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4 # Gut microbiome structure and adrenocortical activity in dogs with aggressive and
5 phobic behavioral disorders

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27 # Abstract

28 Accompanying human beings since the Paleolithic period, dogs has been recently regarded as a
29 reliable model for the study of the gut microbiome connections with health and disease. In order to
30 provide some glimpses on the connections between the gut microbiome layout and host behavior,
31 we profiled the phylogenetic composition and structure of the canine gut microbiome of dogs with
32 aggressive (n = 17), phobic (n = 15) and normal behavior (n = 17). According to our findings,
33 aggressive behavioral disorder was found to be characterized by a peculiar gut microbiome
34 structure, with high biodiversity and enrichment in generally subdominant bacterial genera. On the
35 other hand, phobic dogs were enriched in *Lactobacillus*, a bacterial genus with known probiotic and
36 psychobiotic properties. Although further studies are needed to validate our findings, our work
37 supports the intriguing opportunity that different behavioral phenotypes in dogs may be associated
38 with peculiar gut microbiome layouts, suggesting possible connections between the gut microbiome
39 and the central nervous system and indicating the possible adoption of probiotic interventions aimed
40 at restoring a balanced host-symbiont interplay for mitigating behavioral disorders.

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43 **Keywords**: microbiome, behavioral disorders, aggressive dogs, phobic dogs, hormones, HPA-axis

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52 # Introduction

53 Descending from the gray wolf (*Canis lupus*), dogs were domesticated during the Paleolithic
54 period, accompanying humans across the transition from hunting-gathering to rural agriculture of
55 the Neolithic, to post-industrialized Western lifestyle [1-3]. The frequent sharing of food resources
56 with human beings has been a selective force able to drive changes in the digestive and metabolic
57 system of dogs, enabling them to efficiently adapt to a more starch-enriched diet compared to their
58 wild ancestor, and ultimately influence canine behavior [4,5]. The canine gastrointestinal tract
59 harbors a complex and highly biodiverse microbial ecosystem, whose predominant taxa resemble
60 those typically found in the gut of other omnivorous mammals. However, in comparison to both
61 mice and pigs, the canine gut microbiome (GM) result the most similar to humans [6,7]. Thus, in
62 dogs, the GM-host mutualistic exchange well approximate what has been observed in humans [8-
63 11]. Indeed, the peculiar patterns of dysbiosis observed in dogs with IBD are generally comparable
64 to variations typically found in humans, suggesting that bacterial responses to inflammatory
65 conditions are conserved among the two [12,13]. As observed in humans, the eubiotic and stable
66 configuration of the canine GM is therefore of fundamental importance for the maintenance of a
67 homeostatic gut environment and of the overall host health.

68 Several recent studies have shown the ability of the mammalian GM to communicate with the host
69 central nervous system (CNS) through several parallel channels, involving the vagus nerve,
70 neuroimmune and neuroendocrine signaling mechanisms, and the production of neuroactive
71 chemicals – i.e. gamma-aminobutyric acid (GABA), serotonin (5-HT), norepinephrine and
72 dopamine [14-17]. Conversely, the CNS can influence GM structure and metabolome, influencing
73 the gut environment, acting on motility, secretion and permeability via the autonomic nervous
74 system (ANS) [18]. It is thus a matter of fact that the GM can influence the host behavior and *vice*
75 *versa*, exerting a key role in the modulation of the gut-brain axis. The development of researches

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76 during the last decades indeed suggests the presence of a bidirectional communication between gut
77 and brain.
78 Despite the high variability and severity of behavioral disorders observed in dogs, the aggressive
79 behavior has been found to be the most common, followed by separation anxiety and phobia [19].
80 Aggressive, anxiety and phobia behavioral disorders can be considered as stress responses with an
81 increase in glucocorticoid (GC) secretion mediated by the hypothalamic-pituitary-adrenal (HPA)
82 axis [20,21]. Although recent works on canine microbiome have investigated potential interactions
83 with aggression, these studies have focused on the variations of their GM profile after targeted
84 dietary interventions to reduce aggressive behaviors [22,23]. To the best of our knowledge, no study
85 has focused on the comparison of the GM structure between dogs exhibiting aggressive, phobic and
86 normal behavior, with specific associations with adrenocortical activity. In order to provide some
87 glimpses in this direction, in our work, 49 dogs – 31 males and 18 females – of different breed, age,
88 and weight, housed in individual boxes were enrolled. Following the behavioral evaluation
89 performed by a behaviorist veterinary and a dog handler, dogs were classified into three groups:
90 aggressive, phobic and normal behavior. We then profiled the phylogenetic composition and
91 structure of their canine GM, measuring the levels of fecal hormones (i.e. cortisol and testosterone)
92 to evaluate the adrenocortical activity in each study group. Fecal GC and their metabolites may
93 constitute a reliable non-invasive proxy of adrenocortical activity, reflecting long-term stress
94 responses with less interference from acute stressors [24-26].
95 Our data suggest that peculiar microbiome configurations could be connected with dogs' behavioral
96 phenotype, which in turn might be exacerbated by altered proportions of gut symbionts, ultimately
97 triggering a feedback loop. In this scenario, targeted interventions on GM could constitute a
98 valuable tool to restore a proper host-symbiont interplay, finally mitigating behavioral disorders and
99 improving the overall host health.
100

101
Results

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102

103 **Relative abundance of major gut microbiome components in the**
104 **enrolled cohort**

105 In order to evaluate possible differences in GM communities among the behavioral study groups,
106 we collected fecal samples from 49 dogs. In particular, 31 males and 18 females of different breed,
107 aged between 1 and 13 years, were recruited from three animal shelters located in the metropolitan
108 area of Bologna (Italy). Following the behavioral evaluation performed by a behaviorist veterinary
109 and a dog handler, dogs were grouped based on their behavioral phenotype: 17 were classified as
110 aggressive and 15 as phobic, while 17 exhibited a normal behavior. The 16S rRNA sequencing,
111 performed using the Illumina MiSeq platform, yielded a total of 1,611,153 high-quality sequences,
112 with an average of 32,880 ± 6,009 sequences per sample (range 20,664 – 45,486), subsequently
113 clustered in 8,460 OTUs with a 97% identity threshold.
114 The most abundant phyla detected within the normal behavior samples were Firmicutes (68.0 ±
115 4.6%), Bacteroidetes (13.7 ± 3.6%), and Actinobacteria (9.9 ± 1.6%), with Fusobacteria (4.8 ±
116 1.3%) and Proteobacteria (2.1 ± 0.8%) as minor components. The aggressive group showed similar
117 proportions among the dominant phyla when compared to the normal behavior group, except for a
118 reduced relative abundance of Bacteroidetes (P-value = 0.02; Wilcoxon test). No significant
119 differences at phylum level were detected between the phobic and the normal behavior groups, as
120 well as between the phobic and the aggressive groups.
121 At family level, Lachnospiraceae, Erysipelotrichaceae and Clostridiaceae constitute the major
122 components of the normal behavior group (relative abundance > 10%). A depletion in the relative
123 abundance of Bacteriodaceae, Alcaligenaceae and [Paraprevotellaceae], as well as an increase in
124 Erysipelotrichaceae (P-value < 0.05) was observed in the aggressive group compared to the normal
125 behavior group. The phobic group was instead characterized by an increase in the relative
126 abundance of the family Rikenellaceae (P-value = 0.04) when compared to the normal behavior

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127 group. Aggressive and phobic groups were found to be distinguishable due to different proportions
128 in the relative abundance of the bacterial families [Mogibacteriaceae] and Veillonellaceae,
129 respectively depleted and enriched in the aggressive group (P < 0.04) (Fig 1A, 1B).

