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Which microbiome are we talking about? Contrasted diversity patterns and eco-evolutionary processes between gill and intestinal microbiomes of Antarctic fairy shrimps

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Metazoans comprise multiple physical niches ("microenvironments"), each colonized by unique microbiomes that contribute to their hosts' evolutionary dynamics, influencing their health, physiology, and adaptation to changing environments. Most wildlife microbiome studies focus on higher metazoans and multiple host microenvironments, while studies of lower species often concentrate on a single microenvironment, sometimes pooling whole bodies or specimens. This is particularly evident in small-sized animals, such as freshwater meiofaunal invertebrates, thus impeding a holistic understanding of microbiome assembly across host microenvironments and its relation with host population genetics. Leveraging the anostracan fairy shrimp *Branchinecta*, which has easily discernible organs and expected high levels of intraspecific genetic divergence, we aimed to investigate the microbiome assembly processes and test the phylosymbiosis signal in two microenvironments (gill and intestine) across four host populations of *Branchinecta gaini* within Maritime Antarctica, using 16S rRNA metabarcoding. Our results showed that the gill and intestine harbor strikingly different microbiomes resulting from the *B. gaini* ecological filtering of the surrounding environment microbial community. Both microenvironments exhibit their respective core microbiomes, yet the gill's core microbiome is narrower and constitutes a smaller proportion of the overall bacterial community compared to that of the intestine. Within each host population (*i.e.* each sampling site), the microbiome assembles through distinct eco-evolutionary processes in both microenvironments, mostly stochastically (ecological drift) in the gill and deterministically (variable selection) in the intestine. Across different *B. gaini* populations, variable selection dominates in driving compositional divergence of both microenvironment microbiomes, although to a lesser extent in the gill. Lastly, our study reveals robust correlation between host intraspecific genetic structure and intestine microbiome composition, providing evidence of phylosymbiosis in

anostracans. Contrastingly, phylosymbiosis was less pronounced in the gill microbiome. We discuss the potential differences in ecological filtering between each host microenvironment that may underlie the difference in the strength of phylosymbiosis. Our study highlights the relevance of considering host microenvironment and intraspecific levels in testing the phylosymbiosis hypothesis to better understand the intricate eco-evolutionary relationships between hosts and their microbiomes.

KEYWORDS

invertebrate microbiome, gill, intestine, ecological filtering, ecological assembly processes, *Branchinecta*, West Antarctic Peninsula, freshwater ecosystems

1 Introduction

Most macroorganisms exist in close association with diverse microbial partners, colonizing various physical niches provided by their hosts, thereby conforming what is termed metaorganisms (McFall-Ngai et al., 2013; Ludington, 2024). The variability in microbiome composition and function across different body parts has been linked to various facets of the host ecology (Zilber-Rosenberg and Rosenberg, 2008; Alberdi et al., 2022; Ludington, 2024). An iconic example is the human body, where each organ constitutes a distinct ecological niche that hosts a unique microbiome carrying out specific functions essential for host health (Costello et al., 2009; Dekaboruah et al., 2020). However, most of the animal-associated microbiome studies remain confined to compositional description of single-body sites (Rosenberg and Zilber-Rosenberg, 2018; Woodhams et al., 2020; Wang et al., 2023). Considering various body sites within the same host is necessary to gain a holistic understanding of the eco-evolutionary processes driving microbiome assembly (Stegen et al., 2013; Zhou and Ning, 2017). One of these eco-evolutionary processes is the contribution of the host evolution to microbiome assembly. Indeed, the intimate and prolonged ecological interdependency of hosts and their microbiomes can result in continuous mutual adaptation, also known as co-evolution (Wilson and Duncan, 2015; Rosenberg and Zilber-Rosenberg, 2018; Hayward et al., 2021; Mazel et al., 2023). Co-evolution can leave a phylogenetic footprint of the host in the microbiome composition, meaning that closely related host species exhibit more similar microbiomes, which is known as phylosymbiosis (Bordenstein and Theis, 2015; Brooks et al., 2016; Groussin et al., 2020). Although the detection of phylosymbiosis signature does not explicitly underpin a common evolutionary history between hosts and their microbiomes (Moran and Sloan, 2015; Mazel et al., 2018; Mallott and Amato, 2021), it constitutes a relevant hypothesis to delve into the eco-evolutionary mechanisms that shape metaorganisms-associated microbiome (Lim and Bordenstein, 2020; Mazel et al., 2023; Mallott, 2024).

Phylosymbiosis has been extensively tested across a biased fraction of metazoan species (Woodhams et al., 2020; Mazel et al., 2023), primarily vertebrates, including mammals (Groussin et al., 2017; Moeller et al., 2017; Youngblut et al., 2019) and fishes (Doane et al., 2020; Pan et al., 2023; Sadeghi et al., 2023; Schwob et al., 2024a), and to a lesser extent in invertebrates, like insects (Qin et al., 2023), sponges and corals (Pollock et al., 2018; O'Brien et al., 2020; Pankey et al., 2022). Despite a growing body of research, the universality of phylosymbiosis still remains to be fully tackled (Mazel et al., 2018; Lim and Bordenstein, 2020). The frequency and magnitude of phylosymbiosis appear to be inconsistent among taxonomic groups (Lim and Bordenstein, 2020), being relatively common and strong across mammals (Mallott and Amato, 2021), while looser in other vertebrates, such as birds (Trevelline et al., 2020; Bodawatta et al., 2022), and even absent in most (though not all) of the terrestrial and aquatic invertebrates studied (Hammer et al., 2019; Huot et al., 2019; Eckert et al., 2021; Boscaro et al., 2022; Eckert et al., 2022). Consequently, general conclusions about the underlying mechanisms of phylosymbiosis may be skewed by the restricted range of investigated models (Hammer et al., 2019), emphasizing the need to consider understudied species (Leasi et al., 2023). Some authors suggested that the phylosymbiosis signal might be obscured by extended divergence time among studied species (Groussin et al., 2017; Kohl et al., 2018; Ross et al., 2018), as well as by taxonomically rich microbiomes (Mallott and Amato, 2021), which contain a relatively high proportion of transient microorganisms (Ross et al., 2018). Conversely, the phylosymbiosis signal seems to be more frequent in internal rather than external host body sites (Moran et al., 2019). Moreover, considering the intraspecific variability of hosts offers an essential, yet often overlooked, resolution level for generalizing conclusions about the consistency of phylosymbiosis across taxonomic resolution and understanding how intraspecific host genetic variation associates with microbiome variation (Mazel et al., 2018; Couch and Epps, 2022). To the best of our knowledge, no study combines intraspecific resolution and multiple body microenvironments to test the eco-evolutionary processes in general, and phylosymbiosis in particular.

Characterizing the microbiome assembly across multiple body sites can be challenging, particularly in small-sized animals. To date,

the microbiome of meiofaunal freshwater invertebrates like copepod and cladoceran has only been investigated at the whole individual-scale. These invertebrates host a specific microbiome discernable from the surrounding water microbiome (Samad et al., 2020), confirming the host-driven ecological filtering (Mazel et al., 2018). In *Daphnia* (Anamopoda), specific bacteria were consistently associated with geographically and genetically separated host populations, suggesting a stable "core microbiome" shaped by host-microbiome interactions (Qi et al., 2009; Guivier et al., 2018). Additionally, the gut microbiome of *Daphnia magna* is shaped by host genotype and enhances host fitness by providing essential nutrients (Cooper and Cressler, 2020; Macke et al., 2020; Motiei et al., 2020). In the branchiopod genus *Artemia* (Anostraca), host species has been identified as a key determinant of microbiome composition. The *Artemia* microbiome influences host tolerance to low salinity, thereby potentially contributing to its evolutionary trajectory (Nougué et al., 2015; Lee et al., 2024). Despite the ecological significance of branchiopoda-associated microbiomes, their variability across body sites and their eco-evolutionary drivers are unclear (Eckert et al., 2021; Boscaro et al., 2022). In previous phylosymbiosis studies focusing on invertebrates, the relatively small size imposed to use the whole individuals (Eckert et al., 2021; Leasi et al., 2023), or even to pool several individuals for microbiome assembly (Callens et al., 2018; Eckert et al., 2022). Besides ignoring the microbiome variability across host body site and among individuals, these approaches may have masked the impact of host phylogeny on microbiome assembly (Mazel et al., 2018).

