



TITLE:

Stomach and colonic microbiome of wild Japanese macaques

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1 **Stomach and colonic microbiome of wild Japanese macaques**

2 Short title: Gut-site-specific microbiome

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26 **Highlights**

- 27 • Diversity, composition and function vary between stomach and colonic microbiome of
28 Japanese macaques
- 29 • Stomach microbiome is more enriched in microbes that metabolize simple sugar,
30 whereas the colonic microbiome has more fiber-degrading microbes.

31 32 **Abstract**

33 Within the gastrointestinal tract, the physiochemical microenvironments are highly
34 diversified among the different stages of food digestion. Accordingly, gut microbiome
35 composition and function vary at different gut sites. In this study, we examine and compare the
36 compositional and functional potential between the stomach and colonic microbiome of wild
37 Japanese macaques (*Macaca fuscata yakui*) living in the evergreen forest of Yakushima Island.
38 We find a significantly lower microbial diversity in the stomach than in the colon, possibly due
39 to the stomach's acidic and aerobic environment, which is suboptimal for microbial survival.
40 According to past studies, the microbial taxa enriched in the stomach are aero- and acid-tolerant.
41 By functional prediction through PICRUST2, we reveal that the stomach microbiome is more
42 enriched in pathways relating to the metabolism of simple sugars. On the contrary, the colonic
43 microbiota is more enriched with fiber-degrading microbes, such as those from Lachnospiraceae,
44 Ruminococcaceae and *Prevotella*. Our study shows a clear difference in the microbiome
45 between the stomach and colon of Japanese macaques in both composition and function. This
46 study provides a preliminary look at the alpha diversity and taxonomic composition within the
47 stomach microbiome of Japanese macaques, a hindgut-fermenting non-human primate.

48 **Keywords:** primates, gut microbiome, Japanese macaques, gut sites

49 **Introduction**

50 Along the gastrointestinal (GI) tract, the microbiome typically diversifies in relation to
51 the digestive functions (mechanical, chemical, and microbial breakdown) and corresponding

52 physiochemical environment at different gut sites (Gu et al., 2013; Hillman, Lu, Yao, &
53 Nakatsu, 2017; D. Li, Chen, Zhao, Zhang, & Chen, 2019). For example, the microbial
54 community in the upper GI tract is likely suited to the breakdown of simple sugars and proteins,
55 while the microbiome in the lower GI tract is likely suited to complex plant polysaccharides.
56 In addition, how different microbes adapt to the physiochemical environment at different gut
57 sites may determine the acquisition/colonization mechanism of the gut microbiome (Merrell,
58 Goodrich, Otto, Tompkins, & Falkow, 2003; Seedorf et al., 2014; Vega, 2019).

59 Many studies on the gut microbiome-host relationship have focused on the colonic
60 microbiome, which plays a major role in fermentation. In the anaerobic environment of the
61 colon, gut microbes carry out fermentation to transform food materials into short-chain fatty
62 acid and other nutrients, serving as energy and nutritional source for the hosts. It is estimated
63 that the colon alone contains over 70% of the bacteria residing in the body (in the case of
64 humans (Jandhyala et al., 2015)). Compared to the other GI sites, which usually require
65 invasive sampling, it is possible to study the colonic microbiome non-invasively using fecal
66 samples. Therefore, despite the potential differences among GI sites, the gut microbiome
67 studies have mainly focused on the microbial community in the colon/hindgut of animals
68 (Clayton et al., 2019).

69 Compared with the colon, the stomach, which carries out chemical digestion, presents
70 a different environment for most bacteria, including its low-pH environment and short transit
71 time. In past studies on humans and animals (captive rats, swine, mice, baboons and red-
72 shanked doucs), bacterial diversity in the upper sections of the GI tract, such as the stomach,
73 tends to be lower than that in the lower sections, such as the colon (Clayton et al., 2019; Stevens
74 & Hume, 1998). Moreover, the function of microbes in the stomach is potentially different
75 from that in the colon. For example, pathways related to environmental information processing

76 increases in the upper GI tract of house mice, suggesting an active material exchange between
77 gut microbes and the digestive organ (D. Li et al., 2019).

78 Despite the environmental differences between the stomach and colon, there have been
79 few studies devoted to the stomach microbiome. An understanding of the stomach microbiome
80 is, however, important in providing insights into how the animals acquire gut microbes and
81 how the microbes distribute to the lower GI tract. Mammals are generally born with a sterile
82 GI, and thus they acquire gut microbes from the environment. Even after acquisition, microbes
83 vary in their ability to establish a population under various physiochemical environments
84 across the GI tract. While some studies have pointed out the difference in microbiome between
85 the stomach/foregut and colon/hindgut of the animals, the study subjects have only been a few
86 species of nonhuman primates (NHPs), mostly with a focus on the captive foregut-fermenting
87 species (e.g. red-shanked doucs (Amato et al., 2016; Clayton et al., 2019), black and white
88 colobus monkeys, and langurs (Amato et al., 2016)). Despite the fact that most NHPs are
89 hindgut-fermenters, there is clearly a lack of knowledge on the diversity and distribution of
90 microbial communities within the hindgut-fermenting NHPs. Such knowledge would provide
91 basic information regarding the gut microbiome of the hindgut-fermenting NHPs. Furthermore,
92 comparing NHP gut microbiome of different fermentative strategies would improve our
93 understanding of the special digestive adaptations of the foregut-fermenters and thus the
94 evolutionary trajectory of primate feeding strategy.

95 In this work, we studied wild Japanese macaques (*Macaca fuscata yakui*) inhabiting
96 warm-temperate evergreen forest in Yakushima Island, Japan, to understand the spatial
97 difference in the gut microbiome between the stomach and colon. Japanese macaques feed on
98 a considerable amount of mature leaves and other fibrous foods to survive the food-scarce
99 seasons (Hanya, 2004; Hanya, Noma, & Agetsuma, 2003; Hill, 1997; Kurihara, Kinoshita,
100 Shiroishi, & Hanya, 2020). They spend approximately 35% of their annual feeding time on

101 fibrous leaves and shoots (Hill, 1997). Of this, the neutral detergent fiber content of the major
102 food leaves could be as high as 42% (Hanya, Kiyono, Takafumi, Tsujino, & Agetsuma, 2007).
103 It is therefore critical to understand how the gut microbiome contributes to the macaques'
104 nutrition while considering the macaques' intake of fibrous food items.

105 In this study we aimed (1) to examine and compare the microbiome compositions of
106 Japanese macaques at two different gut sites, the stomach and colon, and then (2) to infer and
107 compare the functions of the gut microbiome at different gut sites. Our hypothesis is that the
108 stomach microbiome will be less diverse and related to environmental information processing
109 and simple sugar metabolism, while the colonic microbiome will be more diverse and enriched
110 with pathways involving fiber digestion. This study aims to improve our understanding of the
111 hindgut-fermenting NHPs' gut microbiome, while focusing on the filtering effect imposed by
112 different GI sites on the microbiome diversity and function.

113

114 **Methods**

115 **Sample collection**

116 We collected stomach content and colon samples from a total of 13 individual
117 macaques inhabiting the coastal area of Yakushima Island, Japan (30°N, 131°E): males and
118 females from each of the three troops (Umi A, Umi B and Umi C) during July 11-14, 2017,
119 May 27-30, 2018, and September 25-28, 2019 (Supplementary Table 1). We sampled each
120 monkey only once. In 2017, we only collected colonic samples: one male and one female from
121 Umi A and one female from Umi B. In 2018, we collected both stomach and colon samples
122 from one male and one female from each of the three troops. In 2019, we collected stomach
123 and colon samples from one male and one female from Umi A and Umi C. These individuals
124 were captured for the purpose of attaching GPS collars. One of us (A. K.), as a vet, darted the
125 animals with VARIO 1V ® Telinject and anesthetized them with 40 or 60 mg of ketamine, 0.2

126 or 0.3 mg of medetomidine, 1 or 1.5 mg of midazolam, and 0.5 or 0.75 mg of atipamezole,
127 assuming that body mass is 8 or 12 kg for adult females or males, respectively. We determined
128 the amount of anesthetic based on data from previous captures for this population and the
129 guidelines set by Primate Research Institute, Kyoto University (Cizauskas, 2008; Primate
130 Research Institute, Kyoto University (KUPRI), 2010). After immobilization, we sampled
131 stomach content by inserting a Nelaton catheter from the mouth into the stomach. For colonic
132 (rectal) microbiome, we swabbed an 8-cm sterile cotton swab into the anus. We stored all the
133 samples in 1-ml lysis buffer (0.5% SDS, 100 mM EDTA (pH 8.0), 100 mM Tris-HCl (pH 8.0),
134 and 10 mM NaCl) at room temperature. We obtained permission for the capture of macaques
135 and entry to the study sites from the Yakushima Forest Ecosystem Conservation Center,
136 Kagoshima Prefecture, and the Ministry of Environment, Japan, adhering to the legal
137 requirements of Japan. We followed the approved capture and sampling protocol by the Field
138 Research Committee of Primate Research Institute, Kyoto University (KUPRI) (#2017-009,
139 #2018-002 and #2019-006). The entire project, including capture and sampling, followed the
140 Guidelines for Field Research of KUPRI and the American Society of Primatologists Principles
141 for Ethical Treatment of Non-Human Primates.

142

143 **Sample storage, DNA purification, 16S rRNA amplification and sequencing**

144 Our method followed Hayakawa et al. (2018) with slight modification. After bead-beating
145 and centrifuging at 20,000 x g for 1 min, we mixed each sample with 1000 µl InhibitEX buffer
146 of the QIAamp DNA Stool Mini Kit (QIAGEN GmbH, Hilden, Germany), then centrifuged
147 the samples at 20,000 x g for 1 min. After that, we mixed 600 µl of the supernatant with 25 µl
148 proteinase K and 600 µl Buffer AL. We followed the manufacturer's protocols to purify the
149 fecal DNA. Using the Qubit dsDNA HS Assay Kit and a Qubit fluorometer (Thermo Fisher
150 Scientific), we then estimated the DNA concentration for each sample. We amplified the V3-

151 V4 region of the 16S rRNA gene with primers as follows: S-D-Bact-0341-b-S-17 (forward) 5'-
152 CCT ACG GGN GGC WGC AG-3' and S-D-Bact-0785-a-A-21 (reverse) 5'-GAC TAC HVG
153 GGT ATC TAA TCC-3' (Klindworth et al., 2013). To improve chastity in the Illumina
154 platform, we fused these primers with the specific overhang adapters 5'-TCG TCG GCA GCG
155 TCA GAT GTG TAT AAG AGA CAG-[3-6-mer Ns]-[forward primer]-3' and 5'-GTC TCG
156 TGG GCT CGG AGA TGT GTA TAA GAG ACA G-[3-6-mer Ns]-[reverse primer]-3', where
157 the 3-6-mer Ns (NNN, NNN N, NNN NN, or NNN NNN) were in the same quantity (Lundberg,
158 Yourstone, Mieczkowski, Jones, & Dangl, 2013).

159 We purified the PCR product using Agencourt AMPure XP beads (Beckman Coulter, Inc.,
160 Carlsbad, CA, USA). Using the Illumina Nextera XT Index Kit, we attached specific dual
161 indices and sequencing adapters to each amplicon by PCR. To make the pooled sequencing
162 library, we mixed the PCR products at the same amount of DNA (2 ng/sample). Using an
163 Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., La Jolla, CA, USA), we then estimated
164 the fragment size distribution of the library. After diluting the library to 15 pM, we carried out
165 the sequencing run with 30% PhiX spike-in on an Illumina MiSeq sequencing platform using
166 the MiSeq Reagent Kit v3 (600 cycles). The read lengths from the MiSeq run were 301 bp
167 (forward sequences), 8 bp (forward indices), 8 bp (reverse indices), and 301 bp (reverse
168 sequences). We deposited the raw data in the DDBJ database with accession number
169 DRA009571.

170

171 **Data analysis**

172 We processed the raw sequences with QIIME2-2019.10 (Bolyen et al., 2019). After
173 demultiplexing according to the barcodes, we implemented quality control, denoising, chimera
174 removal, and generation of amplicon sequence variants using the DADA2 pipeline (Callahan
175 et al., 2016). The pipeline filtered out one stomach sample, UMI11, and one colonic sample,

176 UMI21, due to low sequencing quality. We then determined phylogeny of the denoised
177 amplicon specific variants (ASVs) using the q2-fragment insertion. To assign the taxonomy of
178 the ASVs, we used QIIME2 naïve Bayes feature classifier trained against the Greengenes 13_8
179 reference database. Before analysis, we excluded ASVs classified as mitochondria or
180 chloroplast from the dataset. We plotted the rarefaction curves using the “ggrare” function of
181 R package *ranacapa* (Kandlikar et al., 2018). To explore the functional difference between gut
182 sites, we predicted the Kyoto Encyclopedia of Genes and Genome Orthology (KO) pathways
183 through phylogenetic investigation of communities by reconstruction of unobserved states
184 (PICRUSt2) (Langille et al., 2013) following guidelines at
185 <https://github.com/picrust/picrust2/wiki>. By default, PICRUSt2 excluded all ASVs with the
186 nearest sequenced taxon index (NSTI) value > 2 from the output. The average NSTI value of
187 our dataset was $0.1901 \pm \text{SD } 0.1805$.

