

***Bdellovibrio* and Like Organisms are Predictors of Microbiome Diversity in distinct Host Groups**

**Julia Johnke¹, Sebastian Fraune², Thomas Bosch², Ute Hentschel^{3,4}, and
Hinrich Schulenburg^{1*}**

¹Department of Evolutionary Ecology and Genetics, Zoological Institute, CAU Kiel,
Am Botanischen Garten 1-9, 24118 Kiel, Germany

²Department of Cell and Developmental Biology, Zoological Institute, CAU Kiel, Am
Botanischen Garten 1-9, 24118 Kiel, Germany

³RD3 Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel,
Düsternbrooker Weg 20, 24105 Kiel, Germany.

⁴Section of Marine Biology, CAU Kiel, Am Botanischen Garten 1-9, 24118 Kiel,
Germany.

*** Correspondence:**

Hinrich Schulenburg
hschulenburg@zoologie.uni-kiel.de
+49 431 880 4141

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28 **Abstract**

29

30 Biodiversity is generally believed to be a main determinant of ecosystem functioning.
31 This principle also applies to the microbiome and could consequently contribute to host
32 health. According to ecological theory, communities are shaped by top predators
33 whose direct and indirect interactions with community members cause stability and
34 diversity. *Bdellovibrio* and like organisms (BALOs) are a neglected group of predatory
35 bacteria that feed on Gram-negative bacteria and can thereby influence microbiome
36 composition. We asked whether BALOs can predict biodiversity levels in microbiomes
37 from distinct host groups and environments. We demonstrate that genetic signatures
38 of BALOs are commonly found within the 16S rRNA reads from diverse host taxa. In
39 many cases, their presence, abundance, and especially richness are positively
40 correlated with overall microbiome diversity. Our findings suggest that BALOs can act
41 as drivers of microbial alpha-diversity and should therefore be considered as
42 candidates for the restoration of microbiomes and the prevention of dysbiosis.

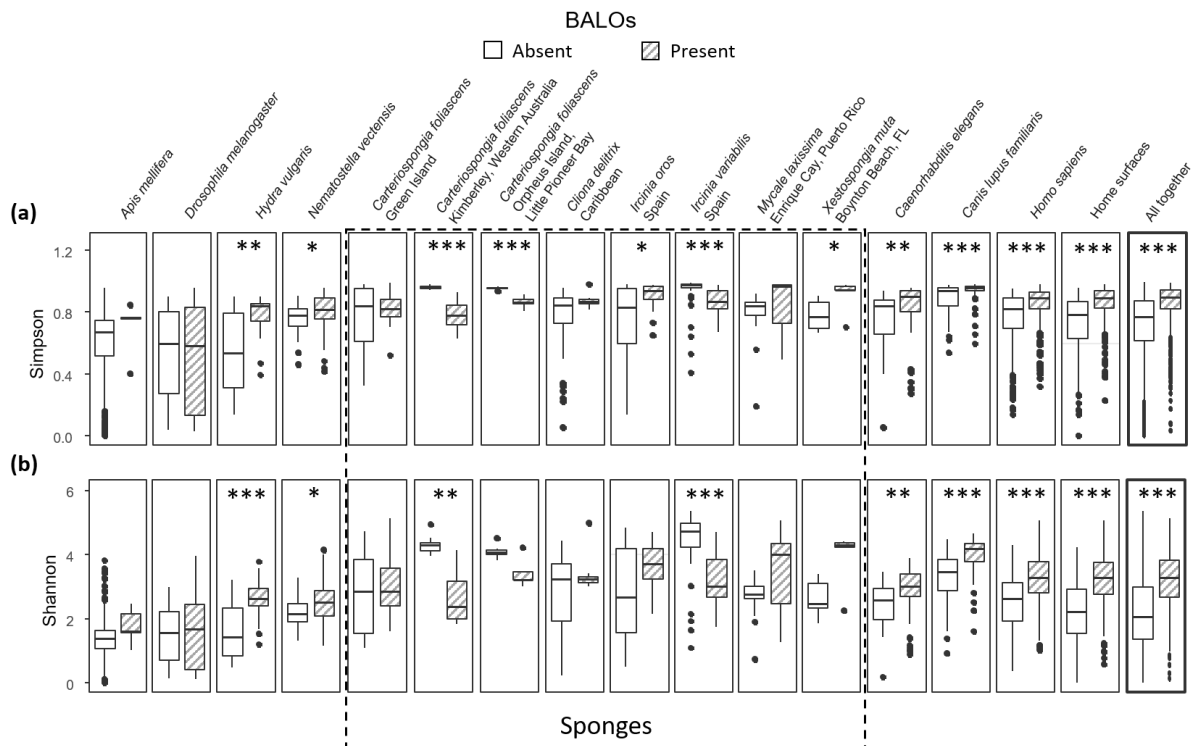
43 Biodiversity is a key attribute of productive [1] and stable ecosystems [2]. This is likely
44 due to the activity of highly productive keystone species [3], which are often more
45 common in species-rich communities [1]. Nevertheless, productivity and stability
46 appear to be mainly driven by diversity itself and not by individual taxa [4]. Species-
47 rich communities exist for example in the human gut and oral microbiome and are
48 usually assumed to consist of functionally redundant species that act as insurance in
49 case of extinctions [5, 6]. Consequently, species-rich communities are more resilient
50 (cf. [7]). To date, most studies on the effect of biodiversity on ecosystem functioning
51 and specifically the effect of microbiome composition on host health have focused on
52 a single trophic level. Yet, changes in the diversity of one trophic level can affect other
53 trophic levels, either directly through consumer-resource interactions or indirectly when
54 the decrease of one species leads to abundance changes of other species [8]. The
55 presence of top predators has particularly strong effects because they can limit
56 dominant species abundance and thereby free niches for rare taxa [9–11]. The impact
57 of predators is likely distinct from environmental stressors, which may similarly free
58 niches and subsequently increase microbiome diversity, as recently documented for
59 the microbiome of *Daphnia* waterfleas after antibiotic exposure [12]. Yet, in this case,
60 the effect on community composition is likely to be random, whereas predators usually
61 target the dominant species.