130 At the genus taxonomic level, *Clostridium*, *Lactobacillus*, *Blautia* and *Collinsella* represent the
131 major portion of the normal behavior group GM (relative abundance > 5%). Several microbial
132 genera were found to be significantly depleted in the aggressive group. The relative abundance of
133 the genera *Oscillospira*, *Peptostreptococcus*, *Bacteroides*, *Sutterella*, and *Coprobacillus* were
134 significantly lower in aggressive compared to normal behavior group, while *Catenibacterium*,
135 *Megamonas* and [*Eubacterium*] showed an opposite trend (P-value < 0.04). At the genus level, no
136 significant differences were detected between the phobic and the normal behavior group. The
137 differences found between phobic and aggressive groups were due to an increased relative
138 abundance of *Catenibacterium* and *Megamonas* in the latter group (P < 0.007), in addition to a
139 slightly depletion of the genus *Epulopiscium* (P = 0.04) (S3 Fig).

140 **Fig 1. Canine gut microbiome profile of the behavior groups.** (A) Relative abundances of
141 family-level taxa in each subject of the enrolled cohort (barplots) and respective average values of
142 each study group (piecharts). (B) Boxplots showing the distribution of the relative abundances of
143 bacterial families enriched or depleted within the gut microbiota of aggressive or phobic groups.

144

145 Comparison of the overall gut microbiome compositional structure 146 between aggressive, phobic and normal behavior dogs

147 The intra-individual diversity of the canine GM was assessed by means of the phylogenetic metric
148 Observed OTUs and the Shannon biodiversity index at the genus level. While according to the
149 Shannon index the intra-individual GM diversity was comparable between aggressive, phobic and
150 normal behavior groups (mean ± SD, 5.2 ± 0.8, 5.14 ± 0.7, and 5.2 ± 0.7, respectively), the
151 Observed OTUs metric highlighted a statistically significant difference between aggressive and

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152 phobic groups (P-value = 0.02; Kruskal-Wallis test). In particular, the aggressive group was
153 characterized by the higher number of different taxa observed (mean ± SD, 583.9 ± 310.6), the
154 phobic group had the lower value (430.07 ± 112.28), and the normal behavior group exhibited
155 intermediate values (454.8 ± 118.3) (Fig 2).

156 According to the Jaccard similarity index, the Principal Coordinates Analysis (PCoA) of the inter-
157 sample variation highlighted a significant separation between the structural composition of the GM
158 among study groups (P-value = 0.02, permutation test with pseudo-*F* ratios) (Fig 2). In order to
159 identify bacterial drivers that contribute to groups clustering (permutation correlation test, P-value <
160 0.001), a superimposition of the genus relative abundance was performed on the PCoA plot. As
161 showed in S1 Fig, major drivers of the normal behavior group segregation were *Faecalibacterium*,
162 *Bacteroides*, *Phascolarctobacterium, Fusobacterium*, *Prevotella*, and [*Prevotella*]. The phobic
163 group was characterized by an enrichment in *Lactobacillus*, while *Dorea*, *Blautia*, *Collinsella*,
164 [*Ruminococcus*], *Slackia*, *Catenibacterium*, and *Megamonas* were more represented within the
165 aggressive group.

166 To dissect discriminant GM component for dogs showing aggressive behavior, we applied the
167 machine learning method Random Forest [27] to the genus level data set. Behavior-discriminatory
168 bacterial genera were identified with distinctive changes in their relative abundances (Fig 3).
169 Specifically, our analysis revealed two 'aggressive-discriminatory' bacterial genera:
170 *Catenibacterium* and *Megamonas*.

171 **Fig 2. Biodiversity of the canine gut microbiome.** Boxplot showing the alpha diversity measures
172 computed with phylogenetic and non-phylogenetic metrics (Shannon diversity index, observed
173 OTUs). Behavior-related groups are identified with colored box and whiskers (orange, Aggressive;
174 blue, Phobic; green, Normal). Significant difference was found between aggressive and phobic
175 groups, according to the observed OTUs metric (P-value = 0.02; Kruskal-Wallis test). Principal
176 coordinate analysis (PCoA) plots showing the beta diversity of the intestinal bacterial communities

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177 of the study groups, based on Jaccard similarity index. A significant separation between aggressive
178 and phobic behavior groups was found (P-value = 0.02, permutation test with pseudo-*F* ratios).
179 **Fig 3. Behavior-related gut microbiome signature.** Top 26 features from the obtained dataset as
180 revealed by Random Forest. Stars denote the bacterial genera discriminant of aggressive group.
181 Boxplots shows the comparison of the relative abundances of these bacterial genera between the
182 study groups.
183

184 Fecal cortisol and testosterone levels

185 Cortisol and testosterone levels were measured in fecal samples through RIAs. The statistical
186 analysis did not evidence any significant differences between the three groups of dogs for hormonal
187 data. Our results showed that the three study groups had similar median value of fecal cortisol and
188 testosterone levels (Fig 4A). However, it should be noticed that the range of testosterone level in
189 aggressive and phobic populations is largest than in normal, so we can assume that there is a greater
190 variability between subjects (Fig 4B). As consequences of the previously described results, the
191 median of testosterone/cortisol (T/C) ratio were similar in all the three groups, only slightly higher
192 in phobic than in aggressive and normal dogs (Fig 4C).
193 **Fig 4. Fecal hormone levels of the behavior groups.** Boxplots showing levels of cortisol (A) and
194 testosterone (B), and testosterone/cortisol ratio (C) detected in stool samples of the study groups.
195 No significant difference was found among study groups (P-value > 0.05, Kruskal-Wallis test).
196

197 Discussion

198 Within the present work, we profiled the GM structure and measured the fecal cortisol and
199 testosterone levels of 49 dogs - 31 males and 18 females - of different breed, age, and weight,
200 housed in individual boxes of three animal shelters located in the metropolitan area of Bologna
201 (Italy). Dogs were classified into three study groups based on their behavioral phenotype:

