

ORIGINAL ARTICLE OPEN ACCESS

Metabarcoding of Broad-Diet Nototheniid Fish Stomachs Reveals Newly Detected Antarctic Parasite Signals and Potential Host Associations

Micaela B. Ruiz^{1,2}  | Jesús Zarcero³  | Bruno Fusaro⁴  | Eugenia Moreira^{4,5}  | Manuel Novillo²  | Charlotte Havermans^{1,6}  | Elisabet Alacid³ 

¹FB2, BreMarE – Bremen Marine Ecology, Marine Zoology, University of Bremen, Bremen, Germany | ²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Diversidad y Ecología Animal (IDEA), Córdoba, Argentina | ³Department of Marine Ecology, Centre for Advanced Studies of Blanes (CEAB), CSIC, Blanes, Catalonia, Spain | ⁴Instituto Antártico Argentino, San Martín, Buenos Aires, Argentina | ⁵Instituto de Biología Funcional y Biotecnología (BIOLAB), INBIOTEC-CONICET, UNCPBA-CICPBA, UNCPBA, Facultad de Agronomía, Azul, Buenos Aires, Argentina | ⁶HYIG ARJEL, Benthic Ecology, Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Bremerhaven, Germany

Correspondence: Micaela B. Ruiz (micaruiz90@gmail.com) | Elisabet Alacid (e.alacid@ceab.csic.es)

Received: 20 February 2026 | **Revised:** 19 June 2026 | **Accepted:** 27 June 2026

Keywords: 18S | Antarctic regions | biodiversity | food web | host–parasite interactions | molecular sequence data

ABSTRACT

Parasites shape marine food-web structure by increasing network connectivity and adding trophic links and include important pathogens that can pose risks to ecosystem health. Despite their growing ecological and economic relevance, parasite diversity remains largely unexplored in Antarctic marine systems, particularly among protist lineages. Here, we present an 18S rDNA metabarcoding survey of parasite diversity in Antarctic fish, using stomach contents from two nototheniid species collected in Potter Cove (Isla 25 de Mayo/King George Island), contributing to the limited number of studies applying this approach in the region. We identified 242 parasitic OTUs, representing 16% of OTU richness and 9% of total reads, with metazoans dominating in terms of read abundance and protists contributing most to overall diversity. Multivariate analyses revealed high inter-individual variability and limited effects of host species, fish length, or sex, whereas depth emerged as the main structuring factor for parasite assemblages, driven primarily by prey-associated protists. This study provides a snapshot integrating potential fish parasites with putative prey-associated and environmentally derived parasite taxa, revealing previously undocumented molecular records in Antarctic benthic-pelagic communities and expanding current knowledge of Antarctic parasite diversity. In addition, sequence-based correlation analysis inferred putative associations between newly detected parasite taxa and local hosts, generating hypotheses of potential food-web interactions. Notably, we detected multiple protistan taxa of known pathogenic relevance rarely or never reported in Antarctic marine systems. This finding underscores the potential of combining broad-diet fish as natural samplers with metabarcoding to survey local parasite diversity and to support surveillance of emerging disease risks in Antarctic ecosystems undergoing rapid environmental change.

1 | Introduction

Parasitism is a fundamental ecological interaction that shapes energy flow, nutrient cycling, and food-web structure and stability across ecosystems (Dunne et al. 2013; Kuris et al. 2008;

Lafferty et al. 2006; Thompson et al. 2013). By increasing network connectivity and adding trophic links, parasites can alter food-web topology and dynamics, influencing community resilience and transmission pathways (Britton 2013; Hudson et al. 2006; Lafferty et al. 2008). These effects are particularly

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important in marine systems, where many parasites are embedded within trophic interactions as trophically transmitted stages or concomitant prey. For instance, planktonic and benthic invertebrates can transmit parasites to pelagic fish through predation, while fish may act as definitive, intermediate, or paratenic hosts, facilitating transmission across higher trophic levels (Marcogliese 2002). Further empirical evidence of the broader influence of parasites on food webs comes from studies of invasive species, as parasite co-introductions can add new nodes and links, displace native host–parasite assemblages, or spill over into native host communities (Britton 2013, and references therein). Moreover, parasites regulate host population dynamics, alter species succession, competition, and evolutionary processes (Hatcher et al. 2006; Hudson et al. 2006; Lafferty et al. 2008; Poulin 2007). Beyond their ecological roles, many parasites pose risks to animal health, underscoring the need for effective surveillance and robust data to understand their distribution, dynamics, and spread (Carlson et al. 2020).

Marine parasites are ubiquitous and taxonomically diverse, ranging from microscopic protists to metazoan helminths (De Vargas et al. 2015; Rohde 2002, 2005; Skovgaard 2014). Historically, research has focused on macroparasites identifiable by visual inspection and microparasites causing conspicuous lesions detectable through histological methods, while cryptic, low-abundance parasites or asymptomatic infections, particularly those caused by microparasites or associated with poorly studied hosts, have often been overlooked (Nadler and De León 2011; Okamura 2016; Poulin et al. 2023; Poulin and Morand 2000). Because traditional microscopy-based techniques generally lack sensitivity to detect low-abundance and microscopic forms compared to molecular methods (Balseiro et al. 2010; Stout et al. 2024; White et al. 2013), parasite diversity in marine hosts has likely been underestimated (Forrester et al. 2021; Miura et al. 2005; Okamura et al. 2018).

In recent years, DNA metabarcoding has emerged as a powerful tool to overcome the limitations of traditional parasite surveys (Bass et al. 2015, 2019, 2023; Bass and Del Campo 2020). By targeting broad-spectrum genetic markers (e.g., 18S rRNA and COI), metabarcoding allows simultaneous detection of a wide range of eukaryotic taxa, including both metazoan and protistan parasites (Bass et al. 2019; Miller et al. 2024). Applications on marine host tissues (e.g., gills and skin) and environmental DNA have revealed hidden parasite diversity in aquatic ecosystems, improving taxonomic resolution, recovery of diversity and the detection of low-abundance parasites (Bass et al. 2023; Jeon et al. 2025; Scheifler et al. 2022; Thomas et al. 2022). These advances highlight the potential of metabarcoding to reveal the “hidden” component of parasite biodiversity including the detection of rare and potentially invasive species (e.g., Holman et al. 2019).

Beyond taxonomic identification, sequence-based correlation analyses offer a powerful approach to infer potential host–parasite associations. By analyzing co-occurrence patterns from community sequencing data, it is possible to identify previously unrecognized interactions or confirm known associations, increasing the understanding of parasite ecology and transmission networks, particularly in poorly studied systems (Faust and Raes 2012; Layeghifard et al. 2017; Lima-Mendez et al. 2015). In

marine ecosystems, gut-content metabarcoding has enabled the simultaneous detection of prey items and associated parasites in fish, revealing potential links between diet and parasite acquisition (Rijal et al. 2024). Similarly, metabarcoding of zooplankton digestive contents has uncovered prey, symbionts, and parasite diversity in eukaryotic plankton, providing insights into potential species interactions (Hanström et al. 2025; Zamora-Terol et al. 2021).

In Antarctic marine ecosystems, parasitological research has been historically biased toward large metazoan parasites detected using morphological approaches, particularly those infecting vertebrate hosts (MacKenzie 2017; Alt et al. 2022; Klimpel et al. 2017; Oğuz et al. 2015). More recently, molecular analyses have been performed to investigate microeukaryotic parasites, such as ciliates, dinoflagellates, and microsporidia (Clarke et al. 2019; Cleary et al. 2025; Cleary and Durbin 2016; Grattepanche et al. 2022; Ilicic et al. 2022; López-Rodríguez et al. 2024). Yet, the diversity, prevalence, and ecological roles of protist parasites remain largely unexplored in Antarctic marine systems. This knowledge gap is particularly concerning given that rapid environmental change and increasing biological connectivity in the Antarctic Peninsula (Chown et al. 2012; Convey and Peck 2019) are likely to alter host–parasite dynamics. Biological invasions and climate-driven range expansions are becoming more frequent, with a growing number of non-native species recently documented in Antarctic waters. These introductions are facilitated by vectors such as global trade, ballast water discharge, and rafting processes, which may increase the risk of co-introduced parasites and associated emerging marine diseases (Hughes et al. 2020; Costello et al. 2021).

Nototheniid fish are key components of demersal food webs in coastal Antarctic marine ecosystems and are among the most studied groups, given their trophic importance and dominance (Marina et al. 2018; Near et al. 2012, 2015). Within this family, *Notothenia coriiceps* and *Notothenia rossii* are both widely distributed in the Atlantic (Scotia Arc) and Antarctic sectors and exhibit a broad, generalist diet encompassing both benthic and pelagic prey, thereby mediating energy and nutrient transfer across trophic levels. These species share similar ecological traits and coexist sympatrically in rocky substrates covered by macroalgae and invertebrates, where they feed primarily on krill, copepods, amphipods (e.g., hyperiids), gelatinous taxa, and macroalgae (summarized in Barrera-Oro 2002; Barrera-Oro et al. 2019; Moreira et al. 2021, 2023; Ruiz et al. 2024). Because of their high trophic position and broad diets, these fish can accumulate a high diversity of parasites, including their own parasites, as well as prey-associated and trophically transmitted parasites (Marcogliese and Cone 1997; Thieltges et al. 2013). This makes them ideal candidates to function as natural samplers of parasite diversity, with their stomach content serving as a snapshot of the local community of potential hosts (prey) and associated parasites (Rijal et al. 2024).

In this study, we applied 18S rDNA metabarcoding to the stomach contents of two keystone Antarctic nototheniid fish species, *N. coriiceps* and *N. rossii*, to characterize local parasite diversity. Specifically, we aimed to: (i) assess the diversity of potential metazoan and protist parasites present in their stomach contents; (ii) compare read-based parasite assemblages

in relation to biological and environmental variables; and (iii) explore putative prey-associated parasites and potential host associations using sequence-based correlation analysis. By doing so, we seek to identify novel or poorly documented parasite taxa and their potential host associations in Antarctic marine ecosystems, contributing to a broader understanding of Antarctic parasite diversity and its potential relevance for ecosystem-health surveillance in a region undergoing rapid environmental change.