62 *Bdellovibrio* and like organisms (BALOs) are obligate predators of Gram-negative
63 bacteria in a wide range of habitats [13, 14]. BALOs were recently linked to a healthy
64 human gut microbiome [15], and proposed as living antibiotics in medical treatment
65 [16] and water remediation [17]. Additionally, a microcosm experiment showed that
66 their predatory activity can exceed phage-induced mortality [18]. We here draw
67 attention to this neglected group of predators and tested their association with
68 microbial diversity as an indicator of a healthy microbiome across distinct animal host
69 groups and environments.

70
71 We analyzed 16S rRNA data from randomly chosen, exemplary host taxa that are
72 representative of distinct animal taxonomic groups, including early branching
73 metazoans, ecdysozoa, selected vertebrates, and additionally home surfaces (Table
74 S1 and Supplementary Methods). We only considered studies, if they included
75 samples with and without BALOs, thereby allowing us to determine the consequences
76 of BALO presence and absence in comparable groups. We determined BALO
77 occurrence (although not necessarily activity) by identifying OTUs that showed 97%
78 sequence identity to members of the BALO-containing taxonomic groups
79 *Bdellovibrionales* (including the families *Bacteriovoracaceae* and *Bdellovibrionaceae*)
80 and *Micavibrionales* (including *Micavibrionaceae*). From these data, we inferred
81 relative BALO abundance and corresponding microbiome alpha- (i.e., Shannon-
82 Wiener diversity, Simpson's diversity, richness) and beta-diversities.

83
84 The presence of BALOs was associated with a significantly higher Simpson and
85 Shannon diversity for the microbiomes of seven and five host species, respectively, as
86 well as the home surfaces (Figure 1, Table S2). The main exceptions referred to two
87 sponge species, *Carteriospongia foliascens* and *Ircinia variabilis*, which showed a
88 significantly higher alpha-diversity in the absence of BALOs. This negative association
89 was not observed for microbiome richness (Figure S1, Table S3). Our subsequent
90 analysis of absolute OTU numbers revealed that microbiome richness is significantly
91 associated with both BALO abundance (Figure 2a, Table S4) and BALO richness
92 (Figure 2b, Table S4) in case of *H. vulgaris* and the sponges. A trend toward this
93 association was additionally observed for *N. vectensis* and *D. melanogaster*.

