




SHORT COMMUNICATION

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Ecological filtering and phylogeographic structuring of *Psychrilyobacter* within two closely related limpet species from the Southern Ocean

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Abstract

Purpose The ecological interdependence between macroorganisms and their microbial communities promotes stable associations over time, potentially leading to their evolutionary co-diversification. The detection of intricate eco-evolutionary interactions between animals and their microbiota is challenging, primarily due to complex bacterial communities related to poorly resolved host population structure. Strikingly, co-diversification in invertebrates, characterized by generally less complex microbiota, remains largely unexplored. Here, we compared the bacterial communities associated with two distinct lineages of *Nacella* limpets, a dominant shallow water patellogastropod of the Southern Ocean shores with a well-described population structure. Our goals were to elucidate the uniqueness of *Nacella* microbiota, resulting from an ecological filter that selectively favors certain bacterial taxa. Additionally, we aimed to depict the genetic structure of bacterial symbiont seeking evidence of co-diversification with *Nacella*.

Methods We sequence the V4-V5 regions of the bacterial 16S rRNA gene in three distinct microenvironments associated with *Nacella*: rock substrate, radula, and whole intestine. These samples were collected from two populations of *Nacella deaurata* and *Nacella concinna*, located in the West Antarctic Peninsula and Falkland/Malvinas Islands, respectively.

Results We assessed ecological filtering patterns in the limpet microbiota, uncovering unique bacterial communities in both radulas and intestines, with specifically enriched bacterial taxa compared to the surrounding environment. By examining microdiversity patterns of core bacterial taxa, we revealed a deep phylogeographic structure of *Psychrilyobacter* in *Nacella* intestines.

Conclusion We highlight the Southern Ocean limpets of the *Nacella* genus as a novel and promising model for studying co-diversification between marine mollusks and their resident microbiota.

Keywords Ecological filtering, Microbiota, Limpet, Microdiversity, Minimum entropy decomposition, *Psychrilyobacter*

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Findings

The intimate interdependence between macroorganisms and their microbial partners promotes stable associations over evolutionary timescales, which could ultimately result in the simultaneous divergence of both host and microbial symbionts (Moran and Sloan 2015; Moeller et al. 2016). This co-divergence, also known as co-diversification, does not necessarily imply obligatory host-restricted symbionts. For instance, repeated horizontal acquisition of certain microbes, through an ecological filtering of the surrounding environment community shaped by host diet or physiology, can lead to host-restricted microbial lineages (Moran and Sloan 2015; Moran et al. 2019). Nevertheless, the high complexity of microbial communities and the poorly resolved host population structure generally challenge the detection of specific host-microbe associations, particularly in vertebrate models (Alberdi et al. 2022).

In contrast to vertebrates, invertebrates generally possess less complex microbiota, making them intriguing models to comprehend the importance of host evolution in structuring animals-microbes associations (Petersen and Osvatic 2018; O'Brien et al. 2019; Groussin et al. 2020). Among invertebrates, mollusks stand out as ecologically significant and diverse phyla, being Gastropoda the richest class regarding species diversity (Neu et al. 2019). Although descriptions of marine gastropod microbiota are accumulating, they mainly focus on commercial and/or northern hemisphere species, excluding most gastropod diversity (Neu et al. 2019; Maltseva et al. 2021b; Panova et al. 2022). Moreover, the contribution of host diversification in shaping gastropod microbiota assembly remains largely unexplored. This knowledge gap widens in the Southern Ocean, where the true limpet genus *Nacella* (Nacellidae) thrives as the most conspicuous patellogastropod along intertidal and subtidal shores. Ecologically, species within this genus are characterized as generalist grazers due to their diverse diet, which includes seaweed, macroalgae, and some invertebrates (Rosenfeld et al. 2018). Biogeographically, the genus *Nacella* contains two major and recently diverged clades, the South American and the Maritime Antarctic/sub-Antarctic Islands, associated with the intensification of the Antarctic Circumpolar Current, which constitutes an oceanographical barrier to species dispersion (González-Wevar et al. 2017). On the premise that host speciation through geographical isolation may trigger its symbionts' diversification (Groussin et al. 2020), we hypothesized that two geographically isolated *Nacella* species shared specific bacterial symbionts whose phylogeographic structure at fine genetic resolution mirrors the host divergence. In this proof-of-concept study leveraging 16S amplicon sequencing, we aimed to assess (i) the

specificity of *Nacella* species microbiota, often referred to as ecological filtering (Mazel et al. 2018), (ii) the presence of a core microbiome between the South American (*Nacella deaurata*) and Antarctic (*Nacella concinna*) species, and (iii) the phylogeographic pattern at fine-genetic resolution (i.e., microdiversity) within core bacterial taxa associated with *Nacella*.

