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NOTE

Feeding ecology and microbiome of the pteropod *Limacina helicina antarctica*

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ABSTRACT: The pteropod (pelagic snail) *Limacina helicina antarctica* is a dominant grazer along the Western Antarctic Peninsula (WAP) and plays an important role in regional food web dynamics and biogeochemical cycling. For the first time, we examined the gut microbiome and feeding ecology of *L. h. antarctica* based on 16S and 18S rRNA gene sequences of gut contents in the WAP during austral summer. Eukaryotic gut contents of *L. h. antarctica* indicate that this species predominantly feeds on diatoms and dinoflagellates, supplementing its diet with ciliates and foraminifera. *Mollicutes* bacteria were a consistent component of the gut microbiome. Determining the gut microbiome and feeding ecology of *L. h. antarctica* aids in identifying the underlying mechanisms controlling pteropod abundance and distribution in a region of rapid environmental change.

KEY WORDS: Zooplankton · Gut microbiome · Southern Ocean · Diatom · Dinoflagellate · *Mollicutes* bacteria · Western Antarctic Peninsula

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1. INTRODUCTION

The abundant pteropod (pelagic snail) *Limacina helicina antarctica*[1] is a dominant grazer among zooplankton, and is an important prey species for other pteropods, fish, and seabirds along the Western Antarctic Peninsula (WAP), a region of rapid climate change (Hunt et al. 2008, Bernard et al. 2012, Mintenbeck & Torres 2017, Henley et al. 2019). However, the feeding ecology of *L. h. antarctica* has not been well characterized, and to our knowledge, its microbiome has not been examined. While *L. h. antarctica* abundance over the past 25 yr has remained stable overall along the WAP, its long-term abundance has increased sub-regionally in the offshore slope region, attributed to a shortened sea ice season in spring/summer that favors longer periods of open water for feeding (Thibodeau et al. 2019). Since pteropod biogeography can be influenced by the availability of food, examining the feeding ecology of *L. h. antarctica* will aid in identifying mechanisms controlling its abundance and distribution in this dynamic region of the Southern Ocean. Furthermore, identification of the gut microbiome contributes to our understanding of marine microbial diversity and host–microbe interactions in marine ecosystems (Troussellier et al. 2017).

Thecosome (shelled) pteropods have typically been considered generalist, suspension-feeding omnivores

[1]*Limacina rangii* (d'Orbigny, 1835), the currently recognized name (Janssen et al. 2019) for the species previously called *Limacina helicina antarctica* Woodward, 1854

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because they use a mucous web to feed (Lalli & Gilmer 1989), with their guts and feces containing large numbers of phytoplankton (Hopkins 1987). The diet of *Limacina* spp. has been previously described via a few qualitative analyses of gut contents, and these analyses suggested that pteropods mainly feed on phytoplankton and small protozoa (Lalli & Gilmer 1989, Hunt et al. 2008). The use of amplicon-based sequencing methods facilitates the determination of the prey field in the water column as well as within the gut immediately prior to capture. Cleary et al. (2018) conducted amplicon sequencing of the 18S rRNA gene for Antarctic krill *Euphausia superba* gut contents and identified diverse prey including phytoplankton, copepods, ciliates, and pteropods. The only prior zooplankton microbiome study in the Southern Ocean analyzed the microbiome of *E. superba* by 16S rRNA gene sequences (Clarke et al. 2019). They found different krill-associated microbes from those in surrounding seawater as well as distinct communities inhabiting the krill molts, digestive tract, and fecal pellets (Clarke et al. 2019). Despite the high abundance and importance of thecosomes, such as *L. h. antarctica*, as grazers in the Southern Ocean, no study has utilized sequencing techniques to examine their feeding ecology or microbiome.

The objectives of this study were to examine *L. h. antarctica* feeding in the WAP by analyzing gut contents and to compare the gut microbiome composition with surrounding seawater communities using high-throughput sequencing of 16S and 18S rRNA genes. Our study provides higher taxonomic resolution of pteropod gut contents compared to prior studies using traditional microscopy and isotopic analysis and represents one of the few studies to examine the microbiome of a gelatinous zooplankton species.

