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Article

Gut Microbiome in Children from Indigenous and Urban Communities in Mexico: Different Subsistence Models Different Microbiomes

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Abstract: The Human Gut Microbiome is an important host's component defining its health. These microorganisms are mutualistic symbionts dependent on factors such as host's age, subsistence models and sociocultural practices, among others. The conjunction of these factors define the microbial ecosystem dynamics. Using a fecal microbiome approach in children, a comparison of two Mexican communities with contrasting lifestyles: "westernized" (Mexico City) and "non-westernized" (Me'phaa indigenous group) was evaluated. The main differences between these two communities are in bacteria associated with different types of diets (high animal protein and refined sugars vs high fiber food, respectively). In addition, the gut microbiome of Me'phaa children showed higher total diversity and the presence of exclusive phyla, such as Deinococcus-Thermus, Chloroflexi, Elusimicrobia, Acidobacteria and Fibrobacteres. In contrast, Mexico City children had less diversity and the exclusive presence of Saccharibacteria phylum which is associated with the degradation of sugar compounds. This comparison allows further exploration of the selective pressures affecting microbial ecosystemic composition over the course of human evolution and the potential consequences of pathophysiological states correlated with westernization lifestyles.

Keywords: Intestinal microbiome, infant microbiota, diet, westernized, non-westernized, lifestyle, microbial diversity, human health.

1. Introduction

The human gastrointestinal tract is colonized by an abundant and diverse assemblage of microorganisms known as gut microbiota (GM) which impacts host physiology [1]. The GM is composed of more than 2000 genera with an incredible diversity of functions that influence host health [2], [3]. GM synthesize a huge number of proteins, more than are encoded in the human genome [4], participate in the biosynthesis of vitamins, fermentation of dietary polysaccharides, absorption of ions and regulation of a number of host metabolic pathways [4], [5]. Moreover, the GM secretes antimicrobial peptides aiding in the state of homeostasis [6], and regulates the development and function of the innate adaptation of the immune system [3]. To date, at least 50 human pathologies have been associated with changes in the abundance, composition and networks of communication of GM [7]. These pathologies are not only associated with intestinal issues such as bowel diseases or gastrointestinal cancer, but also with autoimmune disease, metabolic syndromes and neurological pathologies [7], [8]. To date, it has been reported that one third of our human GM is common to most people [2], [9].

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In this sense, human GM shows a sort of "core" that is commonly composed by 14 out 664 genera, such as Bacteroides, Bifidobacterium, Enterococcus, Prevotella, etc.[10]. Nevertheless, the other two-thirds is specific to each individual, which can result in great microbial genetic dissimilarity, up to 80% [2], [11]. Interestingly, although host-associated microbes are presumably acquired from the environment, the GM is surprisingly different from the environment surrounding the host [11]. This suggests a coevolution process between our species and their gut microbial taxa, which has been adapted to a particular host internal environment [12]. Thus, individuals' lifestyle is a factor that has been identified among the most important in determining the composition, abundance, and stability of the intestinal microbiome [13]. In particular, sociocultural practices such as type of birth (cesarean section or normal delivery), early life feeding, access to allopathic medications and diets have been the most studied [2]. The other important factor is the host's age. From birth, the microbial diversity increases and converges toward an adult-like microbiota around the first 3–5 years of life [14]. Within this period, the gut microbiota starts to resemble and share many similarities such as overall number of taxa and functional genes [14]–[16]. For instance, in comparison to adults, infant microbiota is enriched in Bifidobacterium spp. And Faecalibacterium spp., which could be playing an important metabolic role for the ongoing development during childhood [17]. The period before the stabilization of microbiota is critical for child growth and development since any alteration may influence adult health [14], [18]. Nevertheless, while it may seem paradoxical, the vast majority of these results have been obtained from populations with a so-called "westernized lifestyle". To date, fewer than 15 studies have evaluated the GM in populations that do not conform to the "westernized lifestyle" [13], including hunter-gatherers like the Hazda of Tanzania [19], subsistence farmers in Bassa, Nigeria [20], and Amerindians in South America [21].

Evaluating populations with diverse lifestyles is fundamental for two reasons: First, it allows us to explore how those lifestyle practices impact the structure of the human GM, in particular among bacteria that are associated with human pathologies, that are usually present in westernized societies; second, it provides valuable information on the specific adaptations of the gut microbiota to changes in human lifestyle over the course of human evolution from a hunter-gatherer lifestyle, through small scale agriculture, to post-industrial westernized lifestyle, and how those adaptations modify components of the host's fitness. For instance, one of the reported consequences from lifestyle changes are the low quantities of dietary microbiota-accessible carbohydrates (MACs), the depleted abundance of VANISH taxas (Volatile and/or Associated Negatively with Industrialized Societies of Humans), such as Prevotellaceae, Spirochaetaceae and Succinivibrionaceae, the low total phylogenetic abundance, and a high presence of other taxa positively associated in societies of urbanization/modernization (BIoSSUM), such as Bacteroidaceae, Enterobacteriaceae and Verrucomicrobiaceae groups [13]. Moreover, populations with "non-westernized" styles have higher intestinal microbiota abundance [20], [22], as well as an increment of overall phylogenetic diversity, particularly in those groups with high carbohydrate-active enzymes (CAZyme), which prevents the loss of genes encoding different types of glycoside hydrolase capable of degrading complex plant-derived carbohydrates rich in fiber diets [13].

In Mexico, there are at least 56 independent indigenous groups whose lifestyle practices vary in different degrees from the typical "westernized lifestyle" [23]. Among these, the Me'Phaa people from a region known as "Montaña Alta" in the state of Guerrero, is one of the groups whose lifestyle differs most strongly from the "westernized lifestyle" typical of more urbanized areas [24]–[26]. The Me'Phaa is a Pre-hispanic indigenous group composed of fifty to eighty families, each with five to ten family members. Most people only speak their native language [24], and they are based largely on subsistence farming of legumes including beans and lentils, and the only grain cultivated is corn. Wild edible plants are also collected, and some fruits and vegetables are cultivated in garden plots [27]. Animal protein is acquired by hunting and raising some fowl, but meat is consumed almost entirely during special occasions and is not part of the daily diet [27]. All food resources are completely produced locally, cultivated and harvested nearby the community [27]. Ninety eight percent of births are through natural delivery, and children are breastfed to the age of two. There is almost no access to allopathic medications, and there is no health service, plumbing, or water

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treatment [25], water for washing and drinking is obtained from small wells. These communities represent those with the lowest income in the country and the highest index of child and adult morbidity and mortality [28]. In consequence, the inhabitants of this region have a contrasting lifestyle compared with other regions in the same country like those observed in Mexico City, which is the most urbanized city of the country and the fifth most populous city in the world [29].