We sampled two closely related species, *N. deaurata* ($n=5$) and *N. concinna* ($n=4$), along with the rocks they were associated with, from two biogeographic provinces of the Southern Ocean separated by the Antarctic Polar Front: the Falkland/Malvinas Islands (FAL/MAL) and the West Antarctic Peninsula (WAP) (Additional Information S1: Table S1). The radula and the entire intestine were aseptically retrieved from limpet individuals, while rock surfaces were thoroughly scraped to collect the substrate material ("rock" hereafter). The genomic DNA of each microenvironment was extracted with the DNeasy PowerSoil Pro Kit (Qiagen, CA, USA). A touchdown PCR protocol was carried out to amplify the bacterial 16S V4-V5 region using the modified Bakt_341F/Bakt_805R primer pair (Klindworth et al. 2013) (Additional Information S2: Method S1). Subsequently, the PCR products were sequenced on the Illumina MiSeq PE300 platform. Sequences were then processed using the Mothur pipeline (Schloss et al. 2009), OTUs were assembled based on 97%- and 99%-identity, and low-abundance OTUs were discarded according to Bokulich et al. (2013). Taxonomic classification of OTUs was performed with Mothur using the SILVA database (v. 138) (Quast et al. 2013). Finally, 97%- and 99%-identity OTU tables were rarefied to their corresponding number sequences per sample, specifically 86,714 and 70,390, respectively. All rarefaction curves at both OTU resolution reached saturation, indicating robust coverage of sample bacterial diversity (Additional Information S3: Fig. S1).

By comparing alpha-diversity across microenvironments at 97%-identity OTU resolution, we found a clear reduction in both taxa and phylogenetic diversities from rocks to radulas and intestines (p -values < 0.05 and p -values < 0.001 , respectively, Fig. 1a and b). A similar pattern was observed in beta-diversity, with a strong effect of the microenvironment in shaping bacterial community compositions (p -value < 0.001 , Fig. 1c, Additional Information S1: Table S2). These findings suggest an ecological filtering of the surrounding microbial community, potentially mediated by host traits such as internal physicochemical properties, and leading to a more constrained and evolutionarily convergent set of bacterial taxa in the intestine (Moran and Sloan 2015). The lack of significant difference in both alpha- and beta-diversity between radulas and intestines (Fig. 1 and Additional Information S1: Table S2) suggests that the most selective

