Invasive freshwater snails form novel microbial symbioses

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ABSTRACT

Resident microbes (microbiota) can shape host organismal function and adaptation in the face of environmental change. Invasion of new habitats exposes hosts to novel selection pressures, but the impacts on microbiota and their relationship with hosts after this transition (e.g., how rapidly symbioses are formed, whether microbes influence invasion success) are unclear. We used high-throughput 16S rRNA sequencing of New Zealand (native) and European (invasive) populations of the freshwater snail *Potamopyrgus antipodarum* to find that while invaders do carry over some core microbial taxa from New Zealand, most of their microbial community is distinct. This finding highlights that invasions can result in the formation of novel symbioses. We further show that the native microbiome is composed of fewer core microbes than invasive snails, suggesting that the microbiota is streamlined to essential members. Together, our findings demonstrate that microbiota comparisons across native and invasive populations can reveal the impact of a long coevolutionary history and specialization of microbes in the native host range, as well as new associations occurring after invasion.

Keywords: *Potamopyrgus antipodarum*, invasion, microbiome, coevolution, symbiosis, infection

31 **INTRODUCTION**

32 All organisms are home to numerous microbes. The relationship between hosts and
33 their microbial communities (microbiota) can play a role in key host functions (1, 2) from
34 nutrition (3) to behaviour (4) and immune responses (5). The microbiota has also been shown
35 to impact host evolution (6, 7). Microbial community composition can be determined by host
36 traits such as genotype (8), sex (9), infection of the host by parasites or pathogens (10),
37 environmental conditions (6, 11), and genotype by environment interactions (12). Therefore,
38 host organisms and the microbes on which they depend for vital processes can both be
39 impacted by environmental changes, with potential consequences for host adaptation (13).

40 When species invade novel habitats, they are exposed to new environmental conditions
41 and selection pressures. Because host and microbe fitness can be mutually dependent, invasion
42 is predicted to influence both the host and its microbiome (6). The fitness effects of microbes
43 on their host may affect invasion success and the potential for subsequent adaptation to a new
44 habitat (14). The performance of introduced species could also be altered by the availability of
45 microbes outside their native range if there are core microbes that the host needs to thrive. If
46 host-associated microbes are not all carried over from the native environment, lost upon
47 invasion, and/or unavailable in the new environment, the diversity of the microbiomes of
48 invasive lineages could be low. The invader's microbiome might thus only represent a subset of
49 that residing in the native lineages (15, 16). This pattern is directly analogous to the loss of
50 genetic diversity that often characterizes invasive lineages (15, 17). Whether such subsampling

51 occurs at the microbiome level remains an important open question with implications for host
52 fitness in the new environment.

53 New invasions may also affect the level of specialization of microbiota relative to hosts
54 in the native range. This outcome could be a consequence of the comparatively short time
55 period for coevolution between the invasive host and newly acquired microbes vs. the microbes
56 from the native environment (15, 16). These effects of colonization could influence microbiome
57 structure. These consequences could be harmful for the host, such as in a situation where
58 suitable mutualistic microbes are not in the new habitat. It is also possible that invasive hosts
59 form new symbioses with the microbes available (18). A more diverse microbiota may result if
60 there is decreased immune-mediated control of novel microbes (19). By this logic, we might
61 therefore expect native host individuals to have a less rich microbiota. In particular, established
62 and stable microbial communities, in which novel competitive interactions between microbes
63 have long since played out, are likely to be of lower diversity (20). This prediction might be
64 particularly likely to hold if the host has evolved to cultivate beneficial associations (19).
65 Addressing whether this prediction is met requires characterization of how invasion impacts
66 microbial diversity and composition.

67 While molluscs are one of the most species-rich phyla (21), and represent an
68 ecologically and economically important class of invaders (22, 23), little is known about the
69 relationships between molluscs and their symbiotic microbes with the exception of a handful of
70 recent studies (24, 25). Here, we leveraged a powerful model system for host-parasite
71 coevolution, the New Zealand freshwater snail *Potamopyrgus antipodarum*, to characterize the

72 impact of invasion on host microbiota. There is substantial across-lake population variation in
73 microbiome composition among native *P. antipodarum* (26). *Potamopyrgus antipodarum* in
74 these native New Zealand populations also feature wide individual-level variation in
75 reproductive mode (sexual vs. asexual) and ploidy level (sexual snails are diploid while asexual
76 snails are triploid or tetraploid; (27, 28)), and are coevolving with sterilizing trematode parasites
77 (29, 30). By contrast, invasive lineages of *P. antipodarum* in Europe and North America are
78 polyploid asexuals with reduced genetic variation compared to the native range (17, 31) and
79 have escaped from parasite infection (32, 33).

80 We conducted 16S rRNA sequencing of field-collected *P. antipodarum* from waterbodies
81 across New Zealand and Europe to assess changes in microbiota diversity and composition
82 across and within native and invasive host populations. We predicted that, 1) similar to
83 patterns often described for genetic diversity, invasive *P. antipodarum* would have a subset of
84 native microbial diversity because invaders are often subsamples of native diversity and
85 because key symbionts needed to thrive should be maintained in the new environment; 2)
86 invaders would possess lower microbial diversity due to subsampling and because they have
87 had less time in the new environment to acquire new symbioses; 3) invaders will have fewer
88 conserved microbes than native counterparts as a consequence of relatively recent contact
89 with symbionts in the new environment, and therefore, relatively low time for the evolution of
90 specialization. To our knowledge, our study of *P. antipodarum* is the first comparison of
91 diversification in host-associated microbial communities between native and invasive molluscs.