2. MATERIALS AND METHODS

The Palmer Antarctica Long Term Ecological Research (PAL LTER) study region is located west of the Antarctic Peninsula, spanning approximately 700 km from north to south, and 200 km from the peninsula coast to the continental slope offshore (Waters & Smith 1992) (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/a088p019_supp.pdf). Sampling for this study occurred during the PAL LTER 2017 summer research cruise (30 December 2016 to 10 February 2017) aboard the ARSV 'Laurence M. Gould.' Samples of *Limacina helicina antarctica* were collected with a 2 m, square-frame, 700 µm mesh net, towed obliquely to 120 m as described by Thibodeau et al. (2019). Gut contents of *L. h. antarctica* were sampled from 9 stations during this cruise, based on when animals were available. At least 10 individual guts from pteropods collected at each station (Fig. S1) were immediately dissected once onboard to avoid gut evacuation. Guts were then immediately frozen at −80°C. To compare gut contents with prey available in the seawater, corresponding surface seawater samples were collected from 0.5 m at 6 of the 9 pteropod collection sites and drawn from 20 l Niskin bottles attached to a conductivity–temperature–depth/rosette, with 2 to 4 l of seawater filtered onto a 47 mm 0.45 µm Supor filter (Pall), and frozen at −80°C.

The gut microbiome and prey contents of *L. h. antarctica* along the WAP were determined with high-throughput amplicon sequencing of 16S and 18S rRNA genes of prokaryotes and eukaryotes, respectively. Primers 515F-Y and 926R targeting both 16S and 18S rRNA genes were used (Quince et al. 2011, Parada et al. 2016). This primer pair encompasses both the V4 and V5 hypervariable regions of 16S and 18S rRNA genes. The DNA extraction, PCR setup, and sequencing are described in Text S1 in the Supplement. Amplicon sequences from this study have been deposited to the Sequence Read Archive under accession number PRJNA646234 (https://www.ncbi.nlm.nih.gov/sra).

Bioinformatics of the 16S and 18S sequences were conducted using the DADA2 package (Callahan et al. 2016) and visualized with Phyloseq as amplicon sequence variants (ASVs; McMurdie & Holmes 2013). Diversity was estimated with unrarefied sequences of seawater and pooled gut samples and analyzed separately for 16S and 18S using Phyloseq. Additional information on the bioinformatic analysis is provided in Text S1.

3. RESULTS

The principal coordinate analysis plot of *Limacina helicina antarctica* gut and seawater samples from the same locations showed a significant difference between the gut contents and pelagic microbial communities (permutational multivariate ANOVA, F = 5.03, p = 0.001, Fig. 1); therefore, guts were analyzed separately from pelagic microbial communities for further analyses. Diversity of gut communities was significantly lower than that of water communities (ANOVA, F = 9.31, p = 0.01). Replicate 16S samples from guts revealed relatively similar communities (Fig. S2).

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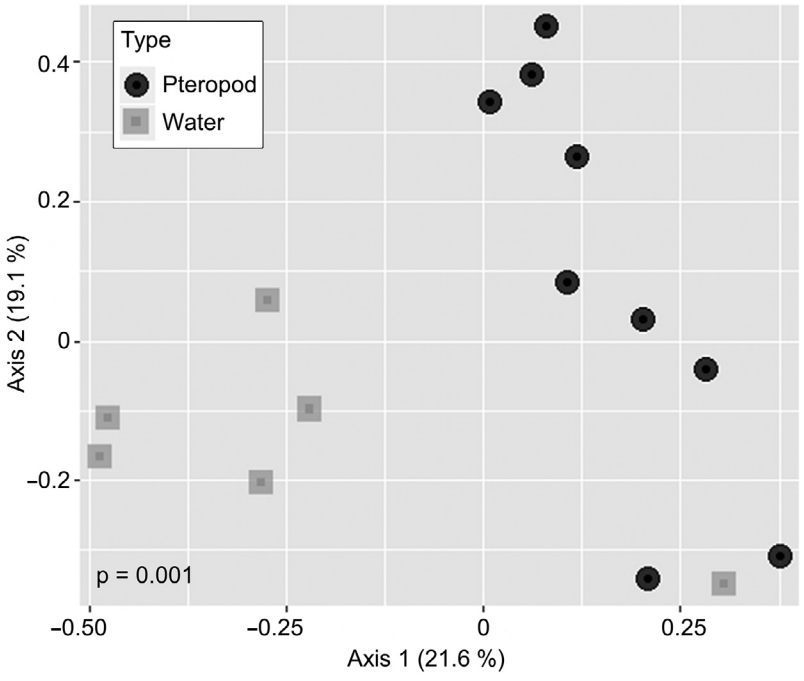

