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Research Article

RESEARCH ARTICLE

Composition and structure of the skin microbiota of rorquals off the Eastern South Pacific

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One sentence summary: Rorquals off the coast of Chile possess a diverse microbiota that is not only influenced by species but also by geographic location.

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ABSTRACT

Recent advances in high-throughput sequencing have enabled the large-scale interrogation of microbiota in the most diverse environments, including host-associated microbiota. This has led to the recognition that the skin microbiota of rorquals is specific and structurally different from that of the ocean. This study reveals the skin microbiome of 85 wild individuals along the Chilean coast belonging to *Megaptera novaeangliae*, *Balaenoptera musculus* and *Balaenoptera physalus*. Alpha diversity analysis revealed significant differences in richness and phylogenetic diversity, particularly among humpback whales from different locations and between blue and humpback whales. Beta diversity was partially explained

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by host and location but only accounting for up to 17% of microbiota variability (adjusted VPA). Overall, we found that microbiota composition was dominated by bacterial genera such as Cardiobacter, Moraxella, Tenacibaculum, Stenotrophomonas, Flavobacteria and Pseudomonas. We also found that no ASVs were associated with the three rorqual species. Up to four ASVs were specific of a location, indicating a great variability in the microbiota. To the best of our knowledge, this is the first report on the composition and structure of the skin microbiota of whales off the coast of Chile, providing a foundational dataset to understand the microbiota's role in rorquals.

Keywords: rorquals; skin microbiota; pacific ocean; amplicon sequencing; bacterial composition; amplicon sequence variants

INTRODUCTION

During the last decade, many studies have shown that changes in the microbiota can affect the health of humans and other animals (Redinbo 2014; Von Hertzen *et al.* 2015). Research has been conducted to understand the implications of these changes on the health and conservation of wild species. However, these have been focused on a limited set of taxa and habitats (Bahrndorff *et al.* 2016). Currently, the influence of environmental factors (e.g. temperature, location, etc.), along with host-related factors (e.g. age, sex, breed, etc.), on diversity and composition of microbial communities in animals has started to become clearer (Rosenberg *et al.* 2010; Wong, Chaston and Douglas 2013). To date, a number of studies have compared the microbiota of individuals from the same species or phylogenetically close species having, among other factors, different diets or inhabiting different geographical locations (Martinson *et al.* 2011; Avena *et al.* 2016). Several studies on marine mammals (e.g. cetaceans) have shed light on the influence of microbiota in topics such as host health, disease and nutrition, yet acknowledging the dearth of information available reviewed by Mootapally *et al.* (2017).

Since the skin of migratory cetaceans is exposed to different ocean basins, where different levels of anthropogenic impact and bacterioplankton composition exist, these marine mammals could become relevant models to increase our understanding of the drivers of wildlife microbiota. There are key advantages that would support the use of cetaceans for this purpose. For instance, (i) cetaceans are the only group of fully-aquatic mammals that are distributed worldwide (Kaschner *et al.* 2012), (ii) cetaceans perform extensive migrations in distinct biotic and abiotic environments (Corkeron and Connor 1999), (iii) cetaceans respond with high sensitivity to environmental changes, which make them useful for health-associated studies (Nelson *et al.* 2015) and (iv) cetaceans represent a unique evolutionary lineage. This latter point is particularly relevant since microbiota is strongly influenced by the phylogeny of the host as a result of millions of years of co-evolution and adaptations to different environments (Teeling *et al.* 2005; Apprill *et al.* 2020).

The role of microbiota in the skin of cetaceans is not yet well understood. Early studies based on culture-dependent approaches using humpback whale (*Megaptera novaeangliae*) skin samples, revealed that the microbiota of the skin is different from that of the surrounding seawater (Appril *et al.* 2011). This was also observed by Bik *et al.* (2016), who showed that skin microbiota of humpback whales was distinct from that of dietary fish and seawater, and also highly diverse and variable according to body site and host species. Apprill *et al.* (2014), described for the first time the skin microbiota of humpback whales from the Northern Hemisphere using amplicon sequencing approaches. This study proposed a core skin bacterial community composed mainly of bacteria from the genera *Tenacibaculum* and *Psychrobacter*, both previously associated with immune defense functions against pathogens and adaptation to extreme environmental conditions, respectively (Apprill *et al.* 2014). In addition, the same study revealed the presence of potentially pathogenic and predatory bacteria (Orders Clostridiales and Bdellovibrionales) in animals under stress, as in the case of entanglement by fishing nets. Recent studies have shown that skin microbiota of humpback whales around the Western Antarctic Peninsula exhibits shifts in average relative abundance and emergence of bacterial groups both temporarily, at the beginning and at end of seasons, and across whale concentration areas (Bierlich *et al.* 2018).

With more than 8000 km in length (4971 miles), the Chilean coast has numerous locations with high concentrations of whales, including rorquals (Gibbons, Capella and Valladares 2003). Among them, key locations for migratory species and feeding grounds for fin, blue and humpback whales are: (i) Pingüino de Humboldt Natural Reserve (PHNR) in Northern Chile (Toro *et al.* 2016; Sepúlveda *et al.* 2018), (ii) Gulfs of Corcovado and Ancud (CHLO; Hucke-Gaete *et al.* 2004) and (iii) Francisco Coloane Marine Park in the Strait of Magellan (MS) (Gibbons, Capella and Valladares 2003). In all of these locations, anthropogenic impact have increased over time, mainly due to activities such as whale-watching, salmon aquaculture and ship traffic (Toro *et al.* 2016; Guzman and Capella 2017; Hucke-Gaete *et al.* 2018; Sepúlveda *et al.* 2018).