92 We also address associations between the fundamental and often-variable organismal traits of
93 sex, reproductive mode, and infection status on microbiota within those host populations.
94
95 **Study System**
96 Native New Zealand *P. antipodarum* are primarily found in freshwater lakes and streams
97 (34). These snails have gained prominence as a model for studying the evolutionary
98 maintenance of sexual reproduction (27, 28) as well as host-parasite coevolution (27, 30).
99 Multiple triploid and tetraploid asexual lineages have been separately derived from diploid
100 sexual conspecifics (27, 28), and New Zealand populations are commonly infected by a
101 sterilizing trematode parasite, *Atriophallophorus winterbourni* (formerly *Microphallus livelyi*)
102 (27, 30, 35). A previous study hinted that the microbiome composition of these snails varies
103 substantially among native populations and between sexuals and asexuals (26). However, these
104 snails were either laboratory cultured or housed in a laboratory for several months before
105 harvest (Maurine Neiman, personal communication) meaning that whether these results hold
106 in snails sampled directly from the field remains unclear.
107 Only asexual lineages of *P. antipodarum* are highly successful invaders; why remains
108 unclear. Nevertheless, *P. antipodarum* can survive a range of harsh conditions that may
109 facilitate travel to and establish in new habitats (22, 36). Invaders are susceptible to few
110 biological enemies (22), passing alive through the digestive systems of trout (37) and are only
111 rarely infected by parasites (36). Once they invade, these snails can influence ecosystems due
112 to rapid population growth (22, 38), high population densities (32, 39), and competitive

113 exclusion of native invertebrates (22, 38). Invasive *P. antipodarum* can even harm the native
114 predators that ingest them (37).
115

116 **MATERIALS AND METHODS**

117 **Sample Collection, Processing, and Sequencing**

118 We collected adult *Potamopyrgus antipodarum* from shallow (lake depth < 1 m) rocks
119 and vegetation from three New Zealand sites in January 2015 (Table 1; Fig. S1) and ten
120 European collection sites (Table 1; Fig. S1) in spring 2016. Snails were maintained in 15 L tanks
121 separated by source population in a 16° C room with a 16:8 hour light:dark cycle for less than
122 one month before dissection. Snails were fed dried *Spirulina* cyanobacteria *ad libitum* (40).
123 All snails were sexed prior to DNA extraction and assessed for *A. winterbourni* infection
124 based on the presence of metacercariae cysts via dissection (41) (Table 1). All metacercariae
125 were removed from infected snails using a micropipette. After dissection, we used flow
126 cytometry (28, 42) to determine ploidy status as a proxy for reproductive mode of New Zealand
127 samples (diploids are sexual and polyploids are asexual). Because it is already established that
128 the European invasive lineages are virtually all polyploid asexuals (17, 43), we did not perform
129 flow cytometry on these samples.
130 We used the Qiagen DNeasy Plant Mini Kit (QIAGEN Inc.) for DNA extractions, following
131 manufacturer protocol, but eluting DNA in 40 µl 100:1 TE buffer. Samples with a 260/280 ratio
132 > 1.6 and containing > 20 ng of total DNA, based on Nanodrop® 1000 (Thermo Fisher Scientific),
133 were analysed on a 1% agarose gel. Samples that reached our quality criteria and produced

134 clear gel bands were shipped to the W.M. Keck Center for Comparative Functional Genomics
135 (University of Illinois at Urbana-Champaign) for sequencing. See Electronic Supplementary
136 Methods for more details about sample collection and processing.

137 The 16S rRNA V4 region was amplified from the *P. antipodarum* microbiome gDNA using
138 the 515F Golay-barcoded primers and 806R primers (44, 45). Samples were prepared in
139 accordance with the standard Earth Microbiome Project 16S rRNA protocol (46). Please see our
140 Electronic Supplementary Methods for PCR reaction mixtures and thermocycler conditions.
141 gDNA was quantified using the Qubit 2.0 fluorometer (Thermofisher Scientific,), amplicons were
142 pooled at equimolar ratios (~240 ng per sample), and amplicon pools were cleaned using the
143 Qiagen PCR Purification Kit (QIAGEN Inc.). The multiplexed library was quality checked and
144 sequenced with the MiSeq 2x250 bp PE v2 protocol at the W.M. Keck Center for Comparative
145 Functional Genomics (University of Illinois).

146

147 **Computational and Statistical Analyses**

148 We removed PhiX sequences from sequencing libraries using Bowtie2 (47) (PhiX genome
149 obtained from: support.illumina.com/sequencing/sequencing_software/igenome.html).
150 Demultiplexed paired-end fastq files were processed using DADA2 in R (3.4.0), with the
151 suggested filtering and trimming parameters, as previously described (48). We then merged
152 paired-end reads and constructed an amplicon sequence variant (ASV) table. We used the
153 native implementation of the DADA2 Ribosomal Database Project (RDP) naïve Bayesian

classifier (49) trained against the GreenGenes 13.8 release reference fasta (https://zenodo.org/record/158955#.WQsM81Pyu2w) to classify ASVs taxonomically.

We used phyloseq v. 1.16.2 to perform estimate_richness and vegan's pd function to calculate alpha diversity measurements of observed ASVs, Shannon's index, PD Whole Tree, Pielou's evenness, and Chao1 (50), and to perform ordinations using PCoA on unweighted and weighted UniFrac distance scores (51). We used the Songbird reference frames approach to calculate taxa differentials (52). Data visualization and statistical analyses were performed in R; see Electronic Supplemental Methods for details.

