bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity.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 4.0 International license.

1

2

3 # Persistence of the ground beetle (Coleoptera: Carabidae)

4 # microbiome to diet manipulation

5 Anita Silver[1], Sean Perez[1], Melanie Gee[1], Bethany Xu[1], Shreeya Garg[1], Kipling Will[1,2*],

6 Aman Gill[1]

7 1. Department of Environmental Science, Policy and Management, University of California

8 Berkeley, Berkeley, California, United States of America

9 2. Essig Museum of Entomology, University of California Berkeley, Berkeley, California,

10 United States of America

11 * Corresponding author.

12 E-mail: kipwill@berkeley.edu (KW)

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity.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 4.0 International license.

13 **ABSTRACT**

14 **Host-associated microbiomes can play important roles in the ecology and evolution**
15 **of their insect hosts, but bacterial diversity in many insect groups remains poorly**
16 **understood. Here we examine the relationship between host environment, host traits, and**
17 **microbial diversity in three species in the ground beetle family (Coleoptera: Carabidae), a**
18 **group of roughly 40,000 species that synthesize a wide diversity of defensive compounds.**
19 This study found that the ground beetle microbiome is consistent across different host food
20 sources. We used 16S amplicon sequencing to profile three species that are phylogenetically
21 distantly related, trophically distinct, and whose defensive chemical secretions differ:
22 *Anisodactylus similis* LeConte, 1851, *Pterostichus serripes* (LeConte, 1875), and *Brachinus*
23 *elongatulus* Chaudoir, 1876. Wild-caught beetles were compared to individuals maintained in the
24 lab for two weeks on carnivorous, herbivorous, or starvation diets. Soil environment but not diet
25 had a significant effect on bacterial diversity and composition. The three carabid species have
26 patterns of microbial diversity similar to those previously found in other insect hosts.
27 Metagenomic samples from two highly active tissue types — guts, and pygidial gland secretory
28 cells (which produce defensive compounds) — were processed and sequenced separately from
29 those of the remaining body. The observed similarity of the pygidial gland secretory cell
30 microbiome across hosts suggests the possibility that it may be a conserved community, possibly
31 due to functional interactions related to defensive chemistry. These results provide a baseline for
32 future studies of the role of microbes in the diversification of defensive chemical biosynthesis in
33 carabids.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

34 **KEYWORDS** 16S, Carabidae, beetle, insect, diet, resident microbiome, microbiome,
35 microbial ecology, defensive chemistry, guts, pygidial gland, secretory cells, amplicon,
36 metagenomics

37 **SHORT TITLE** Host diet and microbial diversity in ground beetles

38

39 INTRODUCTION

40 Insects are by far the most diverse group of animals (1) (2), and it is becoming clear that
41 the success of several major insect groups is due in part to their resident microbiomes (2) (3).
42 However, microbiomes and their possible symbiotic functions remain understudied in many
43 major groups of insects, including Carabidae ground beetles. Carabidae consists of around
44 40,000 described species, making it one of the most species-rich animal families on earth (4).
45 Moreover, the variety of defensive chemicals produced in the carabid pygidial gland system is an
46 impressive example of evolutionary diversification (5). Secretory cells of the pygidial gland
47 system produce such diverse classes of molecules as carboxylic acids, formic acid, quinones,
48 hydrocarbons, and aromatics; chemical diversity exists even within some genera (5). Whether
49 microbes play a functional role in carabid chemical diversity has not yet been studied.

50 Interactions between insects and their associated microbiomes can contribute to insect
51 diversification (3). Microbiomes can benefit host insects in many ways, such as producing
52 vitamin B (6), regulating host metabolism in response to stress (7), and contributing to host
53 development (8). Notable examples of microbial symbionts supporting nutrient acquisition in
54 insects include *Buchnera* bacteria producing essential amino acids allowing aphids to live on a
55 nutrient-poor diet (9) and highly diverse termite gut microbes digesting cellulose for their wood-
56 feeding hosts (10) (11). Unlike aphids and termites, carabids tend to be dietary generalists, and

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

57 microbial species are also known to contribute to other host phenotypes, including nutrient
58 acquisition and detoxification. In ants (12), *Harpalus pensylvanicus* (Degeer, 1774) (Carabidae)
59 (13) and *Cephaloleia* (Coleoptera: Chrysomelidae) (14), microbial symbionts assist their hosts in
60 metabolizing different food sources. It is known that bacterial symbionts enable several beetle
61 species to thrive in chemically hostile environments. For example, the mountain pine beetle
62 *Dendroctonus ponderosae* (Hopkins, 1902) (Coleoptera: Curculionidae) can inhabit pine trees
63 because its microbes break down defensive terpenes produced by the trees (15). The
64 microbiomes of *Nicrophorus vespilloides* Herbst, 1783 (Coleoptera: Silphidae) and other carrion
65 beetles protect their hosts from toxins and speed up host digestion, making it easier for these
66 beetles to feed on decaying carcasses (16) (17). Insects are well known to benefit from defensive
67 and protective symbioses. *Lagria villosa* (F.) (Coleoptera: Tenebrionidae) beetles live in
68 symbiosis with *Burkholderia gladioli* that protect their host's eggs from pathogens by producing
69 the antifungal compound lagriamide (18). *Paederus* (Coleoptera: Staphylinidae) beetles are well
70 known for producing toxic hemolymph that causes severe dermatitis; the toxin, pederin, is
71 produced by a *Pseudomonas*-like symbiont (19). The Asian citrus psyllid *Diaphorina citri*
72 (Kuwafyama, 1908) (Hemiptera: Liviidae), an invasive pest in the U.S. that causes citrus disease,
73 harbors endosymbiotic *Candidatus* Profftella armatura (Betaproteobacteria) that produce
74 diaphorin, a toxin similar to pederin (20). Given the diversity of established insect-bacterial
75 associations as well as the diversity of ground beetle defensive chemistry, it is worth examining
76 the hypothesis that carabid microbiomes are associated with their defensive chemical production.
77 If bacteria indeed contribute to carabid host functions, we would expect a non-random
78 microbiome composition corresponding to the functional role they play — in other words,
79 microbial diversity should correspond to functional diversity. This is not necessarily

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

80 straightforward, since insect microbiome composition can be explained by several factors such
81 as host phylogeny (2) (21) (22) (23), dietary guild (23) or sampling locality (24). Although many
82 insects have persistent host-associated communities, some do not, highlighting the potential for
83 stochastic changes in microbial diversity; for example, some lepidoptera caterpillar microbiomes
84 consist entirely of microbes ingested with leaves (25). If the carabid microbiome is similarly
85 transient, it would be most closely resemble that of the community of the recent diet (12) (26) or
86 other local environment (11), and not obviously correlate with other factors such as host
87 phylogeny, chemistry, or tissue type. Host-associated microbiome composition can also be
88 influenced by changes to host diet, as has previously been found in some Coleoptera and
89 Lepidoptera species (14) (27). Provided that some of the variation in microbiome diversity and
90 composition can be explained by factors outside of the transient aspects of the environment,
91 however, it is possible that these microbes serve some function that benefits their host.

92 As insects have an open circulatory system that allows hemolymph to flow throughout
93 the body, microbial communities are found in many insect tissues (28); but as in other animals,
94 insects often have distinct microbial communities in different tissues (10) (12) (28). Some of this
95 diversity may relate to the variety of conditions found within insect anatomy, including aerobic
96 and anaerobic regions and extreme pH gradients (11) (28). Tissue-specific diversity could also be
97 explained by a co-evolutionary relationship between hosts and symbiotic microbiota, in that
98 hosts can harbor functionally useful bacteria in specialized tissues. For example, termites
99 regulate unique microbiomes in each of several gut pouches (11), and many insect species
100 maintain useful symbionts in specialized cells called bacteriocytes (28). The present study
101 focuses on the pygidial gland secretory cells (hereafter just secretory cells) and the gut. We
102 hypothesize that these tissues are most likely to host microbially-mediated functions because

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

103 these organs are responsible for defensive chemical synthesis and digestion of food respectively,
104 both known to involve bacterial symbionts in other insect taxa.

105 In this study, we used 16S metagenomic amplicon sequencing to quantify the bacterial
106 diversity hosted by three carabid species under several dietary treatments. Each host species
107 produces distinct primary defensive compounds: *Anisodactylus similis* produces formic acid,
108 *Pterostichus serripes* carboxylic acids, and *Brachinus elongatulus* quinones (29)(Will &
109 Attygalle, unpublished data). *Anisodactylus similus* has a distinct natural feeding preference from
110 the other two species, so together these three species represent two different trophic types.
111 *Brachinus elongatulus* and *P. serripes* are naturally generalist predator-scavengers, preferring
112 animal matter but observed in nature and in the lab to eat a wide variety of sugar and protein rich
113 plant and animal material; in contrast, *A. similis* is typically observed feeding on fallen fruits,
114 seeds, and pollen (30) (Will unpbl.). In addition to sequencing wild-caught beetles preserved at
115 the time of collection, we also subjected live beetles of each species to three specific dietary
116 treatments, and dissected tissues of interest from each specimen.