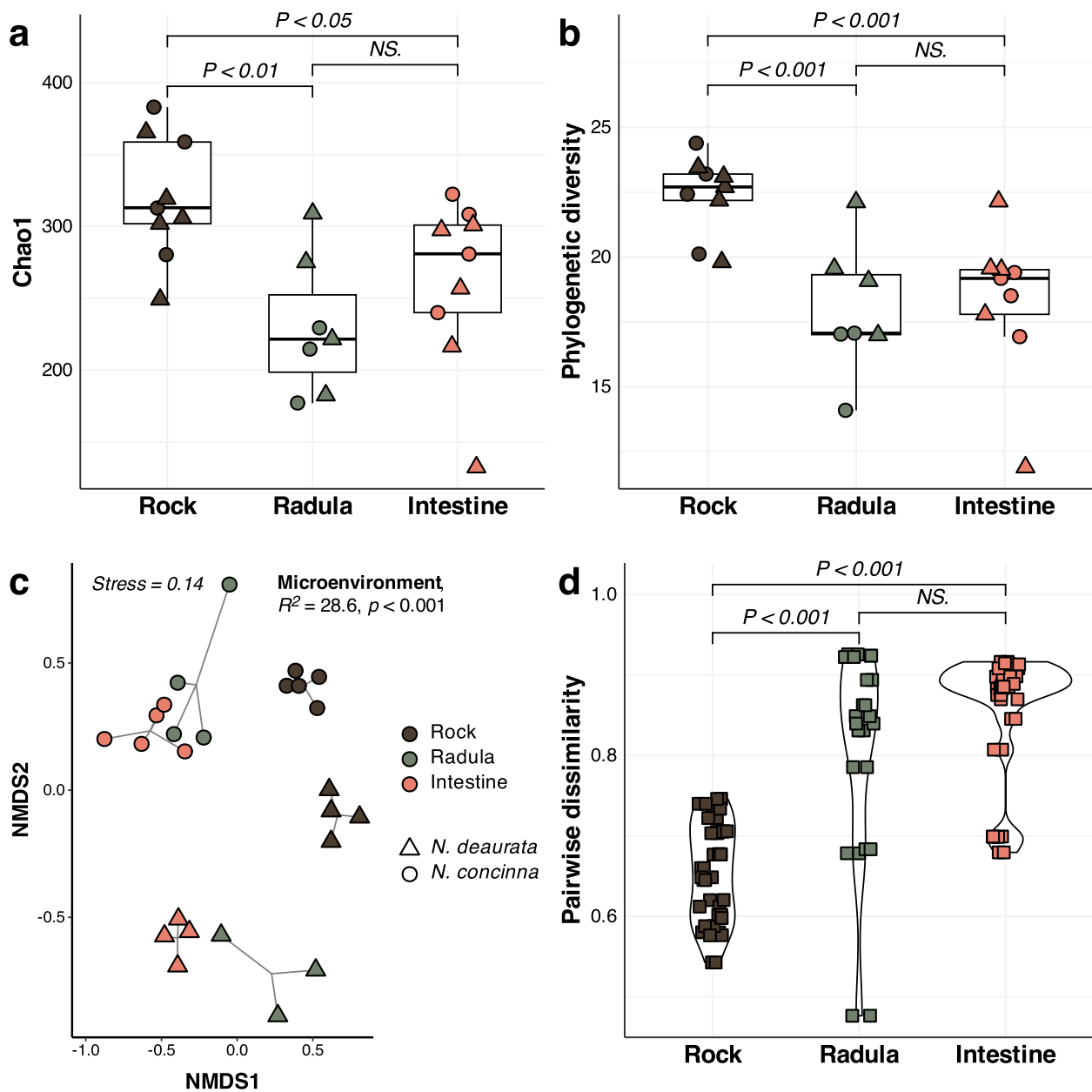


Fig. 1 Bacterial alpha- and beta-diversity at 97%-identity OTU resolution across *Nacella* spp. microenvironments. Colors are assigned to the microenvironment. Triangles and circles refer to *N. concinna* and *N. deaurata*, respectively. Squares represent interspecies pairwise dissimilarity of beta-diversity. Chao1 (**a**) and phylogenetic diversity (**b**) comparisons across microenvironments, independent of *Nacella* species. **c** Non-metric multidimensional scaling (NMDS) profiling revealing the bacterial community composition variations among *Nacella* microenvironments and species. **d** Comparisons of bacterial community dissimilarities among microenvironments. The effect of the microenvironment factor on sample grouping in the ordination and tested by PERMANOVA, as well as the p -values associated with the Wilcoxon test pairwise comparisons among microenvironments, are provided

filtering probably takes place right after radula scraping, as reported in the freshwater snail *Pomacea canaliculate* (Li et al. 2019). These findings mark the initial characterization of radula microbiota in marine snails. Moreover, detecting a microbial community in radulas, as diverse as in intestines (Fig. 1a and b), challenges the initial

assumption of this microenvironment being inhospitable to bacteria. Additionally, the bacterial communities in radulas and intestines were more heterogeneous than in rocks (p -values < 0.001 , Fig. 1d), highlighting the host individual influence in bacterial community composition (i.e. microbiota individualization), as observed in other

invertebrate models (Schwob et al. 2020; Gafarova et al. 2023). Alpha-diversity and beta-diversity patterns across microenvironments were globally consistent between 97%- and 99%-identity OTU resolutions, with sole exception being bacterial community dissimilarities among microenvironments (Fig. 1 and Additional Information S3: Fig. S2). Specifically, dissimilarities between radula and intestine were significant at 99%-OTU but not at 97%-OTU (Fig. 1d and Additional Information S3: Fig. S2d). For subsequent comparisons between microenvironments and the phylogeographic analysis of core taxa, we focused on the 97%-identity resolution.

We further explored the community differentiation drivers within microenvironments using Linear discriminant analysis (LDA) effect size (LEfSe) (Yang 2020). We identified 146, 39, and 29 bacterial OTUs significantly enriched in rock, radula, and intestine, respectively (Fig. 2a). A larger number of OTUs sit at the intersection of radula with intestine and rock, suggesting radula as an ecotone. Contrarily, very few OTUs were found at the rock-intestine intersection, further supporting the higher specificity of the intestine microenvironment. The taxonomic assignment of enriched OTUs was aggregated at the genus level, with each microenvironment characterized by its own set of taxonomic groups. Specifically, the rock bacterial community was enriched in *Granulococcus*, *Illumatobacter*, *Leucothrix*, *Litoreibacter*, *Yoonia-Loktanella*, the radula in unclassified *Bacilli* and *Mycoplasmataceae* genera (Fig. 2b), and the intestine in *Psychrilyobacter* and *Psychromonas* (Fig. 2b and Additional Information S1: Table S3). These two latter genera have been consistently found as key components of the gut microbiota of snail taxa like *Littorina*, *Rubyspira*, and *Haliotis* (Aronson et al. 2017; Gobet et al. 2018; Maltseva et al. 2021b), implying their strong adaptation to gut conditions and their significance in the gastropods' ecology. To evaluate the conservativeness level in *Nacella*-associated bacterial communities and detect bacterial taxa probably important for *Nacella* ecology, we established a 100% threshold core microbiota for each microenvironment. Of the 130 identified core OTUs, 64 were shared across the three microenvironments. Conversely, 19, 1, and 10 were exclusively observed within rock, radula, and intestine, respectively (Fig. 2c), pointing to lower conservatism of radula and intestine bacterial communities, likely due to host individual-related factors (Pisaniello et al. 2023). Notably, five dominant core taxa (i.e., OTU1–3, OTU8, and OTU10) accounted for 36% of the dataset abundance, including *Psychrilyobacter* and *Psychromonas* (γ -Proteobacteria), *Sulfitobacter* and *Ahrensia* (α -Proteobacteria), and *Bastopirellula* (Planctomycetes) (Fig. 2c). Relative abundance patterns of these core OTUs substantially varied according to the host

and the microenvironment (Additional Information S1: Table S4), suggesting that setting an arbitrary abundance cut-off in core definition may inadvertently excluded ecologically relevant bacteria in the context of single temporal sampling (Neu et al. 2021).

