Gut microbiota features of the geographically diverse Indian population

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Abstract

Population-level microbial profiling allows for identifying the overarching features of the microbiome. Knowledge of population specific base-line gut microbiome features is important due to the widely reported impact of geography, lifestyle and dietary patterns on the microbiome composition, structure and function. Here, the gut microbiota of more than 1000 subjects across the length and breadth of India is presented. The publicly available 16S rRNA gene profiling data of faecal microbiota from the Landscape Of Gut Microbiome - Pan-India Exploration (LogMPIE) study representing 14 major cities, covering populations from northern, southern, eastern and western part of India analyzed. Majority of the dominant OTUs belonged to the Firmicutes, Bacteroidetes and Proteobacteria phyla. The rarer fraction was comprised of OTUs mainly from the phyla Verrucomicrobia and Spirochaetes. The median core size was estimated to consist of 12 OTUs (>80% prevalence) dominated by representing genera *Prevotella*, *Faecalibacterium*, *Bacteroides*, *Roseburia*, *Megasphaera*, *Eubacterium* and *Gemmiger*. Geographic location explained majority of the variation in the gut microbiota community structure. The observations of the present study support the previous reports of *Prevotella* dominance in the Indian population. The *Prevotella*/*Bacteroides* ratio was high for the overall population irrespective of geographic location and did not correlate with BMI or age of the participants. Despite a rapid transition towards a western lifestyle, high prevalence of *Treponema* in the Indian gut microbiota suggests that the urban population still harbors signatures of the traditional gut microbiome. The results presented here improve the knowledge of baseline microbiota in the Indian population across the length and breadth of the country. This study provides a base for future studies which need to incorporate numerous other confounding factors and their impact on the observed characteristics of the Indian gut microbiome.

53 **Keywords:** Population-level, gut microbiota, core microbiota, Prevotella, Indian gut
54 microbiome

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Introduction

Numerous population-level studies have been conducted to investigate base-line as well as population specific characteristics of the human gut microbiome. These included human populations from the USA, Netherlands, Belgium, Denmark, Spain, Africa, Venezuela, China, Mongolia, Fiji, Israel and Papua New Guinea (Qin *et al.*, 2010, Jalanka-Tuovinen *et al.*, 2011, Huttenhower *et al.*, 2012, Qin *et al.*, 2012, Yatsunenko *et al.*, 2012, Lahti *et al.*, 2014, Li *et al.*, 2014, Zhang *et al.*, 2014, Martínez *et al.*, 2015, O'Keefe *et al.*, 2015, Yano *et al.*, 2015, Falony *et al.*, 2016, Rothschild *et al.*, 2018). These studies have uncovered a vast diversity of the gut microbial communities as well as identified several factors influencing the microbiome, including age, ethnicity, dietary patterns, geographical location, consistency of faecal samples (Bristol stool chart), lifestyle, etc. It is commonly observed that *Bacteroides* is associated with high protein diet while *Prevotella* is associated with high fibre diet (David *et al.*, 2014, Gorvitovskaia *et al.*, 2016). Several bacteria have been identified as part of the core microbiota in diverse populations as well as common core functions have been reported (Turnbaugh *et al.*, 2009, Jalanka-Tuovinen *et al.*, 2011, Huse *et al.*, 2012, Li *et al.*, 2014, Falony *et al.*, 2016). These studies have directed mechanistic studies and clinical trials for identifying health and disease related diagnostic biomarkers and development of strategies for modulation of the microbiome for health benefits (De Filippo *et al.*, 2010, Cotillard *et al.*, 2013, David *et al.*, 2014, Schubert *et al.*, 2014, Zeller *et al.*, 2014, O'Keefe *et al.*, 2015, Baxter *et al.*, 2016, Desai *et al.*, 2016).

However, similar information on population-level characteristics of the gut microbiota in a Indian subjects with representative sampling across its geography are limited (Ghosh *et al.*, 2013, Shetty *et al.*, 2013, Dehingia *et al.*, 2015, Bhute *et al.*, 2016). Previously, the importance of understanding the complexity and diversity of the gut microbiome in the Indian population was reviewed (Shetty *et al.*, 2013). Several features that make the subjects in the Indian sub-

continent different such as dietary habits, socio-economic situations, societal traditions of dietary habits, vast genetic diversity as well as prevalence of diseases not associated with altered gut microbiome was documented (Shetty *et al.*, 2013). The YY- paradox is an important differentiating factor of human populations in the Indian sub-continent, where Indians with same body mass index as a Western individual have three times the fat content (Yajnik & Yudkin, 2004). This makes the application of BMI to classify obese and non-obese status debatable for the Indian population (Yajnik & Yudkin, 2004, Shetty *et al.*, 2013). A first step towards better understanding the role of gut microbiome on health is to catalogue the population specific microbial diversity, composition and structure using a large representative sample. This "stamp-collection" process has been a driving factor for several of the currently known disease and health associations and development of potential microbiome biotherapeutic candidates (Qin *et al.*, 2012, Everard *et al.*, 2013, Lahti *et al.*, 2014, Dao *et al.*, 2015, Falony *et al.*, 2016, Plovier *et al.*, 2017, Shetty *et al.*, 2017). These features can be further linked to several populations specific features as well as individual-specific microbiota features using extensive phenotyping and measurement of environmental covariates (Falony *et al.*, 2016).

Here, results from the largest standardized collection of the gut microbiota profiles of heterogeneous Indians subjects across geography is presented. The primary focus of the study was to identifying compositional variation, similarities and dissimilarities in gut microbial community structure and identifying the core microbiota across geographic landscape. Furthermore, the underlying variation across the gut microbial community structure was found to be associated with the *Prevotella*/*Bacteroides* ratio.

