The microbiome of *Calasterella californica* is shaped mostly by the genetics of the host thalli

Ixchel González-Ramírez[1,2,a], Michael J. Song[3,a], Brent D. Mishler[1,2]

[1]University and Jepson Herbaria, University of California, Berkeley, CA

[2]Department of Integrative Biology, University of California, Berkeley, CA

[3]Skyline College, San Bruno, CA

[a]These authors contributed equally

Keywords: Liverwort, Bryophyte, Microbiome, California Conservation Genomics Project, CCGP, *Methylobacterium*, Metagenomics

Short title: *Calasterella californica* microbiome composition

Manuscript received _____; revision accepted _____.

ABSTRACT

Premise of the study: Plant microbiomes have been shown to affect essential life processes like plant growth, defense mechanisms, and stress responses. Much of the work done on this topic has focused on vascular plants and model organisms. Liverwort biology is very different from that of vascular plants, making generalizations difficult. Here we characterize the microbiome of the liverwort *Calasterella californica* and explore whether climatic, geographic, and genetic factors are potential drivers of the microbiome composition.

Methods: We utilized the genomic data obtained through the California Conservation Genomics Project, which densely sampled individuals of *C. californica* across California. We selected 34 samples and utilized the MetaPhlAn pipeline to characterize the composition of the microbial communities for each sample.

Key results: We characterize the core microbiome of *Calasterella,* which is dominated by *Methylobacterium*. There is a weak association between the relative abundance of bacteria in the Hyphomicrobiales and liverwort size. Genetic distance was the most important factor in shaping beta diversity of the *Calasterella californica* microbiome. Environmental distance was only weakly predictive and geographic distance was not significant.

Conclusions: Leveraging genomic data obtained from field samples of a liverwort species allowed us to characterize the microbial diversity of *C. californica* and its variation across space. There is a group of taxa that are consistently associated with this plant. And the variation observed across samples seems to be linked to the genetic similarity of the liverworts.

INTRODUCTION

The community of bacteria, archaea, and small eukaryotes associated with an organism, known as the microbiome, has been shown to have a considerable role in shaping plant morphology, ecology, and evolution. Plant microbiomes play a central role in some of the most basic aspects of terrestrial plant life such as: mycorrhizae (Taylor et al., 1995), specialized nitrogen-fixation structures (Song et al., 2024), hormone signaling (reviewed in Li et al., 2023), and growth-promotion and disease protection (reviewed in Trivedi et al., 2020). Bryophytes have many well-characterized microbiome interactions, including the presence of mycorrhizae in liverworts and hornworts (reviewed in Pressel et al., 2010) and nitrogen-fixing cyanobionts in hornworts (Li et al., 2020). Thalloid liverworts in particular have been studied as proxies for early land-plant microbiomes (Alcaraz et al., 2018), which are hypothesized to have included nitrogen-fixing bacteria and methanotrophs (Knack et al. 2015).

Thalloid liverworts have been shown to associate with many members of *Methylobacterium* who live off of methanol excreted by the liverwort pores and in exchange promote host growth by secreting cytokinins (Alcaraz et al., 2018; Kutschera and Koopmann, 2005). Similarly, Alcaraz and colleagues (2018) found many other potentially symbiotic bacteria in the *Marchantia* microbiome that promote pathogen defense through the production of plant hormones in exchange for plant-produced polymers including growth-inducing genera like *Rhizobium* and organic degrading bacteria like *Lysobacter*. These bacteria may be actively recruited by the liverwort by inhibiting the growth of competing bacteria (Mewari and Kumar, 2008).

While the factors that shape the composition of the bryophyte microbiome are not well understood, some patterns have emerged. For example, it has been observed that bryophytes have a unique microbiome that is consistent over development (Bragina et al., 2012) and independent from geography (Opelt et al., 2007). Some studies suggest evidence for vertical

transmission of some beneficial *Burkholderia* taxa in moss (Bragina et al., 2013). Wicaksono et al. (2023) found that *Riccia* liverworts recruit specific bacteria and that the surrounding soil is not an important reservoir for them. However, they also found that enrichment of *Methylobacterium* was dependent on field type (Wicaksono et al., 2023). Similarly, the bacteriome of *Marchantia inflexa* liverworts were found to be highly shaped by habitat (Marks et al., 2017).