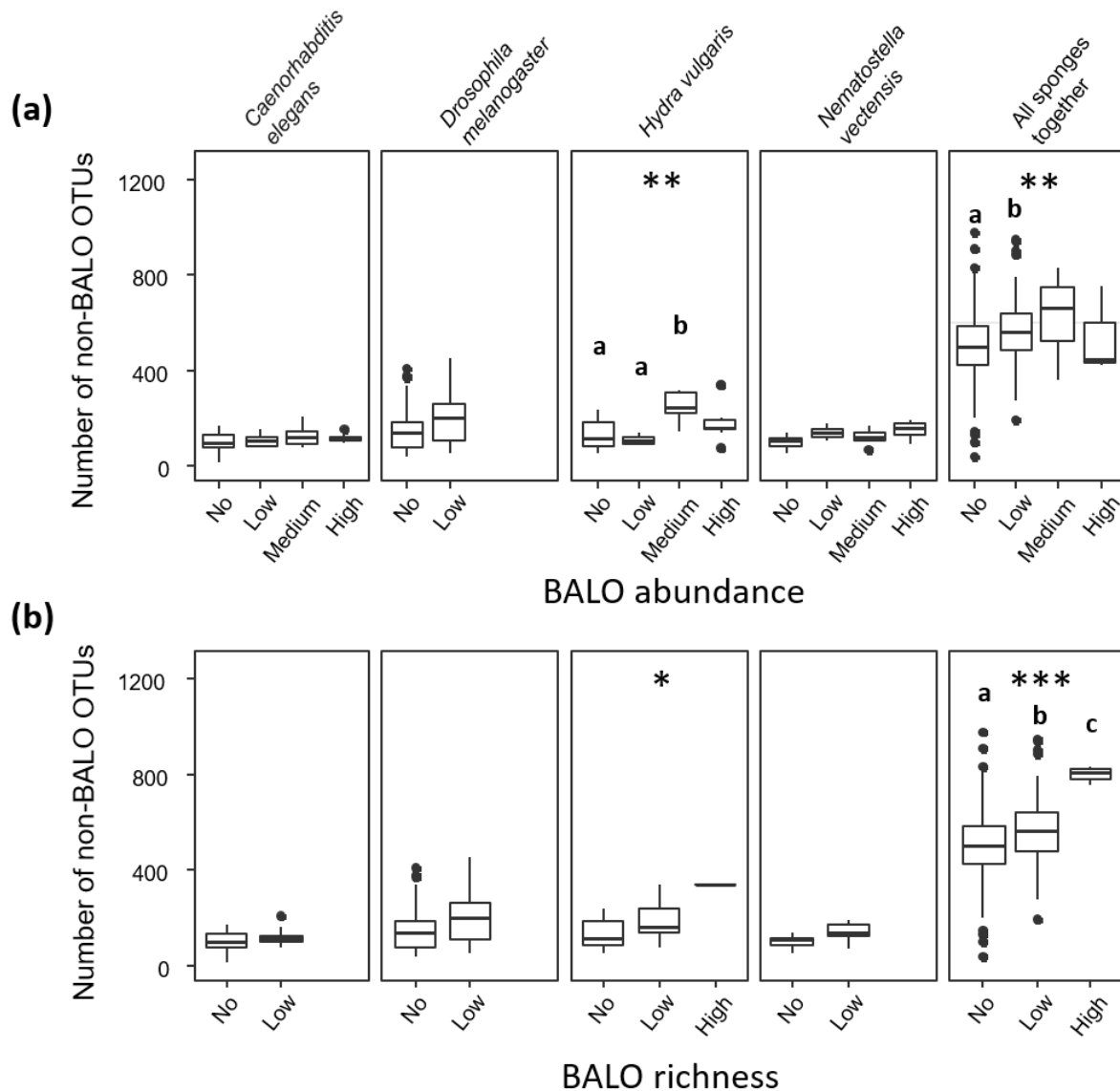
94 Interestingly, for both host systems, OTU richness was highest with medium BALO
 95 abundance, which possibly indicates that BALO richness rather than abundance
 96 influences microbiome richness.
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100 **Fig. 1.** Microbiome alpha-diversity in the presence and absence of BALOs. The
 101 Simpson (a) and Shannon (b) diversity is shown for a set of different hosts. Significant
 102 differences are indicated by asterisks and were calculated using the Wilcoxon rank
 103 sum test. P-values: $p < 0.001$: '***', $0.0011 > p < 0.01$: '**', $0.011 > p < 0.05$: '*'. P-values are
 104 given in the Table S2.

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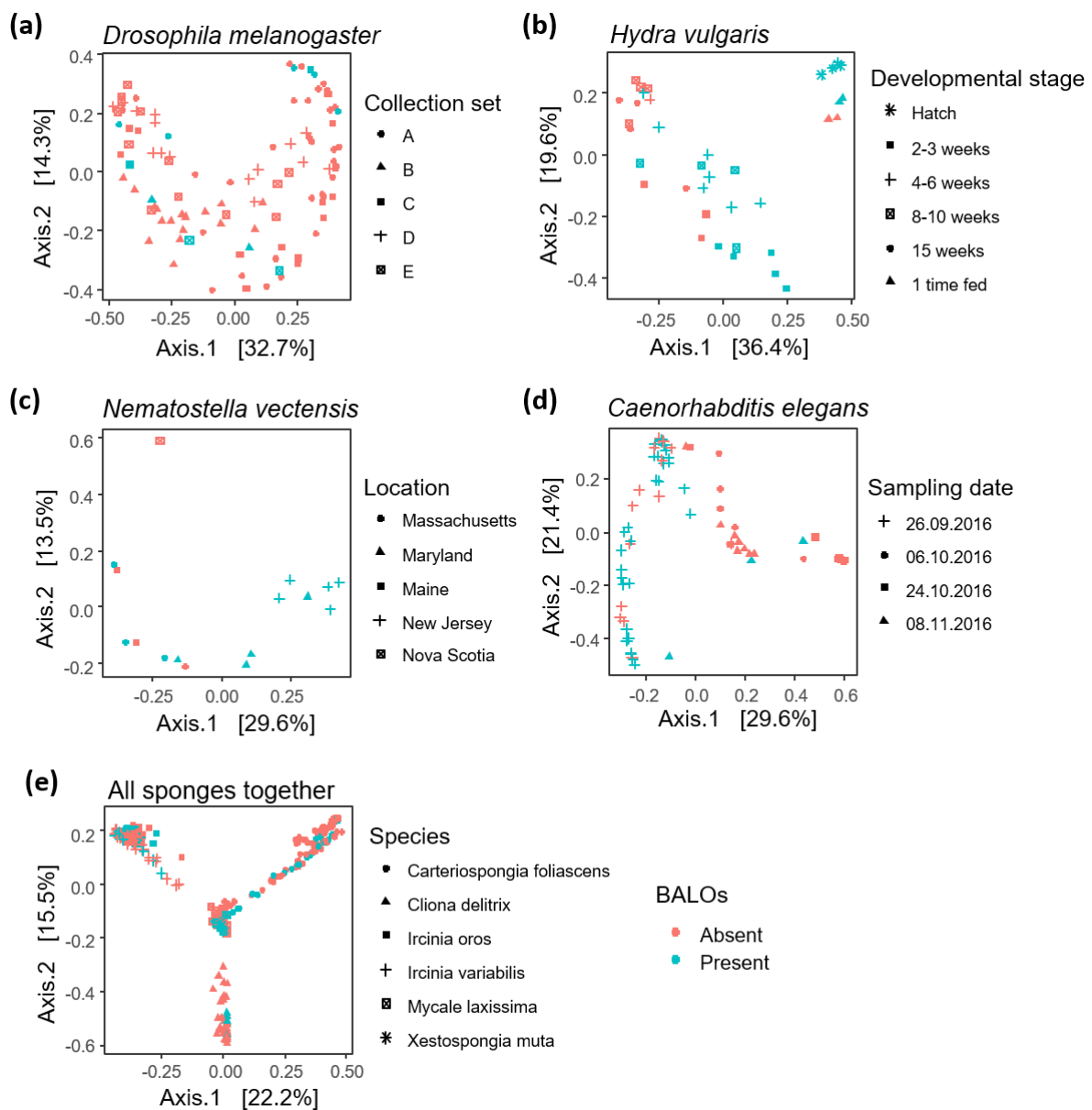