The anostracan fairy shrimp *Branchinecta gaini* Daday, (1910) is the largest (adult body size c. 16 mm) and most abundant crustacean species of Antarctic lacustrine habitats. *B. gaini* is widely distributed across Maritime Antarctica (*i.e.*, South Shetland Islands, the Antarctic Peninsula, and the islands of the Scotia Arc) and southern South America (Hawes, 2009). It plays a key role in the trophic chain of these freshwater ecosystems (Peck, 2004; Hawes, 2009). *B. gaini* is considered as a primarily non-selective detritivore and herbivorous filter feeder (Lukic et al., 2023), although occasional predation on copepods like *Boeckella poppei*, larval stages of *Parochlus steineni* and even conspecific organisms has been reported (Paggi, 1996). *B. gaini* is characterized by two main microenvironments: a prominent branchial lamina (referred to as gill) and a digestive tract (referred to as intestine) easily discernable through body transparency (Lynch, 1964), facilitating the dissection of these tissues despite the host's small size. The microbiome of *B. gaini* remains unexplored to date. Similarly, the intraspecific genetic variation of *B. gaini* in the Maritime Antarctica is uncharacterized, although other Branchinectidae species exhibit high intraspecific genetic divergences (Aguilar et al., 2017). Given the relative isolation of freshwater lentic ecosystems in Maritime Antarctica (*i.e.*, patchiness of suitable habitat), *B. gaini* is likely to exhibit geographic and genetic structuring at an intra-species level, as previously reported in other freshwater invertebrates [*e.g., B. poppei*, Maturana et al. (2020)]. Thus, the fairy shrimp *B. gaini* represents a suitable model for comprehensively investigating the microbiome assembly processes and testing the phylosymbiosis signal across different host microenvironments in relation with the intraspecific genetic structure of host populations.

This study aims to (i) evaluate the distinguishability of *B. gaini* microbiomes between gill and intestine, (ii) identify the core microbiomes of gill and intestine, and their keystone Operational Taxonomic Units (OTUs), across geographically-distant sites in Maritime Antarctica, (iii) compare the eco-evolutionary processes governing gill and intestine microbiome assembly at local and regional scales, and (iv) elucidate the relative effects of host phylogenetic diversity, geography and climate in shaping both *B. gaini* microbiomes.

2 Methods

2.1 Host and environmental samples recollection and preparation

Specimens of *B. gaini* were sampled from Maritime Antarctica lake water between 2018 and 2023, during the austral summer (January), using a hand-crafted net, with mesh size < 1mm (specimen body size of 15–20 mm) (Table 1). The sampling sites encompass four shallow (0.5–1 m-deep) freshwater lakes, located in Signy Island (PUM) in the South Orkney Islands, King George Island (KIT, WUK) in the South Shetland Islands, and Avian Island (ANT) in the West Antarctic Peninsula (Figure 1). Immediately following sampling, entire individuals of *B. gaini* were preserved in absolute ethanol with one 50-ml Falcon tube dedicated per sampling site, for subsequent morphological and molecular analyses. The ethanol-preserved *B. gaini* individuals (8 specimens per site) were dissected under binocular magnifier to separate the head, the gill and the entire intestine with its content. Due to logistical constraints, superficial sediment was collected at a single site (ANT) in sterile Ziploc® bags. Samples from all the microenvironments (*i.e.*, sediment, head, gill and intestine) were conserved into 2-ml sterile cryotubes at -20°C until further molecular analyses (Table 1).

2.2 Molecular procedures

Prior to molecular processing, host individuals were carefully rinsed with sterile water to remove the ethanol and minimize the risk of cross-contamination. For host analysis, the genomic DNA from *B. gaini* head samples was extracted using the DNAeasy Blood & Tissue Kit (Qiagen, CA, USA) protocol for small tissue samples with previous optimizations, including a preliminary incubation in proteinase K+ATL buffer solution overnight at 37°C (Maturana et al., 2020). One segment of the mitochondrial cytochrome c oxidase subunit I (cox1) was amplified using the universal primer LCO1490 and HCO2198 (Folmer et al., 1994). Detailed PCR protocol is given in Supplementary Material (Supplementary Material S1). The obtained PCR products obtained were sequenced in both directions at Macrogen Chile, using Sanger technology.

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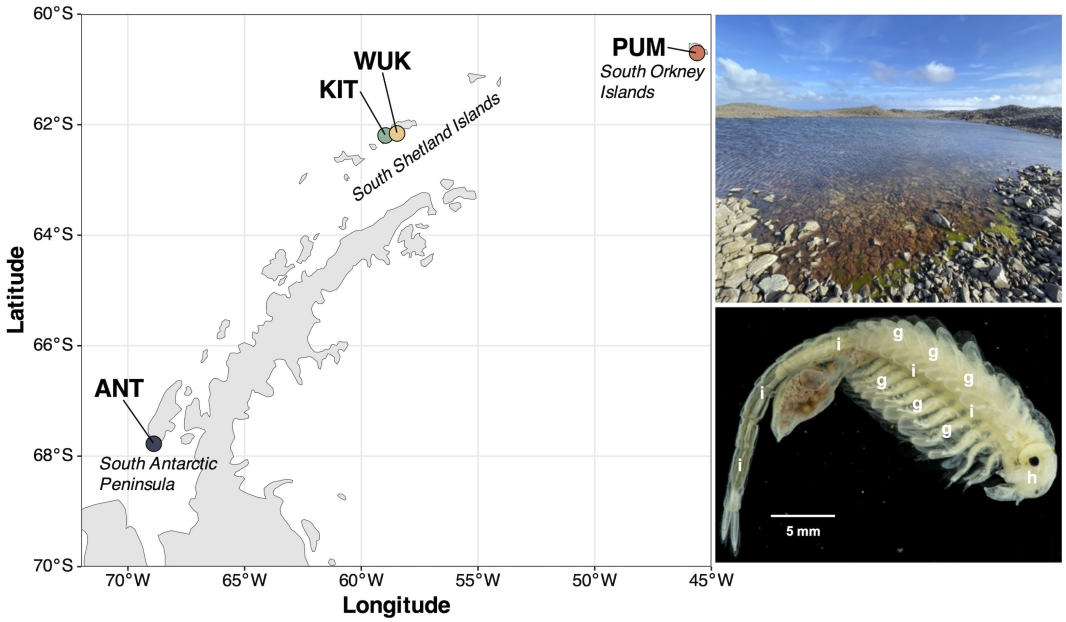

FIGURE 1
Localization of the sampling sites of *Branchinecta gaini* across Maritime Antarctica. From North to South, PUM: Lake 'Pumphouse' from South Orkney Islands, WUK and KIT: Lakes 'Wukja' and 'Kitiesh' from South Shetland Islands, and ANT: Lake 'Antena', from South Antarctic Peninsula (see Table 1 for details). The photos illustrate the characteristic habitat of *B. gaini* (above) and an individual of *B. gaini* (below). White letters "i", "g" and "h" indicate the intestine, the gill and the head, respectively. More detailed photos are provided in Supplementary Figure S1.

For microbiome analysis, the genomic DNA from gill and intestine samples was extracted using the DNAeasy PowerSoil Pro Kit (Qiagen, CA, USA) with a previous FastPrep-24® homogenization step (20 s at 4 m s^{-1}) (MP Biomedicals, USA) according to manufacturer's recommendations. Amplification, library preparation, and amplicon sequencing was carried out by Novogene (Beijing, China). Briefly, the hypervariable V3-V4 region of the 16S rRNA gene was amplified using the 341F-806R primers (Klindworth et al., 2013), and the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, USA), following the PCR protocol detailed in Supplementary Material (Supplementary Material S2).

2.3 Bioinformatic treatment of sequencing data

Host cox1 partial sequences were manually quality controlled, assembled and edited using GENEIOUS 10.2.2 (Kearse et al., 2012).

TABLE 1 Sampling locations of *Branchinecta gaini* and overview of metabarcoding sequencing data.

Region	Locality	Site	Date	GPS coordinates	Microenv.	N	Nseq. (Relat. Abund.)
South Orkney Islands	Signy I.	Lake Pumphouse (PUM)	01-2018	60°42'05.00"S 45°36'51.31"W	Gill	4	681,376 (8.3%)
					Intestine	7	1,251,377 (15.2%)
South Shetland Islands	Fildes Peninsula, King George I.	Lake Wujka (WUK)	01-2019	62°09'32.25"S 58°28'01.77"W	Gill	6	722,924 (8.8%)
					Intestine	8	1,007,827 (12.3%)
South Shetland Islands	Fildes Peninsula, King George I.	Lake Kitiesh (KIT)	01-2019	62°11'41.14"S 58°57'40.71"W	Gill	6	1,023,860 (12.4%)
					Intestine	8	1,246,796 (15.2%)
South Antarctic Peninsula	Margarite Bay, Avian I.	Lake Antena (ANT)	01-2023	67°46'32.59"S 68°53'21.44"W	Water	1	147,072 (1.8%)
					Sediment	3	345,612 (4.2%)
					Gill	7	822,516 (10.0%)
					Intestine	8	975,076 (11.9%)

Microenv., microenvironment; N, number of samples successfully sequenced; Nseqs. (Relat. Abund.), total number of sequences per sample type, and relative abundance in the whole dataset, after sequence cleaning; I., island.

Alignments were obtained using MUSCLE (Edgar, 2004) with standard setting. The host phylogenetic tree was reconstructed from cox1 sequences with NGPhylogeny (Lemoine et al., 2019), using the HKY85+I model recommended by the implemented Smart Model Selection tool (SMS), and complemented by non-parametric SH-like branch supports. Subsequently, the pairwise phylogenetic distances between the pairs of host cox1 sequences within the host were calculated with the *cophenetic.phylo* function implemented in the APE R package (v5.7-1) (Paradis and Schliep, 2019).