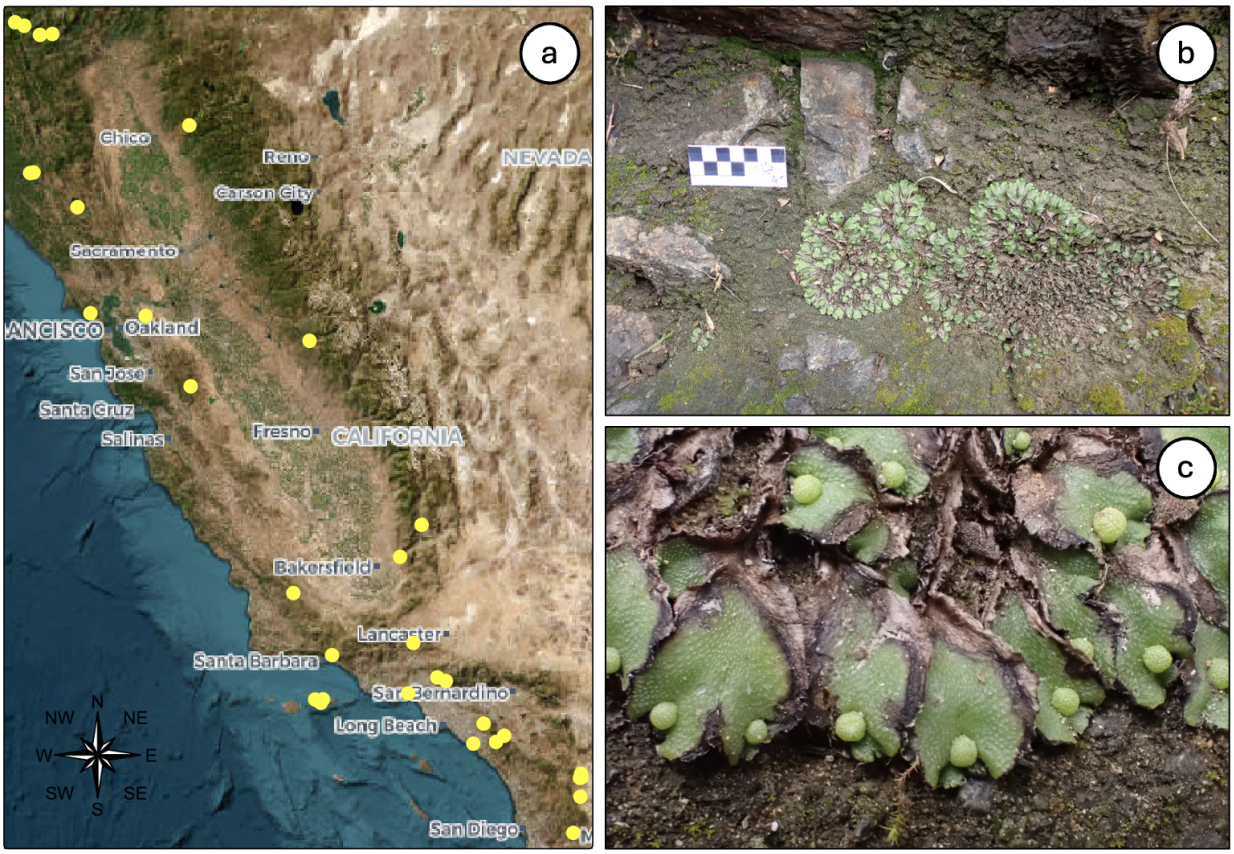


Figure 1. **a**. Collection locations of *Calasterella californica* for this study. **b**. Typical environment and morphology of *C. californica*. **c**. Close up to the female thalli of *C. californica*.

Calasterella californica is a complex thalloid liverwort endemic to the west coast of North America, extending from the southernmost part of Oregon to Baja California Norte in Mexico (Fig. 1). This species is outstanding in the diversity of ecosystems in which it occurs, from humid forests (*e.g.* in the Cascades region) to the desert (*e.g.* Anza Borrego). In all these ecosystems,

81 *C. californica* occurs in exposed areas, over bare soil or rock and plays an important role in soil
82 retention and secondary succession. In this paper, we characterize and compare the bacterial
83 metagenome of 34 samples of *C. californica* collected across the state of California. We explore
84 whether differences in microbiome composition across samples are associated with factors like
85 liverwort genetics, variation in environmental variables, or geographic distance between
86 samples. To achieve our goals, we utilize *C. californica* samples obtained as a part of the
87 California Conservation Genomics Project (CCGP), a state-funded conservation project to
88 provide high quality reference genomes for over 100 species and population-level resequencing
89 to study the effects of climate change and to inform conservation efforts (Shaffer et al., 2022;
90 Toffelmier et al., 2022; Fiedler et al. 2022; Beninde et al. 2022). While the stated goals of the
91 CCGP focus on landscape genomics of the host organisms," we propose that this population-
92 level sampling across the state also provides an opportunity to study metagenome variation and
93 bacterial ecology, and we provide a case study to demonstrate its potential.

94 **METHODS**

95 We selected thirty-four samples of *Calasterella californica* collected as part of the CCGP project
96 (Fig 1). The selection aimed to represent the geographic range, the environmental niche space,
97 and the genetic diversity of the 110 samples collected for the CCGP project. For each of the
98 samples, total genomic and symbiont DNA of one to four thalli was extracted using the Qiagen
99 DNeasy Plant Pro kit (California, USA). Library preparation and sequencing was carried out at
100 the UC Davis DNA Technologies & Expression Analysis Core Laboratory. Libraries were
101 sequenced using an Illumina NovaSeqX 300 (PE150) flow-cell.
102 **Characterization of *C. californica* microbiome composition**. For each sample, the paired-
103 end reads were mapped to the *Calasterella californica* isolate IGR150-G1 genome (González-
104 Ramírez et al., 2024) using bwa-mem (Li, 2013). We used samtools (Li et al., 2009) to filter
105 reads that mapped to the genome and reads that did not align to the genome were kept for

106 downstream analysis. We used MetaPhlAn on the Galaxy webserver (Galaxy Version
107 4.1.1+galaxy3; Giardine et al., 2005; Blanco-Míguez et al., 2023) to profile the composition of
108 microbial communities using these short-read data. MetaPhlAn can process raw reads without
109 the need for adapter trimming and quality filtering (Nicola Segata, MetaPhlAn developer; online
110 communication) and therefore the raw reads were used to maximize the amount of information.
111 Results were visualized using Krona (Galaxy Version 2.7.1+galaxy0; Ondov et al., 2011) and
112 with R (R Core Team 2021).
113 In order to compare the microbiome of field-collected samples vs tissue cultivated in-
114 vitro, we assessed the microbiome composition of the tissue used for the reference genome of
115 *C. californica*, which was grown from a spore in the lab. PacBio HiFi reads from González-
116 Ramírez et al. (2024) were run through the BugSeq pipeline (Fan et al., 2021). Metagenomic
117 classification was visualized using Krona (Ondov et al., 2011). This in-vitro sample required a
118 different pipeline, that can analyze long-read data, than the field-collected samples.
119 **Exploring potential drivers of microbiome composition.** To assess whether the microbiome
120 composition is correlated with (1) liverwort genetics, (2) environmental conditions, or (3) sample
121 geographic proximity, we performed mantel and partial-mantel tests using the ape package
122 (Paradis and Schliep, 2019) in R with 999 permutations. First, we calculated pairwise distance
123 matrices for each of the three variables. The liverwort genetic distance matrix was constructed
124 based on 23180 bi-allelic and uncorrelated SNPs using Manhattan distance.
125 To characterize the macro-environment of each sample, we obtained bioclimatic variables
126 values for 19 climatic variables from the WorldClim2 database (Fick et al., 2017) and used these
127 values to compute the pairwise euclidean environmental distance matrix. The geographical
128 distance matrix was constructed with the geographic coordinates of the collection points using
129 the `distm` function of the geosphere R package (Hijmans, 2024). Next, we constructed a
130 pairwise dissimilarity matrix based on the microbiome species relative abundance data from

131 MetaPhlAn using the Bray-Curtis dissimilarity index. Finally, we performed mantel tests between
132 the microbiome composition distance matrix and each of the three variables mentioned.