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 108 **Fig. 2.** Host microbiome richness measured as number of different non-BALO OTUs
 109 with increasing BALO abundance (a) and BALO richness (b). Significant differences
 110 are indicated by asterisks and were calculated using the Kruskal-Wallis rank sum test.
 111 P-values: $p < 0.001$: '***', $0.0011 > p < 0.01$: '**', $0.011 > p < 0.05$: '*'. Significant differences
 112 between single categories of BALO abundance and BALO richness are indicated by
 113 different letters and were calculated with Dunn's post hoc test. All P-values are given
 114 in the Table S4.

115
 116
 117 In contrast, variation in microbiome beta-diversity was not linked to the BALOs (Figure
 118 3). At the same time, our PCoA analysis indicated an influence of BALOs on sample
 119 clustering for several hosts (especially cnidarians and *C. elegans*). However, the
 120 clustering was not independent of sample type, making it impossible to infer the exact
 121 cause of clustering from the current data.

122 To exclude that BALO presence is caused by high microbiome diversity as a
 123 consequence of sampling effects, we analyzed the complete sponge dataset,
 124 additionally including species without BALOs [19]. We found that alpha-diversity *per*
 125 *se* does not predict the presence of BALOs (Table S5 and S6), which is therefore
 126 unlikely caused by sampling effects alone.

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130 **Fig. 3.** PCoA of microbiome samples from different hosts using Bray Curtis distances.

131 Samples are color-coded by presence and absence of BALOs. Different shapes

132 indicate different sample subsets as indicated by the respective legends.

133

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135 The loss of top predators has comprehensive effects on community structure [9, 10].

136 We tested this idea by comparing microbiome alpha-diversities for distinct animal hosts

137 and environments that either lacked or contained a prominent group of microbial

138 predators, the BALOs. With the exception of the considered insects and most sponge

139 species, we found that microbiomes containing BALOs were characterized by a

140 significantly higher alpha-diversity.

141 In contrast to the overall results, two sponge species showed a negative correlation

142 between BALO presence and microbiome diversity, although not when considering

143 microbiome richness. These results may suggest that BALO-containing sponges

144 harbor a more species-rich, but less even microbiome. Notably, sponges in general

145 possess a comparatively species-rich microbiome (Figure S1). In these cases,
146 evenness may be negatively correlated to richness, consistent with previous
147 observations for plant communities [20] and possibly due to sampling effects, where a
148 superior competitor is more likely present in species-rich communities [1]. A niche-
149 preemption model was previously identified to be the best predictor for the patterns in
150 plant communities [20]. Niche-preemption should favor resource use plasticity among
151 the less competitive species, resulting in lower growth and consequently reduced
152 evenness. In case of the sponges, the negative richness-evenness-relationship might
153 then overshadow the effect of BALOs on microbiome diversity. Temporal effects could
154 additionally explain the higher sponge microbiome diversity in the absence of BALOs.
155 As the sponge data used in this study came from single time point samples, we cannot
156 exclude subsequent changes in the community structure, for example a delayed effect
157 of BALO loss or gain on microbiome diversity. However, the longitudinal data on
158 surface microbiomes [21] indicates that changes in BALO presence/absence are
159 associated with more or less simultaneously occurring changes in OTU richness (Fig.
160 S2).

161 We found that BALO OTU richness, rather than abundance, is significantly associated
162 with microbiome richness in *H. vulgaris* and the combined set of sponges. Moreover,
163 this significant association between BALO and microbiome richness was only
164 observed when the high BALO richness category could be included. Considering that
165 different BALO strains are known to vary in their range of suitable prey [22], the above
166 results may suggest that a more diverse BALO community is able to prey on a more
167 diverse set of bacteria and thereby reduces the predation pressure on single species,
168 thus increasing microbiome diversity.

169 Our additional analysis of beta-diversity did not reveal a strong BALO influence on
170 microbiome community structure. Together with the results on alpha-diversity, this may
171 imply that BALO presence is not correlated with a specific community composition and
172 that BALOs survive in a range of differently assembled communities.

