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Respiratory bacteriome and its predicted functional profiles in blue whales (*Balaenoptera musculus*)

Carlos A. Domínguez-Sánchez[1,2], Diane Gendron[3], Roberto C. Álvarez-Martínez[1] & Karina Acevedo-Whitehouse[1,4]✉

The respiratory microbiome plays a critical role in the health of organisms and studying it in natural populations can reveal interactions between hosts and their environment, as well as help predict responses to environmental stressors. We characterized the core respiratory bacteriome and functional profiles of Eastern North Pacific blue whales (*Balaenoptera musculus*) sampled in the Gulf of California using next-generation sequencing. Our compositional analysis identified 15 dominant bacterial phyla in the respiratory tract, with Proteobacteria (34.44%), Firmicutes (26.98%), Bacteroidota (20.26%), Fusobacteriota (7.61%), and Actinobacteria (5.55%) as the most abundant. Nineteen ASVs, representing 12 bacterial genera (primarily *Corynebacterium*, *Oceanivirga*, *Tenacibaculum*, and *Psychrobacter*), were shared by over 60% of whales, with a relative abundance greater than 0.02%. These bacteria, proposed to be the core respiratory bacteriome of blue whales, contributed to functional pathways associated with metabolism, environmental information processing, and cellular processes. Notably, two whales with high relative abundance of *Mycoplasma* spp. and of *Streptococcus* spp., exhibited overrepresented pathways related to nucleotide metabolism and translation, suggesting a suboptimal immune status or dysbiosis. To our knowledge, this is the first functional profiling of the bacteriome in any cetacean. Future studies are needed to explore how the blue whale respiratory bacteriome may vary over time, seasonally or across geographical locations. This study establishes a baseline for future research on the plasticity of the bacteriome, its associations with other microbiome components, the impact of environmental changes on its diversity, and its relevance for health. Our novel approach underscores the ecological and physiological importance of the bacteriome and its potential for long-term monitoring of a sentinel marine species in a rapidly changing ocean.

Keywords 16S rRNA, Balaenoptera musculus, Blue whale, Microbiome, Bacteriome, Functional profiling

The advent of modern technologies that allow for the identification of bacteria in environmental or clinical samples[1] has led to a surge in studies examining the abundance, diversity, and structure of microbiomes across species[2]. Increasing our understanding of the microbiome is crucial because microbial communities associated with specific organs or tissues can significantly impact host physiology[3] and health[4]. For instance, respiratory infections may arise when opportunistic microorganisms—normally part of a healthy respiratory tract—proliferate under certain conditions[1], disrupting the diversity and composition of the microbial community in a phenomenon known as dysbiosis[5], which can contribute to disease. Additionally, respiratory disease can result from exposure to non-commensal microorganisms with pathogenic potential. This underscores the importance of microbiome composition as a potential predictor of health and disease progression, often more so than the mere presence of specific microorganisms commonly associated with disease. Understanding how microbiomes differ between individuals could, therefore, become a valuable tool for assessing health[6].

When using the microbiome to assess health status, it is important to distinguish between commensal, opportunistic, and transient bacteria[7]. This distinction is complex, as the symbiotic relationships of bacteria can vary both across species and among individuals[8] To help differentiate potential commensal and mutualistic bacteria, it is necessary to identify the core microbiome—the microbial taxa that predominate within a community

[1]Unit for Basic and Applied Microbiology, School of Natural Sciences, Autonomous University of Queretaro, 76010 Queretaro, Mexico. [2]Mingan Island Cetacean Study (MICS), 378 Rue du Bord de la Mer, Longue-Pointe-de-Mingan, QC G0G 1V0, Canada. [3]Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, 23096 La Paz, Baja California Sur., Mexico. [4]Carr. a Chichimequillas S/N, Ejido Bolaños, 76140 Santiago de Querétaro, Qro, Mexico. ✉email: karina.acevedo.whitehouse@uaq.mx

and are common in apparently healthy individuals[9]. Defining the core bacteriome (the bacterial community of the microbiome) involves setting the detection threshold (relative abundance) and determining the minimum occurrence percentage (prevalence) of bacterial taxa to include[10]. However, because biological justifications for these prevalence and threshold values are often lacking[11], it is important to exercise caution when interpreting results[10]. Despite varying definitions, the core bacteriome tends to be relatively stable, particularly when samples from closely related individuals are analyzed[11,12].

Microbial taxonomic composition provides a basic understanding of the microbiome, but it does not fully capture the intricate microbial contributions to host health[13]. Bacteria within the mammalian microbiome exist in composite communities[1], whose diversity and abundance result from complex interactions between species[14]. The metabolic contributions of these communities are important to the host and depend on their composition[15]. This is where functional profiling becomes important, as it reveals the metabolic and ecological roles of microbial communities[16,17]. Combined taxonomic and functional analyses offer a deeper understanding of the microbiome's dual nature, as both a diverse community and a functional unit that essential for the holobiont's processes[18]. Functional predictions are based on genetic data derived from sequencing the 16 S rRNA gene[18,19], which is mapped to reference databases, correlating specific taxa with known functional abilities[20]. These functions are organized hierarchically, from broad functional categories to specific metabolic pathways[21]. This approach allows researchers to infer the ecological and metabolic roles of the microbiome without the need for whole-genome sequencing, making it a powerful and accessible tool for microbiome research[19]. By integrating taxonomic and functional analyses, we can gain deeper insights into a host's microbiome and its role in holobiont resilience, particularly in the context of health[22].