188 We performed statistical analyses in R v 3.6.1 with an alpha level of 0.05, with R
189 packages *phyloseq* (McMurdie & Holmes, 2013), *vegan* (Oksanen et al., 2019), and
190 *microbiome* (Lahti & Shetty, 2012). For analysis, we transformed the dataset to compositional
191 abundance (i.e. % of total sequences per sample) using the “transform” function in package
192 *microbiome*. We calculated alpha diversity through the “alpha” function in package
193 *microbiome*. To test the effect of gut sites in alpha diversity (observed richness and Shannon
194 index), we used the pairwise Wilcoxon rank sum test with P-adjustment using the false
195 discovery rate (FDR) method. For beta diversity (weighted and unweighted UniFrac), we
196 constructed principal coordinate analysis (PCoA) plots based on unweighted and weighted
197 UniFrac distances calculated using ASVs. We then used the PERMANOVA test with the
198 “adonis” function in package *vegan* (permutation = 999). To detect the bacterial taxa and KO
199 pathways that were significantly different between stomach and colonic microbiota (log linear
200 discriminant analysis (LDA) score > 2.0, $P < 0.05$), we carried out linear discriminant analysis

201 of effect size (LEfSe) with the default parameters (Segata et al., 2011) available at
 202 <http://huttenhower.sph.harvard.edu/galaxy/>. We conducted the LEfSe on bacterial taxa at level
 203 6 (i.e. genus level).

204

205 **Results**

206 **Sequencing result and basic characteristics of stomach and colonic microbiome**

207 After quality filtering, we acquired 1,296,806 reads from 9 stomach and 12 colonic
 208 samples of Japanese macaques (Supplementary Table 1). For the stomach samples, the average
 209 reads obtained per sample was $15,448 \pm \text{SD } 13,119$. For the colon samples, the average reads
 210 obtained per sample was $100,081 \pm \text{SD } 118,889$. The rarefaction plot for the samples showed
 211 that the sequencing depth was sufficient (Supplementary Figure 1).

212 The stomach and colon did not share any ASVs. In the colon, the 1290 ASVs uncovered
 213 were from 14 phyla, 23 classes, 29 orders, and 46 families. The top three abundant phyla of the
 214 colonic microbiome were Firmicutes ($74.78 \pm \text{SD } 9.90 \%$), Bacteroidetes ($12.04 \pm \text{SD } 10.12 \%$),
 215 and Proteobacteria ($4.61 \pm \text{SD } 3.24 \%$) (Figure 1; Supplementary Table 2). In contrast, the top
 216 three abundant phyla of the stomach microbiota were Proteobacteria, Firmicutes, and
 217 Bacteroidetes, making up $70.07 \pm \text{SD } 17.09 \%$, $20.79 \pm \text{SD } 13.16 \%$, and $1.96 \pm \text{SD } 2.29 \%$ of
 218 the stomach microbiome (Figure 1; Supplementary Table 2). The 240 ASVs uncovered in the
 219 stomach were from 6 known phyla, 12 classes, 17 orders, and 21 families.

220

221 **Alpha diversity and beta diversity differed significantly between stomach and colon**

222 Alpha diversity indices (observed richness and Shannon index) were significantly
 223 higher in the colon than in the stomach (pairwise Wilcoxon signed rank-sum test, P-adjustment
 224 by FDR: observed richness: $V=27$, $P = 0.0313$, Figure 2a; Shannon index: $V=28$, $P = 0.0156$,
 225 Figure 2b). The average observed richness and Shannon index of the stomach microbiome were

226 $30 \pm \text{SD } 9.23$ and $2.92 \pm \text{SD } 0.29$, respectively. On the contrary, average observed richness and
 227 Shannon index of the colonic microbiome were $119.55 \pm \text{SD } 109.88$ and $4.16 \pm \text{SD } 0.86$.

228 PCoA plots based on unweighted (Figure 3a) and weighted UniFrac distance (Figure
 229 3b) revealed that the samples form two distinctive clusters based on the gut site. In both plots,
 230 the colon samples were more scattered compared with the stomach samples. Adonis tests also
 231 suggested a significant effect of gut sites to the gut microbiome (Adonis: unweighted UniFrac:
 232 $R^2 = 0.1029$, $P = 0.001$; weighted UniFrac: $R^2 = 0.2408$, $P = 0.001$). Microbiota of the stomach
 233 and colon were different in both composition and abundance. The difference in microbial
 234 composition between gut sites overrode the difference caused by seasonal variation and/or
 235 identity of the individuals (i.e. troop and sex). We did not find any effect of the troop (Adonis:
 236 unweighted UniFrac: $R^2 = 0.1144$, $P = 0.310$; weighted UniFrac: $R^2 = 0.1306$, $P = 0.249$) or
 237 sex of the individuals (Adonis: unweighted UniFrac: $R^2 = 0.0567$, $P = 0.354$; weighted UniFrac:
 238 $R^2 = 0.0308$, $P = 0.831$). Since we collected the samples at different seasons/times of different
 239 years, unweighted UniFrac, but not on weighted UniFrac, was marginally significantly related
 240 to the year of collection (Adonis: unweighted UniFrac: $R^2 = 0.0671$, $P = 0.048$; weighted
 241 UniFrac: $R^2 = 0.0620$, $P = 0.313$). Close examination of datasets containing only stomach or
 242 colon samples, however, suggested little difference based on the year of collection (Adonis:
 243 stomach: $R^2 = 0.1533$, $P = 0.298$; colon: $R^2 = 0.1023$, $P = 0.318$). This marginal effect may be
 244 a result of the small sample size.

245

246 **Taxonomy-based comparison between stomach and colon of Japanese macaques**

247 Through the LEfSe test, we detected the bacterial genera whose relative abundance
 248 differs significantly between the colonic and stomach microbiomes of Japanese macaques. In
 249 total, 133 genera were significantly enriched at specific gut sites (LEfSe: log LDA score > 2.0 ,
 250 $P < 0.05$; Figure 4; Supplementary Table 3). Of these taxa, 26 were enriched in the stomach,

251 including orders Pasteurellales and Enterobacteriales (class Gammaproteobacteria),
252 Lactobacillales and Gemellales (class Bacilli), Neisseriales (class Betaproteobacteria), and
253 Fusobacteriales (class Fusobacteriia). In the colon, 107 genera were enriched, mainly from
254 phyla Verrucomicrobia, Tenericutes, and Bacteroidetes and orders Clostridiales (phylum
255 Firmicutes) and Bacteroidales (phylum Bacteroidetes) (Supplementary Figure 2). In particular,
256 the top 15 enriched genera were mostly from families Ruminococcaceae and Lachnospiraceae
257 of the order Clostridiales.

258

259 **Predicted functional difference between stomach and colon**

260 Overall, PICRUST2 identified 154 KO pathways (average NSTI: $0.1901 \pm \text{SD } 0.1805$)
261 (Douglas et al., 2019). Based on LEfSe analysis, we defined 75 differentially abundant
262 pathways between the stomach and colon (LEfSe: log LDA score > 2.0 , $P < 0.05$). Among
263 these, 36 pathways were enriched in the colon and 39 were enriched in the stomach (Figure 5;
264 Supplementary Table 4). Most of the differentially abundant pathways (54/75) were related to
265 metabolism. Specifically, the top enriched metabolic pathways in the colon microbiome were
266 related to the metabolism of multiple nutrients such as terpenoids, polyketides, amino acid and
267 glycan. Other than the metabolic pathways, multiple pathways related to cellular processes and
268 genetic information processing were also enriched in the colonic microbiome. On the other
269 hand, the stomach microbiome was especially enriched with metabolic pathways related to
270 carbohydrates e.g. ascorbate and aldarate metabolism and citrate cycle. Furthermore, pathways
271 related to the metabolism of other amino acids, e.g. glutathione metabolism, were enriched in
272 the stomach microbiome. Other than metabolic pathways, stomach microbiome was also
273 enriched in pathways related to environmental information processing, such as the
274 phosphotransferase system and ABC transporters.

275

276 **Discussion**277 **Stomach microbiome is less diverse than colonic microbiome**

278 Our study found that wild Japanese macaques' stomach microbiome was less diverse
279 than their colonic microbiome, supporting findings in the previous studies on mammals (red-
280 shanked doucs (Clayton et al., 2019), Abert's and fox squirrels (Reed, Pigage, Pigage,
281 Glickman, & Bono, 2019), and pikas (H. Li et al., 2017)). Such a difference in diversity
282 revealed the strong effect exerted on the microbiota by the physiochemical environment in the
283 stomach. The stomach generally has a rapid flow of low-pH gastric acid, causing strong
284 disturbance for the survival and growth of microbes (Lambert, 1998; Savage, 1977). As a result,
285 the stomach not only has lower microbial diversity but also may have lower microbial biomass
286 than the colon. The indigenous microbes in such an environment are likely have a tolerance to
287 the acidic and aerobic environment in the stomach and could reproduce rapidly as a way to
288 maintain an active population in the stomach. Though not able to colonize the stomach, some
289 microbes presumably could pass through the stomach and eventually colonize the lower GI
290 tract, such as the colon. The colon provides a rather different environment for bacterial growth:
291 it is characterized by an anaerobic and neutral-to-alkaline condition. Together with the
292 extended transit time, microbes are able to establish populations and form complex interactions
293 within the colon (Müller et al., 2019; Roager et al., 2016). In the case of humans, the half-
294 emptying time of the colon (ca 400 min) could double that of the stomach (ca 165 min)
295 (Camilleri et al., 1989). The physiochemical environment and fast transit of the stomach may
296 present as a bottleneck for bacterial growth, "selecting" the gut microbes passing down to the
297 lower GI tract. However, the gut microbes may then be able to establish a population once they
298 pass through.

299

300 **Taxonomic difference between stomach and colon microbiome of Japanese macaques**

301 As adaptive characteristics to the acidic and aerobic conditions, the stomach
302 microbiome is enriched by acid- and aero-tolerant microbes. Our results revealed that
303 Proteobacteria were especially abundant in the stomach (70.07%) in comparison with their
304 proportion in the colon, which is just 4.61%. Unlike the majority of gut microbes,
305 Proteobacteria are often facultatively anaerobic, and thus are competitive in surviving in the
306 oxic environment of the stomach (Moon, Young, Maclean, Cookson, & Bermingham, 2018;
307 Shin, Whon, & Bae, 2015). By LEfSe analysis, we also identified Lactobacillales enriched in
308 the stomach microbiome. In addition to their ability to withstand an oxic condition, they are
309 also acid-tolerant, which may allow the species to flourish in the stomach (Walter, 2008).
310 Residing in the epithelial surface of the stomach, Lactobacillales species are able to maintain
311 a community even under the continuous disturbance of gastric acid (Savage, 1977; Walter,
312 2008). As opposed to the stomach microbiome, we found colonic microbiota enriched in
313 anaerobic microbes that actively involved in fiber degradation. For example, families
314 Lachnospiraceae and Ruminococcaceae and genus *Prevotella* were more abundant in the colon.
315 These bacterial taxa are active plant degraders with key carbohydrate-active enzymes, sugar
316 transport mechanisms, and metabolic pathways (Biddle, Stewart, Blanchard, & Leschine, 2013;
317 Chen et al., 2017). The presence of fiber-degrading bacterial taxa such as families
318 Lachnospiraceae and Ruminococcaceae and genus *Prevotella* corroborates the major role of
319 colonic microbiota as fiber fermenters. Nevertheless, the absolute abundance of these bacterial
320 taxa would possibly be higher in the colonic microbiome if the biomass of the stomach
321 microbiome were really low.

322 Interestingly, we found that the bacterial taxa enriched in the stomach were related to
323 the oral cavity in other mammals, including humans. For example, genera *Veillonella* and
324 *Streptococcus*, the oral nitrate-reducing bacteria, are common in the mouth or throat of feral
325 horses and humans (Abranches et al., 2019; Doel, Benjamin, Hector, Rogers, & Allaker, 2005;

326 Meyer et al., 2010). Hence, the community we observed in the stomach may have represented
327 the transient microbes that were swallowed during food intake of Japanese macaques. Japanese
328 macaques usually store food in their cheek pouch for an extended duration (Yumoto, Noma, &
329 Maruhashi, 1998). The microbes in the oral cavity may colonize the food surface before the
330 macaques actually swallow the food. This partly supports the notion that the gut microbes enter
331 from the oral cavity but then the GI sites “selects” out a part through the varied physiochemical
332 environments. It would be interesting to further study how the microbes transfer from the oral
333 cavity to the stomach and the lower GI tract.

