous communities in Latin America are acknowledged as socially vulnerable populations, confronting enduring impacts from historical events and ongoing challenges stemming from racism at both individual and institutional levels. Despite governmental responsibilities to safeguard their rights and needs, these communities persist in facing racial prejudices. Their ongoing struggle revolves around achieving recognition as traditional communities and securing access to fundamental human rights. These communities find themselves marginalized in economic and political spheres, experiencing disparities within the healthcare system and encountering obstacles in accessing essential services (Cabeza de Baca et al., 2020). This enduring legacy underscores the ongoing challenges these groups confront, emphasizing the importance of addressing historical injustices for a more equitable future.

In terms of health, indigenous peoples in Latin America experience significantly poorer health conditions compared to the general population, as noted by the Economic Commission for Latin America (ECLA in English and CEPAL in Spanish) (CEPAL, 2014).

Indigenous households, especially those residing in rural areas, frequently confront elevated health risks due to inadequate living conditions and restricted access to essential services such as healthcare, water, and sanitation.

Moreover, the impact of educational attainment on health cannot be overstated. The SES-health gradient, a phenomenon where individuals with lower socioeconomic status (SES), particularly those from racial and ethnic minorities, experience health challenges and chronic issues at a disproportionate rate compared to those with higher SES, underscores the intricate interplay of social factors in health disparities (Adhikari et al., 2017, De Oliveira et al., 2023). For instance, research indicates that indigenous communities experience elevated infant mortality rates compared to non-indigenous populations, including rates of 19/1,000 in Colombia, 22/1,000 in Ecuador, 99/1,000 in Peru, and 106/1,000 in Brazil. The impact of these high mortality rates can be devastating for small indigenous populations, potentially leading to the demise of entire communities (De Oliveira et al., 2023).

Additionally, heightened interactions with other populations since the colonization process (for instance, the introduction of new transmissible pathogens) or more recently driven by economic activities like mining exploration (e.g.,in Argentina producing alarming concentrations of heavy metals in the hair of native communities), further heightened the vulnerability of Native American populations, compounding the challenges they already confronted. Other diseases such as sexually transmitted infections and infectious diseases such as malaria, yellow fever, intestinal parasites, and coronavirus disease 2019 (COVID-19) are also a concern for these populations (De Oliveira et al., 2023). Finally, the transition from an agricultural to a more industrialized food source makes indigenous populations more susceptible to complex diseases such as high blood pressure, obesity, and anemia (For an extensive review see De Oliveira et al., 2023).

- 1.8.2 Efforts in genetic research

In general, scientific research involving participants from native human populations is much less common when compared to studies involving non-native groups. The lack of scientific studies involving indigenous peoples occurs for various reasons, including challenges in obtaining samples and resources. Additionally, these groups are often neglected in the design of studies, especially in biomedical and life sciences, including genomics. This raises concerns that without corrective actions, precision genomic medicine would be available only for "privileged few" populations (Bustamante et al., 2011; Popejoy and Fullerton, 2016).

As already highlighted in this Thesis, recognition should be given to the efforts of research groups, among which is the one led by Prof. Francisco Mauro Salzano, who passed away on September 28, 2018, leaving an enormous legacy intertwined with the history of Brazilian and global science and founded the now-called Laboratory of Human and Molecular Evolution (LEHM) at Universidade Federal do Rio Grande do Sul (UFRGS), to which he remained affiliated until his death. Over seven decades, Salzano, with indispensable assistance from collaborators at UFRGS and other institutions and indigenous leaders, collected an invaluable collection of biological and documentary samples from various Brazilian indigenous peoples. A vast scientific production was generated from data collected in these expeditions. For example, a total of 89 theses and dissertations were defended under his supervision, with a significant portion focusing on the evolutionary and demographic history of American indigenous peoples.

From this scientific production and others, crucial findings have contributed to a comprehensive understanding of the overall diversity pattern of Native Americans and shed light on some rare genetic conditions. These insights initially emerged through studies on blood protein groups and other proteins, later advancing to the examination of DNA. Despite

these notable strides and additional research endeavors, knowledge about the genetic profile of Native American peoples remains comparatively limited, especially in the age of genomics and its various branches, including proteomics and microbiota metagenomics. This parallels the challenges faced by other indigenous groups.

Although as mentioned in the previous sections, several studies have provided valuable context they have not fully addressed the ongoing obstacles and disparities faced by Native American populations in contemporary genetic research. Unique regional complexities exacerbate the situation, including the difficulty of access, given that a large portion of these communities reside in geographically remote areas of South America, such as the Amazon. Furthermore, the small and isolated nature of many communities, coupled with their reliance on subsistence strategies like hunting and gathering, along with the use of lesser-known native languages, further compounds the complexity of genetic research.

Over time, Native American populations have been victims of violence, resulting in robust reactions upon interactions with non-indigenous individuals, stemming from historical trauma. The concept of historical trauma has been conceived and utilized to illustrate the detrimental impact of colonization on Native Americans, shedding light on profound and intergenerational collective traumas, compounded by experiences of discrimination, racism, and oppression (Brave Heart et al., 2011). Recent incidents in Brazil, highlighting the absence of public policies safeguarding the health and well-being of the Yanomami people, vividly portray the ongoing challenges faced by Brazilian indigenous populations. This emphasizes the pressing need to explore effective avenues to definitively break the cycle that perpetuates historical trauma. The events involving the Yanomami people have been extensively documented by both national and international media.

Additionally, as highlighted by Tsosie et al. in 2020, the genomic data collected over decades from Indigenous peoples and their ancestors may not have offered immediate benefit

to the contributing Indigenous groups. Evenmore, in some cases some of this information has helped to perpetuate stereotypes and other harms (Arango-Isaza et al., 2023). While recent scientific studies offer unprecedented insights into the human past, the rapid advancement in this field raises significant concerns regarding the ethical balance and expectations for community engagement (Tsosie et al., 2020). Whilst ethical guidelines for genomic research have received significant attention in high-income countries such as the United States, New Zealand, and Australia, there is a notable lack of literature addressing the specific context and challenges faced by researchers in the Global South (Arango-Isaza et al., 2023).

In this context, it is crucial to underscore the importance of empowering indigenous researchers to assume a prominent role in genetic studies. By providing indigenous researchers with the tools and opportunities to assume a prominent role in genetic studies, there is a potential to address the existing disparities and biases in research. Additionally, it involves establishing research collaborations that respect and integrate the cultural perspectives of local communities, which provide detailed accounts of their own histories and ethnographic data (Tsosie et al., 2020).

Promoting indigenous representation in scientific research and discarding neocolonialist practices requires transparent research protocols and active community participation (Arango-Isaza et al., 2023). Involving Indigenous communities in aDNA research for example, can assist in recognizing their ancestors, a crucial aspect for repatriation efforts. Collaborative efforts have the potential to enhance our global comprehension of diversity in both ancient and contemporary populations (Tsosie et al., 2020).

For instance, in the United States, members of the Navajo Nation are exploring the possibility of developing a genomics research policy, discussing its potential implications, including in biomedical areas (Claw et al., 2021). The cited study aimed to identify the main

concerns, needs, and desires of the Navajo people regarding genetic research, with Assistant Professor Katrina Claw from the University of Colorado, who is a member of the Navajo Nation, serving as the first author. This indigenous-led approach is a step toward dismantling historical biases and ensuring that research aligns with the specific needs and perspectives of these communities.

Similarly, delivering tangible results to communities is of utmost importance. However, this task comes with its own challenges, particularly in translating genetic concepts such as admixture and emphasizing the distinction between identity and biology. In Chile, researchers collaborated with Mapuche communities, adopting a strategy that involved sharing results with the communities before publishing the scientific paper. This approach facilitated the incorporation of community perspectives. To ensure broad outreach, they disseminated study results to individual community members, cultural representatives, and high schools. They emphasized the region's pre-European contact history using didactic materials and a Spanish report written in non-specialized language (Arango-Isaza et al., 2023).

The implementation of specific public policies to grant indigenous individuals access to higher education and postgraduate studies is another crucial step. In Brazil, this initiative holds the potential to reshape expectations by fostering the training of qualified human resources within indigenous communities, enabling active participation in the discussion, design, and implementation of research in the field of genetics and related areas.

1.9 Oral microbiota in indigenous groups

In light of the concerns related to indigenous communities, particularly regarding their healthcare and historical neglect, one thriving area of research in recent years is the study of the microbiota. The microbiota is defined as the community of microorganisms that inhabit various sites or habitats, including those within other living organisms. Given that evidence strongly suggests the pivotal role of oral microbiota in human health and disease, its examination in diverse human populations carries significant advantages.