To explore the microdiversity structuration of these OTUs across microenvironments and *Nacella* species, we used the Minimum entropy decomposition algorithm (Eren et al. 2015), following the procedure described in a previous work (Schwob et al. 2021). Remarkably, the OTU3 assigned to *Psychrilyobacter* was the sole OTU displaying unique oligotypes within each *Nacella* species intestine (Additional Information S1: Table S5). We then reconstructed OTU3 oligotype networks in each microenvironment using the Median Joining method in the PopART software (Leigh and Bryant 2015), estimating genetic and phylogeographic differentiation indices (F_{ST} and Φ_{ST} , respectively) in Arlequin software (Schneider et al. 2000). Intriguingly, unlike rock and radula, OTU3 microdiversity in intestine exhibited strong phylogeographic structure, forming two distinct clusters specific to each *Nacella* species (Fig. 3). The disparity between differentiation indices in the intestine ($F_{ST} < \Phi_{ST}$, Fig. 3) suggests that the structuration among the oligotypes associated with *N. deaurata* and *N. concinna* primarily stemmed from an evolutionary divergence between the oligotypes within each host-species (Maturana et al. 2020). The phylogenetic analysis of these oligotypes using Bayesian inference further supported the hypothesis that these fine-scale genetic clusters represent at least two strongly diverged evolutionary units with different host specificity (Additional Information S2: Method S2 and Additional Information S3: Fig. S2) (Martiny et al. 2023).

Additionally, the observation of identical *Nacella* species-specific oligotypes in both intestines and rocks indicates that hosts might acquire *Psychrilyobacter* horizontally by selectively associating with particular strains from their environment. The fine-scale phylogeographic patterns in *Nacella* intestines suggest host-specific regulatory mechanisms, either passive (e.g., internal condition filtering) or active (e.g., immune system), controlling the association with *Psychrilyobacter* strains (Cicala et al. 2018; Maltseva et al. 2021b). These host-specific regulatory mechanisms, understudied in *Nacella*, are likely to vary among species. Previous studies have consistently demonstrated that closely related gastropod species exhibited variations in their diets, proteomics, and metabolomics, thereby triggering the association with divergent microbiomes (Maltseva et al. 2021a, 2021b).

A recent genomic study of an abalone-associated strain of *Psychrilyobacter* provided evidence of functions potentially crucial for the host's physiology, such as synthesizing various vitamins or maintaining an acidic gut