In cetaceans, characterizing the microbiome offers a unique opportunity to link microbial community structure and function with host ecology, physiology, and responses to environmental change, providing valuable insights for conservation and health monitoring[23,24]. Whales, as long-lived animals that play a critical role in the ocean's carbon movement and storage, are vital to marine ecosystems[25], and are often considered sentinels of ocean health[26]. The study of the cetacean microbiome is still in its early stages. Microbial diversity has been assessed for a few species[1,27–31], and some opportunistic pathogens in the respiratory tracts of free-ranging cetaceans have also been described[32,33]. However, to our knowledge, no study has yet combined taxonomic and functional profiling of the microbiome in any cetacean species. Blue whales, among the world's largest and most iconic animals, play an essential role in marine ecosystems[34]. Their long migrations and diverse habitats make them valuable indicators of ocean health[35]. Despite this, only one published study has examined the respiratory microbiome of blue whales in the wild[36]. Given the growing importance of understanding blue whale health in their natural environment, studying their respiratory microbiome is both timely and relevant. Not only would it provide insights into their exposure with potential pathogens[32], but it would also establish a baseline of core bacteria and functional profiles in apparently healthy individuals. This baseline could facilitate the identification of dysbiosis, help predict potential diseases and ultimately inform conservation strategies and management plans for the species[37]. This is a pressing need, especially in light of the global and local environmental changes currently affecting oceans[38]. Here, we characterized the common core and functional profiles of the respiratory bacteriome in Eastern North Pacific blue whales from the Gulf of California using next-generation sequencing on blow samples collected from 17 adult blue whales via a non-invasive drone-based technique[39].

Results

A total of 19 samples were analysed, including 17 photo-identified blue whales, one technical control, and one seawater sample. Exhaled breath was collected from the whales using a drone-based method previously described[39], with no adverse behavior observed before, during, or after sampling. After filtering, denoising, merging, and chimera elimination (2.38% of reads), we obtained 68,922 sequences (mean per sample: 3514.8 [SD = 1998.3]), which corresponded to 1304 amplicon sequence variants (ASVs). We removed 51 ASVs classified as Archaea (n = 2), chloroplasts (n = 27), or mitochondria (n = 7), as well as those not classified at the phylum level (n = 15), and 22 ASVs identified as contaminants using the *Decontam* algorithm based on the LabControl sample reads. This left 1231 ASVs remained, with 500 ASVs classified as "*Others*" (representing less than 0.02% relative abundance).

Species richness (S) in the blow samples ranged from 62 to 404 (mean = 189.63.06 [SD = 113.71]), and Simpson's diversity index (D) ranged from 0.49 to 0.98 (mean = 0.94 [SD = 0.11]). The compositional analysis identified 15 bacterial genera (Fig. 1, Table S1) with *Psychrobacter* spp. (mean = 12.07% [SD = 6.09%]), *Oceanovirga* spp. (mean = 10.93% [SD = 4.42%]), *Tenacibaculum* spp. (8.87% [SD = 6.39%]), and *Streptococcus* spp. (6.79% [SD = 20.13%]) being the most abundant. Notably, two blow samples Bm057 and Bm044) exhibited a high relative abundance of the opportunistic pathogens *Mycoplasma* spp. (27.22%), and *Streptococcus* spp. (74.37%). In addition, we identified *Bacteroides* sp. in sample Bm042 at a high relative abundance of 13.96% compared with the other samples (mean = 0.02% [SD = 0.04%]). For this whale, mucus was also retrieved during blow sampling, which had a noticeable bad smell and a yellowish coloration; features that were not observed in any other samples.

Functional profiling, at 97% similarity, was possible for 32.53% of the ASVs. At KEGG Level 1, the most predominant pathways were associated with metabolism (mean = 74.88% [SD = 4.36%]), followed by environmental information processing (mean = 9.98% [SD = 1.82%]), cellular processes (mean = 5.54% [SD = 1.67%]), and genetic information processing (mean = 5.40% [SD = 1.57%]). At KEGG Level 2, the top subcategories included global and overview maps (mean = 38.37% [SD = 2.96%]), carbohydrate metabolism (mean = 9.65% [SD = 1.14%]), amino acid metabolism (mean = 7.23% [SD = 1.45%]), and membrane transport (mean = 6.55% [SD = 1.32%]). At KEGG Level 3, the most abundant pathways were metabolic pathways, biosynthesis of secondary metabolites, ABC transporters, and microbial metabolism in diverse environments (Fig. 2).

