

## **SUPPLEMENTARY INFORMATION**

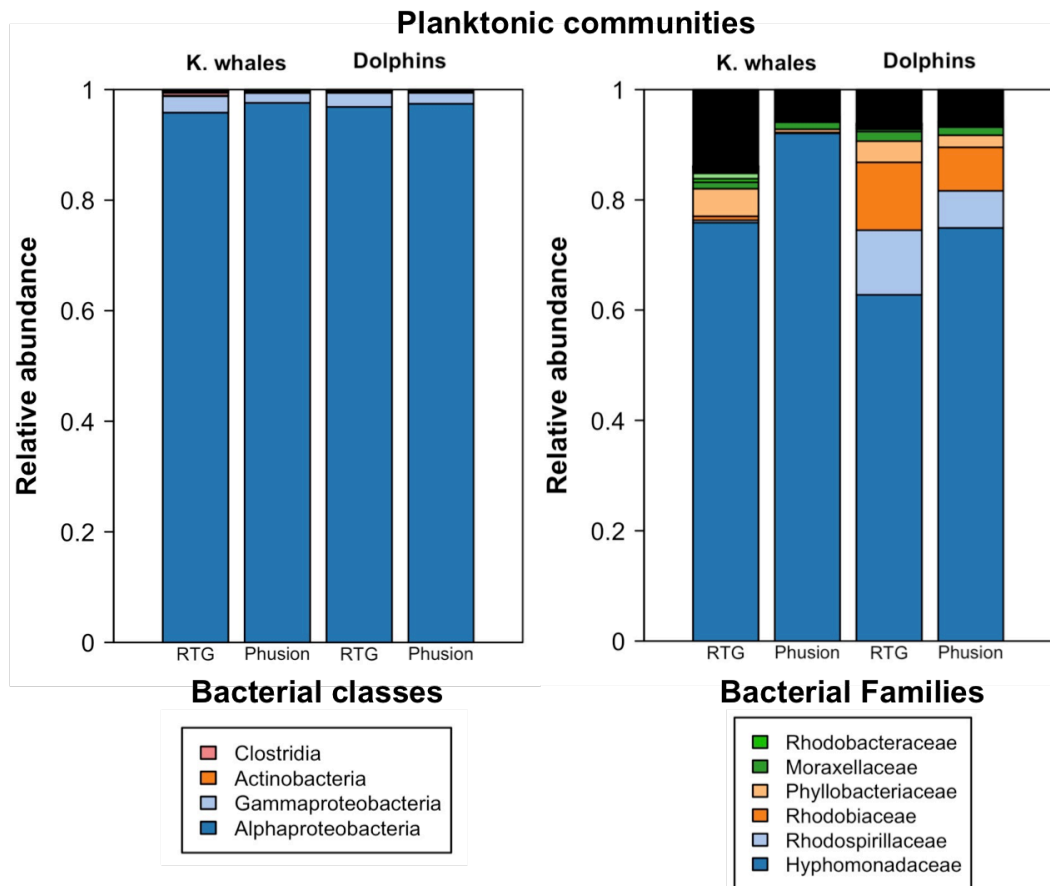
### **Captive bottlenose dolphins and killer whales harbor a species-specific skin microbiota that varies among individuals**

Chiarello M., Villéger S., Bouvier C., Auguet JC., and Bouvier T.

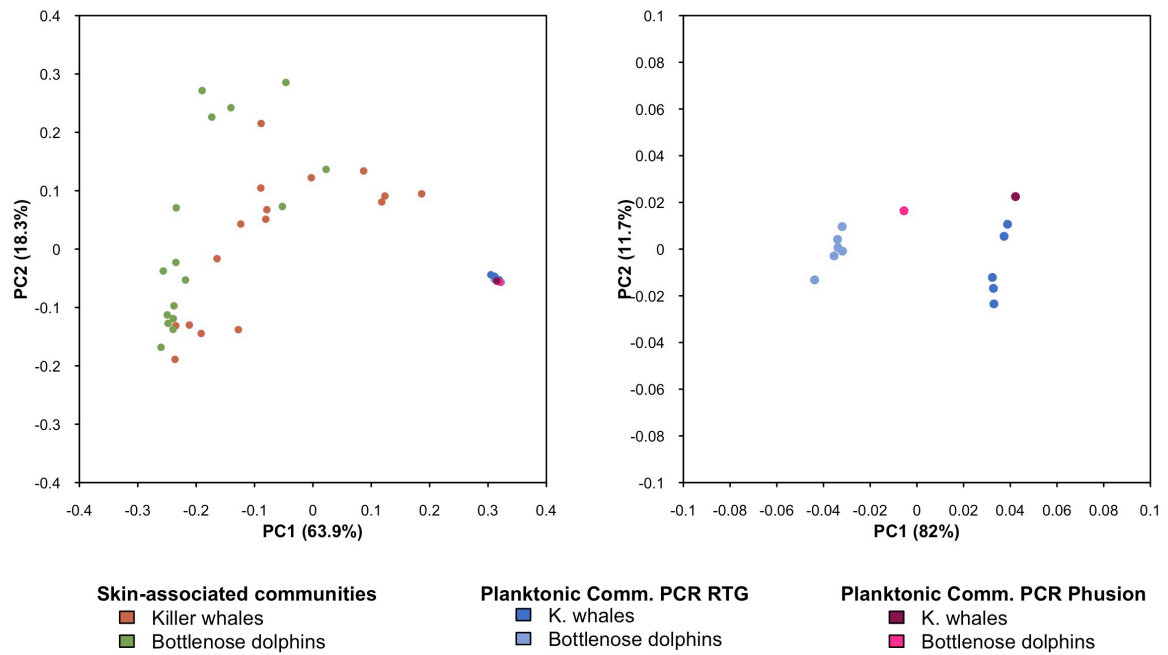
**Supplementary Information S1: Description of the two PCR protocols used in this study and comparison of bacterial composition on water samples**