However, as previously emphasized, not all populations receive equal representation in scientific studies, particularly in genetic research. For a more comprehensive exploration of this issue regarding the microbiota, especially the oral microbiota, within indigenous populations, please refer to Chapter 3. Results: Marcano-Ruiz et al. (2023). Oral Microbiota, Co-Evolution and Implications for Health and Disease: The Case of Indigenous Peoples (*Genet Mol Biol* 45:e20210309).

CHAPTER 2

JUSTIFICATIVE AND OBJECTIVES

2.1 Justificative and general objective

The study of Native American peoples is of paramount significance due to their diverse demographic structures, historical legacies, and contemporary challenges. Understanding these dynamics is crucial for addressing both historical questions and the specific needs of different Native American groups.

Exploring mating patterns in Southern Native American populations offers insights into genetic diversity and cultural resilience, shedding light on indigenous cultures. Meanwhile, the enduring impacts of assimilation, stemming from colonization, require examination to understand their lasting effects on present-day South American societies.

Additionally, this research strives to address persistent health disparities in Indigenous communities. It highlights the fundamental role of oral microbiota in oral and systemic health, especially among populations that have undergone rapid dietary changes and face social vulnerabilities. Thus, we aimed to provide scientific data that supports the development of culturally sensitive health strategies designed to improve health services, promote social justice, and empower Native American populations, ultimately promoting equity.

2.2 Specific objectives

1) To review studies on the oral microbiota in indigenous peoples and identify issues of inequity (Chapter 3).
2) To explore evolutionary phenomena involving the symbiosis between microorganisms in the human oral cavity and between them and the eukaryotic host (Chapter 3).
3) To analyze mitogenomes, including an original dataset from Andean populations from a broad and diverse sample of Native Americans across different regions of South America (Chapter 4).
4) To evaluate the nature of mtDNA diversity using native lineages in admixed (mestizo) populations and compare it with that of lineages in Native American populations from South America (Chapter 4).
5) To determine if the findings of Tavares et al. (2019) can be replicated in other regions (Chapter 4).
6) To discuss whether the contribution of indigenous women to the formation of national populations in South America can be considered a case of cultural genocide (Chapter 4).

CHAPTER 3

RESULTS

Oral Microbiota, Co-Evolution and Implications for health and disease: The case of Indigenous peoples (Marcano-Ruiz et al. 2024. *Genet Mol Biol*. 46:e20230129).

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Review Article
60 years of the PPGBM UFRGS – Special Issue

Oral microbiota, co-evolution, and implications for health and disease: The case of indigenous peoples

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Abstract

Evidence indicates that oral microbiota plays a crucial role in human health and disease. For instance, diseases with multifactorial etiology, such as periodontitis and caries, which cause a detrimental impact on human well-being and health, can be caused by alterations in the host-microbiota interactions, where non-pathogenic bacteria give way to pathogenic orange/red-complex bacterial species (a change from a eubiotic to dysbiotic state). In this scenario, where thousands of oral microorganisms, including fungi, archaea, and phage species, and their host are co-evolving, a set of phenomena, such as the arms race and Red or Black Queen dynamics, are expected to operate. We review concepts on the subject and revisit the nature of bacterial complexes linked to oral health and diseases, as well as the problem of the bacterial resistome in the face of the use of antibiotics and what is the impact of this on the evolutionary trajectory of the members of this symbiotic ecosystem. We constructed a *16SrRNA* tree to show that adaptive consortia of oral bacterial complexes do not necessarily rescue phylogenetic relationships. Finally, we remember that oral health is not exempt from health disparity trends in some populations, such as Native Americans, when compared with non-Indigenous people.

Keywords: Oral microbiota, co-evolution, Native Americans.

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The microbiota, coevolution, and implications for human health and disease

The microbiota is the community of microbes that colonize sites (or habitats), including within other living organisms. This means that symbiosis, a biological phenomenon where two or more species interact evolutionarily with different implications for each other, is necessarily present in the microbiota.

The microbiota was largely ignored as a determinant factor of health and disease in living beings, partly due to methodological limitations that hindered the identification of its members and prevented a full understanding of their complex interactions. However, advances in large-scale sequencing techniques have produced a wealth of data that can now be analyzed using sophisticated bioinformatics tools, generating robust evidence that the microbiota plays a crucial role in the health and disease of living organisms. These methodologies have also been used to reveal how the members of this complex ecosystem evolve.

The oral cavity of an animal contains diverse sites, including teeth, the gingival sulcus, gingiva, tongue, cheek, lip, and palate, as well as the dental biofilm (also known as plaque), and dental calculus (a form of hardened dental plaque) that forms at the teeth and the tooth-gum interface. In these sites, microorganisms inhabit and co-evolve with each other and with the host organism. This sensitive and complex ecosystem promotes a state of healthy balance (eubiosis) but also triggers disease in the host when an imbalance occurs, characterized by pathogenic microorganisms dominating over non-pathogenic ones (dysbiosis) (Kang *et al.*, 2021; Radaic and Kapila, 2021).

For example, periodontal diseases (PDs), including gingivitis and periodontitis, have a multifactorial etiology, with genetic and environmental factors contributing to their development. PDs are characterized by dysbiosis in the oral microbiota, with chronic inflammation promoted by pathogenic microorganisms and their metabolic products leading to the destruction of the supporting tissues of the teeth. This destruction results in progressive loss of connective tissue attachment and bone resorption, significantly affecting chewing function and the appearance of affected individuals (Ferreira *et al.*, 2017; Mohanty *et al.*, 2019; Kang *et al.*, 2021).

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Periodontal diseases represent a global public health concern, with a detrimental impact on human well-being and development. The PDs high incidence, prevalence, and life years lost/impacted, measured by disability-adjusted life years, have increased by ~8% from 1990 to the present, with particular impact in countries with low human development indices (Cui *et al.*, 2022).

The microbiota within the oral cavity is also a gateway to other colonizable sites in the body. Some oral microbiota species are considered risk factors for various systemic diseases, including certain types of cancer, cardiovascular, neurodegenerative, and metabolic diseases (Zhang *et al.*, 2018; Dioguardi *et al.*, 2020). The relationship between the oral microbiota and diabetes is particularly interesting, as research has shown that periodontitis-associated bacteria can produce lipopolysaccharide, leading to insulin resistance, inflammation, and impaired blood sugar control. In addition, poorly controlled diabetes can contribute to oral disease, highlighting the two-way relationship between diabetes and periodontitis (Zhang *et al.*, 2018). Furthermore, microbes can metabolize a wide range of different medications and have the potential to alter their mechanism of action, indicating that drugs can influence microbiota composition, and vice versa (DeClercq *et al.*, 2021).

In African bats, Lutz *et al.* (2022) found a link between dysbiosis in the oral microbiota and susceptibility to the Protozoan malarial parasites (*Plasmodium* spp). Specifically, the presence of *Pantoea agglomerans* bacteria can increase susceptibility to these parasites in bats. On the contrary, other authors showed that a protective microbiota suppresses parasite infection, reducing the need for host-based defenses. These results suggested that the microbiota can alter host-parasite co-evolutionary patterns and processes (Rafaluk-Mohr *et al.*, 2022).

Arms race, Red Queen, and Black Queen dynamics operating

It is important to note that natural selection can operate in various ways in a context where there are symbiotic relationships. For instance, natural selection can favor mutualism, a long-term codependency, when there is a benefit for each of the involved partners. However, mutualism requires that all members of the symbiosis maintain fitness, which makes them vulnerable over time to the evolution of "cheaters" or other forms of destabilization (Nelsen *et al.*, 2020). Therefore, despite the fact that interactions involving mutualism can be stable for long periods, it can also be seen as an evolutionarily transitory condition since it can evolve into parasitism, where one organism, the parasite, lives at the expense of the other, the host. On the one hand, commensalism describes a symbiotic relationship in which one organism benefits without causing harm to the other species in the ecological relationship. Commensal bacteria and their products have been shown to play a crucial role in regulating the development, homeostasis, and function of innate and adaptive immune host cells, which makes it context indistinguishable from mutualism. Besides, under certain conditions, it may be challenging to distinguish between commensalism and parasitism because the protagonist member may exact a high cost that cannot be compensated by other members of the association (Leung and Poulin, 2008). The gradual emergence of microbial mutualists from parasitic ancestors is also well documented (Drew *et al.*, 2021).

Moreover, a momentary loss of adaptive value in one of the species participating in the symbiotic relationship can trigger an evolutionary arms race or "Red Queen" dynamic (Van Valen, 1973). For example, in the case of antagonists, the pathogen and the host evolve to respond to the infection and defense strategies of each other, respectively, resulting in a situation where antagonistic organisms exert reciprocal selective pressure. This confrontation between antagonists will continue indefinitely unless one species goes extinct or when antagonists become evolutionary partners, ending the arms race or the Red Queen dynamic.