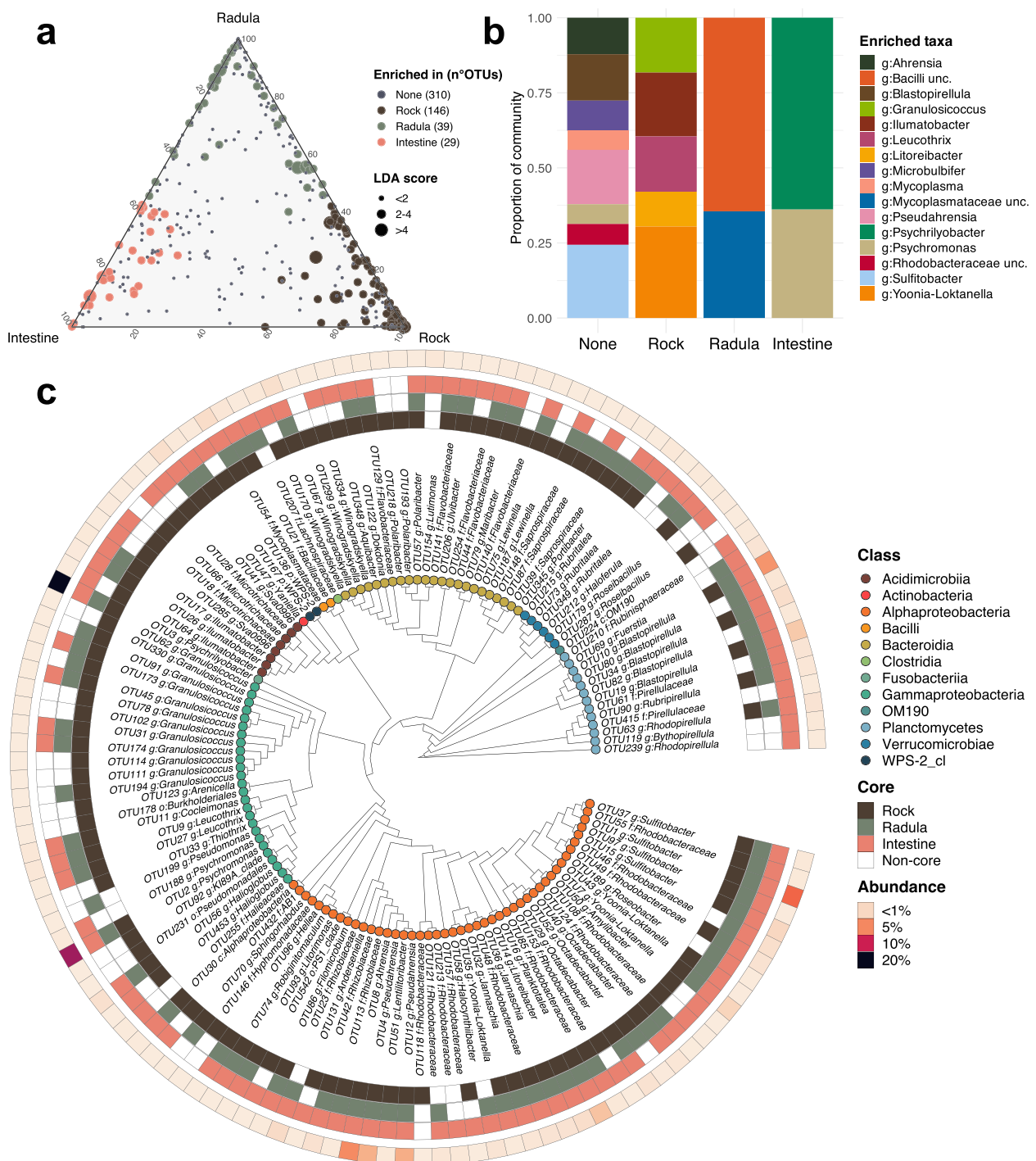


Fig. 2 Shared and specific bacterial taxa across the *Nacella* microenvironments. **a** Ternary plot showing the significantly enriched OTUs in each microenvironment. Size is scaled on the LDA scores from the LEfSE analysis. **b** Compositional barplot revealing the genus taxonomic assignment of the enriched OTUs (unc., unclassified). **c** Phylogenetic tree of the 130 OTUs shared across either rock, substrate, or intestine samples. The colors of the tree tips correspond to the core OTUs assignment at the class level. The adjacent heatmaps indicate the membership of the core OTUs to each microenvironment and its relative abundance within the core microbiota dataset

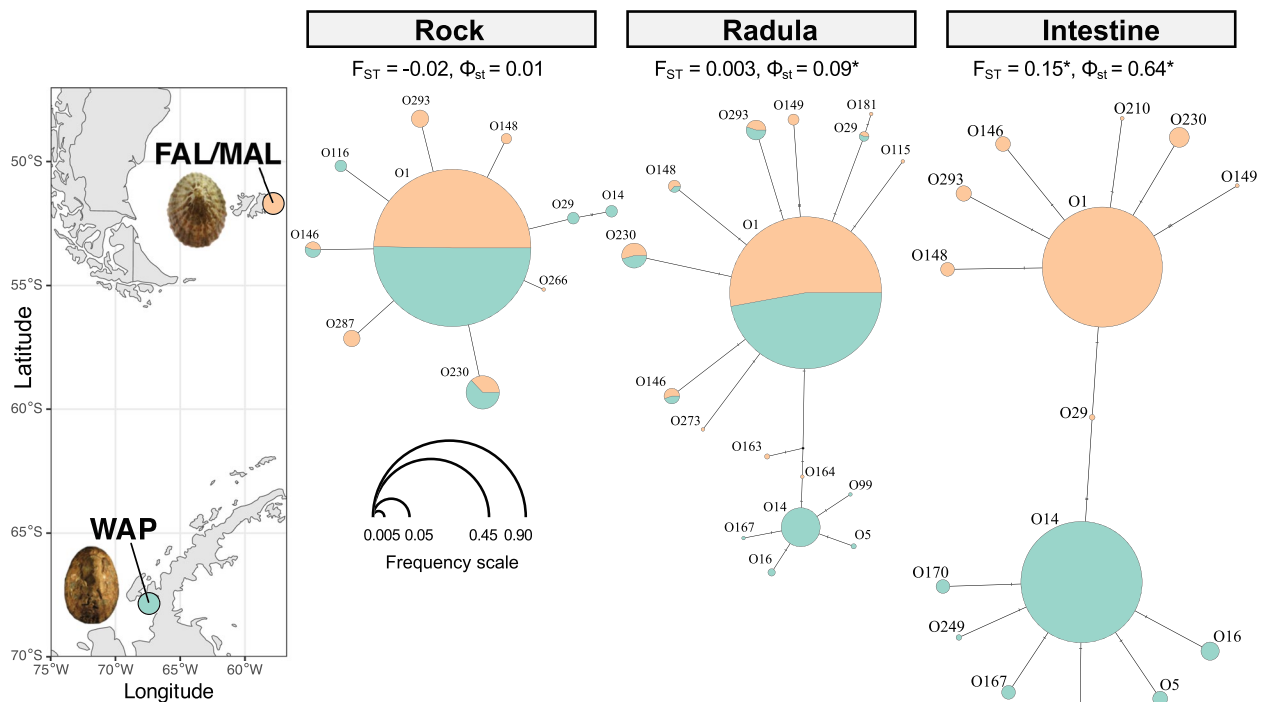


Fig. 3 Oligotype networks of 16S microdiversity within the OTU3 assigned to *Psychrilyobacter* genus. Networks are constructed for each *Nacella* microenvironment. The colors are assigned to the biogeographic provinces (West Antarctic Peninsula, WAP, and Falkland/Malvinas Islands, FAL/MAL). Oligotype numeration encompasses the three microenvironments. The asterisk indicates significance based on 10,000 permutations