	<b>Skin samples</b>	<b>Water samples</b>
Kit	Phusion High-Fidelity	PuRe <i>Taq</i> Ready-To-Go PCR Beads
Total vol. ( $\mu$ L)	20	25
DNA vol. ( $\mu$ L)	2	5
Initial denaturation	1 min 98°C	2 min 94°C
PCR cycle	1 min 94°C; 40s 57.8°C; 30s 72°C	1 min 94°C; 40s 57.8°C; 30s 72°C
Nb. of cycles	35	35
Final extension	10 min 72°C	10 min 72°C

**S1-Table 1:** PCR reagents and conditions used for the two sample types studied. Skin DNA and water DNA were respectively amplified using the Phusion High-Fidelity DNA polymerase (Biolabs, Ipswich, USA) and PuRe *Taq* Ready-To-Go PCR Beads (Amersham Biosciences, Freiburg, Germany) following manufacturer's instructions.

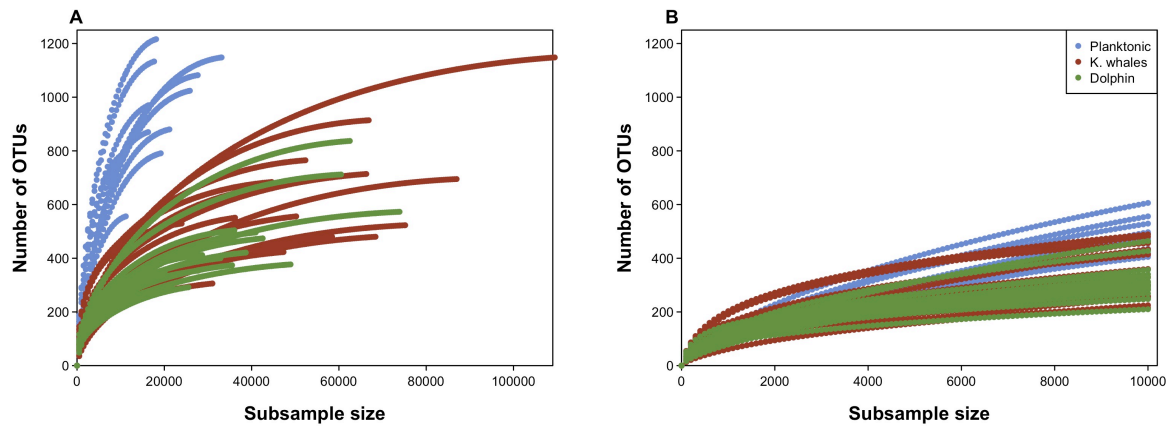


**S1-Fig 1: Most abundant classes and families in planktonic communities analyzed using Phusion and Ready-To-Go kits.** Both PCR types were performed on the same DNA extracted from animals' surrounding water. Class-level bacterial composition was very similar between both PCR types.