Results and Discussion

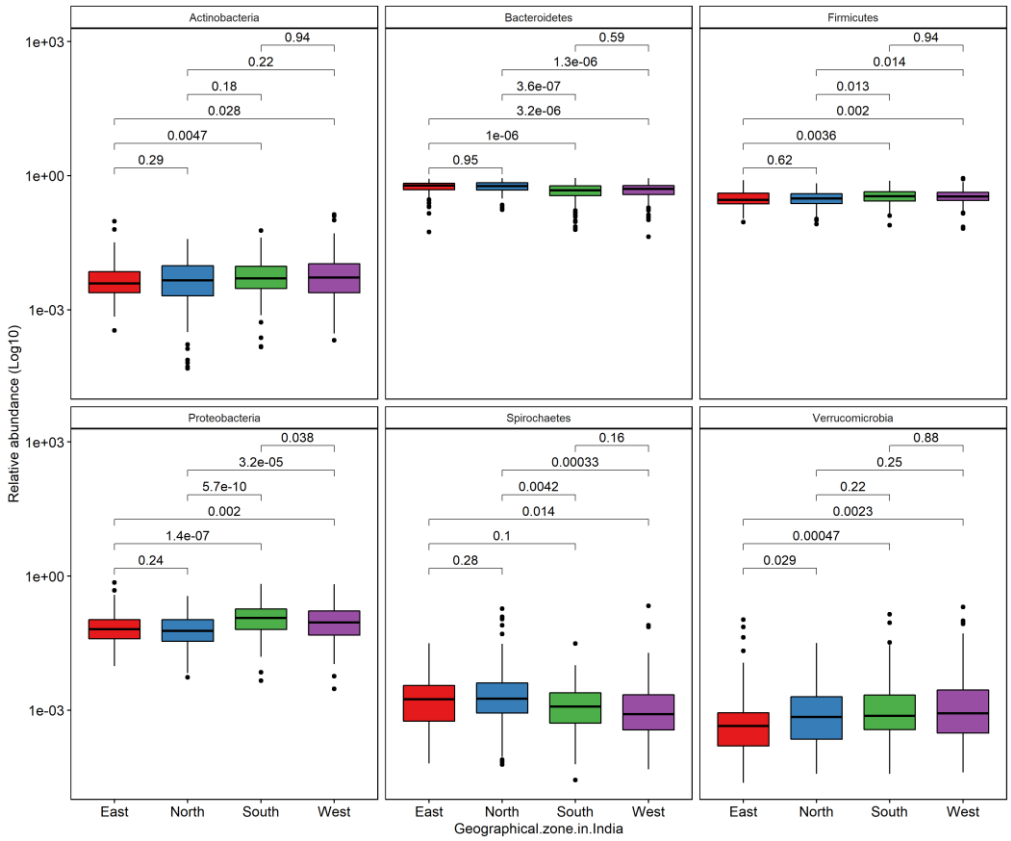
Brief description of the study population

The detailed the subject data is described in the original article reporting the LogMPIE study (Dubey *et al.*, 2018). Briefly, the study reported microbial profile of 1004 Indian individuals

residing in 14 cities different cities. These broadly covered the populations representative of north, west, east and south geographic areas of the country. Data on lifestyle, body mass index (BMI), age and gender were reported. The mean and standard deviation for age was 37.2 ± 11.9, for BMI was 27.9 ± 4.9 represented by 420 females and 584 males. Out of the 1004 subjects, 556 were categorised as non-obese and 448 as obese based on the BMI. The subjects were further categorised following a sedentary and non-sedentary lifestyle. The metadata was limited to these factors and other important metadata such as dietary intake (vegetarian/non-vegetarian, ratio of carbohydrates to protein in diet, consumption of yogurt with live bacterial cultures, etc.), stool consistency, history of medications was not reported. Therefore, the preliminary analysis here does not address the effects and/or contribution of these factors to the variation in gut microbiota.

Microbial composition and community level variation across geography

The microbiota composition showed differences at phylum level in individuals from the different geographic zones (Figure 1). Prominent differences in relative abundance were observed in the phyla Bacteroidetes, Firmicutes and Proteobacteria. Individuals from east and north harboured higher abundances of Bacteroidetes compared to west and south. Individuals from north and south harboured relative higher abundance of Spirochaetes (Figure 1).



123

124 **Figure 1:** Comparison of relative abundances of major phyla in the gut microbiota of Indians. The p-values were
125 calculated using Wilcoxon test.

126 At genus level, *Prevotella* was abundant across the geographic landscape, followed by
127 *Faecalibacterium* (Figure 2). Genus *Bacteroides*, *Megasphaera*, *Parasutterella*, *Haemophilus*
128 showed variable abundances, where few individuals had more than 0.4 (proportional)
129 abundance. Comparison of microbiota of Indians with other populations has reported the
130 enrichment of *Prevotella* and *Megasphaera* (Bhute *et al.*, 2016). The observation in a large
131 population here provides further support for their association with Indian gut microbiota.
132 *Megasphaera* is a butyrate and propionate producer both of which are known for anti-
133 inflammatory properties (Hosseini *et al.*, 2011, Lin *et al.*, 2012, Louis & Flint, 2017). The
134 observation of variable abundances of *Parasutterella* and *Haemophilus* is intriguing as these
135 are hardly reported to be highly prevalent and/or abundant in gut microbiota of healthy western
136 adults (Human Microbiome Project, 2012, Falony *et al.*, 2016). However, abundance of

Parasutterella was associated with urban Mongolian microbiota (Zhang *et al.*, 2014). The physiological and metabolic characterization is currently focused on the anaerobic lifestyle of bacteria from Bacteroidetes phyla, Lachnospiraceae and Ruminococcacaea families (Barcenilla *et al.*, 2000, Sonnenburg *et al.*, 2010, Flint *et al.*, 2012, Flint *et al.*, 2012, Reichardt *et al.*, 2014). All of which have been reported to be dominant in the Western population. However, microbiota analysis of non-western populations advocates the need to focus on obligate anaerobic bacteria from phyla Proteobacteria and Spirochaetes to understand their role in health of non-western adults (Martínez *et al.*, 2015, Bhute *et al.*, 2016, Das *et al.*, 2018).