133 **RESULTS**

134 **Composition of *Calasterella californica* microbiome.** A total of 161 bacterial genera were
135 found in the in-vitro grown sample; this value was 177 across the 34 samples collected in the
136 field (Supp. Table S1). Fifty bacterial genera were shared between the in vivo and in vitro
137 samples: *Achromobacter, Acidovorax, Actinoplanes, Agreia, Agrobacterium, Alsobacter,*
138 *Asticcacaulis, Aureimonas, Azospirillum, Belnapia, Brevundimonas, Burkholderia, Caulobacter,*
139 *Dankookia, Devosia, Duganella, Dyadobacter, Flavobacterium, Geodermatophilus,*
140 *Hansschlegelia, Herbaspirillum, Herbiconiux, Hymenobacter, Klebsiella, Massilia,*
141 *Mesorhizobium, Methylobacterium, Methylophilus, Microbacterium, Mucilaginibacter,*
142 *Mycolicibacterium, Neorhizobium, Nostoc, Novosphingobium, Paraburkholderia, Pedobacter,*
143 *Phenylobacterium, Polaromonas, Pseudomonas, Pseudorhodoferax, Rhizobium, Rhodoferax,*
144 *Sphingobium, Sphingomonas, Spirosoma, Tardiphaga, Variovorax, Williamsia, Xanthomonas,*
145 and *Xylophilus*. Nonetheless, the proportions of these taxa vary substantially between the field-
146 collected and in-vitro samples (Supp. Fig. S1).

147 Microbial communities associated with individual *C. californica* samples were composed
148 on average of 27.85 genera (range: 15 – 50). Across the 177 genera represented in the 34 field-
149 collected samples, there are six genera of bacteria that were present in more than 70% of the
150 samples: *Methylobacterium* (97%), *Spirosoma* (85%), *Pseudomonas* (79%), *Inquilinus* (0.74%),
151 *Dankookia* (74%), and *Sphingomonas* (71%). All of these genera, with the exception of
152 *Inquilinus,* were also found in the liverwort grown from a spore in the lab. Furthermore, except
153 for *Dankookia*, all these genera have been reported to be endophytic in seed-plants (Afzal et al.
154 2019), often having beneficial effects on their plant hosts. The presence of these bacteria in the
155 microbiome across the *C. californica* samples suggests that these bacteria may represent the

156 core microbiome associated with *C. californica*, and that they could be vertically transmitted,
157 since the spore-grown plant had them too.
158 **Drivers of variation in microbiome composition.** When we compared the microbiome
159 composition across samples, we found that members of the Hyphomicrobiales were abundant in
160 the majority of samples, especially taxa in the Methylobacteriaceae (Fig. 2). *Methylobacterium*
161 was found in high abundance in many but not all of our samples. Notably, these taxa did not
162 constitute a large proportion in the *in vitro* sample (Supp. Table S1). Since it has been
163 hypothesized that members of the Hyphomicrobiales (syn. Rhizobiales), which contains many
164 nitrogen-fixing taxa and methanogens, promote growth, we tested whether there was an
165 association between thallus width (as a proxy for thallus size) and the proportion of the
166 microbiome constituted by members of the Hyphomicrobiales (Figure 4). We found a weak yet
167 significant association (F-statistic = 5.223 on 1 and 31 DF, $p = 0.0293$, adjusted r squared =
168 0.1166). Interestingly, Hyphomicrobiales were found to be relatively abundant across all
169 samples of C. californica (Supp. Fig. S1).

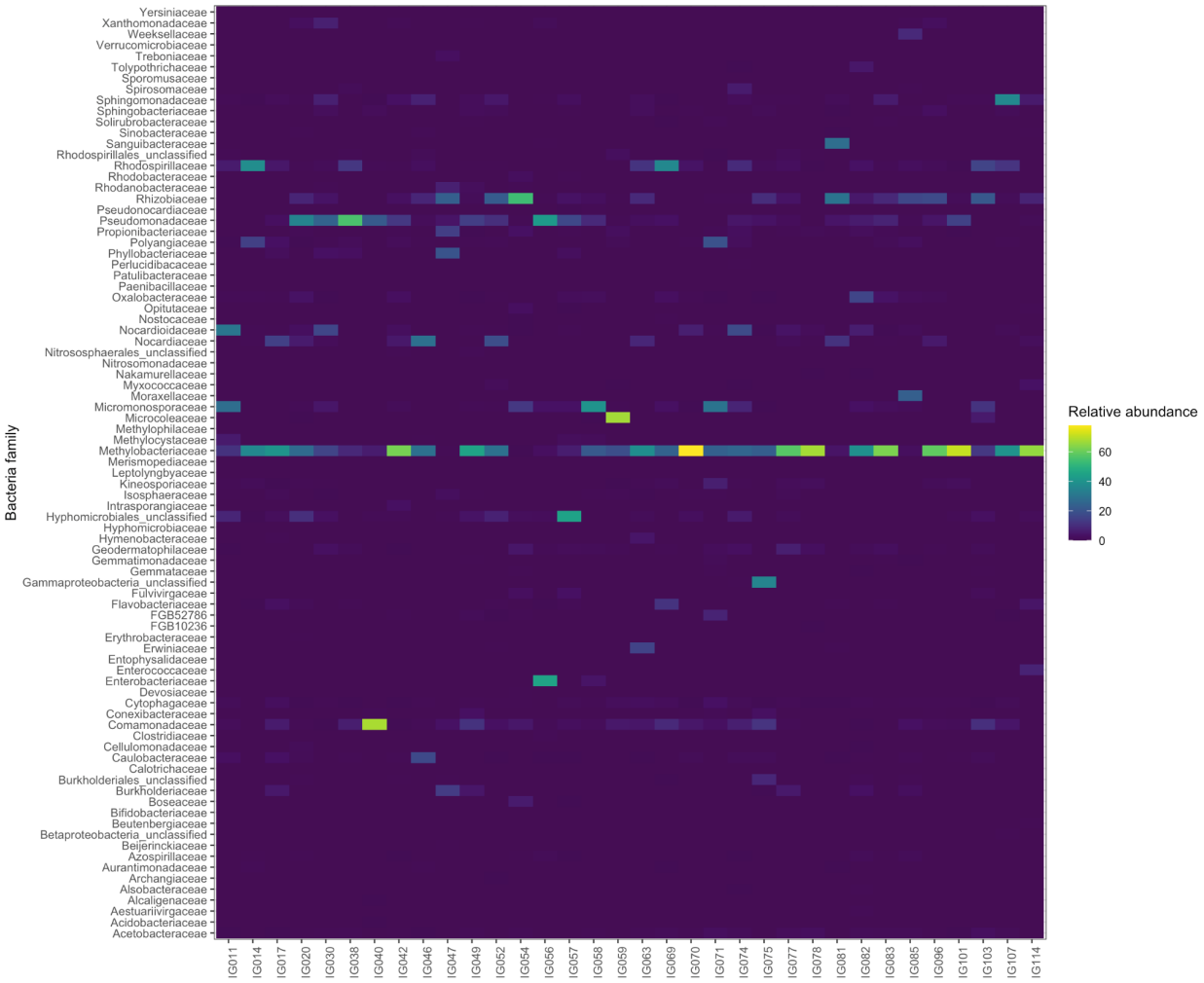


Figure 2. Relative abundance (in percentage of bacterial reads identified) of different bacteria families across *C. californica* samples.

