

## Pipeline optimization for metabarcoding analysis of prokaryotic community harbored in the respiratory tract of short-finned pilot whales.

*Globicephala macrorhynchus*



**BLOW**

suitable matrix to discover the microbial communities within cetaceans' respiratory tract

1. Sampling



2. Storage  
-80°C

3. DNA extraction



4. Quality and quantitative DNA assessment

5. Illumina MiSeq  
Metabarcoding  
(Amplicon sequencing V3-V4 and V4-V5 regions of 16S rRNA)

6. Microbial Communities analysis

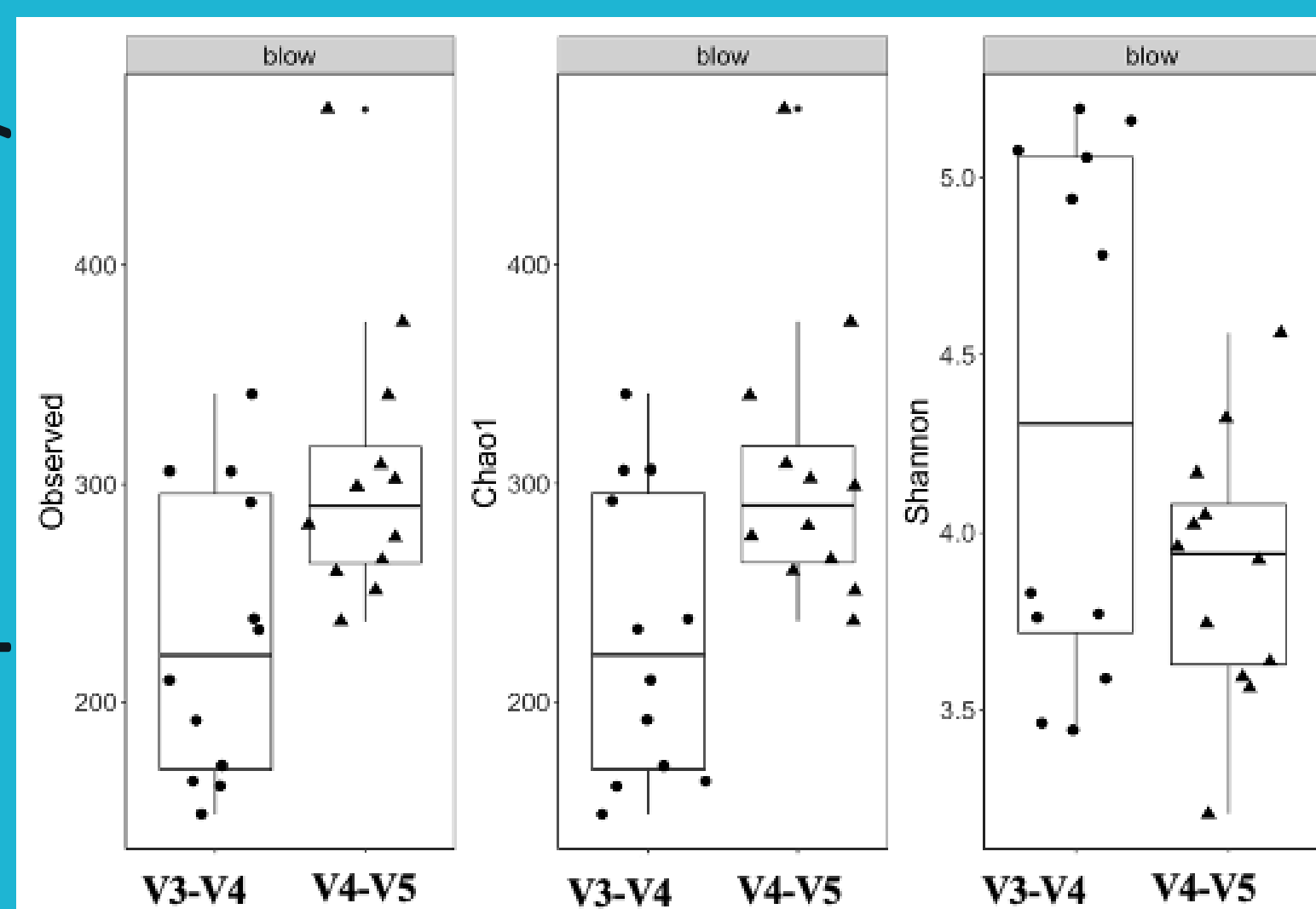


## COMPARISON of 16S rRNA primers (Blow Samples)

**V3-V4 16S rRNA gene**  
Primers: 341F/806R

**V4-V5 16S rRNA gene**  
Primers: 515F-Y/926R

Alpha Diversity



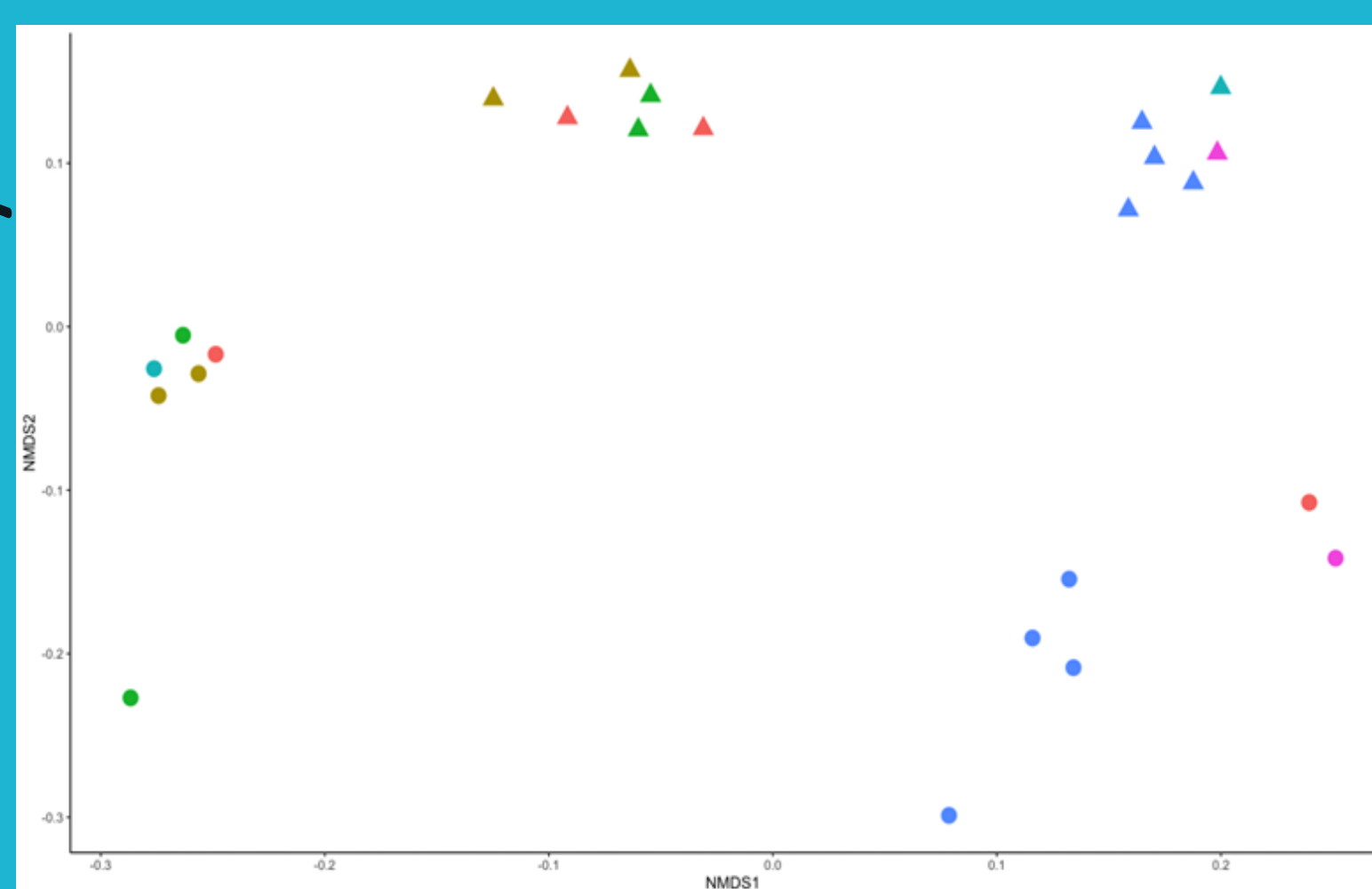
Blow community richness (ASV observed; Chao1) and diversity (Shannon)

significantly different between the two primer sets.