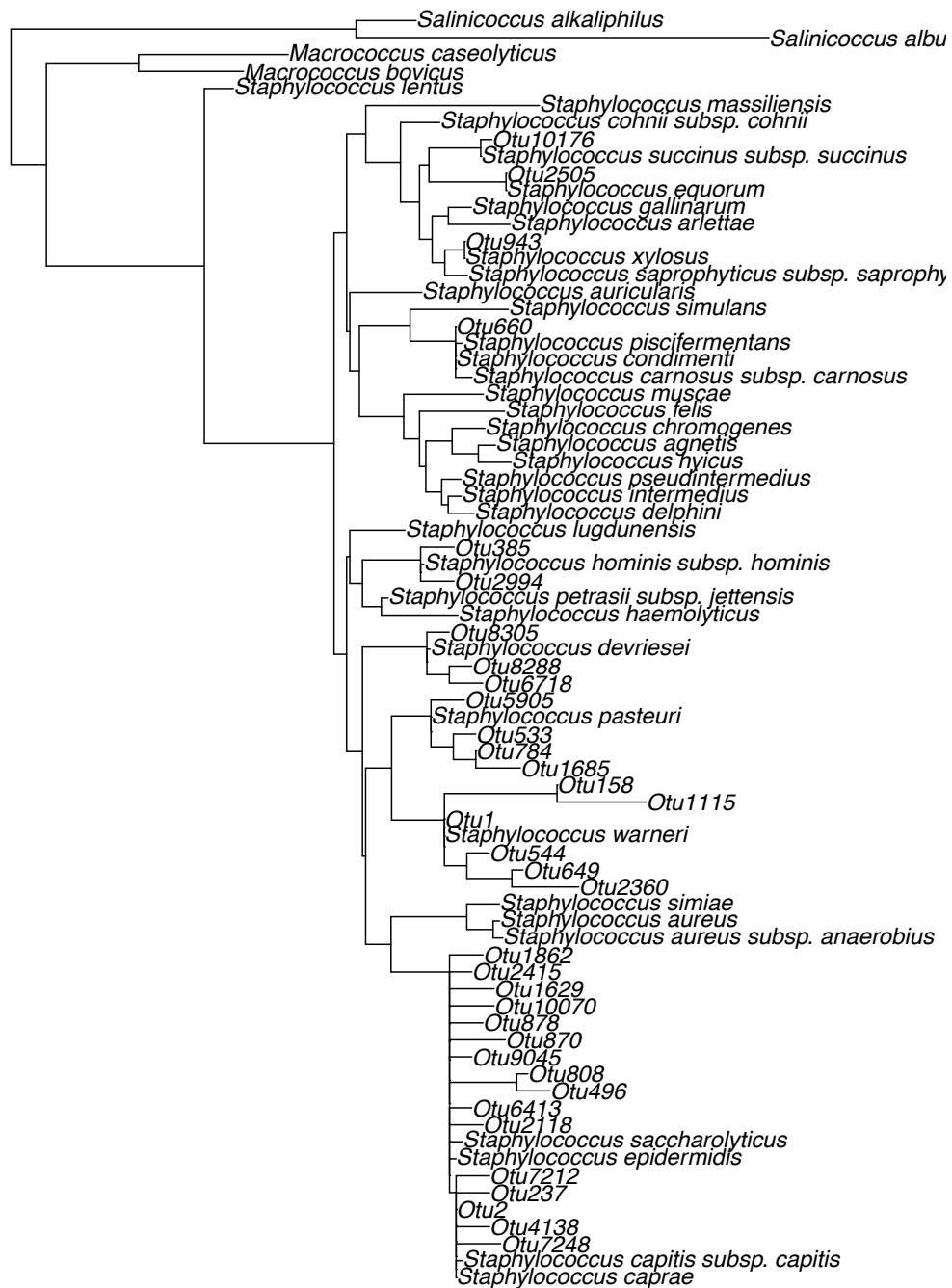
**S1-Fig 2:** PCoAs based on Weighted Unifrac, showing planktonic communities analyzed using both PCR types. On (A) panel, all samples included in this study *plus* water replicates that could be amplified using Phusion kit. On (B) panel, only planktonic communities were displayed. Water replicates from both animals' pools clustered together, whatever the PCR type used for amplification.

## Supplementary Information S2: Assessing the effect of sequences subsampling



**S2-Figure 1: Rarefaction curves** obtained on raw sequencing outputs (*i.e.* with uneven number of sequences read for each sample) (A) and after random subsampling of 10,000 reads for each sample, and (B) number of OTUs at each rarefaction size. Subsampling did not affect the ranking of OTUs richness between planktonic and skin-associated communities (See results in main text).