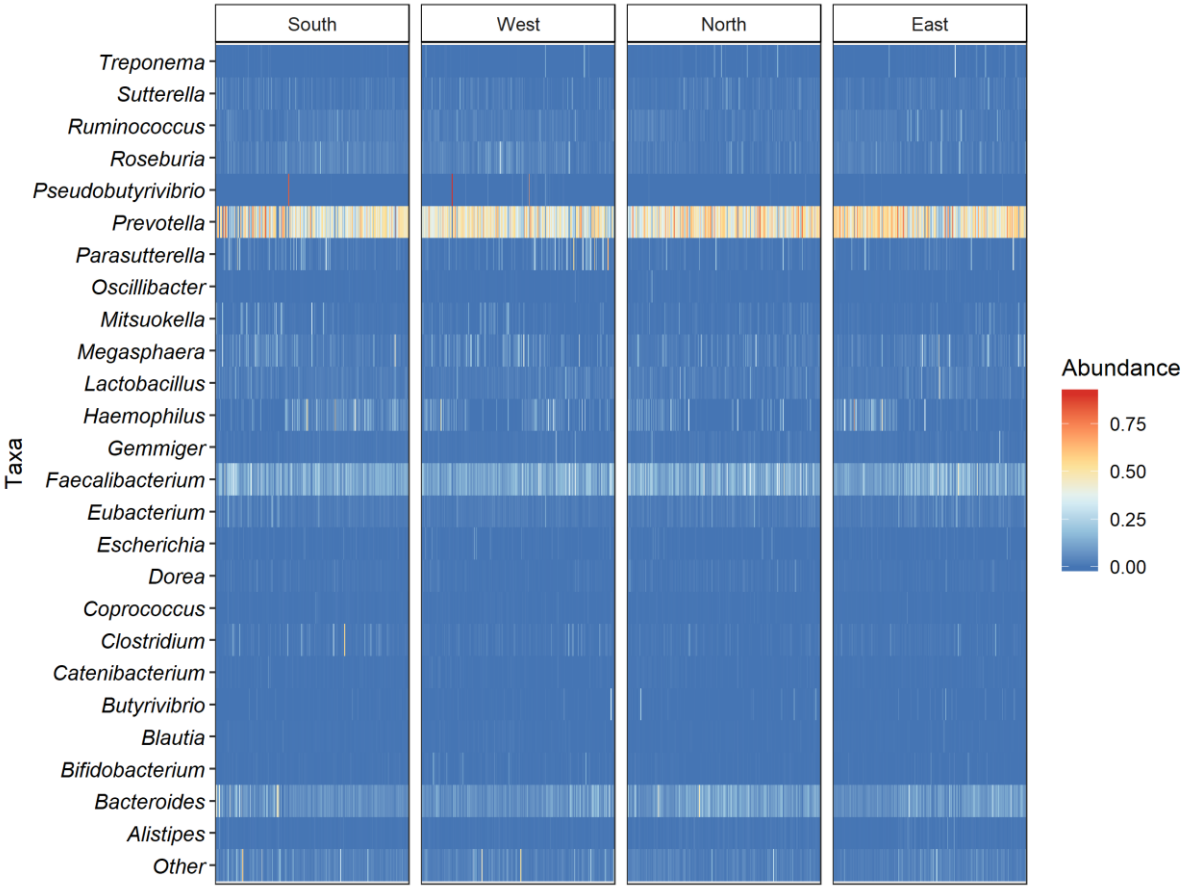


Figure 2: Inter-individual variation in relative abundance of top 25 gut microbial genera in subject from different geographical zones in India.

Based on unconstrained principal coordinate analysis (PCoA) analysis of OTU-level, no major separation was observed between the populations from different broadly classified geographic

locations *i.e.* north, west, east or southern part of the country (Figure 3). The microbial community structure was not significantly associated with obesity status of the individuals within the population (PERMANOVA, P = 0.585). Geographical location (city of residence) explained the most variation (PERMANOVA, R2=0.10, Pr(>F) = 0.001), followed by geographical zone (PERMANOVA, R2 = 0.02, Pr(>F) = 0.001), gender (PERMANOVA, R2=0.002, Pr(>F) = 0.009) and lifestyle pattern (PERMANOVA, R2 = 0.005, Pr(>F) = 0.001).

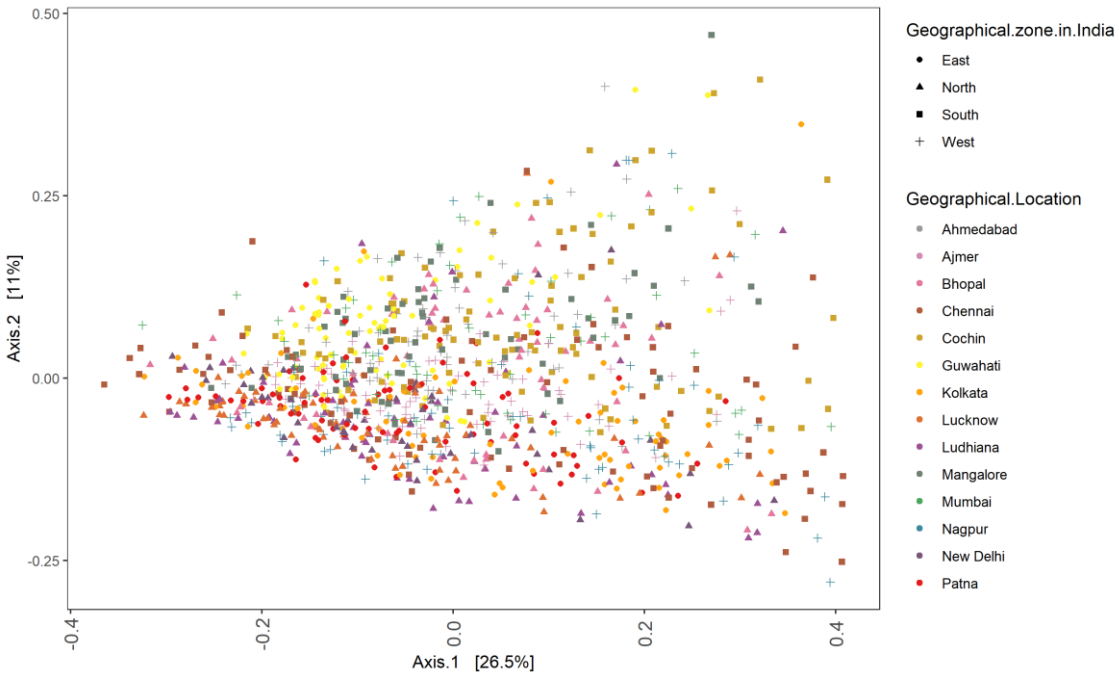


Figure 3: Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity based on OTU relative abundances.