117 This study examined how the transient factor of diet treatment, and more permanent
118 factors including host species and tissue, contribute to the observed variation in carabid-
119 associated microbiomes. We assume that if a given factor (e.g., food type or environment) does
120 not impact the microbiome then the composition and diversity of the microbiome will appear
121 random with respect to the state of that factor. On the other hand, if a factor is influential, we
122 expect changes to that factor to significantly explain microbiome composition and diversity.
123 Specifically, microbiome composition and diversity within treatments should be more similar
124 than expected by random chance. We predicted that 1] If carabid beetles harbor non-transient
125 host-associated microbes, then diet and local environmental factors would be insufficient to

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

126 explain overall microbial variation across species. 2] Compared to diet treatment, host species
127 would explain a greater share of microbial diversity — i.e. microbial communities would cluster
128 more by host species than diet treatment. 3] A significant proportion of carabid microbial
129 diversity would be explained by host tissue type. If microbial communities are random with
130 respect to host tissue, that would undermine the hypothesis that microbes are involved in tissue-
131 specific host functions like defensive chemical synthesis in secretory cells.
132

133 **METHODS**

134 **Beetle husbandry and dissection.** Twelve individuals each of *Anisodactylus similis*
135 LeConte, 1851, *Pterostichus serripes* (LeConte, 1875), and *Brachinus elongatulus* Chaudoir,
136 1876 were collected (total 36 specimens). *Pterostichus serripes* and *A. similus* were collected
137 from U.C. Berkeley's Whitaker's Forest, Tulare County, CA (36.7022°, -118.933°). *Brachinus*
138 *elongatulus* were collected from national forest land in Madera Canyon, Santa Cruz County, AZ
139 (31.72°, -110.88°). For each species, three wild-caught specimens were preserved in 95% ethanol
140 immediately upon collection, and the remaining beetles were transported live to laboratory
141 facilities on the U.C. Berkeley campus. For each species, in addition to wild-caught specimens,
142 three diet treatments (banana, mealworm, and starvation) were tested in triplicate. Diet-treated
143 beetles were kept in sterile containers with sterilized soil and water for 17 days in July, 2018.
144 Banana-fed (Trader Joe's, Dole Banana Ecuador) and mealworm-fed (Timberline, Vita-bugs
145 Mini Mealworms 500 count) beetles were fed on the first day, and subsequently fed and watered
146 every three days using heat-sterilized forceps and autoclaved water. All feeding portions were
147 0.04g (+/- 0.01g). Starved beetles received water, but no food. Banana and mealworm bacterial
148 communities were sequenced as controls and were removed from the analysis after confirming

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

149 samples were not contaminated. On the last day, beetles were quickly killed by placing them for
150 one minute at -80°C in their plastic containers. All specimens, including wild-caught beetles,
151 were dissected as described by McManus et al. (31). Each beetle was dissected into three groups
152 of tissues: secretory cells, gut (including foregut, midgut, and hindgut), and the rest of the body
153 minus the secretory cells and gut (subsequently referred to as 'partial body'). Parasitic worms
154 (Nematomorpha) found to be infecting one starved beetle and one mealworm-fed beetle were
155 removed from those specimens and the worm tissues not included in downstream analysis.

156 **DNA extraction, PCR, and next generation sequencing.** Tissues were incubated
157 overnight in a 9:1 ratio of buffer ATL and proteinase K (Qiagen DNeasy Blood & Tissue Kit) at
158 55°C on a rocking tray. Lysate from overnight incubation was transferred to sterile 1.5ml O-ring
159 tubes containing 0.25g (+/- 0.02g) of 0.1mm diameter zirconium beads and bead beat at 2000rpm
160 for 3 minutes in a PowerLyzer to lyse bacterial cells. DNA was extracted from the lysed
161 homogenate using Solid Phase Reversible Immobilization (SPRI) magnetic beads made
162 following the method of Rohland (32): 100µL lysate was mixed with 180µL of well-mixed,
163 room temperature SPRI beads, incubated for approximately 5 minutes on the bench, then
164 transferred to a magnetic rack. After the SPRI beads pelleted, 200µL 80% ethanol was added.
165 After 30 seconds the supernatant was removed, the ethanol wash was repeated a second time and
166 the supernatant was removed again. Then, the tubes containing SPRI bead tubes were removed
167 from the magnetic rack and allowed to air dry completely. DNA was eluted by adding 50µL TB
168 solution (10mM Tris) directly onto the beads and incubating for 5 minutes, then returning
169 samples to the magnetic rack to pellet the SPRI beads and retrieve the DNA-containing
170 supernatant.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

171 The V4 region of the 16S rRNA gene was PCR amplified in duplicate in 25µL reactions
172 using GoTaq Green Master Mix (Promega), and the resulting PCR products were subsequently
173 pooled. During the first round, previously described primers (33) 515FB_in (5'-ACA CTC TTT
174 CCC TAC ACG ACG CTC TTC CGA TCT GTG YCA GCM GCC GCG GTA A-3') and
175 806RB_in (5'-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TGG ACT ACH
176 VGG GTW TCT AAT-3'), which were adapted to be complementary to the second round
177 primers (34), were added to the ends of all 16S genes with the following conditions (BioRad
178 thermocycler): initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 45 sec,
179 50°C for 1 min, 72°C for 1:30 min, and a final extension step of 72°C for 10 min. A second
180 round of PCR was performed using unique combinations of barcoded forward (5'-AAT GAT
181 ACG GCG ACC ACC GAG ATC TAC ACX XXX XXX XAC ACT CTT TCC CTA CAC GA-
182 3') and reverse (5'-CAA GCA GAA GAC GGC ATA CGA GAT XXX XXX XXG TGA CTG
183 GAG TTC AGA CGT G-3') primers (34) to create a dual-index amplicon library for Illumina
184 sequencing (position of barcodes indicated by 'X' characters). The conditions for the second PCR
185 reaction were: initial denaturation at 94°C for 3 min, followed by 10 cycles of 94°C for 45 sec,
186 50°C for 1 min, 72°C for 1:30 min, and a final extension step of 72°C for 10 min. All pooled
187 duplicate PCR products were run on a 1% agarose gel for 30 min at 100V, and imaged under UV
188 light to verify successful PCR. DNA concentration was quantified using a Qubit fluorometer,
189 and equimolar amounts were pooled. The pooled library was purified (Qiagen Qiaquick PCR
190 Purification Kit) and sent for Illumina MiSeq sequencing at the U.C. Berkeley Genomics
191 Sequencing Laboratory.

192 **Analysis.** Amplicon reads for the V4 region of 16S were de-multiplexed with deML (35)
193 and processed using DADA2 (36), including quality filtering with maxEE=2. Reads were de-

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

194 replicated into unique 16S amplicon sequence variants (ASVs, also referred to as phylotypes)
195 using a read error model parameterized from the data. Paired-end reads were merged and
196 mapped to ASVs to construct a sequence table. Chimeric sequences were removed. Taxonomic
197 assignments for exact matches of ASVs and reference strains were made using the Ribosomal
198 Database Project database (37). Sequence tables and taxonomic assignments were imported into
199 R version 3.5 (38) for downstream analysis and combined into a single phyloseq (39) object for
200 convenience. To account for variation in sequencing effort across samples, samples were scaled
201 according to variance stabilized ASV abundances using DESeq2 (40) (41). ASV alignments
202 made using DECIPHER (42) (43) were used to construct a neighbor-joining tree, and this tree
203 was then used as the starting point for deriving a maximum likelihood tree from a generalized
204 time-reversible model with gamma rate variation, implemented with the phangorn package in R
205 (44). The tree was rooted using QsRutils (45). For comparative analysis between beetles, ASV
206 data from all three tissues of each specimen were combined into an aggregate bacterial
207 community. Alpha diversity measures were calculated using the packages phyloseq (39) and
208 picante (46). Non-metric multidimensional scaling (NMDS) plots of beta diversity were created
209 using phyloseq (39), and analysis of similarities (ANOSIM) tests were run using the package
210 vegan (47). Bray-Curtis distances were calculated both for aggregate community data and for the
211 original dataset. Venn diagrams of phylotypes present by diet were rendered by VennDiagram
212 (48). To control for possible sequencing errors, only phylotypes occurring at least twice in the
213 entire dataset were included in venn diagram analysis. Hierarchical clustering of communities
214 was performed with the package ape (49). Secretory cells were tested for differential abundance
215 of microbe phylotypes using an equivalent method to RNA-seq differential expression analysis,
216 implemented using DESeq2 (40) (39).

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

217 **Ethics Statement.** No permits were required for the described study, which complied
218 with all relevant regulations.

219 **RESULTS**

220 **Sequencing results:** After quality filtering, the mean number of reads per sample was
221 19,868, and the median number of reads per sample was 17,532.

222 **Alpha diversity:** There was a median of 95 and a mean of 98.6 ASVs present per
223 sample.

224 ***Diet.*** Phylogenetic diversity (PD) of aggregate communities was not associated
225 significantly with diet treatment. When tissues were considered individually, only PD of the
226 partial body varied significantly across diet treatments (Fig. 1). Richness results were similar to
227 PD. Neither evenness nor Shannon diversity showed any significant effect of diet in either
228 individual tissues or pooled microbiomes. Compared to wild-caught beetles, keeping the host in
229 captivity subjected to any of the diet treatments had a minor effect on PD but not on other alpha
230 diversity measures of the microbiome. Host diet did not correlate with community alpha
231 diversity.

232 **Fig 1. Boxplots of phylogenetic diversity (PD), with outliers depicted as points.**
233 (A) Plots grouped by host species. PD of partial bodies varied significantly by host
234 species (chi-squared = 8.11, p = 0.017), but PD of all other tissues and of aggregate
235 communities did not. (B) Plots grouped by diet treatment. PD of partial bodies varied
236 significantly by diet treatment (chi-squared = 8.96, p = 0.030), but PD of all other tissues
237 and of aggregate communities did not.

