**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*) | BlowOral cavityOral cavityOral cavity | Dominguez-Sanchez et al 2022Soares-Castro et al 2019Soares-Castro et al 2019Soares-Castro et al 2019 |
| Proteobacteria--Gammaproteobacteria--Pseudomonadales--Moraxellaceae—*Psychrobacter—uncultured bacterium* | Bottlenose dolphin [W] (*Tursiops truncatus*)Humpback whale [W] (*Megaptera novaeangiliae*) | Mouth, blowholeSkin, blowhole | Smies et al 2022Apprill et al 2017 |
| Proteobacteria--Gammaproteobacteria--Pseudomonadales--Moraxellaceae—*Psychrobacter—uncultured bacterium* | Bottlenose dolphin [W] (*Tursiops truncatus*)Humpback whale [W] (*Megaptera novaeangiliae*) | Mouth, blowholeSkin, blowhole | Smies et al 2022Apprill 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 cavityOral cavityOral cavity | Soares-Castro et al 2019Soares-Castro et al 2019Soares-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 dolphinTursiops truncatus [H] | SkinSkin | Li et al 2019Smirnova 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 cavityOral cavityOral cavity | Soares-Castro et al 2019Soares-Castro et al 2019Soares-Castro et al 2019 |
| Proteobacteria--Alphaproteobacteria--Rhodobacterales--Rhodobacteraceae—*Paracoccus sp.* | Killer whale [H] (*Orcinus orca*)Bottlenose dolphin [H]*Tursiops truncatus* | SkinSkin | Chiarello et al 2017Chiarello et al 2017 |
|  |  |  |  |  |
| 8 ASVs found in M1, F1, F2 and Habitat | Campilobacterota--Campylobacteria--Campylobacterales--Arcobacteraceae--*uncultured bacterium* | Seawater, widespread throughout aquaticsSouthern resident killer whale (*Orcinus orca*) | Water columnMucus | Venancio et al 2022Rhodes et al 2022 |
| Proteobacteria--Gammaproteobacteria--Oceanospirillales--Saccharospirillaceae*--uncultured bacterium* | Marine waters, coastal sediment, tidal flatsBottlenose dolphins [W] (Tursiops truncatus) (T. aduncus) | Blowhole | Chen et al., 2009;  Choi et al., 2011; Labrenz et al., 2003; Pinhassi et al., 2007Lima 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*) | Scalesskin, oral | Eisenberg et al . 2016Palmer et al 2020 |
| Bacteroidota--Bacteroidia--Flavobacteriales--Flavobacteriaceae—*Tenacibaculum sp.* | pelagic fish (parasitic) Humpback whales (Megaptera novaeangliae) | Scales, external parasiteSkin | Avendaño-Herrera et al 2006Apprill et al 2016 |
| Campilobacterota--Campylobacteria--Campylobacterales--Campylobacteraceae—*Campylobacter sp.* | grey seals (*Halichoerus grypus*)terrestrial species (65+) | GutGut  | Baily et al 2015Brooks et al 2023 |
| Proteobacteria--Gammaproteobacteria--Vibrionales--Vibrionaceae—*Vibrio sp.* | Highly common in marine systemsShellfishPacific pilot whales [W](*Globicephala macrorhynchus)*Bottlenose [H] *(Tursiops truncatus*)Snapper (Lutjanus campechanus)  | Water columnGillsGutStomach tissueGills | Howard and Bennett 1993Howard and Bennett 1993Buck and Spotte 1986Buck and Spotte 1986Sehnal et al 2021 |
| Proteobacteria--Gammaproteobacteria--Pasteurellales--Pasteurellaceae—P*hocoenobacter sp.* | Striped dolphin [W] (*Stenella coeruleoalba)*Harbour porpoise [W] (*Phocoena phocoena*) | Oral cavityUterus | Foster et al 2000Godoy-Vitorino et al 2017 |
| Fusobacteriota--Fusobacteriia--Fusobacteriales--Fusobacteriaceae—*Fusobacterium sp.* | Bottlenose dolphin [H] (*Tursiops truncatus*)California sea lions [H] (*Zalophus californianus*) | Oral and rectal cavityOral, rectal, gut  | Bik et al 2016Bik et al 2016 |

**s**

**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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