334

335 **Functional difference between stomach and colon microbiome**

336 According to the functional prediction by PICRUSt2, the main functional differences
337 between the stomach and colonic microbiomes were related to metabolism. Such differences
338 may be related to the different digestive roles of the stomach and the colon. The stomach
339 microbiome was more enriched in the metabolic pathways involving carbohydrates, especially
340 simple sugar. For example, we found glycolysis/gluconeogenesis and citrate cycle (TCA)
341 enriched in the stomach microbiome. The microbes may utilize part of the simple sugar that
342 was not digested by the enzyme in the stomach. However as mentioned above, the stomach
343 microbiome may be less abundant and less diverse. While the stomach microbiome may have
344 functions supplementing the digestive role of the stomach, the overall effect remains limited.
345 On the other hand, metabolic pathways related to terpenoids, polyketides, amino acid and
346 glycan increased in the colonic microbiome. Glycan biosynthesis and metabolism are also
347 abundant in the gut microbiome of Tibetan macaques during winter (Sun et al., 2016). These
348 pathways are related to the digestion of glycan produced by the breakdown of cellulose and
349 hemicellulose. Since the colonic microbiome is the main fermentation site, it makes sense that
350 the enriched pathways are related to the digestive efficiency of the fibrous foods eaten by the

351 macaques. Overall, the differentially enriched pathways implied that the microbial
352 communities in both gut sites are equipped to supplement the digestive functions of these gut
353 sites.

354

355 **Stomach microbiome of Japanese macaques compared to foregut microbiome of colobus**

356 Compared with the foregut-fermenting NHPs, the relative difference in diversity
357 between the gut sites was great in this study (Table 1). Given the biases caused by varied
358 sampling and analysis methods across studies (Asangba et al., 2019; Hayakawa, Sawada, et al.,
359 2018), we only made comparisons of diversity across different host species in the form of
360 stomach to colon ratio, instead of the absolute number of ASVs or any other index. In our study,
361 the observed richness of the macaques' stomach microbiome is nearly a quarter that of the
362 colonic microbiome. On the other hand, the red-shanked douc's foregut microbiome is about
363 half as diverse as the hindgut microbiome (Clayton et al., 2019). Again, this may be due to the
364 difference in gut physiology between foregut- and hindgut-fermenting animals. The colobines
365 are anatomically unique in having evolved a large, sacculated foregut for extended
366 fermentation (Matsuda, Chapman, & Clauss, 2019). Compared to the hindgut fermenters like
367 Japanese macaques in the present study, the foregut of the colobines is relatively alkaline for
368 the optimal fermentation condition (Lambert, 1998). The relatively alkaline stomach
369 environment of colobines may allow a more diverse foregut microbiota and thus maximize
370 energy harvest from their nutritionally poor folivory-based diet. Despite the biases caused by
371 variations in sampling, storage and analysis methods across studies, the relative difference in
372 alpha diversity indices between the foregut- and hindgut-fermenting NHPs is apparent.
373 However, again, the current study remains preliminary, and further studies, including more
374 species and a larger sample size, would greatly improve our knowledge of the stomach/foregut
375 microbiome of NHPs overall.

376 In comparing composition at the phylum level, the top two dominant phyla in the
377 stomach microbiome of wild Japanese macaques and the foregut of captive red-shanked doucs
378 (Clayton et al., 2019) were Proteobacteria and Firmicutes, different from those of the wild
379 proboscis monkeys (Hayakawa, Nathan, et al., 2018) studied, which are dominated by
380 Firmicutes and Bacteroidetes. Notably, the dominance of Firmicutes and Bacteroidetes rather
381 than Proteobacteria is a more common pattern found in the colonic microbiome of mammals
382 including NHPs (Amato et al., 2015; Clayton et al., 2018; Lee, Hayakawa, Kiyono, Yamabata,
383 & Hanya, 2019; Ley, Lozupone, Hamady, Knight, & Gordon, 2008). As mentioned above,
384 Proteobacteria are competitive in surviving the relatively oxygen-abundant environment of the
385 stomach. The foregut of wild proboscis monkeys may present an environment similar to the
386 colon, thus harboring a colonic microbiome-like community. Alternatively, the enriched
387 Proteobacteria found in the stomach/foregut microbiome of Japanese macaques and red-
388 shanked doucs may be replaced by functionally redundant microbial species from the phyla
389 Firmicutes and Bacteroidetes in the foregut microbiome of the proboscis monkeys. The
390 difference between the foregut microbiomes of the two colobines may be related to the
391 simplified captive diet that includes more easily digestible foods. The foregut microbiome of
392 captive proboscis monkeys was less diverse than and compositionally different from that of the
393 wild proboscis monkeys which forage on diverse types of plants (Hayakawa, Nathan, et al.,
394 2018). Hence, the foregut of the captive red-shanked doucs may be different from that of
395 proboscis monkeys through divergence in macronutrient intake. To clarify the general pattern
396 of the dominant phyla and species in the stomach/foregut microbiome as well as the related
397 factors, data from more species and a larger sample size are needed. In the present study, the
398 stomach microbiome composition of Japanese macaques was marginally related to the dietary
399 variation across seasons. However, our examination of the effect of seasons, sex and other host
400 factors remains preliminary due to the limited sample size. It would be interesting to carry out

401 a detailed study to examine the response of the foregut/stomach microbiome to environmental
402 factors.

403

404 **Conclusion**

405 Overall, the stomach and colonic microbiome of the Japanese macaques are distinctive
406 from each other in diversity, composition and function. Compared with the foregut-fermenting
407 NHPs, the stomach of hindgut-fermenting NHPs potentially present a harsher physiochemical
408 environment for microbial acquisition and survival. Our result revealed the filtering effect
409 imposed by different GI sites on the gut microbiome, shedding light on how microbes adapt to
410 different physiochemical GI environments and distribute along the GI tract.

411

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429

430 **References**

- 431 Abranches, J., Zeng, L., Kajfasz, J. K., Palmer, S., Chakraborty, B., Wen, Z., ... Lemos, J. A.
432 (2019). Biology of oral Streptococci. In *Gram-Positive Pathogens* (pp. 426–434).
433 John Wiley & Sons, Ltd. DOI: 10.1128/9781683670131.ch26
- 434 Amato, K. R., Leigh, S. R., Kent, A., Mackie, R. I., Yeoman, C. J., Stumpf, R. M., ... Garber,
435 P. A. (2015). The gut microbiota appears to compensate for seasonal diet variation in
436 the wild black howler monkey (*Alouatta pigra*). *Microbial Ecology*, 69(2), 434–443.
437 DOI: 10.1007/s00248-014-0554-7
- 438 Amato, K. R., Metcalf, J. L., Song, S. J., Hale, V. L., Clayton, J., Ackermann, G., ... Braun,
439 J. (2016). Using the gut microbiota as a novel tool for examining colobine primate GI
440 health. *Global Ecology and Conservation*, 7, 225–237. DOI:
441 10.1016/j.gecco.2016.06.004
- 442 Biddle, A., Stewart, L., Blanchard, J., & Leschine, S. (2013). Untangling the genetic basis of
443 fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut
444 communities. *Diversity*, 5(3), 627–640. DOI: 10.3390/d5030627
- 445 Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ...
446 Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome
447 data science using QIIME 2. *Nature Biotechnology*, 37(8), 852–857. DOI:
448 10.1038/s41587-019-0209-9

- 449 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S.
 450 P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data.
 451 *Nature Methods*, *13*(7), 581–583. DOI: 10.1038/nmeth.3869
- 452 Camilleri, M., Colemont, L. J., Phillips, S. F., Brown, M. L., Thomforde, G. M., Chapman,
 453 N., & Zinsmeister, A. R. (1989). Human gastric emptying and colonic filling of solids
 454 characterized by a new method. *The American Journal of Physiology*, *257*(2), G284-
 455 290. DOI: 10.1152/ajpgi.1989.257.2.g284
- 456 Chen, T., Long, W., Zhang, C., Liu, S., Zhao, L., & Hamaker, B. R. (2017). Fiber-utilizing
 457 capacity varies in *Prevotella*-versus *Bacteroides*-dominated gut microbiota. *Scientific*
 458 *Reports*, *7*(1), 2594. DOI: 10.1038/s41598-017-02995-4
- 459 Cizauskas, C. A. (2008). Zoo animal & wildlife immobilization and anesthesia. *Journal of*
 460 *Wildlife Diseases*, *44*(2), 528–530. DOI: 10.7589/0090-3558-44.2.528
- 461 Clayton, J. B., Gomez, A., Amato, K., Knights, D., Travis, D. A., Blekhman, R., ... Johnson,
 462 T. J. (2018). The gut microbiome of nonhuman primates: Lessons in ecology and
 463 evolution. *American Journal of Primatology*, *80*(6), e22867. DOI: 10.1002/ajp.22867
- 464 Clayton, J. B., Shields-Cutler, R. R., Hoops, S. L., Al-Ghalith, G. A., Sha, J. C. M., Johnson,
 465 T. J., & Knights, D. (2019). Bacterial community structure and function distinguish
 466 gut sites in captive red-shanked doucs (*Pygathrix nemaeus*). *American Journal of*
 467 *Primatology*, *81*(10–11), e22977. DOI: 10.1002/ajp.22977
- 468 Doel, J. J., Benjamin, N., Hector, M. P., Rogers, M., & Allaker, R. P. (2005). Evaluation of
 469 bacterial nitrate reduction in the human oral cavity. *European Journal of Oral*
 470 *Sciences*, *113*(1), 14–19. DOI: 10.1111/j.1600-0722.2004.00184.x
- 471 Douglas, G. M., Maffei, V. J., Zaneveld, J., Yurgel, S. N., Brown, J. R., Taylor, C. M., ...
 472 Langille, M. G. I. (2019). PICRUSt2: An improved and extensible approach for
 473 metagenome inference. *BioRxiv*. DOI: 10.1101/672295

- 474 Gu, S., Chen, D., Zhang, J.-N., Lv, X., Wang, K., Duan, L.-P., ... Wu, X.-L. (2013).
475 Bacterial community mapping of the mouse gastrointestinal tract. *PLOS ONE*, 8(10),
476 e74957. DOI: 10.1371/journal.pone.0074957
- 477 Hanya, G. (2004). Diet of a Japanese macaque troop in the coniferous forest of Yakushima.
478 *International Journal of Primatology*, 25(1), 55–71. DOI:
479 10.1023/B:IJOP.0000014645.78610.32
- 480 Hanya, G., Kiyono, M., Takafumi, H., Tsujino, R., & Agetsuma, N. (2007). Mature leaf
481 selection of Japanese macaques: Effects of availability and chemical content. *Journal*
482 *of Zoology*, 273(2), 140–147. DOI: 10.1111/j.1469-7998.2007.00308.x
- 483 Hanya, G., Noma, N., & Agetsuma, N. (2003). Altitudinal and seasonal variations in the diet
484 of Japanese macaques in Yakushima. *Primates*, 44(1), 51–59. DOI: 10.1007/s10329-
485 002-0007-7
- 486 Hayakawa, T., Nathan, S. K. S. S., Stark, D. J., Saldivar, D. A. R., Sipangkui, R., Goossens,
487 B., ... Matsuda, I. (2018). First report of foregut microbial community in proboscis
488 monkeys: Are diverse forests a reservoir for diverse microbiomes? *Environmental*
489 *Microbiology Reports*, 10(6), 655–662. DOI: 10.1111/1758-2229.12677
- 490 Hayakawa, T., Sawada, A., Tanabe, A. S., Fukuda, S., Kishida, T., Kurihara, Y., ... Agata, K.
491 (2018). Improving the standards for gut microbiome analysis of fecal samples:
492 Insights from the field biology of Japanese macaques on Yakushima Island. *Primates*,
493 59(5), 423–436. DOI: 10.1007/s10329-018-0671-x
- 494 Hill, D. A. (1997). Seasonal variation in the feeding behavior and diet of Japanese macaques
495 (*Macaca fuscata yakui*) in lowland forest of Yakushima. *American Journal of*
496 *Primatology*, 43(4), 305–320. DOI: 10.1002/(SICI)1098-2345(1997)43:4<305::AID-
497 AJP2>3.0.CO;2-0