These two co-evolutionary types depend on whether there is reciprocal positive selection for host defenses and parasite infectivity (arms race) or balancing selection acting on the populations of the species in confrontation (Red Queen), which might be due to frequency-dependent selection or heterozygote advantage. Both models have been most often used to explain co-evolution between pairs of antagonists (*e.g.,* prey-predator, parasite-host interactions) (Rafaluk-Mohr *et al.*, 2022), or when an escalatory co-evolution results in a shift from mutualism to parasitism. However, they are not the only ones. For instance, the microbiota members in caries and periodontal diseases can display a sophisticated structural and functional interdependence which could also be explained by the "Black Queen" hypothesis (Lamont *et al.*, 2018). This hypothesis postulates, among other things, that pathogen agents that provide subversion of host immunity may discard expensive functions since they are maintained by other members of the bacterial community (Morris *et al.*, 2012). In this case, one set of dominant pathogenic microorganisms does not necessarily eliminate the others; conversely, they recruit them as helpers.

It should be noted, however, that these models/hypotheses are not mutually exclusive and do not claim to explain everything, particularly considering multispecific symbiosis, since the nature of the neutral, harmful, or beneficial interactions can change rapidly. They also do not include the role of the powerful purifying selection, drift, among other evolutionary processes.

In summary, the oral microbiota significantly influences human health and well-being and vice-versa. The interactions between microorganisms and the immune and inflammatory responses of the host are intricately correlated and can be affected by various other factors, including anthropogenic environmental conditions (*e.g.*, oral hygiene, healthcare, use of antibiotics, dental visits, diet, and smoking) and genetic factors. As a result, diseases can develop, leading to associated clinical outcomes. Furthermore, poor oral health results in debilitating pain, limited social interactions, difficulties in eating and speaking, embarrassment, and a dramatic loss of quality of life. From an evolutionary perspective, the oral microbiota provides fascinating examples of the co-evolution of thousands of microorganisms and a particularly notable host, the primate *Homo sapiens*.

Other evolutionary dynamics operating in the microbiota are commented on in the Supplementary Material (Additional information regarding the main topics of the review).

Oral microbiota, its composition, and its complexity

How to identify bacterial taxonomic categories

The human oral cavity is home to approximately 1,000 different species, with bacteria being the most predominant (Zhang *et al.*, 2018; Radaic and Kapila, 2021). Recent investigations have identified ultra-small bacteria belonging to the newly classified group known as "candidate phyla radiation" (CPR), as well as species belonging to the domains of life Archaea and Eukarya (Fungi and Protozoa), and viruses, including bacteriophages.

Due to the prevalence and significance of bacteria in the human oral microbiota, this review provides a more detailed exploration of their diversity and role in health and disease contexts. Relevant information on other oral microorganisms, such as fungi and viruses, can be found in the Supplementary Material section.

Classifying life forms into a hierarchical system (taxonomy) and applying names to that hierarchy (nomenclature) is a challenging task. This challenge becomes particularly difficult in the case of microbes, such as bacteria. However, it is at a turning point due to modern research and bioinformatics methodologies.

According the "Human Oral Microbiota Database" (HOMD, 2023), the oral human bacterial community is dominated by 18 Phyla. Among these are Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Fusobacteria, which account for 94% of the detected taxa but the species-level abundance for each oral site is variable.

HOMD indicates that there are 774 oral bacterial species, 58% of which are formally named and classified, 16% have not been classified/named yet, although cultivated. In contrast, 26% are known only as non-cultivated phylotypes, *i.e.*, an active group of individuals that are phylogenetically similar but have not been reproduced in culture yet.

The taxonomy adopted by HOMD provides a tentative naming scheme for currently unnamed taxa based on the *16SrRNA* sequence phylogeny so that strain, clone, and probe data from any laboratory can be directly linked to a reference scheme with a stable nomenclature. The 16SrRNA molecule, is required for the initiation of bacterial protein synthesis and the stabilization of correct codon-anticodon pairing in the 30S ribosomal subunit during mRNA translation (Wimberly *et al.*, 2000).

For decades, hypervariable regions of *16SrRNA* have been used to identify microorganisms, particularly bacterial and archaeal species, due to their evolutionary rate and other characteristics that can provide specific phylogenetic signatures. However, it is important to note that the classification of microbes based on *16SrRNA* sequence relationships has limitations, particularly in accurately representing phylogeny at different taxonomic levels. Nevertheless, culture-independent techniques are now producing thousands of metagenome-assembled genomes. In addition, metagenome-based identification facilitates the assessment of functional dynamics within the microbial community.

By combining vertically inherited single-copy protein genes, which are increasingly available from metagenome data sets, excellent resolution can be achieved, resulting in a more comprehensive representation of microbial diversity. Although concatenated protein trees have their limitations due to some level of lateral gene transfer, varying rates of evolution, and recombination, they have been used and considered the best approach for a reference bacterial phylogeny (Parks *et al.*, 2018; GTDB taxonomy is publicly available at the Genome Taxonomy Database (2023)). Other initiatives can be found in "The Human Microbiome Project Consortium" (2023). The Supplementary Material in this review also explores other aspects of the methodologies used to classify microorganisms.

The bacterial complexes

Although there is individual variation, most unrelated healthy people are believed to share a core oral microbiome (Caselli *et al.*, 2020), the collective genome of the oral microbiota. The co-evolution of this "healthy" core microbiome and the host genome, and their corresponding adaptive phenotypes, promotes an evolutionary state of apparent equilibrium, another way to define eubiosis. As already mentioned, this momentary stability can be disrupted when a group of bacteria triggers microbial imbalance (dysbiosis), provoking host diseases. These pathogenic microorganisms can evade the host immune response, surviving and reproducing in an oxidative stress-rich environment, such as the periodontal pocket, or thrive under an acidic environment found on supragingival biofilm associated with carious lesion development. In this scenario, a set of microorganisms cooperates in a co-evolutionary relationship. However, they harm the host, triggering a new cycle of arms race or Red Queen dynamics, considering the host and the pathogen, which can be extended to other members of the microbiota that pose as antagonists. The Black Queen hypothesis can also be evoked between the microbes to explain at least parts of these findings since, in many cases, there is no complete overlap among the species.

The observation that there is a succession of bacterial prevalence from a healthy oral state to a diseased oral state led Socransky *et al.* (1998) to utilize various available techniques to classify subgingival biofilm bacteria into color groups, as shown in Table S1.

The "red complex" is an adaptive consortium of pathogenic bacteria species present in the human oral microbiota that are strongly linked to oral diseases. These bacterial species include *Tannerella forsythia, Treponema denticola*, and *Porphyromonas gingivalis*, and their concomitant appearance is mainly due to their synergistic interaction (Socransky *et al.*, 1998). Recent studies have shown that these bacteria exhibit metabolic interdependence (Nayak *et al.*, 2018), signaling a scenario of obligatory mutualism between them.

Bacteria such as *Campylobacter rectus, Fusobacterium nucleatum,* and *Eubacterium nodatum,* belong to the "orange complex" and are considered pathogenic agents (Table S1). They also serve as a bridge between early colonizers and the red complex bacteria. The oral colonization sites and the number of the red complex bacteria increase with an increase

in colonization by the orange complex (Mohanty *et al.*, 2019; Kang *et al.*, 2021). Subjects who did not respond adequately to antibiotic treatment ("refractory" patients) may have 80% of their bacterial microbiota composed of red and orange species (Socransky and Haffajee, 2005).

Dental caries is another multifactorial oral disease, and it is considered the most prevalent human disease. Dental caries is caused by an overgrowth of acid-tolerant bacteria in the oral cavity, such as *Streptococcus mutans,* but the presence of red complex bacteria has been found to be positively associated with an increased risk of this pathology in different populations (Ozga *et al.*, 2016; Inquimbert *et al.*, 2019).

Species of the genus *Streptococcus* make up the "Yellow complex", while the "Green complex" is composed of species of the genera *Campylobacter, Capnocytophaga, Aggregatibacter*, or *Eikenella*. The "purple complex" comprises *Veillonella parvula* and *Actinomyces odontolyticus* (*Schaalia odontolytica*), while the species of genus *Actinomyces* are recognized members of the "blue complex" (Socransky and Haffajee, 2005) (Table S1). These last four conglomerates are early colonizers of the tooth surface and firmly related to periodontal health.