2 | Material and Methods

2.1 | Sampling

To investigate parasite diversity in Antarctic nototheniid fish, we re-analyzed the 18S rDNA stomach-content dataset published by Ruiz et al. (2024), which was generated to characterize the diet composition of *Nothotenia rossii* and *N. coriiceps*. Briefly, nototheniid fish were collected in the coastal waters of Potter Cove at Isla 25 de Mayo/King George Island, South Shetland Islands (62°14' S and 58°40' W). A total of 46 specimens of *N. rossii* and 46 *N. coriiceps* were caught during the austral summer of 2022 (January–March). For sampling, trammel nets (15 m long, 1.5 m deep, 2.5 cm inner mesh, 12 cm outer mesh) were deployed on a rocky bottom with red and brown macroalgal beds. For each fish specimen, total and standard length, weight, sex, and collection depth were recorded. Stomachs were isolated, weighed, and stored at −20°C until further processing (Ruiz et al. 2024).

2.2 | Sample Processing, DNA Extraction, Library Preparation and Sequencing

Stomach samples were dissected to extract their content and homogenized under sterile conditions to avoid cross-contamination. DNA was extracted in triplicate from ~25 mg of stomach content using a Blood and Tissue DNA extraction kit (Qiagen) following the manufacturer's protocol and including a negative control for every 24 extractions. Only high-quality extracts, with a 260/280 ratio over 1.5 and concentrations higher than 10 ng/μL (assessed using a Nanodrop ND-1000 spectrophotometer, Thermo Fisher Scientific) were used for sequencing. Triplicates were pooled before library preparation (Ruiz et al. 2024).

We performed metabarcoding of the nuclear 18S gene v1v2 region, which covers a broad-spectrum of eukaryotes, including protists and parasitic metazoans (Miller et al. 2024; Ruiz et al. 2024). DNA metabarcoding library preparation and sequencing were carried out by AllGenetics and Biology SL (www.allgenetics.eu). Libraries were prepared by amplifying ~450 bp of the 18S rDNA the F04/R22mod primer pairs (Blaxter et al. 1998; Sinniger et al. 2016). Primers included Illumina sequencing adapters at their 5' ends. Two-step PCRs were performed without replicates, and negative controls were included throughout the process. The first PCR was carried out in 12.5 μL reactions with 1 μL of template DNA and 35 cycles of thermocycling. Details of reaction mixtures concentrations and cycling conditions are specified in Ruiz et al. (2024).

A second PCR was used to add indexing primers directly to PCR1 products (no purification step), using 5 cycles at 60°C. Library sizes were checked with agarose gels to verify the length of the DNA fragments before sequencing. Libraries were then purified using magnetic beads and pooled in equimolar amounts. The final pool was sequenced on an Illumina NovaSeq platform (2 × 250 bp), with 5% PhiX added, aiming for a total of 12 Gbp of output.

2.3 | Bioinformatic Analysis

To generate a curated OTU table for downstream analyses, bioinformatic processing of the 18S sequencing data involved two consecutive steps. First, we used Cutadapt v2.8 (Martin 2011) to remove primers and residual adapter sequences. Second, we applied functions from the R package dada2 v1.18.0 (Callahan et al. 2016) to perform sequence trimming and filtering, denoise reads using the Divisive Amplicon Denoising Algorithm (DADA), merge paired-end reads, identify and remove chimeras, and generate amplicon sequence variants (ASVs). The resulting ASVs were subsequently clustered into operational taxonomic units (OTUs) using the program swarm2 (Mahé et al. 2015) with an iterative local threshold $d=4$ (Brandt, Pradillon, et al. 2021; Brandt, Trouche, et al. 2021; Günther et al. 2021; Ruiz et al. 2024). Produced OTUs were taxonomically annotated against the PR2 reference set v.4.14.0 (<https://doi.org/10.1093/nar/gks1160>) and manually curated using the World Register of Marine Species (WoRMS; <https://www.marinespecies.org>) and BLASTn searches against the NCBI database. Only OTUs were used in subsequent analyses.

Non-target taxa (bacteria, archaea and terrestrial taxa) and contaminants (predator and human DNA) were removed from the curated OTU dataset. We removed every OTU for which the read abundance in the blanks or negative controls was higher than 10% of the total reads of that OTU, and those with less than 4 reads. Finally, we removed samples with a read depth of less than 10,000 reads (Drake et al. 2022; Siegenthaler et al. 2022).

2.4 | Identification and Annotation of Potential Parasite OTUs and Their Hosts

To identify potential parasite OTUs from the curated dataset and annotate their likely host associations, we classified OTUs based on the information available in the literature following the approach of Alacid et al. (2025). Briefly, the taxonomic classification of OTUs was thoroughly examined to identify and annotate parasitic groups or taxa *ad hoc*, assuming that the taxonomic assignment was correct and selecting them for further analysis in our study (File S1). First, we pre-selected phyla known to include parasites from the literature (Adl et al. 2007, 2019; Poulin and Morand 2000; Weinstein and Kuris 2016). For OTUs identified to species/genus level, we searched Google Scholar, PubMed and NCBI databases. If sources described them as parasites (including facultative/opportunistic or pathogens), we kept them and annotated their host(s). For higher rank assignments, we retained all OTUs when the group was reported as mostly or exclusively parasitic; otherwise, each OTU was

BLASTed against NCBI, with uncultured/environmental sample sequences excluded from the search. We inspected the top 10 hits with 100% query cover and $\geq 90\%$ identity. OTUs were retained only if matched metadata indicated parasitism, and classified as non-parasitic otherwise. Finally, OTUs known to infect terrestrial hosts (including humans) were excluded from subsequent analyses.

2.5 | Statistical Analyses

For statistical analyses of the curated metabarcoding dataset (File S2), we primarily used the vegan package v4.2.3, and all graphs were generated using the packages ggplot2 v3.5 and ggpurbur v0.6.1 from Rcran (Kassambara 2016; Oksanen et al. 2001; R Core Team 2013; Wickham 2009). To describe overall parasite diversity and its contribution to the stomach-content community, the entire dataset was used to assess the number of OTUs and the relative read abundance of parasite taxa compared with the whole community and within each individual fish sampled. The subset of all potential parasite OTUs was used for all further analyses, hereafter referred to as parasite assemblage dataset. Alpha diversity metrics (Shannon, Simpson and rarefied richness) of the parasite assemblage were calculated for each individual sample using diversity and rarefy functions of the vegan package. Group differences between the two fish species for each diversity metric were tested with Wilcoxon rank-sum tests using the package stats version 4.3.3. Venn diagrams were created to determine the shared and unique parasite OTUs among fish species using R package VennDiagram v1.7.3 (Chen 2011).

To assess variation in parasite assemblage composition among biological and environmental groups, we performed multivariate statistical analyses. Parasite read counts were converted to relative read abundances per sample using the decostand function, and then subjected to a fourth-root transformation prior to computing Bray–Curtis dissimilarities with the vegdist function. Biological and environmental variables were categorized into three levels: total fish length (small: 21.0–29.9 cm; medium: 30.0–38.0 cm; large: ≥ 38.1 cm), stomach weight (≤ 10 g; 10.1–25.0 g; ≥ 25.1 g), and sampling depth (≤ 10 m; 11–20 m; ≥ 20.1 m). These categories, along with fish species *N. coriiceps* (NOC), *N. rossii* (NOR) and sex (female (F), male (M)), were used as factors in the analysis. To assess differences in parasite assemblage composition among groups, a Permutational Multivariate Analysis of Variance (PERMANOVA) with 9999 permutations was performed using the adonis2 function from the vegan package. *p*-values from pairwise comparisons were adjusted using the Holm correction method. Homogeneity of multivariate dispersion was tested using PERMDISP (betadisper followed by permutest in vegan), and pairwise post hoc comparisons were conducted with the pairwiseAdonis function (Martinez Arbizu 2020; Oksanen et al. 2001; R Core Team 2013). Similar percentage (SIMPER) analysis was performed to determine the contribution of individual species (OTUs) to the observed dissimilarity between depth categories using the simper function in vegan. Bray–Curtis dissimilarities were visualized using Principal Coordinates Analysis (PCoA) using the cmdscale function.

To test for differences in parasite read abundance for each categorical factor, total parasite read abundance was calculated as the sum of raw reads counts across all OTUs annotated as parasites within each sample. Violin plots were generated and differences in the mean abundance were tested using Wilcoxon test (when factors had two levels) and Kruskal–Wallis (if ≥ 3 levels) using stats package from R base. If the latter was significant, we performed post hoc pairwise comparisons using Dunn's test from FSA R package v0.10.1 (Ogle et al. 2026). To test if the read abundance of parasites increased with fish size, total length was used as a continuous predictor. A simple linear regression using the package stats (lm function) was fitted on $\log_{10}$ -transformed counts, reporting the fitted equation, R^2 , and the model *p* value, and Spearman's rank correlation ρ was calculated with the function cor.test.

To visualize the distribution of parasite-associated relative read abundance, we generated heatmaps from the curated OTU table using the pheatmap R package (Kolde 2025). Parasite OTUs were first split into two groups according to their literature-based host annotation (Files S1 and S2): (i) fish-associated parasites, defined as parasite taxa reported to infect fish and for which *N. rossii* and *N. coriiceps* potentially act as definitive hosts; and (ii) prey-associated parasites, defined as parasite taxa reported to infect organisms that constitute the nototheniids diet. In the present analysis, candidate prey were defined as non-parasitic OTUs detected in the stomach contents and assigned to dietary organisms or ingested through-diet items. For each parasite group, OTUs were aggregated at the species level and visualized as heatmaps of relative read abundance across individual fish samples. In addition, to examine which hosts within the fish diet contributed most to the prey-associated parasite signal, prey-associated OTUs were further aggregated by their host type (File S2). Relative read abundance was then calculated per sample and visualized as a heatmap. For fish-associated parasites, we also estimated parasite prevalence for each fish species, defined as the percentage of individuals in which a parasite taxon was detected from the total number of individuals collected.