We found no significant correlation of microbiome composition with geographic distance or environmental distance (Fig. 3). We did, however, find a significant correlation with genetic distance (z-stat = 1076314, p-value = 0.03). This correlation was still significant when accounting for geographic distance (r statistic = 0.205, p-value = 0.023; partial mantel test). When visualizing the similarity of samples following their microbiome community and genetics of the host liverworts (Supp. Fig. S2), while the clustering is not identical, large groups remain congruent. We also found a weakly significant association between microbiome community

distance and ecological distance when accounting for geographic distance (r statistic = 0.133, p-value = 0.095; partial mantel test).

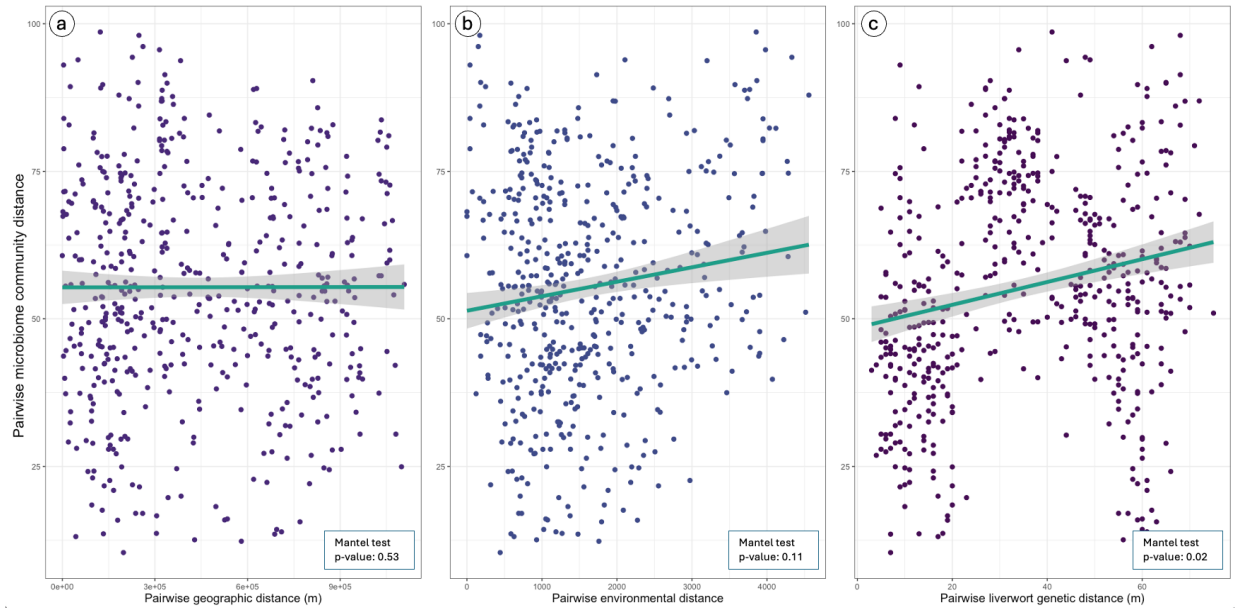
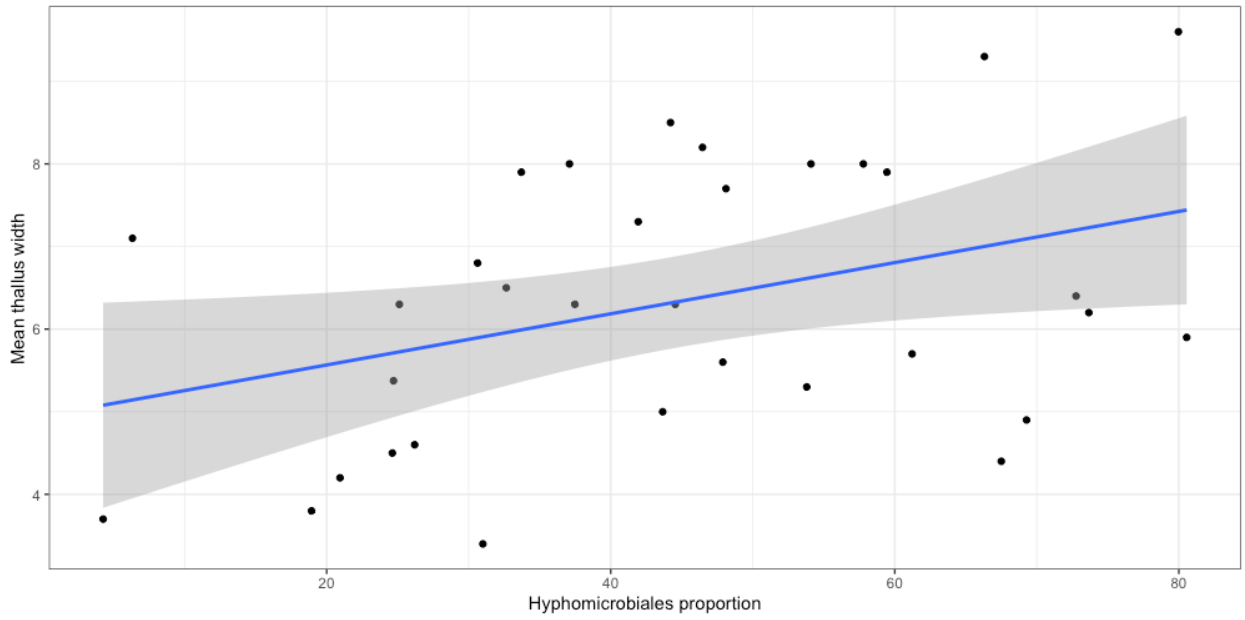


Figure 3. Correlation of pairwise distances between microbiome composition and three potentially explanatory variables. **a.** Geographic distance, **b.** Environmental distance, and **c.** Genetic distance.



188 **Figure 4.** Mean thallus width by proportion of Hyphomicrobiales for *Calasterella* samples. A line
189 of best fit was added using a linear regression model (standard error in grey).