pH (Liu et al. 2023). The microdiversity structuration we reported, if indicative of *Nacella*'s metabolism reliance on *Psychrilyobacter*, may result from stable associations over time and space, potentially culminating in the specialization of *Psychrilyobacter* strains to their respective hosts and their diversification mirroring the phylogenetic divergence of *N. concinna* and *N. deaurata*. Thus, the diversification of *Psychrilyobacter* strains is likely to be coupled with functional divergence, which remains to be fully explored through genome-resolved metagenomics, transcriptomics, or metabolomics approaches to fully understand their ecological role within *Nacella*.

In conclusion, our study marks a promising starting point in exploring the intricate evolutionary relationships between *Nacella* and its microbiome, which raises intriguing questions regarding whether the divergence of metaorganisms in the Southern Ocean may have triggered their microbiome co-diversification.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13213-024-01751-0>.

Additional file 1: Table S1. Sampling locations of *Nacella* spp. and overview of metabarcoding sequencing data. N: number of samples, Nseqs. (Relat. Abund.): total number of sequences per sample type and host species, and relative abundance in the whole dataset after the relative

abundance filtration step. **Table S2.** Pairwise PERMANOVA comparisons of Bray-Curtis dissimilarity across *Nacella* spp. microenvironments.

Table S3. Mean abundances and standard errors (se) of the enriched bacterial taxa across microenvironments. **Table S4.** Microdiversity distribution of the five most abundant core OTUs across *Nacella* spp. microenvironments.

Additional file 2: Method S1. The 50- μ L touchdown PCR reactions comprised 25 μ L of Q5[®] High-Fidelity DNA Polymerase (New England BioLabs[®] Inc.), 2.5 μ L forward primer (10 μ M), 2.5 μ L reverse primer (10 μ M), 5 μ L template DNA (about 150 ng/ μ L) and 17 μ L PCR-grade water. The PCR conditions were an initial denaturation step of 3 min at 98°C, followed by 15 cycles of 10 s at 98°C, 20 s at 67–60°C (touchdown PCR annealing) and 15 s at 72°C. After the initial touchdown cycles, an additional 15 cycles were performed, for 10 s at 98°C, 20 s at 57°C, 15 s at 72°C and a final extension of 2 min at 72°C. **Method S2.** The phylogenetic analysis of the oligotypes corresponding to the OTU3 of *Psychrilyobacter* was conducted using Bayesian inference (BI) with MrBayes v.1.3.1 (Huelsenbeck and Ronquist 2001). The nucleotide substitution model for the dataset was determined through jModelTest 2 (Darriba et al. 2012) using the Bayesian information criterion (BIC). Nodal support values BI reconstructions were estimated through 10,000,000 generations with 4 Markov chains, and trees were sampled every 1000 generations. The first 25% of trees sampled were discarded as burn-in. Posterior probability densities were plotted as a maximum clade credibility tree using TreeAnnotator v.1.6.1 (<http://beast.bio.ed.ac.uk/TreeAnnotator>) and visualized using FigTree v.1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Additional file 3: Fig. S1. Accumulation curves of *Nacella* species and microenvironment conditions' richness based on different alpha diversity metrics at 97%- and 99%-identity OTU resolution. The samples were rarefied to 86,700 and 70,390 reads for 97%- and 99%-identity OTU datasets, respectively. Colors correspond to *Nacella* species and micro-environment conditions. **Fig. S2.** Bacterial alpha- and beta-diversity

at 99%-identity OTU resolution across *Nacella* spp. microenvironments. Colors are assigned to the microenvironment. Triangles and circles refer to *N. concinna* and *N. deaurata*, respectively. Squares represent interspecies pairwise dissimilarity of beta-diversity. (a) Chao1 and (b) phylogenetic diversity comparisons across microenvironments, independent of *Nacella* species. (c) Non-Metric Multidimensional Scaling (NMDS) profiling revealing the bacterial community composition variations among *Nacella* microenvironments and species. (d) Comparisons of bacterial community dissimilarities among microenvironments. The effect of the microenvironment factor on sample grouping in the ordination and tested by PERMANOVA, as well as the *p*-values associated with the *Wilcoxon* test pairwise comparisons among microenvironments, are provided. **Fig. S3.** Bayesian inference of the phylogenetic relationships among the oligotypes conforming OTU3 assigned to *Psychrilyobacter* genus. The size of circles in the adjacent bubble plot is scaled on oligotype relative abundance (%). Colors indicate the *Nacella* species (orange; *N. deaurata*, green; *N. concinna*). The support of the tree basal node is provided.