Within each of the geographic zones *i.e.* north, south, east and west, there are differences in the microbiota structure between the cities (Supplementary Figure 1). The above observations demonstrate that environmental factors are a major driver of the gut microbiota, especially the location of residence in the cohort investigated in this study. Further highlighting the effect of geographic locations and related confounding factors as an important challenge in identifying health and disease associated biomarkers for the Indian population. A major metadata lacking in the LogMP study is the dietary intake. Each of the cities sampled in the Log MP study is

166 separated at least 200km, while most are separated by a distance of more than 500 km. Each of
167 these cities has distinct lifestyle as well as culinary traditions. A comprehensive characterisation
168 of the gut microbiota and its association with disease will require incorporating information on
169 diet and lifestyle related cofounding factors in future studies.

170 ***Prevalent dominant and rare bacteria in the Indian gut microbiota***

171 Both the dominant and rare fractions of the microbiome play an important role in stability and
172 resilience of the microbial community (Shade *et al.*, 2014, Lynch & Neufeld, 2015, Shetty *et*
173 *al.*, 2017, Delgado-Baquerizo *et al.*, 2018, Jia *et al.*, 2018). Identifying bacteria that comprise
174 the dominant and rare fractions is important to better understand their potential role and
175 consequent impact on the functioning of the microbiome. Based on the abundance-occupancy
176 analysis, OTUs from phyla Firmicutes, Bacteroidetes and Proteobacteria were identified as
177 covering the abundant fractions in the Indian gut microbiota (Figure 4). The most abundant
178 OTU was from the Firmicutes phyla was *Faecalibacterium prausnitzii* (OTU000444; 0.14,
179 100%), from Bacteroidetes was *Prevotella copri* (OTU000745; 0.4, 99%), from phylum
180 Actinobacteria was *Bifidobacterium bifidum* (OTU000175; 0.002, 68%), from Proteobacteria
181 was *Haemophilus parainfluenzae* (OTU000484; 0.03, 87%), from Spirochaetes was
182 *Treponema succinifaciens* (OTU000961; 0.004, 73%) and from Verrucomicrobia was
183 *Akkermansia muciniphila* (OTU000067; 0.002, 61%). Three OTUs from Proteobacteria were
184 present in more than 90% of the samples (OTU000703:Parasutterella, OTU000468:Gemmiger
185 and OTU000935:Sutterella with 0.02, 0.01 and 0.009 mean proportional abundance). A detailed
186 list is given in supplementary table 1.

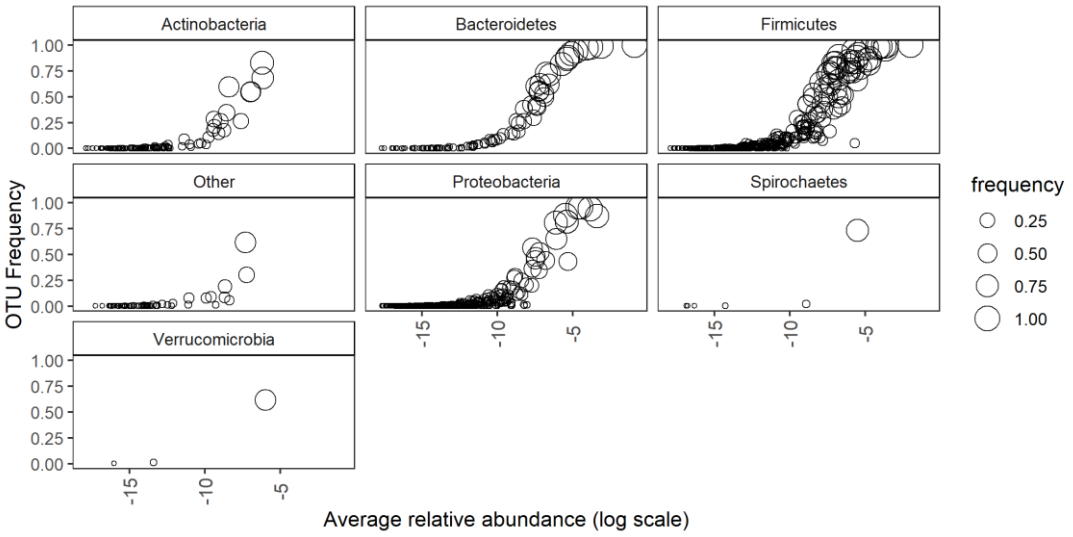


Figure 4: Occupancy-Abundance relationship for OTUs from major phyla in the Indian gut microbiota (n=1003). The x-axis is log transformed for clarity.

In the present study, re-analysis of the data was done to gain detailed insight into the core microbiota following the bootstrap approach as reported previously (Jalanka-Tuovinen *et al.*, 2011, Salonen *et al.*, 2012, Shetty *et al.*, 2017). The change in core size with respect to various abundance and prevalence thresholds is shown in Figure 5A. The median core size and the core OTUs were estimated to consist of 12 OTUs (minimum relative abundance threshold of 0.0001 and presence in at least 80%). These included otu000745 (*Prevotella copri*), OTU000444 (*Faecalibacterium prausnitzii*), OTU000162 (*Bacteroides plebeius*), OTU000756 (*Prevotella stercorea*), OTU000542 (*Lactobacillus rogosae*), OTU000814 (*Roseburia faecis*), OTU000149 (*Bacteroides coprophilus*), OTU000834 (*Ruminococcus gnavus*), OTU000594 (*Megasphaera elsdenii*), OTU000426 (*Eubacterium eligens*), OTU000468 (*Gemmiger formicilis*), OTU000148 (*Bacteroides coprocola*). Investigation of varying abundance and prevalence thresholds for inclusion of core microbiota aided in identifying both abundant and rare members of the core microbiota in the Indian population (Figure 5B). The *Prevotella copri* was identified as the most prevalent and dominant core bacteria across a range of abundance and prevalence thresholds (Figure 5 A and B). This is in accordance with recent report on the gut microbiota

of tribal as well as urban Indian populations (Dehingia *et al.*, 2015, Bhute *et al.*, 2016, Das *et al.*, 2018, Tandon *et al.*, 2018). At the genus level, the core microbiota contributed to a large fraction of the total microbiota across geographies and gender (Supplementary figure 2).