Therefore, the aim of the present study is to explore whether these contrasting lifestyles influence the ecosystemic dynamics of GM in childhood, an important age for the GM stability. To evaluate this, we determined the GM abundance, composition and interpopulation differences in children from 5-10 years old who inhabit in Mexico and whose lifestyle practices are opposed; Children from Mexico City that have grown following a "westernized" lifestyle, and children from the Me´phaa ethnic group which have grown in a "non-westernized" lifestyle..

2. Materials and Methods

Study site

Children feces from Mexico City (18.102"W 19°12'36.36"W) and two Me'phaa communities, Plan de Gatica (17°7' 49.5552"N 99.7', EASL: 510 m) and El Naranjo (17°9' 54.0036"N 98°57' 50.9832"W, EASL: 860 m) were obtained. The distance between the two indigenous communities is almost 30 km, and the socio-economic and cultural patterns are similar [27].

In this work the "westernized" population is represented by children that inhabit the south of Mexico City and that are part of a federal pedagogical program at the National Pedagogical University (Fig. 1). This population corresponds to a medium-high economic level with abundant diets characterized by high animal protein consumption, refined vegetable oils, cereal grains, and sugars (e.g. soda, biscuits, snacks, etc.), as well as low fiber and vegetables intake.

Fig 1. Maps of Sample Populations and Lifestyle conditions in the Me´Phaa population. A) Sampling locations for this study. Map displaying the geographical locations taking as reference the

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south central region of Mexico in the state of Guerrero (Black points) and Mexico City (white point). B) A representative Indigenous family from the Me´Phaa community sampled. C)The typical house construction observed in Me´Phaa community, and D) Male and Females children from 5-8 years old dressed in traditional clothes. Photos by I.G.-S.

Sample collection

Fecal samples were obtained from children between 5-10 years old. For the Me' phaa community and Mexico city; 33 children (15 male and 18 female) and 13 children (four male and nine female) were collected respectively. Each participant collected a fecal sample in a sterilized plastic jar. Each jar had a unique nomenclature designated to the participant written on the lid. All fecal samples were frozen with liquid nitrogen to a posterior storage at -20ºC until DNA extraction. Before the DNA extraction, fecal samples of approximately 100µl were collected with a pipette tip and placed in a 1.5-milliliter sterile microtube.

Additionally to fecal samples, the study included anthropometric measurements (i.e. height, weight, BMI) from all participants, as well as a questionnaire applied to the corresponding mother about the children nutritional status and information on pregnancy, childbirth and length of breastfeeding. For the Me'phaa community, the assistance of a translator was needed since this community does not speak the Spanish language.

Fecal DNA extraction

Each sample (~100 µl of fecal DNA) was extracted using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA was resuspended within 30 µl of molecular grade water and stored at -20°C until PCR amplification.

16S rDNA gene amplification and sequencing

The hypervariable V4 region of the 16S rDNA gene was amplified with universal bacterial/archaeal primers 515F/806R following the procedures reported by Caporaso et al. (2012) [30]. The PCR mix was done in 25 µl reactions by triplicate per samples as follows: 2.5 µl Takara ExTaq PCR buffer 10X, 2 µl Takara dNTP mix (2.5 mM), 0.7 µl bovine serum albumin (BSA, 20 mg ml-1), 1 µl primers (10 µM), 0.125 µl Takara Ex Taq DNA Polymerase (5 U µl-1) (TaKaRa, Shiga, Japan), 2 µl of DNA and 15.67 µl nuclease-free water. The PCR protocol included an initial denaturation step at 95°C (3 min), followed by 35 cycles of 95°C (30 s), 52°C (40 s) and 72° C (90 s), followed by a final extension (72°C, 12 min). Triplicates were pooled and purified using the SPRI magnetic bead, AgencourtAMPure XP 214 PCR purification system (Beckman Coulter, Brea, CA, USA). The characterization of the fecal purified 16S rDNA fragments (~20 ng per sample) were sequenced on an IlluminaMiSeq platform (Yale Center for Genome Analysis, CT, USA), generating ~250 bp paired-end reads. All sequences obtained were uploaded to the NCBI database under the Bioproject number PRJNA593240.

Analysis of the sequence data

The paired-end 2x250 reads were processed in QIIME2. The reads were denoised with the DADA2 plugin to resolve the amplicon sequence variants (ASVs) [31]. Both forward- and reverse-reads were truncated at 200 pb, and chimeric sequences were removed using the "consensus" method. Representative ASVs sequences were taxonomically assigned using the "classify consensus-vsearch pluggin" [32], using the SILVA 132 database as a reference [33]. An alignment was performed with the MAFFT algorithm [34]. After masking positional conservations and gap filtering, a phylogeny was built with the FastTree algorithm [35]. The abundance table and phylogeny were

 doi:10.20944/preprints202007.0668.v1

exported to the R environment to perform the statistical analysis with the phyloseq [36] and ggplot2 packages. Plastidic ASVs were filtered out of the samples, which were rarefied to a minimum sequencing effort of 21 000 reads per sample. The total diversity (alpha diversity) of the ASVs was calculated using Faith's Phylogenetic Diversity Index (PD), Shannon's Diversity Index and Observed ASV´s.

Statistics Analyses

To determine whether alpha diversity is different between community and sex, we performed a Welch two sample t-test to evaluate Faith´s PD index and Shannon index; a Wilcoxon rank-sum test with continuity correction to evaluate Observed ASV´s. Comparisons were done in all possible combinations between sex (i.e. male and female) and community (indigenous and westernized).

Beta diversity analysis between sex and community was estimated by computing weighted, unweighted UniFrac and Bray-Curtis distances. Statistical differences were determined by a Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA). Additionally, we performed a differential abundance analysis with the DESeq2 library [37] to determine the main discriminant ASV´s between community and sex.

We additionally explored the particular differences on the most characterized VANISH and BIoSSUM families; Prevotellaceae, Spirochaetaceae and Succinivibrionaceae as the VANISH group, and Bacteroidaceae, Enterobacteriaceae and Verrucomicrobiaceae as the BIsSSUM gruop [13]. Hence, we performed a zero inflated beta regression model (ZIBM) considering community and sex of the children as additive predictors. If abundance was 0 in >=90% of the studied samples, a Wilcoxon-rank sum test was performed instead of ZIBM. Statistically significant comparisons in all alpha and beta diversity analysis were considered with $p < 0.05$. Comparative statistical analysis was performed in R 3.5.0 [38] implementing the following packages: vegan [39], MASS [40], and GAMLSS [41].

3. Results

3. 1. Microbiome Taxonomic Characterization

This study generated a dataset of 42 fecal samples: 29 children (ages 5-10 years) from the Me'phaa indigenous group (Montaña Alta de Guerrero) and 13 children (ages 5-10 years) from Mexico City. A total of 336,000 sequences were recovered after performing the quality filtering and removing chimeras. Firmicutes and Bacteroidetes were the dominating phyla in both populations (Fig. 2A). Firmicutes, Bacteroidetes and Actinobacteria were higher in children from Mexico City than those from the Me'phaa (77.10 % - 68.04%, 14.84% - 12.07% and 5.34% - 2.44% respectively). In contrast, some phyla were only present within the indigenous community including Deinococcus-Thermus (0.079%), Chloroflexi (0.01%), Elusimicrobia (0.01%), Acidobacteria (0.0071%), Fibrobacteres (0.004%), and only Saccharibacteria (0.0003%) was present in urban GM.