238 ***Tissue.*** Tissue explained a large portion of the variance in PD of aggregate communities
239 (Kruskal Wallis chi-squared = 55.5, p < 0.0001), so results were plotted separately for each

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

240 tissue (Fig. 1). Secretory cell microbiomes had higher PD than gut microbiomes (Fig. 1).
241 Richness, Shannon diversity, and evenness all varied significantly by tissue ($p < 0.0001$) as well.
242 ***Species.*** Evidence of an effect of host species on microbial community diversity was
243 relatively weak, and varied by tissue. Overall PD and evenness did not vary significantly by
244 species. Richness ($p = 0.043$) and Shannon diversity ($p = 0.041$) varied only slightly significantly
245 by species. Secretory cells had a very consistent alpha diversity level across species, only
246 varying significantly by the measure of evenness ($p = 0.030$). Gut alpha diversity varied
247 significantly across species by richness ($p = 0.027$), Shannon diversity ($p <$ 1e-05), and evenness
248 ($p <$ 1e-05) but not PD. Differences in gut alpha diversity appear to be driven by the
249 exceptionally low evenness in *A. similis* guts. The partial body microbiome had significantly
250 different PD (Fig. 1) and richness ($p = 0.0033$), but no change in evenness, across host species.
251 PD of partial bodies was highest in *P. serripes.*
252 **Community diversity distance analysis:** Bray-Curtis distances for aggregate
253 community data (Fig. 2a) and for the original dataset (Fig. 2b-d) reveal that community
254 similarity is associated with several of the factors tested. Results of ANOSIM performed with
255 Unifrac distances were consistent with results using Bray-Curtis distances reported below.
256 ***Diet.*** Aggregate communities did not cluster by diet (Fig. 2a). They did cluster by captive
257 versus wild-caught beetles (ANOSIM R statistic = 0.3336, $p < 0.001$). The only tissue that
258 clustered significantly by diet was secretory cells, but these clustered with a lower R statistic by
259 diet ($R = 0.23$) than by species ($R = 0.28$). Clustering by diet was explained by significant
260 differences between captive and wild-caught beetles. Phylotypes present in aggregate
261 communities were compared across diet treatments (Fig. 3). A total of 1003 phylotypes were
262 present across all diet conditions, 613 of which were present in wild-caught beetles. Of the

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

263 phylotypes present in wild-caught beetles, 78% were present in at least one other diet condition.
264 Just over a quarter of phylotypes were shared across all four diet conditions.

265 **Fig 2. Bray-Curtis ordination of microbiome beta diversity using non-metric**
266 **dimensional scaling.**

267 (A) Aggregate communities clustered significantly by species (ANOSIM R statistic =
268 0.92, $p < 0.001$) and tissue ($R = 0.66$, $p < 0.001$) only. (B) Secretory cell microbiomes
269 clustered by species ($R = 0.28$, $p < 0.001$) and diet ($R = 0.23$, $p < 0.001$). (C) Gut
270 microbiomes clustered clearly by species ($R = 0.96$, $p < 0.001$), and not by diet. (D)
271 Partial body microbiomes are also clustered clearly by species ($R = 0.95$, $p < 0.001$), and
272 not by diet.

273 **Fig 3. Venn diagram of phylotypes present in aggregate communities by diet**
274 **treatment.**

275 ***Tissue.*** Microbial communities clustered clearly and significantly by host tissue.

276 ***Species.*** Aggregate communities clustered by host species, with the *B. elongatulus*
277 microbiome being the most distinct (Fig. 2a). Individual tissues also clustered by species. The
278 secretory cells had a much lower clustering statistic than the other tissues, indicating that
279 microbial diversity in secretory cells is less differentiated than other tissues.

280 **Community Composition:** The most abundant phyla across all samples were
281 Proteobacteria (mean abundance 48.7%), Bacteroidetes (mean abundance 17.8%), Tenericutes,
282 and Firmicutes (Fig. 4). Together, these four phyla comprised a mean of 94.6% of the bacteria in
283 each sample. Communities in all beetle species and tissues had similar phylum-level
284 compositions. Differences by host species arose more clearly at the level of bacterial genera, so
285 community composition of each beetle species was plotted separately at this level (Fig. 4).

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

286 Bacterial genera with median relative abundance across all samples of 1.5% or above were, in
287 descending order of median relative abundance: *Acinetobacter, Spiroplasma, Yersinia,*
288 *Flavobacterium, Pseudomonas, Enterobacter,* and *Enterococcus.*

289 **Fig 4. Relative abundances of prevalent bacterial taxa by host tissue and species.**
290 (A) Mean abundance in the secretory cells of the ten bacterial genera that were most
291 abundant on average in all samples (n = 36, 12 per species). (B) Mean abundance of these
292 bacterial genera in the guts (n = 36, 12 per species). (C) Mean abundance of these
293 bacterial genera in the partial bodies (n = 36, 12 per species). (D) Mean relative
294 abundance, across all host species, of the ten most abundant bacterial phyla (n = 108, 36
295 per tissue). Upper left legend applies to panels A-C and lower left to D.

296

297 ***Diet.*** Community composition was not significantly different across diet treatments (Fig.
298 5).

299 **Fig 5. Relative abundances of prevalent bacterial taxa by host diet treatment.**

300 (A) Mean abundance in *A. similis* of the ten most abundant bacterial genera across
301 all samples (n = 36), grouped by diet treatment (n = 9, each). (B) Mean abundance in *B.*
302 *elongatulus* of these bacterial genera, grouped by diet treatment. (C) Mean abundance of
303 these bacterial genera in *P. serripes,* grouped by diet treatment. Genera included are the
304 same as in Fig 4. Photographs of beetles depict typical host morphology.

305

306 ***Tissue.*** Differential abundance analysis of secretory cells versus all other tissues revealed
307 that four phylotypes associated with two families were differentially expressed ($p < 0.002$). Two
308 Flavobacterium phylotypes were more abundant in secretory cells than other tissues by factors of

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

309 10.22 and 16.23. Two Comamonadaceae phylotypes of unknown species were 9.38 and 9.92 fold
310 more abundant in secretory cells. Secretory cell community composition is relatively conserved
311 at the level of bacterial genera (Fig. 4). Compared to other tissues, gut microbiomes were more
312 dominated by the ten most abundant bacterial genera; these ten genera composed over 50% of
313 microbial abundance in all host species' guts, over 60% of abundance in *B. elongatulus* guts, and
314 less than 50% of abundance in other tissues' microbial communities (Fig. 4).

315 ***Species.*** Hierarchical clustering of community similarity showed that community
316 differences corresponded with host species for all tissues. *Brachinus elongatulus* guts have more
317 Firmicutes, and less Tenericutes and Actinobacteria, than the other two host species. Breaking
318 down community composition to the genus level confirmed the status of *Brachinus* as the most
319 distinct host species (Fig. 4).

320 **DISCUSSION**

321 The present study assessed the degree to which diet and local environment shape
322 microbiome composition and diversity in three carabid host species and among two active tissue
323 types in each. Local environment was controlled for by comparing wild-caught and lab-reared
324 beetles, and differences between these two conditions highlighted its importance. We
325 hypothesized that if carabid beetle microbiomes play functional roles in chemical defense or
326 other host traits, then a portion of microbial diversity should be non-transient; conversely, if
327 carabid microbial diversity is entirely explained by diet and local environment, then it is unlikely
328 microbes play a consequential role in the diversification of carabid defensive chemistry or other
329 phenotypes. We found that patterns in microbiome composition and diversity are largely
330 explained by the intrinsic factors of host species and tissue type. In contrast, shifts in host diet to
331 carnivory, herbivory, or starvation had no significant effect on bacterial species diversity or

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

332 composition. These findings demonstrate that carabid microbiomes are highly persistent to
333 changes in host diet, paving the way for future efforts to decipher the ecological patterns and
334 metabolic interactions that underlie non-transient host-associated microbial diversity in ground
335 beetles. These results contribute to broader efforts to understand how the microbial diversity
336 hosted by insects relates to insect evolution and ecology.

337 **Explanatory Power of Factors Tested**

338 By subjecting beetles to differential dietary treatments in a controlled, sterile
339 environment, this study quantified how transitory factors like host diet and soil environment
340 predict variation in carabid microbial diversity, compared to intrinsic factors like tissue type and
341 host taxonomy. The robustness of microbial community composition and diversity to diet
342 changes, including to restricted herbivorous and carnivorous diets (Figs 1-3, 5), supports our first
343 hypothesis of non-transient microbial communities. Controlling for local environment showed
344 that soil is an important source of microbes for carabid beetles. As predicted in our second
345 hypothesis, host species explains our results better than transient factors, both in terms of
346 community composition (Fig. 4) and distance ordination. The finding that microbiome
347 composition and diversity are associated most closely with tissue type strongly supports our third
348 hypothesis. Tissue identity explains much of the variation between communities, in composition
349 (Fig. 4) and distance ordination, and most of the variation in PD (Fig.1). Previous research has
350 found that factors such as environmental filtering (3) and routes of microbe dispersal (22) can
351 shape microbiome composition, and the especially strong association with tissue type could be
352 related to these factors.