However, the structure and composition of the skin microbiota of whales along the Chilean coast is still unknown, which prevents any microbiota study on whales' health and potential anthropogenic impacts. Therefore, in this study we aimed to test whether microbial composition and structure is driven primarily by host species or location, and to what extent the microbiota is homogeneously distributed among whale species. For this, we sampled along the Chilean coast from 30 to 53°S the skin of blue (*Balaenoptera musculus*), fin (*Balaenoptera physalus*) and humpback whales that migrate to Chilean continental waters in the Southern Hemisphere Summer season and analyzed it using 16S rRNA amplicon sequencing.

MATERIALS AND METHODS

Skin sample collection, storage and processing

Samples were collected from three locations off the coast of Chile: (i) A feeding and nursing ground in the Gulfs of Corcovado and Ancud (CHLO) during the Summer seasons of 2015, 2016 and 2017; (ii) the Strait of Magellan (MS) during the Summer seasons of 2010, 2011, 2016 and 2017 and (iii) the waters around the island of Pingüino de Humboldt Natural Reserve (PHNR) in the north of Chile during the Summer season of 2017 (Fig. 1). Rorqual skin tissue samples were obtained for 96 individuals from the three locations. Samples were collected by means of biopsy dart procedures from the upper flank, close to the dor-

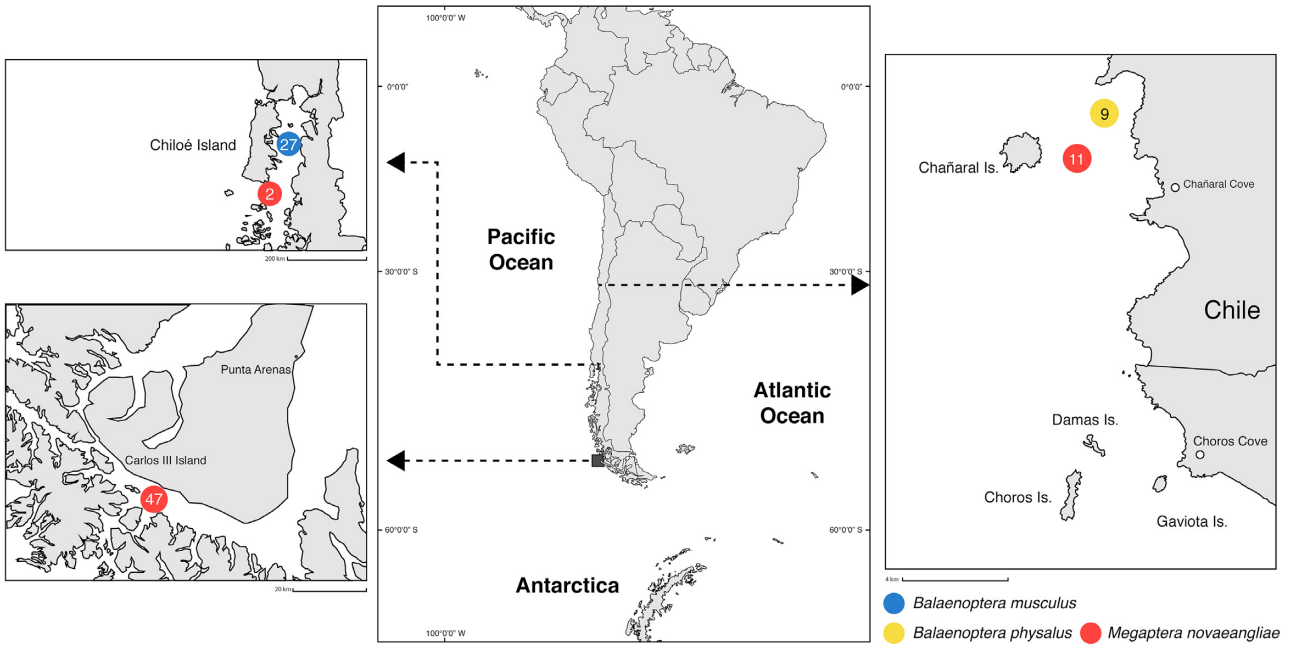

Figure 1. Sampling locations along the coast of Chile. Samples were obtained by using biopsy darts for remote tissue collection from the rorqual specimens. Colored dots indicate rorqual species. PHNR = Pingüino de Humboldt Natural Reserve; CHLO = Gulfs of Corcovado and Ancud; MS = Magellan Strait and the number inside the circles are the number of samples for rorquals species for location.

sal fin, using a crossbow (Krützen *et al.* 2002; Lambertsen 2006). Individual photo-identification was used to avoid resampling of individuals based on comparison to photo ID catalogs of dorsal fins (for fin whales), dorsal zones (for blue whales) and dorsal and ventral fluke patterns (for humpback whales) according to recommendations by Urian *et al.* (2015).

A total of 29 samples were taken in the CHLO sampling location, 27 of which corresponded to blue whales (*Balaenoptera musculus*), including five samples taken from the suction cups used for acoustic tag fixing, and the remaining two corresponded to humpback whales (*Megaptera novaeangliae*). In the area surrounding the PHNR island 20 samples were taken, 11 of which corresponded to humpback whales and nine to fin whales (*Balaenoptera physalus*). In the MS sampling location, 47 samples were taken, all of them corresponding to humpback whales. All samples were handled using sterile material and deposited in Eppendorf tubes. Samples from CHLO were kept in liquid nitrogen and stored at −80°C. Samples from the MS were suspended in 20% DMSO and stored at −80°C until processing. Samples from PHNR were suspended in RNAlater (Qiagen) and stored at −80°C.