The presence of certain species of oral microbiota, such as *P. gingivalis* (red) and *Prevotella intermedia* (orange), has also been associated with an increased risk of cancer (Pignatelli *et al.*, 2022), while some studies show that the nature of the oral microbiota can vary considering diabetic and non-diabetic subjects, although the specific differences are not clear (Zhang *et al.*, 2018). However, some studies reveal that subjects with good glycemic control have a lower detection of *Filifactor alocis* as compared to fair- and poor-glycemic-control subjects. The co-occurrence of this microorganism with the red *T. forsythia* in diabetic subjects with chronic periodontitis indicates a synergistic collaboration between them (Pandian *et al.*, 2023).

Additionally, studies have linked PDs and *P. gingivalis* to Alzheimer disease. This red complex bacterial species was detected in the brains of Alzheimer patients and have shown to increase amyloid plaque production in mice (Abbayya *et al.*, 2015; Teixeira *et al.*, 2017; Dioguardi *et al.*, 2020; Radaic and Kapila, 2021). The inflammatory process associated with PDs may also intensify inflammation in the central nervous system, contributing to the occurrence of Alzheimer disease (Dioguardi *et al.*, 2020).

In addition to the oral bacteria mentioned above, some opportunistic pathogens found in the oral cavity and other body sites can also significantly threaten overall health. For instance, *Staphylococcus aureus*, which is often an asymptomatic colonizer, can turn into a virulent and multiresistant pathogen that causes infection in the oral cavity and can spread throughout the body, making it of great clinical significance (Howden *et al.*, 2023). Noteworthy, *S. aureus* isolates prevented the growth of *T. denticola* and *P. gingivalis,* suggesting a certain level of antagonism with these red complex bacteria (Suzuki *et al.*, 2013).

Interestingly, the studies show that the microbial community in the context of poor oral health is enriched with species traditionally classified as red-complex bacteria. However, they do not entirely replace the non-pathogenic ones. In other words, the virulence and pathogenicity of the red/orange species against the host are thought to be enhanced not only for their synergistic interactions with each other but also with other members of the oral microbiota. This finding indicates that the Black Queen hypothesis can be part of the phenomena that explain the co-evolution of these oral microorganisms.

Diversity of the oral microbiota in humans and other primates

Some studies with non-human primates, extinct hominins (*e.g., Homo neanderthalensis*), and ancient *Homo sapiens* populations have been carried out, introducing temporal depth and a macroevolutionary perspective regarding the nature of the oral microbiota.

For example, chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*) that inhabit different African sanctuaries had a salivary microbiota that was more similar to each other compared to the humans (employees of each sanctuary). Furthermore, the two human groups compared also showed similarities in their microbiota, a result consistent with the phylogenetic relationships and physiology of the hosts (Li *et al.*, 2013).

Other studies also revealed that the dental calculus microbiota of chimpanzees has a higher frequency of bacteria of phyla Bacteroidetes and Fusobacteria, while humans have higher proportions of Firmicutes and Proteobacteria species (35% and 19%, respectively, of the species already identified in the oral cavity), but the causes of these differences remain unknown (Ozga *et al.*, 2019; HOMD). Ozga *et al.* (2019) did not find a significant association between one particular bacterial genus and the presence of caries or the absence of teeth in chimpanzee. Besides, PDs have been documented in captive and wild great apes, but the connection between these and the red complex bacteria in the oral cavity of *Pan* spp. is not known yet (Ozga *et al.*, 2019). Another example can be found in the Supplementary Material section.

Analysis of dental calculus from five Neanderthals revealed that meat consumption contributed to substantial variation in their oral bacterial community (Weyrich *et al.*, 2017). Weyrich (2021) used modern farmers and hunter-gatherers as proxies to understand how changes in diet have been affecting the oral microbiota of *Homo sapiens* over time. The author suggests that in Europe, the evolution of the oral microbiota has been shaped by interactions with Neanderthals in ancient times as well as adaptation to agriculture and industrialization in more recent times.

Genetic data in the context of the human oral microbiota

Within an ecosystem where powerful evolutionary forces are at play, signatures in the microbiome and host genome are expected to occur. Below are some illustrative examples.

Resistome as an example of the bacterial response

Antibiotics are vital for treating infectious diseases, including oral pathologies. However, antibiotics act as agents of natural selection. Their excessive and inappropriate use has led to the rapid development and spread of microbial resistance,

which is a serious global public health challenge. If nothing is done, the number of human annual deaths attributable to antimicrobial resistance will rise from the current ~700,000 to ~10 million in the year 2050 (O'Neill, 2016).

Therefore, identifying the "resistome" of the microbiota, which refers to the set of functional genes responsible for antibiotic resistance (ARGs) present in bacteria, become critical due to accelerated emergence of antibiotic-resistant oral pathogens. In addition to antibiotics, metal and metal oxide nanoparticles have been studied due to their antibacterial properties in dentistry. Consequently, genes responsible for metal resistance (MRGs) are investigated due to the decay of their antibacterial activity over time (Kang *et al.*, 2021). The co-occurrence of ARGs and MRGs signals the existence of multiple selective pressures. Kang *et al.* (2021) conducted a metagenomics study, considering dental plaque samples from healthy and periodontitis subjects and those after successful treatment. Genes that confer resistance to Tetracycline [such as *tet(32)* and *tetW*] and multiple drugs (such as *emrA, emrB,* and *mdtG*) were highlighted by the authors, but the number of ARGs in patients was significantly higher than in the group of healthy volunteers. Furthermore, they found a significant change in the profiles of ARGs and MRGs of the microbial community present in dental plaque due to treatment for periodontitis (Kang *et al.*, 2021).

ARGs and MRGs are often transferred between bacteria via mobile genetic elements, such as conjugative plasmids and transposons, promoting bacterial genome mutability and evolution. For example, transposons belonging to the Tn916 family can transfer resistance to various pathogens, including commensal and pathogenic oral bacteria (Kang *et al.*, 2021).

Sukumar *et al.* (2023) investigated the oral resistome and its role in dental caries in 221 twin children at three different time points T1 (6.7±2.7 months, absence of teeth), T2 (1.6±0.4 years, primary/deciduous/baby teeth only), and for T3 (8.5±1.2 years, mixed dentition). They found the co-occurrence of Tn916 transposase and ARGs in 50%, 32%, and 35% of individuals at T1, T2, and T3, respectively. This co-occurrence was predominantly observed in *Streptococcus* species across all time points (78%). Moreover, the study showed that over time, Tn916 was detected in a greater number of species and in combination with more ARGs. For instance, at T1, the co-occurrence of Tn916 with *tet(M)* and *ermB* was found in *Streptococcus oralis*, but by T3, the same species was associated with three additional ARGs: *mefA, msrD*, and *lsa(C)*. The investigation revealed a decrease in both ARGs and species abundance in dental caries compared to healthy teeth (Sukumar *et al.*, 2023).

It is noteworthy that ARGs, MRGs, and other lines of bacterial defense mechanisms predate the existence of synthetic antibiotics produced/used by humans. These adaptive traits have been shaped by billions of years of evolution since the appearance of the first bacteria (Figure S1). In addition to the mobile resistome, bacterial species possess a resistome consisting of genes in their single circular chromosome.

Surveillance of resistomes is a crucial tenet of the One Health initiative in combating antimicrobial resistance (Sukumar *et al.*, 2023; WHO, 2023). One Health is an integrated, unifying approach to balance and optimize the health of people, animals, and the environment (WHO, 2023).

Table S2 provides examples of oral bacteria that have developed resistance to common antibiotics, such as β-lactam and Tetracycline, as well as other selected bacterial-resistance information.

Host-defense peptides and other examples of the *Homo sapiens* response

There are known host-defense peptides (HDPs), originally described just as antimicrobial, but now have renewed significance as curators of the pervasive microbial loads required to maintain homeostasis and manage microbiome diversity (Meade and O'Farrelly, 2019). One of the best known and studied HDP families is the β-defensins which are produced in diverse combinations by epithelial and immune cells. β-defensins members, such as DEFB1, are peptides produced in diverse combinations by epithelial and immune cell populations, which manage the microbial colonization. Some alleles in polymorphic *DEFB1* loci are risk factors for PDs and caries (Table S3).

Host cytokines also modulate the immune response, altering its efficiency in the competition against pathogens and increasing PDs susceptibility. Baker *et al.* (2021) found in the saliva of children with caries a significantly higher concentration of ten salivary immunological markers, such as interleukins (Baker *et al.*, 2021). Furthermore, some allele combinations in polymorphic loci in Interleukin-10 gene (single-nucleotide polymorphisms SNPs: IL10; A-1082G rs1800896, C-819T rs1800871, and C-592A rs1800872) may reduce IL-10 production and has been associated with oral pathologies, such as periodontitis. In contrast, other allele combinations promote the anti-inflammatory immune response, which is important for protection against the microbes that cause periodontitis (Lopes *et al.*, 2017).