**Supplementary Information S3: phylogenetic analysis of *Staphylococcus* sp.**

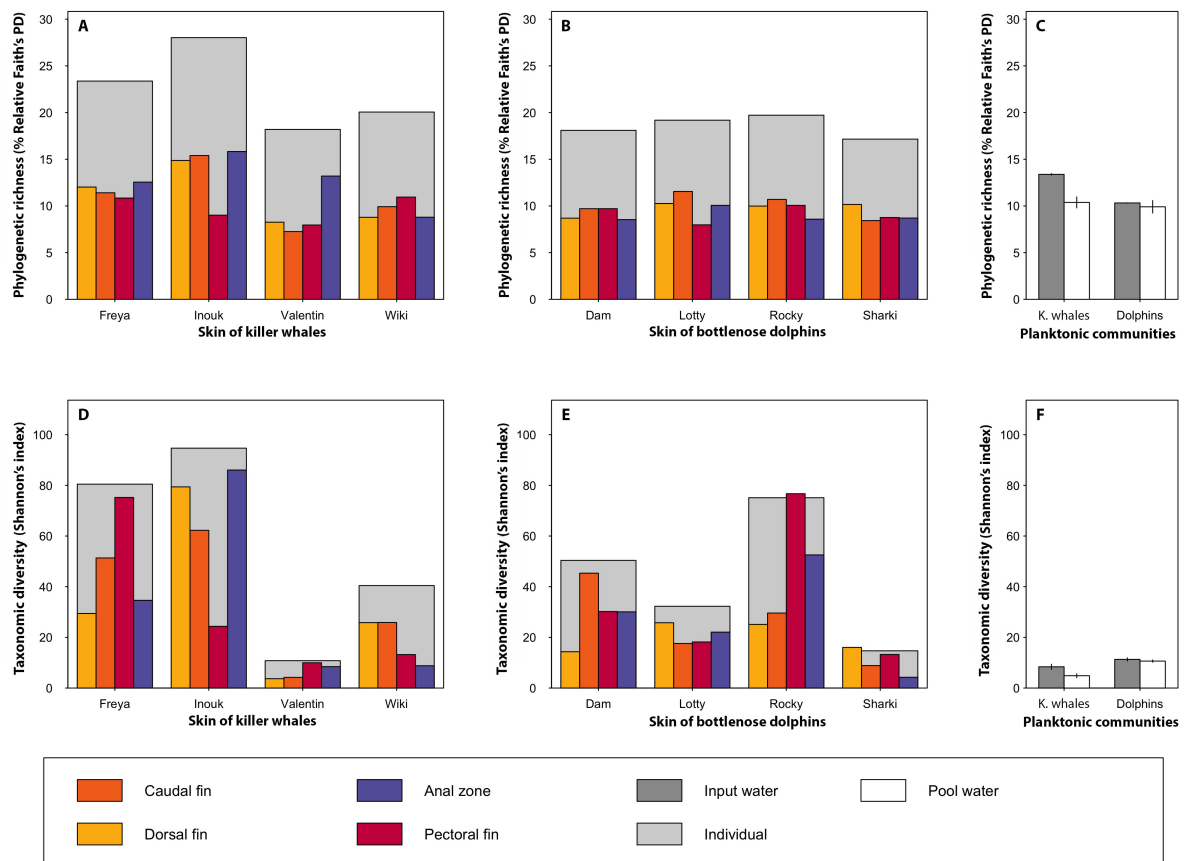


**S3-Fig 1: Phylogenetic relationships of sequences affiliated to *Staphylococcus* sp.** Sequences from this study are labelled as «OTUXX», while other sequences were downloaded from SILVA database. Macrocooccus sp and Salinicoccus sp were used as outgroup. Accession numbers from downloaded sequences are available in the table below.

**S3-Table 1:** Accession numbers of sequences downloaded from SILVA database used to make phylogenetic analysis of Staphylococcus-affiliated sequences.

Species	Accession
<i>Macrococcus bovicus</i>	Y15714.1.1546
<i>Macrococcus caseolyticus</i>	KP191046.1.1466
<i>Salinicoccus albus</i>	EF177692.1.1478
<i>Salinicoccus alkaliphilus</i>	AF275710.1.1459
<i>Staphylococcus agnetis</i>	HM484980.1.1409
<i>Staphylococcus arlettae</i>	AB009933.1.1494
<i>Staphylococcus aureus</i>	L36472.3194.4748
<i>Staphylococcus aureus subsp. anaerobius</i>	D83355.1.1476
<i>Staphylococcus auricularis</i>	D83358.1.1475
<i>Staphylococcus capitis subsp. capitis</i>	L37599.1.1469
<i>Staphylococcus caprae</i>	AB009935.1.1492
<i>Staphylococcus carnosus subsp. carnosus</i>	B009934.1.1493
<i>Staphylococcus chromogenes</i>	D83360.1.1475
<i>Staphylococcus cohnii subsp. cohnii</i>	D83361.1.1477
<i>Staphylococcus condimenti</i>	Y15750.1.1545
<i>Staphylococcus delphini</i>	AB009938.1.1493
<i>Staphylococcus devriesei</i>	FJ389206.1.1537
<i>Staphylococcus epidermidis</i>	D83363.1.1475
<i>Staphylococcus equorum</i>	AB009939.1.1494
<i>Staphylococcus felis</i>	D83364.1.1475
<i>Staphylococcus gallinarum</i>	D83366.1.1477
<i>Staphylococcus haemolyticus</i>	X66100.1.1544
<i>Staphylococcus hominis subsp. hominis</i>	X66101.1.1544
<i>Staphylococcus hyicus</i>	D83368.1.1476
<i>Staphylococcus intermedius</i>	D83369.1.1476
<i>Staphylococcus lentus</i>	D83370.1.1480
<i>Staphylococcus lugdunensis</i>	AB009941.1.1492
<i>Staphylococcus massiliensis</i>	EU707796.1.1477
<i>Staphylococcus muscae</i>	FR733703.1.1537
<i>Staphylococcus pasteurii</i>	AB009944.1.1494
<i>Staphylococcus petrasii subsp. jettensis</i>	JN092118.1.1444
<i>Staphylococcus piscifermentans</i>	Y15754.1.1544
<i>Staphylococcus pseudintermedius</i>	AJ780976.1.1512
<i>Staphylococcus saccharolyticus</i>	L37602.1.1527
<i>Staphylococcus saprophyticus subsp. saprophyticus</i>	AP008934.743716.745270
<i>Staphylococcus simiae</i>	AY727530.1.1478
<i>Staphylococcus simulans</i>	D83373.1.1476
<i>Staphylococcus succinus subsp. succinus</i>	AF004220.1.1548
<i>Staphylococcus warneri</i>	L37603.1.1470
<i>Staphylococcus xylosus</i>	D83374.1.1477