In the male from the city, Firmicutes (66.5%) were found in lower proportion in relation to Bacteroidetes (26.24%) abundance, in contrast to females from the city and children from the indigenous population (Fig. 2 E-F). Moreover, females from Mexico City were the group that presented the highest proportion of Firmicutes (81.7%). In addition, the abundance of Tenericutes (11.50% and 7.55%) and Proteobacteria (5.34% - 3.05%) was higher within females and males of the indigenous population when compared to urban children GM (0.70% - 0.27%; 0.80% - 0.61% respectively) (Fig. 2 C-D). Overall, the diversity in the indigenous children was greater than for urban children. The Venn diagram (Fig. 2B) revealed that only a quarter (23.64 %) of the total ASV's were shared between the indigenous and urban populations sampled. Specific ASV's from the city and the indigenous community (25% and 51. 3% respectively) indicated that the shared diversity is lower than specific ASV's from each location.

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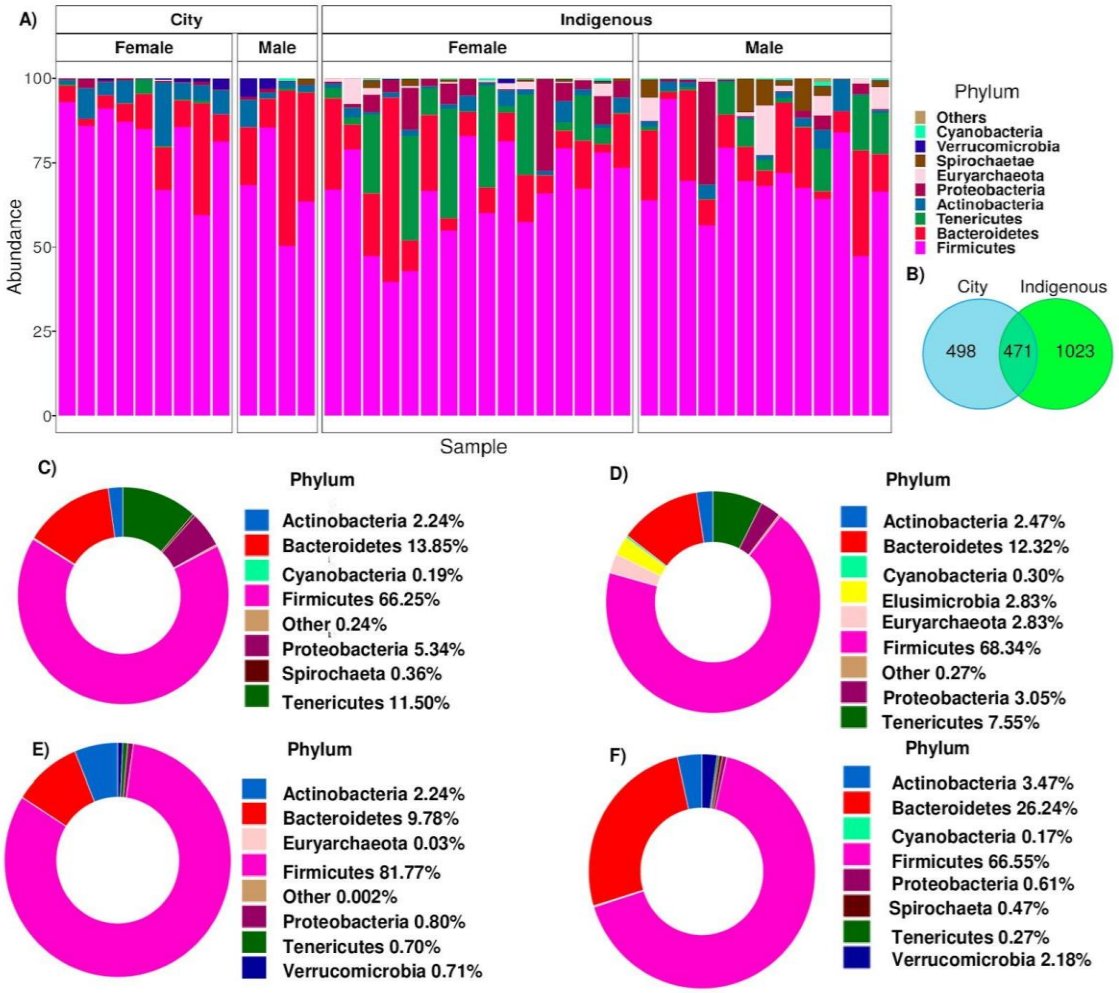

Fig 2. Relative abundance of microbiota. A) Distribution of bacterial composition. A) Distribution of bacterial composition (16S rDNA V4) at phylum level of male and female children from Mexico City and the Me' Phaa community , phyla with relative abundances < 1 % were agglomerated in the "Others" category. B) Venn diagram of ASV 's from Mexico City and the Me' Phaa community. C) Relative abundance of female children from Me' Phaa community D) Relative abundance of male children from the Me' Phaa community. E) Relative abundance of female children from Mexico City. F) Relative abundance of male children from Mexico City community.

3. 2. Comparison of GM in children from Mexico City and Me'phaa communities

The difference of Faith´s PD index, between communities are statistically significant (t = -3.54, p-value < 0.01), but not between sexes within urban or indigenous communities (t = -1.75, df = 29.70, p-value = 0.092). Children from the Me'phaa community showed a greater phylogenetic diversity than children from Mexico City (Fig. 3A). A similar relation is found with the Shannon index, the Me'phaa community has greater alpha diversity than children from Mexico City (Fig. 3B; t = -1.87, df = 29.8, p-value < 0.07), which is more accentuated between sexes; males showed a greater diversity than females in both communities (t = -2.1983, df = 28.075, p-value = 0.03; Fig. 3B). The difference between sexes was also present in the number of observed ASVs (W = 134.5, p-value = 0.04), although there were no significant differences between communities (Fig. 3C; W = 131.5, p-value = 0.12).