190 **DISCUSSION**

191 These findings corroborate previous studies that found that liverworts recruit specific bacteria,
192 especially *Methylobacterium* (Alcaraz et al., 2018; Kutschera and Koopmann, 2005), and that
193 there is an effect of the environment on microbiome composition (Wicaksono et al., 2023; Marks
194 et al., 2017). We also found evidence of a correlation between the relative abundance of
195 Hyphomicrobiales microbes in an individual's microbiome and its thallus size. Given that there
196 could be an association between genetic similarity and environment due to selection or other
197 factors, we also compared genetic distances among the host plants to test whether genetic
198 factors or environment were more strongly correlated with microbiome community composition.
199 We found that genetic distance was the most important factor in shaping beta diversity of the
200 *Calasterella* microbiome, but that the environment was also weakly predictive. The joint
201 importance of both environmental factors and host genetics in shaping the plant microbiome has
202 also been found in duckweeds (Wei and Tan, 2023), *Boechera* (Wagner et al., 2016), and maize
203 (He et al., 2024). Notably, these patterns are contrary to the major patterns observed in humans
204 in which the environment is more important than genetics in shaping the gut microbiome
205 (Rothschild et al., 2018).

206 There has been much recent work on vascular plants to investigate the role of host
207 genetics in shaping the root and leaf microbiome (reviewed in Zhang et al., 2023). However,
208 liverwort morphology is very different from that of vascular plants, making these assessments
209 hard to generalize. A recent study on the microbiome of the liverwort *Riccia* suggests that
210 bacterial communities associated with liverworts are highly stable, showing little variation across
211 environmental variables like soil type (Wicaksono et al. 2023), which is consistent with our
212 results. With the recent publication of high-quality liverwort genomes (González-Ramírez et al.,
213 2024; Diop et al., 2020; Linde et al., 2023), future work can now begin to assess how genetics

214 shapes the bryophyte metagenome and similarities and differences with vascular plants.
215 Additionally, our methodological approach does not allow us to make inferences about whether
216 the bacteria identified here are endophytic or epiphytic; further studies could categorize these
217 groups.

218 This demonstration of the power of the CCGP dataset to elucidate the ecology of the
219 liverwort microbiome using metagenomic techniques suggests that this approach should be
220 applied to all of the taxa of the CCGP that have a microbiome. Song et al. (2024) has previously
221 used similar approaches focusing on the *Azolla* endosymbionts, in a well characterized
222 system—the leaf pocket. However, many of the taxa of the CCGP may also have microbiomes
223 that could be studied, including taxa for which leaf or whole animal sampling was performed:
224 *Quercus tomentella* (Mead et al., 2024), *Linanthus parryae* (Anghel et al., 2022), *Hetaerina*
225 *americana* (Grether et al., 2023), *Branchinecta lynchi* (Kieran Blair et al., 2023), *Polyergus*
226 *mexicanus* (Cash et al., 2024), and many others (see https://www.ccgproject.org/reference-
227 genomes-tracking). We encourage future collaboration to address the multiple avenues of
228 research that these data from large scale genomic approaches can facilitate.
229

230 **ACKNOWLEDGEMENTS**

231 The authors thank Carl Rothfels for his constructive comments and edits to the manuscript.
232 Thank you to the California Conservation Genomics Project, with funding provided to the
233 University of California by the State of California, State Budget Act of 2019 [UC Award ID RSI-
234 19-690224]. IGR was supported by a UC Mexus-CONACyT fellowship (number 709967), a
235 Plant Science Fellowship by Oak Spring Garden Foundation, and the Philomathia Graduate
236 Fellowship in Environmental Sciences, at UC Berkeley.
237

AUTHOR CONTRIBUTIONS

IGR and MJS designed the study, performed the analyses and wrote the first draft of the manuscript. BDM supervised the work and edited the manuscript.

DATA AVAILABILITY STATEMENT

All the data and code used for the analyses is hosted in the GitHub repository: https://github.com/ixchelgzlzr/microbiome_Ccal.git

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412 # Supplemental Information

413 **Supplemental Table S1.** Genera of bacteria in the microbial community associated with in-vitro
414 and field-collected specimens of *C. californica*.
415

In vitro	In vivo	Unique in vitro	Unique in vivo	Shared
Aaosphaeria	Achromobacter	Aaosphaeria	Acinetobacter	Achromobacter
Acanthamoeba	Acidovorax	Acanthamoeba	Aeromicrobium	Acidovorax
Achromobacter	Acinetobacter	Acidobacterium	Altererythrobacter	Actinoplanes
Acidobacterium	Actinoplanes	Actinomadura	Amnibacterium	Agreia
Acidovorax	Aeromicrobium	Acytostelium	Anaerospora	Agrobacterium
Actinomadura	Agreia	Afipia	Aquabacterium	Alsobacter
Actinoplanes	Agrobacterium	Agromyces	Aquincola	Asticcacaulis
Acytostelium	Alsobacter	Alternaria	Arcicella	Aureimonas
Afipia	Altererythrobacter	Ancylobacter	Azohydromonas	Azospirillum
Agreia	Amnibacterium	Ankyra	Beijerinckiaceae_unclassified	Belnapia
Agrobacterium	Anaerospora	Aphanomyces	Betaproteobacteria_unclassified	Brevundimonas
Agromyces	Aquabacterium	Aquibium	Bifidobacterium	Burkholderia
Alsobacter	Aquincola	Arenibacter	Blastococcus	Caulobacter
Alternaria	Arcicella	Aspergillus	Bosea	Dankookia
Ancylobacter	Asticcacaulis	Aureococcus	Burkholderiales_unclassified	Devosia
Ankyra	Aureimonas	Bacidia	Caballeronia	Duganella
Aphanomyces	Azohydromonas	Bacillus	Calothrix	Dyadobacter
Aquibium	Azospirillum	Bartonella	Cellulomonas	Flavobacterium
Arenibacter	Beijerinckiaceae_unclassified	Beauveria	Chiayiivirga	Geodermatophilus
Aspergillus	Belnapia	Bipolaris	Chimaeribacter	Hansschlegelia
Asticcacaulis	Betaproteobacteria_unclassified	Blastochloris	Chlorogloea	Herbaspirillum
Aureimonas	Bifidobacterium	Bodomorpha	Chryseobacterium	Herbiconiux
Aureococcus	Blastococcus	Botrytis	Clostridium	Hymenobacter
Azospirillum	Bosea	Bradyrhizobium	Conexibacter	Klebsiella
Bacidia	Brevundimonas	Cadophora	Corallococcus	Massilia
Bacillus	Burkholderia	Caenimonas	Curtobacterium	Mesorhizobium
Bartonella	Burkholderiales_unclassified	Canariomyces	Cutibacterium	Methylobacterium
Beauveria	Caballeronia	Candida	Cylindrospermum	Methylophilus
Belnapia	Calothrix	Candidatus Methylopumilus	Desmonostoc	Microbacterium
Bipolaris	Caulobacter	Capnocytophaga	Emticicia	Mucilaginibacter
Blastochloris	Cellulomonas	Capronia	Enterobacter	Mycolicibacterium
Bodomorpha	Chiayiivirga	Ceraceosorus	Enterococcus	Neorhizobium
Botrytis	Chimaeribacter	Cercospora	Erwinia	Nostoc
Bradyrhizobium	Chlorogloea	Chaetomium	Friedmanniella	Novosphingobium
Brevundimonas	Chryseobacterium	Chlorella	Frigoribacterium	Paraburkholderia