We corrected for effects of snail sex, infection status, and reproductive mode (Table 1) when testing for geographic associations with microbiota by only conducting analyses on female uninfected asexual (polyploid) snails, allowing us to directly compare native and invasive snails. For alpha diversity analyses, we rarefied samples to 10,000 ASVs per sample and discarded two samples that had fewer reads than this threshold. To test covariate effects on microbiota alpha diversity, we used Welch's Two Sample t-tests and adjusted *p*-values ("adj-*p*") with a Bonferroni correction for multiple tests. We conducted beta diversity analyses on all ASVs after removing singletons. We normalized ASV counts by adding one and then $\log_e$-transforming ASV counts (48). To evaluate beta diversity we performed PCoA on the distance matrices built on the unweighted and weighted UniFrac scores of each sample (51).

We used Analysis of Similarity (ANOSIM; 999 permutations, R-statistics (R^2) and exact *p*-values reported in Results) to evaluate effects of geographic location (sample site) on microbiota beta diversity . Because all European snails were female uninfected asexual snails

175 and many New Zealand snails were sexual, infected, and/or male, we only performed the
176 ANOSIM comparing Europe and New Zealand on female uninfected asexual snails.
177 To analyse population specificity, we focused on the core microbiome of snails within
178 locations, defined as taxa found in at least 90% of samples from a given location. We calculated
179 the proportion of shared core microbiome taxa among all sampling locations and also
180 compared all European samples vs. all New Zealand samples. We used a t-test to compare the
181 proportion of reads that mapped as core microbiome taxa between the combined Europe and
182 combined New Zealand sample groupings.
183 We performed a Permutational Multivariate Analysis of Variance Using Distance
184 Matrices analysis (ADONIS) to test the effects of reproductive mode, sex, and infection status
185 on microbiota beta diversity. The ADONIS analysis was limited to the Lake Alexandrina (New
186 Zealand) sample site as it was the only site for which we were able to obtain all conditions
187 (sexual, asexual, male, female, infected, and uninfected). ADONIS tests were conducted with
188 999 permutations.
189 We employed the Analysis of Composition of Microbes (ANCOM) algorithm to conduct a
190 differential count analysis (53). These analyses were limited to the Lake Alexandrina (New
191 Zealand) sample site for the reasons noted above. We tested the effect of sex in uninfected
192 male vs. uninfected female sexual snails, tested the effect of reproductive mode on uninfected
193 female sexual vs. uninfected female asexual snails, and tested the effect of infection status on
194 asexual female trematode-infected vs. asexual female uninfected snails. We used machine
195 learning to model microbiome classification by geography using female uninfected asexual

196 snails and ASVs agglomerated phylogenetically using default settings (phyloseq tax_glom; h =
197 0.2). Machine Learning was trained on a random forest model using the caret package (v6.0-81)
198 with a test split of 80:20 and fit a random forest classifier over the tuning parameter of snail
199 origin (New Zealand or Europe). Please see the Electronic Supplemental Methods for more
200 details on bioinformatic and statistical analyses.
201

202 **RESULTS**

203 Variation in *P. antipodarum* microbiota was in large part driven by whether a snail was
204 from Europe or New Zealand (Fig. 1; ANOSIM; R = 0.97*;* $p < 0.01$; Permutations: 999; ADONIS; R^2
205 = 0.33, $p < 0.01$; weighted UniFrac dissimilarity distances are presented in Fig. S2). We did not
206 find significant differences among microbiomes among sample sites within European or within
207 New Zealand samples. We thus attribute the geographic distance-to-microbiome distance
208 correlation to major differences between the most geographically distant European and New
209 Zealand microbiomes. As validation, we performed a Mantel test on coordinate and microbiota
210 distance matrices and again found geographic distance was significantly associated with
211 microbiome distance (Mantel test; Observation = 0.13; $p < 0.01$).
212 To identify taxa that significantly predicted ecosystem clustering, we used machine
213 learning classification via a random forest model to classify snails as sampled from Europe or
214 New Zealand based on microbiome composition. The model classified samples based on
215 relative microbial abundance into European or New Zealand ecosystems with 99.6% accuracy.
216 We corroborated these results using a reference frames approach and identified taxa that are

217 associated with snails from Europe or New Zealand (52). There were eight phyla that were
218 exclusively associated with snails from Europe and none that were exclusively associated with
219 snails from New Zealand (Table 2; Fig. 1). However, there were more Firmicutes taxa associated
220 with snails from New Zealand (9 taxa) than Europe (6 taxa) (Table 2; Table S1).

221 To test the degree to which microbiota exhibit host population-specific distributions, we
222 calculated the core microbiome of snails from Europe and New Zealand, defined at the tip
223 agglomerated level. European snails had a core microbiome (found in >=90% of samples)
224 consisting of 30 taxa while snails from New Zealand contained a core of one taxon (Fig 2A),
225 *Arenimonas*. This taxon, which defined the New Zealand snail core microbiome, was also a part
226 of the European snail core microbiome. Our results departed from this prediction of higher core
227 microbiome specificity within New Zealand sites by showing relatively consistent core
228 microbiome composition, containing fewer taxa, across the New Zealand sample sites
229 compared to European sample sites. We found that the core microbiome of European snails
230 constituted, on average, 13.8% of their microbiome. By contrast, the core microbiome of New
231 Zealand snails constituted 70.1% of their microbiome, a marked and statistically significant
232 difference between native and invasive snails (Fig 2B; T-test; $p < 0.01$). Proteobacteria were the
233 main constituent of the core microbiota in both snail populations, making up 100% (1/1) of the
234 New Zealand snail core and 68.9% of the taxa in the European snail core (20/29).