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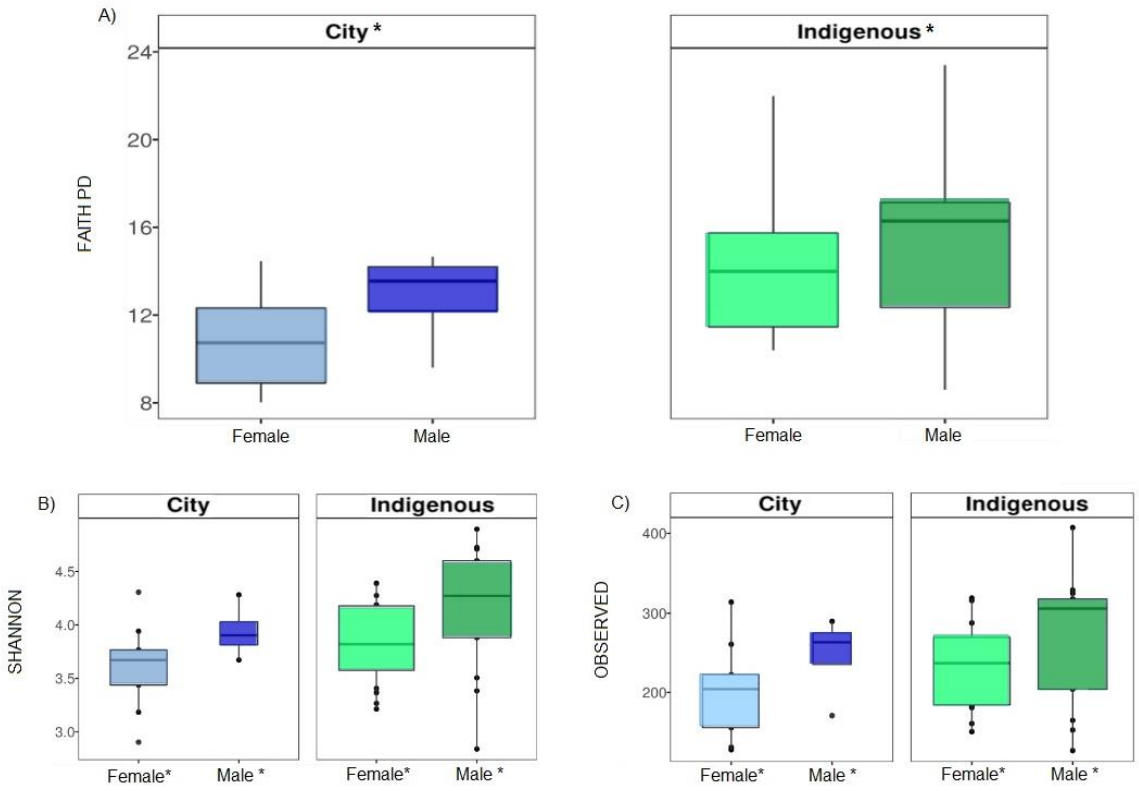

Fig 3. Alpha Diversity. Alpha diversity indexes of children GM by population and sex. Alpha diversity indexes of children GM by population and sex. The Box-plot with median of (A) Faith PD, (B) Shannon Index and (C) Observed ASV's. * corresponds to significant differences ($p < 0.05$).

The GM composition in children shows a clear separation between the urban and indigenous communities (Fig. 4A; PERMANOVA: F=5.72, R2=0.13, p <0.001), which is independent of sex (adonis: F= 1.16, p = 0.21). Bray-Curtis dissimilarity was also evident between communities (Fig. 4B; PERMANOVA: F= 6.37, R2= 0.14, p < 0.01), regardless of sex (PERMANOVA: F= 1.41, p = 0.09).

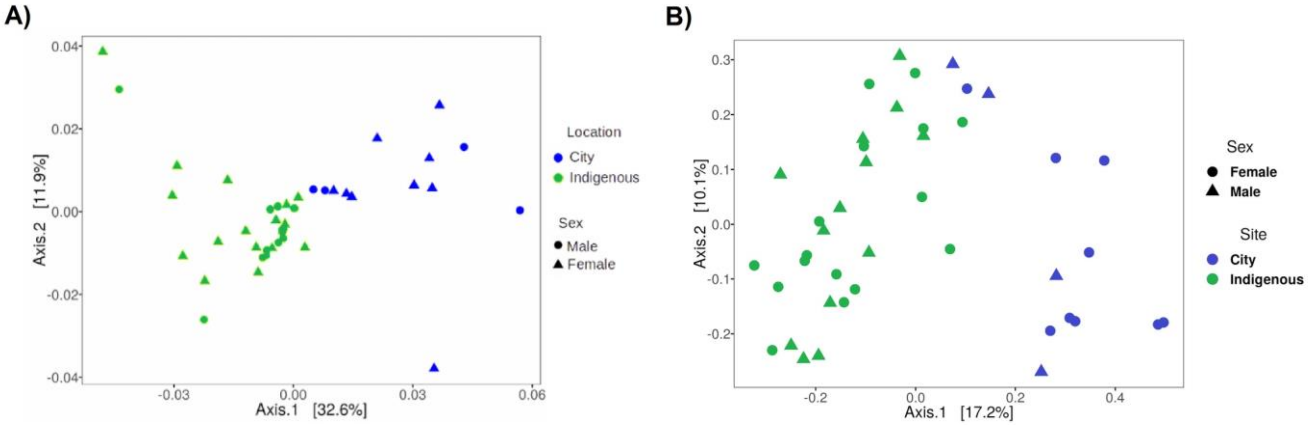

Figure 4. Unweighted Unifrac and Bray-Curtis Analysis. The GM of two communities represented in this study (City = Mexico City; indigenous = Me´phaa) were separated by A) Unweighted UniFrac and B) Bray-Curtis analysis: Me'phaa (green), Mexico City (blue). Sex is showed with a circle (male) and a triangle (females). Differences between both communities were statistically significant in the PERMANOVA test at a level of p <0.01 with both distances metrics.

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Each location had specific ASVs, detected in the log2 fold change analysis (Fig. 5). ASVs of Akkermansia, Ruminococcus 1, Coprostanoligenes and Phascolarctobacterium genera were mostly associated with Mexico City children. In contrast, Prevotella 7 and 9, Treponema 2, Catenibacterium, Christensenellaceae R-7 group, Faecalibacterium, Ruminococcaceae UCG-009 and Ruminococcaceae UCG-014 were associated to the Me'phaa community.