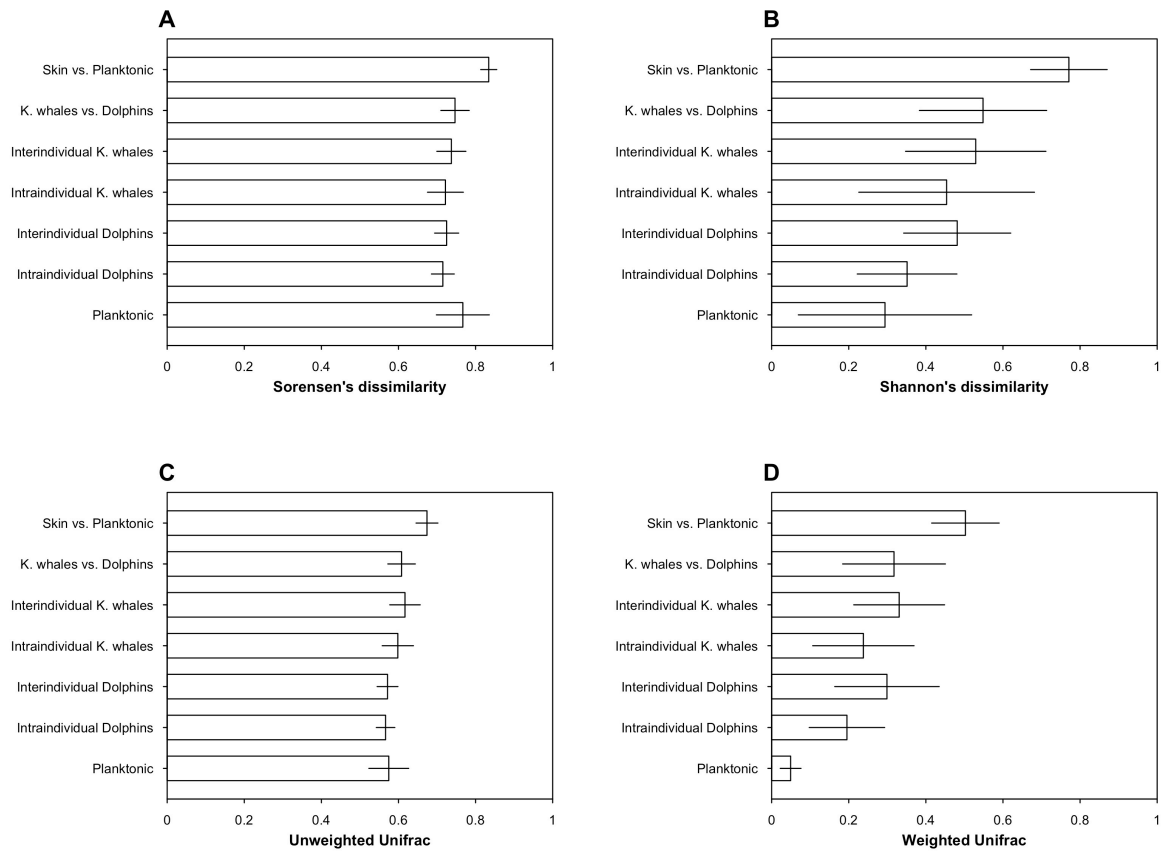
## Supplementary Information S4: Alpha-diversity recovered from samples.



**S4-Fig 1: Diversity of skin-associated communities of killer whales (A and D), dolphins (B and E), and of planktonic communities (C and F).** The first row of plots (A-C) illustrates the phylogenetic richness observed in a sample, measured with relative Faith PD, *i.e.* the sum of the height of all branches from the phylogenetic tree recovered in the sample corrected by the total height of the phylogenetic tree, and converted into percentage. The second row of plots (D-F) illustrates taxonomic diversity, measured by the index of Shannon, which is based on the relative abundances of OTUs recovered in a sample. Total diversity of each individual (*i.e.* accounting for all body zones sampled) is illustrated with larger light-gray bars. Bars on panels C and F represent the mean (and associated standard deviation) of OTU richness and phylogenetic diversity for planktonic communities ( $n=3$  water samples). “Pool” refers to animal’s surrounding water, and “Input” refers to the water sampled at the exit of filtering system.