Fig. 1. Principal coordinate analysis representing the beta-diversity of prokaryote and eukaryote communities within seawater (Water) and pooled *Limacina helicina antarctica* guts (Pteropod) based on 16S and 18S amplicon sequence variants

There was adequate coverage of all samples for 16S and 18S sequences as determined by rarefaction curves and the number of observed ASVs (Fig. S3, Table S1). A total of 52 271 and 662 129 trimmed 18S and 16S rRNA gene sequences, respectively, were obtained from 6 seawater samples and 25 gut samples of *L. h. antarctica*. To obtain an adequate number of eukaryotic sequences (18S), samples were combined by station for downstream analyses and yielded sequence averages ± SE of 43 506 ± 15 328 for 16S and 30 063 ± 6294 for 18S (Table S1). When pteropod host 18S sequences were subtracted from those in the gut, a total of 23 699 sequences were available for analysis of 18S (mean 2633 ± 933 per sample); hence gut samples were pooled for subsequent 16S and 18S sequence analyses.

Pooled 16S gut samples of *L. h. antarctica* indicated that *Mollicutes*, *Alphaproteobacteria*, and *Bacteroidia* comprised the largest proportion of the prokaryotic community and were present within all samples (Fig. 2). *Gammaproteobacteria* were also present in all seawater samples but did not contribute to a substantial proportion of the community. Eukaryotic gut contents of *L. h. antarctica* as determined with 18S sequences comprised diatoms (Bacillariophyceae, Coscinodiscophyceae, Mediophyceae), dinoflagellates (Dinophyceae),