Considering large-scale genomic association studies (GWAS) and periodontitis or caries data, the first results were published more than 20 years ago (reviewed in Morelli *et al.*, 2020). Other lines of research, including investigations with twins or families, and approaches with candidate genes, helped build a solid argument on the role of host genetic factors in oral diseases. For example, Schaefer *et al.* (2010) showed the *GLT6D1* SNP rs1537415 association with aggressive periodontitis. Bevilacqua *et al.* (2018) found association between the *CRACR2* (*EFCAB4B*) rs242016 and periodontitis in a small Italian population (Table S3 provides other examples). See also Morelli *et al.* (2020) and Weyrich (2021).

We compiled allele frequencies in human populations, considering selected genes with SNPs previously associated with PDs and/or caries like those mentioned above (Tables S3-S4). A statistical test revealed that the major geographical groups significantly differ in their risk/or protection alleles (Table S5), suggesting differential susceptibilities across human populations.

Indigenous peoples, neglected peoples

The United Nations (UN) recognize that indigenous peoples are among the most vulnerable and disadvantaged

groups in the world, emphasizing the need for special measures to protect their rights, preserve their unique cultures and ways of life, and address their health (UN, 2023). Despite this recognition, indigenous communities often remain invisible and marginalized, as exemplified by recent events, such as the Yanomami case in Brazil (Boadle, 2023).

The scarcity of scientific studies on indigenous peoples is just one aspect of this invisibility problem. A 2009 survey found that 96% of participants in GWAS were Europeans or European descendants (Need and Goldstein, 2009). More recent data from Sirugo *et al.* (2019) showed that this bias remains strong, with 78% of people included in GWAS studies being Europeans or having European ancestry, only 10% being Asians or having Asian descent, 2% being Africans or having African descent, 1% being Latin Americans, and less than 1% being from other populations. This bias hinders the ability of the researchers to understand the genetic basis of common diseases with multifactorial inheritance (*e.g.*, PDs and dental caries), which are characterized by many small-effect alleles whose frequencies can vary significantly across human populations. The lack of information from neglected and invisible populations prevents the use of methods for predicting polygenic genetic risk alleles for these common diseases, which affect millions of people from these populations worldwide.

The same can be said for rare and Mendelian genetic diseases. Advances in genomic technologies are transforming the diagnosis of these diseases, but indigenous populations often experience inequities in diagnostic and therapeutic access (D'Angelo *et al.*, 2020).

Without action to address these distortions, genomic medicine will soon be available only to a few privileged people (Bustamante *et al.*, 2011; Popejoy and Fullerton, 2016).

Oral health is not exempt from health disparity trends among Indigenous populations; untreated dental caries, less restored teeth, and PDs have been reported to be higher in Indigenous than non-Indigenous populations (Nath *et al.*, 2021). As a result, these neglected people experience amplified damage caused by worse oral health.

The indigenous people of the American continent (or Native Americans) can be considered neglected regarding genetic/genomic and oral cavity health studies when they are compared with non-natives. Nevertheless, some efforts that resulted in publications already signal some trends. For example, it is known that the specific combination of social, historical, environmental, economic, and ethnic-cultural circumstances in which Indigenous and non-Indigenous individuals interact can create varying protective or risk factors that affect oral health outcomes in distinct ways for Indigenous groups (Arantes *et al.*, 2021).

The study performed by Arantes *et al.* (2021) found that Indigenous individuals from Guarani-Kaiowá, Guarani (unspecified regarding partiality), Terena, and Kadiwéu populations (Mato Grosso do Sul Brazilian State) had a lower incidence of tooth decay compared to non-Indigenous individuals, but they faced greater challenges in accessing restorative dental services.

Figueiredo *et al.* (2013) and Ribeiro *et al.* (2016) investigated a native community (Kiriri) in northeastern Brazil, whose members have been influenced by external sociocultural habits, including a diet rich in sugar, despite striving to preserve part of their traditions. The population has a high prevalence of destructive periodontal disease (98%), and individuals with this pathology had higher systolic blood pressure than controls (Ribeiro *et al.*, 2016).

Gaetti-Jardim *et al.* (2015) investigated the oral microbiota of individuals from the Umutina Indigenous Territory (IT), where an ancestral lifestyle is observed and there has been no admixture with non-native populations. The Umutina IT is home to 480 individuals of the Umutina, Paresi, Bororo, Bakairi, Kayabi, Irantxe, Nambikwara, and Terena peoples, whose occupation of the territory dates back at least six generations (Gaetti-Jardim *et al.*, 2015). The occurrence of pathogenic bacteria was similar to that previously described for other populations worldwide, but with some notable differences. The red complex *T. denticola* has a more restricted distribution in Native American populations, being observed in only 5% of the Guatemalan Maya and 13-17% of the natives of the Xingu IT, a number similar to that found in the Umutina IT (Gaetti-Jardim *et al.*, 2015). Likewise, *Actinobacillus actinomycetemcomitans* belonging to the green bacterial complex (Socransky and Haffajee, 2005), but currently recognized as one of the agents of PDs (Gholizadeh *et al.*, 2017), had reduced prevalence in natives of the Umutina IT when compared to non-native Brazilians (Gaetti-Jardim *et al.*, 2015).

Clemente *et al.* (2015) characterized the oral bacterial microbiota of 34 individuals from an isolated Yanomami village. Their oral microbiota was compared with that found in urbanized non-native individuals living in the United States. The yellow bacteria genus *Streptococcus* dominated both populations, but the Yanomami had higher proportions of two other yellow genera, *Prevotella* and *Fusobacterium*. The authors found that despite any known exposure to antibiotics, bacteria in the Yanomami participants had ARGs that confer resistance to synthetic antibiotics. Clemente *et al.* (2015) emphasized the need for a broad characterization of the microbiota and its resistome, considering that indigenous peoples are still living according to ancestral lifestyles before modern practices promoted potential dysbiosis.

Lopes *et al.* (2017) investigated three *IL10* polymorphisms in urban non-indigenous individuals living in Belém (Pará, Brazilian State), and found that those with a higher contribution of Native American ancestral alleles had an increased risk of developing periodontitis.

Regarding other American countries, Ozga *et al.* (2016) used *16SrRNA* to access the microbiota of Cheyenne and Arapaho members living in Oklahoma (USA) and compared them with their non-indigenous neighbors. They found that the natives had a higher frequency of bacteria implicated in systemic disorders, such as those of the orange complex genus *Prevotella*. In another study, Agnello *et al.* (2017) sought to understand why Indigenous children in North America suffer with a higher degree of severe early caries (S-ECC) than the general population. They found that the S-ECC group had extremely higher level of cariogenic *Streptococcus mutans*, and 9-fold higher level of red complex genus *Porphyromonas* than the caries-free counterparts.

Bravo-Lopez *et al.* (2020) reported the first direct evidence of the presence of red complex bacteria (*T. forsythia, P. gingivalis,* and *T. denticola*) in dental calculus samples from archaeological skeletons spanning from the pre-Columbian to the colonial period in Mexico. *T. forsythia* was the dominant bacteria, and the phylogenetic relationships of the strains showed that some of them arrived with the first migrants to America, while others arrived with Europeans and Africans in the 16th century. The authors also identified that the *tetQ* gene involved in resistance to Tetracycline was absent in all ancient *T. forsythia* strains.

Honap *et al.* (2023) investigated the skeletal remains of ancestors of the Wichita, a southern Plain Native American people from Oklahoma. They also found the presence of the three red complex species, regardless of oral disease, in the sampled tooth. The researchers corroborated that there were pre-Columbian strains of those bacteria, which were prevalent until Europeans arrived.

Additional instances of indigenous peoples from other continents and ethical considerations regarding microbiota studies in these communities are available in the Supplementary Material section.

Exploring bacterial complexes

Socransky *et al.* (1998) used a range of techniques available at the time of their studies, including DNA-DNA hybridization, to show contextual similarities between species within complexes and their relationship to clinical outcomes (Table S1). Basically, bacteria belonging to the blue, yellow, green, and purple complexes are linked to oral health. Conversely, bacteria from the orange and red complexes are associated with dysbiosis of the microbiota, most commonly leading to the development of diseases in the oral cavity and also in other organs and tissues of the body.

