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Differences in the bacterial microbiome between larvae and adults of Antarctica's only endemic insect, Belgica Antarctica (Diptera: Chironomidae)

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Belgica antarctica (Diptera: Chironomidae) is the Antarctic continent's only endemic insect. In this study we characterized the bacterial microbiomes of *B. antarctica* larvae and adults, and archaea from larvae only. All samples were collected from Orne Harbor in the western Antarctic Peninsula. We compare microbial community between life stages and compare these microbial communities with those previously reported from other Antarctic invertebrates. Possible links to gut-associated processes or microbial functions are discussed. The most abundant bacterial phylum in larvae was Actinobacteriota, and those in adult flies were Actinobacteriota and Proteobacteria. The relative abundance of Actinobacteriota decreased from 76-88% in larvae to 8–44% in adults. The relative abundance of some bacterial groups was higher in adults than in larvae, particularly Proteobacteria, from 3-15% in larvae to 28–86% in adults. Genus-level bacterial community composition differed significantly between life stages (Bray–Curtis PERMANOVA; R^2 = 0.492, p = 0.009, n = 5 per life stage), with corresponding differences also detected at the phylum level (R^2 = 0.748, p = 0.009). These differences may reflect several other factors, including developmental stage, environmental exposure, differences in host physiology and the presence of surface-associated microorganisms. However, the current study design does not allow microbial turnover during metamorphosis to be directly assessed. Preliminary archaeal-targeted sequencing identified 56 ASVs from larval samples, although low read recovery and the absence of negative controls limit interpretation. Phylogenetic analysis was performed on these unresolved archaeal ASVs, and three were identified as forming a distinct clade closely associated with a crenarchaeotal sequence previously recovered from the related sub-Antarctic midge, *Eretmoptera murphyi*. Following taxonomic filtering, no archaeal sequences from adult samples were retained for downstream analysis. The *B. antarctica* microbiome showed clear similarities with that of *E. murphyi* (seven shared phyla and three shared genera), a species palaeoendemic to sub-Antarctic South Georgia. Further work examining Antarctic invertebrate microbiomes

requires targeted approaches to separate gut microorganisms from the broader microbiome as well as the utilization of -omics technologies to help clarify possible functional and evolutionary roles.

KEYWORDS

archaea, invertebrate, metamorphosis, microbial community composition, microbial ecology, polar

1 Introduction

Insects are the most diverse, widespread and successful clade of animals, with over one million species currently described (Engel and Moran, 2013; Ioannidis et al., 2017). Their evolutionary success can, in part, be attributed to their associated microbial communities, known as the microbiome (Gupta and Nair, 2020; Łukasik and Kolasa, 2024). The microbiome includes microorganisms that are present on the external surface of the cuticle, known as ectosymbionts, those that live within the host's cells, known as endosymbionts, and those that live within the body but extracellularly (Gupta and Nair, 2020), with the gut microbiome generally being the most studied. The microbiome of insects can include algae, archaea, bacteria, fungi, protozoa and viruses (Worland et al., 2000; Gurung et al., 2019), with bacteria being the most abundant group (Ling et al., 2022). In the present study, we use the term "microbiome" to refer specifically to the archaeal and bacterial communities. As with other animals, the microbiome can play important roles in maintaining the health of insect hosts, including providing nutrition (Zhang et al., 2023), resisting pathogens (Jing et al., 2020), improving reproductive success (Muratore et al., 2020) and enhancing cold tolerance (Ferguson et al., 2018; Morgan-Richards et al., 2023) by producing cryoprotectants (Raza et al., 2020). The microbiome can also enhance nutrient acquisition in nutrient-limited environments, as seen in termites, whose microbiome allows them to recycle excretory products, fix gaseous nitrogen from the atmosphere and break down lignocellulose (Engel and Moran, 2013).

In holometabolous insects, the microbiome can change markedly from larvae to adults following metamorphosis (Kucuk, 2020). During this process, the insect's morphology is substantially restructured, including the gut epithelium in the pupal phase (Manthey et al., 2022). For these insects, metamorphosis ensures that each life stage is specialized for specific tasks like growth and development in larvae (Hammer and Moran, 2019), versus mating and oviposition in adults (Müller and Müller, 2015). This morphological reorganization also typically results in a shift in microbial communities (Manthey et al., 2022). There is often a decline in microbiome diversity from larvae to adults, possibly due to physiological and biochemical changes (Hammer and Moran, 2019) and, in some species, the complete cessation of feeding in the imago (Sela et al., 2020). There can also be anatomical changes to the gut that affect microbiomes, for example, in the dung beetles *Euoniticellus intermedius* and *E. triangulates,* only larvae possess a compartmentalized hindgut, and this structure is absent in adults (Shukla et al., 2016). One risk of metamorphosis is the loss of useful microorganisms (Manthey et al., 2022). To guard against this, some microbes are maintained across metamorphosis (Kucuk, 2020) via various structures such as bacteriocytes, antennal glands and crypts. After adult emergence, microbial communities can also be re-established from environmental substrates, particularly in species where adults feed (Schmidt and Engel, 2021).

The existence of distinct microbiomes across life stages can facilitate changes in dietary requirements (Manthey et al., 2022). For example, larvae may have a higher proportion of microbial species involved in cellulose digestion and nitrogen fixation in comparison to adults (Hammer and Moran, 2019), enabling them to acquire the nutrients needed to survive metamorphosis and accumulate reserves for oviposition in the adult stage (Arrese and Soulages, 2010). Overall, while changes in the microbiome across life stages are a direct consequence of metamorphosis, this process also acts as an ecological reset, decoupling larval and adult stages, facilitating microbiome turnover and allowing adaptation with shifts in habitats, diet and behavior (Manthey et al., 2022). In chironomids, known as "non-biting midges," studies have reported significant alteration of the associated bacterial community across the four developmental stages of various freshwater species such as *Chironomus transvaalensis* (Sela et al., 2020) and *C. circumdatus* (Laviad-Shitrit et al., 2021). In *C. transvaalensis*, bacterial community composition differed substantially between larvae and adults, with analysis indicating a 90.25% dissimilarity between these two life stages (Sela et al., 2020). In *C. circumdatus,* developmental stage explained 41% of the variation in bacterial community composition (Laviad-Shitrit et al., 2021).

Belgica antarctica Jacobs 1900 (Diptera: Chironomidae) (Figure 1) is a brachypterous midge (Convey and Block, 1996) and is the only insect endemic to the Antarctic continent (Chown and Convey, 2016). Its distribution includes coastal areas of the western Antarctic Peninsula to ~69°S and extends north to Elephant Island in the South Shetland Islands (Allegrucci et al., 2012; Kozeretska et al., 2021). *Belgica antarctica* is holometabolous with four larval instars and a typically 2-year lifecycle (Yoshida et al., 2025). The larvae are generally found in moist substrates, primarily feeding on algae, mosses, microorganisms, and detritus (Lee and Denlinger, 2015). Adult emergence occurs synchronously during the summer months where mating takes place in aggregations (Benoit et al., 2007). Adults do not have functional mouthparts and are unable to feed (Finch et al., 2020). The larvae must, therefore, accumulate the necessary energy reserves for reproduction in the adult stage (Devlin et al., 2022).

Using metagenomic screening, Maistrenko et al. (2023) analyzed existing *B. antarctica* datasets (larvae and adults), together with PCR analysis (of adults), to investigate the fly's bacterial microbiome, with a focus on possible endosymbionts. Endosymbionts such as *Wolbachia* and *Spiroplasma* were not detected through PCR analysis, and this metagenomic study revealed only 14 potential associated bacterial species in the microbiome. The non-reviewed preprint of Kovalenko et al. (2025) used a more targeted approach to characterize the bacterial microbiome of *B. antarctica* larvae and their surrounding substrate. This work suggested the presence of known endosymbiotic genera including *Wolbachia*, *Cardinium* and *Rickettsia*, although these were all in very low abundances (e.g., the putative *Wolbachia*'s relative abundance ranged from 0.06–0.11%). The most dominant phyla were Proteobacteria (up to 52%) and Bacteroidota (up to 17%). *Belgica antarctica*'s sister species, *Eretmoptera murphyi*



FIGURE 1
Fourth instar larvae (left) of *Belgica antarctica* and an adult male (right). Larvae are 7–8 mm in length (Michailova et al., 2021) and this adult is ~3 mm. Adult males usually range from 1.6–2.5 mm in length and the adult females are often larger, ranging from 1.5 to 3.2 mm. Photos: Richard E. Lee Jr.

