**Supplementary Material**

**Supplementary Video SV1 (supplementary digital media).** Live recording of dysteriid ciliates sampled on petri dish from blowhole exhalation of M1 on July 14, 2021, observed under a dissecting microscope (Olympus SZX16; 3.5–90X).

**Table S1. Relative abundances of the major groups of eukaryotic taxa in the blow microbiome.** Relative abundance numbers represent percentages, and are the averages from each sample set; H=Habitat.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Anti-metazoan 18S rDNA Relative Frequencies** | **M1** | **F1** | **F2** | **H** |
| Unassigned eukaryota | 77.68 | 86.63 | 94 | 8.54 |
| Alveolata, Ciliophora, Phyllopharyngea, Cyrtophoria, Dysteriidae | 22.22 | 12.10 | 6.02 | 0.0 |
| Alveolata, Dinoflagellata, Dinophyceae, Peridiniales, Thoracosphaeraceae | 0.0 | 1.04 | 0.0 | 59.85 |
| Stramenopiles , Gyrista, Bacillariophyceae | 0.0 | 0.0 | 0.0 | 27.10 |
| Archaeplastida, Chlorophyta\_X, Chlorodendrophyceae, Chlorodendrales, Chlorodendraceae*.* | 0.15 | 0.57 | 0.0 | 4.24 |
| Eukaryota, Obazoa, Opisthokonta, Metazoa, Arthropoda, Hexapoda, Insecta | 0.0 | 0.0 | 0.0 | 0.27 |

**Table S2. Relative abundances of 19 core ASVs.** A: 11 ASVs found in all M1, F1, and F2 samples. B: 8 ASVs found in M1, F1, F2, and Habitat samples. Relative abundance numbers represent percentages, and are the averages from each sample set; H=Habitat.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **A** |  | **M1** | **F1** | **F2** | **H** |
| **11 ASVs unique to M1, F1, F2** | Proteobacteria--Gammaproteobacteria--Cardiobacteriales--Cardiobacteriaceae--unc. bacterium | 15.82 | 18.24 | 5.90 |  |
| Bacteroidota--Bacteroidia--Flavobacteriales-Flavobacteriaceae—*Gangjinia sp.* | 8.50 | 5.33 | 0.33 |  |
| Proteobacteria--Gammaproteobacteria--Cardiobacteriales--Cardiobacteriaceae—*Suttonella sp.* | 2.13 | 1.80 | 2.98 |  |
| Proteobacteria--Gammaproteobacteria--Pseudomonadales--Moraxellaceae—*Psychrobacter—unc. bacterium* | 0.43 | 1.49 | 10.24 |  |
| Proteobacteria--Gammaproteobacteria--Pseudomonadales--Moraxellaceae—*Psychrobacter—unc. bacterium* | 0.31 | 0.34 | 3.50 |  |
| Bacteroidota—Bacteroidia—Flavobacteriales—Weeksellaceae—*uncultured bacterium* | 0.40 | 1.41 | 0.59 |  |
| Bacteroidota—Bacteroidia—Bacteroidales—Porphyromonadaceae—*Porphyromonas sp.* | 0.59 | 0.77 | 0.18 |  |
| Bacillota—Clostridia—Lachnospirales—Lachnospiraceae—*Buytrivibrio*--unc. bacterium | 0.76 | 0.73 | 0.05 |  |
| Proteobacteria--Gammaproteobacteria--Alteromonadales--Shewanellaceae—*Shewanella sp.* | 1.08 | 4.13 | 30.81 |  |
| Bacteroidota--Bacteroidia--Bacteroidales--Marinifilaceae—*Marinifilum sp.* | 0.05 | 1.17 | 0.05 |  |
| **B** |  |  |  |  |  |
| **8 ASVs found in M1, F1, F2 and Habitat** | Campilobacterota--Campylobacteria--Campylobacterales--Arcobacteraceae--unc. bacterium | 14.37 | 6.80 | 0.93 | 0.10 |
| Proteobacteria--Gammaproteobacteria--Oceanospirillales--Saccharospirillaceae*--unc. bacterium* | 11.57 | 19.97 | 2.10 | 0.22 |
| Fusobacteriota--Fusobacteriia--Fusobacteriales--Leptotrichiaceae—*Oceanivirga sp.* | 14.53 | 4.47 | 0.70 | 0.09 |
| Bacteroidota--Bacteroidia--Flavobacteriales--Flavobacteriaceae—*Tenacibaculum sp.* | 8.83 | 6.95 | 6.47 | 0.16 |
| Campilobacterota--Campylobacteria--Campylobacterales--Campylobacteraceae—*Campylobacter sp.* | 1.84 | 2.60 | 0.28 | 0.15 |
| Proteobacteria--Gammaproteobacteria--Vibrionales--Vibrionaceae—*Vibrio sp.* | 0.27 | 3.54 | 16.1 | 0.36 |
| Proteobacteria--Gammaproteobacteria--Pasteurellales--Pasteurellaceae—P*hocoenobacter sp.* | 0.20 | 0.15 | 3.39 | 0.09 |
| Fusobacteriota--Fusobacteriia--Fusobacteriales--Fusobacteriaceae—*Fusobacterium sp.* | 0.06 | 0.15 | 0.05 | 0.04 |