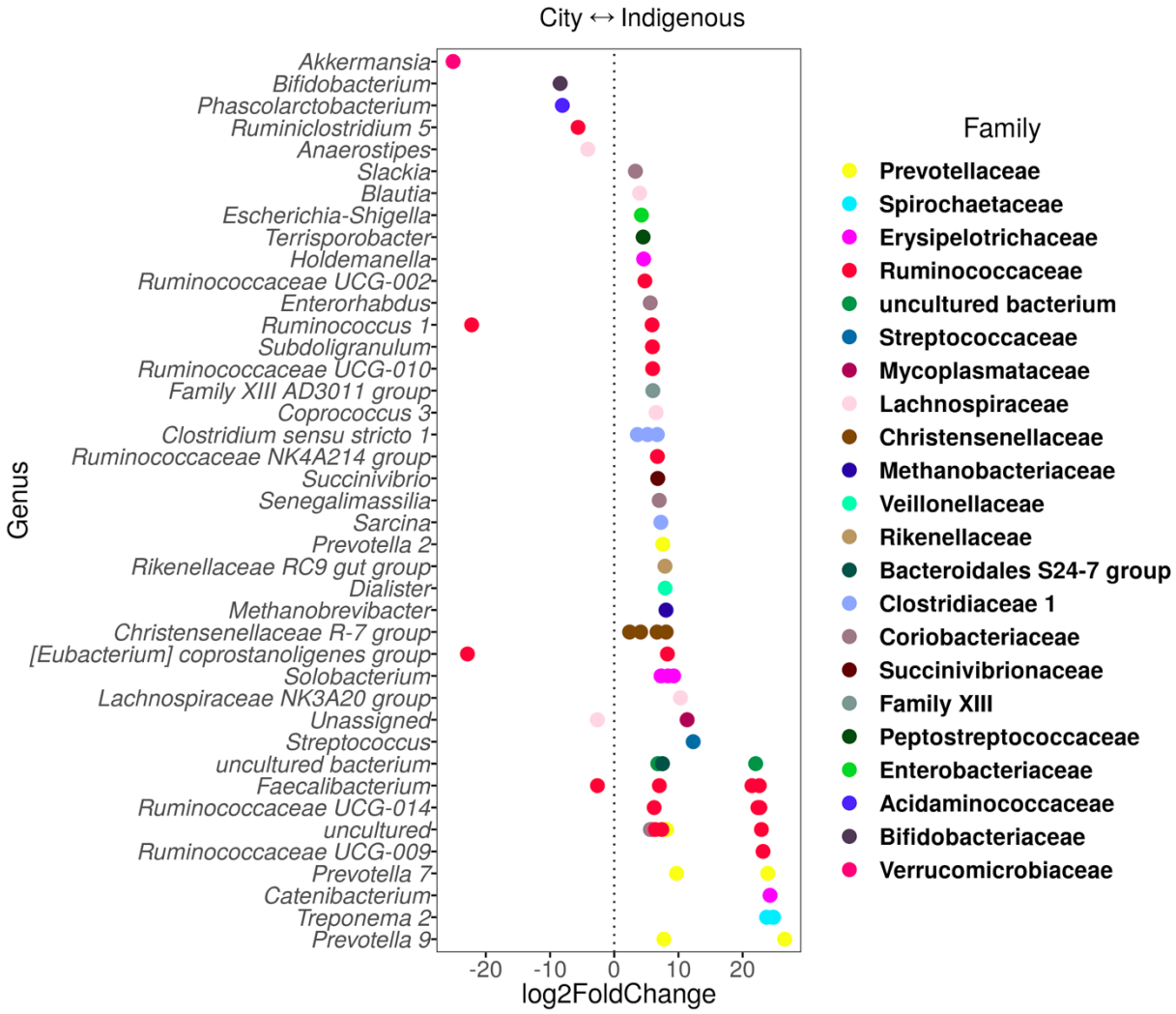

Figure 5. Log2fold change analysis. Fecal-prokaryotic ASVs grouped by genus and colored by family (legends). Log2fold change values indicate the strength and direction of the association to City (<0) and Me'phaa (>0) children. Significance ($p < 0.01$) based on p-values corrected with the FDR (false discovery rate) method.

3. 3. *VANISH and BloSSUM taxa in children from Mexico City and Me' Phaa*

In addition, to determine whether there are differences in the abundance of VANISH taxa (volatile and/or associated negatively with industrialized societies of humans) as well as those positively associated to societies of urbanization/modernization (BIoSSUM), we considered community and sex of the children as predictors of the following families: Bacteroidaceae,

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Enterobacteriaceae, Verrucomicrobiaceae, Prevotellaceae, Spirochaetaceae and Succinivibrionaceae (Fig. 6). These groups were selected according to Sonnenburg and Sonnengurg (2019).

Infant GM from Mexico City exhibits a greater abundance in two of three BIoSSUM groups Bacteroidaceae ($t = -4.78$, $p < 0.01$) and Verrucomicrobiaceae ($t = -2.81$, $p < 0.01$) when compared to Me´phaa community (Fig. 6 D, E). This was independent of the sex of the children ($t = -0.32$, $p = 0.71$). Although the Enterobacteriaceae family was more abundant in the Me´phaa community (0.03 vs 0.01), the difference was not statistically significant ($t = 1.86$, $p = 0.07$; Fig. 6 F). In contrast, the VANISH groups Prevotellaceae ($t = 2.97$, $p < 0.01$), Spirocheataecae ($W = 81$, $p < 0.01$) and Succinivibrio ($W = 104$, $p < 0.01$) were more abundant in children from the Me´phaa community than in children from Mexico City (Fig. 6. A-C).

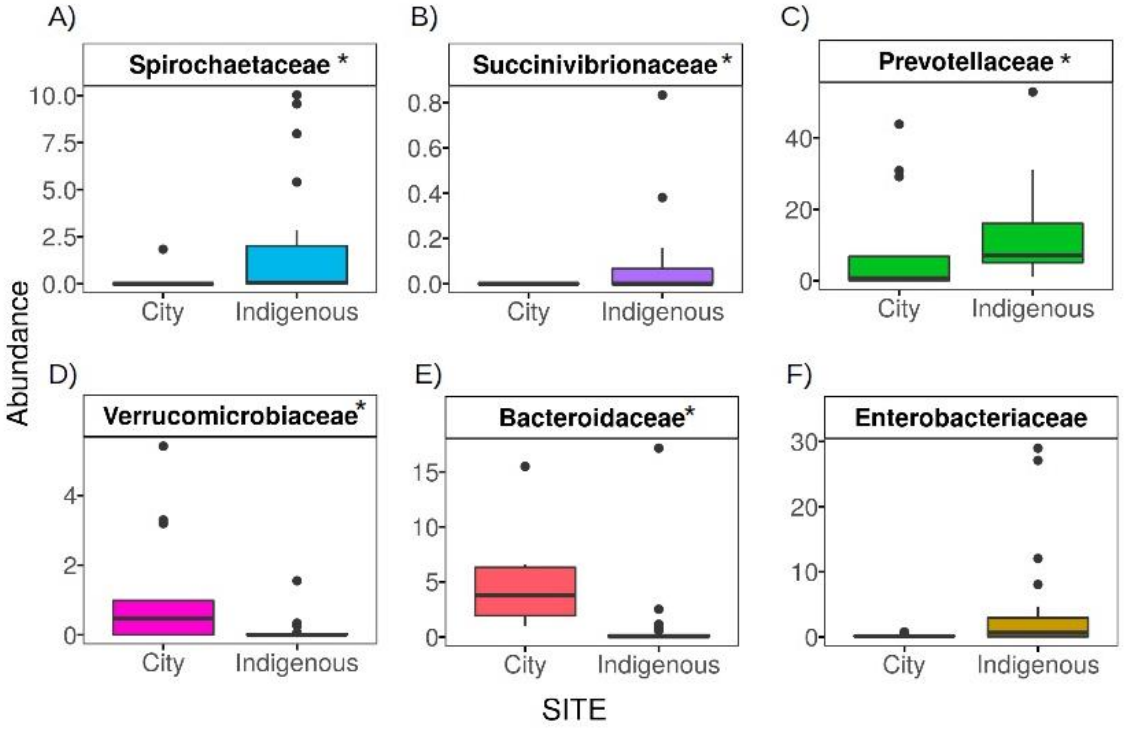

Figure 6. VANISH and BloSSUM Box-plots. VANISH and BIoSSUM Box-plots showing family taxa between Mexico City and Me'phaa children's fecal microbiota. The VANISH taxa are composed by Spirochaetaceae, Succinivibrionaceae and Prevotellaceae families (A-C). BIoSSUM family taxa are Verrucomicrobiaceae, Bacteroidaceae and Enterobacteriaceae (D-F). *Corresponds to significant differences ($p < 0.01$).