235 Because the majority of predictive taxa were in higher relative abundance in European
236 samples and most taxa from the core microbiome of European samples were not found in New
237 Zealand, we expected that European snail microbiomes would have higher species richness

238 than the New Zealand snails. Indeed, we found that European snails (mean: 116 ASVs, s.e.:
239 2.51) had on average 3.52x more ASVs than New Zealand snails (mean: 32.9 ASVs, s.e.: 2.56)
240 (Fig. 3; T-test; adj-$p < 0.01$; t = -23.1). There was significantly higher Chao1 and PD Whole Tree
241 diversity amongst European snail microbiota, suggesting higher richness of rare species at
242 relatively low abundance and higher phylogenetic diversity in European snail microbiota
243 compared to New Zealand snail microbiota (Fig. 3; T-test; adj-p values < 0.01; Chao1 t = -25.2;
244 PD t = -24.8). Shannon diversity was also significantly higher in European snail microbiota (Fig.
245 3; T-test; adj-$p < 0.01$; t = -4.52). Our subsequent prediction that New Zealand snail microbiota
246 may have higher Pielou's evenness than the European snails was upheld (Fig. 3; T-test; adj-p <
247 0.01; t = 22.4). There were thus more species, more rare species, and higher phylogenetic
248 richness in European snail microbiota, as well as higher species evenness in New Zealand snail
249 microbiota.

250 Our comparisons of snails from Lake Alexandrina show that sex ($R^2 = 0.147$; adj-p <
251 0.05), reproductive mode ($R^2 = 0.0467$; adj-$p < 0.05$), and infection status ($R^2 = 0.0330$; adj-p <
252 0.05) all are associated with microbiota composition (Table 3; ADONIS; Permutations: 999). We
253 corrected for these covariate effects and highlight taxa that were significantly different by sex
254 and reproductive mode (Fig. S3; ANCOM; adj-$p < 0.05$). No taxa significantly differed by
255 infection status. We identified ten ASVs (all Xanthomonadaecae) that were significantly higher
256 in abundance in asexual vs. sexual snails (Fig S3). 50 ASVs (*Niabella* (n = 16), *Bacillus* (n = 15),
257 and OM60 (n = 19)) were significantly more abundant in male vs. female snails. There was
258 significantly higher ASV richness in uninfected compared to infected snails (T-test; adj-$p < 0.01$;

259 Fig S4). There were no significant differences in observed ASV richness when comparing
260 reproductive mode or sex (Fig. S4).
261

262 **DISCUSSION**

263 There is a potential for loss of mutualistic microbes upon invasion and the reduced time
264 for host-microbiota coevolution in the new range. Thus, we predicted that invasion of an
265 organism would be reflected in its microbiota. The native New Zealand and invasive European
266 snails had distinct microbiotas. Whilst invasive snails carried the core taxa from New Zealand,
267 they harboured more core taxa overall and higher phylogenetic diversity. These results suggest
268 that invaders seem to readily acquire new symbionts, which may play a role in the success of
269 this worldwide invader. Our results also suggest that the symbionts associated with invasive
270 lineages may be more opportunistic and less specialized. This pattern may be a consequence of
271 the comparatively short time for host-microbe coevolution such that host-control mechanisms
272 have not yet evolved to keep microbial diversity in check (19).

273 The core microbe from native New Zealand snails was maintained in European invasive
274 snails. This result suggests that there could be selection to maintain certain symbiont
275 associations in both ranges, which may be important to host biology. Because we were unable
276 to obtain environmental samples, and it remains unclear whether some core microbes are
277 inherited, we cannot say whether core microbes found in European and New Zealand
278 populations were present in both environments, and thus carried over from the latter.
279 Nevertheless, European samples had 30 times more core taxa overall, and their most predictive

280 taxa were absent or relatively infrequent in native snails. In other systems, it has been shown
281 that invaders have lower microbial diversity and/or a subset of microbes relative to that
282 present in native populations (15, 16). Invasive *P. antipodarum* populations feature low
283 genotypic diversity relative to the native range (31, 54, 55) and mitochondrial haplotypes of
284 invaders represent genetic subsets of native mitochondrial haplotype diversity (17). However,
285 this genetic subsampling in invasive *P. antipodarum* populations is not reflected in their
286 microbiota diversity.

287 Across both ranges, proteobacteria dominate among the core microbes. Proteobacteria
288 and the majority of the other core microbial taxa observed here are consistent with Takacs-
289 Vesbach et al. (26). These results suggest a shared coevolutionary history between snails and
290 microbial symbionts has driven the loss of non-essential partners, and ultimately streamlined
291 the microbiome down to specialized taxa (19). A similar phenomenon occurs in a number of
292 insect systems, whereby the host harbours few core microbes that are key players in
293 organismal function (56, 57). Conversely, other insect taxa are host to relatively diverse core
294 microbial populations (58).