- 498 Hillman, E. T., Lu, H., Yao, T., & Nakatsu, C. H. (2017). Microbial ecology along the
499 gastrointestinal tract. *Microbes and Environments*, 32(4), 300–313. DOI:
500 10.1264/jsme2.ME17017
- 501 Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Reddy, D.
502 N. (2015). Role of the normal gut microbiota. *World Journal of Gastroenterology*,
503 21(29), 8787–8803. DOI: 10.3748/wjg.v21.i29.8787
- 504 Kandlikar, G. S., Gold, Z. J., Cowen, M. C., Meyer, R. S., Freise, A. C., Kraft, N. J. B., ...
505 Curd, E. E. (2018). ranacapa: An R package and shiny web app to explore
506 environmental DNA data with exploratory statistics and interactive visualizations.
507 *F1000Research*, 7, 1734. DOI: 10.12688/f1000research.16680.1
- 508 Kurihara, Y., Kinoshita, K., Shiroishi, I., & Hanya, G. (2020). Seasonal variation in energy
509 balance of wild Japanese macaques (*Macaca fucata yakui*) in a warm-temperate
510 forest: A preliminary assessment in the coastal forest of Yakushima. *Primates*, 61(3),
511 427–442. DOI: 10.1007/s10329-020-00797-3
- 512 Lahti, L., & Shetty, S. (2012). *Microbiome R package*. Retrieved from
513 <http://microbiome.github.io/microbiome>
- 514 Lambert, J. E. (1998). Primate digestion: Interactions among anatomy, physiology, and
515 feeding ecology. *Evolutionary Anthropology*, 7(1), 8–20. DOI: 10.1002/(SICI)1520-
516 6505(1998)7:1<8::AID-EVAN3>3.0.CO;2-C
- 517 Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., ...
518 Huttenhower, C. (2013). Predictive functional profiling of microbial communities
519 using 16S rRNA marker gene sequences. *Nature Biotechnology*, 31(9), 814–821.
520 DOI: 10.1038/nbt.2676

- 521 Lee, W., Hayakawa, T., Kiyono, M., Yamabata, N., & Hanya, G. (2019). Gut microbiota
522 composition of Japanese macaques associates with extent of human encroachment.
523 *American Journal of Primatology*, 81(12), e23072. DOI: 10.1002/ajp.23072
- 524 Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., & Gordon, J. I. (2008). Worlds within
525 worlds: Evolution of the vertebrate gut microbiota. *Nature Reviews Microbiology*,
526 6(10), 776–788. DOI: 10.1038/nrmicro1978
- 527 Li, D., Chen, H., Zhao, J., Zhang, H., & Chen, W. (2019). Potential functions of the
528 gastrointestinal microbiome inhabiting the length of the rat digest tract. *International*
529 *Journal of Molecular Sciences*, 20(5), 1232. DOI: 10.3390/ijms20051232
- 530 Li, H., Li, T., Berasategui, A., Rui, J., Zhang, X., Li, C., ... Li, X. (2017). Gut region
531 influences the diversity and interactions of bacterial communities in pikas (*Ochotona*
532 *curzoniae* and *Ochotona daurica*). *FEMS Microbiology Ecology*, 93(12), fix149.
533 DOI: 10.1093/femsec/fix149
- 534 Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D., & Dangl, J. L. (2013).
535 Practical innovations for high-throughput amplicon sequencing. *Nature Methods*,
536 10(10), 999–1002. DOI: 10.1038/nmeth.2634
- 537 Matsuda, I., Chapman, C. A., & Clauss, M. (2019). Colobine forestomach anatomy and diet.
538 *Journal of Morphology*, 280(11), 1608–1616. DOI: 10.1002/jmor.21052
- 539 McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive
540 analysis and graphics of microbiome census data. *PLoS ONE*, 8(4), e61217. DOI:
541 10.1371/journal.pone.0061217
- 542 Merrell, D. S., Goodrich, M. L., Otto, G., Tompkins, L. S., & Falkow, S. (2003). PH-
543 regulated gene expression of the gastric pathogen *Helicobacter pylori*. *Infection and*
544 *Immunity*, 71(6), 3529–3539. DOI: 10/frxv7z

- 545 Meyer, W., Kacza, J., Schnapper, A., Verspohl, J., Hornickel, I., & Seeger, J. (2010). A first
546 report on the microbial colonisation of the equine oesophagus. *Annals of Anatomy*,
547 *192*(1), 42–51. DOI: 10.1016/j.aanat.2009.10.004
- 548 Moon, C. D., Young, W., Maclean, P. H., Cookson, A. L., & Bermingham, E. N. (2018).
549 Metagenomic insights into the roles of Proteobacteria in the gastrointestinal
550 microbiomes of healthy dogs and cats. *MicrobiologyOpen*, *7*(5), e00677. DOI:
551 10.1002/mbo3.677
- 552 Müller, M., Hermes, G. D. A., Canfora, E. E., Smidt, H., Masclee, A. A. M., Zoetendal, E.
553 G., & Blaak, E. E. (2019). Distal colonic transit is linked to gut microbiota diversity
554 and microbial fermentation in humans with slow colonic transit. *APSSelect*, *7*(2),
555 G361–G369. DOI: 10.1152/ajpgi.00283.2019@apsselect.2020.7.issue-2
- 556 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., ... Wagner,
557 H. (2019). *vegan: Community ecology package*. Retrieved from [https://CRAN.R-](https://CRAN.R-project.org/package=vegan)
558 [project.org/package=vegan](https://CRAN.R-project.org/package=vegan)
- 559 Primate Research Institute, Kyoto University (KUPRI). (2010, June 9). *Guidelines for care*
560 *and use of nonhuman primates*. Retrieved from [https://www.pri.kyoto-](https://www.pri.kyoto-u.ac.jp/research/sisin2010/Guidelines_for_Care_and_Use_of_Nonhuman_Primates20100609.pdf)
561 [u.ac.jp/research/sisin2010/Guidelines_for_Care_and_Use_of_Nonhuman_Primates20](https://www.pri.kyoto-u.ac.jp/research/sisin2010/Guidelines_for_Care_and_Use_of_Nonhuman_Primates20100609.pdf)
562 [100609.pdf](https://www.pri.kyoto-u.ac.jp/research/sisin2010/Guidelines_for_Care_and_Use_of_Nonhuman_Primates20100609.pdf)
- 563 Reed, A., Pigage, J. C., Pigage, H. K., Glickman, C., & Bono, J. M. (2019). Comparative
564 analysis of microbiota along the length of the gastrointestinal tract of two tree squirrel
565 species (*Sciurus aberti* and *S. niger*) living in sympatry. *Ecology and Evolution*,
566 *9*(23), 13344–13358. DOI: 10.1002/ece3.5789
- 567 Roager, H. M., Hansen, L. B. S., Bahl, M. I., Frandsen, H. L., Carvalho, V., Gøbel, R. J., ...
568 Licht, T. R. (2016). Colonic transit time is related to bacterial metabolism and

- 569 mucosal turnover in the gut. *Nature Microbiology*, *1*(9), 16093. DOI:
570 10.1038/nmicrobiol.2016.93
- 571 Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual Review of*
572 *Microbiology*, *31*(1), 107–133. DOI: 10.1146/annurev.mi.31.100177.000543
- 573 Seedorf, H., Griffin, N. W., Ridaura, V. K., Reyes, A., Cheng, J., Rey, F. E., ... Woebken, D.
574 (2014). Bacteria from diverse habitats colonize and compete in the mouse gut. *Cell*,
575 *159*(2), 253–266. DOI: 10.1016/j.cell.2014.09.008
- 576 Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., &
577 Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome*
578 *Biology*, *12*, R60. DOI: 10.1186/gb-2011-12-6-r60
- 579 Shin, N.-R., Whon, T. W., & Bae, J.-W. (2015). Proteobacteria: Microbial signature of
580 dysbiosis in gut microbiota. *Trends in Biotechnology*, *33*(9), 496–503. DOI:
581 10.1016/j.tibtech.2015.06.011
- 582 Stevens, C. E., & Hume, I. D. (1998). Contributions of microbes in vertebrate gastrointestinal
583 tract to production and conservation of nutrients. *Physiological Reviews*, *78*(2), 393–
584 427. DOI: 10.1152/physrev.1998.78.2.393
- 585 Sun, B., Wang, X., Bernstein, S., Huffman, M. A., Xia, D.-P., Gu, Z., ... Li, J. (2016).
586 Marked variation between winter and spring gut microbiota in free-ranging Tibetan
587 macaques (*Macaca thibetana*). *Scientific Reports*, *6*(1), 26035. DOI:
588 10.1038/srep26035
- 589 Vega, N. M. (2019). Experimental evolution reveals microbial traits for association with the
590 host gut. *PLoS Biology*, *17*(2), e3000129. DOI: 10.1371/journal.pbio.3000129
- 591 Walter, J. (2008). Ecological role of Lactobacilli in the gastrointestinal tract: Implications for
592 fundamental and biomedical research. *Applied and Environmental Microbiology*,
593 *74*(16), 4985–4996. DOI: 10.1128/AEM.00753-08

594 Yumoto, T., Noma, N., & Maruhashi, T. (1998). Cheek-pouch dispersal of seeds by Japanese
595 monkeys (*Macaca fuscata yakui*) on Yakushima Island, Japan. *Primates*, 39, 325–
596 338. DOI: 10.1007/bf02573081
597
598
599
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Table 1. Foregut/stomach microbiota of the colobines and Japanese macaques

Common name	Scientific name	Captive/Wild	Observed richness (Stomach)	Observed richness (Colon)	Reference	Most abundant phylum in stomach		
						1	2	3
Japanese macaque	<i>Macaca fuscata</i>	Wild	30.0 ± SD 9.23	119.55 ± SD 109.88	Present study	Proteobacteria	Firmicutes	Bacteoidetes
Red-shanked douc	<i>Pygathrix nemaeus</i>	Captive	606.5 ± 166.52	1239.5 ± 146.57	Clayton et al., 2019	Firmicutes	Proteobacteria	Bacteroidetes
Proboscis monkey	<i>Nasalis larvatus</i>	Captive, Provisioned and Wild	501 - 962	N.A.	Hayakawa et al., 2018	Bacteroidetes	Firmicutes	Proteobacteria

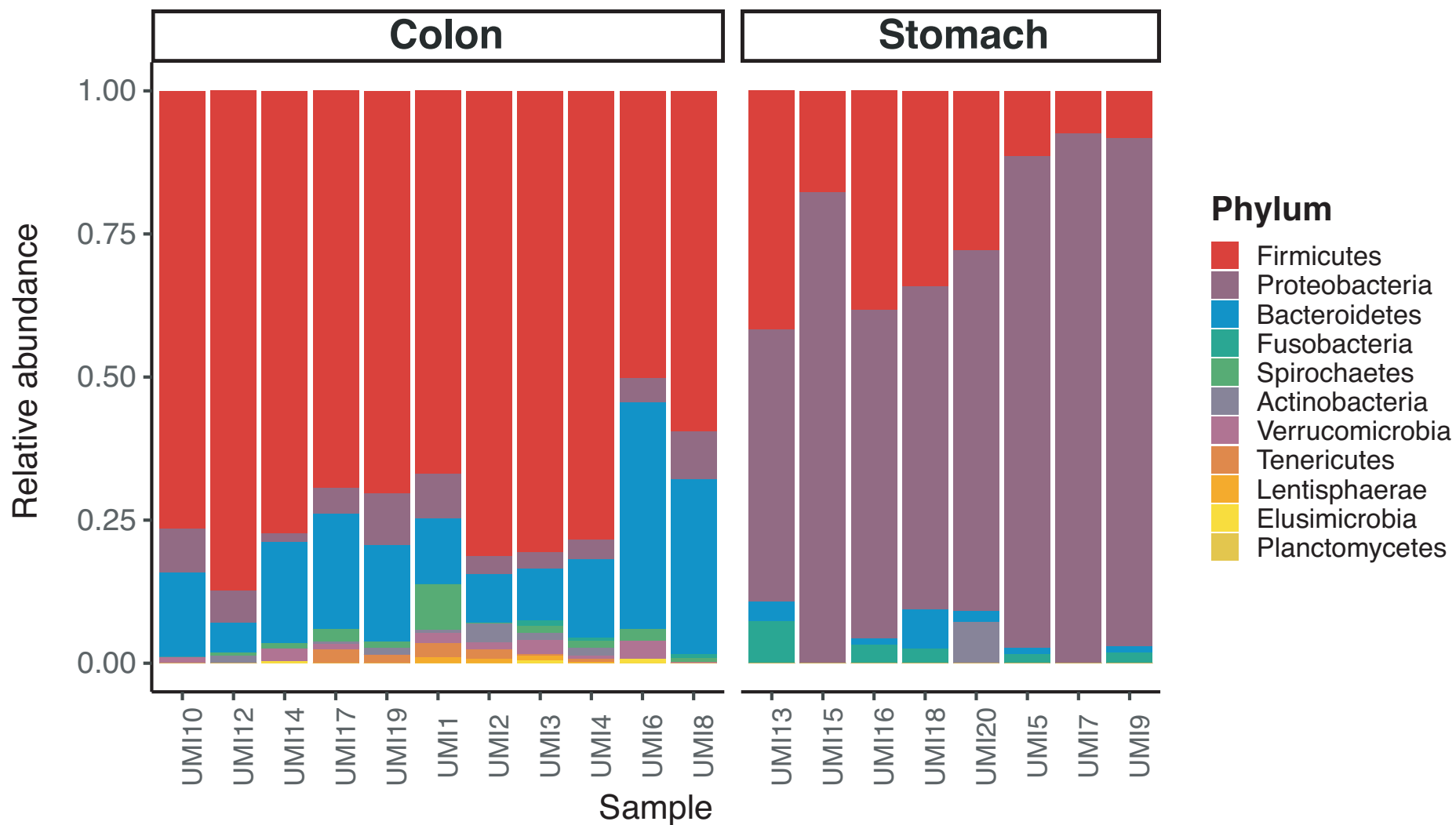


Figure 1. Relative abundance of gut bacterial taxa at phylum level (% of total sequences per sample)