Burkholderia	Clostridium	Chromohalobacter	Frondihabitans	Pedobacter
Cadophora	Conexibacter	Cladophialophora	Fulvivirgaceae_unclassified	Phenylobacterium
Caenimonas	Corallococcus	Cokeromyces	Gammaproteobacteria_unclassified	Polaromonas
Canariomyces	Curtobacterium	Colletotrichum	Gemmataceae_unclassified	Pseudomonas
Candida	Cutibacterium	Comamonas	Gemmatirosa	Pseudorhodoferax
Candidatus Methylopumilus	Cylindrospermum	Coniophora	Geodermatophilaceae_unclassified	Rhizobium
Capnocytophaga	Dankookia	Coniosporium	GGB35955	Rhodoferax
Capronia	Desmonostoc	Cordyceps	GGB44695	Sphingobium
Caulobacter	Devosia	Cryptococcus	GGB52803	Sphingomonas
Ceraceosorus	Duganella	Cryptosporidium	GGB53655	Spirosoma
Cercospora	Dyadobacter	Cupriavidus	GGB64492	Tardiphaga
Chaetomium	Emticicia	Daldinia	GGB64512	Variovorax
Chlorella	Enterobacter	Deinococcus	GGB65415	Williamsia
Chromohalobacter	Enterococcus	Delftia	GGB68992	Xanthomonas
Cladophialophora	Erwinia	Diaphorobacter	GGB69028	Xylophilus
Cokeromyces	Flavobacterium	Diaporthe	GGB71872	
Colletotrichum	Friedmanniella	Dichotomopilus	GGB72163	
Comamonas	Frigoribacterium	Dickeya	GGB77784	
Coniophora	Frondihabitans	Dictyostelium	GGB78124	
Coniosporium	Fulvivirgaceae_unclassified	Didymosphaeria	GGB78370	
Cordyceps	Gammaproteobacteria_unclassified	Dioszegia	GGB79878	
Cryptococcus	Gemmataceae_unclassified	Dissoconium	Granulicella	
Cryptosporidium	Gemmatirosa	Drechmeria	Hungatella	
Cupriavidus	Geodermatophilaceae_unclassified	Drepanopeziza	Hyalangium	
Daldinia	Geodermatophilus	Dyella	Hyphomicrobiales_unclassified	
Dankookia	GGB35955	Eimeria	Ideonella	
Deinococcus	GGB44695	Emericellopsis	Inquilinus	
Delftia	GGB52803	Endocarpon	Intrasporangiaceae_unclassified	
Devosia	GGB53655	Epidermidibacterium	Janibacter	
Diaphorobacter	GGB64492	Exophiala	Kamptonema	
Diaporthe	GGB64512	Exserohilum	Kibdelosporangium	
Dichotomopilus	GGB65415	Falsiroseomonas	Kineococcus	
Dickeya	GGB68992	Ferranicluibacter	Kineosporia	
Dictyostelium	GGB69028	Fomitopsis	Kocuria	
Didymosphaeria	GGB71872	Fusarium	Larkinella	
Dioszegia	GGB72163	Gaeumannomyces	Leifsonia	
Dissoconium	GGB77784	Geminella	Luteibacter	
Drechmeria	GGB78124	Gemmobacter	Marmoricola	
Drepanopeziza	GGB78370	Glaciihabitans	Methylopila	
Duganella	GGB79878	Gynurincola	Methylorubrum	
Dyadobacter	Granulicella	Hafniomonas	Microbacteriaceae_unclassified	

Dyella	Hansschlegelia	Halomonas	Microcoleus
Eimeria	Herbaspirillum	Hartmannibacter	Microlunatus
Emericellopsis	Herbiconiux	Herminiimonas	Microvirga
Endocarpon	Hungatella	Heterobasidion	Modestobacter
Epidermidibacterium	Hyalangium	Homo	Myxococcus
Exophiala	Hymenobacter	Hydrogenophaga	Naasia
Exserohilum	Hyphomicrobiales_unclassified	Hyphopichia	Nakamurella
Falsiroseomonas	Ideonella	Hypoxylon	Nevskia
Ferranicluibacter	Inquilinus	Ilyonectria	Nibricoccus
Flavobacterium	Intrasporangiaceae_unclassified	Janthinobacterium	Nitrososphaerales_unclassified
Fomitopsis	Janibacter	Kalmanozyma	Nitrosospira
Fusarium	Kamptonema	Kickxella	Nocardioides
Gaeumannomyces	Kibdelosporangium	Labilibacter	Noviherbaspirillum
Geminella	Kineococcus	Labilithrix	Ohtaekwangia
Gemmobacter	Kineosporia	Lasiodiplodia	Paludisphaera
Geodermatophilus	Klebsiella	Lasiosphaeria	Panacagrimonas
Glaciihabitans	Kocuria	Lentinula	Pantoea
Gynurincola	Larkinella	Leptographium	Patulibacter
Hafniomonas	Leifsonia	Letharia	Pedococcus
Halomonas	Luteibacter	Leucobacter	Perlucidibaca
Hansschlegelia	Marmoricola	Ligilactobacillus	Phormidesmis
Hartmannibacter	Massilia	Lipomyces	Pseudarthrobacter
Herbaspirillum	Mesorhizobium	Lithohypha	Pseudonocardia
Herbiconiux	Methylobacterium	Litorilituus	Pseudorhizobium
Herminiimonas	Methylophilus	Malassezia	Pseudosporangium
Heterobasidion	Methylopila	Martelella	Pseudoxanthomonas
Homo	Methylorubrum	Maudiozyma	Rhizobacter
Hydrogenophaga	Microbacteriaceae_unclassified	Meira	Rhizobiaceae_unclassified
Hymenobacter	Microbacterium	Melampsora	Rhodanobacter
Hyphopichia	Microcoleus	Mesoflavibacter	Rhodanobacteraceae_unclassified
Hypoxylon	Microlunatus	Metarhizium	Rhodococcus
Ilyonectria	Microvirga	Metarhizobium	Rhodocytophaga
Janthinobacterium	Modestobacter	Methylicorpusculum	Rhodoplanes
Kalmanozyma	Mucilaginibacter	Methylobacillus	Rhodospirillaceae_unclassified
Kickxella	Mycolicibacterium	Methylogaea	Rhodospirillales_unclassified
Klebsiella	Myxococcus	Methyloradius	Roseococcus
Labilibacter	Naasia	Methylotenera	Roseomonas
Labilithrix	Nakamurella	Methylovorus	Saccharibacillus
Lasiodiplodia	Neorhizobium	Microdochium	Sanguibacter
Lasiosphaeria	Nevskia	Micromonospora	Serinibacter
Lentinula	Nibricoccus	Mixia	Simulacricoccus
Leptographium	Nitrososphaerales_unclassified	Mollisia	Skermanella