Schaeffer 1914 (Diptera: Chironomidae) (Allegrucci et al., 2012) is an insect native and palaeoendemic to sub-Antarctic South Georgia, whose larval microbiome was recently characterized (Brayley et al., 2026). Dominant bacterial phyla included Actinobacteriota (up to 52%), Proteobacteria (up to 31%), Chloroflexi (up to 18%), and Planctomycetota (up to 10%), with one archaeal group identified and assigned to Crenarchaeota.

The available studies investigating *B. antarctica's* microbiome (Maistrenko et al., 2023; Kovalenko et al., 2025) did not characterize the archaeal communities, the microbiome of adults, or attempt to link the findings to the fly's reproduction or development. Therefore, the aims of this 16S rRNA gene amplicon sequencing study were (1) to identify key archaeal and bacterial taxa associated with both larvae and adults of *B. antarctica* (whole insects), together with preliminary archaeal findings from larvae, (2) to explore whether there is an association between the microbial community and life stage, and (3) to compare the fly's microbiome with those available from other Antarctic invertebrates.

2 Methods

2.1 Sample collection

Larval and adult *B. antarctica* were hand-collected by PC from Orne Harbor (64° 37' S, 62° 32' W) located in the western Antarctic Peninsula, during the austral summer in February 2024. Individuals were immediately preserved in 96% ethanol, which would also help remove microbes from the cuticle (Crippen and Sheffield, 2006) and stored until return to the United Kingdom and analysis at the University of Birmingham. Residual ethanol was not specifically removed prior to DNA extraction. Seven third-instar larvae of similar size were selected for metagenomic analysis to minimize variation associated with larval development (Sela et al., 2020; Sela and Halpern, 2022). This instar was selected because it was the most abundant stage at time of collection. Seven adults were also selected for analysis. It is not possible to sex larvae, so it is assumed the samples were a mix of males and females. Adults show clear sexual dimorphism (Kozeretska et al., 2021), and so a range of sizes were selected to provide a mixed sex sample.

2.2 Microbial DNA extraction and sequencing

Total DNA was extracted from whole individual larvae or adults separately using the DNeasy PowerSoil Pro Kit (QIAGEN). Initially, individuals were manually broken apart with a sterile pipette tip in solution C1 (lysis buffer) before the other extraction steps were carried out following the manufacturer's protocol. The extracted DNA was quantified using a NanoDrop ND-2000 (Wilmington) and was then used as a template for PCR amplification. Universal primers were used to target and amplify the bacterial V4 region of the 16S rRNA; 515F (5′ - GTGCCAGCMGCCGCGGTAA - 3′) and 806R (5′ - GGACTACHVGGGTWTCTAAT - 3′) (Caporaso et al., 2010). Customized primers were used to target and amplify the archaeal V4 region; 519F (5′ - CAGYMGCCRCGGKAAHACC - 3′) and 806R (5′ - GGACTACNSGGGTMTCTAAT - 3′) (Zhang et al., 2023). Other PCR reagents were prepared using the NEBiolabs Q5 PCR kit following the manufacturer's protocol. PCR reactions were prepared to a final volume of 25 μL, comprising 5 μL 5 × Q5 Reaction Buffer, 0.5 μL 10 mM dNTPs, 1.25 μL 10 μM forward primer, 1.25 μL 10 μM reverse primer, 2.5 μL template DNA, 0.25 μL Q5 High-Fidelity DNA Polymerase, 5 μL 5 × Q5 High GC Enhancer and 4.25 μL nuclease-free water. Two separate PCR reactions were carried out for each extraction, with 28 cycles for bacterial DNA and 32 cycles for archaeal (95 °C for 30s, 53 °C for 30s, extension step of 72 °C for 30s). The concentration of DNA was quantified again, and the five larvae and adults (out of the seven original individuals) providing the highest concentrations of DNA were selected for archaeal and bacterial sequencing to maximize sequencing success. DNA concentrations of the selected bacterial samples ranged from 4.2–6.7 ng μL^{-1} in the larvae and 7.3–18.1 ng μL^{-1} in the adults. DNA concentrations of the selected archaeal samples ranged from 4.1–5.4 ng μL^{-1} in the larvae and 4.4–6.1 ng μL^{-1} in the adults. Due to variability in DNA yield following PCR amplification of bacterial and archaeal 16S rRNA gene regions, the individuals selected for sequencing differed between the two datasets. Within each life stage, four of the five larval individuals

and three of the five adult individuals were represented in both the bacterial and archaeal datasets. Library preparation and sequencing were carried out commercially on an Illumina PE250 platform by Novogene (UK).

2.3 Sequencing analyses

A total of 281,985 larval and 241,274 adult bacterial sequences, and 345,665 larval and 341,179 adult archaeal sequences were generated. The DADA2 pipeline (V1.32.0; Callahan et al., 2016) was used for quality trimming, error rate estimation, merging, chimera removal and amplicon sequence variant (ASV) feature table construction. The final bacterial dataset comprised five larval and five adult samples. The larval archaeal dataset comprised of five individuals. Primer sequences were removed from the 5′ region of forward and reverse reads (19 and 20 bp, respectively), and reads were truncated at the first instance of a quality score ≤10. Following dereplication, merging and chimera removal, 39.1–91.4% of larval bacterial, 44.6–80.9% of adult bacterial, 49.2–72.4% of larval archaeal and 72.8–90.9% of adult archaeal reads were retained for further analysis. ASVs classified as chloroplast, mitochondria or archaea were removed from bacterial samples. ASVs classified as chloroplast, mitochondria or bacteria were removed from archaeal samples. This resulted in almost all sequences being removed from the adult archaeal samples, possibly indicating high levels of contamination or low archaeal biomass and, thus, this dataset was not subjected to further downstream analyses. Taxonomy was assigned to genus level using SILVA 138.1 prokaryotic SSU taxonomic training data, formatted for DADA2 and Zenodo (McLaren and Callahan, 2021). To remove sequences potentially arising from the host, a local BLAST search was performed using the genomic sequence data available for *Belgica antarctica* (Kelley et al., 2014). ASVs with greater than 90% sequence similarity across 90% of the query length were removed (Lagunas et al., 2023). Inspection of the larval bacterial ASV sequences revealed differences in amplicon length between samples, with one sample predominantly containing 271-bp sequences and the remaining four predominantly containing 276-bp sequences. To ensure consistent sequence-level comparisons, larval bacterial ASVs were standardized to a common 271-bp region by truncating longer sequences at the 3′ end. Sequences shorter than 271 bp were excluded, and ASVs with identical sequences following standardization were merged by summing their read counts. This standardization was applied before downstream diversity analyses. Bacterial diversity and taxonomic composition were subsequently analyzed using unrarefied ASV count data to retain the available sequencing information. For analyses of community composition and beta diversity, ASV counts were converted to relative abundances to account for differences in sequencing depth between samples. ASVs that represented > 1% of the relative abundance in at least one of the samples were classed as relatively abundant. This threshold was selected to focus on the more abundant taxa within the microbial communities. Lower-abundance taxa were grouped as "Other" and unclassified taxa were retained separately.

Larval archaeal ASVs were recovered at very low frequency, so sequencing-depth checkpoints and rarefaction decisions were evaluated on the complete ASV output of the archaeal sequencing run (i.e., all non-bacterial ASVs retained after filtering), across all ASVs generated in the archaeal pipeline to characterize complete recovery. Due to low archaeal read counts per sample after quality control (263–1,698 reads), rarefaction and quantitative diversity metrics were not applied to the larval samples. The complete sequence dataset is available through the NCBI SRA database, BioProject ID: PRJNA1468618.