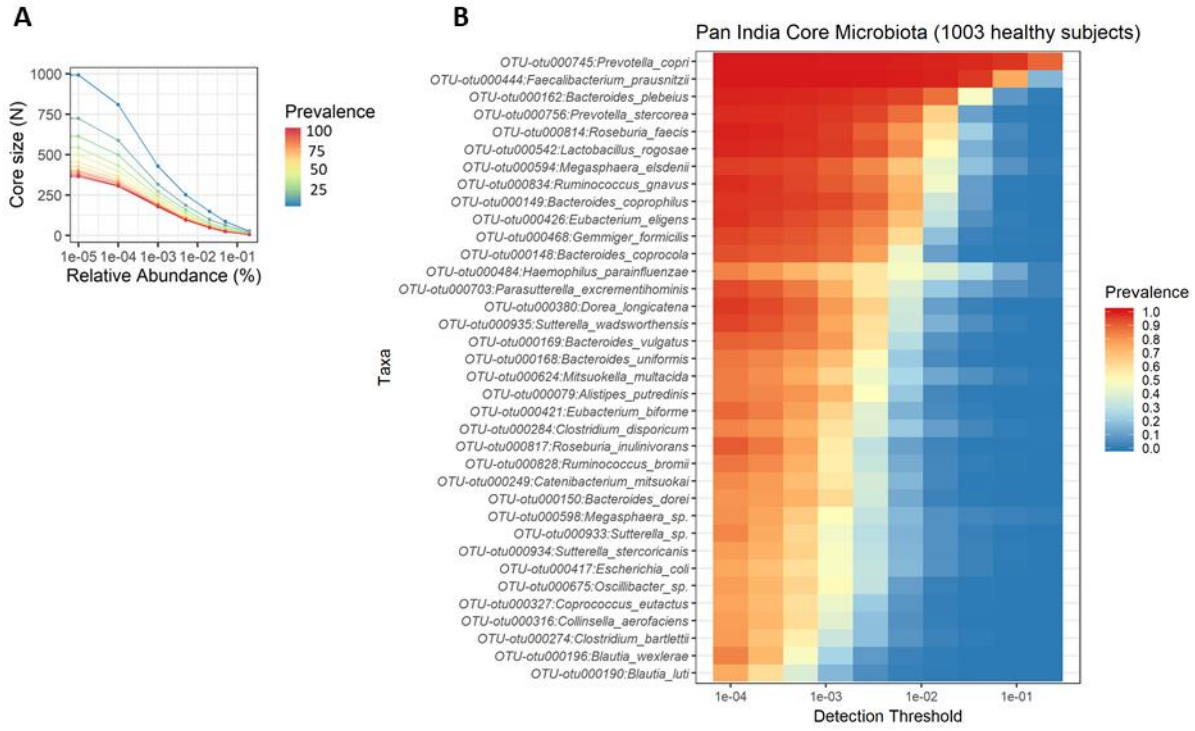


Figure 5: Core microbiota in Indian population. A] The difference in number of core OTUs and their prevalence at different abundance thresholds. B] Heatmap depicting the core OTUs, their prevalence at different detection thresholds (relative abundance).

The dominance and prevalence of *Faecalibacterium* is associated with both western and non-western populations (Falony *et al.*, 2016, Shetty *et al.*, 2017). Prevalence and abundance of *Prevotella* is associated with gut microbiota of non-western populations (Falony *et al.*, 2016). In our study, we identify both of these genera as a part of the Indian core microbiota. These bacteria have a range of metabolic traits related to degradation of complex polysaccharides (David *et al.*, 2014, Heinken *et al.*, 2014). However, there is a lack of direct evidence of complex fibre degradation ability for *Prevotella copri*, the most abundant and prevalent species detected in the gut microbiome. This species is known to have β -Galactosidase, α-

Arabinofuranosidase and β-Glucosidase activity (Hayashi *et al.*, 2007). On the contrary numerous evidence exists for polysaccharide degradation ability in species from the genus *Bacteroides* (Sonnenburg et al., 2010). Further investigation of physiology and polysaccharide degrading ability of *Prevotella* and its species/strains across human populations will be crucial to better understand its role in the gut microbiome.

Prevotella dominance is hallmark of Indian gut microbiota irrespective of geographic location, age, gender and BMI

The dominance of *Prevotella* or *Bacteroides* is an important property of the human gut microbiome as these bacteria are known to be biomarkers of diet and lifestyle (Gorvitovskaia *et al.*, 2016). Hence, the *Prevotella* versus *Bacteroides* (P/B) ratio in the microbiota of Indian subjects was investigated in all the subjects (n=1003). The obese individuals were also included in this analysis because there no strong effect of obesity status was observed on the microbiota community composition (see above). A continuum was detected irrespective of the BMI values where only a few subjects had exhibited high P/B ratio (Figure 6A). The subjects across geographies had a microbiota characterised by high P/B ratio (Figure 6B). Additionally, no significant correlation was observed between BMI and age with P/B ratio in the study cohort (Supplementary figure 3). The differences of P/B ratio between genders (male/female) was also not significant (Supplementary figure 4). P/B ratio showed significant correlation with the PCoA axis 1 which explained 30.6% of the variation in the microbial community in the study cohort (Supplementary figure 5).