173 Our results from a range of distinct animal hosts and environments point to BALOs as
174 potential drivers of microbiome alpha-diversity, possibly by actively preying on highly
175 abundant species, thereby favoring rare species. Thus, BALOs may be of particular
176 importance for our understanding of the stability and resilience of microbiome
177 ecosystem functions. Our current meta-analysis is, however, based on associations,
178 which can only be indicative of possible causal relationships. An important next step
179 should therefore be a detailed experimental analysis of the exact causal role of BALOs
180 on microbiome diversity and resulting functions. It would be of similar high interest to
181 assess to what extent other kinds of bacterial antagonists, such as phages, or
182 environmental stressors may also influence microbiome diversity and the associated
183 effects. Moreover, it is worth testing whether the interaction between BALOs and other
184 bacteria is additionally shaped by the host immune system, which could cause different
185 dynamics of the BALO-mediated effects within rather than outside host organisms.

186 Considering that BALOs are not pathogenic to higher organisms [23], have a likely
187 stronger effect on community structure than phages [18], and appear to enhance
188 microbial diversity, they are highly promising candidates for probiotic therapy [24] that
189 aims at restoring disturbed microbiomes and improving host health or ecosystem
190 productivity and stability.

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200 **Conflict of interest statement**

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202 The authors declare no conflict of interest.

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Supplementary Material

291 ***Bdellovibrio* and Like Organisms are Predictors of** 292 **Microbiome Diversity across Diverse Host Groups**

293 **Julia Johnke¹, Sebastian Fraune², Thomas Bosch², Ute Hentschel^{3,4}, and Hinrich**
294 **Schulenburg^{1*}**

295 ¹Department of Evolutionary Ecology and Genetics, Zoological Institute, CAU Kiel, Am
296 Botanischen Garten 1-9, 24118 Kiel, Germany

297 ²Department of Cell and Developmental Biology, Zoological Institute, CAU Kiel, Am
298 Botanischen Garten 1-9, 24118 Kiel, Germany

299 ³RD3 Marine Microbiology, GEOMAR Helmholtz Centre for Ocean Research Kiel,
300 Düsternbrooker Weg 20, 24105 Kiel, Germany.

301 ⁴Section of Marine Biology, CAU Kiel, Am Botanischen Garten 1-9, 24118 Kiel, Germany.

302 * **Corresponding author:** Hinrich Schulenburg
303 hschulenburg@zoologie.uni-kiel.de

304

305 **1 Supplementary Methods**

306 For our analysis, we randomly selected exemplary host taxa that are representative of distinct
307 taxonomic animal groups, ranging from very simple to more complex hosts and including early
308 branching invertebrates, ecdysozoa, and also vertebrates (Table S1). In addition, we only
309 considered host taxa, for which a single study included at least five samples with and without
310 BALOs - with the exception of the *Nematostella* dataset with only four samples without BALOs.
311 This preselection was performed in order to allow a direct comparison of samples with and
312 without BALOs for each host system or environment. Further, only studies with publicly
313 available OTU tables were selected. Moreover, we considered one study with longitudinal data
314 generated from human mucus, sebum, skin swabs, as well as from different surfaces from
315 their family homes [10]. This data set served to test the stability of the association of BALO
316 presence and bacterial community diversity across time within the same environment.

317 Several datasets were from microbiomeDB (<http://microbiomedb.org/mbio/>) and only included
318 relative abundance data, while the remaining data sets also had information on absolute
319 frequencies. The *Caenorhabditis elegans* dataset was produced by us for this study by
320 sampling worms from the Kiel Botanical garden in 2016 at four consecutive time points (one in
321 October, two in September, and one in November). Worm samples were prepared as
322 described previously [1] by using the protocol for “natural worm” microbiome extraction. DNA
323 was sequenced using the Miseq platform and the primers 515f-806r to sequence the V4 region
324 of the 16S rRNA gene. Original sequence data are available from the European Nucleotide
325 Archive (accession number PRJEB30476). Sequence reads were analyzed using Mothur v.
326 1.39.5 [2] as described in the Miseq SOP (https://www.mothur.org/wiki/MiSeq_SOP) and the
327 SILVA reference database version 128. OTU clustering was based on 97% sequence identity.
328 Samples with BALOs were categorized based on their abundance (i.e., high (11-227 reads),
329 medium (6-10), low (1-5), and no reads) and richness (high (5-7), low (1-4), and no). We
330 compared microbiome alpha-diversity in the presence and absence of BALOs using two-
331 sample Wilcoxon rank sum tests to account for outliers. We assessed the influence of BALO
332 abundance or richness on microbiome richness with the Kruskal-Wallis rank sum test and
333 Dunn’s post hoc test with p-value adjustment using *fdr*. Beta-diversity was measured using
334 Bray Curtis distance on relative abundance and visualized using PCoA of the 500 most
335 abundant OTUs. Sponge samples were analyzed using Fisher’s exact test and the Wilcoxon
336 rank sum test to test for an association between BALO presence/absence and microbiome
337 alpha-diversity, either as categorical or continuous variable. All statistical analyses were
338 performed in R [3] using phyloseq [4] and vegan [5].