For prey-associated parasites, the host-type heatmap showed that parasites associated with mollusks and crustaceans represented the dominant prey-associated parasite groups, consistent with the relevant contribution of these groups to nototheniids diet reported by Ruiz et al. (2024) using the same dataset. Based on this result, we assessed potential host–parasite associations for the two dominant host categories (mollusks and crustaceans) based on read-abundance co-occurrence analyses across samples. We divided the curated OTU table into putative parasites and candidate hosts. Non-parasitic OTUs assigned to Mollusca and Crustacea were selected as candidate hosts and correlated with parasite OTUs whose potential hosts were mollusks or crustaceans, respectively. For each parasite–host pair, we extracted their sample-wise abundance and tested their association using Spearman's rank correlation using stats implemented in base R (cor.test function, method = “spearman”). Only positive significant associations ($p < 0.05$; correlation > 0.5) were retained and subsequently annotated at species level. Correlation results were visualized as chord diagrams using the R package circlize v 0.4.17 (Gu et al. 2014).

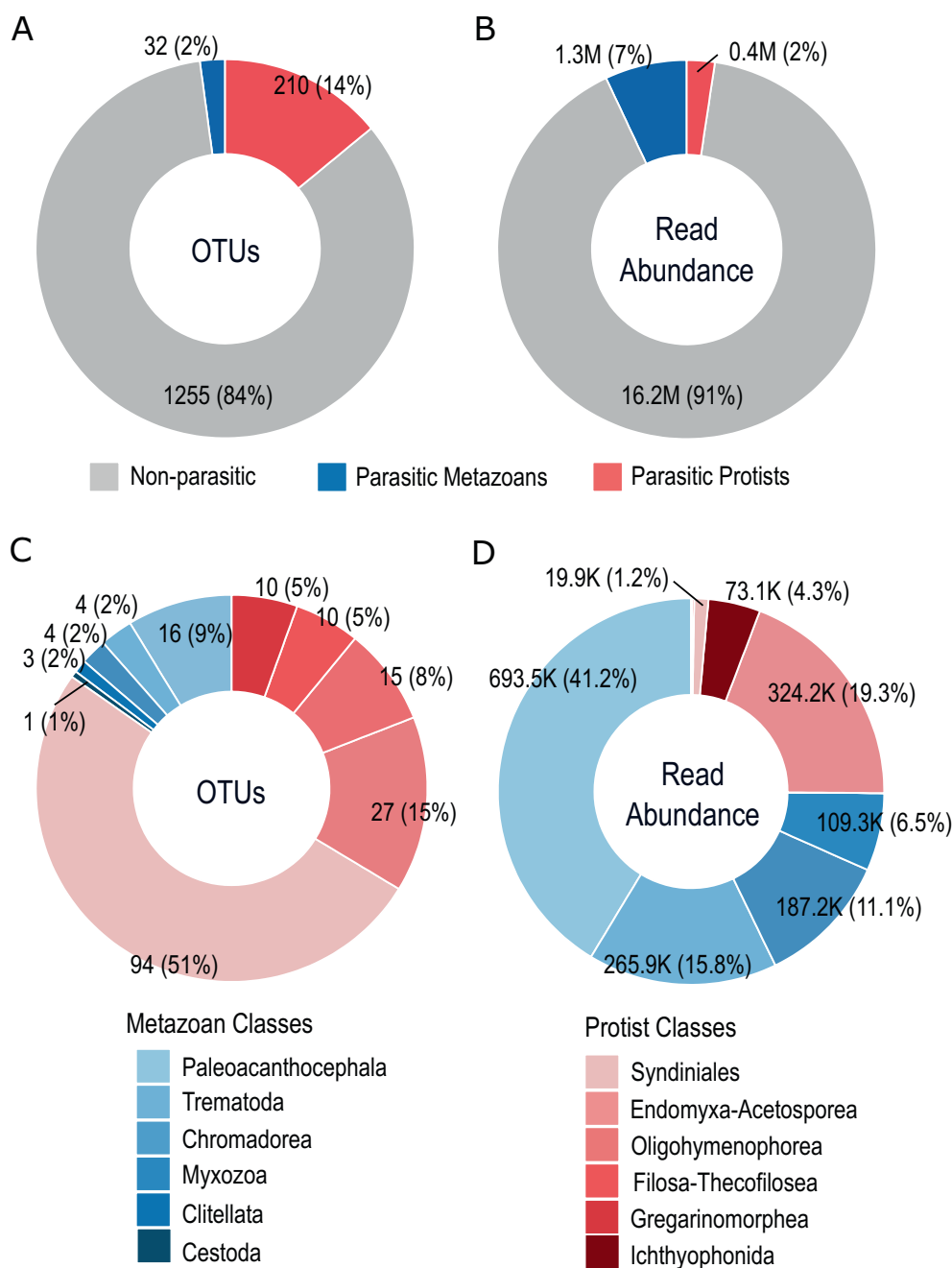


FIGURE 1 | Overview of parasite composition in the curated 18S rDNA stomach-content metabarcoding dataset from Antarctic nototheniid fish. Donut charts show the contribution of non-parasitic OTUs (gray), metazoan parasite OTUs (blue), and protist parasite OTUs (red) to the total curated community based on OTU richness (A) and sequencing read abundance (B). Bar plots show the most represented parasite classes among metazoan and protist parasites based on OTU richness (C) and relative read abundance (D).

3 | Results

3.1 | Overview of the Parasitic Assemblage in Nototheniid Fish Stomach Contents

The Novaseq sequencing run produced 47,106,864 paired-end raw reads, which were clustered into 1757 OTUs (Ruiz et al. 2024). After quality filtering, two *Notothenia coriiceps* samples were excluded from the analysis because they were represented by less than 10,000 reads, leaving a final dataset of 44 *N. coriiceps* and 46 *N. rossii* samples. The curated OTU table comprised 17,929,365 reads and 1497 OTUs (File S2). Of

these 1497 OTUs, 1255 were classified as non-parasitic and were not included in the parasite-specific downstream analyses. The remaining 242 OTUs were identified as potential parasites, including 32 metazoan OTUs and 210 protist OTUs, representing 2% and 14% of the curated dataset, respectively (Figure 1A). In terms of read abundance, parasites accounted for only 9% of the entire fish stomach-contents community (Figure 1B), with metazoan parasites being more abundant than protists, representing 1.3 (7%) and 0.4 (2%) million reads, respectively. At class level, within the metazoan parasites, those belonging to Paleacanthocephala had the highest number of OTUs (16), representing 9% of the parasitic OTUs detected in nototheniid fish

stomachs, followed by Trematoda and Chromadorea (4 OTUs, 2%), Clitellata (3 OTUs, 2%), Myxozoa and Cestoda (1 OTU, 1%) (Figure 1C). In terms of relative read abundance, the four classes primarily contributing to the parasitic assemblage were Paleacanthocephala, with 693 K reads representing 41.2% of the parasite dataset (Figure 1D), followed by Trematoda (269 K reads, 15.8%), Chromadorea (187 K reads, 11.1%), and Myxozoa (109 K reads, 6.5%). Regarding protist parasites, the highest number of OTUs was observed in the class Syndiniales, with 94 OTUs detected, representing 51% of the parasite dataset. It was followed by Endomyxa-Acetosporea (27 OTUs, 15%), Oligohymenophorea (15 OTUs, 8%), Filosa-Thecofilosea (10 OTUs, 5%), and Ichthyophonida (10 OTUs, 5%) (Figure 1C). The most abundant protist parasite class was Endomyxa-Acetosporea, representing 19.3% of the relative read abundance (324.2 K reads), followed by Ichthyophonida (73.1 K reads, 4.3%) and Syndiniales (19.9 K reads, 1.2%) (Figure 1D).

3.2 | Proportion of Parasites, Taxonomic Composition and Diversity Metrics in the Stomachs of *N. coriiceps* and *N. rossii*

Comparison of the proportion of parasitic vs. non-parasitic OTUs within each individual fish showed that non-parasitic OTUs dominated the overall community composition across most individuals (Figure 2, top panels). However, in a few individuals of both fish species, including both metazoan and protist parasites, contributed substantially to the total relative read abundance. Individuals in which parasites reads accounted for more than 50% of total reads were generally dominated by metazoan parasites, with the exception of one specimen of *N. rossii* (NOR_116), in which protist parasites comprised 70% of total reads. The general tendency appeared similar between species, showing high inter-individual variability, although *N. rossii* had a slightly thicker upper tail (i.e., a few more individuals with comparatively high parasite proportions). The balance between metazoan and protist parasite reads also varied among individuals, with no clear species-specific bias.

The taxonomic composition of parasites at phylum level for each fish (Figure 2, bottom panels) showed that both species exhibited high inter-individual variability and no consistent pattern of dominance was observed between the two hosts. Metazoan parasites of the phyla Acanthocephala and Platyhelminthes were frequently dominant, followed by Nematoda, Cnidaria, and Annelida. Regarding parasitic protists, some individuals harbored mostly protistan parasites, including mainly taxa belonging to Rhyzaria and Opisthokonta, followed by Chlorophyta, Alveolata, and Stramenopiles.