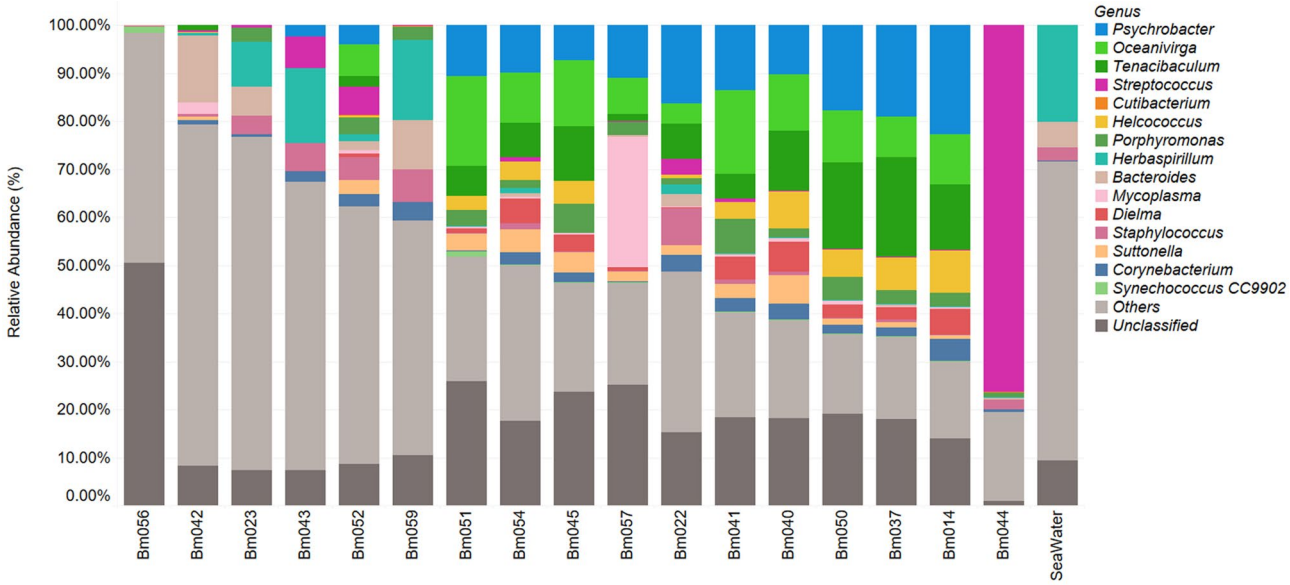

Fig. 1. Stacked bar plot depicting relative abundance of the top 15 bacterial genera. Each vertical bar depicts the relative abundance of adjusted sequence variants (ASVs) and associated taxa that were recovered per sample. Plot shows the top fifteen identified bacterial genera, unclassified, and "others" (sum of bacteria that did not reach the detection threshold of 0.02%).

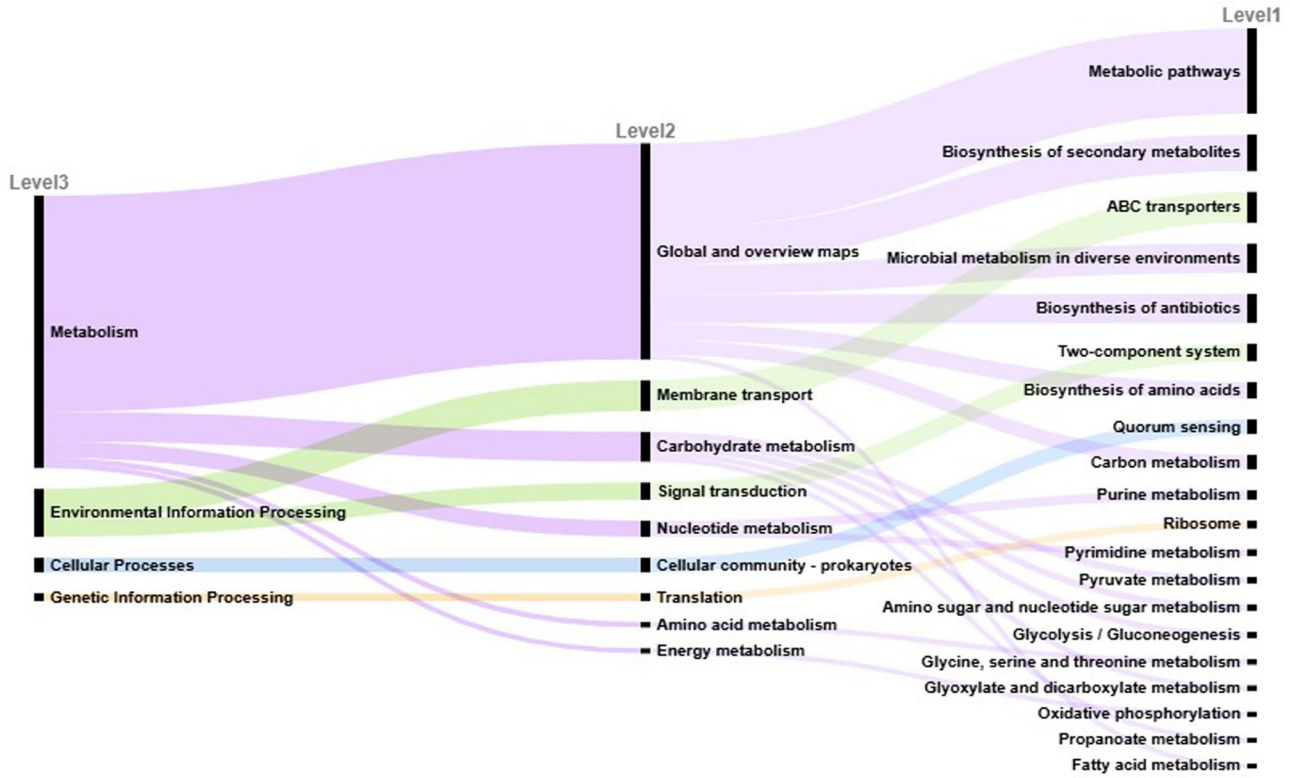

Fig. 2. Alluvial diagram of the top 20 predicted functional pathways (at different KEEG levels) associated with the bacteriome in the respiratory tract of blue whales.