295 It is well established that antagonistic coevolutionary interactions such as between
296 hosts and pathogens can drive rapid evolution and diversification (59, 60). In the case of
297 mutualisms, theory has predicted relatively low rates of evolution compared to antagonistic
298 relationships (61, 62), although empirical evidence in support of this conjecture remains mixed
299 (63). For example, mutualisms can drive diversification, such as that observed in plant-animal
300 mutualisms (64). Even in the absence of host evolution, beneficial resident microbes can evolve

301 within days (65). Furthermore, competitive interactions between microbes that overlap in
302 resource use can also drive diversification (20).
303 Coevolution between *P. antipodarum* and *A. winterbourni* is well documented (27, 29,
304 30). Reciprocal antagonistic selection between *P. antipodarum* and *A. winterbourni* is expected
305 to be strong because infected snails are completely sterilized (66), and parasites die if they
306 encounter resistant individuals (67). In other systems, the potential for mutualist microbes to
307 be involved in host defence and even host-parasite coevolution has been demonstrated (68,
308 69). However, the extent to which coevolution operates in diverse communities that feature
309 both mutualists and antagonists is unclear. Our results do not suggest that the microbiota is a
310 dominant player in this host-parasite coevolutionary relationship: some taxa differed only
311 marginally, but not statistically significantly, in relative abundance between infected and
312 uninfected snails, although we did observe significantly higher ASV richness in uninfected snails
313 compared to infected snails.
314 In other systems, it does seem that microbial diversity could confer some degree of
315 parasite resistance (70), perhaps analogous to the host genetic diversity conferred by
316 outcrossing in *P. antipodarum* (71). However, that asexual *P. antipodarum* are more successful
317 invaders strongly suggests that this diversity cannot outweigh the costs of being sexual. Here,
318 we found that reproductive mode was associated with microbiome composition and ten ASVs
319 that were significantly more abundant in asexuals than sexuals. Takacs-Vesbach et al. (26)
320 observed significant differences in microbiome composition between sexuals and asexuals and
321 among lake populations of *P. antipodarum*. Because the mechanism(s) leading to transitions

322 from diploid sexuality to polyploid asexuality in *P. antipodarum* are still unclear, future work
323 could focus on the possible links between microbes and reproductive mode and/or
324 polyploidization. For example, *Wolbachia*-mediated transitions to asexuality are well
325 characterized in arthropods (72). Besides a handful of studies (73, 74), however, very little is
326 known about potential links between host ploidy level and microbiome composition, especially
327 in animals.

328 While it is established that symbiotic microbes have important effects on host fitness,
329 the relationship between microbes and molluscan invaders has received little attention. Here,
330 we find that the process of invasion is associated with some carryover of a core microbiota, but
331 mostly involves the formation of numerous new symbiotic relationships, suggesting that
332 invasive *P. antipodarum* are able to take advantage of readily available microbes. Despite some
333 impact of biological trait variation on the microbiota in native snail populations, there is a
334 consistent and strong pattern suggesting that host-microbiota coevolution (or host evolution
335 alone (19)) streamlines the microbiota of native snails to essential members. Future research
336 will need to establish the fitness impacts of those new microbes on invaders, and whether the
337 invading snails have adapted to accommodate them. Notwithstanding this possibility, we show
338 that the potential for invasion to alter the microbiome is clear.

339

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DATA AVAILABILITY

Raw sequencing data will be made publicly available on NCBI Sequence Read Archive and accession numbers will be added upon article acceptance.

AUTHOR CONTRIBUTIONS

KCK conceived the project. KCK, LB, DD, MN, and GH designed project. LB, MN, and KCK conducted field collections. LB, DD, CAT, and CF performed DNA extraction, flow cytometry, and prepared sequencing libraries. DD carried out the bioinformatics analysis. The manuscript was written by LB, DD, and KCK with input and approval from the other authors.

FIGURE AND TABLE CAPTIONS

Fig 1. Snail microbiota ecosystem clustering. Biplot PCoA on unweighted UniFrac dissimilarity distances between snail microbiota profiles across European and New Zealand sample sites. Each point represents a single snail and are colour coded based on whether a point is a microbial taxon (grey filled with outline colour indicating sample site) or snail (colour filled by sample site) and indicates collection site of the snail. Taxa represented on the plot are the top 20-ranked differentials in predicting whether a snail was from New Zealand or Europe and are annotated at the deepest available taxonomic level. Density plot below PCo1 shows snail sample density across PCo1 labelled by sample site. Density plots to the left of PCo2 show snail sample density across PCo2 labelled by sex (male or female), infection status (infected or uninfected), and reproductive mode (sexual or asexual).

Figure 2. Core microbiota of European and New Zealand snails. (A) 16S rRNA phylogeny of core microbiota (>90% samples) of European (EU) and New Zealand (NZ) snails. Fractional abundance averaged across samples within countries is represented by dot size. (B) Violin plot summarizing the proportion of amplicon reads mapped to core. Significance is denoted as *** = $p < 0.001$ (T-test).

Fig 3. Alpha diversity measurements across sample sites. Figures are faceted by diversity metric. X-axis shows sampling site. Y-axis shows value of diversity metric. NZ = New Zealand samples and EU = European samples.

Table 1. Sample collection sites. The coordinates and location names of sample sites for both native and invasive snails are list below. Ploidy is a proxy for reproductive mode; diploids are sexual, polyploids are asexual. We also include the sex, infection status, and number of individuals sequenced from each condition and location and condition.

Table 2. Europe and New Zealand microbiome phyla predictors. We used a multinomial regression to predict whether snails were from Europe or New Zealand samples sites. Represented here is a summary of counts of phyla that were associated with snails from EU or NZ.

Table 3. ADONIS testing effects of sex, ploidy, and infection status on microbiome UniFrac distances. Results from Analysis of Variance Using Distance Matrices (ADONIS) testing of the effects of sex, ploidy, and infection status on unweighted microbiome UniFrac distances between snails. All tests were performed on snails from Lake Alexandrina with 999 permutations.