353 The random variation of microbial communities across diet treatments, together with the
354 patterns in microbial community variation by host species and tissue type, indicates that carabid

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

355 beetles possess non-transient, host-associated microbiomes. This is in accordance with our
356 hypothesis that if carabid beetles harbor non-transient host-associated microbes, then diet and
357 local environmental factors would not be sufficient to explain overall microbial variation across
358 species. The finding that microbial communities are more similar within samples from the same
359 host species supports our second hypothesis that microbes may be transmitted between
360 individuals of the same species. The consistency of microbial communities within host species
361 and tissue groups also raises the possibility that they co-evolved with their carabid hosts.

362 One transient factor that does explain a significant amount of the variation in our data is
363 the impact of taking the host from the wild and keeping it under laboratory conditions on a
364 common mix of sterile soil. Husbandry in a lab was previously found to have reduced
365 microbiome diversity in carrion beetles (17) and lepidopteran species (27), indicating that a
366 portion of these microbiomes are continuously acquired from the hosts' surrounding
367 environment. In the case of carabid beetles, soil may be an important source of microbial
368 diversity. In our study, host captivity influences PD (Fig. 1) and the number of bacterial
369 phylotypes present (i.e., richness) in individual microbiomes.

370 Factors other than those that were manipulated in this study could also exert influence on
371 carabid microbiomes throughout the carabid life cycle. Our study does not address how juvenile
372 carabids acquire microbes during their egg, larval, or pupal stages. Acquisition may occur by
373 selective uptake from the environment as in leeches, hydra, and vibrio (11), or via direct
374 transmission from a parent as in dung beetles (8). The relatively short time-frame of our study
375 means it also cannot address the possible effects of long-term diet or environmental changes on
376 carabid microbiomes, e.g., over the course of a season or year. Microbiome variability is
377 explained by individuals' long-term environment in drosophila (11) and houseflies (24). Finally,

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

378 this study was not designed to disentangle the effects of host chemistry and host species identity,
379 as each of the included species has different defensive chemical products.

380 **Comparison to other insect microbiomes**

381 Given that related insect hosts sometimes share a subset of their microbiome, such as the
382 conserved core microbiome of two dung beetles species (50), we anticipated that the three
383 carabid species we tested might harbor distinct but overlapping microbial communities.
384 Community distance ordination (Fig. 2), species- and genus-level composition, and similarity
385 clustering showed that microbiomes were indeed distinct across *A. similis*, *P. serripes*, and *B.*
386 *elongatulus*. The microbiomes of these three species also had many overlapping phylotypes.

387 A previous study of microbiome compositions showed that the resident gut microbiomes
388 of two species of carabids, *Harpalus pensylvanicus* (Degeer, 1774) and *Anisodactylus*
389 *sanctaecrucis* (Fabricius, 1798), had different composition and species richness from each other
390 (51), and our results show a similar pattern of diversity across host species. The prevalence of the
391 genus *Spiroplasma* in our results agrees with the findings of previous studies of carabid
392 microbiomes (31) (51). *Dysgonomonas*, which has previously been found to be prevalent in *B.*
393 *elongatulus* (31), was one of the most abundant genera, and also had higher relative abundance
394 in *B. elongatulus* than in other beetle species, especially in the guts (Fig. 3). Another abundant
395 genus in our study was *Enterococcus,* which is known to be associated with digestive tracts of
396 several organisms including *B. elongatulus* (31). In *B. elongatulus* and *P. serripes,* we found
397 *Enterococcus* to be more abundant in the guts than in the other tissues (Fig. 3).

398 The results of our small-scale study give additional preliminary evidence that carabid
399 microbiomes are similar to other Coleoptera microbiomes. Several highly abundant bacterial
400 genera in these samples were previously found in *Cephaloleia* (Chrysomelidae) beetles (14). The

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

401 phylogenetic diversity of these carabid microbiomes is within the compass of previous studies in
402 Coleoptera and in omnivorous insects (23). The phylogenetic diversity of the pygidial gland
403 secretory cells, which are homologous structures found in carabids and other Adephaga, was
404 unusually high. The lack of notable similarity in community composition between these results
405 and previous studies of Coleoptera microbiomes confirmed past research that found that insect
406 microbiome correspondence to phylogeny is not apparent when examined at deeper phylogenetic
407 levels, typically corresponding to high-level taxonomy such as order (11) (21).

408 Comparing our results to past results across Insecta, we find that the five most abundant
409 bacterial phyla in this study (Proteobacteria, Bacteroidetes, Actinobacteria, Tenericutes and
410 Firmicutes) have previously been found to be the five most highly prevalent bacterial phyla in
411 insects generally (2). Unlike what is known from gut samples from many other insect species (2),
412 *Wolbachia* and *Rickettsia* are not among the most abundant bacterial genera in carabid guts.
413 Insect gut microbiomes are more diverse across species than mammalian gut microbiomes (2),
414 and past studies have shown that gut microbiomes group more loosely in beetles than in
415 hymenopterans and termites (23). Comparison between the microbiomes from the carabid gut
416 samples in our study and other insect gut microbiomes confirms this relatively high diversity.
417 Gut phylotype richness in these samples was commensurate with the known range of richness
418 among insect microbiomes (23), but higher than what has previously been found to be typical
419 across several studies of insects (28).

420 **Possible functions of the microbes**

421 Based on past studies of microbial symbiont functions, we hypothesized that microbes
422 could play a role in carabid defensive chemical synthesis or nutrient metabolism. Specifically,
423 that a significant proportion of carabid microbial diversity could be explained by host tissue type.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

424 The association found between host tissue type and patterns of microbiome composition and
425 diversity tentatively support this hypothesis. This association, however, could also be a result of
426 other functional relationships with the host, such as commensalism or parasitism, or simply an
427 artifact of how the microbes are acquired.

428 There are no clear patterns in microbiome diversity specifically associated with guts (Fig.
429 4), so we cannot draw any conclusions about whether carabid gut microbes are involved in
430 nutrient metabolism.

431 Microbial communities associated with secretory cells have much more similar
432 composition (Fig. 2, Fig. 4) across host species than the composition associated with any other
433 tissue. This similarity is noteworthy because secretory cells are a part of the pygidial gland
434 system, an organ system which plays a conserved functional role across all host species. Possible
435 explanations for this finding include coevolution or characteristics of the environment within
436 secretory cells. This similarity is also consistent with the possibility that certain bacterial
437 phylotypes play a symbiotic role in host chemical defense. To further investigate this last
438 possibility, we checked for bacterial phylotypes that were differentially abundant in the secretory
439 cells compared to other tissues. We found four such differentially expressed phylotypes: two
440 from the genus *Flavobacterium* and two from an unidentified genus within the family
441 Comamonadaceae. Although our study design limited our ability to draw definitive conclusions,
442 it is distinctly possible that species that are especially abundant in the secretory cells could be
443 involved in defensive chemical biosynthesis. Interestingly, some *Flavobacterium* species can
444 produce quinones (52); *Flavobacterium* species are also known endosymbionts of giant scale
445 insects (53).

446 **Future directions**

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

447 This study found that patterns in microbial diversity and composition in carabid beetles
448 are not random, and that the parameters that best explain them include host tissue and species.
449 Transient changes in host diet have no significant effect on carabid microbiome diversity,
450 although maintaining host beetles in sterile soil does have a modest but significant effect.
451 Microbiome composition and diversity within the current, limited sample from across the carabid
452 phylogeny appears to agree with previous findings regarding the microbiomes of Coleoptera and
453 other insects.

454 Our results suggest that symbiosis may be a possibility, particularly in the secretory cells.
455 Given the limitations of 16S amplicon data in assessing functional microbe-host interactions,
456 future efforts to understand the nature of carabid microbiomes should consider shotgun
457 metagenomic or other approaches that more directly quantify functional genes and metabolic
458 pathways. Corroborating genetic data with experiments that confirm the metabolic activity of
459 bacterial isolates from carabid tissues could also be useful. Additional future studies might use
460 antibiotics to determine whether the presence of symbiotic microbes is essential for carabid host
461 nutrition or defensive chemistry. Due to the great diversity among carabids, understanding the
462 role of microbiomes in carabid hosts will be a key step toward understanding the diversity of
463 possible host-microbiome interactions in insects and other animals.

464 **ACKNOWLEDGMENTS**

465 We thank Wendy Moore and her lab group at the University of Arizona, Tucson, for
466 providing the *Brachinus elongatulus* for the study.