339 **2 Supplementary Figures and Tables**

340 Table S1: Summary of the considered and analyzed studies.

Study	Host body site	Seq. platform	Environment of host	N samples	Normalization	Further information
<i>Caenorhabditis elegans</i> (Nematoda)						
This study*	Gut	Miseq (V4)	Natural isolates from compost heaps	73	4986 reads per sample	Time series
<i>Nematostella vectensis</i> (Cnidaria)						
[6]	Whole animals	454 (V2)	Natural isolates, but maintained in the lab for 10 years as clonal lines	16	3000 reads per sample	Microbiome diversity of species sampled along the US east coast
Six sponge species						
[7]	Random sponge pieces	Hiseq (V4)	Natural isolates from different sites	315 samples	No normalization	Different species and different sampling sites
<i>Drosophila melanogaster</i> (Insecta, Diptera)						
[8]	Whole flies	Miseq (V3-V4)	Samples taken from various kitchen	79	1200 reads per sample	Only adult flies
<i>Apis mellifera</i> (Insecta, Hymenoptera)						
Unpublished, Dominguez-Bello	whole head, whole larva, whole pupa, whole gut	Unknown	Unknown	383	Unknown	From microbiomeDB, different functional guilds and developmental stages, effect of Tetracycline application
<i>Canis lupus familiaris</i> (Vertebrata, Mammalia)						
[9]	Sebum	Illumina GAIIx (V2)	Different homes	145	5000 reads per sample	From microbiomeDB, most BALOs in sebum und mucus
Home surfaces						
[10]	Different surfaces from family homes	Hiseq (V4)	Different homes	690	2500 reads per sample	From microbiomeDB
<i>Homo sapiens</i> (Vertebrata, Mammalia)						
[10]	Mucus, sebum, skin swabs	Hiseq (V4)	Different homes	910	2500 reads per sample	From microbiomeDB
<i>Hydra vulgaris</i> (Cnidaria)						
[11]	Whole animals	454 (V1-V2)	Lab-kept animals	36	No normalization	Developmental data

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342

343 Table S2: Test statistics of the comparison of microbiome alpha-diversity in the absence and
 344 presence of BALOs as shown in Fig. 1.

diversity measure	host	W	P
Simpson	<i>Apis mellifera</i>	594	0.1541
Simpson	<i>Caenorhabditis elegans</i>	774	0.001617
Simpson	<i>Canis lupus familiaris</i>	1382	< 0.001
Simpson	<i>Drosophila melanogaster</i>	506	0.9089
Simpson	<i>Homo sapiens</i>	57463	< 0.001
Simpson	<i>Nematostella vectensis</i>	736	0.03724
Simpson	Family homes	27095	< 0.001
Simpson	<i>Hydra vulgaris</i>	68	< 0.001
Simpson	<i>Carteriospongia foliascens</i> _Green Island	91	0.589
Simpson	<i>Carteriospongia foliascens</i> _Kimberley, Western Australia	48	< 0.001
Simpson	<i>Carteriospongia foliascens</i> _Orpheus Island, Little Pioneer Bay	50	< 0.001
Simpson	<i>Cliona delitrix</i> _Caribbean	140	0.3135
Simpson	<i>Ircinia oros</i> _Spain	148	0.01725
Simpson	<i>Ircinia variabilis</i> _Spain	425	< 0.001
Simpson	<i>Mycale laxissima</i> _Enrique Cay, Puerto Rico	35	0.1653
Simpson	<i>Xestospongia muta</i> _Boynton Beach, FL	5	0.04798
Simpson	All together	486490	< 0.001
Shannon	<i>Apis mellifera</i>	557	0.1151
Shannon	<i>Caenorhabditis elegans</i>	792	0.0025
Shannon	<i>Canis lupus familiaris</i>	1019	< 0.001
Shannon	<i>Drosophila melanogaster</i>	487	0.9348
Shannon	<i>Homo sapiens</i>	49350	< 0.001
Shannon	<i>Nematostella vectensis</i>	729	0.0327
Shannon	Family homes	22200	< 0.001
Shannon	<i>Hydra vulgaris</i>	58	< 0.001
Shannon	<i>Carteriospongia foliascens</i> _Green Island	85	0.4231
Shannon	<i>Carteriospongia foliascens</i> _Kimberley, Western Australia	45	0.004662
Shannon	<i>Carteriospongia foliascens</i> _Orpheus Island, Little Pioneer Bay	41	0.05528
Shannon	<i>Cliona delitrix</i> _Caribbean	159	0.6025
Shannon	<i>Ircinia oros</i> _Spain	185	0.1247
Shannon	<i>Ircinia variabilis</i> _Spain	417	< 0.001
Shannon	<i>Mycale laxissima</i> _Enrique Cay, Puerto Rico	35	0.1653
Shannon	<i>Xestospongia muta</i> _Boynton Beach, FL	6	0.07323
Shannon	All together	415580	< 0.001

345

346 Table S3: Test statistics of the comparison of microbiome richness in the presence and
347 absence of BALOs as shown in Fig. S1.