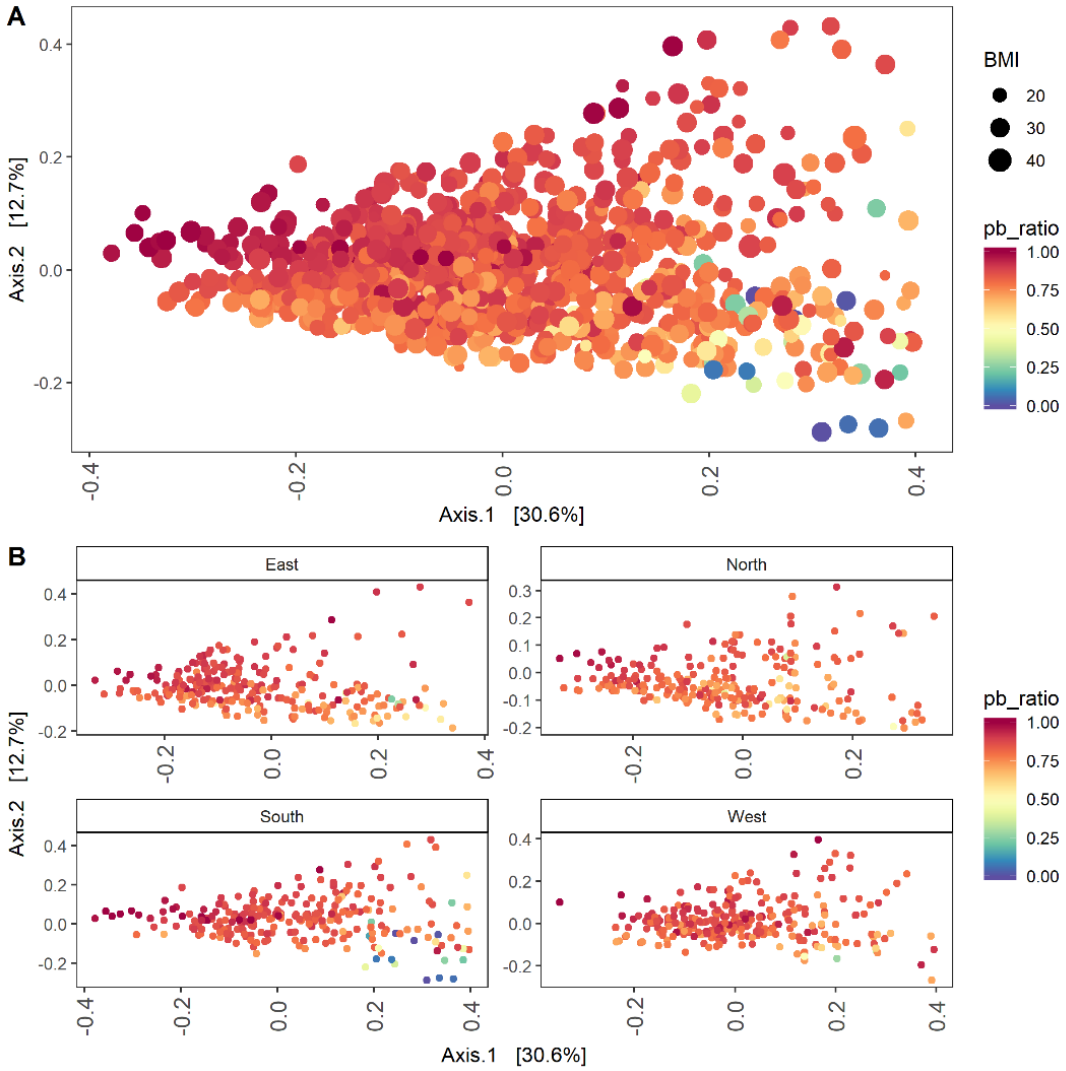


Figure 6: Principal coordinates analysis based on Bray-Curtis dissimilarity based on genus level relative abundances. **A]** PCoA depicting the gradient of *Prevotella/Bacteroides* ratio and distribution of body mass index (BMI) in 1003 Indian subjects. **B]** Same PCoA as in panel A, but coloured and facetted for depicting the distribution of *Prevotella/Bacteroides* ratio in the Indian gut microbiota in different geographical zones (East, n = 250; North, n = 243; South, n = 250; West, n = 260).

Summary

The gut microbiome of Indian subjects differs in composition at phylum level across the four geographical zones. Overall variation in the gut microbial community structure in Indians is mostly driven by city of residence. Despite the large differences in the geographic location, there exists a core of 12 OTUs that are shared among 80% of the subjects. These core OTUs

252 are classified as members of genera that are known for their ability to degrade complex
253 polysaccharides (*Prevotella*, *Bacteroides*), produce butyrate and propionate (*Faecalibacterium*,
254 *Megasphaera*) as well as ability to degrade mucin (*Ruminococcus gnavus*). Compared to the
255 Westernized urban populations, the Indian population still harbours features of non-
256 industrialized gut microbiota such as *Treponema*, which was present in 73% of the subjects at
257 a low mean relative abundance of 0.004. Previously, *Treponema* was found to be characteristic
258 of a traditional microbiome (Obregon-Tito *et al.*, 2015). Therefore, efforts need to made for
259 cultivating and preserving human gut origin *Treponema* isolates from diverse populations that
260 are undergoing rapid transition towards a Western lifestyle. The majority of variation in the
261 microbial community structure was correlated with the ratio of *Prevotella* versus *Bacteroides*.
262 However, due to lack of information on dietary habits, no concrete associations could be made
263 to explain the high P/B ratio observed in the Indian population. Since both *Bacteroides* and
264 *Prevotella* are capable of degrading complex polysaccharides, there is need to identify the trade-
265 off between *Prevotella* or *Bacteroides* domination in the Westernized and urban Indian gut
266 microbiota.

267 ## Methods

268 ***Data from LogMPIE***

269 The data analysed in this study was obtained from figshare (Dubey *et al.*, 2018). Detailed
270 information on sample collection and processing for DNA extraction, 16S rRNA gene
271 amplification and sequencing are provided in the original publication (Dubey *et al.*, 2018).
272 Here, some key points are described. The samples were collected by participants using sterile
273 OMNIgene®•GUT stool collection kit. DNA extraction was done using the QiaAmp DNA
274 Stool Mini Kit (Qiagen, Hilden, Germany). Two primer pairs one of V3 and one for V4
275 hypervariable region of the 16S rRNA gene was used for amplification (Milani *et al.*, 2013,
276 Dubey *et al.*, 2018). The sequencing was done Ion S5 System (Thermo Fisher Scientific,

Carlsbad, CA, USA). OTU tables were obtained by processing raw reads following the QIIME workflow on the Ion Reporter Server. OTU picking was done using the *pick_closed_reference_otus.py* command in QIIME (Caporaso *et al.*, 2010).

Microbial community data handling, analysis and visualisation

The relative abundance microbial profiling data and metadata were obtained from (Dubey *et al.*, 2018)(https://doi.org/10.6084/m9.figshare.c.4147079.v1). The taxonomy was corrected to make it compatible with *read_phyloseq* function of microbiome R package (Lahti & Shetty, 2018). The resulting phyloseq object was analysed in R (v3.5.1) using the phyloseq (v1.24.1) and microbiome R package (v1.2.1) (McMurdie & Holmes, 2013, Lahti & Shetty, 2018). One subject, Subject-8032 was removed since initial ordinations revealed it to be highly divergent and thus the analysis was limited to 1003 subjects. Data visualisation was done using a combination of ggplot2 (v3.1) and ggpubr (v0.1.8) packages.