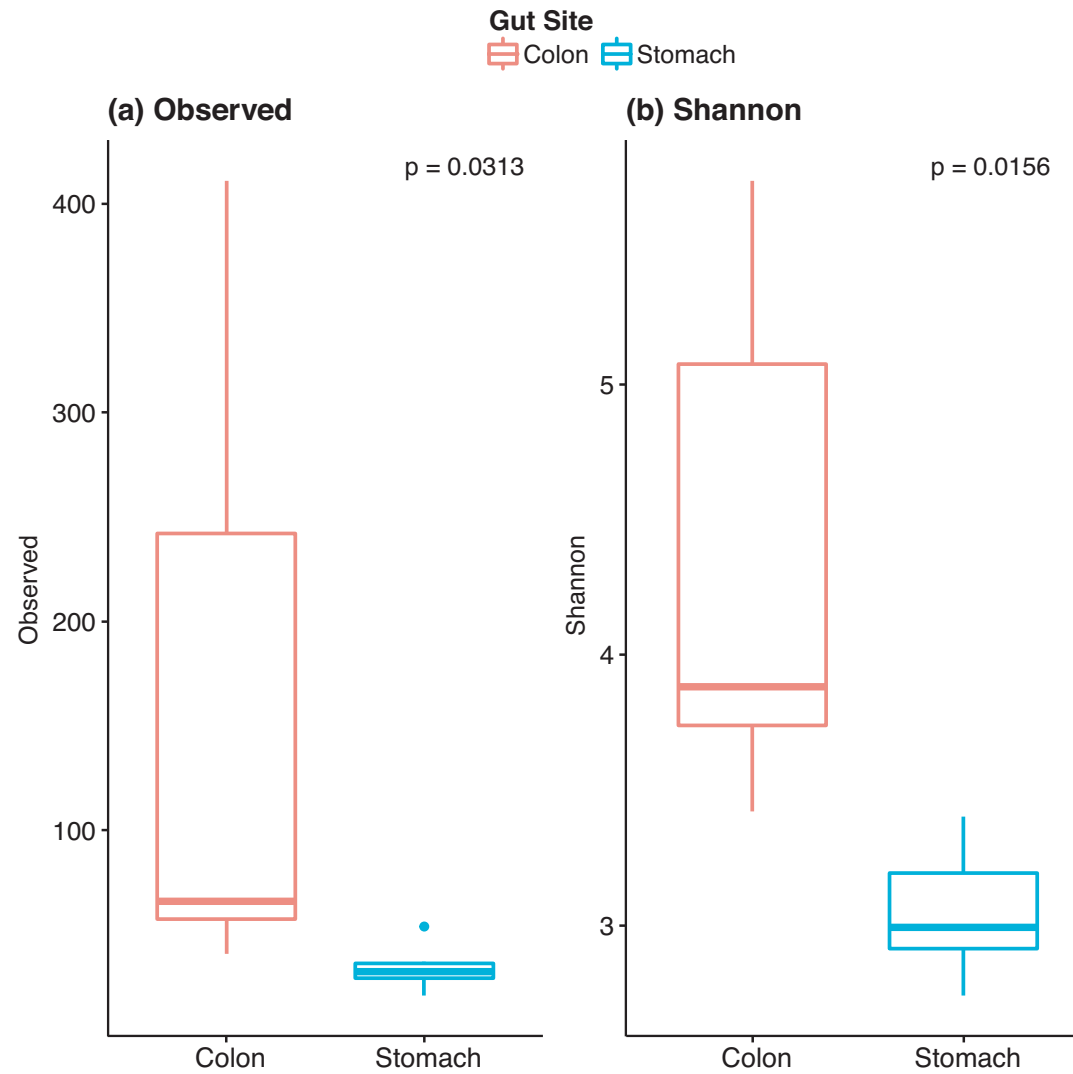


Figure 2. (a) Observed richness and (b) Shannon diversity index of stomach and colonic microbiomes of Japanese macaques

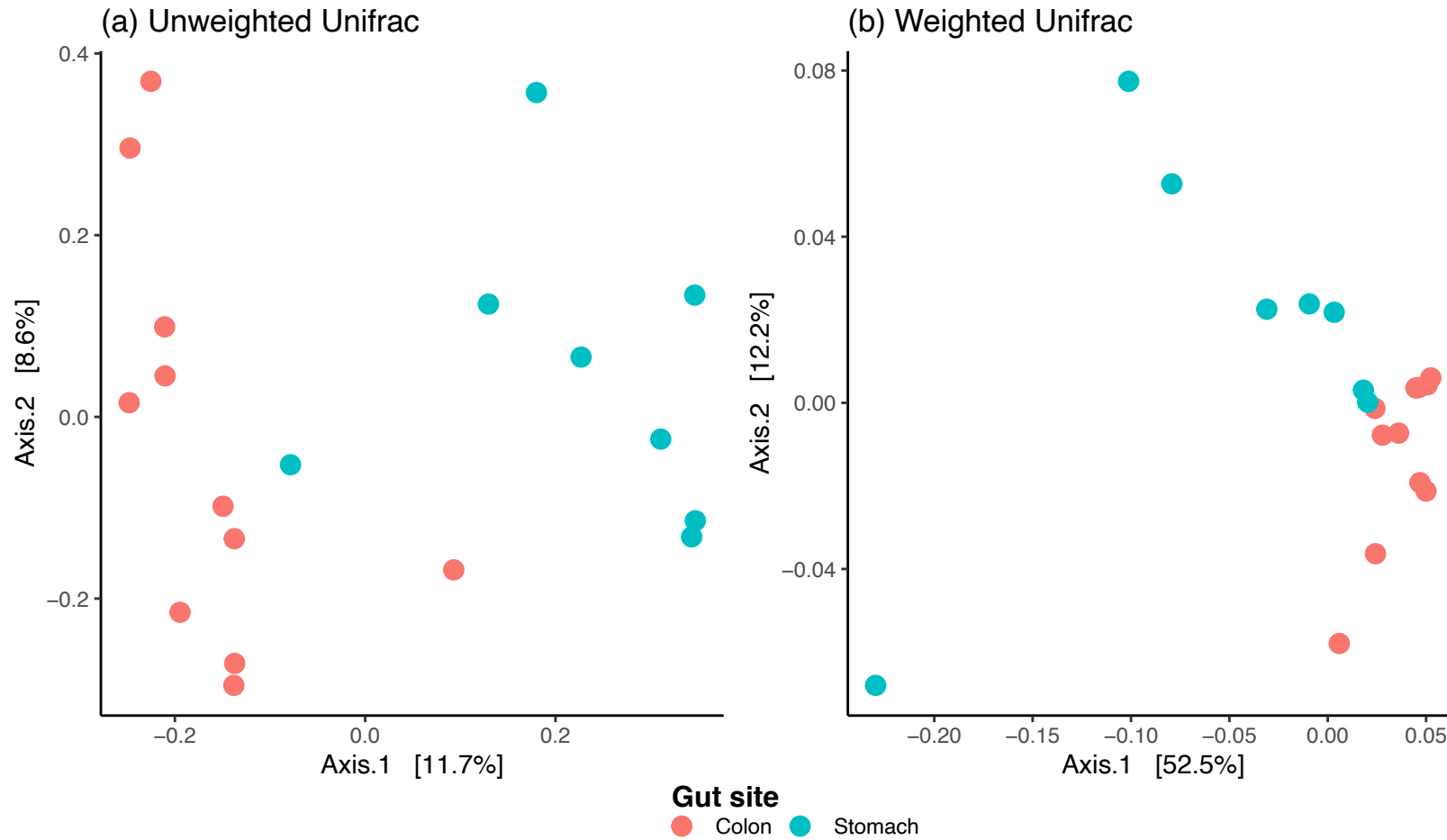


Figure 3. Principal coordinate analysis plots based on (a) unweighted and (b) weighted UniFrac distance for macaques' gut bacterial communities

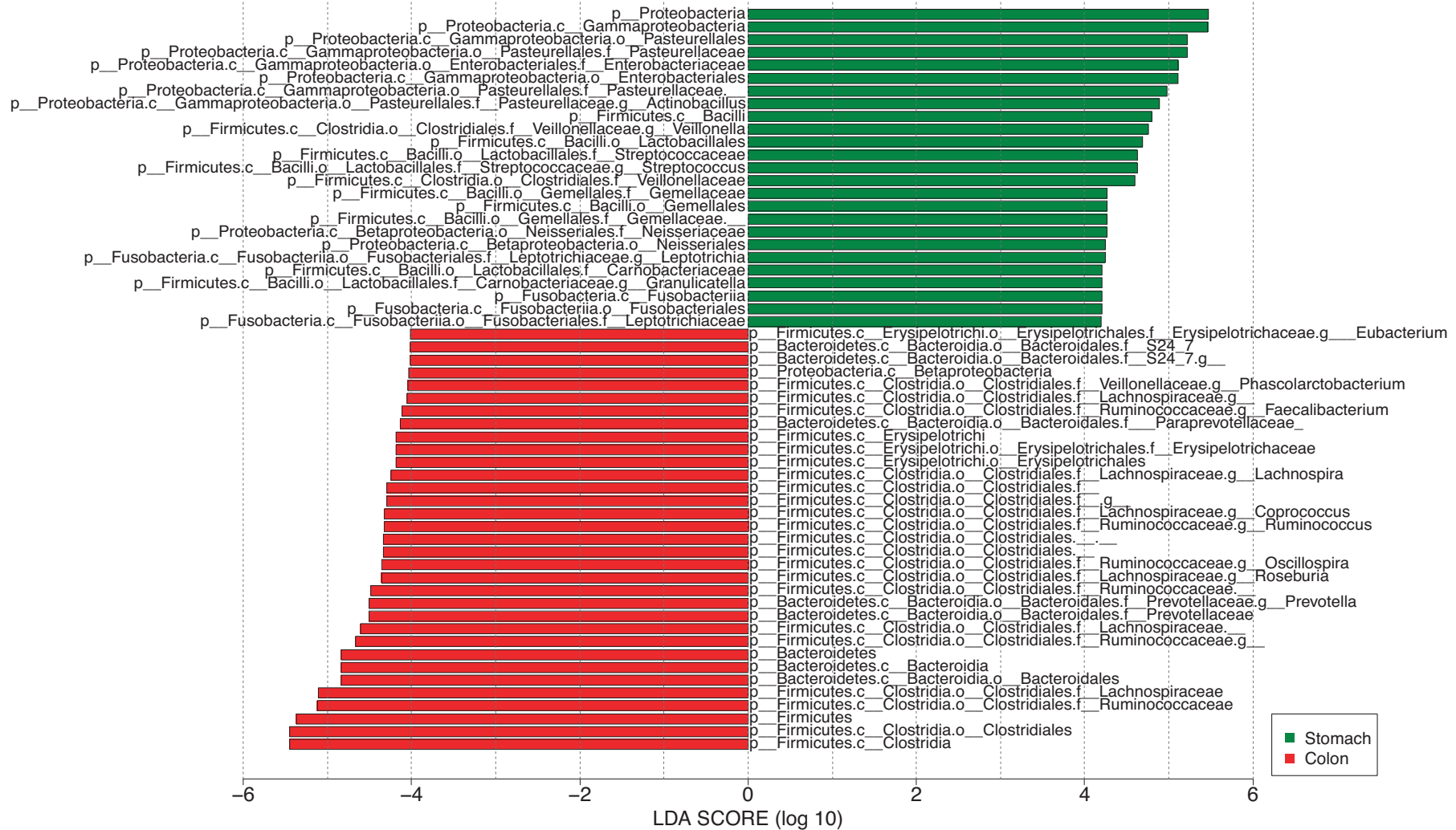


Figure 4. Gut microbial genera differentially abundant in the stomach and colonic microbiome. Plot showing the histogram of linear discriminant analysis (LDA) scores computed for differentially abundant bacterial genera (log LDA score > 5.0, $p < 0.05$)