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202 aggressive, phobic or normal behavior. The phylogenetic profiles of the canine GM observed in our
203 cohort were found to be in line with those already reported in literature for healthy dogs [9,28,29],
204 but with a slightly higher abundance of Firmicutes and Actinobacteria, as well as a corresponding
205 lower abundance of Bacteroidetes and Proteobacteria. According to our results, the aggressive
206 group GM is characterized by a higher number of observed OTUs compared to both phobic and
207 normal behavior groups. Interestingly, the GM structure of our cohort segregate according to the
208 behavioral disorder of the host, showing a stronger separation of the aggressive group. The latter
209 group seems to be defined by a higher abundance of typically subdominant taxa, such as *Dorea*,
210 *Blautia*, *Collinsella*, [*Ruminococcus*], *Slackia*, *Catenibacterium*, and *Megamonas*. Conversely, the
211 phobic group is characterized by an enrichment of *Lactobacillus*, a bacterial genus comprising well-
212 known GABA producers, the main CNS inhibitory neurotransmitter able to regulate emotional
213 behavior in mice via the vagus nerve [30,31]. The major drivers for the normal behavior group
214 segregation are *Faecalibacterium*, *Bacteroides*, *Phascolarctobacterium*, *Fusobacterium*, *Prevotella*
215 and [*Prevotella*], reflecting the predominance of bacterial genera commonly associated with the
216 GM of healthy dogs [12,32]. Finally, according to the literature, no correlation was observed
217 regarding the phobic behavior in relation to sex or age [19].
218 According to our results, fecal cortisol and testosterone levels of aggressive dogs did not
219 significantly differ from those of phobic and normal dogs. Aggressive dogs are well known to
220 possess higher blood concentrations of cortisol and lower serotonin levels than non-aggressive dogs
221 [20]. However, fecal cortisol levels are not influenced by the activation of HPA axis during the
222 sampling procedure, which is itself stressful for the animal [33]. This can possibly explain the
223 differences in the observed cortisol levels between studies carried out in blood or fecal samples,
224 Testosterone is often correlate with aggressive behaviors in many species [34], but this association
225 is not completely demonstrated in dogs. Indeed, some studies have evidenced that the castration
226 reduce only mildly aggressiveness and that neutered dogs can be more aggressive [35,36]. In

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227 contrast with results of Terburg *et al.* [37], who suggested that a high value of T/C ratio may be a
228 predictive factor of aggressive behavior, we found no significant differences between groups.
229 Studies about cortisol in phobic state are confusing, and it seems that cortisol does not increase, or
230 increase only slightly in phobic subjects compared to normal ones [38]. Our results did not show
231 any significant differences between phobic and normal dogs for cortisol concentration. However,
232 the cortisol level of phobic dogs is slightly lower than normal ones. Phobic state can cause a chronic
233 and excessive stress [39], and scientific literature about phobic dogs suggests that they can tend to a
234 depressive state. In human beings and in dogs, depression is characterized by lower cortisol and
235 serotonin levels [40].
236 Our results suggest that dysbiotic GM configurations in a long-term stress levels scenario might
237 influence the local gut environment through the release of potentially neuroactive microbial by-
238 products, probably affecting the behavior of the host mainly as a side effect. In particular, dogs
239 exhibiting aggressive behavioral disorders were characterized by a peculiar GM structure, a high
240 biodiversity and an enrichment in generally subdominant bacterial genera. We then applied a
241 machine learning method (Random Forest) to our genus level data set, identifying *Catenibacterium*
242 and *Megamonas* as bacterial discriminants of aggressive behavior. Respectively belonging to the
243 families Erysipelotrichaceae and Veillonellaceae, *Catenibacterium* and *Megamonas* have been
244 recently correlated with primary bile acid metabolism and abdominal pain in humans [41-43],
245 suggesting a possible connection between a dysbiotic GM profile and behavioral disorders. We
246 found no alteration within the GM of dogs with phobic behavioral disorder except for an increase in
247 *Lactobacillus*, bacterial genera with well-known probiotic properties. Interestingly, chronic
248 treatment with *L.rhamnosus* can influence anxiety- and depression-related behavior by modulating
249 GABA receptor mRNA expression in specific brain regions [30]. Even if it is impossible to dissect
250 the factors supporting the increase of *Lactobacillus* observed in phobic dogs, it is tempting to
251 speculate that a higher abundance of this psychobiotics [44,45] could contribute to the
252 establishment of peculiarities of the phobic behavioral phenotype. However, further studies are

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253 required to validate our findings. Indeed, the limitations due to the small number of enrolled
254 animals imply a limited statistical power. Further, invasive blood sampling will be necessary,
255 allowing to match blood and fecal cortisol and testosterone levels to find robust connections with
256 the GM state. Nonetheless, our study support the intriguing opportunity that different behavioral
257 phenotypes in dogs associate with peculiar GM layouts. Particularly, aggressive dogs possess
258 dysbiotic GM configuration, possibly exacerbating the host aggressiveness and supporting the
259 adoption probiotic interventions aimed at restoring a balanced host-symbiont interplay, improving
260 the overall host health and eventually mitigating behavioral disorders. This preliminary research can
261 thus be considered a starting point for future studies of clinical interest, deepening the
262 understanding of the mechanisms underlying the relationship between behavioral disorders and the
263 GM, ultimately providing new insight into veterinary behavioral medicine and facilitating a
264 predictive diagnosis of canine behavior.
265

266 Materials and methods

267

268 Enrolled animals, behavioral evaluation and sample collection

269 The entire study was previously evaluated and approved by the Scientific Ethic Committee for
270 Animal Experimentation (University of Bologna). All the procedures were monitored by the
271 responsible of the Department of Veterinary Medical Science (DIMEVET) for animal welfare.
272 In the study, 49 dogs (31 males and 18 females) of different breed, age, and weight, housed in
273 individual boxes of three different animal shelters located in the metropolitan area of Bologna
274 (Italy) were enrolled (S1 Table). The animals were fed on a mixed diet, including wet and dry
275 commercial feed and additional homemade food.

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276 For each animal, a behavioral evaluation was performed by a behaviorist veterinary and a dog
277 handler, classifying the dogs enrolled based on their behavioral phenotype. Seventeen animals were
278 classified as aggressive and 15 as phobic, while 17 animals exhibited a normal behavior.
279 Fecal samples were collected from each animal immediately after the evacuation, between 7:00 and
280 12:00 am, avoiding debris and cross-contaminations. Specimens were frozen with liquid nitrogen
281 and transported to the laboratory, then stored at -80°C until DNA extraction and sequencing. The
282 remaining part of fecal samples was picked up through non-sterile bags and frozen at -20°C until
283 cortisol and testosterone assay.
284