Analysis of the parasitic assemblage in the fish stomach contents revealed similar diversity metrics between *N. coriiceps* and *N. rossii* (Figure S1,S2). No statistical differences were found regarding Shannon, Simpson and richness indexes, although *N. coriiceps* exhibited slightly higher variability, with one individual showing a higher Shannon diversity value. Comparing shared and unique OTUs between the two nototheniids, *N. coriiceps* harbored many more unique OTUs than *N. rossii* (165 vs. 28). The two species had 49 OTUs in common (Figure S1; Table S1), assigned to 17 classes and comprising both metazoan

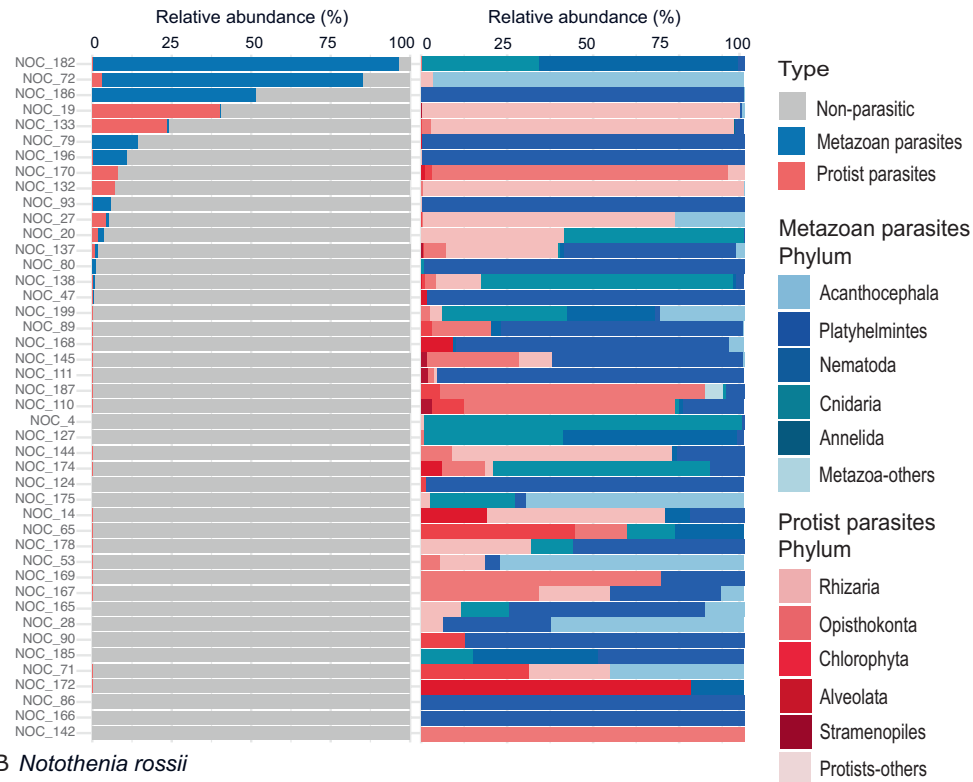
and protist parasites spanning a wide range of hosts (Table S1). Among the shared OTUs, metazoan parasites infecting fish were generally the most abundant, including several Acanthocephala (e.g., *Aspersentis*, *Echinorhyncus*), Platyhelminthes (*Ascarophis*, *Hysterolecithoides*), and the myxozoan *Kudoa*. Among the shared taxa associated with prey-ingested parasites, the most abundant were cercozoans within Endomyxa-Ascetosporea, including species parasitic on molluscan hosts (e.g., *Bonamia* and *Minchinia*; Table S1).

3.3 | Multivariate Analysis: Relationships of the Parasite Assemblage With Environmental and Biological Variables

To explore patterns in parasite assemblage composition across biological and environmental variables, Principal Coordinates Analysis (PCoA) was performed based on Bray–Curtis dissimilarities (Figure S2). The ordination plots showed considerable overlap among most groups, although partial separation was apparent between sampling depth categories, particularly between the shallowest (≤ 10 m) and intermediate depths (11–20 m). The multifactorial PERMANOVA analysis revealed significant effects of both depth and stomach weight categories on parasite assemblage composition (Table S2). The results suggest that sampling depth and stomach weight significantly contributed to the differences observed in the parasite assemblages. However, single-factor PERMANOVA analysis (Table S2) revealed that only sampling depth categories had a significant effect on parasite assemblage composition ($F = 2.20$, $p = 0.0017$, adjusted $p = 0.0085$). In contrast, species, fish total length, and stomach weight showed marginally significant effects ($p < 0.05$), but these effects were not statistically significant after adjusting for multiple comparisons (adjusted $p = 0.135$ for all three factors). Sex had no significant effect (adjusted $p = 0.466$), indicating no influence on parasite assemblage composition. Additionally, the test for homogeneity of multivariate dispersion showed no significant differences among groups, supporting the PERMANOVA results except for sex, indicating differences in within-group variability (Table S2). Pairwise comparisons among depth categories revealed that shallow (≤ 10 m) and intermediate depths (11–20 m) differed significantly in parasite assemblage composition ($p = 0.0036$, Table S2). Similarity percentage (SIMPER) analysis revealed that these differences were caused primarily by *Minchinia* sp. and *Olpidiopsis* sp., which were particularly notable for their high read abundance in intermediate depths, whereas species like *Hematodinium perezii*, *Acrochaete leptochaete*, *Olpidiopsis pyropiae* and *Bonamia ostreae* were more sparsely represented but still contributed to the dissimilarity between the two depths (Table S3). However, there was no significant difference between shallow and deep ($p = 0.0782$), neither intermediate and deep ($p = 0.0566$).

Analysis of total parasite read abundance in relation to different biological and environmental factors revealed no significant differences for species, sex, sampling depth, or fish length (Figure S3A–C,E, Table S4). However, stomach weight categories showed statistically significant effects on parasite read abundance (Kruskal–Wallis test, chi-squared = 7.35, $df = 2$, $p = 0.03$). To explore these differences further, a post hoc Dunn's test was performed. The results indicated significant differences

A *Notothenia coriiceps*



B *Notothenia rossii*

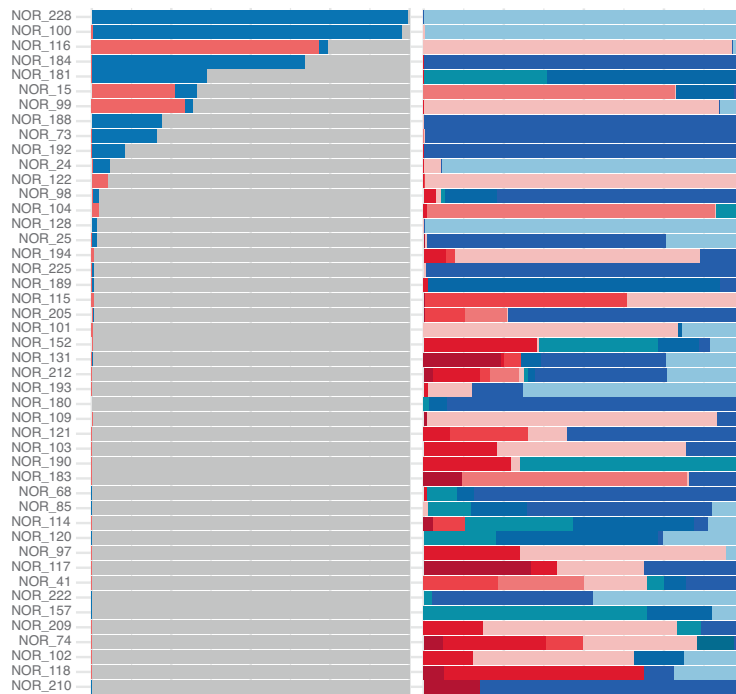


FIGURE 2 | Relative contribution and broad taxonomic composition of parasite-associated reads across individual Antarctic nototheniid fish based on the curated 18S rDNA stomach-content metabarcoding dataset. Panels correspond to *Notothenia coriiceps* (A) and *N. rossii* (B). Left panels show the relative read contribution of non-parasitic taxa (gray), metazoan parasite OTUs (blue), and protist parasite OTUs (red), calculated relative to the total community in each individual fish. Right panels show stacked bar plots representing the composition of parasite-associated reads at phylum-level for metazoan and protist parasites within the parasite-only dataset.

in parasite read abundance between low stomach weight (≤ 10 g) and both intermediate (10.1–25 g) and high (> 25 g) weight categories ($p = 0.0096$ and $p = 0.0059$, respectively), where lowest stomach weight showed higher parasite read abundance

(Figure S3D). No significant difference was observed between the intermediate and high categories ($p = 0.3451$). The linear regression analysis for the continuous variable revealed a non-significant relationship between parasite read abundance and

TABLE 1 | Detection prevalence of fish-associated parasite taxa identified in the stomach-content 18S rDNA metabarcoding dataset of *Notothenia coriiceps* (NOC) and *N. rossii* (NOR).

Phylum	Class	Order	Species	Prevalence (%)	
				NOC	NOR
Acanthocephala	Paleacanthocephala	Echinorhynchida	<i>Echinorhynchus</i> sp.	38.6 (17)	61 (28)
			<i>Aspersentis</i> sp.	18.2 (8)	34.8 (16)
		Polymorphida	<i>Corynosoma</i> sp.	11.4 (5)	2 (1)
Annelida	Clitellata	Acanthobdellida	<i>Acanthobdella peledina</i>	2 (1)	N.D
		Euhirudinea	<i>Notobdella nototheniae</i>	N.D.	2 (1)
Platyhelminthes	Trematoda	Plagiorchiida	<i>Hysterolecithoides epinepheli</i>	79.5 (35)	76 (35)
			<i>Ectenurus virgula</i>	6.8 (3)	17.4 (8)
			Plagiorchiida_XXX_sp.	11.4 (5)	13 (6)
			<i>Azygia</i> sp.	N.D	2 (1)
	Cestoda	Diphylobothridea	<i>Diphylobothrium latum</i>	N.D	2 (1)
Nematoda	Chromadorea	Rhabditida	<i>Ascarophis</i> sp.	37.3 (12)	30.5 (14)
Cnidaria	Myxozoa	Multivalvulida	<i>Kudoa</i> sp.	43.2 (19)	50 (23)
Ichthyosporea	Ichthyosporea_X	Dermocystida	<i>Dermocystidium</i> sp.	2 (1)	N.D
		Ichthyophonida	<i>Ichthyophonus</i> sp.	9.1 (4)	15.2 (7)
Microsporidia	Microsporidia_X	Microsporida	<i>Desmozoon lepeophtherii</i>	4.5 (2)	N.D
			<i>Enterospora nucleophile</i>	N.D	4.3 (2)
Apicomplexa	Coccidiomorpha	Eimeriida	<i>Eimeria anguillae</i>	4.5 (2)	N.D.
			<i>Eimeria nemethi</i>	4.5 (2)	2 (1)
Perkinsozoa	Perkinsea	Perkinsida	<i>Gadixcellia</i> sp.	2 (1)	N.D.