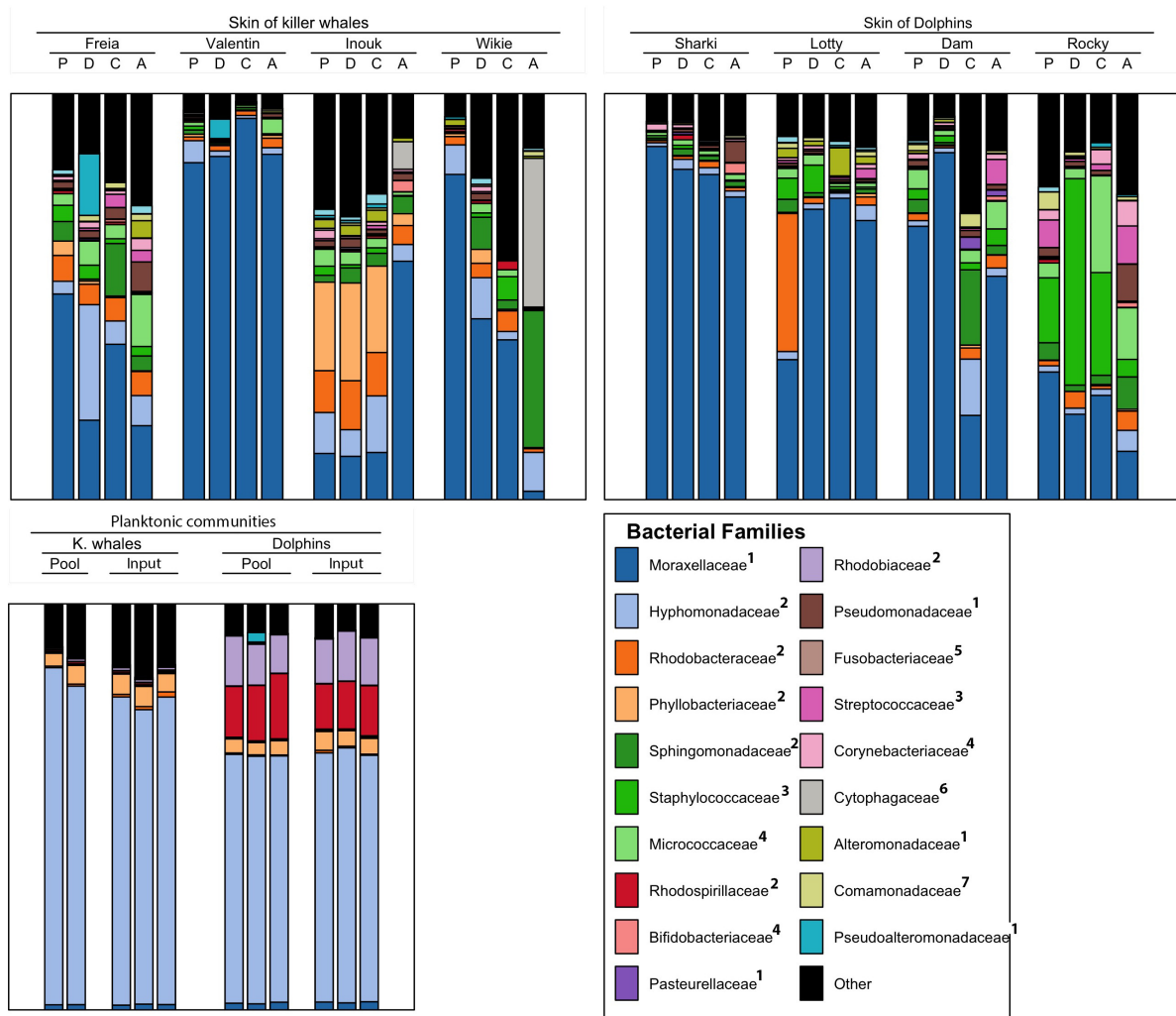


## Supplementary Information S5: Dissimilarity between microbiotas



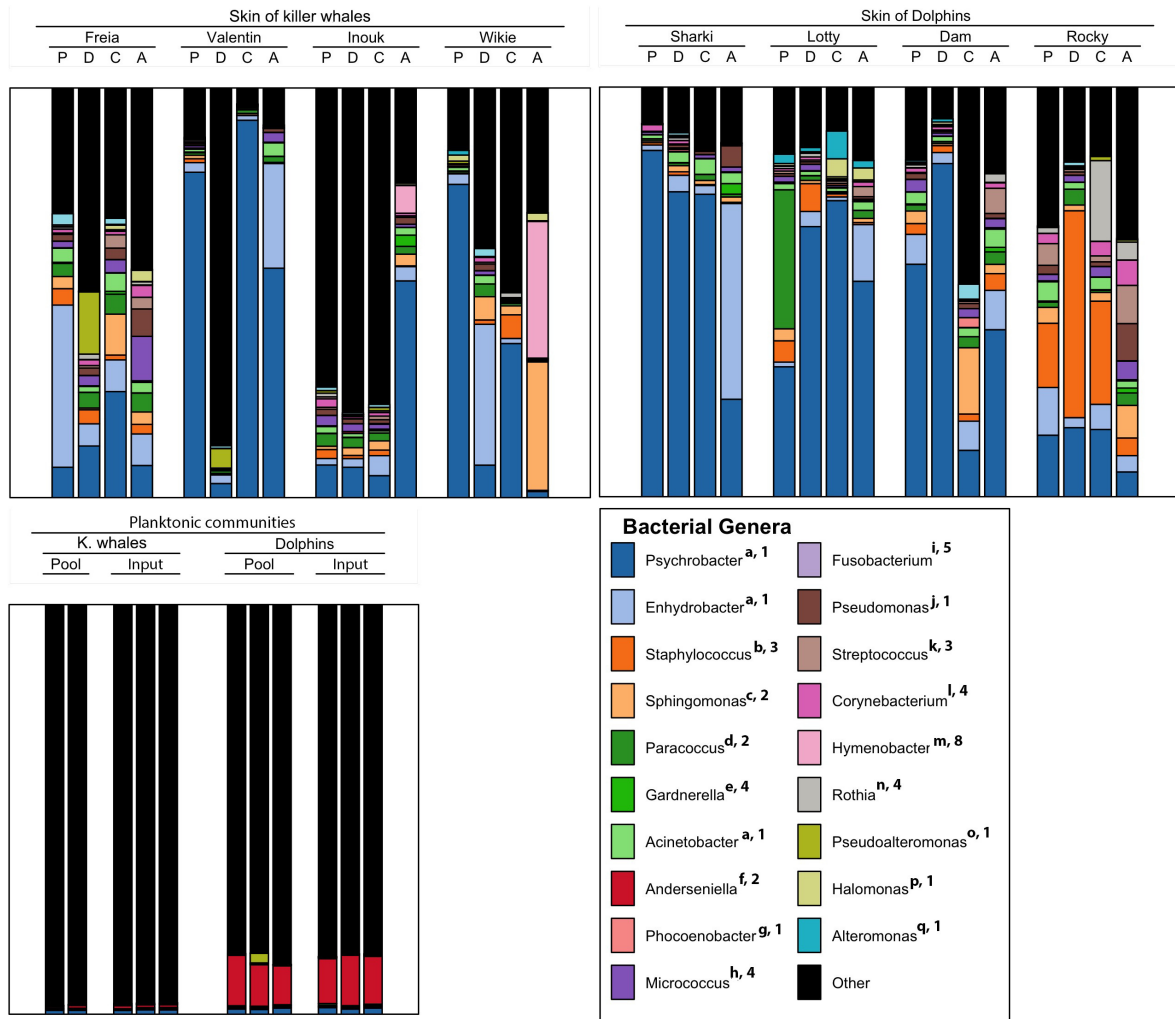
**S5-Fig 1: Average of pairwise dissimilarity between sets of microbiotas for two facets and two components of biodiversity.** A: Taxonomic (based on OTUs) compositional (insensitive to relative abundances of OTUs) dissimilarity. B: taxonomic structural (weighted by OTUs relative abundances) dissimilarity. C-D: phylogenetic compositional and structural dissimilarities. Error bars represent the standard deviation of dissimilarity indices among pairs of microbiotas.

## Supplementary Information S6: Structure of microbiotas



**S6-Fig 1: Mean relative abundance of bacterial families** in skin-associated communities of common bottlenose dolphin and killer whales, and planktonic communities. P: upper side of pectoral fin, D: dorsal fin, C: upper side of caudal fin, A: anal zone. “Pool” refers to animal’s surrounding water, and “Input” refers to the water sampled at the exit of pipe from filtering system.

<sup>1</sup>: Gammaproteobacteria; <sup>2</sup>: Alphaproteobacteria; <sup>3</sup>: Bacilli; <sup>4</sup>: Actinobacteria; <sup>5</sup>: Fusobacteria; <sup>6</sup>: Sphingobacteria; <sup>7</sup>: Betaproteobacteria.



**S6-Fig 2: Mean relative abundance of bacterial genera** in skin-associated communities of common bottlenose dolphin and killer whales, and planktonic communities. P: upper side of pectoral fin, D: dorsal fin, C: upper side of caudal fin, A: anal zone. “Pool” refers to animal’s surrounding water, and “Input” refers to the water sampled at the exit of pipe from filtering system.