DNA extraction and sequencing

DNA was extracted using the UltraClean Tissue & Cells DNA Isolation Kit (Cat No. 12334-S, MoBio Laboratories, Inc., Carlsbad, CA). Samples were homogenized on a horizontal vortex adapter (MoBio Laboratories) and the DNA concentration was quantified by fluorimetry in a Qubit® 3.0 Fluorometer (Invitrogen, Waltham, MA), using the Qubit dsDNA HS Assay Kit (Invitrogen, Waltham, MA). The V4 hypervariable region (515F and 806R primers) of the 16S rRNA gene was amplified from the extracted DNA using the barcoded dual-index primers developed by Kozich *et al.* (2013). Primers were chosen because they have been tested extensively, provide good taxonomic coverage and are used by global surveys of microbiome diversity, e.g. Earth Microbiome Project (Thompson *et al.* 2017). PCR products were prepared for sequencing using the Illumina TruSeq DNA Library Preparation Kit (Illumina Inc., San Diego, CA), according to the manufacturer's instructions. Paired-end (2 × 250 bp) Illumina MiSeq sequencing was conducted at The Microbial Systems Molecular Biology Laboratory (MSMBL) from The University of Michigan. Raw sequence data were deposited in NCBI's Sequence Read Archive database under BioProject PRJNA428495.

Inference of amplicon sequence variants

Demultiplexed paired-end reads were processed in R v4.0.3 (R Development Core Team 2017) using the package DADA2 v1.18.0 (Callahan *et al.* 2016) for inference of amplicon sequence variants (ASVs). Forward and reverse reads were quality trimmed/filtered and truncated at 240 nt and 200 nt, respectively. Ambiguous bases were not allowed, and a maximum of two expected errors was set. Subsequent steps, including error rates learning, dereplication, denoising and merging of paired reads were performed using adjusted parameters according to DADA2 Pipeline Tutorial (1.16). After building the ASVs table and removing chimeras, taxonomic assignment was performed with the Ribosomal Database Project's (RDP) naive Bayesian classifier (Wang *et al.* 2007), natively implemented in DADA2, using the Silva v138 database (Quast *et al.* 2013) as reference. A phylogenetic tree was inferred under the Maximum Likelihood optimality criterion as implemented in FastTree v2.1.10 (Price, Dehal and Arkin 2010). Sequences assigned to taxa Eukarya, Archaea, Chloroplast or Mitochondria were discarded. The decontam package was used to identify and remove contaminants using the 'frequency' and the 'prevalence' methods. Finally, samples with less than 1000 reads were discarded to avoid relying in taxonomic classification with low support. Water blanks and mock communities (Zymo microbiomics standard) were used to assess contamination and accuracy.

Community analyses

All statistical analyses were conducted in R v4.0.3 and RStudio v1.3.1073 using the packages phyloseq v1.34.0 (McMurdie

and Holmes 2013), microbiome v1.12.0 (Lahti and Shetty 2017), ampvis2 2.6.5 (Andersen *et al.* 2018), ggpubr v0.4.0 (Kassambara 2018), stats v4.0.3 (R Development Core Team 2017), agricolae v1.3–3 (De Mendiburu and Simon 2015), btools v0.0.1 (www.github.com/twbattaglia/btools) and vegan v2.5–6 (Oksanen *et al.* 2016). Plots were generated using ggplot2 v3.3.2 (Wickham 2016) and base R functions.

The relative effect of sampling procedure and sample preservation method was tested using ANOVA and the Wd∗-test for alpha and beta diversity, respectively. Read counts were normalized using the variance stabilizing transformation as implemented in the DESeq2 package (Love *et al.* 2014), which were used for downstream analyses.

Alpha diversity was assessed with the R package phyloseq using Chao1 and Shannon indices, plus Faith's Phylogenetic Diversity index as implemented in the R package btools. The Shapiro–Wilk test implemented in ggpubr was used for evaluating normality of alpha diversity indices. Significant differences in alpha diversity estimates among whale species and sampling locations were analyzed with the R package stats using ANOVA and pairwise comparisons between groups with Tukey's honest significance test with the agricolae R package.

Beta diversity was assessed by a Double Principal Coordinate Analysis (DPCoA) on Bray–Curtis distances as implemented in the phyloseq R package (Pavoine *et al.* 2004). Ampvis2 was used to generate heatmaps for the 25 more abundant taxa (best-hit taxonomic assignment) by rorqual species and geographical location, and Venn diagrams for visualization of the core microbiota among species and locations (frequency cutoff 90%). To identify drivers of beta diversity, we first tested whether the dispersion among groups was homogeneous using the vegan functions *betadisper* and *permutest*, to then assess statistical significance of the beta diversity among rorqual species and sampling locations by means of a Wd∗-test using the function WdS.test implemented in the R package MicEco (https://github.com/Russel88/MicEco) on a Bray–Curtis dissimilarity matrix. The Wd∗ test is robust to heteroscedasticity and unbalanced designs (Hamidi *et al.* 2019).

The R package ampvis2 was used to perform Principal Component Analysis (PCA) on a Hellinger transformed ASVs table, adding the envfit argument for geographical location. A cluster dendrogram was calculated using vegan's *hclust* and *mrpp* functions to visualize similarity in bacterial composition among rorqual species. Significance of ASVs agglomerated at family level was tested using vegan's *envfit* function on the first two principal components using a maximum estimated *P*-value of 0.001. Based on Bray–Curtis dissimilarities, a variance partitioning analysis (VPA) was performed using vegan's *varpart* function to distinguish the proportion of variation in bacterial communities explained by the variables 'geographical location' and 'rorqual species', as well as their combined effect. A Similarity Percentage (SIMPER) analysis, implemented in R package vegan, was used to identify the specific ASVs with the greatest contribution to the differences observed between rorqual species.