Note: Fish-associated parasites were defined based on literature-derived host annotations as parasite taxa reported to infect fish hosts (Files S1 and S2). Prevalence was calculated separately for each fish species as the percentage of individuals in which each parasite taxon was detected, relative to the total number of individuals analyzed (*N. coriiceps*: $N=44$; *N. rossii*: $N=46$). The number of positive individuals is shown in parentheses. N.D. indicates that the taxon was not detected. Taxonomic assignments are shown at phylum, class, order, and lowest available taxonomic level. Detection in stomach contents should be interpreted as metabarcoding evidence of parasite-associated DNA and not as confirmation of active infection.

fish length (Figure S3F) ($R^2=0.04$, t -statistic = -1.89 , $p=0.092$), although the line indicates a negative correlation, with larger fish having fewer parasite reads.

3.4 | Detection, Prevalence and Relative Read Abundance of Fish-Associated Parasites

A total of 19 distinct fish-associated parasite taxa, 12 metazoans and 7 protists, were detected in the stomach contents of nototheniid fish (Table 1). The most prevalent parasite in both hosts was the trematode *Hysterolecithoides epinepheli*, detected in 79.5% of *N. coriiceps* and 76.0% of *N. rossii* individuals. Other trematodes were detected at lower prevalences (6.8%–17.4%). Acanthocephalans showed contrasting host patterns, with *Echinorhynchus* sp. and *Aspersentis* sp. being more frequent in *N. rossii*, whereas *Corynosoma* sp. appeared more frequently in *N. coriiceps*. Several parasite taxa were detected exclusively in one host species, although always at low prevalences (2%–4.5%). The myxozoan *Kudoa* sp. and the nematode *Ascarophis* sp. were highly prevalent in both species (30%–50.0%), whereas protistan parasites, such as *Ichthyophonus* sp. and *Dermocystidium* sp.

occurred at lower frequencies (2.0%–15.2%). Overall, while both fish species hosted a broad and overlapping spectrum of parasites, *N. coriiceps* exhibited higher prevalence for a greater number of parasite taxa.

Regarding the relative read abundance of detected fish-associated parasites, metazoans clearly dominated the parasitic assemblages in both *Notothenia* species, while protistan parasites showed comparatively lower relative read abundance and occurred in fewer individuals (Figure 3). The metazoan *Hysterolecithoides epinepheli* (Platyhelminthes) showed particularly high relative read abundance in numerous individuals of both species, frequently representing the majority of parasite-associated reads. *Kudoa* sp. (Myxozoa) and *Ascarophis* sp. (Nematoda) were consistently detected in both fish species and exhibited moderate to high relative read abundances in a subset of individuals, particularly in *N. coriiceps*, where some individuals exhibited notable peaks. Within the Acanthocephala phylum, *Echinorhynchus* sp. and *Aspersentis* sp. showed higher relative read abundances in *N. rossii*, while in *N. coriiceps* their presence was more limited, with lower relative read abundances overall. In contrast, protistan groups,

such as Ichthyosporidia, Microsporidia, Apicomplexa and Perkinsida were detected at low read abundance and in a limited number of fish hosts, reflecting their lower contribution to the overall parasitic assemblage.

The heatmap representing the relative read abundance of potential parasites of fish prey revealed a predominance of protist taxa over metazoans in both *N. coriiceps* and *N. rossii* (Figure 4). Only 5 metazoan parasite species were detected and they were occasionally present, with most individuals showing low to moderate read abundances. In contrast, protists, especially those belonging to the phyla Cercozoa and Dinoflagellata, were consistently more represented in terms of read abundance across multiple individuals. At species level, the cercozoans *Minchinia* and *Bonamia* showed particularly high relative read abundances in many individuals of both fish species. Other protists, such as *Hematodinium*, *Olpidiopsis*, *Labirynthula*, and the apicomplexan Lecudinidae_X showed higher read abundances in multiple samples, albeit more variable across individuals. When comparing the two fish species, *N. coriiceps* exhibited a higher diversity and relative read abundance of prey-associated parasites than *N. rossii* (Figure 4), with one individual showing a broader signal across multiple protistan taxa, indicating a more complex and diverse parasite assemblage.

parasites of mollusks showed the highest read representation in both fish species, accounting for approximately 45% of the mean relative read abundance, followed by those associated with crustacean hosts (12%–5%) (Figure 5, Table S5). Parasites potentially associated with macroalgae also displayed substantial read representation, ranging from 8% to 11% of mean relative read abundance, contributing to a broad and diverse parasite profile in around 30% of individuals of both fish species. Similarly, putative polychaete-associated parasites were detected with moderate relative read abundance in both fish species, albeit with slightly higher occurrence in *N. coriiceps*. In contrast, parasites associated with protist hosts potentially ingested through the diet, such as dinoflagellates and diatoms, appeared at lower read abundance and were more sporadically distributed among individuals of both species (Figure 5, Table S5). Parasites associated with the rest of host groups were detected at much lower relative read abundances and in fewer fish, with limited contribution to the prey-associated parasite assemblage.

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FIGURE 4 | Heatmap of putative prey-associated parasite taxa detected in individual stomach-content samples of *Notothenia coriiceps* and *N. rossii*. Prey-associated parasites were defined from literature-derived host annotations as parasite taxa reported to infect organisms occurring in the nototheniid diet (Files S1 and S2). Rows represent parasite taxa aggregated at the species level, and columns represent individual fish samples grouped by host species. Values correspond to $\log_{10}$ -transformed relative read abundance calculated relative to the total curated community in each sample. Row annotations indicate parasite type (Metazoa or Protist) and phylum, while column annotations indicate fish host species.

whereas *Haplosporidium* sp. was related to *Mytilus* sp. and to another bivalve from the order Pectinida. Another protist parasite associated with these two bivalve mollusks was *Aplanochytrium* sp. (Sagenista). Among the metazoan parasites associated with fish prey, the nematode *Punctodora ratzeburgensis* and the cnidarian *Monobrachium parasitum* were both correlated with the Antarctic bivalve *Cyamiomacra laminiifera*. However, *M. parasitum* showed a broader

bivalve host range and exhibited a stronger association with *Gaimardia trapesina*.

Regarding crustacean-associated parasites, positive correlations were detected between several parasite classes and different candidate crustacean hosts (Figure 6B). Within the Alveolata, Syndiniales of the genus *Hematodinium* showed strong associations with several Malacostraca and Ostracoda

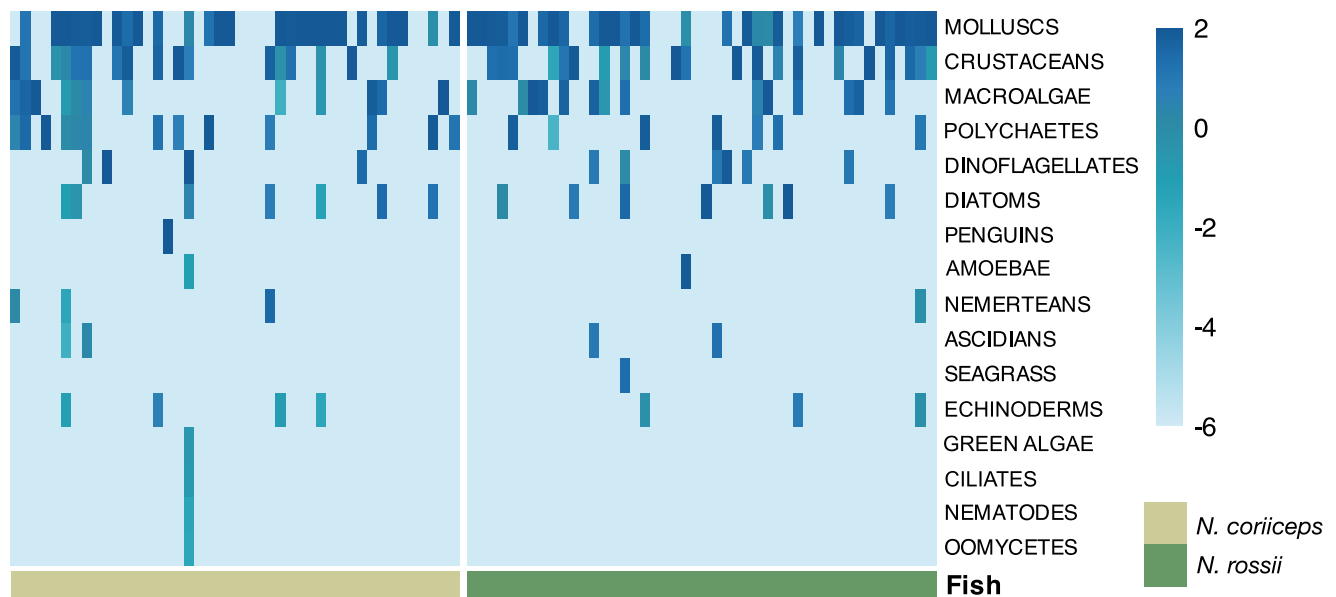


FIGURE 5 | Heatmap of host categories associated with putative prey-associated parasite OTUs detected in the stomach contents of *Notothenia coriiceps* and *N. rossii*. Prey-associated parasite OTUs were grouped according to their literature-derived host annotations, and host categories represent the organisms reported as potential hosts of those parasites (Files S1 and S2). Values correspond to $\log_{10}$ -transformed relative read abundance of parasite reads assigned to each host category, calculated relative to the total curated community in each individual fish sample. Rows correspond to host categories, and columns correspond to individual fish samples grouped by fish species.

taxa, whereas the ciliate family Lynchellidae was more strongly correlated with Malacostraca, showing a potential preference for that host group. However, Oligohymenophorea ciliates exhibited a broad range of associations with several Malacostraca and copepods. Another parasitic group also associated only with Malacostraca was those belonging to the Paradinidae family (Endomyxa-Acetosporea). Finally, a single OTU classified as *Microsporidium* sp. was correlated to the isopod *Ianthopsis nasicornis*.