467 **REFERENCES**

468 1. Stork NE. How Many Species of Insects and Other Terrestrial Arthropods Are There on
469 Earth? Annu Rev Entomol. 2018;63(1):31–45.
470 2. Yun J-H, Roh SW, Whon TW, Jung M-J, Kim M-S, Park D-S, et al. Insect Gut Bacterial
471 Diversity Determined by Environmental Habitat, Diet, Developmental Stage, and

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

472 Phylogeny of Host. Drake HL, editor. Appl Environ Microbiol. 2014 Sep 1;80(17):5254–
473 64.
474 3. Vavre F, Kremer N. Microbial impacts on insect evolutionary diversification: from patterns
475 to mechanisms. Curr Opin Insect Sci. 2014 Oct;4:29–34.
476 4. Lorenz W, 1950-. Systematic list of extant ground beetles of the world [Internet]. W.
477 Lorenz; 2005 [cited 2019 Sep 27]. Available from: http://agris.fao.org/agris-
478 search/search.do?recordID=US201300114567
479 5. Will KW, Attygalle AthulaB, Herath K. New defensive chemical data for ground beetles
480 (Coleoptera: Carabidae): interpretations in a phylogenetic framework. Biol J Linn Soc.
481 2000 Nov;71(3):459–81.
482 6. Douglas AE. The microbial dimension in insect nutritional ecology. Funct Ecol. 2009
483 Feb;23(1):38–47.
484 7. Wong AC-N, Dobson AJ, Douglas AE. Gut microbiota dictates the metabolic response of
485 Drosophila to diet. J Exp Biol. 2014 Jun 1;217(11):1894–901.
486 8. Schwab DB, Riggs HE, Newton ILG, Moczek AP. Developmental and Ecological Benefits
487 of the Maternally Transmitted Microbiota in a Dung Beetle. Am Nat. 2016
488 Dec;188(6):679–92.
489 9. Douglas AE. Nutritional Interactions in Insect-Microbial Symbioses: Aphids and Their
490 Symbiotic Bacteria *Buchnera*. Annu Rev Entomol. 1998 Jan;43(1):17–37.
491 10. Breznak JA. Intestinal Microbiota of Termites and other Xylophagous Insects. Annu Rev
492 Microbiol. 1982 Oct;36(1):323–323.
493 11. Engel P, Moran NA. The gut microbiota of insects – diversity in structure and function.
494 FEMS Microbiol Rev. 2013 Sep;37(5):699–735.
495 12. Russell JA, Moreau CS, Goldman-Huertas B, Fujiwara M, Lohman DJ, Pierce NE.
496 Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. Proc Natl
497 Acad Sci. 2009 Dec 15;106(50):21236–41.
498 13. Lundgren JG, Lehman RM. Bacterial Gut Symbionts Contribute to Seed Digestion in an
499 Omnivorous Beetle. Van der Heijden M, editor. PLoS ONE. 2010 May 26;5(5):e10831.
500 14. Blankenchip CL, Michels DE, Braker HE, Goffredi SK. Diet breadth and exploitation of
501 exotic plants shift the core microbiome of *Cephaloleia* , a group of tropical herbivorous
502 beetles. PeerJ. 2018 May 17;6:e4793.
503 15. Adams AS, Aylward FO, Adams SM, Erbilgin N, Aukema BH, Currie CR, et al. Mountain
504 Pine Beetles Colonizing Historical and Naïve Host Trees Are Associated with a Bacterial
505 Community Highly Enriched in Genes Contributing to Terpene Metabolism. Appl Environ
506 Microbiol. 2013 Jun 1;79(11):3468–75.
507 16. Vogel H, Shukla SP, Engl T, Weiss B, Fischer R, Steiger S, et al. The digestive and
508 defensive basis of carcass utilization by the burying beetle and its microbiota. Nat
509 Commun. 2017 Aug;8(1):15186.
510 17. Kaltenpoth M, Steiger S. Unearthing carrion beetles' microbiome: characterization of
511 bacterial and fungal hindgut communities across the Silphidae. Mol Ecol. 2014
512 Mar;23(6):1251–67.
513 18. Flórez LV, Scherlach K, Miller IJ, Rodrigues A, Kwan JC, Hertweck C, et al. An antifungal
514 polyketide associated with horizontally acquired genes supports symbiont-mediated defense
515 in Lagria villosa beetles. Nat Commun. 2018 Jun 26;9(1):1–10.
516 19. Kellner RLL. Molecular identification of an endosymbiotic bacterium associated with
517 pederin biosynthesis in Paederus sabaeus (Coleoptera: Staphylinidae). Insect Biochem Mol

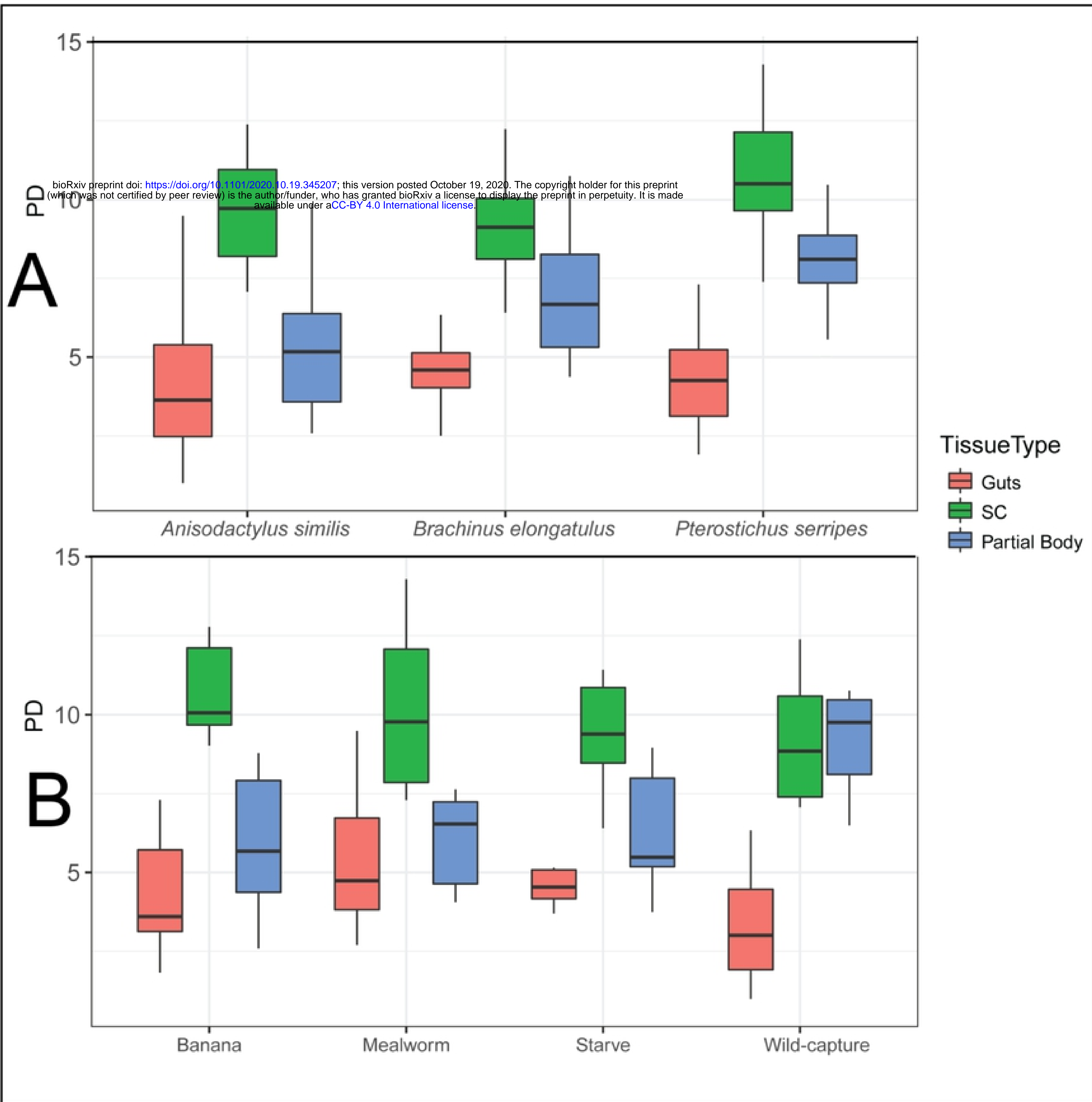
bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

518 Biol. 2002 Apr;32(4):389–95.
519 20. Nakabachi A, Ueoka R, Oshima K, Teta R, Mangoni A, Gurgui M, et al. Defensive
520 Bacteriome Symbiont with a Drastically Reduced Genome. Curr Biol. 2013 Aug
521 5;23(15):1478–84.
522 21. Jones RT, Sanchez LG, Fierer N. A Cross-Taxon Analysis of Insect-Associated Bacterial
523 Diversity. Gilbert JA, editor. PLoS ONE. 2013 Apr 16;8(4):e61218.
524 22. Brooks AW, Kohl KD, Brucker RM, van Opstal EJ, Bordenstein SR. Phylosymbiosis:
525 Relationships and Functional Effects of Microbial Communities across Host Evolutionary
526 History. Relman D, editor. PLOS Biol. 2016 Nov 18;14(11):e2000225.
527 23. Colman DR, Toolson EC, Takacs-Vesbach CD. Do diet and taxonomy influence insect gut
528 bacterial communities? Mol Ecol. 2012 Oct;21(20):5124–37.
529 24. Bahrndorff S, de Jonge N, Skovgård H, Nielsen JL. Bacterial Communities Associated with
530 Houseflies (Musca domestica L.) Sampled within and between Farms. Wilson BA, editor.
531 PLOS ONE. 2017 Jan 12;12(1):e0169753.
532 25. Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP, Fierer N. Caterpillars lack a resident gut
533 microbiome. Proc Natl Acad Sci. 2017 Sep 5;114(36):9641–6.
534 26. Scully ED, Geib SM, Carlson JE, Tien M, McKenna D, Hoover K. Functional genomics
535 and microbiome profiling of the Asian longhorned beetle (Anoplophora glabripennis)
536 reveal insights into the digestive physiology and nutritional ecology of wood feeding
537 beetles. BMC Genomics. 2014;15(1):1096.
538 27. Tang X, Freitak D, Vogel H, Ping L, Shao Y, Cordero EA, et al. Complexity and
539 Variability of Gut Commensal Microbiota in Polyphagous Lepidopteran Larvae. Dale C,
540 editor. PLoS ONE. 2012 Jul 17;7(7):e36978.
541 28. Douglas AE. Lessons from Studying Insect Symbioses. Cell Host Microbe. 2011
542 Oct;10(4):359–67.
543 29. Attygalle AB, Xu S, Moore W, McManus R, Gill A, Will K. Biosynthetic origin of
544 benzoquinones in the explosive discharge of the bombardier beetle Brachinus elongatulus.
545 Sci Nat. 2020 Aug 1;107(4):1–11.
546 30. Larochelle A. The food of carabid beetles (Coleoptera: Carabidae, including Cicindelinae).
547 Fabreries. 1990;Supplément 5:1–132.
548 31. McManus R, Ravenscraft A, Moore W. Bacterial Associates of a Gregarious Riparian
549 Beetle With Explosive Defensive Chemistry. Front Microbiol. 2018 Oct 5;9:2361.
550 32. Rohland N, Reich D. Cost-effective, high-throughput DNA sequencing libraries for
551 multiplexed target capture. Genome Res. 2012 May 1;22(5):939–46.
552 33. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-
553 high-throughput microbial community analysis on the Illumina HiSeq and MiSeq
554 platforms. ISME J. 2012 Aug;6(8):1621–4.
555 34. Lange V, Böhme I, Hofmann J, Lang K, Sauter J, Schöne B, et al. Cost-efficient high-
556 throughput HLA typing by MiSeq amplicon sequencing. BMC Genomics. 2014 Jan
557 24;15:63.
558 35. Renaud G. grenaud/deML [Internet]. 2019 [cited 2019 Sep 25]. Available from:
559 https://github.com/grenaud/deML
560 36. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2:
561 High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016
562 Jul;13(7):581–3.
563 37. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