RESULTS

Amplicon sequence variants inference

After quality trimming/filtering, reads were fed into the DADA2 pipeline and 2222 ASVs were inferred. A total of 32 ASVs were removed after discarding mock community, water and two samples from *Eubalaena australis* (species not part of this work). Also, we removed potential contaminants (29 ASVs; decontam: 'frequency' and 'prevalence' methods), non-bacterial taxonomic assignments (467 ASVs from Eukarya, Chloroplast, Mitochondria, Archaea) and 16 ASVs were discarded by removing two humpback whale samples from CHLO, which resulted in 1678 ASVs. After additional filtering of samples with less than 1000 reads, 85 samples were left for analysis. The potential contribution of confounding variables regarding sampling procedure and preservation method was discarded since the variables were not significant drivers of alpha or beta diversity, either individually or in interaction with other variables (ANOVA and Wd∗-test; *P*-value > 0.05). Raw read numbers and reads after quality control, denoising, merging and chimera removal for whale samples, as well as blanks and mock community samples are provided in the supplementary file 1.

Bacterial diversity on whale skin samples

We calculated three alpha-diversity indices (Chao1, Shannon and Faith's Phylogenetic Diversity) to determine diversity differences among the three rorqual species and among the three sampling locations. We evaluated normality for alpha diversity indices and tested whether covariables, including sampling and preservation method, were significantly associated with diversity. We found no significant associations between alpha diversity and sampling or preservation methods on humpback whale sample location (ANOVA; *P*-value > 0.1 for Shannon index; *P*-value > 0.7 for Chao1 index; *P*-value > 0.8 for PD) or rorqual species (ANOVA; *P*-value > 0.1 for Shannon index; *P*-value > 0.8 for Chao1 index; *P*-value > 0.2 for PD).

When comparing bacterial diversity among humpback whale sample locations, we observed significant differences in Chao1 index and Faith's phylogenetic diversity between the PHNR and MS locations (Fig. 2A; *P*-value $= 0.003$ for Chao1 and 0.002 for PD). When comparing bacterial diversity among whale species, we observed significant differences in Faith's Phylogenetic diversity index between blue and fin and blue and humpback whales (Fig. 2B; *P*-value $= 0.005$). No significant differences in richness or evenness were detected between whale species.

Microbiota dissimilarity among species and geographical sampling sites

Beta diversity analyses of the skin microbiota showed significant differences associated with rorqual species and sampling geographical location. Double principal coordinate analysis (DPCoA) revealed that the axis1 accounts for 52.3% and the axis2 for 14.8% of the total variance observed in the microbial communities (Fig. 2C). The analyses also revealed significant heterogeneity in community composition (*betadisper* and *permutest*; $P = 0.001$), being the variables rorqual species and sampling geographical location significantly predictive of community structure (Wd∗-test with: *P*-value $= 0.001$ for both variables). The multi-response permutation procedure (Mrpp) analysis showed a significant difference between blue whales and humpback whales ($P = 0.000999$), while *hclust* showed that fin whales are more similar in overall community composition to humpback whales (Fig. 4E). We also used variance partitioning analysis (VPA) to quantify the effects of two or more groups of variables representing some distinct, ecologically interpretable phenomena. In this case, 7% of the skin microbiota variation was explained by sampling geographical location and 1% by rorqual species. When combined, both variables influence 17% of the variability in whale skin microbiota, while the remaining 75% is being influenced by other unaccounted variables.