We have performed a phylogenetic analysis using *16SrRNA* sequences (see Additional information section/ Material and Methods; Tables S6-S7) of the bacteria belonging to these complexes, and the topology is present in Figure 1. The tree illustrates that the species in the complexes do not necessarily cluster together. Figure S2 shows a network with the same data set, another way to illustrate the differences and similarities. This result may be indicating some level of evolutionary convergence that leads bacteria not closely related phylogenetically to have similar characteristics (*e.g.*, virulence or non-virulence), when the consortium between them confers an adaptive advantage under others and the host. For instance, the three main members of the red complex, *T. forsythia, P. gingivalis,* and *T. denticola* belong to two different phyla, predicted by HOMD and GTDB consortium methodologies (Table S1), and are equally capable of producing various virulence factors that assist in their survival and contribute to the development and progression of the oral disease in the host: *P. gingivalis* produces, for example, heat shock proteins, while *T. forsythia* and *T. denticola* utilize proteases and dentilisin, respectively, among others, as virulence factors. *In vivo* studies have shown that these bacteria have nutritional interdependency, and the ability to regulate the virulence factors of each other (Nayak *et al.*, 2018). These phyla are separated by about 2.8 billion years (Figure S1).

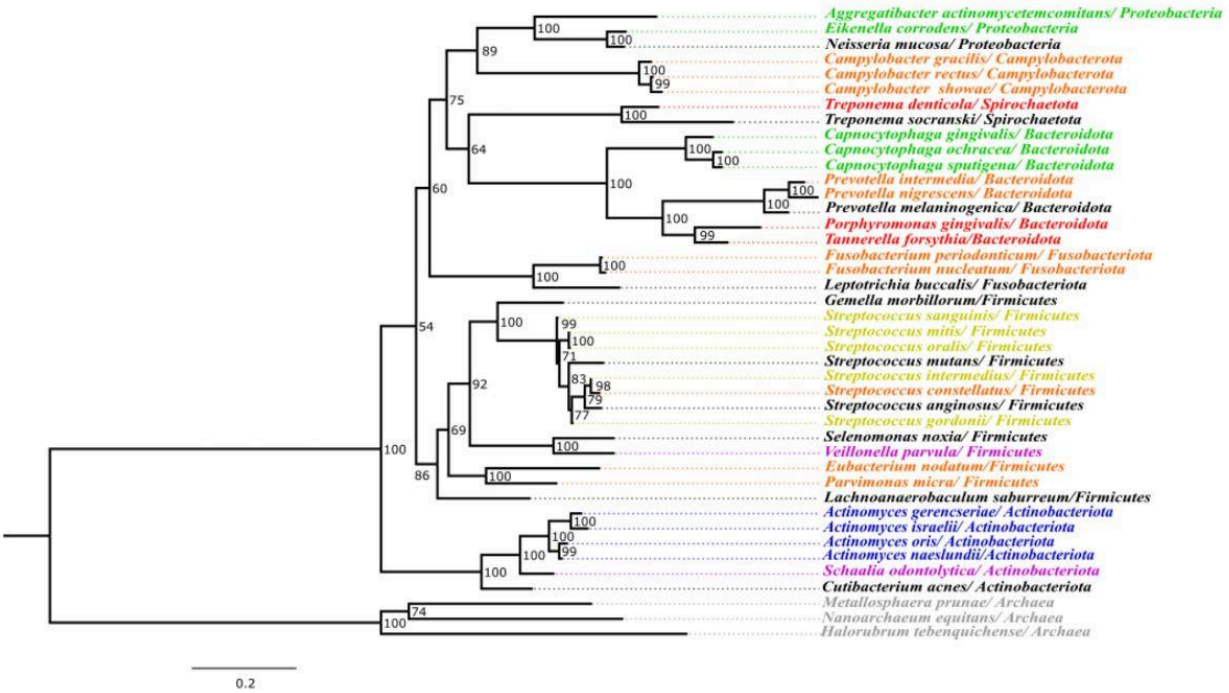

Figure 1 – Maximum Likelihood phylogeny based on *16srRNA* sequences from 39 bacterial taxa. The colors in each taxon indicate the representation of complexes and groups. The Phyla nomenclature is according to the GTDB.

Noteworthy, the notion of complexes to indicate the level of periodontal health and pathogenicity, is being challenged. For example, in addition to the primary members of the red and orange complexes, there are other bacteria that have been suggested to be involved in PDs. These include: *Filifactor alocis, Fretibacterium fastidiosum, Eikenella corrodens, Capnocytophaga* spp., and *Aggregatibacter actinomycetemcomitans*, the last three genera already associated with the yellow and green complexes, respectively, whose members are more commonly associated with periodontal health contexts (Gholizadeh *et al.*, 2017; Kang *et al.*, 2021; Ozuna *et al.*, 2022; Pandian *et al.*, 2023). Kang *et al.* (2021) concluded that beyond the red complex *T. forsythia* and the orange complex *C. rectus*, *F. fastidiosum* is also a key driver during microbiota alteration in the progression of periodontitis.

Valdebenito *et al.* (2018) analyzed the effects of competition between two prevalent species in individuals with healthy teeth (*Streptococcus sanguinis;* Yellow complex) or with caries (*Streptococcus mutans*). These two species occupy the same ecological site and have similar metabolic needs. The study focused on characterizing the differences in their genomes to explore the potential genetic advantages of one over the other, since they are antagonist species. For instance, *S. sanguinis* produces hydrogen peroxide (H_2O_2; gene *spxB*) with antimicrobial activity, but this bacterium has also an enzymatic machinery involved in H_2O_2 detoxification. *S. mutans* itself does not produce significant amounts of H_2O_2, and it is highly susceptible to H_2O_2 because it lacks an efficient detoxification machinery. The authors found that *S. sanguinis* has three additional detoxification enzymes, which could be advantageous over *S. mutans*. In contrast, mutacin is involved in a system responding to environmental changes, including the ability to incorporate foreign DNA (competence) and biofilm development. Mutacin allows *S. mutans* to adapt and survive under stress (extremely acidic conditions), an unfavorable condition for *S. sanguinis*. This example illustrates that even within the same genus, there are bacteria associated with a healthy state and those related to oral pathology, indicating an antagonistic condition, which potentially triggers a systematic arms race or Red Queen dynamics between them. Interestingly, *S. sanguinis* is predominantly isolated in healthy children, whereas non-detectable levels were found in individuals with carious lesions, suggesting a replacement. In this case, the Black Queen hypothesis loses strength, at least considering the evolutionary relationships between these two streptococcal species.

Conclusions

General microbiota patterns are observed in healthy individuals and those with oral pathologies, although with some individual variation. Microbiota variability is also notable among human populations. Bacteria from different phyla can also form adaptive consortia, while those from the same genus can result in different outcomes for the host, indicating antagonism. Furthermore, many lines of evidence reinforce the idea that the genetic profile of the human host is related to the dynamics of its oral microbiota and vice versa (Weyrich, 2021).

The influence of host and microbiota genetics, evolution, environmental exposures, treatments, and bacterial resistance to antibiotic stresses the need to consider the problem within multidisciplinary or even translational approaches, such as the One Health initiative proposed by World Health Organization.

Despite the challenges faced by this complex theme, reversing the global trend of deaths and loss of quality of life caused by oral infectious diseases, particularly in countries with low human development indices and neglected populations, is essential.

We conclude with the hope that in a future review it will no longer be necessary to refer to Native American peoples as neglected groups when compared to others.

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Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

Authors Contributions

MM-R. Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing; GMT. Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing; MTSM. Data curation, Investigation; LSK. Investigation; LS-F Funding acquisition, Investigation; TL Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing; MCB Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Supervision, Project administration, Writing.

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Supplementary material

Supplementary Material – the following online material is available for this article:

Table S1 – Species belonging to bacterial complexes used for the phylogenetic analysis.

Table S2 – Examples of oral bacteria with strains resistant to common antibiotics.

Table S3 – Examples of SNPs associated with caries and/or PDs.

Table S4 – Selected SNPs associated to the PDs and/or caries and allele frequencies.

Table S5 – χ^2statistical test calculated for major human groups based on allele frequencies.

Table S6 – Bacterial species analyzed for the *16SrRNA* gene.

Table S7 – Microbial composition of the supra and subgingival plaque classified by complex..

Figure S1 – Divergence time tree considering 39 bacterial taxa.

Figure S2 – *16srRNA* sequence-based haplotype network considering 39 bacterial taxa.

Additional information regarding the main topics of the review.

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Supplementary material can be found in:

https://www.scielo.br/j/gmb/a/rPGTgSncfc6r5NCBcrbLyGb/?lang=en

CHAPTER 4

MANUSCRIPT IN PREPARATION (American Journal of Human Biology).

South America Natives and the impact of European colonization: Evidence from mitogenomes.