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<sup>a</sup>: Moraxellaceae; <sup>b</sup>: Staphylococcaceae; <sup>c</sup>: Sphingomonadaceae; <sup>d</sup>: Rhodobacteraceae;

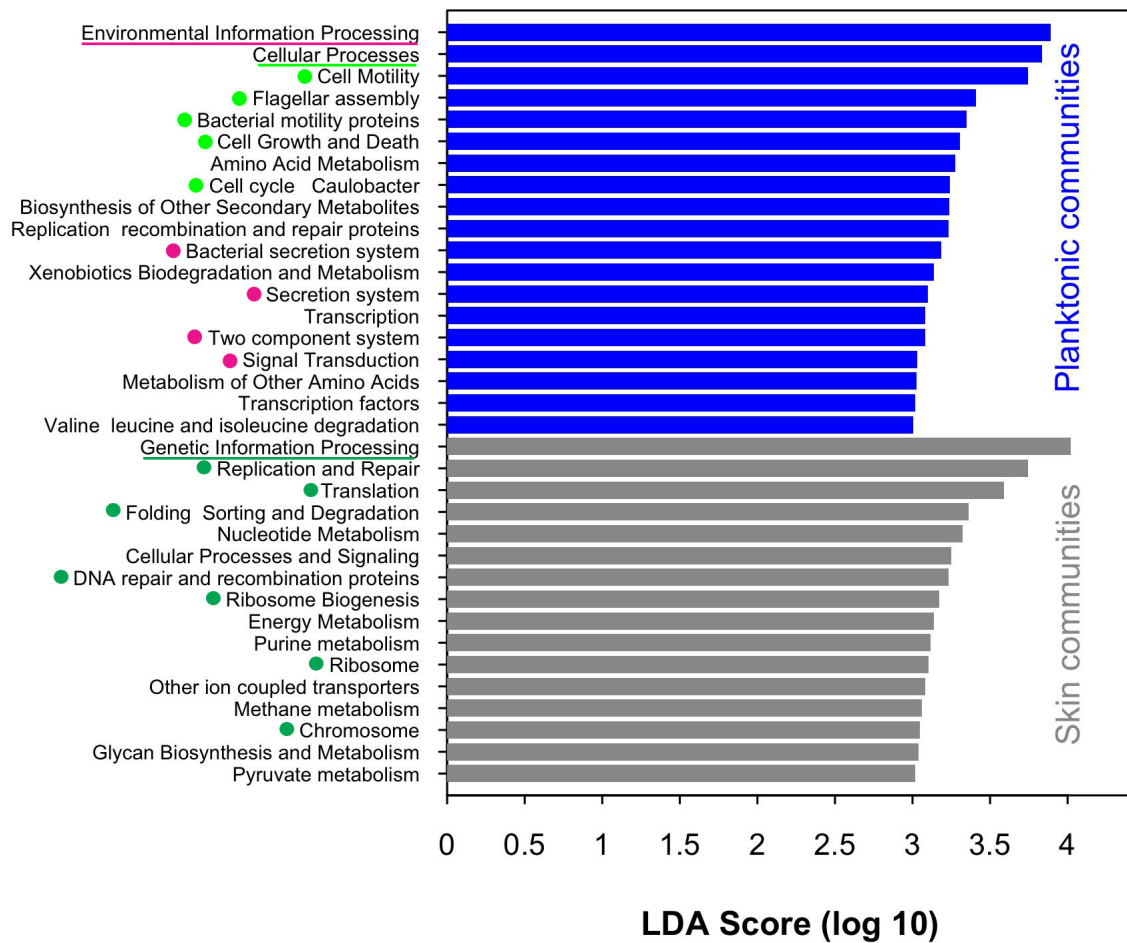
<sup>e</sup>: Bifidobacteriaceae; <sup>f</sup>: Rhizobiaceae; <sup>g</sup>: Pasteurellaceae; <sup>h</sup>: Micrococcaceae;

<sup>i</sup>: Fusobacteriaceae; <sup>j</sup>: Pseudomonadaceae; <sup>k</sup>: Streptococcaceae; <sup>l</sup>: Corynebacteriaceae;

<sup>m</sup>: Flavobacteriaceae; <sup>n</sup>: Actinomycetaceae; <sup>o</sup>: Pseudoalteromonadaceae; <sup>p</sup>: Halomonadaceae;

<sup>q</sup>: Alteromonadaceae.

**Supplementary Information S7: LefSe analysis performed on KEGG pathways**



**S7-Fig 1: LefSe analysis performed on KEGG pathways**, showing the most discriminating pathways (higher LDA score) between skin-associated and planktonic communities. functional categories associated with highest LDA score for each type of microbial communities are underlined, and pathways belonging to these categories are illustrated with dots of corresponding colors.