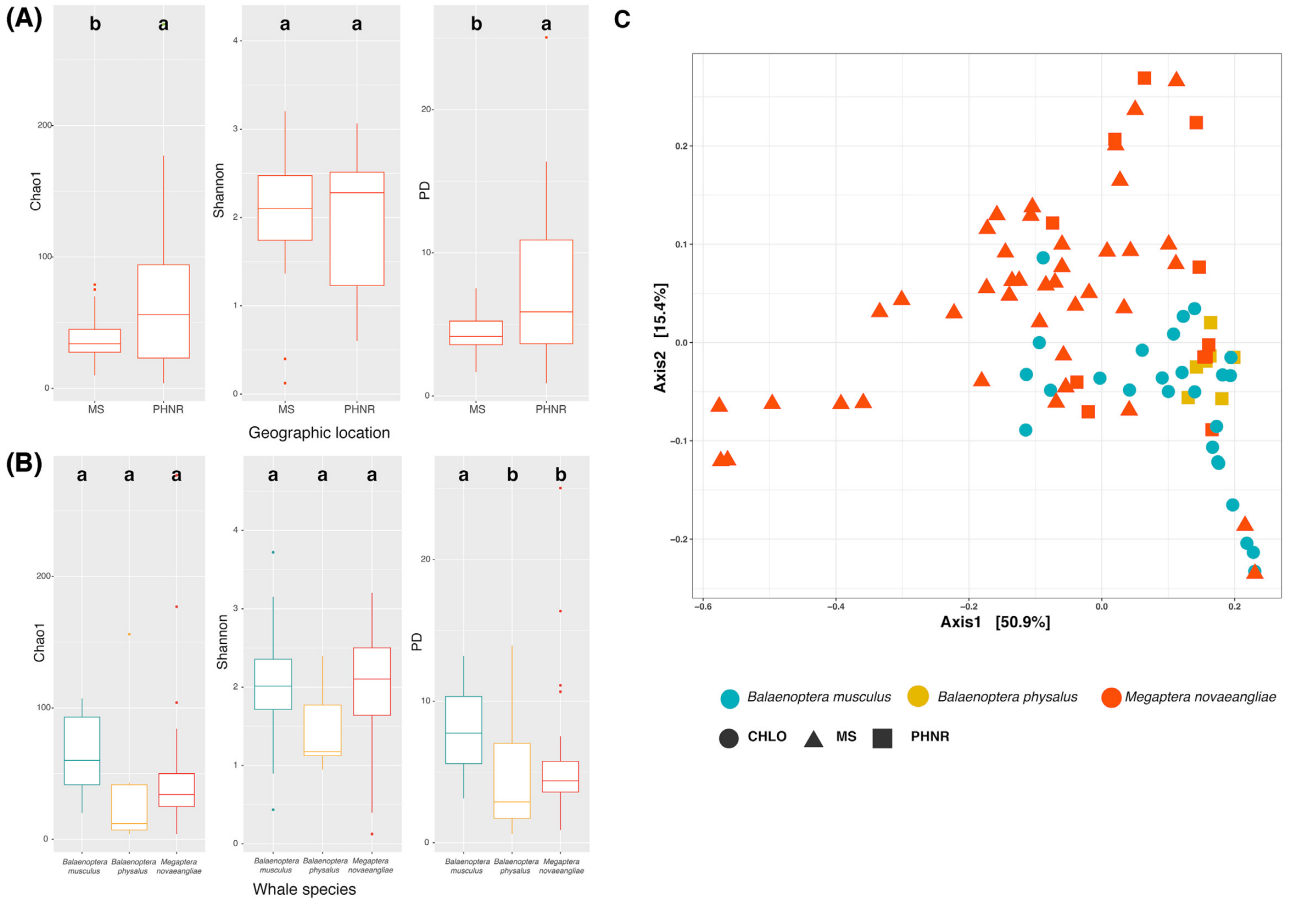

Figure 2. Alpha and Beta diversity of bacterial communities in rorqual skin samples. A total of three alpha diversity indices were calculated for evaluating differences in richness and diversity for **(A)** sampling locations of humpback whales and **(B)** species of rorquals. A one-way ANOVA was performed and the labels 'a', 'b' or 'ab' correspond to significant groups determined by Tukey's test. **(C)** Double principal coordinates analysis (DPCoA) on Bray–Curtis dissimilarity matrix. PHNR = Pingüino de Humboldt Natural Reserve; CHLO = Gulfs of Corcovado and Ancud; MS = Magellan Strait.

Bacterial community composition

The total composition of bacterial communities at the phylum level was mainly dominated by Proteobacteria (70.6%) and Bacteroidota (23.3%) as the most representative phyla in the skin microbiota of the three rorqual species. Firmicutes were found in a much lower abundance (3.8%). In particular, we observed that for fin whales 94.1% were Proteobacteria and 1.7% Bacteroidota. In humpback whales, the phylum Proteobacteria has 63.7% whereas Bacteroidota was 31% of the total, averaged over the two locations. For blue whales we found that Proteobacteria is found in 77.9% and Bacteroidota in 13.2% of the total microbiota.

We also calculated the relative abundances of the 25 most representative bacterial genera by rorqual species and geographical location (Fig. 3). Interestingly, an undetermined genus belonging to the family Cardiobacteriaceae was the most abundant in blue whales, accounting for 23.9% of the abundance. Other abundant bacterial genera found in blue whales were *Klebsiella*, *Pseudomonas* and an unidentified genus of the family Moraxellaceae. In fin whales, the most abundant bacterial genus detected was *Stenotrophomonas* (47.1%), followed by *Acromobacter* (11%) and a member of the Cardiobacteriaceae family (7.6%). Likewise, in humpback whales from PHNR the most abundant genera were *Stenotrophomonas* (28.5%), *Moraxella* (12.5%) and *Psychrobacter* (12.3%); while for humpback whales from the MS the most abundant genera were *Psychrobacter* (22.6%), *Tenacibaculum* (14.8%) and a member of the family Flavobacteriaceae (12.3%; Fig. 3).

Core microbiota and ASVs associated with each rorqual species

To evaluate the commonness and ubiquity of taxa present in the microbiota, we determined the core set of ASVs, agglomerated by best-hit taxonomic assignment, occurring in the different rorqual species and sampling locations. All ASVs present in at least 90% of the samples were considered to be part of the core. No significant number of shared ASVs were found among the sampling locations for humpback whales. In particular, only four ASVs (43.26% of total abundance) were found to be shared among humpback whales from MS and three ASVs (28.3% of total abundance) from PHNR (Fig. 4A). Pairwise comparisons between rorqual species revealed few shared ASVs (Fig. 4B and C). Specifically, only two ASVs (Moraxellaceae and *Psychrobacter*) were shared between blue and humpback whales, though representing 28.2% of the relative abundance (Fig. 4B). Other pairwise comparisons yielded no shared ASVs and few species-specific ASVs, again all accounting for high proportions of the relative abundance (Fig. 4B). In all core microbiota analyses, most of the ASVs were non-core, suggesting they were not highly prevalent or highly abundant (Fig. 4B).