Bacterial paired-end raw sequences were analyzed through the Mothur pipeline (v1.48.0) (Schloss et al., 2009), following the previously described procedure (Schwob et al., 2020). Chimeras were removed using Uchime implemented in Mothur (Edgar et al., 2011). Cleaned sequences were clustered into OTUs at 97%-identity, and low-abundance (< 0.0001%) OTUs were discarded according to (Bokulich et al., 2013). The final OTU table, representing a total of 688 OTUs, was rarefied at 80,000 sequences per sample and converted into a Bray-Curtis dissimilarity distance matrix using the *vegdist* function of the VEGAN R package (v2.6-2) (Oksanen et al., 2011). The representative sequences of the OTUs were used to calculate the phylogenetic tree with NGPhylogeny (Lemoine et al., 2019), using the GTR+G+I model recommended by the implemented Smart Model Selection tool (SMS), and complemented by non-parametric SH-like branch supports. The bacterial phylogenetic tree was rooted on the branch separating Cyanobacteria from the rest of the sequences (Coleman et al., 2021).

2.4 Microbiome diversity analysis

The observed richness, the Chao1, and the Shannon indices were estimated using the *plot_richness* function from the PHYLOSEQ R package (version 1.46) (McMurdie and Holmes, 2013), and Faith's phylogenetic diversity was estimated using the *estimate_pd* function from the BTOOLS R package (v0.0.1) (Battaglia, 2021). Mean values of alpha-diversity indices among microenvironments were compared using a non-parametric pairwise Wilcoxon test through the *geom_signif* function of the GGPUBR R package (Kassambara, 2020).

The beta-diversity pattern of *B. gaini* microbiomes across sites and microenvironments was visualized through a hierarchical clustering and a non-metric multidimensional scaling (NMDS) ordination, using the *hclust* function from the DENDEXTEND R package and the *plot_ordination* function in the PHYLOSEQ R package, respectively. Differences in community structure among sites and microenvironments were tested with PERMANOVA and pairwise PERMANOVA using the *adonis2* and *pairwise.adonis* functions (VEGAN and FUNFUNS R packages, respectively). The contribution of individual OTUs to the distinguishability of the microbiome among microenvironments was assessed using Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis, performed via the *run_lefse* function in the MICROBIOMEMARKER R package (Cao et al., 2022).

The ecological niche breadth (B_N) was computed for each OTU within the gill and intestine microbiome using the Levins' method implemented in *levins.Bn* function of the MICRONICHE R package (Finn et al., 2020). To classify OTUs as generalists, specialists, or neutralists within gill and intestine microbiomes, their Levins' B_N values were compared to a null distribution, following the methodology described in Gao et al. (2023). The proportions of neutralists, generalists, and specialists were statistically compared between gill and intestine microenvironments using the DUNN.TEST R package (Dinno, 2017). The mean community-level niche breadth was calculated for each sample and the significance of the difference in means between the two microenvironments was assessed using the Wilcoxon test.

To characterize the core microbiome of *B. gaini* gill and intestine, we applied the 'common core' definition (Risely, 2020), which encompasses the OTUs present in a large proportion of hosts (*i.e.*, in > 75% of all replicates from each site), including rare OTUs (*i.e.* no abundance criteria) (Lahti et al., 2017). OTUs with an occurrence frequency of less than 75% were classified as part of the flexible microbiome. The OTU tables of gill and intestine core microbiomes were transformed into relative abundances (normalized by each total core abundance). Co-occurrence networks were computed for each core community using the SPIEC-EASI algorithm with default parameters in R (Kurtz et al., 2015). Nodes' features, including closeness centrality (*i.e.*, the proximity of a node to all other nodes in the network), betweenness centrality (*i.e.*, the extent to which a node lies on the shortest paths among other nodes in the network), and degree count, were used to assess their potential hub taxa status (Berry and Widder, 2014). The microbiome assembly processes within the gill and intestine of *B. gaini* were analyzed both at each individual site and across all sites, and then averaged by microenvironments, using an in-house R function of the framework from Stegen et al. (2015), and previously published in Delleuze et al. (2024).

For phylosymbiosis analysis, Mantel tests with 9,999 permutations were conducted for each microenvironment (gill, intestine) to independently test the correlation between the microbiome Bray-Curtis dissimilarity distance and the host phylogenetic distance matrix using the cophenetic function implemented in the APE R package. The Mantel correlations were visualized under scatterplots generated with the GGPLOT R package (Wickham, 2016). Tanglegrams were generated using the *tanglegram* function in the DENDEXTEND R package to illustrate the correspondence of samples' order between the dendrograms of gill and intestine microbiomes and the host tree. Partial Mantel tests were employed to determine the contribution of host phylogeny to beta-diversity in gill and intestine microbiomes, while controlling for geographic and climatic distances (Martiny et al., 2006). Geographic distances among sites were computed from the longitude and latitude coordinates using the *earth.dist* function of the FOSSIL R package (Vavrek, 2011). Climatic variables, including precipitation amount (pr), mean daily maximum air temperature (tasmax), mean daily minimum air temperature (tasmin), mean daily air temperature (tas), and surface downwelling shortwave flux (rsds) were obtained from the CHELSA database v2.1 (Karger et al., 2017), selecting January 2018 data as the most coincident with our sampling time. Standardization of climatic variables was performed using the *decostand* function (VEGAN). Next, automatic stepwise

model selection for redundancy analysis was performed through the *ordiR2step* function (Vegan) to select the climatic variables that explained most of the variance in the OTU composition of gill and intestine samples. To address collinearity among explanatory factors, principal component analysis (PCA) was conducted on the selected climatic variables (*i.e.*, pr, tasmax, and rsds), and the PCA axis scores were transformed into Euclidean distances using the *vegdist* function (Vegan) that will be further used as the climatic distance matrix.

3 Results

3.1 Significant alpha-diversity variations across *B. gaini* microenvironments

Microbiome alpha diversity varied significantly across *B. gaini* microenvironments. Except for the WUK site, the observed and Chao1-estimated OTU richness was approximately twice higher in intestinal samples compared to gill samples (Figure 2; Supplementary Figure S2). Likewise, greater phylogenetic diversity was observed in intestine than in gill (Figure 2). Contrarily, the Shannon diversity values were more homogenous between gill and intestinal samples across sites (Supplementary Figure S2). At the ANT site, alpha diversity indices of the lake sediment (excluding the Shannon index) were comparable to those of the intestine and significantly higher than those of the gill samples (Supplementary Figure S3).

3.2 Unique microbiome features in gill vs. intestine of *B. gaini*

Within each site, clear dissimilarities in microbiome compositions were observed between gill and intestinal microenvironments (pairwise PERMANOVA, p-values < 0.005, Table 2; Figures 3A, B). For ANT and KIT, the microbial communities clustered primarily by microenvironments and secondly by site, indicating that microbiome compositions among samples from the same microenvironment (despite coming from different sites) were more similar than among samples from the same site (Figures 3A, B). Intestinal communities were on average more homogeneous than gill communities (mean pairwise dissimilarity in gill: 0.71 ± 0.006, vs. in intestine: 0.56 ± 0.006,