4. Discussion

In this study, we compared children's fecal GM from the Me'phaa indigenous community and from Mexico City ("Non-westernized" vs "westernized lifestyles" respectively). The results followed a predictive pattern of dissimilarity that mirror the traditional pre-Columbian indigenous conditions versus a westernized society. Previous reports have illustrated similar differences in gut microbiomes, suggesting a general impact of life practices usually present in westernized societies [21], [22], [42]–[47]. Both GM richness and abundance was higher in the Me'phaa community than in Mexico City (Fig. 3), where this decrease is reflected as a loss of microbial organisms and genes [22], [42]. Similar to our results, other studies in "Non-westernized" populations from Africa and South America have previously found higher GM abundance than in westernized societies [21], [43]. This increase in the diversity of fecal microbiota may be associated due a higher intake of plant-derived carbohydrates with a rich diet in fiber and grains which are common in the Me'phaa communities

 doi:10.20944/preprints202007.0668.v1

[13], [48]. Similar to the Mossi ethnic group from Burkina Faso [43], the Me'phaa diet is also low in fat and animal protein. Furthermore, the use of antibiotics as well as high fat diets is often associated with a reduction in biodiversity within westernized populations [49] and a shift from Bacteroidetes to Firmicutes [50], [51].

The Me'phaa community has not been exposed to allopathic antibiotics, a dramatic difference to the urban population included in this study, which on average have received >2 doses of antibiotics throughout the last 3 years [8]. In addition to diet and antibiotics, the gut microbiome is also driven by other factors that are contrasting between the two communities explored, such as sanitation, social behavior, climate, type of birth, breast feeding, parental care, etc. [13], [21], [22], [43]–[47], [52].

Although it is clear that microbial diversity and abundances change worldwide, mainly due to all of the factors mentioned above, Firmicutes, Bacteroidetes, Actinobacteria and Verrucomicrobia are common phyla reported for human gut diversity [53]. Excluding Verrucomicrobia, these taxa were also highly representative in the two communities, which may tell us about the general "core" present in the human GM (Fig. 2C). Nevertheless, the microbial diversity was highly contrasted regarding the abundance of other less representative phyla. GM of children from Mexico City showed a greater dominance of Firmicutes, Bacteroidetes, Actinobacteria and Verrucomicrobia, while GM from Me'phaa children was additionally represented by other phyla, including Tenericutes, Proteobacteria, Actinobacteria, Euryarchaeota, Spirochaete, Cyanobacteria and Elusimicrobia. A recent study observed similar results after founding that two phyla, Tenericutes and Cyanobacteria, were also present in higher proportions in the GM of adults from traditional indigenous societies compared with adults inhabiting westernized societies [54]. In addition, Me'phaa children not only had higher abundance of these phyla, but also had other exclusive groups. Eleven phyla were exclusively present in Me´phaa people and some in low abundances (under 0.01 %); Chloroflexi, Deinococcus-Thermus, Acidobacteria, Fibrobacteres, Planctomycetes, Gemmatimonadetes, Latescibacteria, Nitrospirae, Lentisphaerae, Hydrogenedentes and Aminicenantes (Fig. 2. C-D). The presence of this great diversity is important because several low-abundance taxa are crucial for the homeostasis and maintenance of functions in the human GM [53]. For instance, another study documented a high prevalence and diversity of Planctomycetes in GM from communities in Senegal [55], and this phylum is characterized by production of antimicrobial compounds and antimicrobial activity [56]. The GM metabolic reconstruction from Senegal communities suggests an anaerobic fermentative pathway and the capability to degrade multiple polysaccharides and glycoproteins from these exclusive groups [54], [56], [57]. Acidobacteria is a phylum particularly reported in traditional communities and capable of breaking-down sugars [54], [58]. In contrast, Saccharibacteria is associated with the degradation of sugar compounds [59] and were only present in Mexico City children. Finally, we identified, to our knowledge, few phyla that were not reported previously for human GM: Hydrogenedentes, Fibrobacteres and Nitrospira. The phylum Hydrogenedentes is a versatile carbon and energy-yielding chemotrophic metabolic group, associated with nitrogen, carbon, and sulfur pathways [60]. Fibrobacteres are primary degraders of cellulosic plant biomass in herbivore guts which has prompted the suggestion that cellulose degradation may be a unifying feature of the phylum [61]. Nitrospira phylum is a nitrite-oxidizing bacterium group that are commonly found in fresh/marine environments and soil but also reported for mammalian guts [62], [63].

On the other hand, although Firmicutes, Bacteroidetes, Actinobacteria and Verrucomicrobia are the most reported phyla in the human gut [53], these groups were higher in children from Mexico City than those from the Me'Phaa location. Interestingly, Actinobacteria is associated with gastrointestinal and systemic diseases and their potential in therapeutic uses. In fact, Bifidobacteria are widely used as probiotics in many pathological conditions [64]. The Verrucomicrobia phylum is composed of environmental microorganisms and commonly abundant in the human gut after antibiotic exposure [65]. In turn, the Firmicutes and Bacteroidetes abundance ratio (F/B) has been used as a biomarker of several health conditions in westernized societies, providing important information on the host's lifestyle, diet and somehow, its metabolic function [66]–[68]. For instance, it has been found that westernized children with an approximate F/B ratio of 3:1 (or more) might be