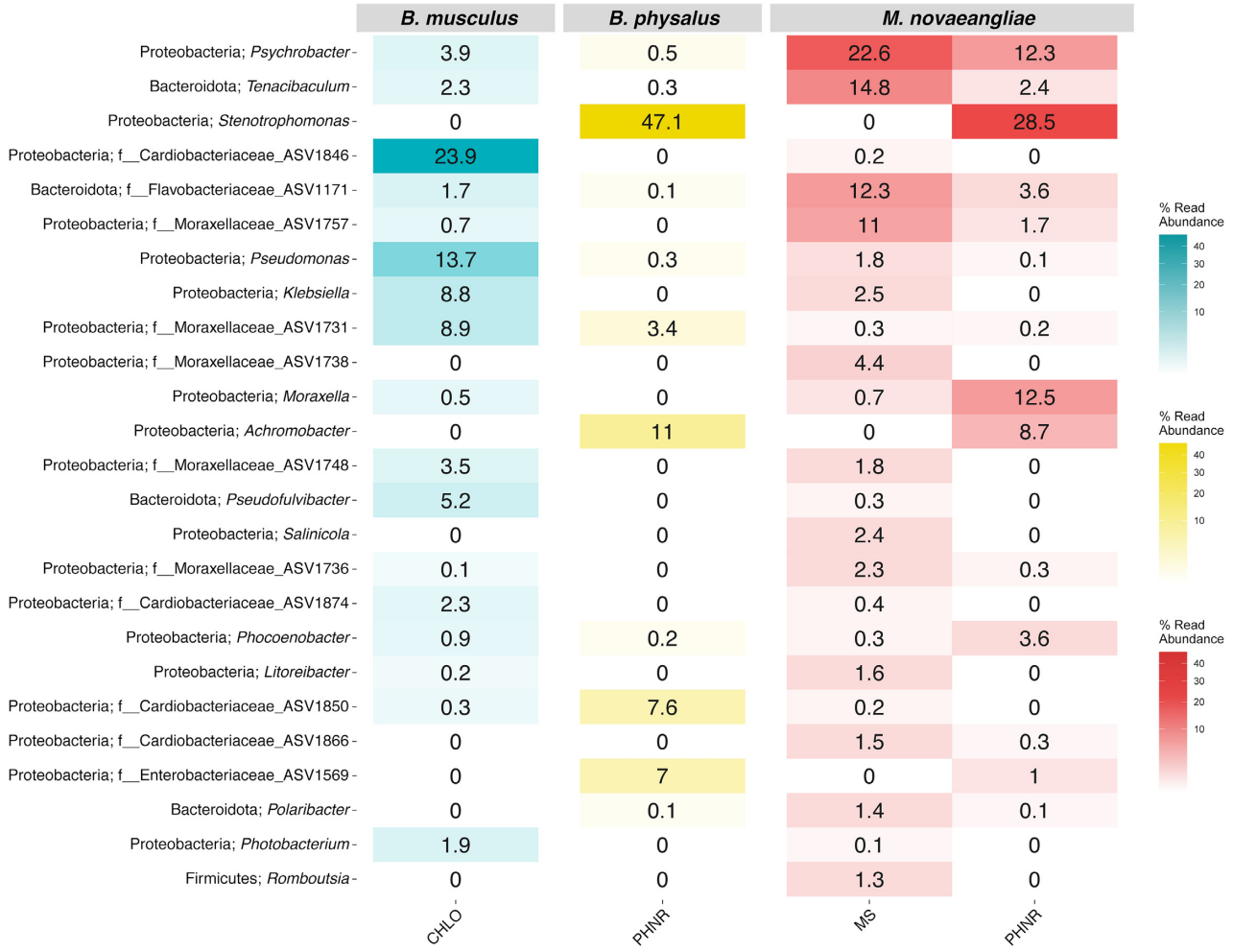

Figure 3. Composition and structure of rorquals skin microbiota. Heatmap showing the 25 most abundant ASVs (bacterial lineages) in the skin microbiota for the three rorqual species. Taxonomic ranks 'class' (c_) or 'family' (f_) are indicated when no genus-level taxonomic assignment was available.

We also investigated the potential relationship between the skin microbiota of rorquals and specific taxa to identify potential species-specific taxonomic markers (Fig. 4D). Using PCA analysis, we calculated vectors representing taxa with a clear relationship to whale species (Fig. 4D). In the case of fin and humpback whale samples from PHNR, the most significant ASVs belong to the families *Xanthomonadaceae, Alcaligenaceae, Burkholderiaceae, Bacillaceae, Planococcaceae* and *Yersiniaceae*. For the humpback whale samples, the most significant ASVs belong to the families *Moraxellaceae* and *Flavobacteriaceae* and *Solimonadaceae* and *Chitinophagaceae*. For the blue whale samples, the most significant ASVs belong to the families *Mycoplasmataceae, Promicromonosporaceae, Pseudomonadaceae, Cardiobacteriaceae Exiguobacteriaceae* and *Enterobacteriaceae*. Additionally, we performed a SIMPER analysis to infer the cumulative contribution of ASVs and found largely congruent results (Supplementary file 2). These results suggest that some bacterial taxa are preferentially associated with specific rorqual species, which might be linked to host factors modulating the bacterial composition of the rorqual skin microbiota.

DISCUSSION

To the best of our knowledge, this is the first comparative characterization of the skin microbiota of three rorqual species (*Balaenopteridae*) from the Eastern South Pacific, and the first description of the skin microbiota of blue and fin whales from the South Pacific Ocean. Our results suggest that (i) the structure and composition of the microbiota depends on both rorqual species and geographical location, which is consistent with reports in other marine mammals (Apprill *et al.* 2014; Bierlich *et al.* 2018), (ii) the most abundant taxa found in this study represent well-known members of the whale skin microbiota (e.g. *Cardiobacter, Stenotrophomonas, Tenacibaculum)* and (iii) microbial communities are highly structured within and among whale species with few or no shared ASVs.

Drivers of alpha and beta diversity

We found that alpha and beta diversity are modulated by geographic location and whale species. For marine mammals, the skin microbiota varies significantly among species and geographic location, both influencing the composition of bacterial communities (Apprill *et al.* 2020). For humpback whales, our results suggest that skin microbiota composition is influenced by geographical location at the time of sampling. This has also been observed for other species such as North American bats, red squirrels, vultures, different ecotypes of orcas and humpback whales of the Northern Hemisphere and Antarctica (Apprill *et al.* 2014; Ren *et al.* 2017; Bierlich *et al.* 2018; Hooper *et al.* 2019).