In two blow samples (Bm057 and Bm044) with the highest relative abundance of opportunistic pathogens, functional profiling revealed overrepresentation of pathways such as nucleotide metabolism, membrane transport, translation, folding, sorting and degradation, and carbohydrate metabolism; while pathways related to amino acid metabolism, cofactor and vitamin metabolism, lipid metabolism, and biosynthesis of secondary metabolites were underrepresented (Fig. 3). Among the bacterial genera identified, *Psychrobacter* (26.83%) contributed most to the functional pathways predicted in the blue whale respiratory tract, followed by *Tenacibaculum* (17.48%) and *Porphyromonas* (13.01%). Genera such as *Suttonella* and *Streptococcus* contributed less (4.07% and 3.25%, respectively; Fig. S1). Despite variation in taxonomic composition, functional profiles across individuals were consistent (Fig. S2).

The core bacteriome analysis identified 19 ASVs from 12 bacterial families (Fig. 4, Table S2), with *Tenacibaculum* (ASV3) and *Oceavivirga* (ASV7) the being the most abundant genera (30.01% [SD = 15.94] and 28.78% [SD = 9.76], respectively]). The core functional profile derived from these core ASVs was composed

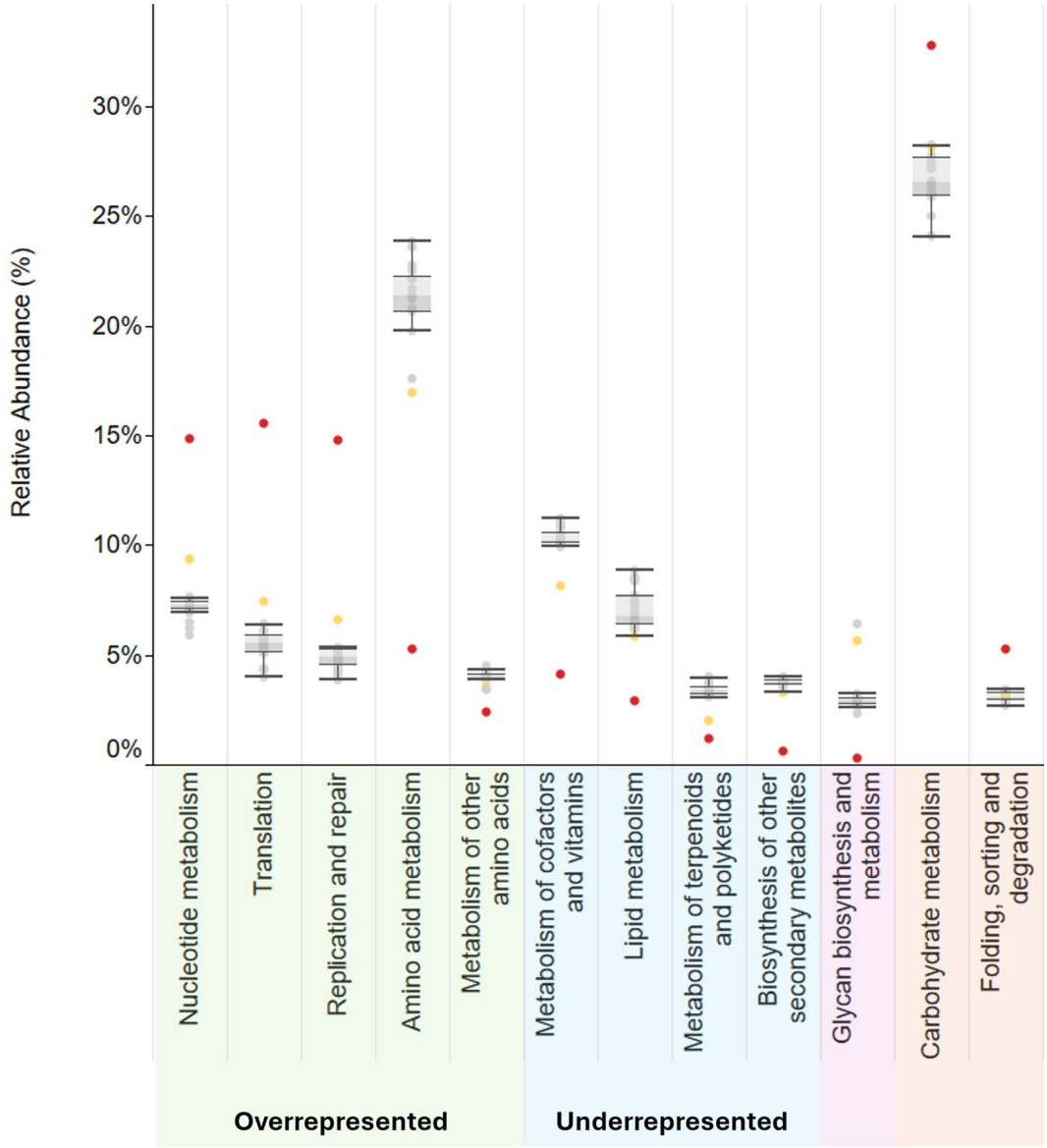

Fig. 3. Boxplot of the relative abundance of functional pathways (at KEGG Level 2) across all blow samples. Red dots represent blow sample Bm057 (the whale that had a high relative abundance of *Mycoplasma* sp.), while yellow dots correspond to blow sample Bm042 (the whale that had a high relative abundance of *Streptococcus* sp.). Functional pathways that were overrepresented in both Bm057 and Bm044 compared to all other samples (grey) are highlighted as light green columns, while underrepresented pathways are shown as light blue columns. The functional pathway that was underrepresented in the bacteriome of Bm057 but over represented in Bm042 is highlighted as light purple columns, and the functional pathways were over represented only in Bm057 are shown in as light orange columns.