285 Fecal cortisol and testosterone radioimmunoassays

286 Cortisol and testosterone concentrations were determined by radioimmunoassays (RIAs) based on
287 binding of ^{3}H-steroid by competitive adsorption [46]. All concentrations were expressed in pg/mg
288 of fecal matter. Extraction methodology was modified from Schatz & Palme [21]. Cortisol and
289 testosterone were extracted from fecal specimens (500 mg, wet weight) with methanol-water
290 solution (5 ml, v/v 4:1) and ethyl ether (5 ml). The portion of ether was vaporized under an
291 airstream suction hood at 37° C. Dry residue was finally dissolved again into 0.5 ml PBS (0.05 M,
292 pH 7.5), adding 125, 250, 500, or 1000 pg of ^{3}H-cortisol or ^{3}H-testosterone to 500 mg of feces and
293 incubating for 30 min at room temperature, performing a recovery test on five replicates. The
294 extraction was performed as described before, yielding a mean percentage recovery of 87.5 ± 2.4
295 and 89.3 ± 2.1 for cortisol and testosterone, respectively. Cortisol and testosterone metabolites
296 assay in feces were carried out according to Tamanini [47] and Gaiani [48], respectively. Analysis
297 were performed in duplicates. The cortisol RIA was performed using an antiserum to cortisol-21-
298 hemisuccinate-BSA (anti- rabbit), at a working dilution of 1:20 000 and ^{3}H-cortisol (30 pg/tube
299 vial) as tracer. The testosterone RIA was performed using an antiserum to testosterone-3-
300 carboxymethyloxime-BSA (anti- rabbit), at a working dilution of 1:35 000 and ^{3}H-testosterone (31
301 pg/tube vial) as tracer. Fecal cortisol and testosterone samples containing high concentrations of

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302 endogenous steroids (100 μl) were serially diluted through PBS (0.05 M, pH 7.5) in volumes of 50,
303 25, 10 and 5 ml, in order to determine the parallelism between cortisol and testosterone standards.
304 Parallelism was assessed between these serial dilutions of standards (ranging from 7.8 to 1000
305 pg/100 ml tube vial). Validation parameters of analysis were: sensitivity 0.19 pg/mg, intra-assay
306 variability 5.9%, inter-assay variability 8.7%, for cortisol; sensitivity 1.1 pg/mg, intra-assay
307 variability 6.2%, inter-assay variability 9.6%, for testosterone. Radioactivity was determined using
308 a liquid scintillation β counter and a linear standard curve, ad hoc designed by a software program
309 [49].
310

311 **Bacterial DNA extraction from stool samples**

312 Total microbial DNA was extracted from each fecal sample using the DNeasy Blood & Tissue kit
313 (QIAGEN), with the modified protocol described by Turroni *et al.* [50]. Briefly, 250 mg of feces
314 were resuspended in 1 ml of lysis buffer (500 mM NaCl, 50 mM Tris-HCl pH 8, 50 mM EDTA, 4%
315 SDS). Fecal samples were added with four 3-mm glass beads and 0.5 g of 0.1-mm zirconia beads
316 (BioSpec Products, Bartlesville, USA) and homogenized with 3 bead-beating steps using the
317 FastPrep instrument (MP Biomedicals, Irvine, CA) at 5.5 movements/s for 1 min, keeping the
318 samples on ice for 5 min after each treatment. Samples were subsequently heated at 95°C for 15
319 min and centrifuged to pellet stool particles. Supernatants were added with 260 μl of 10 M
320 ammonium acetate, centrifuged for 10 min at full speed, and incubated in ice for 30 min with one
321 volume of isopropanol. Nucleic acids were collected by centrifugation, washed with 70% ethanol
322 and resuspended in 100 μl of TE buffer. RNA and protein removal was performed by incubating the
323 samples with DNase-free RNase (10 mg/ml) at 37°C for 15 min and protease K at 70°C for 10 min,
324 respectively. Subsequently, DNA purification with QIAmp Mini Spin columns were performed as
325 per manufacturers instruction. The extracted bacterial DNA was quantified using the NanoDrop
326 ND-1000 spectrophotometer (NanoDrop Technologies).

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327

328 ## PCR amplification and sequencing

329 The V3-V4 region of the 16S rRNA was amplified with PCR using 200 nmol/l of S-D-Bact-0341-
330 b-S-17/S-D-Bact-0785-a-A-21 primers [51] with Illumina overhang adapter sequences, in a final
331 volume of 25 µl containing 12.5 ng of genomic DNA and 2X KAPA HiFi HotStart ReadyMix
332 (Kapa Biosystems). PCR reactions were performed in a Thermal Cycle T gradient (Biometra
333 GmbH) using the following thermal program: 3 min at 95°C for the initial denaturation, followed
334 by 25 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for
335 30 sec, and a final extension step at 72°C for 5 min. PCR products of about 460 bp were purified
336 using a magnetic bead-based system (Agencourt AMPure XP; Beckman Coulter) and sequenced on
337 Illumina MiSeq platform using the 2 x 250 bp protocol, according to the manufacturer's instructions
338 (Illumina). The libraries were pooled at equimolar concentrations, denatured and diluted to 6 pmol/l
339 before loading onto the MiSeq flow cell.

340

341 ## Bioinformatics and statistics

342 Raw sequences were processed using a pipeline combining PANDAseq [52] and QIIME [53]. The
343 UCLUST software [54] was used to bin high-quality reads into operational taxonomic units (OTUs)
344 through an open-reference strategy at a 0.97 similarity threshold. Taxonomy was assigned using the
345 RDP classifier and the Greengenes database as a reference (release May 2013). Chimera filtering
346 was performed discarding all singleton OTUs. Alpha rarefaction was evaluated by using the
347 Observed OTUs metric, and the Shannon biodiversity index, which aims to measure diversity based
348 on evenness, while beta diversity was estimated according to the Jaccard similarity. Random
349 Forests and all statistical analysis was computed using R software (version 3.1.3) and the packages
350 randomForest, vegan and made4. The significance of data separation on the PCoA was tested using
351 a permutation test with pseudo-*F* ratios (function adonis of vegan package). Non-parametric and

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352 correlation tests were achieved with Wilcoxon rank-sum or Kruskal-Wallis test and the Kendall tau,
353 respectively. Cortisol and testosterone concentrations, as well as T/C ratio was analyzed using the
354 normality test of Shapiro-Wilk, in order to establish the distribution of each variable in the
355 population. P-values < 0.05 were considered statistically significant.
356

357 # References

358 1. Frantz LA, Mullin VE, Pionnier-Capitan M, Lebrasseur O, Ollivier M, Perri A, et al.
359 Genomic and archaeological evidence suggest a dual origin of domestic dogs. Science.
360 2016;352(6290):1228-31. doi: 10.1126/science.aaf3161. PubMed PMID: 27257259.
361 2. Wang GD, Zhai W, Yang HC, Fan RX, Cao X, Zhong L, et al. The genomics of selection in
362 dogs and the parallel evolution between dogs and humans. Nat Commun. 2013;4:1860. doi:
363 10.1038/ncomms2814. PubMed PMID: 23673645.
364 3. Larson G, Karlsson EK, Perri A, Webster MT, Ho SY, Peters J, et al. Rethinking dog
365 domestication by integrating genetics, archeology, and biogeography. Proc Natl Acad Sci U S A.
366 2012;109(23):8878-83. doi: 10.1073/pnas.1203005109. PubMed PMID: 22615366; PubMed
367 Central PMCID: PMCPMC3384140.
368 4. Ollivier M, Tresset A, Bastian F, Lagoutte L, Axelsson E, Arendt ML, et al. Amy2B copy
369 number variation reveals starch diet adaptations in ancient European dogs. R Soc Open Sci.
370 2016;3(11):160449. doi: 10.1098/rsos.160449. PubMed PMID: 28018628; PubMed Central
371 PMCID: PMCPMC5180126.
372 5. Axelsson E, Ratnakumar A, Arendt ML, Maqbool K, Webster MT, Perloski M, et al. The
373 genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature.
374 2013;495(7441):360-4. doi: 10.1038/nature11837. PubMed PMID: 23354050.
375 6. Coelho LP, Kultima JR, Costea PI, Fournier C, Pan Y, Czarnecki-Maulden G, et al.
376 Similarity of the dog and human gut microbiomes in gene content and response to diet.