a significant indicator for childhood obesity [69]. More specifically, it has been reported that high levels of Firmicutes such as Ruminococcaceae and depleted levels of Bacteroidetes such as Bacteroidaceae and Bacteroides are associated with obesity [67]. Interestingly, in the Me'phaa children, the relation of Firmicutes/Bacteroidetes (F/B) was similar to that associated with western societies. Nevertheless, only 2 infants (6% of the group) were obese (Supp. Table 2). Evidence suggest that it is possible for the gut microbiome to coevolve within the indigenous communities while maximizing energy intake from fibers while also protecting them from inflammations and noninfectious colonic diseases [43]. The lifestyle of the Me'phaa is characterized by low access to food, this could indicate that a configuration that allows nutrients to be absorbed more efficiently would be beneficial in this context, without necessarily meaning excessive calorie intake. Nevertheless, the inference of functionality from a phylum level or even lower taxonomic levels is complicated due to the great diversity and complexity of the species and this association needs further research in bigger samples; nowadays, we are just beginning to describe and understand the microbiota in many worldwide traditional populations, which is opening new perspectives in the definition of what is a "good" microbiome for the human health. Some species and families have been cataloged as emerging groups for new bioindicators of human health in these traditional communities. For instance, the family christensenellaceae is a relatively recently described bacterial family, which is highly heritable and it links the microbial ecology of the human gut and several diseases such as obesity or inflammatory bowel disease [70]. Here, we found that the relative abundance from all species of this family were significantly higher in children from the Me'phaa community than from Mexico City (Fig. 5). The magnitude of this difference has not been reported before in communities from the same country with contrasting lifestyles. This opens new questions about whether this family is providing some services to the health of these indigenous people, and if so, can the lack of these taxa impact health in a westernized lifestyle? A possibility is that this group was present in Pre-Columbian populations but is currently lost from Mexico City children. In contrast, another possibility is that the group is replaced by others but with similar functionality, an event known as functional redundancy [2]. For instance, the genus Akkermansia, a BioSUMM group that belongs to the Verrumocrobia family, was more abundant in Mexico City fecal samples than the Me'phaa children (Fig. 5), which was expected. This genus is related to the reduction of weight gain and fat accumulation, similar to what occurs with the Christensenellaceae family. It is also characterized by improving glucose tolerance, and reducing inflammation and metabolic endotoxemia in diabetic and obese model animals [71]. However, Akkermansia has also a great capacity of degrading the mucus of the intestinal epithelium, a key element in maintaining the equilibrium and overall health of the organism. In a healthy state, the intestinal epithelium together with the mucus layer act as a physical barrier to bacteria and foreign antigens [72]. Given that Akkermansia can also have a role in diverse diseases because their abundance increases in patients with Parkinson's disease, multiple sclerosis, Alzheimer's disease, among others [71]. Hence, although both groups participate in similar inflammatory and metabolic functions, there could be "hidden" indirect costs over other physiological ways, affecting the long-term host´s health. Nevertheless, this also needs future research.

Besides the family Verrucomicrobia, we also observed contrasting GM composition in the rest of the VANISH and BioSUMM families proposed in the present study. For instance, Bacteroidetes was also higher in Mexico City children (Fig. 6), while the Me'phaa fecal microbiota was characterized by a great presence and diversity of Prevotellaceae (Fig 5; Fig 6C). The high abundance in Prevotella correlates to previous reports from worldwide traditional communities [22], [47]. This is also similar to research which compared the Yanomami (Amerindians) and the BaAka (Africans) with U.S. subjects [44], [73]. It also correlates to the results from Guahibo Amerindians, Malawians, Nicobarese, African hunter-gatherers and more [21], [44]–[46]. It has been suggested that Prevotella and Treponema in Burkina Faso children's and Hadza gut microbiota are associated with a high fiber intake, maximizing metabolic energy extraction from ingested plant polysaccharides [43], [49]. In addition to Prevotella, Eubacterium has also been associated with vegetarian diets within traditional communities [22], [46], [74] and were more abundant in Me'phaa children. Further, Faecalibacterium

 doi:10.20944/preprints202007.0668.v1

and Dialister have been described as enriched taxa in the gut microbiome of traditional populations [47]. Similar to these results, genera Streptococcus, Clostridium sensu-stricto, members of the Erysipelotrichaceae family and more (Fig. 5), were only present in the Me'phaa community. In contrast to previous reports [22], [49], Bacteroides and Blautia found in non-industrialized communities are taxa associated with animal proteins in diet and were enriched genera in Me'Phaa children, in comparison to Mexico City. The diversity in Mexico City children was lower in comparison to the Me' phaa, although specific genera from the families Ersypelotrichaceae, Bifidobacteriaceae, Methanobacteriaceae, Ruminococcaceae and Lachnospiraceae were only found in Mexico City children (Fig. 5).

Since the industrial revolution, there have been a lot of diet changes in westernized communities [75]. Western diets shift the microbiota away from fiber degraders in favor of species that thrive on mucus [74]. In contrast, in non-westernized communities decreases the animal-based diet, reflects a reduction in the abundance of bile-tolerant microorganisms and increases the levels of specific Firmicutes (e.g. R. bromii) that metabolize dietary plant polysaccharides [74], [76]. Hence, the association with bacteria provides advantages for obtaining energy [77]. For the Me'phaa, a diet rich in fiber based on vegetables, fruits and legumes is a constant throughout the year. This consumption is associated with Microbiota-accessible carbohydrates (MACs) [78]. In contrast, western populations have lost microbiota-accessible carbohydrates which is the cause of a substantial depletion and functionality of gut microbiota taxa (e.g. VANISH) [13]. A microbial diversity provides also a diverse microbial enzymatic capacity needed to degrade nutrients and many forms of complex polysaccharides in human diets [79], [80]. Thus, taxa transferred less efficiently to the offspring and potential extinction occurs in subsequent generations [13], [81]. This loss of microbiota diversity is likely involved in the increasing propensity for a broad range of inflammatory diseases, such as allergic disease, asthma, inflammatory bowel disease (IBD), obesity, and associated no communicable diseases (NCDs). Therefore, specific taxa, their metabolic pathways, and their interactions to human health should be considered in future microbiome research [70]. In this sense, dominant phyla from the human gut microbiome are globally distributed, and independent of geographic location. However, the tendency of more diversity and abundance microbial groups within non-westernized communities is a constant worldwide. Nowadays, most non-westernized lifestyle communities are in decline. Although their presence is crucial to reintroduce bacterial lineages that have been eradicated in westernized human populations. Currently, community composition alone is not a good predictor of disease state. Hence, the contribution of specific taxa, their metabolic pathways, their networks of communications and their interactions to human health is a new priority for microbiome research. For future research, we strongly recommend the use of OMICS approaches to study more non-westernized populations. This, in order to understand how geography, climate, diet, age, gender and environment affect the gut microbiome and its function. OMICS techniques have the potential to reveal functional consequences of these changes, considering the microbial ecology of the gut and its impact on human biology and health.