4 | Discussion

A high diversity of potential parasites was detected in Antarctic fish, with both metazoan and protistan taxa recovered from the stomach contents of two nototheniid species. While metazoan parasites dominated in terms of read abundance, likely reflecting their larger body size and higher DNA yield, a known bias of metabarcoding approaches (Govindarajan et al. 2023), protistan parasites accounted for most of the OTU richness and diversity. This parasitic diversity included taxa potentially infecting nototheniid fish as well as putative parasites associated with their diet (prey), thereby providing a broader view of the local parasitic community than conventional techniques such as histology (Muñoz and Rebolledo 2019; Palm et al. 1998). The comprehensive detection of parasite diversity in fish gut contents enabled by metabarcoding represents a valuable methodological advancement (Berry et al. 2015; Miller et al. 2024; Rijal et al. 2024; Scheifler et al. 2019) and has also been successfully applied to fish skin and gills (Scheifler et al. 2022). These results highlight a substantial diversity of protistan taxa, expanding current knowledge of Antarctic marine biodiversity and revealing an overlooked component of Antarctic parasitic assemblages.

Although metabarcoding is a well-established tool for describing the diversity of eDNA communities, its quantitative interpretation remains controversial (e.g., Ershova et al. 2021; Martin et al. 2022). A meta-analysis of 22 metabarcoding studies that targeted a wide variety of organisms and genetic markers found a significant but weak quantitative relationship between organism biomass and sequence read number (Lamb et al. 2019). Read counts may be influenced by multiple taxon-specific and methodological factors, including organism biomass, DNA yield, marker copy number, primer affinity, amplification efficiency, and DNA extraction success. In parasite metabarcoding, these biases may be further amplified by differences in life-cycle stages present in the gastrointestinal tract, including eggs, larvae, cysts, or resistant stages, which may differ in DNA extraction efficiency and generate differences in read signal independently of the number of parasite individuals. Accordingly, metabarcoding results must be taken as a semi-quantitative assessment of the importance of the different taxa. Relative and total read abundance reported here should be interpreted as semi-quantitative indicators of parasite signal rather than as direct measures of organism abundance, biomass, infection intensity, or parasite load. Likewise, correlations between putative prey-associated parasites and candidate hosts should be interpreted as potential sequence-based associations rather than direct evidence of infection or ecological interaction.

4.1 | Structure of Fish Parasite Assemblages and Ecological Drivers

Fish parasite assemblages and parasite read abundance showed high inter-individual variability, consistent with generalist feeding strategies of nototheniids (Moreira et al. 2021, 2023; Ruiz et al. 2024) and thus, the resulting heterogeneity in exposure to

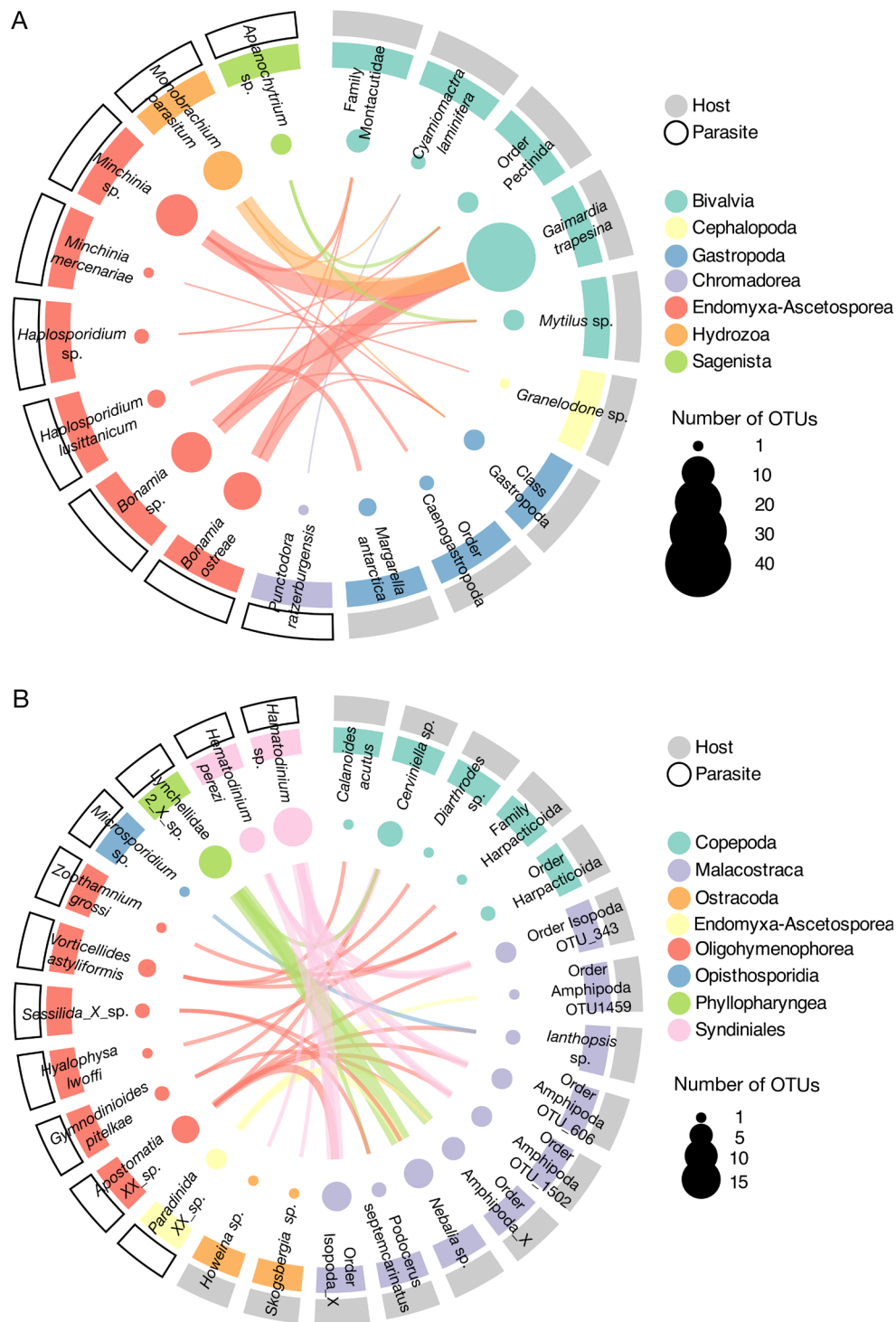


FIGURE 6 | Positive read-abundance co-occurrence associations between putative prey-associated parasite taxa and candidate host/prey OTUs detected in the stomach-content metabarcoding dataset of *Notothenia coriiceps* and *N. rossii*. Chord diagrams show significant pairwise Spearman correlations (File S3) between parasite OTUs and non-parasitic OTUs assigned to molluscan candidate hosts/prey (A) and crustacean candidate hosts/prey (B). Parasite OTUs were selected based on literature-derived host annotations indicating mollusks or crustaceans as potential hosts (Files S1 and S2). Gray sectors indicate candidate host/prey taxa, and white sectors indicate parasite taxa; colors denote broad taxonomic affiliation. Chords represent significant positive correlations across individual fish samples ($p < 0.05$; $p > 0.5$), with chord width proportional to the number of significant OTU-level correlations supporting each association. Circle size reflects the number of OTUs associated with each taxon. Associations should be interpreted as potential prey–parasite links, not definitive evidence of infection source or ecological interaction.

trophically-transmitted and prey-associated parasites (Lester and McVinish 2016; Poulin and Leung 2011). These individual differences may also reflect variation in host traits and other

intrinsic factors affecting susceptibility, such as body size, physiological condition, degree of exposure, or recent feeding history (Iyaji et al. 2010). In Antarctic nototheniid fish, parasite

abundance and richness have been shown to increase with host size, particularly in *N. rossii* (Muñoz and Rebolledo 2019), illustrating how intrinsic characteristics can mediate individual parasite loads. Opposite to the literature, we did not find a relationship between body size (total length) and parasite read abundance. However, our analysis was restricted to stomach contents, which may account for this discrepancy. Notably, we observed a higher parasite read signal in smaller stomachs, which may reflect a dilution effect associated with metabarcoding, whereby larger stomachs contain greater biomass of prey and host tissue, reducing parasite detectability (Rijal et al. 2024).

Regarding the parasite assemblage composition, we did not detect major differences in relation to most environmental or biological variables examined (fish species, total length, stomach weight, and sex). The small differences observed may be explained by the strong ecological similarity between *N. coriiceps* and *N. rossii* (Muñoz and Rebolledo 2019). Despite some differences in spatial distribution and adult foraging behavior, they share several key life-history traits (Cali et al. 2017) and exhibit a substantial diet overlap throughout their ontogeny (Barrera-Oro 2002; Moreira et al. 2021; Ruiz et al. 2024). Antarctic parasites in *N. rossii* and *N. coriiceps* have been described as more generalists than subantarctic parasites, allowing the coexistence of multiple parasite species in the same host (Muñoz and Cartes 2020). These results align with those of Muñoz and Rebolledo (2019), which also found that *N. rossii* and *N. coriiceps* shared most of the parasite taxa.