**Table S3. Group pairwise significance ANOSIM results** for pairwise comparison groupings based on Bray-Curtis values.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | **Pairwise ANOSIM** | | |  |  |
| **Group Significance** | | | | | | |
| **Group 1** | **Group 2** | **Sample size** | **Permutations** | **R *statistic*** | **p-value** | **q-value** |
| M1 | F1 | 7 | 999 | 0.029 | 0.029 | 0.105 |
| M1 | F2 | 7 | 999 | 0.035 | 0.035 | 0.105 |
| M1 | Habitat | 5 | 999 | 0.213 | 0.213 | 0.261 |
| F1 | F2 | 6 | 999 | 0.104 | 0.104 | 0.208 |
| F1 | Habitat | 4 | 999 | 0.239 | 0.239 | 0.261 |
| F2 | Habitat | 4 | 999 | 0.261 | 0.261 | 0.261 |

**Table S4. Overall group pairwise significance ANOSIM results** for pairwise group significance.

|  |  |
| --- | --- |
|  | **ANOSIM** |
| permutations | 999 |
| sample size | 11 |
| number of groups | 4 |
| R test statistic | 1.0 |
| p-value | 0.001 |

**Table S5. Group pairwise significance of alpha diversity.** Pairwise comparison groupings based on Kruskal-Wallis tests.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Alpha Diversity** | | | | |
| **Kruskal-Wallis Pairwise** | | | | | |
| **Group 1** | | **Group 2** | **H** | **p-value** | **q-value** |
| M1(n=4) | | F1 (n=3) | 0.5 | 0.480 | 0.480 |
| M1(n=4) | | F2 (n=3) | 4.5 | 0.034 | 0.149 |
| M1(n=4) | | Habitat(n=1) | 2.0 | 0.157 | 0.216 |
| F1(n=3) | | F2 (n=3) | 3.9 | 0.049 | 0.149 |
| F1(n=3) | | Habitat(n=1) | 1.8 | 0.180 | 0.216 |
| F2 (n=3) | | Habitat(n=1) | 1.8 | 0.180 | 0.216 |

**Table S6. Overall group significance in alpha diversity.** Based on Kruskal-Wallis tests.

|  |  |  |
| --- | --- | --- |
|  | **Alpha Diversity** | |
|  | *Kruskal-Wallis (all groups)* | |
| H | | 7.4848 |
| sample size | | 0.0579 |
| number of groups | | 4 |