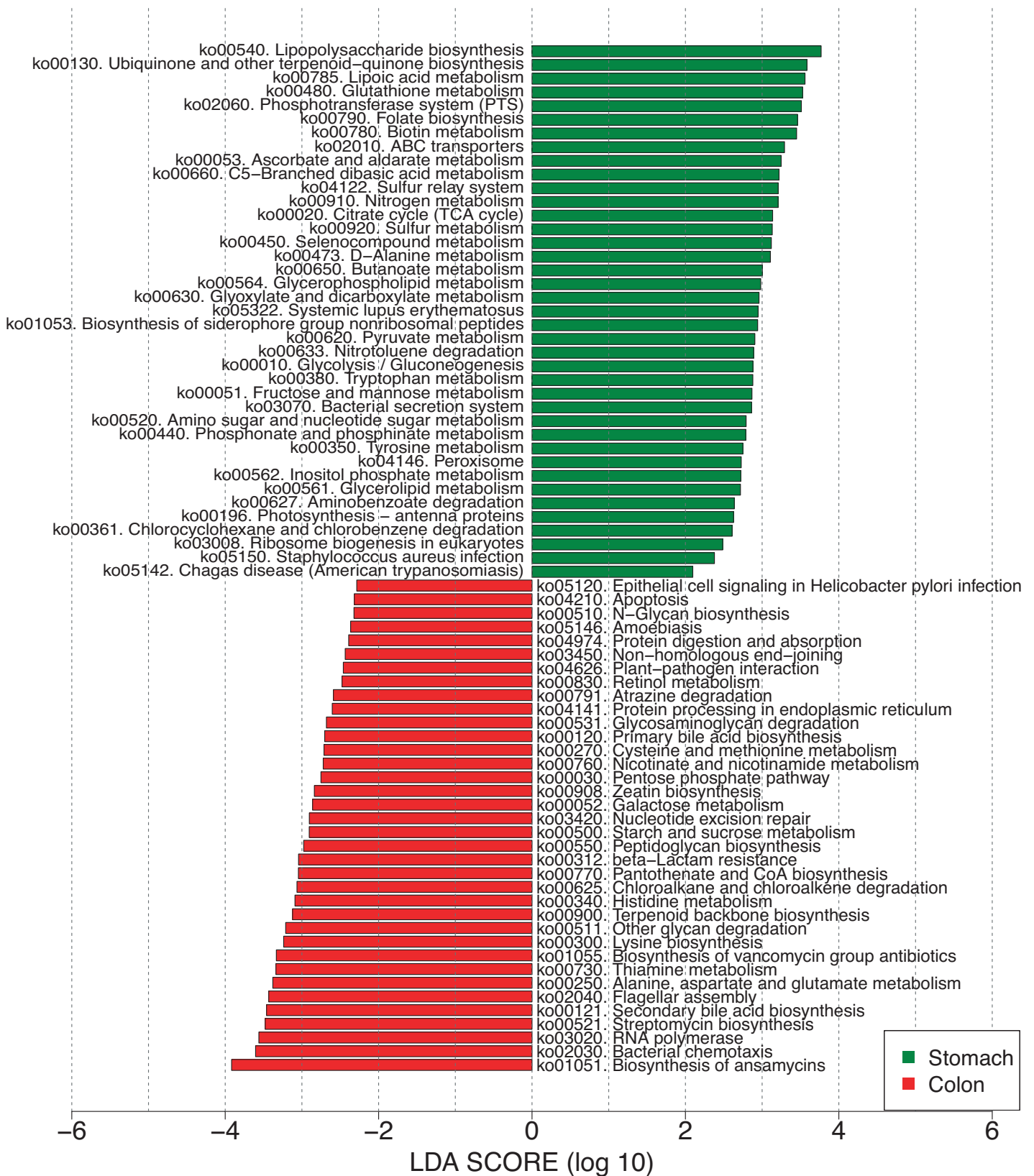
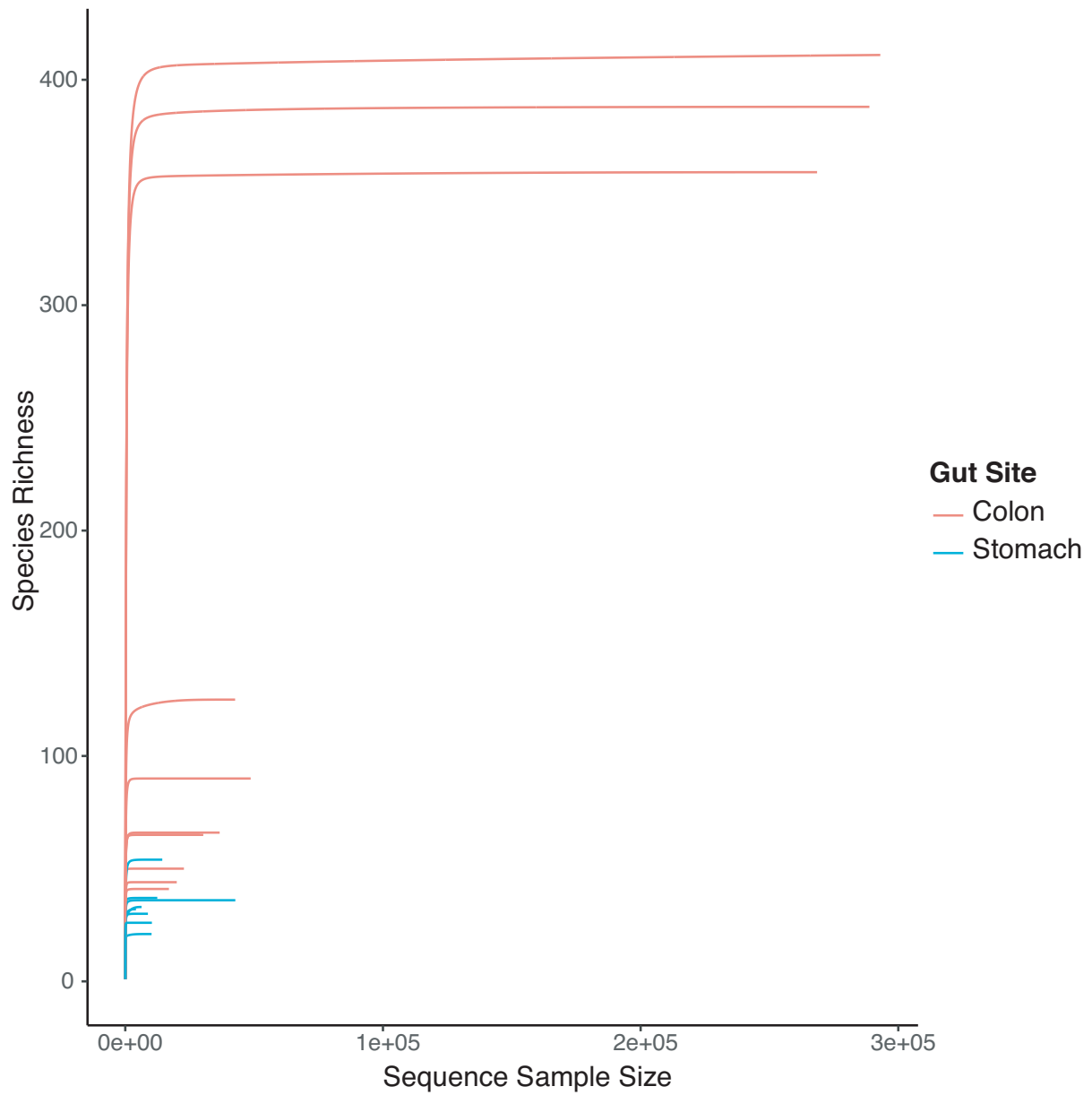
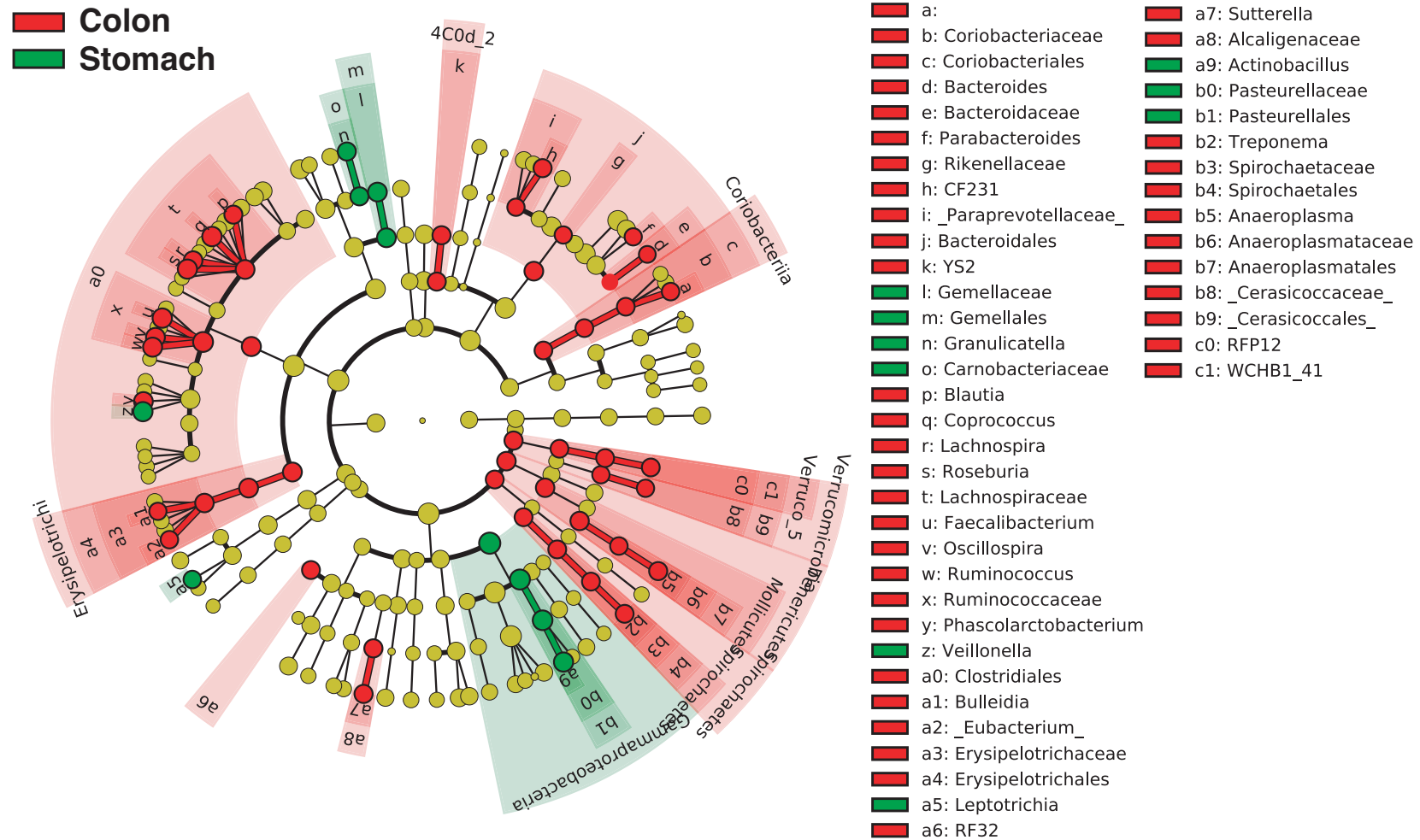


Figure 5. Histogram of LDA scores computed for differentially abundant Kyoto Encyclopedia of Genes and Genome Orthology (KO) pathways in the stomach and colonic microbiome (log LDA score > 2.0, $p < 0.05$)



Supplementary Figure 1. Rarefaction curve of stomach and colonic samples



Supplementary Figure 2. Cladogram plotted from LefSe showing the taxonomic levels represented by rings with phyla in the outermost ring and genera in the innermost ring. Each circle is a member within that level. Those taxa in each level are colored by the gut sites in which the taxa are more abundant (log LDA score >2.0, $p < 0.05$)

Supplementary Table 1. Sample information

Sample ID	Group	Sex	Collected year & month	Gut Site	PairedID	Seuqncing depth
UMI1	umia	male	2017 July	colon	2017AM	42628
UMI2	umia	female	2017 July	colon	2017AF	269882
UMI3	umib	female	2017 July	colon	2017BF	289151
UMI4	umia	female	2018 May	colon	2018AF	294209
UMI6	umia	male	2018 May	colon	2018AM	17427
UMI8	umib	female	2018 May	colon	2018BF	48753
UMI10	umib	male	2018 May	colon	2018BM	19976
UMI12	umic	female	2018 May	colon	2018CF	23050
UMI14	umic	male	2018 May	colon	2018CM	30265
UMI17	umia	male	2019 Septmenber	colon	2019AM	36631
UMI19	umia	female	2019 Septmenber	colon	2019AF	28918
UMI5	umia	female	2018 May	stomach	2018AF	46398
UMI7	umia	male	2018 May	stomach	2018AM	4221
UMI9	umib	female	2018 May	stomach	2018BF	10529
UMI13	umic	female	2018 May	stomach	2018CF	15547
UMI15	umic	male	2018 May	stomach	2018CM	6658
UMI16	umia	male	2019 Septmenber	stomach	2019AM	11950
UMI18	umia	female	2019 Septmenber	stomach	2019AF	15528
UMI20	umic	male	2019 Septmenber	stomach	2019CM	12760