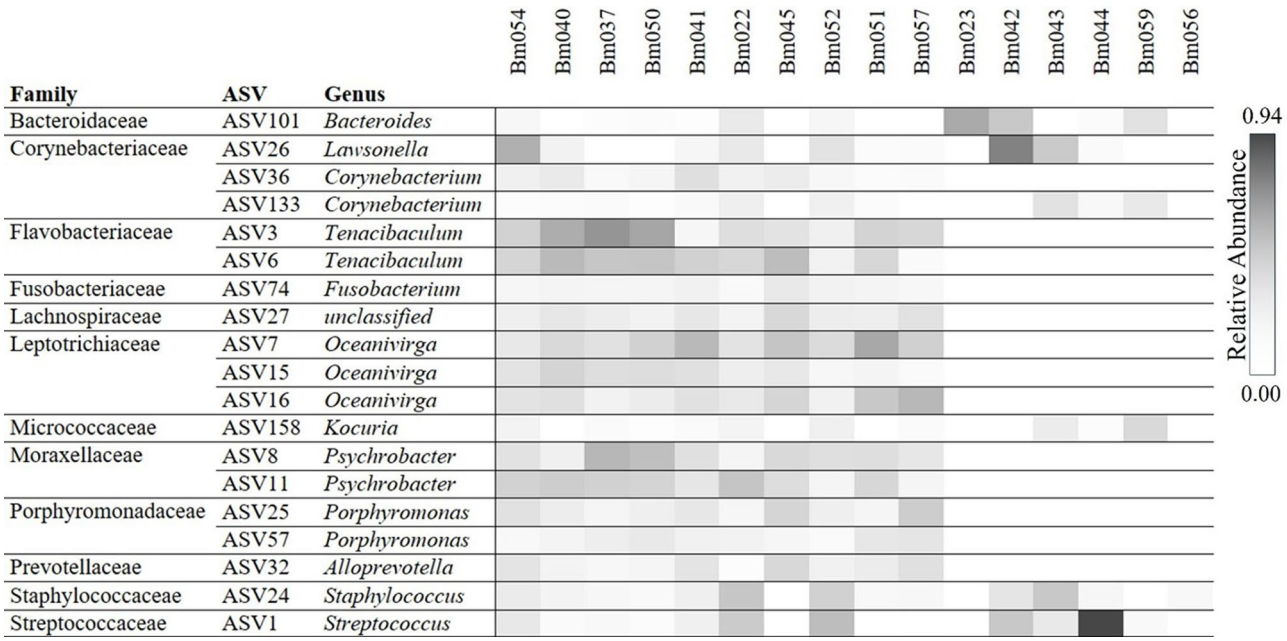

Fig. 4. Relative abundances of bacterial genera that constitute the core respiratory bacteriome of the blue whale. The figure includes the seven ASVs that were present in more than 60% of the samples and that had a relative abundance of over 0.02%. The relative abundance of each ASV shown in this plot is confined to the core microbiome members and not the entire microbiome of each sample.

mainly by metabolic pathways (24.99%), biosynthesis of secondary metabolites (11.09%), biosynthesis of antibiotics (8.73%), and microbial metabolism in diverse environments (8.34%) (KEEG level 1; Fig. S3).

The Bayesian approach used to estimate the contribution of seawater diversity to blow samples indicated that seawater contributed on average of 1.68% (SD = 0.81). *Herbaspirillum* sp., the most common genus in seawater (20.23% relative abundance; Fig. 1), was also detected in blow samples, albeit at a lower average abundance (3.39%; SD = 5.97). Interestingly, three whale blows (from individuals Bm023, Bm043, and Bm059) exhibited notably higher levels of *Herbaspirillum* sp. (9.44%, 15.65%, and 16.82%, respectively).

Discussion

A healthy microbiome is generally characterized by high diversity, which helps both the microbiome and the host cope with external challenges[30]. In our study, the respiratory bacteriome of the blue whale exhibited considerable diversity, with significant variation in bacterial richness and abundance across samples. These fluctuations may arise from several factors, including bacterial immigration from the environment during inhalation, mucociliary clearance, and community growth rates[40], all of which can vary among healthy individuals[27]. However, variations could also stem from sampling techniques, such as differences in the number of blows, volume of sample collected, whale size and behavior (e.g. dive depth and duration) [39,41]. Notably, the bacterial diversity observed in blue whale blow samples was similar to that reported for humpback whales and bottlenose dolphins[41,42], although the blue whale blow showed greater taxonomic richness. This may be attributed to differences in methods used to resolve taxonomy[43–45] or the identification of rare bacterial species[44,45], which play an important role in microbiome resilience, given their contribution as a seed bank of genetic resources that can lead to the restoration of the core microbiome[46].