Statistical analysis

The dissimilarity in gut microbiota composition between the subjects were investigated using the Bray-Curtis dissimilarity index calculated at OTU level and genus level relative abundance data using the phyloseq R package. The unconstrained principal coordinates analysis PCoA ordinations were visualised using the *plot_ordination* function. The contribution of each of the metadata categories, geographical location (city of residence), geographical zone, gender, and lifestyle pattern was investigated using PERMANOVA (999 permutations) (*adonis* function, vegan (v2.5-3) R package). Pair-wise comparisons were done using Wilcoxon test. Correlations between Prevotella versus Bacteroides ratios with age, BMI and PCoA axis 1 were based on Pearson's correlations and done using the *stat_cor* function and visualised using *ggscater* function in ggpubr.

Core microbiota analysis

The core microbiota analysis was done using the blanket approach (Salonen *et al.*, 2012). In this approach, the random sub-samples are drawn and the frequency of an OTU to be present in user defined samples (here, 810 samples) at a minimum relative abundance threshold (here, 0.0001) was calculated. Using 1000 bootstrap (boot, v1.3-20, R package) the median core size was estimated (Canty & Ripley, 2012). The effect of prevalence and abundance thresholds as well as the abundance and prevalence distribution of core OTUs were visualized using the microbiome R package (Lahti & Shetty, 2018).

Prevotella/Bacteroides ratio analysis

The *Prevotella*/*Bacteroides* ratio were analysed using the approach described previously (Gorvitovskaia *et al.*, 2016). The OTU data was aggregated at genus level and used for further analysis.

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Conflict of interest

The author declares no conflict of interest.

Data and code availability

The raw sequencing files are made available by the authors at European nucleotide archive (ENA) under the primary accession code, PRJEB25642, and secondary accession code, ERP07577 (Dubey *et al.*, 2018). The codes used for the analysis done in this manuscript will be made available at the following GitHub repository (https://github.com/microsud/Indian-gut-microbiota).

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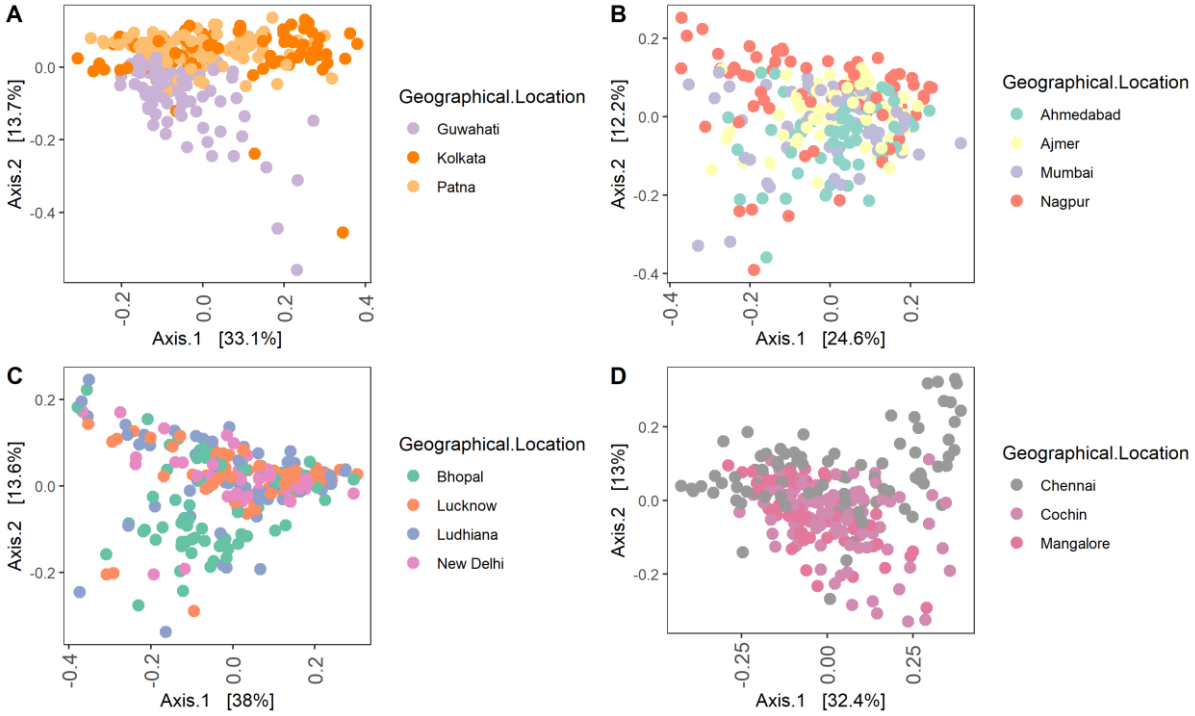
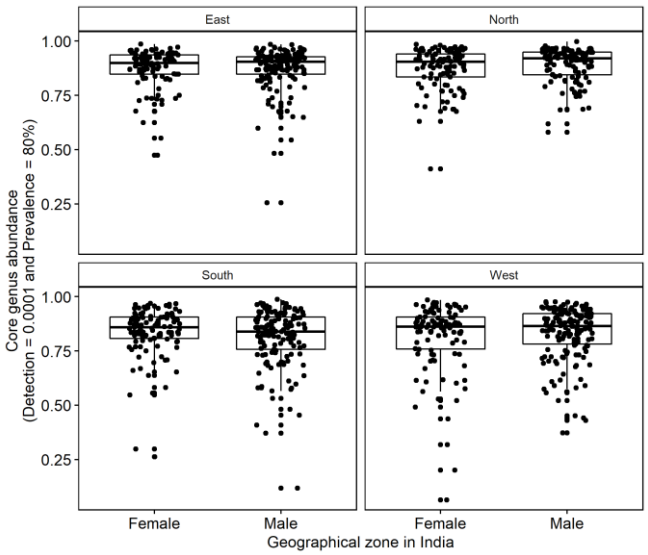
Supplementary data



Supplementary figure 1: Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity based on genus-level relative abundances. **A]** East; **B]** West; **C]** North **D]** South.