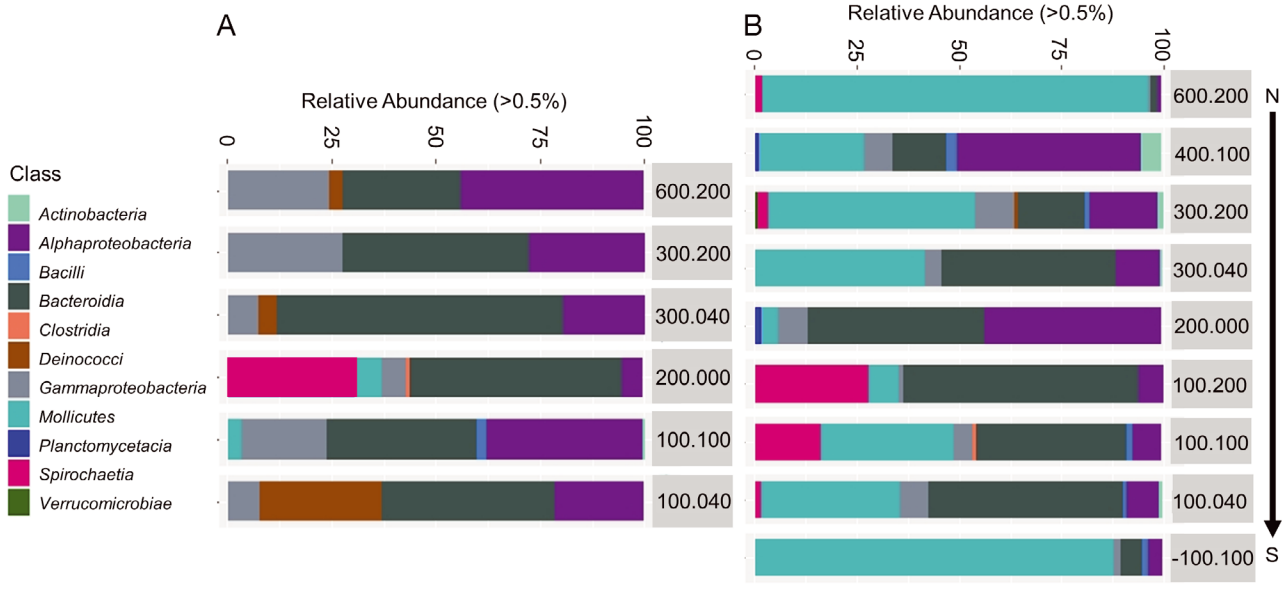

Fig. 2. Prokaryotic community composition at the class level present (A) in surface seawater and (B) within pooled *Limacina helicina antarctica* pteropod guts at each sampling station as determined by 16S amplicon sequence variants. Numbers in grey represent sampling station numbers, arranged from north (N) to south (S) (see Fig. S1 for station locations). Cyanobacteria are not included here because their taxonomies could not be resolved to class level

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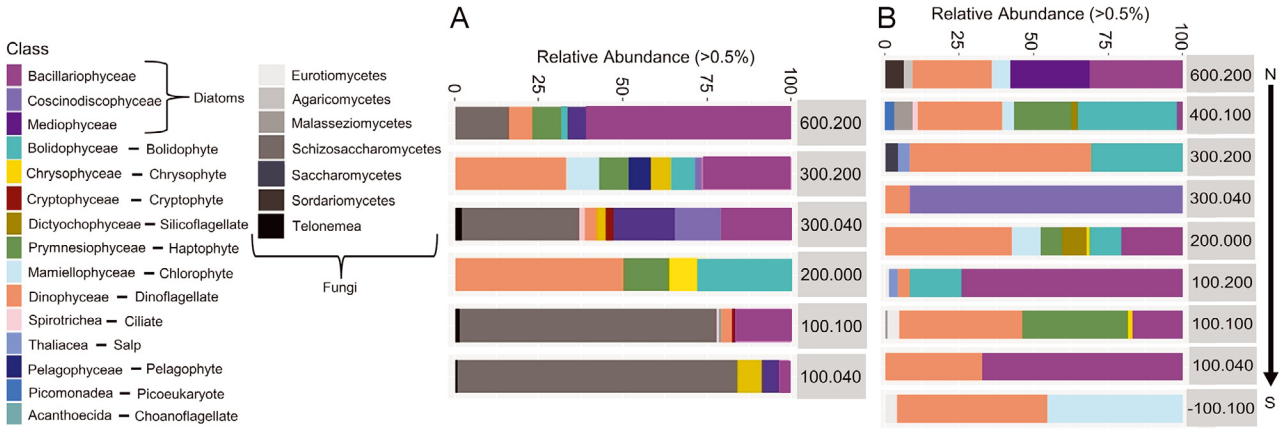

Fig. 3. Eukaryotic community composition at the class level present (A) in surface seawater and (B) within pooled *Limacina helicina antarctica* pteropod guts as determined by 18S amplicon sequence variants. Other details as in Fig. 2

chlorophytes (Mamiellophyceae), and haptophytes (Prymnesiophyceae) (Figs. 3 & S4). Analysis of chloroplast 16S further confirmed the presence of diatoms and haptophytes (Fig. S5). Dinoflagellates were the only eukaryotic group present in all gut samples. Haptophytes and chlorophytes were also present in guts throughout the sampling grid. Other eukaryotic groups, including salps (Thaliacea), and protozoa such as ciliates (Spirotrichea) and foraminifera (Picomonadea), and fungi only occurred in a few samples.