The presence of a complex respiratory bacteriome is beneficial for a host, as higher microbial diversity supports vital ecosystem functions[47]. Functional analysis of the blue whale bacteriome revealed overrepresentation of pathways related to macromolecular metabolism and environmental information processing and signal transduction, indicating a potential role in adapting to environmental changes[48,49]. This result reinforces the idea that bacteriome diversity serves a protective role for the host[18], as these pathways are critical for maintaining host health and epithelial immune function[50,51] by enabling microbial communication with host immune cells via molecular signals that activate pattern recognition receptors, triggering cytokine production and immune cell recruitment[52], including dendritic cells[53].

Our findings indicate that the respiratory bacteriome of blue whales is dominated by members of Proteobacteria, Firmicutes, Bacteroidota, Actinobacteria, and Fusobacteriota, which are common bacterial phyla in the respiratory microbiome of other mammals[1]. Particularly noteworthy is the consistent presence of *Psychrobacter* sp. and *Tenacibaculum* sp., which are known commensal bacteria[54,55] that contribute to respiratory and skin health[4,27,29,30,56], although they can also be implicated in pathological conditions in other organs[57,58]. Additionally, the respiratory core bacteriome included *Oceanivirga* sp., a bacterium common to the respiratory tract of various marine mammals from different geographical locations[59], and identified as part of the core respiratory bacteriome of humpback whales[41]. Given that *Oceanivirga* sp., was present in most of the blue

whales sampled, it is reasonable to consider it a key member of their respiratory bacteriome, reflecting a healthy respiratory epithelium.

It is important to recognize that while the bacterial taxa in the blue whale's respiratory bacteriome share similarities with those found in the oropharynx and nasopharynx of terrestrial mammals[60], cetaceans lack anatomical connections between the mouth and nasopharynx[41]. Thus, the bacteria identified in this study are more likely associated with the respiratory tract rather than the oral cavity of the blue whales. In addition, it is important to note that this composition may vary over time and space, and could be influenced by factors such as fasting, reproductive stage[6,61], or other physiological variables[1,62,63].

Interestingly, four bacterial genera (*Psychrobacter*, *Tenacibaculum*, *Staphylococcus*, and *Corynebacterium*) identified in the blow samples are typically found in the skin of humans and other terrestrial mammals[64,65]. These genera were also identified in the skin microbiota of both captive and free-ranging cetaceans[1,6,28,55,56]. Given that strict protocols were followed to minimize contamination during sampling, processing or sequencing, their presence in whale blow suggests that they colonize the epithelial lining of the blowhole and are forcefully expelled during exhalation[41]. Moreover, *Psychrobacter* and *Tenacibaculum*, contributed significantly to metabolic and environmental processing pathways, suggesting their role in maintaining microbial and host homeostasis. We hypothesize that these bacteria establish a commensal or mutualistic associations with the blue whale, potentially offering a protective role against dysbiosis and environmental stressors. Furthermore, it is possible that these taxa play a crucial role in maintaining respiratory health in this species, and more detailed functional analyses will be necessary in the future to clarify their ecological and physiological roles.

Our study also found that approximately 2% of the microbial diversity in blow samples overlapped that of seawater, indicating some influence of the marine environment on the respiratory bacteriome, possibly as carryover during diving immersions. However, this overlap should be interpreted with caution, as seawater sampling was limited in number and not conducted for every breath sample. The absence of more water samples restricts our ability to fully assess the extent to which environmental microorganisms contribute to the respiratory bacteriome composition. Regardless, the detection of *Psychrobacter*, *Oceanivirga*, *Tenacibaculum*, *Helcococcus*, *Porphyromonas*, *Mycoplasma*, *Dielma*, *Synechococcus*, and *Suttonella*, in blue whale blow, but not in seawater, adds support to the notion that these taxa are intrinsic to the blue whale's respiratory microbiome. Variations in the relative abundance of *Herbaspirillum* sp. in certain samples suggest that whale diving behavior, environmental factors and technical sampling conditions may also influence bacterial detection.

We identified *Bacteroides* spp. in blow Bm042 at a relative abundance of 13.96%. *Bacteroides* spp. can influence airway immune responses by inducing regulatory T cells and associated cytokines and has been shown to promote transient PD-L1 expression and modulate general aeroallergen responses[66]. This genus has also been reported in increased abundance during tracheobronchitis, suggesting potential roles in modulating respiratory immune function[66,67]. Interestingly, whale Bm042 also presented mucus with a yellowish coloration, which may indicate a high concentration of airway mucin, which is associated with various pulmonary diseases[68]. The excessive synthesis of mucin can result from increased neutrophil recruitment, reflecting an acute inflammatory response to bacterial infection in the airways[69,70]. Given its immunomodulatory capacity, the elevated abundance of *Bacteroides* spp. in the blow of whale Bm042 may reflect a role in host immune regulation during localized airway infection or inflammation. Its presence alongside signs of mucus suggests a potential microbial shift and underscores the need to consider both protective and pathogenic roles of *Bacteroides* spp. in the respiratory tract.