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Authors' contributions

GS and SR conceived and designed the study. JO and CGW provided funding for the field expedition. SR collected the samples. GS, SR, and JO processed the samples. GS extracted the DNA, organized sequencing, and managed analysis. SR contributed to phylogenetic and phylogeographic analysis. GS specialized in figure generation. GS, SR, and JO jointly interpreted the findings. JO secured sequencing funding. GS wrote the manuscript draft. GS, SR, CGW, and JO authors read, edited, and approved the final manuscript.

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Availability of data and materials

The datasets generated for this study can be found in the National Center for Biotechnology Information (NCBI) repository, under the accession number PRJNA1025002.

Declarations

Ethics approval and consent to participate

No approval of research ethics committees was required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Alberdi A, Andersen SB, Limborg MT, Dunn RR, Gilbert MTP (2022) Disentangling host-microbiota complexity through hologenomics. *Nat Rev Genet* 23:281–297. <https://doi.org/10.1038/s41576-021-00421-0>
- Aronson HS, Zellmer AJ, Goffredi SK (2017) The specific and exclusive microbiome of the deep-sea bone-eating snail, *Rubyspira osteovora*. *FEMS Microbiol Ecol* 93. <https://doi.org/10.1093/femsec/fiw250>
- Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG (2013) Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat Methods* 10:57–59. <https://doi.org/10.1038/nmeth.2276>
- Cicala F, Cisterna-Céliz JA, Moore JD, Rocha-Olivares A (2018) Structure, dynamics and predicted functional role of the gut microbiota of the blue (*Haliotis fulgens*) and yellow (*H. corrugata*) abalone from Baja California Sur Mexico. *PeerJ* 6:e5830. <https://doi.org/10.7717/peerj.5830>
- Eren AM, Morrison HG, Lescault PJ, Reveillaud J, Vineis JH, Sogin ML (2015) Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. *ISME J* 9:968–979. <https://doi.org/10.1038/ismej.2014.195>
- Gafarova E, Kuracji D, Sogomonyan K, Gorokhov I, Polev D, Zubova E, Golikova E, Granovitch A, Maltseva A (2023) Gut bacteriomes and ecological niche divergence: an example of two cryptic gastropod species. *Biology (Basel)* 12(12):1521. <https://doi.org/10.3390/biology12121521>
- Gobet A, Mest L, Perennou M, Dittami SM, Caralp C, Coulombet C, Huchette S, Roussel S, Michel G, Leblanc C (2018) Seasonal and algal diet-driven changes of the digestive microbiota of the European abalone *Haliotis tuberculata*, a generalist marine herbivore. *Microbiome* 6:60. <https://doi.org/10.1186/s40168-018-0430-7>
- González-Wevar CA, Hüne M, Segovia NI, Nakano T, Spencer HG, Chown SL, Saucède T, Johnstone G, Mansilla A, Poulin E (2017) Following the Antarctic Circumpolar Current: patterns and processes in the biogeography of the limpet *Nacella* (Mollusca: Patellogastropoda) across the Southern Ocean. *J Biogeogr* 44:861–874. <https://doi.org/10.1111/jbi.12908>
- Grossin M, Mazel F, Alm EJ (2020) Co-evolution and co-speciation of host-gut bacteria systems. *Cell Host Microbe* 28:12–22. <https://doi.org/10.1016/j.chom.2020.06.013>
- Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glockner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 41:e1. <https://doi.org/10.1093/nar/gks808>
- Leigh JW, Bryant D (2015) POPART: full-feature software for haplotype network construction. *Methods Ecol Evol* 6:1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Li L-H, Lv S, Lu Y, Bi D-Q, Guo Y-H, Wu J-T, Yue Z-Y, Mao G-Y, Guo Z-X, Zhang Y (2019) Spatial structure of the microbiome in the gut of *Pomacea canaliculata*. *BMC Microbiol* 19:1–9. <https://doi.org/10.1186/s12866-019-1661-x>
- Liu M, Wei G, Lai Q, Huang Z, Li M, Shao Z (2023) Genomic and metabolic insights into the first host-associated isolate of *Psychrilyobacter*. *Microbiol Spectr* 11:e03990–03922. <https://doi.org/10.1128/spectrum.03990-22>
- Maltseva AL, Varfolomeeva MA, Ayanka RV, Gafarova ER, Repkin EA, Pavlova PA, Shavarda AL, Mikhailova NA, Granovitch AI (2021a) Linking ecology, morphology, and metabolism: niche differentiation in sympatric populations of closely related species of the genus *Littorina* (*Neritrema*). *Ecol Evol* 11:11134–11154. <https://doi.org/10.1002/ece3.7901>
- Maltseva AL, Varfolomeeva MA, Gafarova ER, Panova MAZ, Mikhailova NA, Granovitch AI (2021b) Divergence together with microbes: a comparative study of the associated microbiomes in the closely related *Littorina* species. *PLoS One* 16:e0260792. <https://doi.org/10.1371/journal.pone.0260792>
- Martiny JBH, Martiny AC, Brodie E, Chase AB, Rodriguez-Verdugo A, Treseder KK, Allison SD (2023) Investigating the eco-evolutionary response of microbiomes to environmental change. *Ecol Lett*. <https://doi.org/10.1111/ele.14209>