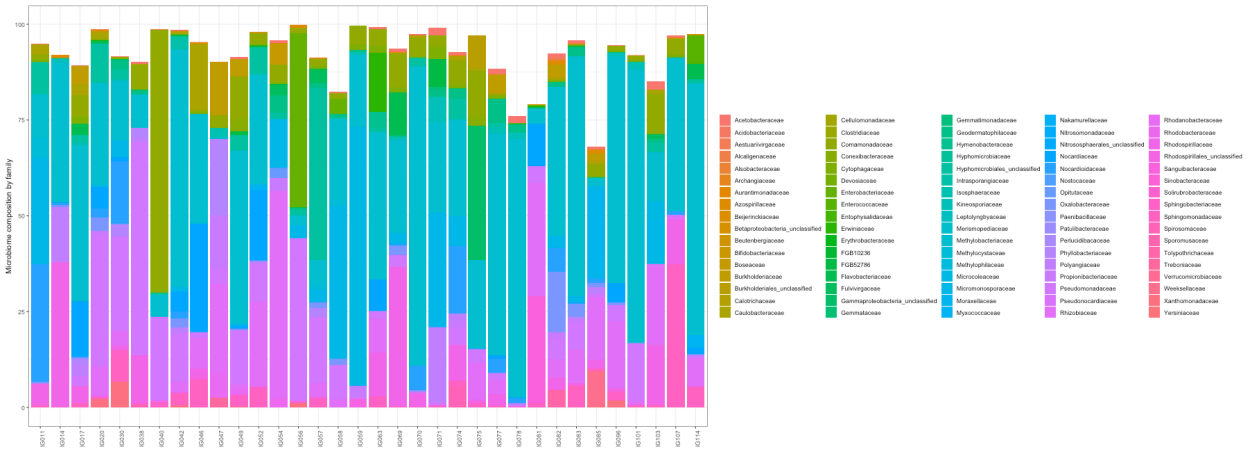
Letharia	Nitrosospira	Mucor	Solirubrobacter
Leucobacter	Nocardioides	Mycoavidus	Sphingobacteriaceae_unclassified
Ligilactobacillus	Nostoc	Mycobacterium	Stenotrophomonas
Lipomyces	Noviherbaspirillum	Nannizzia	Synechocystis
Lithohypha	Novosphingobium	Nannocystis	Tahibacter
Litorilituus	Ohtaekwangia	Neocystis	Tolypothrix
Malassezia	Paludisphaera	Neurospora	Urbifossiella
Martelella	Panacagrimonas	Niabella	Verrucomicrobium
Massilia	Pantoea	Nocardia	Virgisporangium
Maudiozyma	Paraburkholderia	Nocardiopsis	
Meira	Patulibacter	Nonomuraea	
Melampsora	Pedobacter	Oscillibacter	
Mesoflavibacter	Pedococcus	Paenibacillus	
Mesorhizobium	Perlucidibaca	Paeniroseomonas	
Metarhizium	Phenylobacterium	Pandoraea	
Metarhizobium	Phormidesmis	Paracidovorax	
Methylicorpusculum	Polaromonas	Paracoccus	
Methylobacillus	Pseudarthrobacter	Paraphaeosphaeria	
Methylobacterium	Pseudomonas	Pararhizobium	
Methylogaea	Pseudonocardia	Parietochloris	
Methylophilus	Pseudorhizobium	Penicilliopsis	
Methyloradius	Pseudorhodoferax	Penicillium	
Methylotenera	Pseudosporangium	Pestalotiopsis	
Methylovorus	Pseudoxanthomonas	Photorhabdus	
Microbacterium	Rhizobacter	Phyllobacterium	
Microdochium	Rhizobiaceae_unclassified	Phytophthora	
Micromonospora	Rhizobium	Pleomorphomonas	
Mixia	Rhodanobacter	Podospora	
Mollisia	Rhodanobacteraceae_unclassified	Polychytrium	
Mucilaginibacter	Rhodococcus	Porogramme	
Mucor	Rhodocytophaga	Priceomyces	
Mycoavidus	Rhodoferax	Pseudophaeobacter	
Mycobacterium	Rhodoplanes	Pseudoroseomonas	
Mycolicibacterium	Rhodospirillaceae_unclassified	Pseudovirgaria	
Nannizzia	Rhodospirillales_unclassified	Pseudozyma	
Nannocystis	Roseococcus	Psychrobacter	
Neocystis	Roseomonas	Puccinia	
Neorhizobium	Saccharibacillus	Purpureocillium	
Neurospora	Sanguibacter	Pyrenophora	
Niabella	Serinibacter	Pyricularia	
Nocardia	Simulacricoccus	Rahnella	
Nocardiopsis	Skermanella	Ramlibacter	
Nonomuraea	Solirubrobacter	Recurvomyces	

Nostoc	Sphingobacteriaceae_unclassified	Rhizophagus
Novosphingobium	Sphingobium	Rhodoalgimonas
Oscillibacter	Sphingomonas	Rhodofomes
Paenibacillus	Spirosoma	Rhodophyticola
Paeniroseomonas	Stenotrophomonas	Rhodopseudomonas
Pandoraea	Synechocystis	Rhodotorula
Paraburkholderia	Tahibacter	Roseicella
Paracidovorax	Tardiphaga	Rosenbergiella
Paracoccus	Tolypothrix	Saxophila
Paraphaeosphaeria	Urbifossiella	Scheffersomyces
Pararhizobium	Variovorax	Schizophyllum
Parietochloris	Verrucomicrobium	Shewanella
Pedobacter	Virgisporangium	Shimia
Penicilliopsis	Williamsia	Shinella
Penicillium	Xanthomonas	Siccirubricoccus
Pestalotiopsis	Xylophilus	Sinorhizobium
Phenylobacterium		Sphaerulina
Photorhabdus		Sporisorium
Phyllobacterium		Sporothrix
Phytophthora		Stanieria
Pleomorphomonas		Streptomyces
Podospora		Sugiyamaella
Polaromonas		Suillus
Polychytrium		Taibaiella
Porogramme		Talaromyces
Priceomyces		Terriglobus
Pseudomonas		Tetrahymena
Pseudophaeobacter		Thalassotalea
Pseudorhodoferax		Thermomonospora
Pseudoroseomonas		Thermothelomyces
Pseudovirgaria		Thermothielavioides
Pseudozyma		Thiothrix
Psychrobacter		Tilletiopsis
Puccinia		Trametes
Purpureocillium		Trichoderma
Pyrenophora		Trichophyton
Pyricularia		Trichosporon
Rahnella		Truncatella
Ramlibacter		Tuber
Recurvomyces		Ustilaginoidea
Rhizobium		Ustilago
Rhizophagus		Vanrija
Rhodoalgimonas		Verticillium

