

Effect of access to human-subsidized food on bacterial gut microbiome of the Neotropical cormorant (*Phalacrocorax brasilianus*).

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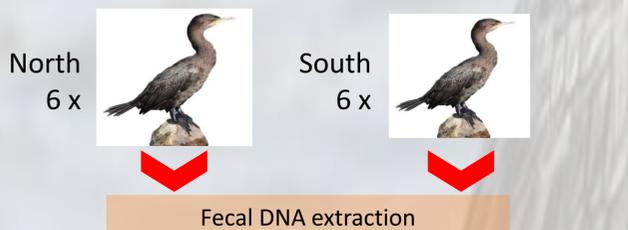
ABSTRACT. Aquatic birds represent one of the most abundant and ubiquitous avian species and are natural reservoirs for a large variety of emerging pathogens. The Neotropical cormorant (*Phalacrocorax brasilianus*) is one of the most abundant marine birds of the Americas, using different natural as well as urban habitats. In the present study, we employed DNA metabarcoding to explore bacterial gut microbiome from two cormorant populations in Chile: Valdivia population, which uses natural and urban spaces and is subsidized by local fish market; and Chillan population, which feeds exclusively on fishes from natural habitats. After MiSeq sequencing and QIIME analysis, we found 2,633 bacterial operational taxonomic units (OTUs) using the 16S rRNA: 79% of the OTUs were shared between both populations, whereas 14% were exclusive for Chillan and 7% were exclusive for Valdivia. We also found that 615 from 2,633 OTUs (23.4%) showed significant differences in their relative abundances between populations. Interestingly, *Campylobacter* exhibited a higher relative abundance in Valdivia (75.6%), whilst *Pepstreptococcus*, *Coprococcus* and *Clostridium* were more abundant in Chillan. Richness and phylogenetic diversity did not differ between populations, but community structure did. PICRUSt analysis revealed that functional composition differed between populations, and genes related to immune diseases and biosynthesis of secondary metabolism were more abundant in Chillan population, whereas genes related to catabolism and signal transduction were more abundant in Valdivia population. DNA metabarcoding provides a straightforward approach to study microbiomes associated to wildlife. The Neotropical cormorant seems to be a natural reservoir of *Campylobacter*, a foodborne bacterial pathogen, which has an astonishing abundance in the human-subsidized Valdivia population. Antibiotic use by aquaculture industry can speed up the development of antibiotic resistance in *Campylobacter*. Consequently, antibiotic resistance could have significant effects on community structure of gut microbiome in wild birds and on the dispersal of antibiotic-resistant bacteria.

INTRODUCTION

Aquatic birds represent one of the most abundant and ubiquitous avian species, which can be found both in natural as urbanized areas. The use of different habitats can influence the quantity and variability of food items at which a bird could have access. For example, birds can exploit resources influenced by wastewaters, and agriculture or aquaculture activities. It is widely known that diet changes gut microbiota (i.e. bacterial community associated with animal gut), and these changes can be more significant if individuals exploit food resources exposed to contaminants such as heavy metals, pesticides and antibiotics.

In the present study, we studied the gut microbiota of two Chilean populations of the Neotropical cormorant (*Phalacrocorax brasilianus*) separated by 450 km. These population differ in the habitat use and the food source: the northern population (Chillán) catch sea fishes; whereas the southern population (Valdivia) feed river fished as well as fish carcasses from the local fish market (salmon, snoek). Therefore, we expect that both populations harbor different gut microbiota because they feed on different food items. To characterize the gut microbiota, we used DNA metabarcoding using the 16S rDNA as molecular marker to estimate bacterial composition as well as to determine the community structure associated with each cormorant population.

METHODOLOGY



Using QIIME, sequences were clustered into OTUs (Operational Taxonomic Units) with 97% nucleotide identity. Representative OTU sequences were used for taxonomic assignments using Greengenes database and to build a phylogenetic tree.