- Maturana CS, Segovia NI, González-Wevar CA, Díaz A, Rosenfeld S, Poulin E, Jackson JA, Convey P (2020) Evidence of strong small-scale population structure in the Antarctic freshwater copepod *Boeckella poppei* in lakes on Signy Island, South Orkney Islands. *Limnol Oceanogr* 65:2024–2040. <https://doi.org/10.1002/lno.11435>
- Mazel F, Davis KM, Loudon A, Kwong WK, Groussin M, Parfrey LW (2018) Is host filtering the main driver of phyllosymbiosis across the tree of life? *Msystems* 3:e00097–e18. <https://doi.org/10.1128/msystems.00097-18>
- Moeller AH, Caro-Quintero A, Mjungu D, Georgiev AV, Lonsdorf EV, Muller MN, Pusey AE, Peeters M, Hahn BH, Ochman H (2016) Cospeciation of gut microbiota with hominids. *Science* 353:380–382. <https://doi.org/10.1126/science.aaf3951>
- Moran NA, Ochman H, Hammer TJ (2019) Evolutionary and ecological consequences of gut microbial communities. *Annu Rev Ecol Syst* 50:451–475. <https://doi.org/10.1146/annurev-ecolsys-110617-062453>
- Moran NA, Sloan DB (2015) The hologenome concept: helpful or hollow? *PLoS Biol* 13:e1002311. <https://doi.org/10.1371/journal.pbio.1002311>
- Neu AT, Allen EE, Roy K (2019) Diversity and composition of intertidal gastropod microbiomes across a major marine biogeographic boundary. *Environ Microbiol Rep* 11:434–447. <https://doi.org/10.1111/1758-2229.12743>
- Neu AT, Allen EE, Roy K (2021) Defining and quantifying the core microbiome: challenges and prospects. *Proc Natl Acad Sci* 118:e2104429118. <https://doi.org/10.1073/pnas.2104429118>
- O'Brien PA, Webster NS, Miller DJ, Bourne DG (2019) Host-microbe coevolution: applying evidence from model systems to complex marine invertebrate holobionts. *mBio* 10:e02241–02218. <https://doi.org/10.1128/mbio.02241-18>
- Panova MAZ, Varfolomeeva MA, Gafarova ER, Maltseva AL, Mikhailova NA, Granovitch AI (2022) First insights into the gut microbiomes and the diet of the *Littorina* snail ecotypes, a recently emerged marine evolutionary model. *Evol Appl*. <https://doi.org/10.1111/eva.13447>
- Petersen JM, Osvatic J (2018) Microbiomes in natura: importance of invertebrates in understanding the natural variety of animal-microbe interactions. *Msystems* 3:e00179–e117. <https://doi.org/10.1128/msystems.00179-17>
- Pisaniello A, Handley KM, White WL, Angert ER, Boey JS, Clements KD (2023) Host individual and gut location are more important in gut microbiota community composition than temporal variation in the marine herbivorous fish *Kyphosus sydneyanus*. *BMC Microbiol* 23:275. <https://doi.org/10.1186/s12866-023-03025-2>
- Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41:D590–596. <https://doi.org/10.1093/nar/gks1219>
- Rosenfeld S, Marambio J, Ojeda J, Rodríguez JP, González-Wevar C, Gerard K, Contador T, Pizarro G, Mansilla A (2018) Trophic ecology of two co-existing sub-Antarctic limpets of the genus *Nacella*: spatio-temporal variation in food availability and diet composition of *Nacella magellanica* and *N. deaurata*. *ZooKeys* 738:1–25. <https://doi.org/10.3897/zookeys.738.21175>
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75:7537–7541. <https://doi.org/10.1128/AEM.01541-09>
- Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol Bioinform Online*. 1:47–50. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2658868/>
- Schwob G, Cabrol L, Poulin E, Orlando J (2020) Characterization of the gut microbiota of the Antarctic heart urchin (Spatangoida) *Abatus agassizii*. *Front Microbiol* 11:308. <https://doi.org/10.3389/fmicb.2020.00308>
- Schwob G, Segovia NI, González-Wevar C, Cabrol L, Orlando J, Poulin E (2021) Exploring the microdiversity within marine bacterial taxa: toward an integrated biogeography in the Southern Ocean. *Front Microbiol* 12:703792. <https://doi.org/10.3389/fmicb.2021.703792>
- Yang C (2020) microbiomeMarker: microbiome biomarker analysis toolkit. R package version 0.99.0. <https://doi.org/10.5281/zenodo.3749415>

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