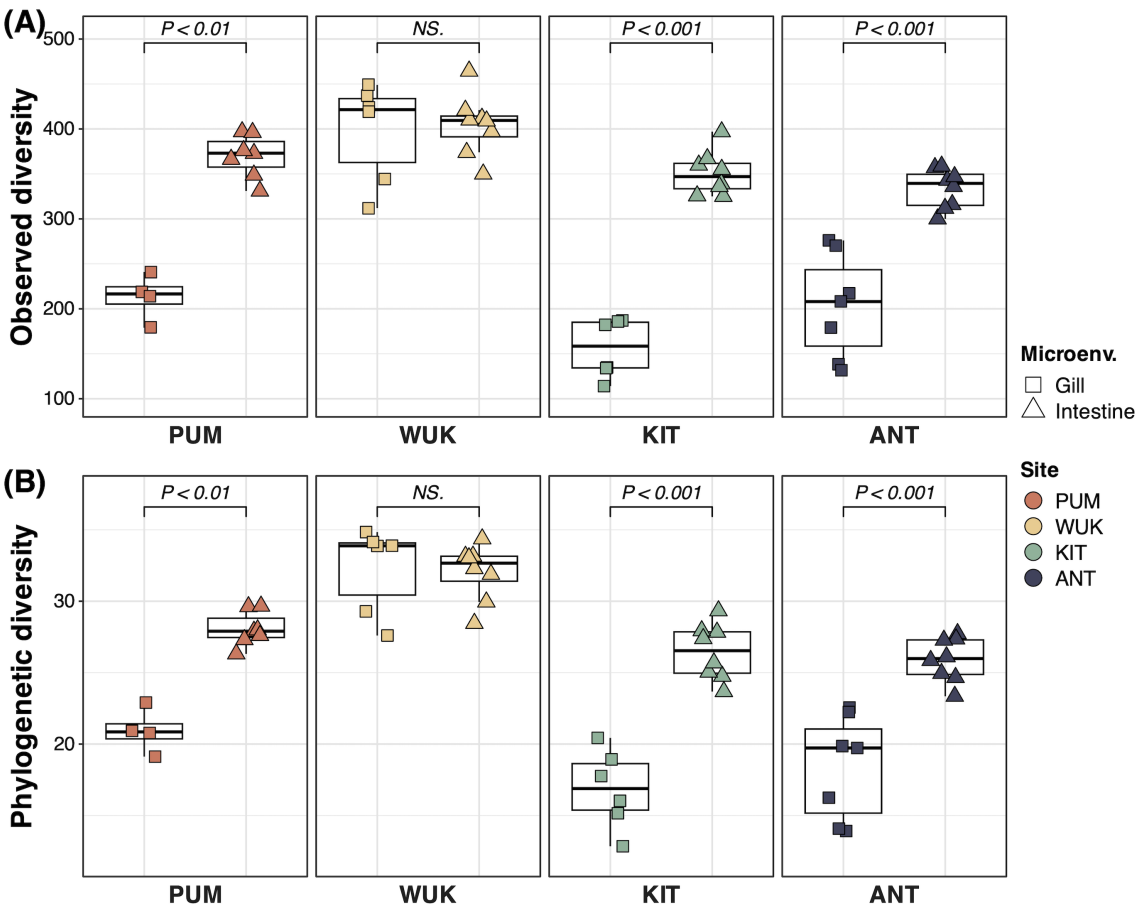

FIGURE 2
Bacterial alpha diversity in gill and intestine microbiomes of *Branchinecta gaini*. The shape and color correspond to the microenvironment and to the site, respectively. The significance of the differences was inferred through Wilcoxon tests, and p-values (*P*) are displayed. '*NS.*' indicates non-significant differences. **(A)** Observed diversity; **(B)** Chao Index.

Wilcoxon test, $p < 0.001$, Supplementary Figure S4). The effect of the sampling site in shaping *B. gaini* microbiome composition was twice as strong as that of the microenvironment ($R^2 = 0.39$, $p = 0.001$ and $R^2 = 0.19$, $p = 0.001$), and was about 25% stronger in intestine than in gill ($R^2 = 0.78$, $p = 0.001$ and $R^2 = 0.51$, $p = 0.001$, respectively, Table 2; Figure 3B). The effect of the host microenvironment on community composition differed depending on the levels of the site factor, as evidenced by the significant interaction in the PERMANOVA ($R^2 = 0.13$, $p = 0.001$, Table 2), and by the hierarchical clustering in which communities clustered either according to the microenvironment (*i.e.*, KIT and ANT) or by site (*i.e.*, PUM and WUK, Figure 3A). At the ANT site, sediment microbiome composition was more similar to intestine than gill samples (Figures 3A, B).

The partitioning of gill, intestinal, and sediment microbiomes was explained by the significant enrichment of discriminant bacterial taxa within each microenvironment, with a total of 375 discriminant OTUs identified through LEfSe analysis. The taxonomic affiliations of the top 20 OTUs with highest LDA scores within each microenvironment encompassed 12 bacterial classes (Figure 4). In the gill microbiome, discriminant OTUs were mainly from the *Bacteroidia* class, predominantly represented by *Flavobacterium*. In the intestine microbiome, most of discriminant OTUs were assigned to the *Clostridia* and *Alphaproteobacteria* classes, represented by *Clostridium* and *Tabrizicola*, respectively (Figure 4). The discriminant OTUs of sediment microbiomes predominantly belonged to the *Gammaproteobacteria* and *Bacteroidia* classes, represented by various taxa such as *Rhodoferax* and *Lentimicrobiaceae*, among others.

The Levins' niche breadth index (Bj) was roughly 30% higher in the intestine compared to the gill microenvironment (Wilcoxon test, $p < 0.001$, Figure 5, left panel). The proportion of specialist OTUs was strongly higher in the gill community (96%) than in the intestine community (52%). Generalist OTUs were nearly absent in both microenvironments (< 0.01%), and around half of the intestinal OTUs were neutralists (Figure 5, right panel).

3.3 Core microbiome detected but no hub taxa in gill and intestine of *B. gaini*

All core OTUs detected had a minimum prevalence of 87.5% across all samples and variable abundances, particularly within the intestine, as indicated by the abundance-occupancy curves (Figure 6A). Gill samples revealed a more flexible microbiome with only 12 core OTUs detected, whereas intestinal samples displayed 107 core OTUs (Figure 6B). In both microenvironments, the core OTUs accounted for substantial fractions of the entire bacterial communities, with cumulative relative abundances of 45.5% (gill) and 73.3% (intestine) of the community (Figure 5). The core composition was mostly distinct between both microenvironments. In line with the microenvironment-specific OTUs detected in the preceding section, the gill core microbiome was mostly dominated in abundance by OTUs belonging to genera like *Polaromonas* and *Flavobacterium*. Conversely, the intestine core microbiome was primarily dominated by OTUs belonging to *Intrasporangiaceae* and *Tabrizicola* (Figure 4; Figure 6C). The only bacterial genera (with relative abundance > 0.015) represented in both gill and intestine were *Clostridium sensu stricto 9* and *13* (Figure 6C), previously detected as significantly enriched solely within intestinal samples (Figure 4).

Co-occurrence networks computed for both gill and intestine core microbiomes did not reveal any hub taxa in either microenvironment. In the gill core microbiome, a single significant interaction was identified between *Acinetobacter* and *Microbacteriaceae* taxa, with betweenness centrality values below the threshold to be considered as hub taxa. Contrastingly, in the intestine microbiome, co-occurrence interactions among core

TABLE 2 Individual and pairwise PERMANOVA on Bray-Curtis dissimilarities assessing the effect of microenvironment and site on microbiome composition.

Dataset	Sample grouping	Df	Sums of square	F-statistics	R^2	*p*-value
ALL†	Microenv.	1	2.56	29.70	0.19	0.001
	Site	3	5.20	20.12	0.39	0.001
	Microenv. * Site	3	1.68	6.51	0.13	0.001
ANT	Microenv.	2	2.80	17.58	0.70	0.001
	↳ Sediment vs. Gill	–	–	13.51	0.63	0.010
	↳ Sediment vs. Intestine	–	–	22.85	0.72	0.010
	↳ Gill vs. Intestine	–	–	13.90	0.52	0.001
KIT	↳ Gill vs. Intestine	–	–	12.59	0.51	<0.001
WUK	↳ Gill vs. Intestine	–	–	10.49	0.47	<0.001
PUM	↳ Gill vs. Intestine	–	–	10.50	0.54	0.005
Gill	Site	3	3.97	32.26	0.51	0.001
Intestine	Site	3	2.92	6.47	0.78	0.001

Significance was inferred through 999 permutations. † Without sediment samples from ANT. Microenv., microenvironment.

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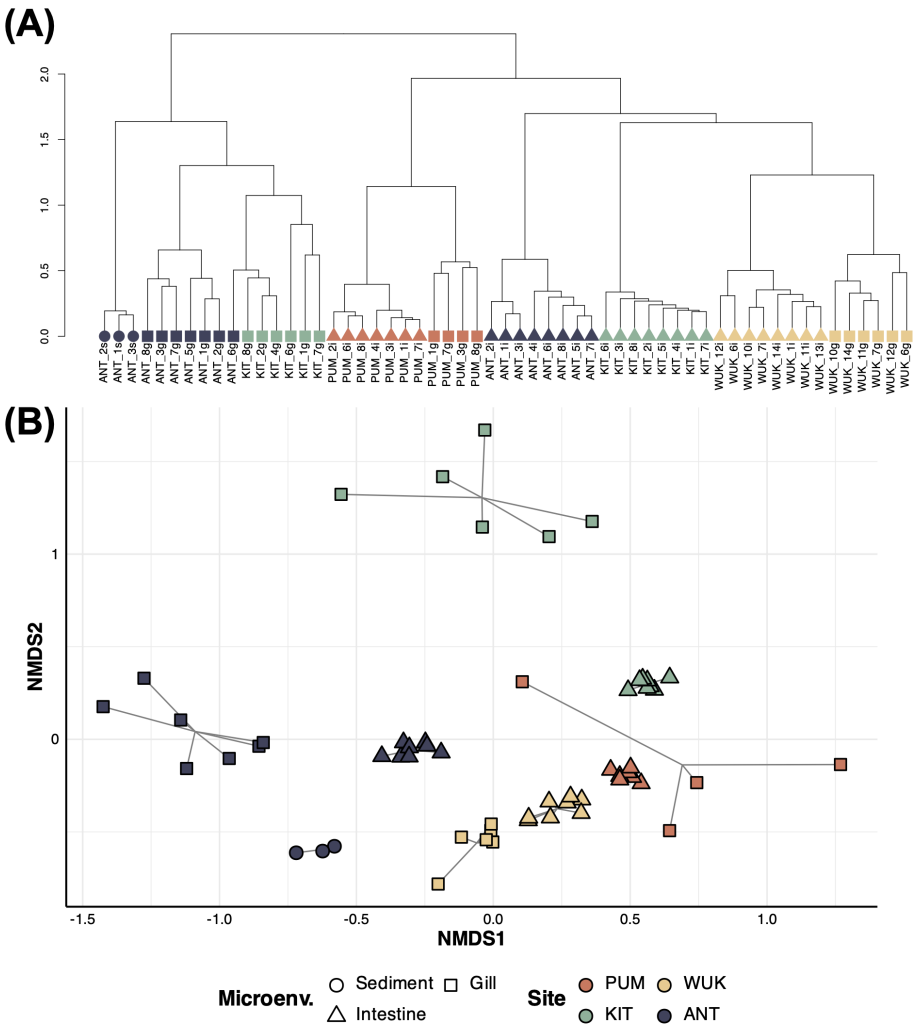