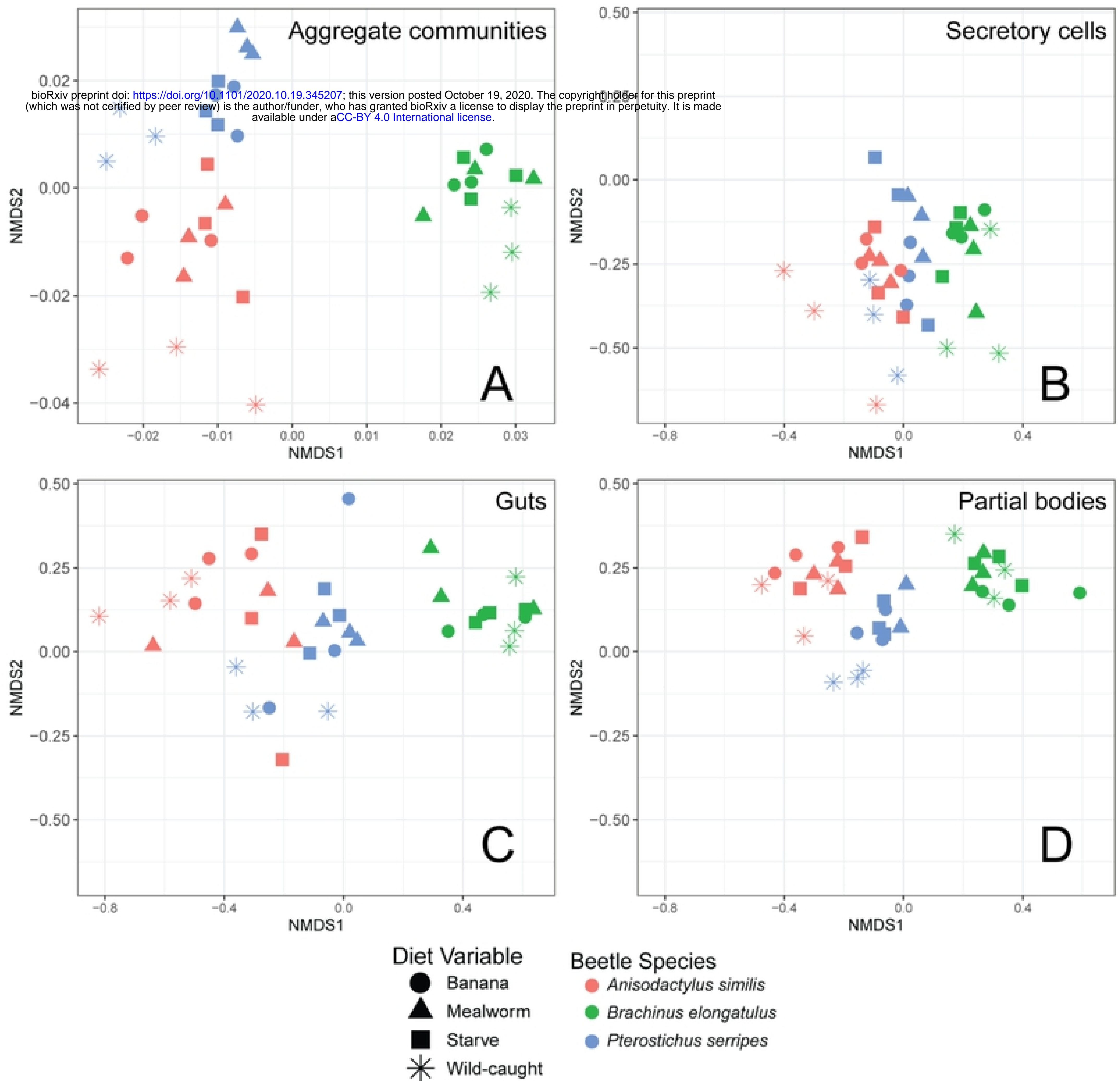
564 Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 2014 Jan
565 1;42(Database issue):D633–42.
566 38. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna,
567 Austria: R Foundation for Statistical Computing; 2018 [cited 2019 Sep 25]. Available from:
568 https://www.R-project.org/
569 39. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis
570 and Graphics of Microbiome Census Data. PLOS ONE. 2013 Apr 22;8(4):e61217.
571 40. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for
572 RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
573 41. McMurdie PJ, Holmes S. Waste Not, Want Not: Why Rarefying Microbiome Data Is
574 Inadmissible. PLOS Comput Biol. 2014 Apr 3;10(4):e1003531.
575 42. Wright ES. DECIPHER: harnessing local sequence context to improve protein multiple
576 sequence alignment. BMC Bioinformatics. 2015 Oct 6;16(1):322.
577 43. Wright E S. Using DECIPHER v2.0 to Analyze Big Biological Sequence Data in R. R J.
578 2016;8(1):352.
579 44. Schliep KP. phangorn: phylogenetic analysis in R. Bioinformatics. 2011 Feb 15;27(4):592–
580 3.
581 45. Quensen J. QsRutils: R Functions Useful for Community Ecology [Internet]. 2019 [cited
582 2019 Sep 25]. Available from: https://github.com/jfq3/QsRutils
583 46. Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, et al.
584 Picante: R tools for integrating phylogenies and ecology. Bioinformatics. 2010 Jun
585 1;26(11):1463–4.
586 47. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan:
587 Community Ecology Package [Internet]. 2019 [cited 2019 Sep 25]. Available from:
588 https://CRAN.R-project.org/package=vegan
589 48. Chen H. VennDiagram: Generate High-Resolution Venn and Euler Plots [Internet]. 2018
590 [cited 2019 Sep 25]. Available from: https://CRAN.R-project.org/package=VennDiagram
591 49. Paradis E, Schliep K. ape 5.0: an environment for modern phylogenetics and evolutionary
592 analyses in R. Bioinformatics. 2019 Feb 1;35(3):526–8.
593 50. Franzini PZN, Ramond J-B, Scholtz CH, Sole CL, Ronca S, Cowan DA. The Gut
594 Microbiomes of Two Pachysoma MacLeay Desert Dung Beetle Species (Coleoptera:
595 Scarabaeidae: Scarabaeinae) Feeding on Different Diets. Forster RJ, editor. PLOS ONE.
596 2016 Aug 17;11(8):e0161118.
597 51. Lundgren JG, Lehman RM, Chee-sanford J. Bacterial Communities within Digestive Tracts
598 of Ground Beetles (Coleoptera: Carabidae). Ann Entomol Soc Am. 2007 Mar
599 1;100(2):275–82.
600 52. Collins MD, Ross HNM, Tindall BJ, Grant WD. Distribution of Isoprenoid Quinones in
601 Halophilic Bacteria. J Appl Bacteriol. 1981 Jun;50(3):559–65.
602 53. Matsuura Y, Koga R, Nikoh N, Meng X-Y, Hanada S, Fukatsu T. Huge Symbiotic Organs
603 in Giant Scale Insects of the Genus Drosicha (Coccoidea: Monophlebidae) Harbor
604 Flavobacterial and Enterobacterial Endosymbionts. Zoolog Sci. 2009 Jul;26(7):448–56.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.