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595

Fig. 1

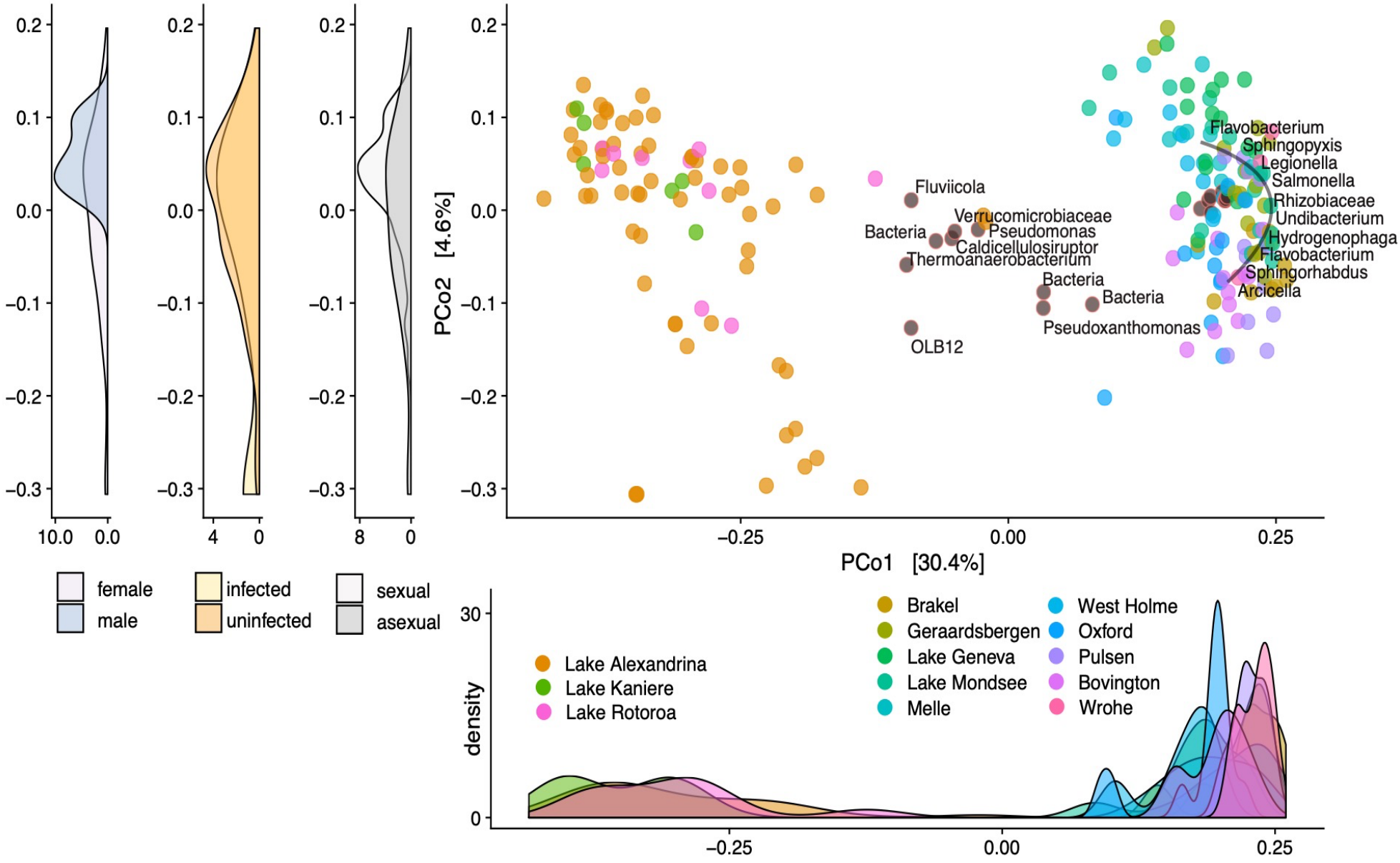
PCo2 [4.6%]
PCo1 [30.4%]
Flavobacterium
Sphingopyxis
Legionella
Salmonella
Rhizobiaceae
Undibacterium
Hydrogenophaga
Flavobacterium
Sphingorhabdus
Arcicella
Fluviicola
Verrucomicrobiaceae
Pseudomonas
Bacteria
Caldicellulosiruptor
Thermoanaerobacterium
Bacteria
Bacteria
Pseudoxanthomonas
OLB12
female
male
infected
uninfected
sexual
asexual
density
Lake Alexandrina
Lake Kaniere
Lake Rotoroa
Brakel
Geraardsbergen
Lake Geneva
Lake Mondsee
Melle
West Holme
Oxford
Pulsen
Bovington
Wrohe