FIGURE 3
Bacterial beta diversity in *Branchinecta gaini*-associated and lake sediment microbiomes. Hierarchical clustering **(A)** and non-metric multidimensional scaling ordination **(B)** are based on Bray-Curtis dissimilarities among *B. gaini* microenvironments. Clustering is performed using the ward. D2 agglomeration method. The shapes and colors correspond to the microenvironment and to the site, respectively. In sample nomenclature, the first three letters, the number, and the last lowercase letter correspond to the site, the sample number, and the microenvironment, respectively.

OTUs were more abundant, with average betweenness and closeness centrality of 209.2 ± 20.0 and 0.002 ± 2.5e^{-5}, respectively, and a degree count of 3.3 ± 0.2; however, none of the nodes in the core co-occurrence network reached the 97th percentile of closeness and betweenness centrality values, precluding them from being classified as hub OTUs (Supplementary Figure S5).

3.4 Divergence-driving processes dominate in *B. gaini* microbiome assembly

Ecological processes leading to microbiome divergence (*i.e.*, ecological drift, dispersal limitation, and variable selection) accounted for most (> 95%) of the community composition turnover in both microenvironments, regardless of the geographical scale (*i.e.*, intra- and inter-site) (Figures 7A, B). However, the respective contributions of these ecological processes were markedly different between the gill and intestine. Notably, at the intra-site scale, variable selection was the dominant process within the intestinal microbiome across *B. gaini* individuals, accounting for 60% of the community composition turnover, while it only explains about 6% of the turnover observed in the gill microbiome. In contrast, ecological drift was the most important process in the gill microbiome, driving more than 70% of the community composition turnover but only 36% in the intestine microenvironment. Additionally, the dispersal limitation contribution, absent in the intestine microbiome assembly processes, drove 18% of the intra-site turnover of the gill bacterial community (Figure 7A).

When examining the inter-site turnover, the contribution of variable selection increased to 95% and 58% in the gill and intestine microbiomes, respectively. The contribution of dispersal limitation

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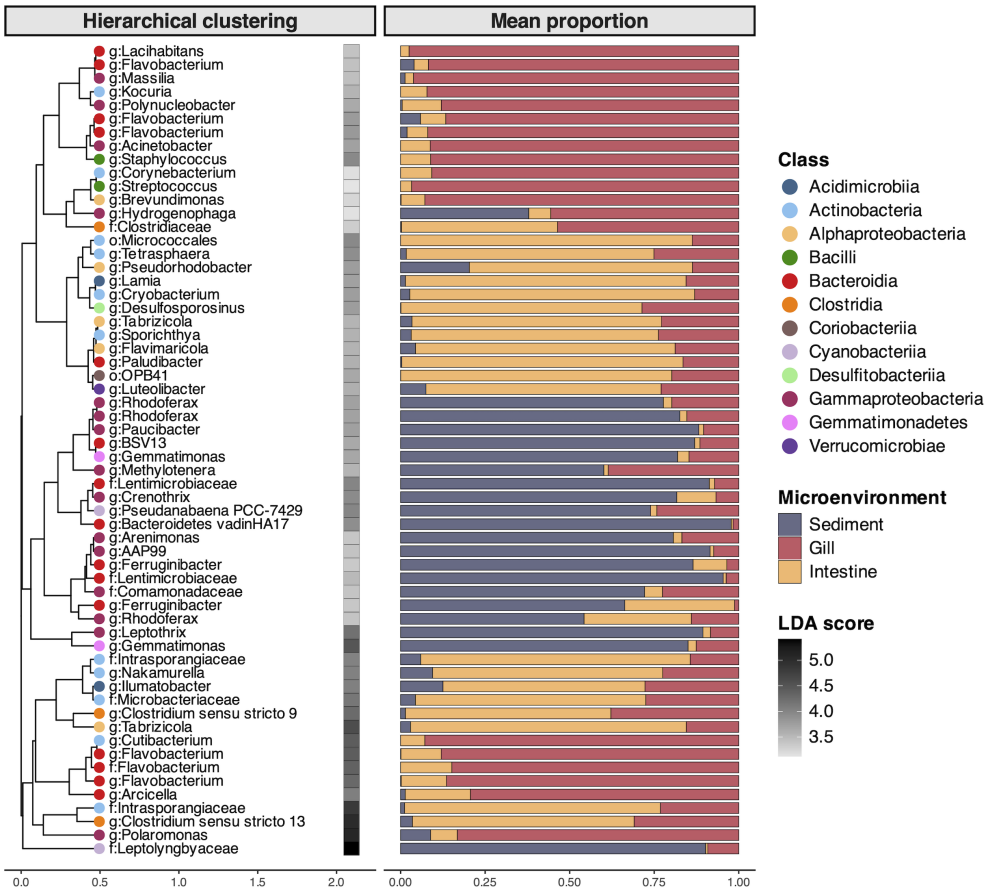

FIGURE 4
The twenty most enriched bacterial OTUs within each *B. gaini* and lake sediment microenvironments identified through Linear discriminant analysis (LDA) effect size (LEfSe) analysis. Taxonomic affiliations at the genus level (g): or at the family level (f): when genus was undetermined, and LDA score are presented for each OTU. The LDA score is represented by the color gradient in the heatmap. The barplot indicates the mean relative abundance of the indicator bacterial OTUs to the microbial community within each microenvironment (Microenv.). The hierarchical clustering is based on complete method from OTU relative abundance across microenvironments.

slightly increased, accounting for 21% and 5% in the gill and intestine microbiomes, respectively. Meanwhile, the contribution of ecological drift decreased to 16% and 0% in the gill and intestine microbiomes, respectively (Figure 7B).

3.5 Stronger host phylogenetic footprint in intestinal vs. gill microbiome of *B. gaini*

A substantial congruence was detected between microbiome composition dissimilarity and *B. gaini* phylogenetic distances, with a stronger correlation in the intestine ($R^2 = 0.64$, $p = 0.0001$) compared to the gill ($R^2 = 0.49$, $p < 0.0001$) (Figure 8A). Upon controlling for either climatic or geographic variability, the correlation strength slightly weakened for both microenvironments, but remained higher in the intestine than in the gill microbiome (Supplementary Table S3). In addition, while the *p*-values of partial Mantel tests remained largely significant in case of the intestine, they became less significant for the gill, and even marginally significant in the case of climatic distance-controlled correlation (Supplementary Table S3). Finally, the degree of alignment between the microbiome dendrogram and the host phylogenetic tree labels (*i.e.*, entanglement) was twice higher in the intestine compared to the gill (Figure 8B).

4 Discussion

We have limited understanding of how microbiome assembly vary across host microenvironments and among intraspecific populations of the same species. In this study, we surveyed the microbiome associated with two microenvironments of the fairy shrimp *B. gaini* across four populations distributed throughout Maritime Antarctica, and explored the eco-evolutionary mechanisms influencing the assembly of these microbiomes. Our results refine previous findings on meiofaunal host-microbiome relationships and verify several general hypotheses regarding the detection of phylosymbiosis (Woodhams et al., 2020).