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377 Microbiome. 2018;6(1):72. doi: 10.1186/s40168-018-0450-3. PubMed PMID: 29669589; PubMed
378 Central PMCID: PMCPMC5907387.
379 7. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, et al. Evolution of
380 mammals and their gut microbes. Science. 2008;320(5883):1647-51. doi: 10.1126/science.1155725.
381 PubMed PMID: 18497261; PubMed Central PMCID: PMCPMC2649005.
382 8. Honneffer JB, Minamoto Y, Suchodolski JS. Microbiota alterations in acute and chronic
383 gastrointestinal inflammation of cats and dogs. World J Gastroenterol. 2014;20(44):16489-97. doi:
384 10.3748/wjg.v20.i44.16489. PubMed PMID: 25469017; PubMed Central PMCID:
385 PMCPMC4248192.
386 9. Swanson KS, Dowd SE, Suchodolski JS, Middelbos IS, Vester BM, Barry KA, et al.
387 Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals
388 similarities with humans and mice. ISME J. 2011;5(4):639-49. doi: 10.1038/ismej.2010.162.
389 PubMed PMID: 20962874; PubMed Central PMCID: PMCPMC3105739.
390 10. Song SJ, Lauber C, Costello EK, Lozupone CA, Humphrey G, Berg-Lyons D, et al.
391 Cohabiting family members share microbiota with one another and with their dogs. Elife.
392 2013;2:e00458. doi: 10.7554/eLife.00458. PubMed PMID: 23599893; PubMed Central PMCID:
393 PMCPMC3628085.
394 11. Hayward JJ, Castelhano MG, Oliveira KC, Corey E, Balkman C, Baxter TL, et al. Complex
395 disease and phenotype mapping in the domestic dog. Nat Commun. 2016;7:10460. doi:
396 10.1038/ncomms10460. PubMed PMID: 26795439; PubMed Central PMCID: PMCPMC4735900.
397 12. Vazquez-Baeza Y, Hyde ER, Suchodolski JS, Knight R. Dog and human inflammatory
398 bowel disease rely on overlapping yet distinct dysbiosis networks. Nat Microbiol. 2016;1:16177.
399 doi: 10.1038/nmicrobiol.2016.177. PubMed PMID: 27694806.
400 13. Minamoto Y, Otoni CC, Steelman SM, Buyukleblebici O, Steiner JM, Jergens AE, et al.
401 Alteration of the fecal microbiota and serum metabolite profiles in dogs with idiopathic

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402 inflammatory bowel disease. Gut Microbes. 2015;6(1):33-47. doi: 10.1080/19490976.2014.997612.
403 PubMed PMID: 25531678; PubMed Central PMCID: PMCPMC4615558.
404 14. Forsythe P, Bienenstock J, Kunze WA. Vagal pathways for microbiome-brain-gut axis
405 communication. Adv Exp Med Biol. 2014;817:115-33. doi: 10.1007/978-1-4939-0897-4_5.
406 PubMed PMID: 24997031.
407 15. Fung TC, Olson CA, Hsiao EY. Interactions between the microbiota, immune and nervous
408 systems in health and disease. Nat Neurosci. 2017;20(2):145-55. doi: 10.1038/nn.4476. PubMed
409 PMID: 28092661.
410 16. Neuman H, Debelius JW, Knight R, Koren O. Microbial endocrinology: the interplay
411 between the microbiota and the endocrine system. FEMS Microbiol Rev. 2015;39(4):509-21. doi:
412 10.1093/femsre/fuu010. PubMed PMID: 25701044.
413 17. Lyte M. Microbial endocrinology in the microbiome-gut-brain axis: how bacterial
414 production and utilization of neurochemicals influence behavior. PLoS Pathog.
415 2013;9(11):e1003726. doi: 10.1371/journal.ppat.1003726. PubMed PMID: 24244158; PubMed
416 Central PMCID: PMCPMC3828163.
417 18. Mayer EA. Gut feelings: the emerging biology of gut-brain communication. Nat Rev
418 Neurosci. 2011;12(8):453-66. doi: 10.1038/nrn3071. PubMed PMID: 21750565; PubMed Central
419 PMCID: PMCPMC3845678.
420 19. Yalcin E, Batmaz H. Signalment Factors, Comorbidity in Behavior Diagnoses in Dogs in
421 Bursa Region, Turkey (2000 - 2004) Acta Vet. Brno. 2007; 76: 445-450. doi:
422 10.2754/avb200776030445.
423 20. Rosado B, Garcia-Belenguer S, Leon M, Chacon G, Villegas A, Palacio J. Blood
424 concentrations of serotonin, cortisol and dehydroepiandrosterone in aggressive dogs. Appl Anim
425 Behav Sci. 2010;123(3-4):124-30. doi: 10.1016/j.applanim.2010.01.009. PubMed PMID:
426 WOS:000275677500006.

bioRxiv preprint doi: https://doi.org/10.1101/573865; this version posted March 19, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

427 21. Schatz S, Palme R. Measurement of faecal cortisol metabolites in cats and dogs: a non-
428 invasive method for evaluating adrenocortical function. Vet Res Commun. 2001;25(4):271-87.
429 PubMed PMID: 11432429.
430 22. Kirchoff NS, Udell MAR, Sharpton TJ. The gut microbiome correlates with conspecific
431 aggression in a small population of rescued dogs (Canis familiaris). PeerJ Preprints. 2018;
432 6:e26990v1. doi: 10.7287/peerj.preprints.26990v1.
433 23. Re S, Zanoletti M, Emanuele E. Aggressive dogs are characterized by low omega-3
434 polyunsaturated fatty acid status. Vet Res Commun. 2008;32(3):225-30. doi: 10.1007/s11259-007-
435 9021-y. PubMed PMID: 17891468.
436 24. Van der Weyde LK, Martin GB, Paris MC. Monitoring stress in captive and free-ranging
437 African wild dogs (Lycaon pictus) using faecal glucocorticoid metabolites. Gen Comp Endocrinol.
438 2016;226:50-5. doi: 10.1016/j.ygcen.2015.12.022. PubMed PMID: 26723188.
439 25. Kersey DC, Dehnhard M. The use of noninvasive and minimally invasive methods in
440 endocrinology for threatened mammalian species conservation. Gen Comp Endocrinol.
441 2014;203:296-306. doi: 10.1016/j.ygcen.2014.04.022. PubMed PMID: 24798579.
442 26. Sheriff MJ, Krebs CJ, Boonstra R. Assessing stress in animal populations: Do fecal and
443 plasma glucocorticoids tell the same story? Gen Comp Endocrinol. 2010;166(3):614-9. doi:
444 10.1016/j.ygcen.2009.12.017. PubMed PMID: 20051245.
445 27. Breiman, L. Random Forests. Mach Learn. 2001; 45: 5-32.
446 28. Omatsu T, Omura M, Katayama Y, Kimura T, Okumura M, Okumura A, et al. Molecular
447 diversity of the faecal microbiota of Toy Poodles in Japan. J Vet Med Sci. 2018;80(5):749-54. doi:
448 10.1292/jvms.17-0582. PubMed PMID: 29643280; PubMed Central PMCID: PMCPMC5989017.
449 29. Sandri M, Dal Monego S, Conte G, Sgorlon S, Stefanon B. Raw meat based diet influences
450 faecal microbiome and end products of fermentation in healthy dogs. BMC Vet Res. 2017;13(1):65.
451 doi: 10.1186/s12917-017-0981-z. PubMed PMID: 28245817; PubMed Central PMCID:
452 PMCPMC5331737.