Supplementary Table 2. Relative abundance of microbial phyla

Phylum	Colon											Average	SD
	UMI1	UMI10	UMI12	UMI14	UMI17	UMI19	UMI2	UMI3	UMI4	UMI6	UMI8		
Actinobacteria	0.55%	0.00%	1.10%	0.00%	0.36%	1.04%	2.37%	1.03%	1.05%	0.00%	0.00%	1.21%	0.73%
Bacteroidetes	11.30%	15.50%	4.69%	22.07%	21.96%	16.05%	7.03%	9.27%	12.70%	38.17%	29.69%	12.04%	10.12%
Cyanobacteria	4.20%	0.00%	2.61%	1.04%	0.00%	0.00%	1.25%	0.85%	3.22%	1.31%	0.00%	1.65%	1.44%
Elusimicrobia	0.00%	0.00%	0.00%	0.26%	0.00%	0.00%	0.00%	0.31%	0.00%	0.72%	0.00%	0.10%	0.23%
Euryarchaeota	0.13%	0.00%	0.00%	0.37%	0.00%	0.00%	0.90%	0.00%	0.64%	0.00%	0.00%	0.40%	0.31%
Firmicutes	61.06%	71.73%	83.91%	70.21%	67.90%	71.79%	80.15%	75.98%	75.02%	50.98%	56.89%	74.48%	9.90%
Fusobacteria	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.73%	0.41%	0.00%	0.67%	0.33%	0.29%
Lentisphaerae	0.89%	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.71%	0.20%	0.00%	0.00%	0.40%	0.33%
OD1	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.02%	0.00%	0.00%	0.00%	0.01%	0.01%
Planctomycetes	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Proteobacteria	7.29%	11.80%	7.20%	3.38%	4.36%	8.67%	3.55%	4.18%	3.54%	3.96%	11.79%	4.61%	3.24%
Spirochaetes	6.53%	0.00%	0.49%	0.72%	2.12%	1.07%	0.06%	0.84%	0.97%	1.98%	0.65%	0.94%	1.83%
Tenericutes	4.34%	0.00%	0.00%	0.11%	2.34%	1.38%	2.19%	1.84%	0.60%	0.00%	0.18%	1.47%	1.40%
Verrucomicrobia	1.47%	0.97%	0.00%	1.86%	0.96%	0.00%	0.87%	2.02%	0.62%	2.88%	0.14%	1.12%	0.92%
WPS-2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%	0.18%	0.20%
NA	2.24%	0.00%	0.00%	0.00%	0.00%	0.00%	1.13%	2.22%	0.36%	0.00%	0.00%	1.04%	0.90%

Phylum	Stomach								Average	SD
	UMI13	UMI15	UMI16	UMI18	UMI20	UMI5	UMI7	UMI9		
Actinobacteria	0.00%	0.00%	0.00%	0.00%	7.22%	0.00%	0.00%	0.00%	0.67%	2.55%
Bacteroidetes	3.13%	0.00%	0.93%	6.91%	1.86%	1.12%	0.00%	0.78%	1.96%	2.29%
Cyanobacteria	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Elusimicrobia	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Euryarchaeota	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Firmicutes	39.47%	18.77%	33.84%	34.18%	27.77%	11.40%	7.46%	6.13%	20.79%	13.16%
Fusobacteria	6.99%	0.00%	2.86%	2.47%	0.00%	1.53%	0.00%	1.38%	2.16%	2.34%
Lentisphaerae	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
OD1	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%	0.00%	0.21%	0.19%
Planctomycetes	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Proteobacteria	45.12%	81.23%	50.85%	56.44%	63.10%	85.10%	92.54%	66.41%	70.07%	17.09%
Spirochaetes	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Tenericutes	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Verrucomicrobia	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
WPS-2	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
NA	5.29%	0.00%	11.51%	0.00%	0.05%	0.33%	0.00%	25.31%	4.14%	9.07%

Supplementary Table 3. Bacterial genera identified by LEfSe analysis different between stomach and colonic microbiota (log LDA score >2.0, $p < 0.05$)

Differentially abundant taxa	Class	log ₁₀ (LDA score)	p-value
p__Firmicutes.c__Clostridia	colon	5.4511	0.0003
p__Firmicutes.c__Clostridia.o__Clostridiales	colon	5.4511	0.0003
p__Firmicutes	colon	5.3684	0.0003
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae	colon	5.1202	0.0002
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae	colon	5.1121	0.0002
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales	colon	4.8410	0.0004
p__Bacteroidetes.c__Bacteroidia	colon	4.8410	0.0004
p__Bacteroidetes	colon	4.8357	0.0004
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__	colon	4.6670	0.0002
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.___	colon	4.6074	0.0002
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Prevotellaceae	colon	4.5093	0.0015
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Prevotellaceae.g__Prevotella	colon	4.5073	0.0015
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.___	colon	4.4849	0.0002
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Roseburia	colon	4.3561	0.0005
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Oscillospira	colon	4.3527	0.0013
p__Firmicutes.c__Clostridia.o__Clostridiales.___	colon	4.3365	0.0002
p__Firmicutes.c__Clostridia.o__Clostridiales.___.	colon	4.3365	0.0002
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Ruminococcus	colon	4.3207	0.0005
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Coprococcus	colon	4.3207	0.0013
p__Firmicutes.c__Clostridia.o__Clostridiales.f__g__	colon	4.2950	0.0005
p__Firmicutes.c__Clostridia.o__Clostridiales.f__	colon	4.2950	0.0005
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Lachnospira	colon	4.2466	0.0002

p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales	colon	4.1820	0.0002
p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales.f__Erysipelotrichaceae	colon	4.1820	0.0002
p__Firmicutes.c__Erysipelotrichi	colon	4.1820	0.0002
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Paraprevotellaceae__	colon	4.1328	0.0005
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Faecalibacterium	colon	4.1131	0.0005
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__	colon	4.0554	0.0013
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Veillonellaceae.g__Phascolarctobacterium	colon	4.0432	0.0002
p__Proteobacteria.c__Betaproteobacteria	colon	4.0296	0.0277
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__S24_7.g__	colon	4.0184	0.0002
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__S24_7	colon	4.0184	0.0002
p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales.f__Erysipelotrichaceae.g__Eubacterium__	colon	4.0108	0.0331
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Paraprevotellaceae.g__Prevotella__	colon	3.9777	0.0033
p__Cyanobacteria.c__4C0d_2.o__YS2.f__	colon	3.9256	0.0076
p__Cyanobacteria	colon	3.9256	0.0076
p__Cyanobacteria.c__4C0d_2.o__YS2.f__g__	colon	3.9256	0.0076
p__Cyanobacteria.c__4C0d_2	colon	3.9256	0.0076
p__Cyanobacteria.c__4C0d_2.o__YS2	colon	3.9256	0.0076
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Paraprevotellaceae.g__	colon	3.9231	0.0163
p__Spirochaetes	colon	3.9203	0.0005
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Christensenellaceae.g__	colon	3.9092	0.0076
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Christensenellaceae	colon	3.9092	0.0076
p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales.f__Erysipelotrichaceae.g__	colon	3.9026	0.0013
p__Tenericutes	colon	3.8969	0.0033
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.___.___	colon	3.8807	0.0013
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.___	colon	3.8807	0.0013
p__Spirochaetes.c__Spirochaetes	colon	3.8774	0.0033

p__Spirochaetes.c__Spirochaetes.o__Spirochaetales.f__Spirochaetaceae.g__Treponema	colon	3.8774	0.0033
p__Spirochaetes.c__Spirochaetes.o__Spirochaetales	colon	3.8774	0.0033
p__Spirochaetes.c__Spirochaetes.o__Spirochaetales.f__Spirochaetaceae	colon	3.8774	0.0033
p__Proteobacteria.c__Betaproteobacteria.o__Burkholderiales	colon	3.8751	0.0013
p__Proteobacteria.c__Betaproteobacteria.o__Burkholderiales.____	colon	3.8604	0.0331
p__Proteobacteria.c__Betaproteobacteria.o__Burkholderiales.__	colon	3.8603	0.0331
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea	colon	3.8424	0.0076
p__Verrucomicrobia	colon	3.8352	0.0013
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__	colon	3.8300	0.0033
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__.g__	colon	3.8300	0.0033
p__Proteobacteria.c__Betaproteobacteria.o__Burkholderiales.f__Alcaligenaceae.g__Sutterella	colon	3.8294	0.0013
p__Proteobacteria.c__Betaproteobacteria.o__Burkholderiales.f__Alcaligenaceae	colon	3.8294	0.0013
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia	colon	3.8272	0.0033
p__Proteobacteria.c__Gammaproteobacteria.o__Aeromonadales.f__Succinivibrionaceae	colon	3.8051	0.0033
p__Proteobacteria.c__Gammaproteobacteria.o__Aeromonadales.f__Succinivibrionaceae.g__Succinivibrio	colon	3.8051	0.0033
p__Proteobacteria.c__Gammaproteobacteria.o__Aeromonadales	colon	3.8051	0.0033
p__Proteobacteria.c__Epsilonproteobacteria.o__Campylobacteriales.f__Helicobacteraceae.g__Flexispira	colon	3.7996	0.0163
p__Proteobacteria.c__Alphaproteobacteria.o__Rickettsiales.f__.g__	colon	3.7749	0.0331
p__Proteobacteria.c__Alphaproteobacteria.o__Rickettsiales.f__	colon	3.7749	0.0331
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Bacteroidaceae.g__Bacteroides	colon	3.7680	0.0331
p__Verrucomicrobia.c__Opitutae	colon	3.7645	0.0331
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae	colon	3.7611	0.0076
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Bacteroidaceae	colon	3.7577	0.0331
p__Actinobacteria.c__Coriobacteriia.o__Coriobacteriales.f__Coriobacteriaceae.g__	colon	3.7569	0.0163
p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales.f__Erysipelotrichaceae.g__RFN20	colon	3.7558	0.0331
p__Verrucomicrobia.c__Opitutae.o__Cerasicoccales__	colon	3.7534	0.0331

p__Verrucomicrobia.c__Opitutae.o__Cerasicoccales.f__Cerasicoccaceae.g__	colon	3.7534	0.0331
p__Verrucomicrobia.c__Opitutae.o__Cerasicoccales.f__Cerasicoccaceae_	colon	3.7534	0.0331
p__Actinobacteria.c__Coriobacteriia.o__Coriobacteriales.f__Coriobacteriaceae	colon	3.7450	0.0163
p__Actinobacteria.c__Coriobacteriia	colon	3.7450	0.0163
p__Actinobacteria.c__Coriobacteriia.o__Coriobacteriales	colon	3.7450	0.0163
p__Proteobacteria.c__Alphaproteobacteria.__.__.__	colon	3.7305	0.0163
p__Proteobacteria.c__Alphaproteobacteria.__.__.__	colon	3.7305	0.0163
p__Proteobacteria.c__Alphaproteobacteria.__	colon	3.7305	0.0163
p__Tenericutes.c__Mollicutes	colon	3.7230	0.0076
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Paraprevotellaceae.g__CF231	colon	3.7057	0.0076
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Rikenellaceae	colon	3.7040	0.0033
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Rikenellaceae.g__	colon	3.7040	0.0033
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Mogibacteriaceae_	colon	3.6991	0.0076
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Mogibacteriaceae.g__	colon	3.6991	0.0076
p__Tenericutes.c__Mollicutes.o__Anaeroplasmatales	colon	3.6984	0.0076
p__Tenericutes.c__Mollicutes.o__Anaeroplasmatales.f__Anaeroplasmataceae	colon	3.6984	0.0076
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Porphyromonadaceae.g__Parabacteroides	colon	3.6944	0.0076
p__Tenericutes.c__RF3.o__ML615J_28.f__	colon	3.6869	0.0331
p__Tenericutes.c__RF3	colon	3.6869	0.0331
p__Tenericutes.c__RF3.o__ML615J_28.f__g__	colon	3.6869	0.0331
p__Tenericutes.c__RF3.o__ML615J_28	colon	3.6869	0.0331
p__Verrucomicrobia.c__Verruco_5	colon	3.6757	0.0033
p__Verrucomicrobia.c__Verruco_5.o__WCHB1_41.f__RFP12	colon	3.6757	0.0033
p__Verrucomicrobia.c__Verruco_5.o__WCHB1_41.f__RFP12.g__	colon	3.6757	0.0033
p__Verrucomicrobia.c__Verruco_5.o__WCHB1_41	colon	3.6757	0.0033
p__Proteobacteria.c__Alphaproteobacteria.o__RF32	colon	3.6503	0.0033