**Table S7. Core microbiota. Top:** List of ASVs that are shared among all whale samples (M2, F2, and F3) but not found in Habitat. **Bottom**: List of ASVs shared among all samples (M2, F2, F3, and Habitat). Columns at right indicate previously reported studies. [W] represents wild animals, [H] represents animals under professional human care.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| This Study | | Previously Reported | | |
|  |  | **Animal** | **Body site** | **Reference** |
| 11 ASVs unique to M1, F1, F2 | Proteobacteria--Gammaproteobacteria--Cardiobacteriales--Cardiobacteriaceae--*uncultured bacterium* | Bottlenose dolphin (*Tursiops truncatus*) [W] | Blow | Apprill et al 2017 |
| Bacteroidota--Bacteroidia--Flavobacteriales-Flavobacteriaceae—*Gangjinia sp.* | Southern resident killer whale (*Orcinus orca*) [W] | Mucus | Rhodes et al 2022 |
| Proteobacteria--Gammaproteobacteria--Cardiobacteriales--Cardiobacteriaceae—*Suttonella sp.* | Blue whale (*Balaenoptera musculus*) [W]  Short-beaked common dolphin *(Delphinus delphis*) [W]  Harbour porpoise[W] (*Phocoena phocoena*)  Striped dolphin [W] (*Stenella coeruleoalba*) | Blow  Oral cavity  Oral cavity  Oral cavity | Dominguez-Sanchez et al 2022  Soares-Castro et al 2019  Soares-Castro et al 2019  Soares-Castro et al 2019 |
| Proteobacteria--Gammaproteobacteria--Pseudomonadales--Moraxellaceae—*Psychrobacter—uncultured bacterium* | Bottlenose dolphin [W] (*Tursiops truncatus*)  Humpback whale [W] (*Megaptera novaeangiliae*) | Mouth, blowhole  Skin, blowhole | Smies et al 2022  Apprill et al 2017 |
| Proteobacteria--Gammaproteobacteria--Pseudomonadales--Moraxellaceae—*Psychrobacter—uncultured bacterium* | Bottlenose dolphin [W] (*Tursiops truncatus*)  Humpback whale [W] (*Megaptera novaeangiliae*) | Mouth, blowhole  Skin, blowhole | Smies et al 2022  Apprill et al 2017 |
| Bacteroidota—Bacteroidia—Flavobacteriales—Weeksellaceae—*uncultured bacterium* | Bottlenose dolphin [H] (*Tursiops truncatus*) | Blow | Biancani et al 2021 |
| Bacteroidota—Bacteroidia—Bacteroidales—Porphyromonadaceae—*Porphyromonas sp.* | Short-beaked common dolphin *(Delphinus delphis*) [W]  Harbour porpoise[W] (*Phocoena phocoena*)Striped dolphin [W] (*Stenella coeruleoalba*) | Oral cavity  Oral cavity  Oral cavity | Soares-Castro et al 2019  Soares-Castro et al 2019  Soares-Castro et al 2019 |
| Bacillota—Clostridia—Lachnospirales—Lachnospiraceae—*Buytrivibrio*--u*ncultured bacterium* | Pygmy sperm whale [W] (*Kogia breviceps)* | Gut | Erwin et al 2017 |
| Proteobacteria--Gammaproteobacteria--Alteromonadales--Shewanellaceae—*Shewanella sp.* | Bryde’s whale [W]  *Balaenoptera brydei*  Bottlenose dolphin  Tursiops truncatus [H] | Skin   Skin | Li et al 2019  Smirnova et al 2017 |
| Bacteroidota--Bacteroidia--Bacteroidales--Marinifilaceae—*Marinifilum sp.* | Short-beaked common dolphin *(Delphinus delphis*) [W]  Harbour porpoise[W] (*Phocoena phocoena*)  Striped dolphin [W] (*Stenella coeruleoalba*) | Oral cavity  Oral cavity  Oral cavity | Soares-Castro et al 2019  Soares-Castro et al 2019  Soares-Castro et al 2019 |
| Proteobacteria--Alphaproteobacteria--Rhodobacterales--Rhodobacteraceae—*Paracoccus sp.* | Killer whale [H] (*Orcinus orca*)  Bottlenose dolphin [H]  *Tursiops truncatus* | Skin  Skin | Chiarello et al 2017  Chiarello et al 2017 |