bioRxiv preprint doi: https://doi.org/10.1101/573865; this version posted March 19, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

453 30. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion
454 of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a
455 mouse via the vagus nerve. Proc Natl Acad Sci U S A. 2011;108(38):16050-5. doi:
456 10.1073/pnas.1102999108. PubMed PMID: 21876150; PubMed Central PMCID:
457 PMCPMC3179073.
458 31. Lyte M. Probiotics function mechanistically as delivery vehicles for neuroactive
459 compounds: Microbial endocrinology in the design and use of probiotics. Bioessays.
460 2011;33(8):574-81. doi: 10.1002/bies.201100024. PubMed PMID: 21732396.
461 32. Garcia-Mazcorro JF, Dowd SE, Poulsen J, Steiner JM, Suchodolski JS. Abundance and
462 short-term temporal variability of fecal microbiota in healthy dogs. Microbiologyopen.
463 2012;1(3):340-7. doi: 10.1002/mbo3.36. PubMed PMID: 23170232; PubMed Central PMCID:
464 PMCPMC3496977.
465 33. Farca AM, Cavana P, Badino P, Barbero R, Odore R, Pollicino P. Measurement of faecal
466 corticoid metabolites in domestic dogs. Schweiz Arch Tierheilkd. 2006;148(12):649-55. doi:
467 10.1024/0036-7281.148.12.649. PubMed PMID: 17263082.
468 34. Archer J. The Behavioural Biology of Aggression, Vol. 1. Cambridge (UK): CUP Archive.
469 Cambridge University Press. 1988.
470 35. Neilson JC, Eckstein RA, Hart BL. Effects of castration on problem behaviors in male dogs
471 with reference to age and duration of behavior. J Am Vet Med Assoc. 1997;211(2):180-2. PubMed
472 PMID: 9227747.
473 36. Guy NC, Luescher UA, Dohoo SE, Spangler E, Miller JB, Dohoo IR, et al. Demographic
474 and aggressive characteristics of dogs in a general veterinary caseload. Appl Anim Behav Sci.
475 2001;74(1):15-28. doi: Doi 10.1016/S0168-1591(01)00153-8. PubMed PMID:
476 WOS:000170357100002.

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477 37. Terburg D, Morgan B, van Honk J. The testosterone-cortisol ratio: A hormonal marker for
478 proneness to social aggression. Int J Law Psychiatry. 2009;32(4):216-23. doi:
479 10.1016/j.ijlp.2009.04.008. PubMed PMID: 19446881.
480 38. Fyer AJ. Current approaches to etiology and pathophysiology of specific phobia. Biol
481 Psychiatry. 1998;44(12):1295-304. PubMed PMID: 9861472.
482 39. Schull-Selcer EA, Stagg W. Advances ad phobias. An update on separation anxiety and
483 noise aversion. Vet. Clin. Small Animal 1991; 38:1081-1106. doi: 10.1016/j.cvsm.2008.04.012.
484 40. Cocchi M, Gabrielli F, Tonello L, Delogu M, Beghelli V, Mattioli M, Accorsi PA.
485 Molecular contiguity between human and animal consciousness through evolution: some
486 considerations. J. Phylogen. & Evolution Biol. 2013; 1:1-8. doi: 10.4172/2329-9002.1000119.
487 41. Aleman JO, Bokulich NA, Swann JR, Walker JM, De Rosa JC, Battaglia T, et al. Fecal
488 microbiota and bile acid interactions with systemic and adipose tissue metabolism in diet-induced
489 weight loss of obese postmenopausal women. J Transl Med. 2018;16(1):244. doi: 10.1186/s12967-
490 018-1619-z. PubMed PMID: 30176893; PubMed Central PMCID: PMCPMC6122649.
491 42. Sakon H, Nagai F, Morotomi M, Tanaka R. Sutterella parvirubra sp. nov. and Megamonas
492 funiformis sp. nov., isolated from human faeces. Int J Syst Evol Microbiol. 2008;58(Pt 4):970-5.
493 doi: 10.1099/ijs.0.65456-0. PubMed PMID: 18398204.
494 43. Yusof N, Hamid N, Ma ZF, Lawenko RM, Wan Mohammad WMZ, Collins DA, et al.
495 Exposure to environmental microbiota explains persistent abdominal pain and irritable bowel
496 syndrome after a major flood. Gut Pathog. 2017;9:75. doi: 10.1186/s13099-017-0224-7. PubMed
497 PMID: 29255490; PubMed Central PMCID: PMCPMC5729606.
498 44. Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. Biol
499 Psychiatry. 2013;74(10):720-6. doi: 10.1016/j.biopsych.2013.05.001. PubMed PMID: 23759244.
500 45. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. Psychobiotics and the
501 manipulation of bacteria-gut-brain signals. Trends Neurosci. 2016;39(11):763-81. doi:

bioRxiv preprint doi: https://doi.org/10.1101/573865; this version posted March 19, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

502 10.1016/j.tins.2016.09.002. PubMed PMID: 27793434; PubMed Central PMCID:
503 PMCPMC5102282.
504 46. Fenske M, Schonheiter H. Thin-layer chromatography on silica-coated aluminium sheet as
505 an adjunct to radioimmunoassay of steroids. J Chromatogr. 1991;563(1):178-83. PubMed PMID:
506 1648105.
507 47. Tamanini C, Giordano N, Chiesa F, Seren E. Plasma cortisol variations induced in the
508 stallion by mating. Acta Endocrinol (Copenh). 1983;102(3):447-50. PubMed PMID: 6681927.
509 48. Gaiani R, Chiesa F, Mattioli M, Nannetti G, Galeati G. Androstenedione and testosterone
510 concentrations in plasma and milk of the cow throughout pregnancy. J Reprod Fertil.
511 1984;70(1):55-9. PubMed PMID: 6694152.
512 49. Motta M, Esposti AD. A computer program for mathematical treatment of data in
513 radioimmunoassay. Comput Programs Biomed. 1981.
514 50. Turroni S, Rampelli S, Biagi E, Consolandi C, Severgnini M, Peano C, et al. Temporal
515 dynamics of the gut microbiota in people sharing a confined environment, a 520-day ground-based
516 space simulation, MARS500. Microbiome. 2017.
517 51. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of
518 general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based
519 diversity studies. Nucleic Acids Res. 2013;41(1):e1. doi: 10.1093/nar/gks808. PubMed PMID:
520 22933715; PubMed Central PMCID: PMCPMC3592464.
521 52. Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. PANDAseq: paired-
522 end assembler for illumina sequences. BMC Bioinformatics. 2012;13:31. doi: 10.1186/1471-2105-
523 13-31. PubMed PMID: 22333067; PubMed Central PMCID: PMCPMC3471323.
524 53. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al.
525 QIIME allows analysis of high-throughput community sequencing data. Nat Methods.
526 2010;7(5):335-6. doi: 10.1038/nmeth.f.303. PubMed PMID: 20383131; PubMed Central PMCID:
527 PMCPMC3156573.