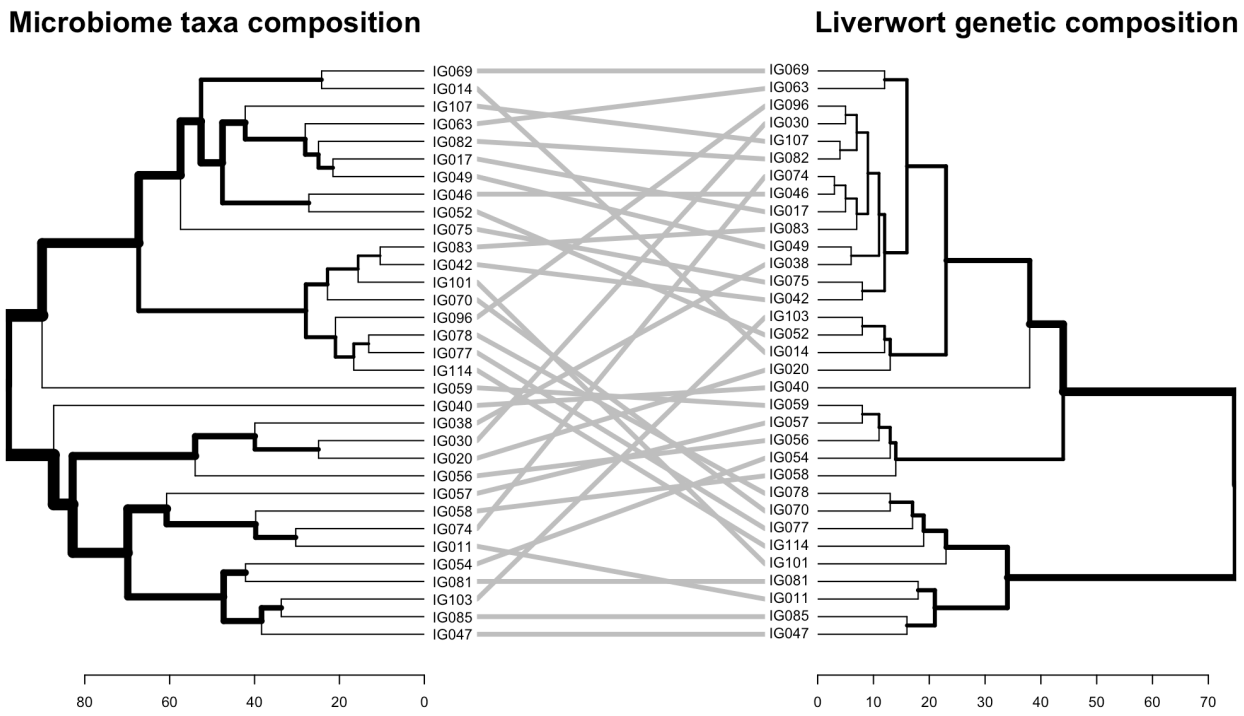
Rhodoferax	Vibrio
Rhodofomes	Winogradskyella
Rhodophyticola	Xenophilus
Rhodopseudomonas	Zavarzinia
Rhodotorula	
Roseicella	
Rosenbergiella	
Saxophila	
Scheffersomyces	
Schizophyllum	
Shewanella	
Shimia	
Shinella	
Siccirubricoccus	
Sinorhizobium	
Sphaerulina	
Sphingobium	
Sphingomonas	
Spirosoma	
Sporisorium	
Sporothrix	
Stanieria	
Streptomyces	
Sugiyamaella	
Suillus	
Taibaiella	
Talaromyces	
Tardiphaga	
Terriglobus	
Tetrahymena	
Thalassotalea	
Thermomonospora	
Thermothelomyces	
Thermothielavioides	
Thiothrix	
Tilletiopsis	
Trametes	
Trichoderma	
Trichophyton	
Trichosporon	
Truncatella	
Tuber	
Ustilaginoidea	
Ustilago	

Vanrija
Variovorax
Verticillium
Vibrio
Williamsia
Winogradskyella
Xanthomonas
Xenophilus
Xylophilus
Zavarzinia

Supplemental Figure S1. Microbiome composition of samples of *C. californica* at the family taxonomic level.



Supplemental Figure S2. Dendrograms reflecting the clustering of samples by the similarity of their microbiome composition (left) and by the similarity of the liverwort genetics (right).


Microbiome taxa composition
Liverwort genetic composition
IG069
IG014
IG107
IG063
IG082
IG017
IG049
IG046
IG052
IG075
IG083
IG042
IG101
IG070
IG096
IG078
IG077
IG114
IG059
IG040
IG038
IG030
IG020
IG056
IG057
IG058
IG074
IG011
IG054
IG081
IG103
IG085
IG047
IG069
IG063
IG096
IG030
IG107
IG082
IG074
IG046
IG017
IG083
IG049
IG038
IG075
IG042
IG103
IG052
IG014
IG020
IG040
IG059
IG057
IG056
IG054
IG058
IG078
IG070
IG077
IG114
IG101
IG081
IG011
IG085
IG047
80
60
40
20
0
0
10
20
30
40
50
60
70

428 **Figure legends**

429 **Figure 1**. **a**. Collection locations of *Calasterella californica* for this study. **b**. Typical environment
430 and morphology of *C. californica*. **c**. Close up to the female thalli of *C. californica*.

431

432 **Figure 2.** Relative abundance (in percentage of bacterial reads identified) of different bacteria
433 families across *C. californica* samples.

434

435 **Figure 3.** Correlation of pairwise distances between microbiome composition and three
436 potentially explanatory variables. **a.** Geographic distance, **b.** Environmental distance, and **c.**
437 Genetic distance.

438

439 **Figure 4.** Mean thallus width by proportion of Hyphomicrobiales for *Calasterella* samples. A line
440 of best fit was added using a linear regression model (standard error in grey).

441