p__Proteobacteria.c__Alphaproteobacteria.o__RF32.f__g__	colon	3.6503	0.0033
p__Proteobacteria.c__Alphaproteobacteria.o__RF32.f__	colon	3.6503	0.0033
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Veillonellaceae.g__Dialister	colon	3.6481	0.0331
p__Tenericutes.c__Mollicutes.o__Anaeroplasmatales.f__Anaeroplasmataceae.g__	colon	3.6393	0.0163
p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales.f__Erysipelotrichaceae.g__Bulleidia	colon	3.6107	0.0163
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__RF16.g__	colon	3.6099	0.0331
p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__RF16	colon	3.6099	0.0331
p__Proteobacteria	stomach	5.4670	0.0003
p__Proteobacteria.c__Gammaproteobacteria	stomach	5.4632	0.0003
p__Proteobacteria.c__Gammaproteobacteria.o__Pasteurellales	stomach	5.2180	0.0012
p__Proteobacteria.c__Gammaproteobacteria.o__Pasteurellales.f__Pasteurellaceae	stomach	5.2179	0.0012
p__Proteobacteria.c__Gammaproteobacteria.o__Enterobacteriales.f__Enterobacteriaceae	stomach	5.1109	0.0074
p__Proteobacteria.c__Gammaproteobacteria.o__Enterobacteriales	stomach	5.1092	0.0074
p__Proteobacteria.c__Gammaproteobacteria.o__Pasteurellales.f__Pasteurellaceae.___	stomach	4.9740	0.0074
p__Proteobacteria.c__Gammaproteobacteria.o__Pasteurellales.f__Pasteurellaceae.g__Actinobacillus	stomach	4.8884	0.0008
p__Firmicutes.c__Bacilli	stomach	4.8013	0.0003
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Veillonellaceae.g__Veillonella	stomach	4.7553	0.0012
p__Firmicutes.c__Bacilli.o__Lactobacillales	stomach	4.6845	0.0035
p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Streptococcaceae	stomach	4.6254	0.0023
p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Streptococcaceae.g__Streptococcus	stomach	4.6225	0.0023
p__Firmicutes.c__Clostridia.o__Clostridiales.f__Veillonellaceae	stomach	4.5965	0.0475
p__Firmicutes.c__Bacilli.o__Gemellales.f__Gemellaceae	stomach	4.2689	0.0010
p__Firmicutes.c__Bacilli.o__Gemellales	stomach	4.2689	0.0010
p__Firmicutes.c__Bacilli.o__Gemellales.f__Gemellaceae.___	stomach	4.2676	0.0034
p__Proteobacteria.c__Betaproteobacteria.o__Neisseriales.f__Neisseriaceae	stomach	4.2652	0.0320
p__Proteobacteria.c__Betaproteobacteria.o__Neisseriales	stomach	4.2471	0.0320

p__Fusobacteria.c__Fusobacteriia.o__Fusobacteriales.f__Leptotrichiaceae.g__Leptotrichia	stomach	4.2449	0.0109
p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Carnobacteriaceae	stomach	4.2049	0.0109
p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Carnobacteriaceae.g__Granulicatella	stomach	4.2049	0.0109
p__Fusobacteria.c__Fusobacteriia	stomach	4.2045	0.0345
p__Fusobacteria.c__Fusobacteriia.o__Fusobacteriales	stomach	4.2023	0.0345
p__Fusobacteria	stomach	4.2012	0.0345
p__Fusobacteria.c__Fusobacteriia.o__Fusobacteriales.f__Leptotrichiaceae	stomach	4.1920	0.0194

Supplementary Table 4. KO pathways identified by LEfSe analysis different between stomach and colonic microbiota(log LDA score >2.0, $p < 0.05$)

KO pathway	Level 3	Gut site	\log_{10} (LDA score)	p -value	Level1	Level 2
ko01051	Biosynthesis of ansamycins	colon	3.917	0.001	Metabolism	Metabolism of terpenoids and polyketides
ko02030	Bacterial chemotaxis	colon	3.604	0.008	Cellular Processes	Cell motility
ko03020	RNA polymerase	colon	3.561	0.000	Genetic Information Processing	Transcription
ko00521	Streptomycin biosynthesis	colon	3.479	0.000	Metabolism	Biosynthesis of other secondary metabolites
ko00121	Secondary bile acid biosynthesis	colon	3.461	0.000	Metabolism	Lipid metabolism
ko02040	Flagellar assembly	colon	3.433	0.026	Cellular Processes	Cell motility
ko00250	Alanine, aspartate and glutamate metabolism	colon	3.380	0.000	Metabolism	Amino acid metabolism

ko00730	Thiamine metabolism	colon	3.339	0.000	Metabolism	Metabolism of cofactors and vitamins
ko01055	Biosynthesis of vancomycin group antibiotics	colon	3.333	0.001	Metabolism	Metabolism of terpenoids and polyketides
ko00300	Lysine biosynthesis	colon	3.238	0.000	Metabolism	Amino acid metabolism
ko00511	Other glycan degradation	colon	3.212	0.001	Metabolism	Glycan biosynthesis and metabolism
ko00900	Terpenoid backbone biosynthesis	colon	3.122	0.005	Metabolism	Metabolism of terpenoids and polyketides
ko00340	Histidine metabolism	colon	3.091	0.000	Metabolism	Amino acid metabolism
ko00625	Chloroalkane and chloroalkene degradation	colon	3.064	0.033	Metabolism	Xenobiotics biodegradation and metabolism
ko00770	Pantothenate and CoA biosynthesis	colon	3.046	0.008	Metabolism	Metabolism of cofactors and vitamins

ko00312	beta-Lactam resistance	colon	3.044	0.000	Human Diseases	Drug resistance: antimicrobial
ko00550	Peptidoglycan biosynthesis	colon	2.977	0.005	Metabolism	Glycan biosynthesis and metabolism
ko00500	Starch and sucrose metabolism	colon	2.905	0.048	Metabolism	Carbohydrate metabolism
ko03420	Nucleotide excision repair	colon	2.903	0.001	Genetic Information Processing	Replication and repair
ko00052	Galactose metabolism	colon	2.861	0.006	Metabolism	Carbohydrate metabolism
ko00908	Zeatin biosynthesis	colon	2.838	0.005	Metabolism	Metabolism of terpenoids and polyketides
ko00030	Pentose phosphate pathway	colon	2.752	0.021	Metabolism	Carbohydrate metabolism
ko00760	Nicotinate and nicotinamide metabolism	colon	2.722	0.003	Metabolism	Metabolism of cofactors and vitamins
ko00270	Cysteine and methionine metabolism	colon	2.712	0.002	Metabolism	Amino acid metabolism

ko00120	Primary bile acid biosynthesis	colon	2.703	0.000	Metabolism	Lipid metabolism
ko00531	Glycosaminoglycan degradation	colon	2.681	0.013	Metabolism	Glycan biosynthesis and metabolism
ko04141	Protein processing in endoplasmic reticulum	colon	2.605	0.000	Genetic Information Processing	Folding, sorting and degradation
ko00791	Atrazine degradation	colon	2.589	0.008	Metabolism	Xenobiotics biodegradation and metabolism
ko00830	Retinol metabolism	colon	2.477	0.033	Metabolism	Metabolism of cofactors and vitamins
ko04626	Plant-pathogen interaction	colon	2.460	0.008	Organismal Systems	Environmental adaptation
ko03450	Non-homologous end-joining	colon	2.436	0.001	Genetic Information Processing	Replication and repair
ko04974	Protein digestion and absorption	colon	2.390	0.001	Organismal Systems	Digestive system
ko05146	Amoebiasis	colon	2.365	0.004	Human Diseases	Infectious disease: parasitic

ko00510	N-Glycan biosynthesis	colon	2.321	0.001	Metabolism	Glycan biosynthesis and metabolism
ko04210	Apoptosis Epithelial cell signaling in Helicobacter pylori infection	colon	2.317	0.005	Cellular Processes	Cell growth and death
ko05120	Lipopolysaccharide biosynthesis	colon	2.285	0.039	Human Diseases	Infectious disease: bacterial
ko00540	Ubiquinone and other terpenoid-quinone biosynthesis	stomach	3.769	0.000	Metabolism	Glycan biosynthesis and metabolism
ko00130	Lipoic acid metabolism	stomach	3.585	0.000	Metabolism	Metabolism of cofactors and vitamins
ko00785	Glutathione metabolism	stomach	3.559	0.000	Metabolism	Metabolism of cofactors and vitamins
ko00480	Phosphotransferase system (PTS)	stomach	3.528	0.000	Metabolism	Metabolism of other amino acids
ko02060		stomach	3.512	0.000	Environmental Information Processing	Membrane transport

ko00790	Folate biosynthesis	stomach	3.462	0.000	Metabolism	Metabolism of cofactors and vitamins
ko00780	Biotin metabolism	stomach	3.449	0.000	Metabolism	Metabolism of cofactors and vitamins
ko02010	ABC transporters	stomach	3.292	0.000	Environmental Information Processing	Membrane transport
ko00053	Ascorbate and aldarate metabolism	stomach	3.249	0.001	Metabolism	Carbohydrate metabolism
ko00660	C5-Branched dibasic acid metabolism	stomach	3.221	0.002	Metabolism	Carbohydrate metabolism
ko04122	Sulfur relay system	stomach	3.209	0.000	Genetic Information Processing	Folding, sorting and degradation
ko00910	Nitrogen metabolism	stomach	3.208	0.000	Metabolism	Energy metabolism
ko00020	Citrate cycle (TCA cycle)	stomach	3.137	0.000	Metabolism	Carbohydrate metabolism
ko00920	Sulfur metabolism	stomach	3.132	0.000	Metabolism	Energy metabolism

ko00450	Selenocompound metabolism	stomach	3.120	0.000	Metabolism	Metabolism of other amino acids
ko00473	D-Alanine metabolism	stomach	3.108	0.002	Metabolism	Metabolism of other amino acids
ko00650	Butanoate metabolism	stomach	3.003	0.000	Metabolism	Carbohydrate metabolism
ko00564	Glycerophospholipid metabolism	stomach	2.982	0.000	Metabolism	Lipid metabolism
ko00630	Glyoxylate and dicarboxylate metabolism	stomach	2.960	0.021	Metabolism	Carbohydrate metabolism
ko05322	Systemic lupus erythematosus	stomach	2.948	0.016	Human Diseases	Immune disease
ko01053	Biosynthesis of siderophore group nonribosomal peptides	stomach	2.942	0.037	Metabolism	Metabolism of terpenoids and polyketides
ko00620	Pyruvate metabolism	stomach	2.905	0.003	Metabolism	Carbohydrate metabolism
ko00633	Nitrotoluene degradation	stomach	2.890	0.010	Metabolism	Xenobiotics biodegradation and metabolism
ko00010	Glycolysis / Gluconeogenesis	stomach	2.880	0.001	Metabolism	Carbohydrate metabolism

ko00380	Tryptophan metabolism	stomach	2.879	0.008	Metabolism	Amino acid metabolism
ko00051	Fructose and mannose metabolism	stomach	2.867	0.017	Metabolism	Carbohydrate metabolism
ko03070	Bacterial secretion system	stomach	2.862	0.006	Environmental Information Processing	Membrane transport
ko00520	Amino sugar and nucleotide sugar metabolism	stomach	2.791	0.001	Metabolism	Carbohydrate metabolism
ko00440	Phosphonate and phosphinate metabolism	stomach	2.787	0.001	Metabolism	Metabolism of other amino acids
ko00350	Tyrosine metabolism	stomach	2.749	0.008	Metabolism	Amino acid metabolism
ko04146	Peroxisome	stomach	2.728	0.000	Cellular Processes	Transport and catabolism
ko00562	Inositol phosphate metabolism	stomach	2.723	0.017	Metabolism	Carbohydrate metabolism
ko00561	Glycerolipid metabolism	stomach	2.717	0.005	Metabolism	Lipid metabolism
ko00627	Aminobenzoate degradation	stomach	2.638	0.005	Metabolism	Xenobiotics biodegradation and metabolism

ko00196	Photosynthesis - antenna proteins	stomach	2.628	0.028	Metabolism	Energy metabolism
ko00361	Chlorocyclohexane and chlorobenzene degradation	stomach	2.609	0.013	Metabolism	Xenobiotics biodegradation and metabolism
ko03008	Ribosome biogenesis in eukaryotes	stomach	2.486	0.000	Genetic Information Processing	Translation
ko05150	Staphylococcus aureus infection	stomach	2.378	0.001	Human Diseases	Infectious disease: bacterial
ko05142	Chagas disease (American trypanosomiasis)	stomach	2.096	0.001	Human Diseases	Infectious disease: parasitic