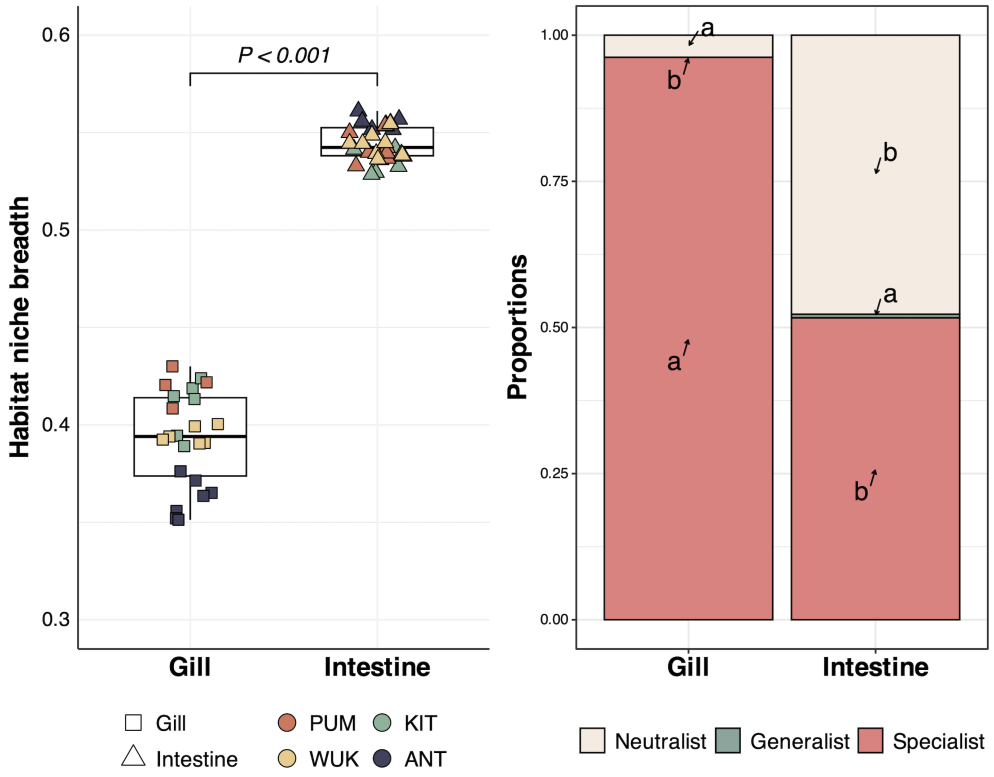

FIGURE 5
Habitat niche breadth (left) and proportions of habitat neutralists, generalists, and specialists (right) of the microbiome of *Branchinecta gaini* microenvironments. In the left panel, means of the Levins niche breadth index (Bj) were calculated for each OTU within a community (*i.e.*, sample). Differences between microenvironments were assessed for significance using the Wilcoxon test. In the right panel, arrows are used to indicate the different category within each microenvironment. Different lowercase letters between gill and intestine for the same category (*e.g.*, neutralists) indicate significant difference, as determined by the Dunn test. All comparisons were statistically different (p-values < 0.001).

4.1 *B. gaini* microbiome is shaped by host microenvironments

Assessing the distinguishability of bacterial community across host microenvironments and between the host and the surrounding environment is an essential first step to further unravel the microbiome significance in host ecology and evolution (Hammer et al., 2017; Schwob et al., 2024b). The composition of gill and intestine microbiomes of *B. gaini* significantly differed from each other. This finding demonstrates that fairy shrimps host body-site specific microbiomes, as previously observed in bigger crustaceans from Malacostraca class such as shrimps and crabs (Zhang et al., 2016; Cornejo-Granados et al., 2018). Our results underscore the importance of examining distinct organs individually, even in small and seemingly simple organisms like branchiopods. Moreover, the gill and intestine microbiomes were distinct from the lacustrine sediment environment. This first evidence of host ecological filtering in *B. gaini* suggests a selective enrichment of specific bacterial taxa able to colonize the gill and intestine tissues within *B. gaini* (Moran and Sloan, 2015; Mazel et al., 2018). A similar pattern has been previously reported through whole individual analysis in other small and microscopic freshwater organisms (Samad et al., 2020; Eckert et al., 2021; Boscaro et al., 2022).

4.2 Greater specificity and stochasticity in gill versus intestine microbiome assembly of *B. gaini*

Based on alpha-diversity results, host filtering seems to be stronger in the gill than in the intestine, limiting its colonization to a fewer number of, and more phylogenetically homogeneous, bacterial taxa compared to the intestine samples. This suggests that the gill may be a more selective habitat with a lower microbial carrying capacity compared to the intestine. Consistently, almost all the gill OTUs were identified as specialists according to the Levin's niche breadth framework. For instance, the most enriched and prevalent bacterial taxa in the gills is *Polaromonas*, a psychrophilic genus previously isolated from saline pond water, and various glacial environments, including lake microbial mat and glacier surfaces in Antarctica (Kapardar et al., 2010). Some authors propose that this genus can form dormant cells, facilitating its survival and dispersion over time and space (Darcy et al., 2011). Moreover, the *Polaromonas* genus is described as metabolically versatile due to high levels of horizontal gene transfer, allowing it to thrive during transient periods of higher temperatures and substrate availability (Yagi et al., 2009). This "opportunitroph" lifestyle (Polz et al., 2006) echoes the ecological strategy observed in *B. gaini*, which can survive extreme environmental fluctuations (Peck, 2004).

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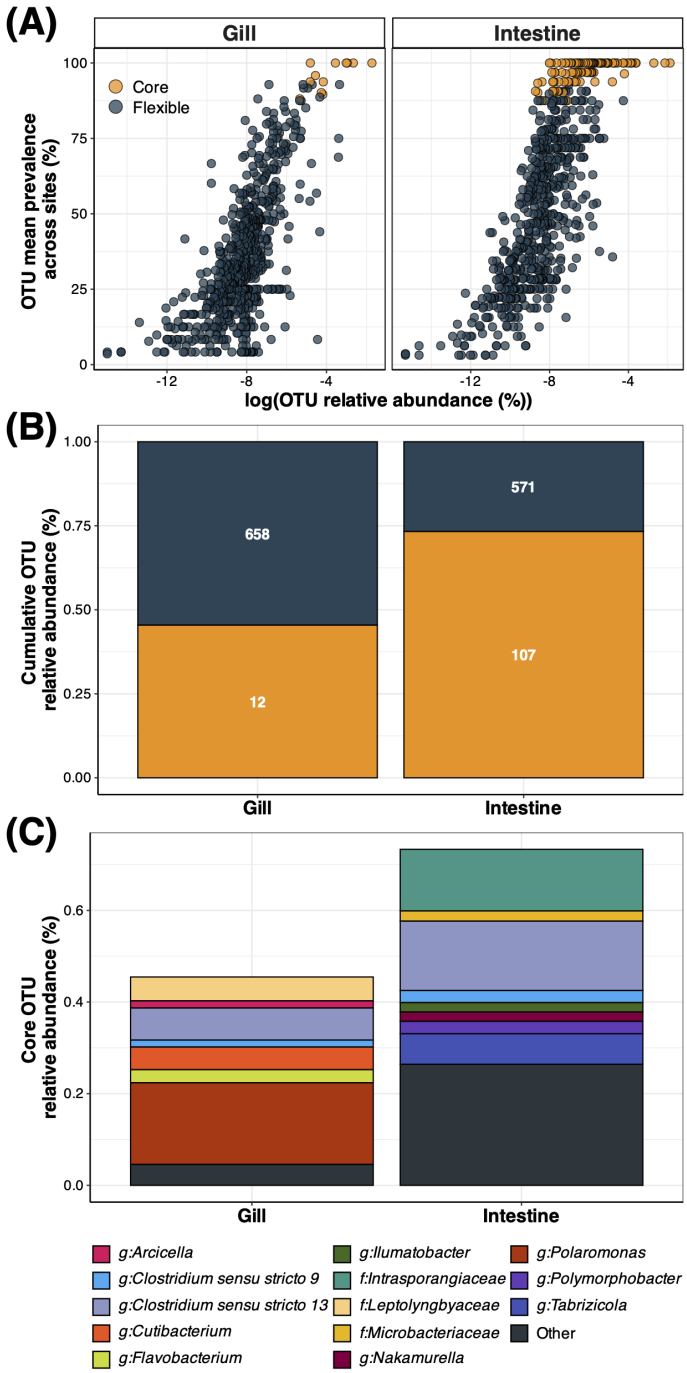

FIGURE 6
Bacterial core and flexible microbiomes of *B. gaini* gill and intestine. **(A)** represents the abundance-occupancy curves of all OTUs in *B gaini* gill and intestine. Colors indicate either the core (carrot orange) or flexible OTUs (charcoal blue). **(B)** represents the cumulative relative abundance of core (carrot orange) and flexible OTUs (charcoal blue) in the gill and intestine. The number inside each bar section indicates the number of OTUs. **(C)** displays the taxonomic composition at the genus level (or family level when genus information was not available) of the gill and intestine core microbiome OTUs. Core OTUs with relative abundance < 0.015 are represented under the label "Other".

B. gaini gills were also enriched in *Flavobacterium*, a genus associated with gill rot symptoms in various cold freshwater fish species (Farkas, 1985). The enrichment of these genera in the gills of freshwater invertebrates from the surrounding habitat is unprecedented and warrants further dedicated study to uncover their potential ecological roles.

The apparently stronger specificity observed in the gill versus the intestine aligns with the greater contribution of dispersal limitation processes in the gill, as previously shown in other organ-associated microbiomes of invertebrates and mammals (Mazel et al., 2024). This suggests that gill-associated OTUs possess phenotypic bacterial traits associated with reduced dispersal abilities (Mallott, 2024).