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528 54. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics.
529 2010;26(19):2460-1. doi: 10.1093/bioinformatics/btq461. PubMed PMID: 20709691.

530

531

532 Supporting information

533 **S1 Table. Metadata of the enrolled cohort.**

534 **S1 Fig. Superimposition of the genus relative abundance on the PCoA plot.** Arrows represent
535 the direction of significant correlations (permutation correlation test, P-value < 0.001).

536 **S2 Fig. Main bacterial genera represented within the canine gut microbiome.**

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A

Aggressive

WOOLF WILLY REXPT REX RUPERT SNOOP TYSON NETTUNO PEPE RE JAGO POLDO SASHA LILITH CI2A PLUTO SPANK

Phobic

CG4F CG3F CI4F CI5F ROGER DINA DIDI FRANCO LOLLO TAO CG2F CI3F LUISA OLIVIA CASH

Normal

LIO GIORGIO DASH CI6N MOLLICA AVON RUDY MIA TASHI LEA SALLY SHILA CI1N ISOLA CG1N CI7N NIKITA

Other families
Enterobacteriaceae
Succinivibrionaceae
Alcaligenaceae
Fusobacteriaceae
Erysipelotrichaceae
[Mogibacteriaceae]
Veillonellaceae
Ruminococcaceae
Peptostreptococcaceae
Peptococcaceae
Lachnospiraceae
Clostridiaceae
Clostridiales Unclassified
Turicibacteraceae
Streptococcaceae
Lactobacillaceae
Enterococcaceae
[Paraprevotellaceae]
Prevotellaceae
Bacteroidaceae
Coriobacteriaceae
Bifidobacteriaceae
Other Unclassified

B

Bacteroidaceae

p.value = 0.05

[Paraprevotellaceae]

[Mogibacteriaceae]

p.value = 0.03

Rikenellaceae

Erysipelotrichaceae

p.value = 0.05

Alcaligenaceae

Veillonellaceae

normal
phobic
aggressive

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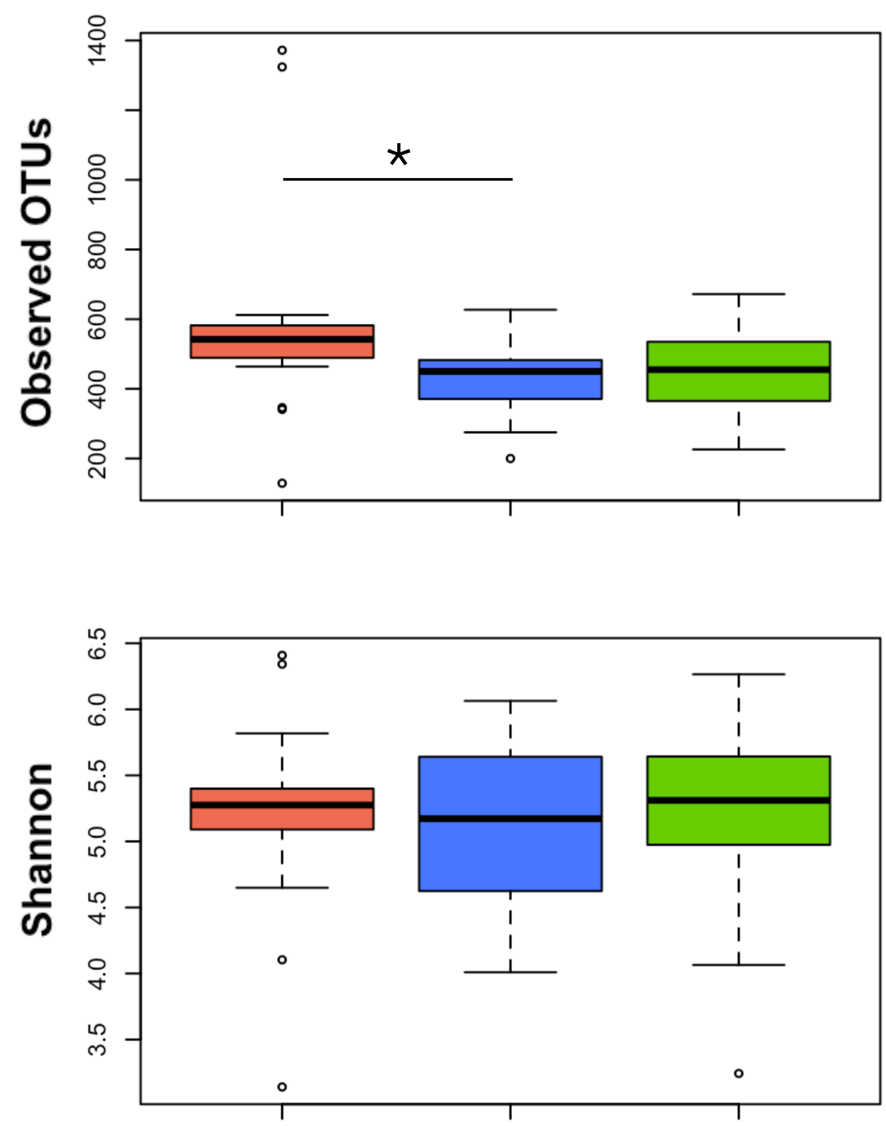