2.4 Phylogenetic analysis of archaeal ASVs

Due to a high number of larval archaeal ASVs that could not be classified, all 56 recovered ASVs were investigated further against the NCBI database using the BLASTn tool to assess the possible identity of these unresolved sequences. The results were not used to formally reassign the SILVA taxonomy and low-coverage, or low-identity matches, were interpreted cautiously. The maximum number of target sequences was increased to 500, the expected (E) value threshold was set to 10 and filtering for low complexity regions was disabled. These permissive parameters were deliberately used to allow for greater detection of potential archaeal matches (Sharon et al., 2005) from these poorly resolved ASVs. BLAST results were evaluated using percentage sequence identity and query coverage, and these values, alongside the closest match for each ASV are reported in Table 1. Phylogenetic analysis was subsequently used to provide an independent assessment of the relationships between these ASVs and various archaeal reference sequences. For phylogenetic tree construction, *Methanococcoides burtonii* (Allen et al., 2009) and *Haloferax volcanii* (Hartman et al., 2010) were chosen as reference sequences. *Methanococcoides burtonii* represents a highly studied methanogenic archaea (particularly in cold environments) and has also previously been reported in Antarctica (Franzmann et al., 1992). *Haloferax volcanii* is a well-studied halophilic archaeon, and similar species have also been reported in Antarctica (Williams et al., 2014). Additionally, Haloarchaea can be used as model organisms when investigating the phylogenetics of extremophiles (Hartman et al., 2010; Tschitschko et al., 2018). A Crenarchaeaota reference sequence from the sister species of *B. antarctica*, *E. murphyi* (Brayley et al., 2026), was used to compare archaeal phylogenies between Antarctic insects. The ASVs and reference sequences were aligned using the AlignSeqs() function in the DECIPHER package. To ensure comparability across sequences, full-length archaeal reference sequences were trimmed to the same amplicon region as the ASVs using alignment-based column filtering prior to phylogenetic construction. The resulting multiple sequence alignment was converted to a phylogenetic data object using phyDat() from the phangorn package, and a pairwise genetic distance matrix was computed with dist.ml() based on a maximum likelihood model of nucleotide substitution using the BIONJ algorithm. Tree-based (patristic) genetic distances between ASVs and reference sequences were calculated from the resulting phylogeny, with lower values indicating greater genetic similarity.

2.5 Statistical analyses

Statistical analyses were carried out using RStudio, version 2024.12.0 + 467. Due to the small sample sizes, differences in alpha and beta diversity between life stages were assessed using exploratory statistical analyses, while variation among individual samples was interpreted descriptively. Alpha-diversity indices (Shannon and Simpson diversity) were calculated from unrarefied bacterial ASV count data using the diversity function in the vegan package (Oksanen et al., 2026), to characterize within-sample microbial diversity. Both indices incorporate richness and evenness, while the

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TABLE 1 BLAST results of larval archaeal ASVs and their closest phylogenetic reference sequence based on genetic distances.

Closest BLAST match	Identity	Query cover	Accession	Match type	Closest phylogenetic reference	Genetic distance
Rosenvingiella polyrhiza isolate Rpol2	99.77%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.724
Xanthonema sp. 395 strain 395	99.09%	100%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.809
Rosenvingiella polyrhiza isolate Rpol2	100.00%	99%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.724
Xanthonema sp. 395 strain 395	99.09%	99%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.810
Uncultured archaeon clone LA_06B	**100.00%**	**99%**	**JX099248.1**	**Archaeal**	*Eretmoptera murphyi* (16S rRNA)	0.000
PREDICTED: *Conger conger* E3 ubiquitin-protein ligase-like	80.39%	22%	XM_061229912.1	Non-archaeal (low hit)	*Eretmoptera murphyi* (16S rRNA)	1.148
Uncultured eukaryote clone TE106B	99.77%	100%	KM870643.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.775
PREDICTED: *Conger conger* E3 ubiquitin-protein ligase-like	80.39%	22%	XM_061229912.1	Non-archaeal (low hit)	*Eretmoptera murphyi* (16S rRNA)	1.148
Xanthonema sp. 395 strain 395	99.77%	100%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.814
Uncultured archaeon clone LA_06B	**99.62%**	**98%**	**JX099248.1**	**Archaeal**	*Eretmoptera murphyi* (16S rRNA)	0.017
Rosenvingiella polyrhiza isolate Rpol2	98.83%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.732
Botrydiopsis callosa strain SAG 30.83	100.00%	99%	AJ579340.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.832
Xanthonema sp. 395 strain 395	98.41%	99%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.813
Rosenvingiella polyrhiza isolate Rpol2	99.05%	99%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.731
Xanthonema sp. 395 strain 395	98.87%	100%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.809
Uncultured eukaryote clone TE106B	98.59%	100%	KM870643.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.776
Xanthonema sp. 395 strain 395	98.86%	100%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.810
Tribonema viride strain SAG 23.94	99.56%	100%	AM490825.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.812
Xanthonema sp. 395 strain 395	98.41%	100%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.816
Tribonema viride strain SAG 23.94	99.56%	100%	AM490825.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.813
Rosenvingiella polyrhiza isolate Rpol2	98.82%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.735
Rosenvingiella polyrhiza isolate Rpol2	99.53%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.729

(Continued)

TABLE 1 (Continued)

Closest BLAST match	Identity	Query cover	Accession	Match type	Closest phylogenetic reference	Genetic distance
Xanthonema sp. 395 strain 395	98.41%	100%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.817
Amoeboradix gromovi strain K-1	95.50%	100%	MF370974.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.849
Onychostoma macrolepis dynein, cytoplasmic 1	81.36%	15%	XM_058749802.1	Non-archaeal (low hit)	*Eretmoptera murphyi* (16S rRNA)	1.018
Uncultured *Glomeromycotina* clone CHTACBSHOTU1211	97.94%	69%	MZ480961.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.839
Uncultured eukaryote clone TE104H	96.74%	100%	KM870634.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.756
Xanthonema sp. 395 strain 395	98.87%	100%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.817
Rosenvingiella radicans isolate Rrad1	96.67%	98%	EF200514.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.760
Xanthonema sp. 395 strain 395	98.39%	100%	AM491615.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.812
Rosenvingiella radicans isolate Rrad1	96.69%	99%	EF200514.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.760
Uncultured eukaryote clone TE106B	98.82%	100%	KM870643.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.776
Uncultured eukaryote clone TE104H	96.50%	100%	KM870634.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.749
Teberdinia sp. CP-WI-1	99.76%	99%	GU004239.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.759
Rosenvingiella polyrhiza isolate Rpol2	98.83%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.733
Uncultured archaeon clone hsdk08-a14	**98.59%**	**87%**	**KF818490.1**	**Archaeal**	*Eretmoptera murphyi* (16S rRNA)	0.146
Uncultured eukaryote clone TE106B	98.83%	100%	KM870643.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.781
Uncultured eukaryote clone TE106B	98.82%	100%	KM870643.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.774
Botrydiopsis callosa strain SAG 30.83	97.50%	99%	AJ579340.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.828
Uncultured eukaryote clone TE106B	97.89%	100%	KM870643.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.777
Uncultured eukaryote clone TE106B	98.59%	100%	KM870643.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.776
Uncultured eukaryote clone TE106B	98.59%	100%	KM870643.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.775
Uncultured *Chlorophyta* gene for 18S rRNA	98.83%	100%	AB257661.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.723
Rosenvingiella polyrhiza isolate Rpol2	99.06%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.731
Rosenvingiella polyrhiza isolate Rpol2	98.83%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.732

(Continued)

TABLE 1 (Continued)

Closest BLAST match	Identity	Query cover	Accession	Match type	Closest phylogenetic reference	Genetic distance
Uncultured eukaryote clone s3_27	89.26%	100%	GQ462637.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.729
Chlorella sp. Esh	99.53%	100%	X72706.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.716
Rosenvingiella polyrhiza isolate Rpol2	98.36%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.729
Rosenvingiella polyrhiza isolate Rpol2	99.29%	98%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.736
Rosenvingiella polyrhiza isolate Rpol2	97.89%	100%	EF200519.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.731
Uncultured eukaryote clone TE104H	97.16%	98%	KM870634.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.755
Uncultured archaeon clone BUNVGAR6	**91.70%**	**100%**	**JN205348.1**	**Archaeal**	***Eretmoptera murphyi* (16S rRNA)**	**0.740**
Micractinium sp. partial sequence KNUA036	99.53%	99%	KT883906.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.747
Planophila laetevirens strain partial sequence SAG 2008	95.08%	100%	MF034638.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.091
Prasiola crispa isolate Pcri1	98.12%	99%	EF200530.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.739
Paraucinula septata partial sequence isolate MUMH585	98.50%	71%	AB183530.1	Non-archaeal	*Eretmoptera murphyi* (16S rRNA)	0.785

ASVs in bold are those assigned to archaea using both the SILVA database and BLAST search.

Simpson index is more sensitive to dominant taxa (Morris et al., 2014), which were the primary focus of the present study. Differences in Shannon and Simpson diversity between larval and adult samples were assessed using exploratory two-sided exact permutation tests, comparing mean diversity values between life stages. Beta diversity was examined descriptively using Bray–Curtis dissimilarities calculated from bacterial ASV relative abundances using the vegdist function in vegan.