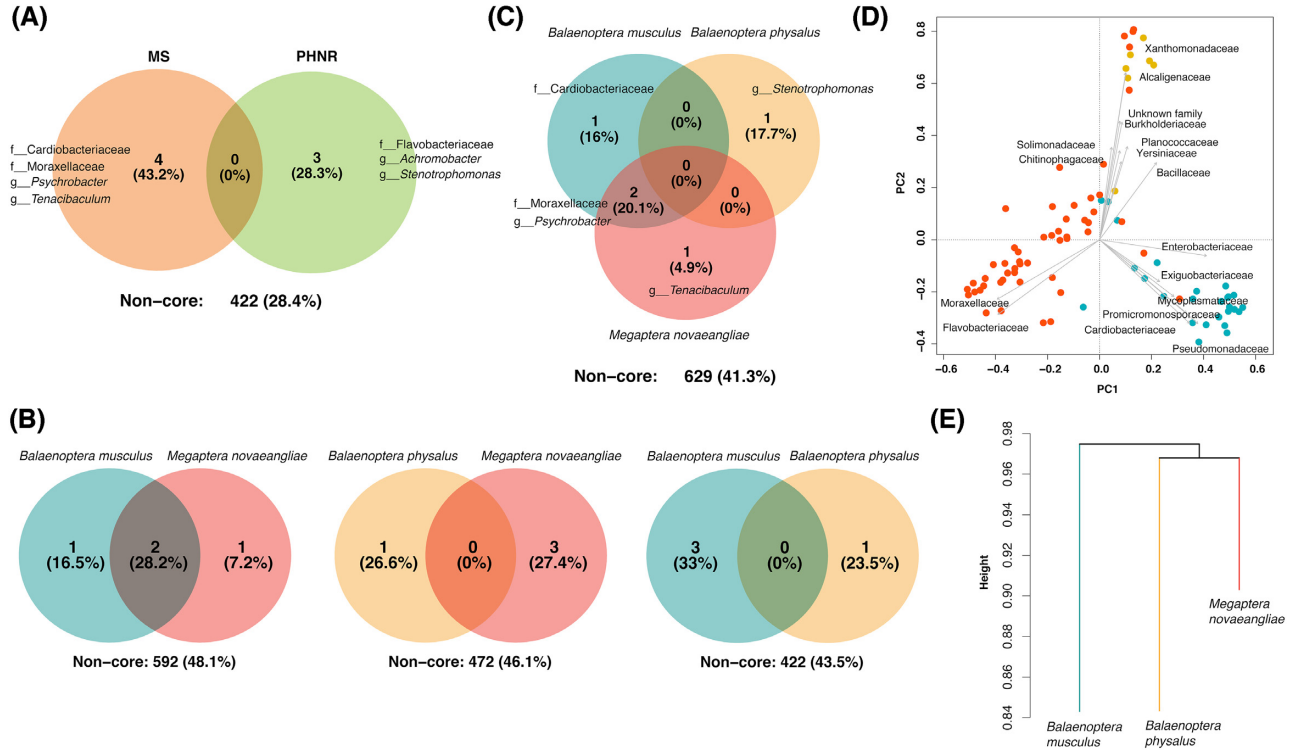

Figure 4. Core microbiota distribution and main bacterial families by geographical location and rorqual species. The Venn diagrams show core best-hit ASVs among **(A)** geographical locations and **(B and C)** rorqual species determined at 90% frequency. Colors indicate sampling locations and species. Numbers represent the amount of unique or shared ASVs and percentages indicate percentage of total abundance. **(D)** Principal Component Analysis (PCA) on Hellinger transformed ASVs abundances with calculated vectors for ASVs agglomerated to family level with a maximum estimated *P*-value of 0.001. Colors represent rorqual species. **(E)** Cluster dendrogram for microbial community dissimilarity between the three species of rorquals.

In belugas (*Delphinapterus leucas*), for instance, the skin microbiota varied significantly between different populations, season and health status (Van Cise *et al.* 2020). Furthermore, the skin microbiota composition also revealed species-specific differences between the rorquals studied, phenomenon previously reported for marine fishes and amphibians (Fitzpatrick and Allison 2014; Kueneman *et al.* 2014; Larsen, Mohammed and Arias 2014).

High-level microbiota composition in the whale species under study

The most abundant phyla found in the three rorqual species were Proteobacteria and Bacteroidota, followed by Firmicutes and Fusobacter, being the former the phylum found in highest relative abundance (63.3%). This result is in agreement with previous observations on skin microbiota studies performed on different vertebrate species (Avena *et al.* 2016), with exception of human skin studies where the most abundant phylum is Actinobacteria (Grice and Segre 2011). In humpback whale populations, researchers have reported an average abundance of 43.8% of Bacteroidota and 45.3% of Proteobacteria from skin tissue (Nelson *et al.* 2015; Bierlich *et al.* 2018). When analyzing differences by rorqual species, we observed that in humpback whales the dominant phylum was Proteobacteria (93.1%), being Bacteroidota (2.4%) the least abundant compared to the other two rorqual species. This has also been observed in captive orcas and bottlenose dolphins where Proteobacteria was the dominant phylum (Chiarello *et al.* 2017).

Microbiota composition of fin whales

The microbiota of fin whales was represented in a large percentage by *Stenotrophomonas* (47.1%; Class: Gammaproteobacteria), a bacterial genus that has been isolated from soil, rhizosphere and aquatic environments, which also includes several pathogenic strains for humans (nosocomial infections; Patil *et al.* 2018). In different Antarctic ecotypes of orcas, the bacterial species *Stenotrophomonas maltophilia* have been found highly represented in most of the individuals (Hooper *et al.* 2019). Several taxa belonging to *Stenotrophomonas spp.* have antibacterial and antifungal activities, inhibiting the growth of pathogens (Barnes *et al.* 2020). Whether this feature could explain the lower microbiota richness and diversity within fin whales remains to be tested. Another abundant genus was *Achromobacter* (11%), commonly found in various environments (including oceans). Opportunistic pathogenic species of this genus have also been found in immunosuppressed humans (Cools *et al.* 2016).