Depth appeared to be the only structuring factor for parasite assemblages, with distinct communities characterizing shallow and intermediate depths. The taxa driving these differences were protist parasites, including representatives of the genera *Minchinia*, *Bonamia*, *Olpidiospsis*, *Acrochaete*, and *Hematodium*, which were more abundant and frequent at intermediate depths (11–20 m). These parasites are known to infect mollusks (Bearham et al. 2008; Burrenson and Ford 2004; Ford et al. 2009), macroalgae (Gunnarsson and Nielsen 2016; Huber 1892; Klochkova et al. 2012; Sekimoto et al. 2008), and crustaceans (Li et al. 2021). This depth pattern could be explained by the distribution of their hosts at the sampling location, where mollusks, macroalgae, and crustaceans are particularly abundant between 10 and 20 m depth (Klöser et al. 1996; Lagger et al. 2018). There may be a distinction between parasites that are intrinsic to the fish host and those associated with ingested prey. Fish parasites are unlikely to vary across the tested biological variables, given the similar ecology of the two nototheniid species (Muñoz and Rebolledo 2019). In contrast, prey-associated parasites may vary with the recent feeding history (Lester and McVinish 2016; Muñoz and Cartes 2020). Because stomach contents provide a snapshot of recent feeding, prey-associated parasite assemblages may differ among individuals according to recent prey consumption and foraging depth shortly before sampling.

4.2 | New Insights Into Potential Parasites of Antarctic Nototheniid Fish Detected by Metabarcoding

A total of 19 parasite taxa potentially infecting fish, including metazoans and protists, were identified in the stomach contents

of *N. rossii* and *N. coriiceps*. Parasite assemblages detected by metabarcoding were dominated by metazoan helminths, particularly trematodes, acanthocephalans, and nematodes, consistent with traditional parasitological surveys of Antarctic nototheniids and other benthic fish (Kuzmina et al. 2020, 2022; Laskowski et al. 2012, 2014; Muñoz and Rebolledo 2019; Palm et al. 1998; Zdzitowiecki and Laskowski 2004). This indicates that these parasite groups constitute the core helminth fauna of Antarctic fish and supports an overall consistency between metabarcoding and conventional approaches. Still, differences were observed at lower taxonomic resolution, with only some coincidences at the genus/species level, including *Aspersentis* and *Corynosoma* previously reported from fish stomachs (Amin et al. 2021; Muñoz and Rebolledo 2019; Palm et al. 1998). Oğuz et al. (2015) reported a higher diversity of helminth parasites in *N. coriiceps* and *N. rossii*. However, direct comparisons with our results are difficult because that review included parasites from multiple tissues (e.g., skin, stomach, liver, intestine, lumen and muscle) without distinction, whereas our analysis was restricted to stomach contents. Noteworthy, our metabarcoding approach detected several taxa not previously reported in nototheniids, including *Acanthobdella peledina*, *Hysterolethodes epinepheli*, *Ectenurus virgula*, and *Azygia* sp. In addition, we found that the myxozoan *Kudoa* sp. was highly prevalent in both fish species. Although myxozoans have been previously reported in Antarctic nototheniids (Brickle et al. 2001; Kovaleva et al. 2002), their high read abundance and frequency observed here suggests that they represent an important, yet often underestimated component of Antarctic fish parasite assemblages.

Among metazoans, several parasites showed high read abundance and frequency in both nototheniid species, including trematodes (*Hysterolethodes*), acanthocephalans (*Aspersentis* and *Echinohynchus*), and nematodes (*Ascarophis*). These helminths have indirect life cycles that depend on trophic transmission through infected intermediate hosts (prey) (e.g., mollusks and crustaceans) to complete their development (Benesh et al. 2017; Rocka 2006; Sures et al. 2025). Sharing dominant trophically transmitted parasites between these two congeneric and sympatric nototheniids was also reported by Muñoz and Rebolledo (2019), who concluded that this pattern reflects their overlapping diets and shared ecological niches. Despite this overall similarity, we observed differences between the two host species in the less abundant helminth taxa, such as the higher prevalence of *Corynosoma* sp. in *N. coriiceps*, and the occurrence of annelids and some plathyelminths restricted to one host species. Such variation is expected for less abundant trophically transmitted parasites (Lester and McVinish 2016; Marcogliese 2002; Poulin and Leung 2011), and likely reflects differences in parasite life cycles, local availability of intermediate hosts, subtle variations in microhabitat use, depth distribution, or recent feeding history (Barrera-Oro 2002; Barrera-Oro et al. 2019; Eastman et al. 2011; Moreira et al. 2021, 2023; Sures et al. 2025). However, because these patterns are based on metabarcoding data, differences in read representation may also reflect methodological biases, including primer mismatches or poor amplification efficiency for particular taxa (Lamb et al. 2019).

Protistan fish parasites were detected at much lower prevalence and read abundance than metazoans, but spanned

several major parasitic lineages, including ichthyosporans (Dermocystida, Ichthyophonida), microsporidians, apicomplexans, and a perkinsid. Although these groups are well-recognized fish parasites in marine systems (Freeman et al. 2017; Lom and Nilsen 2003; Mendoza et al. 2002; Molnár 2006), reports from Antarctic fish remain scarce (Davison 1997; Desvignes et al. 2022; Evans and Tupmongsol 2014; Polyakova and Gordeev 2021). Indeed, among 374 Antarctic and Sub Antarctic fish species, protistan parasites were reported only from six hosts, representing less than 1% of the fish fauna (Polyakova and Gordeev 2021). Previous records mainly include apicomplexan species of the families Eimeriida and Haemogregarinida and microsporidians of the family Glugeidae (Barber et al. 1987; Polyakova and Gordeev 2021; Rodyuk 1988). More recently, several perkinsid X-cell lineages have been described from Antarctic fish, including the genera *Xcellia* (Evans and Tupmongsol 2014), *Notoxcellia* (Desvignes et al. 2022, 2025), and *Cryoxcellia* (Evans et al. 2023). Notably, no ichthyosporans have been formally reported to date from Antarctic fish hosts (Gozlan et al. 2014; McVicar 2011; Polyakova and Gordeev 2021). The detection here of *Gadixcellia* (Perkinsid), together with *Ichthyophonus* and *Dermocystidium* (Ichthyosporae), expands the known diversity and biogeographic range of these parasites in Antarctic fish. Importantly, these taxa cause important fish diseases worldwide in a wide range of wild and farmed species (Freeman et al. 2017; Gozlan et al. 2014; McVicar 2011). Their detection in the Antarctic food web raises important questions regarding their potential impact on fish populations.

4.3 | Putative Prey-Associated Parasites: Nototheniids as Natural Samplers of Local Parasite Diversity

The present metabarcoding study of fish gut contents expands the range of parasite taxa detectable beyond those directly associated with fish hosts. We detected a wide diversity of putative prey-associated and environmentally linked parasite taxa spanning 17 major eukaryotic lineages, capturing a wider component of local Antarctic parasite diversity. These results highlight the value of broad-diet fish as effective biological samplers of parasite signals circulating through local food webs.

The putative prey-associated parasite assemblage was dominated by protistan taxa, while only a small number of metazoan parasites were detected and generally at low relative read abundance. This predominance of protists likely reflects both their high diversity and the ecology of these groups, which include free-living and environmentally dispersed life-cycle stages, as well as parasites of planktonic and benthic organisms that may be ingested through trophic interactions (Rückert 2025; Skovgaard 2014). In contrast, metazoan parasites with complex life-cycles and tissue-specific infections may be less likely to be consistently captured through gut content metabarcoding (Creer et al. 2016; Deagle et al. 2019; Pompanon et al. 2012).

The most frequently detected prey-associated parasites and those with the highest read representation were linked to crustaceans, bivalves, and macroalgae. This pattern is consistent with prey composition inferred from dietary analyses based on COI

metabarcoding by Ruiz et al. (2024), which indicated that 52.7% of reads were assigned to Arthropoda, 25.5% to Rhodophyta, and 12.8% to Mollusca. The higher occurrence of parasites associated with these taxa is therefore most likely a consequence of their prevalence in the diet of *N. rossii* and *N. corriceps*. This dietary composition is also consistent with the benthic assemblage described for Potter Cove (e.g., Sahade et al. 2015) and the ongoing benthic community surveys at the sampling site, where macroalgae dominate in terms of areal coverage, while bivalves and gastropods are among the most abundant invertebrates in terms of density (Barrera-Oro et al. 2019; L. Ferrero, pers. comm.). Together, these patterns support the idea that prey availability strongly structures the prey-associated parasite signal detected in fish stomach contents, integrating information from multiple trophic compartments.

Among protists, Syndiniales represented the most diverse group detected. Members of this group are widespread in the ocean, often dominating 18S ribosomal DNA surveys, including marine Antarctic systems (Clarke et al. 2019; Cleary and Durbin 2016). Syndiniales have been reported to infect a wide range of planktonic and benthic organisms, including dinoflagellates, cercozoans, radiolarians, copepods, fish eggs, and many crustaceans (Guillou et al. 2008). Planktonic organisms serve as a diet to filter-feeders, such as mollusks, which were reported as one of the main prey groups for the studied nototheniid fish together with crustaceans (Ruiz et al. 2024). Thus, the high diversity of Syndiniales detected in the fish stomach contents likely reflects indirect ingestion through prey consumption. Similarly, the broad representation of cercozoans, encompassing parasites of mollusks (Arzul and Carnegie 2015), Labyrinthulomycetes, and *Olpidiopsis* infecting macroalgae (Badis et al. 2020; Raghukumar 2017), and other protistan lineages, suggests that nototheniids integrate parasite signals from multiple compartments of the marine trophic network.

4.4 | Detection of Ecologically Relevant Crustacean and Mollusk Parasite Signals: Implications for Antarctic Benthic Communities

Within the protistan diversity detected in this study, several parasite taxa globally recognized as important pathogens of crustacean and mollusk hosts were consistently frequent across fish individuals. Several of these taxa showed high read representation among prey-associated parasites and displayed positive significant read-abundance correlations with specific Antarctic invertebrates, suggesting their distribution within the Antarctic benthic food web.