Two unidentified species from *Mycoplasma* and *Streptococcus* were found in the blow of two whales. As 16 S rRNA gene sequencing does not allow reliable species-level resolution, our assignments were limited to the genus level, and we acknowledge that the detected *Streptococcus* and *Mycoplasma* taxa may include both commensal and opportunistic members. This taxonomic uncertainty underscores the importance of continued monitoring, since shifts at the genus level can still provide meaningful indicators of host health. Various species within these bacterial genera are known respiratory tract opportunists in mammals[71,72] and have been detected in the lungs of stranded marine mammals[73,74], although their presence does not necessarily indicate disease since they can also occur in healthy hosts[72]. This is essential to consider when studying the bacteriome of individuals, as the type of relationship between host and bacteria can depend on different factors, including the status of the immune system[8,71]. The low prevalence of these pathogens in our study likely suggests that they are not common members of the respiratory bacterial community and highlights the natural diversity of the blue whale respiratory microbiome. As the blue whales migrate through coastal areas, they could become exposed to transient bacteria which do not normally manage to colonize the respiratory epithelium. However, the intense maritime traffic and potential human interactions[75] could act as stressors that affect immune regulation of bacterial communities in susceptible hosts and favor the growth of transient or opportunistic bacteria[68–70,76,77]. Therefore, it is plausible that the detection of these bacteria could indicate underlying health conditions, a suboptimal immune status, or chronic stress in these individuals[78]. We have some support for this argument as the respiratory bacteriome of the two whales that harbored *Mycoplasma* spp. and *Streptococcus* spp. exhibited distinct functional pathway patterns than the other whales, whose bacteriome functional profiles remained largely stable across individuals. Namely the bacteriome of these two whales showed overexpression of nucleotide metabolism, translation, and replication and repair pathways, which have been associated with various diseases in humans[79]. In contrast, pathways involved in lipid metabolism and biosynthesis of other secondary metabolites were underrepresented in these whales, suggesting possible vulnerabilities in their immune responses, as has been shown for humans[50,80]. As these functional profiles were inferred from 16 S rRNA gene data, incorporating functional analyses based on transcriptomics or other omics approaches in future studies would provide a more comprehensive understanding of the microbiome's functional potential. The identification of these bacterial genera and the distinct functional profile of the bacteriome of the whales that harbored them, highlights the need for ongoing monitoring specific microbial taxa, regardless of their perceived roles as commensal, mutualistic or opportunistic in other mammals,

and underscores the importance of considering natural fluctuations in the respiratory bacteriome when assessing the health of blue whales.

Given the current threats facing marine ecosystems, that include habitat degradation, pollution, and other anthropogenic stressors[26], the taxonomic and functional study of the blue whale respiratory bacteriome offers valuable insights into their health and resilience. Respiratory microbiome data can serve as an early warning system by detecting shifts associated with environmental change, disease, or human activities[41]. Monitoring such changes in bacterial composition and functionality over time can help inform conservation efforts and management strategies[23] to protect these iconic species and the ecosystems they inhabit. While our study is based on a modest number of individuals, it represents a meaningful fraction of the population migrating through the Loreto area. Future studies incorporating multiple blow samples per individual could capture temporal variability more effectively, reduce potential sampling bias, and further strengthen the value of microbiome monitoring for conservation and health assessments.

Methods

Sample collection

Using a small Phantom 3® quadrocopter drone (DJI Innovations, China) with floaters and sterile Petri dishes, we collected 17 blows samples from 17 different individual blue whales sampled between February and March 2016 and 2017 in Loreto Bay National Park (25° 51′ 51″ N, 111° 07′ 18″ O) within the Gulf of California, Mexico. The number of sampled whales represents 17% of the estimated 100 blue whales that reside during winter/spring in the southwestern Gulf of California (mark-recapture data from 1994 to 2006[81]. Each whale was photo-identified prior to sample collection[81]. The approach of the drone to the whale was done from the caudal fin towards the head to minimize disturbance, and sampling was conducted at a height between 3 and 4 m above the blowhole[39]. We observed the whale body condition (see Supplementary Material) for each individual and recorded characteristics of their blow, such as color and odor when we were sufficiently close to the whale during sampling.

For each sample, blow droplets were swabbed directly from the Petri dish using one sterile cotton-tipped swab per individual. These were then transferred to a sterile 1.5 mL cryogenic microtube containing 500 µL of 96% molecular grade ethanol and kept frozen in a liquid nitrogen container until processing. To address potential contamination, all necessary precautions were taken, always including the use of sterile gloves and face masks during sample processing. In addition, we included a technical control, termed "LabControl" (a template-free DNA negative extraction control), to identify any contaminants during sample processing. Furthermore, we included a seawater sample, termed "seawater" (a DNA sample extracted from 1mL water collected at a depth of 0.10 m in the same area where we sampled the whale blows), to consider potential sources of bacterial diversity for the blow samples.