MDS2 - 14.7%

MDS1 - 19.9%

1.0 0.5 0.0 -0.5 -1.0 -1.5 -2.0

-1.5 -1.0 -0.5 0.0 0.5 1.0

normal
phobic
aggressive

Sex - male
Aggressive

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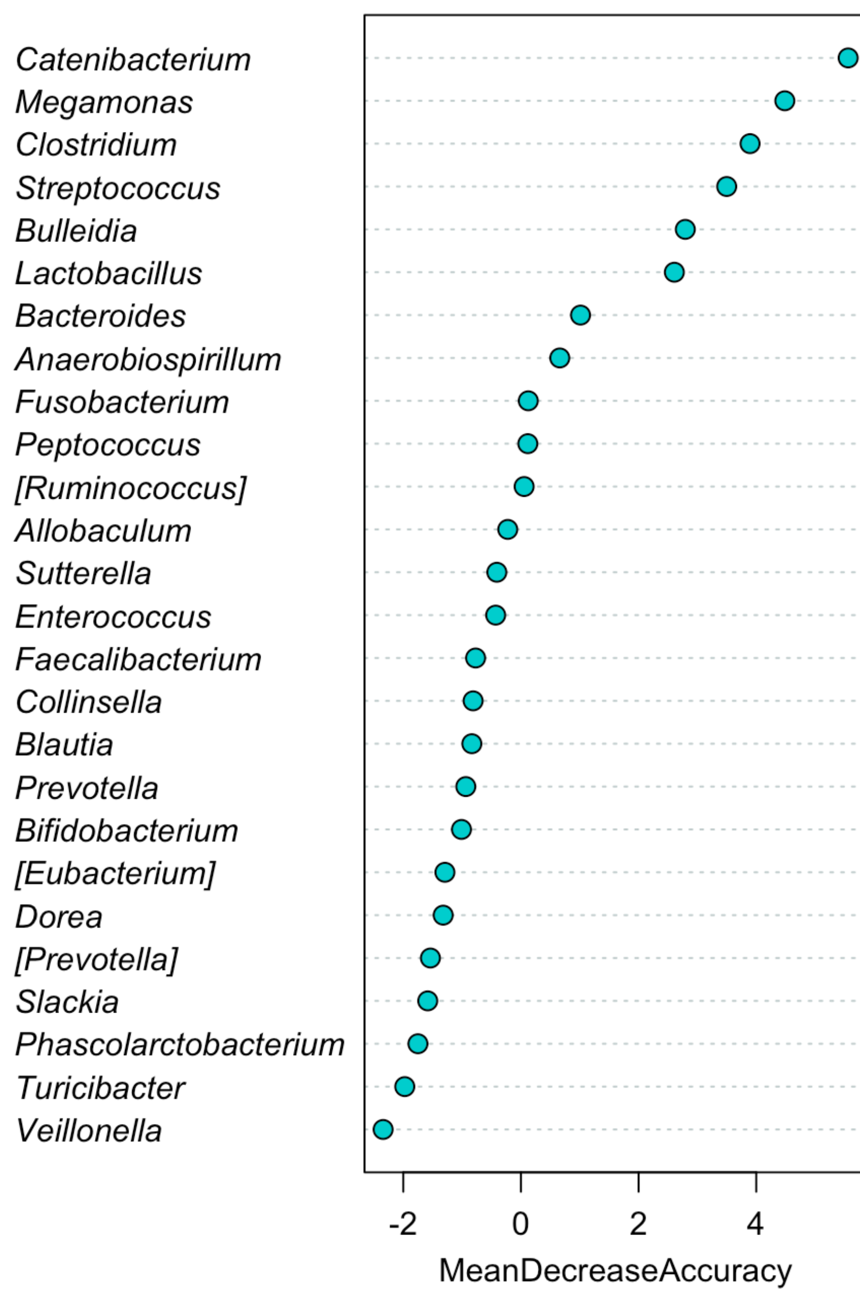

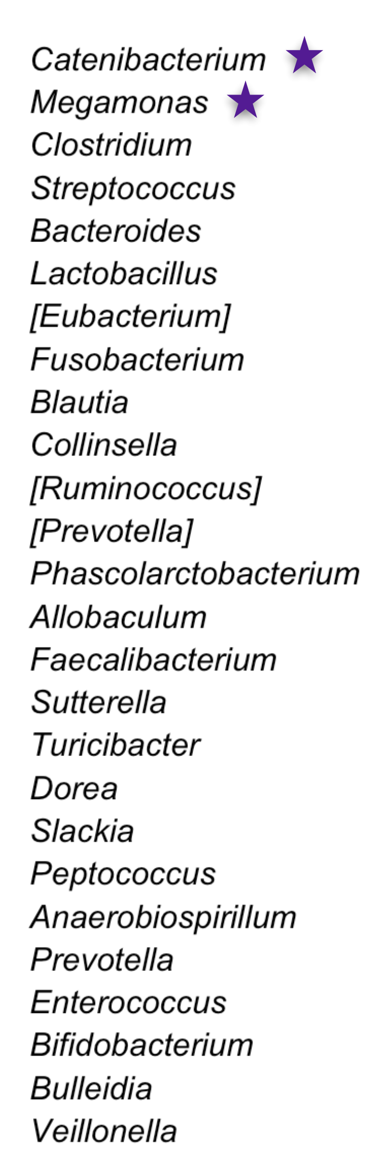

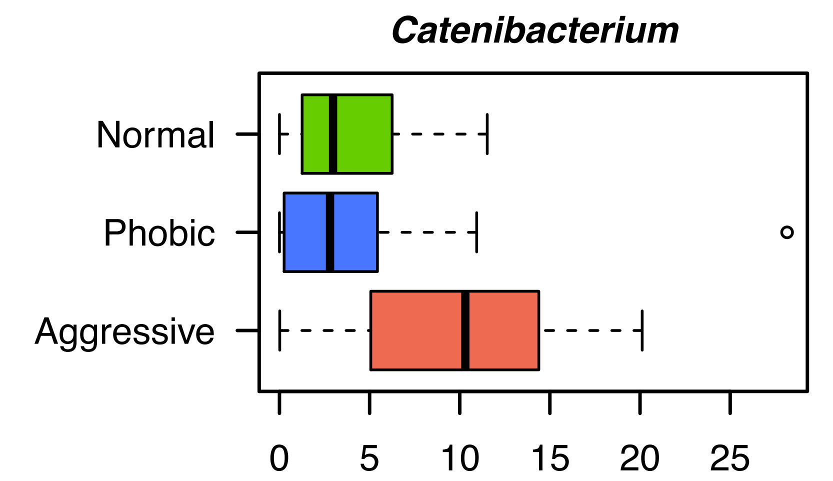

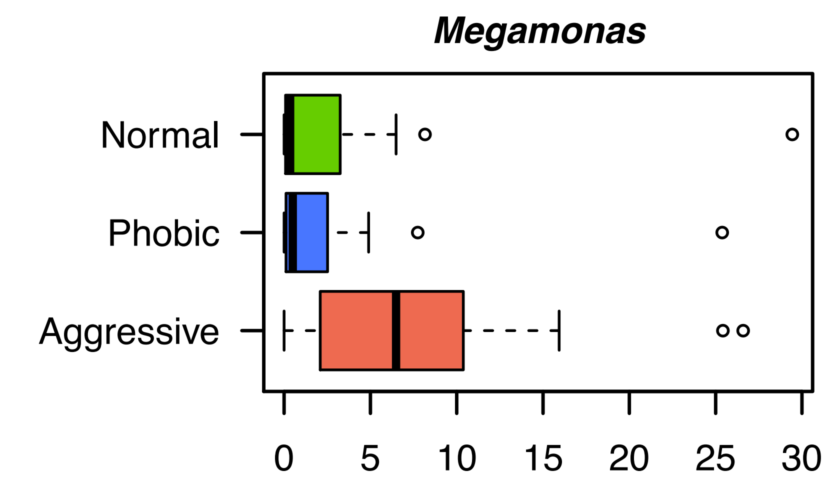

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