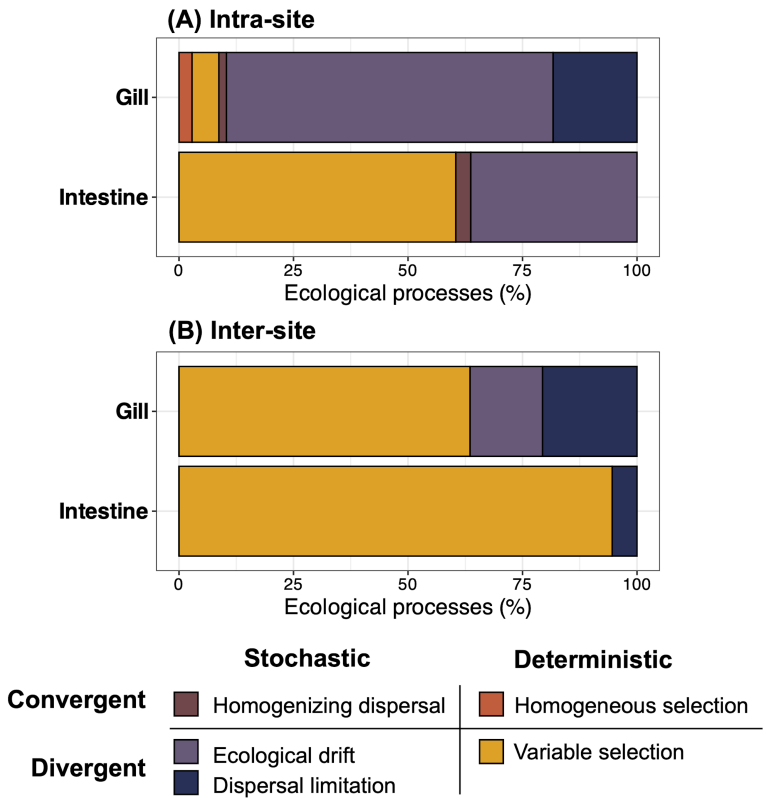

FIGURE 7
Estimation of the ecological processes governing the microbiome of *Branchinecta gaini* microenvironments within **(A)** and among **(B)** sites. The significance of the contribution changes in ecological processes was determined using a permutation test. The 'ecological processes' axis refers to the mean contribution of the community assembly processes.

Additionally, we identified a narrow core microbiome in the gill consisting of only 12 OTUs, yet accounting for almost 50% of all reads in the gill. This finding suggests that at least a fraction of the gill microbiome may be less transitory, reflecting some degree of host-symbiont fidelity over time and space (Risely, 2020).

However, the relatively higher variability in gill microbiome composition compared to the intestine, coupled with the greater proportion of flexible microbiome, challenges the notion of a highly specific gill microenvironment and prompts distinct scenarios to explain this discrepancy. First, in line with observations from other crustacean species, we propose that the microbial community colonizing *B. gaini* gill is periodically eliminated and gradually re-establishes after each host molting event (Corbari et al., 2008; Middlemiss et al., 2015; Zhang et al., 2021). Although the molting rate in *B. gaini* remains uncertain, estimates for the closely related species *B. giga* suggest approximately 18 molts per life cycle (Daborn, 1975). It is thus plausible that asynchronicity in molting and differences in the gill recolonization outcomes across individuals introduce variability in the microbiome composition.

Alternatively, the unique anatomical structure of the anastrocan gills, which lack a protective gill chamber unlike larger invertebrates, results in a more direct and continuous exposure to the environment (Paggi, 1996). Consequently, the gill microbiome may be more influenced by the lake water column than the intestine, leading to greater variability across sampling sites, potentially reflecting environmental differences among lakes. This aligns with the stronger increase of variable selection from intra-site to inter-sites in the gill compared to the more stable intestine microbiome. The high flexibility of microbiome in response to the external environment has been previously described in freshwater zooplankton, including rotifers, copepods, and cladocerans (Eckert et al., 2021). Although the lack of direct water sampling in this study limits our understanding of the water microbiome's influence on the gill microbiome, *Branchinecta* species primarily inhabit the surface of the nutrient-rich substrates at the bottom of Antarctic lakes, rather than the water column. These sediments can be considered as their primary habitat, as *Branchinecta* actively plow through superficial sediments using their thoracopods (Cáceres and Rogers, 2015). Additionally, the interstitial water present in the sediment samples provides an indirect representation of the water environment, compensating to some extent for the absence of explicit water samples in this study.

Finally, stochastic processes, primarily ecological drift, dominates in *B. gaini* gill microbiome assembly at a local scale (intra-site), suggesting that the microbiome is mostly acquired horizontally through pervasiveness uptake of environmental bacteria (Hammer et al., 2017, 2019; Rosenberg and Zilber-Rosenberg, 2021). Ecological assembly processes have not been specifically estimated in the past in crustacean models, but this finding intuitively contrasts with the typical pattern described in gill of other crustaceans, such as crabs, where the microbiome coating

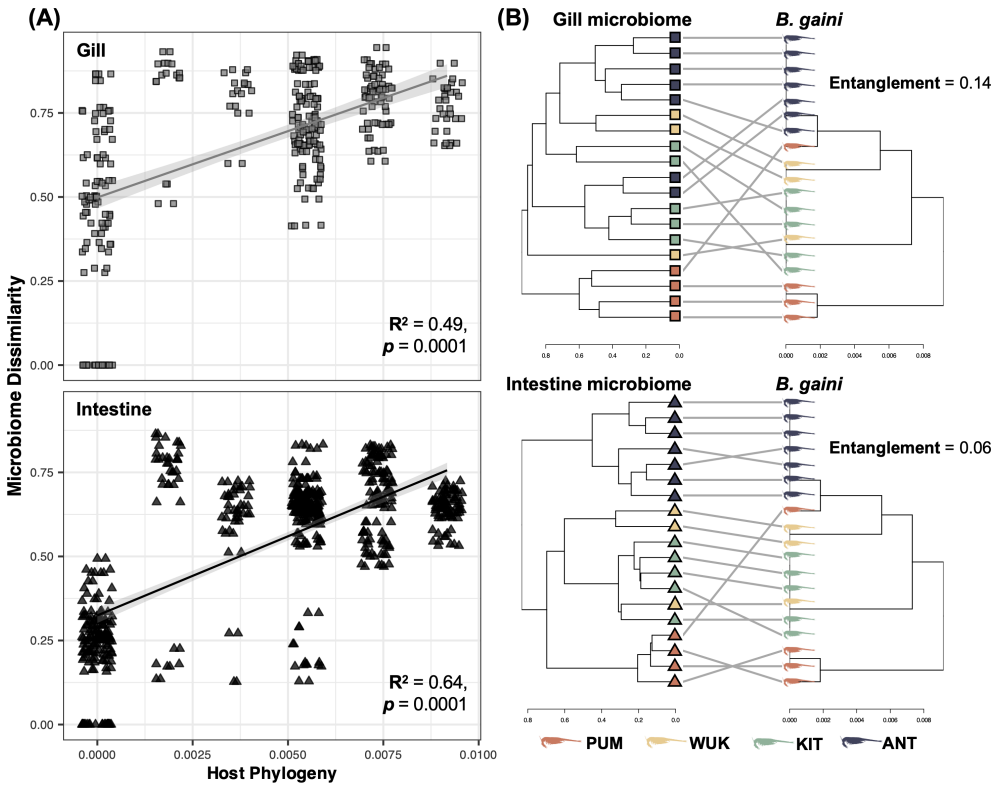

FIGURE 8
Congruence between *Branchinecta gaini* phylogeny and its microbiome composition. **(A)** Scatter plots of partial Mantel test results based on microbiome dissimilarity of *B. gaini* gill (gray square) and intestine (black triangle), using Bray-Curtis distances and host phylogeny. R^2 and *p*-values based on 10,000 permutations are provided. **(B)** Tanglegrams showing the association between the estimated *B. gaini* phylogeny and its gill (square) or intestine (triangle) microbiome composition. The scale and direction of the divergence are indicated by the scale bars, representing Bray-Curtis dissimilarity and pairwise phylogenetic distances for microbiome and host, respectively. The degree of alignment between the dendrograms is presented under a value of entanglement (0: fully aligned labels, 1: fully mismatched labels). Colors represent the sampling site as follows: Terra cotta; PUM, goldenrod yellow: WUK, asparagus green; KIT, dark slate blue; ANT.

the gills lamella is dominated by stable bacterial taxa across host populations (Bacci et al., 2023; Fusi et al., 2023). The importance of ecological drift during establishment of symbioses has been demonstrated to weaken/counteract deterministic selection through mechanisms such as priority or founder effect (Hagen and Hamrick, 1996; Kohl, 2020). Thus, the colonization outcome (*i.e.*, symbiont community composition) of a selective microenvironment may vary within and between host populations after the coarse ecological filter against taxa unable to colonize the tissue, even in the case of highly specific and vertically-transmitted symbionts (Vega and Gore, 2017; Lange et al., 2023; Chen et al., 2024). Such mechanisms may explain the detection of relatively variable community composition, the detection of stochastic assembly processes in the gill, in spite of the specificity of this microenvironment, which initially seemed contradictory.