Supplementary figure 2: Contribution of 13 core genera towards the total abundance in the Indian female (n = 419) and male (n = 584) gut microbiota. The core microbiota was determined based on a minimum relative

500 abundance of 0.0001 in present in minimum of 80% subjects (1000 bootstraps). The core genera were *Prevotella*,
501 *Faecalibacterium*, *Bacteroides*, *Eubacterium*, *Roseburia*, *Ruminococcus*, *Lactobacillus*, *Megasphaera*, *Sutterella*,
502 *Gemmiger*, *Blautia*, *Clostridium*, *Dorea*

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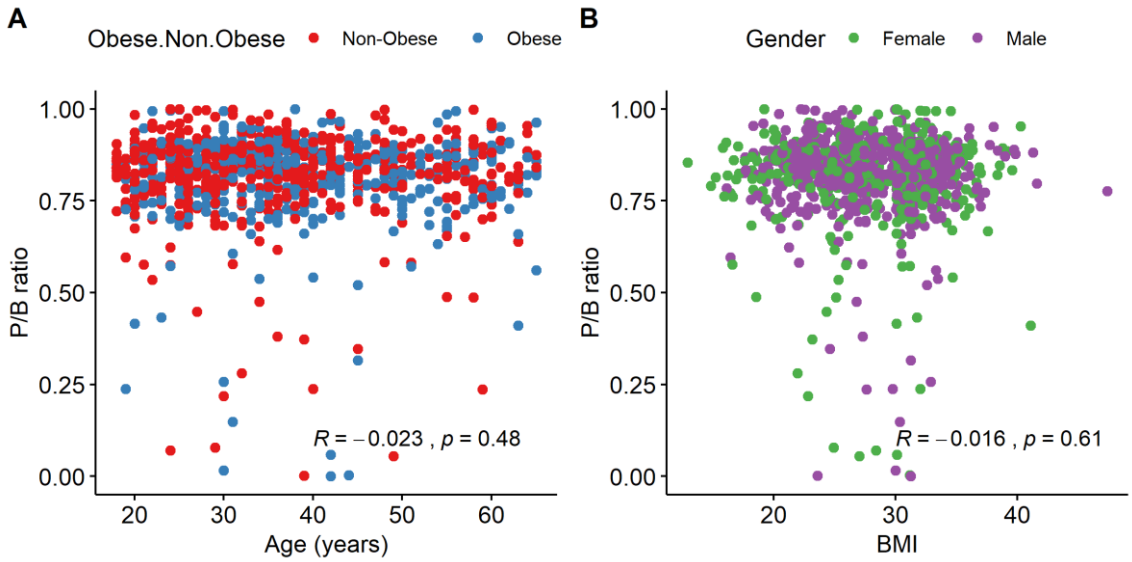
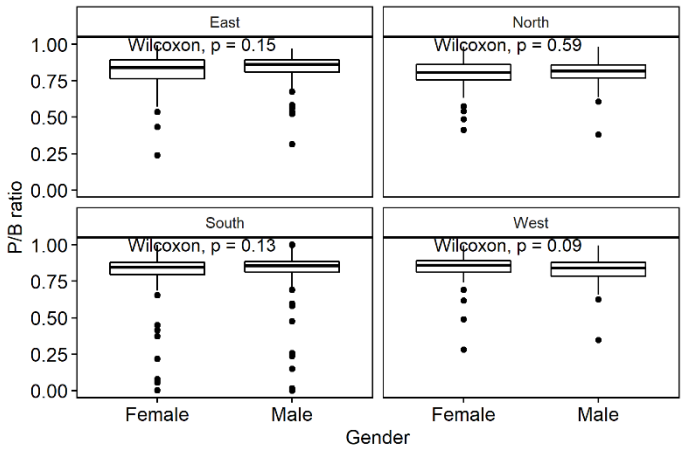


504

505 **Supplementary figure 3:** Pearson's correlation analysis. **A]** Relationship between *Prevotella*/*Bacteroides* ratio
506 and Age. **B]** Relationship between *Prevotella*/*Bacteroides* ratio and body mass index (BMI).



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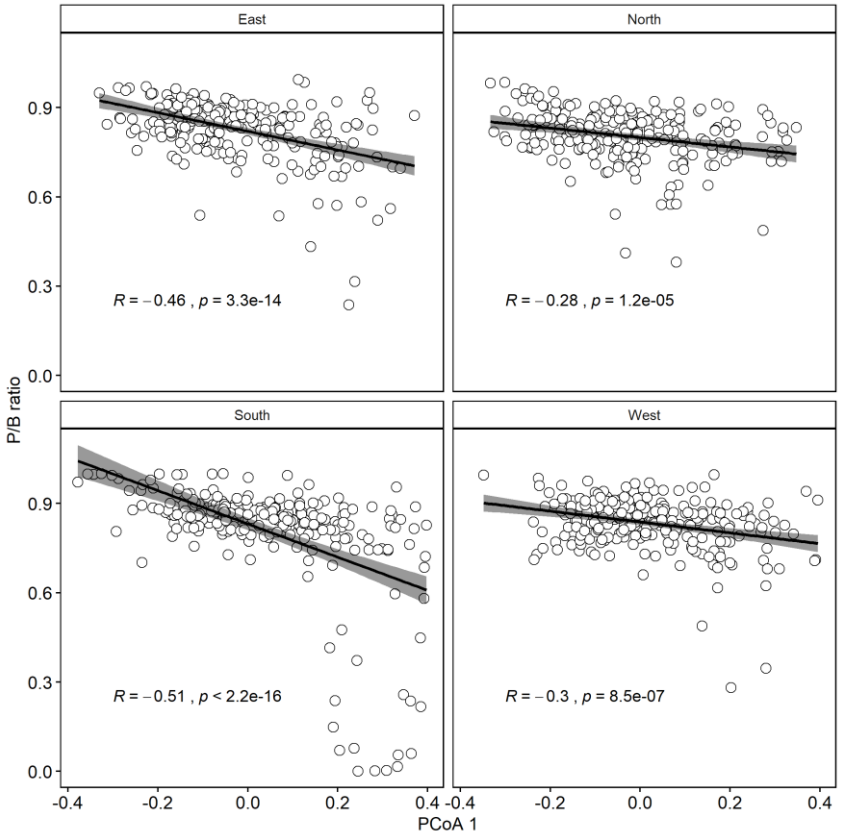
508 **Supplementary figure 4:** Comparison of P/B ratio between genders from different geographical locations. The p-
509 values were calculated using Wilcoxon test.

510



511

512 **Supplementary figure 5**: Pearson's correlation between PCoA axis 1 and P/B ratio.

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