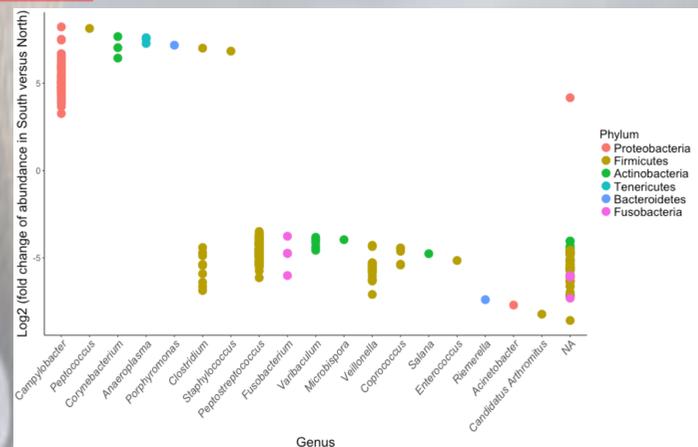
OTU	Species	Sample1	Sample2	Sample3
1	E.coli	17	0	335
2	S.aurus	231	11800	45
3	unknown	30	0	0
...

Counts of OTUs per sample

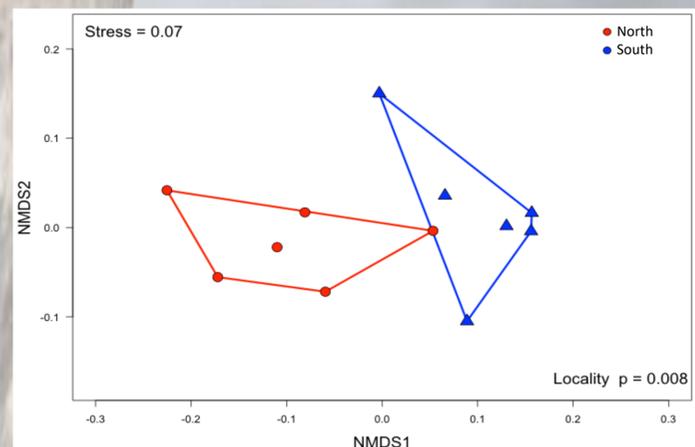
PICRUSt

Functional predictions based on sequenced genomes and phylogenetic relationships.

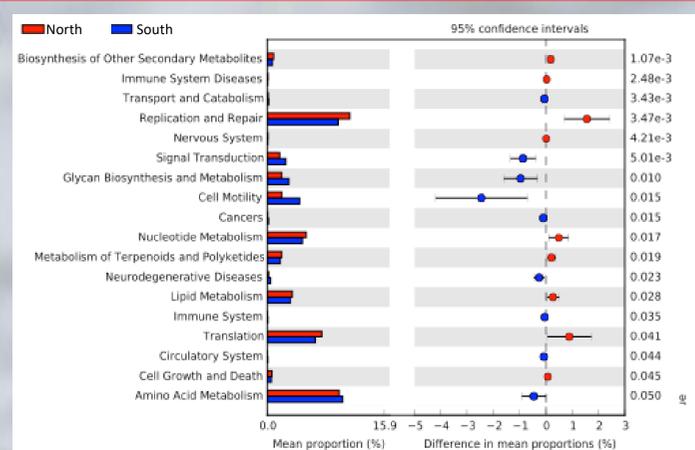
RESULTS



R1. Comparison (DESeq analysis) to OTUs showing differential abundances (after FDR p-adjustment) between southern (positive log₂ values) and northern populations (negative log₂ values). Gut microbiota of southern bird population is dominated by *Campylobacter*, whilst *Pepstreptococcus*, *Coprococcus* and *Clostridium* were more abundant in guts of birds from the northern population.



R2. NMDS plot representing the weighted-Unifrac distances (phylogenetically-corrected ecological distances) among samples. Northern and southern populations establish separated clustered, which is statistically supported (PERMANOVA test). This result indicates that gut microbiota community structure differ between bird populations.



R3. Functional categories that exhibit significant differences (after FDR p-adjustment) between bird populations. This approach can be useful to determine differences in the metabolic functions between bacterial communities as showed here. However, the real impact on pathogenicity or metabolic coupling with bird host must to be tested through metatranscriptomic approaches.

CONCLUSIONS

- Birds using urbanized areas and feeding human-subsidized food have a particular gut microbiota compared to birds using natural habitats.
- High prevalence of *Campylobacter*, a known pathogenic bacteria, in human-subsidized birds could be because they feed on aquaculture-farmed fished (e.g. salmon), which has been exposed to antibiotics as part of antimicrobial treatments.
- Effects of pathogenic bacteria on normal gut microbiota and dispersal of antibiotic resistance should be investigated to know their impact on human and wildlife health.

REFERENCES

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