**V4-V5** ca. 32% more bacterial ASVs

**V3-V4** Shannon index 11% higher

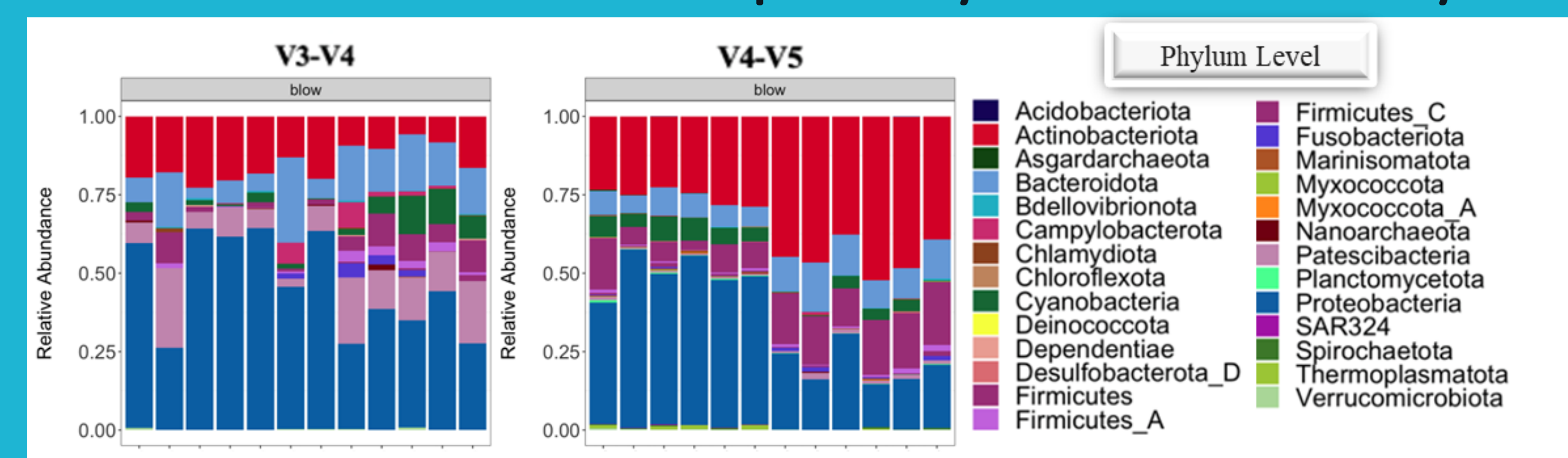
Beta Diversity



Distinction of primer sets communities

Individuals traveling together when they were sampled, appeared in the same cluster in the V4-V5 dataset.

## Characterization of the prokaryotic community



All samples from both datasets shared a main core microbiota in their blow

**Actinobacteriota, Bacteroidota, Firmicutes, Proteobacteria**

**V4-V5** Detected a higher number of taxa however, the majority of them had a relative abundance <1%.

## Core Microbiota

All samples of the V4-V5 dataset in the same cluster (at Genus Level)

## CONCLUDING REMARKS

1<sup>st</sup> study describing the blow microbial diversity in short-finned pilot whales.  
The selection of the primer set to use to analyze blow samples depends on the main goal of the study:

- capture more diversity: **V3-V4** primers set;
- higher microbial richness (microbial rare biosphere): **V4-V5** primers set.

Preliminary evidence that cetaceans host a core group of bacteria associated with the respiratory system.  
The characterization of the respiratory-associated microbial communities can be used as a **future biomarker for the physiological state of the airways in cetaceans.**

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