Supplementary Materials:

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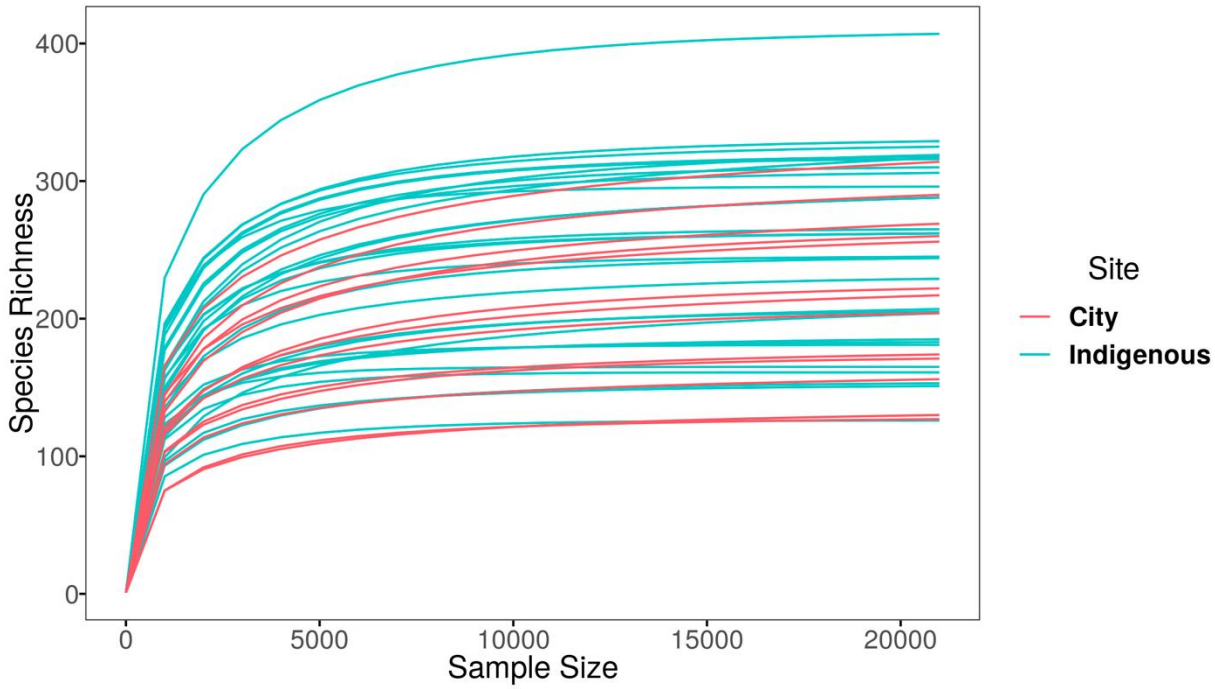

S Figure 1. Accumulation curve of 16S amplicon libraries from each individual in the study. Mexico City and Me′ phaa children are represented in pink and blue respectively. Phylotypes were based on ASV sequence identity.

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Mexico City	
Phylum	Relative abundance
Firmicutes	77.103613522
Bacteroidetes	14.8497693691
Actinobacteria	5.3471919457
Verrumicrobia	1.1493073747
Proteobacteria	0.744833246
Tenericutes	0.5744705016
Spirochaetae	0.1458158544
Cyanobacteria	0.0560548385
Euryarchaeota	0.027477862
Fusobacteria	0.000732743
Elusimicrobia	0.0003663715
Saccharibacteria	0.0003663715
Indigenous (Me'phaa)	
Phylum	Relative abundance
Firmicutes	68.0491496599
Bacteroidetes	12.0734693878
Tenericutes	8.7302721088
Proteobacteria	4.4933673469
Actinobacteria	2.4447278912
Euryarchaeota	2.1181972789
Spirochaetae	1.6964285714
Cyanobacteria	0.2472789116
Verrumicrobia	0.0826530612
Chloroflexi	0.0163265306
Elusimicrobia	0.0120748299
Deinococcus-Thermus	0.0079931973
Acidobacteria	0.0071428571
Fibrobacteres	0.0049319728
Planctomycetes	0.0040816327
Gemmatimonadetes	0.0035714286
Fusobacteria	0.0034013605
Latescibacteria	0.0022108844
Nitrospirae	0.0011904762
Lentisphaerae	0.0010204082
Hydrogenedentes	0.0003401361
Aminicenantes	0.000170068

Supplementary Table 1. Relative abundances of the most abundant phyla found in fecal samples from children in Mexico City and the Me'phaa communities.

Individual Code	Group	Sex	Age	Height (m)	Weight (kg)	BMI
A27	Mexico City	M	6	1.26	22.7	14.29
A36	Mexico City	M	9	1.34	28.2	15.7
A09	Mexico City	F	7	1.21	27.4	18.71
A07	Mexico City	F	6	1.22	23.9	16.05

 doi:10.20944/preprints202007.0668.v1

A35	Mexico City	M	11	1.43	37	18.09
A13	Mexico City	F	9	1.37	42.6	22.69 *
A31	Mexico City	F	5	1.14	17.4	13.38
A20	Mexico City	F	10	1.44	42.6	20.54
A26	Mexico City	F	9	1.46	37	17.35
A16	Mexico City	F	8	1.34	25.9	14.42
A05	Mexico City	F	6	1.16	22.9	17.01
A29	Mexico City	F	9	1.39	42.6	22.04 *
A24	Mexico City	M	6	1.16	21	15.6
103	Me'phaa	F	7	1.07	17.6	15.37
82	Me'phaa	F	5	1.02	15.4	14.8
101	Me'phaa	M	9	1.2	21.5	14.93
136	Me'phaa	F	8	1.19	20.5	14.4
151	Me'phaa	F	6	0.9	14.7	18.14
96	Me'phaa	M	8	1.19	19.7	13.9
114	Me'phaa	M	6	1.04	18.1	16.73
76	Me'phaa	M	6	1.18	20.7	14.86
135	Me'phaa	M	10	1.26	23	14.48
92	Me'phaa	F	6	1.09	16.2	13.63
117	Me'phaa	F	9	1.26	26.4	16.62
131	Me'phaa	M	9	1.27	27.7	17.17
109	Me'phaa	F	9	1.22	21.2	14.24
102	Me'phaa	M	8	1.12	17.8	14.19
95	Me'phaa	M	10	1.34	29.1	16.2
27	Me'phaa	F	7	1.07	16.6	14.49
50	Me'phaa	M	7	1.11	22.3	18.09
6	Me'phaa	F	8	1.02	18	17.3
49	Me'phaa	M	9	1.15	24.7	18.67
28	Me'phaa	M	6	0.97	15.1	16.04
24	Me'phaa	F	6	1.03	19	17.9

 doi:10.20944/preprints202007.0668.v1

4	Me'phaa	M	5	0.86	17.3	23.39 *
51	Me'phaa	M	5	0.99	17.8	18.16
13	Me'phaa	F	8	1.17	21.5	15.7
63	Me'phaa	F	6	1.09	15.6	13.13
45	Me'phaa	F	10	1.24	25.2	16.38
22	Me'phaa	F	10	1.31	44	25.63 *
23	Me'phaa	F	8	1.05	18.3	16.59
10	Me'phaa	F	7	1.07	21	18.34

Supplementary Table 2. Table containing anthropometric data, including height, weight and BMI from children of both groups [82]. * corresponds to BMI of children with obesity

Author Contributions I.G.-S., conceived and designed this study A.S.-Q. and I.G.-S., wrote the first draft of the manuscript, I.G.-S., S.M.-C., and J.N. collected biological samples, A.S.-Q.,L.I.F.,S.M.-C., and O.G performed DNA extraction, amplification and sequencing, D.C.-G. and I.G.-S. analyzed all data. All authors contributes to the writing and editing the final version. All authors approved the final version of the manuscript and gave approval for publication.

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