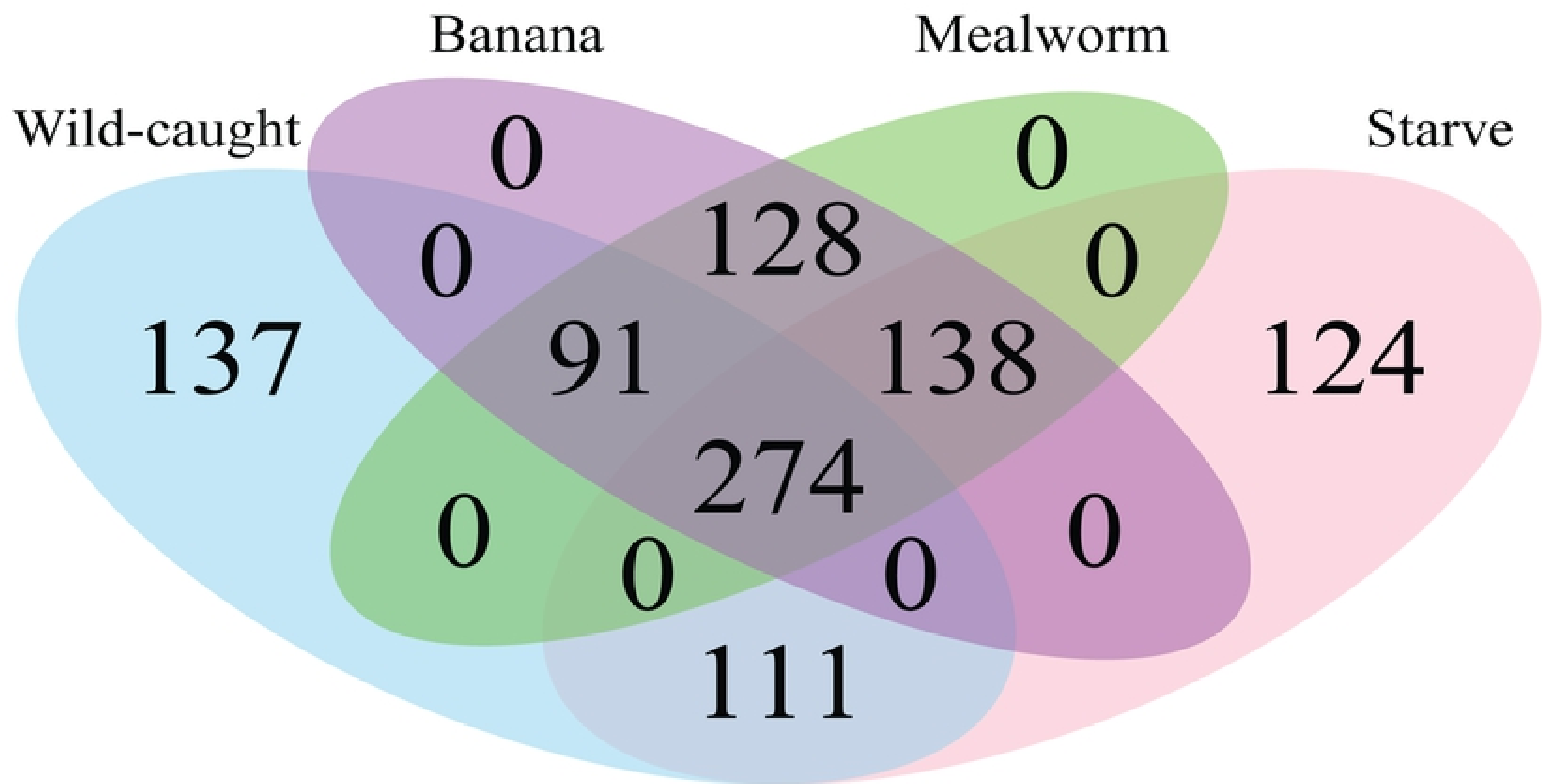


Figure

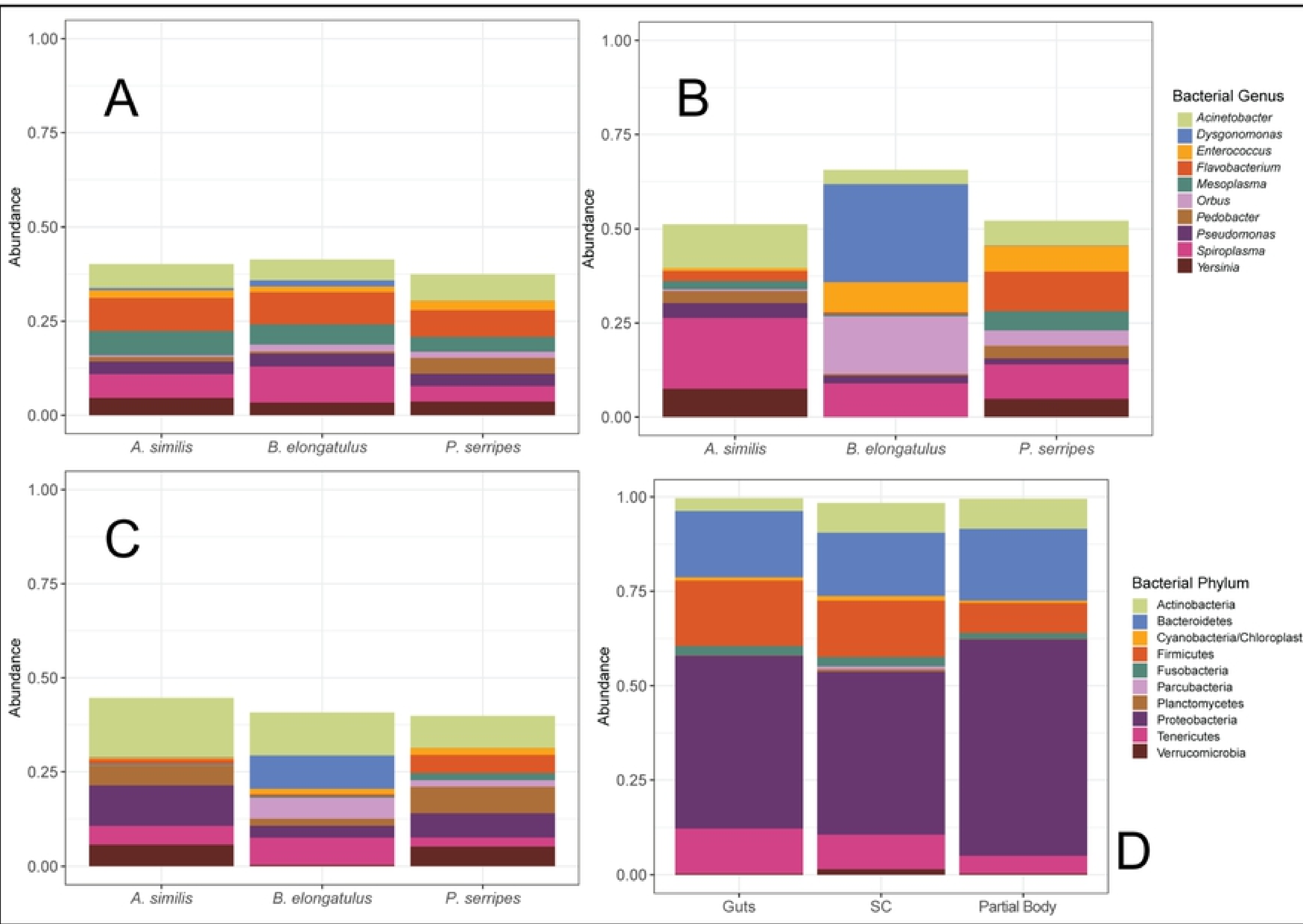
bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.