Within Syndiniales, *Hematodinium* spp. were strongly associated with multiple malacostraca and ostracod taxa. *Hematodinium* species have been reported infecting over 40 species of marine crabs, shrimps, lobsters, and amphipods worldwide (Stentiford and Shields 2005), causing epidemics in farmed and wild crustacean populations and leading to important losses in local economies (Small 2012). Although marine molecular surveys already detected this genus in Antarctica (Cleary and Durbin 2016), confirmed host records are scarce. The correlations observed here provide insights into potential interactions between *Hematodinium* parasites and Antarctic crustaceans,

highlighting the need to evaluate their host range, distribution, and ecological impact in the Antarctic marine system.

We revealed the molecular presence of haplosporidian parasites belonging to the genera *Bonamia*, *Minchinia*, and *Haplosporidium*, which, to our knowledge, have not been formally reported from Antarctic marine ecosystems (Arzul and Carnegie 2015; Burrenson and Ford 2004; Engelsma et al. 2014), making their detection here particularly noteworthy. In our dataset, haplosporidians were among the most represented prey-associated parasites in terms of read abundance, and showed consistent positive correlations with several molluscan taxa, including the bivalve *Gaimardia trapesina*, gastropods such as *Margarella antarctica*, and mussels related to *Mytilus* spp. These associations are ecologically coherent, as they are well-known parasites infecting a wide range of bivalve hosts globally, where they are responsible for severe diseases and large-scale mortality events in both wild and cultured populations (Carnegie et al. 2016). Their detection in Antarctic food webs has important ecological and biogeographic implications. The high read representation of these taxa in the fish stomach contents, together with their significant correlations with specific prey, suggests that haplosporidians may be present within local benthic communities and being incorporated into fish diets through consumption of infected prey.

The detection of haplosporidian signals is particularly relevant in the context of biological invasions and climate-driven range expansions in the Antarctic Peninsula (Chown et al. 2012). Haplosporidians are globally distributed in temperate regions and have been associated with the movement of bivalves through global trade, ballast water, and rafting processes (Costello et al. 2021; Hill-Spanik et al. 2015). Recent studies have documented a growing number of invasive species in Antarctica (Hughes et al. 2020), including the first settlement of *Mytilus* spp. on Isla 25 de Mayo/King George Island (Cárdenas et al. 2020), highlighting the growing potential for non-native bivalves to establish in Antarctic systems. Moreover, kelp-rafting has been demonstrated to transport bivalves such as *Gaimardia trapesina* over long distances in the Southern Ocean (Helmuth et al. 1994; Puccinelli et al. 2018). These studies demonstrate plausible mechanisms for the arrival of mollusks and their associated parasites into Antarctic coastal ecosystems (Hughes et al. 2020).

Although parasite DNA in stomach contents does not demonstrate active infection, their high relative read abundance and the potential host associations observed here raise important questions regarding their host range, disease dynamics, and ecosystem-level consequences. Given the severe impacts these parasites exert in temperate marine systems, their apparent incorporation into Antarctic food webs warrants targeted investigation using histology, in situ diagnostics, and host-focused molecular surveys.

5 | Conclusions

This study adds to emerging metabarcoding-based surveys of parasite diversity in Antarctic fish, showing that nototheniids harbor parasite assemblages broadly consistent with classical

parasitological studies while also revealing a hidden component of Antarctic parasite diversity. By analyzing stomach contents, we show that broad-diet fish can act as natural samplers of local diversity, integrating potential parasites of fish, putative prey-associated parasites, and environmentally derived taxa. Metabarcoding notably expanded the detectable parasite diversity, particularly among protistan lineages historically overlooked in Antarctic fish. In addition, read-abundance correlation analysis provided potential associations between detected parasites and candidate local hosts. The detection of molecular signals from taxa of known pathogenic relevance highlights the value of trophic metabarcoding to help improve our understanding of parasite biogeography and to support future surveillance of emerging disease risks and biological invasions in Antarctic marine ecosystems.

Author Contributions

Conceptualization: M.B.R., E.A. (lead), and C.H.; methodology: M.B.R., B.F., E.M., M.N., C.H., J.Z., and E.A.; investigation: B.F., E.M., M.N., and C.H.; formal analysis and visualization: M.B.R., J.Z., and E.A.; writing – original draft: M.B.R. and E.A. (lead); writing – review and editing: M.B.R., B.F., E.M., M.N., C.H., J.Z., and E.A.

Acknowledgments

We thank the reviewers for their work, which has helped us improve our work.

Funding

This work was partially funded by the European Union under the Horizon Europe Marie Skłodowska-Curie Actions grant agreement No. 101110770 awarded to E.A. Logistical and financial support for sample collection and processing was provided by the Dirección Nacional del Antártico, Instituto Antártico Argentino. M.B.R. was supported by the Deutsche Forschungsgemeinschaft (DFG) in the framework of the priority program SPP 1158 “Antarctic Research with comparative investigations in Arctic ice areas” by the following grant HA 7627/3-1, LE 2323/11-1. C.H. was supported by the Helmholtz Young Investigator Group “ARJEL – Arctic Jellies” with the project number VH-NG-1400, funded by the Helmholtz Society and the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequences were submitted to the Sequence Read Archive (SRA) (<https://www.ncbi.nlm.nih.gov/sra>) under accession numbers BioProject ID PRJNA1047314 and BioSamples ID SAMN38529455–SAMN38529651 (Ruiz et al. 2024).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **File S1:** Annotation of potential parasite OTUs and their known host groups, including taxonomic classification, NCBI BLAST information, and supporting references. **File S2:** Curated OTU table including read counts per sample, taxonomic assignment of each OTU, parasite/non-parasite classification, parasite type (metazoan or protist), and known host group. Individual sample IDs are coded with the prefixes NOC for *Notothenia coriiceps* and NOR for *Notothenia rossii*. **File S3:** Potential sequence-based associations between putative mollusk and crustacean parasites and candidate mollusk and crustacean hosts detected in fish stomach contents, including parasite and host taxonomic assignments, correlation coefficients and *p*-values. **Figure S1:** Alpha diversity metrics and shared OTUs of parasite assemblages in the two fish species. Violin plots show the distribution of Shannon diversity, Simpson index, and rarefied richness based on parasite OTUs detected in individual stomach-content samples of *Notothenia rossii* (NOR) and *Notothenia coriiceps* (NOC). Box plots indicate medians and interquartile ranges, and jitter points represent individual samples. Differences between fish species were tested using Wilcoxon rank-sum tests, with corresponding *p*-values and effect sizes (*r*) shown in each panel. The Venn diagram depicts the number of shared and unique parasite OTUs detected in each fish species. **Figure S2:** Principal Coordinates Analysis (PCoA) plots based on Bray–Curtis dissimilarities showing parasite assemblage composition across categories of five explanatory variables: fish species (*Notothenia coriiceps* (NOC) and *N. rossii* (NOR)), sex (female and male), total length (small: 21.0–29.9 cm; medium: 30.0–38.0 cm; large: ≥ 38.1 cm), stomach weight (≤ 10 g; 10.1–25.0 g; ≥ 25.1 g), and depth (≤ 10 m; 11–20 m; ≥ 20.1 m). Each point represents an individual fish, and ellipses represent the 95% confidence interval for each group. Axes represent the first two dimensions of variation (PCoA1 and PCoA2), which together explain 30.3% of the total variation in parasite assemblage composition. Permanova results are reported in Table S2. **Figure S3:** Violin plots with overlaid box plots and jitter dots (A–E) showing total parasite read abundance (fourth-root

transformed) across categorical factors. Global p -values from Wilcoxon rank-sum tests (two levels) or Kruskal–Wallis tests ($\geq$ three levels) are shown in each panel. (F) Linear regression model of total parasite read abundance (fourth-root transformed) against fish total length (mm) shows the fitted regression line (black), regression equation, R^2 , and model p -value. **Table S1:** Shared parasite OTUs between *Notothenia coriiceps* (NOC) and *Notothenia rossii* (NOR), including parasite division, class and species-level assignment, the potential host, total read counts and relative read abundances for each fish species. **Table S2:** Statistical results of multifactorial and single-factor PERMANOVA analyses testing differences of parasite assemblage composition based on Bray–Curties dissimilarities calculated from fourth-root transformed relative read abundances, including pairwise comparisons. Results include degrees of freedom (df), R^2 values, F -statistics, unadjusted p -values, adjusted p -values (Holm correction), number of levels in each factor, and sum of squares (Sum of Sqs) and dispersion statistics (PERMDISP) results where applicable. **Table S3:** Summary of SIMPER analysis identifying parasite OTUs contributing to differences in parasite assemblage composition between shallow (≤ 10 m) and intermediate (11–20 m) depths. For each OTU, the average relative read abundance (average), the contribution of each OTU to the dissimilarity between depths (ratio), the cumulative contribution to the dissimilarity (cumsum), and the statistical significance (p -value) are shown. Mean read abundance, frequency of occurrence (%), and total number of samples (n) at each depth category are also provided for each depth category. **Table S4:** Non-parametric statistical analyses of fourth-root transformed parasite read abundance across categorical factors. The upper section reports Wilcoxon rank-sum or Kruskal–Wallis test statistics, including the test statistic (W or χ^2) and p -values. The lower section summarizes the post hoc Dunn's test results for stomach weight categories, including Z values, p -values, and adjusted p -values for all pairwise comparisons. **Table S5:** Mean relative abundance (MRA, %), standard deviation (SD), and frequency (%) of parasite OTUs grouped according to their potential host category in *N. coriiceps* and *N. rossii*. MRA represents the percentage of relative abundance assigned to parasite OTUs within each host category, SD indicates the variability of the relative abundance among samples, and frequency represents the percentage of samples in which parasite OTUs assigned to each host category were detected. N.D indicates not detected.