Fig. 2

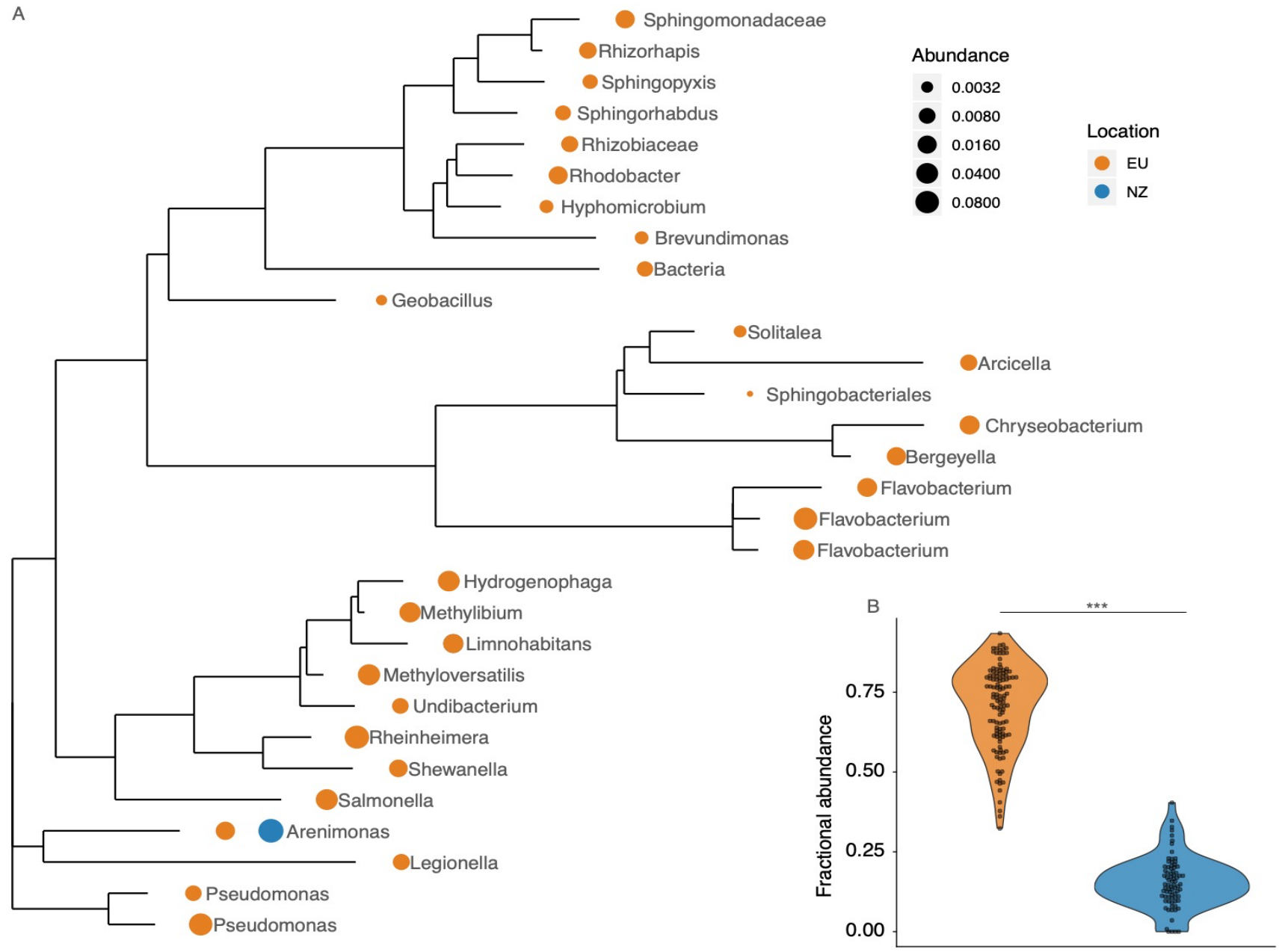
A
Sphingomonadaceae
Rhizorhapis
Sphingopyxis
Sphingorhabdus
Rhizobiaceae
Rhodobacter
Hyphomicrobium
Brevundimonas
Bacteria
Geobacillus
Solitalea
Arcicella
Sphingobacteriales
Chryseobacterium
Bergeyella
Flavobacterium
Flavobacterium
Flavobacterium
Hydrogenophaga
Methylibium
Limnohabitans
Methyloversatilis
Undibacterium
Rheinheimera
Shewanella
Salmonella
Arenimonas
Legionella
Pseudomonas
Pseudomonas
Abundance
0.0032
0.0080
0.0160
0.0400
0.0800
Location
EU
NZ
B
***
Fractional abundance
0.75
0.50
0.25
0.00