To assess differences in bacterial community composition between larvae and adults, unrarefied ASV counts were aggregated according to their phylum- and genus-level taxonomic assignments. ASVs lacking assignments at the corresponding taxonomic level were excluded from each analysis. Community composition was assessed using Bray–Curtis dissimilarities and Aitchison distances at both taxonomic levels. As microbiome abundance data are compositional and contain zeros, zero values were replaced using count zero multiplicative (CZM) replacement in the zCompositions package. Taxa containing more than 80% zero values across samples were excluded during this procedure. The resulting compositions were centered log-ratio (CLR) transformed, and Aitchison distances were calculated as Euclidean distances between CLR-transformed compositions. Differences in bacterial community composition between life stages were assessed using PERMANOVA (Anderson, 2001), with life stage as the grouping variable and 999 permutations. The R^2 value was used to estimate the proportion of variation in community composition associated with life stage. Homogeneity of multivariate dispersion was assessed using PERMDISP (betadisper in the vegan package), with significance determined using 999 permutations. Bacterial community composition was visualized using principal coordinates analysis (PCoA), based on Bray–Curtis dissimilarities at the phylum level and Aitchison distances at the genus level.

3 Results

3.1 Alpha and beta diversity

Bacterial alpha diversity varied among larval samples (Supplementary Figure S1). Bacterial alpha diversity also varied among adult samples (Supplementary Figure S2). Mean Shannon diversity was higher in adult samples (4.330) than in larval samples (3.300), although this difference was not statistically significant (exact permutation test, $p = 0.056$). Mean Simpson diversity was also higher in adults (0.962) than in larvae (0.843), with a statistically significant difference between life stages (exact permutation test, $p = 0.016$). However, these exploratory results should be interpreted cautiously given the small sample size ($n = 5$ per life stage). PCoA based on

Bray–Curtis dissimilarity showed variation in bacterial community composition among individual samples within both life stages (Supplementary Figures S3, S4).

3.2 Differences in bacterial microbiome composition between larvae and adults

There was a significant difference between the assigned bacterial ASV composition of larvae and adults at the genus level based on Bray–Curtis dissimilarities (PERMANOVA; $R^2 = 0.492$, $F = 7.759$, $p = 0.009$), with life stage explaining 49.2% of the variation in bacterial community composition. Multivariate dispersion did not differ significantly between life stages at the genus level (PERMDISP; $F = 2.753$, $p = 0.114$). At the phylum level, bacterial community composition also differed significantly between larvae and adults based on Bray–Curtis dissimilarities (PERMANOVA; $R^2 = 0.748$, $F = 23.687$, $p = 0.009$), with no significant difference in multivariate dispersion (PERMDISP; $F = 2.376$, $p = 0.131$). PCoA based on Bray–Curtis dissimilarities showed separation between larval and adult bacterial communities along the first axis, which explained 71.63% of the total variation (Figure 2).

Analysis using Aitchison distances also identified significant differences in bacterial community composition between life stages at both the genus level (PERMANOVA; $R^2 = 0.368$, $F = 4.653$, $p = 0.009$) and phylum level ($R^2 = 0.372$, $F = 4.737$, $p = 0.009$). At the phylum level, multivariate dispersion did not differ significantly between life stages (PERMDISP; $F = 1.044$, $p = 0.346$). However, genus-level multivariate dispersion differed significantly (PERMDISP; $F = 6.707$, $p = 0.033$), with adult samples exhibiting greater mean distances to their group centroid than larval samples (21.45 and 17.84, respectively). This indicates that differences in within-group dispersion may have contributed to the significant genus-level Aitchison PERMANOVA result. PCoA based on Aitchison distances also showed separation between larval and adult bacterial communities along the first axis at the genus level, with the first two axes explaining 49.02% of the total variation (37.71 and 11.31%, respectively; Figure 2).

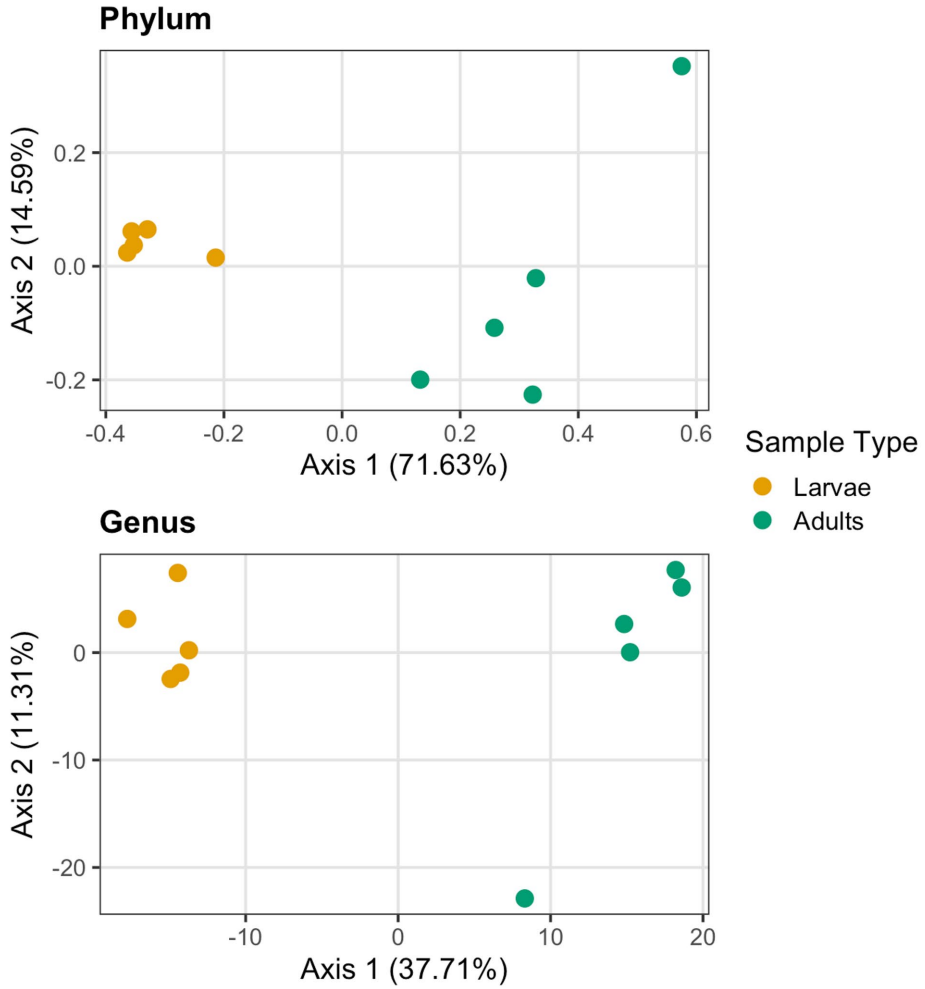

FIGURE 2
Principal coordinates analysis (PCoA) of bacterial community composition at the phylum (above) and genus (below) levels in larval and adult *Belgica antarctica*. The phylum-level plot is based on Bray–Curtis dissimilarities calculated from relative abundances, whereas the genus-level plot is based on Aitchison distances calculated from centered log-ratio (CLR)-transformed abundances following count zero multiplicative (CZM) replacement. At the phylum level, Axis 1 and Axis 2 explain 71.63 and 14.59% of the total variation, respectively. At the genus level, Axis 1 and Axis 2 explain 37.71 and 11.31% of the total variation, respectively. Each point represents an individual sample ($n = 5$ per life stage), with larvae shown in yellow and adults in green. Separation of samples along the first axis indicates differences in bacterial community composition between developmental stages.

3.3 Bacterial microbiome taxonomic composition

A total of 701 bacterial ASVs were recovered from larval samples and 1,521 from adult samples. Eight relatively abundant (>1%) bacterial phyla were identified in larvae and seven in adults (Figure 3A). The larval bacterial microbiome was dominated by Actinobacteriota (76–88% relative abundance), whereas Proteobacteria was the most relatively abundant phylum in adults (28–86%). Actinobacteriota was also relatively abundant in adults (8–44%). Relatively abundant phyla identified in both life stages were Actinobacteriota, Bacteroidota, Chloroflexi, Deinococcota, Firmicutes and Proteobacteria. Cyanobacteria and Planctomycetota were detected among the relatively abundant taxa only in larval samples, whereas Acidobacteriota was detected only in adult samples.

Twelve relatively abundant (>1%) bacterial classes were identified in each life stage (Figure 3B). Actinobacteria was the most abundant class in larvae (73–84%) and was also relatively abundant in adults (8–43%), whereas Gammaproteobacteria was the most abundant adult class (27–86%). Acidimicrobiia, Actinobacteria, Alphaproteobacteria, Bacilli, Bacteroidia, Chloroflexia, Clostridia, Deinococci, Gammaproteobacteria, and Thermoleophilia were detected among the relatively abundant classes in both life stages. Cyanobacteriia and Planctomycetes were detected among the relatively abundant classes only in larval samples, whereas Anaerolineae and Blastocatellia were detected among the relatively abundant classes only in adult samples.