Discussion

Considering the high frequency of Native American mtDNA lineages in admixed populations resulting from the Latin American asymmetric mating pattern (indigenous women with European men) and the relatively short evolutionary time of the admixture process (524 years; approximately 21 generations), it has been suggested that current South American admixed populations serve as a reservoir of Native American genetic lineages. This suggests that they reflect the patterns of Native American mtDNA diversity during the pre-contact period between colonizers and conquered peoples.

In fact, Tavares and colleagues (2019) found that some admixed populations in Uruguay and Brazil carried a more accurate demographic signal from the past than contemporary Native populations. Furthermore, several mtDNA studies, involving both current and archaeological Native South American populations, have revealed a strong ancient expansion signal with variable dates around the Last Glacial Maximum (~22ka). This event is interpreted as the arrival in Beringia and subsequent entry into the American continent (Llamas et al, 2016; Arias et al., 2018a; Barbieri et al., 2017; Brandini et al., 2018; Tavares et al., 2019). However, using mtDNA data, except Tavares et al. (2019), it is not easy to find more comprehensive studies using natives and admixed populations from the same geographic regions.

The phylogenetic results led us to believe that we face different demographic patterns throughout the continent. On one hand, it seems that the indigenous populations in the south, many of which were tragically eradicated, managed to endure through the integration of women into society. This suggests that the extermination was potentially more cultural in nature rather than solely physical. Thus, here we have to evolve the concept of cultural extermination, or in other words, “cultural genocide”.

On the other hand, in regions like the Amazon, despite also suffering significant population declines, they succeeded in preserving considerable ancient genetic diversity among their present-day representatives, among which in groups that still relatively present their ancestral cultures. Noteworthy that, unlike the Southern Cone where few indigenous ethnic groups exist today (Guarani and Kaingang, speakers of the Tupi and Ge languages, respectively; live on legal indigenous lands demarcated in the 19th and early 20th centuries), and even in the past, Amazonia is a region with many different groups, some of which have preserved their hunter-gatherer diet habits to this day. All analyzed admixed populations showed either population stability or ongoing growth patterns (such as the Andean populations) closer to the present, similar to the archaeological samples, while all Native populations showed signs of decline. Altogether, these results are consistent with the intensity of the colonization process in different parts of the continent. This raises an important question: to what extent did the genocide experienced by some indigenous populations have a greater impact on their cultural destruction rather than physical extermination? and what does genetics have to say about it? We propose that cultural assimilation by indigenous women in the south could explain the pattern found. However, while admixed populations can be a very informative reservoir of Native American genetic diversity such as the case mentioned above, caution must be taken depending on the local demographic history.

The Truth and Reconciliation Commission of Canada has defined cultural genocide as the deliberate dismantling of structures and practices that enable a group to persist cohesively. This typically involves the seizure of land, forced relocation of populations, and constraints on their mobility (2023). Cultural genocide, while distinct from genocide in the sense of directly targeting people, can nonetheless yield physical consequences. It is important to note that the boundary between physical and cultural genocide is often blurred. In settler societies like the Americas, Australia, and South Africa, indigenous peoples endured significant suffering and fatalities due to diseases, starvation, and associated physical and cultural pressures (Hitchcock & Twedt, 2009).

Within international law, cultural genocide lacks a precise and universally accepted definition. This concept has never been conclusively defined, acknowledged, or codified by any nation in the world. Its inception in the 1940s was contentious, and its status remains contentious to this day (Luck, 2018). As a legal construct within international law, cultural genocide was introduced as a sub-category or facet of genocide, working alongside physical genocide and biological genocide. It encompassed the destruction of both tangible elements (e.g., places of worship) and intangible components (e.g., language) of cultural structures (Bilsky & Klagsbrun, 2018). Without necessarily using the words "cultural genocide" or "ethnocide," diplomats, international judges, United Nations special rapporteurs, and various experts have continuously warned about the diverse threats that a culture may face. The Inter-American Commission on Human Rights (CIDH) and the International Criminal Tribunal for the former Yugoslavia (ICTY) have certainly played a significant role in this campaign (Novic, 2016), fully endorsing the possibility of group destruction through the elimination of their culture and drawing noteworthy conclusions in terms of state responsibility and obligations.

In the context of settler colonialism, the concept of biocultural assimilation is significant. It highlights how assimilation programs, particularly in settler-colonial societies, can paradoxically serve as a method of elimination. These programs are designed to integrate indigenous populations into the dominant culture while erasing their unique cultural and biological identities, as could be the case of some populations in America (e.g Uruguay) (Short, 2010).

Our analysis underscores the relevance of cultural genocide. This concept, distinct from physical genocide, highlights the comprehensive state-led efforts to erase the cultural identity of targeted groups. In the case of Southern America, the biocultural assimilation of women highlights the intricate workings of assimilation programs, especially in settler-colonial settings where prolonged and consistent mechanisms unintentionally preserved elements of ancestral diversity in present-day admixed populations. These intricacies call for ongoing scrutiny and a fresh perspective when integrating these examples into various genetic studies in the region.

The present study provides further evidence that admixed populations in the Southern Cone of South America are a crucial genetic reservoir of Native American mtDNA lineages and support the idea that these lineages retain part of the general past demographic history of Native Americans. The results also highlight the importance of considering different factors, such as specific geographic locations (and distinct historical and colonial backgrounds), in the analysis of mitogenome data throughout South America.

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CHAPTER 5

FINAL CONCLUSIONS

Studying population genetics and demography in human populations allows us to understand how genetic variation is distributed among them, providing insights into human migration patterns, population histories, and adaptation to different environments. Moreover, studying evolution in genetics, anthropology, and health is significant for understanding human diversity, history, and disease susceptibility. This knowledge is crucial for addressing public health challenges and designing targeted interventions tailored to specific populations.

The focus on indigenous people in these fields is crucial due to their unique genetic diversity, cultural heritage, and health disparities. Indigenous populations offer valuable insights into human evolution, as they often harbor ancient genetic lineages and adaptations to diverse environments. Additionally, studying indigenous populations provides an opportunity to address health inequities and disparities, as they often face greater health risks and limited access to healthcare compared to non-indigenous populations. By prioritizing research on indigenous communities and engaging them in scientific studies, we can gain a more comprehensive understanding of human evolution, health, and cultural diversity while working towards promoting health equity and social justice.

Scientific studies examining the settlement of America, demography, and evolution had shed light on Native American ancestry and migration patterns. Pioneering research by Professor Francisco Mauro Salzano revealed the genetic landscape of Native Americans, evolving from blood systems to DNA analysis. In South America environmental transitions influenced demographic and cultural changes, particularly in the Andes. In other regions, settlement led to the formation of small, isolated groups, shaping distinct genetic patterns (Tarazona-Santos et al.,2001).

Mitochondrial DNA (mtDNA) analysis has been crucial for understanding population dynamics, genetic diversity, and historical migrations. With its high mutation rate and maternal inheritance, mtDNA traces maternal lineage and reveals population history. Recent

studies using mitogenomes have refined our understanding of colonization patterns and demographic events in the Americas (*e.g*. Llamas et al; 2016; Arias et al., 2018; Brandini et al., 2018; Tavares et al., 2019) incorporating Bayesian methods with mtDNA datasets to assess population size changes and migration patterns.

The arrival of Europeans in South America in the 15th century marked the beginning of significant demographic changes and the decline of indigenous populations. The formation of modern South American populations involved a mixing of Native American, European, and African populations. However, this mixing was asymmetric with European settlers predominantly intermarried with indigenous or african women. This blending created mestizo societies with complex ancestries. Regional differences in colonization and admixture patterns are influenced by geography, historical events, and social dynamics. Some areas, like Northeast Brazil, Cuba, and Venezuela, have higher African ancestry, while others, like Southeast/South Brazil, Argentina, and Uruguay, have higher European ancestry.

Since then, Native Americans have suffer different forms of genocide which has led to the loss of much of their pre-Columbian diversity. European colonization imposed cultural elements, displacing and diminishing local populations. This decline was particularly severe in areas with smaller pre-Columbian populations, such as the Antilles. Factors contributing to the decline include forced labor, the spread of European diseases, and atrocities of conquest. Some regions were profoundly disrupted by European intrusion, leading to decimation even before epidemics struck. The decline also had a gender bias, disproportionately affecting males of reproductive age. The European-dominated patriarchal structure led to the assimilation of indigenous and African cultures, often through forced assimilation or extermination.