Fig. 3

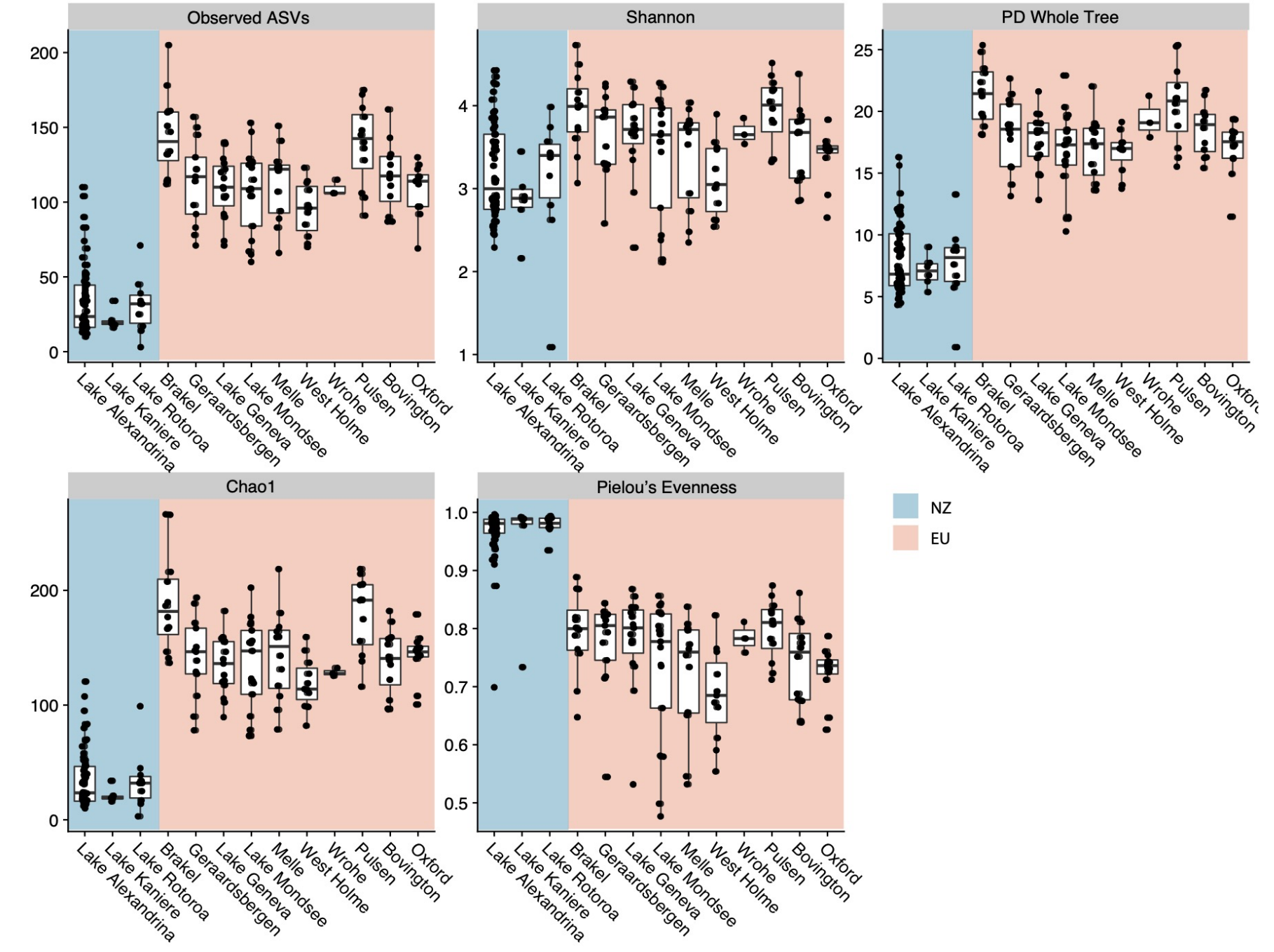
Observed ASVs
Shannon
PD Whole Tree
Chao1
Pielou's Evenness
Lake Alexandrina
Lake Kaniere
Lake Rotoroa
Brakel
Geraardsbergen
Lake Geneva
Lake Mondsee
Melle
West Holme
Wrohe
Pulsen
Bovington
Oxford
NZ
EU

Table 1. Sample collection sites.

Country	Location	Geographic Coordinates	Ploidy	Infection	Sex	No. Snails
New Zealand	Lake Alexandrina	43° 56' 12.9" S, 170° 27' 35.4" E	Diploid	Infected	Female	9
			Diploid	Infected	Male	2
			Diploid	Uninfected	Female	22
			Diploid	Uninfected	Male	19
			Polyploid	Unknown	Female	16
			Polyploid	Infected	Female	10
			Polyploid	Uninfected	Female	20
New Zealand	Lake Kaniere	42° 48' 21.8" S, 171° 7' 40.5" E	Diploid	Infected	Female	2
			Diploid	Uninfected	Female	6
New Zealand	Lake Rotoroa	41° 47' 44.5" S, 172° 36' 22.4" E	Diploid	Infected	Female	1
			Diploid	Uninfected	Female	10
			Polyploid	Infected	Female	1
			Polyploid	Uninfected	Female	10
Austria	Lake Mondsee	47° 50' 37.7" N, 13° 20' 30.7" E	Polyploid	Uninfected	Female	17
Belgium	Brakel	50° 48' 12.1" N, 3° 45' 20.9" E	Polyploid	Uninfected	Female	12
Belgium	Geraardsbergen	50° 47' 30.4" N, 3° 54' 15.0" E	Polyploid	Uninfected	Female	13
Belgium	Melle	51° 1' 11.6" N, 3° 48' 59.6" E	Polyploid	Uninfected	Female	12
Germany	Wrohe	54° 16' 7.4" N, 9° 57' 35.9" E	Polyploid	Uninfected	Female	3
Germany	Pulsen	54° 19' 17.3" N, 10° 27' 9.8" E	Polyploid	Uninfected	Female	12
Switzerland	Lake Geneva	46° 30' 45.2" N, 6° 36' 27.4" E	Polyploid	Uninfected	Female	15
United Kingdom	West Holme	50° 40' 20.1" N, 2° 10' 29.03" W	Polyploid	Uninfected	Female	11
United Kingdom	Bovington	50° 41' 26.1" N, 2° 13' 29.02" W	Polyploid	Uninfected	Female	12
United Kingdom	Oxford	51° 45' 6.5" N, 1° 13' 0.35" W	Polyploid	Uninfected	Female	12

Table 2. Europe and New Zealand microbiome phyla predictors.

Phylum	Europe	New Zealand
Acidobacteria	3	0
Actinobacteria	4	0
Armatimonadetes	2	0
Bacteroidetes	31	13
Chlamydiae	0	0
Chloroflexi	0	0
Cyanobacteria	4	3
Deinococcus-Thermus	1	0
Epsilonbacteraeota	1	0
Firmicutes	6	9
Gemmatimonadetes	0	0
Lentisphaerae	0	0
Nitrospirae	1	1
Planctomycetes	2	0
Proteobacteria	65	32
Spirochaetes	1	0
Tenericutes	1	0
Verrucomicrobia	1	2

Table 3. ADONIS testing effects of sex, ploidy, and infection status on microbiome UniFrac distances.

Condition	Df	SS	MS	F Model	R2	P
Sex	1	1.2899	1.28991	14.5946	0.14665	0.001
Reproductive mode	1	0.4105	0.41049	4.6444	0.04667	0.001
Infection	1	0.29	0.29	3.2813	0.03297	0.005
Residuals	77	6.8055	0.08838	0.77371		
Total	80	8.7959	1			