Twenty relatively abundant (>1%) bacterial orders were identified in larvae and 23 in adults (Figure 3C). Micrococcales was the most abundant order in larvae (52–71%) and was also abundant in adults (7–36%), whereas Pseudomonadales reached a relative abundance of 86% in adults. Relatively abundant orders common to both larvae and adults were Burkholderiales, Chitinophagales, Corynebacteriales, Deinococcales, Flavobacteriales, Frankiales, Micrococcales, Microtrichales, Propionibacteriales, Rhizobiales, Solirubrobacterales, Thermomicrobiales, and Xanthomonadales. Chloroflexales, Isosphaerales, Kineosporiales, Leptolyngbyales, and Rhodobacterales were detected among the relatively abundant orders only in larval samples, whereas Bacillales, Blastocatellales, Caldalkalibacillales, Caulobacterales, Clostridiales, Cytophagales, Pseudomonadales, RBG-13-54-9, and Sphingobacteriales were among the relatively abundant orders only in adult samples.

Twenty-five relatively abundant (>1%) bacterial families were identified in larvae and 22 in adults (Figure 3D). Intrasporangiaceae was the most abundant family in larvae (36–61%) and was also abundant in adults (5–31%). Pseudomonadaceae reached a relative abundance of 86% in adults, while Diplorickettsiaceae accounted for up to 29%. Relatively abundant families identified in both larvae and adults were Chitinophagaceae, Comamonadaceae, Deinococcaceae, Dermacoccaceae, Diplorickettsiaceae, Intrasporangiaceae, JG30-KF-CM45, Micrococcaceae, Nakamurellaceae, Nocardiaceae, Nocardioidaceae, and Weeksellaceae. The families 67–14, Beijerinckiaceae, Geodermatophilaceae, Iamiaceae, Isosphaeraceae, Kineosporiaceae, Leptolyngbyaceae, Microbacteriaceae, Rhodobacteraceae, Roseiflexaceae, Solirubrobacteraceae, Sphingomonadaceae, and Xanthomonadaceae were detected among the relatively abundant families only in larval samples, whereas Bacillaceae, Blastocatellaceae, Caldalkalibacillaceae, Caulobacteraceae, Clostridiaceae, Hymenobacteraceae, Pseudomonadaceae, Rhizobiaceae, Spirosomaceae, and Sphingobacteriaceae were detected among the relatively abundant families only in adult samples.

Twenty-four relatively abundant (>1%) bacterial genera were identified in larvae and 21 in adults (Figure 3E). *Humibacillus* was the most abundant genus in larvae (29–60%) and was also detected in adults (0–19%). *Pseudomonas* reached a relative abundance of 86% in adults, while *Rickettsiella* accounted for up to 29%. Relatively abundant genera identified in both larvae and adults were *Chryseobacterium*, *Deinococcus*, *Humibacillus*, *Nakamurella*, *Nocardioides*, *Rhodococcus,* and *Rickettsiella*. *Aeromicrobium*, *Allobranchiibius*, *Antricoccus*, *Conexibacter*, *Cryobacterium*, *Galbitalea*, *Iamia*, *Knoellia*, *Luteimonas*, *Marmoricola*, *Oryzihumus*, *Paeniglutamicibacter*, *Polymorphobacter*, *Pseudarthrobacter*, *Quadrisphaera*, *Taibaiella,* and *Terrabacter* were detected among the relatively abundant genera only in larval samples, whereas *Blastocatella*, *Branchiibius*, *Brevundimonas*, *Caldalkalibacillus*, *Clostridium sensu stricto 13*, *Dermacoccus*, *Ferruginibacter*, *Hymenobacter*, *Nesterenkonia*, *Pedobacter*, *Phyllobacterium*, *Polaromonas*, *Pseudomonas,* and *Spirosoma* were detected among the relatively abundant genera only in adult samples.

3.4 Archaeal taxonomic composition and phylogenetic analysis

Due to poor sequencing quality, archaeal reads from the adult samples were not analyzed further. A total of 56 ASVs were recovered from larval samples. Of these, four were classified as Archaea by SILVA and were all assigned to the phylum Crenarchaeota (class Nitrososphaeria, order Nitrososphaerales, family Nitrososphaeraceae, genus *Candidatus Nitrocosmicus*). Three ASVs remained unassigned to any phylum, and the remaining 49 ASVs were classified as Eukaryota. All the Eukaryota-assigned ASVs could not be resolved to a specific phylum and were therefore considered "unclassified." No ASVs were classified to species level.

BLAST analysis of the larval archaeal ASVs showed that 52 shared high similarity with various non-archaeal matches (80.39–100%; Table 1), most of which matched eukaryotic taxa. Of the ASVs assigned to archaea using the SILVA database, the three most consistently supported archaeal ASVs by BLAST analysis (ASV_5, ASV_10, ASV_36), showing high sequence similarity (98.59–100%) to uncultured archaeal clones. ASV_52 was identified as archaeal by BLAST but not by SILVA, so its identify remains uncertain. ASV_54 was assigned to archaea by SILVA but showed a non-archaeal BLAST match, so its identity also remains uncertain. Phylogenetic analysis of the larval archaeal ASVs showed that the majority formed two large clusters that were phylogenetically distant from all archaeal reference sequences (Figure 4). These ASVs were characterized by long branch lengths and high genetic distances to archaeal references (typically ~0.7–0.85 substitutions per site; Table 1), consistent with their predominantly non-archaeal BLAST assignments. A subset of ASVs (ASV_6, ASV_8, and ASV_25) was positioned nearest to *H. volcanii* and *M. burtonii* among the included archaeal references. Despite this placement, the ASVs exhibited very large genetic distances (0.84–1.15 substitutions per site) and were assigned non-archaeal identities by BLAST. In contrast, three ASVs (ASV_5, ASV_10, and ASV_36) formed a distinct, tightly clustered clade closely associated with the *E. murphyi* crenarchaeotal reference sequence. These ASVs exhibited markedly lower genetic distances (0.000–0.146 substitutions per site). ASV_54 was also positioned near the *E. murphyi* reference (genetic distance = 0.091 substitutions per site), but its non-archaeal BLAST

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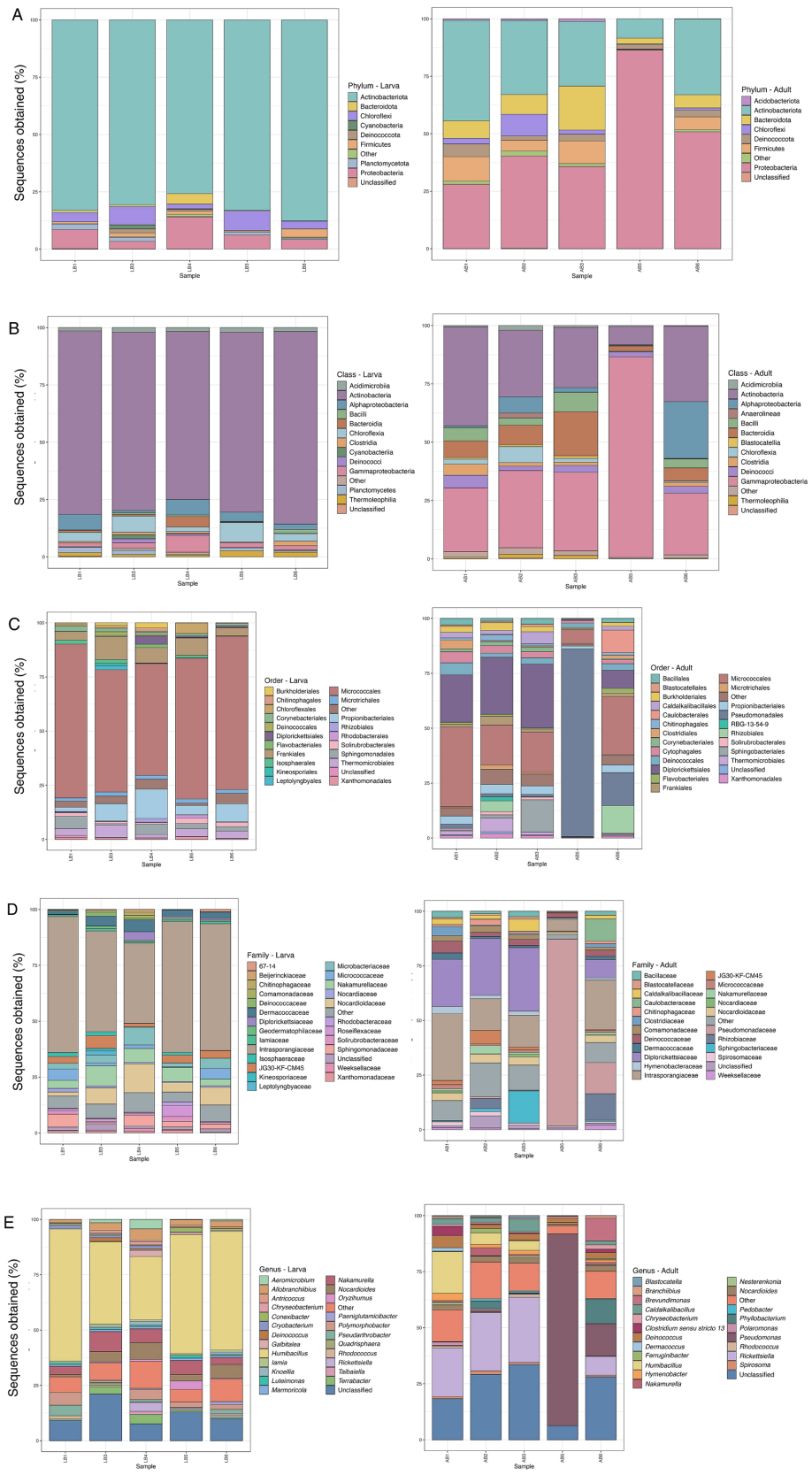