4.3 Greater consistency and determinism in intestine versus gill microbiome assembly of *B. gaini*

We detected a predominant core microbiome across the four populations of *B. gaini* accounting for almost two thirds of the total community abundance, suggesting the existence of stable, permanent, and abundant microbial partners in the intestine of *B. gaini* (Astudillo-Garcia et al., 2017). Among the core microbial taxa, we reported the *Intrasporangiaceae* family, which has been characterized as highly abundant in the intestine of terrestrial earthworms and correlated with the digestive capacity of the host (Liu et al., 2023). This family has also been repeatedly detected in Antarctic ecosystems, mainly in soils and seawater (Giudice et al., 2007), but also associated with metazoan hosts such as tardigrades living on the surface of glaciers (Zawierucha et al., 2022). The *Clostridium sensu stricto 13* and *9* genera were also particularly prevalent and enriched in *B. gaini* intestines. Representatives of the *Clostridia* class, including *Clostridium*, are typically detected in the gut of freshwater invertebrates and fish (Zhao et al., 2018; Weingarten et al., 2019; Savard et al., 2023). *Clostridium* is a fermentative anaerobe known for its chitinolytic activity (Olsen et al., 1999). We suggest that the enrichment of these genera in the intestine of *B. gaini* might be attributed to their chitinolytic activity, enabling them to leverage the chitin-rich peritrophic matrix.

As in the gill microenvironment, the microbiome assembly processes within the intestine of *B. gaini* revealed a predominance of processes leading to a divergent microbiome. Yet, in contrast with the gill, these processes were mostly deterministic, dominated

by variable selection and ecological drift at intra-site scale, and almost exclusively by variable selection at inter-sites scale. Our findings contrast with several studies of the macrofauna gut microbiomes, which have identified either deterministic processes leading to phylogenetically convergent gut microbiome (*i.e.*, homogenizing selection) (Xiong et al., 2017; Li et al., 2019), or mostly stochastic processes leading to divergent microbiome (*i.e.*, mainly ecological drift) (Yan et al., 2016; Ge et al., 2021; Schwob et al., 2021). Our results also diverge from the typical pattern observed in the micro-eukaryotic community of freshwater lakes on the King George Island (South Shetland Islands, Maritime Antarctica) (including the same sampling site as ours), which are dominated by homogenizing dispersal process (Zhang et al., 2022). In our case, the high contribution of variable selection, which causes the variability of intestinal microbiome, might be somewhat linked to *B. gaini* host-related factors (*e.g.*, ecology, diet, genetics, physiology) that selected, enriched or maintained bacteria adapted to the intestine microenvironment with site-specific differences (Stegen et al., 2013, 2015). We speculate that the trophic plasticity of *B. gaini*, coupled with potentially significant inter-sites variations in nutrient resources, may drive the gut microbial diversity toward various directions associated with different dietary intakes. This could lead to distinct enrichments within the intestine and consequently high intraspecific variations in microbiome compositions, similar to patterns observed in Tibetan herdsmen and birds (Li et al., 2018; Bodawatta et al., 2021). In line with this, the niche breadth in the intestine was wider than in the gill, with half of the OTUs being either neutralists or generalists, suggesting that the diet flexibility of *B. gaini* promotes a metabolically more flexible microbial community in the intestine (Jiao et al., 2020).

4.4 Intestinal microbiomes exhibit stronger phylosymbiosis than the gill microbiomes

Our study addressed three significant gaps in the current phylosymbiosis literature by (i) comparing the strength of phylosymbiosis in two host microenvironments, (ii) through host intraspecific resolution, and (iii) within an unexplored taxonomic group (Brachiopoda). To date, only a single phylosymbiosis study has been reported in this region (Schwob et al., 2024a). We detected robust correlations between within-species phylogenetic distances of *B. gaini* and its microbiomes' compositions. This result formally confirms the feasibility of detecting phylosymbiosis among genetically-structured intraspecies populations of freshwater invertebrates, thereby highlighting the importance of preserving the individual identity when considering different populations (Mazel et al., 2018; Lim and Bordenstein, 2020). Our results contrast with previous findings that mostly report the absence of phylosymbiosis in meiofaunal invertebrates, including rotifers, crustaceans, and flatworms, among others (Turgay et al., 2020; Eckert et al., 2021; Boscaro et al., 2022; Eckert et al., 2022; Rosa and Loreto, 2023). Several major methodological variances may explain the difference in phylosymbiosis detection with these previous works. First, ancient divergence time among different hosts tend to obscure the phylogenetic signal within their microbiomes, thus limiting the detection of phylosymbiosis (Brooks et al., 2016; Groussin et al., 2017). Consistently, Leasi et al. (2023) did not detect phylosymbiosis signal among the microbiomes of seven marine interstitial nemertean genera, but the correlation was positive when tested within the single genus *Ototyphlonemertes*. Secondly, we distinguished two different host microenvironments within *B. gaini*, while all the aforementioned studies used complete individuals for inspecting phylosymbiosis association. Mixing different microbiomes from whole individuals may lead to spurious conclusions about phylosymbiosis. Here, the strength of the phylosymbiosis pattern was markedly different between gill and intestine microbiomes. Specifically, the phylosymbiosis signal was weaker (and even marginally significant) in the *B. gaini* gill compared to intestine microenvironment when controlling for climatic and geographic distances. These findings suggest that, independently of climatic and geographic variables, host genetics shape more strongly the intestine microbiome than the gill microbiome, reflecting deterministic assemblages of bacteria. This is in accordance with the predominance of the variable selection process observed for the intestine microbiome assembly across sampling sites. This pattern is consistent with the general trend in phylosymbiosis studies showing that internal microbiomes (*e.g.*, gut) tend to harbor stronger and more frequent phylosymbiosis signals than external microbiomes (*e.g.*, skin), as reported in fishes (Minich et al., 2022), due to likely more direct influence of host physiology, immune system, and trophic diet in shaping the microbial communities (Moran and Sloan, 2015; Mazel et al., 2018; Woodhams et al., 2020).

We cannot discard a non-adaptive origin of the phylosymbiosis through host deterministic ecological filtering. Indeed, more phylogenetically closely related hosts might share more similar habitat, ecology and physiology, thus indirectly selecting similar microbial taxa from the surrounding environment (Moran and Sloan, 2015; Mazel et al., 2018). This hypothesis likely applies in the case of the intestine microenvironment, as the phylosymbiosis may result from diverging dietary intakes, related to diet preferences and/or the available resources (Hammer et al., 2020), which tend to be more homogenous within and between geographically closer sites, as observed in lake plankton (Soininen et al., 2011). This is in line with previous microscopic observations in another branchiopod genus (*Artemia*), suggesting that the intestinal microbial community is mostly transient and associated with the ingested material (Martin et al., 2020). The localization and density of the microbes in the intestine, as well as the functional host-symbiont complementarity remain to be fully resolved in *B. gaini*, and constitute promising avenues of research to further understand the gut microbial roles in freshwater crustaceans inhabiting lacustrine ecosystems in the rapidly changing region of Maritime Antarctica.

5 Conclusions

This study examines the microbiome assembly processes and phylosymbiosis signal in the Antarctic fairy shrimp *Branchinecta gaini*, relating to the host's intraspecific genetic structure. We found that the gill and intestine harbor distinct microbiomes, each with specific core OTUs that form site-dependent interaction networks,

assembled through eco-evolutionary processes that result in divergent microbial communities. The host genetic structure correlates with the composition of both intestine and gill microbiomes of fairy shrimp, validating the phylosymbiosis hypothesis. The stronger phylosymbiosis signal in the intestine is linked to deterministic microbiome assembly processes associated with site-specific dietary variations that reflect the host's genetic structure. In contrast, the greater variability observed in the gill microbiome–due to stochastic assembly processes likely influenced by environmental exposure and/or random colonization–obscures this signal. These findings emphasize the importance of distinguishing between host microenvironments, even in small organisms, when studying the microbiome assembly.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA1090016 https://www.ncbi.nlm.nih.gov/, PP506203-PP506230.

Ethics statement

The animal study was approved by Comité de Bioética, Instituto de Ecología y Biodiversidad (IEB), Santiago, Chile. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

GS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration, Resources. LC: Writing – review & editing, Validation. PV: Methodology, Writing – review & editing. YT: Methodology, Writing – review & editing. FM: Visualization, Writing – review & editing, Methodology. TC: Writing – review & editing, Funding acquisition, Project administration, Resources. JO: Funding acquisition, Project administration, Resources, Writing – review & editing, Validation. CM: Project administration, Resources, Writing – review & editing, Validation, Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2024.1438057/full#supplementary-material

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