|  |  |  |  |  |
| 8 ASVs found in M1, F1, F2 and Habitat | Campilobacterota--Campylobacteria--Campylobacterales--Arcobacteraceae--*uncultured bacterium* | Seawater, widespread throughout aquatics  Southern resident killer whale (*Orcinus orca*) | Water column  Mucus | Venancio et al 2022    Rhodes et al 2022 |
| Proteobacteria--Gammaproteobacteria--Oceanospirillales--Saccharospirillaceae*--uncultured bacterium* | Marine waters, coastal sediment, tidal flats  Bottlenose dolphins [W] (Tursiops truncatus) (T. aduncus) | Blowhole | Chen et al., 2009;  Choi et al., 2011; Labrenz et al., 2003; Pinhassi et al., 2007  Lima et al 2012 |
| Fusobacteriota--Fusobacteriia--Fusobacteriales--Leptotrichiaceae—*Oceanivirga sp.* | Atlantic salmon (*Salmo salar*),  common dolphin (*Delphinus delphis*) striped dolphin (*Stenella coeruleoalba*)  harbour seals (*Phoca vitulina*) | Scales    skin, oral | Eisenberg et al . 2016  Palmer et al 2020 |
| Bacteroidota--Bacteroidia--Flavobacteriales--Flavobacteriaceae—*Tenacibaculum sp.* | pelagic fish (parasitic)   Humpback whales (Megaptera novaeangliae) | Scales, external parasite  Skin | Avendaño-Herrera et al 2006  Apprill et al 2016 |
| Campilobacterota--Campylobacteria--Campylobacterales--Campylobacteraceae—*Campylobacter sp.* | grey seals (*Halichoerus grypus*)  terrestrial species (65+) | Gut  Gut | Baily et al 2015  Brooks et al 2023 |
| Proteobacteria--Gammaproteobacteria--Vibrionales--Vibrionaceae—*Vibrio sp.* | Highly common in marine systems    Shellfish  Pacific pilot whales [W](*Globicephala macrorhynchus)*  Bottlenose [H] *(Tursiops truncatus*)  Snapper (Lutjanus campechanus) | Water column    Gills  Gut  Stomach tissue  Gills | Howard and Bennett 1993  Howard and Bennett 1993  Buck and Spotte 1986  Buck and Spotte 1986  Sehnal et al 2021 |
| Proteobacteria--Gammaproteobacteria--Pasteurellales--Pasteurellaceae—P*hocoenobacter sp.* | Striped dolphin [W] (*Stenella coeruleoalba)*   Harbour porpoise [W] (*Phocoena phocoena*) | Oral cavity  Uterus | Foster et al 2000  Godoy-Vitorino et al 2017 |
| Fusobacteriota--Fusobacteriia--Fusobacteriales--Fusobacteriaceae—*Fusobacterium sp.* | Bottlenose dolphin [H] (*Tursiops truncatus*)  California sea lions [H] (*Zalophus californianus*) | Oral and rectal cavity  Oral, rectal, gut | Bik et al 2016  Bik et al 2016 |

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**Supplementary Figures**

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**Figure S1. Taxonomic bar plots.** Relative frequencies of the bacterial taxa present in the blow microbiome of all three animals and habitat water on all sampling dates (M1=male 1; F1= female 1; F2= female 2). Different plots represent taxonomic levels: **A.**

*Class level:* 24 classes identified; **B.**  *Order level***:** 66 bacterial orders identified.

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**Figure S2.** **Alpha rarefaction for all groups.** A. Shannon index analysis; B. Faith’s Phylogenetic Diversity (PD) index; C. Alpha rarefaction of the observed features (i.e representative sequences) for each group. Sequencing depth is represented on the x-axis for all indices.

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