FIGURE 3
Stacked barplots showing the relative abundances (expressed as percentages of assigned bacterial sequences) of the dominant bacterial phyla **(A)**, classes **(B)**, orders **(C)**, families **(D)**, and genera **(E)** (>1% relative abundance in at least one sample) detected in larval and adult *Belgica antarctica*. Taxa with relative abundances below 1% are grouped as "Other." Consistent colors are used for corresponding taxa across larval and adult samples to facilitate comparisons of bacterial community composition between life stages.

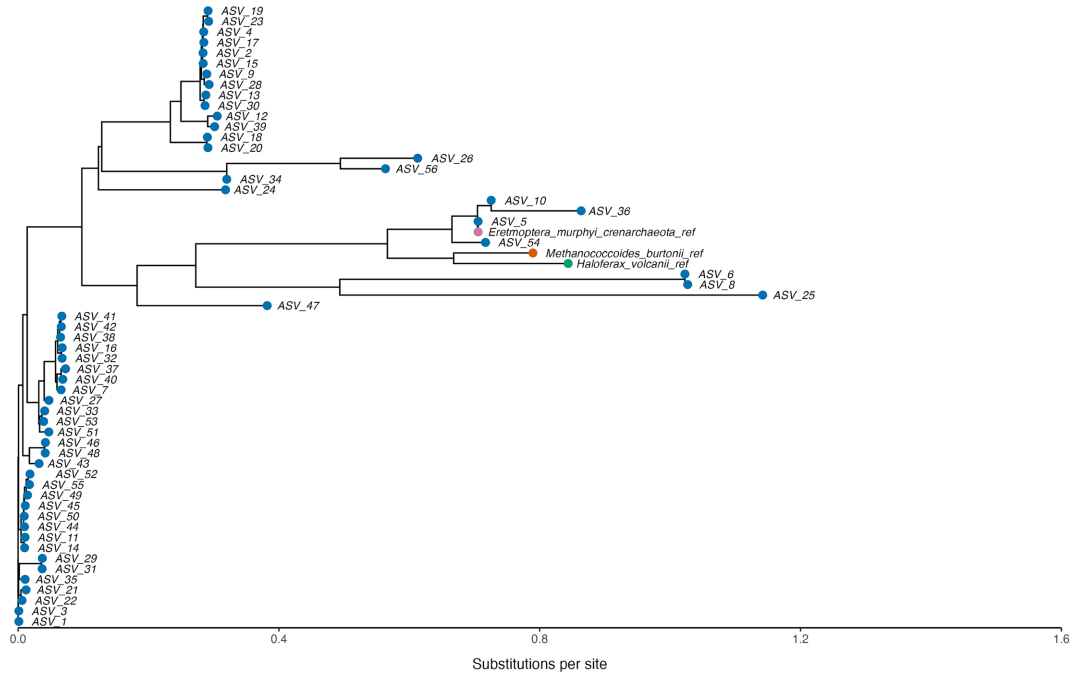

FIGURE 4
Neighbor-joining phylogenetic tree showing 52 amplicon sequence variants (ASVs; blue circles) recovered from archaeal-targeted sequencing of *Belgica antarctica* larvae, alongside representative archaeal reference sequences: *Eretmoptera murphyi* Crenarchaeota (purple), *Haloferax volcanii* (green) and *Methanococcoides burtonii* (orange). Branch lengths represent estimated nucleotide substitutions per site, with shorter genetic distances indicating greater sequence similarity. Taxonomic assignments of the ASVs were evaluated separately using SILVA and BLAST, and not all ASVs were confirmed to be archaeal.

match conflicted with its SILVA assignment, preventing confident classification as archaeal.

4 Discussion

4.1 Comparison of the bacterial microbiomes of larval and adult *B. antarctica* and their possible functional roles in development and reproduction

As DNA was extracted from whole insects, the microbial communities characterized here represent the whole-insect microbiome, and it is not possible to distinguish gut-associated microorganisms from those associated with other host tissues or surfaces. Therefore, any proposed links between the observed microbial differences and gut restructuring during metamorphosis should be considered only as hypotheses, and we are unable to directly establish microbial turnover within individuals. The observed differences may also reflect several non-exclusive factors, including life stage, environmental exposure and surface-associated microorganisms. The microbiomes of larvae differed significantly from those of adults in *B. antarctica* (at the genus level), a finding that is consistent with other studies of chironomids (Sela et al., 2020; Laviad-Shitrit et al., 2021) and dipterans more generally, including mosquitoes (Yin et al., 2025) and *Drosophila melanogaster* (Wong et al., 2013). This alteration in microbial communities is common amongst holometabolous insects that undergo complete metamorphosis, in which their body parts are entirely restructured (Manthey et al., 2022). The adults of *B. antarctica* lack functional mouthparts and do not feed (Devlin et al., 2022), meaning any retention within microbial communities after metamorphosis may represent persistent host-associated microorganisms, or may represent ectosymbiont or commensal groups that are acquired through contact with environmental substrates by the mobile adults. However, the present study cannot distinguish between these possibilities. There are currently few insect microbiome studies that have examined developmental change in species with non-feeding adults. In the chironomid, *C. transvaalensis*, the relative abundance of Proteobacteria increased in adults (Sela et al., 2020), in common with our findings in *B. antarctica*. In black soldier flies (*Hermetia illucens*), where the pupae are non-feeding and relatively immobile, the bacterial diversity also decreased at this point in development, only sharing two ASVs with the feeding adults (Klammsteiner et al., 2026).

Although the adults of *B. antarctica* do not feed (Finch et al., 2020) and are wingless (Kelley et al., 2014), they are very active, moving across diverse habitat surfaces and substrates (Benoit et al., 2007; Ajayi et al., 2021). Thus, the adult insects will likely adsorb microorganisms from the substrates they encounter onto their cuticle (Devlin et al., 2022). Although samples were stored/washed in ethanol, this does not ensure complete removal of surface-dwelling microorganisms (Binetruy et al., 2019). Potts et al. (2020) highlighted that *B. antarctica* is exposed to a diverse range of substrates including mosses, lichens, grasses, algae, sand, guano near nesting birds and waste products from other marine vertebrates such as seals. All the abundant bacterial phyla in the larvae and adults of *B. antarctica* have previously been detected in Antarctic substrates (Doytchinov and Dimov, 2022; Varliero et al., 2024), although no information on the terrestrial microbial communities in or near Orne Harbor is available. Thus, the higher bacterial alpha diversity observed in adults,

particularly as indicated by the Simpson index may, in part, reflect the environmental microbiome rather than those that are strictly insect-associated. Future studies should include steps to isolate endosymbionts specifically, perhaps using bleach, as this has been determined to be the most effective strategy for removing external bacterial DNA (Binetruy et al., 2019). Additionally, future research should characterize the microorganisms in both invertebrates and their surrounding substrates to suggest which taxa are commensals and which unique to the host.