DNA extraction, PCR amplification and sequencing

Total DNA was isolated from the whale blow, seawater, and LabControl samples in one batch using a QIAamp® DNA Mini Kit (QIAGEN, Germany). The primers used for sequencing the 16S rRNA V3 and V4 regions were 341F (5′-CCTACGGGNGGCWGCAG) and 785R (5′-GACTACHVGGGTATCTAATCC), which amplified a single product of 444 bp[82]. The PCR program used an initial denaturation step at 95 °C for 3 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension step at 72 °C for 5 min. Each 25 µL-reaction contained 12.5 ng of extracted DNA, 5 µM of barcoded primers and 2x KAPA HiFi HotStart Ready Mix (KAPABIOSYSTEM, Cape Town, South Africa). 1 µl of each sample was run on a 2100 Bioanalyzer (Agilent Technologies, CA, USA) with an Agilent DNA 1000 chip (Agilent Technologies, CA, USA) to verify amplicon size. AMPure XP beads (New England BioLabs, USA) were used to remove unused primers and primer dimers. Amplicons were sequenced over 2- by 250-bp MiSeq at the Unit of Sequencing and Identification of Polymorphisms of the National Institute of Genomic Medicine (Instituto Nacional de Medicina Genómica, Unidad de Secuenciación e Identificación de Polimorfismos, INMEGEN) in Mexico. Dual index barcodes were used to avoid index hopping[83]. The protocol used by INMEGEN can be seen in: https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf.

16 S rRNA sequence data processing

A quality control overview was performed using FASTQC[84]. This allowed us to obtain a quick impression of the data and avoid downstream problems. The raw sequences were then imported into R v.4.2.1[85], where all subsequent analyses were carried out. We used the Divisive Amplicon Denoising Algorithm 2 (*dada2*) v.1.26.0[44] to infer exact ASVs. This approach is preferable over the rough and less precise 16 S rRNA OTU clustering approach[86] that groups the sequences with a 97% identity[87]. First, we filtered by quality (trunQ = 25) and discarded the sequences that presented more than two Ns (maxN = 0) or more than two expected errors (maxEE = 2). Next, the forward and reverse reads for each sample were combined into a single merged contig sequence, and we grouped all identical reads into unique sequences to determine their abundance. After building the ASVs table and removing chimeras (detected using self-referencing), sequences were classified and identified with *Decipher* v.2.26.0[88], using the SILVA rRNA sequence database v.138.1 as the taxa reference[89]. We used *phyloseq* v.1.42.0[90] to classify and remove any sequence not classified at the kingdom and Phylum level or belonging to Archaea, Eukarya, chloroplasts, or mitochondria.

Contamination assessment

At present, there is no standard approach for minimizing or controlling potential contaminants in 16 S rRNA gene sequencing experiments[91]. In our study, we employed two methods to limit and eliminate contaminant

sequences from downstream analyses. First, we used *metagMisc* v.0.5.0[92] to eliminate ASVs with less than ten reads (minabund = 10[93]). Next, we used *Decontam* version 1.18.0[94] to identify sequences that had a negative relationship with DNA concentration. We classified ASVs found in the LabControl sample as potential contaminants if they were identified as true contaminants by the Decontam algorithm. To ensure result accuracy, we then removed the identified contaminant sequences from the analysis.

Respiratory bacteriome analysis and identification of functional pathways

To get a sense of the bacterial community composition of the samples, we used *phyloseq* to identify the distribution of read counts from all the samples and to plot the relative abundance stacked bar plot at genus level. In addition, we used *SourceTracker*[95], a Bayesian approach that allowed us to estimate the proportion of the bacterial community in the blue whale blows samples that are also detected in the seawater sample. Using *microbiome* v.1.20[96], we identified the common core bacteriome (threshold detection set at ≥ 0.02%, prevalence set at ≥ 60%). We selected these values because we wanted a more conservative approach. Finally, we calculated alpha diversity indices: richness (S) and Simpson's diversity index (D) using *vegan* v.2.6.4[97]. Bacterial functional profiles and pathways were inferred from 16 S rRNA gene sequencing data and annotated at a 97% similarity threshold using the ref99NR database as a reference, employing the Tax4Fun2 package[19], which is based on the Kyoto Encyclopedia of Genes and Genomes (KEGG; [20]). All graphs were rendered using Tableau v.2024.3[98] and RAWGraphs v 2.0[99].

Use of animals in research

All methods were performed in accordance with the relevant international guidelines and regulations of Mexican authorities. See ethical approval.

We confirm that our manuscript complies with the ARRIVE Essential 10 guidelines. The study design, experimental groups, and units are clearly described, with exact sample sizes reported. All outcome measures, including the primary outcome, are clearly defined. Statistical methods and assumptions are detailed, along with the software used. Comprehensive information on the animals, including species and probable health status, is provided. Experimental procedures are described with sufficient detail to allow replication, including what was done, how, when, where, and why. Results are presented with descriptive statistics and measures of variability, along with confidence intervals where appropriate.

Data availability

Data from the Sequence Read Archive (SRA) submission will be released upon publication. Accession ID: PRJNA977688.

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Author contributions

C.A.D. collected the samples, performed molecular analyses, analyzed the data, and drafted the manuscript. R.C.A. conducted statistical programming for microbiome analysis and helped interpret the results. D.G. conducted fieldwork, collected samples, and co-supervised the research. K.A.W. conceived, designed, and supervised the research. All authors read and commented on the final draft of the manuscript and gave approval for publication.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
This study complied with the recommendations and methods for approaching blue whales provided by Mexican legislation (NOM-059-SEMARNAT-2010). All procedures were approved by the Bioethics Committee of the Universidad Autónoma de Queretaro (Mexico), and sampling was conducted under permits SGPA/DGVS/00255/16 and SGPA/DGVS/01832/17 issued by the Dirección General de Vida Silvestre to D. Gendron.

Additional information
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Correspondence and requests for materials should be addressed to K.A.-W.