Host	W	P
<i>Caenorhabditis elegans</i>	178.5	0.06802
<i>Drosophila melanogaster</i>	348	0.1102
<i>Hydra vulgaris</i>	88.5	0.003041
<i>Nematostella vectensis</i>	11	0.1293
All sponges together	4531.5	< 0.001
<i>Carteriospongia foliascens</i> _Green Island	144	< 0.001
<i>Carteriospongia foliascens</i> _Kimberley, Western Australia	70	0.9321
<i>Carteriospongia foliascens</i> _Orpheus Island, Little Pioneer Bay	116	0.4946
<i>Cliona delitrix</i> _Caribbean	468	0.021
<i>Ircinia oros</i> _Spain	518	< 0.001
<i>Ircinia variabilis</i> _Spain	664	0.01112
<i>Mycale laxissima</i> _Enrique Cay, Puerto Rico	220	0.9319
<i>Xestospongia muta</i> _Boynton Beach, FL'	42	0.1058

348

349 Table S4: Test statistics of the comparison of microbiome richness and BALO abundance
 350 and BALO richness as shown in Fig. 2.

host	category	Kruskal-Wallis Chi-squared	Df	P	Significant Dunn's
<i>Caenorhabditis elegans</i>	BALO abundance	3.3508	1	0.06717	
<i>Drosophila melanogaster</i>	BALO abundance	6.3712	3	0.09488	
<i>Hydra vulgaris</i>	BALO abundance	12.795	3	0.005102	Medium:Low P = 0.014, No:Medium P = 0.011
<i>Nematostella vectensis</i>	BALO abundance	3.7776	3	0.2865	
All sponges together	BALO abundance	14.573	3	0.002221	No:Low P = 0.0033
<i>Caenorhabditis elegans</i>	BALO diversity	4.2926	3	0.2316	
<i>Drosophila melanogaster</i>	BALO diversity	2.5684	1	0.109	
<i>Hydra vulgaris</i>	BALO diversity	6.7652	2	0.03396	
<i>Nematostella vectensis</i>	BALO diversity	2.489	1	0.1146	
All sponges together	BALO diversity	17.87	2	< 0.001	No:Low P = 0.0032, No:High P = 0.0053, Low:High P = 0.0349

351

352 Table S5: Test statistics of the comparison of sponge microbiome alpha-diversity category
 353 and BALO presence. Contingency tables are based on the average of the respective value
 354 (given in brackets for the different categories) for each species.

	Microbiome richness ^a		Fisher's Exact Test	
	high (≥ 600 - 809.25)	low (306.67 - < 600)	P	Odds ratio
BALOs present	10	38	0.7356	0.79247
BALOs absent	4	12		

	Microbiome Simpson diversity		Fisher's Exact Test	
	high (≥ 0.9)	low (0.38 - < 0.9)	P	Odds ratio
BALOs present	17	31	1	1.20297
BALOs absent	5	11		

	Microbiome Shannon diversity		Fisher's Exact Test	
	high (3.5 - 4.74)	low (1.7 - < 3.5)	P	Odds ratio
BALOs present	23	25	1	1.17976
BALOs absent	7	9		

355 ^a Microbiome richness is treated as a categorical variable, being either high or low. Cut-offs
 356 for the two groups are indicated.

357

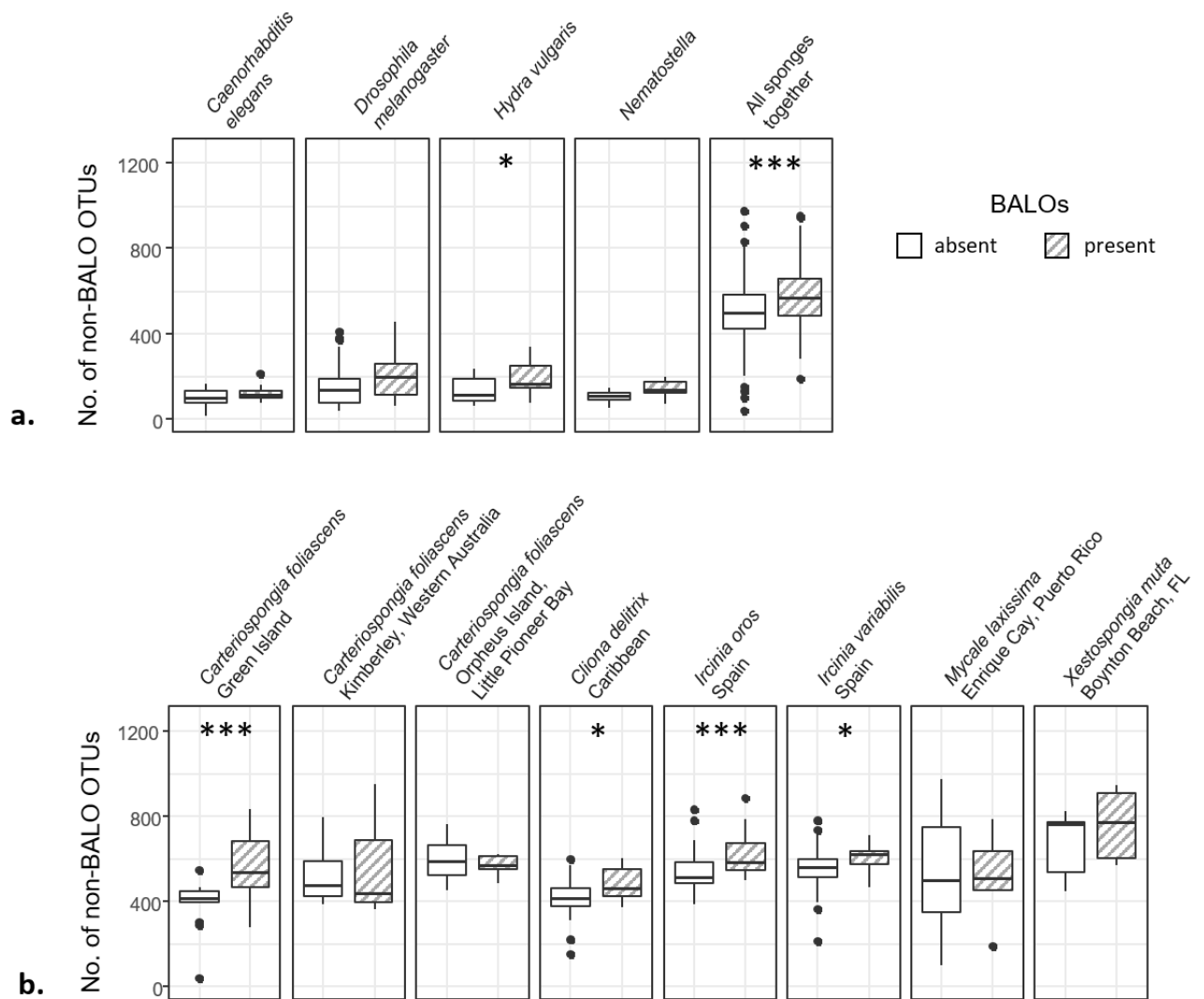
358 Table S6: Wilcoxon rank sum test statistics of the comparison of sponge microbiome alpha-
359 diversity in samples either with BALO presence *versus* BALO absence.