Visual comparisons of the dominant phyla suggested that *B. antarctica* larvae exhibited a slightly more diverse microbiome than those of the adults, including one additional group, Acidobacteriota (Figure 3A). Notably, larval microbiomes were largely composed of Actinobacteriota, whereas adults had a lower proportion of this phylum. Our findings support those of both Maistrenko et al. (2023) and Kovalenko et al. (2025), with both studies reporting this phylum in *B. antarctica*. This phylum was also identified in our recent microbiome study of the sub-Antarctic sister species, *E. murphyi* (Brayley et al., 2026). A higher prevalence of Actinobacteria in larvae compared to other developmental stages has been reported in the moth, *Etiella zinckenella* (Lepidoptera: Pyralidae) (7% in larvae versus almost absent in adults, which is a much smaller percentage compared to *B. antarctica* larvae, 77–88%) (Chen et al., 2025). The authors suggested that this phylum might aid in the digestion of plant material in the larvae (by producing enzymes), compared to the adults which feed on nectar. Given that *B. antarctica* adults do not feed, efficient nutrient acquisition during the larval stage may be particularly important, especially in nitrogen-limited conditions, which typify many Antarctic terrestrial habitats (Convey et al., 2014). However, whether Actinobacteriota perform similar functions in *B. antarctica* remains unknown and cannot be determined from the present taxonomic data. Amongst chironomids, Actinobacteriota have been found in the egg mass and larval microbiome of *C. circumdatus*, albeit at very low abundance (<5%; Laviad-Shitrit et al., 2021) and in the egg mass (4.1%) and larvae (6.5%) of *C. transvaalensis* (Senderovich and Halpern, 2013). This phylum was found in larger relative abundances in the larvae of *E. murphyi* (~50%) and was hypothesized to play a role in host detritivory (Brayley et al., 2026). *Humibacillus*, a genus within the phylum Actinobacteria, was found at relatively large and comparable relative abundance in both larvae and adults. This genus has not been widely reported in insect microbiomes, however it is commonly found in soil (Saxena et al., 2020), therefore its detection here, as well as the report of one OTU by Maistrenko et al. (2023), may indicate environmental presence on the surface of the insects or in consumed detritus in the larvae, with no specific functional relationship.

While the relative abundance of Actinobacteriota was lower in adults (though remaining a prominent phylum), those of Bacteroidota, Firmicutes and, particularly, Proteobacteria, were higher than in larvae (Figure 3A). Proteobacteria have been reported in various dipterans and often dominate the host gut (Sontowski and van Dam, 2020). In chironomids, this phylum has been found in *C. transvaalensis* across all life stages from egg mass to adult. Similar to our findings, the relative abundance of Proteobacteria was highest in the adults, approximately 93–95%, versus 33–36% in larvae (Sela et al., 2020). Proteobacteria was also the most abundant phylum across egg mass, larva and pupa in *C. circumdatus*, and also generally increased in abundance across development, with an average of ~22.9% in pupae versus ~4.3% in larvae across all sampling sites (Laviad-Shitrit et al., 2021).

A bacterial genus of note (Figure 3E) is *Rickettsiella*, which was more prevalent in the adult samples compared to larvae. This genus is a known intracellular parasite/symbiont in other insects including the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) (Vecherskii et al., 2022) and the sand fly, *Phlebotomus chinensis* (Diptera: Psychodidae) (Li et al., 2016). This genus is present in the green lacewing, *Chrysoperla sinica* (Tjeder) (Chrysopidae: Neuroptera), where the relative abundance increased from 76.16% in eggs to 85.57% in adults (Zhao et al., 2019).

Wolbachia was not detected in the samples analyzed, a common bacterium found in around half of all insect species (Ross et al., 2020) being associated with *B. antarctica*. This bacterium often alters reproductive strategies in its host, including the development of parthenogenesis (Stouthamer et al., 1999), as well as influencing multiple other aspects of host biology. While Kovalenko et al. (2025) reported some *Wolbachia* sequences in their bacterial assessment of *B. antarctica* larvae, our data are consistent with the conclusion of Maistrenko et al. (2023), which did not identify *Wolbachia* from *B. antarctica* samples, and also with the wider synthesis of Serga et al. (2024) who concluded that *Wolbachia* has not yet been found in any Antarctic invertebrate. Unlike Kovalenko et al.'s study or our earlier characterization of *E. murphyi* (Brayley et al., 2026), we also did not identify any *Cardinium* or *Rickettsia* sequences in *B. antarctica*, genera which are also known to be reproductive manipulators in invertebrates (Weinert et al., 2009; Mathieson et al., 2025). Targeted molecular approaches and further sampling would be required to assess the occurrence of these potential endosymbionts in *B. antarctica*.

Although some of the bacterial taxa detected have been previously associated with nutritional or stress-related functions in other organisms, the present data do not provide direct evidence of microbial function in *B. antarctica*. Any potential functional associations therefore remain untested hypotheses and areas for future work.

4.2 Preliminary evidence of archaeal sequences in *B. antarctica* larvae

Given the low recovery of archaeal reads, and the absence of negative controls during sample processing and sequencing, it was not possible to exclude background DNA or contamination as a potential source of some of the recovered sequences. There was also a substantial loss of archaeal reads following quality filtering. Therefore, these results should be interpreted cautiously and as preliminary observations. In the larval samples, four ASVs were assigned to Crenarchaeota using SILVA, which was also detected in *B. antarctica*'s sister species, *E. murphyi* (Brayley et al., 2026). However, only three ASVs (ASV_5, ASV_10, and ASV_36) were consistently supported as archaeal by both SILVA and BLAST. These sequences clustered closely with the *E. murphyi* crenarchaeotal reference in the phylogenetic analysis (Figure 4). In contrast, ASV_52 and ASV_54 had conflicting taxonomic assignments between SILVA and BLAST, preventing confident classification. Most remaining ASVs had non-archaeal BLAST matches and were phylogenetically distant from the included archaeal references, suggesting substantial non-target amplification. Archaeal-targeted primers can amplify non-archaeal ribosomal sequences (Mori

et al., 2014), which may partly explain these findings. Further targeted sequencing, appropriate negative controls and expanded reference databases are required to establish the identity and occurrence of archaea associated with *B. antarctica*. Increased effort is also required to isolate and culture these Antarctic microorganisms to improve the coverage available in reference databases (Lambrechts et al., 2019).

4.3 Comparison of the microbiomes of *B. antarctica* and those of other Antarctic invertebrates

A comparative overview of microbiome composition across the available studies of Antarctic invertebrates is given in Figure 5. Owing to methodological differences among studies and the restriction of comparisons to the phylum level, these results should be interpreted cautiously and do not offer definitive insights into microbiome diversity. *Belgica antarctica*'s microbiome is more diverse than those of most of Antarctic invertebrates for which data are available. The phyla reported in the reconstructed *B. antarctica* microbiome of Maistrenko et al. (2023) were almost all present in larvae and adults in the current study, with the exception of Fusobacteria. However, our analyses identified additional phyla not reported by Maistrenko et al. (2023), including Chloroflexi, Cyanobacteria, Deinococci, and Planctomycetota. At the genus level, only *Humibacillus* was shared between our study and Maistrenko et al. (2023). Kovalenko et al. (2025) data on the bacterial microbiome of *B. antarctica* show some striking differences with our study. Their study suggested that Proteobacteria and Bacteroidota were the most abundant phyla (up to 52%), whereas the current study showed that Actinobacteria were the dominant phylum in the larvae (up to 88%). Deinococci and Chloroflexi were also abundant phyla in our study but were not reported by Kovalenko et al. (2025). Across the available studies, Chloroflexi have been reported in only one other species, in the larvae of *E. murphyi* (Brayley et al., 2026). Deinococci has only been found in males, females and tritonymphs of the Antarctic mite, *Alaskozetes antarcticus* (Holmes et al., 2019). From the available literature, this phylum appears to be unique to these two Antarctic invertebrates.

At the genus level, only three genera of bacteria were shared between our study and that of Kovalenko et al. (2025), *Chryseobacterium, Galbitalea* and *Nakamurella*, suggesting that some of these may be components of the core microbiome of *B. antarctica* [although these genera were not reported in the untargeted study of Maistrenko et al., 2023]. These differences in microbiome composition across studies suggests that the environment and surrounding substrate may have a profound impact on insect microbiomes. Kovalenko et al. (2025) sampled from Galindez Island in the Wilhelm Archipelago, and all sampling sites had significant ornithogenic influence. They also sampled the surrounding moss substrate of the larvae and showed that the microbiome present was strongly associated with substrate type, particularly for the Actinobacteria. As previously noted, there were also bioinformatic differences between the two studies. Kovalenko et al. (2025) utilized OTU clustering at 97% similarity to assign sequences to taxonomy, rather than directly assigning ASVs, as undertaken in the current study.