4. DISCUSSION

Mollicutes, *Alphaproteobacteria*, and *Bacteroidia* comprised the largest proportion of the prokaryotic community in the gut of *Limacina helicina antarctica* and were present within all samples. *Bacteroidia* and *Alphaproteobacteria* were equally frequent within the pteropod gut and in surrounding seawater, suggesting that those bacteria were acquired passively through feeding and were not endemic to the host pteropod. In contrast, *Mollicutes* were a key component, representing the most relatively abundant prokaryotic class (>40% of relative abundance) in all gut samples, but they rarely occurred or were absent in seawater samples. However, we are not able to directly distinguish between host-specific microbiomes and those associated with its ingested prey since *L. h. antarctica* was not starved prior to gut extraction. Differences between gut and pelagic microbial communities in seawater may also be due to variation in sampling depth.

Mollicutes were a common component of the *L. h. antarctica* microbiome as they occurred in all gut samples, indicating that the gut microbial community primarily comprises a few highly abundant taxa (i.e. *Mollicutes*, *Alphaproteobacteria*, and *Bacteroidia* at >70%). Low diversity within the gut microbiome has also been observed in Southern Ocean lanternfish, revealing that low diversity may be attributed to the occurrence of digestive enzymes and acidic pH (Gallet et al. 2019). In addition, *Mollicutes* are dominant components of other mollusk and aquatic invertebrate gut microbiomes, including jellyfish, but their function remains uncertain (Boyle et al. 1987, Daley et al. 2016). Expanding the application of sequencing analyses to determine the metabolic functions (i.e. transcriptomics) of these gelatinous zooplankton gut microbiomes is needed.

The eukaryotic gut contents of *L. h. antarctica* as determined with 18S sequences were consistent with previous studies using traditional methods and indicate that pteropods are mainly herbivorous in summer, consuming predominantly diatoms and dinoflagellates but also supplementing their diet with ciliates. At stations where diatoms and dinoflagellates were not the dominant prey, chlorophytes, bolidophytes, and haptophytes, specifically *Phaeocystis*, were present in the gut. These results generally agree with those of Rozema et al. (2017), who found that nanophytoplankton (cryptophytes and haptophytes) dominated surface waters in Marguerite Bay (WAP) in summer during low phytoplankton biomass years. A taxon surprisingly absent from pteropod guts was cryptophytes, which in the WAP are most abundant during summer months (December and January) after the seasonal retreat of sea ice (Schofield et al. 2017). The absence of cryptophytes as a prey item may be due to a combination of local envi-

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ronmental heterogeneity and variations in short-term phytoplankton bloom dynamics.

Other eukaryotic groups, including salps, and protozoa such as ciliates and foraminifera, only occurred within 1 or 2 gut samples, respectively, but confirm that *L. h. antarctica* is not solely an herbivorous feeder. Fungi were also present in a majority of gut samples at relatively low abundance and support the presence of marine fungi as a potential food source for zooplankton (Hassett et al. 2020). Fungi have been found in gut contents of both *Euphausia superba* and copepods, but the function of fungi in most marine invertebrates is still uncertain (Cleary et al. 2018, Hassett et al. 2020, Yeh et al. 2020). The increased presence of phytoplankton over ciliates or other protozoa in *L. h. antarctica* guts is to be expected, as our study was conducted during the productive austral summer when phytoplankton are abundant, more so than protozoa (Garzio et al. 2013, Schofield et al. 2017). Thus, *L. h. antarctica* diets in summer are dominated by diatoms and dinoflagellates and supplemented by other phytoplankton (e.g. haptophytes) and microzooplankton (e.g. ciliates). We recognize that some variation may be attributed to differences in 18S rRNA gene copy number among eukaryotes (Gloor et al. 2017). In addition, as we did not distinguish mixotrophic or heterotrophic dinoflagellates from those that are autotrophic, omnivory could be even more important. We show that *L. h. antarctica* is a generalist feeder and, given its high grazing rates and abundance in the region, its feeding likely plays a key role in affecting lower trophic level dynamics along the WAP.

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