Diversity measure ^a	W	P
Simpson	324	0.3598
Shannon	329	0.4018
OTU richness	364	0.7647

360 ^a Microbiome diversity is used as a continuous variable and compared among the two
361 groups, which are either defined by the presence or the absence of BALOs.

362

363

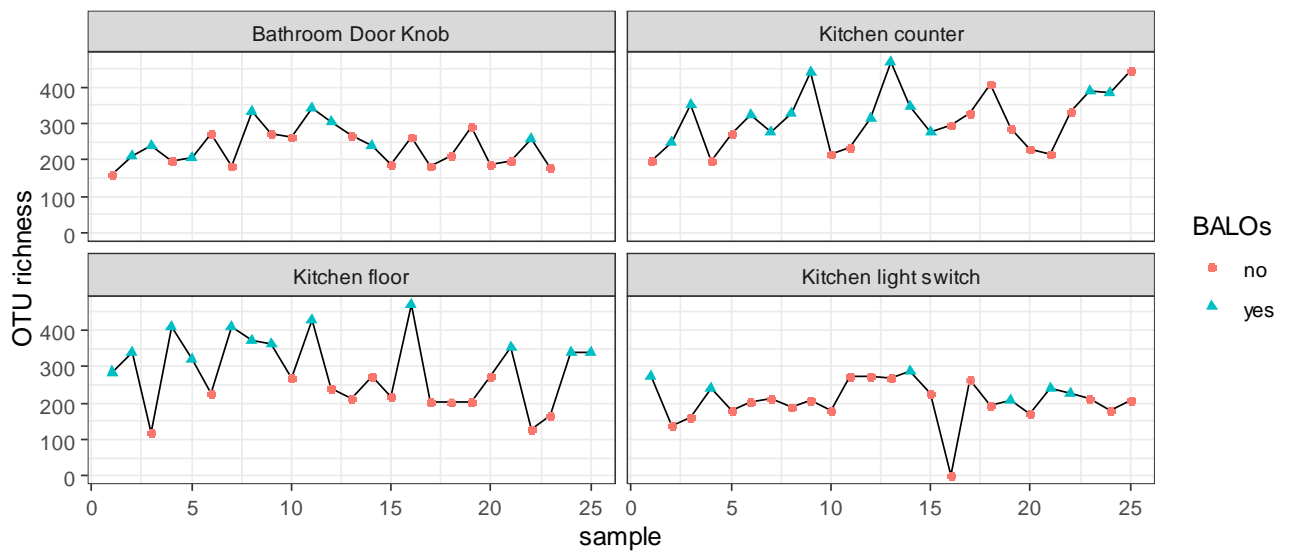


364

365 Fig. S1: Microbiome richness of different hosts (a) and particular sponge species (b)
 366 measured as number of different non-BALO OTUs in the presence and absence of BALOs.
 367 Significant differences are indicated by asterisks and were calculated using the Wilcoxon
 368 rank sum test. P-values: $p < 0.001$: '***', $0.0011 > p < 0.01$: '**', $0.011 > p < 0.05$: '*'. P-values are
 369 given in the Table S3.

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373 Fig. S2: Microbiome richness of longitudinal samples from house 05b from [10]. Samples
374 were taken every other day. Data points are colored and shaped according to the presence
375 or absence of BALOs.

376

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