The microbiomes of both larval and adult *B. antarctica* appear to be most similar to those of its sister species, *E. murphyi* (Brayley et al., 2026). For example, they are both dominated by the seven phyla, Acidobacteriota, Actinobacteriota, Bacteroidetes, Chloroflexi, Firmicutes, Planctomycetota, and Proteobacteria. The similarities in phyla composition between the microbiomes of *B. antarctica* and *E. murphyi* may reflect their shared phylogeny. However, it should be noted that the two species have a

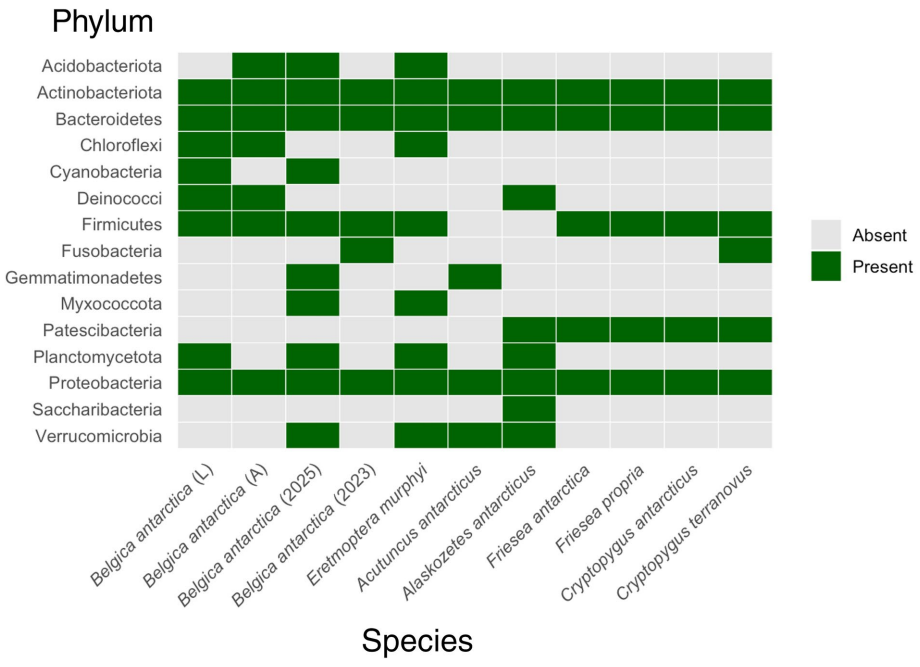

FIGURE 5
Presence/absence heat map comparing the most abundant phyla in the microbiome of larval (L) and adult (A) *Belgica antarctica* (in the current study) with those available from other Antarctic terrestrial invertebrates. "*Belgica antarctica* (2023)" indicates the phyla reported in Maistrenko et al. (2023) non-targeted whole genome study, which combined both larvae and adults. "*Belgica antarctica* (2025)" indicates the phyla reported in Kovalenko et al. (2025) targeted study of larvae.

multi-million year evolutionary and geographic distribution separation (Allegrucci et al., 2012), as well as inhabiting different habitats in their native ranges (maritime Antarctic South Shetland Islands and western Antarctic Peninsula versus sub-Antarctic South Georgia). Indeed, at the genus level, only *Humibacillus*, *Nakamurella,* and *Jatrophihabians* are shared between the larvae of these two species.

Compared to the relatively few available microbiome studies of other Antarctic terrestrial species, some differences and similarities are apparent (Figure 5). Available studies include the tardigrade, *Acutuncus antarcticus* (Parachela, Hypsibiidae), from Victoria Land (continental Antarctica) (Vecchi et al., 2018), the oribatid mite, *Alaskozetes antarcticus* (Oribatida, Trhypochthoniidae), from Cormorant Island, south of Anvers Island off the western Antarctic Peninsula (Holmes et al., 2019), four springtail species, *Cryptopygus antarcticus* (Collembola, Isotomidae) from the South Shetland Islands (maritime Antarctic), *Friesea antarctica* (Collembola, Neanuridae) from the South Shetland Islands and Lagoon Island (Marguerite Bay), and *C. terranovus* (Collembola, Isotomidae) and *F. propria* (Collembola, Neanuridae) from continental Antarctica (North Victoria Land) (Leo et al., 2021). Both *Belgica antarctica* larvae and adults share some dominant phyla with other Antarctic invertebrates, including Actinobacteriota, Bacteroidetes, Firmicutes, and Proteobacteria. At the order level, *B. antarctica* larvae from our study only share Cornebacteriales, Propionibacteriales, and Sphingomonadales with Antarctic springtails (Leo et al., 2021). There is a more substantial overlap of *B. antarctica* adults and the springtails, sharing the orders Burkholderiales, Corynebacteriales, Diplorickettsiales, Frankiales, Micrococcales, Propionibacteriales, and Thermomicrobiales. At the genus level, compared with *Alaskozetes antarcticus* tritonymphs and adults at the genus level, *B. antarctica* larvae only share *Aeromicroium, Chryseobacterium, Deinococcus,* and *Rhodococcus* (Holmes et al., 2019). There is significant overlap with *B. antarctica* adults, where most genera are shared between the two species. Holmes et al. (2019) found that the bacterial microbiomes were similar across life stages in the Antarctic mite (even at generic level), but relative abundances differed. This is similar to our results at phylum level; however, our study revealed large differences between larvae and adults at lower taxonomic ranks. It is possible that this difference reflects their different developmental strategies, as *A. antarcticus* is ametabolous (Escribano-Álvarez et al., 2022), whereas *B. antarctica* is holometabolous (Finch et al., 2020). Compared with the tardigrade, *Acutuncus antarcticus*, only one genus is shared with *B. antarctica* larvae, *Chryseobacterium* (Vecchi et al., 2018). *Belgica antarctica* adults share *Chryseobacterium, Pseudomonas, Halomonas, Dokdonella, Ferruginibacter, Niabella, Luteolibacter,* and *Gemmatimonas* with the tardigrade species.

To advance understanding of the evolutionary and functional roles of Antarctic invertebrate microbiomes, and to evaluate the composition and role of potential endosymbionts, future research is needed to characterize microbiomes across life stages and taxa, coupled with targeted approaches to assess the composition of possible endosymbionts and gut associated microorganisms via gut dissection and culturing of microorganisms (Auger et al., 2025). Expansion of Antarctic microbial databases is also required to identify microorganisms to species level, to enhance understanding of the diversity of microbial communities in this region and also to facilitate identification of any non-native species being introduced. Finally, application of multi-omics approaches will be important to investigate the functional roles of the *B. antarctica* microbiome in host nutrition, development and reproduction (Wei and Li, 2022).

5 Conclusion

This study provides the first characterization of adult *B. antarctica* microbiomes and includes a direct comparison with the larval stage. The bacterial microbiome of larvae was dominated (up to 88%) by members of the phylum Actinobacteriota. Adult samples were dominated by Proteobacteria (28–86%), with Actinobacteriota (8–44%) also being abundant. While larvae and adults shared many phyla, differences in bacterial community composition were also evident at lower taxonomic levels. We speculate that these differences may in part reflect adsorbed microorganisms acquired from the diverse substrates occupied by the more mobile adults. Larvae and adults shared few dominant genera and there was a significant difference between life stages at this taxonomic level, potentially reflecting several other factors, including developmental stage, environmental exposure and surface-associated microorganisms. The *B. antarctica* microbiome includes a higher diversity of bacterial phyla in comparison to other Antarctic invertebrate microbiomes that are available, with this variation potentially associated with differences in feeding habits and environment. However, methodological differences across studies limit robust comparisons. Our analyses provided preliminary evidence of a small number of archaeal ASVs in larvae, with Crenarchaeota being the only assigned archaeal phylum. However, low archaeal read recovery, the absence of negative controls and the lack of an interpretable adult archaeal dataset limit further conclusions. The *B. antarctica* microbiome exhibited the greatest similarity with its sister species, *E. murphyi*, perhaps reflecting their phylogenetic relationship and the use of comparable methodology. To assess possible functional roles of the Antarctic invertebrate microbiomes, as well as explore which microorganisms may be endosymbionts or commensals, more targeted approaches are required to isolate the internal invertebrate environment, as well as an expansion of the currently limited reference microbial databases from this region.

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 at: https://www.ncbi.nlm.nih.gov/, PRJNA1468618.

Ethics statement

Ethical approval was not required because this study involved insect specimens rather than vertebrate animals or higher

invertebrates requiring institutional ethical review. All specimens were collected in accordance with the relevant permits and institutional requirements.

Author contributions

OB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing. SL: Investigation, Methodology, Software, Writing – review & editing. PC: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. YC: Methodology, Project administration, Writing – review & editing. SU: Supervision, Writing – review & editing. NT: Writing – review & editing. SH: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2026.1936441/full#supplementary-material

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