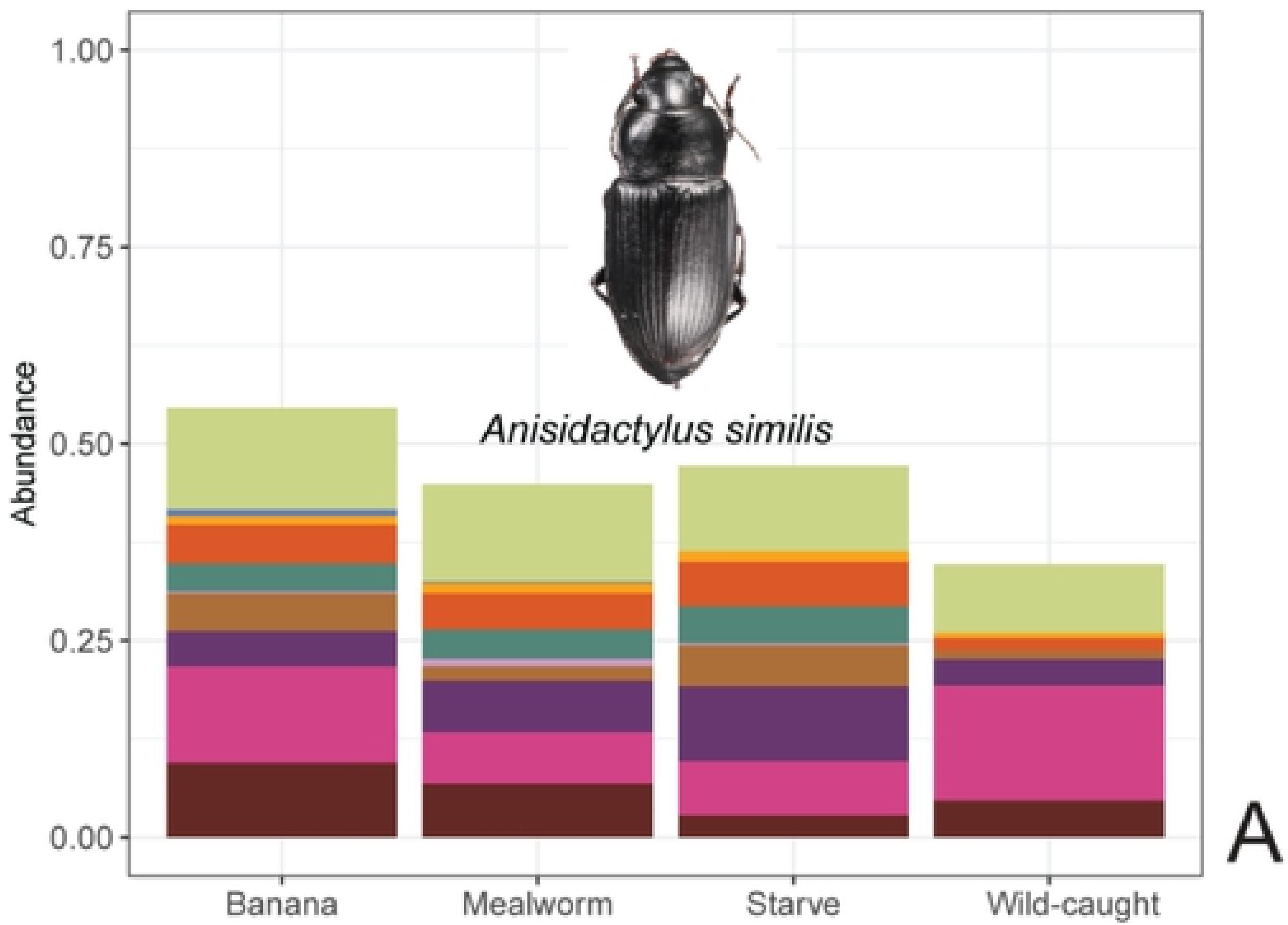
Figure



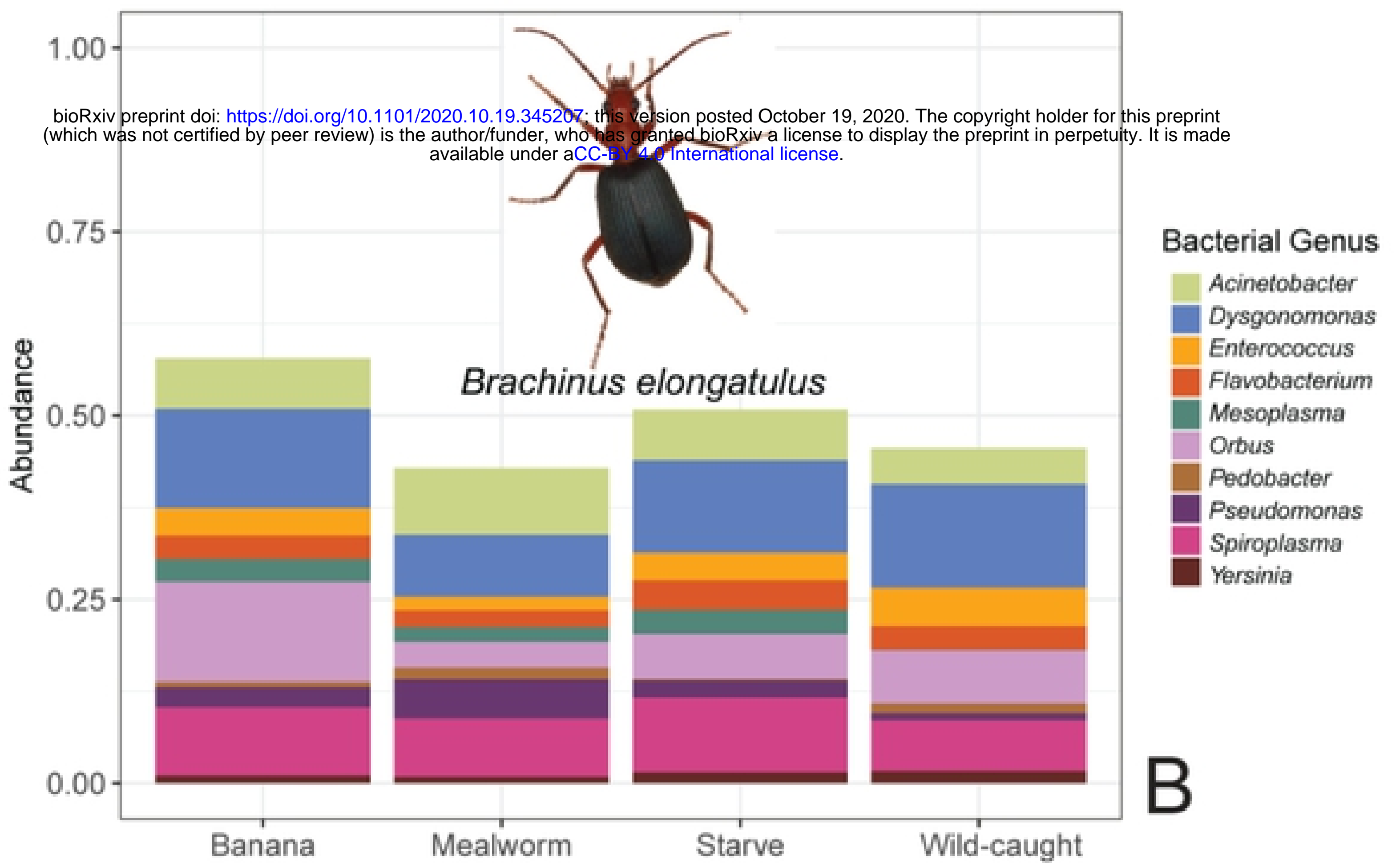
Figure

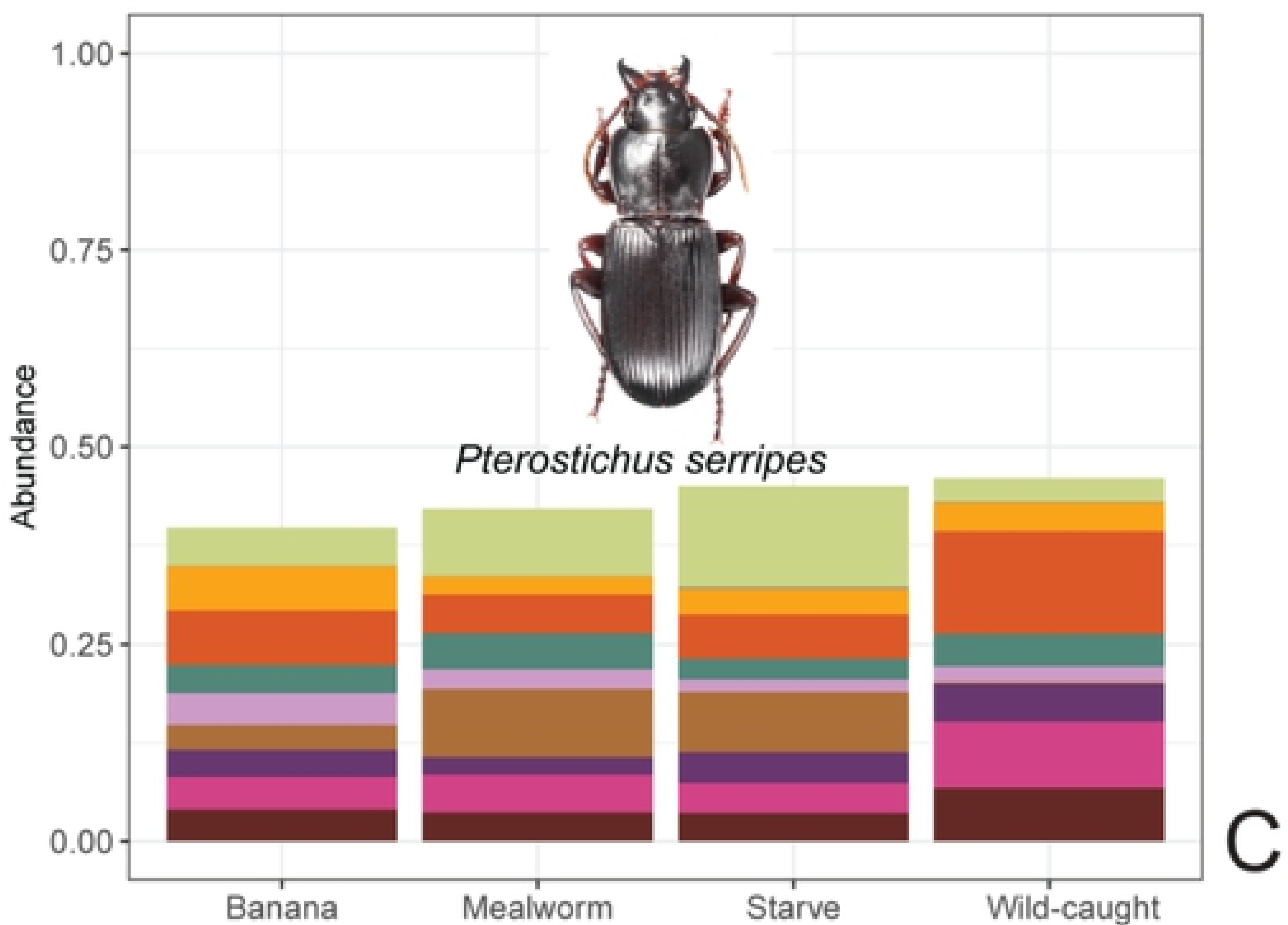


Figure



bioRxiv preprint doi: https://doi.org/10.1101/2020.10.19.345207; this version posted October 19, 2020. 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 4.0 International license.





Figure