Microbiota composition of blue whales

The microbiota of blue whales was predominantly represented by an unidentified genus from the family Cardiobacteriaceae (23.9%), *Pseudomonas* (13.7%), *Klebsiella* (8.8%), *Pseudofulvibacter* (5.2%) and to a lesser extent *Tenacibaculum* (2.3%). Cardiobacteriaceae species have been previously isolated from the blow of humpback whales and mouth and blowhole of bottlenose dolphins (Zasloff 2011; Apprill *et al.* 2017), suggesting that these bacteria could be stable members of the skin microbiota of blue whales. The case of *Klebsiella* is interesting since species such as *Klebsiella oxytoca* have been isolated from skin lesions of an

Atlantic bottlenose dolphin, while other *Klebsiella* spp. has been isolated from goosebeak whales (*Ziphius cavirostris*), suggesting that they are potential pathogens for cetaceans. Nevertheless, more extensive studies are needed to verify the pathogenic potential of these genera in blue whales (Buck *et al.* 1991).

Microbiota composition of humpback whales

In humpback whales, the dominant genera were *Tenacibaculum* (14.8%), *Moraxella* (11%) an unidentified genera of the family Flavobactereacea (12.3%) and *Psychrobacter* (22.6%). These genera have been described by Apprill *et al.* (2014) and Bierlich *et al.* (2018) as part of humpback whale skin microbiota from feeding and breeding locations where this species is found. Apprill *et al.* (2014) suggested that the core microbiota of humpback whales should be comprised of the bacterial genera *Tenacibaculum* (which have defense functions against other microorganisms) and *Psycrobacter* (a group resistant to extreme environmental conditions). In our study, members of the *Psycrobacter* genus were found in high abundance (22.6 and 12.3%), which might be linked to the nutritional status of the individuals, since the ecological condition of the epidermis can change during the foraging period given that the availability of nutrients changes the lipid composition of the dermis, thus modulating the microbiota. This has also been observed in humans, where differences have been described in skin microbiota associated with diet and sebaceous areas of the skin (Ziboh *et al.* 2002; Grice and Segre 2011).

Potential variables influencing microbial community structure and composition

Since all the samples were taken from three feeding zones described for this species in the Southeast Pacific in a similar time period, it is highly probable that the physiological condition of the individuals may have influenced the presence of the genus *Psychrobacter*, as observed by Bierlich *et al.* (2018), who reported a significant decrease in the abundance of *Psychrobacter* at the end of the feeding season in humpback whales from Antarctica linked to environmental and physiological changes. In addition, a possible influence of sea temperature should not be discarded, since sampling locations in these studies exhibit differences in sea temperature with water surface temperatures ranging from 6.5 to 18°C for both areas (Thiel *et al.* 2007; Haro, Aguayo-Lobo and Acevedo 2013). Another important factor to consider, that could explain the differences in the microbiota, are the different areas in which the individuals are distributed, being the humpback whale the only species found in the three zones with different degree of residence of the whales for the different locations and different human impact for the three zones (Capella *et al.* unpublished data). The genus *Tenacibaculum* was found in a greater abundance in whales from the MS (14,8%), while those from the PHNR exhibited a smaller proportion. The low prevalence of this genus has been associated with whales under stress (Apprill *et al.* 2014), for this area it is known that the high number for boats of whale-watching, this cause behavioral stress for these species (Toro *et al.* unpublished data) so this activity could have an effect on bacterial communities. Researchers have shown that orcas (*Orcinus orca*) with prominent yellow coloration show a higher abundance of diatoms in Antarctica, which was positively correlated to the abundance and presence of *Tenacibaculum dicentrarchi*, suggesting that the presence of this unicellular algal group along with cold water environments can influence the proliferation of bacteria of the genus *Tenacibaculum* (Anderson 2000). We also observed taxa belonging to the genus *Moraxella* in greater proportion in the PHNR area than in MS area. This genus is associated with the normal core microbiota of healthy humpback whales (Apprill *et al.* 2011) and to the mouth and blow core microbiome of dolphins. Nonetheless, *Moraxella* species have also been detected in skin lesions of right whales from Greenland (Shotts *et al.* 1990). In humpback whales of PHNR, a high percentage of *Stenotrophomonas* and *Achromobacter* was found, both genera being highly represented in fin whales of the same area. This result suggests that both species sharing the same environment would also share part of their microbiota, similar to what occurs between humans and domestic animals that coinhabit and have a similar skin microbiota (Song *et al.* 2013). In addition, it has been reported that some strains of *Stenotrophomonas* and *Achromobacter* isolated from other hosts carry antibiotic resistance genes (Abbott and Peleg 2015).

Overall similarity in microbiota composition

Recent studies have suggested that phylosymbiosis is an important factor where the phylogenetic and evolutionary characteristics of cetaceans influence modulation of the skin microbiota (Bierlich *et al.* 2018; Apprill *et al.* 2020). Among the three rorqual species studied, humpback and fin whales showed a greater similarity between their microbiomes than with that of blue whales. Recent molecular studies have suggested that fin and humpback whales are closely related to each other, which could possibly explain the similarity between their microbiota (Bérubé and Aguilar 1998). Despite this, blue and fin whales are species closely related too (hybrids have been described from the cross between them) and their ecology is more similar to that of humpback whales (Árnason *et al.* 2018).

Finally, our results highlight the need for further studies regarding rorqual microbiota, since more exhaustive and balanced sampling could shed light on the relative contributions of variables such as gender, age class, kinship, social behavior and non-sampled areas of whale concentration, including breeding areas. This would help us gain a better understanding of the relative contributions and functions of the microbiota on the host health and physiology.

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SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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