Despite efforts to erase indigenous cultures, the genetic legacy of indigenous peoples persists, particularly through matrilineal lines. For example, genetic studies in urban areas like Porto Alegre reveal significant indigenous genetic heritage (mtDNA) among self-identified "white" individuals (Guerreiro et al., 2009). Overall, despite the disappearance of indigenous populations and cultures in some regions, the substantial genetic legacy of indigenous peoples endures. The assimilation of indigenous women into emerging societies has contributed to the erosion of their culture, representing a form of cultural genocide alongside their physical survival. Cultural genocide, distinct from physical genocide, involves

deliberate actions to destroy the structures and practices allowing a group to continue as a distinct entity. It often occurs in the context of assimilation policies and settler colonialism, aiming to replace indigenous cultures with those of the colonizers permanently.

The colonization of the American continent has had profound and enduring impacts on indigenous communities, leading to disparities in wealth, health, and access to resources. Despite efforts to address these issues, indigenous peoples continue to face significant challenges, including poverty, marginalization, and limited access to healthcare and essential services. Indigenous communities in Latin America are particularly vulnerable, grappling with historical injustices, ongoing discrimination, and health disparities (CEPAL, 2014). Factors such as inadequate living conditions, restricted access to healthcare, and low educational attainment contribute to their poorer health outcomes, including elevated infant mortality rates and increased susceptibility to infectious diseases. Additionally, economic activities like mining exploration exacerbate their vulnerability by introducing health risks such as heavy metal contamination (De Oliveira et al., 2023).

Efforts in genetic research involving indigenous populations have been limited, with challenges in obtaining samples and resources and neglect in study design. Despite the great effort of some research groups, specifically in Brazil led by prof. F.M. Salzano and other LEHM researchers, as well as those in the group led by prof. Sergio D. Pena at UFMG, there is still much to be done to address disparities and biases in genetic research, including empowering indigenous researchers and promoting community engagement as pointed out by the indigenous researchers Tsosie and colleagues (2020).

In this context, the present Thesis aimed to address critical issues within two key areas: indigenous health, focusing primarily on an initial exploration of oral microbiota (Chapter 3), and demography, variability, and cultural genocide among Southern American Native populations (Chapter 4).

Chapter 3 delves into the first scope of this thesis by exploring the oral microbiota, marking our research group's entry into this area. Our aim was to provide an overview of the existing knowledge base, identify areas where further research is needed, and inform the development of research questions or hypotheses for future studies within our research group. Objective 1 was achieved through a non-systematic review, or a scope review, where we observed a limited representation of Indigenous populations in studies regarding oral

microbiota and oral genomics in general. For instance, by searching in PubMed database with 5 key terms (oral microbiota; indigenous; natives; native american; oral microbiome) from 2002 to 2024, we could only find 4 articles related to oral microbiota in Native populations or indigenous health. We highlighted the disparities in oral health and microbiota studies between Native Americans and non-Indigenous populations, such as Europeans, underscoring the importance of continuing our research in this area. This perspective motivates our future endeavors, with the hope of contributing to this exciting field and including Brazilian indigenous populations in the near future.

For objective 2, we examined evolutionary dynamics within the microbiota and its interaction with the host, including the host genetic response to dysbiosis and bacterial response through the resistome. Additionally, we revisited bacterial complexes linked to oral health and diseases. One significant finding was the revelation, through a 16S rRNA tree analysis, that adaptive consortia of oral bacterial complexes do not necessarily align with phylogenetic relationships, suggesting other underlying mechanisms.

In Chapter 4, objectives 3 through 6 were addressed. Our analysis of several mitogenomes within Southern American populations through bayesians analysis corroborated the findings of Tavares et al. (2019) revealed differentiation between mitogenomes of Native populations in the Southern cone of South America compared to those in other regions, achieving objectives 3, 4, and 5. For example, populations from the Amazon, both Native and Admixed, have preserved the signal of the initial migration and dispersion to the continent within a similar timeframe, while the Native populations from the Southern Cone appear to have their signal eroded. Regarding objective 7, we hypothesize that the cultural genocide experienced by certain populations of the continent, particularly concerning the assimilation of indigenous women in the formation of certain national societies during the conquest and colonization of the American continent, could explain the patterns observed in our analyses. This prompts reflection on how to further investigate and confirm this hypothesis through different genetic approaches, emphasizing the need to expand the sample size as more mitogenomes of American populations are sampled.

In conclusion, we hope that in the not-so-distant future, researchers, indigenous communities, and native investigators can participate together in genetic studies that may

contribute to understanding their diversity and eliminating the historical disparities so often suffered by them.

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ANNEX I

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Research Article
Human and Medical Genetics

Evolutionary history of the SARS-CoV-2 Gamma variant of concern (P.1): a perfect storm

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Abstract

Our goal was to describe in more detail the evolutionary history of Gamma and two derived lineages (P.1.1 and P.1.2), which are part of the arms race that SARS-CoV-2 wages with its host. A total of 4,977 sequences of the Gamma strain of SARS-CoV-2 from Brazil were analyzed. We detected 194 sites under positive selection in 12 genes/ORFs: *Spike, N, M, E, ORF1a, ORF1b, ORF3, ORF6, ORF7a, ORF7b, ORF8,* and *ORF10*. Some diagnostic sites for Gamma lacked a signature of positive selection in our study, but these were not fixed, apparently escaping the action of purifying selection. Our network analyses revealed branches leading to expanding haplotypes with sites under selection only detected when P.1.1 and P.1.2 were considered. The P.1.2 exclusive haplotype H_5 originated from a non-synonymous mutational step (H3509Y) in H_1 of *ORF1a*. The selected allele, 3509Y, represents an adaptive novelty involving *ORF1a* of P.1. Finally, we discuss how phenomena such as epistasis and antagonistic pleiotropy could limit the emergence of new alleles (and combinations thereof) in SARS-COV-2 lineages, maintaining infectivity in humans, while providing rapid response capabilities to face the arms race triggered by host immuneresponses.

Keywords: Gamma, P.1, evolution, COVID-19.

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Introduction

According to the World Health Organization (WHO) and multiple researchers, the estimated average mortality rate, considering detectable/reported cases, for COVID-19 is lower (2.72%) than the disease caused by MERS-CoV (34.4%) and SARS-CoV (9.6%) (Xiao *et al*., 2020; ECDC, 2021a,b; Krishnamoorthy *et al*., 2021; Awadasseid *et al*., 2021). This number remains low even considering that the number of deaths caused by COVID-19 may be underestimated by 50%, as seen in Wuhan, China (Liu, J. *et al*., 2021). Despite this relatively low mortality rate, SARS-CoV-2 infection has led to the deaths of 4,219,578 people (WHO, 2021a). Over the past 18 months since the first reported COVID-19 case, the WHO still recognizes that the global public health risks associated with COVID-19 remain very high (WHO, 2021b). Comparatively, SARS-CoV and MERS-CoV infected 8,098 and 2,566 people and killed 774 and 866 people, respectively (WHO, 2003; Alfaraj *et al*., 2019; WHO, 2020; Petersen *et al*., 2020).

SARS-CoV, MERS-CoV, and SARS-Cov-2 have high mutation rates ($0.80–2.38 \times 10^{-3}$ substitutions per site per year (Zhao *et al*., 2004; Cotten *et al*., 2014; Li R *et al*., 2020). These mutation rates are of the same order of magnitude as other RNA viruses, and can lead to the acquisition of enhanced virulence and high evolvability, favoring changes in the host and rapid dispersion. A successful zoonotic spillover also depends on the vulnerability of the new host's defenses, and ecological and climatic conditions. Human populations also have cultural habits, with some of them facilitating the transmission of pathogens, *i.e*., hugs, kisses, sharing food (Olival *et al*., 2017; Duffy, 2018). Thus, *Homo sapiens* has become a potentially easy target of new pathogens in modern times because of its large population size, urbanization, ease of mobility of people between cities, countries, and continents, and close contact with wild, semi-wild, and domesticated animals. These conditions were in place for SARS-CoV, MERS-CoV, and SARS-CoV-2 so that the interspecific barriers were overcome, and related diseases have been reported (Kan *et al*., 2005; Zaki *et al*., 2012; Hedman *et al*., 2021).

However, there is a notable difference in the outbreak trajectories associated with these β-COVs, specializing in infecting humans and causing severe respiratory syndrome symptoms, as mentioned above. No complete scenario explaining such differences is well understood. However, it is possible to suggest that certain potential drivers, shaped by microevolutionary phenomena, can turn a local epidemic into a global pandemic, as found with SARS-CoV-2: stronger tropism involving host cells, high transmissibility, elevated transmission rates from asymptomatic individuals, substantial viral load, and relatively low lethality all powerful triggers for the emergence of evolutionarily successful viral lineages. All these conditions/factors together represent a "perfect storm".

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